Third scientific report on the investigations of the Imperial Cancer Research Fund / by E.F. Bashford.

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Bashford, E. F. 1873-
Imperial Cancer Research Fund (Great Britain)

Publication/Creation

London : Taylor and Francis, 1908.

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THIRD SCIENTIFIC REPORT

ON THE INVESTIGATIONS

OF

THE IMPERIAL CANCER RESEARCH FUND.

Under the direction of the Royal College of Physicians of London
and the Royal College of Surgeons of England.

BY

Dr. E. F. BASHFORD,
General Superintendent of Research,
and
Director of the Laboratory.

3

Published by the Authority of the Executive Committee.

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Professor Benjamin Moore
with kind regards
E. F. Bashford.

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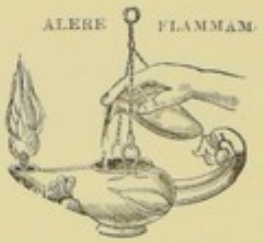
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INTRODUCTION.

The First Scientific Report was prepared in the spring of 1904, and the Second a year later. Thus over three years have intervened between the appearance of the Second and the Third Scientific Report.

The three years devoted to the work on which this Report is based, will not appear excessive to those who realise how many detailed observations have to be collected, in order that a wide and safe basis may be assured on which to build up the experimental study of cancer. We are still in the beginnings of the systematic experimental investigation of cancer, in that preliminary phase where lengthened deliberation, the patient repetition, the augmentation and confirmation of seemingly important primary observations are required as guarantees of the soundness of its foundations. Caution is as necessary as pertinacity and enthusiasm, and, although progress is slow, the following pages are evidence that since the Second Scientific Report was issued a great deal of work has been going on in the laboratories continuously and profitably.

The Second Scientific Report was followed by many criticisms. Much controversy has been spared by refraining as far as possible from rejoinders, and by waiting till the investigation of more extensive material necessitated modification, or justified the attitude taken up in that

Report. It was based chiefly upon the study of a relatively small number of sporadic cases of cancer in mice and on a very detailed investigation of Jensen's tumour, the value of which, as material for the experimental study of cancer, was then not generally recognised, and its very nature even disputed, both at home and abroad.

With the lapse of time we have been able to extend the conclusions arrived at in 1905 to a most extensive and varied material which includes seventy propagable malignant new growths of the mouse. These tumours comprise not only epithelial growths—carcinomata and malignant adenomata—but also connective tissue growths, spindle, polymorphous, and osteo-chondro-sarcomata. Our conclusions have also been confirmed by many independent investigators; while for our part we have been able to confirm observations first made elsewhere. The importance we attached to the study of Jensen's tumour as a true and highly malignant carcinoma capable of propagation has been confirmed.

While in the First and Second Scientific Reports the investigations recorded were of a more general kind, and at every point involved the collaboration of the whole staff, the last three years have seen a progressive division of labour, due to the expansion of the investigations, the necessary concomitant specialisation, and the increased amount of administrative work. The subject matter of this report therefore is dealt with in separate papers by those more directly responsible for special investigations.

In the Second Scientific Report the more likely theories and hypotheses advanced in explanation of cancer were reviewed, and discarded owing to their being incompatible with the results of comparative and experimental study. As was to be expected, all of them still find advocates, but it is not now proposed to discuss them again in detail. With the exceptions referred to below, they will only be alluded to incidentally in the course of the descriptions of special investigations.

All the experimental evidence which was cogent in leading us to discard these hypotheses three years ago, still retains its cogency. Ribbert has considerably modified his hypothesis that carcinoma is due to dislocation of epithelium from its normal association with surrounding epithelium in consequence of chronic inflammatory changes in the connective tissues, whereby they are prepared beforehand to become a soil suitable for the growth of epithelium. Ribbert's own papers [1] and the careful contributions of Victor Bonney [2] in this country are mere reiteration of the observations upon which the hypothesis was originally based. All the additional and new experimental observations made during the past three years, point to the connective-tissue modifications described by Ribbert and Bonney, *e. g.*, around early epitheliomata, as being secondary and not primary fundamental changes.

Much of the pessimism with which the future of the investigation of cancer is still regarded is due to the persistence of the widely disseminated but ill-defined idea, that malignant new growths, as a whole, are of "congenital origin." This idea arises from a generalisation of the fact that certain forms of cancer are undoubtedly associated with congenital abnormalities.

In the Second Scientific Report attention was called to the association of cancer with peculiar and very different forms of irritation, and to the impossibility of reconciling this fact with a congenital origin in all cases. As the data have increased from year to year the mediate relation between various irritants and cancer in particular sites of the body, has become more and more significant.

The study of the incidence of cancer as determined by irritants in man, demonstrates absolutely that the generalisation of the idea of a congenital or embryonic origin is incorrect and this conclusion agrees

[1] Beiträge zur Entstehung der Geschwülste, i. ii. iii. Ergänzung zur "Geschwulstlehre," Bonn, 1906-8.

[2] The connective tissue in carcinoma and in certain inflammatory states that precede its onset. 'Lancet,' May 23rd, 1908.

with the results of experiments, and notably with the experimental production of sarcoma referred to below.

Neglecting cancer in animals—although generalisations to carry any weight at all must be extended to them—and to take but three examples in the case of man, cancer of the skin of the abdomen is practically unknown in Europe, yet it is most extraordinarily frequent in Kashmir ; this is not due to a distribution of congenital "germs" in the skin of the abdomen of Europeans and natives of India other than that in Kashmiris, but to the fact that the latter irritate the abdominal wall by wearing a charcoal oven round the waist and the former do not. Cancer of the floor of the mouth is rare in European women, although not uncommon in men ; but in Ceylon and India generally, the women suffer to a high degree from cancer of the inside of the mouth. Again, this is not due to developmental differences between women in England and those in India, but to the fact that Indian women chew betel-nut and sleep with the plug in the cheek at the exact spot where cancer starts. In needle-women melanotic sarcoma often develops in the fingers at the site of frequent puncturing by the sewing-needle.

Instances for other sites of the body need not be quoted ; suffice it to say that if all forms of cancer are to be explained by such speculations, and the intervention of congenital "germs" is to hold good for all cases, then it must also be assumed that such germs are as uniformly distributed, *e. g.*, over the surface of the body of all vertebrates as the skin is itself, and thus the explanation becomes no explanation at all.

Analogies between infective neoplasms and malignant new growths would appear to be more easy to establish for the sarcomata than for the carcinomata. Whereas in the Second Scientific Report our experimental observations were restricted to carcinoma, all of them that are of fundamental importance have now been extended to sarcomata

of varied histological structure. The parallel nature of the phenomena for the epithelial and connective tissue new growths is most striking, *e. g.*, experimental transference is effected only by implantation of living cells, and is limited to animals of the same species as that in which the primary growth started; the amount of growth under artificial propagation is unlimited and alternates in its rate; the age of the animals into which implantation is made has an important influence, young animals being much more favourable for growth; the specific nature of the phenomena of immunity to inoculation reveals no evidence in favour of a virus common to sarcoma in different species, indeed, it appears to exclude such a possibility. These, and many other points of agreement in the behaviour of epithelial and connective tissue new growths under experimental conditions, make it as certain for the true sarcomata as for the carcinomata, that no analogy exists with any known form of infective disease.

The question will doubtless be asked "if infection and congenital germs cannot be made responsible, is it not possible that hereditary predisposition plays a part in determining the frequency of cancer"? The most recent returns of the Registrar-General show, that in 1906, out of a total of 141,241 deaths of males above 35 years of age, 12,695 died of cancer, and out of a total of 140,607 deaths of females over 35 years of age, 17,671 also died of cancer. Thus the chance that a man over 35 years will die of cancer is one in eleven, and the chance for a woman above the same age is one in eight. On the basis of a similar approximation for 1905 the following table given in the Fifth Annual Report shows how often, taking the proportions * as 1 : 12 and 1 : 8 no death, or one, two, three, etc., deaths from cancer may be expected to be recorded in 100 families, half the members of which are men and half women, no hereditary tendency being assumed, and excluding all persons dying under 35 :—

* The proportion of deaths from cancer was calculated in a similar way by Mr. Harrison Cripps in the 14th Vol. of St. Bartholomew's Hospital Reports, and similar tables were given by Dr. Ogle in the Report of the Registrar General for 1889.

Number of cancer deaths in family.	Per 100 families of 6 members, viz., 3 men, 3 women.	Per 100 families of 8 members, viz., 4 men, 4 women.	Per 100 families of 10 members, viz., 5 men, 5 women.
None	51	41	33
One	36	39	39
Two	11	16	20
Three or more .	2	4	8
	100	100	100

The frequency of cancer as a cause of death is so great that few families of large size escape.

The great frequency of cancer as a cause of death as revealed by the above figures requires to be further analysed. From the national mortality figures above quoted, in one of two cases either a parent or a grandparent will, on an average, have died of cancer, supposing such parents and grandparents to have died after 35 years of age. Suppose a man and wife both of whom died of cancer 60 years ago; further suppose, that of their children, three males and three females, all survived and married, and, two of them, one male and one female, married children of parents who died of cancer like their own parents, while the others married into families with no history of cancer. Were it possible to follow the fate of all descendants of these six families, the comparative frequency of cancer in those of double cancerous heredity and in those of single cancerous heredity would show whether a tendency to the disease is transmitted. It is evident that such observations would have to be continued for many years until the descendants should become numerous enough to exclude the fallacies inherent in small numbers, or, that they should be multiplied by investigations on a large number of families with similar histories. In any case the observations could not be completed within the life-time of one generation of investigators.

If such detailed analyses of the incidence of cancer in a large number of families were practicable, and if they showed great variations above and below the average given in the preceding table, the possibility of the existence of a family susceptibility would be enhanced. In man such an analysis is impracticable because of the length of life, and low fecundity, and because of the progressive alteration in the value to be attached to records of the occurrence of cancer. Resort to experiments is necessary in order to define more accurately the circumstances associated with the spontaneous appearance of cancer.

The difficulty referred to can be obviated in the case of short-lived animals. In the mouse the question is now in a fair way to be definitely settled by means of breeding and in-breeding experiments on a large scale. As in man, so in the mouse, the total number of cases of cancer occurring in different strains appears to vary. The disease has been so frequent as to lead some observers to assert the occurrence of epidemics in certain cages. Our prolonged observations on the occurrence of cancer in the many thousands of mice bred for our investigations have given no support to this interpretation of the greater frequency of cancer in some communities of mice as compared with others, or in the same breeding establishments at different times. The frequency of cancer in mice obeys the general law of age-incidence *.

The surgical removal of spontaneously occurring mammary tumours has enabled us to prolong the life of many mice and to breed from them. In this way we have already obtained upwards of 1000 mice of known cancerous parentage. By successively crossing other spontaneously affected animals with the offspring of cancerous parents, strains are being obtained in which the cancerous heredity is $\frac{1}{2}$, $\frac{3}{4}$, or $\frac{15}{16}$, and even higher. The concentration of a hypothetical hereditary factor in a *known amount*, and in large numbers of animals of *known age*, should in the course of a few years definitely settle, whether there is a family, or only an individual, liability to the disease. As yet we have

* *Cf.* "Age-incidence of Cancer." The Statistical Investigation of Cancer. Second Scientific Report of the Imperial Cancer Research Fund, part i, March 1905.

obtained no evidence that the liability to carcinoma of the mamma has been enhanced by in-breeding.

Leaving the negative side of the report and turning to its constructive aspects, it may be well to point out at once, that no generalisation attempting a unification of a mass of new and old facts is brought forward. Although our experience is extensive, it is in reality only relatively so, both with reference to the new facts which are being accumulated rapidly by a large number of other investigators, and with reference to the field of inquiry and the new points of attack opened up by the successful application of the experimental method. At the present time it appears unwise to entertain exaggerated opinions of the importance of the working hypotheses which are formulated successively as the work proceeds. No new generalisation is likely to be permanent till the present temporary specialisation in the experimental study of cancer has passed away. Still, the following papers exhibit that harmony and mutual connection which makes them form a whole.

The papers in the report are arranged in sequence as logically as is possible. Starting with more general papers giving the evidence on which the comparative biological and experimental study of cancer is based, there follow others dealing with particular problems, the experimental production of sarcoma, the alternations in the biological qualities of tumour cells, the means whereby their growth may be prevented or modified, the relations of the tumours to the animal, and the constitutional conditions in organisms suffering from cancer.

An introductory chapter deals with matters of general interest on the incidence of cancer, and its association with very different forms of chronic irritation in mankind. Dr. Seligmann contributes an account of his observations on the occurrence of new growths in British New Guinea, the natives of which are, as he puts it, "but just emerging from the stone-age."

The zoological distribution of cancer is reviewed and new observations are recorded by Dr. Murray : to that account is appended a paper

showing some of the fallacies which account for the apparent occurrence in cancer, of forms of cell division characteristic of reproductive tissues during the ripening of the sexual elements.

After these papers of general interest, the report is concerned principally with the study of cancer in small laboratory animals. The features of spontaneous cancer in mice are dealt with by Dr. Murray on the basis of a very extensive and varied material, the study of which has occupied him for four years. In the course of this paper, Dr. Murray demonstrates the baseless nature of the assertion that surgical interference causes a tumour to become more malignant or necessarily to disseminate. At the same time he breaks new ground in studies on the relations between an organism and the malignant new growth it supports.

Special groups of tumours are described in greater detail in the papers of Dr. Gierke on the hæmorrhagic mammary carcinomata of mice, of Dr. Haaland on the experimental production of sarcomata, and of Dr. Murray on a transplantable squamous-celled carcinoma.

In these papers attention is drawn to important mutations of histological structure in malignant epithelial new growths, which throw light upon the subsidiary importance of histological minutiæ in the study of malignant new growths of man. These papers illustrate how the experimental method allows us to follow the life history and biological behaviour of a tumour over prolonged periods of time, with the result that the study of cancer has been unburdened of much needless histological lumber. The study of cancer in man had become purely histological, and histological studies in turn had become mere necrology—notes of deaths from tumours of this or that histological order.

Dr. Gierke records observations of importance in reference to the dissemination of cancer once a tumour is present, confirming observations we made in 1904, and adding new facts.

Mr. W. H. Bowen describes experiments on surgical interference with transplanted carcinomata and sarcomata, particularly interference with the blood supply, and records results bearing on the malignant nature of carcinoma and sarcoma in mice, as well as upon the surgical treatment of cancer in the human subject.

Dr. Haaland has made a most valuable contribution to the definition of the circumstances which determine the experimental development of sarcomata during the propagation of carcinomata. The process has occurred nine times in one of our tumour-strains and has been followed step by step. It is recorded with an exactness and detail which have not yet been attempted, and throws much new light upon the mediate relation of the irritation connected with transplantation into new animals, to the onset of a malignant new growth. Observations on this material enable us to substitute an objective study of the inception of cancer for the abstract speculations which have held the field for so long. The experimental study of cancer in animals has come to have a direct bearing on one of the most conspicuous aspects of the ætiology of the disease in man, as referred to above in alluding to the world-wide association of cancer with very varied irritants having nothing in common beyond this association.

The remaining papers deal mainly with the biology of the tumours of mice, the natural features of their growth, and the experimental means by which it can be modified or inhibited. The papers on the experimental analysis of growth and on the propagation of cancer, discuss alternations in the rate of proliferation of the cells of malignant new growths. These alternations have appeared in their true perspective, only as a result of experimental transference. It is made more certain that the biological qualities of cancer-cells vary in a way that finds expression in an alternation between positive and negative phases of growth. In clinical language these phases are fluctuations in malignancy, and a diminution in the size of a tumour will in all probability be followed by renewed increase. They give indications that

surgical removal should be attempted even though a tumour shows signs of diminished growth, and they help towards a better comprehension of the reasons for the unexpected disappearance of tumours after partial operation, or even when they have been held to be inoperable. In the negative phase of growth the cancer-cell is more vulnerable to those modifications which are induced in the living animal as the result of the absorption of normal or cancerous tissue referred to below.

The paper on the natural and induced resistance of mice to the growth of cancer marks the important advance which was made by the discovery announced from the laboratory in July 1906, that animals could be rendered resistant to cancerous inoculations by preliminary treatment with normal tissue. The relation between normal and cancerous tissue thus established by accurate bio-chemical methods, and carried further, in other papers, by observations on the metabolism of sound animals as compared with others bearing tumours, suggests an historical digression.

At the end of the eighteenth and the beginning of the nineteenth centuries, English investigators were striving to elucidate the nature of cancer by anatomical studies on the relations between cancerous and healthy tissues. Percival Pott, Jacob, Abernethy, James Wardrop, John Hunter, Astley Cooper, Carswell, Walshe, Everard Howe, Hodgkin and others were closely associated with the endeavour to segregate separate types of cancer, *e. g.*, chimney-sweeps' cancer, rodent ulcer, fungus hæmatodes, and what we now know as lymph-adenoma. The many resemblances of cancerous to healthy tissues received emphasis from some of them. This relation was fully recognised in Germany by Johannes Müller and his pupil Virchow, and, in England by Sir Samuel Wilks. In 1868 Sir Samuel Wilks who published his first paper in which he makes reference to cancer in 1846, indicated how this similarity differentiated the disease from infections. It has been my privilege to enjoy much intercourse with Sir Samuel Wilks, and through his keen interest in the progress of these investigations, modern experimental study is linked up with the results distinguished English

investigators foreshadowed a century ago. It has taken one hundred years to advance from the exact anatomical to the precise bio-chemical study of cancer as recorded in this report.

Dr. Russell has investigated the difference between the processes at the site of inoculation in normal and in resistant animals respectively, and shows that absence of growth in the latter is due to the failure of the resistant animal to furnish a new connective-tissue and vascular scaffolding for the introduced cancer-cells.

In the paper on resistance and susceptibility to transplanted cancer, these investigations are carried a step further. By accurate quantitative methods the delicate gradations of specific resistance are revealed and the results recorded in easily intelligible graphic form, showing, *e. g.*, that the protection which normal tissues induce is most effective against cancers arising from them, for example, skin protects best against skin cancer.

The remaining three papers deal in different ways with the relation of a malignant new growth to its host, viz., with the processes of cancer metabolism. In an earlier paper this subject is opened by Dr. Murray's account of the variations in body weight in spontaneously affected mice, and the results of operative removal and re-inoculation with transplantable carcinoma. Dr. S. Monckton Copeman, and Dr. J. Wilson Hake show, that the rapid building up of cancerous tissue calls forth a compensatory response on the part of the digestive system of the host animal. This has been measured by estimating the amount of HCl secreted by the stomach during digestion. Their results were in the first instance obtained on mice with transplanted tumours. They have controlled and confirmed them by corresponding investigations of the stomachs of normal mice and of mice spontaneously affected with cancer, and by investigating test-meals from human patients. It is hoped that the results of this comparative investigation may contribute materially to a settlement of a much disputed clinical question. At the same time they offer another demonstration of the parallel obtaining

between the relations of the organism as a whole to the malignant new growth it supports, under natural and experimental conditions respectively. Dr. Haaland and Dr. Cramer carry this line of investigation further in contributions on glycogen, fat, and respiratory metabolism.

Dr. Cramer's paper shows how precise bio-chemical methods can now be applied to the study of the growth of cancer, and brings new and exact information on the nature of the relations existing between a tumour and the animal bearing it. The effect which a growing tumour produces on a normal organism is a problem of nutrition similar to the growth of a fœtus in a pregnant animal ; it cannot be explained by assuming the formation of pathogenic "cancer-ferments" or "cancer-toxins."

The parallel between the growth of tumours under natural and experimental conditions, drawn in the Second Scientific Report, can now be extended to constitutional conditions in animals, as accompaniments of the disease whether naturally or artificially produced, *e. g.*, constitutional conditions favourable to growth, and therefore to dissemination, where a primary tumour is present ; the response of the digestive system to a growing tumour both in natural and experimental cancer, as well as the relation of the metabolism of a tumour to the metabolism of its host. Thus it comes about that the experimental investigation of cancer is being conducted under what we might almost call ideal conditions, and it is perhaps an advantage rather than otherwise, that these experiments cannot be conducted "in vitro" but only "in vivo," for, as I pointed out some years ago in reference to the study of the phenomena of immunity, convenient as the test-tube experiment is, grave fallacies may be involved in directly transferring the results to the living animal[1]. The living mouse has to take the place of the test-tube, even for maintaining an adequate supply of cancer-cells. This complicates the nature of the mere routine of experimentation

[1] On toxic and antitoxic action *in vitro* and *in corpore*. 'Journal of Pathology,' March 1902, and earlier and later papers.

and increases enormously the number of inoculations which, as distinct from experiments proper, require to be made in the conduct of the work.

If the Report be reviewed as a whole, it will be obvious that the experimental study of cancer has already enabled us to approach many —practically all—clinical and pathological aspects of the disease in man, and to throw new light upon each of the aspects of the disease to which it has been applied.

Cancer is ubiquitous in man and vertebrate animals. It has been shown that cancerous tissue of a species of animal retains the characters of that species, whereas in infective tumours (*e. g.* tubercle) occurring naturally in separate species, the biological characters of the newly formed tissue are determined by the common infective agent and not by the tissues of the affected animal. Within a species the individual tissues after they have become cancerous retain characters corresponding to those which distinguish the several normal tissues from one another. There are fluctuations in the rate of proliferation of cancer cells the discovery of which appears of be of theoretical and practical importance. The demands of cancerous tissue for food upon which it may grow, is responded to by the digestive apparatus of normal animals. The growth of experimental tumours can be prevented in its inception by treatment with normal tissues, *e. g.*, by skin in the case of skin cancer. It is a remarkable fact, that whereas the inoculation of skin will protect practically every mouse against a primary inoculation of squamous-celled carcinoma, there is the greatest difficulty once transplantation has been successful, in immunising such a mouse by the inoculation of skin against a second inoculation of the same tumour. It is probable that the presence of an inoculated tumour modifies the animal constitutionally in a direction which favours dissemination. The future definition of the circumstances which determine the relations obtaining here, may be expected to yield a promise that dissemination and the formation of secondary growths in distant organs may be prevented.

These facts, elicited by using the living cancer cell as an indicator of changes in the living animal, and *per contra* the living animal as an indicator of changes in cancer cells, can therefore only be studied by experiments on the living animal. A practical outcome is not yet in sight, and an emphatic *caveat* must be entered against their premature application to treatment of the disease in man. There are no indications that an antitoxic or other serum will be obtainable with curative powers, or that a prophylactic vaccine may be produced; but further investigation is indicated in the direction of preventing dissemination of a malignant new growth by enhancing the resistance of the organism, and by endeavouring to take advantage of the negative phase in the proliferation of cancer-cells, *i. e.*, the time at which experiment has already proved they are most vulnerable.

Meantime the rationale of the early surgical removal of primary tumours is amply justified by experiments, and no substitute for it has been found either by their means, or in alleged empirical remedies.

Apart from the subject matter, each of the papers dealing with experiments contains a detailed account of how the results were obtained and recorded. This has been done of set purpose in order that other investigators may be able to repeat our observations or to compare their results with our own. In the hope of securing some uniformity in experimental methods, there are given in the following pages details of those employed in the Laboratory.

It is essential if comparisons are to be made, that in all experiments uniformity should be aimed at both as regards the manner in which they have been carried out, and in the way in which the results are recorded. There is no reason why other investigators should not repeat the investigations of the Imperial Cancer Research Fund in these respects. Particular attention is directed to a method of studying and recording the course of spontaneous cancer in mice as regards the tumour, the animal itself, and the results of surgical

or other interference. All experiments are recorded with detailed reference to age of animals used, race, dosage, intervals of time, size or weight of tumours, and percentages of successful inoculations. Graphic methods are used to show the sizes of tumours by reproducing them to scale from protocols where they have been drawn as silhouettes of natural size. Percentage curves of the successful inoculations during prolonged propagation will be used to compare and illustrate the biological behaviour of different tumours. Where necessary, complete genealogical trees of the descent of all propagated tumours are also given.

Our experiments during the past six years can all be compared, and repeated if necessary, owing to the early adoption of certain elementary rules for making experiments and recording the results. Our papers have been purposely burdened with statements of doses, weights of tumours and of animals, statements of age, of time, number of animals inoculated as well as of mere percentages of positive results. We have therefore given every facility to others who may desire to repeat our observations. We have also given away our material freely to all serious workers who have asked for it, in the belief that free independent investigation and discussion should be encouraged.

Some sources of confusion in the results recorded by various investigators are avoidable for they are due to disregard of the necessity for employing standards which are especially important in "in vivo" experiments before comparisons can be drawn. It is strange how discrepancies which depend upon different dosage give rise to confusion again and again, whenever a new line of biological investigation is instituted. So far as my knowledge goes the minimal lethal dose of a drug was determined for the first time by Fraser between 1863 and 1868 for Physostigma venenosum and its active principle, and made the basis of important experiments on drug antagonism [1]. Its importance has been

[1] On the characters, actions, and therapeutic uses of the ordeal bean of Old Calabar. Edinburgh Medical Journal, vol. ix. 1863; *cf. also* Proceedings Roy. Soc. Edin., 1868-9, pp. 587-590; Trans. Roy. Soc. Edin., xxvi. 1872, pp. 529-713.

consistently insisted on by him since, *e. g.*, in his later work on the antidotes of venoms and toxins, and in other investigations on the action of various medicinal substances. Nevertheless for nearly another forty years controversy continued to rage as to whether or not atropine could abolish the lethal action of morphine : not one of the disputants on either side made the minimal lethal dose of morphine the basis of his arguments. At Fraser's instigation I was able to prove, after determining the minimal lethal doses of the two drugs, that atropine did abolish the lethal action of morphine, and to show that the most contradictory statements could all be harmonised as incidents in a scale of events determined by differences in the physiological effects, according to the quantitative relations between the doses of the two alkaloids[1]. Similar inaccuracies confused many of the earlier results of investigations in bacteriology, and into the relations between toxin and anti-toxin, all of which Ehrlich did so much to remove. They have also been potent already in causing discrepancies in the results of the experimental investigation of cancer, especially in estimations of the rate of growth of tumours, the extent to which protection can be induced against inoculation, and the specific or universal application of the results.

The papers of many authors do not contain the details necessary for the accurate repetition of their experiments. It is therefore impossible to appraise independently the value of their results, or to harmonise them with our own when divergent. Knowledge would be more certainly advanced if authors, instead of merely reaffirming their conclusions, gave the requisite details which would permit of independent estimations as to the comparative rapidity of growth of the tumours in their possession. An objective statement can be substituted so easily for the loose description of a tumour as "virulent" or "avirulent." The progressive growth of a tumour can be charted in silhouette at regular intervals, or, it can be stated, that from the inoculation of a

[1] Untersuchungen über das Bestehen eines gegenseitigen Antagonismus zwischen Atropin und Morphin. Archives internationales de Pharmacodynamie, vol. viii 1901, pp. 311-351.

certain weight of tumour or of a certain quantity of tumour emulsion, *e. g.*, for tumour 32, 0·02 gram or 0·025 c.c. a tumour of a certain weight, *e. g.* 1·5 grams to 2 grams developed in 10 days, or for tumour 27 only 0·6 to 0·75 gram, and after an interval of six weeks. These statements imply rapid growth and slow growth respectively, and permit of other investigators judging for themselves in the light of their own experience. Further, the size of the initial dose largely determines the size and weight the resulting tumour will reach in a given time. Its size may vary directly or inversely with the dose, according to differences between different tumours, and between the cells of one and the same tumour at different times. Therefore, when the initial dose is unknown or not stated, no judgment as to the rate of increase is possible.

The report is illustrated by drawings and photographs of the appearances described. The drawings have been made by Mr. Richard Muir, of the Pathology Department, University of Edinburgh, or in the laboratory by Mr. J. R Ford, of the firm of Shiells & Ford. The microphotographs have been made by Mr. Richard Muir and Mr. W. Imboden, F.R.M.S. The coloured plates have been prepared from drawings made by Mr. Thornton Shiells.

Special recognition is due to the publishers, Messrs. Taylor & Francis, and Mr. Whitehouse of that firm, for the expeditious and accurate manner in which the letterpress and figures have been prepared for publication. The attention they have devoted to the preparation of the report has largely contributed to its appearance at the present time.

A bibliography of all the communications made from the laboratory is appended, the papers being arranged in chronological order.

It has seemed desirable to add an index, in order that the scattered references to the same subject may be made more easily accessible to those interested in particular topics.

Three of the papers in this report are reprinted from the Proceedings of the Royal Society by permission of the Council, to whom the thanks of the Executive Committee are due.

I desire to place on record the zeal with which my colleagues have worked in harmony, in sharing monotonous routine, and in conducting special investigations often overlapping one another. To Mr. Hall I desire to make acknowledgement for valuable assistance in the general supervision of the laboratory service and the care of animals, and to Mr. Miller and Mr. Chapman for much careful histological and other work.

With the completion of this Report all the points raised in the provisional scheme of inquiry drawn up for the Executive Committee in October 1902 and given as an appendix, have been submitted to investigation. The important investigations made by workers elsewhere and also by my colleagues in the laboratory since that date, have naturally had much influence on the conduction of the work. I desire to thank the Members of the Executive Committee and of the various Sub-Committees for the constant encouragement which they have given to the Staff.

E. F. BASHFORD.

June 30th, 1908.

IMPORTANT CORRIGENDA.

Page 2.—19 lines from top: *for* Sandwich Islands *read* Fiji Islands.

„ 80.—Fig. 16: *for* $\times \frac{25}{1}$ *read* $\times \frac{250}{1}$.

„ 86.—Six lines from bottom: *for* fig. 25 *read* fig. 26.

„ 98.—Six lines from top: *for* fig. 45 *read* fig. 44.

„ 101.—Fig. 43: *for* Tumour $\frac{30}{0}$ *read* Tumour $\frac{63}{0}$.

„ 131.—Fig. 15: *for* Tumour $\frac{2}{0}$ *read* Tumour $\frac{25}{0}$; figs. 18 & 19: *for* Tumour $\frac{90}{0}$ *read* Tumour $\frac{19}{0}$.

„ 153.—In lines 1, 3, and 14 from top: *for* fig. 6 *read* fig. 4.

„ 232.—Fig. 76: *for* 37/10 Y *read* $37/10_2$ Y.

„ 268.—Fig. 4, at second needle: *for* A *read* A′.

„ 395.—In fifth column of Table: blank space in fourth line should read figure "5."

THE ETHNOLOGICAL DISTRIBUTION OF CANCER.

By E. F. BASHFORD, M.D.

The continuation of the statistical investigations on the occurrence of cancer in man has confirmed the results already published. The statistical investigations will form the subject of a separate report, as on a former occasion ; but some general statements of the occurrence of cancer in man may precede an account of the occurrence and study of the disease in animals.

The desire for data relating to various races of mankind was no doubt born of the expectation that some would be found to be highly afflicted with cancer, and others altogether exempt. A statistical investigation of this kind is really a direct attempt to solve the problem of the causation of the disease in man, as contrasted with the experimental study of cancer in animals, which is an indirect method of approaching the same problem. The straightforward admission at the inception of our work, that this indirect method of attack is an indispensable auxiliary to the study of cancer in man, has provoked criticism on the part of persons who would have the investigation of cancer limited by the opportunities for clinical and pathological study in the human subject. Impartial critics are invited to compare the advance in knowledge which has been possible during six years' strenuous exertion under the direct and indirect methods.

Quite unsuspected difficulties have been met in searching for cancer in remote parts of the world. But, great importance has been attached to a continuation of the search in widely separated geographical areas, and in divergent races of mankind in accordance with a uniform plan, and over a period of time sufficient to provide adequate evidence of the occurrence of the disease, or, to yield an explanation for its real or

apparent rarity. Unfortunately these ends have not been attained after five years' investigation, notwithstanding the whole-hearted support the Foreign, India and Colonial Offices have sought from their representatives abroad, and in most instances have obtained. Nevertheless, much information has been collected, of value in controlling the direction of experimental work and supplementing its results, as well as bearing upon various pre-suppositions as to the pathology of cancer.

The assertion has often been made that cancer is a disease peculiar to Europeans and their offspring, and, when occurring in the natives of other parts of the globe, is due either to communication of the disease from them, on the supposition that cancer is infectious, or due to an acquired tendency to developmental anomalies in consequence of the noxious influences of European civilisation.

The supposition that cancer is an infectious disease has received no support during our ethnological studies. As is well known, the introduction of an infective disease among aboriginal populations by Europeans, has frequently given rise to its most wide-spread and epidemic occurrence in an aggravated and fatal form, as *e. g.*, measles, which more than decimated the inhabitants of the Sandwich Islands, or, to take an infective disease of different category, syphilis which has followed in the wake of Europeans with most disastrous consequences in other regions.

The supposition of communication of the disease has been invoked, for example, to explain the frequency of records of malignant new growths among the negroes of America, and their infrequency in Africa. Our investigations have revealed no indication of endemic foci or of epidemic occurrence of malignant new growths in savage races, nor has it been possible to establish any relation between the frequency with which cancer is recorded in aboriginal races and the degree of exposure to contact with Europeans. Cancer, *i. e.* Carcinoma and Sarcoma have been discovered in regions where the degree of contact with Europeans is still at its minimum. Isolated cases of cancer have been reported in Europeans living in parts remote from civilisation as well as in savages. The latter facts show, on the one hand, that Europeans may be attacked by cancer although living for many years under a strange environment, and, on the other hand, that the natives were not naturally exempt, but offered as it were a favourable soil for the disease, and therefore also for its communication from Europeans, if this be possible. Nevertheless, far from there being any evidence of epidemics as the result of the introduction of the disease by Europeans

into virgin soil, there has been the greatest difficulty in obtaining evidence of its frequent occurrence in native races long in close contact with Europeans, *e. g.*, in India, Africa, New Zealand, and Australia.

The second supposition, viz. that savage races living under their natural primitive conditions are not liable to developmental anomalies is contradicted by the fact that such anomalies are frequent, as Aschoff has already pointed out. As regards developmental anomalies, there can be no doubt that certain forms of malignant new growth, most commonly met with in the early years of life, are associated with them. It is a matter of moment that congenital malignant new growths of this nature also occur among the offspring of aboriginal races (fig. 1) still practically free from the influence of European civilisation.

Although no fluctuations in the occurence of cancer, sufficiently striking to arouse even so much as the suspicion of the introduction of an epidemic of cancer among aboriginal races previously exempt, have been discovered, still, there are very considerable differences in the numbers of cases recorded in various races of mankind. It is impossible to explain them satisfactorily at present; but they merit passing consideration here. In a preliminary discussion of this subject elsewhere it has been indicated why some of these differences may be only apparent, while others may or may not be real.

Those who would draw far-reaching conclusions from the frequency of cancer in Europe and the scanty evidence of its occurrence in savage races, may be warned that comparisons may be made only between a few European countries, and then not without reservations. Outside of Europe the reservations are probably of more importance in the case of America, Australia, Canada, Japan, New Zealand, and South Africa. In India and in the various races inhabiting British Colonies and Protectorates no basis whatsoever exists for statistical comparisons. The student of cancer has to be satisfied with the significance of the positive results of attempts to ascertain whether or not cancer occurs at all in savage races.

Recorded Differences in the Incidence of Cancer in Europe.

There are differences in the number of deaths assigned to cancer in different countries in Europe. The crude death-rate, *i. e.*, the number of deaths to 1000 of population living, varies from 0·11 in Servia and 0·4 in Hungary to 1·29 in Switzerland. At the outset of a study of



Fig. 1.—Congenital glio-sarcoma of orbit in a child 5 weeks old, native of Gold Coast, West Africa.
From a Photograph forwarded by W. R. Henderson, C.M.G. late P.M.O.

European statistics it becomes evident that no basis for accurate statistical comparisons exists. In 1903 we pointed out the necessity for different countries adopting a uniform method of investigating cancer statistically before comparisons could be made, but at the same time we acknowledged the improbability of devising a scheme likely to secure this end. In France, Denmark, Sweden, and Bulgaria the causes of death are not tabulated except for the towns. In Norway only 50 per cent. of the causes of death were stated in 1881 as against 85 per cent. in 1901. To those who would fain draw far-reaching conclusions from the difference in the number of deaths recorded in civilised and savage man, it may come as a revelation that comparisons of the data for different countries, even those of Europe, are quite unjustified. But the untenability of all such too hasty conclusions may reassure those members of the public who have been unduly agitated by the wide dissemination of alarmist opinions in the medical and lay press.

It may be more than a coincidence that the largest number of deaths assigned to cancer occurs in Switzerland, where medical inspection of the dead body is customary, and where in 1900 in fifteen of the largest towns autopsies were made already in so high a proportion as 25·7 per cent. of the total deaths.

Stated generally, the number of deaths assigned to cancer increases from one country to another in a manner parallel with the increasing accuracy of the vital statistics of the several countries, and the low death-rates in Servia, Hungary, and Spain are probably the result of under-statement. Thus, to take two instances which differ less markedly from the figures for England, the statistics for Ireland are known to be less reliable than those compiled for England and Wales, and the recorded death-rate from cancer is 0·79 as compared with 0·92, although one would expect the opposite relation from the high proportion of the Irish population which attains to old age, for, as is well known, the frequency of cancer increases with advancing years. The recorded death-rate from cancer in Prussia is also lower than in England and Wales, being 0·70, *i. e.*, less than that of Ireland; but here again the Prussian statistics are acknowledged to be not so accurate. The Prussian statistics have greatly improved in recent years, since many Prussian towns introduced certification of the causes of death by medical men, and the population of the towns has increased *pari passu*, both relatively and absolutely, with the growth of the population.

Throughout Europe disturbing factors come in play, minimising the number of the records of deaths from cancer available for statistical

purposes in some instances, and augmenting them in others. The prejudices of superstition and ignorance, of religious beliefs (not necessarily *quâ ecclesia*), combined with the ease or difficulty of access to educated medical advice, play no unimportant part in determining the number of cases of cancer recognised and recorded. The pathological value of the records of death from cancer is likewise by no means uniform.

If one be justified in dismissing the differences between the rates of mortality in Ireland or Prussia, and England and Wales, as expressions of apparent rather than of real differences in the absolute incidence of cancer, the differences obtaining in the case of other European countries, *e. g.*, Catholic Spain with a death-rate of 0·44, and Protestant Holland with one of 1·01, cannot have any greater importance attached to them. The records of deaths from cancer are obtained by ways so dissimilar as registration of deaths by the layman or laywoman, the priest and the doctor ; moreover different statistical methods of unequal accuracy are employed in different countries. Therefore great caution is necessary in assuming that the final statistical results are comparable and reveal real differences. In any case, the magnitude of the recorded differences loses much of its apparent significance.

Owing to the supposition that sarcoma may differ essentially from carcinoma, or, from mere striving after accuracy, the attempt is made in some statistics to deal separately with the deaths falling under the two categories, *e. g.*, in the German Cancer-Census of October 15th, 1900. This endeavour probably introduces grave errors. With Murray we have shown that the one form of malignant new growth is as difficult to recognise clinically in hospital patients as the other, and also that the probability is great that carcinoma and sarcoma both increase with advancing years : a conclusion to which Weinberg has come independently. Experiment has also shown that sarcomata and carcinomata obey other general laws distinguishing them from the known infective diseases.

In other statistics regarded as highly accurate, new sources of fallacy are introduced, which point to an over-estimate of the frequency of cancer, *e. g.*, new growths may be grouped together with other causes of deaths from a false idea of adding to their completeness, as in Switzerland, where for males, all fatal diseases of the prostate gland are grouped under the heading of "cancer" of that organ. An enlarged prostate is a common disease of old men, but it is not necessarily a "malignant" enlargement, and its sequelæ of cystitis, infection of the kidneys, systemic consequences, and death when beyond treatment, should not be charged invariably to cancer.

Two factors seriously diminish the value of the records of death from cancer. One factor is the manner in which the certification of deaths is effected, and the extent to which this is solely the duty of medical men, as in some States, or, merely the ignorant opinion of a layman, after viewing the body, in others. Prinzing cites two instructive cases. In one, an ignorant peasant who performed this duty returned all deaths as due to cardiac failure, while another returned fifty deaths from diphtheria in Tilsit, at a time when even illness from diphtheria was quite rare. The other factor is the variation in the number of persons surviving to higher ages in different communities and the extent to which the actuarial corrections necessary to render comparisons valid are made, always of course provided the records of deaths from cancer and the other vital statistics of the population are sufficiently numerous and accurate.

The improvements Drs. Farr, Ogle, and Tatham have effected in our own national statistics of cancer, by inquiring into vague statements of the cause of death, of themselves serve to show how much room there is for improvement in countries where laymen are, or were till recently, *e. g.*, in Germany, entrusted with the declaration of the cause of death in the case of cancer—a disease often presenting insuperable difficulties to recognition by the most skilled clinician. The difficulty of diagnosing cancer has been pointed out previously with reference to hospitals in England, Scotland, and Ireland, and a study of the clinical diagnoses of over 8000 cases has confirmed its statistical importance.

The impression made by a study of the death-rates from cancer in European countries is, that comparisons of the results of the statistical tabulations of the records of death from cancer in different countries, with a view to establishing differences in its absolute incidence, appear to be entirely fallacious. As regards more limited areas in single countries, and differences between towns and country districts, the accuracy of the registration of the causes of death is everywhere behind-hand in rural districts as compared with towns, and some countries do not even attempt to compile statistics outside of the towns, as, for example, Bulgaria, France, Denmark, Russia, and Sweden. The remarks made on differences obtaining in the rate of mortality from cancer in separate countries, appear to be applicable also, if in modified form, to the statistics of restricted areas in individual countries. The dimensions of the differences are not so great that they are incapable of explanation by (1) the varying difficulties in the way of obtaining accurate records of the numbers of deaths from the disease,

i. e., of making them approximate to the absolute number ; (2) the divergences in the methods of utilising the data for statistics ; (3) the varying extent to which actuarial correction is made for the age-constitutions of the respective populations ; (4) the different age-constitutions of the populations themselves ; (5) the futility of conclusions based on too small numbers, which are liable to frequent reversal when the statistical results from one year to another over a long period of time are compared, *e. g.*, in small districts, and in populations restricted in numbers or sparsely scattered over very extensive areas as in some British Colonies and Islands under British Protection.

We must guard ourselves from the charge that we deny the existence of real differences in different European countries and even in the less extensive areas comprised in them, or, that we intend to imply that with sufficiently accurate statistics all differences would disappear, and, the deaths from cancer appear as a constant function of the age of the population, more correctly of the senescence of the respective tissues of the individuals in any selected population.

It is obvious that the incidence of cancer in the organs of representatives of the different zoological classes of the vertebrates must differ, since structures peculiar to mammals are absent in the lower vertebrates. From our studies in animals it appears probable that different species of the mammalia are liable to special forms of cancer, from which others are exempt, *e. g.* the variations in the frequency of cancer of the mamma in different species of the mammalia are most remarkable.

As regards the incidence of special forms of cancer, we know the disease is not inevitable even when life is prolonged to its utmost limits, both in man and in animals. Why some individuals escape and others do not, it is impossible to say. It would be folly to assert without demonstration, or indeed without any evidence whatsoever, that the proportion of those escaping to those attacked is constant and invariable, and, that the fact of its being so is obscured only by the imperfections of our methods of recording the deaths from cancer and using them statistically.

The number of deaths assigned to cancer has increased from year to year in practically all countries. This increase appears most alarming when it is taken up as a national problem, *e. g.* in England, Germany, or the United States, without due regard to the universality of the phenomenon. Reference has been made above to the irregularities in the methods of compiling statistics from one country to another and the diversity of the attempts which are being made to fulfil the require-

ments of accuracy. The almost universal endeavour to improve the accuracy of statistics, has an important bearing upon the alleged "increase of cancer" of which mention will be made again below with reference to Japan.

The fact that cancer has been found in races of mankind formerly believed to be exempt and to be common in others in which it was said to be rare, shows how much the number of cases recorded depends upon the care exercised in searching for a disease of slow evolution, which does not obtrude itself upon the superficial observer by a striking and peculiar symptomatology. The study of the alleged increase of cancer involves a very complex statistical investigation. It can only be properly investigated on the basis of accurate and detailed mortality statistics of single countries, and it will be reverted to again in the special statistical report. Mention must, however, be made of the valuable contribution to its settlement which is being made in the Reports of the Registrar-General for England and Wales, by Dr. Tatham's tabulation of the cases of cancer for different sites of the body.

It may be added here that when the investigations of the Imperial Cancer Research Fund were commenced and the resolution come to, to study cancer in animals, doubts were expressed by some as to whether the difficulty of finding a sufficient number of cases would not prove insurmountable. The large number of cases of cancer, over a 1000, which have been observed in mice by various investigators during the past five years does not mean an increase of cancer in mice, but simply that it has been looked for with sufficient care and found. The increase in the number of cases recorded throughout the vertebrates has the same significance.

All it is intended to convey by a brief survey of the statistics of European countries is that the recognisable sources of fallacy in the data at present available, appear to carry more weight than the evidence that the differences they reveal between different countries, and in one and the same country at different times, are real. They have not the fundamental importance for the experimental investigation of the nature of cancer possessed by the results of those other statistical and biological studies which have moulded its present form.

Differences in the Recorded Incidence of Cancer outside Europe.

Complete or relative exemption from cancer on the part of the inhabitants of various parts of the world has been alleged on mere hearsay evidence. Such indefinite reports are principally relied on by those who assert that selected factors of European civilisation are causally connected with the recorded increase in the number of deaths from cancer in England.

The facts ascertained confirm the conclusion previously arrived at, that, taking England and Wales as a standard, there is a gradual diminution in the precision and completeness with which cancer is recorded, Scotland and Ireland showing less precision and completeness, and various outlying parts of the Empire still less of these qualities, till a minimum is reached among the natives of certain tropical colonies, e. g., British Central Africa and New Guinea, and in the case of islands with very small populations, e. g., St. Helena. The diminution proceeds *pari passu* with the increase in the difficulty of making observations on the occurrence of the disease, or of collecting a sufficient number of reliable preliminary data of the numbers of the population, the relative proportions of males and females, the distribution of the population at different age-periods, the crude birth- and death-rates. These considerations modify the importance to be attached to differences in the recorded incidence of cancer in Japan, India, Egypt, and among uncivilised or savage races.

The fact that cases of cancer have been found to occur where previously the disease had not been observed is of importance—the occurrence even of an isolated sporadic case being significant. Since accurate statistical comparisons are impossible, it is permissible to make others of a tentative and cruder kind which, although open to grave fallacy, are as instructive as any which can be made at the present time.

The frequency of cancer in Japan is of special interest. In 1901, the German Cancer Committee seriously accepted the statements of Baelz that useful statistics did not exist in Japan, but that he would try to create them, and he asserted cancer was, without doubt, much rarer in Japan than in Europe. The informant went on to state: "This is at once evident from the relatively large number of cases in the small number of European inhabitants, as contrasted with the rarity with which the disease is observed in natives." Without referring to

the statistical fallacies which vitiate such a conclusion, it is sufficient to point out that, as a matter of fact, the official statistics of the Japanese Empire for that same year (1901) show, that out of a total of 959,126 deaths, 24,598 were returned as due to "cancer" and "other malignant growths" *.

We have to thank Baron Takaki for access to the Japanese statistics. The average death-rate from cancer for the five years 1899–1903 was 0·49, higher than in Servia, Hungary, and Spain. Baron Takaki modestly confessed that cancer was not so well diagnosed in Japan as in England †, nor were the mortality statistics of Japan as yet comparable to those of this country. The approximation of the Japanese figures to those for some European countries and British Colonies, for the statistics of which accuracy is claimed, is thus made all the more striking. The crude figures would indicate that cancer is twice as frequently a cause of death in England as in Japan. It must be evident, without further discussion, that this conclusion might require drastic revision if our information as to the "age-constitution" of the Japanese population were more complete, and if the registration of deaths were carried out as in this country. The future course of the cancer death-rate in Japan, as statistical methods improve there, should throw a most instructive light on the cause or causes of the increase which has taken place in the number of deaths attributed to cancer in European countries. In view of the exceptional position Japan occupies in relation to this problem, owing to differences of race, diet, climate, and perhaps also in the age-constitution and habits of the population, it is desirable to obtain more details as to the death-rate from cancer at different periods of life. The tabulation of the sites of the disease in different parts of the body as now published in the Reports of the Registrar-General for England and Wales should yield very valuable information. Fortunately, the Japanese recognise the national importance of the frequency of cancer among them, and there is every prospect of these improvements being effected. In Japan there is at the present time not only very active investigation of cancer, but of the two journals in the world devoted solely to cancer, one is published in Japan under the editorship of Professor Yamagiwa, Professor of Pathology in Tokio.

Exaggerated importance, not justified by fuller knowledge, has been

* The population of Japan in 1901 was 45,227,464.

† In Japan there are still a large number of unqualified practitioners of medicine.

attached also to assertions that cancer is practically absent in India, in Egypt, and among aboriginal races. In the course of the past three years over 2,000 cases have been reported from hospitals in India. Occurring in a population of 300,000,000, this number appears to imply



FIG. 2.—Carcinoma (scirrhus) of breast in woman born in the Sudan, where she had spent all her life. From a photograph by Capt. Ensor, E.M.C., forwarded by Dr. A. Balfour, Khartoum.

great relative exemption. Such a conclusion loses in cogency when regard is paid to the small number of hospitals, the onerous duties which a relatively small number of officials undertaking this additional work have to discharge, and the circumstances which restrict the number of native applicants for hospital advice. The patients belong

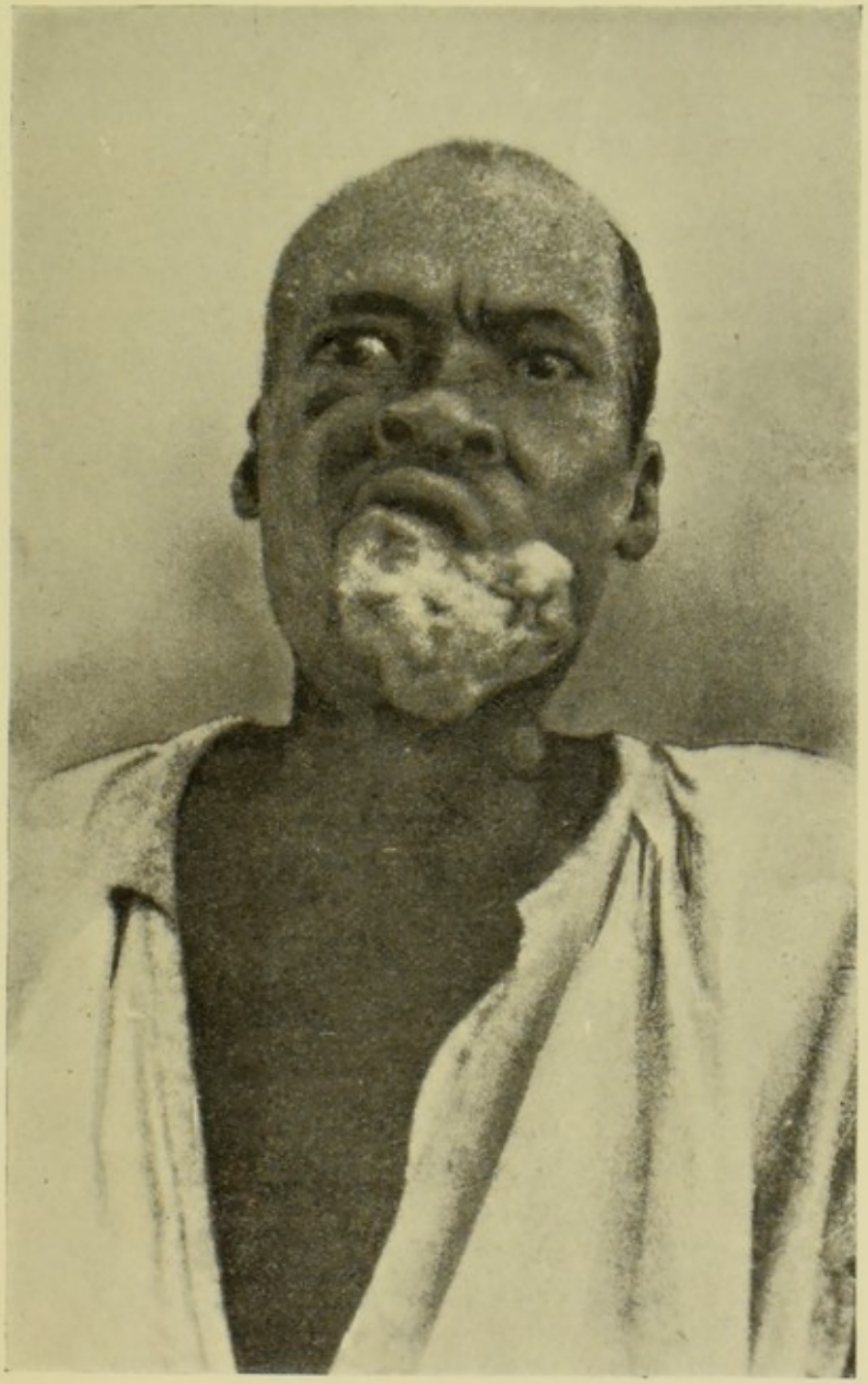


FIG. 3.—Dinka from Fashoda with epithelioma of jaw, secondary glandular involvement. From a photograph by Capt. Ensor, E.M.C., forwarded by Dr. A. Balfour, Khartoum.

to vegetarian as well as other castes, and their distribution throughout India suffices to dispel any hopes of discovering races exempt from the disease in that country.

Dr. Keatinge and his coadjutors have reported 297 cases of cancer among the patients treated in the Government Hospitals of Egypt during the past three years. Dr. Balfour of Khartoum has reported sporadic cases from the Sudan (figs. 2 and 3). In short, reports of the occurrence of cancer have been obtained wherever the search has been made, with certain exceptions where the importance of their absence up to date is discounted either by the population being small in numbers, or sparsely scattered over very wide areas. In some instances the apparent absence of cancer loses significance by the fact that investigators in adjacent regions have succeeded in finding it, as, e. g., in the case of some parts of tropical Africa and various Polynesian islands. Dr. Glanville Corney and his colleagues have forwarded a number of specimens from Fiji.

Attempts have of course been made to obtain some computation of the average age attained by aboriginal races among whom cancer appears to be so rare. Dr. Watkins Pitchford states, on behalf of the Natal Cancer Research Committee, "that the estimated native population of Natal in 1906 was 930,000," and commenting on the rarity of cancer among this population he adds, "that whereas 25·7 per cent. of the population of England and Wales were of the age of 40 and over, only 13·7 per cent of the Natal natives had reached this period of life." For Natal the definite statement is made that native deaths are reported to the Magistrates by "Informants" who are sent at periodic intervals by the chiefs for this purpose, the cause of the death being usually given as "pain in the head" or "sick inside." Although the Residents and the medical and other officials having long experience in tropical and sub-tropical Africa and, e. g., New Guinea, express somewhat contradictory views, the balance of opinion favours the surmise that the natives of these regions have not the same expectation of life as is enjoyed in England. The women in many regions have numerous burdens to bear which are unknown in civilised lands and they especially appear to age much in advance of their years. While it is possible that the age-constitution of native populations has much to do with the difficulty of obtaining numerous records of cancer among them, it is impossible definitely to assert that this is really the case, for some observers have reported that a considerable proportion of the male population is over 45 years of age. Most estimates of age in native races are guesses on the part of

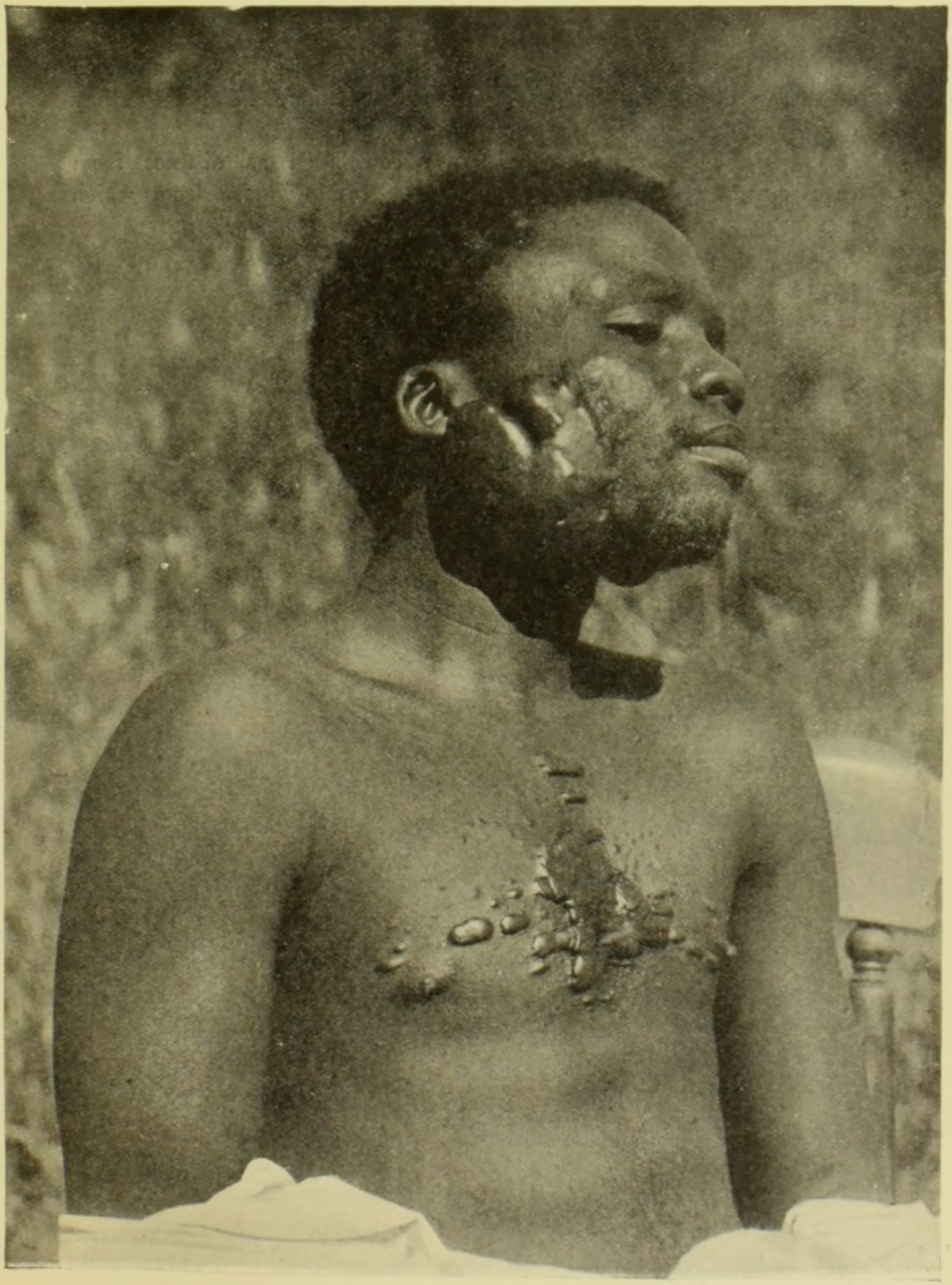


FIG. 4.—Native with large keloid and secondary glandular enlargement resembling sarcoma. Photograph sent by Dr. Long, Maseru, Basutoland.

those who make them, the natives themselves rarely having any idea of the lapse of time. Unless they remember some striking event they can give no assistance.

When the reports of sarcoma are considered as distinct from carcinoma, there is practically no part of the Empire from which reports of the sporadic occurrence of sarcoma have not been received. As pointed out already, the occurrence of sarcoma is as important for the investigation of cancer as the occurrence of carcinoma. The greater ease with which sarcoma has been found may be due to the fact that sarcoma occurs in savage races, as in Europeans, more uniformly throughout life, and is already frequent in those earlier years of life during which the occurrence of carcinoma is still rare.

In dealing with sarcoma in aboriginal races caution is necessary owing to our imperfect knowledge of diseases among them, which may resemble yet be distinct from sarcoma pathologically. Many races exhibit the most extraordinary cheloid growths developing in injuries to the skin either accidentally incurred or deliberately inflicted with a view to adornment. When the adjacent glands become enlarged, as is often the case, the clinical picture may be indistinguishable from that of sarcoma (fig. 4).

It would serve no useful purpose at present to pursue the subject of the ethnological distribution of cancer further. The investigations of the Imperial Cancer Research may be said to have settled once and for all that cancer is a disease common to mankind throughout the world. The questions of its occurrence or non-occurrence having been definitely settled, the problem now presented is its relative frequency in this or that population and area, which overlaps the much discussed question of the alleged increase of cancer, and savage races are unsuited for its study. By the time the natives of Central Africa have so organised their communal life that reliable vital statistics are forthcoming, the conditions of life among them will no longer be what they are to-day, and no doubt they will be interested in what to them may be a new phenomenon, "the alleged increase of cancer." We have seen this stage reached at successive intervals in different countries with the development of their national statistics, and since the work of the Imperial Cancer Research was started Europeans have passed from the belief that cancer was rare or did not occur in Japan, to the minor question of its real or apparent increase in that country.

Like other direct methods of attempting to elucidate cancer problems, it appears as if the study of ethnological distribution has brought us so

far, and then left us face to face with the same pathological problems which limit statistical methods when applied to the cancer-data of England or Europe.

The "Organ-incidence" of Cancer in Different Countries.

Although the limits of accurate statistical conclusions are quickly reached in reviewing the available data of the incidence of the disease in native races of tropical and subtropical countries, the facts when regarded from another standpoint are full of interest. It is a commonplace of pathology that some organs of the body are more frequently the seat of malignant new growths than others, and, when large numbers of cases are reviewed, the organs of the body can be arranged in a fairly definite order according to the frequency with which cancer originates in them. More than 90 per cent. of the cases reported from tropical and subtropical countries are recorded for sites on the surface of the body or for others very accessible to physical examination. The rarity in the records, of cases from internal organs does not necessarily imply that cancer has a predilection for the surface of the body. The difficulty of diagnosing internal cancer even in well equipped hospitals in London, Scotland, and the Provinces, has already been referred to as of statistical importance; and the same difficulties must be greatly enhanced in degree under the circumstances met with in India, and to a still higher degree among savage races. Various races have the greatest objection to the performance of post-mortem examinations, e. g., Mohammedans. In other regions the belief in sorcery and witchcraft is widespread and places many obstacles in the way of the medical observer. When a savage is afflicted with a painful internal complaint he is not likely to vouchsafe any useful information such as might lead to the diagnosis of advanced cancer. The important additions surgical operations and post-mortem examinations make to the number of cases of internal cancer recorded in Europe have already been referred to.

An accurate comparison of the English data with those available from India is not possible. Nevertheless a consideration of the crude figures appears to warrant certain tentative conclusions. In India, as in England, cancer is more frequently recorded from some sites than others. The latest Report of the Registrar General includes a table giving the relative quota which the different organs of the body

contribute to 10,000 deaths from cancer in males and females respectively in England and Wales. Thus out of 10,000 deaths from cancer of males, 148 are due to cancer of the penis and testis, 926 to cancer of the lip, tongue, buccal mucous membrane, and cheek. In females in every 10,000 deaths from cancer, 2,259 are due to cancer of the uterus, 1,656 to cancer of the breast, and only 83 to cancer of the lip, tongue, cheek, etc. Similar mortality statistics for India do not exist, but we are in the fortunate position of possessing records of over 10,000 cases of cancer from males and females together, which have been subjected to complete pathological examination and reported from English hospitals. These selected cases show a quite different frequency of attack on the organs mentioned, and while indicating the necessity for great reservations, they furnish a transition to the much less numerous data from hospitals in India. In English hospitals, cases of cancer of the testis and penis are twice as frequent as in the mortality returns, and cases of cancer of the lip, etc., are nearly three times more numerous. In females, although cancer of the uterus is only half as frequent, cancer of the breast is more than three times as frequent in the hospital cases, while cancer of the lip, etc , is nearly four times more abundant than in the national statistics. Several factors probably combine to cause these discrepancies, because, while a certain proportion of the cases operated on recover completely, an enormous number of the cases of cancer which swell the mortality returns have only been recognised after they were beyond hope of surgical relief. The reversal in the figures for hospital practice, of the relation obtaining between the number of cases for the breast and uterus respectively in the national statistics, illustrates the important share easy examination plays in the early selection of cases suitable for treatment in hospitals.

When every possible weight has been given to such considerations, the figures from Indian hospitals present a very striking result. While cancer of the testis and penis is nearly ten times as frequent as in English hospitals, cancer of the lip, tongue, cheek, etc , contributes nearly the same proportion. Among females the same correspondence is found, cancer of the uterus being slightly more frequent than in English hospitals, and cancer of the breast equals more than three-fifths of the English hospital figures. Cancer of the lip, tongue, cheek, etc., is on the contrary more than six times as frequent in Indian female hospital patients as in the corresponding English group.

The slight differences between the figures for English and Indian hospitals of the frequency of cancer of the breast and uterus make it

at least possible that the *mortality* in India from cancer of these organs may not be markedly different from the English mortality as indicated in the statistics of the Registrar General.

Dr. K. S. Wise records the great frequency of carcinoma of the penis in British Guiana among coolies, and also draws attention to frequency of carcinoma of the cervix uteri.

Attention may now be directed to the close correspondence of the frequency of cancer of the lip, tongue, cheek, and buccal mucous membrane in male English and Indian hospital patients, and the discrepancy between the corresponding figures for females among whom in India cancer of the lip, tongue, etc., is seen far more frequently. The association of certain forms of chronic irritation (clay pipes) with the development of cancer of the lip in this country, is well known and is generally held responsible for the higher incidence of cancer at these sites in males than in females. An analogous form of chronic irritation is found in India, in the form of chewing a mixture of betel leaves, areca nut, tobacco, and slaked lime, which is practised extensively by both sexes. The report of Dr. A. J. Chalmers and Dr. Perry on the incidence of cancer in Ceylon shows this association very clearly. In Ceylon more than half of the total number of cases are recorded for sites in or around the mouth.

Similar conclusions are arrived at by a consideration of the occurrence of cancer of the penis, which is extremely frequent in some races and very rare in others living side by side with them. The frequency of cancer of the penis is apparently associated with chronic irritation due to the accumulation of dirt and secretion under the prepuce, whereas it is practically unknown in Mohammedan races which practise circumcision.

The occurrence of epithelioma of the anterior abdominal wall in Kashmir with a frequency met with nowhere else is also most instructive. Some years ago Dr. Neve drew attention to the development of squamous-celled carcinoma in Kangri burns. The Kangri is a small earthenware vessel enclosed in basket-work variously decorated, and used to contain a charcoal fire. The Kangri is suspended round the waist under the flowing robes to assist in maintaining warmth by the natives of the cold hills of Kashmir. Repeated burns and long continued chronic irritation of the skin of the abdomen are the consequences. A common sequel to this long continued chronic irritation is the development of squamous celled carcinoma with metastases in adjacent lymph glands. We are indebted to Drs. Neave and Rawlence for a specimen of the "Kangri" as well as for photographs of natives using it. We



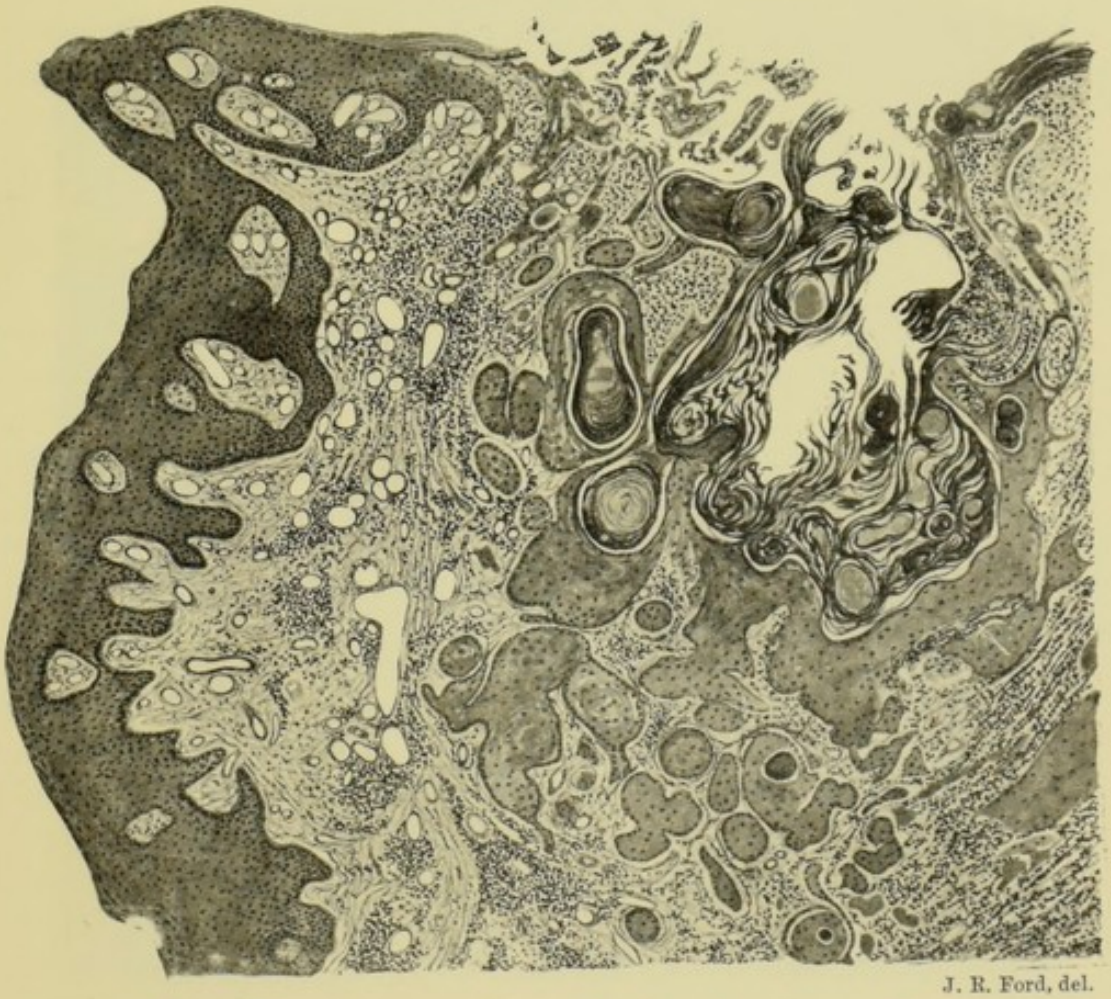
FIG. 5.—Kangri as worn by a youth in Kashmir.



FIG. 6.—Native of Kashmir wearing Kangri under his robes; two others squatting over them.

have examined a large number of "Kangri cancers" in all stages of development. Their characteristic structure leaves no doubt that they are epitheliomata, the accompanying figures (figs. 7 & 8) obviating any need for a detailed description.

In the case of Egypt the apparent association of cancer of the bladder and penis with infection by Bilharzia hæmatobia illustrates the



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FIG. 7.—Kangri ulcer which has become carcinomatous. From anterior abdominal wall. $\times\frac{45}{1}$.

development of the disease as a sequel to a different form of chronic irritation.

The relation of these different forms of chronic irritation to the development of cancer in different parts of the world helps us to comprehend better the part played by other forms of chronic irritation whose association with cancer has long been observed. These irritations

may be mere direct *physical* injury as in fracture of bone or in the "horn core" of cattle in India *, *chemical* as in paraffin, petroleum, arsenic, and aniline cancer, *actinic* as in the case of the short hot clay pipe, the Kangri, the X-ray, or brand cancers (of cattle). Woodhead has observed several cases of squamous celled carcinoma develop in



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FIG. 8.—Metastases in lymph gland from same case as that illustrated in fig. 7. $\times\frac{60}{1}$.

engine-drivers over the shin where the skin had been exposed for years to the direct action of heat. They may be of an *infective nature* as in

* This is a most interesting and instructive form of malignant new growth to which our attention was directed by Capt. Brodie-Mills of Bombay. Squamous cell carcinoma develops at the root of the right horn with great frequency in cattle used for draught purposes—the right horn being used by the natives to attach the animals to agricultural implements or draught-waggons.

Bilharzia for the bladder, the tubercle bacillus where epithelioma develops in an old lupus scar, or Treponema pallidum, as in the association of keratosis linguæ with epithelioma of the tongue. The irritant may be a *larger parasite*. Borrel has recorded the association of cancer with nematodes and cestodes, and we have observed a case of carcinoma of the small intestine in a mouse at the site of attachment of a tape-worm. The irritants cited can readily be augmented by the enumeration of many others having nothing in common, and they can have only a mediate relation to the causation of the disease. This is a conclusion we have expressed already, and it will be reverted to again in discussing the occurrence of cancer in animals, without studying which it would be very unwise to express any opinion on the significance of the association of chronic irritation with the development of cancer in man.

The study of all the circumstances associated with the sporadic occurrence of cancer must remain indispensable so long as we are unable to test the relative importance of each by other than statistical methods. The true importance of some of the circumstances which have been defined as having a mediate relation to the development of cancer, can only be cleared up after its nature has been ascertained, and purely statistical investigations will remain more or less empirical until this end is attained. The statistical investigation of infective diseases was pursued advantageously before the development of bacteriology directly demonstrated the causes of many of them. That demonstration placed old problems in their true perspective, e. g. the geographical distribution of infective diseases, their epidemic and endemic occurrence, the relation between variations in the incidence of infective diseases, and, e. g., water-supply, density of population, rainfall, ground-water, or season. In the case of cancer those who have a serious interest in statistics have not yet advanced from the state of empiricism in which the student of the infective diseases compiled statistics in the days before Pasteur and Koch. It is unjust to minimise the value of the accurate statistics based on the available data, for in our ignorance we do not know that the statistical method has reached its limitations ; but only that it is still of the greatest assistance in helping us to define the objective of our experimental studies.

The increased number of deaths recorded from cancer, its apparent greater frequency in some geographical areas (whether large or small) than others, the assumed importance or unimportance of the influences of race, diet, soil, climate, are all problems of much less importance than, e. g., the established fact that cancer increases in frequency as age

advances in man and animals, and, the infective or non-infective nature of cancer. Still it may not be forgotten, it was impossible for statistics to supply the proofs of the causes of diseases now known to depend on infective organisms, and it is in all probability as much without the province of statistics to supply the answer to these questions, as it is for them to yield a reply to the still more direct question—What is the cause of cancer?

The occurrence of cancer in association with chronic irritation has long been recognised, and has led to conceptions of the nature of the disease more or less out of accord with the hypothesis of a congenital origin as a general explanation of *all* forms of cancer. The facts recorded above seem to show that the different forms of irritation, although they have in themselves nothing in common, are of more moment than the sites to which they are applied. If the hypothesis of a congenital origin is to hold good for the various instances mentioned in the preceding pages, then it becomes necessary to postulate further a uniform and abundant distribution of "embryonic rests" over the body, or to assume a different distribution of "embryonic rests" in Europeans and native races, coinciding with the points they respectively select for the indulgence of various practices involving the application of peculiar irritations. The facts directly refute such a view of the congenital origin of those forms of cancer to which reference has been made above, as developing in consequence of such native customs as, *e. g.*, wearing the Kangri or chewing betel-nut. The same remarks apply also to other better known forms of cancer associated with chronic irritation, *e. g.*, chimney-sweeps-cancer, and to still less known forms, *e. g.*, brand-cancer and "horn-core" in cattle. The difference in the organ incidence of cancer between Europeans and the natives of Kashmir or India and Ceylon is that in Kashmir the abdominal wall is irritated by the Kangri, and the buccal mucous membrane of women in Ceylon and India generally, by chewing betel-nut—it is not in all probability a different distribution of hypothetical "embryonic rests" from that obtaining in Europeans. To these observations and inductions we are now able to add others drawn directly from experiments on animals, and later on in this Report Dr. Haaland will describe the experimental development of sarcomata in mice, under circumstances which appear to throw much light on the reasons why cancer develops in associotion with continuous or intermittent efforts at repair and regeneration. Although at the present time it is necessary to write with great reserve in directly applying the results of experiments to the ætiology of cancer in man, nevertheless their bearing on the mediate causative relation obtaining

between the chronic irritants cited above and the onset of cancer is one of the most suggestive results yet attained.

The advance made by experimental investigations has enabled us to follow in detail the development of *malignant connective tissue new growths* during the artificial propagation of *malignant epithelial new growths*; but we have still much to learn of the nature of the process, and until we are able to start cancerous proliferation in any tissue at will, we shall remain in this same unfavourable position for determining the true importance of many circumstances, which at the same time it is unjustifiable to ignore. Nevertheless the study of the ethnological distribution of cancer cannot be divorced from the comparative and experimental investigations without grave danger of fundamental fallacies. The apparent though temporary specialisation exemplified in the succeeding papers in this report is necessary before conceptions unifying divergent observations can be evolved.

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ON THE OCCURRENCE OF NEW GROWTHS AMONG THE NATIVES OF BRITISH NEW GUINEA.

By C. G. SELIGMANN, M.D., M.R.C.P.

The observations recorded in this paper were made during eleven months of the year 1904 spent in work in British New Guinea and the islands of its dependent archipelagos as a member of the Daniels Ethnographical Expedition. During this time pathological conditions were constantly sought out and, owing to my being able to successfully treat some of the

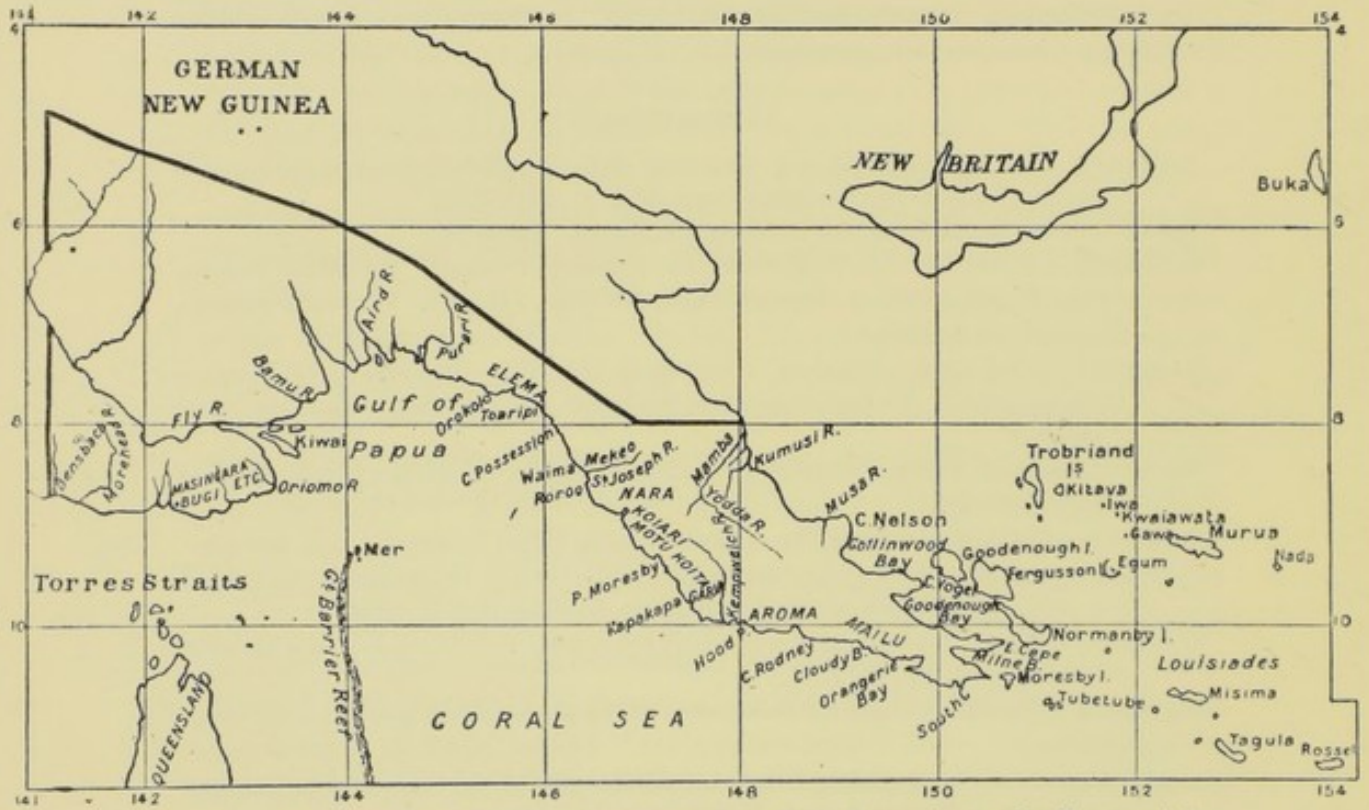


Fig. 1.—The area visited extends coastwise from Cape Possession to the Kempwelch River in the Central Division, Inland the valley of the St. Joseph River, and at the S.E. extremity of the Possession the Trobriands and their dependent Islands.

cases brought to me, a very considerable number of sick people presented themselves for advice.

New Guinea is a country so little known that it is everywhere but

just emerging from the Stone Age : its inhabitants over large areas cannot count beyond five, while it is so uncontaminated by white influence that the exanthemata, with the possible exception of German measles, are unknown, and there are large areas into which venereal diseases have not yet been introduced.

The incidence of new growths among such primitive isolated and uncontaminated folk as these Papuasians * cannot but be of extreme interest, even if the conclusions to be drawn from the knowledge at present available are scanty.

Our knowledge of the occurrence of new growths among the dark-skinned races of the Pacific is too slight to make any general comparison possible ; but since the conditions prevalent in Australia and the Bismarck Archipelago will be briefly alluded to a few pages hence, it seems well at the beginning of the subject to emphasise the necessity of keeping clearly in mind the difference between race and colour. This is especially important in such an area of racial mixture as the Western Pacific.

Thus, to take an example peculiarly apt to the matter under discussion, the Australian and true or Western Papuan, although of about the same colour, vary considerably as to their capacity for the production of hypertrophied scars ; and this in spite of the fact that both races produce scars intentionally. Further, while I have not seen enough Australians to be confident on this point, I am inclined to believe that these hypertrophied scars maintain their prominence for a much shorter time among Papuans than in Australians. As a matter of fact, in spite of the very marked tendency towards hypertrophy of cicatricial tissue in Australians, true tumours among them seem to be even rarer than among Papuans, and although I have made inquiries, I have never heard of a mass suggesting an ovarian tumour or a uterine fibroid occurring in a pure-blooded Australian. Dr. W. E. Roth tells me, moreover, that in all his experience among the natives of North-West Central Queensland he has seen no case which in his opinion was a new growth ; while Professor Stirling, in his report of the work of the Horn Expedition to Central Australia, mentions but one case of new growth—an epithelioma of the foot in a native at Oodnadatta, who was about to submit to amputation †.

* The term Papuasian is used for any native of New Guinea and the neighbouring islands born of parents themselves natives of New Guinea or its archipelagos.

† Report of the Work of the Horn Scientific Expedition to Central Queensland, Part IV. p. 129.

One advantage of the study of the incidence of tumours among such backward races "new" to civilisation as inhabit New Guinea, is that it becomes possible to consider the value of certain factors which at one time or another have been held to be causative in the production of malignant tumours. For this reason the occurrence of new growths was studied not only in Papuasians but in immigrants of the white and Polynesian races; there being a number of the latter in the Possession imported as teachers by the London Missionary Society.

Owing to the courtesy of Dr. Wendland, Chief Medical Officer at Ralum, New Britain, I am also able to adduce some facts relative to the occurrence of tumours among the inhabitants of New Britain, New Ireland, and the Northern Solomons. Further, a series of interesting venereal tumours were obtained from the native dogs of the Central District of British New Guinea. The natives everywhere have the most extreme objection to surgical treatment, so that the number of cases in which microscopical evidence as to the nature of the tumour is forthcoming is very small. As a matter of fact, new growths, whether innocent or malignant, with the exception of small congenital pigmented moles, were rare.

In the following pages an account will be given of the innocent and malignant new growths met with in British New Guinea and Torres Straits among (1) Papuasians, (2) other Melanesians, (3) Europeans and Polynesians.

BENIGN TUMOURS IN PAPUASIANS.

Papillomata.—Cutaneous papillomata are common among the natives throughout the Possession, but are, I think, decidedly less frequent than among Europeans. The largest seen was about the size of a cherry, and sprang from the skin on the front of the leg below the head of the tibia, in a boy of about sixteen.

Fibromata.—I believe that subcutaneous fibromata were not very uncommon, but where there was inflammatory adhesion of the skin, it was not always easy to be sure of the diagnosis, since there can be no doubt that granulomata of an ill-defined nature involving the cutaneous and subcutaneous tissues are common enough. In the following instance it seemed that the diagnosis was reasonably clear :—

Hari Kohu, a man aged about 50, of Akorogo village, near Port Moresby, has a swelling immediately in front of his ear, which is somewhat oblong in shape and about the size of a split apricot. It is subcutaneous

To face p. 29.]



FIG. 2.—Native of Kiriwina with Lipoma.



FIG. 3.—Congenital pigmented moles in a Papuasian.

and freely movable over the deep structures, while the skin over it is nowhere adherent. It is said to have been present since the man was a small boy and to have slowly increased in size. On palpation it is firm and obscurely lobulated, while pressure upon it produces no pain. It seems too firm for a lipoma, so probably it is a fibroma. The patient refused operation.

Lipomata.—Warupi Poni, of Waima, has symmetrical lipomata, the size of a hen's egg, over the outer end of each clavicle. There is also a subcutaneous fibroma, or perhaps an enlarged bursa, over the right acromion and two fibromata or bursæ over the left trochanter, each about the size of a walnut. The patient, who has always been dumb and is partially deaf, is quite intelligent.

A large lipoma sprang from the posterior triangle of the neck of a man of Kiriwina in the Trobriands (fig. 2), in whom it had been present for many years. Although neither of these diagnoses was confirmed by the removal of the tumour, it is impossible that anyone looking at the figure will seriously question the diagnosis of the lump shown in that photograph ; while the masses in the case of Warupi Poni, though symmetrical, were really equally typical.

Osteomata.—A case was seen at Delena of multiple small hard masses springing from the lower end of the femur of an apparently perfectly healthy woman, in whom there was certainly no sign of chronic rheumatoid arthritis or other joint trouble. There can be little doubt that post-mortem examination would have shown that these masses were osteomata, and clinically there seemed no reason to hesitate as to the diagnosis.

At Hulaa a man, Vilai Rakava by name, was seen who was judged to be about 50 and had in the region of the angles of the lower ribs on the right-hand side a hard oval mass between 3 and 4 inches long, which apparently sprang from the ribs; it was not movable upon these, but the skin over it was perfectly free. It caused no pain, and was said to have been of many years' duration. It was probably an osteoma or chondroma.

Angeiomata.—At Iwa a middle-aged woman had a large plexiform angeioma involving the vessels of one temporal region and the forehead on the same side. There was no dilation of the corresponding vessels on the opposite side.

Congenital pigmented moles and pigment spots are extremely common (fig. 3). I can remember only one subject, a girl of about 12,

in whom a tolerably close scrutiny of the exposed parts of her body did not show pigment-flecks ; while of 24 subjects, mostly male, selected at random in the village of Hohodai, 15 had well-marked congenital pigmented moles, in some cases hairy *. This number, equivalent to 62·5 per cent. of the subjects examined, would doubtless have been even larger had the examination of the cases been more complete ; for in no case was the neighbourhood of the genitals examined, while in the women the part of the body covered by the petticoat, *i. e.* from the pelvis to the knees, was necessarily not inspected. The largest congenital pigmented mole or rather series of moles met with is shown in fig. 3, growing behind and in front of the lobule of the ear of a female dwarf, a native of one of the Hanuabada villages.

In spite of the frequency with which they occurred, no case was seen or heard of in which a pigmented mole gave rise to a large tumour or tended to spread.

BENIGN TUMOURS IN OTHER MELANESIANS.

At Herbertshöhe in New Britain, Dr. Wendland, the Government Medical Officer, showed me a fibro-cheloid he had removed from the ear of a native. This growth, which was about the size of a small orange with a tuberose surface, resembled those from the ears of natives of the French Sudan, figured in the Journal of Tropical Medicine by Le Dantec and Boyé †. Like many other Melanesians the folk of New Britain bore the lobule of the ear, and as age advances both the lobule and the hole bored in it may become enormously enlarged owing to the habit of carrying small objects in the pierced lobule.

MALIGNANT TUMOURS AMONG PAPUASIANS.

Malignant tumours are especially observed among the natives of British New Guinea. Sir William Macgregor, who was for nearly ten years Governor of the Possession, first drew attention to their rarity in an address delivered at the London School of Tropical Medicine. Sir William said :

> "For nine and a half years I never saw a case of cancer in British New Guinea, but at the end of that time there occurred an

* Hohodai is one of the component villages of the large village systems formed by the Port Moresby villages and usually known as Hanuabada.

† Journal of Tropical Medicine, May 1901.

example of encephaloid cancer of the tibia in the person of a Papuan that had for seven or eight years lived practically a European life, eating tinned Australian meat daily."

Although constantly on the look-out for new growths I did not myself see any in Papuasians, and for the history and photograph (fig. 4) of the following case I am indebted to Dr. G. V. White of Thursday Island. The patient was a girl of about 16, from the island of Mabuiag in Torres Straits. The history goes that while playing she fell down and hurt her knee but did not break the skin. Shortly after this the knee began to swell and after two or three months, as it continued increasing in size, she was brought into Thursday Island, where she was seen by Dr. White who, finding that the circumference of the joint was 22½ inches, while the limb below was œdematous, and recognising that the tumour was malignant, amputated the leg above the knee. She made a good recovery and was sent back to Mabuiag, where, however, she died in about two months, from what her relatives in the "pidgin" English of the Straits described as "short wind," *i. e.* difficulty in breathing. A portion of the growth was submitted to Dr. M. B. Allen, Professor of Pathology in the University of Melbourne, who stated that the growth was a sarcoma springing from the periosteum.

MALIGNANT TUMOURS OCCURRING IN OTHER MELANESIANS.

The following case was treated in the Thursday Island Hospital, where it was first under the care of Dr. J. L. Waffall and then of Dr. White, and my best thanks are due to these gentlemen for permission to publish the case :—

A Solomon islander, supposed to have been between 25 and 30, who had been working in Torres Straits for some 18 months, but had probably been in Australia much longer, came to Dr. Waffell complaining of a lump about the size of a clenched fist in the middle line of the back between the shoulder-blades, at the level of the 9th and 10th dorsal vertebræ. The lump felt semi-fluctuating except at its upper edge, which was hard. It was apparently of deep origin, and the skin was adherent to it superficially. An attempt was made to remove the growth. At the operation it was found that its deepest parts adhered to the spinous processes of the 6th, 8th, and 10th vertebræ

and the inter-spinous ligaments. The greater part of the mass was removed, and this portion on section presented a central cavity about the size of an egg, in which was a clear mucoid fluid. Around this was a fibroid stroma, with many small cavities containing mucoid substance. The wound healed by first intention, and the patient was discharged from the hospital a fortnight after the operation, with no lump at the site of the wound.

Six weeks later he returned with a swelling, semi-fluctuant to the touch and about the size of an orange at the lower extremity of the scar. Dr. White attempted to operate but found the bleeding was too profuse to allow him to remove the mass, which on section was not cystic but almost jelly-like, and so extremely vascular that the bleeding was stopped with difficulty. The wound never entirely healed and the tumour grew quickly, and bulging through the site of the incision bled slowly but continuously, finally growing to the size of a Rugby football and extending upwards toward the neck. The patient wasted rapidly and developed a severe cough, with slight expectoration, which was not however blood-stained, and died about two months after his second operation. No microscopical examination was made of the growth but that it was malignant is sufficiently obvious, while there can be practically no doubt that the growth was some form of sarcoma.

Dr. Wendland, who is stationed at Ralum, the copra growing centre, as well as the capital of New Britain, has kindly given me his experience in regard to imported Melanesian labourers from the Solomons and New Ireland, for the medical care of whom he is responsible. In some three thousand natives passing through his hands he has seen only two lesions which he regarded as possibly malignant, one of these was an ulcer of the cheek which may have been an epithelioma; the other, the site of which is unfortunately not stated in my notes, he regarded as possibly a sarcoma.

MALIGNANT TUMOURS OCCURRING IN EUROPEANS AND POLYNESIANS IN BRITISH NEW GUINEA.

The two cases of undoubted malignant disease, respectively an epithelioma and sarcoma, have occurred among Europeans resident in British New Guinea, and to these should almost certainly be added a third. viz., the case described on p. 33, which I regard as an instance of rodent ulcer. One case of epithelioma has also occurred in a Polynesian.

The two cases of epithelioma are of special interest in that both

patients had for long resided in New Guinea under circumstances approximating closely to those in which the majority of the coastal natives of the Central Division of British New Guinea live. Further, although the value of the European case is diminished by the fact that the patient had paid a visit to Queensland within a year of his illness, it is noteworthy that the European and Polynesian cases lived only about three miles from each other, though there was an interval between the dates of their illnesses.

Concerning the case of epithelioma in a white man, it affected the face, and was first noted either during a trip to Queensland or at Hulaa in New Guinea, where the patient resided till within a few months of this trip. In any case the patient went to Australia to be operated upon, whether he returned for a short time to Hulaa I am unable to say, but the patient certainly came to England, where he died of exhaustion during the first half of 1903, due to recurrence and the effects of an old standing cardiac lesion.

The second case of malignant disease occurred in the person of a white woman, a missionary who, when the mass was first noted, spent three years in the Possession in a village west of Port Moresby more than 100 miles from Hulaa. The growth was an epulis removed from the upper jaw, and on microscopical examination was seen to be a giant-celled sarcoma.

The following is an account of the case before referred to as being almost certainly a rodent ulcer *.

The patient, aged 48, has spent the last 22 years in New Guinea, except six months in 1888 which he passed in Cooktown in northern Queensland. He has had two attacks of dysentery, reported severe, but has suffered comparatively little from fever. About five years ago he considers that he became unusually sunburnt, after which he noticed a pimple, the size of a small split pea with a rough surface which looked like a small wart, on his face between the left ala nasi and the malar prominence. A "round white head" is described as having formed, and this was treated by Dr. Blayney with fuming acid, probably nitric. As the mass did not improve Dr. Blayney subsequently excised it, and the patient thought this was done some two or three months after it was first noticed. Recurrence took place, and about two years later an oblong flattened mass which did not ulcerate, but formed an elevation in the substance of the cutis, was present. It was only very

* This instance is included here since histologically the mass removed showed malignant characteristics.

slightly raised at first although it became red and looked as if it would inflame. Subsequently, *i. e.*, rather more than two years after the first operation, it slightly increased in size and its centre broke down. It was excised by Dr. A. J. Craigen, when it was noted that there was a certain amount of ulceration at its centre, but no evidence of its having healed in one place while spreading peripherally.

State on Examination.—The patient early in 1903 was a well-nourished, healthy, energetic man, with normal pulse-tension and no evidence of any organic disease of the viscera. There was an irregular cicatrix somewhat raised and in the centre obviously thin-skinned, occupying the region between the left ala nasi and the malar prominence. In one place on the surface of the scar there was a minute scab. With this exception the scar seemed healthy. The thinness noted in the centre of the cicatrix was probably, at any rate in part, due to stretching, healing after the last operation having taken place by granulation.

The Polynesian already referred to as having suffered from epithelioma was a Samoan judged to be about fifty in 1898, when he was a healthy and active but rather fat man whose hair was turning grey. Itama, the patient in question, was a teacher imported by the London Missionary Society in 1882, from which date until he left New Guinea to be operated on for epithelioma he did not leave the country. During the greater part of this time he was stationed at Hulaa in the Central Division, some 60 miles to the east of Port Moresby. Itama noticed a lump on his tongue some time early in 1899, which subsequently broke down and left an ulcer which did not heal. The epitheliomatous nature of the lesion was recognised by Dr. Blayney, who sent Itama to Sydney for operation. He was admitted to the Sydney Hospital under Dr. A. J. Craigen, to whom my best thanks are due for the following extract from the hospital notes :—

> "Itama, age 45, native teacher. Malignant disease of tongue. Admitted January 3rd, 1900; discharged February 22nd, 1900.
>
> "4.1.1900. Ulcer on tongue, had it 6 months. Came as a lump which broke down. Has not been losing weight to his knowledge. Denies any history of syphilis. No scars on penis, papery scars on both shins, especially right. Eyeballs are prominent, but no tumour in region of thyroid. Pulse 119. Heart's sound distant. No cough. Chest clear in front.
>
> "On right side of tongue there is an ulcer, irregular, with hard edges, extending from one inch behind the tip, back to the anterior pillar of the fauces; it is deeply excavated along the lower part of the side of the tongue.

To face p. 35.



FIG. 4.—Sarcoma of knee. Mabuiag Island.

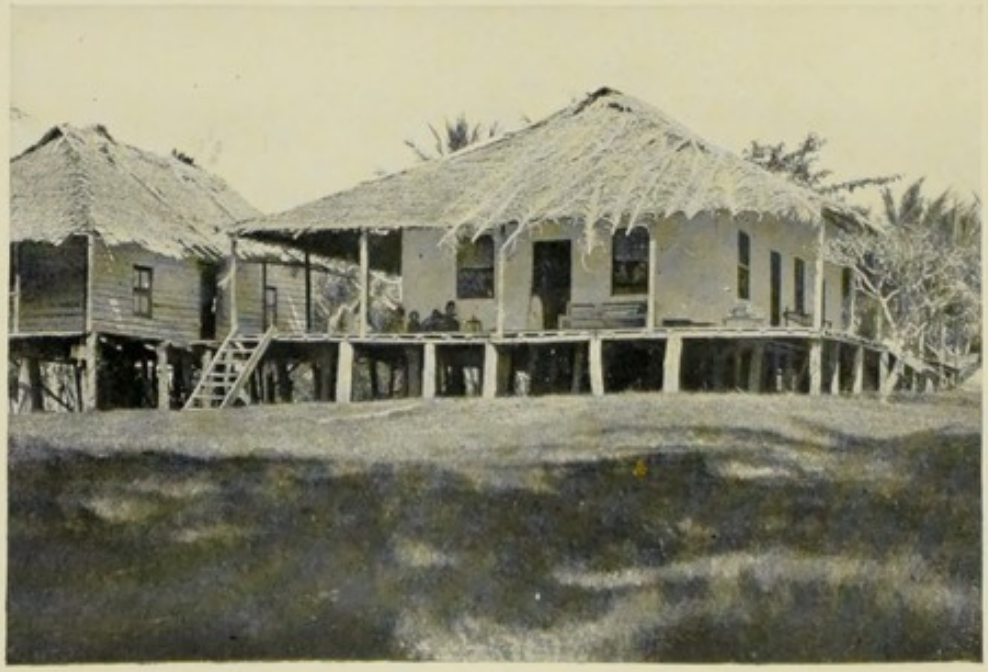


FIG. 5.—House in which Itama lived.

Not firmly attached to the jaw. Right submaxillary gland is enlarged. No other lumps to be felt. No enlarged glands on left side."

A very complete operation was performed, from which the patient recovered well.*

Itama returned to Samoa where he died, as I am informed, within a year, of a local recurrence.

It is interesting to note that Hezekia, the present Polynesian teacher at Hulaa, succeeded Itama, whom he did not however meet. Before coming to Hulaa he had worked for four years at Aroma and for seven near the mouth of the Kemp-Welch River. He said that he is about 47, but does not look more than 40, although his hair is turning grey and a few grey hairs are present in his rather sparse Imperial. His wife is probably about 30. During the past four years Hezekia and his wife have both been quite well; Hezekia has had no fever, his wife has suffered slightly; when seen both appeared in excellent condition.

The house in which Itama became ill (fig. 5) and to which Hezekia succeeded when the latter came to Hulaa after the death of Itama is a lath-and-plaster building, the laths probably consisting of the axes of the frond of the sago-palm. It is a comfortable and airy habitation (fig. 5), with a native-built palm-leaf roof, about 18 feet above the flooring boards, which are supported on piles about 3 or 4 feet above the ground, and within the house are partially covered with native mats. The bedrooms are separated from the sitting-rooms by partitions which only run as high as the walls of the house, leaving a large space under the roof for free circulation of air. The water-supply is from a well about 12 feet

* Extract from Sydney Hospital notes:—

"12.1.1900. Under $CHCL_3$ preliminary tracheotomy done (high operation) and tube tied in. (Anæsthetic then given through the tube.) Incision made in a curved direction from the symphysis to the right angle of the jaw, and then a vertical one downwards along the carotid sheath. The right lingual artery was ligatured and the glands of the anterior triangle cleared out. The left lingual was also ligatured through a small incision. The lower lip was divided and the flap dissected off the jaw, which was sawn through at the symphysis and just in front of the right angle. The whole of the tongue and the floor of the mouth were removed with scissors, in one piece with the portion of jaw attached. The bleeding was free but was easily controlled. The mucous membrane of the mouth was brought together as far as possible, and the skin incisions closed with silkworm gut and horsehair sutures. A drainage tube was placed in the neck. The tracheotomy tube was removed in 3 hours, when there was very little bleeding and the patient's condition was satisfactory. On the 5th or 6th day the patient had a rigor without any apparent cause; these were repeated on several occasions and were evidently of a malarial nature, as he seemed quite accustomed to them, having suffered from New Guinea fever.

deep, the sides of which are faced with corrugated iron. The well itself is usually left uncovered, and is very largely dependent on rain-water which sinks through the porous superficial sandy soil ; it is situated about 100 yards behind the house amidst a number of coconut trees. The house itself is open to the full force of the south-eastern monsoon, and stands about 200 yards above high-water mark at a height of some 8 or 10 feet above the beach, which is composed of sand and a little mud The native houses of Hulaa are all built on piles in the sea, some considerably below high-water mark. The house occupied by the late W. E. G., the European already referred to as having died of epithelioma of the face, was similarly situated, but on less high ground, at a distance of about 200 yards from the beach and some four miles to the west of the native village and the mission dwelling. It is a rough board shanty on piles about 4 feet high, and stands upon sandy soil. It has no windows but many doors and a roof of corrugated zinc. There are coconuts all round the house, which are doing extremely well, while the water-supply is from tanks. This house was inhabited at the time of my visit.

INFECTIVE VENEREAL TUMOURS IN NEW GUINEA DOGS.

A series of infective venereal tumours were observed in dogs in the coast villages of the Central Division in the neighbourhood of Port Moresby. There has been so much crossing with imported dogs of European breeds that it is impossible in any case to say that a dog or bitch is of the pure native breed, but certainly the disease occurs in animals that have every appearance of being so, and according to the natives themselves was endemic before the advent of the white man.

It is interesting to note that although a keen look-out was kept for this disease in the islands of the South-Eastern Archipelagos no case was seen. It was not possible to make experimental inoculations upon sound dogs, but the distribution of the disease and the observations of the natives leave practically no doubt that the masses are infective and are spread by coitus.

The photographs 6 & 7 of the diseased parts removed from two dogs, as well as the following notes of four cases dissected, will make the general character of these growths clear.

Dog A. This was a well-nourished bitch of a yellow-brown colour, a thorough mongrel. All round the entrance of the vagina were masses of ulcerated fungous growth, causing the vulva to appear much enlarged

To face p. 37.]

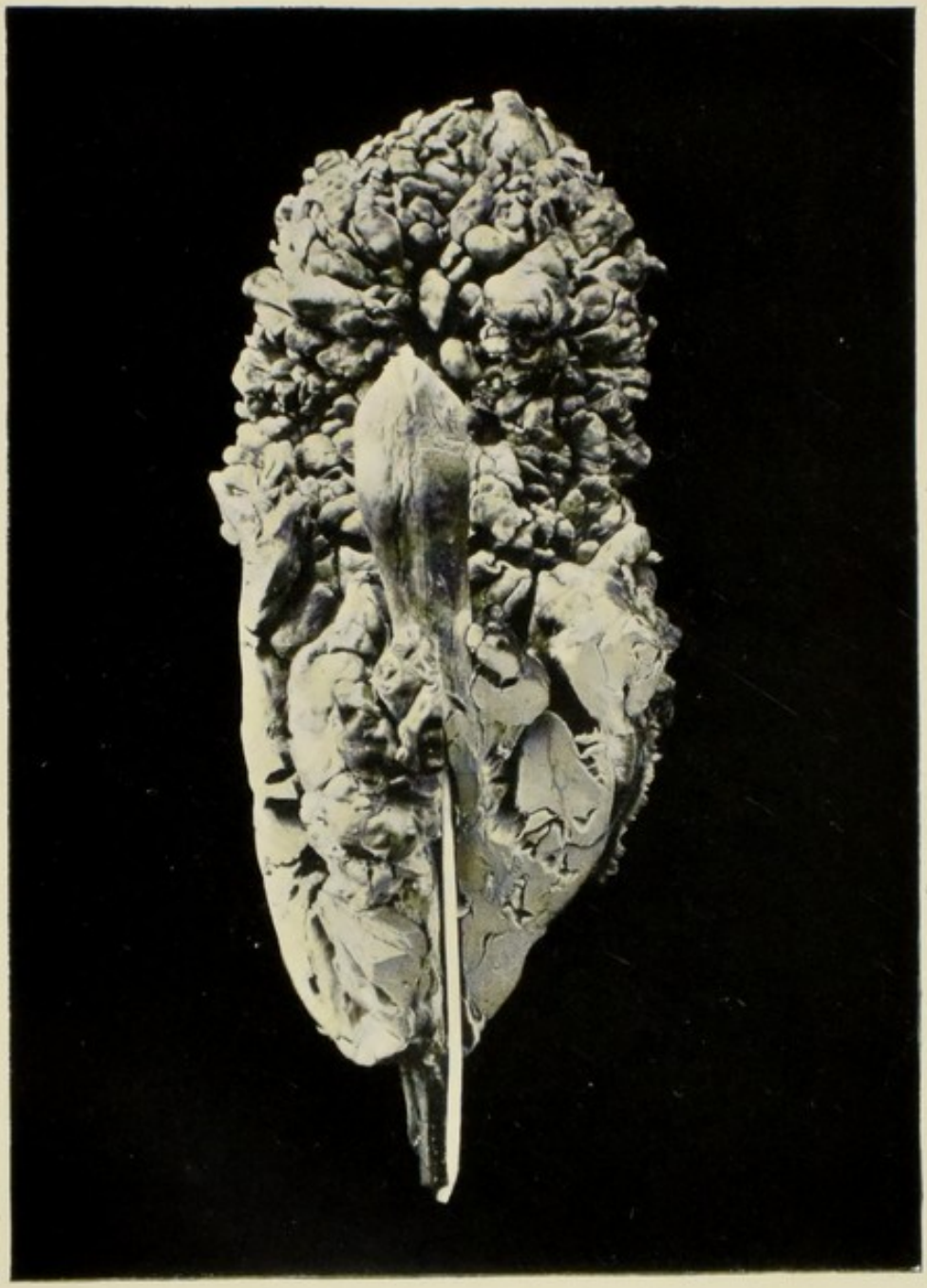


FIG. 6.—Penis of dog showing growth referred to in text, page 37.

and to hang somewhat downwards. The rectum and skin around it were unaffected. From the vulva a thin yellowish fluid dripped continuously. The anterior part of the pelvic girdle was removed after death, when it was found on slitting up the vagina that the growth affected the whole of its length as well as the external surface of the os uteri, but there was no ulceration through the muscular wall of the vagina. The cavity of the uterus was quite unaffected, as were the bladder and ovary ; in fact, there were nowhere any enlarged glands or metastases to the viscera.

Dog B was an extremely emaciated brown bitch. Growth protruded from the vulva as in bitch A, but was larger and was distinctly opaque. Around the vulva and spreading towards the rectum there were scattered patches of ulceration, quite unconnected with the main mass of the tumour, often with bridges of undamaged darkly pigmented skin between them. There were no adhesions to other organs nor was there any invasion of the rectum, though the wall of the vagina was greatly thickened. On slitting up the uterus, the lower part of the unpaired portion was found to be full of bloody slime, and here a separate mass of growth about the size of a split haricot was seen. There were enlarged glands on each side of the belly beneath the superficial fascia, between the groin and the mid-line of the body. There were no metastases in the lungs, liver, pancreas, spleen, or kidneys, but the latter contained a number of minute subcapsular cysts, penetrating certainly not more than from 1 to 2 millimetres into the cortex. The capsules stripped easily. Near both kidneys were certain whitish masses, apparently enlarged glands. They were irregular in shape, and those on the right side lay almost in contact with the vena cava.

Dog C was a thin emaciated male ; the prepuce was much swollen, but there was no obvious ulceration. It was found impossible to press the penis out of the prepuce. On slitting up the prepuce, practically the whole of the surface of the penis was seen to be covered with ulcerated growth (fig. 6). The scrotum was swollen to about the size of a man's fist. The testes appeared to be enlarged and to occupy the whole of the pouch, the surface of which was shiny from distention. The scrotum was not invaded, but both testes were absolutely replaced by tumour-tissue resembling that on the inner surface of the prepuce. The tunica albuginea was thin and distended but nowhere ruptured, and there were no adhesions between its two layers. The whole of the inner surface of the prepuce was occupied by papillary growth, ulcerated

in places. There were enlarged glands in both groins. There were no signs of metastasis to the abdominal or thoracic viscera.

Dog D. This was a large reddish-brown well nourished adult male, more stoutly built than the pure bred native dog generally is, and was doubtless of mixed blood.

The prepuce was swollen and much distended, its skin having a shiny appearance. From its orifice there dropped continually a thin, slightly opaque yellowish fluid. On slitting open the prepuce, the whole of the interior surface was seen to be covered with coarsely papillary masses of whitish growth (fig. 7).

The penis itself was for the most part unaffected ; but near the reflection of the prepuce on its ventral surface there was a papillary growth occupying an area bigger than a shilling, while proximately to this, on the penis in the neighbourhood of the preputial reflection were many separate small elevations, each about the size of a pin's head (fig. 7). There were no enlarged glands in the groin, abdomen, or pelvis, nor were there any signs of metastasis to the liver, spleen. kidneys, intestines, or lungs.

It is noteworthy that in no case did metastasis to organs beyond the genital system occur, while the enlarged glands which were present in two cases showed no secondary growth but only inflammatory swelling. On the other hand, in animals B and D there was discrete growth at some little distance from the main tumour. In every case the masses when cut into were white and soft, suggesting a round-celled sarcoma.

The examination of microscopical sections left no doubt in the mind of the writer that the tumours were histologically round-celled sarcomata. In all of them the mass consists of more or less spheroidal cells of uniform size and character. The amount of connective tissue varies, but is in none of the tumours large, and there is no transformation of the tumour cells to fibroblasts. The capillaries are few and small.

I am indebted to Mr. Shattock—who agrees with me that the tumours are histologically indistinguishable from sarcomata—for the following detailed note of the structure in three of the specimens :—

Dog C. Testis.—"A dense growth of polyhedral cells, in close apposition, and of uniform character. None show any transformation to fibroblasts. Simple capillaries run among the cells, and here and there a thin stream of connective tissue.

"There is a complete absence of polymorphonuclear leucocytes, of

[*To face* p. 38.

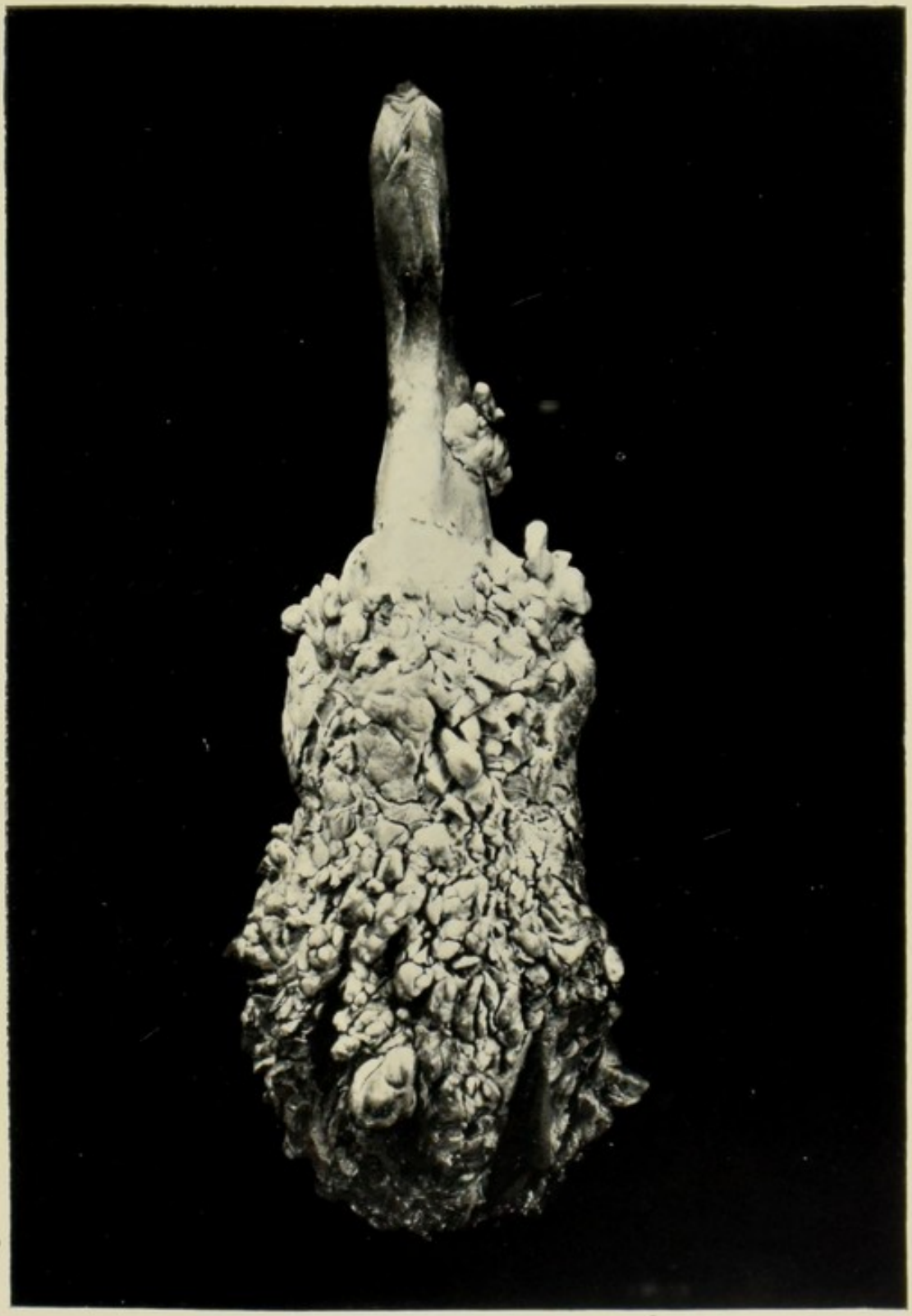


FIG. 7.—Penis of dog showing a single large lobulated mass and many smaller masses of growth referred to in text, page 38.

To face p. 39.]

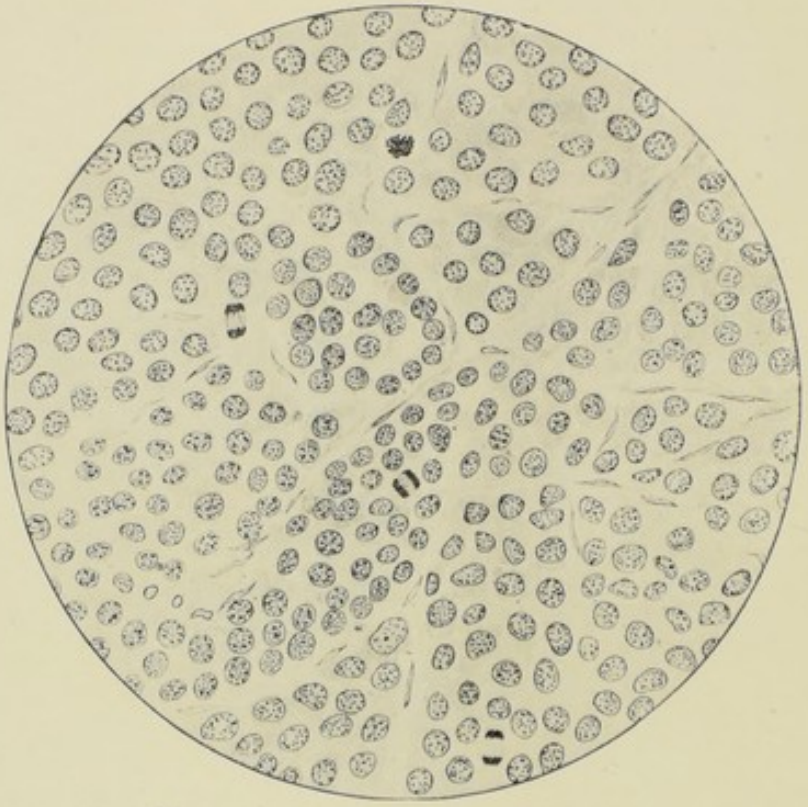


FIG. 8.—Section of Tumour of Bitch

lymphocytes and plasma cells in the growth proper. The nuclei of a number of the tumour cells are undergoing mitosis."

Bitch A. Vagina.—"Precisely the same structure as the preceding. In certain places the vaginal epithelium is intact. In such situations the tumour has invaded the whole of the tissue up to the epithelium which lies directly on the cell-mass of the growth. Mitotic figures occur in no inconspicuous numbers." Fig. 8 is a photograph of a drawing of a section of this tumour *.

Bitch B. Vulval growth.—"The section shows the same class of cell, but the amount of connective tissue intermingled is larger. The surface is excoriated, and the superficial part of the growth infiltrated with leucocytes. Many of the fields, however, completely resemble those in Bitch A."

In none of these tumours are any necrotic changes present.

These tumours of New Guinea dogs are probably identical with those occurring in certain highly-bred dogs in this country, described by Mr. G. Bellingham Smith and the late J. W. Washbourn †. The position and microscopical character of the lesion is identical, and in both series of cases the disease was transmitted by coitus, and invaded the tissues of the vaginal walls, but did not produce metastases to the thoracic and abdominal viscera. Probably the disease is widely spread, since in the tea-growing, hill country of Ceylon I saw a pure bred fox-terrier bitch suffering from what appeared to be the same condition.

CONCLUSIONS.

The writer believes that enough evidence concerning the incidence of benign and malignant new growths in British New Guinea has been presented to warrant the deduction of certain conclusions. Before stating these it is, however, necessary to allude to certain facts which have not been mentioned in this paper, but which must be considered in any description of the geographical and ethnological distribution of new growths. These facts are, in the first place the frequency of chronic ulcerative and irritative process among Papuasians, and secondly, the absence of certain conditions (gout, arterio-sclerosis) due to faulty metabolism. Perhaps, too, syphilis does not occur, or exists only in

* This figure was drawn under $\frac{1}{8}$ in. objective; but the mitotic figures were put in from study with $\frac{1}{12}$ in. objective.

† Transactions of the Pathological Society of London, vol. 48, p. 310.

certain limited areas, while the advent of old age is usually unaccompanied by certain of the retrogressive changes which commonly occur in elderly Europeans.*

Bearing these facts in mind, the writer's conclusions are as follows:—

1. New growths, whether benign or malignant, are rare both among the inhabitants of New Guinea and the closely related Melanesians of the Bismarck Archipelago and the Northern Solomons.

2. When malignant new growths do occur they are, on the present evidence, sarcomata †; these occur in men and infective venereal tumours which histologically are indistinguishable from sarcomata occur in dogs. Carcinomata, if they occur at all, are much rarer.

3. Chronic irritative processes are not *per se* sufficient to produce new growths in Papuasians, among whom wounds are common and practically never heal otherwise than by second intention; while probably every native past middle life bears at least one scar of considerable size.

4. Papuasians and the Melanesians of the Bismarck Archipelago and the Northern Solomons are predominantly vegetable feeders, and do not suffer from gout and arterio-sclerosis; but their immunity to new growths cannot be directly attributed to their diet, since in Australia, where the natives make no gardens and are largely hunters, tumours, whether benign or malignant, are certainly rare and are perhaps as infrequent as among Melanesians.

5. In those rare cases of malignant disease which occur among Melanesians, the incidence of the disease seems to be associated in some obscure way with the adoption of a mode of life which assimilates to that of the white man. Further, the cases of malignant disease occurring in aliens and cited in this paper, show that there is nothing in the environmental conditions prevalent in British New Guinea capable of preventing the development of new growths in aliens resident in the country.

* The evidence on which these statements are founded will be given in detail in that volume of the Report of the Daniels Ethnographical Expedition to New Guinea which deals with Physical Anthropology.

† Since Dr. Seligmann's visit to New Guinea, Dr. Fleming Jones has reported a case of squamous-celled carcinoma of the penis (with specimen) in a native of the Solomon Islands who had lived many years in New Guinea, and a case of epithelioma of the anus in a female Papuan. He also forwarded a specimen of widely disseminated sarcomatous growth from a hen. Dr. Beaumont has reported (with specimen) a case of melanotic sarcoma of the dorsum of the foot in a Papuan.—E. F. B.

THE ZOOLOGICAL DISTRIBUTION OF CANCER.

By J. A. MURRAY, M.B., B.Sc.

In the First Scientific Report we recorded with Bashford, the occurrence of malignant new growths in various classes of vertebrates from mammals to fishes, and drew attention to the important bearing which this wide zoological distribution of cancer had on various conceptions which had been formulated as to the nature and etiology of the disease. Since then we have continued to receive specimens of tumours and tumour-like formations in lower animals. The results of the examination of this material, limited as it has proved in many directions, have confirmed the conclusions advanced in 1903–4 as to the ubiquity, and one may say homogeneity, of malignant new growths wherever they occur.

For this material we have again to acknowledge our indebtedness to many private individuals, members of the Medical and Veterinary professions, to working zoologists and physiologists, and in a very special manner to the Director of the Marine Biological Association's Laboratory at Plymouth, Dr. E. J. Allen and his collaborators, as well as to the authorities of the similar institution at Lowestoft. The small number of specimens described below gives no idea of the mass of material examined or of the labour devolving on those who have so generously supported our endeavours in this matter. Before proceeding to a detailed description of individual tumours of more particular interest, it is important to note that the material at our disposal now embraces a very extensive series of cases of carcinoma of the skin from very widely separated groups of animals. The descriptions and figures which follow will show how very uniformly the characteristic features of malignant new growths of the external covering of the body are reproduced in the representatives of the different classes of vertebrates.

MAMMALS.

The malignant new growths of the mammals are in all respects so similar to those occurring in man that descriptions of individual tumours will not be given, but, as in the First Scientific Report, a tabular statement of all the cases examined since its appearance may suffice. The references to the occurrence of cancer in mammals have increased in frequency in recent years. Thus Pettit described (Bull. Mus. Hist. Naturelle, Paris, 1897, 1900) a sarcoma of the thyroid in a jackal, a squamous-celled carcinoma of the cervix uteri in a gazelle, a small round-celled sarcoma in a bear (abdominal growth), a carcinoma of the parotid in a jackal, and a carcinoma of the thyroid in an opossum. Dr. Burton Clelland, Government Bacteriologist, Western Australia, has forwarded us a carcinoma of the mamma from an old lioness. Lubarsch has described a carcinoma of the kidney in a rabbit, which he transplanted without success. Trotter has shown that malignant adenoma of the liver parenchyma (*cf.* fig. 1) and carcinoma of the uterus is by no means uncommon in cows, and has published a good account of their pathological features. Welsh has recorded the occurrence of malignant new growths in a lioness, a tigress, and in a marsupial (*Dasyurus viverrinus*). Sticker has published an account of a large number of tumours in the dog, cat, sheep, and other domesticated mammals. Loeb has drawn attention to the frequency of squamous-celled carcinoma of the caruncula of the eye in cattle in the Western United States. In addition, a large number of isolated instances of new growths of various organs continue to be described in the veterinary journals, but need not be referred to in greater detail. The occurrence of carcinoma of the mamma (udder) and uterus in cows is a matter of more particular interest, and we desire to thank Mr. A. M. Trotter for supplying us with specimens.

The relative frequency in our own experience of malignant growths on the surface of the body will be apparent at once. The determining factor here is obviously the ease with which the physical examination can be carried out. This consideration has more and more influence on the frequency and situation of the cases recorded as we pass to animals which are more difficult to handle, or in which the lesions occur as miniatures of those in man, as, *e. g.*, in the case of the mouse, which is two to three thousand times smaller than a man by weight, and in which the anatomical lesions of cancer, whether primary or secondary, are correspondingly diminutive and difficult to detect

Malignant New Growths from Domesticated Mammals.

DOG.

Primary Site.	*Age.*	*Sex.*	*Microscopical Examination.*
Mamma	Old	F.	Duct carcinoma.
do.	15	F.	Alveolar carcinoma.
do.	—	F.	Carcinoma cysticum papilliferum.
do.	—	—	Columnar-cell carcinoma.
do.	12	M.	Osteo-sarcoma.
do.	—	F.	Chondro-osteo-sarcoma.
do.	13	F.	Round-cell sarcoma.
do.	4	F.	Sarcoma.
do.	—	F.	Carcinoma.
do.	9	F.	do.
do.	15	F.	do.
do.	Old	F.	Spindle-cell sarcoma.
do.	12	M.	Adeno-carcinoma *.
Pharynx	6	F.	Squamous-cell carcinoma.
do.	—	—	do. do.
do.	—	F.	do. do.
do.	—	F.	do. do.
do.	—	—	do. do.
Palate	—	—	Round-cell sarcoma.
do.	—	—	Alveolar sarcoma.
do.	—	—	Squamous-cell carcinoma.
Tongue	—	—	do. do.
Buccal mucous membrane	10	M.	do. do.
Lip	7-8	—	Alveolar sarcoma.
Ear	—	—	Large round-cell sarcoma.
Scalp	—	—	Round-cell sarcoma.
Skin	Old	—	Squamous-cell carcinoma.
Neck	—	—	Round-cell sarcoma.
Fore leg	—	—	Fibro-sarcoma.
do.	—	—	Myxo-sarcoma.
do.	—	—	Round-cell sarcoma.
do.	—	—	do. do.
do.	10½	M.	Squamous-cell carcinoma.
do.	—	—	Chondro-sarcoma.
do.	Old	—	Spindle-cell sarcoma.
Hind leg	—	—	Melanotic sarcoma.
do.	Old	M.	Mast-cell sarcoma.
do.	10	M.	Melanotic sarcoma.
do.	—	—	Round-cell sarcoma.
do.	—	—	Mast-cell sarcoma.
Body-wall	—	—	Round-cell sarcoma.
Tail	7 mos.	M.	Oval-cell sarcoma.
Liver	Old	F.	Columnar-cell carcinoma.
Intestine	7	F.	Round-cell sarcoma.
do.	—	—	Sarcoma.
Rectum	15	M.	Columnar-cell carcinoma.
Anus	—	—	Adeno-carcinoma.
do.	—	—	Malignant adenoma.
do.	—	—	Squamous-cell carcinoma.

* This tumour was transplantable and was propagated through three generations.

CAT.

Primary Site.	*Age.*	*Sex.*	*Microscopical Examination.*
Œsophagus	15	—	Squamous-cell carcinoma.
Mamma	2½	F.	Spheroidal-cell carcinoma.
Tongue	8–9	M. castr.	Squamous-cell carcinoma.
Small Intestine			Round-cell sarcoma.
Axilla	Old	M.	Myxo-sarcoma.
Mamma	—	—	Alveolar carcinoma.
Mamma	—	F.	Carcinoma.
Mamma (chest-wall)	9	F.	Adeno-carcinoma.
Eyelid	6	—	Spindle-cell sarcoma.
Anus	8–9	M.	Alveolar carcinoma.
Humerus	—	M. castr.	Spindle-cell sarcoma.

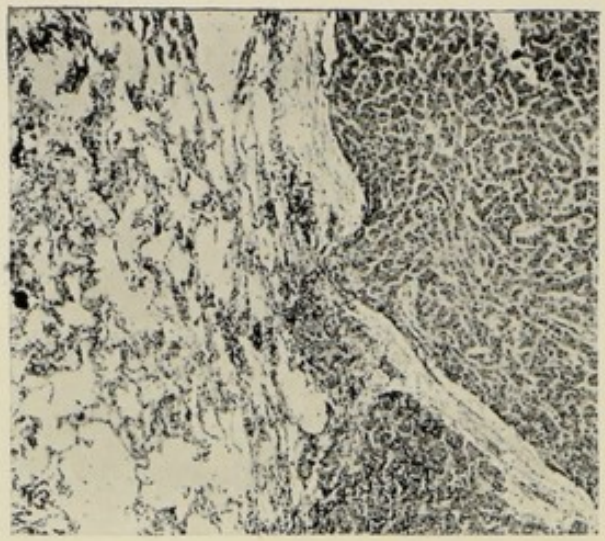
HORSE.

Primary Site.	*Age.*	*Sex.*	*Microscopical Examination.*
Lip	—	—	Squamous-cell carcinoma.
Penis	—	M.	do. do.
Penis	24	M.	do. do.
Eye	—	—	do. do.
Vagina	—	F.	do. do.
Tail	—	—	Melanotic tumour.
Penis	—	M.	Squamous-cell carcinoma.
Tail	12	F.	do. do.
Perineum	7	—	Melanotic sarcoma.
Tail	—	—	Alveolar carcinoma or melanoma without pigment.
Eye	13	M.	Squamous-cell carcinoma.
Face	16	M.	do. do.

COW.

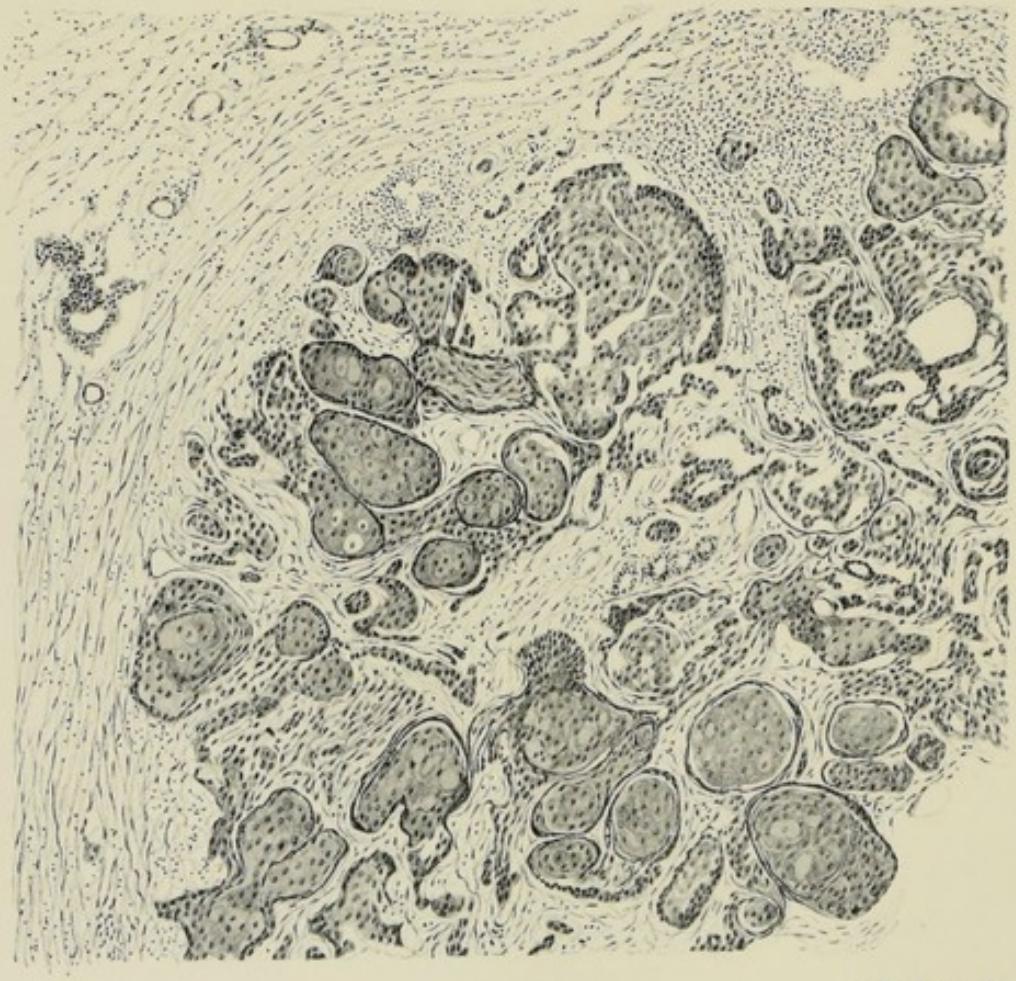
Primary Site.	*Age.*	*Sex.*	*Microscopical Examination.*
Eye	Old	F.	Squamous-cell carcinoma.
Eye	Old	F.	do. do.
Eye	5–6	F.	do. do.
Eye	Old	F.	do. do.
Eyelid	—	F.	do. do.
Skin	Old	F.	do. do.
Horn	—	—	do. do.
Rumen	Old	F.	do. do.
Rumen	Old	F.	do. do.
Rumen	Old	F.	do. do.
Rumen	Old	F.	do. do.
Bowel	Old	F.	Adeno-carcinoma.
Liver	Old	F.	Malignant adenoma. (7 cases.)
Mamma	Old	F.	Papilliferous cystic carcinoma.
Uterus	—	F.	Adeno-carcinoma.
Testis	—	M.	do. do.
Suprarenal	Old	F.	Alveolar sarcoma.
Mediastinum	—	M.	Lymph adenoma.

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Microphoto by W. Imboden.

FIG. 1.—Cow: Primary carcinoma of liver, metastasis in lung. The carcinoma nodule (on right) reproduces minutely the structure of normal liver. The nodules were coloured with bile-pigments. (Material from A. M. Trotter, M.R.C.V.S.) $\times \frac{55}{1}$.



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FIG. 2.—Fowl: Squamous celled carcinoma of buccal mucous membrane, deep surface of growth. (From a preparation by Dr. L. Pick.) $\times \frac{60}{1}$.

BIRDS.

The references to malignant new growths in birds are much scantier than in mammals. Pettit described (Bull. Mus. Hist. Naturelle, Paris, 1900) a carcinoma of the thyroid in a macaw (*Ara macao*). Koch has described a case of squamous-cell carcinoma of the floor of the mouth (fig. 4) in a cock, quite analogous to Pick's case (Berl. Klin. Wochenschr. 1903) (fig. 2). Ehrenreich and Michaelis give a detailed account of two adeno-carcinomata of the small intestine in fowls and of a round-celled sarcoma also of the intestine. In addition they describe two benign tumours in the same animal. Gilruth has added several sarcomata to those already recorded by him. We have received a number of examples of new growths in birds. Many of large size have been due to tubercle, others could not be certainly assigned to diseases of known infective nature, nor yet to the malignant new growths, *e. g.*, a connective-tissue new growth of the liver in a wild blackbird, found dying by Mr. Goodall, exhibited wide dissemination in other organs and may be a sarcoma, but pending more experience of new growths of connective tissue origin in birds we refrain from classifying it as such. Other interesting new growths have been forwarded by Professor White from the rectum of a Rhea, by Dr. Clelland from a duck, and by various dealers in poultry. The occurrence of dermoid cysts in birds in connection with the feather follicles as noted by Koch, is a matter of interest.

Adeno-carcinoma of small intestine of Grouse.

The case described below from the small intestine of the grouse is in many respects similar to the first of the cases of intestinal new growth described by Ehrenreich and Michaelis.

This interesting specimen of a malignant new growth from the only wild bird peculiar to Great Britain, was forwarded to Mr. R. C. S. Edlestone, from the head game-keeper to the Duke of Rutland.

The specimen arrived in a very decomposed condition. In the small intestine a loop was found much thickened and twisted. Microscopical examination showed that a carcinomatous ulcer was present (fig. 3). The base of the ulcer lay at the level of the bases of the crypts of Lieberkuhn, and the submucous layer as well as both muscular layers were replaced by masses of adeno-carcinoma. The infiltration had proceeded through all the coats of the intestine and large nodules had formed on the peritoneal surface. The parenchyma of the growth consisted of acini lined by low cubical epithelium. The lumina having a great tendency

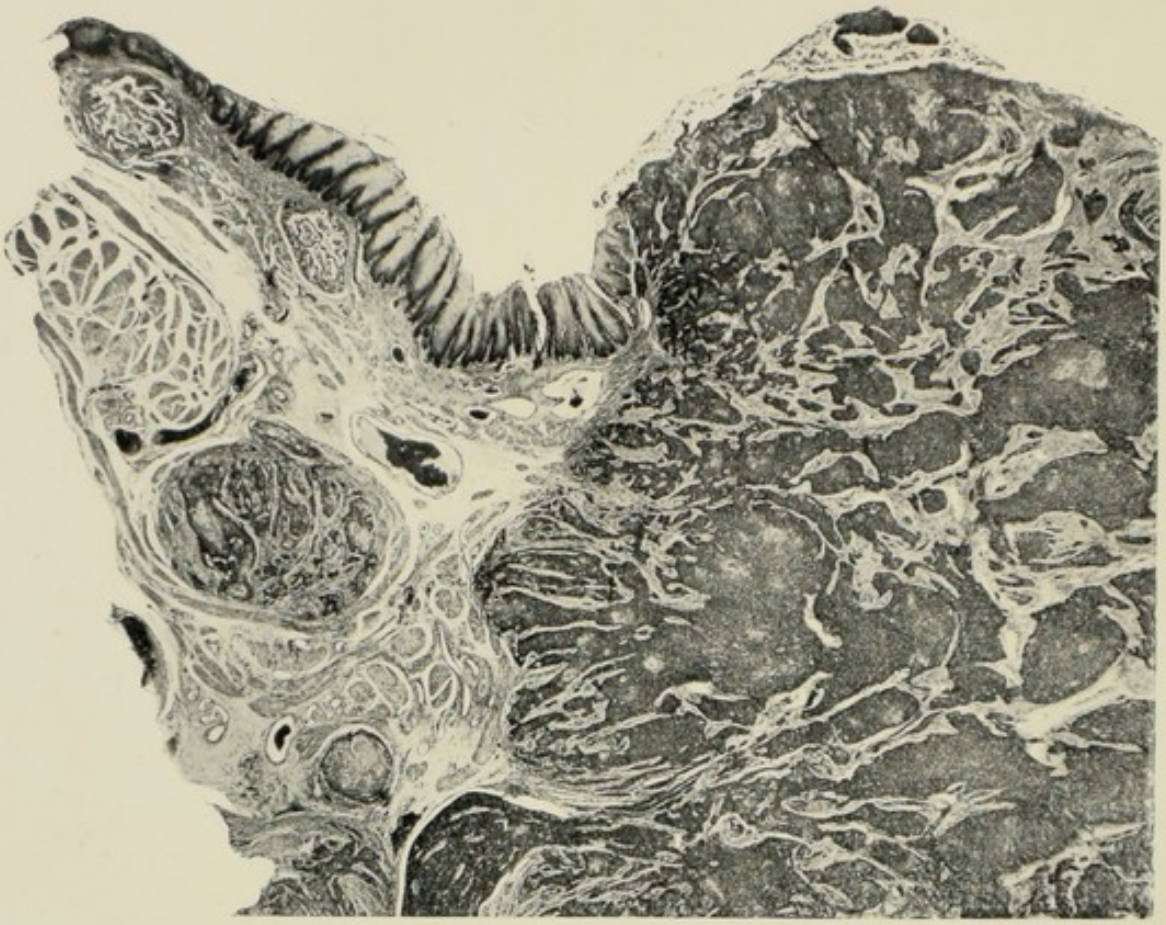
to cystic dilatation without a corresponding multiplication of the cells lining them, many small cysts occur lined by flattened cells forming almost a pavement epithelium. The stroma of this tumour was very abundant and sclerotic, consisting of narrow spindle cells with small



FIG. 3.—Grouse: Primary adeno-carcinoma of small intestine. Infiltrative growth in all coats of intestine: great thickening of circular muscular layer, the surface of which at one part forms the base of the ulcer. $\times \frac{15}{1}$.

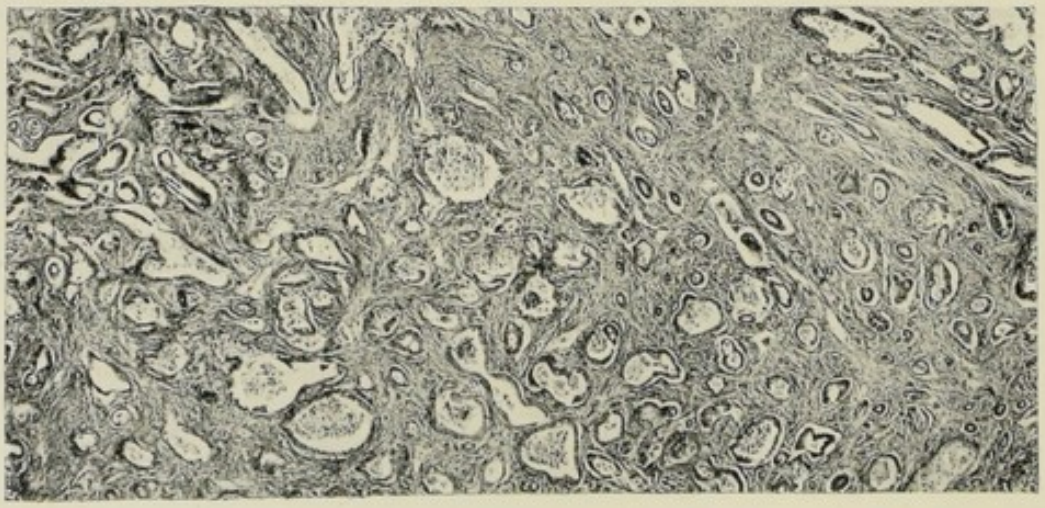
darkly-stained nuclei lying between thick bundles of collagenous fibres. The mode of spread and the relation to the structures of the gut-wall are the same as we have already described in a case of primary

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Microphoto by W. Imboden.

FIG. 4.—Fowl: Squamous cell carcinoma of buccal mucous membrane.
(From a preparation by Dr. M. Koch.) $\times \frac{15}{1}$.



Microphoto by W. Imboden.

FIG. 5.—Canary: Columnar-celled carcinoma. Tumour acini lined by columnar or cubical epithelium, separated from each other by broad strands of fibro-cellular stroma. $\times \frac{55}{1}$.

adeno-carcinoma of the small intestine of the mouse (*cf.* figs. 40 and 41, Second Scientific Report 1905).

Columnar-celled carcinoma of the ovary of Canary.

The relatively enormous tumour (3 cm. diameter) distended the abdominal cavity, and was sent to us by Mr. F. W. Cousens, F.R.C.V.S., who informed us that the peritoneum was covered with secondary growths. On section it was pale with yellowish-white nodules scattered throughout. On microscopical examination (fig. 5) the growth shows large necrotic areas, and between these irregular acini very variable in size lined by columnar epithelium. The connective-tissue stroma consists of broad strands of spindle-cells with abundant collagenous fibrils between. But for the presence of nucleated erythrocytes in the blood-vessels one might imagine that the growth was from the ovary of the human subject.

REPTILES.

Up to the present no malignant new growth has been recorded in reptiles. Pettit (Bull. Mus. Hist. Naturelle, Paris, 1902) has described a fibroma in the stomach of a python. M. Koch demonstrated before the German Pathological Society in 1904, a papilloma of the occipital region of a lizard (*Lacerta agilis*).

AMPHIBIANS.

The new growths from Amphibia are still very limited in number. They have, however, a particular interest from the zoological position of this group, and the clearness of the histological pictures due to the large size of the cells of these animals which have remained the classical objects for cytological study, especially with regard to cell-division, ever since Flemming published his epoch—making studies on indirect (mitotic) cell-division in *Salamandra* in 1882.

Eberth described multiple adenomata of the skin of the frog in 1868, Pettit described a fibro-papilloma of the hand in *Cryptobranchus japonicus* in 1902, and in 1903 Pick described a cystic carcinoma of the testis in the same animal. In 1905 Smallwood described and figured bilateral adrenal tumours in the kidney of the frog, in regard to which some additional points of interest are mentioned below. In 1906 Dr. M. Plehn described a tumour in the ovary of the frog (*R. esculenta*) which she regards as a multiple carcinoma. The tumours were bilateral.

Through the kindness of Mr. Smallwood, of Syracuse University, we have had an opportunity of studying the new growth in a frog's kidney, the naked-eye appearances and histology of which he has described and figured in Anatom. Anzeiger, 1905, fig. 6. To that account it is only necessary to add a short description of the microscopic anatomy of the growth and of its exact relation to the kidney substance. Fig. 7 shows that its structure is that of a columnar-cell carcinoma with extremely elongated slender cells arranged in irregularly shaped acini. Towards the surface of the growth the cells in the younger acini are shorter and almost form a cubical epithelium. Mitotic cell-division is in rapid process throughout thegrowth. The kidney tubules around the growth are compressed and flattened, and there are isolated tubules lying between the tumour acini, and where this is so they are much compressed and distorted, their epithelium showing also distinct granular and fatty degeneration. The expansion of the connective-tissue capsule of the kidney due to the growth of the tumour is accompanied by infiltration of the kidney substance, and, so far as microscopical appearances go, they are conclusive as to the carcinomatous nature of the new growth. Smallwood, reasoning from the position of the growth, concluded that it had arisen from the adrenal bodies, which in the frog lie imbedded in the ventral surface of the kidney substance throughout the whole length of the organ. Histologically the cells do not present the slightest trace of the characteristic granules of the adrenal tissue, and the tumour parenchyma either had arisen from the cortical adrenal tissue or from other epithelial elements of this region.

Two cases of carcinoma of the skin have been obtained from the frog. One of these we owe to our former colleague, Dr. Cramer, of the Physiology Department of Edinburgh University. The growth was situated on the inner aspect of the thigh of an adult male frog. It was hemispherical in form, projecting sharply from the surface, and the skin covering it was stretched over its surface. Microscopically the growth consisted of closely packed alveoli of tumour cells arranged in many layers around central spaces either filled with fluid or cellular débris. Delicate connective-tissue septa separate the alveoli from each other and carry capillary vessels moderately distended with blood. Towards the deep surface of the growth isolated alveoli pass for a short distance between the subjacent muscle-fibres (fig. 8, low power). At one part of the surface the tumour parenchyma is seen in anatomical continuity with the covering epithelium (fig. 9), and in places the cells of the tumour parenchyma are connected to one another as well as

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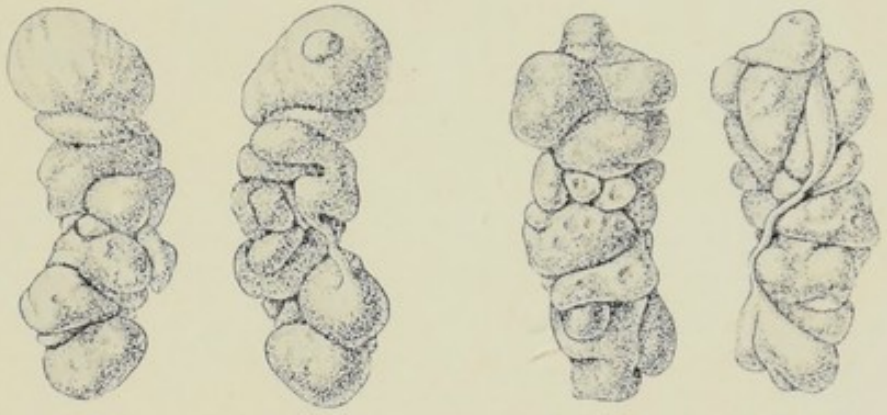


FIG. 6.—Frog: Columnar-celled carcinoma of adrenal invading kidney.
a. Ventral, *b*. Dorsal, aspect of right kidney and growth.
c. Ventral, *d*. Dorsal, aspect of left kidney and growth.
(After Smallwood. Anat. Anz. 1905.) $\times \frac{2}{1}$.

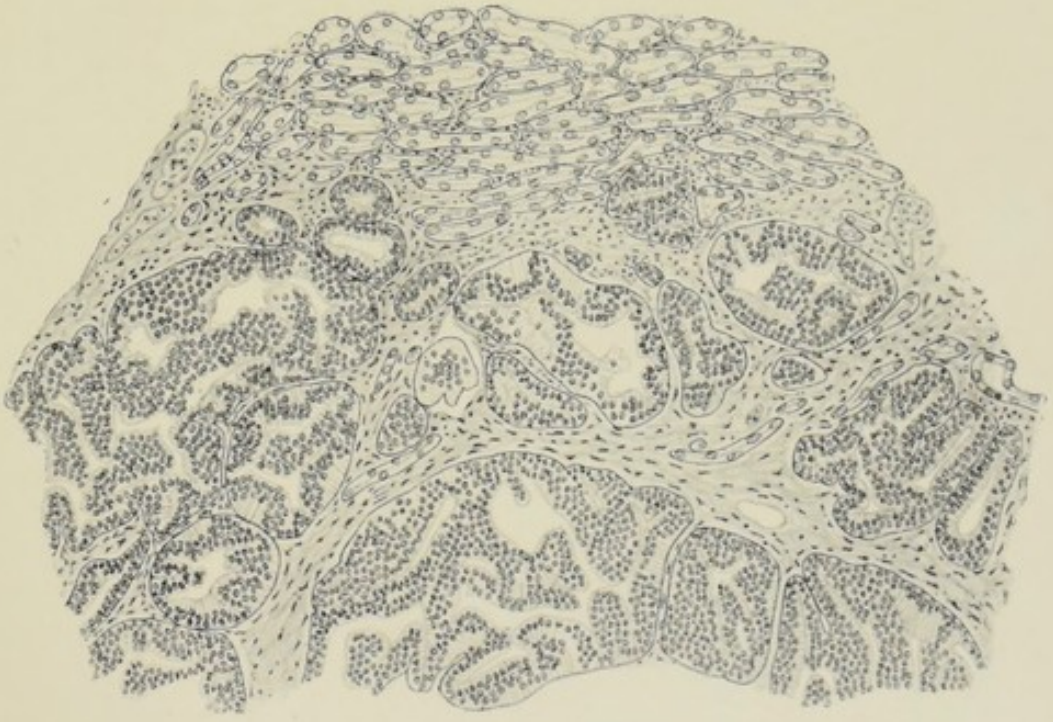


FIG. 7.—Frog: Columnar-celled carcinoma of adrenal invading the kidney.
(From a preparation by Mr. Smallwood.) $\times \frac{65}{1}$.



FIG. 8.—Frog: Malignant adeno-carcinoma of skin glands. The growth has penetrated beneath the dense lamellar layer of the dermis, *a*, and infiltrates the subjacent muscles of the thigh, *b*. $\times \frac{35}{1}$.

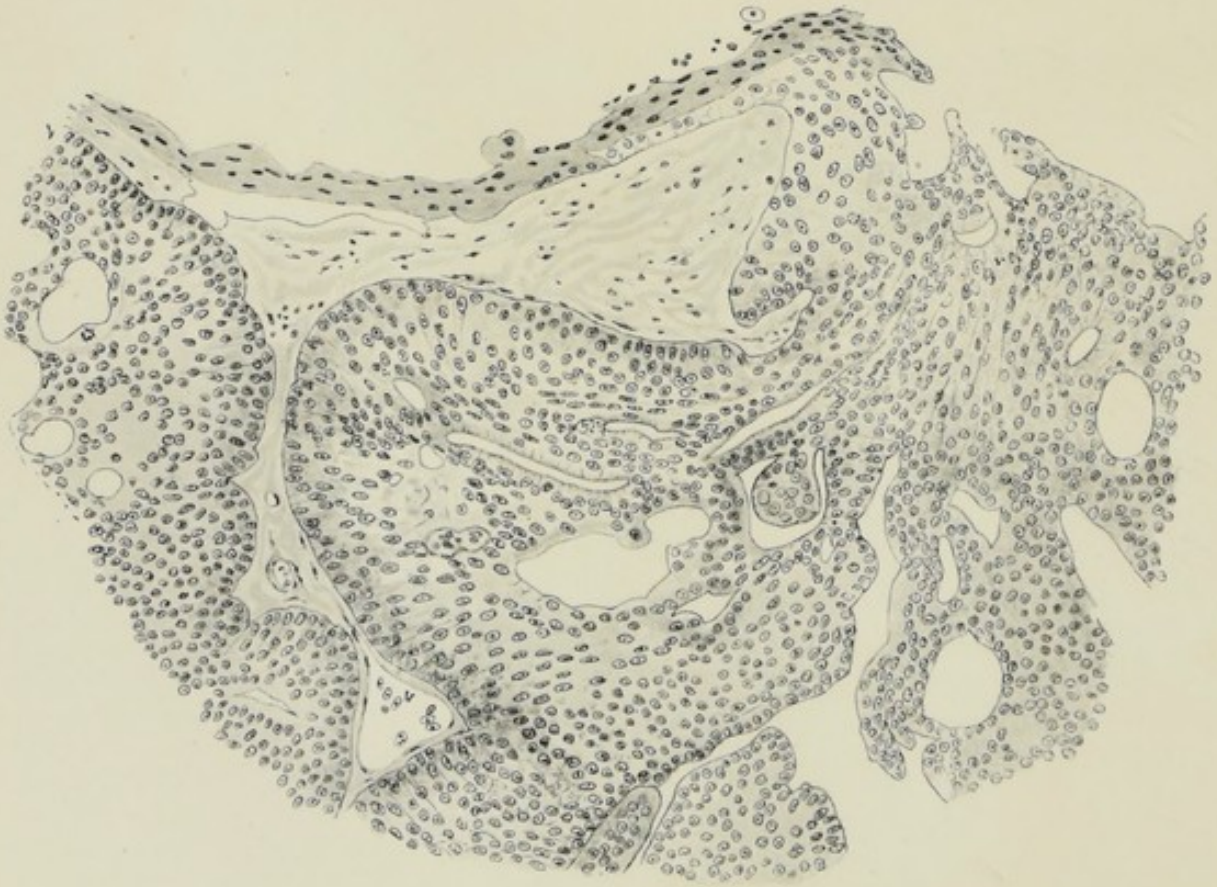


FIG. 9.—Frog: Adeno-carcinoma of skin glands, showing connection with normal skin, the tumour apparently spreading laterally along its deep surface. (*Cf.* Figs. of Triton). $\times \frac{25}{1}$.

to the normal surface epithelium by inter-cellular bridges. Therefore, there can be no doubt that the growth in question is histogenetically connected with the skin. A more difficult question to answer is whether the covering epidermis itself, as is suggested by the occurrence of prickle-cells, or the parenchyma of the accessory glands of the skin, is the tissue from which the growth primarily proceeds. The great tendency which the cells show to the formation of small lumina in the alveoli, speaks in favour of the latter possibility. On the second supposition the growth would be an adeno-carcinoma, and represent a malignant transformation of the skin-glands as contrasted with the benign adenoma of the skin-glands of the frog described by Eberth in Virchow's Archiv, 1868. In Eberth's case the growths were multiple, twenty-eight in number, and lay entirely superficial to the dense lamellar layer of the dermis. They consisted of columnar cells and formed long branching finger-like processes. As can be seen in fig. 8, the growth now under consideration has broken through the deep lamellar layer of the dermis, and, the walls of the lobules enclosing irregular branching spaces, are several cells thick. No metastases could be found in any of the internal organs, and it was unfortunately impossible to attempt transplantation into normal animals at the time. A second case of the same kind was also discovered in the Edinburgh Physiological Department by Dr. Jolly, who favoured us with a histological preparation, but omitted to attempt transplantation. The occurrence of these two cases within a short interval in the animals of one laboratory justifies the hope that in time other cases will be met with, and the attempt made to propagate them by transplantation. Should such endeavours be successful an addition, eminently excellent, will be made to the material on which the experimental and cytological study of cancer can be undertaken. In addition we have received from Dr. Cramer a tumour from the leg of a frog which on microscopic examination was found to consist of large masses of cartilage and osseous tissue in the centre of which the femur can still be made out. It is impossible to decide whether in this case the condition is one of true neoplasm or whether an exuberant formation of callus-tissue has taken place around an old fracture. Transplantation was carried out by Dr. Cramer with negative results.

These three cases, it will be noted, have all been found in anurous amphibia, and may be considered as lending support to v. Hansemann's opinion, expressed in connection with thyroid carcinoma of the trout, that the liability to malignant growths principally falls on the more

differentiated members of any group of animals. The following case of carcinoma of the skin-glands in *Triton cristatus*, for which we are indebted to Dr. B. Zarnik, till lately Assistant to Professor Boveri of Würzburg, of an analogous condition in a urodele amphibian, has therefore an interest apart from the extreme beauty of the material and the clearness of the microscopical pictures it presents. The animal, an adult *Triton cristatus*, was sent to Dr. Zarnik preserved in formalin (fig. 11). As can be seen from the figure there is a mammillated mass at the angle of the jaw, a similar mass at the anterior part of the

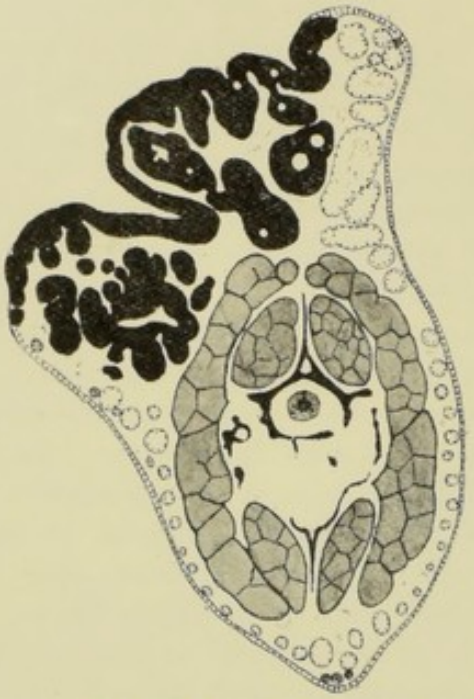
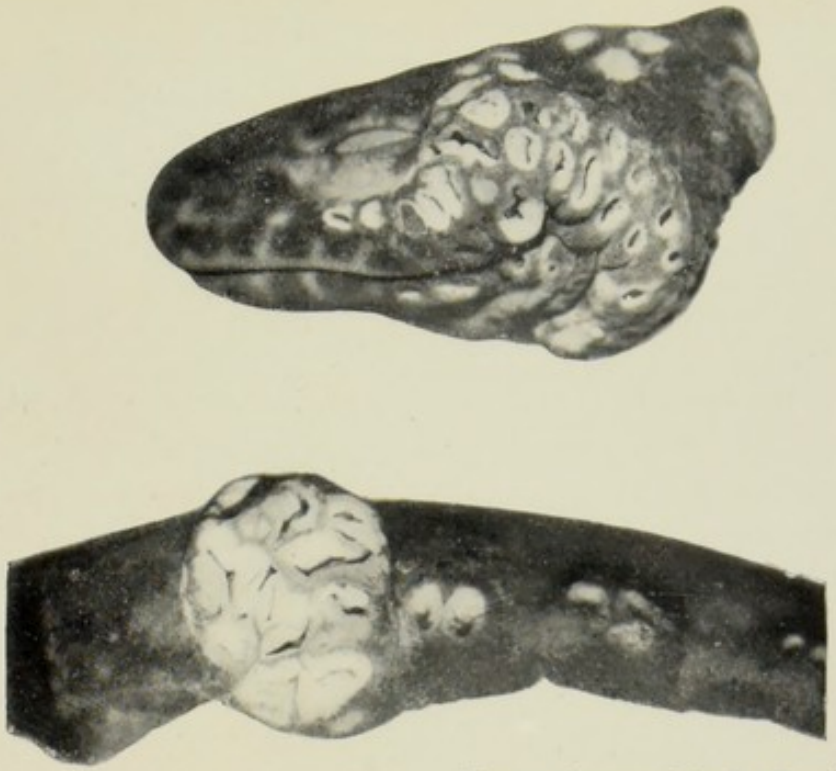


Fig. 10.—Triton: Carcinoma of skin glands, transverse section through tail. Note bending over of mid-dorsal line towards unaffected side and flattening of muscles under main mass of growth. $\times\frac{12}{1}$.

tail, and isolated small nodules posterior to this. The small nodules appear as rounded areas free from pigment, each with a depression in the centre. The larger masses are seen to consist of aggregations of similar nodules, the central depressions of which are usually slit-like or compressed. Transverse sections through the tail show (fig. 10) that the new growth is connected with the skin, and has led to considerable asymmetry. The mid-dorsal line is bent over to the normal side, and the mass of growth has pressed upon and flattened the subjacent trunk-musculature on the affected side. The depressions on the surface lead

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(From a photograph by Dr. Zarnik.)

FIG. 11.—Triton: Carcinoma of skin glands. Macroscopic appearance of growth at angle of mouth and scattered nodules on tail. × 5.



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FIG. 12.—Triton: Carcinoma of skin glands. High power view of left margin of fig. 10. Alveolar part of tumour and commencement of a columnar-celled portion (right upper). Note how sharply the alveolar plugs are marked off from the surrounding normal skin. × $\frac{65}{1}$.

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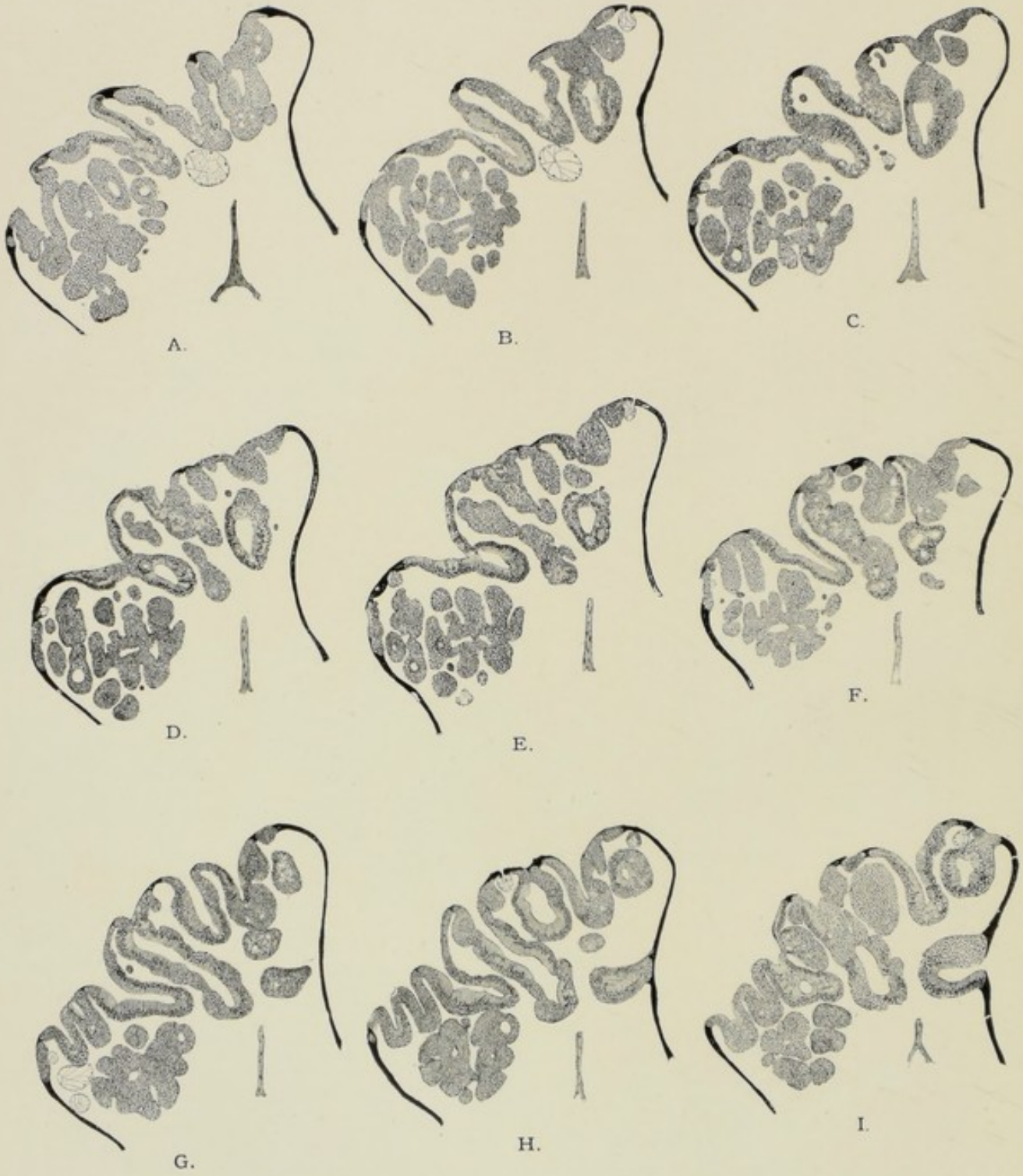


FIG. 13.—A–I. Triton: Carcinoma of skin glands, showing connection of tubules with surface. Selected sections from a series through 1 mm. of the tail. Fig. 10 is intermediate between E and F of this series. $\times \frac{12}{1}$.

into tubules passing downwards from the skin (figs. 10 & 13, A–I). These tubules are lined in places by columnar epithelium, but in many parts the nuclei are arranged irregularly so that an alveolar or carcinomatous condition is produced. Tangential sections of the swollen ends of the tubules have the same alveolar appearance. All the parenchyma masses, whether tubular or alveolar, are connected with the surface epithelium, as is shown in the series of figures (fig. 13, A–I) taken at intervals from a consecutive series of sections through one millimeter's thickness of the growth.

The tumour has arisen by a transformation of a small number of the flask-like glands of the skin on the tail, into enormously hypertrophied tubular and solid structures. In two glands lying between the lobules of the tumour, and still connected with the skin, an early stage of the transformation is in progress (fig. 13, D, left side ; fig. 13, H, right side). A number of the cells towards the fundus of the gland still present an abundant protoplasm containing the characteristic secretion and a flattened elongated nucleus at the base. The cells towards the neck of the gland, however, are much more numerous than in the normal structure, their protoplasm is scantier and the nuclei are relatively and absolutely much larger. The neck of the gland, which normally consists of a delicate tubule lined by one or two cells folded round its periphery, is replaced by a stout cylindrical plug of cells sharply demarcated from the surrounding normal epithelium which is compressed and displaced by their growth. As the proliferation proceeds, the growing cells spread out laterally in the thickness of the epidermis, and separate it from the underlying connective tissue. As this process extends the epidermis between adjacent glands is stripped off in a thin, horny layer till only small wedge-shaped islands of normal epidermis intervene (*vide* figs. 12 & 13). The appearances differ from those described by Eberth for a multiple adenoma in the frog (*l. c.*) in the rapid proliferation of the cells, and associated with this the tendency to an arrangement as in alveolar carcinoma. Thus, while in histological characters these multiple tumours approach very closely to the carcinomata or malignant adenomata, the multiplicity and the absence of infiltration of the surrounding structures (with the exception of the epidermis) associate them with growths of a benign character. There are no facts on which to base any conclusions as to the etiology of the condition.

FISHES.

Since the first authentic case of carcinoma in a fish, viz., of the thyroid gland in a trout, was submitted to us in February 1903 by Mr. Gilruth, over 2000 additonal cases have been reported to us from the same and other hatcheries.

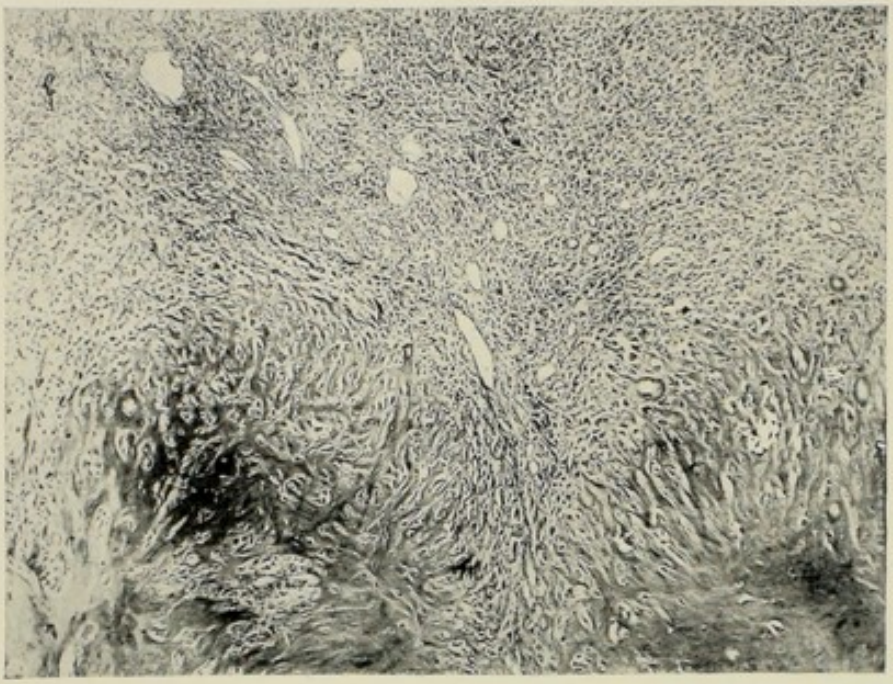
The publication of other cases by Dr. M. Plehn and the monograph of Pick on this subject do not exhaust the problems raised by the frequency of thyroid enlargement often progressing to the development of true carcinoma in trout kept in large hatcheries. Further investigations are necessary to determine its relationships to simple hypertrophy and the relative importance of the external conditions and the hereditary constitution of the animals in the ponds where it occurs. Thus Jaboulay points out that while the indigenous trout in the hatchery at Thonon were free from the disease, the introduction of ova from Germany was followed by the appearance of the characteristic tumours in a large number of the animals raised from the imported ova. Fish of the indigenous stock kept along with the affected race subsequently also developed the disease. Jaboulay concluded somewhat hastily that the disease was both hereditary and contagious.

We have been unable to approach this extremely interesting and important subject from the fact that up to the present we have been unable to find any evidence of its occurrence in the hatcheries in this country at the present time, the last epidemic of the disease appearing in 1888 in Scotland.

In fishes a considerable number of new growths have been recorded. With the exception of the cases of carcinoma of the thyroid in trout, a large proportion of these are either regarded as benign in character or are classified with the sarcomata. In the case of the sarcomata, the pathologist must always be acutely sensible of the difficulties which arise in assigning to this group tumour-like formations of animals in which the pathological processes associated with wound-healing and the reactions of the tissues to infective agents are little known. Even in the domesticated mammals such difficulties make themselves felt, and the necessity for great caution when dealing with new growths in fishes is not by any means lessened by an extensive study of granulomatous and sarcomatous new formations in the higher animals.

In 1906 Dr. M. Plehn described a large number of new growths in fishes and gave an excellent summary of the references in the literature on this subject. In that paper she records the occurrence of myomata, fibromata, lipo-fibromata, and osteoma in various teleosts, and of sarcomata in trout, roach, and minnow.

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Microphoto by R. Muir.

FIG. 14.—Cod: Osteo-sarcoma of operculum. Portions of two osseous nodules are shown with surrounding vascular spindle-celled tissue. $\times \frac{60}{1}$.

(1) *Osteo-sarcoma of the operculum in the Cod (Gadus).*

The specimen was found in a cod caught on the Newfoundland Banks and sent in to the laboratory by order of Sir William McGregor, Governor of Newfoundland. The hemispherical growth of 2 cm. diameter was attached to the subjacent tissues by its flattened surface. It is covered by skin from which all scales have been detached, but the epithelial layer is much thickened and consists of irregular nodular masses of cells arranged in alveoli with projections of the dermal connective tissue between. Subjacent to the latter is the growth itself, of bony hardness and impregnated with lime-salts. Microscopical preparations made after decalcification, show irregularly rounded masses of osseous tissue separated by strands of spindle-cells (fig. 14). At the surface of the nodules these cells become smaller and more oval in shape, and pass by gradual transitions into bone corpuscles imbedded in the characteristic matrix. Thin-walled capillaries run in all directions in the cellular septa between the nodules of bone and occasionally capillaries extend more deeply into the nodules themselves. As the whole fish could not be sent, no data are available as to the presence of metastasis or local extension. The growth is regarded as an osteo-sarcoma from the very cellular character of the septa, and the evident community of origin of the spindle-cells and the cells of the more highly differentiated bone-like tissue developing from them.

(2) *Angioma of pectoral girdle in Cod.*

The flattened dark-coloured growth of about 1·5 cm. diameter was removed from the pectoral girdle of a cod and forwarded to the laboratory by Mr. Crawshay of the Marine Biological Association, Plymouth. From the dark colour of the specimen it was at first surmised that the growth would prove to be a melanotic tumour. Microscopic sections showed, however, that the mass consisted of capillary blood-vessels distended with blood but otherwise of normal appearance. The structure of the tumour differs from an angioma in man only in the histological details, *e. g.*, the elliptical nucleated red blood-corpuscles.

(3) *Squamous-cell carcinoma in Gasterosteus spinachia.*

A specimen of *Gasterosteus spinachia* (a male) measuring 12·5 cm. in length, was sent to the laboratory by Dr. E. J. Allen, Plymouth. The growth is situated behind the anus on the flank (fig. 16), forming

a low conical elevation of the dorsal half of the body-wall. Fig. 15 gives the position and spread of the growth in transverse section as seen under a low magnification (12 diam.). The middle line is marked by the dorsal and anal fins, and the figure shows clearly the great deformity caused by the growth. The centre of the new formation, which has completely

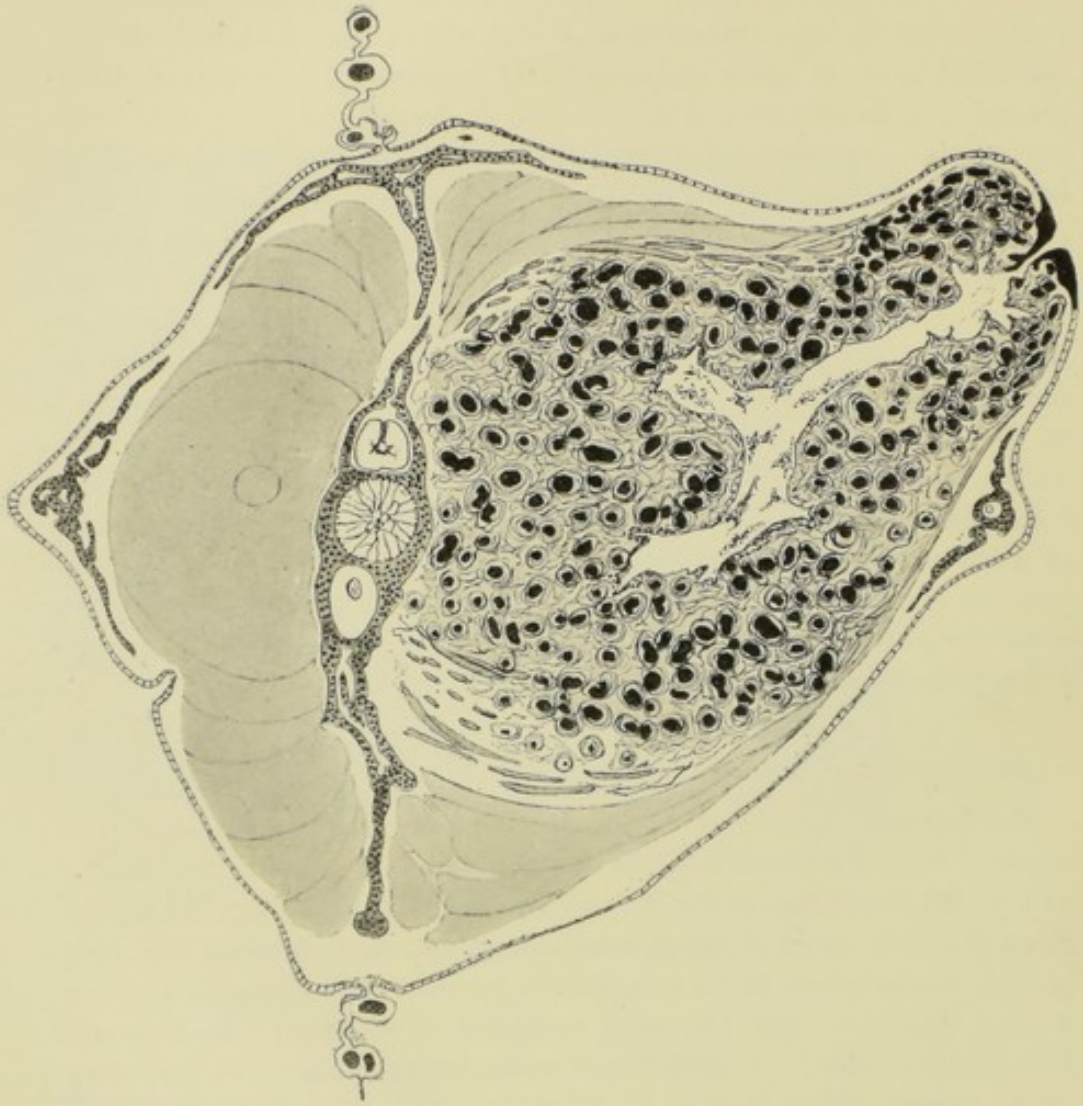


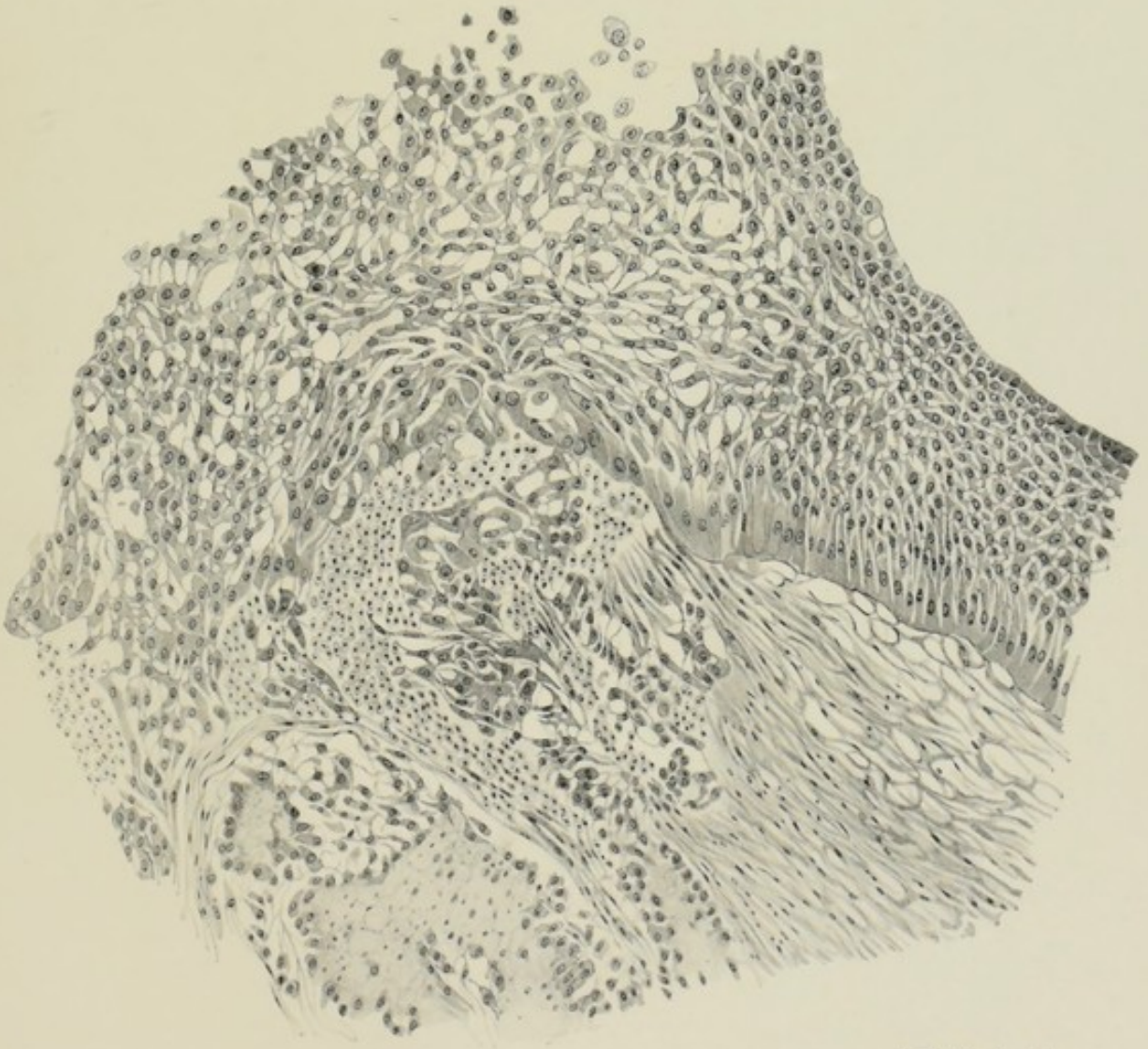
Fig. 15.—Gasterosteus: Squamous-cell carcinoma of skin. Shows infiltration of myotomes, connection with the skin, central degeneration, and dense lamellated stroma. $\times \frac{12}{1}$.

invaded the myotomes of the affected side down to the vertebral column, is occupied by an irregular cavity whose walls are formed of necrotic tissue with ragged projections. The central cavity communicates with the exterior, and at the surface the growth passes apparently continuously into the skin as shown in fig. 17 under a

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FIG. 16.—Gasterosteus: Squamous cell carcinoma of body-wall: natural size.



J. R. Ford, del.

FIG. 17.—Gasterosteus: Margin of growth and adjoining normal skin. $\times \frac{250}{1}$.

higher magnification. The tumour parenchyma is arranged in small solid alveoli, the peripheral cells of which are cubical or low-columnar in form, with scanty protoplasm and densely staining nuclei. Passing towards the centre of the alveoli, cells resembling those at the periphery are almost immediately succeeded by larger vacuolated cells with faintly stained nuclei, and in the most central parts even such indications of structure may be absent, and a granular mass of degenerated protoplasm takes their place. Here and there the peripheral cells of the more healthy alveoli are connected with each other by delicate fibrils as in the normal skin (fig. 17). Having regard to the features of the skin in fish, the growth can be pronounced to be a product of the covering squamous epithelium, and the wide invasion of the muscles as shown in fig. 15 leaves no doubt as to its malignancy. The connective tissue stroma of this growth is very characteristic and peculiar. It consists of very distinct fibrillar laminæ with long spindle-cells between, arranged concentrically around the alveoli. In structure it closely resembles the dense lamellated fibrous layer underlying the normal epidermis elsewhere in this fish. It is impossible to decide whether this character is due to growth of the fibrous layer of the skin *pari passu* with the extension of the tumour, or is of the nature of a specific stroma reaction to the presence of the tumour on the part of the subcutaneous and intermuscular connective tissues of the animal, as has been shown by Bashford and Murray for transplantable mouse tumours.

The ulcerated surface of the growth has already become the nidus for many bacteria and parasitic protozoa, but their presence is obviously an added circumstance, as they are entirely confined to the surface and do not penetrate beyond the most superficial layers.

An interesting tumour of the jaw, having relations in its structure to odontoma on the one hand and squamous-celled carcinoma on the other, has been observed by Dr. Haaland in a specimen of *Sebastes marinus* var. *viviparus* caught by himself off the coast of Norway, and will be described and figured separately at a future date.

With the accumulation of fresh evidence, the conclusions tentatively advanced in the First and Second Scientific Reports as to the ubiquity of malignant new growths in the Vertebrata gain in certainty. The Reptilia, from which no malignant tumour has yet been recorded, still form an exception, due with great probability to the long span of

life of members of this class. The condition for discovering a considerable number of malignant new growths in animals is still that a sufficiently large number of aged individuals should be carefully examined. This conclusion, first clearly enunciated in a paper to the Royal Society and in the First Scientific Report, has been endorsed by Dr. M. Plehn in her studies on cancer in lower vertebrates (Zeitschrift für Krebsforschung, 1906, p. 525) and by v. Hansemann (Berliner Klinische Wochenschrift, 1907).

Thus, although cancer is universal in vertebrate animals escaping the effects of many of the chronic forms of irritation affecting man referred to above (pp. 19–23), its occurrence is frequently associated with other external irritants, and it may not be merely the ease with which lesions on the surface are observed, which has led to the accumulation of our extensive series of skin cancers. As in man, so in animals, no one form of external agency is constantly associated with cancer. The fundamental common factor is the peculiarity of the living cell to exhibit malignant growth under the action of most diverse agencies in divergent forms of life. The wide zoological distribution of malignant new growths, while affording the completest answer to the myriad speculations on the etiological association of conditions peculiar to mankind with the incidence of cancer, cannot be expected to furnish other than the most general indications of the essential, as contrasted with the incidental or subsidiary factors in its development. Nevertheless the surprising homogeneity of the malignant new growths in their general histological characters, points insistently to the cellular aspects and general biological importance of the problems of cancer.

At the time when the First Scientific Report was written (March 1904), it appeared as if the nature of the cellular transformation had been discovered. Farmer, Moore and Walker had described appearances in cancer-cells in man during mitosis, and had interpreted them to mean that the cells of the body had acquired the characteristic forms of cell-division until then only observed in reproductive tissues. We were able to demonstrate in cancer in animals the same morphological appearances described by these observers, and to extend them down the vertebrate scale to the trout. We dissociated ourselves from the other conclusions of these authors, and particularly from their statement that malignant could be distinguished from benign new growths by this means.

In following up this line of investigation discrepancies were met with in the preparations of malignant new growths, when the attempt was made to bridge over the lacunæ between different stages of the

nuclear sequences as met with in reproductive tissues. Extensive enumerations of chromosomes in the mouse gave the average number 36, and raised the suspicion that the accepted number given by Sobotta as 24 was in reality too low. This higher number has also been noted by Michaelis. The examination of the ripening divisions of the egg in this animal (Sobotta generously placed his beautiful material at our disposal) showed that the number was in reality 32. Sobotta himself has recently published the results of a re-examination of his material with this result. The error apparently arose in consequence of the small size of some of the elements and the tendency of others to adhere together.

In these circumstances the amphibian tumours presented an unequalled opportunity for an objective control to the results obtained in human and animal material with smaller cells and chromosomes, such as the mouse and trout. Heterotypical mitotic figures could not be found in any of these growths, and their absence finally disposed of the belief that a reducing division was constant or characteristic of cancer. The conclusion is inevitable that the sources of fallacy and equivocal appearances, some of which are described in the following paper, account for the apparent occurrence of heterotypical mitoses in cancer, and that a true homology does not exist with the maturation processes of reproductive cells.

In this connection it is advisable to refer to certain of the observations of Benjamin Moore, Roaf, and Whitley on the effects of acids and alkalies on developing Echinoderm eggs. Certain peculiarities of the chromosomes met with in the microscopical preparations of these investigators, were regarded by J. E. Salvin-Moore and C. E. Walker as similar to appearances met with in cancer in association with reduction in the number of chromosomes and accelerated cell-division. It is only necessary to compare fig. 18 and fig. 19, reproduced from the paper referred to, and from Boveri's 'Zellen Studien IV.' respectively, to show that the vesicular chromosomes to which so much significance was attached are neither the consequence of altered reaction of the sea-water, nor similar to the heterotypical mitoses of reproductive tissue. They represent the normal process by which the daughter chromosomes reconstitute the daughter nuclei in Echinoderms. The diminution in the number of chromosomes in these experiments is a consequence of multipolar mitosis, and therefore only indirectly of the action of the medium in which the eggs had been placed.

The conclusion we have arrived at is that the gametoid hypothesis is

based on erroneous interpretations of normal and pathological cell-division in no way related to those met with in the maturation of the

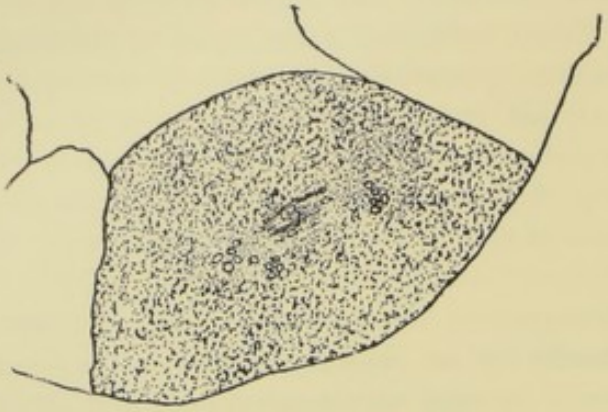


Fig. 18.—Late metaphase of mitosis in cell of Echinoderm blastula. Two groups of vesicular chromosomes. From B. Moore, Roaf, & Whitley, Roy. Soc. Proc. 1906. *Cf.* Fig. 14.

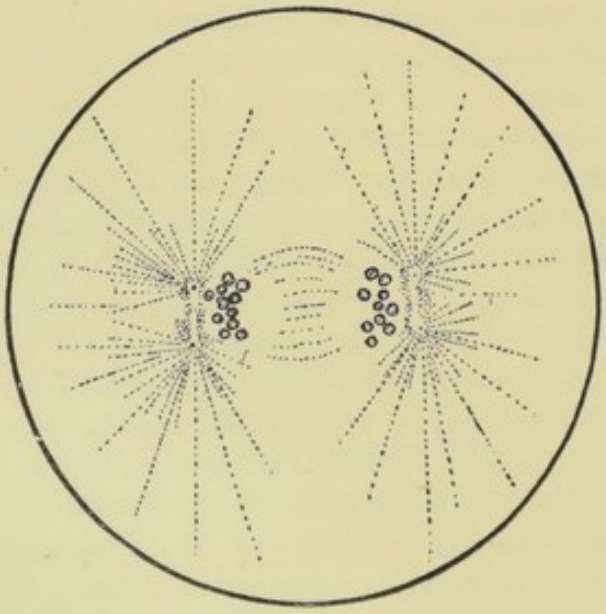


Fig. 19.—Late metaphase of first segmentation spindle of Echinoderm egg after Boveri, Zellenstudien, iv., 1904.

sexual elements, and we have therefore felt no compunction in finally discarding it as a working hypothesis, or the corollary that certain figures

observed in the nuclei of adjacent cancer-cells represented a fusion of nuclei comparable to fertilization.

The retention of the normal number of chromosomes in mouse tumours after propagation for many years shows that an alteration in the number of chromosomes, however brought about, is not an essential feature of cancerous proliferation. Normal bipolar mitosis is by far the commonest mode of cell-division met with under these conditions, and the various abnormalities encountered can only be regarded as of very subsidiary importance, and in all probability outside of the direct line of the continuous proliferation which occurs. The amount and the energy of this proliferation in tissue elements of apparently normal nuclear constitution is the cardinal phenomenon, and we must look for its elucidation to the application and refinement of those methods of biological analysis which have been revealed by the study of experimental cancer, and which are the foundation upon which many of the later papers in this report are based.

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[For references to papers from the laboratory of Imperial Cancer Research Fund, see complete Bibliography in Appendix.]

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ON THE OCCURRENCE OF HETEROTYPICAL MITOSES IN CANCER.

BY E. F. BASHFORD, M.D., AND J. A. MURRAY, M.B., B.Sc.

[Communicated by J. ROSE BRADFORD, M.D., F.R.S. Received November 2; Read November 23, 1905.]

[FIGURES 1 TO 15.]

THE present paper refers to a communication * made to the Royal Society in January, 1904. In that paper and its expansion †, published later, we emphasised the significance of the zoological distribution of cancer; we discussed the unique features of the processes responsible for the experimental transmission of carcinoma from one animal to another and the limitations to its successful attainment: we also published a series of figures depicting the characters of the nuclei of cancer cells during division, in the malignant new growths of fishes and mammals. We shall give a different explanation of the mitoses we figured in our earlier communications as resembling the heterotypical mitoses of reproductive tissue. We have found that those mitoses may be interpreted as somatic mitoses with longitudinally split chromosomes. Their apparent heterotypical form is thus due to variations in the development of the achromatic figure, the peculiar form of the chromosomes and their mode of attachment to the spindle.

Our figures of heterotypical mitoses in cancer confirmed the observations of Farmer, Moore and Walker, communicated ‡ to the Royal Society at the preceding meeting, but we dissociated ourselves from their conclusions on the diagnostic value and the significance of the phenomenon. The amount of chromatin entering into the equatorial plate of the dividing cells of human cancer had long been known to be subject to diminution (von Hansemann §, 1893), but the presence of

* Roy. Soc. Proc., vol. 73.

† First Scientific Report, Cancer Research Fund.

‡ Roy. Soc. Proc., vol. 72.

§ 'Studien über die Spezificität, den Altruismus und die Anaplasie der Zellen,' Berlin, 1893, etc.

heterotypical mitoses appeared to throw a new light on its occurrence and meaning.

We have pointed out that the characteristic changes accompanying the heterotypical mitosis in the reproductive tissues are absent from cancer cells undergoing what we regarded as this form of division, and that the want of correspondence extends to the stages which precede and follow it *. We have also illustrated some of the appearances simulating bivalent chromosomes, but in reality conforming to the type met with in ordinary (somatic) karyokinetic cell-division †. In what follows we shall illustrate other sources of error on the basis of a renewed analysis of the preparations from which the figures of heterotypical mitosis in our previous papers were made and by other figures not yet published.

In the sexual cells of animals the heterotypical mitosis is preceded by a stage known as the "synapsis." In it the chromatic filament is split longitudinally and gathered into a rosette at one part of the nucleus, the nucleolus lying to one side and usually flattened against the nuclear membrane. In this stage the chromosomes are believed to unite in pairs, thus giving rise in the equatorial plate of the heterotypical mitosis to bivalent chromosomes, half as numerous as those characteristic of ordinary somatic cells. The examination of many sporadic and transplanted malignant new growths failed to reveal a corresponding sequence in their nuclei. We therefore undertook a renewed analysis of the preparations of the stages in cell-division already figured from transplanted mouse tumours, and of other preparations resembling them, to determine whether or not their identification as heterotypical were justified. We shall confine our statements mainly to five transplantable mouse tumours because they permit of control observations with varying methods of preservation and staining in a manner not possible with material from sporadic new growths ; but our remarks apply also to the figures we have published from sporadic tumours of the trout and cat.

Von Hansemann ‡ has combated the statements on the presence of heterotypical mitoses in malignant growths, and ascribes the appearances figured to clumping of the chromosomes, and to pathological abnormalities in their form. He adheres to his conclusion that the numerical diminution is not an exact halving of the normal number, but is

* *Loc. cit.*, and 'Lancet,' April 1, 1905. † *Loc. cit.*

‡ 'Biolog. Centralb.' vol. 24, 1905; vol. 25, 1905; 'Verhandl. physiol. Ges., Berlin, 1904.

irregular, and due to (1) asymmetrical mitosis, and (2) casting out and degeneration of chromosomes. Häcker reproduced three of our figures in a paper in which he admitted the striking similarity to heterotypical mitosis, but suggested that adhesion of the chromosomes in pairs*, together with longitudinal splitting of the couples thus produced, might account for the phenomena encountered in cancer. The appearances in some preparations are explicable in the manner suggested by von Hansemann †, but all the forms of cell-division resembling the heterotypical mitoses of reproductive tissue cannot be accounted for in this manner.

Some of the nuclear divisions previously figured have been found on re-examination to be due to an artificial grouping together of distinct chromosomes. Fig. 1, reproducing at higher magnification fig. 3 of our Royal Society Paper, and fig. 27 of the First Scientific Report of the Imperial Cancer Research Fund, affords an example of this source of confusion. The chromosomes seem to be bivalent, *i. e.*, to have the form of rings and loops. This mitosis is not completely depicted in fig. 1, the remainder of the chromosomes being in the next section. The preparation has been carefully restained. The result is shown in figs. 2 & 3. The "rings" and "loops" resolve themselves into a larger number of ordinary short chromosomes, split longitudinally. The dense equatorial plate of the next section shows clearly the presence

* Häcker uses the term "heterotypical" in a purely morphological sense, and embraces in it the mitoses of the cells destined to give rise to the sexual products of Ascaris and the Copepods. In the latter he has shown that exposure to ether ('Anat. Anzeiger,' 1900) may cause all the cells of the developing egg to exhibit this modification (viz., cohesion of the chromosomes in pairs). In the strict sense of the word, the term "heterotype" is applied to the form of mitosis characteristic of the first ripening division of the spermatocytes of amphibia, as first described by Flemming. In the Salamander, ring-shaped chromosomes, half as numerous as the longitudinally split chromosomes of somatic mitoses, are stretched out into elongated ellipses upon the spindle, giving rise to a barrel-shaped figure. Chromosomes of similar form are associated with their numerical reduction in many animals and plants, but it must be borne in mind that different forms of chromosomes occur in the corresponding mitoses of some animals. In Ascaris, *e. g.*, both ripening divisions appear to be effected by a longitudinal splitting of chromosomes arranged transversely on the spindle, and in others ring or loop chromosomes are never formed. It was, of course, conceivable that reduction-divisions might occur in cancer by means of chromosomes unlike those in the reproductive tissues of the same animal. The frequency of cells with diminished numbers of chromosomes led us to examine many tumours for evidence of their occurrence, but without result.

† 'Biolog. Centralb.,' vol. 24, 1904.

of many short chromosomes split longitudinally, and arranged in the manner described below. The mitosis is therefore somatic, and not heterotypical.

The ordinary scheme of karyokinetic cell-division presents, in its phase of equilibrium (amphiaster or equatorial plate), a series of **V**-shaped loops with limbs of equal length, arranged around a central spindle and all lying in a plane at right angles to its axis. This arrangement is by no means universal. Some of the deviations are of great importance to a proper understanding of what occurs in cancer. Frequently the limbs of the **V**-shaped loops are of unequal length. When this is the case the attraction fibres are relatively few in number and attached only to the apex of the **V** and its immediate vicinity, and therefore nearer one end of each chromosome than the other. As a consequence the longer limb does not come to a position of equilibrium in the equatorial plane, but may take up one inclined to the axis of the achromatic figure. When the attraction fibres are attached to the chromosomes in this manner in a cell of elongated form, the longer limbs may even come to lie parallel to the spindle axis, and nuclear divisions closely resembling heterotypical mitoses may result. In such mitoses pairs of distinct chromosomes whose longer limbs lie on opposite sides of the equator, while their apices are closely opposed, simulate bivalent chromosomes, *cf.* figs. 4 to 10. In polar view the apparent halving of the chromosome number due to superposition, and the unusual vertical extension of the free longer limbs, are even more deceptive ; unless the longitudinal splitting of the chromosomes is very clear, the resemblance to heterotypical mitoses may be almost perfect. During the separation of the daughter chromosomes such nuclear divisions are especially deceptive, because the longer limbs adhere for some time after separation of the apices and short limbs. Barrel-shaped forms result, in which the crowding together of the chromosomes renders their enumeration impossible, but at the same time conveys the impression of a diminution in their number.

A much more serious source of error results from individual differences in the size of the chromosomes in one and the same nucleus. Montgomery * and Sutton † have drawn attention to this phenomenon

* T. H. Montgomery, ' Proc. Acad. Nat. Sc., Philadelphia,' 1901 : ' Biol. Bull.,' vol. 4, 1903.

† W. S. Sutton, ' Biol. Bull.,' vol. 4, 1902.

in the sexual cells of invertebrates. We have found it to be present also in many vertebrate mitoses. When the chromosomes attain the position of equilibrium in the equatorial plate the smaller take up a position nearer the axis of the central spindle than the larger or more massive ones. Seen in profile the larger may then completely screen the smaller from view, and lead to an under-estimate of the chromosome number.

Normally, nuclear division takes place by means of bipolar mitosis distributing the halves of the chromosomes to two daughter nuclei. An interesting abnormality results, when, from any cause, this segregation of the daughter elements does not take place. This may result from the centrosome remaining single, or, when after division of the centrosome, one only becomes attached to the chromosomes by the attraction fibres. The chromosomes then remain in one group, but the daughter elements separate slightly from each other before combining to form one large nucleus, containing twice as many chromosomes as the mother nucleus. This form of mitosis, known as a "monaster" (from the presence of only one active attraction sphere), has been studied and described in detail by Th.* and M.† Boveri in invertebrates: figs. 13, 14, and 15, from a squamous cell carcinoma of the tongue (human), represent a cell in this condition. The nuclear membrane has disappeared, but there is no trace of radiations or centrosomes to be seen. The chromosomes are arranged as a hollow sphere around a central clear area, and for the most part consist of two parallel daughter elements in different stages of separation. Some chromosomes in which the separation of the daughter elements is least advanced have a horse-shoe shape. In others, the daughter elements are parallel and widely separated. In a few, the separation is incomplete, the ends only remain apposed, giving the appearance of rings. In fig. 14 this cell has a striking resemblance to a heterotypical mitosis such as occurs in testis. Its cytoplasm is clear and voluminous, and there are no intercellular bridges between it and the surrounding epithelial cells. The number of chromosomes in the cell, however, is not diminished, but amounts to 40 to 50 when both sections are examined. Such a mitosis, instead of reducing the number to half, really results in the original number of

* Th. Boveri, 'Sitz.-ber. Phys.-med. Ges.,' Würzburg, 1897; 'Zellenstudien,' vol. 4, Jena, 1901.

† M. Boveri, 'Jen. Zeitschr. f. Naturwiss.,' vol. 37, 1903.

chromosomes being doubled, because the separated halves of the chromosomes co nbine again to form a single nucleus.

After elimination of these sources of confusion, there remain other apparent heterotypical mitoses which cannot be explained in any of these ways. The chromosomes present an irregular contour and become drawn out like a viscid fluid in the later stages of separation. Individual chromosomes cannot be made out. The achromatic spindle develops at such a rate that the evolution of the chromosomes cannot keep pace with it, and they are drawn towards the spindle and stretched upon it before they have completely contracted and condensed.

All these abnormalities may occur in nuclei possessing the usual number of chromosomes. They also occur in cells with a greater or lesser number associated with the presence of multipolar and asymmetrical mitoses in other cells. In such cases there is no evidence warranting the assumption that the diminution in the number of the chromosomes is due solely to a nuclear division effecting a reduction comparable to that of the sexual cells.

Galleotti* and von Hansemann† have shown that nuclei with diminished numbers of chromosomes (hypo-chromatic) may arise from larger ones by asymmetrical mitosis, in which entire chromosomes pass to one daughter cell, because they are only attached to one or other attraction sphere, and also by "casting out of chromatin." "Casting out of chromatin" is merely an exaggeration of what occurs in asymmetrical mitosis; in it some chromosomes remain unattached to either attraction-sphere, and therefore fail to be included in either daughter nucleus. Krompecher‡ and we ourselves§ have shown that multipolar mitoses may also lead to a diminution in the number of chromosomes. We stated that nuclei with diminished and half the somatic number of chromosomes occur without it being possible to determine whether the diminution has been effected by asymmetrical mitosis, casting out of chromatin, multipolar or heterotypical mitosis.

We have given our reasons for now believing that the mitoses we formerly assumed confirmed the occurrence of a heterotypical reducing division in cancer, are, in reality, somatic mitoses. Although we do not presume to explain in the above manner all the figures which may be brought forward resembling that form of nuclear division, we submit that the occurrence of heterotypical mitoses in cancer requires further

* 'Ziegler's Beitr.,' vol. 14, 1893.
† *Loc. cit.*
‡ 'Centralb. f. Path u. Anat., vol. 13, 1902.
§ *Loc. cit.*

[*To face* p. 66.

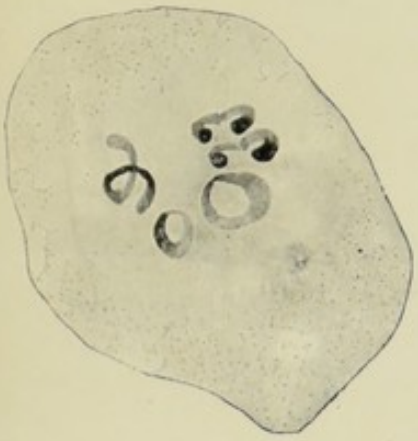


Fig. 1.

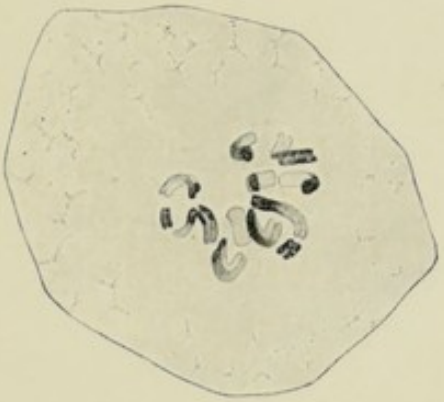


Fig. 2.

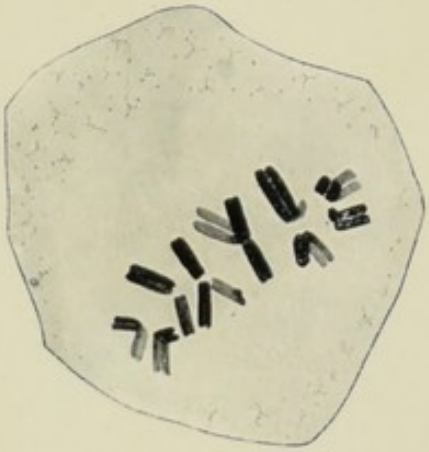


Fig. 3.



Fig. 4.



Fig. 5.



Fig. 7.

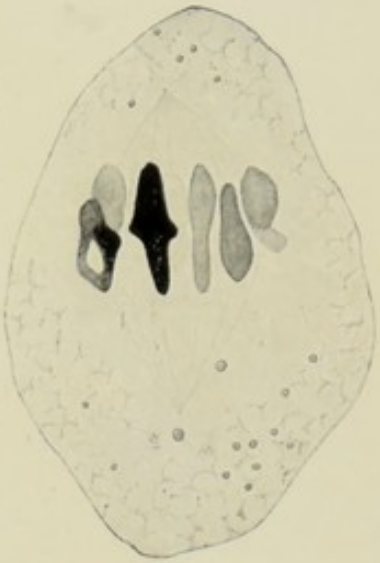


Fig. 6.

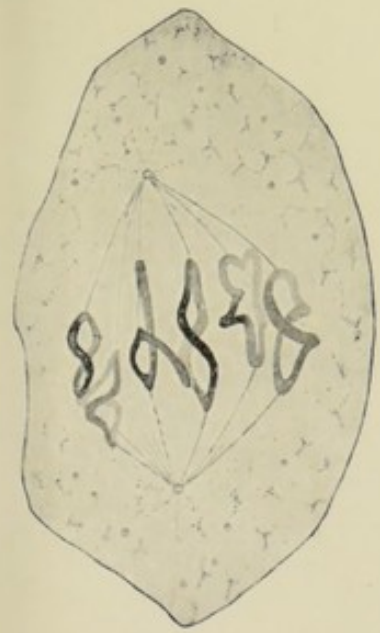


Fig. 9.

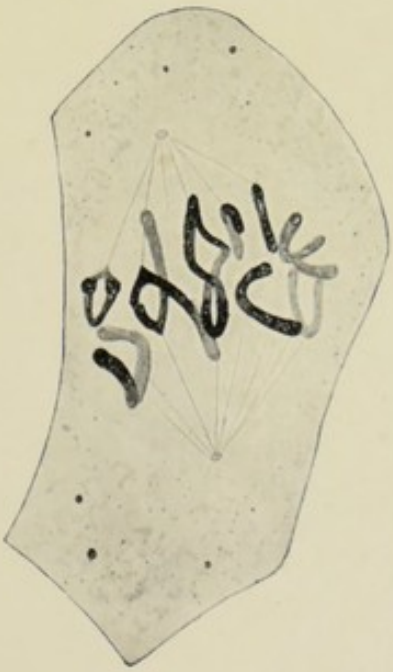


Fig. 10.

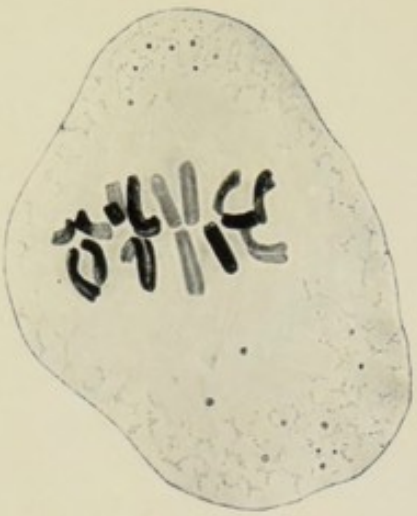


Fig. 8.

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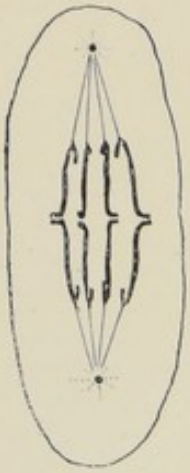


FIG. 11.

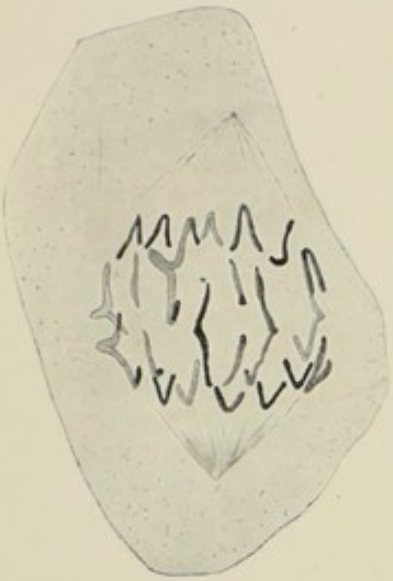


FIG. 12.

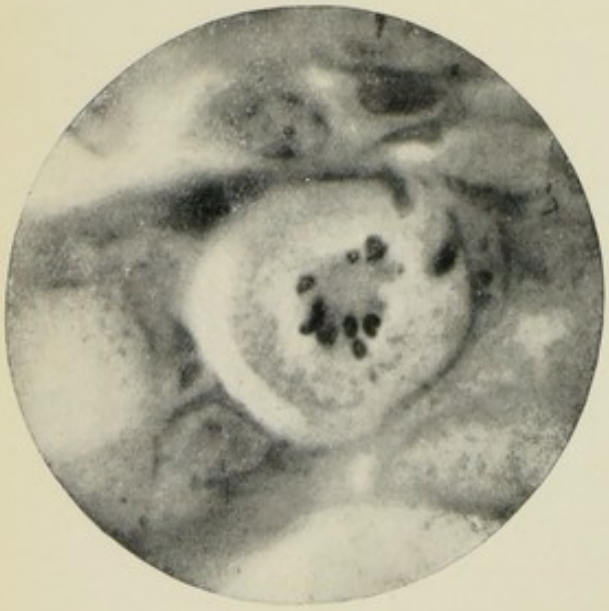


FIG 13.

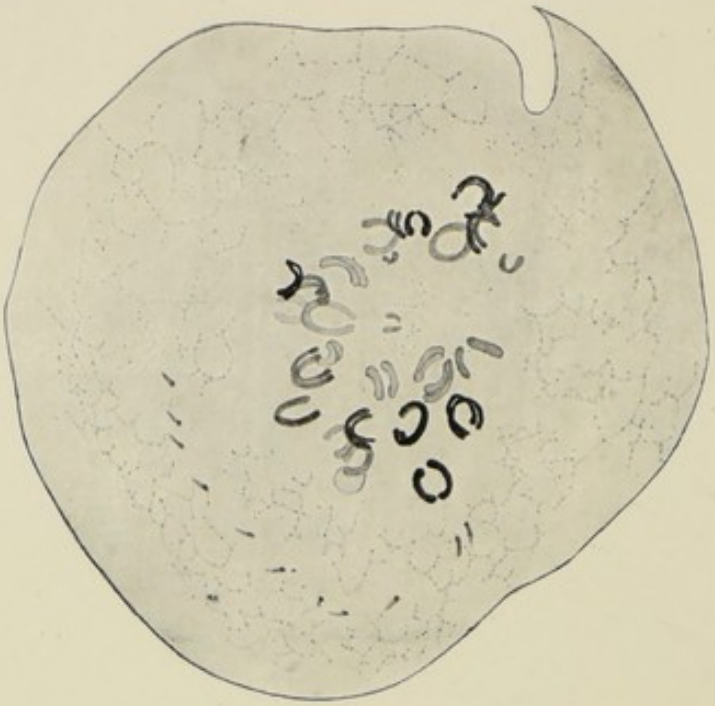


FIG. 14.

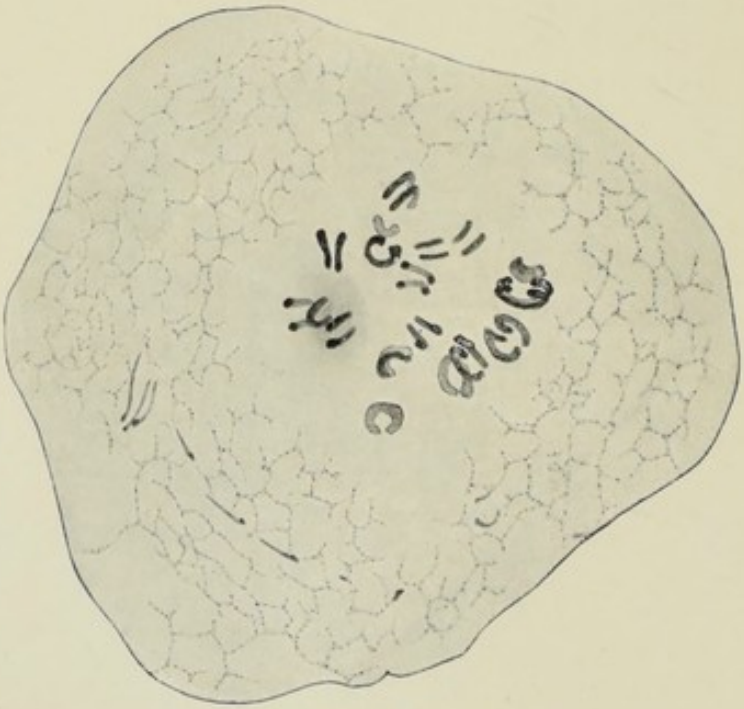


FIG. 15.

proof. Multipolar mitosis and other irregular forms of cell-division occur in cancer, but they do not supervene upon heterotypical mitosis. They are entirely independent of its presence, and, of themselves, suffice to account for the diminutions frequently occurring in the number of chromosomes in cancer throughout the vertebrates.

DESCRIPTION OF FIGURES.

FIG. 1.—Apparent heterotypical mitosis. Transplanted carcinoma of mouse. Analysis of ring, loop, and bivalent chromosomes (heterotypical). Replica of fig. 3 of Royal Society paper, and of fig. 27, First Scientific Report, 1904. × 3000/1.

FIG. 2.—Same section as fig. 1. Analysis after restaining, showing how a fortuitous association of short somatic (longitudinally split) chromosomes gives the appearance of bivalent elements. × 3000/1.

FIG. 3.—Partial analysis of the remainder of the mitosis, of which part only is shown in figs. 1 and 2. Longitudinally split chromosomes with limbs of unequal length lying at various angles to the spindle axis. × 3000/1.

FIG. 4.—Diagram of a somatic amphiaster, in which longitudinally split -shaped chromosomes, with limbs of unequal length, are apparently arranged parallel to the spindle axis. Adjacent chromosomes, with their longer limbs on opposite sides of the equator, if regarded as together forming one chromosome, would convert such a mitosis into a heterotype with half the somatic number of chromosomes arranged longitudinally on the spindle, *e. g.*, figs. 3, 5, 6, 7, 8, 9, & 10.

FIGS. 5 & 6.—Apparent heterotypical mitosis. Fig. 5, replica of fig. 4, Royal Society paper, and of fig. 26 in First Scientific Report, 1904. Transplanted carcinoma of mouse. Chromosomes arranged longitudinally on the spindle. The mitosis is contained in two consecutive sections. × 3000/1.

FIGS. 7 & 8.—Same sections as figs. 5 and 6. Result of analysis after restaining. Longitudinally split chromosomes with unequal limbs projecting above and below the equatorial plane. × 3000/1, *cf.* diagram, fig. 4.

FIG. 9.—Apparent heterotypical mitosis. Transplanted carcinoma of mouse. Loop and figure-of-8 chromosomes arranged longitudinally on the spindle. × 3000/1.

FIG. 10.—Analysis of the same preparation as fig. 9, showing the slight differences in interpretation sufficient to make this mitosis conform to the somatic type. The loop chromosome in the middle of the equatorial plate consists of two distinct V-shaped chromosomes with unequal limbs projecting above and below the equator. The attraction fibres are attached to the apices, and not to the ends of the long limbs as would be the case in a true heterotype. × 3000/1.

FIG. 11.—Diagram of a somatic metaphase in which the limbs of the chromosomes are of unequal length. The longer limbs still cohere after separation of the apices and shorter limbs. The barrel-shaped figure thus produced resembles a heterotype, especially when the compressed form of the cytoplasm crowds the chromosomes together.

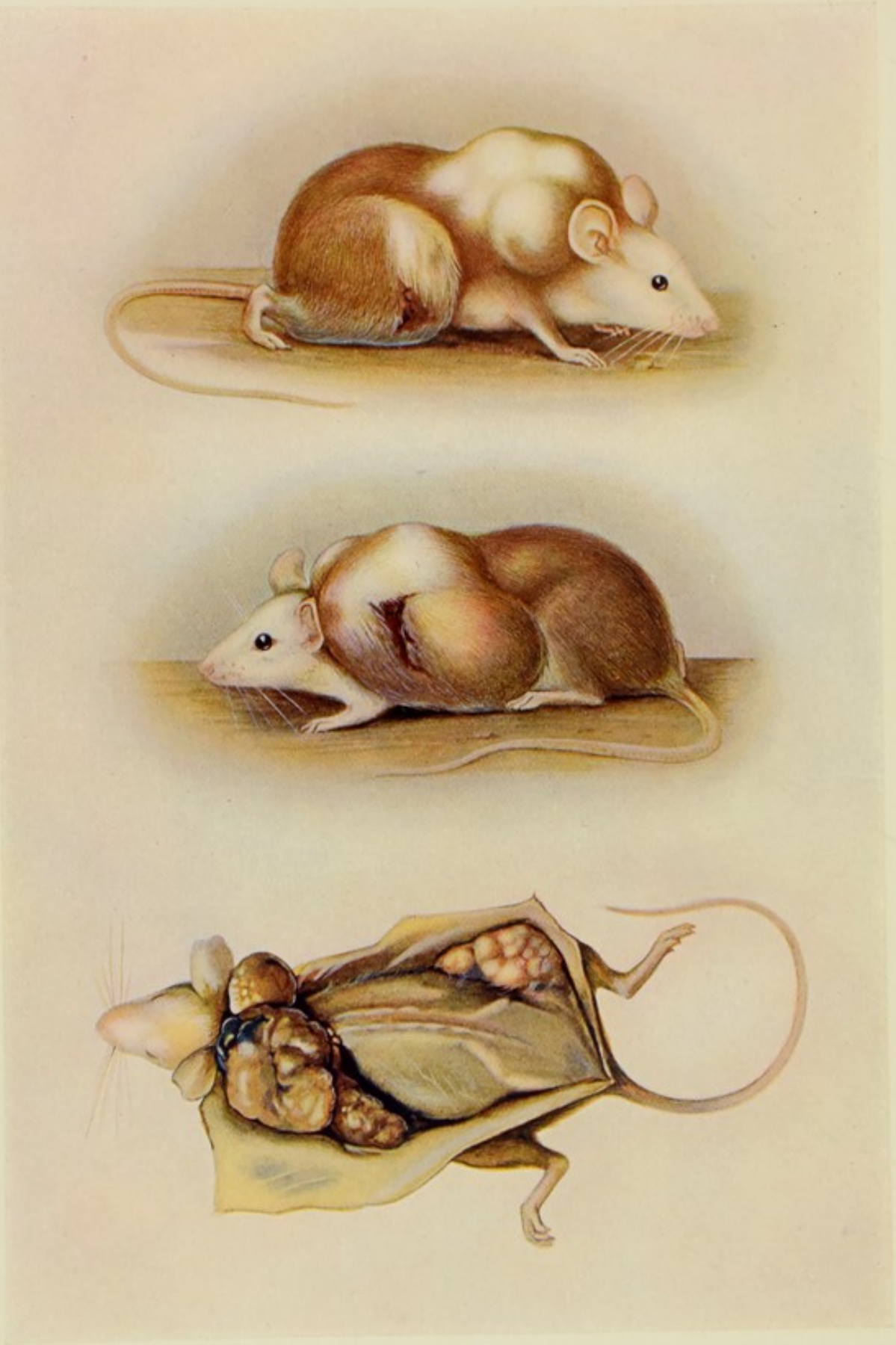
FIG. 12.—Shows the detailed analysis of the mitosis at the upper part of fig. 20, Plate 7, Second Scientific Report, 1905. It illustrates the mode of separation of daughter chromosomes with unequal limbs, as represented diagrammatically in fig. 11. Transplanted carcinoma of mouse. ×3000/1.

FIG. 13.—Microphotograph (untouched) of "monaster" mitosis from squamous-celled carcinoma of the tongue (man). Shows ring and U-shaped chromosomes. × 1000/1.

FIG. 14.—Analysis of same section as fig. 13. Partial separation of the daughter chromosmoes accounts for the presence of rings and U-shaped chromosomes No centrosomes or achromatic figure visible. × 3000/1.

FIG. 15.—Remainder of same cell in next section. Shows large number of chromosomes of ring and U-shape, along with others in which the widely separated daughter-rods are parallel to each other. × 3000/1.

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FIGS. 1–3.—Mouse $\frac{148}{0}$: Three views of a mouse with spontaneous mammary carcinoma from right and left sides, and from back after reflection of the skin. See chart, fig. 45, p. 103.

SPONTANEOUS CANCER IN THE MOUSE; HISTOLOGY, METASTASIS, TRANSPLANTABILITY, AND THE RELATIONS OF MALIGNANT NEW GROWTHS TO SPONTANEOUSLY AFFECTED ANIMALS.

By J. A. MURRAY, M.B., B.Sc.

Among the tumours of mammals a special interest attaches to the new growths of the mouse and rat, because of their suitability for experimental study. The number of these tumours which have been examined with care is now enormously greater than it was even five years ago, after Jensen's and Borrel's work and the investigations of the Imperial Cancer Research awakened renewed interest in the subject first touched upon by Hanau and Morau nearly a decade before. Thus it comes about that the material for a sound classification of the new growths of these small animals is not only fairly extensive, but is also much more varied than it was a few years ago. The confidence felt and expressed by those personally engaged in the experimental study of cancer on this material, that the essential features of cancer as already known and recognised in the new growths of man and other mammals, could be studied in miniature in the mouse with the additional advantage of experiment, is thoroughly justified by later developments.

It is no longer rational, or possible, to oppose a destructive criticism based on a knowledge of human cancer alone, to the results of the study of spontaneous and propagated cancer of animals. One by one the objections which have been raised have been met and refuted by direct observation. Thus the absence of metastases in the case of Jensen's primary tumour and in the inoculated animals, was shown by Haaland (Ann. Inst. Past. 1905), Bashford and Murray (Scient. Rep. 1905), not

to be constant—in fact they were commonly present after an interval of two months. The frequency with which the metastases remained as emboli in the pulmonary artery without invading the lung was regarded as another difference from cancer in man, although malignant infiltration of the lung was a conspicuous feature of the metastases we figured in the lungs in 1905. Then the ground was shifted to the growth of the tumours arising at the site of inoculation, and their encapsuled appearance was adduced as again inconsistent with the diagnosis of malignant new growth. This in turn was disproved by the demonstration of infiltrative growth in the gut-wall and diaphragm (Bashford, Murray and Cramer, 1905). v. Hanseman and after him Lazarus-Barlow contended vehemently that most of the the tumours of the mammary region of the mouse were endotheliomata and not carcinomata, following the views expressed by Eberth. Apolant (1906) and, with Bashford, we ('Lancet' 1907) showed the improbability of this view.

The best reply, however, to all these criticisms has been afforded by the gradual accumulation of a number of growths from other regions in the mouse in which the histological diagnosis is unequivocal, and quite distinct from that of the ordinary mammary growths, which have led to so much dubiety in the minds of advocates for a continuation of histological and pathological study in man without resort to experiments on animals. The mere fact that mice suffer from cancer of other organs of the body and that in those other situations the growths extend in the same way as do those met with in other animals and in man, diminishes the importance of the apparently exceptional characters of the majority of the mammary growths. Furthermore the tumours of the mammary region now include primary growths of unusual type in this animal, such as the squamous-celled carcinoma and the angioma and chondro-osteo-sarcoma described at pp. 78 and 81. It would indeed be remarkable if true carcinoma of the mamma did not occur in the mouse, an animal in which primary carcinoma of the lung (Livingood, Haaland and Tyzzer), the floor of the mouth (Borrel, Haaland), and the small intestine (Bashford, Murray, Cramer, and Twort) have been recorded. Therefore we shall preface the following account of the malignant new growths of the mammary region by giving a description of a number of malignant new growths of the mouse which do not belong to that group. From the standpoint of experiments it is also of moment to know that they are not carried out on an animal with a peculiar idiosyncrasy to a single form of cancer, viz., of the mamma as is too frequently asserted.

To face p. 71.]

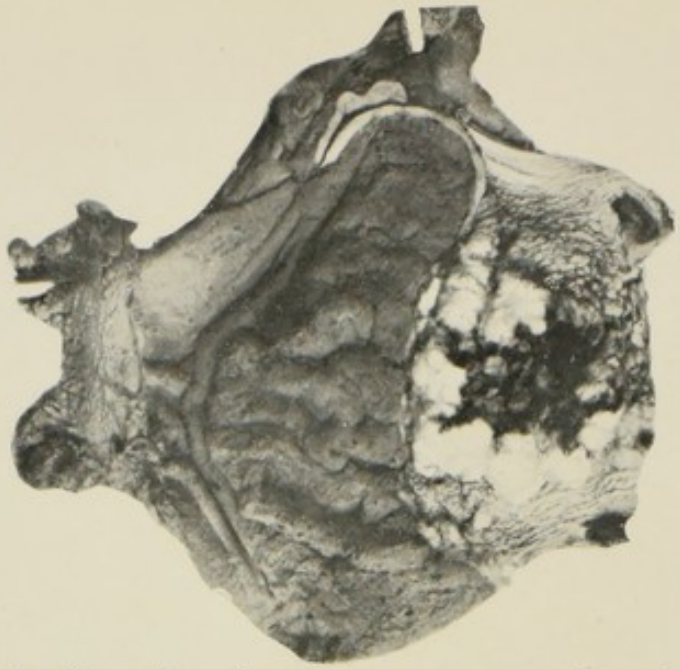


FIG. 4.—Mouse, Stomach: naked eye appearance of an early carcinomatous ulcer of cardiac, squamous celled portion, of stomach. The stomach has been opened along the lesser curvature and the ventral wall turned down. The line of junction of the keratinised and glandular portions is well seen. $\times \frac{4}{1}$.



Microphoto by W. Imboden.

FIG. 5.—Mouse, Stomach: Squamous celled carcinoma of cardiac portion, close to junction with glandular pyloric area, fully developed case. Shews lateral extension under squamous and glandular mucosa at each side of the ulcer, infiltration of the muscular coats, and the formation of a small cyst under the thickened peritoneal coat. $\times \frac{38}{1}$.

Adeno-Carcinoma of Small Intestine.

In the Second Scientific Report (1905) we figured and described an adeno-carcinoma of the small intestine with characteristic infiltration of the gut-wall. In the interval since that Report appeared, a similar case has been discovered by Mr. F. W. Twort. The general features are the same as in our case.

Squamous-cell Carcinoma of the Stomach.

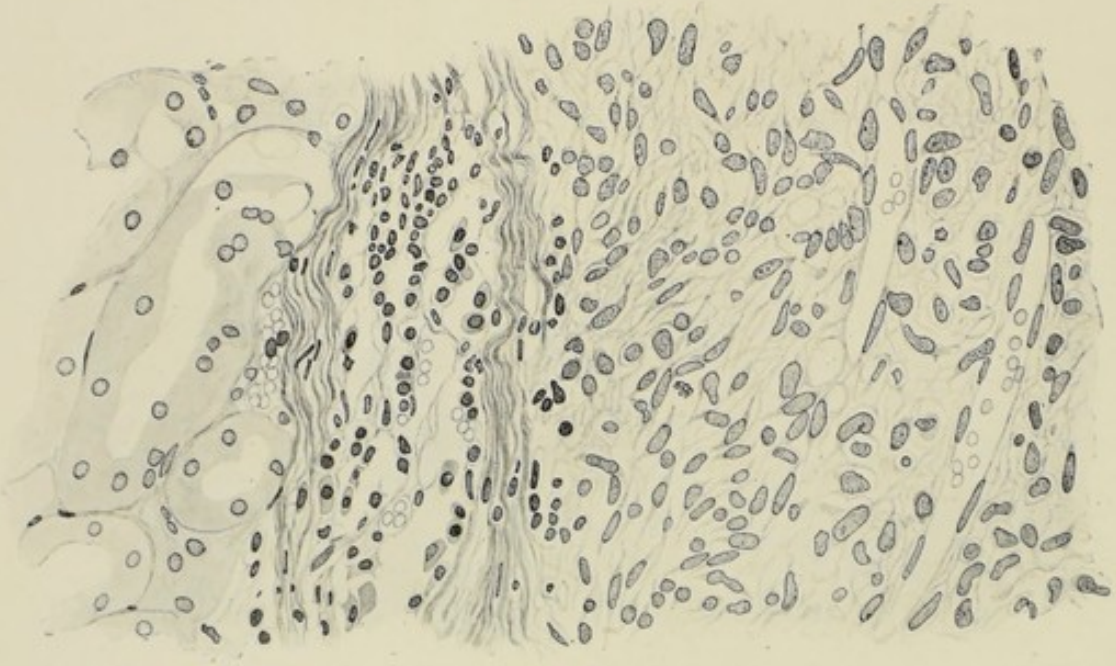
In performing the autopsy of an old male mouse the spleen was seen to be adherent to the greater curvature of the stomach. On opening the stomach (fig. 4) along the lesser curvature, a small ulcer was discovered with thickened edges at the point of adhesion to the spleen. The whole ulcer was cut in serial sections, perpendicularly to the surface of the lining of the stomach. The normal mucous membrane of the stomach in the mouse consists of two parts sharply marked off from each other. The cardiac two-thirds is lined by stratified squamous epithelium directly continuous with that of the œsophagus, and comparable to the rumen of herbivora. The pyloric portion, much smaller in extent, forming one-third of the whole organ, is lined by a glandular epithelium similar to that of other mammals. The ulcer under discussion is situated in the squamous-celled portion immediately adjacent to the glandular part (fig. 5). The squamous-celled alveoli extend laterally under the adjacent glandular and normal squamous mucous membranes. They have infiltrated and expanded the circular muscular coat, extended through the longitudinal layer, and expand under the peritoneal covering into a small cyst. Isolated alveoli lie external to the deeper layer of this flattened cyst between the adjoining lobes of liver and pancreas. The infiltration and broadening of the circular muscle is identical with that described for a primary carcinoma of the small intestine of the mouse in the Second Scientific Report (1905), figs. 40 and 41, and in the carcinoma of the small intestine of the grouse described in the preceding paper, fig. 3, p. 46. An earlier stage of the same condition was discovered at autopsy in an aged female mouse, $\frac{146}{0}$, which presented a carcinoma of the right axillary mamma. The naked-eye appearances are represented in fig. 4, and resemble closely those of the more advanced case.

Adenoma of the Liver.

A mouse with a large mammary carcinoma of the right inguinal region died a few hours after extirpation of the tumour. At the autopsy a mass of growth was found in the right lobe of the liver. The growth measured 1 cm. in its greatest diameter, was slightly flattened and formed a lenticular swelling near the sharp margin of the organ with only a narrow thin edge of liver substance at its free border. On section the growth was white in colour with punctiform hæmorrhages, the cross-sections of blood-vessels. The ordinary brownish coloration of liver was absent, and the oval section of the growth was sharply demarcated from the thin peripheral wedge of liver tissue and from the main mass of the organ. On microscopical examination the white tumour-like mass showed a very close resemblance to normal liver structure. The arrangement of the cells is irregular however, here and there are indications of a radial arrangement as if around a central vein, but portal tracts with bile-ducts are absent. The cells are highly atypical, of very varying size. The reticular fibrils of the protoplasm are much finer than in normal liver cells and are frequently arranged in parallel bundles which stain deeply with the methylene blue of Giemsa's solution and with iron-hæmatoxylin, in this respect resembling the protoplasmic fibrils of the cells of the pancreas. The nuclei present the most bizarre variations. Some are small and dense, others are large and vesicular with enormous nucleoli. In many cells two or more nuclei are present, but mitoses are extremely scanty and evidently highly pathological with clumping of the chromatin.

Where the growth adjoins the surrounding liver substance the cells of the latter are compressed and atrophied. From its relation to the surrounding liver substance, the new formation in question undoubtedly represents a considerable new formation of tissue, reproducing fairly closely the structure of the liver, but it is difficult to assign it definitely to its proper place in a classification system. The amount of the proliferation and the relation to the surrounding normal structures differentiate it from an exuberant regeneration of which, as is well known, the liver is capable. The scarcity of mitoses speaks against a rapid growth, and no metastases were found in the lungs. The liver tissue in the immediate neighbourhood of the growth shows signs of pressure and destruction. Portions were transplanted into 60 normal animals without success. Taking everything into consideration, it seems justifiable to regard the growth as an adenoma of the liver, probably malignant.

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J. R. Ford, del.

FIG. 6.—Mouse. Spindle cell sarcoma, consisting of spindle cells with delicate interstitial collagenous fibrils and thin-walled capillaries, adjoining the kidney cortex (to left). $\times \frac{500}{1}$.

Spindle-Cell Sarcoma of the Kidney Region.

A large intra-abdominal growth surrounding the right kidney was found by Professor Jensen of Copenhagen in an English mouse sent to him by Dr. Bashford two and a half years ago. The growth surrounds the kidney without infiltrating it, and consists of delicate spindle-cells with a varying amount of intercellular collagenous fibrils, and a very abundant supply of thin-walled capillaries (fig. 6). Transplantation was performed by us into 374 normal mice without success. Professor Jensen also transplanted into normal animals with negative results.

Adenoma of Lung.

The tumours which are regarded as adenomata of the lung, were first described by Livingood in 1896. Borrel and Haaland also found these growths in a considerable number (5) of the mice with spontaneous mammary carcinoma described in the latter's paper of 1905. Recently (1907) Tyzzer has given an exhaustive account of twelve cases found in old mice examined by him. Tyzzer notes that half of the animals presented a growth in other parts of the body, while in the remainder the lung tumour was the only one present.

In all these cases the structure of the growths is very uniform. The cells of the growth are usually large, cubical or columnar, and arranged upon the elastic framework of the lung alveoli in a single layer. In the smaller nodules they appear to pass insensibly into the epithelium of the air-alveoli or alveolar passages. Haaland has figured the cells actually loaded with pigment like the familiar catarrhal cells of the lung. In older, *i. e.* large nodules, pressure phenomena occur at the surface, and the tumours are more sharply separated off from the surrounding lung tissue. Tyzzer has described invasion of a small bronchus by a papillomatous ingrowth of tumour. All authors have noted the absence of mitotic figures in the cells of these growths. Tyzzer, however, found mitoses in one of his cases and refers to the frequency of indications of direct nuclear division in the form of cells with two nuclei, an observation we can confirm. In one case of our material mitotic division of a nucleus was seen, but it is in any case extremely rare.

The cases in our material have with two exceptions been found in animals with spontaneous mammary tumours. We are nevertheless inclined to agree with Tyzzer that the association is accidental and due to the greater care with which we have examined the lungs of mice suffering from spontaneous carcinoma of other regions.

Sebaceous Adenoma.

In three aged female mice bearing spontaneous mammary tumours $\left(\frac{71}{0}, \frac{74}{0}, \frac{77\text{–}78}{0}\right)$*, small white glistening nodules were found on the skin. In mouse $\frac{71}{0}$ the nodule was situated in the left groin, in mouse $\frac{74}{0}$ two were present, one in the right axilla and one on the upper lip, and in mouse $\frac{77\text{–}78}{0}$ the nodule was situated behind the right ear. Microscopical examination showed that all had the same structure, that namely of an adenoma of the sebaceous follicles (fig. 7). Those which have been observed grew very slowly, and although that from mouse $\frac{77\text{–}78}{0}$ was inoculated both by subcutaneous implantation and by scarification of epilated skin and rubbing with a piece of the growth, no reproduction of the lesion could be obtained. The interest of the condition lies in the fact that Tyzzer has been able to transplant successfully a similar, but much larger growth.

Malignant Lymphoma or Lymphadenoma.

This peculiar condition was first described by Haaland (1905) from Borrel's laboratory as a generalised hyperplasia of the lymphoid tissue throughout the body. Five of his six cases occurred during two years in mice kept in the same cage. The second case occurred during the life-time of the first. Extended attemps at experimental transmission gave a negative result. Tyzzer (1907) describes a case in which a single gland (right inguinal) was enlarged. Transplantation was attempted without success. Haaland also records a similar result.

Four cases of this condition have occurred in our material. In two practically universal dissemination had occurred as in Haaland's cases. In the other two the growths were more localised. In the first of our generalised cases $\left(\frac{60}{0}\right)$ the main enlargement was in the glands in the neck, those over the shoulder and in the axillæ. The structure is as described by Haaland and Tyzzer, a lymphoid tissue approaching the type of the germinal centres of lymph gland. In the second of these cases $\left(\frac{101}{0}\right)$ all the lymph glands in the body appeared to be enlarged, the liver and spleen were likewise affected and the chest was full of growth. Of our localised cases, one $\left(\frac{119}{0}\right)$ presented a nodule nearly

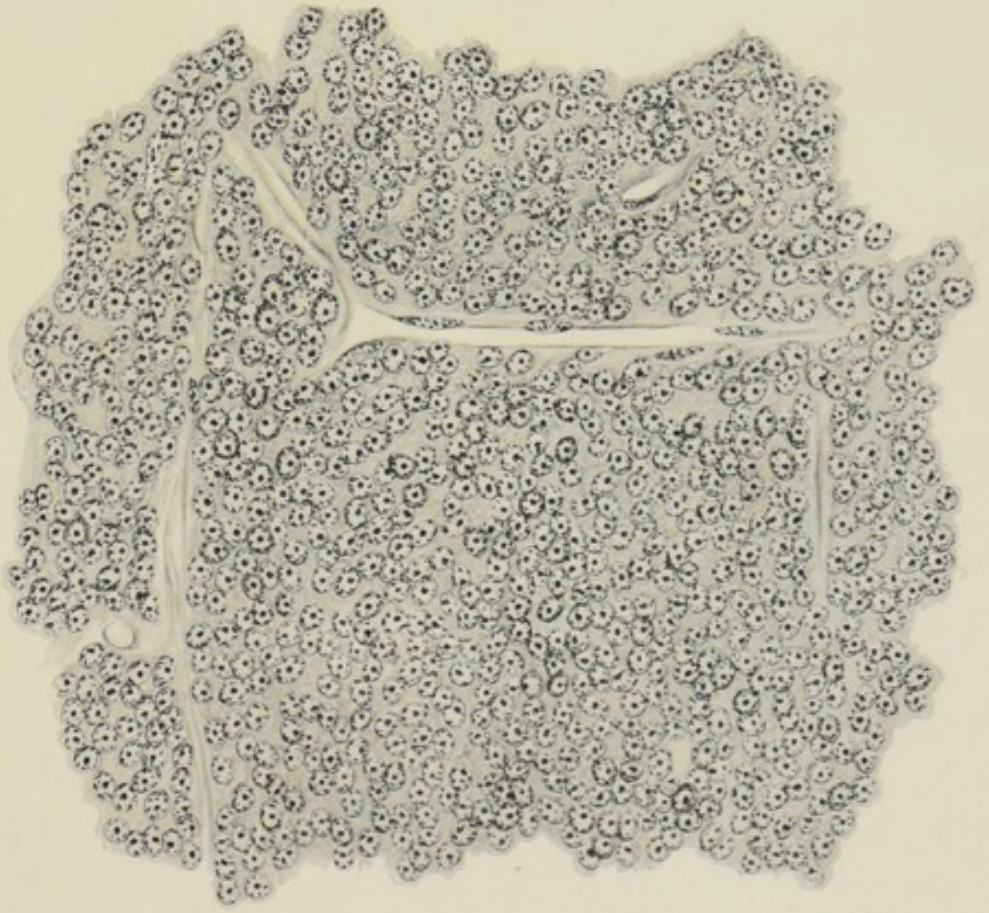
* For explanation of nomenclature of spontaneous tumours, see p. 102.

[*To face* p. 74.



Microphoto by R. Muir.

FIG. 7.—Mouse. Sebaceous adenoma: Case in mouse with spontaneous mammary carcinoma $\frac{77-78}{0}$ The epithelial masses consist of cells with the characteristic appearance of those in sebaceous follicles. $\times \frac{33}{1}$.



R. Muir, del.

FIG. 8.—Mouse. Lymphadenoma: High power figure of thoracic growth replacing right lung in spontaneous mouse $\frac{19}{0}$. $\times \frac{500}{1}$.

1 cm. in diameter in the left groin. This was extirpated and the wound healed promptly. A second growth appeared in the left axilla. This also was extirpated and proved on microscopical examination to have the same structure. The mouse had to be killed a fortnight later, and at the autopsy two masses were found on the right side of the chest. One of these appeared to be from its form and position an enlarged anterior mediastinal gland. The second had the shape of the upper lobe of the right lung, and had apparently replaced that structure entirely.

The other localised example of this condition occurred in a mouse $\left(\frac{19}{0}\right)$ with spontaneous hæmorrhagic-carcinoma of the left axillary mamma. The growth which now concerns us filled the whole upper part of the left side of the thorax, and the pulmonary artery and vein

Fig. 9.

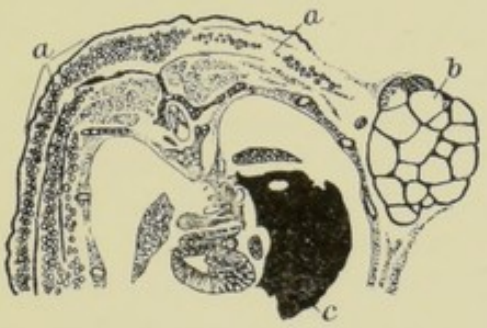


Fig. 10.



Fig. 9.—Transverse section of a mouse with spontaneous carcinoma mammæ $\left(\frac{19}{0}\right)$ and mediastinal lymphadenomatous growths. × 3.

a. Hypertrophied normal mamma (lactation). *b*. Cystic mamma anterior to mammary carcinoma. *c*. Mass of growth, lymphadenoma replacing left lung, lower lobe. × 3.

Fig. 10.—Transverse section of same mouse through growth at level of diaphragm. *a*. Normal mamma. *b*. Mammary carcinoma.. *c*. Posterior end of lymphadenoma in left lung. *d*. Metastasis of mammary carcinoma in right lung, upper lobe.

could be traced into it from the heart. The rest of the lung tissues were completely destroyed, only delicate isolated elastic fibres widely separated from each other, being distinguishable. Extension had also occurred on the right side of the chest along the peri-bronchial lymph spaces. Histologically the tumour consisted of closely packed masses of small polygonal cells with large nuclei (fig. 8). With the exception of this case artificial transmission was attempted with these

growths, both by subcutaneous transplantation, intraperitioneal and intravenous inoculation. In none of the animals was any effect produced.

These four cases came from four separate breeders at long intervals. A development of the disease has not been observed in the animals kept in the laboratory.

Malignant New Growths of the Mammary Region.

The references in the literature to the spontaneous mammary carcinomata of the mouse are closely bound up with the development of experimental cancer research by the successful transplantation of these tumours. The year 1903 marks the modern development of experimental cancer research as exemplified by the papers of Loeb, Jensen, Borrel, Bashford, which rapidly followed one another. Already with the first contribution by Morau in 1894, in which the first successful implantation of mouse tumours was recorded, the structure and classification of the new growths with which he worked received a careful description accompanied by several excellent figures. Five years had then elapsed since Hanau in 1889 had first directed attention to this method of study by his successful transplantation of a squamous-celled carcinoma of the vulva from rat to rat, and had thereby indicated the direction in which further success was to be expected. Nearly ten years elapsed since Jensen in 1903–04 carried the subject a long step forward by his masterly papers on the process of transplantation, studied on the alveolar carcinoma, now generally known by his name and propagated in laboratories all over the world. Borrel simultaneously published an account of successful transplantation of adeno-carcinomata of the mouse. He described, with beautiful figures the structure of the primary and transplanted tumours, and the embolic metastases which they formed in the lungs. In addition he recorded squamous-cell carcinoma of the jaw with lymph gland metastases, and several cases of malignant lymphoma. Bashford's earliest communications followed immediately on those of Borrel and were in time succeeded by those of Michaelis, Ehrlich and Apolant, Clowes and Gaylord. In the interval between Morau and Jensen, pathological-anatomical descriptions of mouse tumours were published by Livingood who recorded primary adenomata of the lung, sebaceous adenomata, and three subcutaneous adeno-carcinomata of the mamma, and by Eberth and Spude who recorded tumours in three mice of the same parentage. These tumours they regarded as endotheliomata, led astray as Apolant was able to show

later by the diffuse distribution of the mamma in the mouse, the acini of which they figured as lymphatic vessels.

Michaelis distinguished three types in the thirteen spontaneous tumours which he had examined in 1905 : alveolar carcinoma, adeno-carcinoma, and malignant adenoma. In 1905 also, Haaland published an account of thirty spontaneous tumours obtained by Borrel and himself in the Pasteur Institute. He described the tumours as adenomata or adeno-carcinomata, and pointed out that alveolar areas frequently occur even in tumours which have a distinct acinous structure. He also described Borrel's and several other cases of squamous-celled carcinoma of the jaw, primary adenomata of the lung, and malignant lymphomata as well as an epitheliomatous tumour of peculiar structure. In addition he gave a very full description of the lung metastases found in animals with spontaneous and transplanted tumours both of the Paris strains and of Jensen's.

In the Second Scientific Report of the Imperial Cancer Research Fund with Bashford and Cramer, we recorded alveolar and adeno-carcinomata as well as a tumour of the cystic and hæmorrhagic type which could be transplanted successfully.

In 1906 Apolant published a monograph on 276 tumours occurring in 221 mice at Ehrlich's Institute. All his tumours were found in adult or aged female mice and corresponded in their distribution to the mamma. He pointed out the great similarity they present in histological structure to the thyroid tumours in man, and gave a classification in which he distinguished two main groups, the adenomata and the carcinomata. In the adenomata he distinguished adenoma simplex, cystadenoma, cystadenoma-papilliferum, and cystadenoma œdematosum seu hæmorrhagicum, in which secondary changes in parenchyma or stroma alter the general characters of the growths. The carcinomata he divided into carcinoma simplex alveolare, cystocarcinoma hæmorrhagicum, carcinoma papillare, and fissure-forming carcinoma. Apolant lays stress on the combination of all these types in one and the same tumour and in single tumours of the same animal when multiple, a condition present in 12 per cent. of the animals. He considers the transformation of the adenomata into carcinomata as of frequent occurrence, and interprets in this sense most of the cases in which both histological types occur in the same tumour. He also holds that direct development of alveolar or other carcinoma occurs, without an intermediate adenomatous stage. There can be no doubt that Apolant recognised in this possibility of transformation of one type of growth

into another the key to the great variability and complicated histology of the mouse tumours. In the sequel the details of such transformation will again be discussed on the basis of personal observations. The papers which have appeared since Apolant's monograph, recognise as established the carcinomatous character of the tumours of the mammary region, and are principally concerned with the phenomena and results of artificial propagation. They will be referred to later so far as they concern the subjects of subsequent chapters along with the corresponding new observations.

Before proceeding to an account of the adeno-carcinomata and alveolar carcinomata of definitely glandular origin, it will be convenient to review briefly, tumours of the mammary region of different histological type, which have occurred in our material.

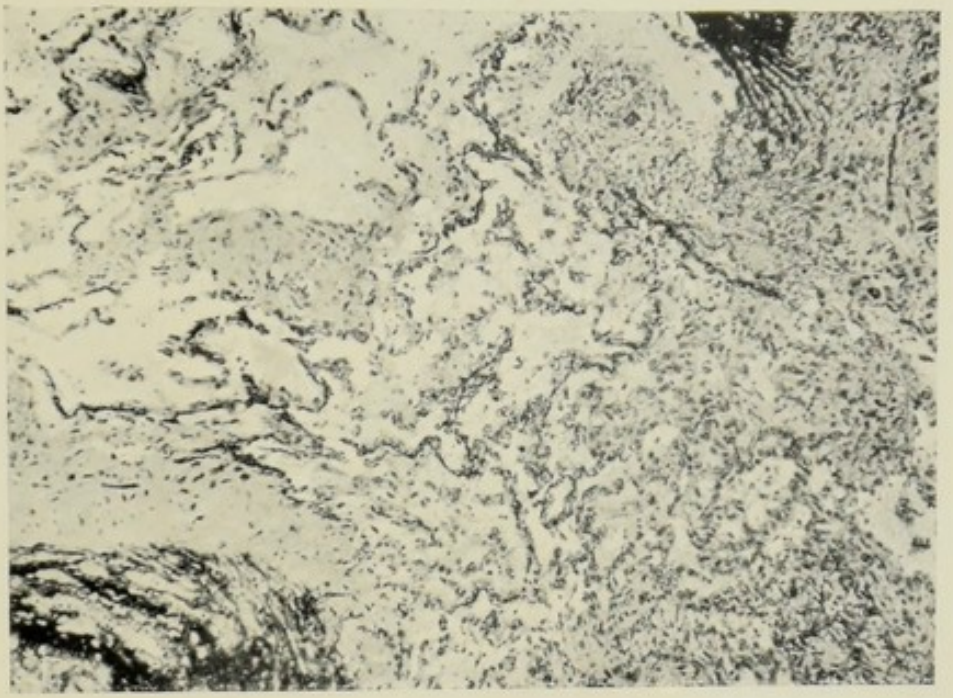
Angioma- or Angio-Sarcoma.

The tumour, of a dark blue colour, was situated in the left inguinal region of an adult male mouse. It was almost entirely formed by a cyst containing blood clot and fluid blood. Microscopical examination (fig. 11) showed no evidence of epithelial elements, but only irregular spaces lined by flattened pavement-like epithelium and filled with blood. In some of the larger spaces were recent thrombi. Small islands of large epithelioid cells lay between adjacent blood-spaces. The whole structure of the growth indicated its probable development from the endothelium of blood-vessels. A small nodule present in the right lung had the structure of an adenoma of the lung. Both tumours were transplanted, the inguinal growth into 82 mice, the lung nodule into 8 only. In both series no tumours developed.

Chondro-Osteo-Sarcoma of Mamma.

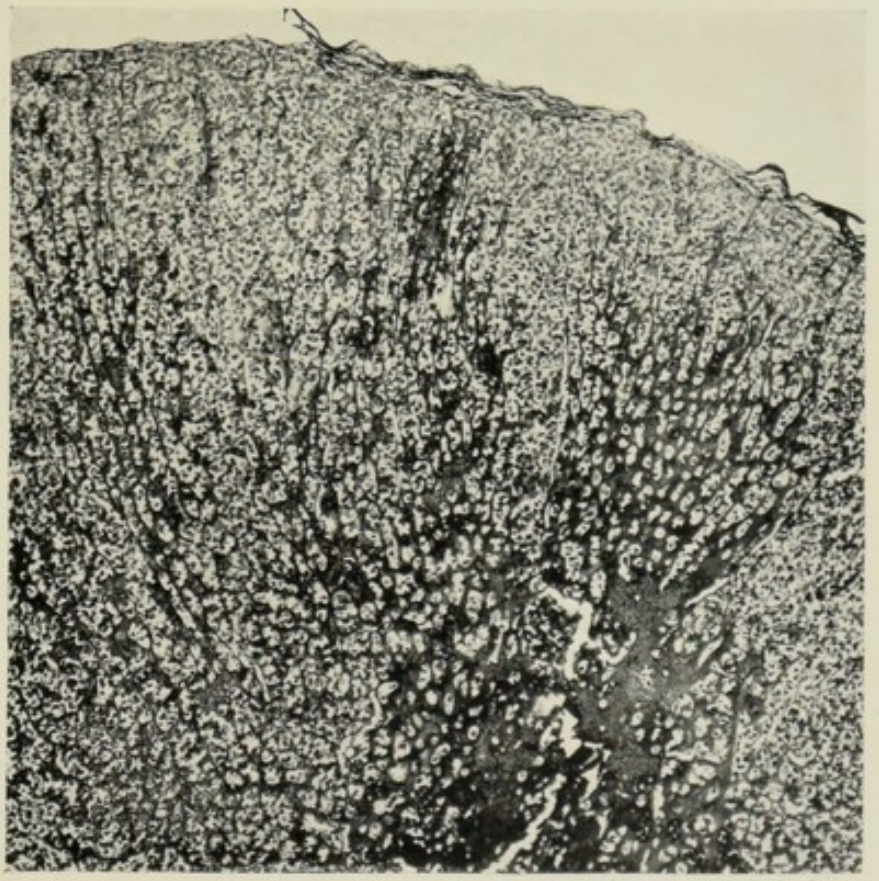
Up to the present, tumours of the mouse in which cartilage or cartilage and bone are present have been described by Ehrlich and by Haaland. In Ehrlich's case the primary tumour was intraperitoneal, and was easily transplantable. Ehrlich regarded an origin from retained fœtal structures or from a congenital foundation as probable. The transplanted tumours consist almost entirely of hyaline cartilage growing as rounded masses into which blood-vessels penetrate. In older tumours these vessels are enormously dilated and hæmorrhages

[*To face* p. 78



Microphoto by R. Muir.

FIG. 11.—Mouse. Angioma or angio-sarcoma of mamma. The growth consists of irregular spaces, partly filled with blood and lined by endothelium. In places solid cell-masses are formed. $\times \frac{90}{1}$.



Microphoto by W. Imboden.

FIG. 12.—Mouse. Transplanted chondroma, from a preparation presented by Professor Ehrlich. Note the arrangement of the cartilage cells in columns, with delicate septa of cartilage matrix between. $\times \frac{40}{1}$.

To face p. 79.]

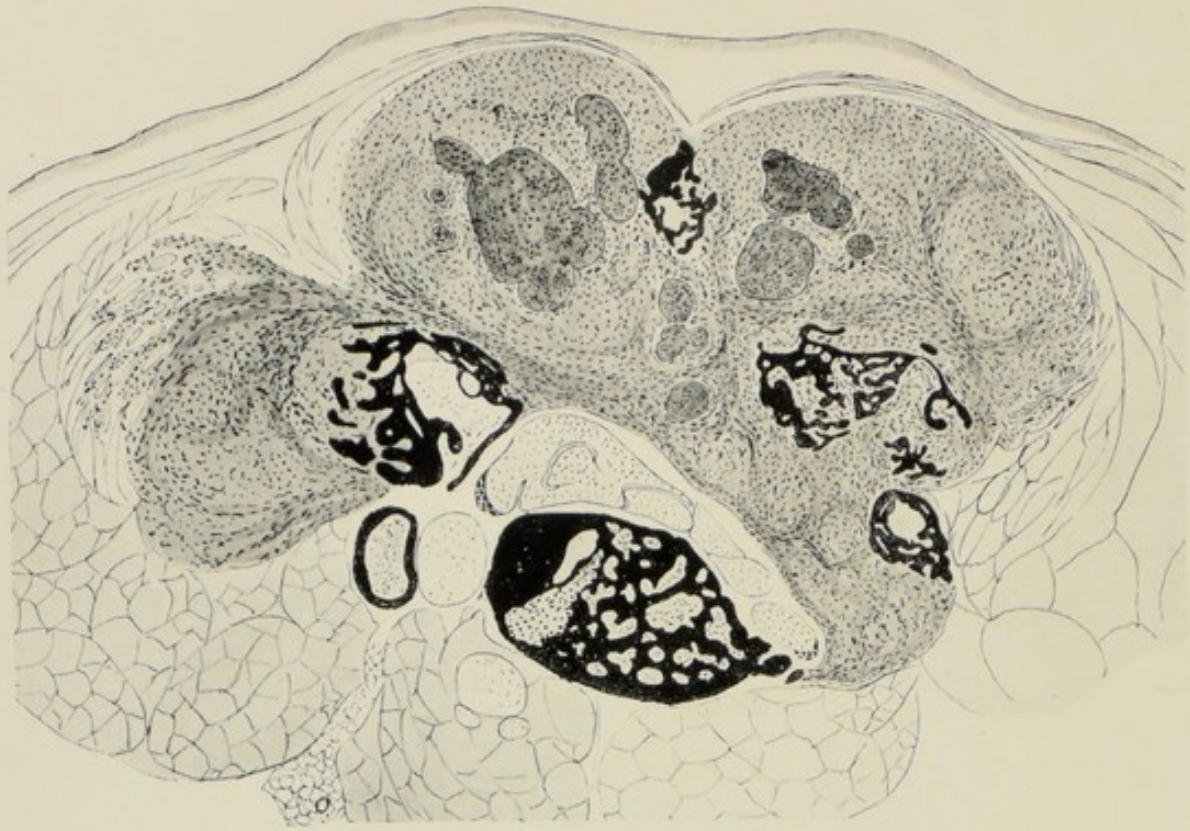
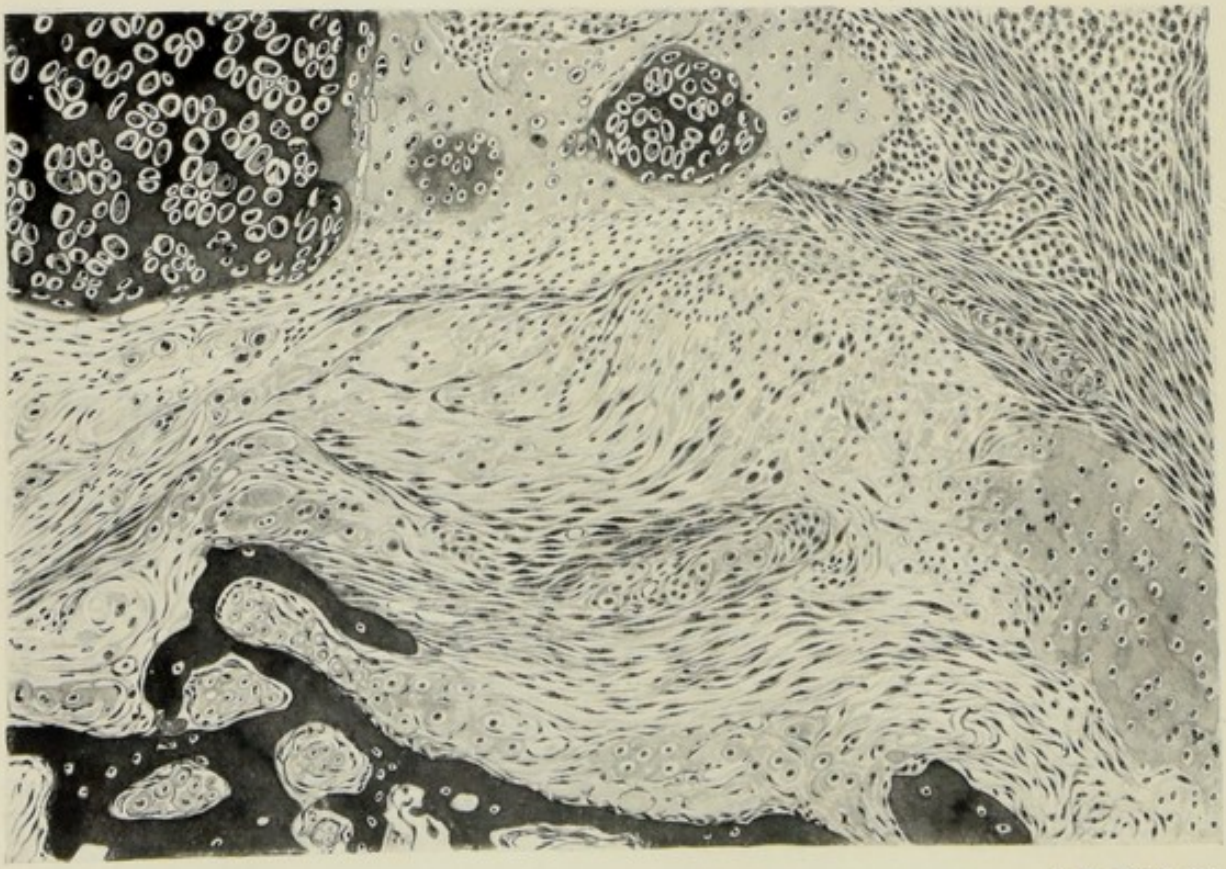


FIG. 13.—Mouse. Chondro-osteo sarcoma of vertebral column (Dr. Haaland's case). Invasion of neural canal, pressure on cauda equina, disruption of neural arch and infiltration of erector spine. $\times \frac{12}{1}$.



J. R Ford, del.

FIG. 14.—Mouse. Chondro-osteo-sarcoma of vertebral column. At the lower part of the figure a spicule of bone from the vertebral arch. Spindle-celled matrix with cartilage nodules. $\times \frac{90}{1}$.

are common. The cells of the nodules present a radial arrangement in columns, similar to that seen in the epiphyses of long bones (fig. 12), a resemblance greatly increased by the dilated blood-sinuses, so that an origin from fœtal bone or corresponding structures in a teratoma is not improbable. Haaland's case occurred in a white mouse at the Pasteur Institute, and involved the vertebral column. The growth had arisen from the bodies and arches of the lumbar vertebræ. The growth had invaded the vertebral canal and compressed the nerve trunks of the cauda equina. Laterally it invaded the extensor muscles of the spine (fig. 13). Histologically the growth consists of a spindle-celled matrix in which nodules of hyaline cartilage are scattered. In some areas an intercellular substance partly hyaline and partly fibrillar is formed, and a new formation of bone has occurred in some places (fig. 14).

The tumour now under discussion was situated in the left groin of an adult female mouse. It was oval in shape, 1·5 cm. in its greatest diameter, and very hard in consistence. It was removed by operation, a thin slice through its greatest diameter preserved for microscopical examination, and the remainder transplanted. Microscopically it consisted of interlacing bundles of spindle cells with many mitoses, between which were delicate collagenous fibrils and capillary blood-vessels. At the surface of the growth (fig. 15) many acini of normal mamma were seen, and the bundles of spindle cells passed diffusely between them so that isolated acini were found for a considerable distance towards the centre of the tumour. The growth was therefore regarded as a spindle-cell sarcoma, and considerable disappointment was felt when the inoculated animals failed to show any proliferation after two months, and the spontaneously affected animal remained free from recurrence. After an interval of three months a small nodule reappeared in the scar, others followed, and the tumour now grew rapidly, so that in five weeks the animal had to be killed and the tumour transplanted. The tumour was now much larger, had infiltrated the muscles of the thigh and had grown inwards and broken through the abdominal wall and projected into the peritoneal cavity as a lobulated glistening white mass. Some of the lobules had the same consistence and appearance as the primary tumour, others were infiltrated with blood and of a dark purple colour, while scattered throughout the growth were small nodules of almost bony hardness and opaque white on section. Microscopical examination showed a correspondingly varied histological structure (fig. 16). The firm elastic portions of the tumour presented the

same structure as the material from the first operation, namely, that of a spindle-celled sarcoma. Hæmorrhage had occurred into some of the smaller nodules, giving the dark purple appearance noted with the naked eye. In other parts minute nodules of hyaline cartilage were found (fig. 16), while the opaque white nodules consisted of bony tissue without calcification, or osteoid tissue. In the centre of some of these osteoid nodules small areas of hyaline cartilage were also encountered. The animals inoculated, especially those which had received portions of the osteoid nodules, developed tumours which grew very slowly but were easily capable of continued propagation. The tumour is noted in the tabular statement of transplanted tumours as No. 92. The propagated tumours show the same great variability of histological structure, some consisting entirely of spindle cells, others showing large masses of cartilage either associated with osteoid tissue or alone ; while in one instance lime salts had been deposited in the osteoid matrix so that true bone had actually been formed. The recurrence after apparently complete removal, infiltration of surrounding structures, and rapid growth, leave no doubt as to the malignancy of the growth and its right to be regarded as a sarcoma. From the variable character of the histological differentiations which it presents some such composite name as " chondro-osteoid-sarcoma " seems appropriate, but all the evidence seems to indicate that we have here not a mixture of several distinct tumours but merely different histological manifestations of a very polymorphous parenchyma.

The occurrence of a connective tissue tumour presenting peculiarities of the matrix not usually found in association with the mamma is not so isolated an observation as might appear at first sight. It is especially in the mammary tumours of the dog that we meet with osseous tissue and cartilage either alone or in intimate association with epithelial elements. The great frequency of complex tumours in the mamma in the dog has been referred to by Sticker, among others, in addition to ourselves. In the present connection it is interesting to note that these tumours, which are relatively benign and multiple in a large proportion of the cases, are nevertheless on occasion malignant. In several cases in our experience in which metastases in distant organs were found in animals with these complex tumours in the mamma, the metastases consisted solely of one only of the components of the primary tumour : in one case the epithelial portion alone seemed capable of metastasis formation ; in another all the metastases had the structure of osteo-sarcoma. Some authors have maintained that a congenital foundation

[*To face* p. 80.



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Fig. 15.—Mouse. Chondro-osteo sarcoma of mamma. Tumour $\frac{92}{0}$. Histology of growth removed at primary operation. Spindle-celled sarcoma with many mitoses. At left side of figure an acinus of mamma lined by columnar cells—mouse nearing end of pregnancy. × $\frac{500}{1}$.

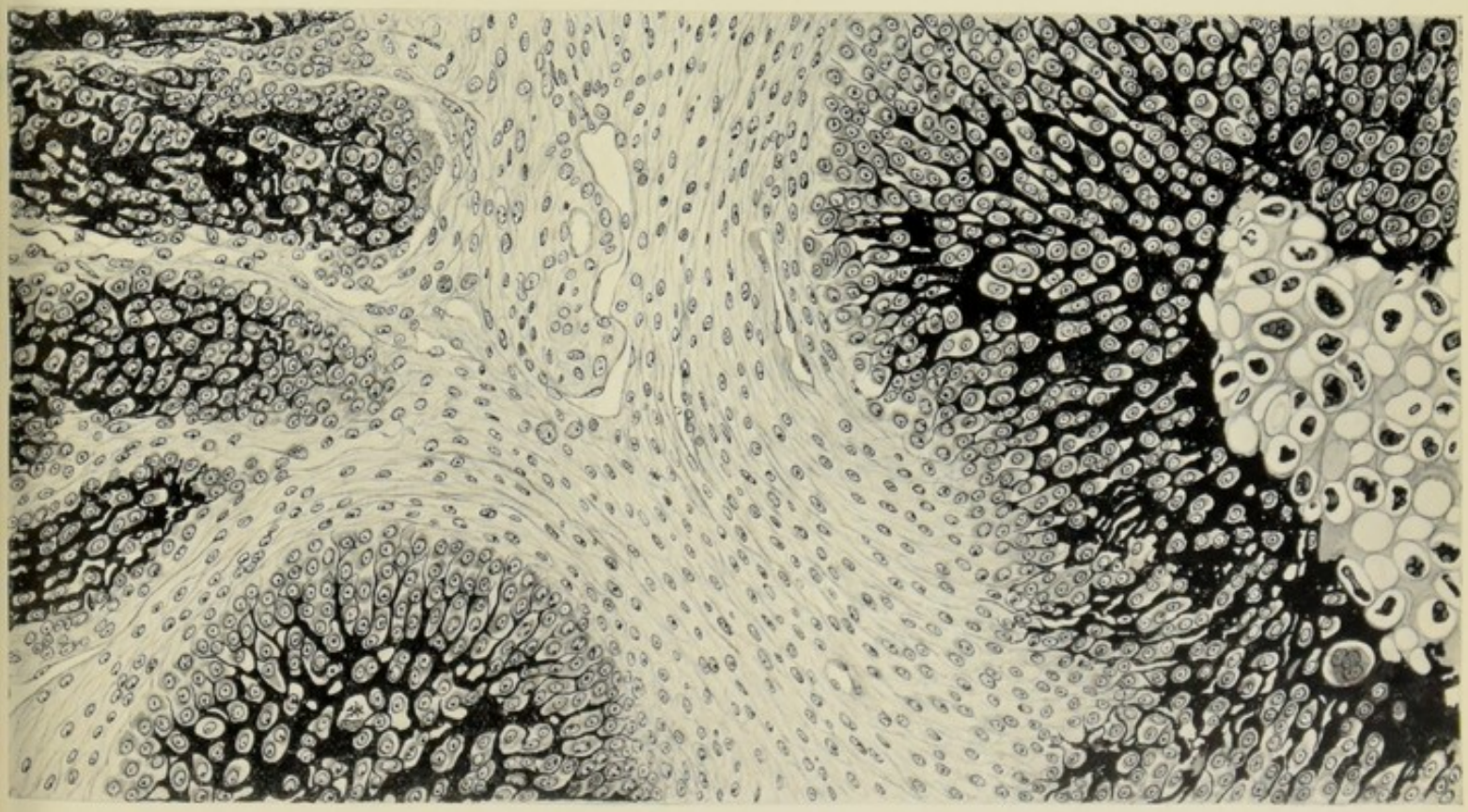
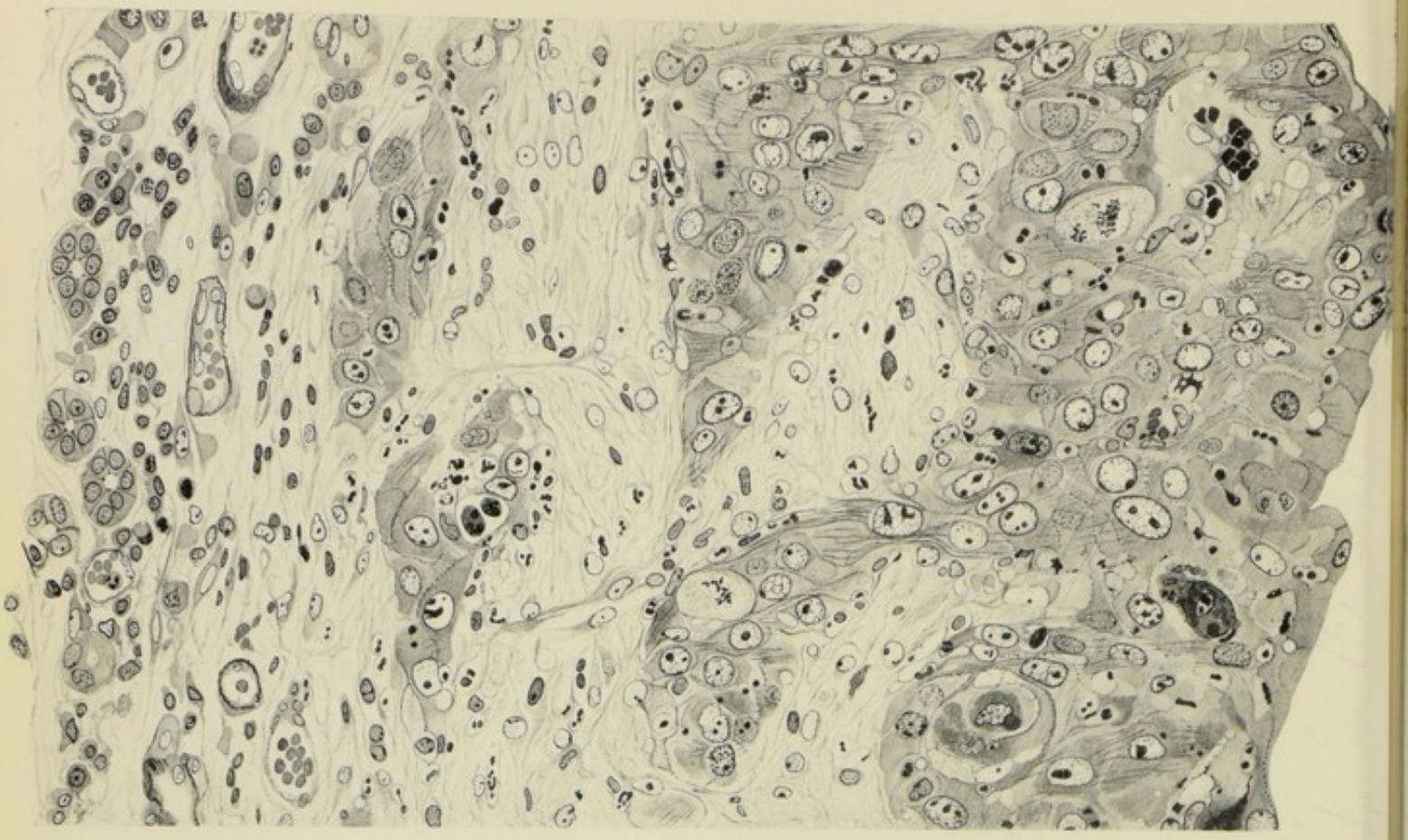
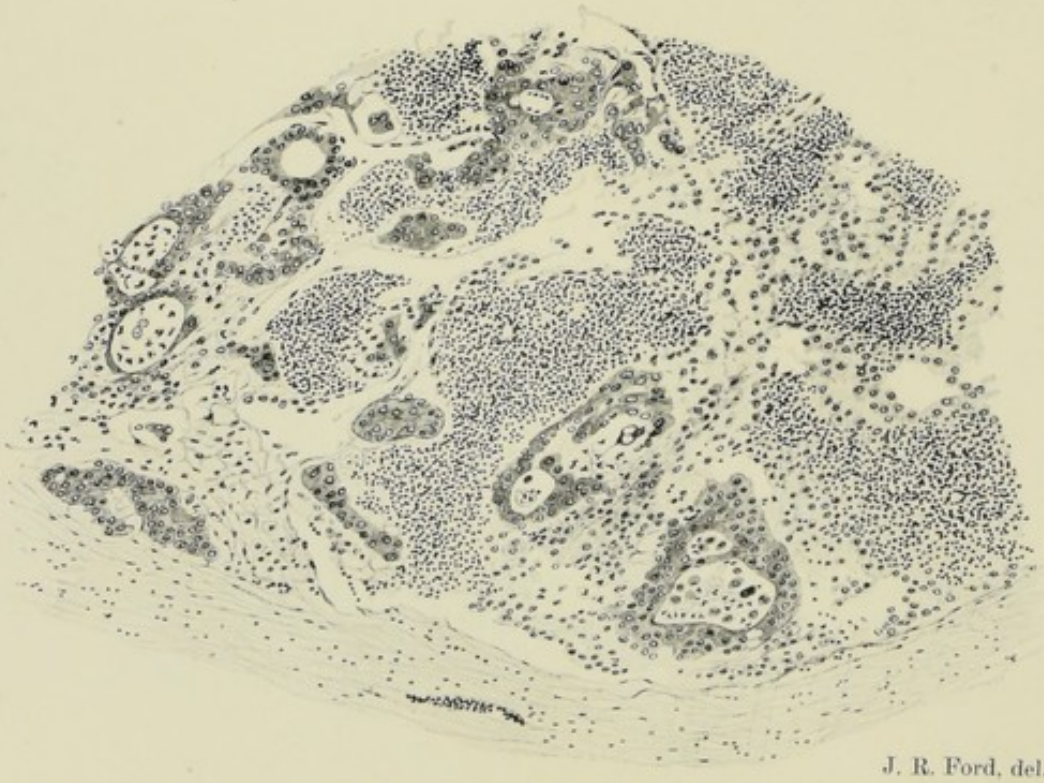


Fig. 16.—Mouse. Chondro-osteo sarcoma of mamma : transplanted tumour of first generation $\frac{92}{1}$. J. R. Ford, del.
Illustrates also the structure of the recurrent spontaneous tumour showing osteoid nodules, spindle cells, and cartilage. × $\frac{25}{1}$.



J. R. Ford, del.

FIG. 17.—Mouse. Squamous-cell carcinoma of neck tumour $\frac{28}{0}$: wall of a cystic alveolus lined by several layers of prickle cells. Many mitoses and formation of concentric epithelial pearls. At the extreme left side of the figure, acini of normal mamma. $\times \frac{330}{1}$.



J. R. Ford, del.

FIG. 18.—Mouse. Squamous cell carcinoma of neck, tumour $\frac{28}{0}$: metastatic squamous epithelial masses in marginal sinus of a lymph gland. $\times \frac{100}{1}$.

must be assumed to account for the presence of cartilage and bone in tumours of the mamma. It is probable that this is unnecessary. It has been shown that cartilage nodules may develop in the wall of the aorta, in the thickened patches produced by long-continued administration of adrenalin. Elastic fibres, another specialised constituent of connective tissue, are developed in the organisation of the pleural exudate of the rabbit produced by injection of aleurone. It is therefore justifiable to assume that the differentiations which may appear in newly formed connective tissues (in the widest sense) are dependent to a high degree on many factors not yet understood, in addition to the predisposition which is apparently implied by that characteristic of the reacting tissue. An analogous phenomenon occurs in the formation of nodules of hyaline cartilage in the callus-tissue of badly united fractures, where cells of the periosteum undergo the allied but quite distinct differentiation into cartilage-cells with their characteristic matrix.

Further investigation must show how far this new transplantable tumour may permit us to elucidate, experimentally, the conditions under which proliferating connective tissue undergoes these varied differentiations.

Squamous-cell Carcinoma of the Neck.

The growth was situated on the right side of the neck a little external to the cervical nipple. It was ulcerated and covered by a scab, and was nearly one centimetre in diameter. It was removed by operation, but proved to be so infected with micro-organisms that all the mice inoculated died or were killed within a few days. The material preserved for microscopical examination (fig. 17) shows that the tumour consists of alveoli each with an irregular central cavity lined by several layers of cells with large vesicular nuclei. Fibrils run between the adjacent cells giving the characteristic appearance of prickles (fig. 17), as seen in normal squamous epithelium. A small lymphatic gland (fig. 18) which is also included in the section shows a number of small nodules lying in the marginal lymph sinus and larger branching masses of epithelioma invading the more central parts.

Squamous-cell Carcinoma of Axilla.

The tumour was situated in the left axilla and measured 2 cm. in its greatest diameter, 1·5 cm. transversely, and 1 cm. thick. Its surface

was covered by a blood-stained scab. The tumour was partially excised under ether and the deeper portions used for transplantation. The superficial part with the adjacent skin was preserved for histological examination. Microscopical examination of sections made perpendicularly to the surface through the centre of this portion shows (figs. 19 & 20) that the growth is connected with the skin. Under the scab the surface epithelium dips down into a depression lined by squamous epithelium and from the deeper surface of this cavity irregular processes proceed in the form of alveoli with central keratinisation. Elsewhere the tumour parenchyma is purely alveolar without keratinisation and at another part close under the skin to one side of the central cavity it presents an adenomatous or adeno-carcinomatous structure (figs. 20 & 22). At one part the preparations show the alveoli of squamous epithelium adjoining an adenomatous area (fig. 21). A metastasis in the lung consists of a spherical mass of closely packed alveoli of polygonal cells in which at several points very perfect keratinisation has taken place (fig. 23). The conclusion to be drawn from these appearances is that the growth is a squamous-cell carcinoma, probably of the region of the nipple. The acinous structure of one part of the tumour is probably only an expression of the tendency which epithelial cells show to arrange themselves as a lining to spaces in which they lie, in this case in the lacunæ of the connective tissue. Ribbert has shown that a similar condition leads to the formation of small cysts lined in the first place by a single layer of cells when normal skin is transplanted into the subcutaneous tissue of the rabbit, an observation we have been able to confirm in the mouse. The cyst-like alveoli of the tumour (28) referred to in the preceding paragraph, the subperitoneal cysts of the epithelioma of the stomach (fig. 5), and analogous formations observed in epitheliomata of man, have probably the same significance. This tumour is referred to as $\frac{32}{0}$ in the tabular summary, and its behaviour on transplantation forms the subject of a separate paper at a later page.

Squamous-celled Carcinoma of Nipple.

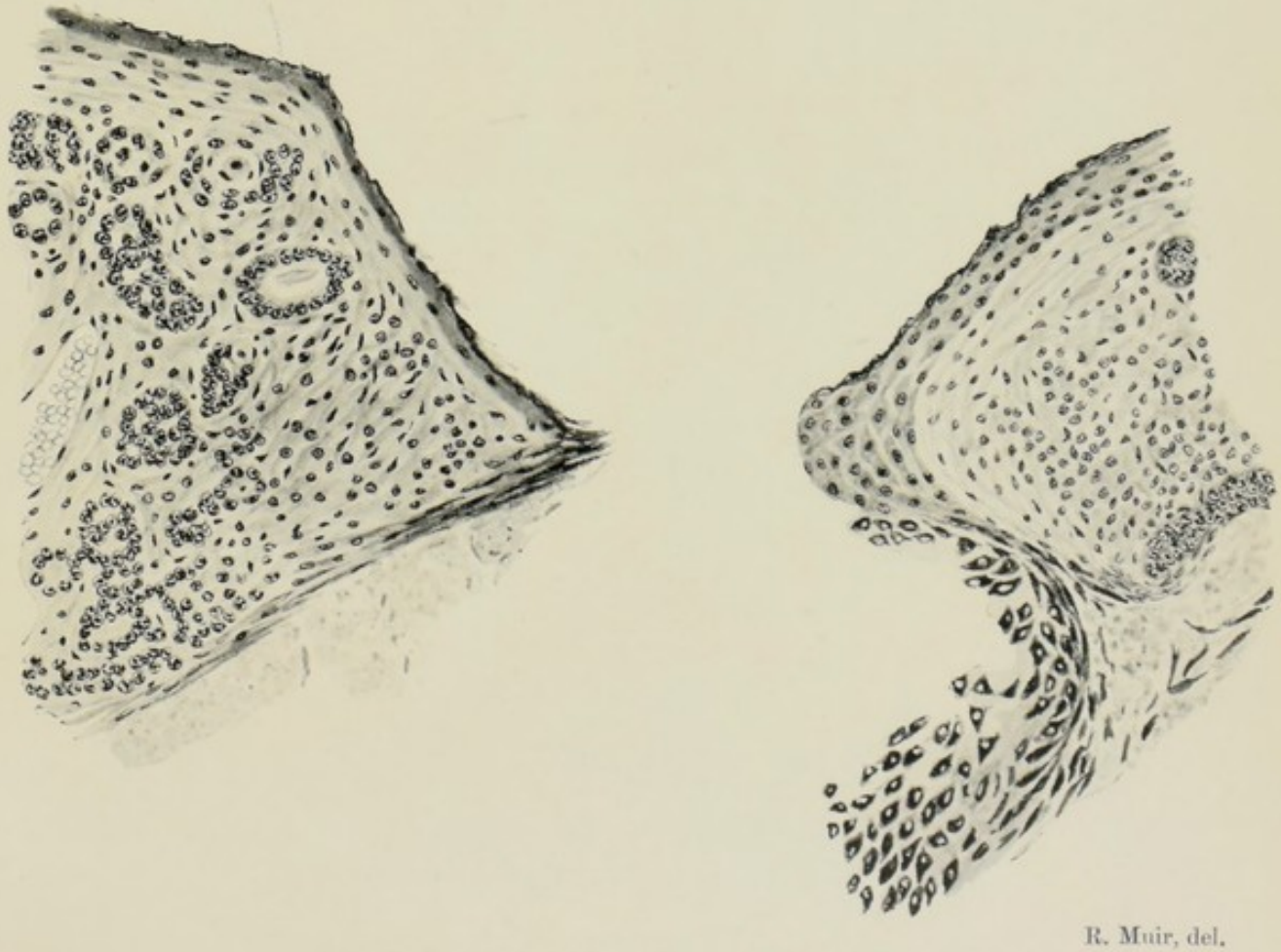
A small elevated disc-shaped tumour nearly 1 cm. in diameter appeared in the region of the left axillary nipple of a mouse $\left(\frac{61}{0}\right)$ from which a hæmorrhagic adeno-carcinoma had been removed from the right side of the vulva three months before. The growth is connected with

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R. Muir, del.

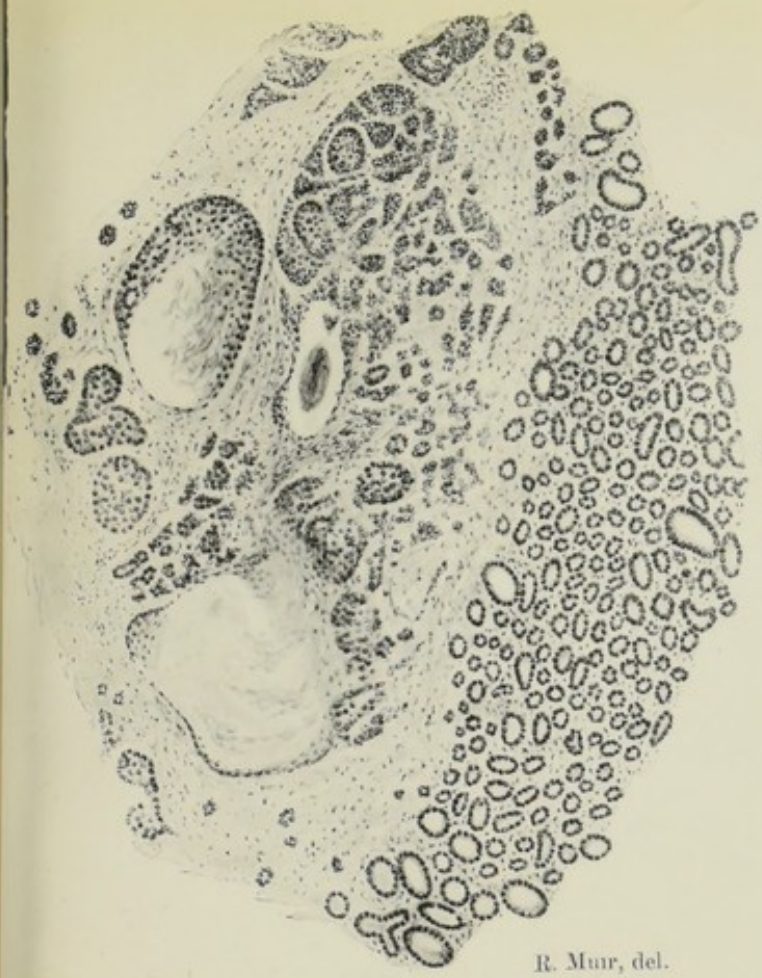
FIG. 19.—Mouse. Squamous-cell carcinoma of axilla: vertical section through superficial part of primary growth showing small cyst filled with masses of keralin, and connection of the epithelium lining it with the skin above and the tumour parenchyma below. $\times \frac{3}{1}$.



R. Muir, del.

FIG. 20.—Mouse. Squamous-cell carcinoma of axilla: higher power view of margins of the growth. Shows also adenomatous structure of the tumour under the skin to the left side. $\times \frac{150}{1}$.

[*To face* p. 82.



R. Muir, del.

g. 21.—Mouse. Squamous-cell carcinoma of axilla: deep surface of growth at junction with adenomatous area. Note varying character of tumour parenchyma. $\times \frac{80}{1}$.



R. Muir, del.

Fig. 22.—Mouse. Squamous-cell carcinoma of axilla: tumour alveoli showing keratinisation in some, solid structure in others, and tendency to formation of lamen-like spaces in others. $\times \frac{80}{1}$.



R. Muir, del.

Fig. 23.—Mouse. Squamous-cell carcinoma of axilla: metastasis in lung, showing solid alveoli and keratinisation. $\times \frac{40}{1}$.

the adjacent hypertrophied skin, is covered by a scab, and the cells are arranged in closely packed alveoli. Keratinisation is absent but prickle-cells are abundant at the surface under the scab, and near the lateral margins. The relation to the adjacent skin, and the histology of the growth leave no doubt that the growth had arisen *in situ* and cannot be regarded as metastatic from the tumour near the vulva.

Glandular Mammary Carcinomata.

Normal Anatomy and Histology of the Mammary Apparatus in the Mouse.

The mammæ of the mouse consist usually of five pairs of glands reaching from the cervical region to the lateral aspect of the anus. Usually one nipple is situated anterior to the fore-leg, one at the same level, and a third behind the fore-leg ventral to the axilla. The fourth nipple lies between the inguinal fold and the urogenital aperture, and here frequently supernumerary nipples are encountered. The fifth nipple lies at the side of the rectum and vulva (or penis) between the middle line and the inner side of the thigh. The mammary glands cover the ventral and lateral aspects of the body with extensions towards the middle line of the back. The most extensive of the latter occur in the shoulder region passing upwards on the side of the body in front of, and behind the fore-leg, and in adult animals usually meet the dorsal extensions of the other side in the middle line. Another extension passes along the inguinal fold to the dorsal end of the iliac crest, and posteriorly a rounded lobe generally is folded round the inner aspect of the thigh. This distribution is represented in fig. 17, where the usual limits of the gland as a whole in the adult female are indicated by the dotted lines and the position of the five pairs of nipples by circles.

Histologically the gland consists of a flattened diffused system of branching tubules loosely arranged in lobules, lined by an epithelium of character varying according to the state of functional activity. In the resting condition the epithelium is cubical or even slightly flattened. Before active secretion of milk commences, as in animals approaching the end of pregnancy, it becomes much deeper, the nuclei remaining at the ends of the cells next the connective tissue as in the portion of the acinus shown in fig. 15, where an ordinary columnar epithelium is found. During lactation the lining epithelium shows great variations,

from the columnar form in which sometimes the individual cells are separated from each other, and appear as flask-shaped elements attached by their necks to the basement-membrane, to flattened pavement-like elements when the acini are distended with milk. The ducts passing from the lobes of the gland to the mammillæ are usually lined by a more columnar epithelium and open into a sinus-like expansion within and below the nipple, lined by stratified squamous epithelium continuous

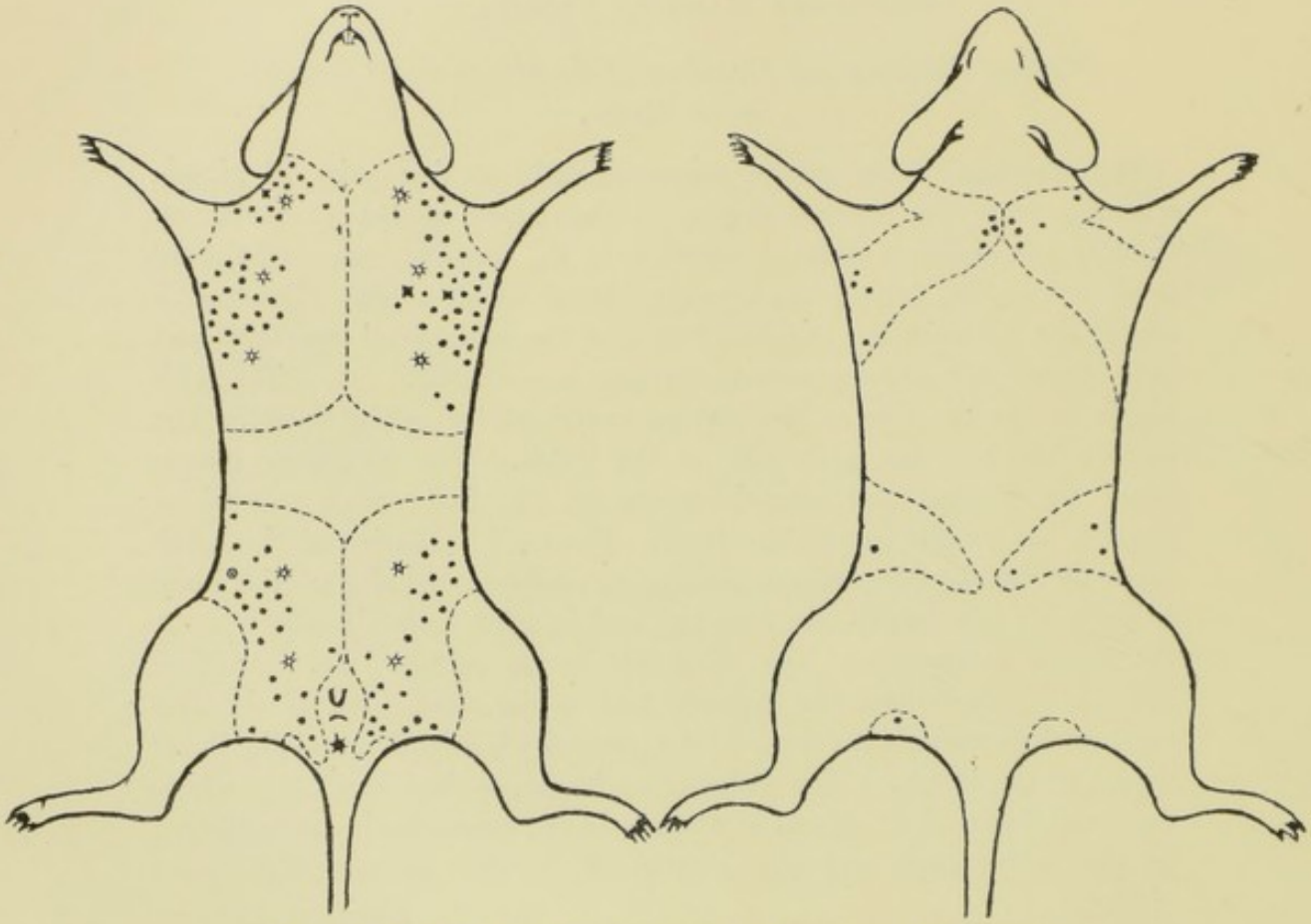


Fig. 24.—Mouse: sites of 142 spontaneous mammary carcinomata.

with the outer integument. The position of the tumours now under discussion corresponds to the distribution just given for the mammary glands, and the site of 142 spontaneous tumours is indicated in fig. 24, where each mammary carcinoma is represented by a dot, the squamous-celled carcinomata by minute squares (■), and the spindle-celled sarcoma of the right groin referred to above (p. 78) by a circle with a cross within it (⊕), to distinguish it from the convention designating

the nipples. From the figure it can be seen that the majority of the growths lie on the ventral or lateral aspects of the body, being principally aggregated in the region of the axilla and groin. Tumours occur also in fewer numbers in the cervical mamma, at the level of the vulva, and less frequently in the dorsal expansions already referred to. Thus the tumours occurring on the nape of the neck and near the sacrum, at one time believed to be irreconcilable with a mammary origin (and referred curiously enough by Pick, among others, to sweat-glands—which are restricted in the mouse to the soles of the feet), in reality corroborate in the strongest manner the conclusion, drawn from their histological structure, that they are all derived from the mamma. These 142 tumours occurred in 119 mice, multiple tumours being present in 18 animals, or 15 per cent., two tumours were present in 14 of these, three simultaneous tumours in three, while in the eighteenth animal the whole inguinal mamma had been transformed into a group of nodules of variable size. From their situation it is clear that all these growths have arisen in close association with the mammary apparatus, and it is interesting to note that they present tumours of different histological types, angioma, sarcoma, squamous-cell carcinoma, and alveolar or adeno-carcinoma in a proportion analogous to that met with in the mammary new growths of the human subject.

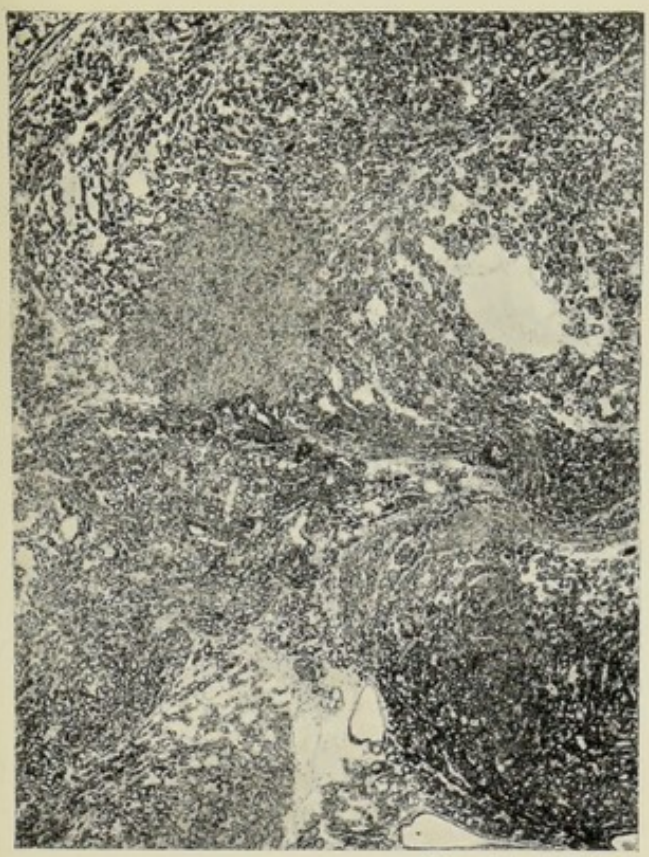
The histological variations presented by the mammary tumours can all be referred, with few exceptions, by easy gradations to the ground type of acinous structure, which leads directly to the structure of normal mamma *. The parenchyma of a single tumour may exhibit several of these modifications, simultaneously or successively. Some of the transitions thus indicated as occurring in a single tumour at different times may appear sufficiently remarkable and bizarre to those versed in the oncology of malignant new growths in the human subject, and it is necessary to point out that they hardly would have been suspected for mouse tumours, without the assistance of experimental propagation. The superiority of this method of investigation is nowhere more clearly demonstrated than in the facility with which the transformations of a

* Throughout this Report the descriptive term alveolar carcinoma (solid cell-masses separated by strands of connective-tissue) is used for solid carcinoma as contrasted with the term adenoma (acinous, glandular, tubular arrangement of cells). Adeno-carcinoma is used to describe intermediate forms in which traces of glandular arrangement can be recognised in larger cell-masses.

definite tumour-parenchyma can be followed for long periods of time, and, the association and sequence of the growth-forms elucidated by comparing the structure of tumours in successive generations of propagation. When we compare the exactness and objectivity of this experimental method, with the laborious nature of the investigations necessary in human pathology, to render probable the derivation of even the most trivial deviations from type, in different parts of a single primary tumour, or in its metastases as compared with the primary tumour, it need not create surprise that the experimental method permits of firmer conclusions on the relationship of types of growth widely different histologically, and of the sequence of intermediate stages. By the experimental method the inter-relation of tumours presenting distinct histological pictures is not only indicated, but practically proved.

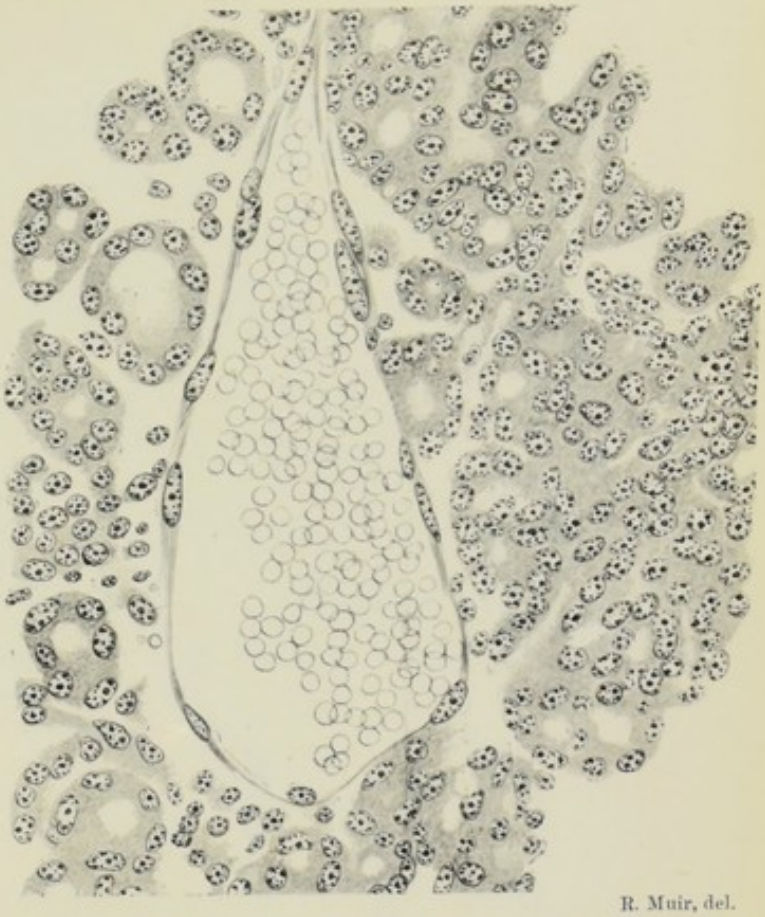
In the first place, it may be pointed out that the purely acinous type of growth, that of a simple adenoma, is extremely rare in these tumours; nevertheless spontaneous tumours in which all traces of acinous structure are absent, are seldom met with. This feature is well brought out in the tabular summary of histology, metastasis, and transplantation, on pp. 91–96. Apolant drew the inference, from the association of adenomatous with other structural types, that the adeno-carcinomatous and the alveolar-carcinomatous areas had arisen directly from pre-existing adenomatous areas. He instances the appearances represented in figs. 9, 10, and 11 of Plate II of his monograph as demonstrating the mode of this transformation in a hæmorrhagic adeno-carcinoma. A careful study of many of these growths, spontaneous and transplanted, has led us to the conclusion that in Apolant's figures the course of the transformation is in the opposite direction and we have to deal here with the splitting up of an alveolar parenchyma into small acini. Figs. 25 and 26 represent at a low magnification and under a higher power the details of such a part of a spontaneous tumour. A whole lobule of the growth in question $\left(\frac{103}{6}\right)$ is seen to consist of radially arranged columns of acini, which end centrally in a small area of alveolar carcinoma. In fig. 25 can be seen the gradual splitting up of the alveolar portion into small acini by ingrowing connective tissue and capillaries. This phase of the transformation of alveolar areas into adenomatous, is very frequent and is represented for another tumour in figs. 27 and 28.

The converse process by which acinous structure is transformed into



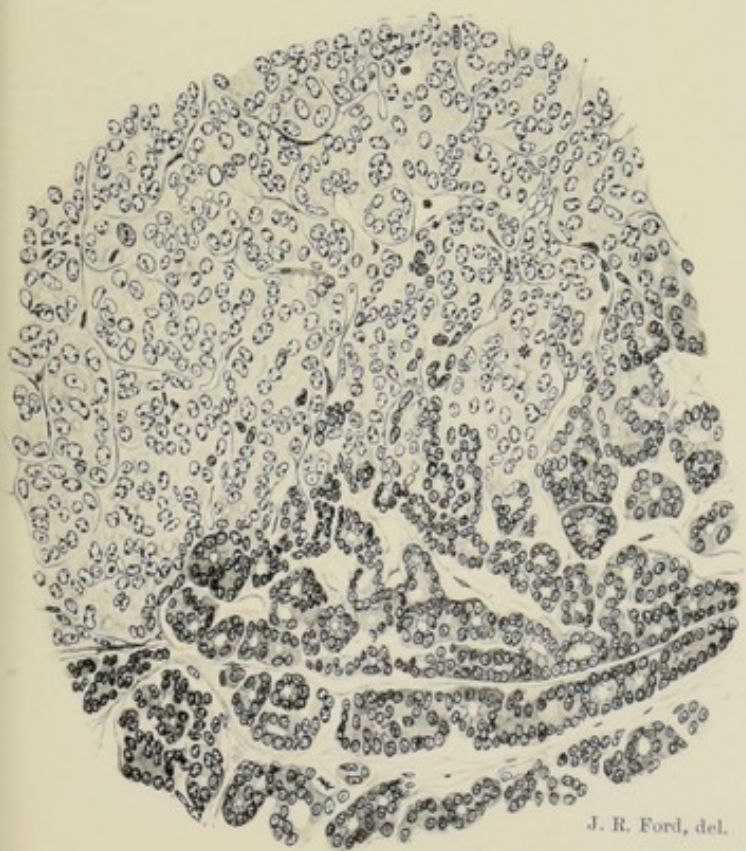
Microphoto by R. Muir.

FIG. 25.—Mouse. Adeno-carcinoma of mamma tumour $\frac{160}{9}$: shows the centre of an otherwise acinous lobule occupied by a small mass of solid or alveolar structure, see next figure. $\times \frac{40}{1}$.



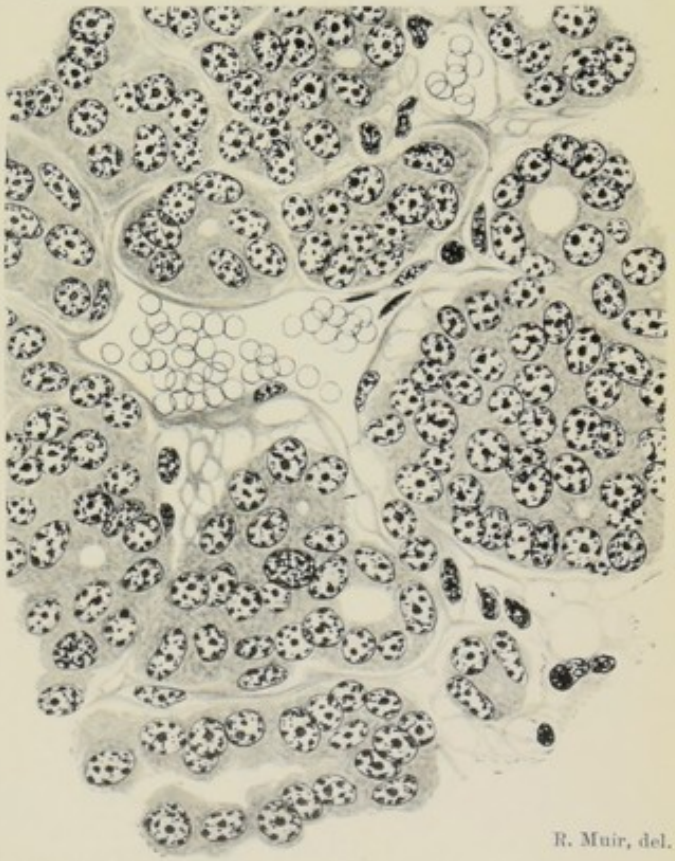
R. Muir, del.

FIG. 27.—Mouse. Hæmorrhagic alveolar carcinoma, tumour $\frac{19}{9}$ transition from solid alveolar to acinous structure. Shows also the delicate-walled dilated capillary blood-vessels. $\times \frac{500}{1}$.



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FIG. 26.—Mouse. Adeno-carcinoma of mamma tumour $\frac{160}{1}$: lower margin of solid alveolar area of fig. 25 showing the details of the process of transformation of solid to acinous structure, by ingrowth of connective tissue and blood-vessels. $\times \frac{250}{9}$.



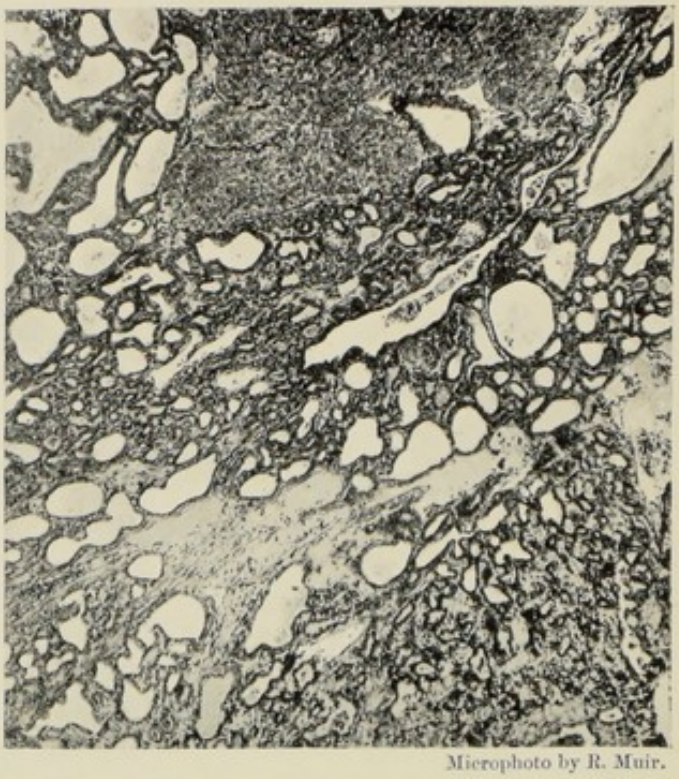
R. Muir, del.

FIG. 28.—Mouse. Hæmorrhagic alveolar carcinoma, tumour $\frac{19}{9}$: transition from alveolar to acinous structure. $\times \frac{200}{1}$.

To face p. 87.]

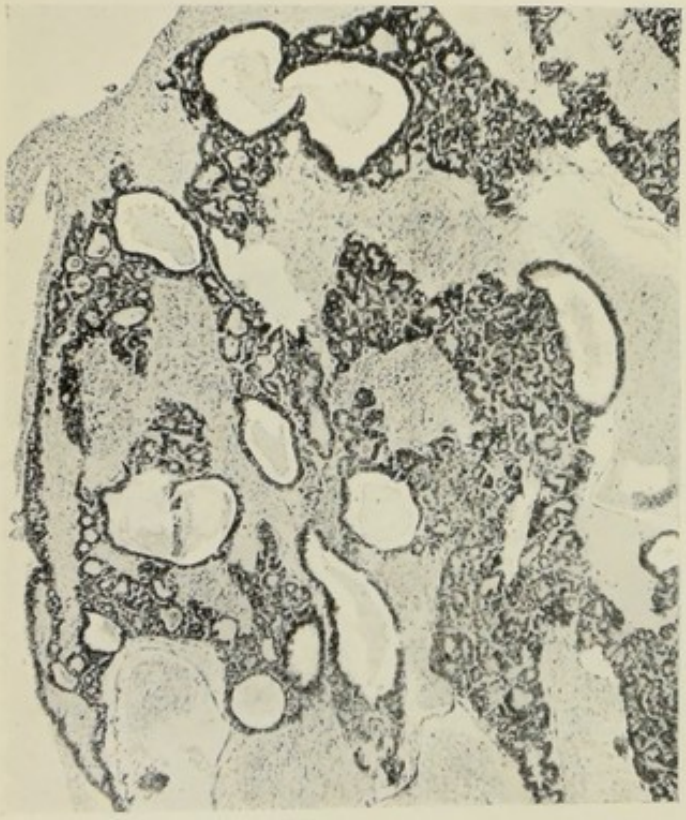


FIG. 29.—Mouse. Hæmorrhagic alveolar carcinoma, tumour $\frac{20}{0}$: late stage of transformation of acinous into alveolar structure, a layer of columnar cells surrounds the central solid mass, the cells of which are in this case smaller and stain more deeply. Small irregular lumina remain. $\times \frac{300}{1}$.



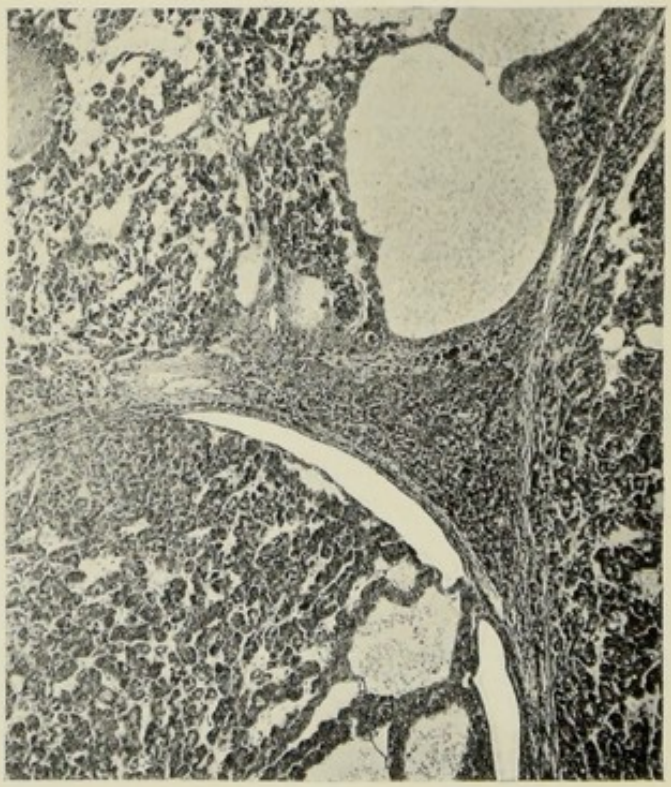
Microphoto by R. Muir.

FIG. 30.—Mouse. Adeno carcinoma, tumour $\frac{2}{0}$: transformation of acinous into alveolar structure, the layer of cells lining the lumen is retained for some time surrounded by solid masses of cells arranged irregularly. $\times \frac{75}{1}$.



Microphoto by R. Muir.

FIG. 31.—Mouse. Hæmorrhagic trabecular alveolar carcinoma with lumina tumour $\frac{20}{0}$: the figure shows an area in which the acinous type prevails with cystic dilation of lumina. The blood-vessels are dilated capillary sinuses separated from the parenchyma by spaces filled with serous fluid. $\times \frac{75}{1}$.



Microphoto by R. Muir.

FIG. 32.—Mouse. Hæmorrhagic cystic adeno-carcinoma, tumour $\frac{70}{0}$: œdema and dilatation of blood-vessels; portions of two spherical lobules are shown in which differentiation into columns of acini has occurred $\times \frac{60}{1}$.

an alveolar condition, or a condition of alveoli with irregular lumina, is frequently seen, but is more difficult to interpret in the spontaneous tumours. It is probable that a mode of transformation occurs in which simultaneously with an increase in size of the cells lining the acini, the lumen is encroached upon and obliterated, and then the solid mass of cells so produced proliferates rapidly to form larger alveoli. Such a condition is however difficult to differentiate from the preceding process of transformation from alveolar to acinous structure. Much more easily interpreted are those cases in which indications of acinous structure are retained alongside of the newly formed alveolar groups. Two modes of transformation can be easily followed. In the first the main proliferation is inwards toward the central lumen. A distinct layer of cells, usually columnar or cubical, remains adjacent to the connective tissue and within this a mass of smaller cells with deeply staining nuclei is formed. Irregular lumina may persist for some time between the elements of the central mass of cells. This condition is illustrated in fig. 29. When the proliferation is mainly outwards from the lumen towards the stroma, solid masses of cells result in which lumina persist for a long time lined by a well marked cubical or columnar layer, which usually stains more darkly than the peripheral cells. Alveoli with several lumina are frequent, in preparations showing this condition, and are partly due to coalescence of adjacent alveoli, partly to concomitant proliferation of the acinous layer. Successive stages of this transformation are shown in fig. 30. Occasionally the transformation of acinous areas into alveolar is disturbed by a more energetic proliferation of the connective tissue occurring at the same time. In such cases crescentic trabeculæ of adenomatous tumour are seen enclosing alveolar masses with clear cells. These are connected usually by a narrow stalk with the more deeply stained adenomatous strands, and at these points the continuity of the cells of the two kinds can be made out. At a later stage the crescentic adenomatous bands have also proliferated and there results a whorled arrangement of alveolar trabeculæ.

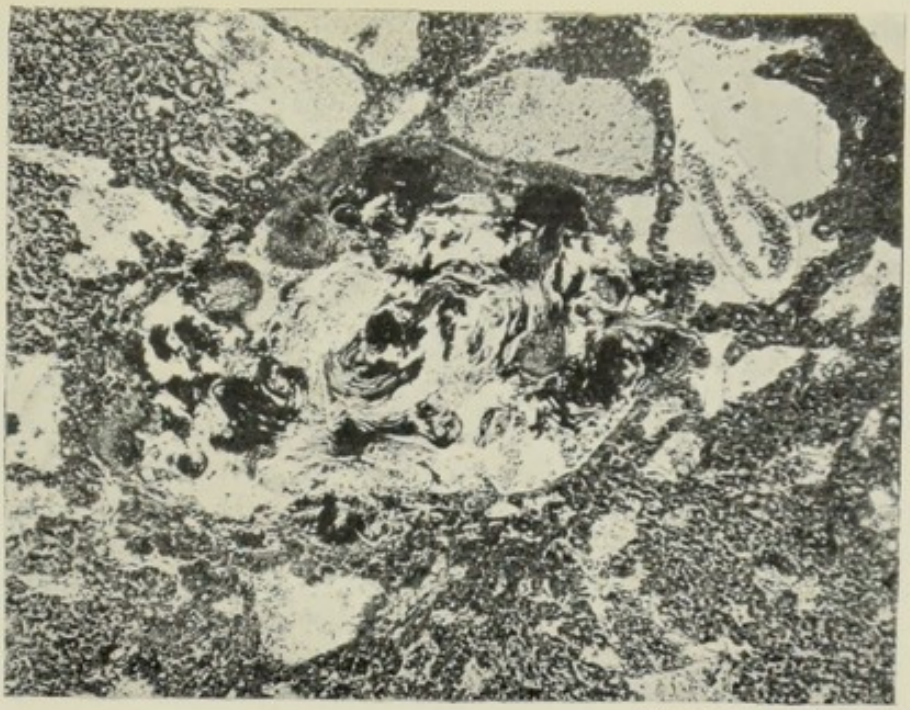
The œdematous and hæmorrhagic changes in the stroma are well illustrated in figs. 31 and 32. These changes which may affect any tumour, alter profoundly the macroscopic appearance and histological structure. The change is particularly liable to occur in those tumours in which an extremely delicate connective tissue is associated with thin-walled capillaries and is dealt with in greater detail in the following paper by Dr. Gierke on the hæmorrhagic tumours. Fluid exudate accumulates between the walls of the capillaries and the adjacent stroma

so that the vessels appear suspended in wide spaces filled with a light flocculent coagulum. Dilatation of the capillaries is generally associated with this condition and points to a condition of partial stasis of the circulation, as the primary change. Hæmorrhages into the dilated lymph-spaces surrounding the vessels frequently occur, and when the acini of the tumour parenchyma are dilated into cysts, such blood extravasations easily pass through the attenuated cyst walls and distend the cystic cavities with corpuscles and clot. The thin trabeculæ of parenchyma which intervene between adjacent cysts when these latter are numerous, are much compressed, and the vascular endothelium may be completely destroyed for long distances. This condition when of long standing as in an old tumour, produces the appearances which have led some observers, and notably v. Hansemann, to classify many of the mouse tumours with the endotheliomata. Careful study of the development of this condition, made possible by routine charting of spontaneous and transplanted tumours, leaves no doubt as to its true significance.

An adeno-carcinoma of the inguinal region which had attained an enormous size $\left(\text{mouse } \frac{129}{0}\right)$ showed an interesting deviation from the usual appearances of mammary tumours. So far as the epithelial component is concerned, the tumour is an adeno-carcinoma of the familiar type. The peculiarity of this tumour consists in the broad bands of spindle-cells which separate the alveoli from each other. These stroma-like septa give to the tumour the appearance described as carcinoma sarcomatodes, and the tumour is regarded as a mixed tumour, composed of carcinoma and sarcoma growing commensally. The tumour was transplantable and Dr. Haaland gives an account with figures of the histology of the primary and transplanted tumours in his paper on sarcoma development in transplanted adeno-carcinoma.

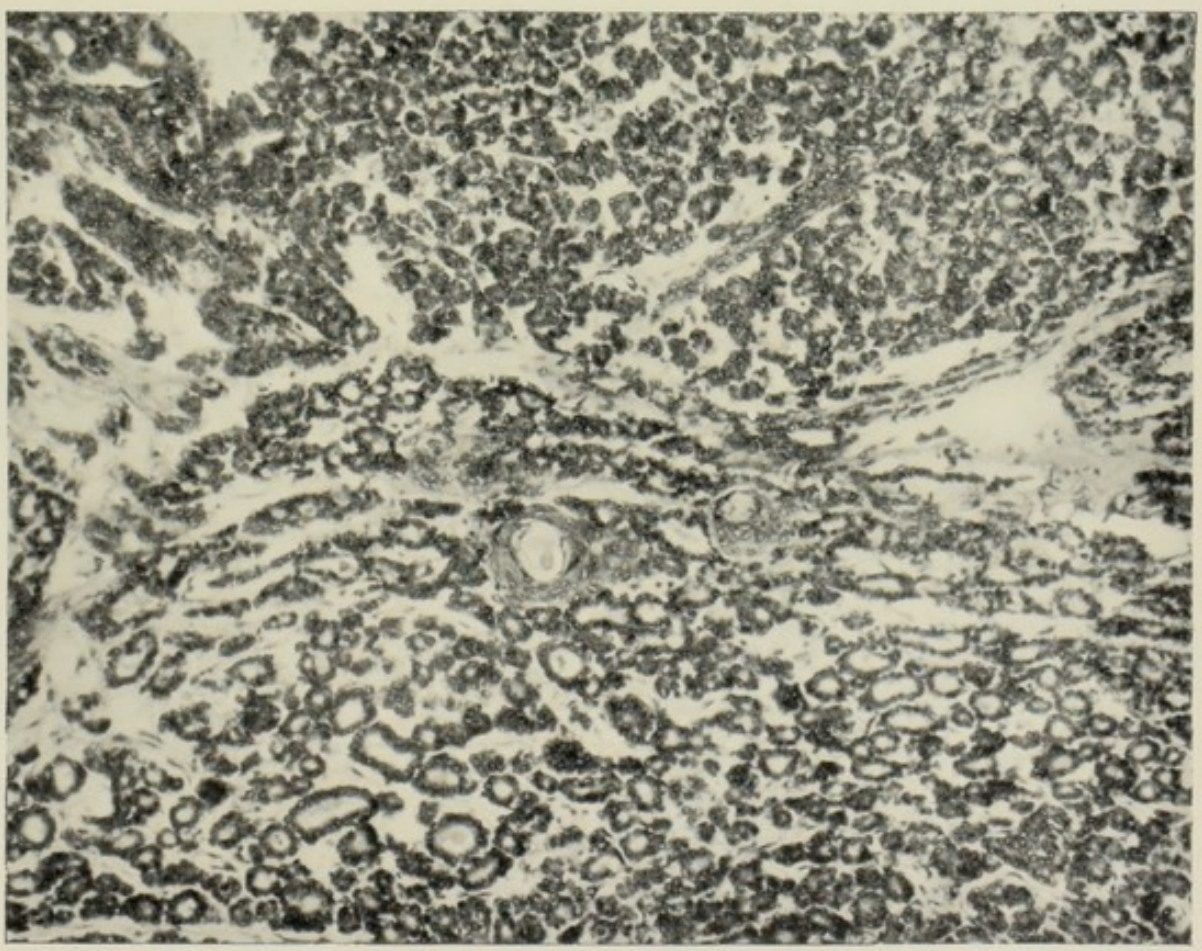
In the Second Scientific Report 1905, it was noted that a spontaneous alveolar hæmorrhagic tumour which we had obtained two years previously presented a small area of keratinisation. Fig. 33 shows the area in question, and while noting its occurrence and the intimate relation which the cells had to surrounding alveoli, we refrained from drawing any conclusion as to its significance for the classification of such growths. It can be seen that the Malpighian layer of the keratinised area is continuous with the surrounding parenchyma which, elsewhere adeno-carcinomatous, is here largely alveolar. Such areas have been met with in several other tumours but always much smaller in

[*To face* p. 88.



Microphoto by R. Muir.

FIG. 33.—Mouse. Hæmorrhagic alveolar carcinoma: area of keratinisation referred to in Second Scientific Report. $\times \frac{90}{1}$.



Microphoto by W. Imboden.

Fig. 34.—Mouse. Adeno-carcinoma, tumour $\frac{103}{0}$: small areas of keratinisation in the course of duct-like tubules. $\times \frac{170}{1}$.

extent. Fig. 34 shows similar nodules in the purely acinous part of tumour $\frac{103}{0}$ to which reference has been made above. In this case such nodules are frequent throughout, but principally in connection with tubules of which the epithelium resembles more closely that of the ducts of normal mamma rather than of the secreting acini. In this respect they recall the similar nodules frequently met with in adenomata of the mamma of the human subject and generally described as cholesteatomata. Henke regards these formations as closely allied to keratinisation, and in the mouse tumour under discussion eleidin granules and prickle-cells have been demonstrated.

These observations have an important bearing on some of the questions raised by the co-existence of acinous and keratinised areas in our transplantable squamous-cell carcinoma $\left(\frac{32}{0}\right)$ and by C. Lewin in his account of a transplantable mammary carcinoma of the rat. This tumour of Lewin presented the structure of an adeno-carcinoma in the primary animal. It was only after propagation through several generations (3rd) that wide-spread keratinisation was observed. Lewin held that the keratinising tumour had developed by infection from the mammary carcinoma of the overlying epidermis covering a subcutaneous nodule. As an alternative he suggested that metaplasia had supervened, transforming the acinous tumour into a squamous-celled carcinoma.

It will be recollected that a transplantable squamous-celled carcinoma in which the connection with the skin of the axilla could be demonstrated as described on p. 81, presented acinous areas in addition to the main mass which was keratinised or alveolar. Whether the primary tumour in Lewin's case had a correspondingly varying structure it is impossible to say, as histological examination was very incomplete. Whether keratinised elements were present in the material used for his primary transplantation or not, seems immaterial in the light of our experience with a transplantable squamous-celled carcinoma which remains alveolar for long periods and at intervals presents wide-spread keratinisation in the daughter-tumours. In addition quite recently acinus-like lumina have appeared in the alveoli of several distinct strains. We may conclude with a high degree of probability that we are in this and in Lewin's case dealing with several growth-forms of a somewhat polymorphous parenchyma, as already suggested for the chrondro-osteoid-sarcoma at pp. 78–79. To speak of metaplasia or anaplasia in this

connection seems inappropriate unless we use these terms with the qualification that they do not necessarily imply any permanent non-reversible alteration in cell-characters. We prefer, therefore, to use the expression "growth-form" for these histological variations, and regard them as indications of the close association of the mammary apparatus with the skin from which it develops. In addition we must recollect that the ampulla which receives the terminal portions of the mammary ducts, is also lined by stratified squamous epithelium and may be regarded as a secondary invagination of the covering epithelium. Therefore, should the cells of a new growth have taken their origin from a part of the gland close to the nipple (and this is frequently the case, see fig. 20), variations in either direction are only to be expected.

Table of Spontaneous Mammary Tumours of the Mouse, giving histology, clinical course, metastasis, and results of transplantation.

No. of Tumour.	Histology.	Number of operations.	Number of recurrences.	Interval between first operation and death.	Metastasis.	TRANSPLANTATION.				
						Number of mice inoculated.	Number of mice which survived.	Number of tumours.	Generation attained.	Duration and result of propagation.
7/0	Hæm. adeno-carc.	—	—	Killed.	None.	133	12	1	3 A	Died out at 4th Gener. 4 months.
9/0	Hæm. alveolar carc.	—	—	Killed.	None.	125	60	2	2 A	1 month.
18/0	Angioma or angio-sarc.	—	—	Killed.	Adenoma of lung.	—	83	0	—	—
19/0	Hæm. alveolar carc.	3	3	92 days.	Lungs.	85 81 81	75 50 70	15 1 0	4 B	4 months.
25/0	Hæm. alveolar and cystic carc.	3	3	37 days.	Lungs and lymph gland.	116 98 58	102 68 29	0 1 0	—	Died out.
27/0	Adeno-carc.	1	1	16 days.	None.	68	26	5	24 A	24 months.
28/0	Squamous-celled carc.	1	1	19 days.	Lymph gland.	Septic.				
30/0	Hæm. and cystic adeno-carc.	—	—	Killed.	None.	72	72	1	—	—
32/0	Squamous-celled carc.	1	1	39 days.	Lung.	201	156	1 (4)	27 B	22 months.
33/0	Hæm. alveolar carc.	1	1	53 days.	Lungs.	126 124	97 65	3 0	2 B	2 months.
34/0	Hæm. adeno-carc., cystic in places; in others alveolar trabeculæ.	1	1	14 days.	—	140 15	98 10	0 0	—	—
37/0	Adeno-carc., alveolar in parts.	1	1	57 days.	None.	162 41	69 38	8 0	23 A	—
38/0	Hæm. adeno carc. cystic and alveolar.	—	—	Killed.	None.	102	86	20	—	All absorbed.
39/0	Hæm. adeno- and alveolar carc.	—	—	Killed.	None.	19	17	3	13 B	16 months.
40/0	Hæm. alveolar carc.	1	—	5 days.	None.	60	49	0	—	—

No. of Tumour.	Histology.	Number of operations.	Number of recurrences.	Interval between first operation and death.	Metastasis.	TRANSPLANTATION. Number of mice inoculated.	Number of mice which survived.	Number of tumours.	Generation attained.	Duration and result of propagation.
41/0	Alveolar carc., lumina in alveoli.	1	0	25 days.	—	61	48	0	—	—
43/0	Adeno-carc.	—	—	Killed.	—	36	29	0	—	—
45/0	Alveolar carc., dilated capillaries.	1	1	20 days.	—	64 62	49 49	2 0	2 B	1 month.
46/0	Hæm. and œdematous alveolar carc.	2	2	25 days.	Lungs & lymph gland (aortic).	51 43 49	10 36 40	0 2 0	18 A	16 months.
47/0	Hæm. adeno-carc., in places alveolar.	2	2	70 days.	Lungs. Sclerosed nodules.	25 55	24 46	0 3	9 A	13 months.
48/0	Hæm. and cystic adeno-carc.	1	1	31 days.	Adenoma of lung.	31 60	1 19	0 1	—	Absorbed.
49/0	Adeno-carc., hæm. & sclerotic areas.	—	—	Killed.	None.	24	19	1	3 A	4 months.
50/0	Hæm. alveolar carc.	2	2	123 days.	Miliary emboli in pulmonary artery.	81 77 118	67 53 87	22 5 5	11 E	14 months.
51/0	Adeno-carc., alveolar locally.	—	—	Killed.	—	146	119	2	3 A	Died out.
52/0	Alveolar carc., lumina in alveoli. Hæmorrhages & broad bands of cellular connective tissue.	1	1	32 days.	—	35 60	27 30	0 1	—	Died out.
53/0	Hæm. and cystic adeno-carc.	2	2	194 days.	Lungs.	17	11	0	—	—
54/0	Hæm. adeno-carc....	1	1	—	—	51	7	0	—	—
55/0	Hæm. and cystic alveolar carc.	1	0	—	—	40	37	0	—	—
56/0	Adeno-carc., in places alveolar with lumina.	1	—	5 days.	—	28	27	1	—	Absorbed.
58/0	Alveolar carc. with lumina.	1	0	9 days.	Emboli in pulmonary artery & infiltrating.	100	40	5	6 C	11 months.

No. of Tumour.	Histology.	Number of operations.	Number of recurrence.	Interval between first operation and death.	Metastasis.	TRANSPLANTATION.				
						Number of mice inoculated.	Number of mice which survived.	Number of tumours.	Generation attained.	Duration and result of propagation.
61/0	Hæm. alveolar carc. Metast.	1	0	135 days.	Lung.	112	101	1	—	Disappeared.
62/0	Hæm. adeno-carc....	0	0	Killed.	—	A 20 B 22	18 9	5 0	4 A	9 months.
63/0	Hæm. adeno-carc. Metast.	1	0	10 days.	Lung, large.	136 62	118 53	20 2	9 B	10 months.
65/0	Adeno-carc. with alveolar islands.	0	0	Killed.	Emboli in pulmonary artery.	A 128 B 35	121 10	14 3	9 A	10 months.
66/0	Hæm. alveolar carc. with lumina.	1	1	54 days.	Lungs, large.	150	147	31	—	—
67/0	.....	0	—	Died.	None.	45	8	0	—	—
71/0	Hæm. and cystic adeno-carc.	1 1	1 1	86 days.	None.	80 30 50	59 29 22	1 0 0	2 A	2½ months.
72/0	Adeno-carc.	1	0	34 days.	Sclerosed embolus in pulmonary artery.	A 129 B 45	120 31	11 0	4 B	11 months.
74/0	Alveolar carc. with lumina.	1	0	46 days.	None.	61	46	0	—	—
75/0	Alveolar carc. with lumina.	1	0	29 days.	None.	80	33	0	—	—
76/0	Hæm. adeno-carc.	1	0	220 days.	None (N.E.).	59	59	5	2 A	5 months.
77/0	Hæm. trabecular alveolar carc. with lumina.	1	0	80 days.	Lungs.	59	50	1	2 A	5 months.
78/0	Do. do.	1	0	67 days.	—	45	37	0	—	—
79/0	Hæm. and cystic alveolar carc.	—	—	Killed.	None (N.E.).	81	61	5	3 A	3 months.
80/0	Adeno-carc., papillary cyst-formation and hæmorrhages.	—	—	Killed.	None (N.E.).	60	41	2	4 A	7 months.
83/0	Adeno-carc.	1	0	15 days.	Lungs, large.	222	197	57	8 A	3 months.

No. of Tumour.	Histology.	Number of operations.	Number of recurrences.	Interval between first operation and death.	Metastasis.	TRANSPLANTATION.				
						Number of mice inoculated.	Number of mice which survived.	Number of tumours.	Generation attained.	Duration and result of propagation.
85/0	Hæm. adeno-carc.	1	0	46 days.	Lungs, large.	110	82	10	5 C	7 months.
88/0	Hæm adeno-carc. Metast.	1	0	17 days.	Large, in Lungs.	87 60	78 53	9 3	2 B	Died out in 2 months.
89/0	Hæm. adeno-carc.	1	0	51 days.	None.	80	65	8	2 C	3 months.
91/0	Hæm. and cystic alveolar carc.	1	0	64 days.	Adenoma of lung.	18	16	1	4 C	7 months.
92/0	Spindle-celled sarc. at P.M. Recurrent tumour chondro-osteoid sarc.	1	1	112 days.	None.	95 324	77 262	0 49	5 A	4 months.
93/0	Hæm. alveolar carc. with lumina.	1 1	1 0	74 days.	Many small nodules in lungs.	52 A 39 *35	50 17 15	4 0 0	4 A	7 months.
94/0	Hæm. adeno-carc., alveolar trabeculæ in places.	1	0	28 days.	None.	125	90	13	6 C	7 months.
95/0	Hæm. alveolar carc.	1	0	11 days.	Lungs.	73	63	1	—	Died out.
96/0	Hæm. and œdematous alveolar carc. A.	...	...	—	—	18	17	1		
	B.	1	0	85 days.	None (N.E.).	26	20	3	3 A	7 months.
97/0	Alveolar carc.	1	1	85 days.	Lungs, large.	35	24	1	—	Absorbed spontaneously.
98/0	Hæm. adeno-carc., alveolar trabeculæ in hæm. areas.	1	0	26 days.	Lungs, large.	100	79	2	2 A	Died out.
99/0	Hæm. adeno-carc. with secretion in acini. Alveolar locally.	—	—	1 day.	—	50	36	1	—	Absorbed spontaneously.
100/0	Hæm. adeno- and alveolar carc.	1	1	54 days.	Lungs. Small nodules.	84 63	57 36	2 11	5 B	4 months.
102/0	Hæm. and cystic adeno-carc.	—	—	Killed.	None (N.E.).	34	22	4	—	Died out.

No. of Tumour.	Histology.	Number of operations.	Number of recurrences.	Interval between first operation and death.	Metastasis.	TRANSPLANTATION.				
						Number of mice inoculated.	Number of mice which survived.	Number of tumours.	Generation attained.	Duration and result of propagation.
103/0	Adeno-carc. with alveolar islands and duct-like structures with keratinised nodules.	—	—	Killed.	None (N.E.).	80	41	0	—	—
104/0	Cystic and hæm. adeno-carc. Secretion in acini.	1	0	27 days.	None (N.E.).	50	43	5	2 C	4 months.
105/0	Adeno-carc. Papilliferous cyst.	2	2	118 days.	Lungs, small nodule.	126	18	0	—	—
106/0	Hæm. adeno-carc. Much secretion and cystic dilatation.	1	0	21 days.	Lungs, large.	47	35	0	—	—
107/0	(A.) Hæm. & cystic adeno-carc.	1	0	85 days.	Lungs, large.	70	57	18	3 D	3 months. Died out.
	(B.) Adeno- & alveolar carc.	1	0	.....	—	157	123	12	3 B	3 months.
110/0	Hæm. and cystic adeno-carc.	—	—	Killed.	Lungs, large.	85	40	6	—	Absorbed spontaneously.
112/0	Hæm. alveolar carc.	1	0	71 days.	None (N.E.).	20	16	2	—	Died out.
113/0	Slightly hæm. alveolar carc. with lumina & ? keratinisation.	—	—	Killed.	Lungs, large.	40	37	3	Still	living.
114/0	Alveolar carc. with lumina in places dilated with secretion.	2	2	89 days.	Liver.	75	56	14	3 B	4 months.
116/0	Cystic hæm. adeno-carc.	—	—	Killed.	None (N.E.).	20	14	0	—	—
117/0	Hæm. & œdematous alveolar carc.	1	0	19 days.	None (N.E.).	23	7	2	2 A	2 months.
119/0	Inguinal lymphadenoma. Axilla 11.	2	0	52 days.	Mediastinal glands.	22	20	0		
121/0	Hæm. and cystic adeno-carc.	1	1	66 days.	Many small nodules in glands.	40	34	2		

No. of Tumour.	Histology.	Number of operations.	Number of recurrences.	Interval between first operation and death.	Metastasis.	TRANSPLANTATION.				
						Number of mice inoculated.	Number of mice which survived.	Number of tumours.	Generation attained.	Duration and result of propagation.
126/0	Hæm. and cystic adeno-carc. (? keratinisation). 2nd operation.	2	0	Killed. 103 days.	Small, in lungs.	32 40	22 30	0 0		
127/0	Hæm. and cystic adeno-carc.	1	0	46 days.	Large, lungs.	44	41	0		
129/0	Alveolar carc. (sarcomatous stroma).	0	—	Killed.	None (N.E.).	115	81	10		
135/0	(A.) Hæm. & cystic adeno-carc.	—	—	Killed.	None (N.E.).	40	34	0		
	(B.) Liver adenoma.	—	—	—	—	60	50	0		
136/0	Hæm. and cystic adeno-carc.	1	1	43 days.	Large, lungs.	40	27	1		
139/3	Alveolar carc.	1	0	11 days.	None (N.E.).	50	40	0		
141/0	Hæm. adeno-carc..	0	—	Killed.	Large, lungs. ? Inguinal gland.					
142/0	Cystic adeno-carc..	1	1	Alive.	—	Not transplanted.				
	Hæm. adeno-carc..									
143/0	Hæm. adeno-carc..	1	—	Alive.	—	40	38	1		
144/0	Hæm. adeno-carc..	1	0	21 days.	None (N.E.).	30	11	0		
146/0	Adeno-carc. (rich stroma).	1	1	41 days.	Large, lungs.	130 40	105 35	32 18		
	Ulcer of stomach.									
148/0	.....	0	—	Killed.	None (N.E.).	See coloured Plate.				
152/0	Alveolar carc. with lumina.	0	—	Killed.	None (N.E.).	50				
155/0	Fissure - forming adeno-carc.	1	1	Alive.	—	82				

Metastases of Spontaneous Tumours.

The formation of metastases is one of the most important diagnostic characters of malignant new growths, of equal importance with local infiltrative growth, of which it is merely one consequence, and therefore the occurrence of metastases in distant organs is of the greatest importance in any discussion of the nature and pathological position of the new growths, spontaneous or transplanted, which form the material of experimental cancer research. Hanau recorded the presence of metastases in the inguinal lymph-glands of a female rat with primary epithelioma of the vulva, which he transplanted successfully, and dissemination over the peritoneum in the transplanted animals. Jensen saw no metastases in the lungs, or elsewhere, in the mouse in which his now well-known tumour occurred. Borrel recorded pulmonary metastases, usually in the form of emboli of the pulmonary artery, in a large proportion of the spontaneously affected mice referred to in his memoir of 1903. Borrel and Haaland recorded lymphatic metastases in cases of squamous-celled carcinoma of the lower jaw in the mouse, and Haaland gave a full description of the pathological anatomy of the condition, with figures of the primary growth and metastases in the lymph-glands. They showed that the metastases of transplanted carcinomata originated in minute emboli of the terminal branches of the pulmonary artery and grew backwards along the artery. Extension may take place from the primary tumour along the veins and ultimately may reach the heart. Lewin and Michaelis recorded metastases in rats inoculated with their transplantable carcinoma, and Flexner and Jobling also recorded metastases in lungs, lymph-glands, and other organs from a transplantable tumour of the vesiculæ seminales of the rat, described as a mixed cell sarcoma which grows infiltratively.

In the following remarks only those cases will be regarded as truly metastatic from primary spontaneous tumours in which the secondary nodules lie either in the lungs or in lymph glands. In the case of the mammary tumours of the mouse, more than one tumour is frequently found in the mammary region of one and the same animal. The general tendency of those who have studied the subject is to regard these cases as instances of multiple tumours, and in many cases there is a sufficient difference in histological structure to make this certain. In other cases, however, this is not so. When animals are kept under observation for relatively long periods, new tumours occasionally develop in parts of the mammary apparatus remote from that occupied

by the tumour first observed, and when the second tumour presents the same histology as the first, and still more when a third, fourth, or even fifth, tumour appears, as in mouse $\frac{77\text{-}78}{0}$ (fig. 44), it is almost impossible to decide the point definitely. Multiplicity certainly occurs (*cf.* e. g. $\frac{61}{0}$, in which a "cancroid" developed after removal of the primary tumour, see p. 102, fig. 45), but when the various tumours agree in histological structure it is almost entirely a matter of individual taste whether those appearing later or of lesser size be regarded as metastatic or not.

The accompanying table (pp. 91–96) shows the frequency with which metastases were found in 68 mice suffering from spontaneous tumours. Metastases were present in the lungs in 27 cases, or rather less than 50 per cent. It is to be noted, however, that the lungs of all these animals have not been completely investigated. In some (indicated by N.E.) naked-eye examination only was made. The lungs of 16 mice in which no metastases could be discerned naked-eye, were examined in serial sections with the result that in eight microscopic metastases were present, while in the remaining eight (50 per cent.) no growth could be found. In three cases metastases were discovered in lymph glands, one of the cases being the squamous-cell carcinoma of the cervical region $\left(\frac{28}{0}\right)$ already referred to (fig. 18). The other two cases of lymph-gland metastasis were found on examining serial sections through entire mice. In the first of these $\left(\frac{25}{0}\right)$ a small nodule (figs. 35 & 37) was discovered in a superficial lymph-gland on the opposite side of the body, the primary growth being a hæmorrhagic and cystic adeno-carcinoma of the right axillary mamma with enormous metastases in the lungs. The other lymphatic metastasis occurred in an aortic gland in mouse $\frac{46}{0}$ and is shown in figs. 36 & 38. It is unnecessary to add a more detailed description to that appended in the figures. Up to the present only five mice suffering from spontaneous tumours have been cut in serial sections, and lymphatic metastasis has been observed in two of them. Whether this represents a higher or lower proportion than would be obtained by a similar investigation of a larger number is of little importance. The drudgery involved in cutting and examining such a number of large sections would be poorly recompensed by a statistical result of this kind. The important fact is that lymphatic



J. R. Ford, del.

FIG. 35.—Mouse. Tumour $\frac{25}{0}$. Spontaneous hæmorrhagic tumour: metastasis in lymph-gland in neck. *Cf.* fig. 37, p. 99. $\times \frac{90}{1}$.

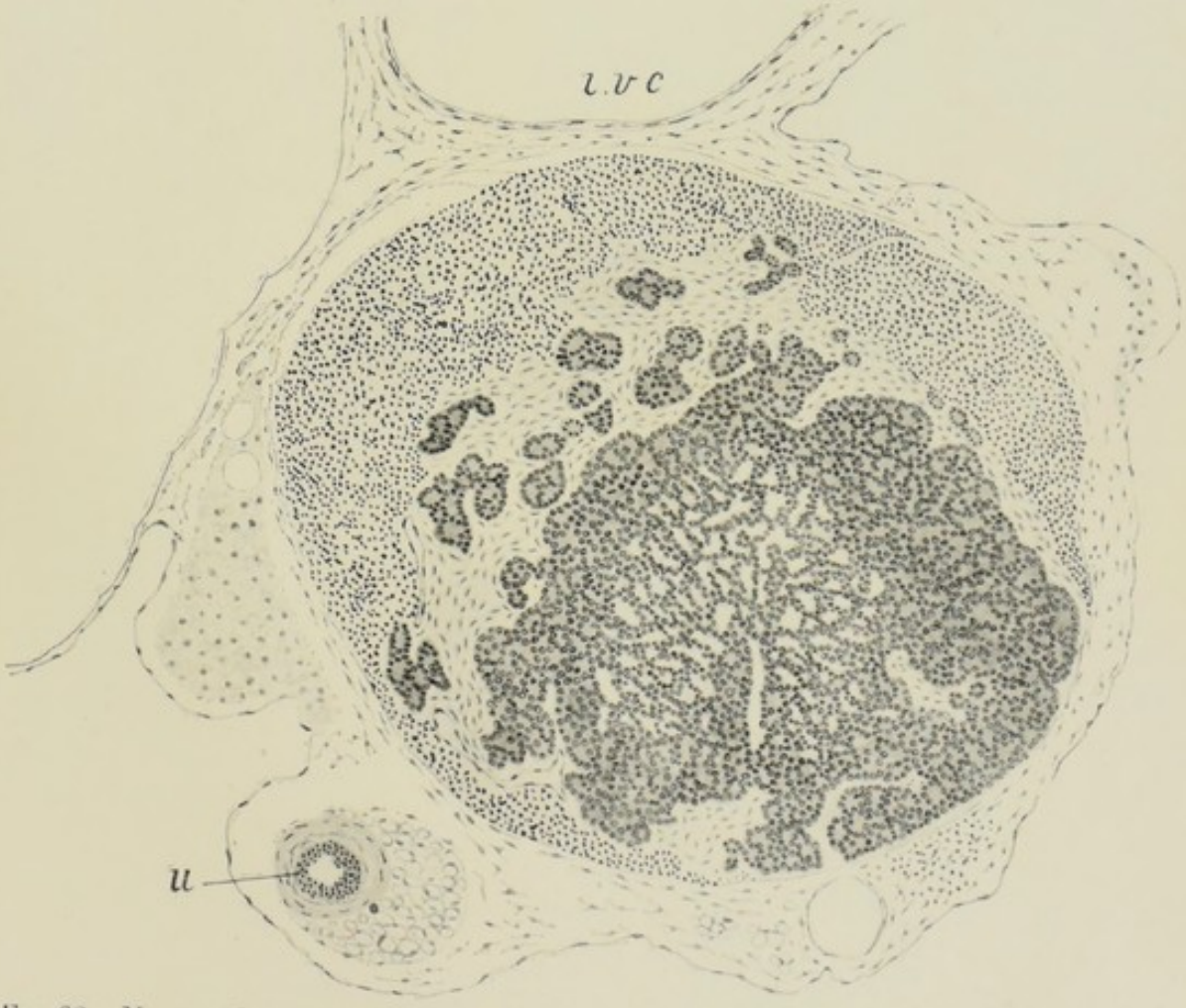


FIG. 36.—Mouse. Alveolar carcinoma of mamma: metastasis in aortic lymph-gland. *i. v. c.* inferior vena cava, *u.* ureter. The growth has expanded the capsule of the gland and the lymphoid tissue is reduced to a crescent. *Cf.* fig. 38, p. 99. $\times \frac{40}{1}$.

metastases do undoubtedly occur, so that in this respect a fundamental difference does not obtain between mouse-tumours and those of other animals and man.

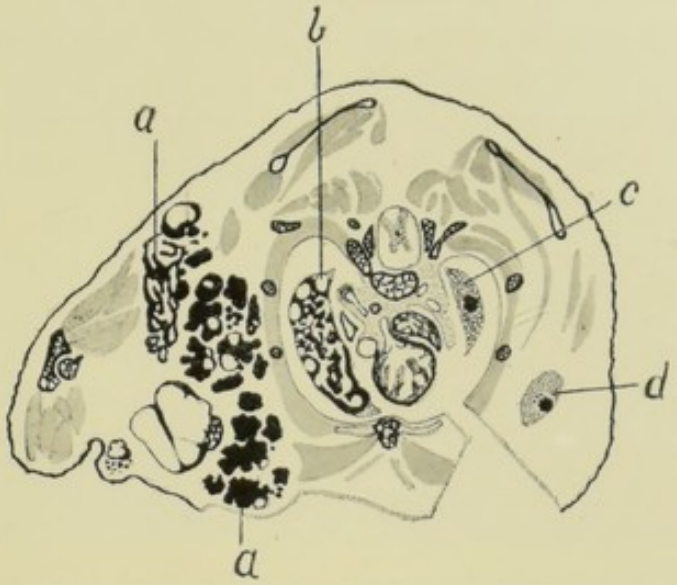


FIG. 37.—Transverse section of a mouse with spontaneous carcinoma mammæ $\left(\frac{25}{0}\right)$. *a*. Primary growth in right axilla. *b*. Right lung replaced by growths. *c*. Small metastasis in upper lobe of left lung. *d*. Lymphatic gland of opposite side with small metastasis. See fig. 35. $\times\frac{3}{1}$.

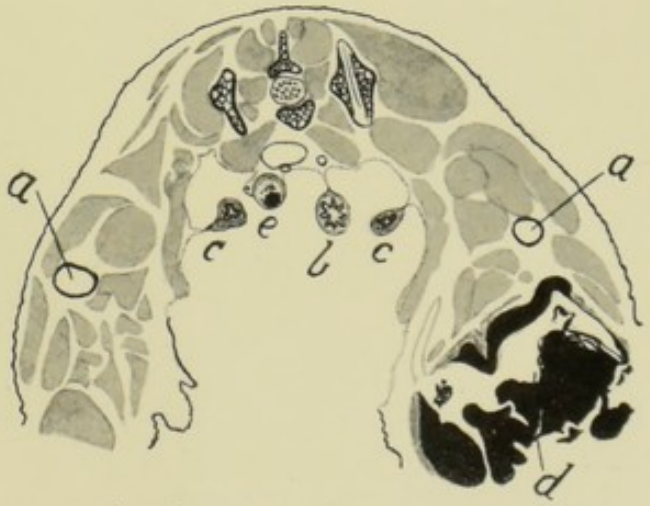
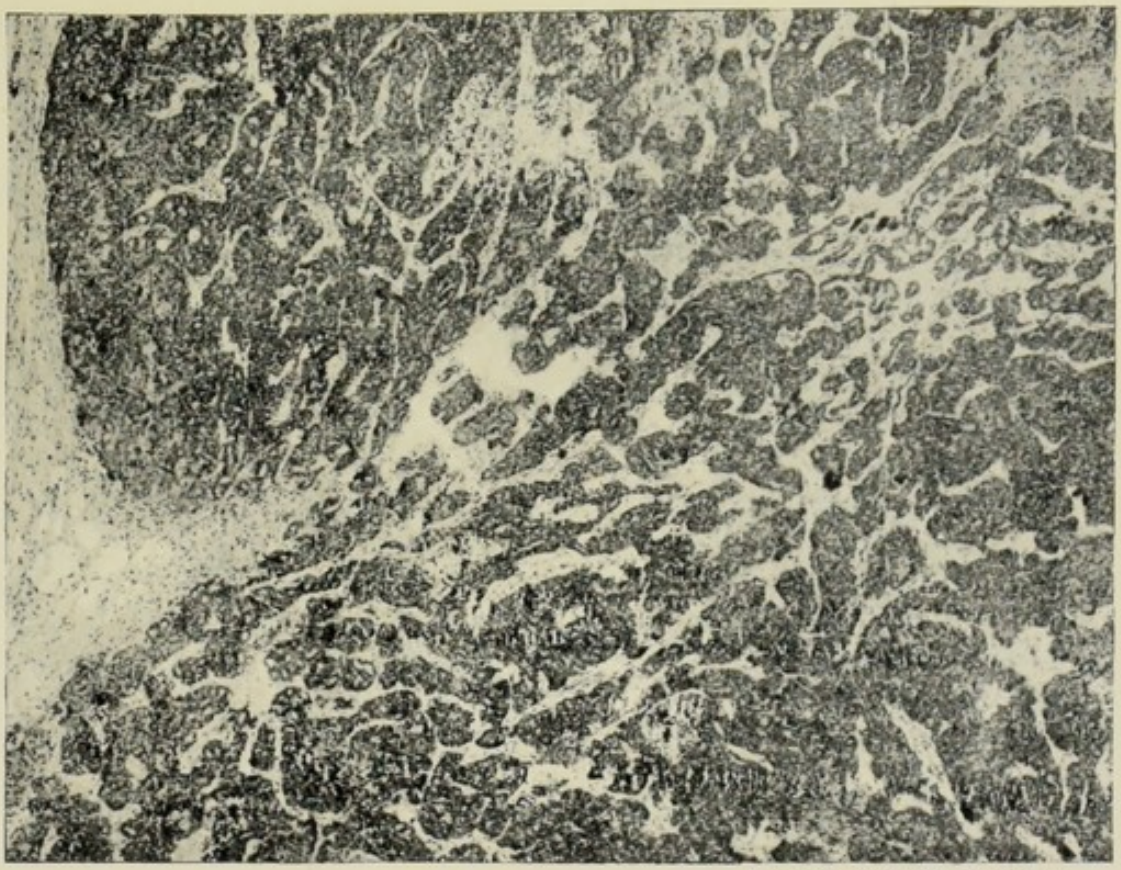


FIG. 38.—Transverse section of a mouse with spontaneous carcinoma mammæ $\left(\frac{46}{0}\right)$ of the inguinal region. *a a*. Femur. *b*. Rectum. *c c*. Uterus. *d*. Extension of primary growth into muscles of thigh; note thickness as compared with right limb. *e*. Aortic lymphatic gland with metastasis. See fig. 36. $\times\frac{3}{1}$

In one case $\left(\frac{114}{0}\right)$ a large metastasis was found in the liver, without involvement of the lungs (figs. 39 & 40). The metastases in the lungs apparently arise in the great majority of cases as the result of the arrest of carcinomatous emboli in the terminal branches of the pulmonary artery. Handley has attempted to show recently that in carcinoma of the mamma in the human subject, growths may occur in the lungs as a result of lymphatic extension or "permeation." However this may be in man, there can be no doubt that in the mouse, extension is mainly by the blood stream. Thus in the case of mouse $\frac{65}{0}$ the nodules in the lungs were all intra-vascular (fig. 42) and had in no instance broken through the internal elastic lamina. Again, in mouse $\frac{50}{0}$ the metastases were mere miliary emboli of the terminal branches of the pulmonary artery (fig. 41). The endothelium and internal elastic lamina seem to present a serious obstacle to the free growth of the cancer cell, and the emboli therefore frequently grow to a considerable size within the vessel. In this way long sausage-shaped masses are formed filling up a considerable part of the pulmonary arterial system, as already described by Borrel, Haaland, Tyzzer, Bashford, Cramer, and Murray. The terminal condition which results takes one of two forms. Healing occurs when the nodules fail to become vascularised, are encapsuled by the proliferation of the endothelium and ultimately degenerate and are replaced by sclerotic connective tissue. Further extension is initiated when capilliaries bud in from the intima of the vessel and the growth being now better nourished, increases rapidly in size, bursts through the walls of the artery and invades the lung tissue. Finally the respiratory area is so much encroached upon that the animal succumbs. Both processes may proceed side by side in the same animal, in the same lung, or even in the same vessel (fig. 43). In this way it is proved that the plasma of the blood does not destroy the cancer cells directly as has been assumed by many. The determining factor in these cases is the cancer cell itself. It is probable that the cells detached from the primary growth at different times vary in their powers of establishing themselves in new sites. As will be shown later, a parallel behaviour is exhibited, when the cells of a primary growth are transferred to new animals at different times by transplantation.

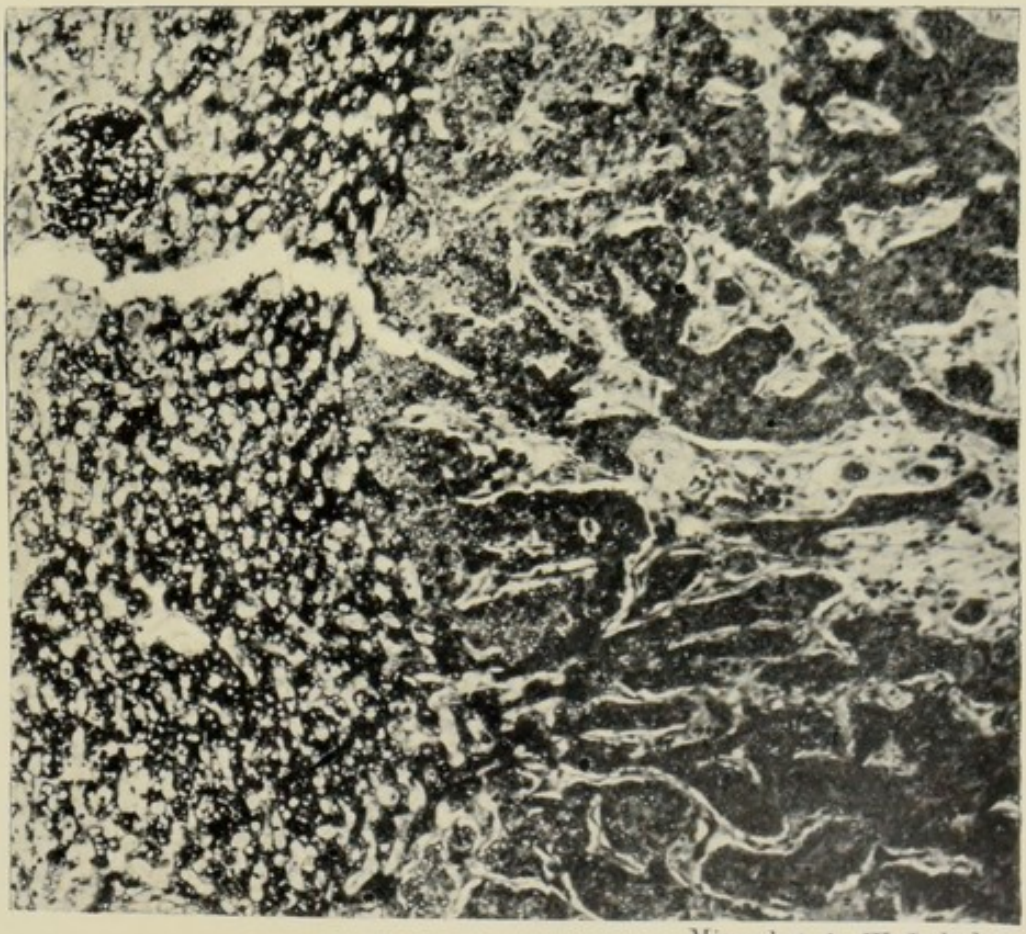
An analogous phenomenon is seen when a tumour, spontaneous or transplanted, after infiltrating the chest or abdominal wall, meets the

[*To face* p. 100.



Microphoto by W. Imboden.

FIG. 39.—Mouse. Alveolar carcinoma of mamma, tumour $\frac{114}{0}$: for comparison with metastasis in liver, fig. 40. $\times \frac{70}{1}$.



Microphoto by W. Imboden.

FIG. 40.—Mouse. Alveolar carcinoma of mamma, tumour $\frac{114}{0}$: metastasis in liver with the same structure as the primary tumour. $\times \frac{150}{1}$.



FIG. 41.—Mouse. Hæmorrhagic alveolar carcinoma of mamma, tumour $\frac{50}{0}$: capillary embol[i] distending the vessel-wall and projecting into the air-cells. $\times \frac{100}{1}$.

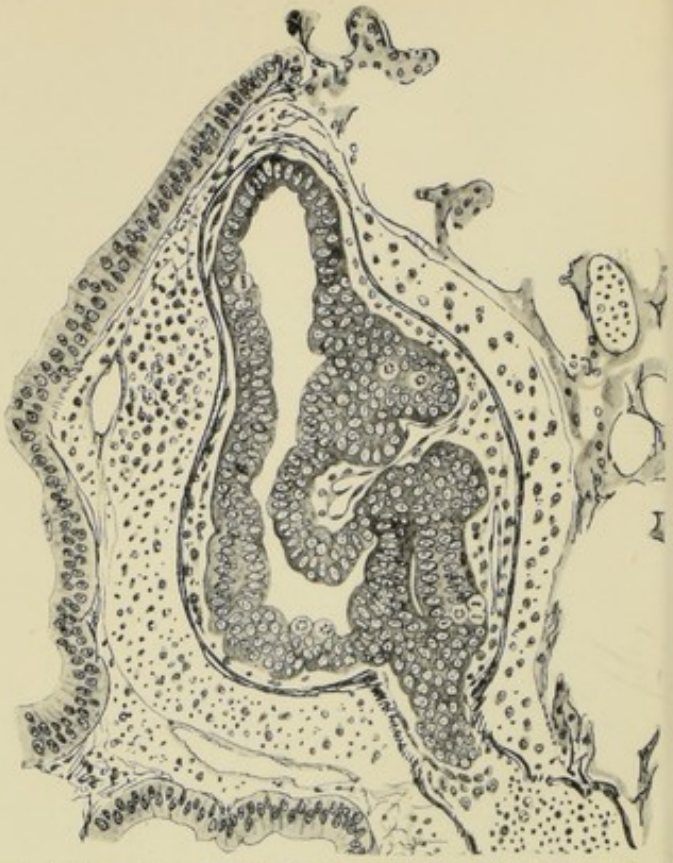


Fig. 42.—Mouse. Adeno-carcinoma of mamma, tumour $\frac{40}{0}$: embolic metastasis in pulmonary artery; the embolus which is entirely within the internal elastic lamina, has become vascularised by capillaries from the intima of the vessel. The artery is completely occluded and the distal part is filled with leucocytes and lymphocytes. $\times \frac{100}{1}$.



FIG. 43.—Mouse. Tumour $\frac{30}{0}$. Metastasis in lung. (*b*) Healed nodule enclosed by sclerosed hyaline connective tissue. (*a*) Healthy embolus breaking through vessel wall. $\times \frac{330}{1}$. (Reproduced from Dr. Gierke's paper.)

endothelial layer of the pleura or peritoneum. The growth frequently projects as a rounded mass covered by the endothelium, although in time the latter usually ruptures and the tumour "fungates" into the serous cavity. The converse process, strictly comparable with the behaviour of intra-vascular emboli, occurs when intraperitoneal transplantation is attempted. The cancer cells are shut off by the endothelium from the connective tissue, the reaction of which, as has been proved by Russell (*vide post*), is essential for successful transplantation. In this way is explained, the long time for which metastases remain intravascular, and incidentally the similarity of the intraperitoneal "rice bodies" of Ehrlich's chondroma with the unvascularised nodules of the same tumour produced in partially immunised mice.

Clinical Course and Results of Operation.

The course of these spontaneous tumours (see figs. 44 & 45) is generally progressive. Although spontaneous absorption is not seldom met with as a localised process in small areas of some tumours (*cf.* fig. 43), the complete disappearance of a spontaneous tumour is a very rare event. On the other hand temporary arrest of growth is frequently observed, and in some cases an actual diminution in size is seen. The tumour in mouse $\frac{57}{0}$ (fig. 44) shows a quite exceptional course in this respect. This mouse was kept under observation for nine weeks without the tumour undergoing any but the most trivial variations in size. As a rule, however, a progressive increase in size takes place. The rate of growth varies considerably. The most rapid increases are met with in the hæmorrhagic adeno-carcinomata, in which sudden increases in volume, although sometimes due to extravasation of blood, are occasionally the result of an almost explosive proliferation of the parenchyma (*cf.* tumour $\frac{100}{0}$ (fig. 45).

For the last two years it has been our custom to obtain the material for transplantation of spontaneous tumours by operation rather than by killing the animals. In this way it has been possible to breed from cancerous mice, stocks of known parentage, to study the biological characters of animals suffering from spontaneous cancer and to note the relative frequency with which these tumours recur.

The technique of these operations merits passing mention. The animal having first been anæsthetised with ether, the skin over the tumour to be removed is cleansed with a pledget of cotton saturated

with ether, after the hair has been clipped short. The field of operation is then moistened with warm sterile saline and an incision made with sharp scissors enclosing so much skin as is adherent to the tumour. The skin is then retracted and the tumour is separated from its surroundings. Wherever large vessels enter or leave the tumour it is advisable to ligature them before they are divided, hæmorrhage being the most serious danger to be apprehended. For smaller vessels and venous oozing from cut surfaces small pressure forceps may be used. When the tumour has been isolated in this manner it is removed, and the wound is closed by interrupted cat-gut sutures. No chemical antiseptics are used, as active solutions are too toxic to the tissues and animals. Healing is almost invariably by first intention, and the mouse itself removes the free ends of the sutures in the course of a week or ten days. The graphic records which accompany this paper are made in the following manner. When an animal with a spontaneous tumour is sent in to the laboratory it receives a number, written as the numerator of a fraction, the denominator of which is zero. This convention, which in the sequel is used indifferently for the mouse or the tumour according to the context, is chosen in order to form the commencement of the series of similar fractions, designating the successive generations of propagation; *e. g.*, spontaneous tumour $\frac{83}{0}$ gave a series of tumours all labelled $\frac{83}{1}$ —the first generation—distinguished from each other by the name of the batch in the succeeding generation to which they give rise. The second generation is therefore labelled $\frac{83}{2}$, and individual series are distinguished by a letter $\frac{83}{2A}$, $\frac{83}{2B}$, $\frac{83}{2C}$; the third generation obtained from these by transplantation is composed of series labelled $\frac{83}{3A}$, $\frac{83}{3B}$, $\frac{83}{3C}$, etc. The number of the mouse with a spontaneous tumour is entered in a large folio protocoll on squared paper, and the site of the tumour recorded by sketching a sufficient area of the adjacent structures, as, for example, the fore or hind limbs, the side of the head, or the anus, vulva and tail, to indicate its position (figs. 44 & 45). In this sketch of the primary site the tumour is drawn as a silhouette of natural size, and the macroscopic appearance of the growth, particularly the presence of hæmorrhage or ulceration indicated by blue or black respectively, on a red ground. The animal is weighed and the weight entered under the same date. Once a week all mice with spontaneous tumours are again examined, the size of the tumours

[*To face* p. 102.

Course of 10 spontaneous mammary carcinomata of the mouse; operation, recurrence, metastasis and suitability of the mice for inoculation with squamous-celled carcinoma.

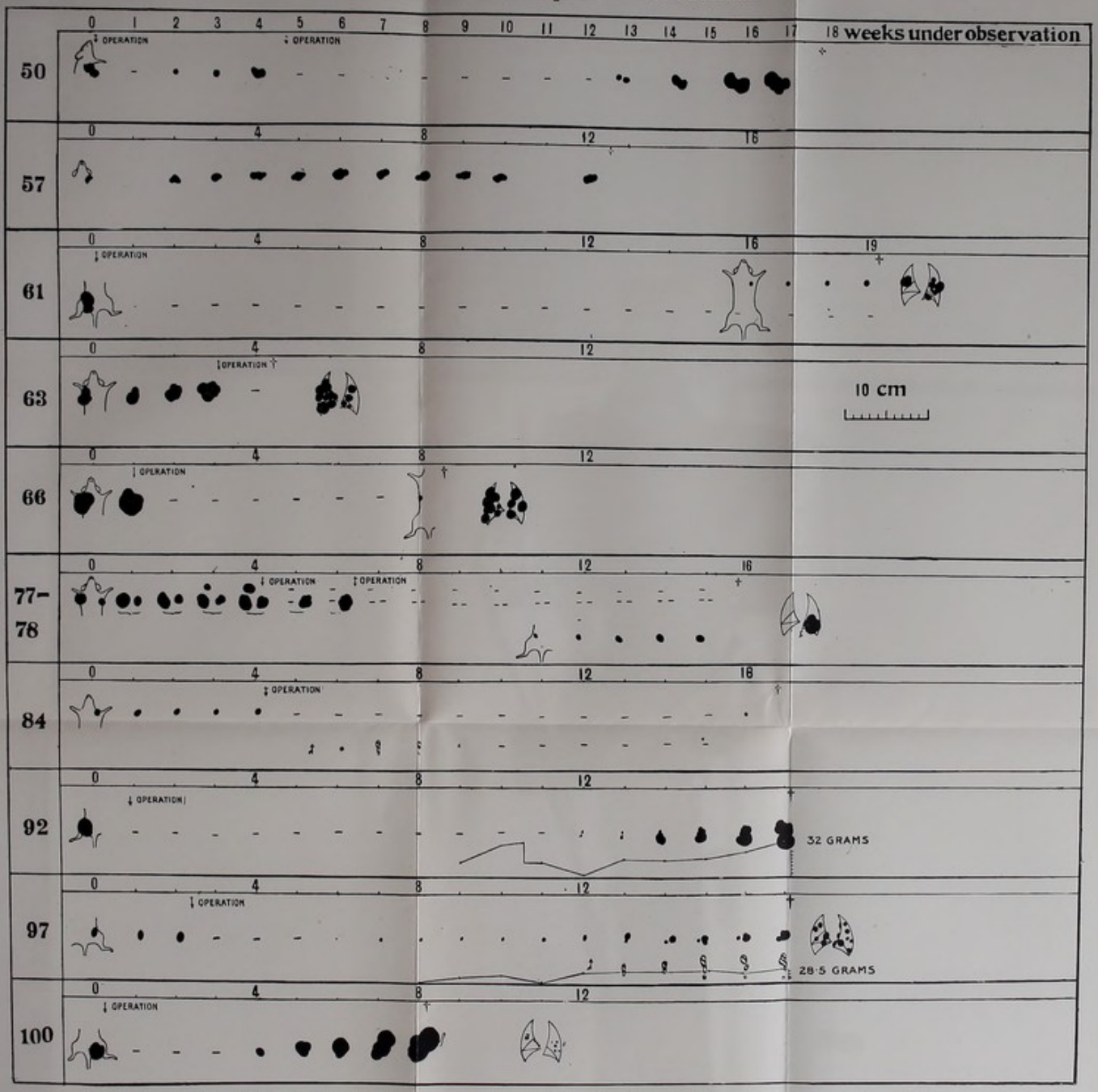


FIG. 44.—Graphic record of the clinical course, effects of operation, recurrence, and metastasis formation in ten mice with spontaneous mammary carcinomata (92 forms an exception, being a spindle-celled sarcoma). The figure shows the sizes of the tumours as silhouettes to scale, at intervals of one week. The time of operation is indicated thus, ‡. In two mice (Nos. 84 and 87) the result of inoculation with transplantable squamous-celled carcinoma (Exp. $\frac{32}{18A}$ *cf.* fig. 47) is also indicated. The time of inoculation with tumour 32 is shown by ↟. The inoculated tumours are shown in outlines and macroscopic metastasis in the lungs are represented diagrammatically in those cases in which they occurred, on an enlarged scale to the right of the record in the corresponding space.

The thin black line in the lower part of the records of tumours 92 and 97 from the 8th to the 16th weeks represent the variations in the weights of the animals, see also fig. 45. The sudden drop during the 11th week in the record of 92 marks the birth of a litter of 5 young. During the interval between operation and recurrence the position of the tumour is indicated by the minus sign.

Course of 13 spontaneous mammary carcinomata of the mouse; operation, recurrence, metastasis and suitability and response of the mice to inoculation with squamous-celled carcinoma.

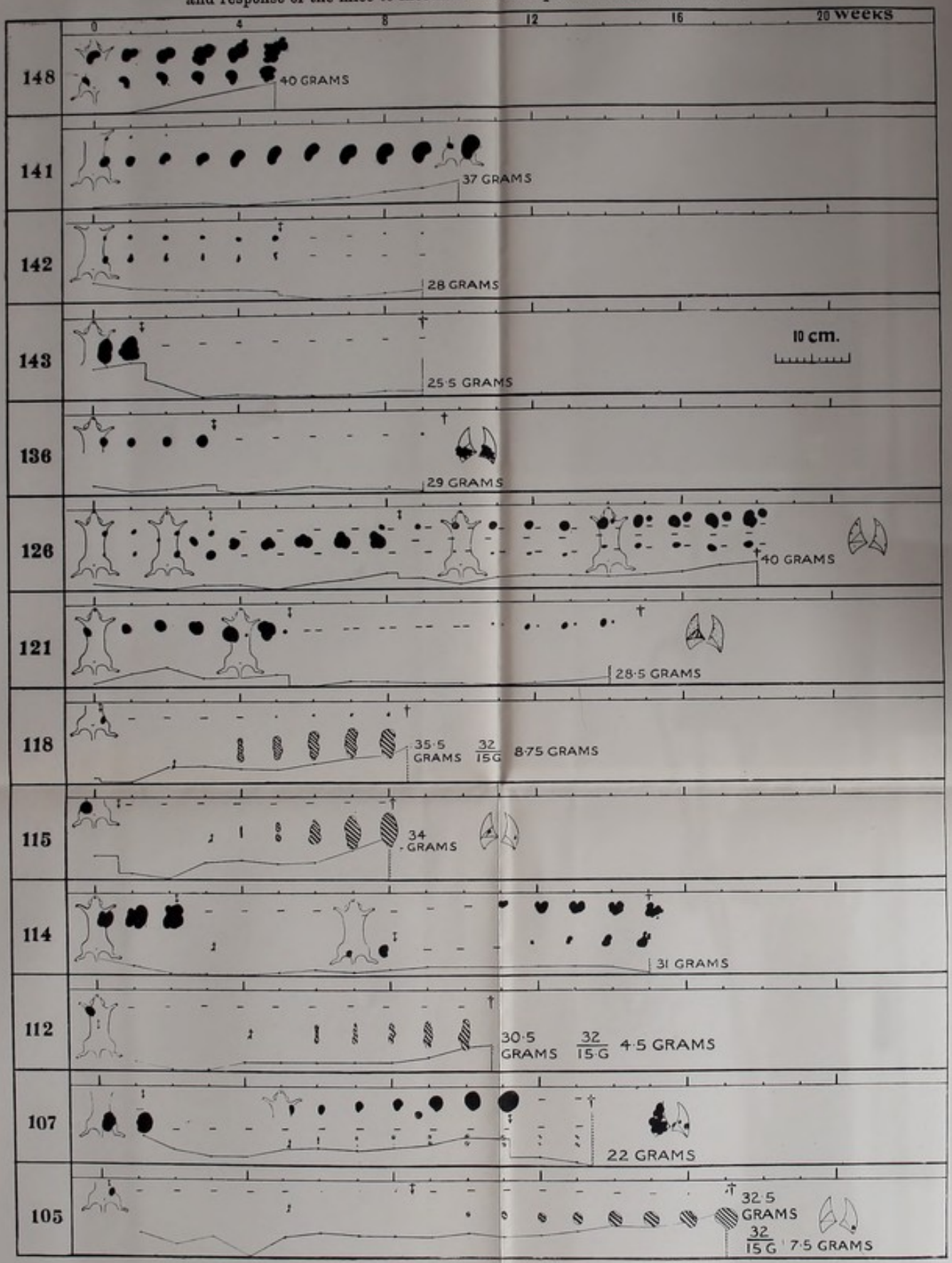


Chart of course of 13 spontaneous mammary carcinomata, showing variations in weight and results of operation.

↕ operation. ✝ death. ↑ inoculation with squamous-cell carcinoma (tumour 32).

Spontaneous tumours, black. Transplanted tumours (32), black and white striped.

FIG. 45.—Graphic record in continuation of fig. 41 of 13 spontaneous mammary carcinomata. (Signs and conventions as in fig. 41.) Tumours 148 and 141 show the progressive increase in size and weight in tumours not operated upon. Tumour 142 shows the constancy of the weight of the animal while the tumour is stationary (upper nodule) or diminishing in size (lower nodule). Tumour 143 shows the great loss in weight following the removal of a large tumour, and the stationary condition of the animal's weight when recurrence does not take place. Tumours 136 and 114 show the terminal fall in weight which precedes death. Mouse 126 shows multiple tumours, and the manner in which when one is removed the others grow (*cf.* also 114); the small growth in the right groin which appeared in the tenth week is probably a glandular metastasis (*cf.* 141). Mice 118 to 105 were inoculated with tumour 32 (squamous-celled carcinoma—exp. $\frac{32}{15G}$ (see fig. 47). The relative rates of growth of transplanted and recurrent spontaneous tumour in the same animal can be compared in mice 118, 107, and 105.

again recorded, and the weight entered as before. The dates of operation are also indicated on the charts at the exact date by means of an arrow or similar sign. The same method is adopted for transplanted tumours, as, *e. g.* in the reduced reproduction of the history of primary tumour $\frac{50}{0}$ and of the daughter-tumours derived from it at the first operation $\frac{50}{1}$ (fig. 46). Practically the same convention is used in the graphic records of the experiments $\frac{32}{16A}$ and $\frac{32}{15G}$ referred to below, representing the results of transplanting a squamous-cell carcinoma $\left(\frac{32}{0}\right)$ into normal mice, and into mice suffering from spontaneous tumours.

The weight of a mouse suffering from spontaneous cancer generally increases slowly with the continued growth of the tumour (figs. 44 & 45). It is only in the later stages when ulceration or hæmorrhage occur, that diminution in weight sets in, and this is always an indication of the gravest import. Seldom does an animal survive more than two weeks after loss in weight has commenced. This sequence forms a parallel in miniature to the progress of the disease in man, for it is now recognised that good general health is quite compatible with the presence of a malignant new growth. Emaciation is, in man, a terminal condition as in the mouse, and the differences between the progress of malignant disease in the two species are almost entirely due to differences in size and in duration of life.

When a large tumour is removed by operation from a mouse, the animal loses weight in excess of that represented by the tumour and blood loss. This is gradually regained in the succeeding week or two weeks, and then the weight remains constant. Minor variations of a half to one gram unless progressive from week to week are of no importance. Inter-current illnesses are always accompanied by loss of weight, sometimes considerable. Should recurrence take place, the animal at first increases in weight, as already noted. Loss in weight generally indicates an approaching lethal issue, either from too great nutritive demands by the recurrent tumour, or from respiratory embarrassment due to extensive pulmonary metastases (fig. 45, mice 114, 136, and 107). The frequency with which recurrence supervenes after apparently complete extirpation, is surprising when the encapsulated appearance of these tumours to the naked eye is borne in mind. Early operation, as in man, gives the best promise of lasting freedom from recurrence. Out of 48 animals operated

on, of which particulars are given in the table on pp. 91–96, recurrence took place in 23, two recurring three times, six twice, and the remainder once. The interval of survival between the first operation and death averaged three to six weeks in the later operations and in five cases was more than 100 days $\left(\frac{50}{0}, \frac{53\text{–}54\text{–}55}{0}, \frac{61}{0}, \frac{76}{0}, \frac{92}{0}\right)$. The number of recurrences and the length of survival, are not strictly comparable with similar data from the human subject, since the prolongation of life is of prime importance in man, but in the case of the mouse other considerations than the prolongation of life, have such importance that the animal may have to be sacrificed before death would have occurred.

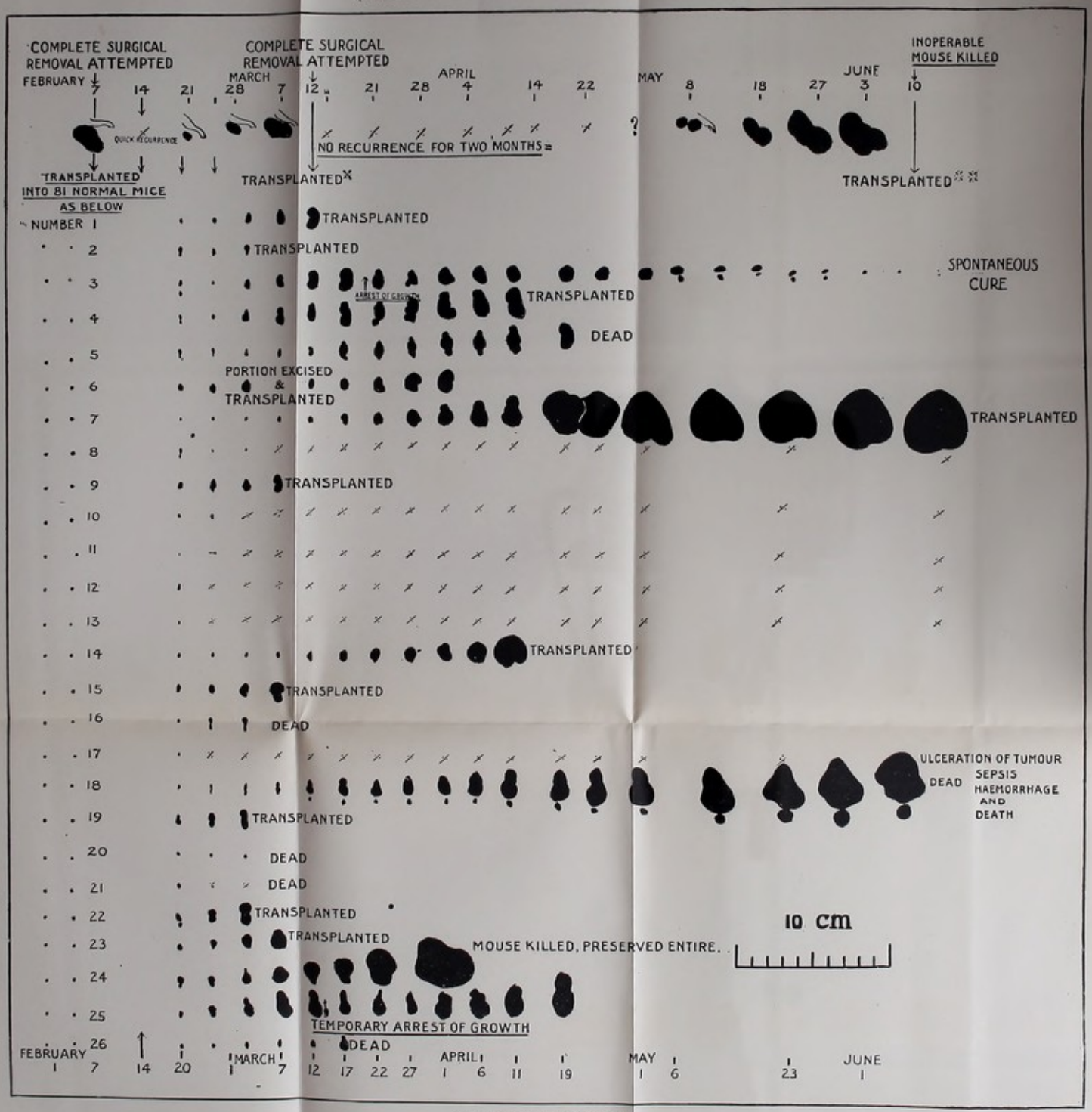
The results have a bearing also on the belief, very widely entertained, that operative interference with a malignant new growth unless complete, increases the malignancy, and particularly the liability to metastases. As is indicated in the tabular summary such an association of operation and dissemination is not constant, metastases of large size occur in animals which have never been operated upon $\left(\frac{113}{0}, \frac{110}{0}\right)$, and those in which several operations have been performed, may show only small nodules $\left(\frac{105}{0}, \frac{50}{0}, \frac{47}{0}\right)$. This question is also suitable for experimental study, and facts will be described in discussing the results of primary transplantation which throw light on it (see p. 105).

The Transplantability of Spontaneous Tumours.

The difficulties met with in effecting successful transplantation of spontaneous mouse tumours have impressed themselves on all who have worked at this subject. Thus Jensen states that he had transplanted several tumours without success, before 1903. Borrel also noted that the first attempts at artificial propagation generally gave very poor results. We made the same experience with Bashford at the commencement of our investigations, and Michaelis experienced the same difficulties. Ehrlich and Apolant attempted a theoretical explanation of the fact that in their experience only about 10 per cent. of all spontaneous tumours could be transplanted at all, and that the hæmorrhagic tumours had in their hands entirely failed to grow. They believed that a real fundamental difference was indicated by their results between the hæmorrhagic tumours on the one hand and the non-hæmorrhagic tumours on the other. They supposed that the tissues of

Spontaneous carcinoma of the mamma of the mouse and results of transplanting it into 81 normal mice.

(Tumours drawn as silhouettes of natural size and reduced to scale.)



Fifty mice did not develop tumours.

FIG. 46.—Chart of clinical course and results of operations on spontaneous tumours $\frac{50}{9}$; growth of 26 tumours produced by primary transplantation (exp. $\frac{10}{1}$). The primary tumour recurred two weeks after the first operation and was again removed after a further interval of two weeks. The mouse remained free from recurrence for two months, after which it reappeared and grew rapidly till death, 123 days after the first operation.

The course of transplanted tumours was either progressive (nos. 1, 4, 5, 6, 7, 9, 15, 15, 16?, 18, 19, 22, 23, 24, and 26) or a temporary proliferation was followed by complete absorption (nos. 8, 10, 11, 12, 13, 17, 21) or a combination of the two processes with a temporary arrest of growth (nos. 3 and 25) accompanied by diminution in size. This temporary arrest of growth of which several of the other tumours give indications coincides in point of time with the cessation of growth in the sister-tumours which disappeared completely and with the long freedom from recurrence after the second operation, just as the initial rapid proliferation of the transplanted tumours synchronises with the rapid recurrence of the primary tumour after the first operation. Mice nos. 6 and 24 are figured in colours and natural size on plate facing p. 115.

the animals in which such non-transplantable ("avirulent" in their nomenclature) tumours occurred, must possess a lower avidity for food-stuffs than do the tissues of normal animals. Following up this line of thought they held that the cells of transplantable spontaneous tumours had a higher avidity for food-stuffs than the cells of normal tissues. We shall discuss this hypothesis on a later page, and merely point out here that in the Second Scientific Report, we recorded with Bashford the successful transplantation of a hæmorrhagic carcinoma through three generations with retention of the hæmorrhagic character. The tabular summary on pp. 91–96 shows the incompleteness of the basis on which Ehrlich's speculations were founded. Of the first 71 tumours with which transplantation was attempted in accordance with a uniform plan, only 16 entirely failed to grow. This is to be compared with Ehrlich's result of 11 successes with 91 tumours. The highest percentage of success was obtained with a hæmorrhagic tumour $\frac{50}{0}$, viz. 30 per cent. (see fig. 46). The conclusion is inevitable that the majority of hæmorrhagic tumours can be propagated experimentally. The conditions of success are mainly technical, and Ehrlich's want of success is to be explained by the differences in technique, and principally by the fact that in the Frankfurt Institute much larger doses of tumour material (0·1 to 0·2 gr.) are introduced into each animal than in our procedure (0·01 to 0·02 gr.). The amount of mechanical injury to the parenchyma involved in this method is also of importance. As a consequence we have been able to inoculate a much larger number of animals, and it is to these two factors combined that one must attribute the discrepancy between the results. In addition the transplantability of most of our tumours has been tested more than once, the material being obtained by operation rather than by necropsy. In this way favourable phases of growth are more likely to be encountered, and in any case the number of animals inoculated has been greatly increased. Hence when the conditions of experiment are carefully attended to, practically every spontaneous tumour can be propagated. No histological distinctions can be discerned at present between the tumours which can be propagated and those which fail to grow.

The table contains data of another kind, which throw light on the belief referred to on a previous page, that operative interference increases the malignancy of a tumour. So far as such an alteration indicates an alteration in cell-character, the evidence of the experi-

ments does not support such a view. Spontaneous tumours have been transplanted at repeated intervals, whenever recurrence necessitated a second or a third operation. It was expected that in these circumstances the results would either become progressively better, or progressively worse. On the contrary the variation is not always in the same direction. Whereas, *e. g.*, with tumour $\frac{19}{0}$ the first transplantation gave 20 per cent., and the second 2 per cent., the third was entirely negative. The same superiority of the results at the first operation over those obtained with material from recurrent tumours is seen in the cases of tumours $\frac{33}{0}$, $\frac{37}{0}$, $\frac{45}{0}$, $\frac{50}{0}$, $\frac{62}{0}$, $\frac{71}{0}$, and $\frac{93}{0}$ The opposite result was obtained with tumours $\frac{25}{0}$, $\frac{46}{0}$, $\frac{47}{0}$, $\frac{48}{0}$, $\frac{52}{0}$, $\frac{92}{0}$, $\frac{96}{0}$, and $\frac{100}{0}$. Tumour 46 is a striking example because, while the transplantations made with material from the first operation and from the second recurrent tumour when the animal was killed were both negative, the transplantations from the second operation gave two tumours in 36 animals. It is as if a favourable phase of growth of short duration had been taken advantage of, for the tumour is capable of unlimited propagation. Lung metastases were transplanted in four cases, $\frac{33}{0}$, $\frac{61}{0}$, $\frac{63}{0}$, $\frac{88}{0}$. Success was obtained twice. These differences in the results of transplanting sporadic tumours at different times are similar to the varying results obtained with propagated tumours as has been pointed out by Bashford, Bowen, and Murray. The chart of Exp. $\frac{50}{1}$ on p. 105, shows that the behaviour of the spontaneous tumour in the animal primarily affected may be almost exactly paralleled by the behaviour of the same tumour growing in normal animals. The rapid recurrence of the primary growth coincides with the initial rapid proliferation of the transplanted tumours. The long period of quiescence after the second operation is reproduced in the transplantation series, where nearly every tumour shows a corresponding cessation of growth and some show diminution in size. The second recurrence and renewed rapid growth of the primary tumour, is practically synchronous with a similar behaviour of the transplanted growths.

The Relations of Malignant New Growths to Spontaneously Affected Animals.

The relation of new growths to the animal spontaneously affected has been the subject of a great deal of speculation. It is unfortunate that these speculations have not always distinguished clearly between the conditions of origin and the conditions of growth in a sufficiently definite manner. The necessity for distinguishing between the two sets of conditions is not diminished by the consideration that any conceptions formed to elucidate those under which malignant new growths arise, inevitably involve a discussion of the nature of the cancerous transformation. The criteria by which speculations on the nature of cancer must be judged are, however, mainly the phenomena elicited by a study of the growth of cancer. The association of the inception of cancer with senescence of the tissues has led to speculation along two distinct lines. Those who regard senescence as a physiological process, inevitable in the normal metazoan life cycle, are forced to assume in cancer a biological alteration conferring relatively or absolutely increased powers of assimilation on the elements of neoplasms. There are others who regard the association with senescence as more casual, and refer cancerous proliferation to a disturbance of intracellular equilibrium, the cells being unable to remain in the resting condition. v. Dungern and Wernher have developed the latter conception at great length. Boveri's suggestion that such a disturbance of intracellular equilibrium may be the result of multipolar mitosis is the most concrete and intelligible example of this view. The localised character of malignant new growths at their commencement, even when multiple, should be a sufficient warning against the fallacy of attempting to account for the origin of malignant new growths by the assumption of general constitutional conditions as essential for the primary cancerous transformation of the tissues. The factors favouring or hindering the growth of tumours once definitely established are of much greater importance from the practical standpoint. Experiment having shown that it is possible to so alter the general condition of animals that transplantable tumours will not grow in them, it seemed rational to assume that a contrary condition, i. e., one favourable to growth, which can also be produced experimentally should be present in animals the subject of spontaneous cancer, and responsible to a certain extent for the progressive growth of the new formation, and its frequent extension by the blood and lymph streams

to distant organs. Such speculations have been put forward by Ehrlich and in a modified form by E. Albrecht. Ehrlich based his conceptions on the fact that the results of primary transplantation of spontaneous tumours are much less successful than those attained with tumours which have been propagated for some time. He has, however, generalised too freely from his own experience with spontaneous tumours, especially those in which the hæmorrhagic type predominates. From what has been detailed above there can be no doubt that he was completely in error in assuming that the hæmorrhagic tumours formed a closely related group, of which a prominent biological characteristic was their low transplantability to normal mice. As has been shown by Bashford, Murray and Bowen, Hertwig and Poll, and others, some hæmorrhagic tumours are transplantable, and the large number which have proved transplantable, as recorded in the present paper, should dispose of the error finally.

These considerations, however, do not necessarily dispose completely of Ehrlich's conceptions of the relation of spontaneous tumours to the animals primarily affected. He assumes that the growth of a tumour not transplantable to normal animals or only transplantable with difficulty, can only take place if the tissues of the animal affected have a lower avidity for nutritive substances than have those of normal animals, and that the low percentage of success of most primary transplantations speaks in the same sense though in less degree. Weighing experiments with transplantable tumours, some of which were performed in conjunction with Dr. Haaland, indicate that the quantities of food-stuffs at the disposal of the tissue and tumour-cells, are not so limited as this assumption implies. In healthy animals a considerable excess of nutritive materials is present, either actually or potentially, and the same conditions obtain in most spontaneously affected mice. E. Albrecht has expressed himself much in the same sense. In these circumstances the relative avidity of the cells of the tissues and the tumour can only find expression in a more or less rapid growth of the tumour, but cannot conceivably result in arrest of growth or starvation of the tumour-cells. When such a result is apparently produced, other factors probably enter, either changes in the tumour-cells or changes in the animals analogous to active immunity. Recently Apolant, 1907, has recognised in part the fallacy underlying this conception, viz., that the growth of a tumour in normal animals is determined only by the energy with which the tumour cells take up nourishment. Another factor is of great importance—what we have called the adaptability of

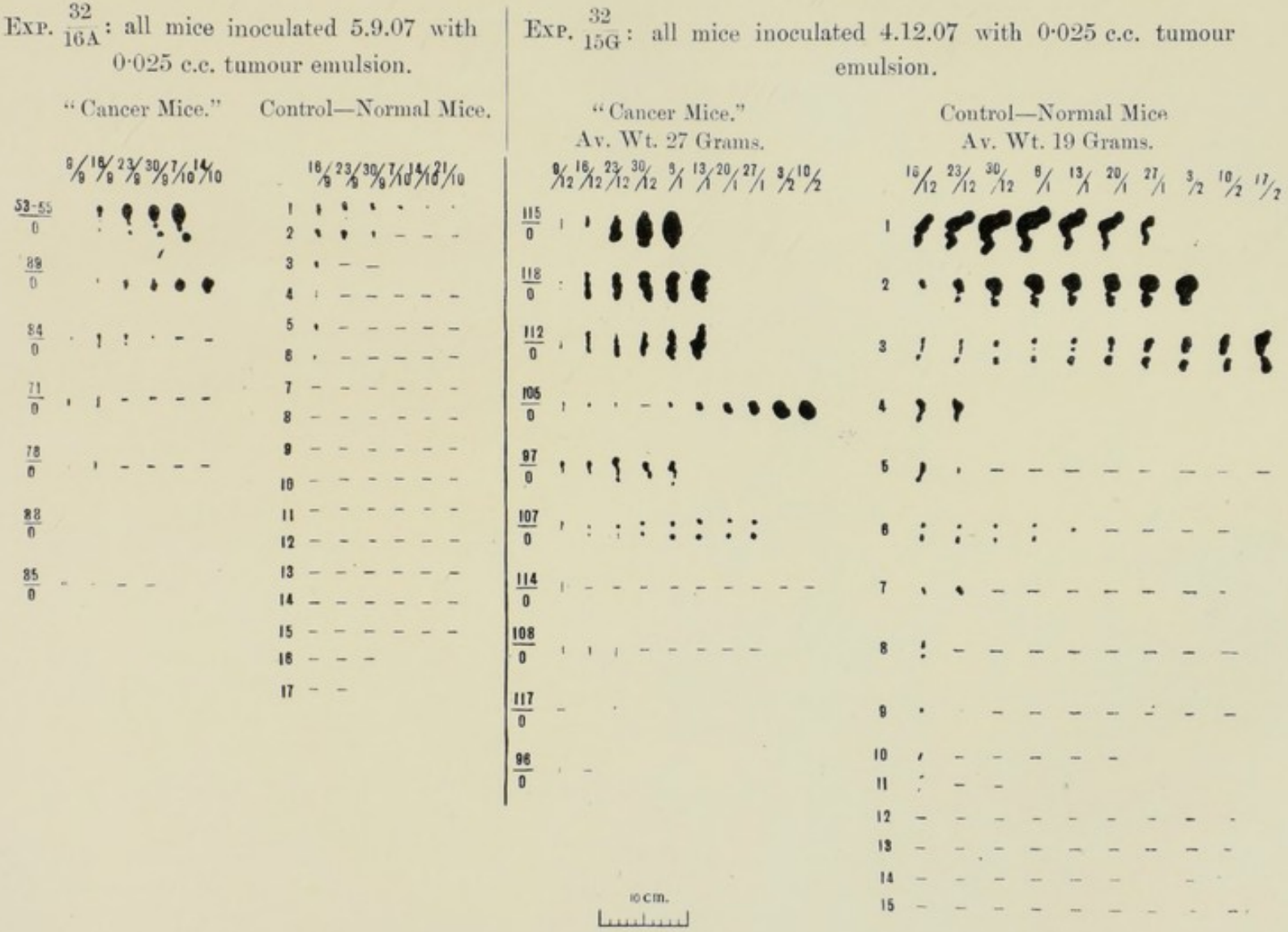


Fig. 47.—Comparison of the suitability of aged normal mice, and of mice suffering from spontaneous cancer, for the growth of squamous-cell carcinoma (tumour 32).

To face p. 109.]

the tumour cells. With Bashford and Cramer we drew attention to its importance in 1906–07. In fact this adaptability, in which may be included for convenience the resistance to such injury as necessarily occurs on transference to new hosts, is probably the most important determining factor in deciding whether a spontaneous tumour shall grow or not. This seems to be the explanation of the otherwise remarkable observation which we have frequently made and which is recorded by Gierke, and Hertwig and Poll, that spontaneous tumours inoculated into animals in which transplanted tumours are already growing, are only positive in them when the spontaneous tumour is also capable of growing in normal mice.

The other problem of the relative avidity of the cells for nourishment as compared with non-cancerous tissue-elements, is nevertheless one of great interest from the standpoint of the nature of the metabolic processes in play in the growth of cancer. In order to obtain more insight into these relations, experiments were devised in the following manner:—Mice spontaneously affected with mammary new growths had these removed by operation. The transplantability of the growths was tested on normal animals, and when a sufficient number of operated animals had been obtained their suitability for the growth of an easily transplantable tumour was tested and compared with a similar or larger number of adult and aged animals. Clean experiments of this kind are extremely difficult to carry out on a large scale. The interval between the operation and the subsequent transplantation necessarily varies between somewhat wide limits, and the number of cases in which the transplantability of the spontaneous tumours has been tested is too small to permit as yet of firm conclusions. The spontaneous carcinomata, as shown on preceding pages, frequently recur after apparently total extirpation, and in those in which local recurrence does not take place, metastases in the lungs are often found after death.

Therefore, even if we discard hypothetical constitutional differences for the present, the animals are not strictly comparable with non-cancerous subjects of similar age. The results obtained in two such experiments are given in the accompanying graphic records (fig. 47). The first of these shows $\frac{(32)}{16\,A}$ that while in normal animals not one tumour grew progressively, in two of the cancer mice, tumours developed and reached a large size. In four others, temporary proliferation was followed by absorption of the small tumours primarily arising.

In the second series $\frac{(32)}{15\,G}$ three mice (112, 115, 118) developed tumours which grew rapidly and progressively to a large size; in a fourth (105) a tumour first appeared five weeks after inoculation at the point of entrance of the inoculating needle and also grew progressively. In two mice (97, 107) tumours developed, but grew very slowly, and after reaching a moderate size remained stationary till death. Finally in three animals no growths appeared, or only a temporary proliferation. The normal animals inoculated at the same time with the same material developed tumours showing a similar gradation; three (4) grew progressively, two remained stationary for a long period, and, in the remainder temporary proliferation was either followed by absorption or the result was negative from the first. It must be noted that the transplantable tumour used for these experiments is one of the most rapidly growing in existence. It is not exceptional to obtain tumours of 1·0 to 1·5 gr. in ten days, starting from a graft of not more than 0·03 gr., an increase in mass of thirty to forty times in this period. For experiments of the kind under consideration this strain (32) has the additional advantage that all animals are not equally suitable, so that it grows progressively in only 50 per cent. of the inoculated animals in most cases. It is therefore particularly suitable for experiments in which slight differences between animals have to be elicited, and shows these differences both by variation in percentage of success and rate of growth. Together the two series of experiments show, that the transplantable tumour adapts itself, on the whole, more readily to the conditions present in animals spontaneously affected than to the conditions in animals of approximately the same age in which cancer has not spontaneously arisen. The further question now arises: what influence does the transplanted tumour exert on the organism as a whole, of a mouse the subject of spontaneous cancer? Does the transplantable tumour grow at the expense of the animal's tissues, or, is sufficient additional nourishment elaborated to supply the materials for the rapidly forming tumour-tissue? Further, how does the transplantable tumour compare in rate of growth with the tumour which has arisen spontaneously in the animal itself, when recurrence—in reality its continued growth—takes place? To these questions the experiments furnish a partial answer. As to the first two, by weighing the animals throughout the course of the experiment at regular intervals and after death determining the weight of tumour arising from the transplantation, it can be shown easily that the tumour does not grow at the expense of

the tissues of its new host in the first instance. On the contrary the animal as a whole increases in weight *pari passu* with the growth of the tumour because food materials are elaborated in sufficient excess for the nourishment of the graft. It is only in the later stages, when the growth has reached a relatively enormous size, that the animal fails to supply food at a quick enough rate. This is seen in the case of mice 105 and 112 of fig. 45, in which the whole increase in weight (7·5 grams in mouse 105, 4·5 grams in mouse 112) is represented by the transplanted tumour. In mice 115 and 118 the transplanted tumour has in the end increased at the expense of the mouse, but the course of the weight-curve shows that this condition is not established at once. It supervenes when the total mass to be fed becomes excessive. Cancerous mice, on the whole, respond better to such calls than do young, still growing normal animals; arrest of growth soon takes place in young animals, and the whole increase in weight which the organism should have exhibited normally at a given age is represented by the weight of the new growth.

It may be concluded that the transplantable tumour establishes itself in the spontaneously affected animal in consequence of its great adaptability to new hosts, that it shares the food supply in common with the normal tissues without starving them, and that its growth results from the capacity its cells have to adapt themselves to the conditions in which they are placed, and to utilise the nourishment presented to them for the formation of new elements, and not because they take up that nourishment with greater avidity. When the spontaneous tumour recurs, its growth progresses as a rule more rapidly than that of the transplanted tumour, even although its energy of growth as tested by transplantation into normal animals may be much inferior to that of the transplantable growth. This fact is illustrated in figs. 44 & 45, in the tumours numbered 97 and 107, in which the spontaneous tumours recurred and grew progressively, though slowly, while the transplanted tumours practically remained stationary. This phenomenon would be quite inexplicable on the assumption of a differential avidity for food material as the principal determining factor in their relative rates of proliferation, but is at once intelligible when the importance of adaptability is considered. The spontaneous tumour requires to make no effort of adaptation; it is in its native environment. The food materials presented to it are those to which it has always been accustomed. It is probable that the factor mainly responsible for its ability to compete successfully with the cells of a growth which, on equal terms (*e. g.*, in

normal animals), would easily outstrip it in energy of proliferation, is that the spontaneous tumour is at home with its surroundings and food supply.

The relation of a spontaneous tumour to the animal in which it is growing cannot be described in the simple manner imagined by Ehrlich by postulating a differential avidity for food-stuffs in accordance with the side-chain theory. The relative avidity with which tissue-elements and tumour-elements take up nourishment must remain vaguely speculative so long as we measure the avidity of the tumour cells by proliferation, *i. e.*, formation of new material, while tissue-elements no longer actively growing, cannot be expected to manifest anything of the kind in adult animals. The experiments referred to above, where young growing animals remain of small size while the tumours grow rapidly, have an apparent bearing on this point. Slow growing tumours do not exert the same influence. In the latter case the animal grows at much the same rate, whether a tumour develops or not. The retardation observed when the transplanted tumour grows with great rapidity, while appearing to speak for a greater avidity of the tumour cells, does not necessarily imply this. The tissue cells, although adding to their numbers during growth, also diminish in their call for new formative material (water, oxygen, etc.) by passing into the differentiated condition. The tumour cells do this to an infinitesimal extent, so that every step forward in new formation of tissue adds to the mass capable of further growth. The extent to which degeneration (frequently extensive in rapidly growing tumours) eliminates their requirements does not seriously invalidate this consideration. With some tumours, notably a transplantable spindle-cell sarcoma of the rat which we have obtained from Professor C. O. Jensen, the weight of tumour produced in a given time is almost exactly proportional to the initial dose, and in this case the condition of equilibrium, in which, while the tumour grows progressively, the total weight of animal and tumour remains stationary, is much more rapidly attained with the large dose than with the small one It is difficult to see why this should be so if the tumour cells extracted food-stuffs in a way and with an energy which leaves an insufficient quantity for the tissues of the host, because as soon as the tumour arising from the small dose had attained the size of that which initiates the growth with a large dose, the weights of animals and tumours in the two groups should exhibit a parallel behaviour.

It is only in the case of different strains of transplantable tumours, or of different series of the same tumour, that a comparison of avidity

can be made with any hope of accuracy. It is then apparent that the energy of assimilation of transplantable tumours is very variable, so that some strains surpass others enormously in this respect. At the same time different series of the same strain may show great differences. Further, as Apolant has pointed out recently, the adaptability of a tumour, which is usually measured by the percentage of success on inoculation, is to a high degree independent of the energy of growth, and he instances tumours which grow in nearly 100 per cent. of normal animals with great slowness. Tumours which grow extremely slowly in high percentage of normal animals seem to show that in them the essential phenomenon is a condition of unstable metabolic equilibrium rather than of increased energy of assimilation, and that the latter property when present is of subsidiary importance. We possess such a carcinomatous strain, placed at our disposal by Mr. F. W. Twort of the London Hospital, and our squamous-cell carcinoma (32) is an example of the converse condition in which a tumour with great energy of growth seldom shows a maximal percentage of success.

The existence of such tumours, the biological characters of which are retained through long periods of propagation, shows that the cellular transformation which initiates carcinomatous growth may take place in varying degrees. The impress which the cells receive at this time, while permitting of great histological variations in their descendants, colours permanently their whole biological behaviour. This biological alteration is of such a kind that the cells are able to take up nourishment, increase in size and multiply indefinitely. They acquire an individuality, and powers of resistance to injurious agencies, superior to those of normal tissue elements. They retain, with probably only apparent exceptions, the chemical equivalent of the histological characters of the elements from which they arise and exhibit a corresponding tendency to an organoid arrangement, in every situation in which they can grow.

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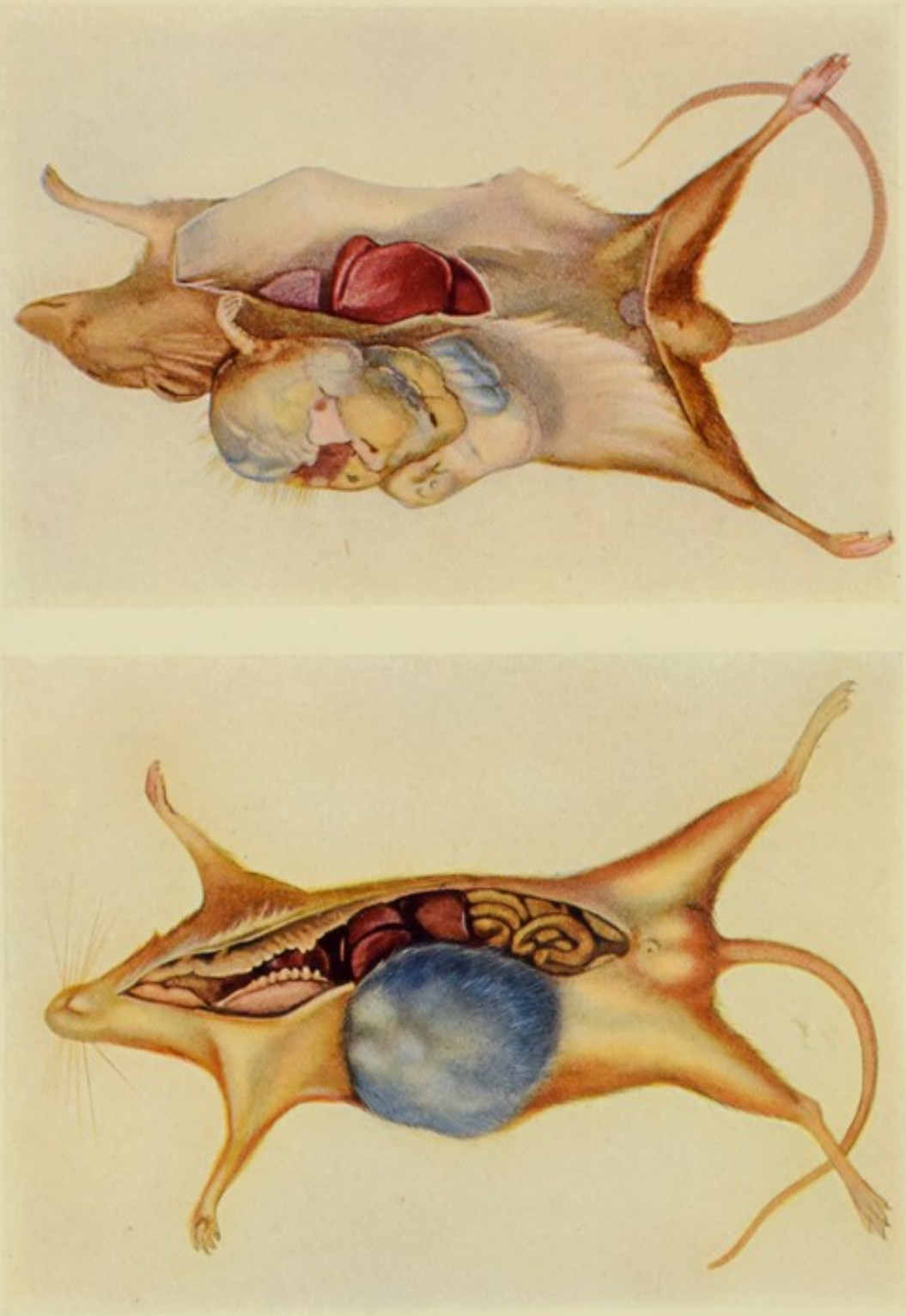
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A complete Bibliography of papers from the Laboratory of the Imperial Cancer Research Fund is given as an Appendix to this Report.]

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FIGS. 1 & 2—Exp. $\frac{50}{1}$. Two mice with transplanted hæmorrhagic mammary carcinoma. No metastases were found in the internal organs.

THE HÆMORRHAGIC MAMMARY TUMOURS OF MICE*, WITH RESULTS OF RESEARCH INTO SUSCEPTIBILITY AND RESISTANCE TO INOCULATION.

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The epithelial tumours of mice have been classified by Apolant, whose classification has been generally adopted. But, although Apolant himself emphasises the fact that individual forms cannot be differentiated sharply, and that an alveolar carcinoma may again assume an adenomatous formation under certain circumstances, as he states in his latest paper, it seems practical to distinguish several groups which, although they retain during transplantation certain structural characteristics as a rule, at the same time present variations from a biological point of view. The majority of epithelial tumours in mice, so far as we are not dealing with squamous-cell carcinoma, may be regarded, according to Bashford and Murray, Apolant, Borrel and Haaland, as mammary tumours. Among these Apolant distinguishes :— (1) *Adenoma*—(*a*) adenoma simplex ; (*b*) cystadenoma simplex ; (*c*) adenoma cysticum œdematosum *sive* hæmorrhagicum ; (*d*) cystadenoma papilliferum: (2) *Carcinoma*—(*a*) carcinoma simplex alveolare; (*b*) cystocarcinoma hæmorrhagicum ; (*c*) carcinoma papillare ; (*d*) fissure-forming adenocarcinoma.

The alveolar, fissure-forming, and papillary carcinomata have been successfully propagated through many generations by a number of investigators. The hæmorrhagic carcinomata, on the contrary, could, at the first beginnings of experimental study in mice, not be transplanted at all, and later only in a few cases were they transplanted with any success, so that Ehrlich estimates the yield of the inoculations as not higher than $\frac{1}{3}$ per cent. But a special interest

* My stay in London being limited, my experiments terminated in September 1907. Thus many of the questions raised can only be definitely settled after a continuance of the enquiry.

thereby attaches to the hæmorrhagic tumours in that Ehrlich inoculated them in extensive experiments in which they served as the preliminary treatment to induce immunity successfully. The preliminary inoculation of such "avirulent" hæmorrhagic tumours protects in most cases against after-inoculation with "virulent" carcinomata and sarcomata.

Ehrlich's opinions on the biology of tumours have been influenced also in another respect by his experiences of the behaviour of these hæmorrhagic tumours in mice. The impossibility of transplantation seemed to him to indicate that tumour-cells do not necessarily possess an increased power of proliferation, *i. e.*, a heightened avidity to foodstuffs in opposition to the normal cells of the body, but that the normal cells of the body had suffered a diminution in their avidity for foodstuffs in certain individuals. In such cases the development of malignant tumours would then be attributable to the general constitutional reduction of the avidity of the body-cells as a whole, and not to a biological alteration of the tumour-cells. The proof adduced in this paper of the propagation of these forms of tumours is evidence of the untenability of Ehrlich's conception.

Nothing essentially new has been reported by other investigators with regard to hæmorrhagic tumours till quite recently, when, after the conclusion of my researches, a communication appeared by Hertwig and Poll respecting a transplantable hæmorrhagic carcinoma. The observations made by the Imperial Cancer Research Fund in London on these forms of new growth which are of extraordinary biological importance, differ in some not unessential points from what has hitherto been known. I was particularly grateful therefore when Dr. Bashford gave me permission to classify results obtained by himself and his colleague, Dr. Murray, and to make extensive inoculations for the purpose of investigating the biological behaviour of hæmorrhagic tumours. In the following paper I shall report on this material and only refer to other forms of tumours for purposes of comparison, either in order to study the mutual relations obtaining between hæmorrhagic tumours and others of different category, or, to test the wider significance of the results by means of tumours of different nature. The most important new point which the London Institute has elicited in advance of previous experience is the fact that even hæmorrhagic tumours can be successfully transplanted, and further, can be propagated through many generations in by no means rare cases. As mentioned above, Hertwig and Poll have reported quite recently a single similar case.

Twenty-nine out of thirty-five hæmorrhagic tumours have proved to be transplantable : of these, fifteen became extinct on further transplanting, as follows :—

8	in the	first	generation	(Nos. 25, 33, 38, 52, 56, 61, 66, 76).
5	„	second	„	(Nos. 9, 30, 48, 49, 51).
2	„	third	„	(Nos. 7, 19).

Fourteen * still continue to live :—

4	in the	first	generation	(Nos. 85, 89, 91, 98).
2	„	second	„	(Nos. 80, 88).
2	„	third	„	(Nos. 72, 79).
2	„	fourth	„	(Nos. 58, 63).
2	„	fifth	„	(Nos. 47, 65).
1	„	eighth	„	(No. 50).
1	„	tenth	„	(No. 39).

In the last two strains (Nos. 39, 50) especially, many series have been obtained, some with high percentage of success and rapid growth.

Altogether upwards of 4000 mice were inoculated with these hæmorrhagic primary tumours, and of these 2851 remained alive long enough to allow one to judge of success, *i. e.* at least three weeks, and 187 tumours developed. Thus about 6·5 per cent. of the first transferences were positive. But in separate instances the result of inoculation varies considerably. Along with the tumours giving quite negative results, there are included those which gave less than 1 per cent. of successful transplantations. The highest result was obtained in tumour 50, giving 22 successes in 67 mice, *i. e.*, 33 per cent. The highest average primary transplantation recorded by Hertwig and Poll is 40 per cent., reckoned however only on 5 mice. In the course of successive transplantations the percentage of tumours developing likewise fluctuates very considerably, often sinking to nil and in other

* Since the termination of my experiments in September 1907, transplantation experiments have been undertaken in twelve more hæmorrhagic tumours. Nine of these proved to be transplantable (Nos. 99, 100, 102, 104, 107, 110, 111, 113, 114), three failed (103, 105, 106). On the whole, 69 tumours occurred in 412 mice, *i. e.* 15·5 per cent. The best result was exhibited in No. 114 with 18 tumours in 54 mice, *i. e.* 33·3 per cent. Metastases were present in 4 mice (Nos. 100, 106, 110 113). Thus the sum total of hæmorrhagic tumours inoculated is brought up to 47, of which 38 could be transplanted.

series rising to 70 per cent. ; but thus far a higher percentage has not been reached with hæmorrhagic tumours. Curves which graphically represent the percentage of results and duration of propagation give indications of the same fluctuations as Bashford, Murray, and Bowen have described for other mouse tumours, particularly for Jensen's tumour, and have referred to periodic fluctuations in the growth of the tumour-cells. This phenomenon is corroborated by Hertwig and Poll as well as by Borrel and Bridré for mouse tumours, and by C. Lewin for rat tumours.

Of the mice with spontaneous hæmorrhagic tumours three developed an additional tumour, which in one case (No. 77/78) was also hæmorrhagic and not transplantable, in the other two cases (Nos. 65, 33) non-hæmorrhagic, and in one instance (No. 65) was transplantable. One mouse (No. 53–55) exhibited even triple tumours which were all hæmorrhagic and could not be transplanted. Thus it appears as if a tendency to primary multiplicity existed, for, the whole circumstances did not point to anything of the nature of metastasis. Hertwig and Poll found also two separate growths in the case of the mouse suffering primarily from their transplantable hæmorrhagic tumour. Apolant calculates that multiplicity occurs in 12 per cent. of all kinds of mammary mouse tumours.

After the extirpation of the primary tumour, two mice (Nos. 72, 74) developed new spontaneous tumours, which owing to their nature and position could not be counted as recurrences.

Genuine recurrences occurred in ten cases after extirpation (Nos. 19, 25, 33, 34, 47, 48 50, 52, 53, 54), four of which (Nos. 19, 25, 47, 50) recurred also after the second apparently complete surgical removal. These recurrent tumours were transplanted six times (Nos. 19, 25, 47, 48, 50) successfully and twice (Nos. 33, 34) in vain.

Macroscopical metastases in the lungs occurred eight times (Nos. 18, 19, 25, 33, 52, 63, 66, 85, 88) and were transplanted three times, once with success (No. 63), and twice without (Nos. 35, 88). Lymphatic glandular metastases were discovered once microscopically (No. 25); but metastases have not been found in any other organ. For a long time metastasis formation was not observed associated with transplanted tumours. More recently, however, lung metastases have also been found, viz. in seven mice (four times in the case of tumour 50, twice for tumour 39, once for tumour 58). The average age of the tumours in these cases was 130 days after inoculation. The rarity seems essentially to rest on the fact that most animals do not live long enough, either

succumbing to ulceration or being killed for the purpose of further transplantation.

Now, how is the fact to be explained that in London the hæmorrhagic tumours were relatively frequently transplantable, while in Frankfurt negative results were constantly obtained? There can be no question of an accidental difference, owing to the abundant material at the disposal of both Institutes, and therefore the distinction must be looked for in the methods applied. The chief methods used for successful transplantation are the following :—

1. In Ehrlich's Institute the tumour is pounded in a mortar and injected by means of Pasteur pipettes.
2. In the Imperial Cancer Research Laboratory a mill is used, as designed and described by Haaland. The tumour matter is injected without any sort of additional fluid by means of a special graduated syringe designed for this purpose.
3. Introduction of a small particle of tumour by means of a hollow needle, which is the routine method for transplanting primary tumours in the laboratory of the Imperial Cancer Research.
4. Hertwig's transplantation of little pieces of tumour in a surgical fashion, which resembles in principle the process adopted by Borrel.

The first two methods have in common the conversion of the tumour into an emulsion, while in the case of the last two methods a small piece of tumour tissue is implanted *in toto* ; so that we may identify the first with the second in principle, and the third with the fourth. At the London Institute the second and third methods are applied. The method with the syringe permits of the introduction and exact measurement of large doses, while transplantation with the needle renders very small doses possible and permits of a more gentle treatment of the tumour tissue, *i. e.* injures it less. Hence, the first tranferences of a spontaneous tumour are always made with the needle which in addition permits of a very great number of experiments being made, for the medium dose in the needle-inoculation may amount to 0·025 grm., although with practice one can give with approximate accuracy small portions of circa 0·01 grm. In the case of later transplantations both methods are used in accordance with the end in view, and I have availed myself of both. With the syringe one can graduate the doses down to even 0·025 ccm. Spontaneous tumours appear to be particularly susceptible to forcible breaking down, as the researches of Bashford and Murray have elicited, while it is obvious during the course of continued propagation that the

resistance against mechanical injury increases correspondingly with the rising transplantability. The size of the dose introduced is perhaps quite equal in importance to the consequences of mechanical injury. Small doses are always used in needle-inoculations, whereas the quantity of tumour emulsion introduced in the form of injections is frequently considerably greater. It is certainly conceivable that small and large doses operate differently, for only a small portion of the cells belonging to the injected tumour matter remain alive; many are killed in the process of making the tumour emulsion, others die during the first days after injection, so that a not inconsiderable quantity of the tumour tissue is absorbed. The absorption of tumour tissue elicits in the mouse that reaction which we must discuss later in more detail under the head of acquired resistance, and which may be directed against the growth of the tumour tissue at the primary inoculation under certain circumstances, in the same way as the absorption of tumour tissue as used by Ehrlich, effectively protects against a secondary inoculation with virulent tumour. Schöne states in the report of his researches in Ehrlich's Institute that a primary dose of 0·3 ccm. effectively induces protection against a subsequent inoculation. This is a very considerable quantity for the organism of a mouse; the equivalent quantity is 1 kg. for the human subject. It seems intelligible that such quantities may have results quite different from those following the inoculation of minimum doses, *i. e.* the implantation of but little injured fragments of tumour tissue by means of the hollow needle.

I am of opinion that the method adopted in the London Institute has not been without influence in contributing to the successful transplantation of hæmorrhagic tumours. At the same time this method possesses a second advantage: by using small doses one is able to inoculate a very great number of mice from spontaneous tumours; in London usually upwards of 100 mice are used. It is evident, therefore, that the chances of positive transference are much greater in the case of representatives of that numerous group of tumours which yield bad results on inoculation. The fact that this influence of methods and doses does not find expression in all kinds of tumours, but that many have been successfully transplanted by means of larger doses, indicates a biological difference between various growths. One cannot speak more exactly at the present time, but one can speculate on the influences exerted by the amount of blood contained in the spontaneous tumour, on a greater immunising power of the tissues of some tumours as compared with others, and, on the slowness and

difficulty with which the cells of the hæmorrhagic tumours establish themselves. The following facts can be stated with certainty :—

1. By the inoculation of large doses of tumour tissue immunity is induced against other tumours.
2. By the inoculation of small doses, consisting of unground tumour tissues, numerous hæmorrhagic tumours, 37 out of 48, have been transferred in London. Hertwig and Poll, who have successfully transplanted a hæmorrhagic tumour, also use only particles of tumour the size of a millet-seed or of a peppercorn.

Perhaps the unfavourable effect of larger doses continues to exist to a certain extent during continued transplantation, if in a less degree than for spontaneous tumours. For, one often notices that in a series of transplantations of the same tumour with large and small doses respectively, the small doses yield the higher percentage of successful inoculations. Retrogression after transitory growth can often be observed with tumours arising from larger doses. In my own experiments, I have seldom exceeded 0·1 to 0·15 ccm., in order not to obtain series too limited in number : perhaps, the susceptibility to the size of dose which exists for spontaneous tumours would manifest itself also for the daughter tumours obtained in the course of continued propagation, by further raising the amount of the dose. Generally speaking, tumours appear to diminish in their susceptibility to dosage during the course of continued transplantation. How important the influence of dosage is in the case of re-inoculations also, will be demonstrated later in more exact detail.

Some statements may be made here of the macroscopic and microscopic features of hæmorrhagic tumours. Apolant's statements with regard to hæmorrhagic spontaneous tumours have been confirmed in essential points and extended to the propagated tumours. The site of these spontaneous tumours is the same as that of the other mammary tumours, viz., mostly on the flanks and the rump, occasionally towards the back. The tumours are more or less movable on the subjacent tissues and raise the skin with which they become adherent when of any size. Their hæmorrhagic nature can be recognised usually by inspection because of their bluish colour. The skin is often greatly stretched through the presence of distension cysts which may be light or dark red in colour. When such cysts rupture reddish serum or a bloody fluid is discharged, an entrance is afforded to bacterial infections,

and local ulceration or general septic conditions may follow. The inoculated tumours behave on the whole in a similar way. At first they are moveable on the subjacent tissues and under the skin. On further growth they become fixed and the skin is thinned. Very frequently quite similar hæmorrhagic and serous cysts develop which are accompanied by the same dangers from infection and ulceration. When we cut through such a tumour, we find it of a very soft consistence for the most part, and a chain of hæmorrhagic and serous cysts running through it and varying both in number and size ; the smallest appear as pin points, the largest about the size of a cherry and containing either fluid or glutinous grumous blood. The tumour tissue appears pinkish-white and very soft in those parts where hæmorrhages are scanty. Should metastases of the lungs be apparent macroscopically, their aspect varies very much. The smallest look reddish-white, the largest, which in some circumstances can comprise a whole lung, are likewise uniformly hæmorrhagic. Naked-eye metastases in other organs have so far not been discovered.

Microscopically, the tumours in question belong to the groups which Apolant has described as *adenoma-cysticum œdematosum* sive *hæmorrhagicum*, and *cystocarcinoma hæmorrhagicum*. The former, which, as Apolant states, frequently contain distended glandular spaces (Apolant's *cystadenoma simplex*) can be shown to be derived from the *adenoma simplex*, by studying the varying degrees of the change presented in the parenchyma and stroma. Distension of the lumina of the alveoli of the parenchyma often occurs through the accumulation of thick opaque liquid or of serous secretion; the epithelium lining the lumina is thereby flattened, and neighbouring cavities often become confluent, thus forming large cysts.

Apolant distinguished several processes in the scanty stroma. Degenerations of the stroma occur. Lymphatic obstruction with resulting œdema is most frequent, then the alveoli are not only separated from each other but the histological picture is also confused, the alveoli being distorted and elongated through the pressure. The pressure frequently causes atrophy and destruction of the alveoli. Cysts likewise occurring as the result of the process just described, may look at a first glance very much like secretion cysts, although in reality they are completely different in nature. The non-epithelial origin of such cysts can be demonstrated before the histological picture is too extensively blurred through modifications, by means of the vessel which traverses the "pseudocyst" and the thin connective tissue lamella which intervenes between the fluid and the epithelial columns.

Changes in the blood-vessels determine more than anything else the histological pictures presented by the tumours we are considering. The thin-walled capillaries which run in the delicate stroma are frequently dilated, becoming, particularly in the œdematous parts, huge blood-sinuses, or, by reason of the limited opportunity for expansion, exhibiting aneurysmal dilatations. Such dilatations of the blood-vessels proceed, here and there, to such an extent that the picture of a cavernous angioma is reproduced, and, in such cases the septa between the sinuses are formed by the compressed adenomatous tissue. All these changes predispose to rupture of vessels and to hæmorrhages, their extent varying greatly in individual cases. A distinct rent may be seen frequently in the capillary wall of those vessels which traverse the œdematous spaces, and through it the blood streams into the pseudo-cysts till eventually it quite fills them, and ultimately contributes to their further distension. The blood then escapes from these pseudo-cysts and courses still farther between the epithelial columns and may even penetrate into the genuine epithelial cysts. Finally, large areas of the normal tumour-structure are completely riddled with hæmorrhage. The constituent parts of the tumour thus cut off and enclosed within a hæmorrhagic area, may completely degenerate as the result of the pressure of blood and the removal of sustenance. The contents of the cysts present all transitions from blood-stained serous liquid to fluid or coagulated blood as the result of the mixing of blood with œdematous fluid and epithelial secretion. According to Apolant a smooth-walled cyst may finally develop from what at the outset had the structure of an adenoma, the contents of such a cyst being a thick chocolate-brown mass consisting of epithelial debris mingled with blood, while only quite insignificant remains of the original adenomatous structure remain attached to the connective tissue of the walls of the cyst.

According to Apolant, the *cystocarcinoma hæmorrhagicum* is derived from an adenomatous tumour which has been modified by hæmorrhage in the way described, and in which atypical epithelial growth has occurred concomitantly with the hæmorrhages. Then compact epithelial complexes or garland-like columns of cells intervene between the hæmorrhagic areas described above, and all transitions exist between the solid and the adenomatous structure. Degenerations and hæmorrhages arise in the manner already indicated for the pure adenoma, and as the end result cysts filled with blood may form in like manner.

Apolant repeatedly asserts that no sharp distinctions exist between

the adenomata and the carcinomata. He found typical carcinomatous epithelial cords in the capsule of hæmorrhagic cysts, the inner wall of which bore remains of adenomatous tissue. Apolant has, as a matter of fact, "comparatively seldom come across a mouse tumour bigger than a cherry that did not in some place or other, but as a rule over wide areas, exhibit a carcinomatous structure."

After my investigations relating to the 35 hæmorrhagic tumours, with which transplantation was attempted in the Imperial Cancer Research Laboratory, I can fully endorse Apolant's histological descriptions and therefore I can omit detailed descriptions of the separate tumours. Nevertheless I wish to emphasise that in my judgment a distinct division between hæmorrhagic adenomata and carcinomata appears impossible, especially after the microscopical examination of hundreds of tumours obtained in transplantation experiments. Useful as Apolant's systematic classification is, we must bear in mind the fact that it is a purely histological division, and insist that it deals with tumours identical biologically, and only slightly varying in their microscopical appearances. In the case of the tumours I examined, like Apolant, I found with great constancy histological pictures which were difficult to reconcile with their purely adenomatous nature. The biological behaviour of the tumours during transplantation, to be mentioned later on, and their formation of metastases, as well as their powers of recurrence, are all evidence that they form one homogeneous group.

The general features of the sporadic hæmorrhagic tumours at my disposal are shown in the microphotographs of tumour 19 (figs. 3 & 4). In many sporadic hæmorrhagic tumours the adenomatous parts preponderate, but, in quite as many, large solid epithelial alveoli are the outstanding feature, and then such solid alveoli often exhibit a curious arrangement of lumina, viz. a sieve-like puncturing of the epithelial mass, owing to the presence of little secretion spaces and the grouping of the cells around them (fig. 5). Portions with an acinous structure are connected at numerous places in the preparation with compact epithelial masses (figs. 6 & 7). At a first glance one might suppose, in agreement with Apolant's presentation of the facts, that we were concerned in such cases with the conversion of what histologically is an adenomatous formation into an alveolar or carcinomatous. But closer examination of the histological appearances shows that their evolution is exactly the reverse. The acinous portions arise from the alveolar, and correspond to a differentiation (maturation) of the tissue of the tumour. This process may be observed

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Microph., R. Muir.

FIG. 3.—Mouse. Microphotograph of a transplantable spontaneous hæmorrhagic tumour ($\frac{15}{0}$). Growth adjoining normal mamma with dilated acini. $\times \frac{75}{1}$.



Microph., R. Muir.

FIG. 4.—Mouse. Microphotograph of transplantable spontaneous hæmorrhagic tumour ($\frac{15}{0}$) showing hæmorrhages. $\times \frac{75}{1}$.

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R. Muir, del.

FIG. 5.—Mouse. Subcutaneous surface of transplantable spontaneous hæmorrhagic tumour $\left(\frac{19}{0}\right)$: mitoses numerous. $\times \frac{500}{1}$.



FIG. 6.—Mouse. Tumour $\frac{19}{1}$. Formation of secretion-spaces in an alveolar portion of a transplanted tumour. $\times \frac{500}{1}$.



FIG. 7.—Mouse. Tumour $\frac{19}{0}$. Transition from alveolar to acinous structure. $\times \frac{500}{1}$.

countless times, not only in the spontaneous tumours but also in those obtained by inoculation. It developes in such a way that precursors of the delicate stroma, and with them young blood-vessels, penetrate into the large disorderly epithelial masses ; thus epithelial groups, between which a hollow space may have arisen already in anticipation as it were, are segregated. The whole epithelial complex becomes split up by the invading connective tissue. Acini may then develop in the alveoli formed by the groups of epithelial cells thus divided up. The process is comparable with the formation of Pflüger's primordial "egg-tubes" in the ovary, or with the development of the embryonic or strumous follicles of the thyroid gland. Apolant has drawn attention to the remarkable similarity which a fully developed adenomatous tumour presents to a struma parenchymatosa of the thyroid gland. It appears to us that the resemblance may be pursued still further, for the development of the follicles of the thyroid gland follows a parallel route. If the epithelial production takes place very rapidly large epithelial accumulations and masses arise of which the partition is due to the ingrowth of invading connective tissue, and in which the appearance of glandular spaces coincides with the occurrence of secretory processes. The whole process may be comprehended by characterising its varying features as different stages in the differentiation of one species of cell which presents a carcinomatous formation at one time in consequence of the accumulation of compact cell-masses, and at another time, an adenomatous formation. Only in this way can we understand the alternations in structure of the same sporadic tumour, alternations which repeat themselves also in the lung metastases, as well as in the propagated daughter tumours. The question of malignancy ought to be decided not according to whether the rapidly proliferating epithelial cells arrange themselves into acini or into solid alveoli, but according to their biological behaviour. As Apolant has observed, variations in the rate of growth determine to a certain degree also variations of structure. This observation in itself assists us materially to understand properly the significance of the histological structure. The epithelial cells will differentiate faultily when growing rapidly, and in consequence assume a garb apparently more malignant histologically than that which they assume when growth is slower. But, I have ascertained by making examination of the histological appearance of my hæmorrhagic tumours in test experiments, that this relation between structure and rate of growth does not always obtain. When I compared the histological structure with the rate of growth anomalies manifested themselves. A

whole series of other factors require consideration, such as the behaviour of the connective tissues and the general constitution of the individual. After influencing the latter by preliminary (immunising) treatment, Apolant observed what he describes as reversion of an alveolar carcinoma to a tumour with adenomatous structure. Whether a biological change is associated with this structural change cannot be decided at present. For my hæmorrhagic tumours I could detect no connection between structure and transplantability ; starting with the same material the same differences in transplantability were revealed in the alveolar tumours as in the adenomatous. One of the most readily transplantable of hæmorrhagic tumours (No. 39) shows, even in later inoculated generations, a structure pronouncedly adenomatous for the most part (fig. 8). Metastasis formation is just as frequent in adenomatous as in alveolar tumours. A partial immunity has not always that effect on the structure assigned to it by Apolant. I compared a tumour growing rapidly in a normal mouse with another of the same series, which by way of exception had developed in a mouse in which a previous inoculation had been negative ; the latter tumour had remained significantly smaller, but on examination was found to have a pronounced alveolar structure in contrast with the markedly acinous structure of the control tumour. Researches on this point are still far too scanty ; we can only say with certainty that the inter-relations between structure and biological behaviour are undoubtedly influenced by very different factors of which only a small portion have hitherto lent themselves to analysis.

As the outcome of researches on human tumours mainly of the thyroid gland, I have already expressed the opinion that the histological structure of a tumour does not of itself permit of a decision as to its innocent or malignant nature. There are thyroid tumours exhibiting an absolutely benign structure when considered from the histological standpoint, and yet in their powers of destructive growth, and tendency to form metastases, etc., they are not surpassed in malignancy by any carcinoma. As I have demonstrated already elsewhere, we must acknowledge such tumours to be carcinomata whose cells retain the capacity for more advanced differentiation. Transitions are provided by tumours which exhibit in some parts a well-marked carcinomatous structure, and in others the structure of a simple goître, as, for example, in metastases in the lungs or lymphatic glands. Eberth has already characterised this condition as "a change for the better." * The

* "Umkehr zum besseren."

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FIG. 8.—Mouse. Acinous structure of tumour 39 in fourth generation. $\times \frac{500}{1}$.



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FIG. 9.—Mouse. Tumour $\frac{50}{0}$. Transplantable spontaneous hæmorrhagic tumour. *Cf.* fig. 5 in Apolant's 'Mammatumoren der Maus.' $\times \frac{45}{1}$.

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Microphoto by R. Muir.

FIG. 10.—Mouse. Microphotograph of inoculated tumour of first generation $\left(\frac{50}{1}\right)$.
Parenchyma acinous, dilated vessels and blood-cysts. $\times \frac{60}{1}$.

unfortunate thing is that this histological "improvement" does not necessarily correspond also to a biological improvement, and, it is their biological behaviour alone which lends to malignant tumours their chief significance, and necessitates, at least in principle, the maintenance of a distinction between them and the benign tumours. We find exactly the same phenomena in the group of mouse tumours we are considering. In their case also we come to the same conclusion, namely, that there are certain interchangeable relations between the histological structure and the biological behaviour of a tumour, but, at the same time, any direct inference from the one to the other may be made only with great caution. On this account Apolant has indicated the difficulty of a pathological-anatomical definition of his *hæmorrhagic cystocarcinoma*: "Ought the hæmorrhagic cystocarcinoma to be counted as among the benign tumours by reason of its thoroughly benign course, or by reason of its structure as among the carcinomatous?" In light of the new facts which I have given in this paper the decision is not difficult. For I have shown that these tumours also may have importance from their very malignant behaviour towards the individual, *e. g.* through metastasis or recurrence after an operation; and, further, they can be propagated to an unlimited extent. As a matter of fact, not only these hæmorrhagic *cystocarcinomata*, but also the tumours distinguished by Apolant as encapsulated hæmorrhagic *cystadenomata* of benign histological structure, display identical qualities. For example, fig. 9 reproduces the features of spontaneous tumour 50, which is easily transplantable. This tumour at the time of writing is in its eighth sub-transplantation, and in its histological features corresponds perfectly to Apolant's fig. 5 of a benign *adenoma cysticum hæmorrhagicum* incapable of propagation. A microphotograph of the first inoculated generation is represented in fig. 10. We are obliged to obliterate still further the distinction, which Apolant himself confesses is undefined, between the two forms *adenoma cysticum hæmorrhagicum* and *cystocarcinoma hæmorrhagicum*, and to assign all these tumours to the same biological group, viz., the hæmorrhagic epithelial tumours of the mouse mamma. As such they form a subdivision of the mouse carcinomata, although we must remember that absolutely definite distinctions cannot be drawn. This impossibility is best illustrated by the tumours resulting from transplantation, which may be free from hæmorrhage occasionally either in the case of individual tumours or in the case of whole series of tumours, whilst their offspring perhaps are again typically hæmorrhagic.

I now pass to a short discussion of the inoculated tumours. In

general it may be remarked of them that they often adhere with astounding persistency to the peculiarities of their mother sporadic tumour. An example of this is shown in figs. 11 & 12. Fig. 11 is taken from the hæmorrhagic primary tumour 88, which showed an adenomatous structure with epithelial cyst formations. The cysts are lined in part with a single layer of low cylindrical epithelium, but in many places there are several layers, and where this is so the epithelium tends to acquire a glandular arrangement and to form cysts. These features are almost exactly reproduced in the small daughter tumour of the first generation, illustrated in fig. 10. Fig. 9, from primary tumour 50, shows the agreement of its structure with that of the daughter tumours of the first inoculated generation, as shown in fig. 10, and, if this resemblance may not be very pronounced in every single instance, the specific property lies latent in the cell, as is proved by the reappearance of the primary structure in later generations. The matter is exactly as Bashford, Murray, and Haaland were able to show in a very excellent manner with their transplantable squamous-celled carcinoma. The tendency to become horny may be less pronounced for generations and then appear again suddenly. This specificity of the cells extends even farther, since they continue to transmit also a specific influence on the connective tissues. The same fact holds good for the hæmorrhagic tumours as has been proved for the rest of the mouse cancers. In transplantation only the epithelial cells remain alive, whereas the connective tissue of the stroma transferred at the same time quickly perishes. The daughter tumour is built up by the transplanted epithelial cells which, besides multiplying themselves, excite the connective tissues of their new hosts to proliferate and make themselves useful as stroma. Cases in which the transferred stroma likewise multiplies and continues to flourish are to be regarded as mixed tumours, and, as has many times been observed, through elimination of the epithelial constituent parts, they may lead finally to pure sarcoma. It is remarkable that this development has not been observed so far in the case of hæmorrhagic tumours, but then the number of tumours of this kind which have been transplanted and propagated for a sufficiently long period of time is not yet extensive enough for final conclusions. Should, however, the future show that the hæmorrhagic carcinomata do not lead to sarcoma formation, this would quite agree with the views expressed of Bashford, Murray, and Haaland. According to them the development of sarcoma is due to a specially strong specific stroma reaction in response to influences proceeding from the epithelium. We shall

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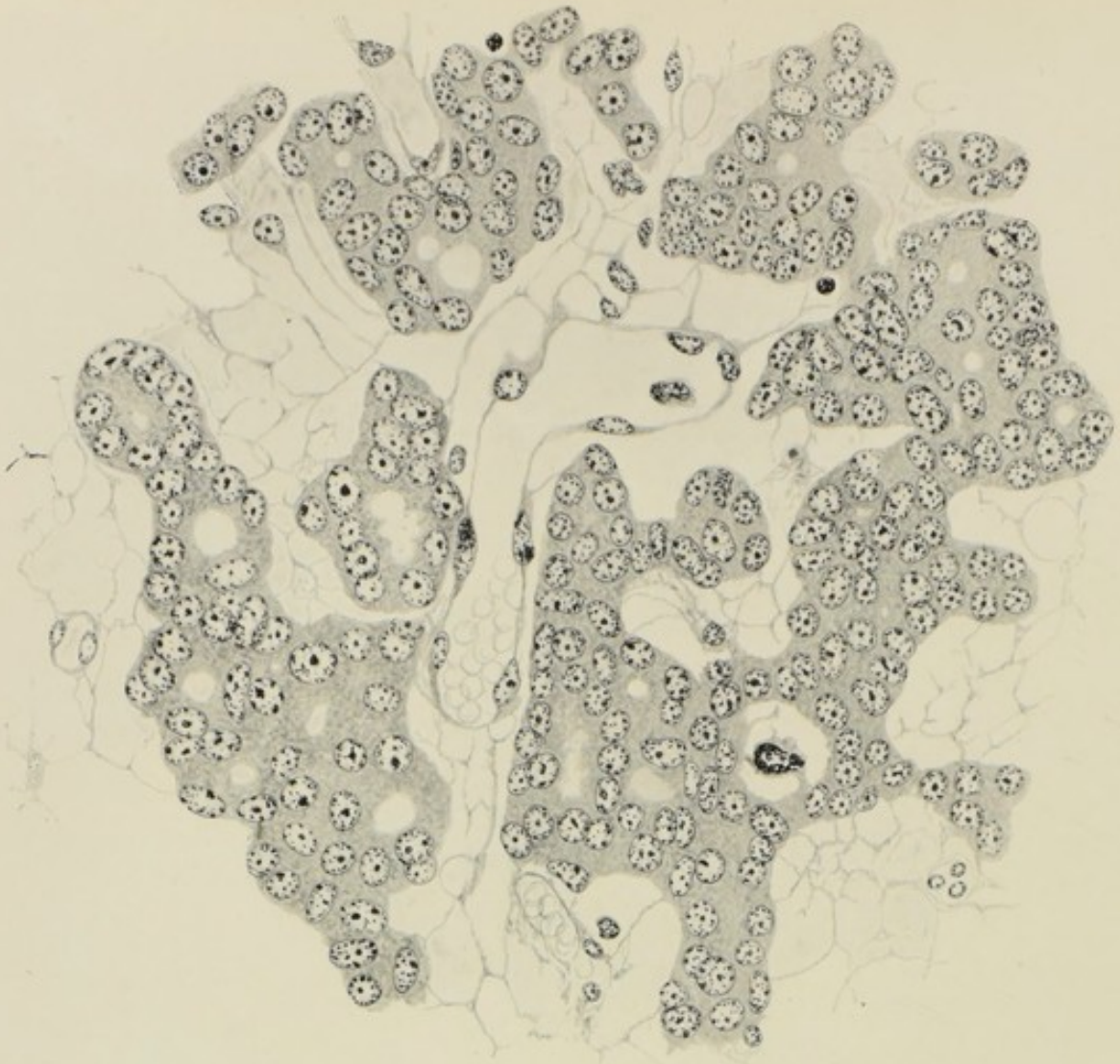
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Fig. 11.—Mouse. Tumour $\frac{88}{0}$. Surface of transplantable hæmorrhagic spontaneous tumour showing cystic spaces and alveolar portions with lumina. *Cf.* fig. 12. $\times \frac{50}{1}$.



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Fig. 12.—Mouse. Tumour $\frac{88}{1}$. First generation of tumour of previous figure showing reproduction of histological features. $\times \frac{50}{1}$.



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FIG. 13.—Mouse. Tumour $\frac{19}{0}$. Delicate reticular and œdematous stroma with dilated capillaries. $\times \frac{700}{1}$.



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FIG. 14.—Mouse. Tumour $\frac{88}{0}$. Infiltration of muscle at the surface of spontaneous tumour.

see directly that the stroma reaction is comparatively weak in the hæmorrhagic tumours, and that most of the characteristics of this group can be explained by this fact. When the transplanted epithelium has a powerful effect on the connective tissue, there is a rich new formation of connective tissue and vessels which contribute to the building up of the tumour and to its nourishment. The amount of this "specific stroma reaction" is to a certain extent characteristic for every tumour, and it is easiest to understand on the hypothesis of chemical substances with chemiotactic actions. It now seems that in analysing this reaction we may differentiate still further in accordance with the varying extent to which the connective tissues and the blood-vessels respond, so that we may speak of a fibro-plastic and an angio-plastic reaction. If the two are in equilibrium, good well-nourished carcinomata with large or small alveoli will arise. If the fibro-plastic influences preponderate, the nourishment of the cancerous cell-nests will suffer, and herein may in part be recognised a reason for a central necrosis of the epithelial masses or of the whole of a tumour as is, for example, so often the case in Jensen's tumour. Should the angio-plastic processes preponderate, there will be formed only a little connective tissue but a rich supply of vessels; and such are the phenomena, as Apolant has already described them, in the case of the hæmorrhagic tumours, for we find in them a very delicate stroma (fig. 13) which can only serve in an insufficient manner as a support and a stay to the numerous vessels. Slight stagnation can then easily lead to œdema of the tissues surrounding the vessel, increased blood-pressure to dilatations and ruptures of the vessels themselves. The nourishment of the tumour will at first be amply provided for through the good blood supply. Interferences with the nourishment first arise as the result of the œdema and hæmorrhages. Thus we can explain all the main features of the hæmorrhagic tumours from the nature of the stroma reaction. It is worthy of note that this reaction is retained most tenaciously through numerous sub-transplantations and together with the specific appearance of the epithelium preserves above all the likeness of the inoculated tumours to the sporadic tumour.

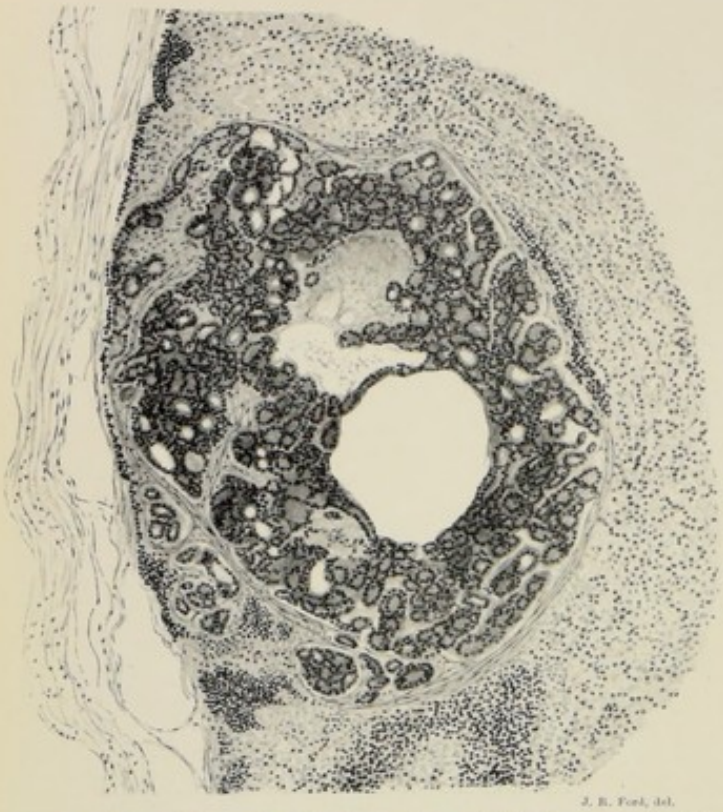
Accordingly, I believe that we may assume a special "specific stroma-reaction" which is an immanent and transmissible quality of some of the epithelial cells and an essential characteristic of this group of hæmorrhagic tumours in mice.

I consider it very possible that the nature of the stroma-reaction plays a prominent part in the establishment of tumour particles after implantation. A too powerful reaction can perhaps lead to absorption, a too

weak, to insufficient nourishment and death of the implanted tissue. The nature of the stroma-reaction may have something to do with the facts that the hæmorrhagic tumours have been found so difficult to transplant and the percentage of successful inoculations has not yet risen to a maximum. With regard to transplantation, we must consider not only the power of multiplication of the epithelial cells, but also the specific stroma-reaction, and since the latter manifests itself in the tissues of the new host, we may presuppose a certain capability of reaction on their part. I think it is conceivable that this is an essential factor in determining individual susceptibility to implantation, and accordingly, that a tumour only continues to grow when it finds adequate power of response in the connective tissue to the stimulus which elicits the specific stroma-reaction. Indeed, it seems to me not impossible that the artificially induced immunity depends upon a change in the capability of the connective tissues to provide the stroma. Since experiments on such resistant animals with a view to demonstrating the presence of bodies specifically directed against the cancer cells have been unsatisfactory up till now, we must entertain such a possibility, although we must always be conscious of the hypothetical character of such representations. This possibility has already been emphasised by Bashford, Murray, and Cramer.

We see similar differences in the effects on the connective tissues in the case of human cancer, and can explain the formation of scirrhous and medullary forms of carcinoma in the same way, while certain other forms of tumour have been termed angio-plastic. Further, there are carcinomata that act directly on the connective tissue of bone causing it to proliferate, so giving rise to the so-called "osteo-plastic carcinomata."

The stand-point has been adopted above that all our hæmorrhagic tumours, even those of adenomatous structure, are malignant. The fact, also mentioned above, that they often recur after operative extirpation favours this view. Infiltrative and destructive growth is as a general rule less prominent in the mouse carcinomata than in their human equivalents, perhaps because the site where they most frequently occur, the subcutaneous tissue, is extraordinarily loose in mice and offers only very insignificant resistance to the growth of the tumours. But it is not altogether lacking, and at the margins of the hæmorrhagic tumours we find places on careful search where the columns of epithelium infiltrate the neighbouring tissue. Thus fig. 14, shows such a margin of tumour 88, infiltrating muscle fibres. In the young marginal undifferentiated



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FIG. 15.—Mouse. Tumour $\frac{2}{0}$. Spontaneous hæmorrhagic tumour: metastasis in lymph-gland in neck. $\times \frac{150}{1}$.



J. R.

FIG. 16.—Mouse. Tumours $\frac{63}{0}$. Metastasis in lung of spontaneously affe mouse. Embolus covered by proliferated endothelium. $\times \frac{350}{1}$.



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FIG. 17.—Mouse. Tumour $\frac{63}{0}$. Metastasis in lung. (*b*) Healed nodule enclosed by sclerosed hyaline connective tissue. (*a*) Healthy embolus breaking through vessel wall. $\times \frac{350}{1}$.



FIG. 18.—Mouse. Tumour $\frac{20}{1}$. Metastasis in lung. Growth filling and distending a branch of the pulmonary artery. Growth has broken through into a small bronchus. See fig. 19. $\times \frac{20}{1}$.



FIG. 19.—Mouse. Tumour $\frac{20}{1}$. Metastasis in lung. A bronchiole filled with growth. The bronchial epithelium is still intact. Lower part of fig. 18 under higher magnification. $\times \frac{500}{1}$.

portions the structure is more alveolar than in the rest of the tumour, which on the whole is adenomatous. The insignificant amount of infiltrative growth represents a difference of degree only and not of principle between the mouse and human tumours. The power of metastasis formation proves to us above all else the biologically malignant character of mouse tumours. It has just been mentioned that metastases in hæmorrhagic tumours were found chiefly in the lungs and only once in the lymphatic glands, and that they are partly of adenomatous, partly of alveolar structure, frequently exhibiting likewise a hæmorrhagic character. A picture of the adenomatous structure of a lymphatic glandular metastasis in hæmorrhagic spontaneous tumour 25 is reproduced in fig. 15.

Metastases of the lung arise as in man through the conveyance of particles of tumour by the blood stream, such as may be discovered occasionally in the branches of the pulmonary arteries. We know especially through the researches of M. B. Schmidt, that by no means every cancerous cell embolus leads to metastasis formation in man. Many are invaded and organised by the connective tissue growing in from the arterial intima in the course of which process the epithelial tumour elements may perish. In hæmorrhagic tumour 63 quite analogous observations were made. Thus in figs. 16 & 17, the branches of the artery in the lung are clearly visible owing to staining the elastic lamina with resorcin-fuchsin. They contain cancerous emboli which are clothed over with new endothelium derived from the vessels. In fig. 17 a place is drawn where such a cancer embolus is completely traversed and organised by hyalin degenerated connective tissue. In another place, however, fig. 17 (*a*), the vessel wall is pierced by the carcinoma which has an adenomatous structure, so that it spreads farther in the lung. These illustrations prove also that in spite of adenomatous structure the tumours have the power of destructive growth, but above all they afford a further important analogy with the facts observed in man. Retrogressive changes in cancer emboli in the lungs in mouse carcinoma have already been mentioned by Lubarsch. If once the artery wall is pierced large lung metastases may follow, in the case cited of tumour 63 they were also present in other lobes of the lung and were successfully transplanted. A similar metastasis with secondary irruption into a small bronchus is shown for the hæmorrhagic tumour 19 (figs. 18 & 19).

With regard to the biological behaviour of the hæmorrhagic mouse tumours, a great number of the observations made on other kinds of

tumours have been confirmed for them also. These observations have thus been put on a still broader basis and acquired more general application. The hæmorrhagic tumours are certainly always "mammary" tumours, and as such almost exclusively found in elderly females, but in spite of that, as in the case of other mouse cancers, experimental transference succeeds just as well to male as to female animals. Old age also does not render animals more suitable for transplantation, youthful animals of 8 to 14 weeks, before puberty, give the same and in many cases better results than the older animals. Even still younger animals may be successfully inoculated, but since they are less capable of withstanding all injurious influences as well as infectious diseases, especially enteritis, series of experiments in which they have been employed often lose in validity through great mortality. Cachectic animals often give a negative result, as Bashford and Murray have found in other forms of cancer.

English mice from various breeding institutions were used, they differ from the German tame mice in that they are procurable in the greatest variety of colours, *e.g.* black, chocolate-brown, yellow, grey, and mixtures with white, and of course pure white : this colouration is very convenient for distinguishing them. On one hand the colour does not appear to exercise any marked influence on the positive or the negative result of transplantations, and on the other hand, no marked differences have manifested themselves between the mice of separate English breeding institutions. All the same, as a rule, mice are preferred, for the object of further transplantation, from the same source as that from which the mouse bearing the spontaneous tumour in question originated. Attempts to transfer the tumours to Berlin mice are being made.

In respect to the influence of the doses, judging from single records, I got the impression that lesser quantities yielded tumours in a greater percentage, but when I add all my reliable records of experiments made to elicit the effects of this influence, there is no difference. It was as follows :—

In large doses (0·3 ccm.) of 18 inoculations, 5 tumours, *i. e.* 28 per cent.

In medium doses (0·15 ccm., 0·1 ccm., 0·025 ccm.) 126 inoculations, 31 tumours, *i. e.* 24 per cent.

In small doses (0·05 ccm., 0·025 and 0·01 ccm.) of 312 inoculations, 76 tumours, *i. e.* 22 per cent.

A preponderance in favour of the large doses is not revealed by such trifling differences, if we take into consideration the smaller number of

experiments with large doses and the fact that they happened to be made with tumours which yielded high percentages of successful inoculations. But these results do not necessarily contradict the explanation given above, of the reason for the failure of earlier attempts leading to the view that the hæmorrhagic tumours could not be transplanted, for, the primary tumours as a whole also behave somewhat differently to those that have been propagated for longer intervals, and it is conceivable that they gradually diminish in their sensitiveness to the results of inoculating large doses.

Since we have become acquainted with the facts, through Bashford and Murray's researches, that an increase of resistance to tumour transplantation can be produced by preliminary treatment with the blood of normal mice, the idea was suggested of bringing the poor transplantability of the hæmorrhagic tumours into relation with the amount of contained blood, and, in practice, as far as possible, portions free from hæmorrhage were chosen for transplantation. On this account I made a series of experiments with tumours 50 and 39, in which as far as was possible I separated the hæmorrhagic from the hæmorrhage-free parts, and inoculated with both an equal number of mice. From the hæmorrhage-free places, in experiment 39/6 I, only one tumour developed among 13 mice, and from the hæmorrhagic parts no tumour developed among 14 mice. With such a low percentage of successful inoculations a conclusion is of course unjustified. In experiment 50/6 E six tumours (*i. e.* 46 per cent.) developed among 13 mice, into which was injected material freed as far as possible from blood, while from 13 injections of hæmorrhagic material, five (*i. e.* 38·5 per cent.) tumours arose. So far as the small number of experiments allow one to judge, no obstruction to the growth of the tumour occurred through the injection of the mixture of fresh and old blood simultaneously with tumour tissue; and we may assume that the protective effect of the blood injection takes its rise not in a direct hindrance of growth, but in secondary reactive phenomena on the part of the organism. Perhaps, too, these reactive phenomena may favour the absorption of tumours. In the case of tumour 50 and Jensen's tumour, I have injected 0·025 ccm. of tumour pulp alone, and together with an equal amount of normal mouse blood. The experiment with 50 is unfortunately not reliable, in consequence of septic infection. In the case of Jensen's tumour (95 G) five tumours developed in 20 control mice, in which the tumour was inoculated without blood, three grew progressively, and two were absorbed quite at the beginning. In the series to which blood had been added, ten tumours occurred in 20 mice, but on reaching

the size of a pea or a bean or over, succumbed to absorption *in toto*. I might add here that I also instituted experiments to ascertain the relation of this blood immunity in the case of hæmorrhagic tumours. For this object I inoculated mice eleven days after injection of 0·5 ccm. blood with tumour 50. There developed in this series (50/7 D) two tumours in eight mice (in the control experiment, 3 in 8), in another attempt (50/7 C) four tumours in 10 mice (control, 5 in 16). The difference is too small to reveal any decided influence from the preliminary treatment with blood. Whether the increase in the resistance demonstrated by Schöne after preliminary treatment with embryonic emulsion holds good also in the case of hæmorrhagic tumours, I must unfortunately leave unsettled, because an experiment in that direction was rendered abortive through septic infection of the controls.

I now turn to results of multiple inoculations, which we can subdivide into simultaneous and successive. Through simultaneous inoculation on each flank of the body, I have been able to corroborate the earlier observations of Bashford and Murray. Recently Bridré has also announced the same results. According to these observations the two inoculations do not influence each other, each yields the same percentage of tumours as when inoculated singly. The tumour with the lower percentage never appears alone, but only in those of the mice in which the tumour with the higher percentage has also developed. If the same tumour is inoculated on either side in varying doses, it appears as if the small dose only gave rise to a tumour when the large dose did so also. Thus in a series of experiments (50/4 C) tumour pulp was introduced, on the right side in seven mice the amount was 0·1 ccm. and in eight mice 0·025 ccm., while on the left side in all fifteen mice minute tumour particles were inserted by the needle in the two series ; in the two groups on the right side there developed respectively 4 and 5 tumours, while in the two groups on the left side there were 1 and 3 tumours respectively. Those on the left side occurred exclusively in mice which had also a tumour on the right side. What seemed most remarkable of all to me was that two of the tumours on the left side first appeared about 40 days after the inoculation and then began to grow, while those on the right side were of considerable size between 14 days and 3 weeks and enlarged progressively. By reason of facts to be mentioned later, relating to multiple inoculations on successive dates, one must conceive it is possible that the small number of cells lying latent on the left side, first found favourable conditions of growth after the stronger development of the tumours on the right side. In all my other experiments

with hæmorrhagic tumours I have not observed a like belated tumour development.

I have carried out a great number of re-inoculations both in the case of mice in which one or more inoculations had been negative as well as in mice in which tumours were already growing. For the sake of brevity I will refer to "50" negatives, "50" positives, to "Jensen" negatives, and "Jensen" positives, and so on, according to the tumour to which they were negative. Owing to the relatively small proportion of successful implantations in the case of hæmorrhagic tumours, I naturally had a good many negatives at my disposal. Conclusions on the protective effects of unsuccessful preceding inoculations are only to be drawn after a careful criticism of the control experiments in untreated mice; the latter ought so far as possible to coincide with the experimental animals in origin, age, etc.—demands which unfortunately are not always easy to satisfy in practice.

As the results of re-inoculation in "negatives," I was able generally to ascertain the following :—

1. Mice which have once been inoculated in vain with small doses (0·025 ccm.) permit only very exceptionally of being reinoculated with the same, or another hæmorrhagic tumour. Thus the re-inoculation of 113 "50" negatives with "50" was only twice positive (controls, 90 mice with 26 tumours). In the figures referring to all hæmorrhagic tumours, I have only obtained three tumours in 153 negatives (*i. e.* 2 per cent.); while in 116 controls 33 tumours developed (*i. e.* 30 per cent.).

2. The re-inoculation of mice which have been negative to hæmorrhagic tumours, with Jensen's tumour is more often positive, even although there is a distinct falling behind the controls. On the one hand, 104 hæmorrhagic "negatives" when re-inoculated with "Jensen" developed 14 tumours, whereas in 56 controls there were 32 tumours. On the other hand, Jensen "negatives" are fairly resistant to re-inoculation with hæmorrhagic tumours; 25 Jensen negatives yielded only one tumour, while in 35 control mice there were 10 hæmorrhagic tumours. If we may draw a conclusion from these figures, it is that the "negatives" are protected in an extraordinarily high degree against the same tumour, and in a somewhat less degree but nevertheless distinctly against different tumours. Hence there exist for these hæmorrhagic tumours exactly the same specific conditions of growth described by Bashford, Cramer, Haaland, and Murray for various other tumours. With such material, viz. tumours yielding a low and very fluctuating proportion of successful inoculations, it is extremely difficult

to obtain certain proof that we are dealing with an artificially induced resistance, and not with mice naturally resistant from the outset which have been picked out through their negative behaviour to previous inoculation as Hertwig and Poll seem to assume. But if we refer to the extensive observations of Ehrlich, who has obtained a high degree of protection against carcinoma with 100 per cent. of successful inoculations, through preliminary treatment with hæmorrhagic tumours, and to the recent results of Bridré, who with minute single doses (by canula) obtained only weak protection and after repeated inoculation and with larger doses complete immunity against tumours with success on inoculation in 100 per cent., we may certainly conclude from our own experiments cited above that the resistance is acquired or artificially increased. In Ehrlich's sense there exists a pan-immunity, viz. a protection which extends also against other sorts of tumours—but this is slighter than that against the tumours used in the preliminary treatment. We can, therefore, in addition to the pan-immunity, speak of an even stronger specific immunity. This is revealed, however, only when the immunisation is not induced by too large doses.

Unexpected results attended the re-inoculation of tumour mice with the same or another tumour. Ehrlich concluded from a long series of experiments that in the case of a mouse with a rapidly growing tumour a re-inoculation is unsuccessful with few exceptions, and he explained this phenomenon by assuming that the tissue implanted at the re-inoculation is deprived of the necessary means of sustenance by the demands made on the food-supply by the tumour already present. Accordingly, Ehrlich concluded this was a special form of immunity which he named "atreptic." As early as 1904, Bashford and Murray (First Scientific Report, p. 15) communicated the fact that 14 days as well as 11 weeks after a primary successful inoculation, re-inoculation was successful—*i. e.*, both in the case of small and large tumours. Later Michaelis also observed no protection in tumour-mice when reinoculated, and he supposed that the low proliferative powers of the tumours used by him might be the cause of his anomalous results, for Ehrlich had expressly stated that his results held good only for tumours of quick growth. I had already obtained all the essential details of the results I record below, when Borrel reported the successful re-inoculation of twelve tumour-mice with the same tumour. In a second series, Borrel had reinoculated with an adenocarcinoma five mice with large tumours, three with medium, and four mice which had been negative to Jensen's

tumour, and observed that tumours developed in a manner exactly parallel to those arising at the first inoculation. Afterwards, Hertwig and Poll announced similar experiments showing that the development of tumours at the first inoculation offers no protection against re-inoculation, that an influence obstructing the growth of the second tumour is not exercised, in many cases the latter even overtakes its twin. Moreover, a third inoculation might be positive in mice in which two tumours were already growing. Quite recently Bridré, in Borrel's laboratory, obtained analogous results, from which he drew the conclusion that mice bearing tumours are at least as receptive to a re-inoculation as normal mice are to a primary inoculation; the percentage of tumours in mice already bearing growths was indeed a little higher than in control normal mice (57 per cent. against 47 per cent.). In one series there were 50 per cent. of successful inoculations in tumour subjects, but in the control normal mice only 8 per cent. Lewin has also lately corroborated this observation in the case of rat tumours.

Having regard to the extensive nature of my experiments, I feel justified in coming to the conclusion that a mouse with a tumour offers conditions far more favourable to subsequent inoculations than those obtaining in normal mice. The numerical differences are naturally more striking when tumours yielding a low percentage of successful inoculations in normal mice are used, as is principally the case with the hæmorrhagic tumours employed by me. Thus, out of a total of 38 mice with hæmorrhagic tumours, 25 (*i. e.* 66 per cent.) developed hæmorrhagic tumours on subsequent inoculation, while out of the corresponding 82 control normal mice 22 (*i. e.* 27 per cent.) developed tumours. Yet this manifestation is not restricted to subsequent inoculations with hæmorrhagic tumours, but is exhibited also on inoculating Jensen tumour into mice already bearing hæmorrhagic tumours. The sum total of such re-inoculations made by me are as follows:—In 82 mice with primary tumours there arose 53 secondary tumours (*i. e.* 64 per cent.), while in the 166 control mice corresponding to them there developed only 60 tumours (*i. e.* 37 per cent.).

The several series thus aggregated together differ very markedly among themselves; for example, in some series the first inoculation was positive in only one or two mice and in them secondary inoculation was again successful with astonishing regularity, while the remaining mice, *i. e.* those negative to the primary inoculation were, as the summary above given shows, nearly invulnerable against re-inoculation.

It is perhaps necessary to emphasise that mice which had previously been inoculated unsuccessfully were of course not used as controls in these experiments, but mice entirely untreated hitherto. In other series of experiments the primary inoculations had yielded good results, and with them the receptivity for re-inoculation of the mouse already bearing a tumour was confirmed. I should like to illustrate such an experiment by inserting here a photographic reduction of the protocol-chart (fig. 20).

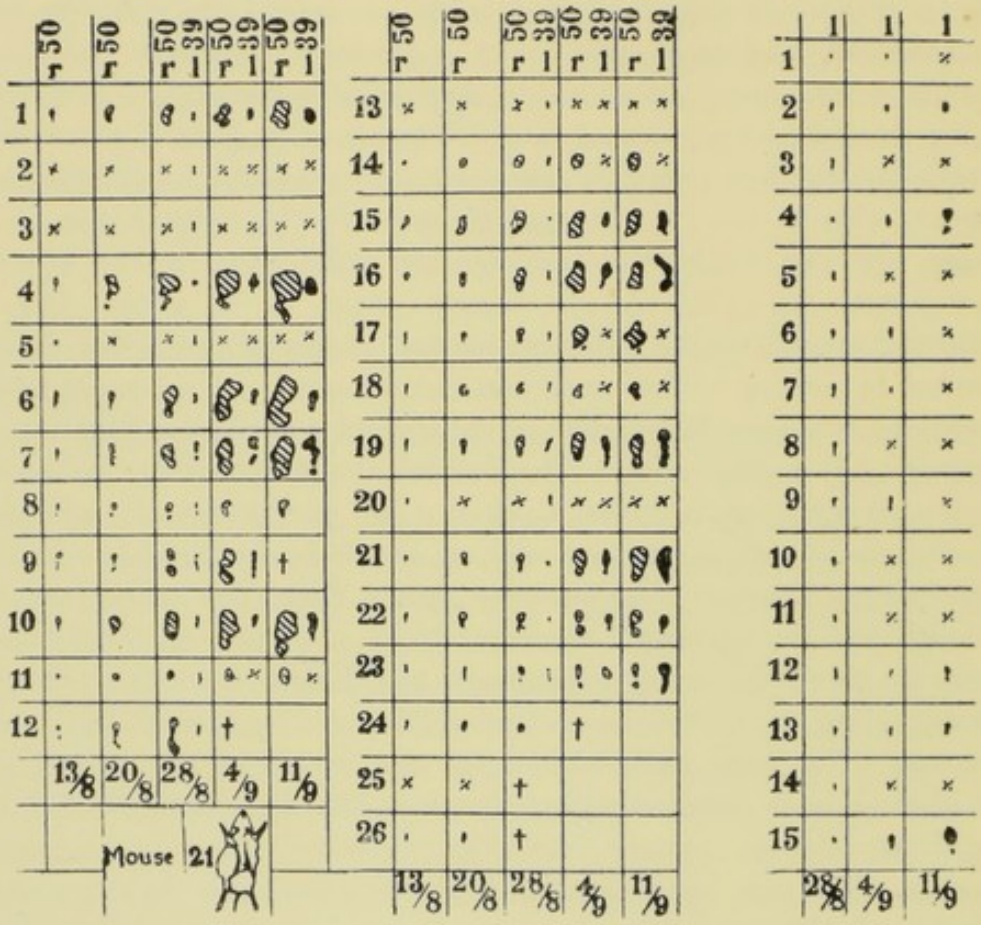


Fig. 20.—Explanation in text. Experiment 50/6 B, and Controls 39, 50.

Experiment 50/6 B.—On 31st July, 1907, twelve mice were inoculated on the right flank with 0·1 ccm. and sixteen mice with 0·025 ccm. of 49 days' growth of tumour pulp obtained from an old tumour (50/5 A, No. 43). The growth of the tumours which developed in the 26 mice remaining alive (Nos. 1 to 12 with the large ; Nos. 13 to 26 with the small dose) is easily followed on the above chart

under the rubric "50 r," the date being printed beneath each column Twenty tumours (*i. e.* 77 per cent.) developed in the 26 mice, one of the highest percentages of successful inoculations I have obtained for hæmorrhagic tumours. It will be perceived that these tumours grew remarkably well. To enable a better judgment it may be added, that the tumours were protocolled by drawing them in their natural sizes. As scale for the reduction mouse 21 was photographed at the same distance on the 11th September, 1907. One can discern in it the bilateral tumours agreeing in size and form with the chart. On the 21st August, 1907, three weeks after the primary inoculation, all the mice were reinoculated on the left side with 0·05 ccm. of another hæmorrhagic tumour 86 days old and of 3·8 grm. weight, viz. 39/4 H, No. 19. The fate of this secondary inoculation can be followed on the table under "39 l" depicted in black. As controls, 15 mice somewhat older were inoculated with the same dose and in the same manner; the tumours developed in them are reproduced photographically in the right column of the chart (Experiment 39/5 Q); in them five tumours developed (33 per cent.) which corresponds to the average result for this tumour ("39"). The secondary inoculation succeeded 12 times (70 per cent.) in the seventeen mice having primary tumours that lived long enough after the re-inoculation, while it failed in all of the five mice remaining alive in which the primary inoculation had failed. In addition to the numerical difference, the greatly accelerated rate of growth as contrasted with that in the controls is most remarkable, and this phenomenon has been observed, if not in so pronounced a degree, in other series of experiments.

From a critical stand-point there are two likely explanations of the undoubted enhanced receptivity to secondary inoculation in the case of mice bearing tumours. Either those animals have been picked out by the primary inoculation, which are naturally suitable for tumour implantation, and, therefore, they are susceptible also to a second inoculation, or, the suitability for implantation is increased in certain cases by the primary growing tumour. In short, we have to do either with a natural or with an artificially induced or increased susceptibility to inoculation. When I incline towards the second opinion, I am conscious that my observations do not afford an absolute demonstration of its truth. Still a number of different facts can be advanced in its favour.

1. Chance must have ruled to an extraordinary extent if such a high percentage of susceptible animals were accidentally grouped together

as in the chart reproduced, while on the same day mice of the same breed under like conditions of life yielded results so very much worse in other series of experiments. Bashford and Murray have given sufficient reasons to justify attributing such divergencies in the success of inoculation to fluctuations in the proliferative energy of the tumour cells themselves ; but, the control experiment of the secondary inoculations shows that the tumour had not a particularly high percentage of successful transplantations at that time, and in spite of this yielded a high percentage in the mice already bearing tumours.

2. The secondary inoculation in mice already bearing tumours is followed by growth so rapid that the secondary tumours not only overtake their fellows developing in the normal mice of the control experiment, but often put the primary tumours in the re-inoculated mouse also in the shade.

3. A mouse which had been negative to the primary inoculation of tumour "50" and was re-inoculated with it, exhibited the exceptional phenomenon of being positive to the secondary inoculation, and also developed a second "50" tumour after a third inoculation. In this case a primary susceptibility was put out of the question by the fact that the primary inoculation remained negative, although the negative result of the primary inoculation may have been partly determined by the nature of the tissue implanted. After the first re-inoculation had succeeded the second was likewise positive (50/4 J, No. 12).

4. In one series (50/5 C) I had collected along with mice that had resisted inoculation with Jensen's tumour, other mice in which large or small Jensen tumours had been spontaneously absorbed. In five of the mice in the latter group a small remainder of the Jensen tumour could be detected. When secondary inoculations were made in them with tumour "50," tumours developed in three of the five, and in each of the other three the remainder of the Jensen tumour began to grow again. In the two remaining mice, in which the re-inoculation with "50" was negative, the Jensen remainder stayed unchanged in one, and, in the other was completely absorbed. It may be added by way of comparison that in the other group of mice of the same series in which there had been complete absorption of Jensen tumour, one positive inoculation of tumour "50" occurred in eight mice, and that in the mice which had resisted inoculation with Jensen's tumour also one tumour arose from the re-inoculation with tumour "50," while in the seventeen control normal mice eight positive results were obtained. From this experiment

I received the impression that through the development of tumour "50" the little "Jensen" remainder was awakened to renewed growth.

5. It should be remembered here in connection with the above mentioned observation, that small doses inoculated on one flank of the body simultaneously with large doses of tumour material on the other flank, after lying latent for a strikingly long period, may suddenly begin to grow after the tumour on the other side has attained a pretty considerable size *.

Whether the facts enumerated above be regarded as evidence sufficient to make it probable that a heightened suitability for growth has been induced in the case of mice already bearing tumours or not, the fact appears incontestable that mice already bearing tumours yield a higher percentage of successful inoculations, and offer a more receptive field for inoculations, with the material and the technique used in London than untreated mice. The question therefore arises as to how the difference in Ehrlich's results is to be explained. As Ehrlich only propounds the theory of atreptic immunity for the rapidly growing tumours, the explanation may be sought in slower growth; but the accompanying table shows, like many other experiments, that rapidly growing tumours were used by me also, and that often the secondary tumour exhibited its greatest power of growth at a time exactly coinciding with the most rapid growth in the primary tumour, and, therefore, the parallelism noticed by Borrel was observed. The primary tumours in each of the five mice in which the secondary inoculation was negative (nos. 8, 11, 14, 17, and 18) exemplify slowness of growth, and the results

* I have learned through a personal communication from Dr. Bashford that since my departure further light of a significant character has been thrown on these questions. It has been shown that mice in which tumours are growing satisfactorily and which therefore have proved their receptivity, can be rendered immune by treatment with badly growing spontaneous tumours, so that they become resistant to virulent tumours. The growing tumour thus does not hinder the bringing about of resistance. These important results contradict above all things the views of Hertwig and Poll, who reject the idea of an acquired resistance and lay weight chiefly on an inborn non-receptivity. Support is thus also given to the view that in special circumstances, in spite of successful transplantations, a resistance to after-inoculation can be induced; hence the question here is one of acquired active resistance, not of "atreptic immunity." Therefore it is very improbable that these animals, so easily rendered resistant and which are used for successive inoculations, should have inherited a quite special disposition to tumour growth. More definite information with regard to that experiment will be issued from the London Institute.

disagree entirely with Ehrlich's conception of atrepsia. It may be remarked that these five mice were reinoculated a second time on the 14th Sept. 1907, and the four that continued to live showed themselves refractory, while the further growth of the primary tumours was inconsiderable and in two there was a decided diminution.

The phenomenon is not confined to the hæmorrhagic tumours but is common to all tumours. In the above calculation I have limited myself to the experiments I myself have executed. But at the London Institute experiments have been carried out with the most divergent varieties of tumour. They permit of the generalisation that, quite independent of the character of the tumour used for primary or secondary inoculation, tumour subjects are for the most part receptive to tumour inoculation in an equal or a higher percentage than normal mice. Should further experiments demonstrate that this is due not merely to an artificial separation of susceptible animals, but really to an artificial increase in susceptibility, we must interpret the increase in suitability to mean that certain hindrances to growth or protective provisions are completely destroyed through the growth of the primary tumour. This view agrees at any rate better with the facts of metastasis formation than the conceptions involved in Ehrlich's atreptic immunity. According to Ehrlich's conceptions the most malignant, most rapidly growing, tumours are those least likely to lead to the formation of metastasis ; as a matter of fact, such a conception is in very marked contradiction to the experience accumulated from human pathology.

Since the facts which have already been detailed in previous pages render it improbable that the difference between Ehrlich's results and mine is due to a difference in material, it is more probable that it arises in the different technique employed. Since, as was the case with Ehrlich's tumours, I have used tumour pulp also for the most part, this aspect of the technique is not the important point of dissimilarity. The influence of the size of the dose appears to me to be of much more weight, and in principle I have formed the same opinion as Borrel and Bridré. Big doses of tumour pulp are absorbed in great part, and only a small proportion of the cells implanted serves as a matrix for the tumour eventually arising from the mass inserted. The absorption of such a great amount of tumour tissue seems to increase the resistance of mice to tumour implantation. Even in the case of mice in which a primary tumour has established itself and is growing, although this increased resistance no longer influences the tumour already established, it affords effective protection against the tumour cells of a new, *i. e.*

secondary implantation establishing themselves. There is nothing strange in this reasoning, for we know that treatments which would induce resistance in normal animals have, as a rule, no influence on a tumour that has already established itself, and started growing: the protection is directed against the establishment of newly introduced grafts only, not against their continued growth once they have established themselves. In some series of experiments it seemed, too, as if the secondary inoculation yielded worse results when the primary tumour had arisen from the introduction of big doses of tumour tissue; but experiments especially directed to elucidating this point have yielded no conclusive proof, because the series of experiments were unsatisfactory. Eight tumours arose from 18 inoculations with 0·3 ccm., and in the eight mice three secondary inoculations were positive. On the other hand, out of 31 inoculations with 0·05 ccm. only nine tumours were obtained, and in these mice the secondary inoculations yielded only two positive results. These experiments must be repeated at a future date on a larger scale †. The theory of atrepsia seems to have received great support through the notable experiments of Schöne. He showed that the resistance to secondary inoculation disappeared after removal of the primary tumour by operation; the tumours had been obtained by inoculation according to the Frankfort technique. Still I maintain that it is not necessarily proved that the mere disappearance of the influence exerted by the tumour effected this revolution in the mouse organism. If the explanation I have given in accordance with my results is right, viz. that the negative result of the secondary inoculation is not referable to atrepsia but to acquired resistance induced by the primary introduction of larger doses, then it is possible to explain Schöne's facts by assuming that resistance can be destroyed by drastic operation; and this is moreover, as a matter of fact, a possibility which Schöne himself entertains.

The fact ascertained with material in London and by the methods there applied, that tumour-subjects offer the best chances for tumour implantation, suggested utilising such mice for practical purposes. It seemed conceivable that they might offer a specially suitable fostering soil for transplanting primary tumours also, which might be of great

* Cf. p. 36.

† Dr. Bashford has recently told me that after experiments by Dr. Russell on other tumours, a distinct effect following the inoculation of big doses was not forthcoming Perhaps a difference exists here too between primary tumours and those which have been propagated for a long time.

advantage in view of the low transplantability often exhibited at the inception of propagation. Experiments made by me for this purpose with two spontaneous tumours did not yield exactly encouraging results. But, in both instances they were tumours such as are generally transplanted with difficulty or not at all. One of them was a sarcoma with spindle-cells (No. 92) of the axillary region, which was analogous to that recently described by Ehrlich and Apolant, in its periphery remains of mammary tissue being enclosed. No tumours developed in the control mice. The implanted tumour tissue was obviously living in one of the six tumour mice employed, 39 days after inoculation. The other primary tumour (No. 91) was hæmorrhagic, and was successfully transferred in two out of eleven control mice and in one out of eight tumour-mice. Hertwig and Poll report some unsuccessful attempts to transplant a primary tumour into mice already bearing inoculated tumours. Further experiments will show whether primary tumours behave differently in this respect from the inoculated tumours, or whether better results can be obtained.

It is also conceivable that in yet another respect the receptivity of tumour-mice may be used, viz., to overcome the difficulties that so often attend transplantation into strange races. But as I have made no experiments in this direction, I only mention the idea by the way.

It is incumbent on me at the conclusion of these observations to express my most respectful thanks to the Committee of the Imperial Cancer Research Fund in London for permission to work in their admirably appointed laboratory. Above all I am indebted to the Director of the Laboratory, Dr. Bashford, for placing the material and the facilities for experiment at my disposal, and for his aid and support and ever ready advice, for which I return my warmest thanks. The Assistants of the Institute, Dr. Murray and Dr. Haaland, have also most courteously helped me in my researches.

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THE EFFECTS OF SURGICAL INTERFERENCE WITH THE BLOOD SUPPLY ON THE GROWTH OF TRANSPLANTED CARCINOMATA AND SARCOMATA.

By W. H. BOWEN, M.S., F.R.C.S.

This Paper deals with an investigation undertaken to elucidate the result of obliterating the blood supply of a tumour. The tumours were of three kinds—first, those resulting from the transplantation of strains of an alveolar carcinoma*; secondly, of similar transplantations of two adeno-carcinomata of special interest because of the tendency to hæmorrhage and formation of cysts containing blood; and thirdly, of a transplantable squamous-celled carcinoma. The observations are being extended to a transplantable spindle-cell sarcoma. These tumours were taken as being comparable to different varieties of malignant growth, characterised by distinctive relations to the blood supply.

For the object of this investigation, the anatomical conditions provided by tumours resulting from artificial propagation seem ideal. From a surgical standpoint the tumours are easily separated from their surroundings at the time of operation and their blood supply can be entirely cut off. I mention particularly that these ideal conditions exist *at the time of operation* since there comes a time when such complete separation is impossible, or attended with such great difficulty as to prevent the attainment of the object of the experiment. In transplanted carcinomata in the mouse infiltration of surrounding tissues, muscle and skin, is usually a late manifestation. In a few cases where we have attempted operation with such conditions present, we have been compelled to kill the mouse during the operation owing to the attendant difficulties. Our aim has always been to completely

* Jensen's tumour.

destroy the continuity of the vessels passing to the growths and to watch the result in a set of uniform experiments.

Some anatomical considerations are necessary to the proper com-

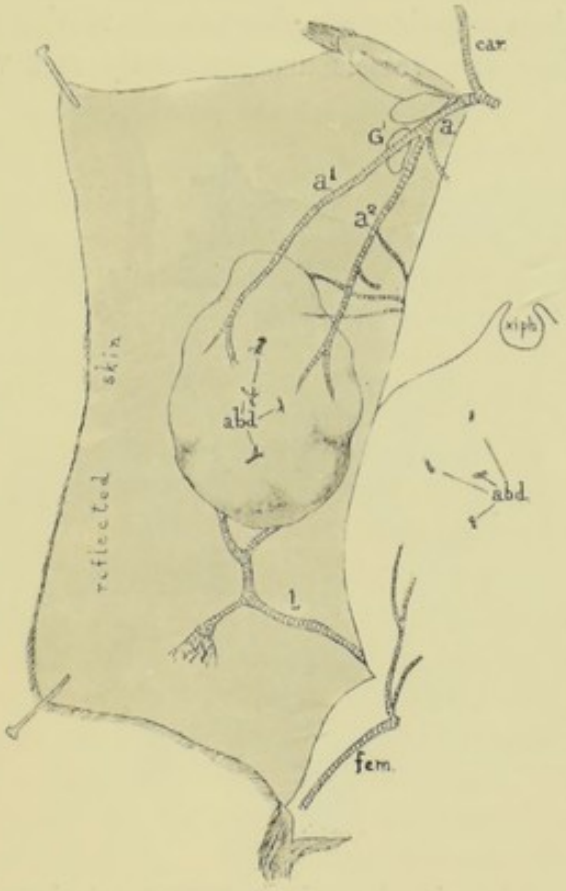


FIG. 1.—Dissection to show blood supply to tumour. The skin over the front of the abdomen is reflected with the tumour attached. In separating the tumour from the abdominal wall some adhesions of its capsule to the muscular parietes have been torn through. Small blood-vessels are divided in effecting this separation. *a*. Main axillary supply to tumour: this comes off from the vessel of the arm. a^1. Anterior branch of main axillary supply. a^2. Posterior branch of main axillary supply. *abd*. Small twigs which ran in the fine fibrous adhesions uniting the capsule of the tumour to the abdominal wall. *Car*. Main vessel of neck. *fem*. Main vessel of thigh. *l*. Main vascular supply to posterior part of tumour coming from a lumbar vessel. *ziph*. Xiphoid cartilage.

prehension of the technique of the operations. The tumours were obtained as the result of inoculation of tumour tissue by a hypodermic needle introduced at the groin pushed forward to the axillary region, the tumour cells thus introduced being deposited in the lax tissues

lying between the skin and the thoracic and abdominal parietes. A number of dissections show that the nourishment of the growth resulting on the side of the body is provided by vessels whose arrangement is shown in the figures drawn from fresh dissections. From the axillary region two vessels pass downwards to the anterior end of the tumour. These two vessels are constantly met with, and result

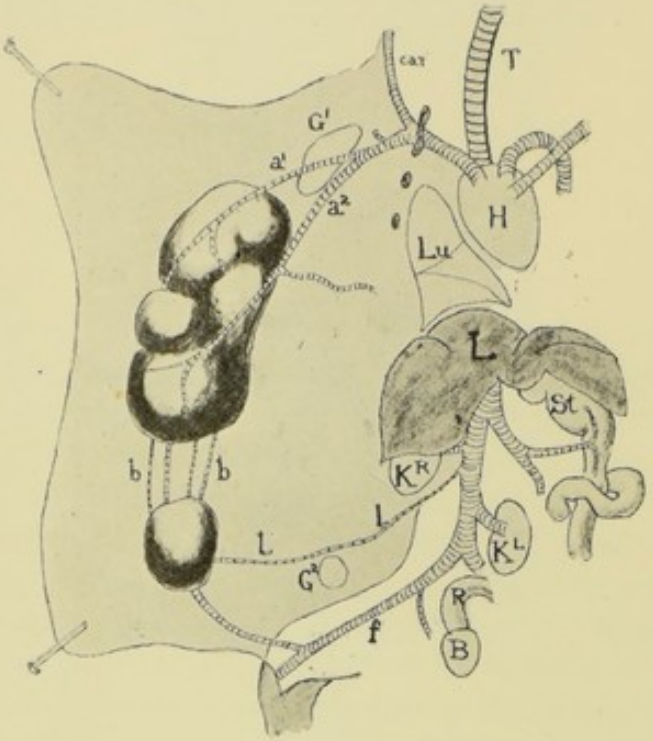


FIG. 2.– Dissection to show blood supply to tumour. a^1. Anterior branch of main axillary blood supply to tumour. a^2. Posterior branch of main axillary supply to tumour. *b.b.* Fibrous bands running between two separate tumours. With these connecting bands run small blood-vessels. *car.* Main blood-vessel of neck. *f.* Main vessel of thigh. *l.* Vascular supply to posterior part of tumour coming from a lumbar vessel. *B.* Bladder. G^1. Axillary gland. G^2. Inguinal gland. *H.* Heart. K^r. Right kidney. K^l. Left kidney. *L.* Liver. *Lu.* Lung. *R.* Rectum. *St.* Stomach. *T.* Trachea. The large vascular supply to the tumour coming from the main vessel of the thigh immediately above the knee-joint is one of the occasional sources of nourishment obtained by these tumours.

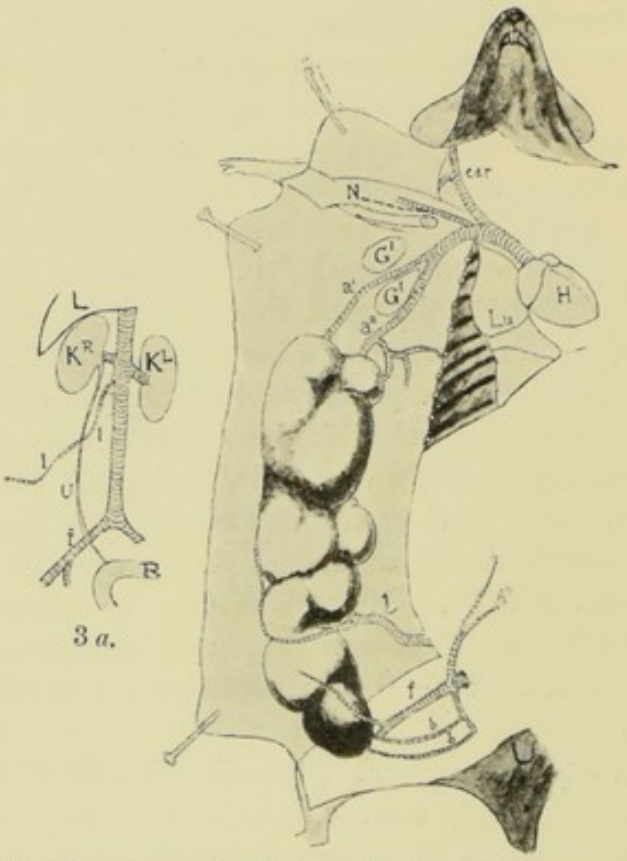
from an increase in size of the two vessels normally present. Sometimes one, sometimes the other, is chiefly instrumental in nourishing the tumour. More usually the inner one of the two (really before dissection the more ventral) provides the chief axillary supply. The nourishment of the posterior part of the tumour is derived from a

lumbar branch of the abdominal aorta arising just immediately posterior to the right renal vessels, and passing downwards and outwards across the muscles of the posterior wall of the abdomen. This also is an enlargement of a vessel normally present. These three vessels, the two axillary and the one lumbar, are the main blood supply of tumours situated on the flank. Nevertheless in the earlier experiments it was found that if they were divided, although the tumour suffered (as shown usually by diminution in size), the effect was only transient and in the course of time renewed growth followed, the reason being that running in the fine areolar tissue between the skin and the panniculus carnosus superficially, and the ensheathing muscles of the thorax and abdomen on the deeper aspect, there are fine vessels which, unrecognised at the time of operation, enlarge afterwards, and are capable of providing an increase in the blood supply to the tumour. To prevent this renewal of the supply of blood it is necessary to perform a more complete operation by dividing not only the main macroscopical blood supply but also the whole of this layer of areolar tissue. The necessity of this step as revealed in the case of tumours in mice, must not be lost sight of in estimating the utility of tying only the main supply of blood to a growth in the human subject.

In addition to the main primary blood supply enumerated, other vascular connections are established by the tumour as it enlarges. They are situated posteriorly as a rule, and come from the iliac or femoral vessels. They have been found coming also from the region of the groin, the middle of the thigh and the knee-joint ; two such cases are figured (figs. 2 & 3). Where the tumours become fixed to the parietes they derive an additional supply of blood from small vessels passing along the fibrous adhesions such as are shown diagrammatically in fig. 1 (*abd*).

In operating on mice our usual procedure is immediately the animal is anæsthetised to carefully cleanse the skin with sterilised warm saline solution. The operation is performed under ether anæsthesia. Its success depends upon three factors : delicacy of manipulation, rapidity in working, and the avoidance of all hæmorrhage. An incision is made to the mesial side of the tumour on the ventral aspect of the mouse from groin to axilla. This incision is made with a pair of sharp scissors and caution has to be exercised on account of the extreme thinness of the abdominal wall. The separation of the tumour from the abdominal and thoracic walls requires delicacy in manipulation and care in dissection.

Especially is this so where the growth has become cystic or very soft. To open the capsule of the tumour is tantamount to failure. The separation of the tumour from the abdominal and thoracic walls is best carried out with dissecting forceps, aided if necessary by fine curved scissors. The blood-vessels do not come clearly into view until this separation of tumour from parietes is complete. Complete separation of the tumour exposes the blood-vessels supplying it. These vessels are each clamped separately between two pairs of Spencer Wells' forceps and divided with scissors between the forceps. The divided ends are then twisted. No ligatures are used. The intervening areolar tissue is



3 *a*.

FIG. 3. Dissection to show blood supply to tumour.

FIG. 3 *a*. Dissection from the same animal to show the origin of the lumbar blood supply.

a^1. Anterior branch of main axillary blood supply to tumour. a^2. Posterior branch of main axillary blood supply to tumour. *b.h.* Fibrous bands connecting tumour to muscular parieties. In these bands small vessels run. *car.* Main vessel of neck. *f.* Main vessel of thigh. *l.* Main vascular supply to post part of tumour coming from a lumbar vessel. G^1. Axillary glands. *H.* Heart. K^r. Right kidney. K^l. Left kidney. *L.* Liver. *Lu.* Lung. *N.* Nerve. *R.* Rectum. *U.* Ureter.

divided with scissors or knife. The whole wound is then washed over with warm saline and the skin sutured. Healing by primary union follows.

Before concluding that the growth of a tumour has been modified by the introduction of experimental conditions, the natural fluctuations in the rate and amount of growth of tumours during artificial propagation must be allowed for. The importance of these fluctuations and their relation to spontaneous healing has been referred to in several papers * from the Laboratory of the Imperial Cancer Research Fund, and their occurrence has been confirmed by the subsequent observations of O. Hertwig and Poll†, Borrel and Bridet, C. Lewin and others, working with entirely different tumours of the mouse and rat. The events following the separation from the blood supply to the tumours have been carefully controlled in two ways. Firstly, in the case of mice bearing tumours on one side only, a large number of mice, inoculated under the same conditions and at the same time as those operated upon, were not interfered with and the behaviour of the tumours carefully observed, the tumours being drawn in natural size in the protocol books. Secondly, in the case of mice bearing bilateral tumours, the precaution just mentioned was observed but further only one of the bilateral tumours was operated upon. The charts (figs 4 & 5) reproduced from the protocols, indicate the nature of the precautions taken. In this way natural fluctuations in growth due to causes inherent in the tumour cells or in the mice, and arising independently of the operation, have been distinguished from the direct results of tying the vessels, and the indirect results arising from the absorption of tumour material. In setting forth the results of the enquiry and tabulating the cases we shall divide the experiments into two groups. Those carried out in mice with unilateral tumours and those carried out with mice with bilateral tumours. This grouping is best seen in the case of the alveolar carcinoma (Jensen) where the distinction is set out and explained. This grouping

* Trans. Medical Soc. of London, vol. xxviii., with discussion on spontaneous disappearance of tumours. Second Scientific Report, Imperial Cancer Research Fund, Part ii. April 1905, pp. 51–68 & p. 10. Roy. Soc. Proc. B. vol. 78, 1906, p. 195, & B. vol. 79, 1907, p. 164 *The Lancet*, Mar. 23, 1907. Berl. klin. Wochenschrift, 1905, No. 46, 1907, Nos. 38 & 39. Zeitschrift für Krebsforschung, 1907. Brit. Med. Journ. Dec. 1, 1906, etc.

† O. Hertwig and H. Poll, Abhandlungen der kgl. preuss. Akademie der Wissenschaften, Berlin, 1907. Borrel and Bridre, Annales de l'Institut Pasteur, 1907. C. Lewin, Zeitschrift für Krebsforschung, Bd. vi. Heft 2, 1908.

is further maintained in dealing with the adeno-carcinomata possessing a tendency to the extravasation of blood into their substance (39 & 50), but in working with the squamous-celled carcinoma we only used mice growing bilateral tumours.

I.

Alveolar Carcinoma (Jensen).

In the first group the tumours in the batch of inoculated mice were watched as a whole, so that the effect of operative interference on some tumours was contrasted with the behaviour of tumours in animals inoculated at the same time, but which were in no way interfered with. In two cases only partial obliteration of the macroscopical blood supply to the tumour was performed.

For these experiments three batches of mice were used, viz. :— 76 F, 91 D, and 93 D. The conditions relating to the tumours operated on in the batches 76 F and 91 D are shown in fig. 4, and in addition, in the case of the batch 76 F two tumours are depicted which although not operated upon are important, since their behaviour throws some light on the response of those which were submitted to operation. A tumour characteristic of the batch 91 D has also been figured in which no interference wtih the blood supply was made.

The method of examination of a batch of tumours from which some were taken for operation purposes may be exemplified by a short description of the experiment 76 F. Twenty mice were inoculated and nineteen of these were living at the end of six days when the sizes of the various tumours were charted for the first time. In 16 of the 19 mice no operation was performed. The history of these 16 mice after inoculation is as follows :—

(*a*) In 7 mice there was no growth at all.

(*b*) In 3 mice transitory tumours appeared ; one of these is figured as No. 4.

(*c*) In 4 mice tumours grew and the mice were ultimately killed and a full post-mortem examination made.

(*d*) One mouse was killed and the tumour transplanted : (it gave the batch 77 K where one tumour appeared in nine mice living. This tumour was again transplanted forming 78 I, in which batch of mice no tumours grew.)

(*e*) In one the tumour was very small and remained stationary.

[*To face p.* 152.

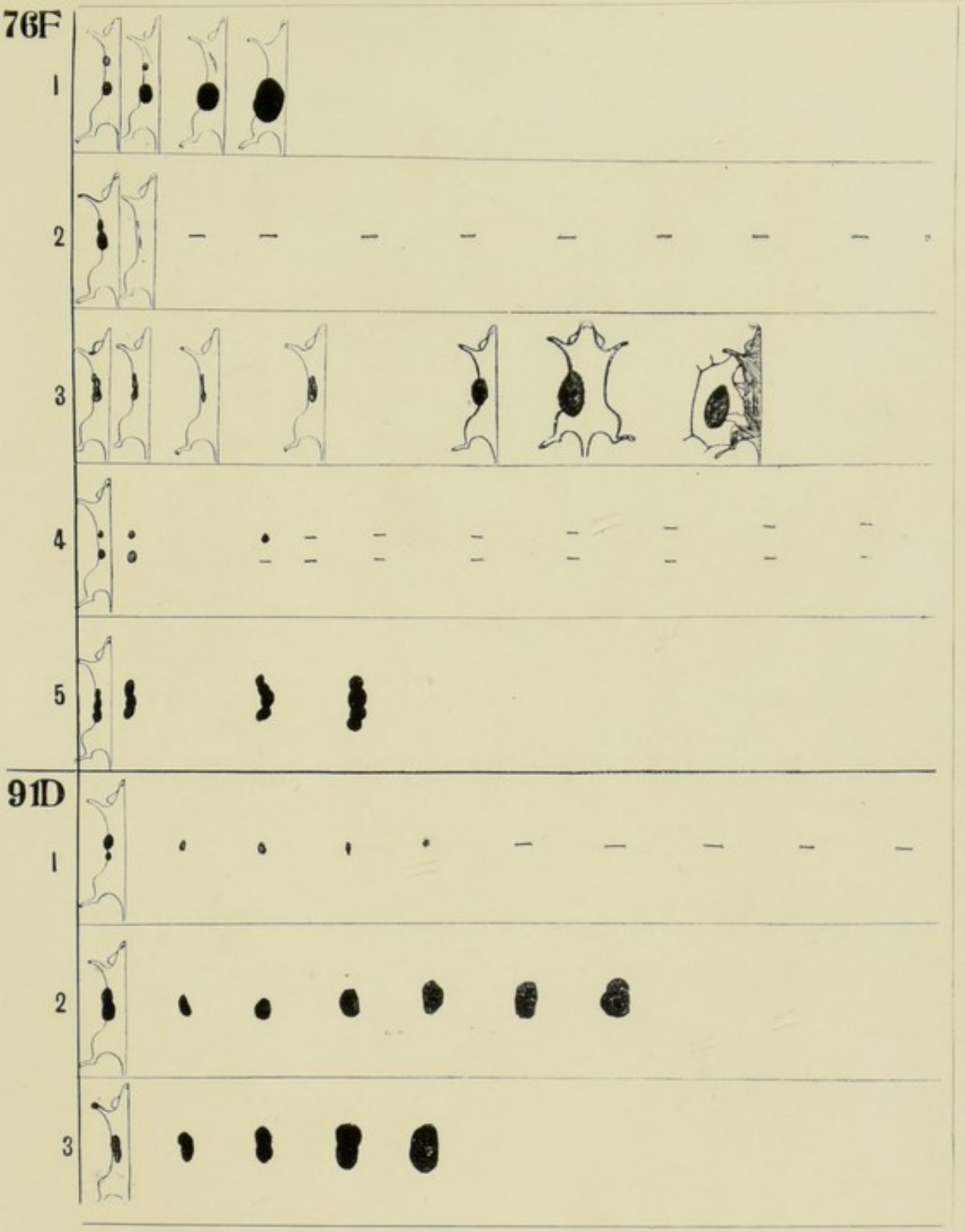


FIG. 4.—Diagrammatic representation of growth of tumours during thirty-two days after operation. The tumours are reduced to $\frac{1}{8}$ of the natural size. 76 F, 1, 2, and 3, and 91 D, 1 and 2, were submitted to operation at the time the first record of the size of the tumour was made. Whilst 76 F, 4 and 5, and 91 D, no. 3 had no operative interference of any sort and are given as examples of the fate of tumours of this batch of which the whole lot of tumours (29 in all) were similarly watched and recorded. 76 F, 1. Occlusion of axillary supply. Disappearance of axillary tumour. Enlargement of posterior tumour. 2. Occlusion of lumbar supply. Complete disappearance of tumour. Mouse living nine months later without any sign of recurrence. 3. Occlusion of axillary and main lumbar supply diminution in size of growth followed by renewed growth. 4. No operation. Spontaneous disappearance of tumour. 5. No operation. Continuous enlargement of tumour. 91 D, 1. Occlusion of axillary and lumbar vessels. Complete disappearance of tumour. Mouse living nine months later without recurrence. 2. Occlusion of axillary and lumbar vessels, slight decrease in size of tumour, followed by renewed growth and early ulceration. 3. No operation. Progressive enlargement of tumour. A comparison may be made between mouse no. 2 submitted to operation and mouse no. 3 without operation of batch 91 D. Whereas the mouse no. 2 was alive and healthy on 20.7.07 which was 31 days after the operation, mouse no. 3 had to be killed on 4.7.07 on account of the large size of the tumour.

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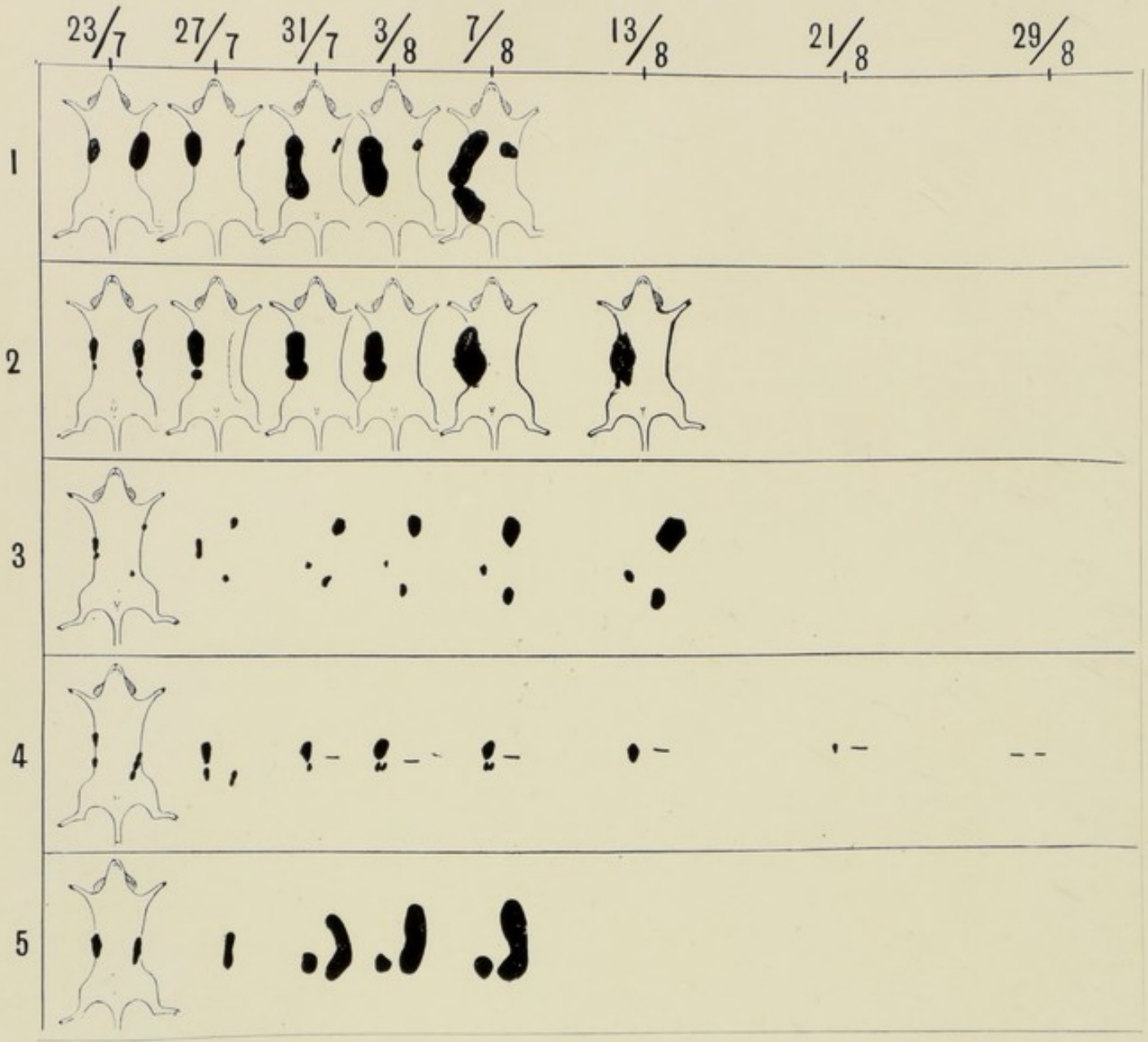


FIG. 5.—Diagrammatic representation of growth of tumours. Tumour reduced $\frac{1}{8}$ of natural size. Batch 95 E. All five mice were submitted to operation. In all the larger of the two tumours had its main vascular supply cut off, whilst the tumour on the opposite side was left alone and watched as a control to the operation. 1. Tumour of left side, being the larger, had main blood supply occluded. Marked diminution in size, followed by recurrence. Control tumour steadily grew to a large size. 2. Tumour on left side submitted to operation. Complete disappearance. No recurrence at time the mouse was killed owing to great enlargement of control tumour. 3. Right side operated on. Diminution in size of tumour, followed by recurrence of growth. Control tumour steadily grew. 4. Left side operated on. Disappearance of tumour, followed by complete disappearance of the control tumour, *i. e.*, the tumour not submitted to operation. 5. Right side operated on. Atrophy of anterior part of tumour, persistence of posterior part and slight enlargement. Progressive growth of left sided tumour.

The tumour no. 5 in fig. 6 was the tumour which reached the largest size in the batch and it was used for transplanting.

The three mice operated on are given in fig. 6 as (1), (2) and (3). In (1) the axillary vessels only were occluded, and the axillary growth disappeared whilst the tumour in the groin grew to a large size. In (2) the lumbar vessel only was divided. The tumour disappeared entirely and the mouse is still living (nine months later) without any trace of a tumour. In (3) both axillary and lumbar supplies were occluded. After a period of shrinking, active growth recurred and was associated with early ulceration. This tumour with its blood supply is shown as demonstrated at post-mortem examination.

The history of the batch 91 D is, that of ten mice inoculated, all lived and seven developed tumours. The four tumours not submitted to operation grew to a large size. One of these is shown in fig. 6 as (3). Of the three tumours where the blood supply was obliterated, two are pictured. In both, the main macroscopical supply was dealt with. In (1) the tumour disappeared entirely and the mouse still lives nine months afterwards without any sign of a growth. In (2) a slight diminution in size was followed by renewed growth and early ulceration. The blood supply to this persistent tumour was found to come from three sources, there being a branch from the main vessel of the thigh which was probably not exposed at the time of operation, a lumbar branch which was probably an enlargement of a fine and unrecognised twig, and small fine twigs coming from the abdominal wall.

It is unnecessary to go fully into the results in the case of the batch 93 D. Two mice were operated on. In one the capsule surrounding the tumour was unfortunately opened during operation. In the other there was great diminution in size, the anterior two-thirds of the tumour disappearing entirely, but the posterior one-third persisted, although it did not grow larger. The blood supply came from a small vessel from the groin running to the posterior end of the tumour.

In the second group the behaviour of all the tumours of the inoculated mice was watched as before, but each mouse was inoculated on both sides of the body, the one tumour being used as a control to the other which was operated on.

This group comprises batches 91 N, 95 E, and 97 A. The larger of the two tumours borne by each animal was operated on in all cases. The tumours operated on in the batch 95 E are shown in fig. 5, but

before turning to them specially we will give a brief summary of the results in the three batches considered collectively.

In all thirty-five mice were inoculated bilaterally and in twenty-two of them bilateral tumours developed. Thirteen mice were submitted to operation. Spontaneous disappearance did not take place in any one of the nine tumours not submitted to operation. Of the thirteen mice operated on, ten lived until they were killed at the completion of the experiment. In one only of these ten did the tumour disappear completely. This is depicted in fig. 5 as (2). In one other mouse, the tumour operated on disappeared, but the control tumour on the opposite side also disappeared. This is no. 4 in fig. 5. In the other eight cases there was ultimately renewed growth ; but in all, the tumours on the side submitted to operation remained much smaller than the control tumours, although, as will be seen by a reference to the chart (fig. 5) the larger of the two tumours was operated on. The reasons for continuation of growth in seven cases were muscular infiltration in four, and the compensatory enlargement of some minute vessel not recognised and occluded at time of operation in three.

Fig. 5 is a record of the results of operating on five mice of the batch 95 E. After what has been said a detailed description of this experiment is unnecessary. The reason for continued growth in mouse (1) was that the pectoral muscle was infiltrated and the anterior part of the tumour got a blood supply through muscular twigs. It will be noted in the case of mouse (2) that only three weeks elapsed from time of operation until the mouse was killed off owing to the large size of the control tumour on the opposite side. Hence it is impossible to regard the disappearance of the tumour as a cure *. The disappearance of the tumour in mouse (4) must be looked upon as a case of spontaneous absorption. In mouse (5) the persistence of growth was due to enlargement of a small lumbar vessel not seen at the time of operation.

II.

It is unnecessary that we should deal at length with the results obtained in the case of the adeno-carcinomata which have a tendency to hæmorrhage and the formation of cysts containing blood. Had we found

* In working with Jensen's tumour there is this objection to using a control tumour in the same animal, that owing to the comparatively rapid growth of such tumours the observation time is very limited.

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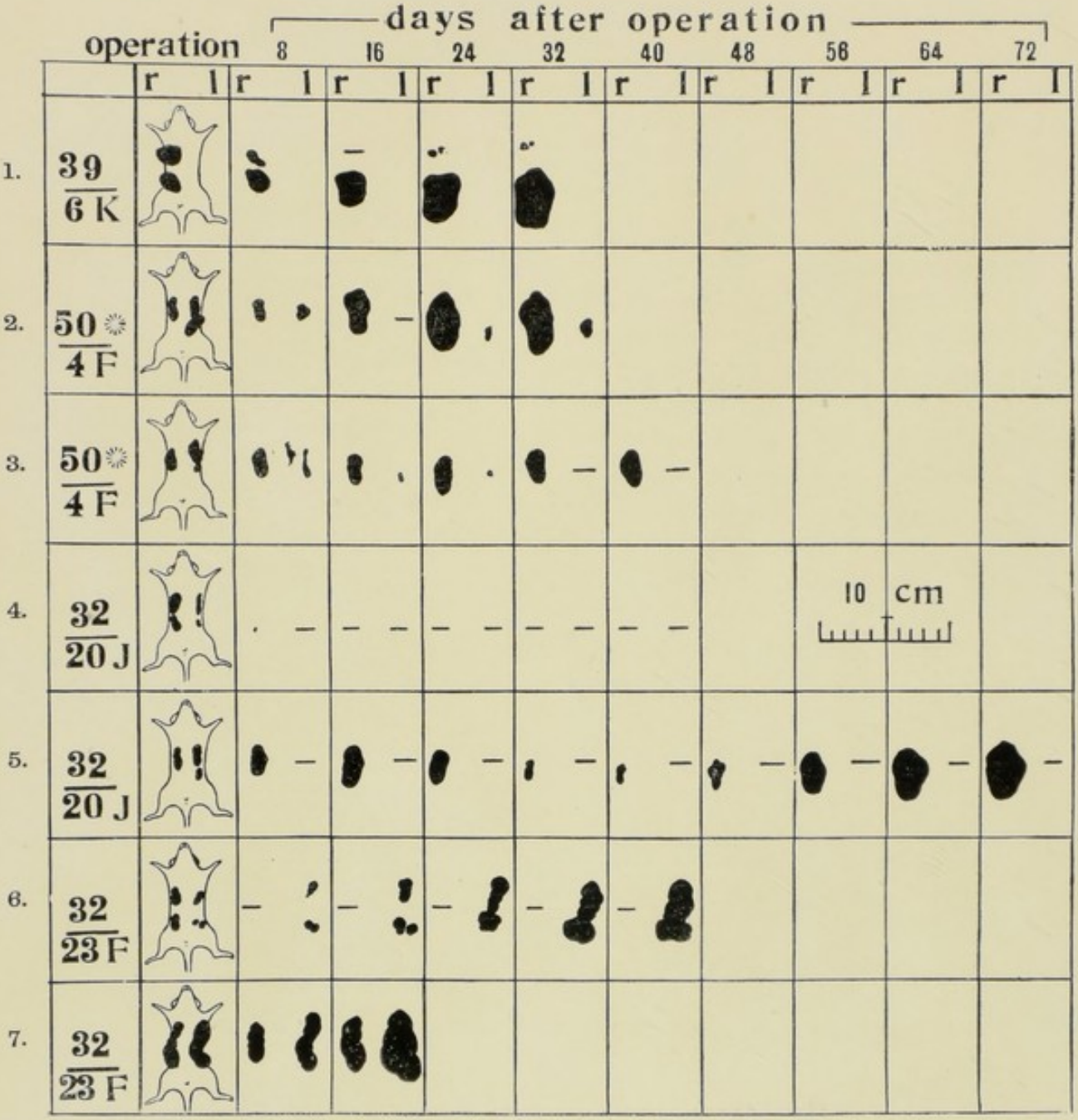


FIG. 6.—Diagrammatic representations of growth of tumours. The batch from which the tumour came is printed before the graphic representation of its growth. Nos. 1, 2, and 3 were hæmorrhagic adeno-carcinomata (tumours 39 and 50), nos. 4–7 squamous-celled carcinoma tumour 32. The mice with the tumours *in situ* are first represented as charted immediately before operation. Following operation the condition of the tumours was charted at intervals of 8 days. In all cases save no. 1 the tumours were on both sides and whilst one side was operated on the tumour on the other side was used as a control. In the case of no. 1 there were two tumours on the same side of the body but quite distinct. The blood supply to the anterior one was tied, the tumour disappeared, but later renewed growth occurred. The posterior tumour steadily enlarged. Nos. 2 and 3 are shown as a contrast: the left side was operated on in both cases, but whereas in no. 3 the tumour disappeared entirely, in no. 2 it took on a renewed growth after an apparent complete disappearance. In no. 4 the left side was operated on but the tumour on both sides disappeared. In no. 5 the left side again was operated on, the tumour disappeared entirely whilst the control tumour first increased in size, then decreased, and then again took on renewed growth until it reached a relatively enormous size. No. 6 had the right side operated on, the tumour on this side disappeared whilst the control tumour steadily enlarged. In no. 7 the right side was operated on but the operation produced little effect and at necropsy the operation was found to be incomplete.

evidence that there was any pronounced difference between the reaction of these tumours and that of the preceding type of growth, we should have set out the results in full. As it is we are justified after operating in 15 cases in stating that the same results are found in the cases of the growths tending to form hæmorrhagic cysts as in the more solid and less vascular alveolar carcinoma. Our first series of experiments were made with a batch of tumours $39\,^{5}/_{M}$. These tumours belonged to the first grouping, *i. e.* unilateral tumours; three of these tumours disappeared entirely, and there were no signs of recurrence some weeks later; in the case of the fourth renewed growth followed and persisted. Complete disappearance of 3 out of 4 of these tumours at first suggested that they might be peculiarly susceptible to interference with their blood supply, but a more complete study in a larger number of cases, especially with bilateral tumours in the manner set out before, showed that no such susceptibility was really present. On the chart forming fig. 6 we have represented the results following operation in two cases of tumours from $50^{*}\,{}^{4}/_{F}$. In this batch, 3 mice grew bilateral tumours out of a total of 24 mice inoculated. These three mice were operated on, the blood supply to the larger tumour being exposed and occluded, the result being that in one the tumour completely disappeared and in two renewed growth occurred. On the same chart we have pictured the result of interference with the blood supply to the anterior of two tumours situated on the same side of the body in a mouse. The figures show the way the tumour thus dealt with apparently disappeared entirely but ultimately recurred again, whereas the posterior tumour steadily grew until it reached a relatively enormous size. At the post-mortem examination in this case secondary growths were found in the lungs.

III.

In considering the results obtained after occluding the blood supply to tumours resulting from the propagation of a squamous-celled carcinoma *, it is necessary to mention a point which is of some importance. This is, that this tumour often infiltrates the neighbouring structures quite early, and if it has already invaded the muscular wall of the abdomen or thorax, successful operation is out of the question. In consequence of this infiltration operation has to be carried out when the growth is quite small, and when it can therefore be very easily separated and all its connections studied and divided without any trouble. It is

* Described in detail in this Report.

possible, therefore, that in the majority of operations carried out with this tumour the occlusion of the blood supply was more complete than is usually the case.

We feel that before attaching any importance to the rather obscure results following some of the operations in this variety of growth, we should like to make a further investigation. In operating, that tumour was exposed which was thought to be free, *i.e.* not fixed to muscle by infiltration. This often meant that the smaller tumour was the one to which the blood supply was occluded, thus differing from the practice in the case of the alveolar carcinoma and hæmorrhagic adeno-carcinomata. In all 7 operations were completed, only bilateral tumours being used. In two of these the tumours on *both* sides disappeared, and no recurrence has taken place over two months later. One of these is charted in fig. 6. In one case the tumour on the side operated on disappeared, whilst the tumour on the unoperated side grew larger, then ulcerated, decreased considerably in size and then again grew to a large size. This has also been figured (fig. 6, no. 5). In one case no recurrence had taken place 16 days after operation, but there had been very little increase in size in the control tumour. In one there was no recurrence on the side operated on, whereas the control tumour grew to a weight of 9·2 grams in a mouse of 17·2 grams. This case is charted as no. 6 in fig. 6. In two cases the tumour operated on rapidly recurred and grew to a large size. One of these is shown as no. 7 in fig. 6.

Similar observations have been made with a transplantable spindle-celled sarcoma described in detail by Dr. Haaland on a later page. With sarcoma the same difficulties are encountered as have been met with for squamous-celled carcinoma, and the results of operating on the tumours in the way described have given no more hopeful results as yet.

From this summary it will be seen that whereas the percentages of successful operations appears high, this is only apparent and unsupported by the closer investigation which reveals a tendency to spontaneous absorption on the part of the batch as shown by complete disappearance of both tumours in two cases. When this feature of the batch as a whole is considered together with the peculiar conditions, as mentioned above, under which operation is carried out, we feel that a more extended investigation of this variety of growth would justify the conclusion that in the absence of a tendency to spontaneous cure any imperfect operation will be followed by persistence of growth.

In concluding this summary of experimental work, we trust that the evidence brought forward may be of some use in estimating the possible

influence on growth of ligature of the main blood supply to malignant tumours in the human subject. We feel the extent of the investigations justify the conclusions appended.

Conclusions.

Complete obliteration of the blood supply to a tumour means sphacelation, separation, and cure.

Complete obliteration is impossible if there is infiltration of neighbouring structures such as is noticeable in the case of the mice experimented on when muscle is infiltrated.

Complete obliteration is only comparable to complete removal of the tumour, and this being so, it must be looked upon as very inferior to the usual mode of treatment by extirpation.

In cases where there is a tendency to spontaneous cure, partial operations may prove successful, and the disappearance of a tumour may be even hastened. Such is the probable explanation of the success of partial operation in the case of mouse no. 2 of the batch 76 F shown in fig. 4. As far as is yet known there is no means of recognising the tendency to spontaneous healing in the living mouse, though by the consideration of the behaviour of a batch of tumours as a whole and of the previous history of the tumours * the possibility of its occurrence may be surmised. Were it possible to follow the natural fluctuations in the growth of tumours of the human subject with the same accuracy as in these experiments, it appears permissible to surmise that valuable indications might be obtained as to when surgical interference could be most advantageously undertaken.

There is no reason to suspect that the interference with these tumours as carried out in this series of experiments in any way increases their malignancy. When continuation of growth takes place it is of no greater rapidity than in the other tumours observed as controls, nor is there greater tendency to metastatic deposit. On the other hand, neither the partial absorption of a tumour nor its complete separation by sphacelation in any way influences the growth on the opposite side of the animal, of a previously established control tumour of the same histological structure. The mice in which tumour material has been

* Those interested in the study of the growth of cancer in mice are referred to a paper "Experimental Analysis of the Growth of Cancer," by E. F. Bashford, J. A. Murray and W. H. Bowen; Proc. Roy. Soc. B. vol. 78, 1906, and "Die Experimentelle Analyse des Carcinomwachstums," Zeitschrift für Krebsforschung, Bd. v. 1907.

absorbed after operation exhibit the specific protection to subsequent inoculation as already described. Whether or not metastases may be prevented from establishing themselves remains a moot point.

Whereas partial operations may result in diminution in size, continuation of growth is the invariable rule and is usually associated with early ulceration owing to interference with the vascular supply of the skin. The continuation of growth proceeds from the maintenance and compensatory increase in the size of the nutrient connections already existing, rather than from the establishment of new sources of supply. It is unnecessary to enlarge upon the difference between obliterating the blood supply in artificially propagated tumours where, as was noted at the beginning, the circumstances are ideal, and doing so in the case of a sporadic growth, not encapsulated but on the contrary infiltrating along fascial and muscular planes, and possessing a blood supply from many sources. The majority of these sources are invisible without a long and unjustifiable dissection.

A TRANSPLANTABLE SQUAMOUS-CELLED CARCINOMA OF THE MOUSE.*

By J. A. MURRAY, M.B., B.Sc.

In the account of spontaneous cancer, the features of a spontaneous squamous-celled carcinoma of the axilla were described on a previous page, and mention made of its transplantability. As this tumour (No. 32 of our transplantable strains), has been made use of frequently in our experiments, it is necessary to give a fuller account than would have been appropriate in the general paper, of the histology of the primary and transplanted tumours and of the course of its experimental propagation.

Squamous-celled carcinoma takes a special position among the malignant new growths of vertebrates. It has apparently a wider zoological distribution than any other form of cancer, and has been observed frequently in many races of mankind in whom cancer is found with difficulty. In addition, the histological appearances of squamous-celled carcinomata are so characteristic and familiar, that this type of new growth has come to stand as a paradigm of all the epithelial new growths. This is due in part to the circumstance, that the majority of the smallest human carcinomata which have been investigated belong to this group, their superficial position rendering early recognition easy. From a consideration of all these points the advantages of a material of this type for experimental work are great and obvious.

* We have given a preliminary account in collaboration with Bashford and Haaland of the main points of interest in connection with this tumour, in a short paper which appeared in the 'Berliner klinische Wochenschrift' in 1907, No. 38. Conjointly with Haaland, it was also the subject of a communication to the Pathological Society of Great Britain and Ireland.

The tumour was situated in the left axilla of an old female mouse. In form it was elliptical, measuring 2 cm. by 1·5 cm. by 1 cm. in thickness. On June 6th, 1906, the mouse having been anæsthetised with ether, the tumour was partially excised, and the wound closed. The skin covering the growth was adherent to its outer surface and was freely removed along with it, Bleeding had occurred from the surface, and, the superficial part of the tumour with the skin attached was preserved for microscopical examination. The microscopical appearances are described along with the other spontaneous tumours, but some additional points will be referred to later. The deeper parts, consisting of softer and firmer areas in which minute opaque white spots were scattered, were used for transplantation, with the exception of several slices preserved for histological examination. Transplantation was carried out by introducing small fragments of 0·01 to 0·02 gr. each, under the skin of the back in 201 young normal mice. In addition a similar fragment was inoculated into the spontaneously affected mouse herself $\left(\text{Mouse } \frac{32}{0}\right)$. During the first five days after inoculation 5 or 6 mice were killed each day, and the graft excised and preserved for examination of the processes at the site of inoculation. Four tumours developed in the 156 mice which were alive three weeks after the inoculation ; they were the size of small shot, hard and movable under the skin. Three of these mice died during the next four weeks, and their tumours, being still too small for transplantation, were preserved and examined histologically. When the spontaneously affected mouse was killed five weeks after operation, the fragment inoculated was found and preserved.

The tumour in the fourth mouse grew very slowly ; after an interval of three months it had attained the size of a pea. During the next month, after having remained of the same size for more than a week, it diminished in size, till, when four months had elapsed since inoculation, it could be felt no longer. Three weeks later (Nov. 10th, 1906) a small nodule could be felt again in the same situation and it grew to the size of a pea in the succeeding four weeks (Dec. 12th, 1906). This tumour now grew more rapidly and had attained a diameter of 1·5 cm. by January 8th, 1907, seven months after inoculation. On this date the mouse was anæsthetised, and the tumour partially excised. Transplantation was effected into 64 mice. Seven tumours developed, and from now onwards the further transplantation was effected without difficulty, compare chart (fig. 1).

The primary tumour grew again after the partial excision on June 6th, 1906. Five weeks later, when the mouse was killed, it weighed 1·5 gram. With part of this recurrent tumour, 53 mice were inoculated by the same method as before, but no tumours developed in the 52 mice which survived. The remainder of the material was preserved *in situ* with the whole mouse.

The tumour which developed in the primary transplantation and was operated upon, did not grow again, and no trace could be found of the nodule left behind at the operation of January 8th, when the mouse was autopsied four weeks later.

Fig. 1—showing graphically the percentage of success of a number of series of experiments commencing with the primary transplantation—illustrates the phenomenon which Bashford and Murray recorded in accustoming Jensen's Danish tumour to English mice (1904) and which Ehrlich has also described (1905) in connection with the first steps in propagation of other spontaneous tumours, and has designated an increase in virulence ("Virulenzsteigerung"). It indicates also the nature of the reservations which must be made, when the growth of transplanted carcinoma cells is compared to the cultivation *in vivo* of a pathogenic micro-organism in a succession of susceptible animals. The increase in percentage of successes and in rapidity of growth, can be referred with a high degree of probability to the rapid increase in adaptability of the parenchyma cells at this period, which is essential for continued transplantation. As a consequence, a progressively larger number of cells survive the injury inseparable from transplantation, and, the size attained by the tumours in equal times is therefore greater. Once this preliminary stage has been passed, the rate of growth and percentage of successes fluctuate between somewhat wide limits, in the manner we have described along with Bashford and Bowen in the "Experimental Analysis of the Growth of Cancer" (v. post.). The limits to which this process of adaptation is subject, and the effects of alterations in the dose of tumour material inoculated on percentage of successes and rate of growth of transplanted tumours, are described in a later paper.

The transplanted tumours grow with great rapidity. Tumours of one to one and a half grams, in weight are usually present after ten days in every series, even when the initial doses do not exceed 0·03 gram. The tumours grow with a great tendency to infiltrate the muscles of the thoracic and abdominal walls and fungate into the serous cavities, features which are illustrated in a later paper in this report. One of the

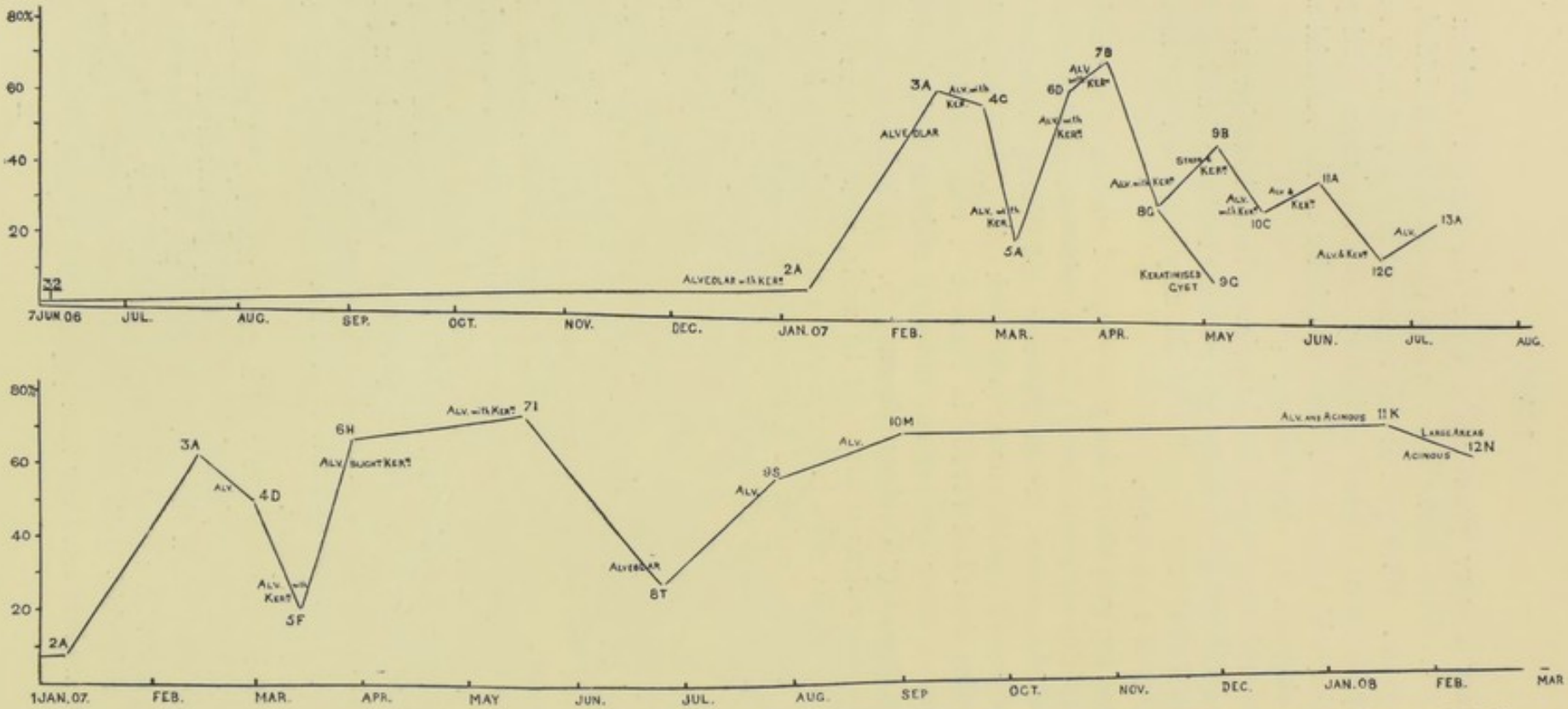


FIG. 1. Percentage curves of early propagation of tumour 32. The upper curve shows the period of excessive keratinisation, and indicates the time at which keratinisation first disappeared in the daughter-tumours. The lower curve shows the mutations of histological type in one strain. Keratinised squamous-celled carcinoma could be demonstrated with two exceptions (3 A, 4 D) at each transference up to the 6th and 7th generation. The alveolar condition which supervened, gave place at the 10th–11th generation to adeno-carcinomatous structure. In the 11th–12th generations the acinous type was very pronounced.

most interesting and important properties of the transplanted tumours of this strain is the frequency, one might almost say constancy, with which it produces large metastases in the lungs (fig. 2). Nearly 50 per cent. of all animals which survive positive inoculation more than six weeks, exhibit them, and extensive infiltration of the thoracic and abdominal walls generally frustrates attempts to remove tumours of any

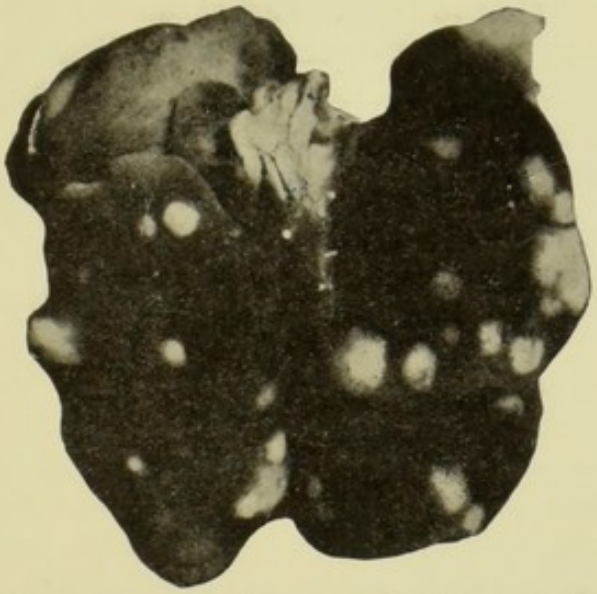


FIG. 2. 32/13 J.—Lungs of a mouse with a transplanted tumour of the 13th generation showing large metastases in the lungs (dorsal aspect), $\times \frac{4}{1}$, two months after inoculation.

size by operation, *cf.* pp. 155, 156. This conjunction of rapid infiltrative growth with great frequency of metastases, enhances the closeness of the parallel which the behaviour of this tumour in normal animals presents to the course of the most malignant spontaneous tumours of the mouse, and of the human subject.

HISTOLOGY OF THE TRANSPLANTED TUMOURS.

Before proceeding to a description of the histology of the transplanted tumours it is necessary to recapitulate briefly the results of the histological examination of the primary growth. As already described, three histological forms were present—keratinised alveoli, solid alveoli, and adenomatous areas. The most intense keratinisation was met with in the lining of the small cyst under the nipple, continuous with the skin of the axilla and side of the thorax. The major part of the tumour consisted of solid alveoli, many of which contained central horny con-

centric masses or epithelial pearls. The adenomatous areas were much less extensive ; the margin of one of these is represented in figure 19, p. 82, in the earlier paper, and another occurred under the skin to one side of the highly keratinised central superficial area (*vide* fig. 20, p. 82). While in these two situations the two forms are fairly sharply separated from each other, this is not by any means invariably the case. Fig. 3 taken from another part of the growth shows the intimate relation subsisting between the two types. Part of the wall of a slightly dilated acinus consists of stratified squamous epithelium with Malpighian layer, prickle cells, and a central keratinised squamous mass. The squamous epithelial layer is continuous at both of its limits with the cubical epithelium of the remainder of the acinus. While the transition is gradual in the lower part, it is more abrupt in the upper. The remainder of the figure shows typical branching acini. Transition areas of this kind are frequent in the adenomatous parts of the growth.

The same intimate association of keratinised and adenomatous areas is encountered in the recurrent tumour, examined in transverse sections of the whole thorax of the spontaneously affected animal. The growth consists of lobules separated from each other by well-marked fibrous septa. The periphery of each lobule is adenomatous, while in the centre more solid cell masses occur with central keratinisation.

The metastasis in the lung, figured on a previous page (fig. 23, p. 82), also contains purely alveolar along with keratinised areas, and, in addition, isolated small acini not shown in the figure.

This tumour therefore consists throughout of adenomatous, alveolar, and keratinised areas intimately associated with each other. Such tumours are already well known to students of human pathology and have received the name of "adeno-cancroid" from Herxheimer who has described several cases from the stomach, cœcum, pancreas, parotid, and the body of the uterus. In his cases keratinisation occurred in situations where normally it is absent, and he argued from the intimate relation of the keratinised alveoli to those which were adenomatous or adeno-carcinomatous, that a single parenchyma was present, developed from an undifferentiated "embryonic foundation" capable of differentiation in two directions. In the case of the mouse tumour now under discussion, the proximity of skin and mamma might seem to justify the assumption of a complex tumour made up of two distinct parenchymata of separate histogenesis. The histological examination of the primary and recurrent tumours, and of the metastases shows, however, so

[*To face* p. 164.



FIG. 3. 32/0. Part of spontaneous tumour showing juxtaposition of acinous and squamous epithelium. The transition is abrupt in the upper wall of the dilated acinus (at the point marked by a mitosis) and more gradual at the lower part. $\times \frac{200}{1}$.



R. Muir, del.

FIG. 4. 32/1. Transplanted nodule in spontaneously affected mouse. The figure shows keratinised, adenocarcinomatous, andadenomatous areas. $\times \frac{200}{1}$.

intimate a relation between the two, that such a complex origin is unlikely, and the results of the histological examination of the transplanted tumours are conclusive for the singleness of the parenchyma.

The individual transplanted tumours have a more homogeneous structure than the primary growth, and are made up, either of solid alveoli in some of which keratinisation is found, or in other cases of similar alveoli which show a tendency to assume the adenomatous type. In the latter case the parenchyma is either arranged as larger masses with acinus-like lumina, or, the process of subdivision has proceeded to the formation of small acini, indistinguishable from those described in the primary tumour. It is only in certain of the nodules obtained from the mice used for the primary inoculation and killed for "early stages," or during the first five weeks (including the graft in the spontaneously affected mouse), that acinous and keratinised areas occur together.

The grafts preserved during the first five days after inoculation from the primary tumour show, as do all such preparations, very extensive central degeneration. The stroma associated with the transplanted parenchyma degenerates completely, and is replaced by a new reaction-tissue from the host. Vascularisation was effected by the fourth day. The peripheral parenchyma cells, while retaining their vitality, do not show much evidence of active proliferation, only a few mitoses being seen. This is in harmony with the low percentage of success. Keratinisation was seen in one of the grafts examined from those preserved on the second day, and was doubtless present in the transplanted tissue. Of the small tumours of the primary transplantation preserved during the first five weeks, one was evidently diminishing rapidly in size. It consisted of several masses of completely keratinised epithelial squames, and of minute compressed epithelial cysts enclosed in dense sclerotic connective-tissue. Another had grown nearly to the size of a split pea ; it consisted of many small solid alveoli separated from each other by bands of collagenous fibrils. In several alveoli the centrally placed cells were keratinised, in others they were necrotic. The nodule arising from the inoculation into the spontaneously affected mouse, shows all three types of growth found in the spontaneous tumour. As can be seen from fig. 4, two large alveoli are almost completely keratinised ; three others of the same size are solid with small punched-out lumina, while the remainder of the minute tumour consists of small acini lined by a single layer of cubical cells.

The tumour from which all the subsequent transplantations took their

origin, $\frac{32}{1}-2\,A$ in our nomenclature *, consisted for the most part of undifferentiated cells arranged in closely aggregated alveoli, separated only by delicate strands of connective tissue carrying blood-vessels. In many alveoli the central cells had undergone necrosis, and towards the centre of the tumour many entire alveoli were completely degenerated. Along the zone of separation of this central necrotic area from the healthy and more superficial part, very perfect keratinisation had occurred in isolated alveoli. They were transformed into concentric epithelial pearls, bounded externally by a single layer of cells with unaltered protoplasm.

For the next few generations the transplanted tumours presented, for the most part, a solid structure with only minute areas of keratinisation, but with considerable necrosis of the central portions of the alveoli. This condition persisted with slight progressive increased keratinisation in the later generations till the sixth. In the seventh generation, however, keratinisation appeared to an even more pronounced degree than in the primary tumour. The tumours at this time reproduced perfectly the appearances of a somewhat highly keratinised epithelioma, as met with in the lip and tongue in the human subject. In the next generation, viz., the eighth, the tumours are strongly keratinised throughout, and figs. 5 and 6 show areas in which the horny epithelial pearls are very perfectly developed.

The process goes a step further in individual tumours of this generation, such as that from which figs. 7, 8, and 9 (low and high powers) are taken. The tumour is completely transformed into a cystic structure filled with scales and flakes of keratin, and lined by a thin cellular layer with the structure of normal skin. Such tumours duplicate an appearance seen in many spontaneous epitheliomata, such as the stomach case from the mouse described on p. 71, and shown here also in fig. 12 ; and in the early case recently described by Ribbert from the human subject. The tumours of the ninth and tenth generations were also keratinised, some very strongly, but usually to a less degree than the tumours of the eighth generation. During this period (seventh, eighth, ninth, tenth, and beginning of eleventh generations) the tumours grew slowly with a lower percentage of success than in the generation immediately before. The period during which excessive keratinisation was a feature of the transplanted

* The nomenclature is fully explained on p. 102, and in a later paper on "The Analysis of Growth."



Microphoto, R. Muir.

FIG. 5. 32/8 G–9 B.—Transplanted tumour of 8th generation, showing extensive keratinisation (stained by Gram's method). $\times \frac{35}{1}$.



Microphoto, R. Muir.

FIG. 6. 32/8 G–9 B.—Transplanted tumour of 8th generation, showing typical epithelial pearls as in ordinary epitheliomata. $\times \frac{35}{1}$.

tumours, was limited in duration, and extended over 5 to 6 weeks ; but traces of keratinisation persisted in the form of minute pearls for a much longer period. The generations to which the tumours belong are apparently entirely a consequence of the exigencies of the experimental method. The whole parenchyma seems to have passed simultaneously through a similar phase of differentiation. The phenomenon recalls the observations we have recorded along with Bashford and Bowen on the experimental analysis of the growth of cancer. We pointed out how parallel strains of Jensen's tumour give simultaneously a high percentage of successes, or, the reverse when spontaneous absorption occurs concomitantly in the mother series. With tumour 32, as with Jensen's tumour in the former experiments, the absolute duration of propagation, not the generation attained, supplies the key to the connection which exists between individual tumours.

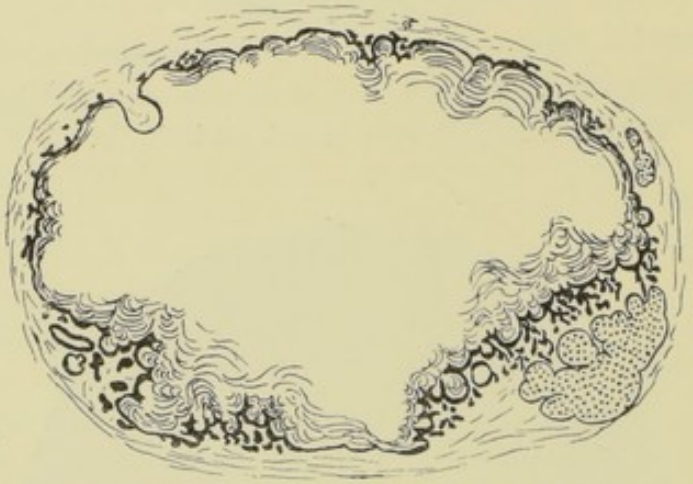
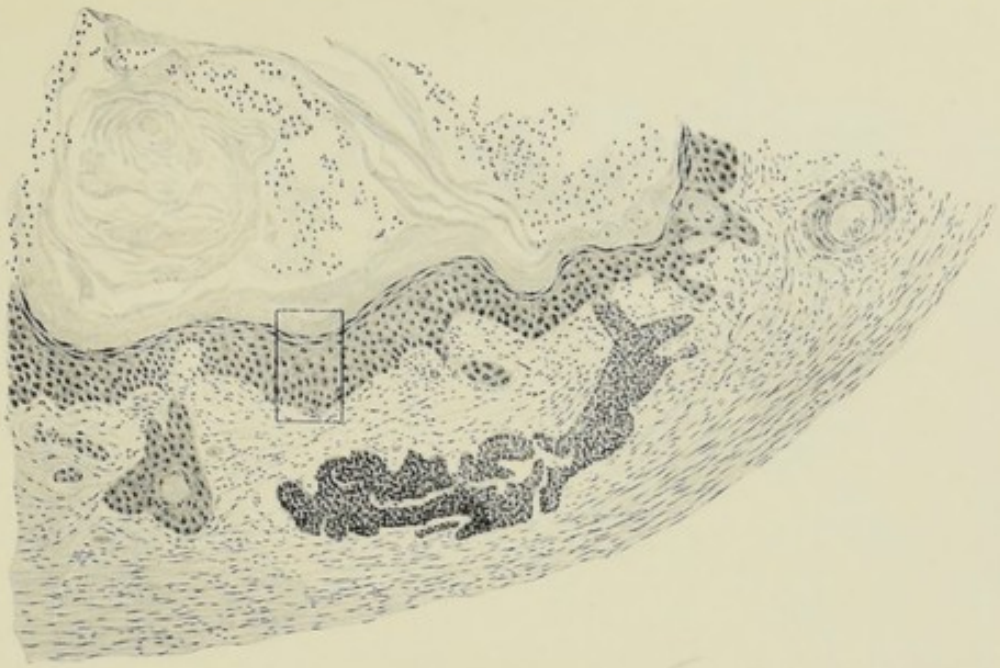


Fig. 7. 32/8 G–9 C.—Schematic figure of whole tumour of 8th generation, consisting of an epithelial cyst filled with keratin scales. A small alveolar area at the right side. $\times \frac{10}{1}$.

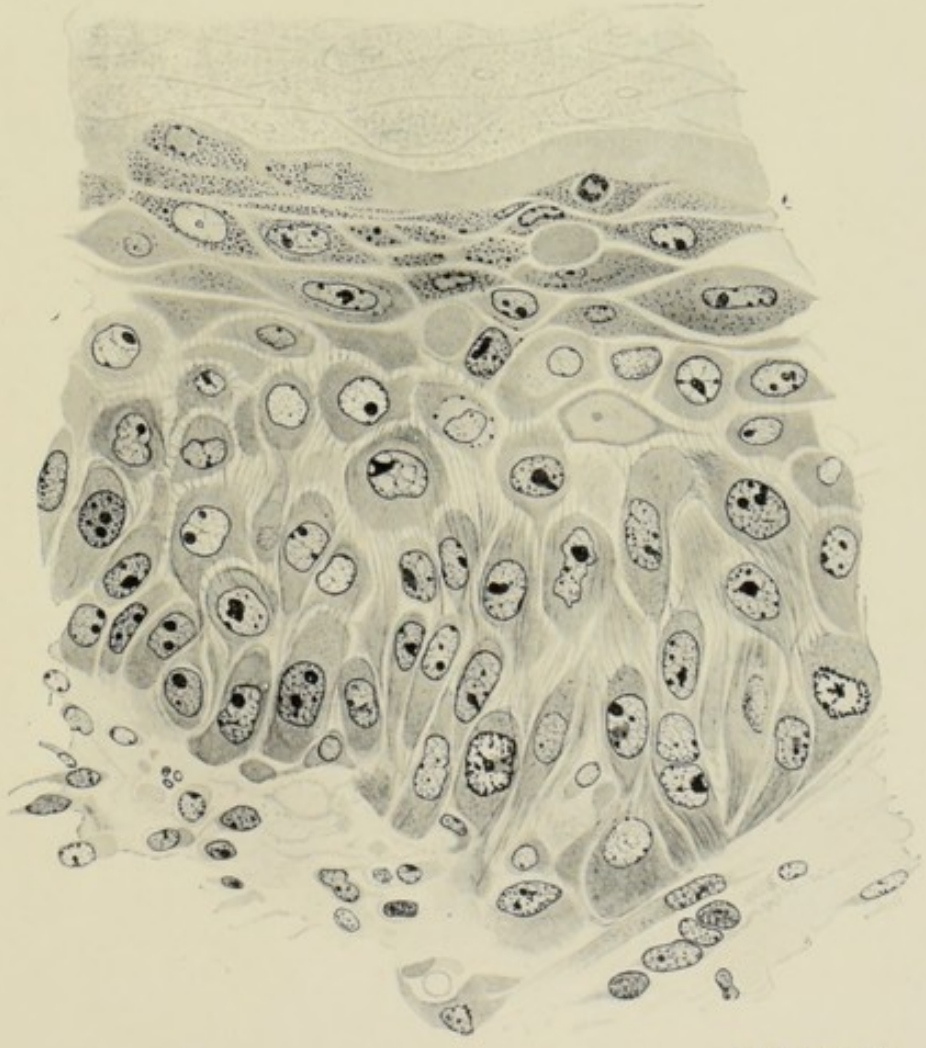
Following on the period during which the transplanted tumours showed extensive keratinization, a phase of greater energy of growth was encountered. The tumours grew rapidly, necrosis was widespread, and keratinisation progressively less, or absent. The tumours at this time presented the most uninteresting histological picture imaginable. Large alveoli of closely packed small cells occur, interrupted only by an occasional capillary blood-vessel (figs. 10 and 11). Necrosis may be very extensive, and hæmorrhage into necrotic areas is not unusual. The intrusion of well-marked adenomatous formations in the daughter-tumours, after this alveolar or medullary condition of the parenchyma

[*To face* p. 168.



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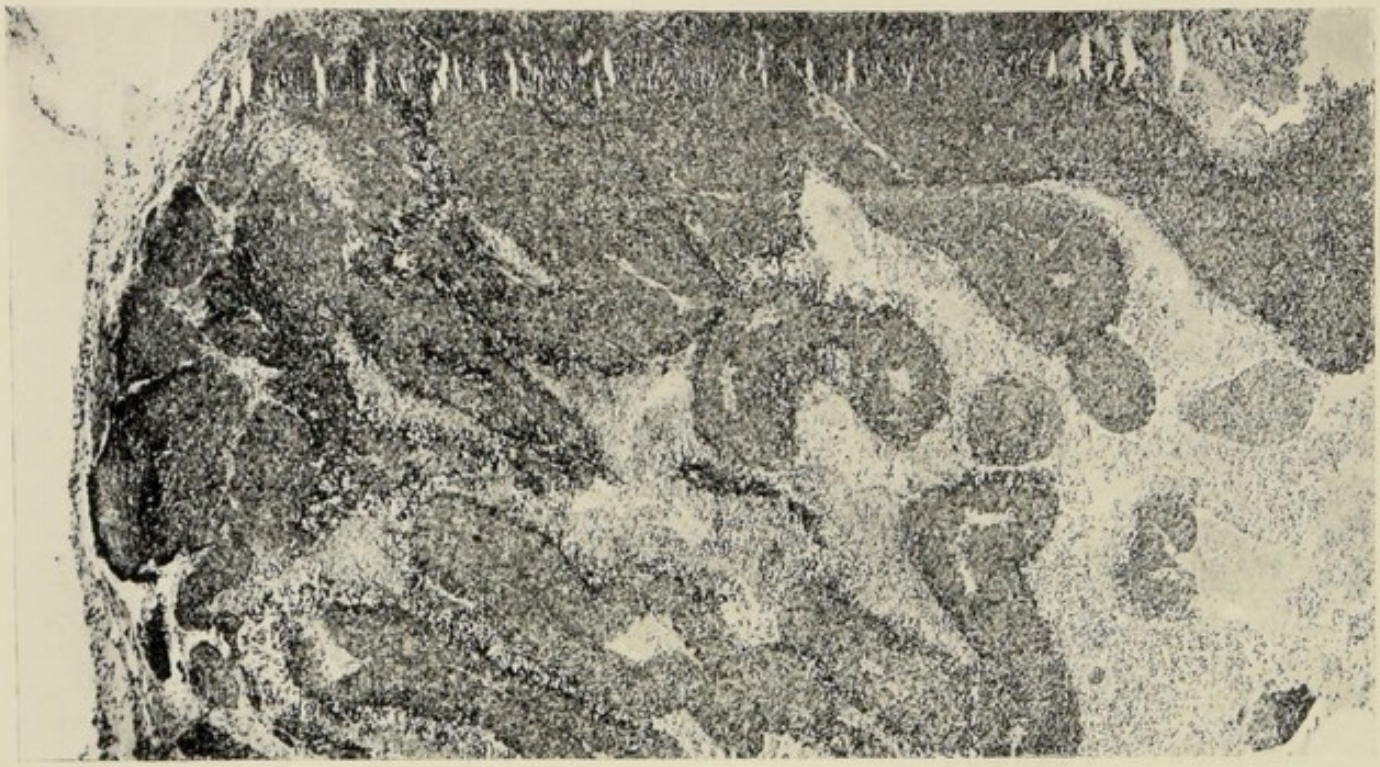
FIG. 8. 32/8 G—9 C. Tumour of 8th generation, right side of fig. 7 at medium magnification. Perfect reproduction of structure of stratified squamous epithelium in the wall of the epithelial cyst, *cf.* fig. 7. $\times \frac{60}{1}$.



J. R. Ford, del.

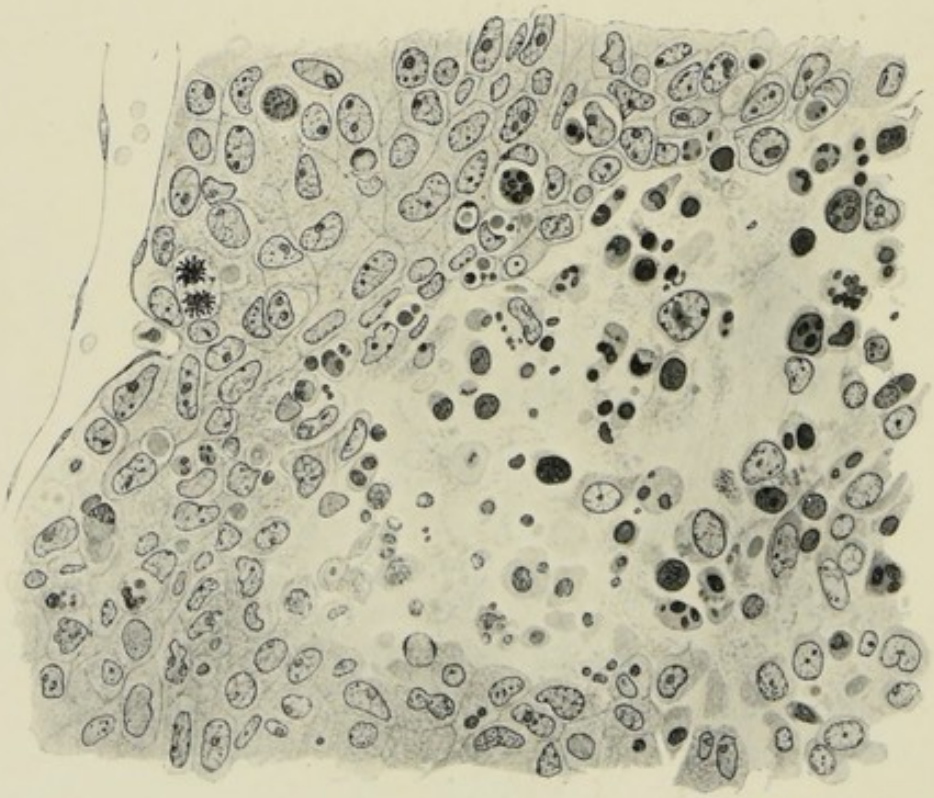
FIG. 9. 32/8 G—9 C. High-power view of area marked in fig. 8. Malpighian layer, prickle cells, eleidin granules and keratinisation as in normal skin. $\times \frac{750}{1}$.

To face p. 169.]



Microphoto, W. Imboden.

FIG. 10. 32/20C—21B. Alveolar tumour of 20th generation, showing apearance of medullary carcinoma and extensive necrosis, surface of tumour to left side. $\times \frac{50}{1}$.



J. R. Ford, del.

FIG. 11. 32/7 B—8 G. Sector of single alveolus of tumour of 7th generation. The necrotic centre of the alveolus is separated from the capillary by a thin shell of healthy dividing cells. $\times \frac{500}{1}$.

had persisted through several generations, was therefore highly remarkable. Fig. 13 shows a portion of one of these tumours in the 11th generation, in which the parenchyma is cut up into small groups of cells arranged in a single layer around a central lumen. Nothing is wanting to make the parallel complete with typical glandular structure, such as is seen in the normal mamma or thyroid. This condition has persisted in the descendants of the tumour figured for several months, and, in addition an analogous transformation has supervened in other strains. In them, however, the process of subdivision of the alveoli has not progressed so far, so that larger masses have resulted with irregular lumina, as in a typical adeno-carcinoma. The process is the same as we have already described in spontaneous tumours, but so far the change remains local, and, the rest of the tumour is solid or alveolar.

The observations on the histological diagnosis of this tumour have a bearing also on the meaning which is to be attached to the terms metaplasia and anaplasia. In the first place attention may be directed to the completeness and reliability of the histological material on which our conclusions are based. It goes without saying that microscopical preparations have been made of all tumours used for transplantation. For this purpose a thin slice is taken through the greatest diameter of every tumour, usually by means of a Valentin's double knife. Therefore the sections examined give as complete a picture as can be desired, of the histology of the material used for propagation. In addition practically all the tumours obtained, and not used for propagation, have been preserved along with the whole animal. We are therefore justified in attaching greater importance to conclusions based upon a material collected systematically in this manner, than would be allowable to the results of examining even a considerable number of transplanted tumours taken at random. It will also be obvious, that the deductions which such an investigation may force upon us, belong to an order, different from that comprising the conclusions based on the examination of spontaneous tumours of man or animals, however numerous. Any connection between different forms of growth, which may be made out by the latter method, must of necessity have only a hypothetical application to the previous history, or later, course of any one particular tumour. In the case of the experimental material, the histological changes have actually taken place during known intervals of time, and in a parenchyma which has never been out of observation since its occurrence in the spontaneously affected animal.

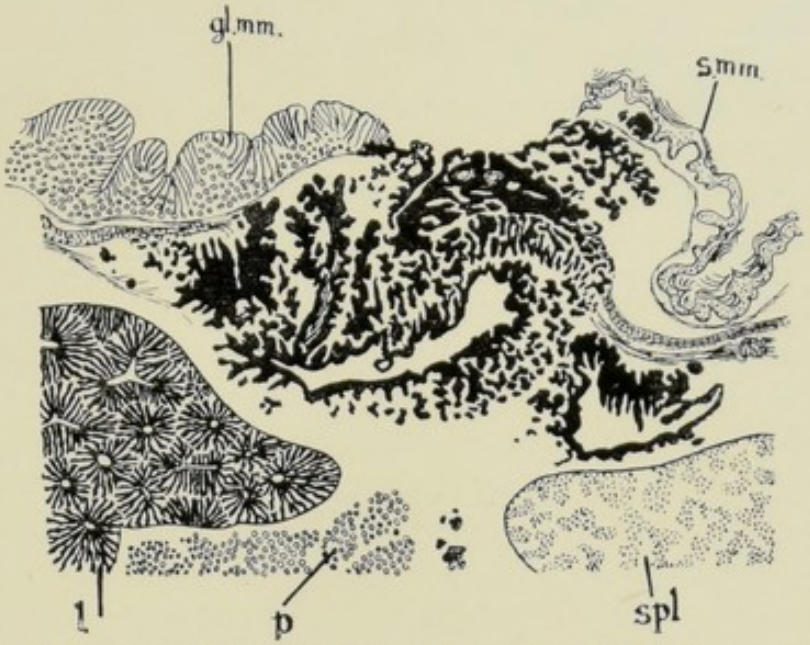
Hence the question arises of the advisability of attempting to describe

and explain the phenomena now under discussion, in the terms current in pathological anatomy. These terms were evolved, and their significance settled, from observations on spontaneous malignant new growths in the human subject, before the possibility had arisen of experimental observations through long periods of time on a constant tumour-parenchyma. In many cases they have been coined because of contradictions which could not be harmonised in the histological and histogenetic classification of tumours in man. If, therefore, it should appear that inordinate violence would be done to the accepted use of these terms by extending them to the results of experimental observations, it might be necessary to consider the advisability of choosing new terms.

The facts for consideration are briefly as follows:—A spontaneous tumour in which keratinised areas were found in association with alveolar masses and adenomatous nodules was transplanted. The whole of the tumours subsequently obtained by artificial propagation, were derived from one tumour of the primary transplantation, in which keratinised areas were found, although the greater part of the section examined was entirely alveolar. The tumours of the generations immediately following were also alveolar, and in most (not all) islands of keratinisation were found. The amount of keratinisation increased enormously, till, in some strains veritable epithelial cysts were formed. After being prevalent for a period of 5 or 6 weeks in 3 or 4 generations, keratinisation diminished in extent in the propagated tumours. The tumours then remained alveolar, some of them till the present time. In others the alveolar parenchyma became subdivided into smaller masses in which lumina appeared, and considerable portions of such tumours presented the appearance of an adenoma. A condition which can be better described as adeno-carcinoma, viz. alveoli with several irregular lumina in each, was met with about the same time in other distinct strains. In some cases this adenomatous type has again given place to the pure alveolar condition.

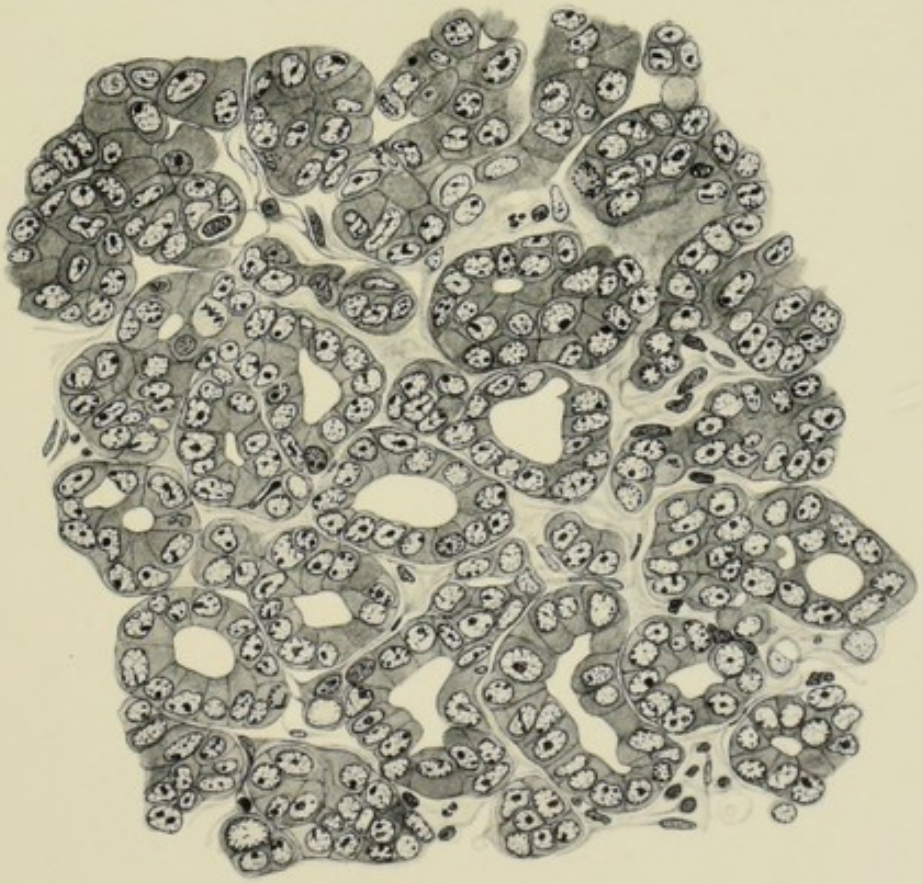
It must never be forgotten that transplanted tumours arise from small fragments only. Since the transplanted tumours do not grow as cultures of free cells but in ordered masses or alveoli, the extent to which any tumour represents separate areas of a preceding tumour, diminishes with each successive transference. This progressive segregation of the offspring of small groups of cells in separate animals, enables us to draw sure conclusions of the greatest importance, from the successive histological alterations of the parenchyma (*cf.* paper on the analysis of growth on a later page).

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gl. mm = glandular mucosa ; sm m = squamous celled mucosa ; l = liver ;
spl = spleen ; p = pancreas.

FIG. 12. Spontaneous carcinoma of stomach in mouse: see p. 71 and figures 4 and 5. Formation of subperitoneal epithelial cysts in deeper part of tumour, *cf.* fig. 7, p. 168. $\times \frac{12}{1}$.



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FIG. 13. 32/11 K—12 N. Tumour of 11th generation showing acinous arrangement of the parenchyma. $\times \frac{350}{1}$.

Thus while it is possible to argue, that the primary growth consists of two separate parenchymata growing commensally in a very intimate manner, the inherent improbabilities of such an assumption are increased, and the assumption rendered unnecessary, by a consideration of the structure of the transplanted tumours. For the latter a composite structure is practically unthinkable. Nevertheless, we find, in a single strain, alveolar structure giving way to widespread keratinisation, so that a typical "cancroid" is produced, and this in turn being replaced by the alveolar form, which in its turn gives place to an adenomatous or adeno-carcinomatous structure.

It is of decisive importance to note, that the keratinised and adenomatous types are separated in point of time by a long interval, with many successive transferences from animal to animal. This proves that the two differentiations, however distinct they may appear, are inherent in cells of one kind. We therefore conclude that the squamous-celled parenchyma may itself grow as a single layer of cells arranged as an epithelium. This is a condition frequently realised in squamous-celled carcinomata of the human subject. The cubical epithelium found in the cases of this sort which we have examined, is usually somewhat irregular, as is also the central space around which they are arranged. Isolated acinus-like formations are found, however, in which the arrangement is perfectly regular. The flattened or cubical epithelium which is found in the early stages of formation of epithelial cysts from transplanted squamous epithelium, as figured by Ribbert, enables us to realise in another way, the manner in which the polarity of the cell thus manifests itself.

From this standpoint it is mainly of academic interest, whether the primary growth be considered as a squamous-celled carcinoma capable of growing as an adeno-carcinoma, or, conversely as an adeno-carcinoma of the mamma, in which excessive keratinisation has occurred, as described and figured in less degree for other spontaneous tumours in an earlier paper (*cf.* figs. 33 and 34, p. 88). The apparent continuity in the primary animal, of the external skin with the margins of the highly keratinised area, as already described, would seem to speak for the former conclusion, and we have given this consideration due weight in describing the tumour as a squamous-celled carcinoma.

The observations of Lewin on a transplantable rat-tumour to which a short reference was made in the paper on spontaneous mouse-cancer, present an interesting parallel to those we have recorded. In Lewin's case—(the initial transplantations were carried out by Michaelis)—the primary tumour, so far as it was examined, had the structure of an

adeno-carcinoma, and it is regarded as an adeno-carcinoma of the mamma by Michaelis and Lewin. Keratinisation appeared in the third generation in tumours arising from subcutaneous transplantation, while the tumours of the same generation obtained by intraperitoneal inoculation remained adeno-carcinomatous. The later generations presented both types, sometimes in combination with transition forms, such as have been described in adeno-cancroid by Herxheimer and others, and in our primary growth. The later development of Lewin's tumour was complicated by the development of a sarcoma.

There can be little doubt that in Lewin's rat-tumour the reverse process—from an adenomatous to a squamous-celled parenchyma—has taken place to that which we have described. He has discussed at great length, the interpretation to be put on his observations, and regards the transformation as essentially a "metaplasia" of adeno-carcinomatous epithelium. His other suggestion of an infection of the overlying skin from a subcutaneous transplanted tumour, need not be discussed here.

These considerations will make clear the limitations, within which we consider it justifiable to describe the mutation of an alveolar carcinoma derived from squamous epithelium, into an adeno-carcinoma, as a "metaplasia." In the usual acceptance of the term, "metaplasia" is applied to changes usually the reverse of this. The transformation of glandular or columnar epithelium into squamous epithelium, or such an association of the two as makes the transformation probable, has been observed frequently in connection with tumours, especially those arising from the bronchial epithelium and less frequently in other organs, as recorded by Herxheiner, Lubarsch, and others. In the cat the vertical fold of epithelium in the trachea which before sexual maturity is columnar and ciliated, becomes squamous after that period. Futterer has shown that the pyloric mucosa of the guinea-pig can be transformed into squamous epithelium by mechanical injury. In most of these cases it is not certain whether the changes are permanent or reversible. If the term metaplasia be used to describe them, and the converse transformation in the tumour under discussion, the reservation must be made that such a reversion can take place. The varying histological appearances would then rank as growth-forms, and they have been described as such in the account of the spontaneous tumour on a previous page.

The alternation of keratinised epithelium (or an acinous arrangement of the cells) with an alveolar or solid carcinomatous condition during the propagation of this tumour, awakens analogous reflexions on the suitability of the term anaplasia, as a description for the transformation

of a highly differentiated tumour parenchyma into a less differentiated one. The term anaplasia was coined by v. Hansemann to describe this transformation, and he regarded it as in some degree inseparable from the conception of a malignant new growth, and as progressive. In particular he had found that the metatases of squamous-celled carcinomata were generally less keratinised than the primary growth, and that they might approximate to the condition of alveolar carcinoma. Jenny, in describing Hanau's transplanted rat-epithelioma, noted that excessive keratinisation was present in the sub-transplantations, and saw that this fact could not be harmonised with a belief in progressive loss of differentiation as constant. Recently v. Hansemann has seen fit to admit the possibility of exceptions to this rule, and, it may be pointed out, that the squamous-celled carcinoma of the mouse's jaw described by Haaland, had formed completely keratinised metastases in the lymph-glands of the neck, while the primary tumour was very slightly keratinised, and in places quite undifferentiated. It is therefore unnecessary to insist further that if the term anaplasia is to be used to describe a loss of differentiation such as occurred in our tumour, it cannot be held to exclude the possibility of a reversion to the differentiated condition.

v. Hansemann's contention that anaplasia is essential and progressive is not borne out by our experience. On the contrary, it is apparently secondary and due in part to rapidity of growth, as Apolant has suggested for glandular carcinomata, and loss of histological differentiation is not only not progressive, but is reversible.

In conclusion we may consider to what extent the histological appearances of this tumour are determined by the conditions of growth. The amount and character of the stroma has varied considerably from one tumour to another. In general terms, tumours which grow slowly have a relatively abundant stroma, and are therefore of firmer consistence, while, rapidly growing tumours are soft and their stroma is reduced to a few cells accompanying the delicate capillaries lying between the alveoli. In the keratinised tumours, which generally grow more slowly than those entirely undifferentiated, a dense sclerotic stroma is usually met with around the keratinised alveoli. Considerable variations have been observed, in the amount of stroma intervening between the alveoli of solid carcinomatous tumours of this strain. Up to the present this alteration has not taken on the definitely progressive character leading to the development of sarcoma, through an intermediate mixed tumour stage.

Apolant has suggested that a reversion of an alveolar tumour to the

adenomatous type may be due to increased resistance of the animal, and he describes several instances in which tumours usually alveolar, have become adenomatous when growing in animals whose resistance had been raised by a preliminary injection of normal mouse-blood, as described by Bashford, Murray, and Cramer. So far as our experience goes, no such association can be made out between histological structure and the resistance of the animals, in our experiments with this squamous-celled sarcoma. Five tumours have been examined which developed exceptionally, in 70 animals treated previously to inoculation with an emulsion of the skin of mouse-embryos, a procedure which produces a high degree of resistance to the growth of this squamous-celled carcinoma. In none of these tumours does the degree of differentiation of the parenchyma differ from that of the tumours in the control animals. In one case the tumour alveoli are small and the stroma correspondingly well developed. A slow-growing tumour in a normal mouse of the same series, exhibits the same peculiarities in even higher degree. These observations therefore do not confirm Apolant's interesting observation, and it has not yet been possible to influence the histological character of the transplanted tumours by experimental methods.

Since the above was written, another spontaneous tumour (No. 164/O) has been obtained, in which widespread keratinisation is present along with adeno-carcinomatous areas. The tumour was entirely subcutaneous, and has been transplanted into 300 normal mice; sufficient time has not yet elapsed, for the result to be available for this Report.

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CONTRIBUTIONS TO THE STUDY OF THE DEVELOPMENT OF SARCOMA UNDER EXPERIMENTAL CONDITIONS.

By M. HAALAND.

I.

With the first successful transference of a carcinomatous tumour from animal to animal of the same species the question arose as to the nature of the process responsible for the development of the new growth, and especially what part was played by the introduced tissues. Hanau[1] inferred from his successful transference of a squamous-cell carcinoma from rat to rat, that the transplantation of the parenchyma cells was the essential factor in the formation of a new tumour. Without directly investigating how the supporting structures of the new tumour arose he discussed the different alternatives, viz., on the one hand the possibility of transference of a connective-tissue germ, and on the other of a formative influence exercised by the carcinomatous cells on the tissues of the new host; he decided in favour of the latter alternative. Morau[2] and Borrel[3] assumed without making further inquiry that the essential part of the process is the grafting of cancer cells. Loeb[4] described observations on the process at the site of inoculation

[1] Hanau: Erfolgreiche experimentelle Uebertragungvon Carcinom. (Fortschritte der Medizin, vol. 7. 1889.)

[2] Morau: Recherches experimentales sur la transmissibilité de certains néoplasmes (Archives de Medicine expérimentale, 1894, p. 677.)

[3] Borrel, A.: Epithélioses infectieuses et epithéliomas. (Annales de l'Institut Pasteur, T. xi. 1903.)

[4] Loeb, Leo: On Transplantation of Tumours. (Journal of Medical Research, vol. vi. 1901.)

—— : Further Investigations in Transplantation of Tumours. (Ibidem, vol. viii. 1902.)

of a rat-sarcoma, but his description of this difficult material is too imperfect to settle definitely what actually took place in his inoculations.

To Jensen[1] the credit is due of having first systematically investigated and clearly described the processes at the site of inoculation. By carefully examining small pieces of tumour at short intervals after transplantation, he proved that the new tumour-parenchyma was derived solely from that introduced. As to the fate of the introduced stroma elements, Jensen expressed himself very carefully. He observed that the hyaline degenerated connective tissue of the graft was penetrated in the course of time by fibroblasts and capillaries from the new animal, and he considered it probable that the old stroma was absorbed by degrees, but he thought it possible that a part of it may remain alive.

Bashford, Murray and Cramer[2] made an exhaustive study of the processes at the site of implantation for Jensen's tumour as well as for numerous other tumours of their own. They stated that the parenchyma alone possessed powers of apparently continuous proliferation in the tumours they examined. The stroma of the grafts underwent degenerative changes as a rule, and was replaced by supporting structures derived entirely from the tissues of the new host. The tissues taking part in this reaction were ultimately transformed into stroma and blood-vessels identical with those of the primary tumour. This stroma reaction is specific, i. e. constant in the several generations of the same tumour but may present peculiarities for different tumours.

Later, Loewenthal and Michaelis[3] studied the processes at the site of implantation for another tumour, and in the main came to the same conclusions.

[1] Jensen, C. O.: Experimentelle Undersögelser over Kraeft hos mus. Copenhagen, 1903.
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[2] Bashford, E. F., Murray, J. A., & Cramer, W.: Source of the Constituent Elements of New Growths obtained by Artificial Propagation. (2nd Scientific Report of the Imperial Cancer Research Fund.—Part II. Taylor & Francis, London, 1905.)
—— : Stroma is a Specific Reaction on the part of the Host. (Ibidem.)
—— : Comparison between the Transmission of an Infective Granuloma of the Dog and Carcinoma of the Mouse. (Ibidem.)

[3] Loewenthal, W., & Michaelis, L.: Ueber den Krebs der Mäuse. (Zeitschrift für Krebsforschung, Band iv. Heft 3, 1906.)

It seems then to be a well established observation that as a rule the stroma degenerates after transplantation, and only the introduced parenchyma is capable of further growth ; the new stroma is formed as a specific reaction on the part of the host. This fact is in perfect agreement with observations in human pathology. The transplantation of tumours from animal to animal is to a certain extent analogous to the formation of metastases in the spontaneously affected individual. It has often been shown that in secondary deposits of carcinoma the new growth proceeds entirely from the parenchyma cells, while the stroma of the metastatic nodule is supplied either from the stroma of the invaded organ or from a newly formed reaction-tissue. From these observations it follows that the stroma of a carcinomatous tumour is to be considered as a normal tissue, while the parenchyma alone possesses malignant properties.

These points having been ascertained it was a most surprising new fact, discovered by Ehrlich and Apolant[1], that the stroma in certain transplantable mouse carcinomata gave rise to a new and distinct kind of tumour-tissue, viz. a transplantable sarcoma. Ehrlich and Apolant observed this phenomenon in three different instances ; in each instance the tumours had been propagated over a prolonged period of time and through numerous generations as carcinomata, without showing any sign of being mixed-tumours. In one case the change occurred in the 9th generation, i. e. after 9 months propagation as a carcinoma ; in another only after 67 generations, i. e. after $2\frac{1}{2}$ years propagation as a carcinoma ; in this latter case, however, the sarcomatous change was preceded by a marked increase in the amount of connective tissue, extending through more than 20 generations. The change consists in a sudden appearance of a sarcomatous tissue between the alveoli of the carcinoma and replacing the original stroma. In succeeding generations the sarcomatous tissue ousts the carcinomatous and entirely supplants it after a shorter or longer time, and ultimately a pure sarcoma is obtained. The time necessary for the completion of the process varied in Ehrlich and Apolant's cases. In one a pure sarcoma was obtained quickly, namely, after one or two generations of mixed tumour; in another after three or four generations; in the third the mixed tumour persisted for more than nine months through ten to fourteen generations before a pure sarcoma

[1] Ehrlich, P., & Apolant, H.: Beobachtungen über maligne Mäusetumoren. (Berliner klin. Wochenschrift, 1905, no. 28.)
Apolant, H.: Die epithelialen Geschwülste der Maus. (Arbeiten aus dem Königlichen Institut für experimentelle Therapie zu Frankfurt a/M. Heft 1, 1906.

was obtained *. The results of the histological examination convinced Ehrlich and Apolant that their tumours were not mixed-tumours from the outset. They also rejected the possibility of the new tumours being infective granulomata. In their first paper they entertained two main possibilities in explanation of the unexpected appearance of a sarcoma :— (1) "In the course of continued propagation the chemical metabolism of the carcinoma cells becomes altered so that substances are formed which have a stimulative action on the connective-tissue cells and incite them to metaplastic growth ; (2) During the continued propagation connective-tissue cells are transplanted along with the carcinoma cells, and in the course of the numerous passages through strange hosts from animal to animal the connective-tissue cells acquire a power of proliferation ultimately attaining to that responsible for tumour formation."

To the second suggested explanation Bashford[1] remarked that as his investigations had shown that the stroma in the transplanted tumours as a rule degenerated and the new stroma was formed *de novo* from the inoculated animal, this hypothesis was in contradiction to the facts. Up to the present no evidence to the contrary has been adduced by other investigators.

In later publications Ehrlich and Apolant seem to have dropped this second explanation and have developed the first a little further. In a paper in the Berliner Klinische Wochenschrift, No. 2, 1906[2], the cause of the sarcomatous change is sought in "a stimulating influence proceeding from the carcinomatous cells, which in a certain stage of the development determines the sarcomatous transformation of the connective tissue scaffolding of the tumour." Ehrlich himself has not defined more clearly what he implies by "a certain stage of the development."

In summarising Ehrlich's and his own observations on this subject, Apolant[3] writes that the life of a transplantable carcinoma may be confined within time limits in the case even of the most virulent carcinomata such as those which had been supplanted by sarcomata

* The absolute time is not stated for the two first cases.

[1] Bashford, Murray, & Cramer: Einige Ergebnisse der experimentellen Krebsforschung. (Berl. klin. Wochenschrift, 1905, no. 46.)

[2] Ehrlich & Apolant: Weitere Erfahrungen über die Sarkomentwicklung bei Mäusecarcinomen. (Ibidem, 1906, no. 2.)

[3] Apolant, H.: Die experimentelle Erforschung der Geschwülste. (Handbuch der pathogenen Mikroorganismen by Kolle and Wasserman. 1ster Ergänzungsband, 1906, p. 456.)

during their investigations. This conception of a primary exhaustion of the carcinoma cells was taken up by Orthner[1] who, starting from abstract speculations on the effects of differences in the assimilative energy of cells in determining the formation of tumours, tried to utilise it to explain the development of sarcoma. In a rejoinder to Orthner, Ehrlich[2] adduced new observations showing that it had been possible in the case of a mixed tumour to isolate the carcinomatous elements from the sarcomatous, and that thereafter the carcinomatous cells could be propagated in numerous generations as any other tumour cells. This fact directly disproves the supposition of a primary exhaustion of the carcinoma.

In a still later publication Ehrlich[3] introduces a new idea. He states that the development of sarcoma depends upon a stimulating influence proceeding from the carcinoma-cells which have undergone some sort of chemical alteration. "Since as a rule all tumours of the same series do not show the same degree of sarcomatous change, I venture to conclu that the several animals respond to the same stimulus with different degrees of proliferation of their connective tissues. This phenomenon we are familiar with in man where the tendency of some individuals to cheloid illustrates it best. The appearance of a sarcoma depends in my opinion on the properties of the host to some extent, viz., on enhanced reaction on the part of the connective tissue progressing to sarcoma."

In their most recent contribution to this subject Ehrlich and Apolant[4] allude to the development of sarcoma from the same standpoint, viz.: that the epithelial cells of a carcinoma occasion, through one or other of their chemical properties, the sarcomatous transformation of the stroma of a predisposed animal.

[1] Orthner, Franz: Das Wesen der Avidität der Zellen zu den Nährstoffen und die Entstehung der Geschwülste aus verlagerten Keimen. (Wiener klin. Wochenschrift, no. 41, 1907.)
—— : Wachstum und Wachstumstillstand gutartiger und bösartiger Geschwülste. (Ibidem, no. 45, 1907.)

[2] Ehrlich: Bemerkungen zu den Aufsätzen des Herrn Dr. Orthner. (Ibidem, no. 49, 1907.)

[3] Ehrlich, P.: Experimentelle Studien au Mäusetumoren. Lecture to the 1st Internat. Congress for Cancer Research, Frankfurt-Heidelberg, 1906. (Zeitschrift für Krebsforschung, Band 5, Heft 1-2, 1907, p. 64.)

[4] Ehrlich & Apolant: Ueber spontane Mischtumoren der Maus. (Berliner klin. Wochenschrift, 1907, no. 44.)

Pathologists (v. Hansemann [1], Schlagenhaufer [2]) criticise Ehrlich and Apolant's observations by objecting that it has not been satisfactorily proved that a sarcomatous element was not already present in the primary tumour. Another criticism by Bashford has been already mentioned (p. 178); furthermore, Bashford [3] objected that the possibility of these sarcomatous tumours being infective granulomata is not excluded so long as the processes at the site of inoculation have not been followed in detail by the systematic examination of "early stages." The final proof, that Ehrlich's tumours are really true malignant new growths, sarcomata, was given by demonstrating their formation of metastases in the pulmonary artery by emboli of tumour cells which continue growing. The presence of collagen fibrils between the cells in intravascular secondary nodules proved the connective tissue origin of the cells [4].

Shortly after Ehrlich and Apolant's first publication, Leo Loeb [5] described another case of sarcoma development in which mixed tumours and spindle-cell sarcomata appeared already in the first transplantation of a sporadic growth. The primary growth is described as an adeno-carcinoma, imbedded in the tissue of the submaxillary gland ; no spindle-cell tissue was found in three pieces examined. It may, however, be objected that where we have a change to sarcoma occurring at the first transference of a primary tumour and the primary tumour only has

[1] v. Hansemann: Verhandlungen der deutschen pathol. Gesellschaft zu Meran, 1905.

[2] Schlagenhaufer: Carcinom und Riesenzellsarkom derselben Mamma. (Centralblatt für allgem. Pathologie u. path. Anat. 1906, no. 10.)

[3] Bashford, E. F.: Einige Bemerkungen zur Methodik der experimentellen Krebsforschung. (Berl. klin. Wochenschrift, 1906, no. 16.)
Ehrlich & Apolant: Erwiederung auf den Artikel des Herrn Dr. Bashford. (Ibidem, 1906, no. 21.)

[4] Haaland, M.: Ueber Metastasenbildung bei transplantierten Sarkomen der Maüs. (Ibidem, no. 34, 1906, and Zeitschrift für Krebsforschung, Band vi. Heft 1–2.)

[5] Loeb, Leo: Further experimental investigations into the Growth of Tumours. Development of Sarcoma and Carcinoma after the inoculation of a carcinomatous tumour of the submaxillary gland in a Japanese mouse. (The University of Pennsylvania Medical Bulletin, July 1906.)
——: Ueber Sarkomentwicklung bei einem drüsenartigen Mäüsetumor. (Berl. klin. Wochenschrift, 1906, no. 24.)
——: Ueber Entwicklung eines Sarcoms nach Transplantation eines Carcinoms. (Deutsche med. Wochenschrift, 1908, no. 1.)
——: American Association for Cancer Research, 1st Meeting, 15th Nov. 1907.) (Extract in the Journal of the Amer. Med. Association, Jan. 4, 1908.)

been investigated, it is more difficult to exclude the possibility of a sarcomatous component having been overlooked in the primary tumour. Loeb's conclusion is that it is most likely that some agency is transmitted from the gland-like structures to the connective tissues, and, accordingly, that the same agency is responsible for the growth of both tumours. He thinks it probable that this agency is transmitted to the connective tissues of the host surrounding the implanted tumour, and not to the connective tissue peculiar to the tumour itself and forming the stroma which lies amongst the glandular parenchyma. His reason for thinking so is, that where the site of inoculation has been examined in mice dying a few days after the inoculation of tumour-emulsion, he noticed a cellular sarcoma-like tissue had developed around the glandular tumour. Everyone familiar with the difficulties of interpreting "early stages" even when the conditions are most favourable, will hardly venture to draw far-reaching conclusions from observations on imperfect material, and for Loeb's instance of sarcoma development the possibility can hardly be excluded, that sarcomatous elements may have existed in the primary tumour.

Last summer, a case of sarcoma development similar to that of Ehrlich and Apolant was observed to occur twice in one of the strains of mammary carcinomata collected in the laboratories of the Imperial Cancer Research Fund. In a preliminary communication, conjointly with Bashford and Murray [1], we showed that the possibility of a mixed tumour from the outset could not be entertained. Not only was our primary tumour and the tumours from the subsequent generations examined with the usual histological methods, but also the behaviour of the stroma after transplantation was tested by examining "early stages." From the observations then at our disposal it appeared most likely that the development of sarcoma was explicable as a new or altered form of specific reaction to influences proceeding from the carcinoma cells, thus agreeing in the main with the opinions of Ehrlich and Apolant.

More recently Liepmann [2] and Lewin [3] have each described a case of sarcoma development in transplanted tumours. Liepmann's case was

[1] Bashford, E. F., Murray, J. A., & Haaland, M.: Ergebnisse der experimentellen Krebsforschung. (Berl. klin. Wochenschrift, 1907, nos. 38 & 39.)

[2] Liepmann: Münchener med. Wochenschrift, 1907, no. 27.

[3] Lewin, C.: Experimentelle Beiträge zur Morphologie und Biologie bösartiger Geschwulste bei Ratten und Mäüsen. (Zeitschrift f. Krebsforschung, Band vi. Heft 2, 1908.)

observed in the 8th passage of a mouse-carcinoma originally studied by Michaelis ; no details of this case have as yet been published. Lewin observed it in the fifth generation of an adeno-carcinoma of the rat, which exhibited simultaneously the characters of squamous cell-carcinoma. Lewin affirms that the four first generations show only a delicate stroma. He assumes without giving any direct evidence, that the sarcoma-cells arise from endothelial cells. He did not investigate the behaviour of the stroma after transplantation. The change subsequently appeared in all his strains, so that there is no pure carcinomatous strain now available for the study of the peculiarities of the stroma of this tumour. Orth[1] has advanced against Lewin's conclusions that the histological preparations do not convince him that a sarcoma is really present, but incline him to regard the growth of connective tissue as granulation tissue. "The presence here and there of spindle cells is also a feature of ordinary proliferations of connective tissue." As to the further transplantation of this sarcoma-like tissue, Orth thinks it requires to be proved that in these presumably pure sarcomatous tumours small remains of carcinoma are not still present and inciting connective tissues to granulomatous growth.

To summarise the present state of the question, in spite of the evidence which has been collected hitherto, some pathologists seem still unconvinced that true sarcomata arise in the course of the propagation of carcinomata in mice, and assume that these tumours may be explained as growth of granulation tissue. Others while admitting their sarcomatous nature, premise that there has been a mixed tumour from the outset of propagation, or attempt to explain the appearance of a sarcomatous tissue after numerous passages of carcinomatous tumours merely as the simultaneous presence of a transplanted carcinoma and a spontaneous sarcoma in the same animal[2]. We shall consider these objections in describing our own investigations.

[1] Orth: Discussion on Lewin's paper, loc. cit.). (Zeitschrift f. Krebsforschung, Band vi. Heft 2, 1908, p. 431.)

[2] Sticker, A.: Discussion on Lewin's paper. (Zeitschrift f. Krebsforschung, Band vi. Heft 2, 1908, p. 431.)

Method of Investigation.

The earlier observations on the development of sarcoma during the propagation of carcinoma were all made more or less unexpectedly, and often a long time after the change had actually occurred. As was to be expected, therefore, the material on which they are based has not been sufficiently complete. None of the other investigators seem to have tried to study the behaviour of the stroma after transplantation, but to have contented themselves merely with the general histological examination of the transplanted tumour.

Bashford pointed out that only through a careful examination of the processes at the site of inoculation would it be possible to come to an understanding on the point as to whether these tumours were granulomata or true sarcomata.

Therefore, once the sarcoma development was observed in one of our strains, it became our aim to collect a material as complete as possible, not only for studying the general histological features of the tumours, but also for a complete study of the processes at the site of inoculation, lest the phenomenon repeated itself in other strains. In this way we hoped to be able to bring forward incontestable evidence of the real nature of the changes and to define when they first occurred. If they are to be considered as real sarcomatous changes, then it is of the greatest importance to establish exactly from which elements the sarcoma cells have originated; whether from cells in tumours of previous generations, or even in the primary growth, or from cells of the reaction-tissue of the host in which the change was first observed.

The importance of this elaborate and detailed study is obvious. If further investigation can meet and refute the criticism advanced by v. Hansemann and by Schlagenhaufer that sarcomatous elements might have existed in the primary tumour, then we witness in the process something quite new, *nothing less than the first development of a malignant new growth from normal cells*, taking it for granted that we can prove beyond all cavil that the tumours obtained are really true sarcomata. All stages in this development take place under experimental conditions so that it is possible to follow the process step by step.

It was hoped that the sarcomatous change would occur again in some other strains and enable us to follow the process throughout, both before and after the alteration had taken place. This happened, but not before about 9 months after the first cases were observed.

We are in a fortunate position with our material for several reasons :—

(1) There are no gaps in the continuity of our earlier material. The primary tumour was examined on two occasions, in both of which several large pieces were preserved. In the following generations slices from *every* tumour which has been transplanted have been preserved for histological examination.

Seeing that the histology of these tumours often varies largely in the periphery and centre of the same tumour, we had made it a part of the routine, long before this change occurred, that it shall not be any haphazard part of the tumour which is taken for histological examination, but systematically a slice through the largest diameter of the whole tumour. In larger tumours several slices through the different parts are preserved by different methods (Zenker, Flemming, Borrel, Alcohol, etc.), or, where convenient, a longitudinal slice has been taken through the whole length of the growth, in order to get as great a surface as possible for examination, including all parts. Besides the tumours which have been transplanted in this strain, up to the present amounting to some 500, we have examined a great many others not transplanted, but preserved for histological study. Furthermore it may be noted, that every mouse with a tumour of this strain, when found dead, is preserved *in toto* in formalin, so that we may be able, if necessary, at any time to go back to our earlier material. As the growth of every tumour is recorded in our protocols ("charted") from 10 days after transplantation every 7 days up to the death of the mouse, we are always able to supplement the histological study of any given tumour by a knowledge of its biological behaviour. The charting of the tumours also allows comparisons of biological peculiarities of the different strains (*cf.* curve fig. 40).

(2) Besides this general histological and biological examination there has been an examination made of the processes at the site of inoculation at short intervals after transplantation, called for brevity's sake "early stages" on two previous occasions, of the primary tumour itself and of a tumour of the 4th generation (4 F giving 5 I). We had thus the opportunity of comparing the behaviour of the tumour from the very start of transplantation with that at any later stage, and the changes that might occur then.

(3) Our carcinoma has not been lost by this development of a sarcoma, as seems to have happened with our predecessors, except in one of Ehrlich's cases, where he succeeded later in recovering the

pure carcinomatous strain again. While the sarcomatous changes occurred in two distinct strains, we were able to continue transplanting 10 other separate strains which did not exhibit this change, and most of them up to the present date remain unaltered. Accordingly we have been able to submit these carcinomatous strains to a thorough study, from the standpoint of the general histological behaviour of the stroma, as well as give especial attention to its behaviour after transplantation, in "early stages." As it became impossible to study every transplanted carcinoma of this strain in this way from the mere multiplication of the number of tumours, we devised a technique, which enables us to make a rapid examination of the tumour before transplantation and, if any local change be present, then to pick out the interesting parts for "early stages" and for transplantation.

The mouse is killed and the tumour laid bare aseptically. While the tumour is still *in situ* three longitudinal slices are cut out through the whole tumour with sterile instruments, while the rest is removed and immediately put on ice in a sterile Petri dish. Of the three slices one is put into 40 per cent. formalin for rapid examination, one into Zenker for later study, and the third put on ice with the rest of the tumour, to be used for "early stages," if required. The first slice remains in the strong formalin till it sinks, *i. e.* for about a quarter of an hour, then it is washed in water for about half an hour and cut on a carbon dioxide freezing microtome. The frozen sections being fastened to a slide by a thin layer of celloidin are stained with Weigert's iron-hæmatoxylin. They allow us to study perfectly the main characteristics of the tumour before we proceed to inoculation. From the slice exactly corresponding, kept on ice, we are able to pick out any places which have thus been found to show interesting changes, and to study their transplantability with the method of "early stages." In this way we have been able to compare the behaviour after transplantation of the normal delicate stroma of these tumours with that of the more cellular or sclerotic parts. In the same way we have compared the behaviour of the stroma of pure carcinomatous strains with that of the tumours showing the first indications of the sarcomatous change, and have followed its advance to the condition of pure sarcoma.

It is the result of these investigations we propose to give in the succeeding pages in the following order :—

(1) Histology of the primary growth and of the subsequent generations of carcinomatous tumours.

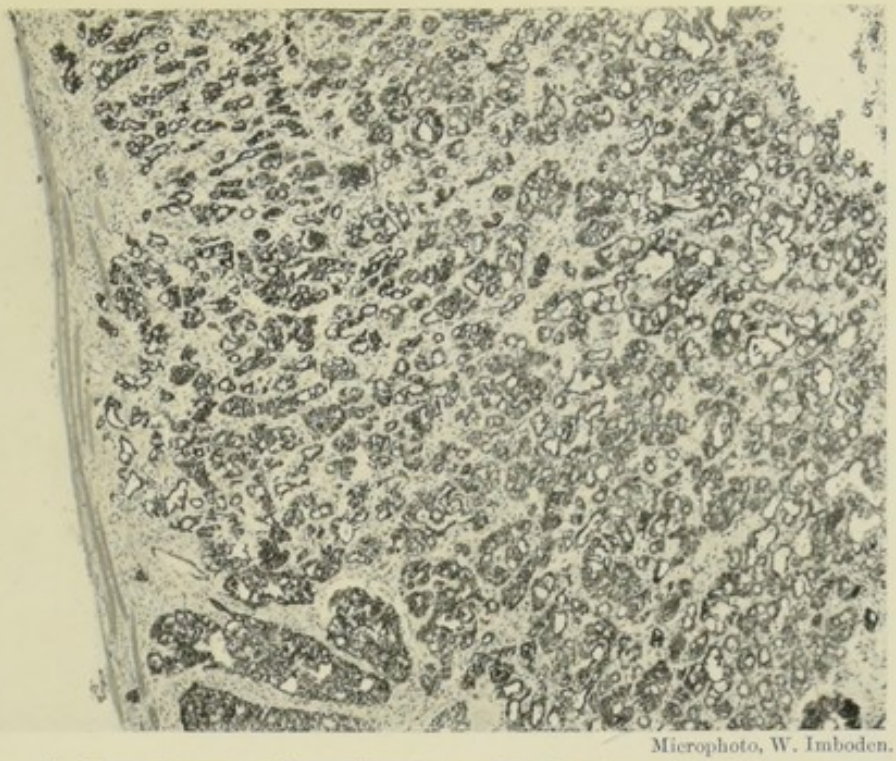
(2) The first cases of sarcoma development.
(3) The progressive advance of the sarcomatous changes as they proceed spontaneously and when the conditions are modified artificially.
(4) Study of changes in the stroma of carcinomatous strains, especially in old tumours.
(5) Later cases of sarcoma development with special reference to examination of the processes at the site of inoculation ("early stages").
(6) Biological characters of carcinomatous, of mixed and of sarcomatous strains, and especially of the carcinomatous strains in which subsequently the development of sarcoma has taken place.
(7) Summary.

(1) Histology of Primary Growth and of the Subsequent Generations of Carcinomatous Tumours.

The primary tumour which forms the starting point of the following observations and experiments was situated on the side of the thorax of an old female mouse, its size being about that of a walnut ($2 \times 1{\cdot}5$ cm.). The tumour was removed by operation on the 25. 9. 06. Two pieces from different parts of the tumour were preserved in Flemming's and Zenker's solutions for histological purposes and the rest inoculated into 162 young mice, about 7 weeks old. A number of these mice were killed on the days following immediately on the inoculation (viz.: on the 2nd, 3rd, 4th, 5th, 7th, and 9th day) for examination of the "early stages" of the processes responsible for the development of the daughter tumours. After three weeks 69 mice survived and of them 8 bore tumours. Of these 8 tumours 4 were transplanted, and their descendants have given rise to a number of parallel strains (see the genealogical tree appended to this paper).

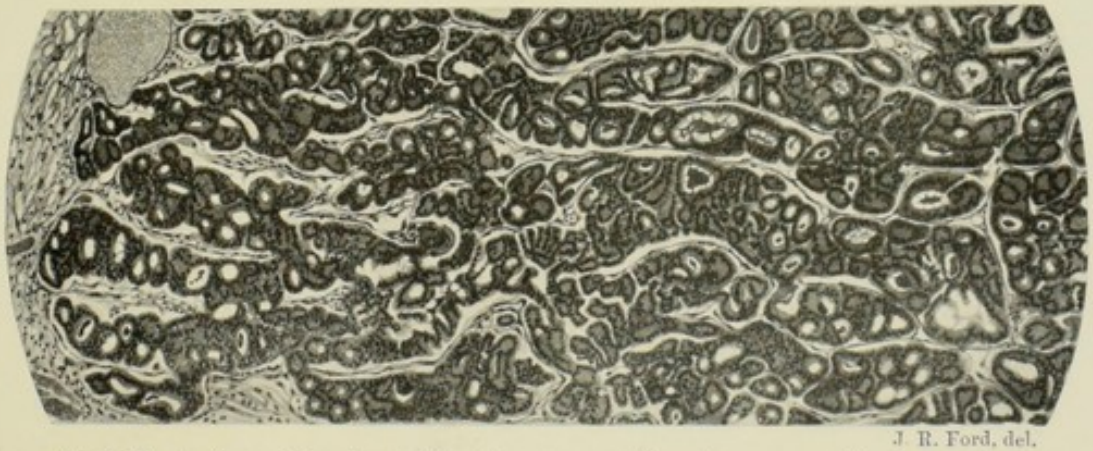
The primary tumour recurred soon after the operation. The recurrent tumour being inoperable the mouse was killed on the 22.11.06, and particles of the tumour were inoculated for the second time, into 41 young normal mice; this time, however, the result was negative. When the mouse was killed 5 pieces of the tumour were preserved in Flemming for histological examination, and the entire mouse preserved in Zenker's fluid and later cut in serial sections.

The histological study of the primary tumour reveals an adenocarcinoma of marked acinous structure, both in the material from the



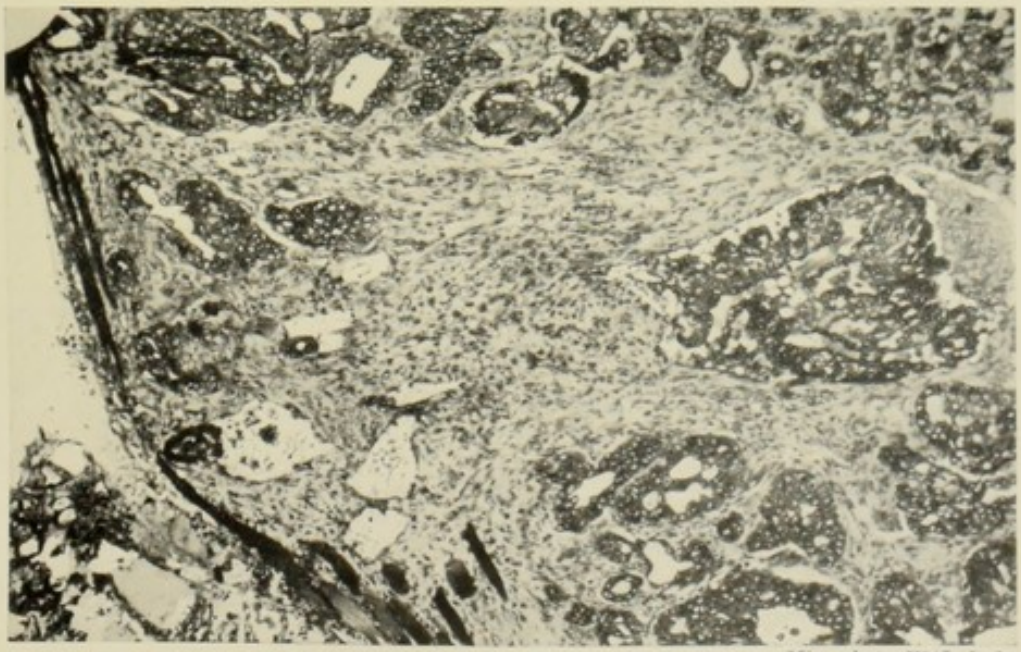
Microphoto, W. Imboden.

Fig. 1.—$\frac{37}{0}$. Primary tumour. Adenocarcinomatous portion of periphery with cellular stroma. Infiltration of panniculus carnosus at surface (left). $\times \frac{20}{1}$.



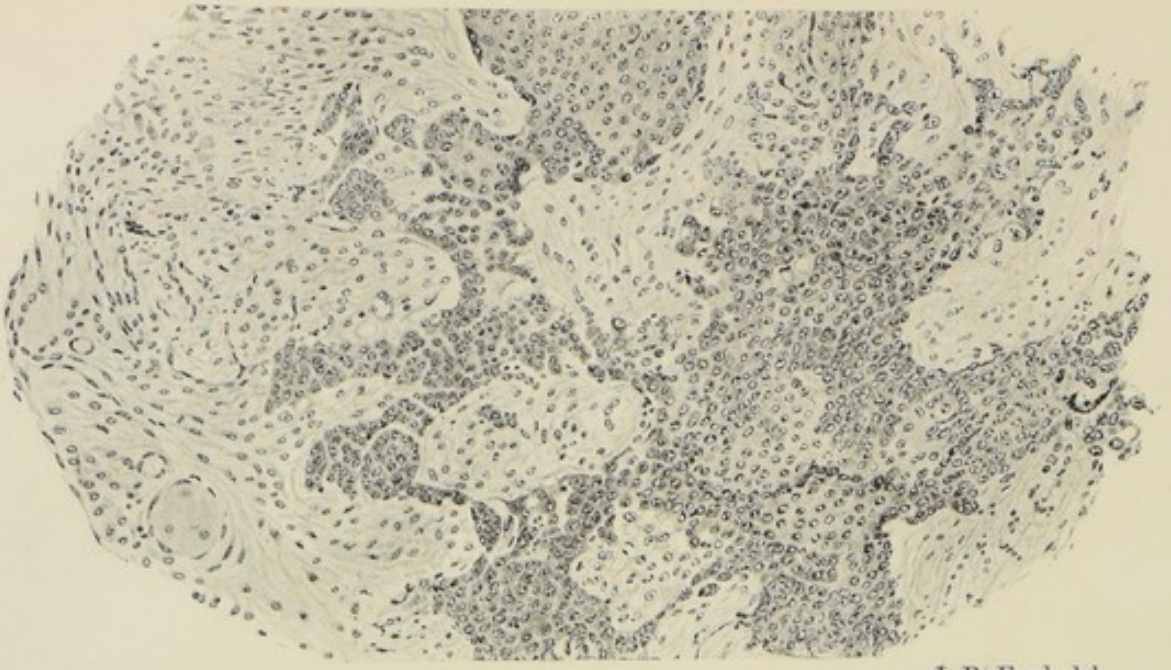
J. R. Ford, del.

Fig. 2.—$\frac{73}{0}$. Another peripheral part of the primary tumour. Parenchyma arranged in columns of acini, many of which contain secretion. Stroma delicate. $\times \frac{40}{1}$.



Microphoto, W. Imboden.

Fig. 3.—$\frac{37}{0}$. Another peripheral part of the primary tumour. Parenchyma mostly alveolar with irregular lumina. Infiltration of panniculus carnosus. The stroma is especially abundant and cellular in this area. $\times$ circa, $\frac{120}{1}$.



J. R. Ford, del.

FIG. 4.—Jensen/50$_2$ C. Tumour in process of spontaneous absorption. The alveoli of parenchyma are separated from each other by broad strands of cellular and fibrous connective tissue. × circa $\frac{150}{1}$.



J. R. Ford, del.

FIG. 5.—37/10$_2$K—11$_2$V.—Tumour of 10th generation (28 days). Pronounced adenomatous parenchyma. Stroma delicate. × $\frac{62}{1}$.



J. R. Ford, del.

FIG. 6.—37/60—7 R. Recurrent tumour of 6th generation, 39 days after operation. Parenchyma alveolar, stroma delicate and scanty. × $\frac{62}{1}$.

first operation and in the recurrent tumour. The parenchyma presents different features in different areas. In some areas it consists of small glandular acini closely resembling the acini of the mamma, the likeness being emphasised by the presence of secretion in some of the acini of the tumour (fig. 1). In other areas the parenchyma is arranged in broad strands of epithelial cells or alveoli in which lumina are present here and there (fig. 2). In others, again, the parenchyma forms solid alveoli without lumina, and packed with cells of identical character. The glandular structure occurs especially in the material from the first operation, as illustrated in figs. 1–3. When the mouse was killed after recurrence the tumour was more alveolar in structure.

The character of the stroma of the primary tumour varies. Throughout the greater part it is delicate as in fig. 2. In one particular area at the periphery of the tumour where at the same time the acinous structure of the parenchyma is also very pronounced, it is rather abundant and somewhat cellular, as fig. 1 shows. Fig. 3 shows a part of this area, under a higher magnification, lying close under and infiltrating the panniculus carnosus. Such localised cellular areas are often met with in sporadic tumours ; and on the whole the cellularity of the stroma is not more pronounced than occurs in most of them. The stroma of the recurrent tumour, examined at the death of the animal, is far less cellular, and is very delicate.

A picture similar to that in fig. 3 is often observed in other propagated tumours, when muscle has been infiltrated ; but it may also occur apart from this, e. g. accompanying processes of spontaneous absorption of tumours. Such a picture is shown in fig. 4 from Jensen's tumour to illustrate transitory changes in the stroma which, however, do not indicate any special properties of its connective tissue cells. They have not persisted on further propagation.

The daughter-tumours obtained by the transplantation of the sporadic growth, as well as their descendants obtained during continued propagation, retain a pronounced acinous structure of the parenchyma as a rule, with a delicate stroma. The details of the histological picture, as far as the parenchyma is concerned, vary within wide limits. In some cases, mostly in the more slowly-growing tumours, we see a marked adenomatous structure, as illustrated in fig. 5 for a 28 days old tumour from the 10th generation. In this tumour the gland-like acini are formed in parts of a single layer of columnar cells ; but more frequently several layers of cells are found. In other cases we get alveolar structures containing lumina here and there, resulting from an irregular

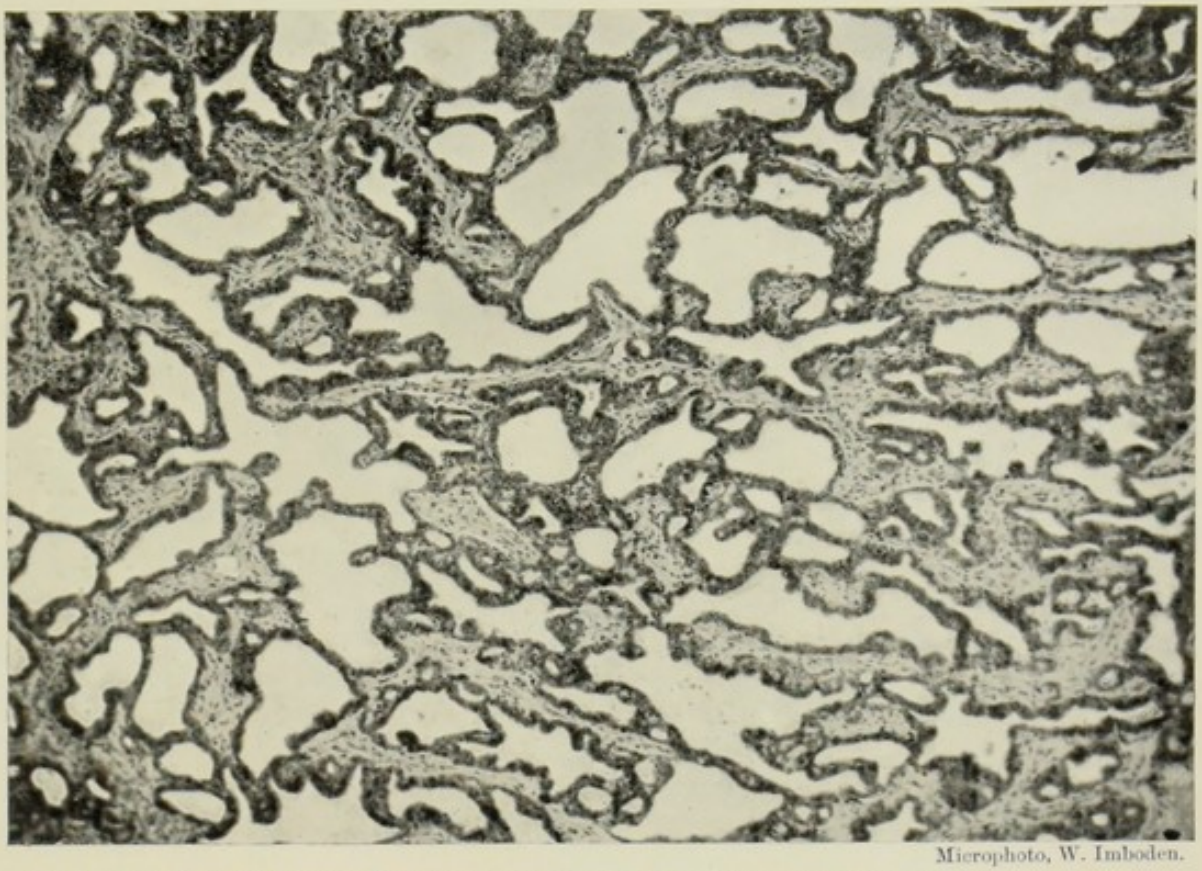
proliferation of the lining epithelium. These lumina are lined with columnar cells, the histological picture being then like that of the primary tumour (fig. 2). This is the most common type. A few strains show a more marked alveolar structure, and in them lumina are met with only occasionally ; such a tumour is shown in fig. 6 from a 39 days old tumour of the 6th generation. Sometimes this alveolar structure is maintained in all tumours of one strain through several generations, and exhibited practically uniformly throughout the whole section of any individual tumour. Still this is not the rule: on the contrary, the younger parts of a tumour, viz., those at the periphery, are usually more solid and alveolar ; while the older parts, viz., those towards the centre, are usually more acinous. However, acinous parts are often intermingled in the same tumour with alveolar areas quite irregularly.

The wide limits within which the appearances in the same tumour vary are shown in figs. 7 and 8. The figures are from photographs of a single tumour in one and the same animal, and from two places in the same section only a few millimetres distant from one another. These two morphological types are sharply distinguished : on the one hand we have the histological picture of a cyst-adenomatous growth with papillary excrescences (fig. 7), and on the other hand that of a typical solid carcinoma (fig. 8). Nobody who is not familiar with the inconstancy of the morphological characters of transplanted tumours would ever have expected two types so different to arise out of the cells of the same tumour when implanted into the same animal.

The stroma of the transplanted tumours is as a rule very scanty (*cf.* figs. 5 and 6). In old tumours there is, however, a marked difference between the stroma of the periphery and that of the centre. Fig. 41 shows a typical old tumour under a low magnification. In the periphery (to the left) the stroma is very delicate and hardly to be seen in the photograph ; it consists mainly of capillaries and delicate collagenous fibrils ; only a few connective-tissue corpuscles are scattered here and there throughout the stroma. In the central part (to the right) the relative proportion of collagenous fibrils is increased, and a sclerotic tissue with very few cells separates the carcinoma-acini from each other. This feature is almost constant in old tumours.

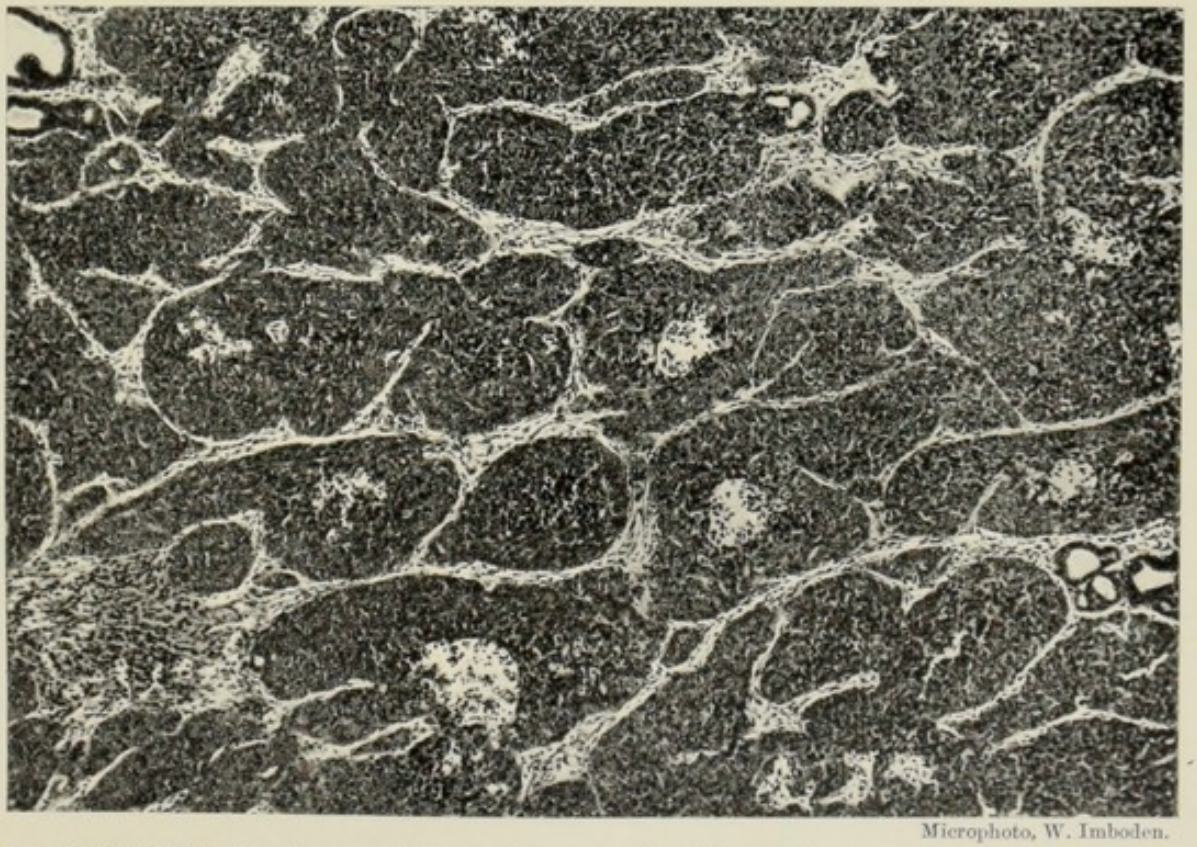
We usually find atrophic and necrotic changes in the carcinoma alveoli which are surrounded by this sclerotic tissue. They proceed from the centre of the alveoli towards the periphery, often leaving only a single layer of carcinoma cells close up to the fibrous tissue, while all the rest

[*To face* p. 188.



Microphoto, W. Imboden.

FIG. 7.—37/11$_3$ O—12$_3$ D. Tumour of 11th generation (41 days). Parenchyma growing as papilliferous cystadenoma. Stroma slightly cellular. × $\frac{100}{1}$.



Microphoto, W. Imboden.

FIG. 8.—37/11$_3$ O—12$_3$ D. Same tumour as fig. 7. Another part at a few millimetres distance. Parenchyma alveolar with central necrosis of alveoli. Stroma slightly cellular. *Cf.* fig. 7. × $\frac{100}{1}$.

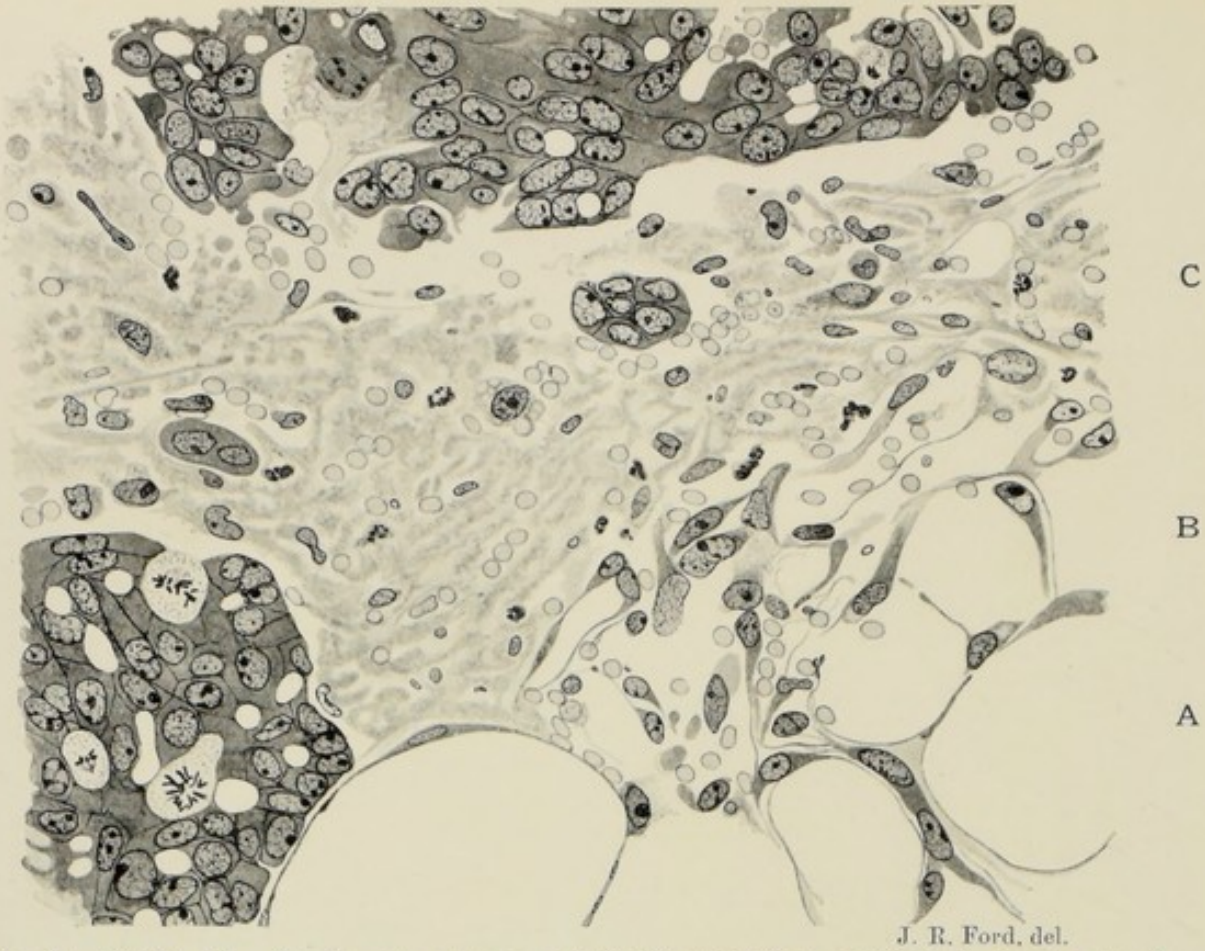


FIG. 9.—37/4 F—5 I. Carcinomatous tumour of fourth generation, examined in "early stages" (48 hours after inoculation). Complete degeneration of stroma. Carcinomatous parenchyma dividing. Early invasion by new capillaries. A = r,tissues of host; B = line separating tissues of host and graft; C = graft. (Zenke, Weigert's hematoxylin.) $\times \frac{500}{1}$.



FIG. 10.—37/9 D—10 V. Carcinomatous tumour, figured in fig. 18, examined in "early stage" (33 hours after inoculation). Rich cellular stroma, mostly undergoing degeneration, no signs of cell division. Carcinomatous parenchyma dividing. A = tissues of host; B = cleft separating tissues of host from graft; C = graft. (Flemming, iron hematoxylin.) $\times \frac{500}{1}$.

of the alveolus is necrotic. In later stages this peripheral layer of carcinoma cells undergoes atrophy also, and in the centre of the tumour we find a sclerotic scar-tissue left without carcinoma. The sclerotic processes in the connective tissue of the central parts of the tumour are analogous to those in an ordinary scirrhus mammæ in the human subject.

This scirrhous character is associated with a relatively slow growth of our tumour, just as in the human subject. At the same time its consistence is firmer, as a rule, than that of the usual alveolar tumour, and it breaks up less easily into an emulsion. Its firmer consistency seems due only in part to a greater abundance of connective-tissue fibrils ; the special structure of the tumour is also contributory. The groups of cells into which the parenchyma is divided, whether acinous or alveolar, are rather small compared with those of other transplantable mammary tumours of the mouse with large alveoli. Since the small acini or alveoli are separately enveloped by connective-tissue fibrils and capillaries, the whole framework of the tumour is stronger, and hence the tumour acquires a firmer consistency than do the alveolar tumours ; the carcinomatous elements seem also better nourished. Necrotic changes due to circulatory disturbances are less pronounced in this tumour than in the alveolar tumours where an early central necrosis of the single alveoli as well as of the centre of the whole tumour is a very characteristic feature.

The average percentage of success of the transplanted series is higher than that of the first transplantation of the primary tumour, as in most other tumours. It has never reached any great height but has usually been about 40 to 50 per cent. Similar fluctuations as have been demonstrated for other tumours by Bashford, Murray and Bowen are met with in this tumour, but are as a rule not so pronounced here as in the more rapidly growing tumours.

Examined in early stages, this tumour does not on the whole markedly differ from others studied by Bashford, Murray and Cramer. The few stroma elements, found between the carcinomatous alveoli, show as a rule definite degenerative changes, and are replaced by a reaction-tissue of angio- and fibroblasts from the host. This seems to be the case both in the primary tumour and also for a tumour of the 4th generation which was examined (used to yield 5 I). There is no direct evidence of participation of the introduced stroma-cells in the formation of the new tumours. Fig. 9 shows a graft of the tumour examined of the 4th generation, preserved 48 hours after transplantation. In the portion

drawn, the degenerative changes of the introduced stroma elements are most marked, and there is no sign of survival or proliferation to be seen. A commencing reorganisation from the host is observed on the periphery of the graft. In sharp contrast to the degenerative changes in the interstitial tissue, the parenchyma has remained alive and shows numerous mitoses. Fig. 10 shows another graft preserved 33 hours after transplantation from a carcinomatous tumour with more cellular stroma than usual (the same tumour as in fig. 18). The stroma-cells show mostly degenerative changes and here also there is no sign of proliferation.

In numerous parallel strains of this tumour the general histological picture has remained essentially unchanged through numerous generations, except for small variations from acinous to alveolar structure and *vice versa*. The stroma in all these tumours has been very delicate as a rule. No especial features have been found sharply distinguishing the stroma from that of other transplanted tumours with the exception of the above mentioned changes advancing to sclerosis in old tumours, and now and then a slightly increased cellularity appearing in one generation and disappearing in the next. In some of the strains, however, the stroma has exhibited unusual features, and we shall discuss them in some detail.

(2) The first cases of Sarcoma Development.

(a) 1*st Case* (7 D–8 H).

Peculiar alterations in the stroma were observed in the first instance simultaneously in four tumours of series D of the 7th generation (7 D). From this series 7 tumours were transplanted in such a way that 4 tumours were mixed together and used as material for the series 8 H; while the other 3, also mixed, gave rise to the series 8 L *. From each of these 7 tumours a slice through the whole was preserved in Zenker's fluid. On histological examination we find in 3 of them the usual picture of an acinous carcinoma in which the stroma is perhaps a little more cellular than normal; in the 4 others, however, a remarkable change has taken place. The nature of the change is illustrated in figs. 11 and 12, drawn from two of the four tumours showing it. We see between the carcinomatous acini in place of the usual delicate stroma a very cellular tissue, consisting of large spindle-cells, rich in

* The mixing together of several tumours has been practised only for these two series and a few of the next generation. It has been our method to transplant each tumour separately, unless exigencies of experiments required an unusual amount of material (immunity experiments).

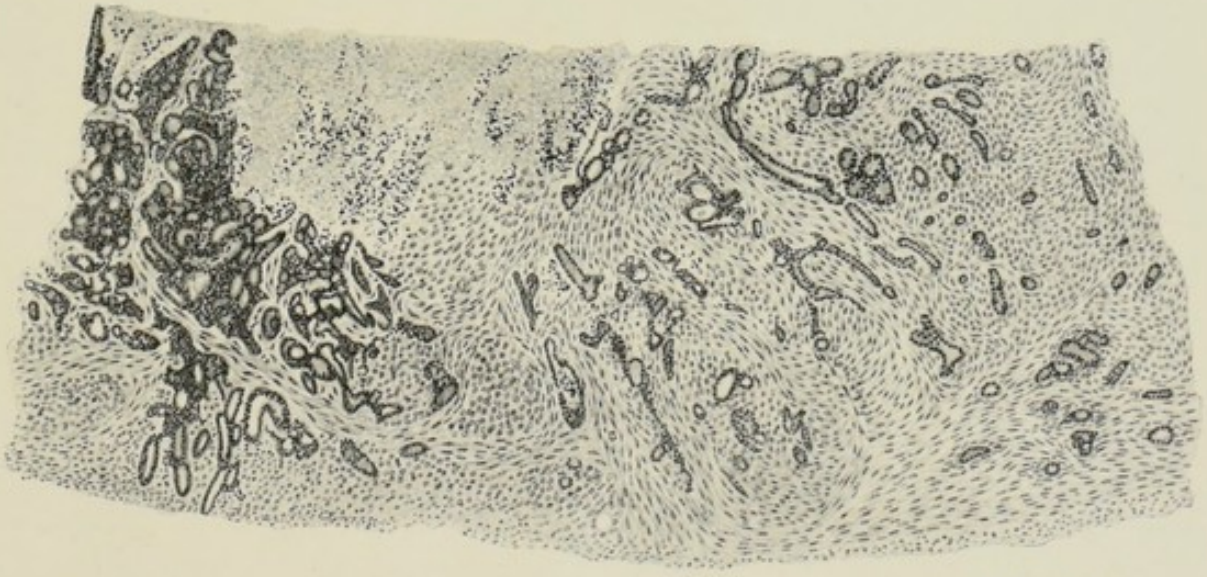
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Figs. 11–16. First case of sarcoma development.



J. R. Ford, del.

Fig. 11.—37/7 D—8 H. One of four tumours (41 days old) of series D of the 7th generation which showed first cases of sarcoma development. Abundant cellular interstitial connective tissue consisting of large spindle cells are present between the islands of acinous parenchyma throughout the whole tumour. $\times \frac{62}{1}$.



J. R. Ford, del.

Fig. 12.—37/7 D—8 H. Another tumour (41 days old) of series D, 7th generation, in which the sarcomatous transformation of the stroma occurs as a localised process, the rest of the tumour only showing a slightly cellular stroma. $\times \frac{62}{1}$.

First case of sarcoma development.

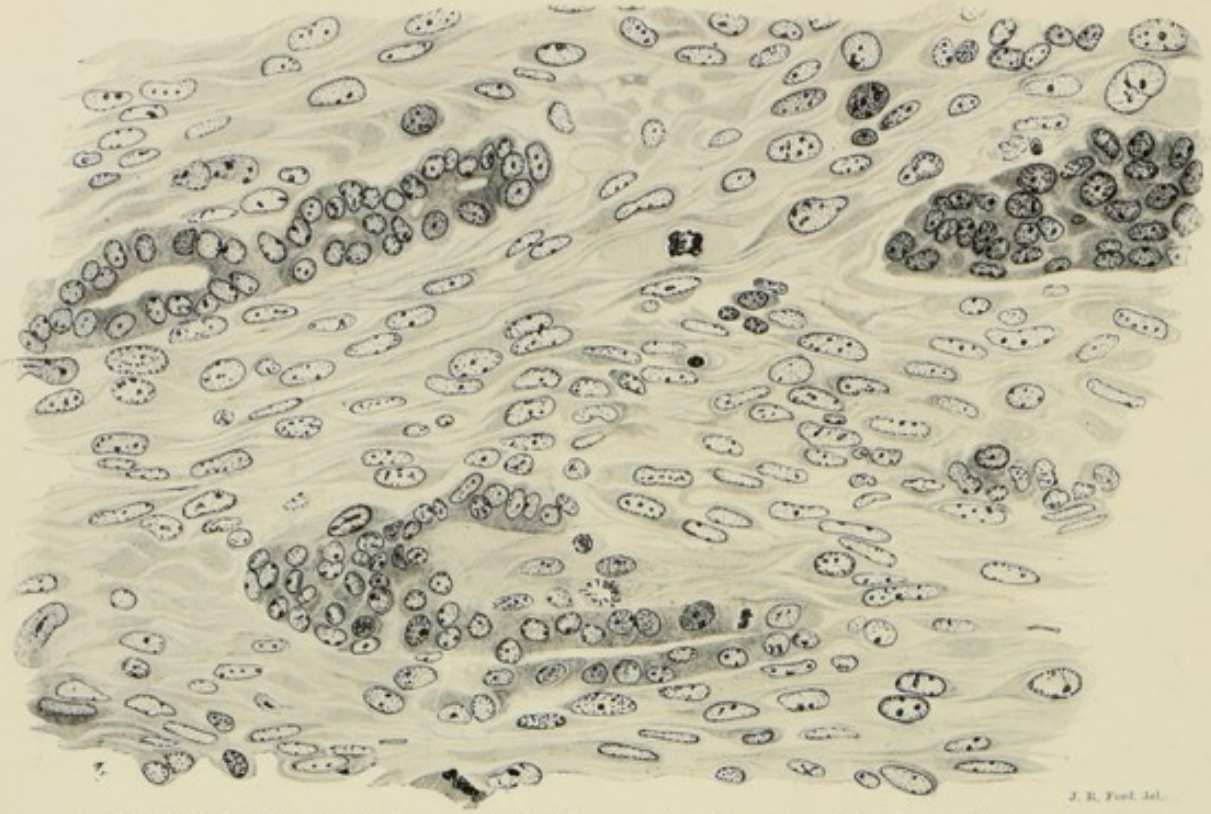


FIG. 13.—37/10 C—11 G. "Mixed tumour" of 10th generation (23 days). More advanced stage of sarcoma development, broad bands of spindle-celled sarcomatous tissue separate the carcinoma-acini. Numerous mitoses in the sarcoma cells. $\times \frac{200}{1}$.



FIG. 14.—37/8 H–9 Q. Mixed tumour from series 8 H examined in "early stages." Peripheral part of a graft 24 hours after transplantation. The graft (C) is separated from the tissues of the host (A) (lower part of figure) by a layer of fibrin (B). Three carcinoma-acini surrounded by rapidly proliferating interstitial sarcomatous tissue. (Flemming; Ironalum hematoxylin. $\times \frac{200}{1}$.

protoplasm with large elongated nuclei. Between these cells and applied to their surface fine collagenous fibrils are to be found, their presence being evidence for the fibroblastic origin of the cells. The same number of capillaries is seen as in the carcinomatous tumour; these capillaries have their own wall of endothelial cells, and the endothelial elements seem to have nothing to do with the change. In three of the tumours the change extends through the whole section; in the fourth, however (fig. 12), it is localised to a single circumscribed area, the rest of the section showing a carcinoma with a somewhat cellular stroma.

In the first instance the alterations were suggestive of a process accompanying spontaneous absorption, similar to those often observed in other tumours and described in detail by Bashford, Murray and Cramer [1] for Jensen's carcinoma, and also by Gaylord and Clowes [2] (*cf.* fig. 4). It was, however, astonishing that the change appeared in so many tumours at the same time and with such uniformity, and the result of transplanting the tumours shows us that we have to deal with a process of a different kind. While series, made with tumours showing signs of spontaneous absorption, usually give a very low percentage of success, if any result at all, these series gave a very high one, about 60 per cent. Further, the histological examination of the daughter-tumours shows that the same changes in the stroma were maintained in the following generation and even increased in degree. Of the 22 tumours developed in 8 H, 20 have been examined histologically (and 11 of them were transplanted). In all of them broad bands of similar large spindle-cells are seen between the carcinomatous acini; and they are dividing rapidly. The same is the case in the next generation. As prototype of this stage in the alteration, a part of a tumour from the 10th generation is shown in fig. 13.

When we examined what was going on at the site of inoculation at this stage, 18–24–36–48 hours, up to five days after transplantation of this changed material, we found that *the cellular connective tissue no longer degenerates after transplantation to anything like the same extent as the scanty stroma in the earlier generations did, but that a large number of its elements are capable of remaining alive, and already after* 24 *hours show numerous mitoses, and continue to proliferate independently* (fig. 14).

[1] Bashford, Murray, & Cramer: Action of Radium on Transplanted Mouse Tumours and its Relation to the Spontaneous Arrest of their Growth. (2nd Scientific Report of the Imperial Cancer Research Fund, April 1905.)

[2] Gaylord, H. R., & Clowes, G. H. A.: On spontaneous cure of Cancer. (Surgery, Gynecology, and Obstetrics, vol. ii. no. 6, June 1906.)

This alteration occurred simultaneously in four different tumours of the same series (7 D). The question that first suggests itself is, what are the characteristics of the mother material of these tumours as well as of the chain of antecedent tumours in this particular strain?

In following this strain from the primary tumour we find merely a simple delicate stroma in the first four generations. In the 5th generation we find a cellular connective tissue round the carcinoma alveoli in a peripheral nodule, like a zone of stronger reaction from the host (fig. 15). The rest of the tumour shows a delicate stroma. The tumour of the 6th generation, which was the mother material for 7 D, presents similar features and it has a delicate stroma throughout almost the entire section, and only in a localised peripheral area is there found a cellular stroma, also apparently implying a zone of stronger reaction round the carcinoma alveoli. This zone of reaction consists mainly of small elongated or round cells (fig. 16). This material has not been examined in "early stages." The difference between the 6th and the 7th generations is very striking. In the latter we find that four out of seven tumours have got an abundant and extremely cellular stroma, the elements of which possess the property of independent continued growth after transplantation. The change seems to be a sudden one, and (apart from the increased cellularity in circumscribed peripheral parts of the tumours in the two previous generations, suggesting an enhanced reaction on the part of the host), there is no indication as to how the new cells have arisen. The fact that the change occurred simultaneously in four tumours of the same series, might suggest that the earliest stages of the process were already present in the mother material as quite localised changes which have escaped our observations, through being absent in the particular slice through the whole tumour, which was preserved and examined histologically.

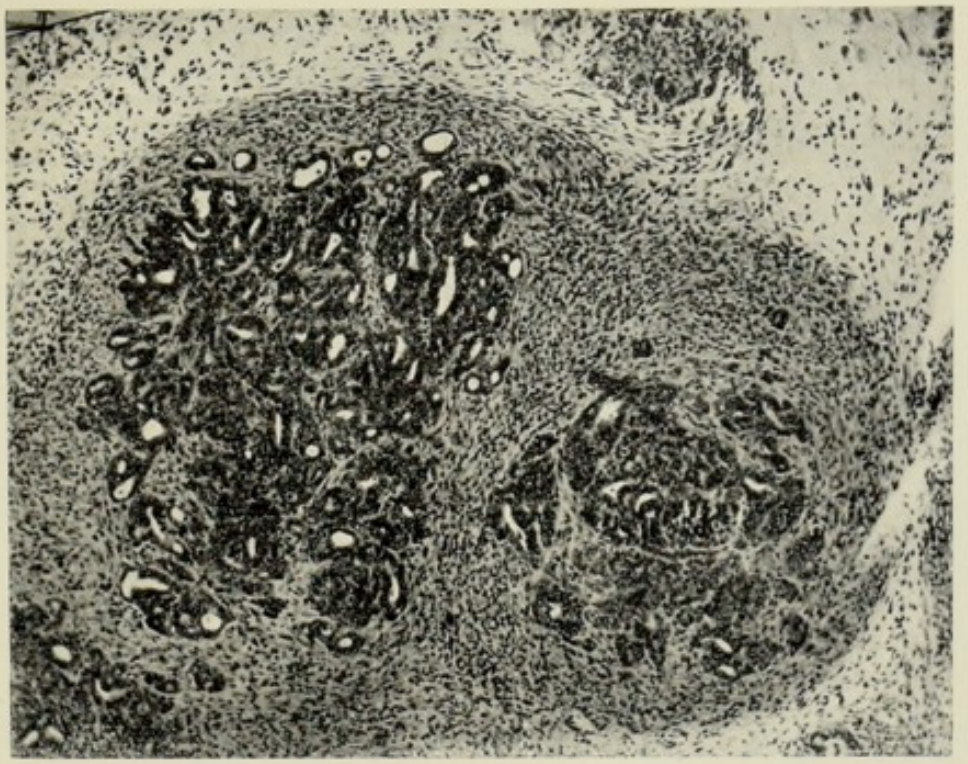
(b) *2nd Case of Sarcoma Development* (8 J–9 H).

Having noticed these changes in one strain, we watched the numerous other strains of the same tumour very closely. Five weeks later the same kind of change was observed in another strain (in a tumour of series J of the 8th generation), which had been propagated independently since the 2nd generation (see the genealogical tree). Up to the 7th generation the tumours transplanted in this strain show the usual delicate stroma without any peculiar feature.

The mother material used to yield the series 8 J shows in the centre

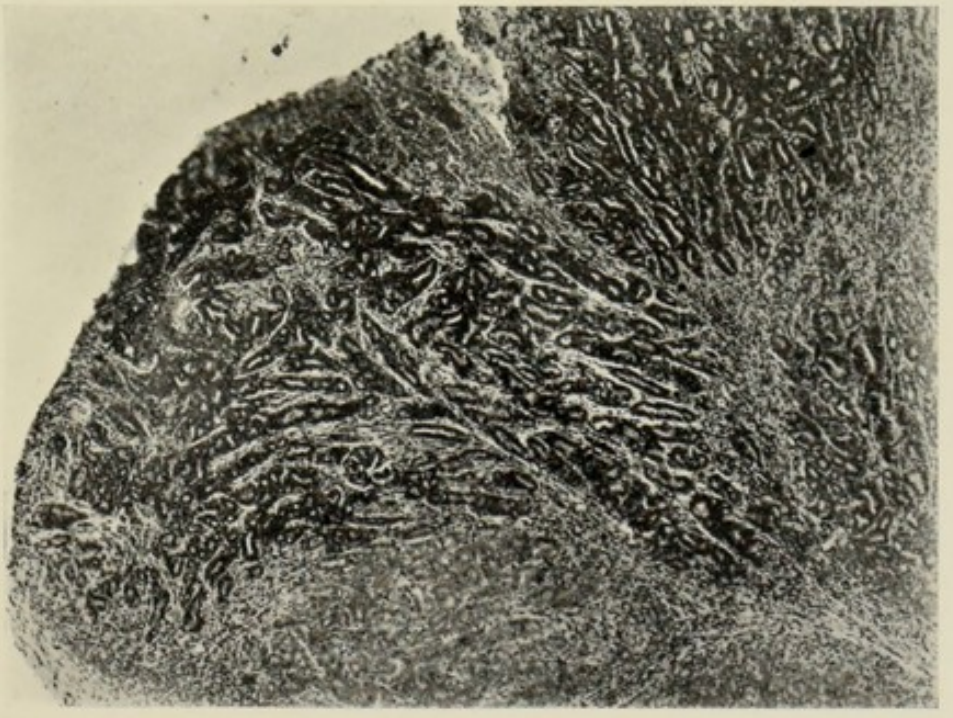
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First case of sarcoma development.



Microphoto, W. Imboden.

FIG. 15.—37/5 F—6 A. Peripheral nodule of a young tumour (23 days) of 5th generation. Parenchyma surrounded by a broad zone of young cellular connective tissue. The stroma in the greater part of the tumour was not so abundant and was delicate. The zone of cellular connective tissue around the nodule figured is indistinguishable from that accompanying spontaneous absorption in other tumours. Two generations later the first cases of sarcoma development occurred in descendants of this tumour (viz. 7 D–8 H). $\times \frac{100}{1}$.

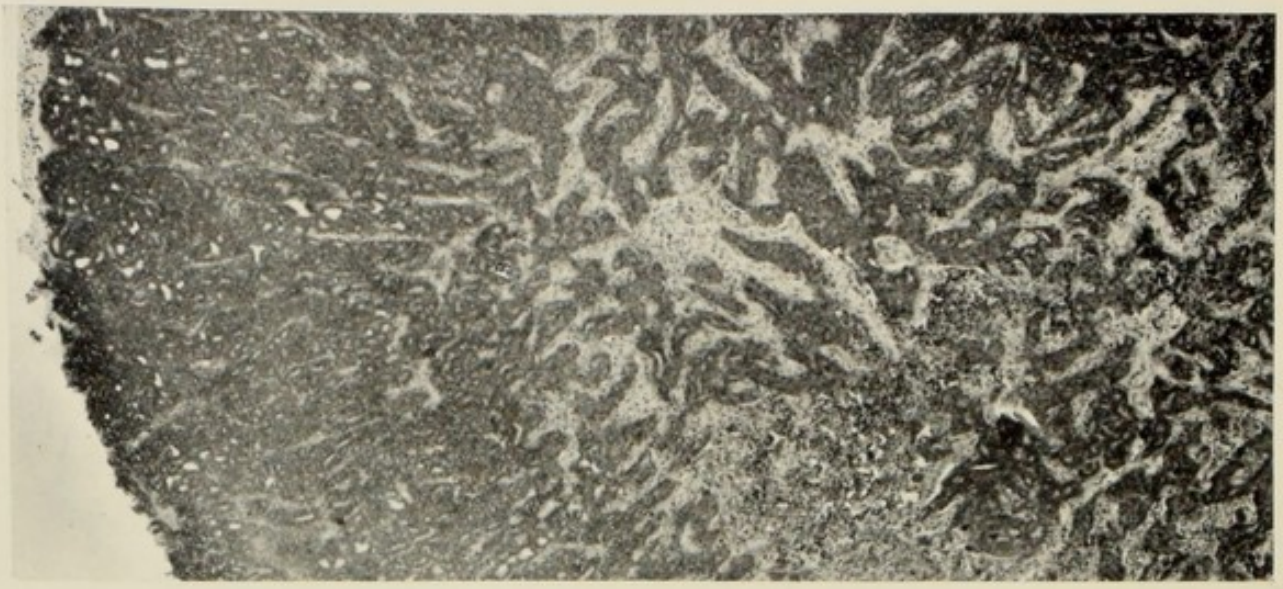


Microphoto, W. Imboden.

FIG. 16.—37/6 A—7 D. Peripheral part of a tumour of 6th generation (27 days); mother-material for the series in which the first cases of sarcoma development occurred. The stroma in the area figured is more cellular than is usual and than it is in the rest of the tumour. $\times \frac{75}{1}$.

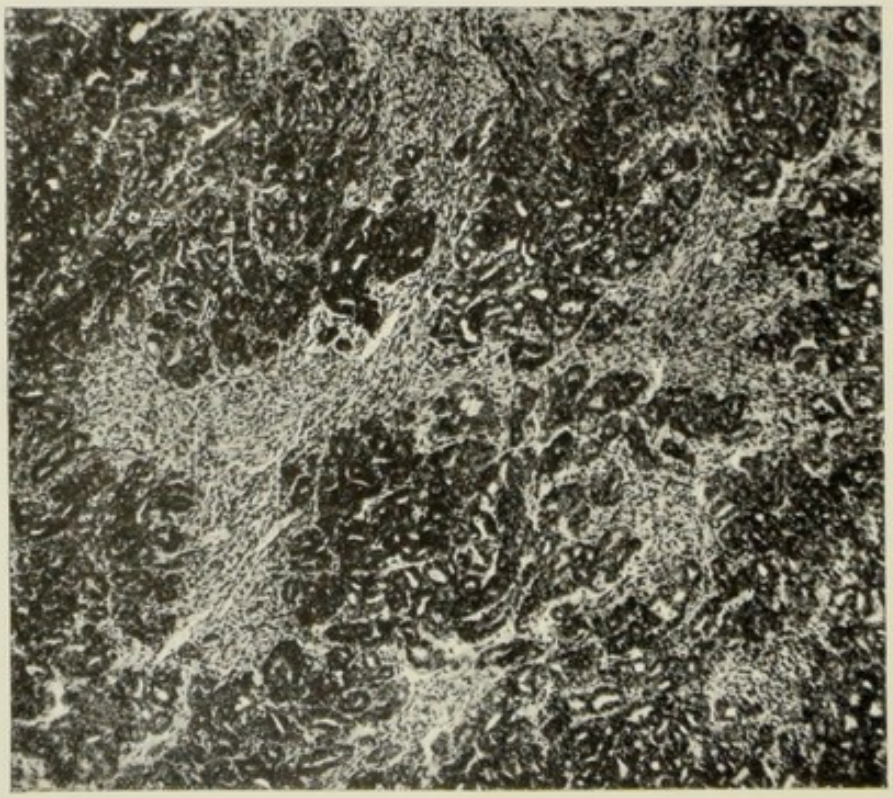
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Figs. 17–20. Tumours associated with second case of sarcoma development.



Microphoto, W. Imboden.

FIG. 17. 37/7 G—8 J. Mother-material of series in which second case of sarcoma development occurred. Section of tumour of 7th generation (40 days). In the centre of the tumour (right) the stroma is sclerotic and slightly cellular. In the peripheral parts the stroma is delicate. $\times \frac{60}{1}$.



Microphoto, W. Imboden.

FIG. 18. 37/8 J—9 D. One of three daughter-tumours (12 days) from series 8 J (the mother-material of which is figured above, fig. 17), showing abundant and cellular stroma in a peripheral area but no sarcomatous changes. The strain derived from this tumour has been further propagated for six generations as pure carcinoma. $\times \frac{80}{1}$.

marked sclerotic change with a slight cellularity (fig. 17), whereas the peripheral parts show a delicate stroma.

In series 8 J there were three tumours, each was transplanted separately and yielded the series 9 D, 9 H, and 9 I, respectively. The tumour used to yield 9 D, shows in a peripheral part a very cellular stroma, as shown in fig. 18. That this cellularity does not signify a sarcomatous change is shown by the fact that the descendants of 9 D up till the present date (through 6 generations) have maintained, more than a year later, their purely carcinomatous character without alteration in the stroma. The tumour which gave 9 I did not show any noticeable change, and its inoculation was negative.

However, the tumour used as material for 9 H showed on histological examination changes similar to those seen in the four tumours of 7 D (figs. 11 & 12). A part of this tumour is shown in fig. 19. The connective-tissue elements have become abundant and form strands of large spindle-cells scattered diffusely through the whole tumour between the carcinomatous acini.

In the strain to which this tumour belongs, a somewhat different technique of transplantation has been employed, compared with that used in the strain in which the case already described occurred. In the first case the method used through several generations was that of mincing down the whole tumour and introducing relatively large quantities, 0·025–0·05 c.c. or more, of the emulsion with a graduated syringe *. The method used from the primary tumour onwards in the second strain in which sarcoma developed is that usually employed for inoculating sporadic tumours in this laboratory, as described by Bashford and Murray, viz.: the insertion of relatively small fragments of the tumour by means of a hollow needle and plunger *, so that the anatomical relations of the components are as little disturbed as possible. At the same time the method of inoculation by the needle results in a progressive subdivision of the tumour, so that later series can be traced back to very minute circumscribed areas in the tumours of preceding generations. The occurrence of the sarcomatous change under both procedures shows that the method of inoculation is of no essential importance in inducing the alteration.

From this tumour yielding series 9 H, thirteen daughter-tumours were obtained, and eleven of them were transplanted separately and examined histologically. In all these tumours and in their descendants,

* Figured on page 268.

the same change is to be found in increasing degree as was also the case in the tumours from 7 D. As an illustration a microphotograph is given of a peripheral part of a tumour of the 10th generation (fig. 20, compare the corresponding stage in fig. 13). In examining early stages from these tumours we find the stroma-cells independently growing, as before illustrated in fig. 14.

The details of all these tumours, both of their histology and of the result of transplantation are given in the genealogical tree, for which reason we need not enter into them here more fully.

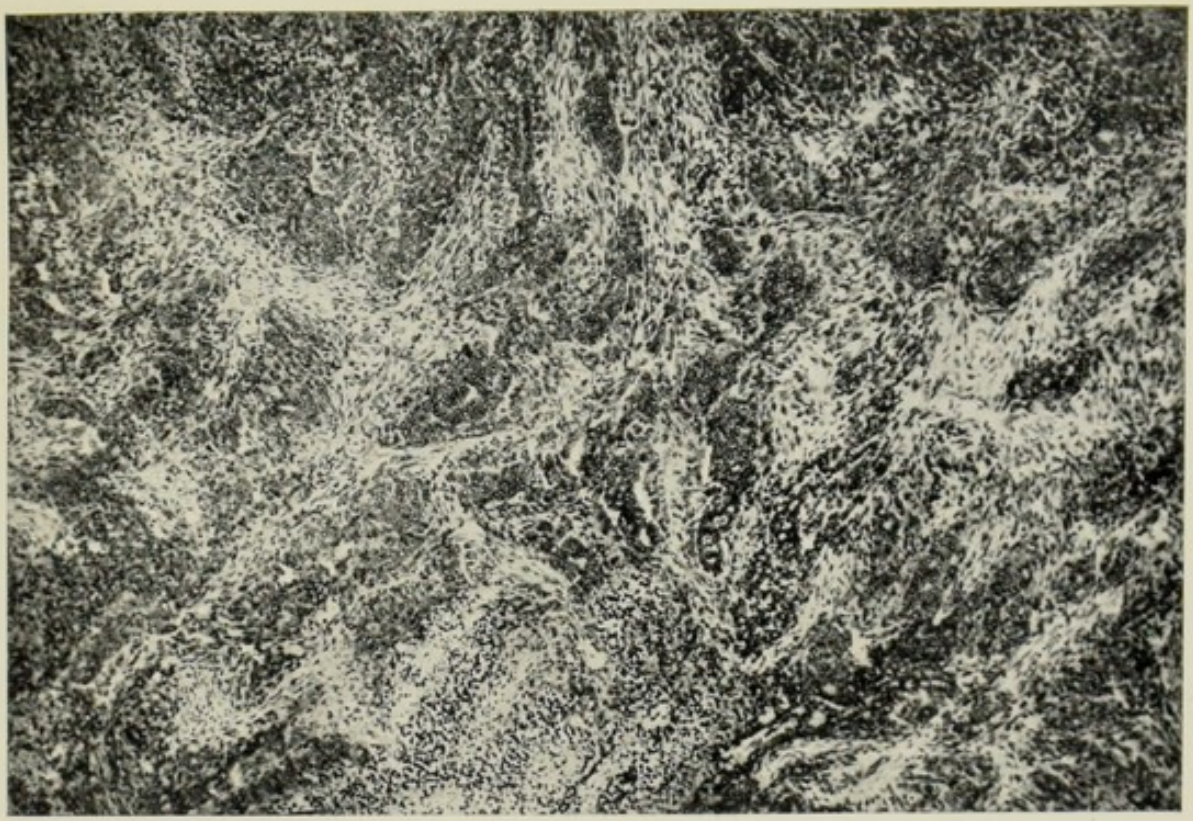
(c) *3rd Case of Sarcoma Development* (?) (2 C).

In examining as many carcinomatous tumours as possible of the earlier generations, we found a third case suggesting the same process, in a series of the 2nd generation (in a tumour in 2 C). In this case a 74 days old tumour weighing 8·8 gr. was completely removed by operation on the 19.1.07. The tumour was not inoculated, but four large slices through the whole tumour were preserved in Zenker. The tumour recurred soon after the operation; on the 22.5.07, on which date a second operation was attempted, it had reached the weight of 7 gr. The tumour was not transplanted but preserved *in toto*. The mouse died shortly after the operation, and as no tumours of 2 C have been transplanted, this special strain has not been continued.

On examining the tumour removed at the first operation, we find the usual histological picture of this carcinoma in which sclerotic changes of the stroma have taken place towards the necrotic centre. The microphotograph, fig. 21, shows a central part of the tumour at the margin of the necrotic focus. The fibrous elements of the connective tissue are increased in quantity but there are remarkably few cells. This non-cellular sclerotic tissue separates the atrophic carcinomatous acini and alveoli widely from each other; the latter show marked necrotic changes in their interior, so that usually only a narrow margin of living parenchyma cells is left. The connective tissue seems to be in an œdematous condition, for the single fibrils are separated from each other and the vessels dilated. This œdematous condition is found frequently accompanying the sclerotic changes in other tumours and adds to the impression of sclerosis, while at the same time it perhaps indicates a connection between the sclerotic

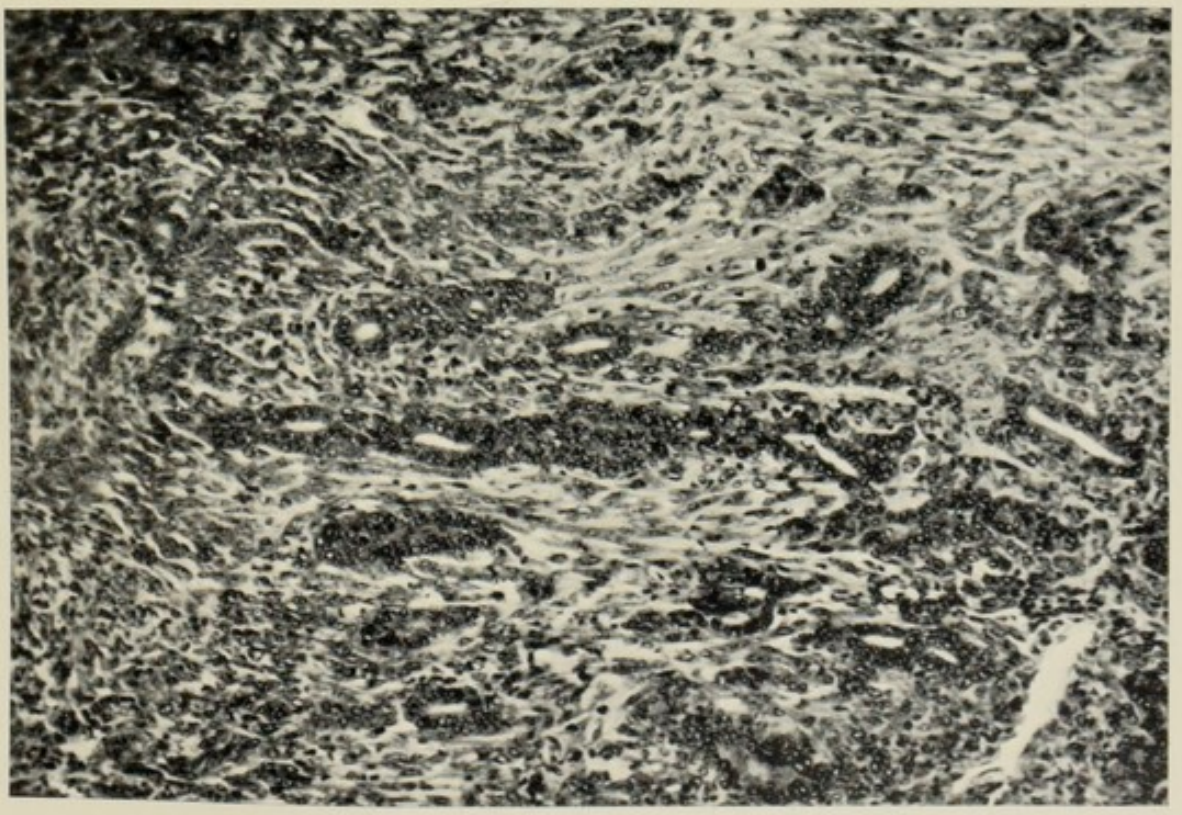
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Second case of sarcoma development.



Microphoto, W. Imboden.

FIG. 19. 37/8 J—9 H. A second daughter-tumour of series 8 J (31 days), showing second case of sarcoma development. Mother-material of this tumour is given in fig. 17. Abundant spindle-celled interstitial tissue as a diffuse change throughout the tumour. The daughter-tumours of this series all show a progressive increase in the amount of sarcomatous tissue (*vide* fig. 20). $\times \frac{100}{1}$.

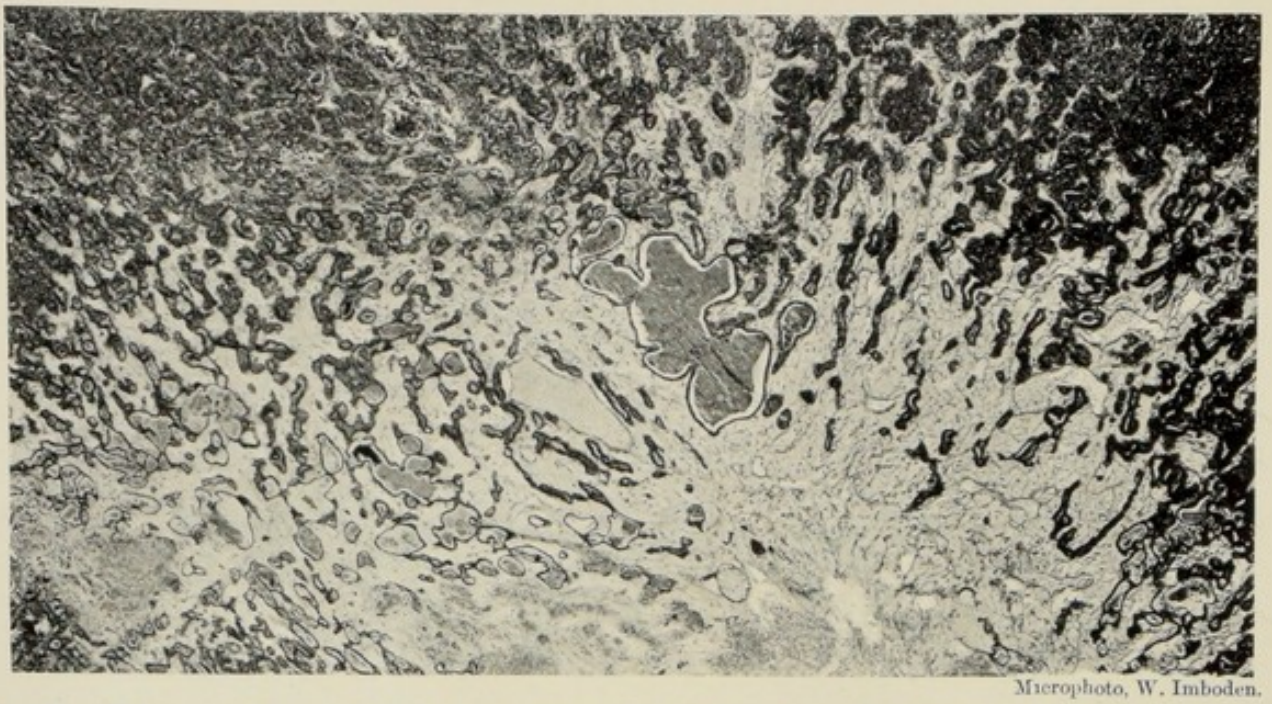


Microphoto, W. Imboden.

FIG. 20. 37/10 R—11_2H. "Mixed tumour" of 10th generation (33 days). Grand-daughter tumour of that shown in fig. 19. Large spindle-celled sarcomatous tissue between carcinoma-acini. $\times \frac{180}{1}$.

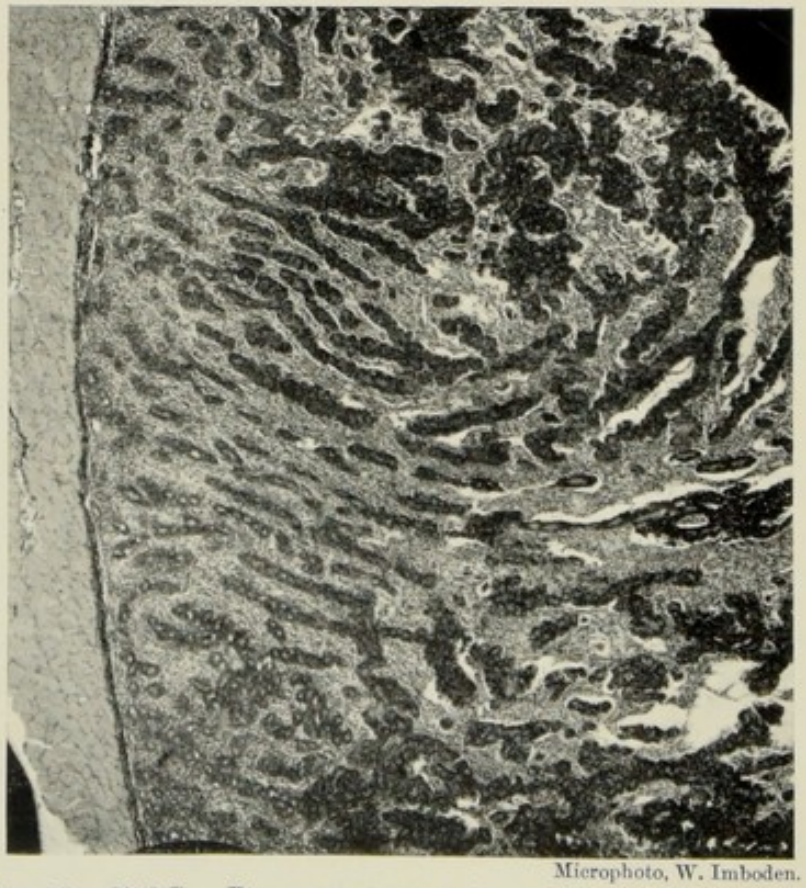
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Third case of sarcoma development.



Microphoto, W. Imboden.

FIG. 21.—37/2 C. Old tumour of 2nd generation (74 days) showing the pronounced degree of sclerotic changes in the stroma from the centre of the tumour; tumour removed at first operation 19.1.07. The peripheral part of the tumour above, the border of the central necrotic area below. Stroma sclerotic and œdematous, with very few cells. Tumour not transplanted. $\times \frac{45}{1}$.



Microphoto, W. Imboden.

FIG. 22.—37/2 C. Recurrent tumour in same animal as that of fig. 21. Removed at second operation 25.5.07. The carcinoma alveoli are now separated by broad bands of cellular connective tissue. Peripheral part of tumour adjoining muscle (left). Tumour not transplanted. $\times \frac{40}{1}$.

changes and circulatory disturbances. The periphery of this tumour shows a delicate stroma like the other carcinomata of this strain.

The tumour removed at the subsequent operation, four months later, shows similar sclerotic changes in the middle of the tumour, but with this difference that the stroma has now become more cellular, not only in the centre but also in some places extending to the periphery. In certain places the stroma is extremely cellular with large spindle-cells, and the picture obtained corresponds exactly to that seen in the tumours of 8 H and 9 H. We give a photograph from the periphery of this tumour where the cellularity of the stroma is most marked (fig. 22). This change has taken place in the same mouse between the first and the second operation on the tumour. We have no means of deciding other than by histological examination whether this change is biologically the same as that found in the two instances already described. This material has not been transplanted, and so we do not know what would have been its fate.

(3) The Progressive Advance of the Sarcomatous Change.

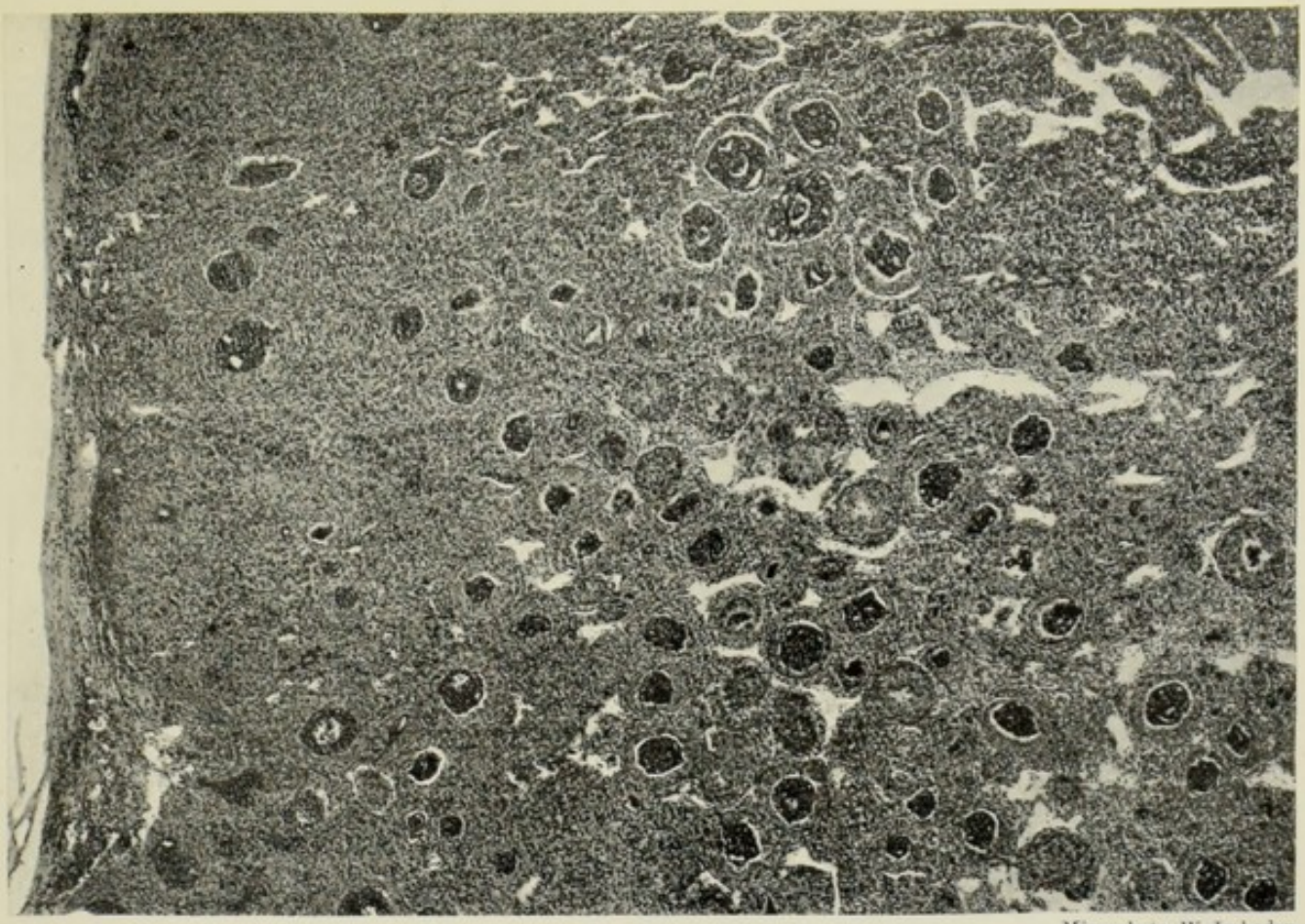
We shall consider the gradual development of the sarcomatous tissue into pure sarcoma, as it has been followed in the two cases mentioned above, before proceeding with the description of the other cases of sarcoma development observed subsequently.

The spindle-cell tissue usually increases in amount from one generation to another. The carcinomatous alveoli become more and more separated from each other by broad bands of sarcomatous tissue which replace the old stroma. The features of the process when thus far advanced are illustrated fully in figures given later in this paper. At this stage, we find slight necrotic changes here and there in the carcinomatous alveoli, showing that their nutrition is to some extent impaired by the changed order of things.

The next step in the process in our case, however, is not what was anticipated nor what has been described by earlier investigators, viz.: a mere increase of the spindle-cell tissue with corresponding diminution in the proportion of carcinoma cells, until a time comes when they are crowded out altogether. A marked intermediate stage occurs in our cases with a different histological picture. In this stage the pronounced spindle-cell picture is mostly lost, and replaced for several generations by a very polymorphous celled tissue. While in the

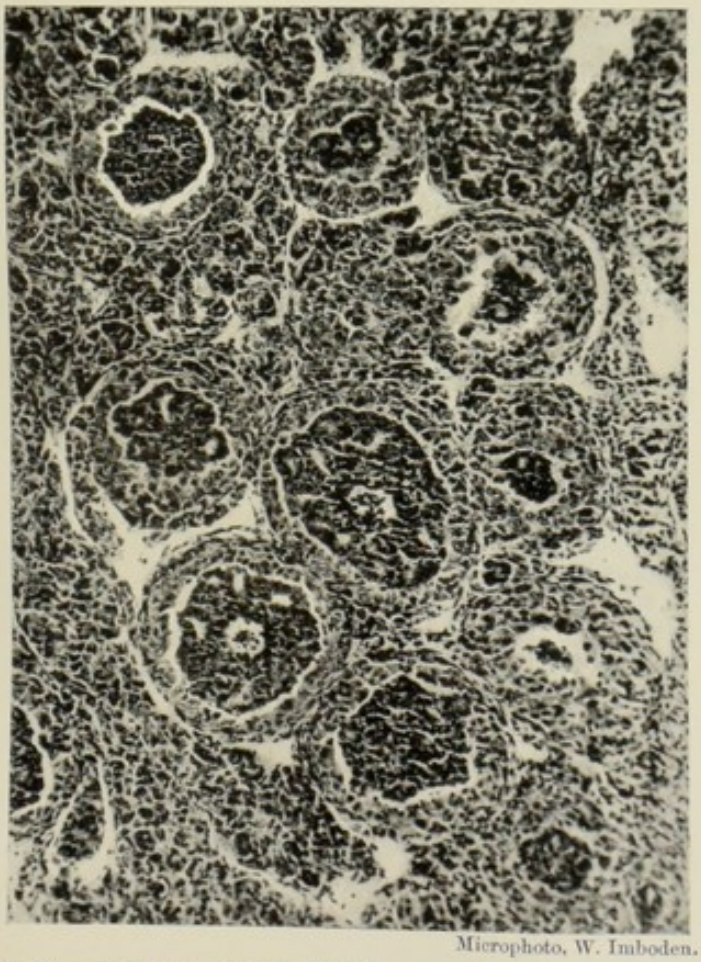
first stage it is very easy to distinguish the darkly stained carcinomatous parenchyma from the lighter sarcoma-cells, this distinction becomes often less marked in the next stage, and frequently we have serious difficulty in distinguishing them from each other. This phenomenon does not indicate any transformation of carcinomatous into sarcomatous cells, but seems to be a process similar in kind to that accompanying changes from acinous to alveolar condition (and *vice versa*) of the parenchyma. Murray has described and illustrated such changes in spontaneous tumours on another page of this Report (figs. 25 & 26, p. 86), similarly they are often found in transplanted tumours. These changes are usually accompanied by slight alterations in the staining reactions. In general, the alveolar condition of the parenchyma is associated with a lighter staining of nucleus and protoplasm ; and when this occurs in a mixed tumour with polymorphous sarcomatous tissue, as is very frequently the case, it may be often difficult to distinguish the two components from each other. In addition we sometimes find evidence of the carcinomatous alveoli being broken up by the sarcomatous tissue. This also adds to the difficulty of distinguishing the two tissues.

As a characteristic feature of this stage peculiar formations are noticed round the carcinomatous acini, consisting of halos of lightly stained extremely polymorphous cells. This phenomenon has not been noticed by our predecessors, but seems to be a constant feature of the process, when it has advanced to a certain stage in our tumour. Then the carcinomatous elements occur as isolated alveoli scattered about in an extremely polymorphous sarcomatous tissue. Round the carcinomatous elements—indifferently whether their structure is acinous or alveolar—zones of lighter cells are present, causing the appearance of halos encircling the darkly stained carcinoma cells, or recalling the areola surrounding the nipple. These halos or areolas are especially well developed in old tumours. Sometimes we find them scattered in circumscribed areas only. In other cases they are very numerous throughout most areas of a section, and then nearly every group of carcinoma cells is surrounded by an areola. Figs. 23–29 reproduce the appearances (*cf.* also figs. 57 and 73). The halo may consist of a single layer of cells, but more usually there are several layers of polymorphous cells with very large nuclei and with a large amount of very lightly staining protoplasm. In some tumours these cells are of approximately uniform size and shape, in other cases they show the most extreme polymorphism. The nucleus is often a true giant nucleus, very rich in chromatin ; sometimes there are several nuclei in one cell ; at



Microphoto, W. Imboden.

FIG. 23.—37/12$_2$ A—13 U. "Mixed tumour" of 12th generation (71 days) in polymorph-celled stage, with halo-formation around remains of carcinoma. (See fig. 24, high power.) $\times \frac{55}{1}$.



Microphoto, W. Imboden.

FIG. 24.—37/12$_2$ A—13 U. High power view of upper part of fig. 23 showing carcinoma alveoli in various stages of degeneration and disappearance. $\times \frac{135}{1}$.



J. B. Ford, del.

FIG. 25.—37/9 C—10 B. Young "mixed tumour" in polymorph-celled stage with halo-formation (24 days). Low power drawing. ×

other times we find the cells forming the halos transformed into typical multi-nuclear giant-cells like those found around foreign bodies, as shown in fig. 27.

The border-line between these cells and the carcinoma cells is generally quite sharp. Towards the sarcomatous interstitial tissue, however, the margin is indistinct for the most part, the cells of the halos showing every stage of transition to the polymorphous sarcomatous cells.

From what kind of cells do those forming the halos arise? It might be suggested that they were transformed epithelial cells, which for an unknown reason had assumed this peculiar appearance. In certain cases the necessity for considering this hypothesis is reinforced by the undoubted occurrence, here and there, of epithelial giant-cells arising from degenerating carcinoma alveoli. However, a thorough histological study shows that the possibility of an epithelial origin for the halo-cells cannot be entertained. On the one hand, their whole appearance, their size, form, and staining reactions are absolutely different from those of the smaller darkly stained carcinoma cells, and the border-line where they adjoin the latter is, as mentioned, usually very sharp. On the other hand, they show great resemblance to the polymorphous cells of the sarcomatous tissue, to which all forms of transition are to be found.

In appearance they remind us of reaction tissue round foreign bodies or necrotic masses. Can these cells be considered as granulation tissue from the host? Numerous mitoses are to be found in them, although this fact does not necessarily reveal anything as to their origin. But the examination of these cells in " early stages " shows that they resist the damaging influences of transplantation much better than do the cells of ordinary granulation tissue. They show numerous mitoses already 24 hours after transplantation and proliferate independently as polymorphous sarcomatous cells (fig. 28). This fact demonstrates that there can be no question of cells of an ordinary granulation tissue, but that they have already acquired malignant properties. In all probability they do not originate from the connective tissue of the new host but are in reality derived from sarcomatous cells previously present, and transferred as components of the tumour tissue from mouse to mouse before the halos were observed. The appearances of the halos may indicate a reaction, but it is a reaction of *sarcomatous* cells.

In young tumours we may find mitoses in the carcinoma cells inside

the halos, as the figure 26 from a 24 days old tumour of 9th generation shows; in this case the carcinoma cells do not seem to have suffered. But in older tumours, where this halo-formation is in an advanced stage, we usually find signs of degeneration of the epithelial elements. The alveoli which are surrounded by halos very often show central necrosis, while otherwise, as already mentioned, central necrosis in the alveoli of this tumour is rather rare. Further, these carcinoma cells look smaller and darker than usual, and in some cases have even a shrunken appearance. The general picture of such a tumour is shown in the microphotograph fig. 23, from a 71 days old tumour of the 12th generation. We see here all stages of these halos; in some the parenchyma looks still quite healthy, in others it shows beginning central necrosis, in others again a complete degeneration has taken place, and the halo encloses only the dead remains of the carcinoma cells. It is shown by examining serial sections of this tumour that the appearances in the figure are not only the result of superficial oblique sections of carcinoma-alveoli. It is in this case obvious that the halo-formation is closely connected with the disappearance of the carcinomatous elements. However, that it is not merely a secondary process, called forth by primary degeneration of the carcinomatous elements, seems to be shown by the fact already mentioned that halos occur in quite young tumours where the carcinomatous parenchyma appears to be in a perfectly healthy condition with dividing cells, as fig. 26 illustrates. The degenerative changes in the carcinoma cells seem to be secondary, and the whole picture suggests a defective nutrition of those carcinoma cells which are enclosed in the halos. The anatomical relation of the carcinoma alveoli to the vessels seems to explain this to a great extent. We find the capillaries in their old place between the carcinoma alveoli (the open spaces between the halos in fig. 24), and we cannot find trace of new capillaries being formed inside the halo. The halo-cells are constantly developed on the surface of the carcinoma alveoli, intercalated between them and the capillaries, and seem thus to separate the carcinoma cells from their nutritive source—the capillaries. Under these conditions it is easily understood that the nutrition of the parenchyma must suffer.

In some cases we see the cells of halos develop into multinuclear giant-cells (fig. 27), similar to those found round foreign bodies. The idea might therefore suggest itself, that we have to deal here with a phagocytic function of the sarcoma cells. That these cells in certain cases can display phagocytic properties does not seem doubtful, and we

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FIG. 26.—37/9 C—10 B. Young "mixed tumour" (24 days). Same preparation as fig. 27 at a higher magnification to show halos around *healthy* carcinoma-alveoli. Many carcinoma cells in division. × circa $\frac{200}{1}$.



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FIG. 27.—37/10 A. Old "mixed tumour" with halo-formation (141 days). The cells of the halos are very polymorphous, those adjacent to the shrunken darkly stained carcinoma alveolus multinucleated with abundant finely-granular protoplasm (giant-cells). × $\frac{332}{1}$.



J. R. Ford, del.

FIG. 28.—37/8 H–9 Q. Graft of mixed tumour with halo-formation, four days after inoculation; halo-cells alive, some proliferating. × $\frac{350}{1}$.

find now and then in the halos, besides the giant-cells, other large cells with vacuolated protoplasm, which are usual when phagocytosis is going on in other places. A quite different question is whether the presence of halos at a certain stage in most of these mixed tumours can be interpreted in this way? The most natural thing seems to be to consider them in the light of their origin as true connective-tissue cells, sensitive to influences from the carcinoma cells. Although in the mixed tumours they seem to be elements growing independently, they at the same time do service as stroma, and we see that they have retained enough of their old properties to be still influenced in their growth by the existing remains of carcinoma. A somewhat similar concentric arrangement of the connective-tissue elements is found, where these have become more abundant in the sclerotic central parts of carcinomatous tumours, when the stroma cannot yet be said to have undergone any sarcomatous transformation (fig. 87). These cases will be mentioned later.

It is an interesting fact that a phenomenon resembling halo-formation, as we have called it for these experimental mouse-tumours, is also found in mixed tumours in the human subject. Without going fully into the literature bearing on this point, we may note in passing that in a quite recent paper on carcino-sarcoma of the uterus H. Albrecht* describes a similar phenomenon round the carcinomatous alveoli :— "All the columns of carcinoma are surrounded by a very thick and broad mantle of round and spindle cells which are indistinguishable from the other sarcomatous cells."

After having considered the halos we return to the other sarcomatous elements in these mixed tumours. As already mentioned, the original spindle-form of the sarcoma cell is usually imperfectly attained in this stage of development. In the majority of the tumours a marked polymorphism prevails. Nuclei are very often met with of extraordinary size, and pathological mitoses seem frequent. The amount of protoplasm is equally liable to great variations. We observe cells much larger in size than are seen in ordinary granulation-tissue. Multinuclear giant-cells are also seen here and there. Apart from the differences in the size of the cells, the picture, as a whole, reminds one very much of a young granulation-tissue, and it is easy to understand the hesitation of experienced pathologists to accept, without more ado,

* Albrecht, H.: Ueber das Karzinosarkom des Uterus. (Frankfurter Zeitschrift f. Pathologie, Band ii. Heft 1, 1908.)

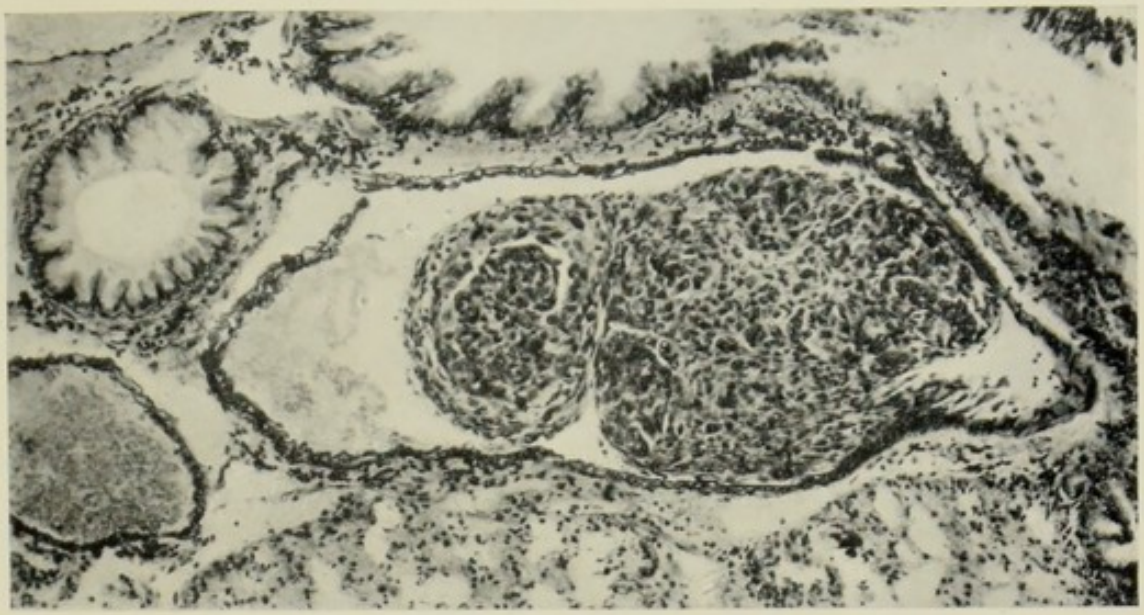
these lesions as sarcomatous. However, it is not difficult to prove in a conclusive manner, that this polymorphous tissue is something more than a pure and simple granulation-tissue. First it forms secondary nodules in the different organs of the body. Especially in the lungs we find metastases in all stages extremely frequently, and can study their origin from the very first. They start from small emboli of the same polymorphous cells, and large metastases develop exclusively by the proliferation of the cells proper to the embolus. The young secondary nodules being usually enclosed by the elastic lamina of the vessels (where this embolus has stuck in a larger vessel) they are easily distinguished from the tissues of the lungs.

The secondary nodules from the mixed tumours are sometimes purely carcinomatous, at others purely sarcomatous and at other times again mixed metastases. The fact that these latter are found frequently shows, that not only single cells may be transported as emboli, but also complexes of cells containing both the carcinomatous and the sarcomatous component. Fig. 29 illustrates such a mixed secondary deposit in a pulmonary vessel, in which the circular arrangement of the sarcomatous elements round the carcinoma as "halo" is very marked. There is here no indication of this halo being formed as reaction from the tissues of the host.

If it still be objected that the formation of metastasis alone does not show anything, as a transported carcinoma cell might call forth the formation of a granulation-tissue, the crucial test is the direct observation of the behaviour of these cells after transplantation. We have shown in fig. 14, and we shall show in several others further on in this paper, that these cells do not behave like ordinary granulation-tissue cells after being introduced into a new animal. Instead of the marked degenerative changes of normal tissues as we see them in the stroma of transplanted tumours generally, we see here a tissue proliferating exuberantly and already showing numerous mitoses 12 to 24 hours after transplantation. The growth following transplantation is not transitory. It does not stop after a short period as in the case of certain normal tissues which proliferate transitorily, but in a number of cases the transplanted cells exhibit a power of continuous growth quite unknown in normal tissues.

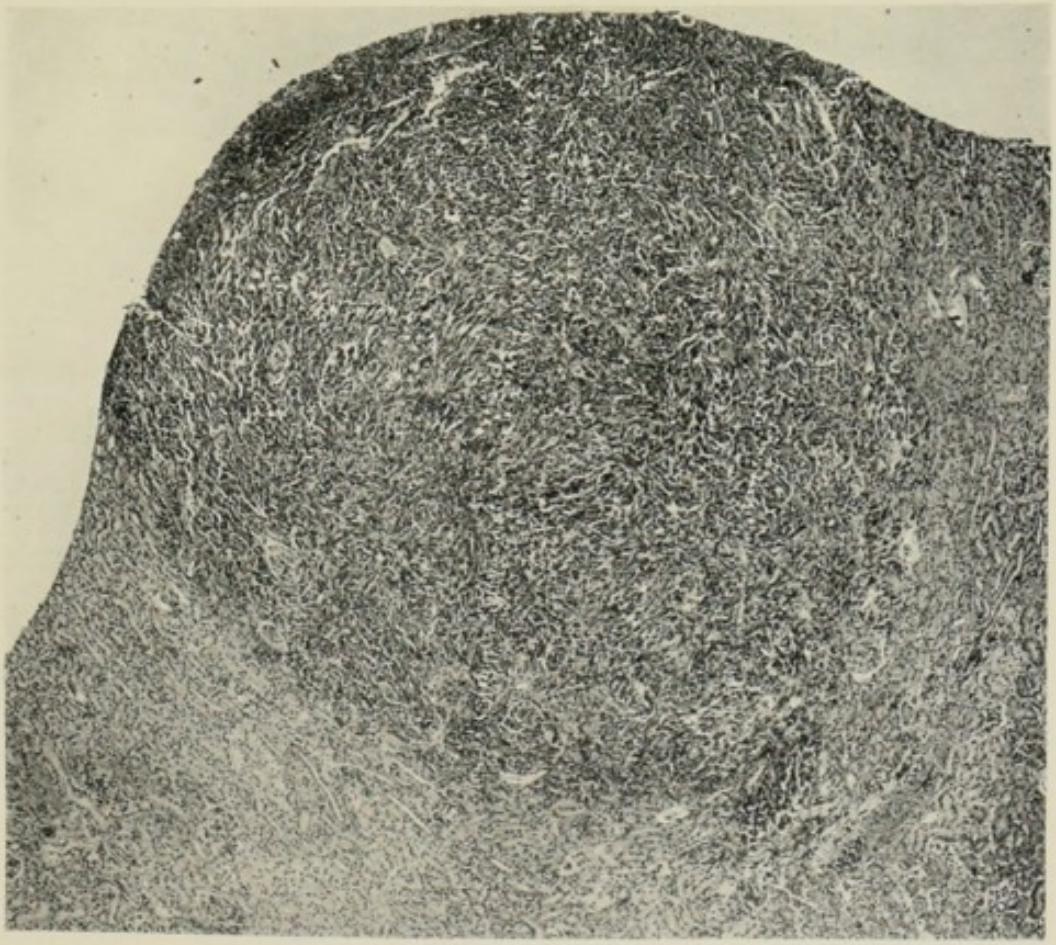
The sarcomatous tissue is very liable to degenerative changes, foci of fatty degeneration and necrosis appearing very early in these tumours. It is more than probable that here also these changes are signs of circulatory disturbances, as already mentioned for the carcinomatous

[*To face* p. 200.



Microphoto, W. Imboden.

FIG. 29.—37/10 A. Mixed tumour (5 months old). Metastasis in pulmonary artery. Combined dissemination of carcinomatous and sarcomatous components. Halo-formations. $\times \frac{120}{1}$.



Microphoto, W. Imboden.

FIG. 30.—37/10 R. Mixed tumour (4 months old). Metastasis in kidney. Dissemination of sarcomatous component only. $\times \frac{58}{1}$.

tumours, probably due to infiltration and obstruction of the vessels of the peripheral portions of the growth. The tumours are very rich in capillary vessels with a distinct continuous wall of endothelial cells. The degenerative changes first take place in the sarcoma cells furthest away from the capillaries, and by their necrosis we obtain a lattice-like arrangement of bands of living cells distributed along the capillaries. Thus in some tumours the arrangement of the living cells may give a picture closely resembling certain angio-sarcomata or peritheliomata in the human subject.

It is clear that the altered interstitial tissue no longer fulfils the same duties to the carcinoma cells as did the scanty stroma in former generations. Instead of a subordinate tissue which only served as a scaffolding and for supplying nutrition, we have an abundant rapidly proliferating tissue which appropriates for its own growth a large part of the nutritive substances conveyed by the circulation. In this competitive growth the sarcoma cells usually show themselves superior to the carcinoma cells. We have seen that in the mixed tumours the sarcoma cells intervene between the carcinoma cells and the capillaries. While a defective nutrition may thus seem to account in part for the disappearance of the carcinoma cells, the primordial cause for the overgrowth of sarcoma is obviously to be sought in progressively altered biological characters of the sarcomatous elements themselves. Consequent upon repeated transplantation these cells seem to acquire a higher energy of growth, which in later stages is superior to that of the carcinomatous parenchyma. This is evidenced both by more rapid growth and by higher percentage of successes in later generations of mixed tumours and by the fact that ultimately it becomes impossible to get the carcinoma and the sarcoma to grow side by side, even when artificially brought together. In the latter case in our experiments the sarcoma invariably outgrows the carcinoma, so that the daughter-tumours are ultimately pure sarcomata and not mixed tumours.

So long as any epithelial components remain in the tumours the surrounding sarcomatous tissue shows marked polymorphism in most of our cases. With the entire disappearance of the carcinoma cells, the sarcomatous elements resume in most cases their first typical aspect of spindle-cells, and this picture is reproduced without any further change for numerous generations. We arrive here at the third stage of the process, viz., that of a pure sarcoma.

Pure Sarcoma.

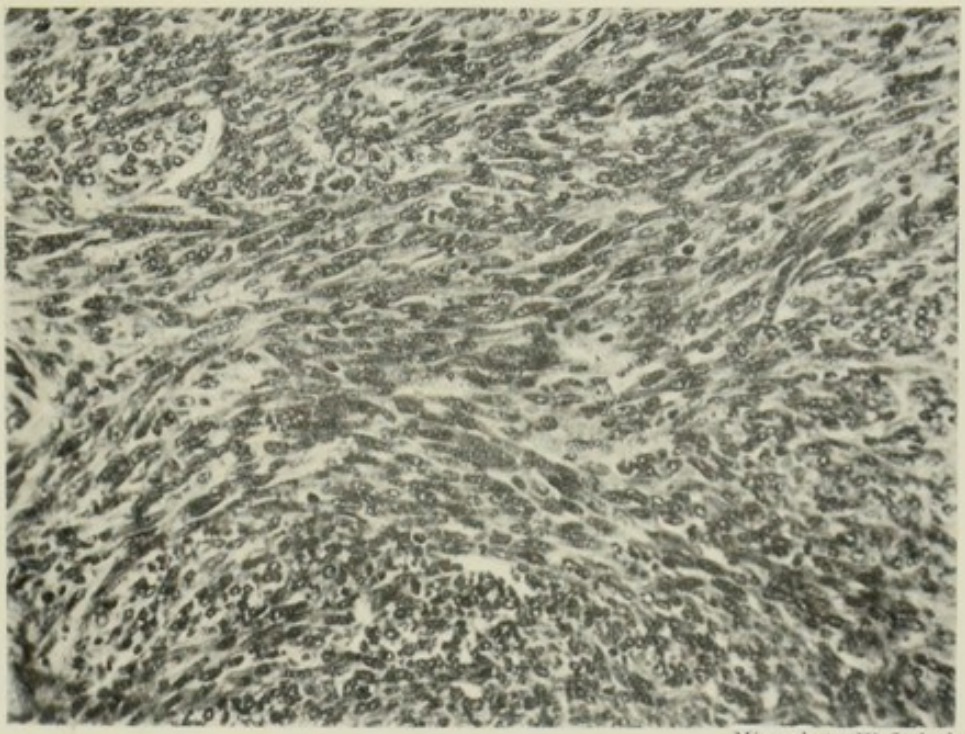
As to the morphology of the pure sarcoma we shall be brief, since in all generations it offers a very uniform picture. Figs. 31–32 illustrate the general aspect of such tumours at a magnification of 250 diameters. The tumour consists of interlacing bundles of spindle-cells; between the individual cells fine collagen fibrils are to be seen. With higher magnification fine fibrils may be found in the protoplasm of the cells (fibroglia, Mallory), exactly like those found in the fibroblasts of young granulation-tissue, thus proving beyond all doubt their connective-tissue origin. Apart from the smaller size of the cells in the mouse sarcoma, the tumours can scarcely be distinguished from very cellular sarcomata in man.

It is of interest to note that the sarcomatous tumours which develop in the several strains are not quite identical morphologically. Thus, the sarcoma developed in our first case has, once it was purified from carcinomatous elements, shown the most regular long spindle-cell type (figs. 31–32), and later, through 15 generations, it has retained for over a year the same histological picture without any change. On the other hand, the main strain of sarcoma developed in our second case (through 9 H) has a somewhat different appearance, as shown in fig. 33, the single elements being either quite short spindles or in most cases showing some degree of polymorphism. This type seems also to remain nearly constant through numerous generations (6 up to the present), although the variability in this latter case is greater, some tumours showing a more marked spindle type and others more pronounced polymorphous elements. It is interesting that we observe here in two different strains from the same spontaneous tumour the phenomena Ehrlich and Apolant observed in two cases of distinct primary origin: one of their cases is a pure spindle-cell sarcoma, while the other shows extremely polymorphous elements.

Fig. 34 shows a microphotograph of a spontaneous sarcoma which has been propagated. This tumour is described by Murray on another page of this Report; we only give the figure to permit of the similarity of the two sarcomata being compared: one a spontaneous tumour, the other developed under experimental conditions (*cf.* fig. 31).

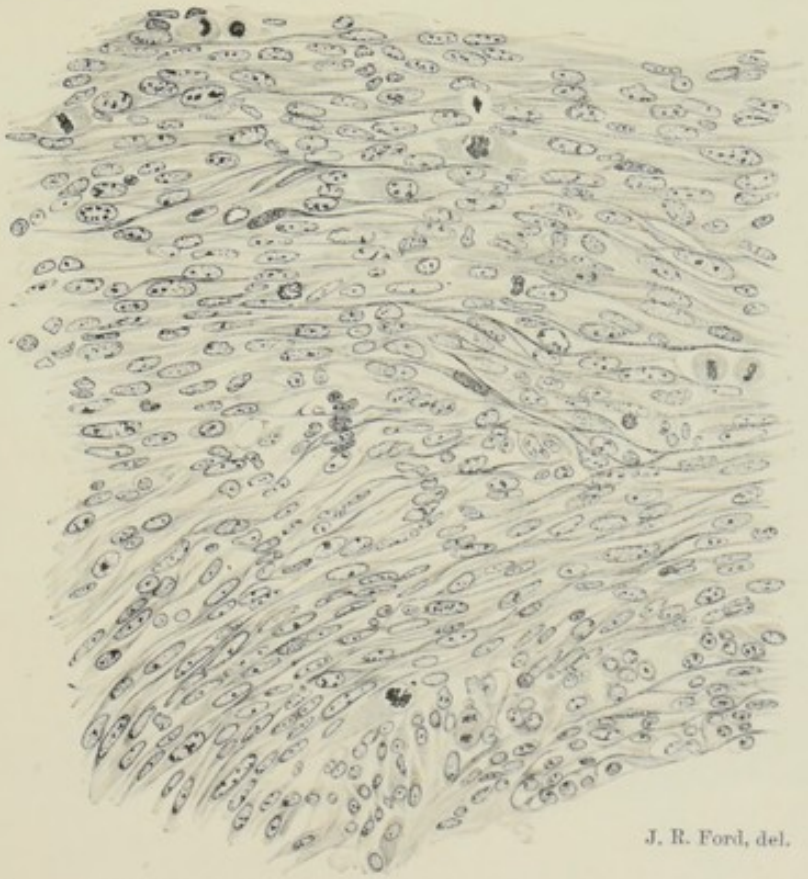
The sarcomata have powers of infiltrative growth much exceeding those usually seen in the carcinomatous tumours. As a result the skin over the tumour is rapidly invaded and ulceration or dry gangrene may take place very early. Furthermore the muscles of the thorax and

[*To face* p. 202.



Microphoto. W. Imboden.

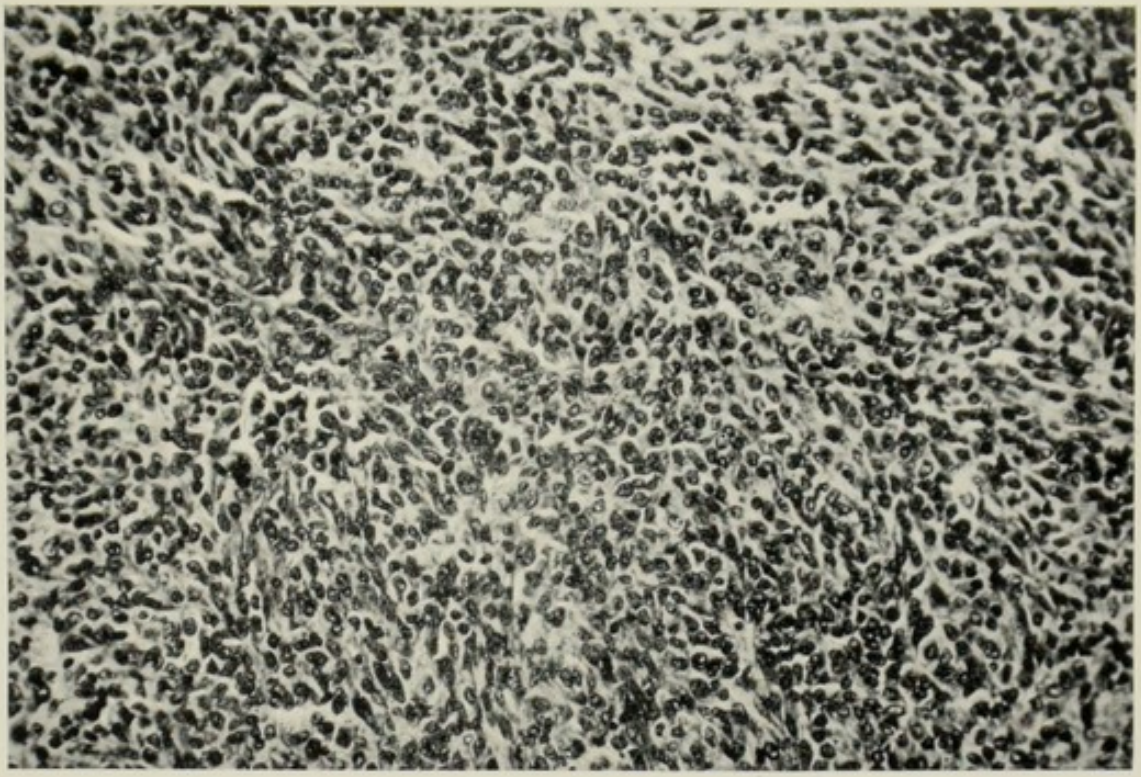
FIG. 31.—37/10$_2$ L—11$_3$ B. Pure spindle-celled sarcoma derived from mixed tumour of 9th generation (61 days). The carcinoma component has completely disappeared and the growth consists entirely of interlacing bundles of large spindle cells. $\times \frac{250}{1}$.



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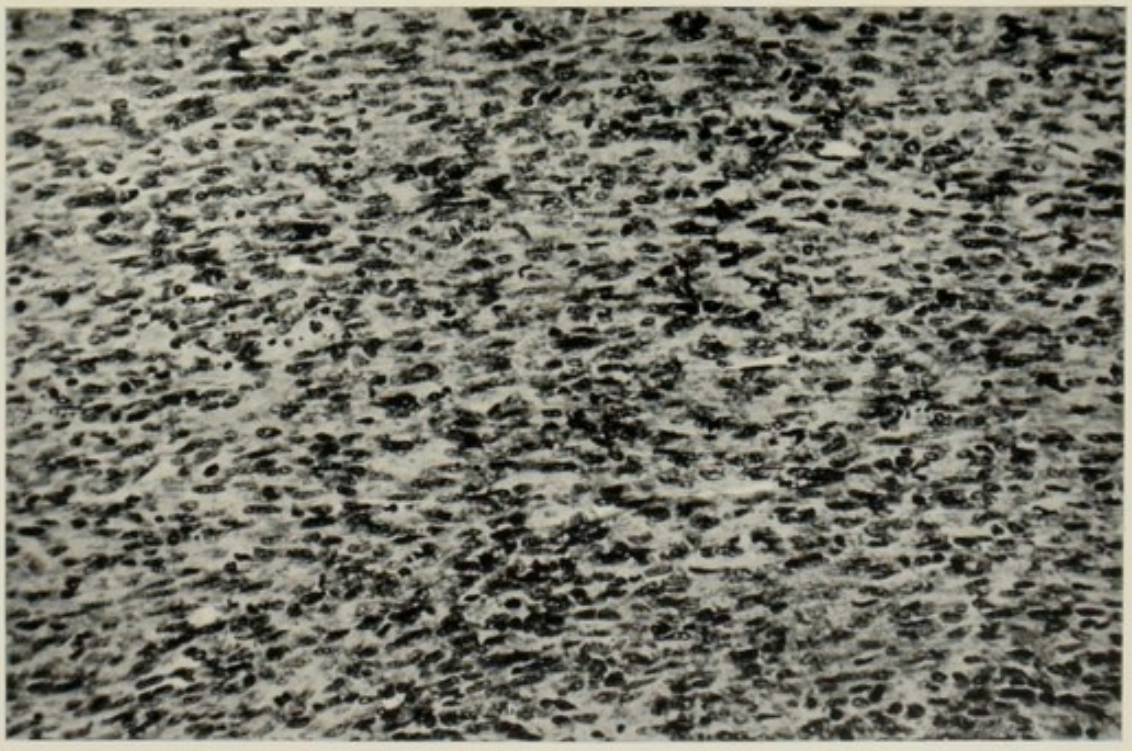
FIG. 32.—37/110—12 P. Pure spindle-celled sarcoma of 10 days, derived from mixed tumour.

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Microphoto, W. Imboden.

FIG 33.—37/16 L–17 G. Polymorph-celled sarcoma (46 days old) after 5 successive transplantations as pure sarcoma. $\times \frac{250}{1}$.



Microphoto, W. Imboden.

FIG 34.—Tumour 92/1–2 E. Spontaneous sarcoma of mouse, first generation. 92 days old. $\times \frac{250}{1}$.

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FIG. 35.—37/13 N. 6 months old mixed tumour. Front view of heart (*h*) and lungs (*l.l.*) from a mouse with inoculated mixed tumour. Note large umbilicated metastases in lungs and heart-muscle. $\times \frac{10}{1}$.

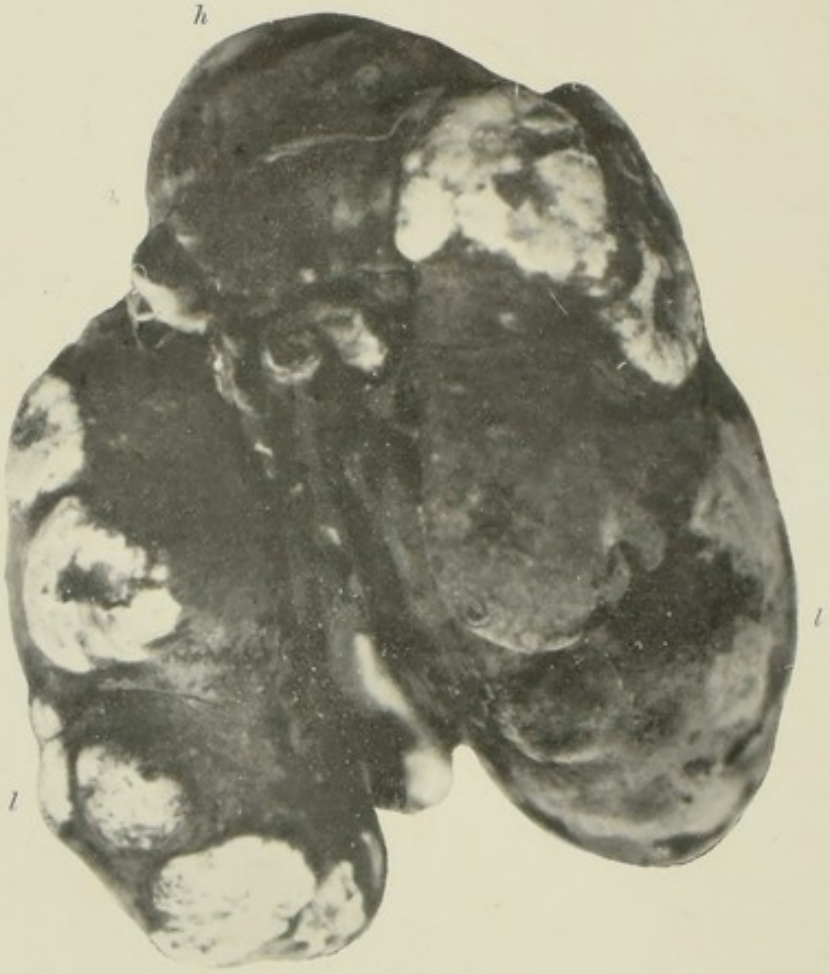


FIG. 36.—Same preparation from the back. $\times \frac{10}{1}$.

abdominal wall are infiltrated. In fig. 3, facing p. 262, a case is shown in which the sarcoma has penetrated the thoracic wall, fungating into the right pleural cavity, infiltrating the diaphragm and with metastases in the lungs.

The sarcomata form secondary growths readily, especially in the lungs ; large metastases are also found remarkably frequently in the myocardium. Figs. 35 and 36 illustrate a case with large secondaries in

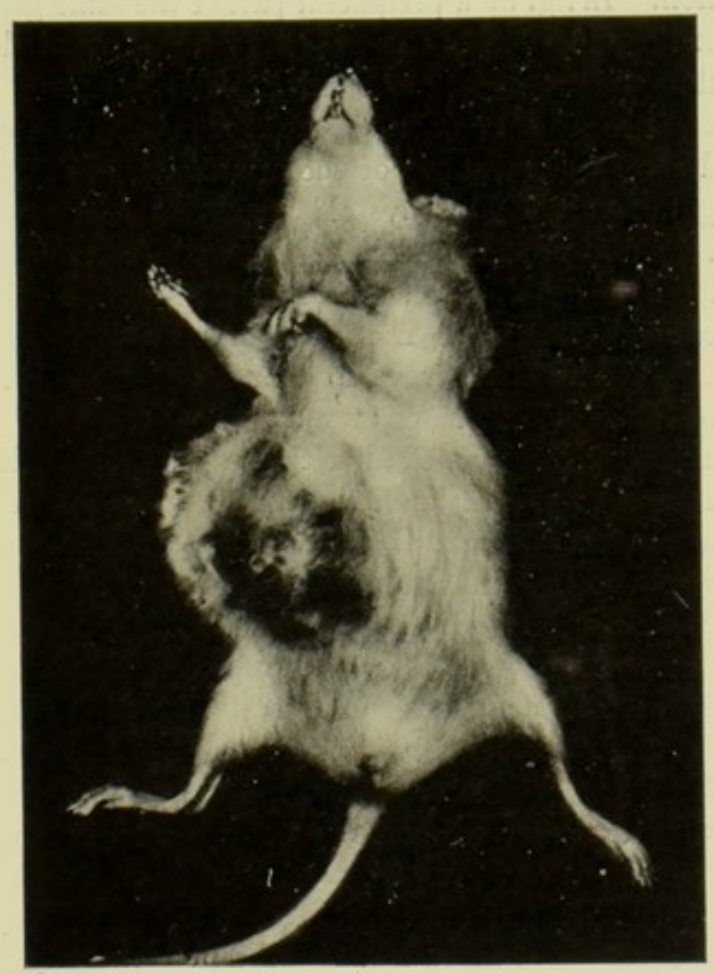


Fig. 37.—37/10$_2$ L. 7 months old mixed tumour. A large secondary nodule of pure spindle cell sarcoma has developed behind left shoulder as result of dissemination from the tumour arising from the inoculation in right flank.

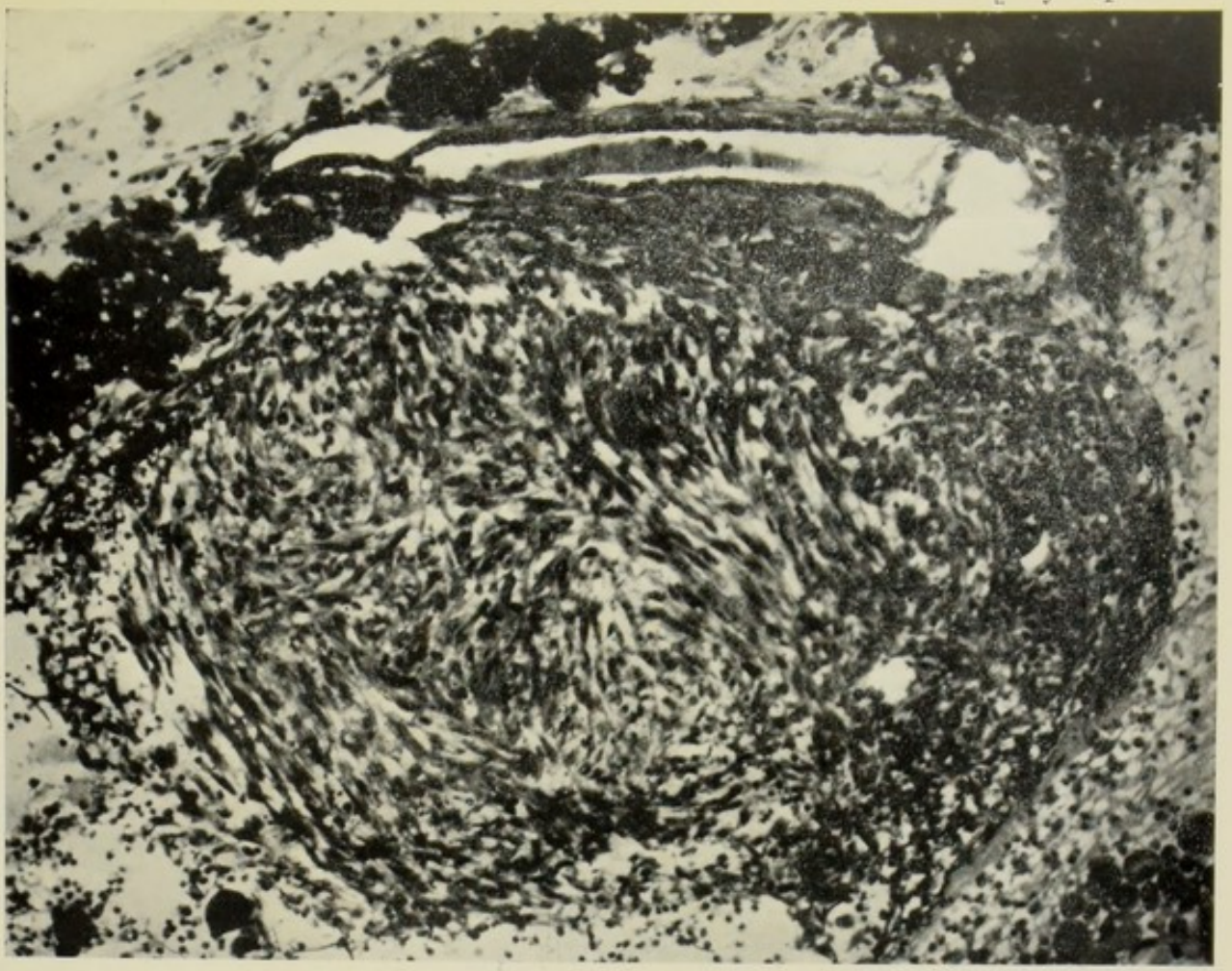
the lungs and heart, magnified about 10 times. The large nodules in the lungs reproduce macroscopically the appearances of the corresponding lesions in man and the same umbilicated centre is seen, as is frequent in secondary nodules in the human subject ; microscopically the lesions are also quite similar. Likewise secondary growths have been found in the liver, spleen and lymph-glands. Fig. 30 illustrates a case of sarcomatous

metastasis in the kidney. Fig. 37 illustrates a mouse in which dissemination has occurred from the tumour developed from the inoculation on the right flank, a large secondary nodule of sarcoma being formed behind the left shoulder.

Examined in "early stages" the sarcomatous cells show themselves capable of remaining alive and continuing growth in the new host. Fig. 38 shows the whole of a small graft preserved 24 hours after transplantation. In fig. 39 a peripheral part of the same graft is drawn under higher magnification. There are very few signs of degenerative changes, but numerous sarcomatous cells are dividing.

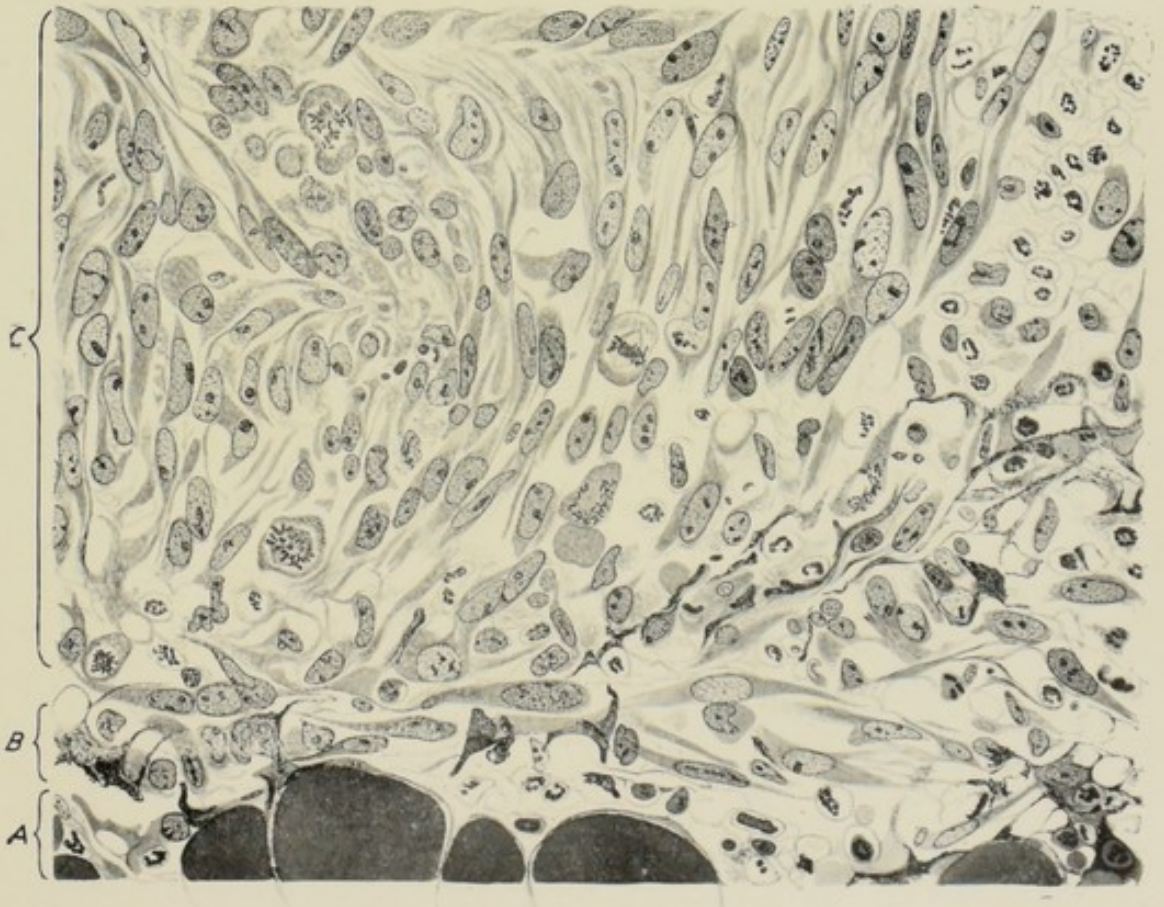
A characteristic feature of these sarcomata is the rapid central necrosis and the special features of the necrotic mass itself. Even in quite young tumours, 10–14 days old, we find the centre necrotic as a rule, and in tumours of three weeks the healthy tumour tissue is usually reduced to a thin shell enclosing a semi-fluid or even fluid brownish hæmorrhagic mass. The fluid brownish contents are characteristic of these strains of sarcoma, in sharp contrast to the firm white necrotic mass in the carcinomatous tumours.

Another remarkable feature of these tumours is their mode of growth, which is extremely rapid in the first week or fortnight in 90–100 per cent. of the animals inoculated, while later, spontaneous absorption occurs very frequently. On the whole they give a higher percentage of continuously growing tumours than do the carcinomatous series. In fig. 40 we attempt to give a graphic representation of the rate of growth of a carcinomatous series of this strain and of a sarcomatous series, both giving about the same percentage of progressively growing tumours (circa 50 %). The curve shows the greater initial rapidity of growth of the sarcomatous tumours as compared with carcinomatous tumours, which generally grow more slowly. This curve is made in the following way : the silhouettes of the tumours developed in each series are carefully drawn each week, the first charting being made 10 days after the inoculation. Counting the number of square-millimetres which the silhouette of each tumour covers, adding them together for each charting and dividing by the number of mice, we obtain figures expressing the average rate of growth from week to week of all the tumours in the series. Such figures approximate only very imperfectly to accuracy ; but provided that the same person always does the charting in a uniform way, we obtain an approximation to the average rate of growth (as exact as it is possible) without killing the mice which we wish to keep alive for continued observation.



Microphoto, W. Imboden.

FIG. 38.—37/14 C. Pure spindle-celled sarcoma, examined in "early stages." Graft preserved 24 hours after transplantation. Very little degeneration of introduced tissue. Leucocyte-infiltration of surrounding host tissues. In the upper part of the figure fatty tissue (black) enclosing a mammary duct cut longitudinally. $\times \frac{180}{1}$.



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FIG. 39.—37/14 C. Pure spindle-celled sarcoma, examined in "early stages." Graft preserved 24 hours after transplantation. Upper left-hand corner of same section as fig. 34. The sarcoma cells are alive and dividing rapidly. Commencing invasion of host tissues by sarcoma cells, no sarcomatous transformation of host tissues. A = host-tissues; B = line separating host tissues and graft; C = graft. $\times \frac{500}{1}$.

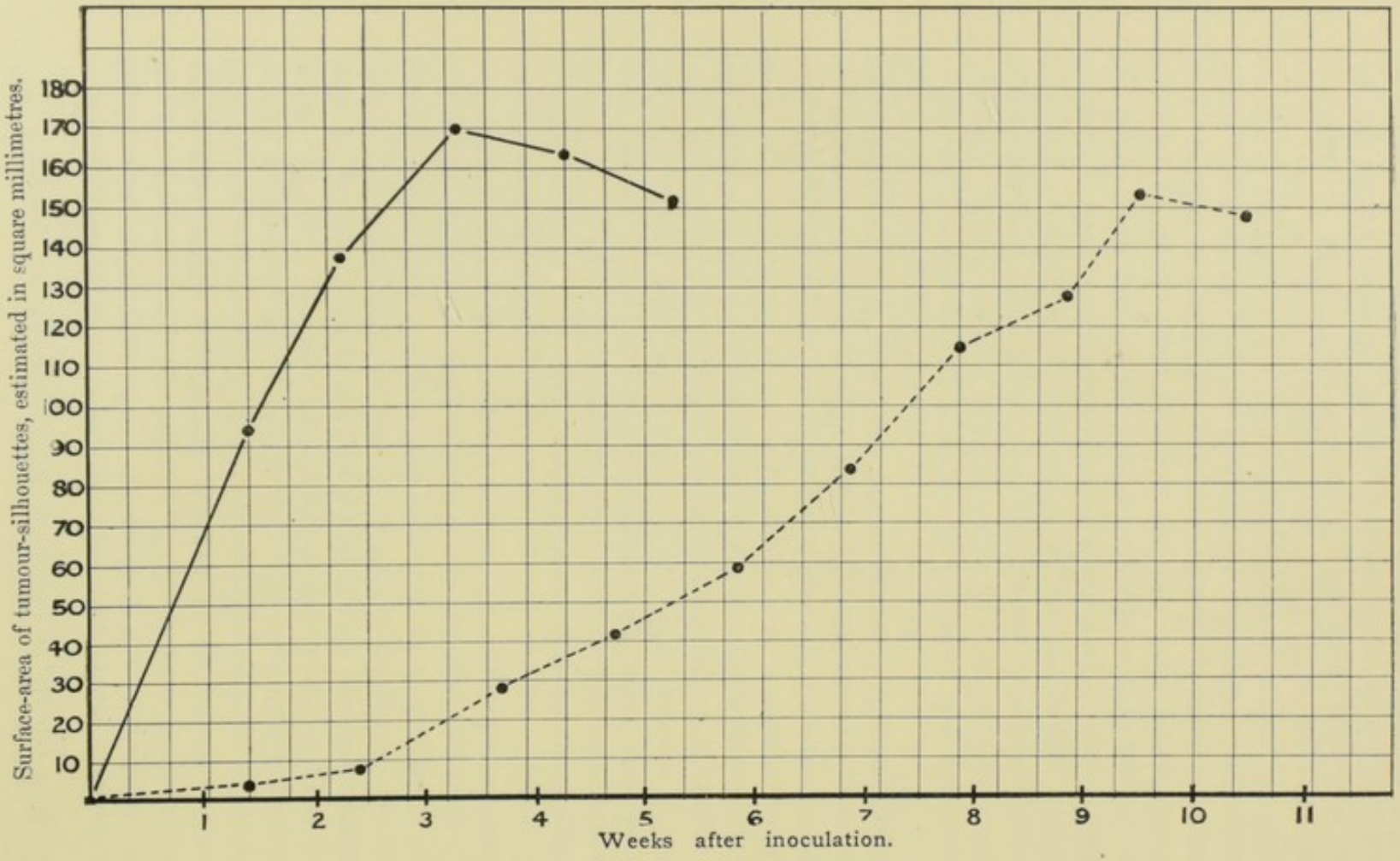


FIG. 40.—CURVES showing the difference in the average rate of growth of the daughter-tumours developing in a carcinomatous and in a pure sarcomatous series. Broken line=Carcinoma (Series 10_3D, 30.1.08, 56 %); unbroken line=Sarcoma (Series 23 A, 6.4.08, 50 % of continuously growing tumours). In both series the same doses (0·025 c.c. of tumour emulsion) were used. Judged merely by the percentage of daughter-tumours developing (ca. 50 %) the two series corresponded.

Attempts at Artificial Purification of Mixed Tumours.

We have shown on previous pages that the mixed tumours tend to become pure sarcomata. As is evident from the detailed data given on the genealogical tree, the rapidity of the process of "purification" varies considerably in different strains; in our tumour it is a slow process, as a rule. Different factors, the influence of which can hardly be foreseen, play a rôle in this process, both individual factors in the different mice and fluctuations in the energy of growth of the cells of the two components, sarcomatous and carcinomatous, respectively. It seems probable that the energy of growth of the two component tissues may fluctuate independently of each other; but it is extremely difficult to settle definitely the relative importance of the soil offered by the new animal, and of biological fluctuations in the tumour cells themselves.

The "purification" needs a certain time, and seems to take place just as well where there has been rapid passage from animal to animal as in some cases in which the passages have been less rapid and the sojourn in each animal correspondingly longer. The process is in both cases the same. It is generally completed first in the centre, where the carcinomatous alveoli are soonest displaced. The rate of the process may vary very much in the different parts of a tumour, so that one part may be altogether free from carcinoma and show the picture of a pure spindle-cell sarcoma, while another part still remains in the stage of a mixed tumour with halos round the carcinoma-alveoli, and polymorph-celled tissue between them.

We have made several attempts to influence this process experimentally. For the above mentioned reasons great caution is necessary in drawing conclusions from these experiments. Various circumstances still beyond control may undoubtedly play a great part in determining the results. According to the stage at which we happen to meddle with the spontaneous process our results vary a good deal; in any case we can only effect an acceleration in the rate with which the spontaneous process is completed. When we take a tumour in an early mixed stage, we are dealing with stroma cells without any very great energy of growth, as is evident from the slow increase in size of the tumours, and in most cases from the rather low percentages of successful inoculations. If we could eliminate the carcinoma cells, it is doubtful whether at this stage the stroma elements alone would be capable of growing continuously and forming a tumour. The possibilities of effecting artificial purification may be more favourable when these

stroma elements show more rapid growth after several passages and tumours develop in a higher percentage of the mice inoculated. Then the sarcomatous component has evidently acquired an increased power of independent growth, at the same time the carcinoma cells have become fewer in number and their nutrition seems to have suffered. When the material in these stages is exposed to influences slightly damaging it, as *e. g.* high or low temperatures, it seems quite natural to expect that these may have certain effects upon the quantitative relation of the two components in the next generation. That this can lead to a more rapid disappearance of the carcinomatous elements in some cases, was shown by some experiments we [1] undertook in 1905 on the suggestion of Professor Ehrlich in his laboratory, in which material from mixed tumours was exposed to heat before inoculation. It seemed from these experiments that heating to 44° for a certain time (more than 25 minutes) might favour the process of purification, without completely suppressing the carcinomatous elements as was shown in later observations. Ehrlich suggests [2] that by heating of the material a "dilution" is obtained, since with higher temperatures fewer and fewer cells will survive. By inoculation of this "diluted" material it is possible to obtain tumours in which, *e. g.*, one half is carcinoma and the other half is sarcoma, where single groups of cells of different kind have remained alive.

We have repeated these experiments with our present material [3], which is not very favourable for such experiments, as the tumour in this stage generally does not grow in a very high percentage and the mixed-tumour stage on the whole is of short and uncertain duration. As we have only one series which can be used, we can hardly draw conclusions. Emulsion of tumour was sucked up into a number of fine graduated syringes; these were aseptically closed and submerged in sterile saline, kept at a constant temperature of 44° for $\frac{1}{4}$–$\frac{1}{2}$ up to 1 hour; then inoculated in doses of 0·025 c.c. into a number of mice. We did not succeed in obtaining tumours from material heated more than 30 min., and up to this point there was no appreciable diminution of the carcinomatous elements to be found. Moreover in the sub-transplantations from these heated and unheated tumours there was no noticeable difference (see genealogical tree).

[1] Experimente an einem Mischtumor. (Berliner klin. Wochenschrift, no. 2, 1906.)

[2] Ehrlich, P.: Experimentelle Studien an Mäusetumoren. (Zeitschrift für Krebsforschung, Band V. Heft 1–2, p. 69, 1907.)

[3] Series 12_2 D, see genealogical tree where it gives details of the mixed tumours from 9 H.

We have tried to purify this mixed tumour through biological methods. As described in another paper in this Report, the increased resistance induced by the inoculation of tumour material seems to be mainly directed against the re-inoculation of the same tumour, in lesser degree against other tumours, especially those of a different histogenesis. This fact led us to try the effect of transplanting our mixed tumour into carcinoma-immune mice, with the hope that perhaps the carcinomatous component would find a less favourable soil in them and the sarcomatous elements more easily prevail. The first experiment of this kind, described conjointly with Bashford and Murray in the Berliner Klinische Wochenschrift, no. 39, 1907, gave a result rather in favour of this view. Three tumours of 8 H were mixed and inoculated into a large number of mice, including ten in which the carcinoma in question had grown transitorily and then been absorbed. All three tumours used for this experiment (experiment 9 F) had the typical structure of mixed growths in an early stage, with spindle- and polymorph-celled sarcomatous tissue. Out of 10 carcinoma-immune mice only one developed a tumour. This tumour also presented the structure of a carcinoma sarcomatodes, but with considerable diminution of the carcinoma-component and with large halos round the alveoli. It was inoculated into normal mice. Five daughter-tumours from this series have been carefully examined. Three of them consist entirely of pure spindle-cell sarcoma. The other two still contain isolated minute carcinomatous islands surrounded by large halos in a polymorphous sarcomatous tissue, but in all in less quantity than in the mother material. All these five tumours were transplanted. In the succeeding generations all the strains propagated from tumours which were already pure spindle-cell sarcomata have preserved this structure without further change. In one of the series from a tumour containing remains of carcinoma (11 O) four tumours were examined and transplanted ; of these two were pure sarcomata while two still contained in one or two places minute traces (single alveoli) of carcinoma surrounded by halos. In the daughter-tumours of these series, however, only pure spindle-sarcomata were found. The details of the experiment are to be found on the genealogical tree (series 9 F and subtransplantations).

While in this way a pure spindle-cell sarcoma was obtained in the 2nd generation (3 months) after passage through an immune animal, and 4 months after the first appearance of the sarcomatous change, the numerous sister-strains kept their characters as mixed tumours considerably longer through numerous generations. In one other

series of this particular strain pure sarcoma was obtained in the 10th generation (10_2L giving 11_3B), but it must be remarked that, although the number of generations is the same, the length of time is double, for it was not till 4 months later, 8 months after the first appearance of the sarcomatous changes that the pure sarcoma stage was reached. In all the other side-branches serving as controls the mixed tumour stage lasted still longer.

This experiment has been repeated several times without conclusive results. Two cases cannot be counted, inasmuch as the mother-material was too far advanced towards pure sarcoma, and the daughter-tumours both in the control series and in the series through immune animals were sarcomata. In one case (series 11_3K) there is no noticeable difference between the two series. From the control series five tumours have been examined ; from the series through immune mice six ; the mixed tumour type is still maintained in all ; here and there in localised parts the sarcomatous elements have ousted the carcinoma cells altogether. In the next generation both series gave pure sarcoma. In another case (series 12_2E, from 8 H), there is decidedly less carcinoma and more differentiated spindle-elements in a tumour which had passed through an immune animal than in the tumours in the control series ; and by further propagation the strain derived from the tumour in the immune animal got rid of the carcinoma after one passage through normal animals, *i. e.* one generation earlier than in the control strain where a sarcoma was obtained after two passages. However, these differences are too small to allow of any final conclusion being drawn.

Summarising we see that in no case has a single passage of a tumour through a partially immunised animal sufficed to obtain a pure sarcoma. In the cases in which a tumour has developed in the carcinoma-immune animals, carcinomatous alveoli have always been found together with the sarcomatous tissue, although the latter forms the greater mass of the tumour. This fact does not surprise us. According to Bashford, Murray and Cramer's investigations, confirmed by Russell's experiments on "early stages" in normal and immune animals, given in another part of this Report, one of the most important factors for determining if a graft is going to live or not seems to be whether it is reorganised from the host. On the other hand, there is no direct evidence that the cells introduced into immune animals are killed by any anti-body in the serum. In the case of carcinoma-immune animals in which the carcinomatous cells alone are generally unable to call forth stroma reaction and therefore incapable of growing, the sarcomatous cells are

still able to grow in a certain proportion and call forth a stroma reaction from which the carcinoma cells may also benefit.

A similar specificity of resistance after inoculation of normal tissues was shown for a squamous-cell carcinoma after previous treatment with normal mouse-skin, as described on a later page of this Report. This observation suggested that it might perhaps be possible through previous treatment with mouse-mamma to make the soil unsuitable for the carcinomatous component of the tumour without interfering seriously with the conditions of growth for the sarcoma. Experiments were made in this direction, and a mixed tumour inoculated in a dose of 0·025 c.c. into mice which 22 days previously had been treated with 0·10 c.c. emulsion of mouse mamma (Series 15 A, from 8 H). The result is similar to that previously mentioned ; a single passage through mice treated with mamma-emulsion is insufficient to cut out the carcinomatous component. But in following the two strains of this mixed tumour of which the one had passed through mice treated with mamma-emulsion and the other (the control series) not, we observe that the tumour strain through the treated mice has become pure sarcoma in the 2nd generation after this passage, one generation earlier than is the case in the control series, where a pure sarcoma appears only in the 3rd generation. As in the similar observations on passage through carcinoma-immune mice we do not feel justified in drawing conclusions, since the differences are too small and there are so many possibilities of error in the erratic progress of spontaneous purification.

4. Study of changes in the Stroma of Carcinomatous Strains.

In the two cases mentioned in which sarcoma developed, the change seems rather sudden, apparently taking place from one generation to the next, or in the third more doubtful case of 2 C even taking place in the same tumour between two operations. That a cellular connective tissue may appear round the carcinomatous alveoli is not extraordinary, for such a change is found frequently, more or less marked (*cf.* fig. 4), generally accompanying the processes connected with spontaneous absorption, as has been shown by Bashford, Murray and Cramer and also by Gaylord and Clowes. The new and striking feature in the present observations is, that the stroma elements in the altered tumours under discussion have acquired an enhanced power of growth, and after transplantation proliferate independently like carcinoma-cells. At which point does this independent growth set in? Is the change really a

sudden one, as it appears to be in the observed cases? In other words, has it originated in the tumour in which marked histological changes are first found, or is it a slow process which can be traced back to previous generations? And from which elements does the sarcoma arise? In these tumours are we dealing with a special stroma which, unlike that of other carcinomata, can be transplanted alongside of the carcinoma-cells and may develop into a sarcoma, or has the reaction-tissue from the new animals acquired malignant properties?

With the intention of contributing something to the solution of these questions we have followed closely as many strains of this tumour as possible. We have studied not only the strains which already showed the change, but also and especially the pure carcinomatous strains in which no such alteration was to be seen. For this purpose we have studied not only young tumours such as are generally used for transplantation, but also old ones, for we have seen that they usually show certain changes in the characters of the central stroma. These old tumours, in which the differentiations of stroma and parenchyma may be supposed to have reached their ultimate limits, present also conditions very analogous to the human tumours we study after post-mortem examination. The transplantable growths, however, have the great advantage that every change in them can be pursued in subsequent generations, just as it can also be followed backwards in the antecedent tumours of previous generations. In this respect, our transplantable mouse-tumours possess an enormous advantage over material from the human subject, in which generally a single stage only of the evolution can be observed, without any possibility of knowing exactly what will become of it in the future or what it has been in the past.

In this study we have paid especial attention to the connective tissues of the tumours. As already mentioned, a study of the characters of the stroma is complete only where its behaviour after transplantation is carefully examined in "early stages." To settle definitely the question when the stroma first becomes transplantable, only one method seems available: viz. to study the processes at the site of inoculation in as many pure carcinomatous strains as possible, watching them carefully from generation to generation in the hope that some one of these strains may develop a sarcoma later, and so place us in the position of having followed the alterations step by step from the very first.

It has been possible to carry out this programme on a very large scale. In the genealogical tree given at the end of this paper all the series of transplantations descended from spontaneous tumour 37 are

recorded. The horizontal lines from left to right indicate the successive generations of tumours obtained by sub-transplantation ; the primary transplantation $\left(\frac{37}{1}\right)$ being to the extreme left. Every horizontal line indicates a tumour which has been transplanted out of the series whose label * is given to the left of the line. The figure and letter at the right end of the horizontal line is the label given to the batch of mice inoculated with this material ; to it the date of inoculation and percentage of success is appended. Above this line the general histological character of the stroma of the tumour is indicated as briefly as possible. The features of the parenchyma are not indicated, because it showed itself to be without importance whether the tumours were acinous or more aveolar, and because on the whole the parenchyma presents, apart from the differences we have already mentioned, a nearly uniform picture of adenocarcinoma. Below the line the age of the tumour used is indicated in days, and whether material has been kept for examination of "early stages" or not†.

The table shows only the transplanted tumours of this strain ; up to the present, about $1\frac{3}{4}$ years after the first transplantation of the primary tumour, they number more than 500 tumours. In addition numerous other tumours have been examined histologically without being transplanted. Those not transplanted, whose number amounts to several hundreds, could not be entered on the table without rendering it too complicated, and only occasionally, in reviewing the most interesting series will mention be made of them.

Excluding the strains in which the sarcomatous change had appeared (8 H and 9 H) and the more recently observed cases described later, the

* The nomenclature is explained in detail in paper on Experimental Analysis of Growth.

† It has been necessary to abridge this information about the histology as much as possible by using numerous abbreviations. Str. del. indicates a delicate stroma, consisting mainly of capillaries with scanty thin collagenous fibres outside them, and connective-tissue corpuscles only very sparsely distributed. When the connective-tissue fibres have increased in quantity without any marked increase in cellularity, as is regularly the case in the centre of old tumours, we speak of a sclerotic stroma (str. scler.) ; the stroma in the peripheral portions of these cases is delicate. Any noticeable increase in the cellularity of the stroma is indicated by the addition of cell., sl. cell. (slightly cellular), or very cell. (very cellular). Where material has been kept for examination of "early stages," this is indicated by E.S. Age of tumours is stated in days (d.).

whole mass of tumours from all the other strains have kept their purely carcinomatous character unchanged, the parenchyma being sometimes more acinous, and at other times more alveolar. In young tumours of these strains the stroma is almost invariably a delicate one, mainly consisting of capillaries with thin collagenous fibres outside the endothelial wall, and with scattered connective-tissue corpuscles. The fact that necrotic changes are less prominent in these relatively slow-growing tumours gives us more opportunity of studying the ultimate development and differentiation of the two tumour-components, parenchyma and stroma, than in most other mouse-tumours, where an early necrosis puts a stop to any further differentiation in the oldest part, viz. the centre of the tumour.

Sclerotic Changes in Old Tumours.

As already mentioned, in old tumours we find, almost constantly, a difference between the central, older parts and the periphery. While, in the latter, the stroma remains delicate as before, in the central parts an increase in the fibrous elements of the stroma takes place, giving a highly sclerotic tissue in the more pronounced cases, somewhat similar to that met with in the central parts of a scirrhus mammæ in the human subject. We find these sclerotic changes more or less pronounced in all strains where old tumours have been examined. Fig. 21 shows that they were already present in a tumour of the second generation (2 C). The reason why these changes are recorded in a less degree for the first generations than later in the genealogical tree seems to be that the tumours used for transplantation in the first generations were on an average much younger than those used later.

Between the thickened strands of fibres the carcinomatous alveoli seem to retrogress and undergo atrophic changes. It is open to argument whether this is due to a primary atrophic process in the carcinomatous alveoli, which degenerate from the centre to the periphery with secondary replacement by a fibrous scar, or, on the contrary, due to an active overgrowth of the connective tissue followed by defective nutrition and secondary atrophic changes of the carcinomatous elements ; or whether both these phenomena are due to a common factor, *e. g.*, the circulatory disturbances in the centre of a tumour. The result of the process is in any case a kind of local healing or local regression, identical with that in the centre of a scirrhus mammæ.

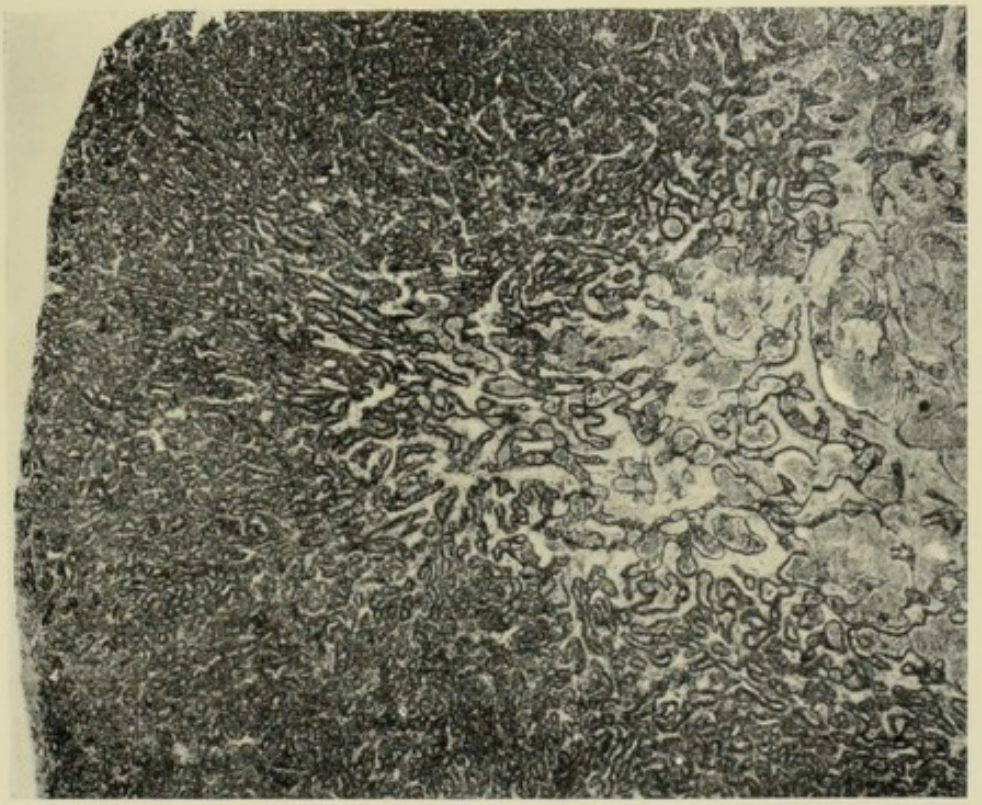
When the sclerotic changes are pronounced the central parts of the carcinoma-alveoli are necrotic or show fatty degeneration, and only a single peripheric layer of carcinoma cells remains alive in the sclerosing tissue which envelops them. Figs. 41 and 21 illustrate this (compare also fig. 61). Where the process is further advanced, the carcinoma cells degenerate completely and are absorbed, and in the centre of the tumour we find only a strongly sclerotic tissue, a scar tissue, without remains of carcinoma. To a certain extent it seems possible to explain this degeneration as due to the defective nutrition of the epithelial cells in the sclerotic tissue. With a higher magnification and with methods staining the fibres, we see that this sclerotic tissue is developed just outside the capillary wall, intercalating itself between it and the carcinoma alveolus; it seems to separate the epithelial cells from the capillaries mechanically, as we have seen the halo cells do in mixed tumours.

Intermingled with the sclerotic process and the atrophy of the epithelium consequent upon it, we find smaller or greater necrotic foci in the centre of the tumour, where carcinoma cells and stroma have undergone complete degeneration *en bloc*, probably owing to circulatory disturbances of a more sudden character. The usual picture in old tumours is a combination of localised necrosis and sclerotic changes in the centre with a more or less thick peripheral layer of healthy epithelial tissue with delicate stroma.

As a rule the sclerotic tissue contains very few cellular elements (see figs. 41 & 21). In some cases, however, we find greater cellularity without its being possible to draw a sharp line between the cellular sclerotic tissue and the ordinary purely sclerotic changes. The cellularity may be due to several causes.

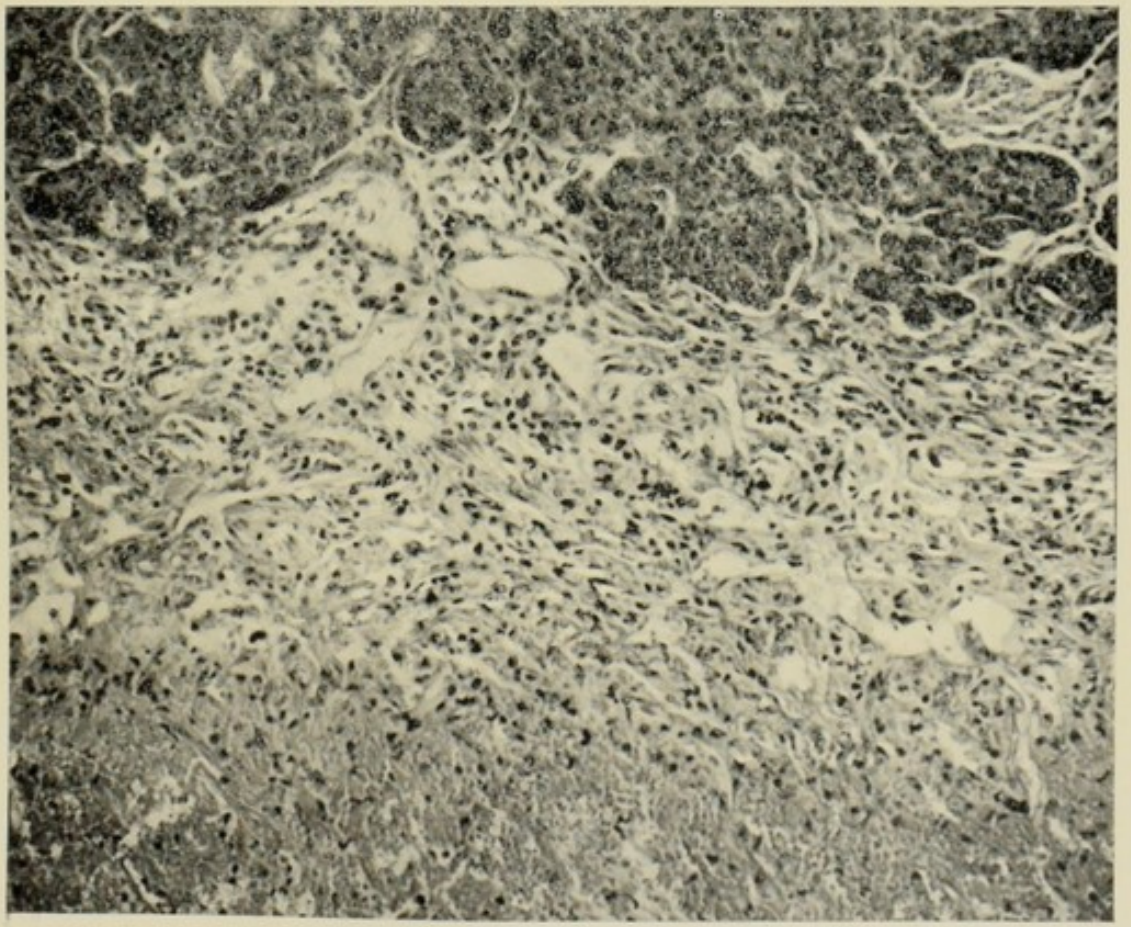
Apart from ulcerated tumours where infection and inflammatory processes may call forth an increased cellularity, the necrotic areas cause in some cases a strong reaction. As shown in fig. 42, we find a zone of very cellular tissue with large capillaries round the necrotic part, and demarcating it from the healthy tissue. From this cellular zone attempts at reorganisation of the necrotic mass appear to be made; in fig. 42 new vessels are seen budding into the dead part from the cellular granulation-tissue surrounding it. In frozen sections stained with sudan we find a zone of large cells, crowded with fatty granules on the border of the dead mass (fig. 63). These elements seem rather to be phagocytic elements devouring the dead material than degenerating

[*To face* p. 214.



Microphoto, W. Imboden.

FIG. 41.—37/9 J—10_2 N. Old tumour of 9th generation (144 days). Parenchyma adenocarcinomatous (usual type for this tumour). Stroma in peripheral parts (left) delicate, in centre sclerotic but not increased cellularity. Margin of necrotic centre at right side of figure. $\times \frac{60}{1}$.



Microphoto, W. Imboden.

FIG. 42.—37/8 Q—9_2 M. Old tumour of 8th generation (147 days). Zone of granulation tissue intervening between healthy parenchyma and necrotic centre of tumour, with commencing organisation of necrotic tissue. $\times \frac{185}{1}$.

cells. In certain cases the necrotic area is reorganised like an infarct and replaced by a kind of scar tissue. The cellularity of the surrounding stroma seems in these cases to be only a normal reaction against the dead tissue indicating a more or less intense process of organisation.

In other cases again we find the sclerotic tissue more uniformly cellular over greater areas, and then it has a rather characteristic appearance. Around the carcinoma alveoli, intervening between the parenchyma and the capillaries, we find large connective tissue cells and fibres in layers of varying thickness. Another system of very strong thick fibres accompanies the capillaries. Fig. 87 gives such a picture from a tumour of the 8th generation. Similar pictures are given in figs. 58, 81 and 86. In some cases the cellular layers surrounding the carcinoma alveolus may, as in fig. 87, remind one of the halo-formation we have described in the mixed tumours.

There seems to be a good deal of difference between the pure sclerosis as illustrated in figs. 41 and 21, and these cellular sclerotic changes. But the examination of a large number of old tumours from different strains, shows that the two forms of sclerosis pass by gradations into one another, and seem to be only different stages or degrees of the same process. The cellularity of the central stroma varies a good deal in the different strains and in the different tumours of the same strain, and between these two extremities we have all intermediate stages.

Generally, when we transplant tumours showing sclerotic changes with more or less cellularity, we do not find any remarkable peculiarity in the stroma of the tumours in the next generations ; they possess the delicate stroma found in most of these strains. In some cases, however, a greater cellularity of the stroma in the mother-material may be maintained in the daughter-tumours, and retained by this strain for many generations as a nearly constant feature. Such stroma has no peculiarities beyond being more cellular and exhibiting more marked sclerotic changes. As we are searching for alterations in the stroma, we have devoted especial attention to some of these strains and shall describe them in some detail, while for most of the other strains we shall refer only to the short data given in the genealogical tree. In one of these strains the sarcomatous change has again appeared, and this time under conditions allowing us to follow the process several generations before the appearance of sarcomatous tissue.

(5) Later Cases of Sarcoma Development.

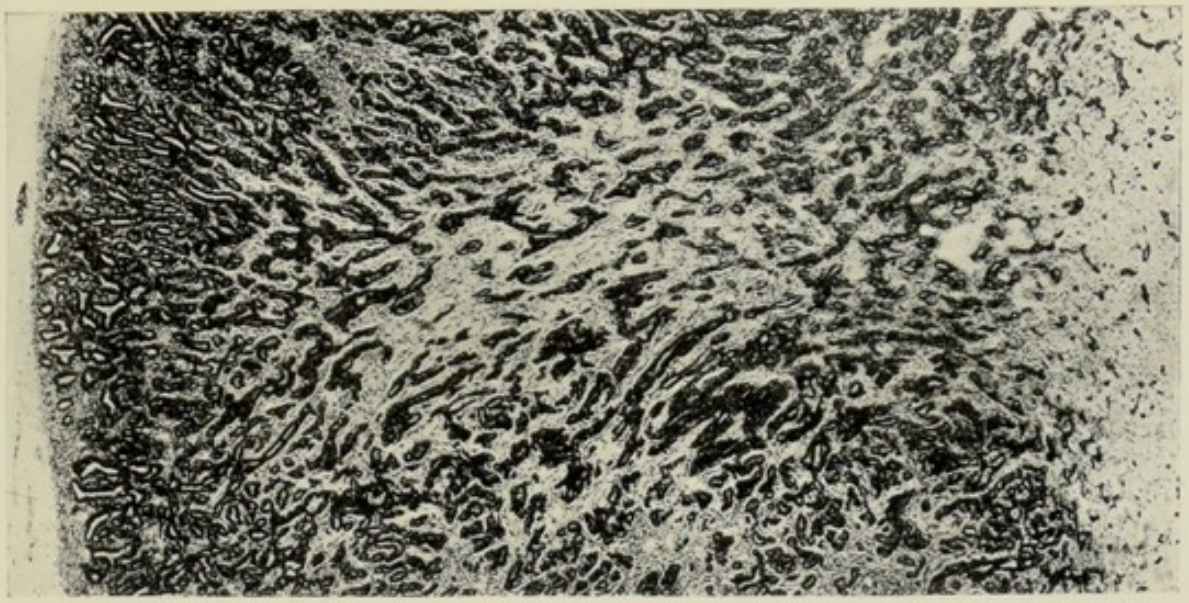
Our third case of sarcoma development (as described on p. 194) appeared after removal by operation of an ordinary carcinomatous tumour. It suggested itself to us, that the operation might possibly play some part in this change, *e. g.*, by prolonging the time during which the carcinoma cells influenced the connective-tissue cells of one animal. We proceeded, therefore, to try the influence of operation systematically. In four cases old tumours were almost completely removed under ether anæsthesia, only the minutest traces of tumour being left behind in order to produce a recurrence: these recurrences were operated upon in the same way. The material removed at each operation was inoculated into a number of mice, of which a certain number were sacrificed for "early stages." A slice through the whole tumour was always preserved for histological examination.

In two of the cases the tumour became inoperable after the second operation and the mouse was killed. In a third case (9 J) we succeeded in removing the tumour five times, and the fifth time so completely that no recurrence took place. In all these cases no sarcomatous change was observed, either in the recurrent tumour or in the sub-transplantations from them. In the fourth case (9 B) we removed the tumour six times, and the mouse was killed after the sixth operation, because the recurrent tumour became inoperable; in this case we inoculated the same tumour seven times and all the seven strains so obtained continue under propagation up to the present. In five of these strains a sarcomatous change has occurred in later generations, and we shall therefore deal with this case more fully.

A Tumour of 9 B, *its recurrences, and the Strains obtained from them.*

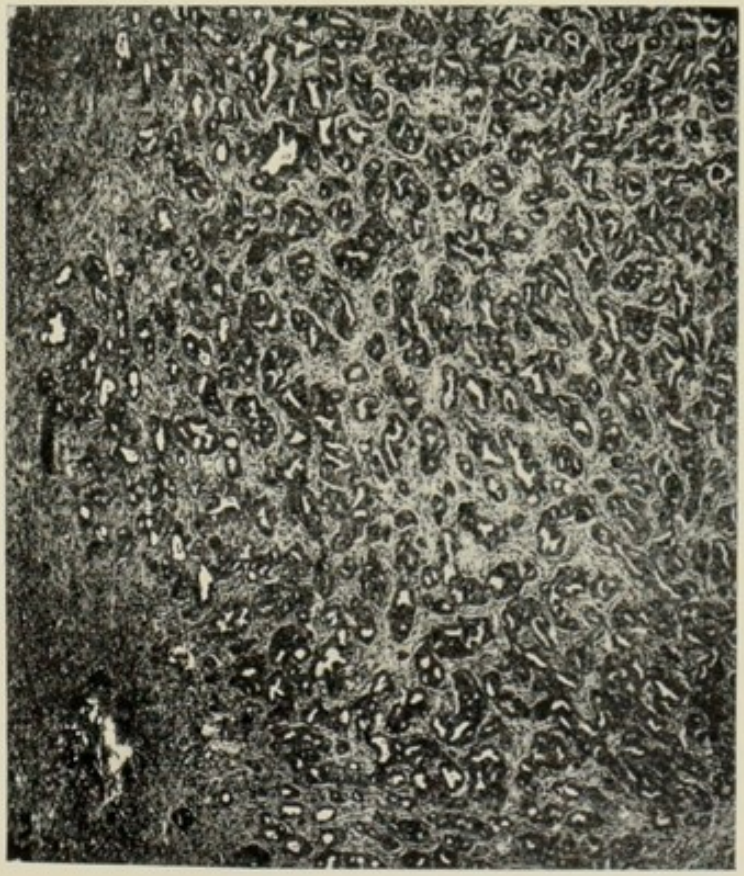
The mother tumour of these strains was a five months old and slowly growing tumour of Series 9 B. Series 9 B arose from inoculations made on 8.5.07, yielding a low percentage (30 per cent.). The tumour selected for successive operations weighed about 3 grm. at the time of the first operation (16.10.07). In its centre there was present a firm, white necrotic mass surrounded peripherally by a zone of healthy tumour-tissue of rather firm consistence. Histologically, the growth shows the usual picture of an adeno-carcinoma with a stroma which is delicate in some parts and slightly cellular in others. Towards the centre it presents marked sclerotic changes. The part of the tumour

[*To face* p. 216.



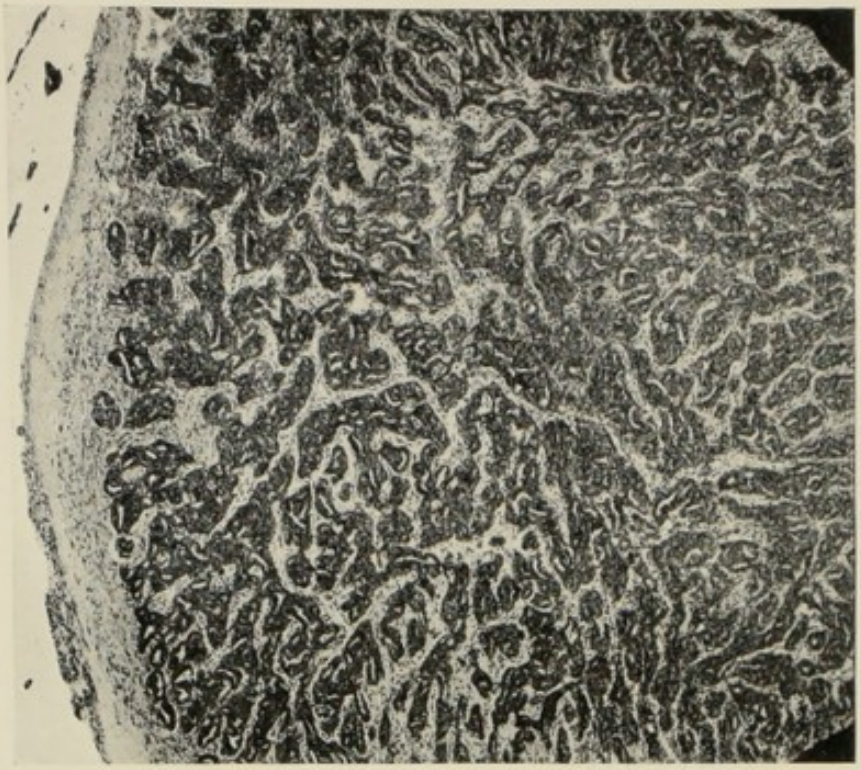
Microphoto, W. Imboden.

FIG. 43.—37/9 B, first operation—10_2 K. Tumour of 9th generation selected for successive operations (161 days old). Sector of growth extending from periphery (left) to necrotic centre (right) through the part of the tumour with most abundant stroma. Stroma sclerotic and cellular. $\times \frac{35}{1}$.



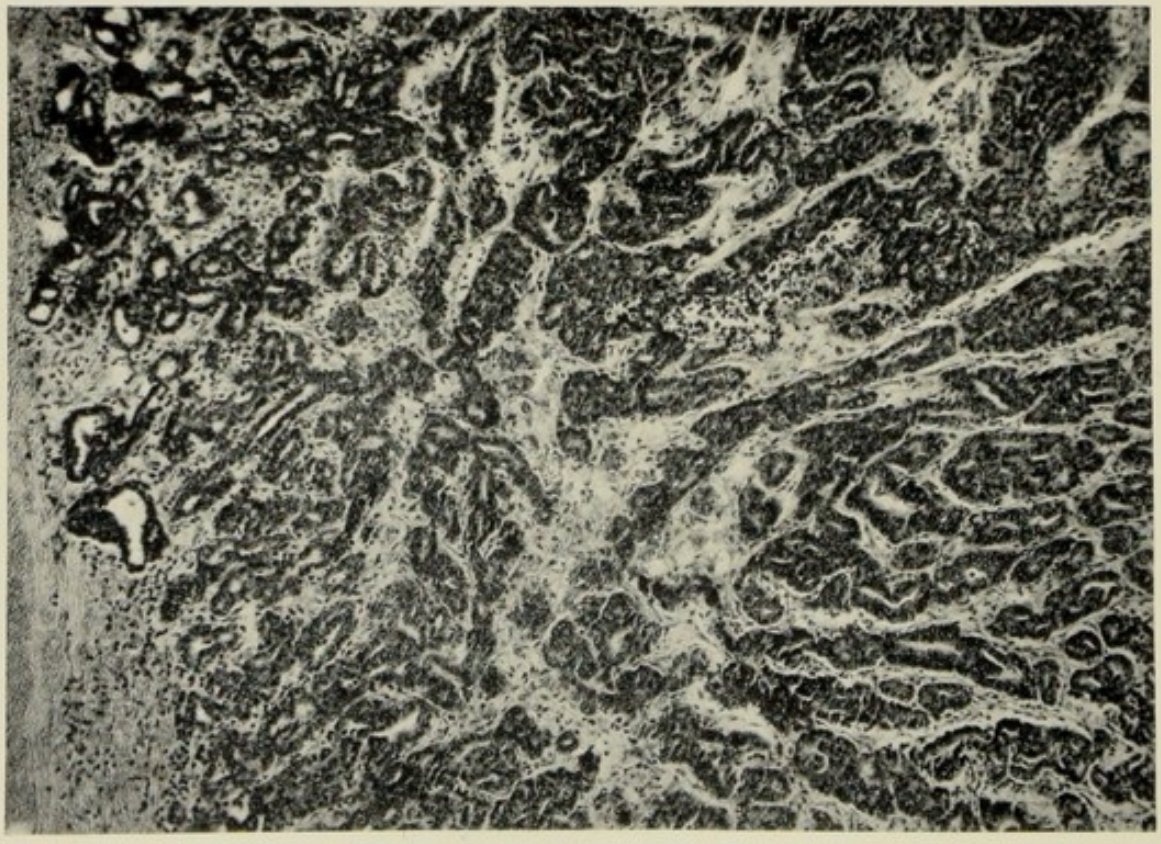
Microphoto, W. Imboden.

FIG. 44.—37/9 B, second operation—10_2 O. Recurrent tumour removed 19 days after first operation Peripheral part of tumour with cellular stroma. Hæmorrhage into capsule of tumour (left). $\times \frac{45}{1}$.



Microphoto, W. Imboden.

FIG. 45.—37/9 B, third operation—10_2 Y. Recurrent tumour removed 46 days after second operation. Peripheral part (left) with cellular stroma identical with that of fig. 44. $\times \frac{45}{1}$.



Microphoto, W. Imboden.

FIG. 46.—37/9 B, fourth operation—10_3 B. Recurrent tumour removed 31 days after third operation. Peripheral part (left) with cellular stroma identical with that of figs. 44 and 45. $\times \frac{100}{1}$.

which shows the most abundant stroma* has been photographed (fig. 43): the photograph reproduces a sector of the tumour from the periphery (at the left) to the necrotic centre (at the right). The stroma is rather cellular and fibrous, with dilated capillaries : towards the centre we see the usual sclerotic changes with atrophy and disappearance of the carcinomatous alveoli.

The tumour was transplanted into 53 young mice labelled Series 10_2K ; in addition 20 mice were inoculated with minute fragments and sacrificed on successive days for the purpose of studying " early stages." The details of the implantations of the tumour itself and of its derivative sub-transplantations are given in the genealogical tree. On a subsequent page we shall consider the further development of this strain (strain from the first operation).

The tumour operated upon recurred shortly after the operation and grew rather rapidly. The second operation was performed 19 days later. The tumour, then weighing about 1·5 grm., had the same firm consistence as the tumour when first operated upon ; but was not necrotic in the centre. Microscopically the picture is remarkably like that exhibited by the tumour at the first operation. The stroma is somewhat more abundant than usual and also cellular, as is shown in fig. 44 from a peripheral part of the tumour. This tumour was likewise transplanted and examined in " early stages." It gave rise to the Series 10_2O (strain from the second operation).

The second operation was also followed by a recurrence, this time somewhat more slowly than after the first operation. In six weeks it had reached the size of a hazel-nut and it was removed. The tumour was firm in consistence ; there was no necrosis. Microscopically the picture was again that of an adeno-carcinoma with abundant and rather cellular stroma (fig. 45). As on the two former occasions the tumour was transplanted (Series 10_2Y) and examined in " early stages " (strain from the third operation).

A third recurrence took place, and 31 days later, when the 4th operation was done, the tumour had grown again to the size of a filbert. The tumour still exhibited the same microscopical picture, and it was especially noticeable that the stroma retained the same degree of cellularity (fig. 46). As before, the tumour was again transplanted and examined : it gave Series 10_3B (strain from the fourth operation).

* For illustrating the appearances of the tumours in successive generations we have invariably selected that part of each in which the stroma is most abundant and cellular.

The 5th removal took place at two sittings : the first (5th operation) 24 days later, when a posterior nodule as big as an almond, infiltrating the abdominal wall, was removed, together with a large piece of this latter, while a smaller nodule in the axilla was left behind. Eleven days later, the axillary nodule, having grown in the meantime to the size of about half a walnut, was removed (6th operation). The histological pictures of these two nodules are in the main the same as those previously mentioned : the stroma is perhaps less rich and cellular in them than in the others.

The recurrence after the 6th operation was firmly adherent both to the thoracic and abdominal walls, so that any further operation was considered impossible. The mouse was killed 310 days after it had been inoculated, and 151 days after the first operation. There were two large masses of tumour—an anterior one about 2 grm. in weight

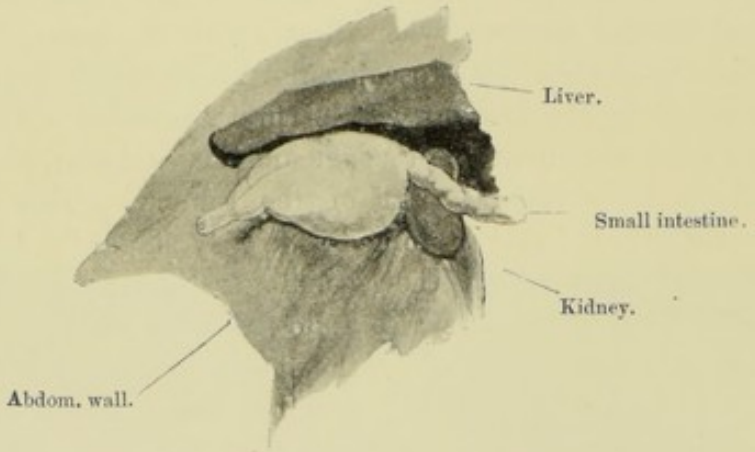
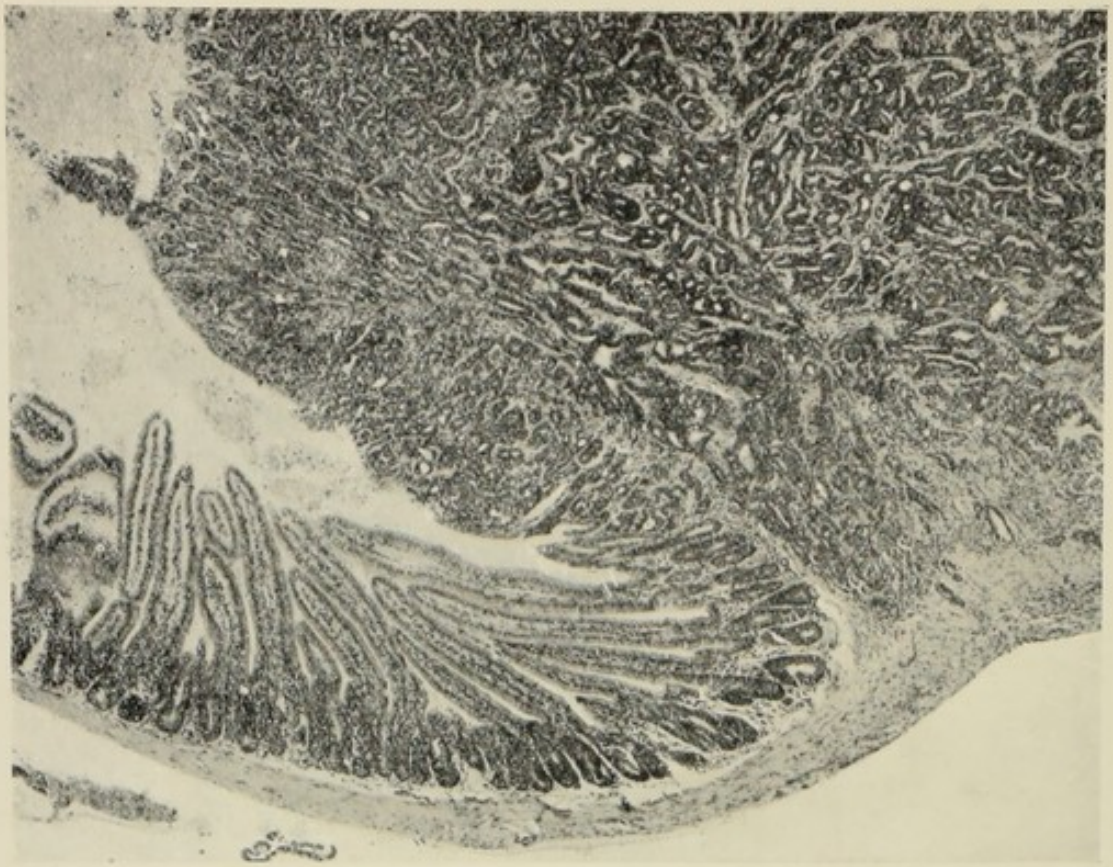


Fig. 47. 37/9 B.—Mouse killed 310 days after inoculation, the tumour had been operated upon and recurred 6 times. Sketch of internal surface of abdominal wall showing a loop of intestine infiltrated and thickened by an extension of the subcutaneous tumour causing adhesions to the abdominal wall and the liver. Nat. size. (*Cf.* figs. 48 & 49.)

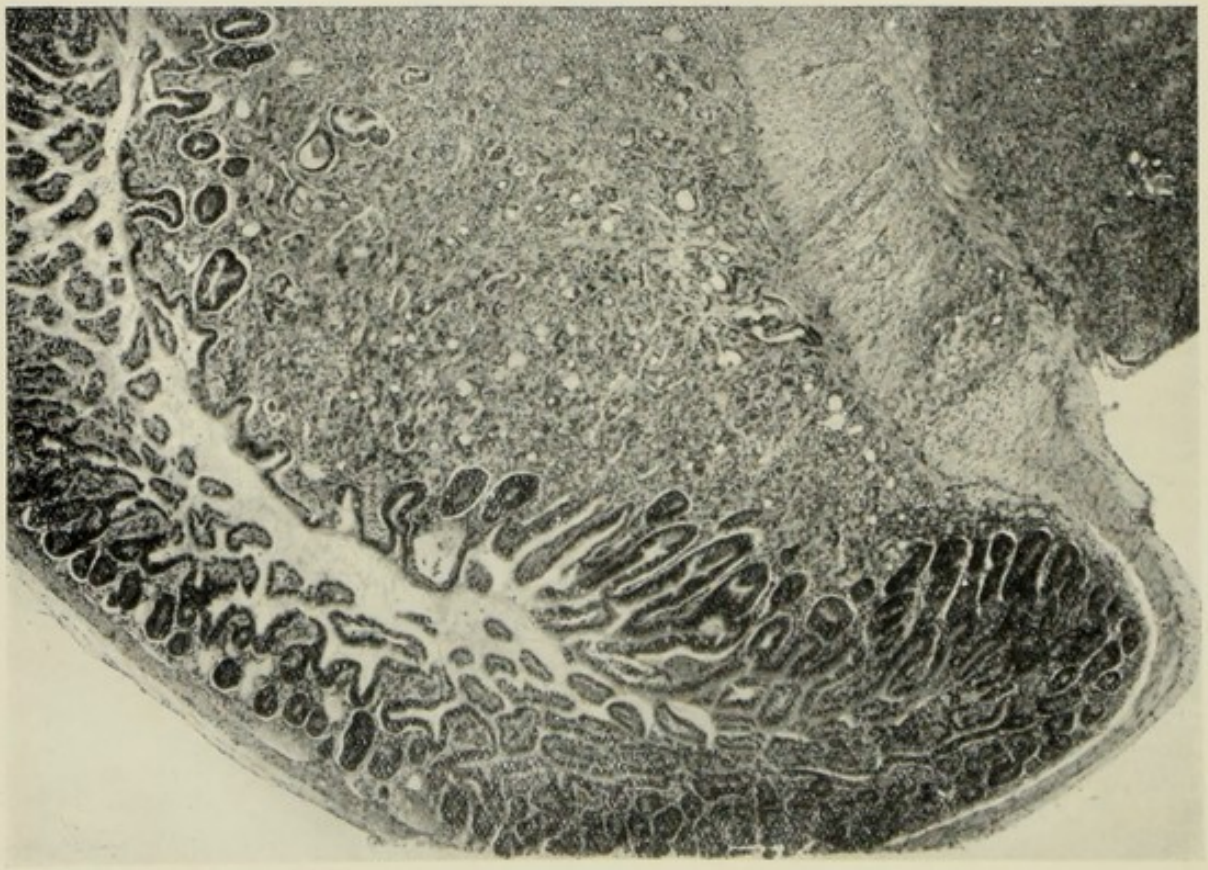
infiltrating the thoracic wall, and a posterior one, ca. 1 grm., which having grown through the abdominal wall had fungated into the abdominal cavity, and enveloped a loop of the small intestine (see fig. 47). Fig 48 illustrates a section through the intra-abdominal nodule, the marked area of which is shown in fig. 49 at a magnification of 50. The tumour has grown infiltratively through the muscular coats. The mucous membrane is completely destroyed over a large area ; thus an

To face p. 219.]



Microphoto, W. Imboden.

FIG. 49.—37/9 B. Tumour invading small intestine. View under higher magnification of area marked in fig. 48. The figure shows infiltration and splitting up of the muscular coats and destruction of the mucosa. $\times \frac{50}{1}$.



Microphoto, W. Imboden.

FIG. 50.—Lateral margin of spontaneous carcinoma of small intestine of the mouse.

ulcer has arisen, the bottom of which is formed of the carcinomatous tissue *. Histologically the tumour does not deviate from the appearances described in the preceding operations.

There were no macroscopic metastases to be seen in the lungs: microscopically one small nodule was found, this, however, in its histo-



FIG. 48. 37/9 B.—Semi-schematic transverse section of the thickened loop of intestine shown in fig. 47. The figure shows the mode of spread in the intestinall wall and the distortion and disturbance of the normal anatomical relations of the structures involved. Normal mucous membrane at bottom of figure. $\times \frac{6}{1}$.

* The infiltrative and destructive growth shown in this specimen corresponds exactly to that found in similar lesions in man, and demonstrates absolutely the baseless nature of the criticisms of those who see in the apparent absence of infiltration of the subcutaneous transplanted tumours an argument against the carcinomatous nature of these growths.

To illustrate how well the lesions of transplanted tumours may correspond to those of spontaneous tumours, we give in fig. 50 a microphotograph at the same magnification of a spontaneous growth of the small intestine of the mouse, described by Bashford, Murray, and Cramer in the Second Scientific Report of the Imperial Cancer Research Fund. The primary site of the tumour in this case is the mucous membrane from which the carcinoma has made its way outwards infiltrating and penetrating the muscular coats. In the experimental case the extension of the carcinoma follows the opposite direction; notwithstanding this fact the resulting anatomical lesions are indistinguishable.

logical features is quite different from the usual secondary growths of this tumour, and undoubtedly must be considered as an adenoma of the lung, like those we have described from Borrel's laboratory, found in mice with spontaneous and inoculated tumours *.

As will be seen from the genealogical tree, tumours from each of these series have been further propagated and studied through several generations. A detailed description of four of the strains obtained follows.

Strain from First Operation.

9 B, 1st operation——10_2K——11_2V——12_2J——**13_2A**——**14 X**——**15 Z**.
(Fig. 43) (Fig. 51) (Fig. 53) (Fig. 54) (Fig. 56) (Fig. 57)

Propagation of material removed at first operation of 9 B.

Carcinoma up to 12_2J ; sarcomatous change in 12_2J–13_2A ; in later generations progressive advance of mixed tumour stage towards pure sarcoma.

9 B, 1st operation, giving series 10_2K. We have already described the histology of the tumour used to yield this series : fig. 43 shows that its connective-tissue scaffolding is stronger than usual and slightly cellular. Following our routine procedure, our first question is : How does the material behave immediately after transplantation in "early stages" ? †

* Later Tyzzer has found the same adenomatous growths occasionally occurring in the lungs of normal mice, and this observation makes it very probable that they are independent processes without any causal relation to the previously existing tumours, where such are found. Murray describes in another paper in this report several cases of the same condition. He comes to the same conclusion as Tyzzer that the association of lung-adenomata with spontaneous or transplanted tumours in other sites is accidental and due to the greater care with which the lungs are examined in these cases.

The elastic framework of the nodule being that of the lung itself is however in this case more strongly developed between the cells of the nodule than between the normal alveoli, thus indicating hypertrophy of the elastic tissue, somewhat similar to that observed in human cancer mammæ and described by Scheel (Ziegler's Beiträge, Bd. 39, 1906) and others. In one of Tyzzer's cases a similar observation is recorded.

† The technique for examination of early stages of the processes at the site of inoculation and their general histological characters need not be referred to in detail here, because it has been previously described by Bashford, Murray, and Cramer (2nd Scientific Report of the Imperial Research Fund) and is again referred to in detail by Russell on a later page of this Report. In this paper we shall only discuss the processes in so far as they diverge from what has been previously ascertained for ordinary carcinomata.

Examination of "Early Stages" of 9 B, *1st operation, giving* 10_2K.—In 24 hours material the usual picture is found: there is complete necrosis of parenchyma and stroma in the centre of the graft, in the periphery here and there islands of parenchyma are alive along the border of the graft, while the stroma elements mostly show signs of degeneration. In examining carefully the serial sections, however, we find here and there close to the periphery places where individual stroma cells have retained their staining reactions and look apparently healthy. We have convinced ourselves that these cells are really cells of the old stroma by studying them in complete serial sections. They occupy exactly the same place as the large fibroblasts in the mother-material, and have the same relation to the collagenous fibres and old vessels. No connection whatever can be found with the tissues of the host, from which polyblasts are already invading the graft, and perhaps there is even a beginning reaction from the fixed elements. We cannot prove that these cells are actively dividing by demonstating mitoses; but from the general impression of their healthy looking nuclei and abundant protoplasm there is no evidence whatever that these individual stroma cells are degenerating.

In 48 hours' material the degeneration of the stroma is further advanced. Where complete necrosis is not evident, we find the nuclei more or less shrunk, darker than usual, and fatty granules in the protoplasm, stained dark with the osmic acid of the fixative. Here and there, however, we see individual connective-tissue cells, belonging to the introduced stroma, which seem to have resisted much better than the others. In one place we see such a cell in division, at the same time presenting brown granules in its protoplasm as an indication of degenerative changes. On account of the numerous cells from the host now invading the graft we cannot follow the connective-tissue cells of the graft any longer, and do not know what becomes of them later. The study of three and four days' material shows complete revascularisation. It is difficult to say how much stress should be laid on the presence of a single mitosis when the great majority of the stroma elements undoubtedly undergo degenerative changes and are replaced by new reaction-tissue.

Five tumours obtained from Series 10_2K have been examined at different ages, from 28 up to 110 days old; three of them have been transplanted (giving 11_2V, 11_2W and 11_3N, see genealogical tree). All these tumours show a slightly greater proportion of connective tissue, compared with that of tumours of earlier generations; in all of them it is most marked in the central parts. The first examined, 28 days after transplantation, is illustrated in figs. 5 and 51. It presents a very pronounced adenomatous structure, and has in the peripheral portions quite a delicate stroma (fig. 5), while the central parts show slight sclerotic changes with some cellularity of the fibrous tissue (fig. 51). This material gave rise to the Series 11_2V, which has been followed further through a series of generations. The tumour next examined, also transplanted, showed in the main the same picture. 110 days after the inoculation, the remaining three mice with tumours of this series were killed for examination. Of these one showed the same picture

with more abundant connective tissue of slightly increased cellularity while the two others show a still more pronounced cellularity of the stroma. In one of these we find in considerable areas of the growth a picture somewhat similar to that in our earlier cases of sarcoma development, and especially to that observed in 2 *c* (see fig. 22). This tumour was transplanted and gave Series 11_3N, which also has been followed in subsequent generations. We shall consider these two strains separately.

(1) We will consider the sub-transplants of the first examined tumour of 10_2K giving 11_2V, and first the material as examined in early stages.

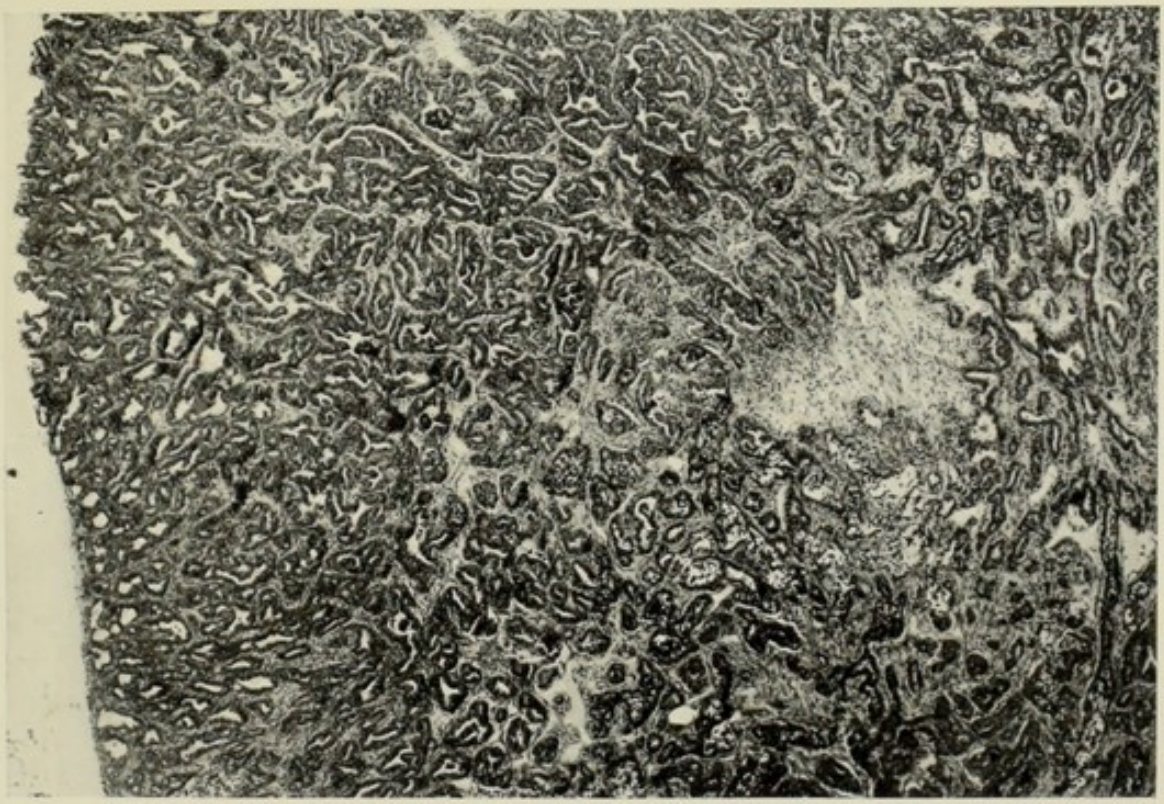
Examination of "Early Stages" of 10_2K, *giving* 11_2V.—In 24 hours' material along with the usual degenerative changes in the graft, acini of parenchyma remain alive at the periphery and show mitoses. Between them several stroma cells are looking healthy, apparently without any degenerative changes. In one place we see a dividing cell, which apparently is a stroma cell (fig. 52). To make quite sure whether we have to deal here with a real stroma cell or an isolated cell of parenchyma, we examined and photographed this place in the sections in front and behind the dividing cell. This examination shows that the dividing cell, which is situated midway between two carcinoma alveoli, is really a cell of the old stroma, and cannot be explained as an isolated carcinoma cell. But as long as only a single mitosis is found, it may be objected that this may be a cell, by chance transplanted in the moment of division. It is known how well mitoses may be preserved in postmortem material, and single mitoses hardly prove anything.

In 48 hours' material the vascularisation is already started, and it is now hardly possible to distinguish the invading cells from the cells of the old stroma, which might still remain alive. That, however, a certain number of stroma cells have been able to escape degeneration up to this time is seen towards the centre of the graft, on the border of the necrotic tissue where several living stroma cells situated exactly as in the normal stroma do not exhibit noticeable degenerative changes: on the other hand we find no evidence of proliferation in them. The connective-tissue elements in this tumour seem therefore to resist degeneration after transplantation to a certain extent better than is usually the case in transplanted carcinomata; on the other hand they do not provide enough evidence to prove a fundamental difference from the latter. Material examined after three and four days is revascularised, and throws no further light on the question of the fate of the stroma cells implanted.

From the Series 11_2V two tumours were transplanted, 70 and 82 days old respectively (giving Series 12_2J and 12_2O). Both these tumours show a marked increase of the connective tissue with slight cellularity. The central parts of the growths show very marked sclerotic changes.

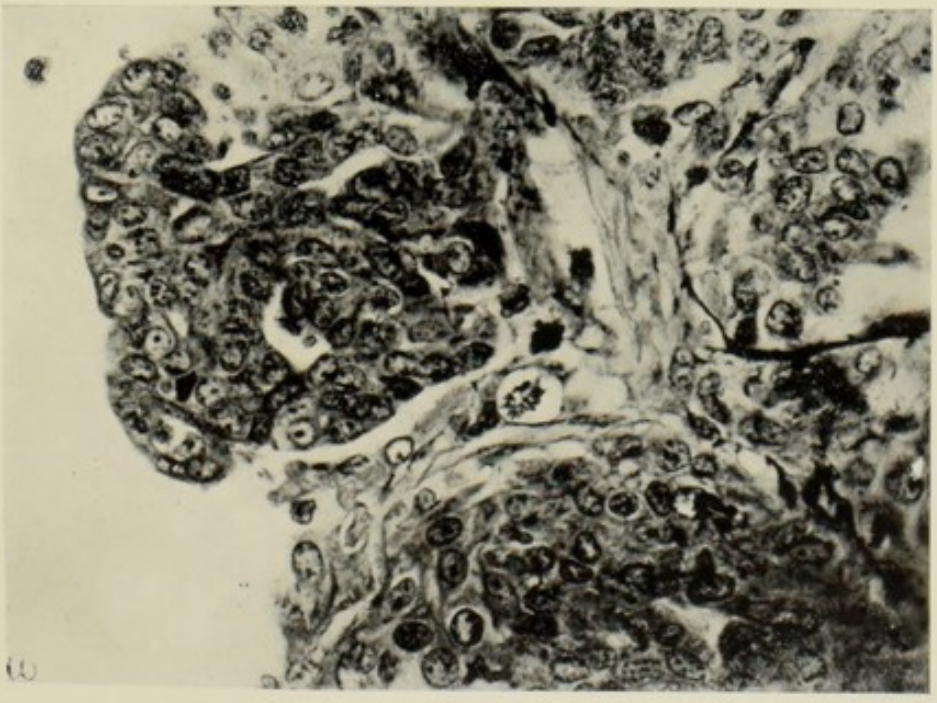
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Strain from first operation on 37/9 B.



Microphoto, W. Imboden.

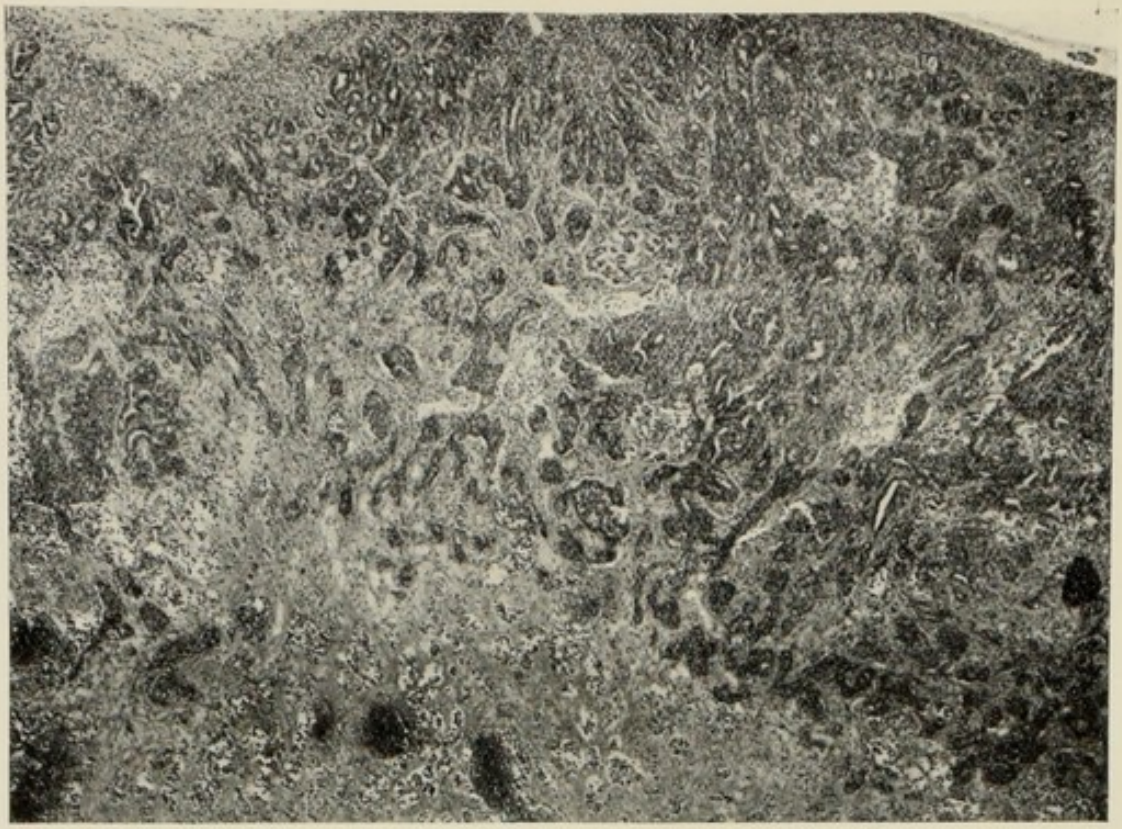
FIG. 51.—37/10$_2$ K—11$_2$ V (28 days old). Tumour from first passage of this strain directly descended from tumour of fig. 43. Stroma slightly cellular. *Cf.* fig. 5, drawn from a peripheral area of the same tumour. × $\frac{50}{1}$.



Microphoto, W. Imboden.

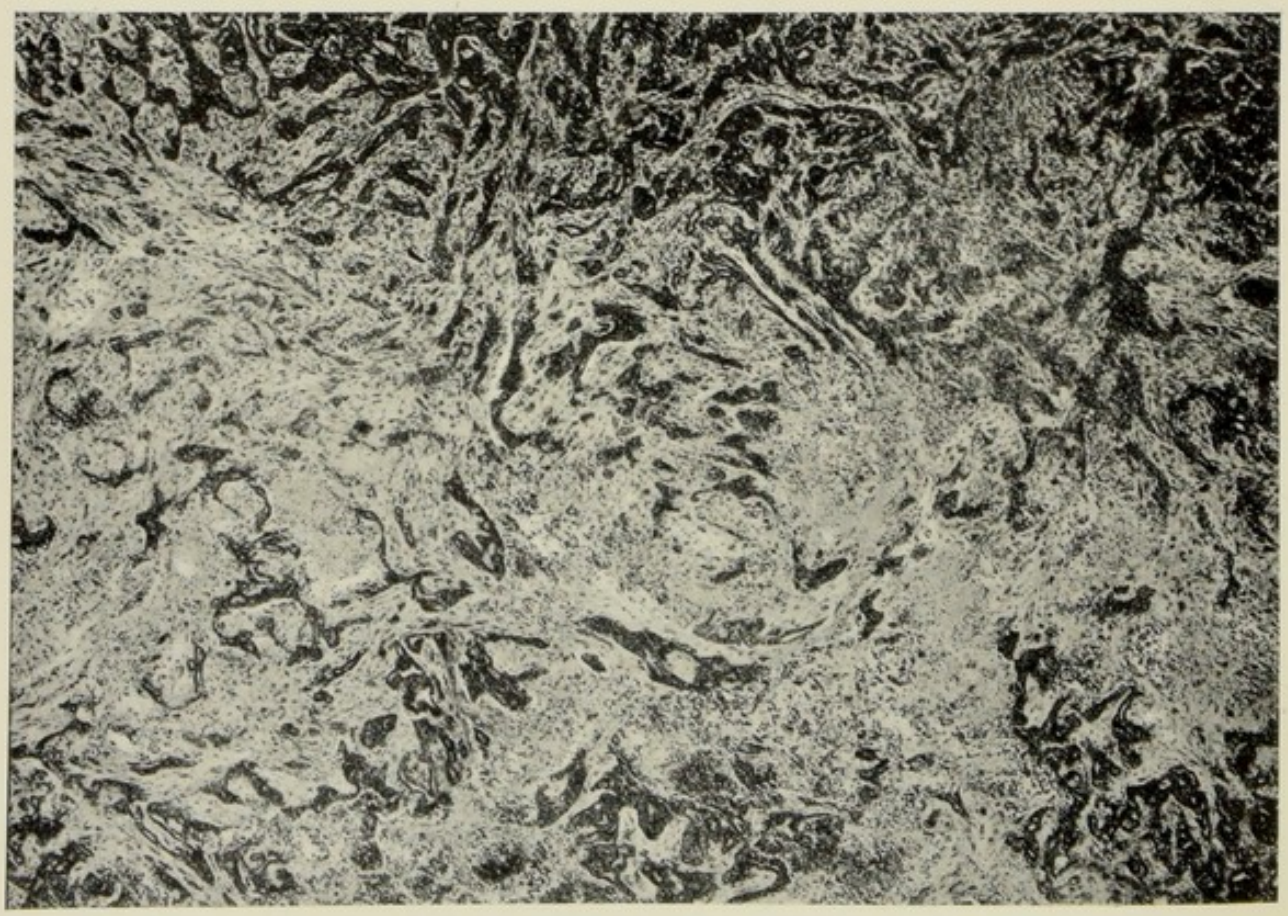
FIG. 52.—37/10$_2$ K–11$_2$ V. Graft preserved 24 hours after transplantation of above tumour (fig. 51). Some of the introduced stroma cells are alive, one in mitosis. × $\frac{500}{1}$.

Strain from first operation on 37/9 B.



Microphoto, W. Imboden.

FIG. 53.—37/11$_2$ V—12$_2$ J (70 days old). Tumour from second passage of this strain directly descended from tumour of fig. 51. Sector from periphery (top of figure) to necrotic centre. Stroma increased in amount, sclerotic and cellular. Broad zone of cellular connective tissue around periphery of tumour. $\times \frac{55}{1}$.



Microphoto, W. Imboden.

FIG. 54.—37/12$_2$ J—13$_2$ A (36 days old). Tumour from third passage of this strain directly descended from tumour of fig. 53. Central part of the tumour. Strands of cellular connective tissue with large spindle-shaped elements. The distribution reminds one of sclerotic changes in the mother-material. $\times \frac{50}{1}$.

In fig. 53 a sector of the tumour used to yield Series 12_2J is shown. Beside the sclerotic and cellular tissue surrounding the necrotic centre of the tumour, another zone of cellular tissue at the periphery suggests a strong reaction from the surrounding tissues, and is very similar in appearance to what is observed when spontaneous absorption is taking place. This material was not examined in early stages. This was done, however, from the sister-tumour giving 12_2O. In this material also some evidence was found of a greater resistance of individual stroma cells, but their active proliferation after transference to new animals could not be definitely proved.

Series 12_2J gave a very low percentage (20 per cent.), only one tumour has been transplanted (giving 13_2A). Fig. 54 shows a central area of this tumour. There seems to be a further development of the sclerosis found in the mother-material, and it now recalls the same process as described for our earliest cases of sarcoma development. Large spindle-shaped stroma-elements are proliferating here and there between the carcinoma alveoli which are compressed and often apparently split up into smaller fragments. Interspersed with the proliferating stroma cells, small necrotic areas are found all over the centre of the section. No "early stage" examination was made of this material.

The further development of the process is very unequal in the tumours of the next generation. In one tumour examined (giving 14 X) the sarcomatous change suggested in the mother-material has progressed very much, as shown in fig. 56. This picture is only found in the middle part of the tumour, while both ends of the elongated growth present carcinomatous structure with a cellular stroma. Two other tumours from this series still show the picture of a carcinoma with a cellular stroma, in the central portions with marked sclerotic changes. A careful examination of one of these apparently carcinomatous tumours shows, however, that its interstitial tissue cannot be considered as equivalent to the ordinary stroma of the earlier generations, but is, at any rate partly, undoubtedly sarcomatous and probably derived from the transplanted and already sarcomatous stroma of the mother-tumour. Fig. 55 shows at a magnification of 180 times one part of this tumour (used to yield 14 V), where the sarcomatous nature of the stroma is evident. It consists of large succulent spindle-cells with numerous mitoses. This fact makes it probable that although sarcomatous cells are present in the mother-material, on transference to new hosts they

do not necessarily continue to proliferate as e. g. in the sister-tumour (fig. 56).

As in the first cases of sarcoma development, the next stage in this process is characterised by the appearance of extremely polymorphous elements. The stroma of the tumour used to yield 14 X (fig. 56) already presents beside the spindle elements, numerous large polymorphous cells. Still more is this the case in the next generation. The two tumours transplanted (giving 15 Z and 15_2A) both present the picture of a polymorph-celled mixed tumour with halo-formation identical to that observed in our earlier cases. Fig. 57 shows a section of one of these tumours. The halo-formation is distributed throughout the whole tumour and exactly corresponds to the pictures given in figs. 23–26, *cf.* also fig. 73. In the next generation the appearances have advanced slightly ; beyond this stage this strain has not been followed as yet.

Examined in " early stages," this polymorphous tissue shows itself capable of further growth in new animals, as we have described it for our earlier cases : this fact, alone, proves the sarcomatous nature of these elements in spite of their polymorphism and morphological resemblance to granulation-tissue.

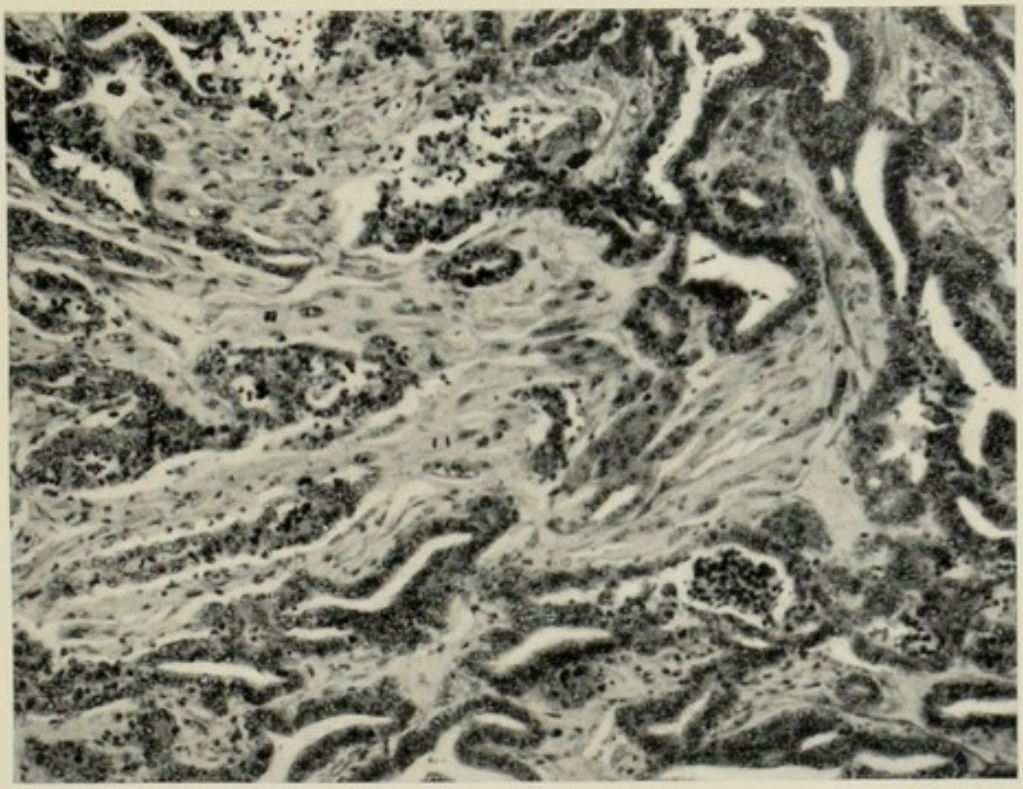
(2) Having considered the main strain from the first operation (through 11_2V), we now return to another tumour out of Series 10_2K (giving 11_3N) and its sub-transplantations. This tumour showed a very cellular stroma (fig. 58), in some places offering a very close resemblance to the earlier cases of sarcoma development. We should have expected this alteration to be progressive in the daughter-tumours of this material. This, however, is not the case.

This material examined in " early stages " shows marked degenerative changes of the abundant stroma elements, already after 24 hours. In the peripheral parts living cells can be found 24 hours after transplantation, and some of them show at this time signs of proliferation. In 48 hours' material, however, there is very little sign of life in the transplanted stroma cells. At the same time the reaction from the host takes place as usual. In agreement with this behaviour of the stroma in " early stages " the next generation from this material shows the picture of a carcinomatous tumour, and not that of a progressive sarcoma-development.

We have examined and transplanted separately three tumours from 11_3N. In all, the carcinoma prevails over the connective tissue which forms a delicate stroma in the periphery of the growth, only in the central parts is the amount of stroma increased and slightly more

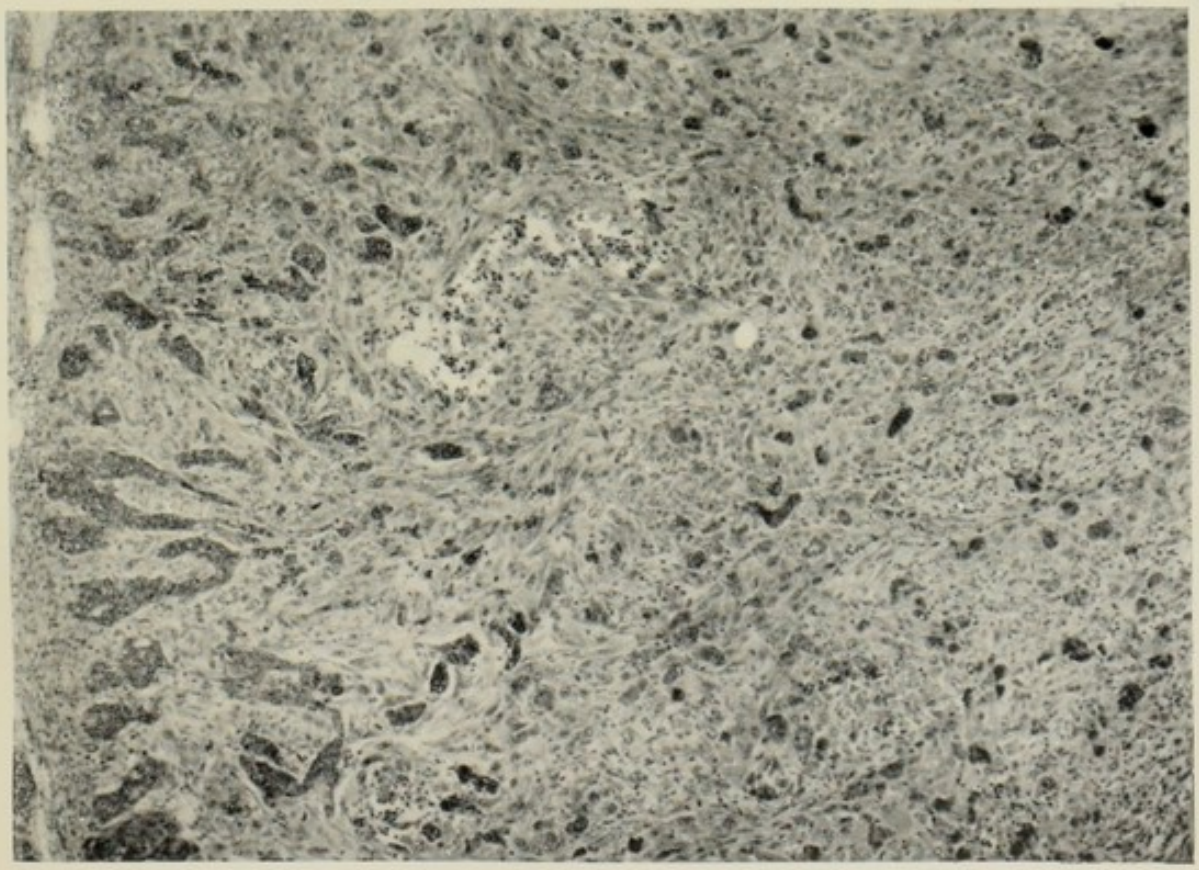
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Strain from first operation on 37/9 B.



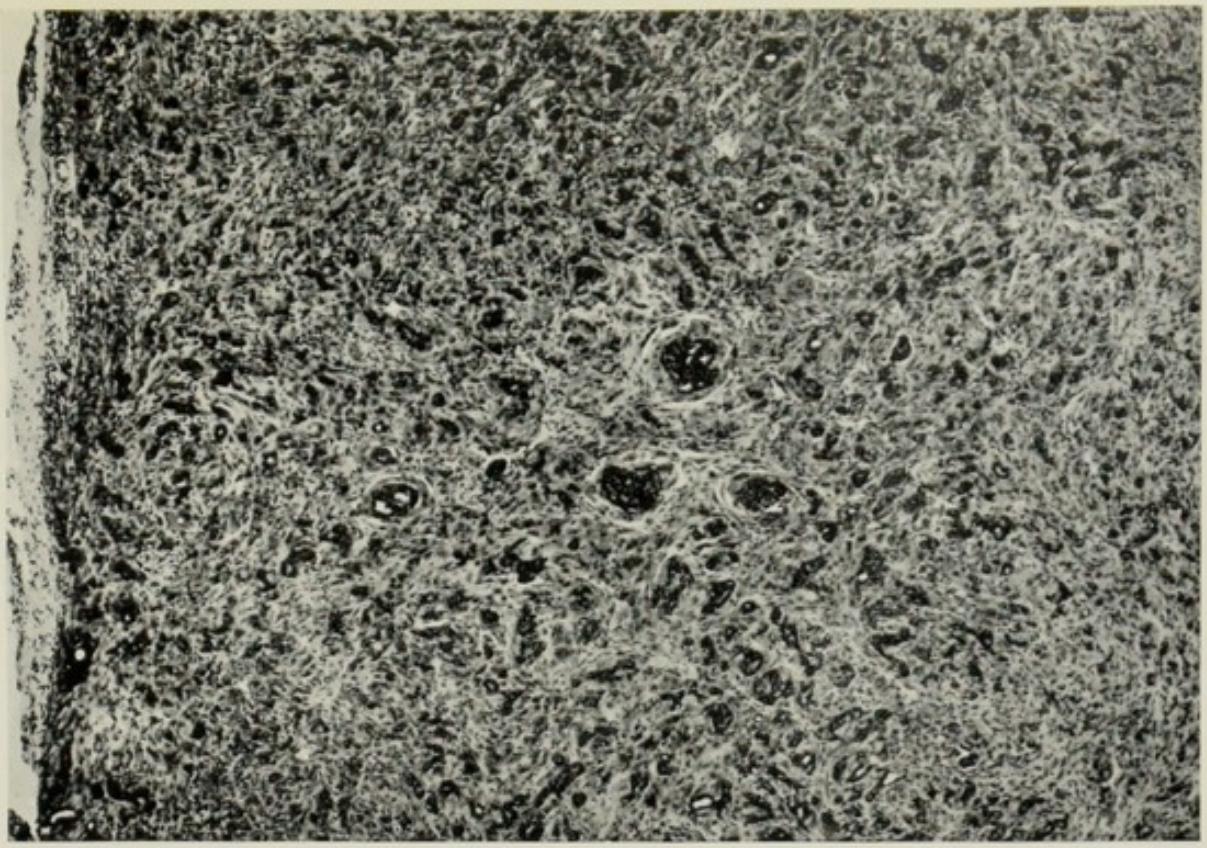
Microphoto W. Imboden.

FIG. 55.—37/13$_2$ A—14 V. A 28 days old tumour from fourth passage of this strain, directly descended from tumour of fig. 54. Small central area in which the stroma is definitely sarcomatous. Note large spindle-cells with mitoses. Elsewhere in this tumour the stroma is not more abundant than in carcinomatous strains, and only slightly cellular. × $\frac{180}{1}$.



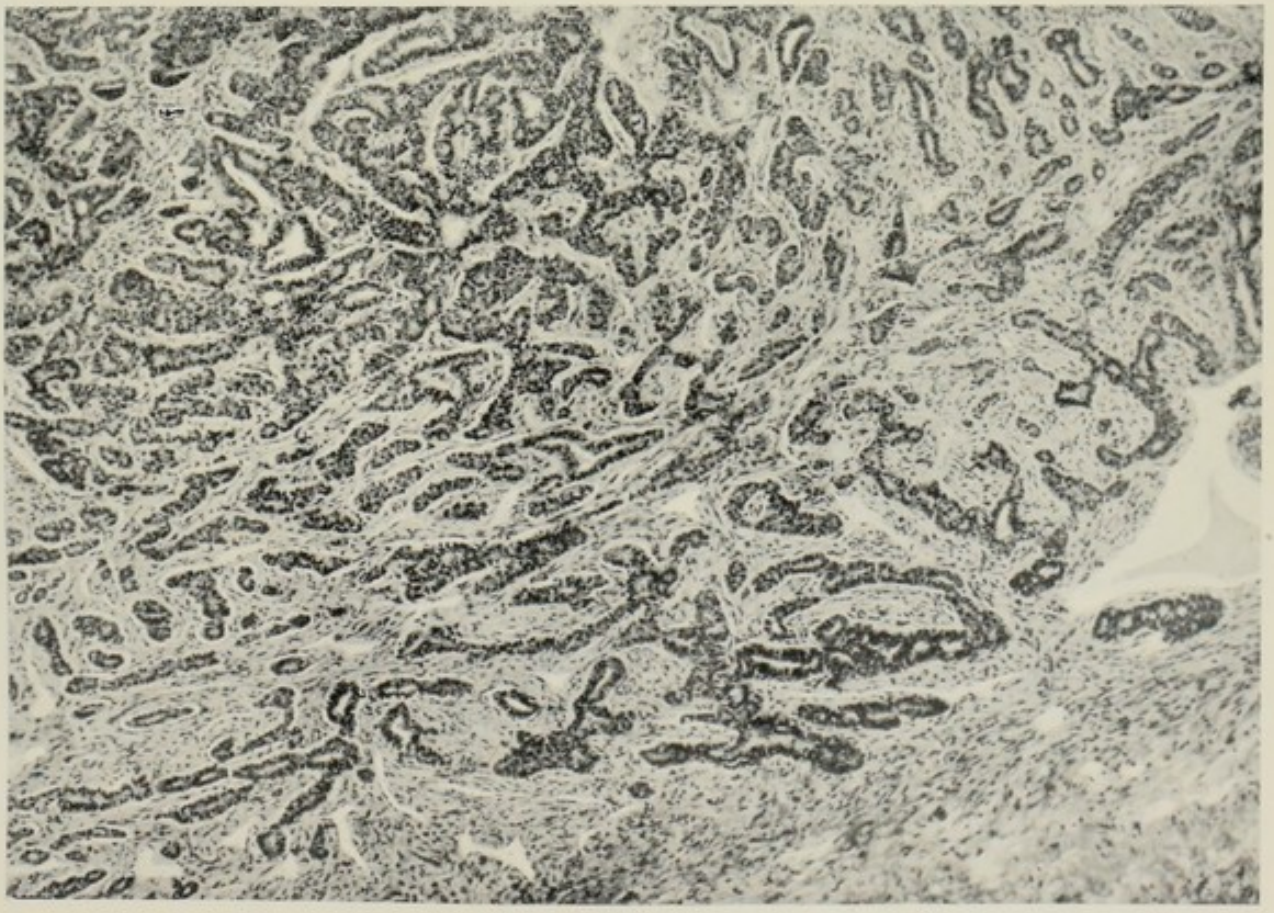
Microphoto. W. Imboden.

FIG. 56.—37/13$_2$ A—14 X. Another tumour (35 days old) from fourth passage of this strain, directly descended from the tumour of fig. 54. In contrast with the sister-tumour of fig. 55 the sarcomatous change has progressed very much and the carcinomatous component is reduced to small isolated alveoli. The sarcomatous tissue shows marked polymorphism of the cells. × $\frac{160}{1}$.



Microphoto W. Imboden.

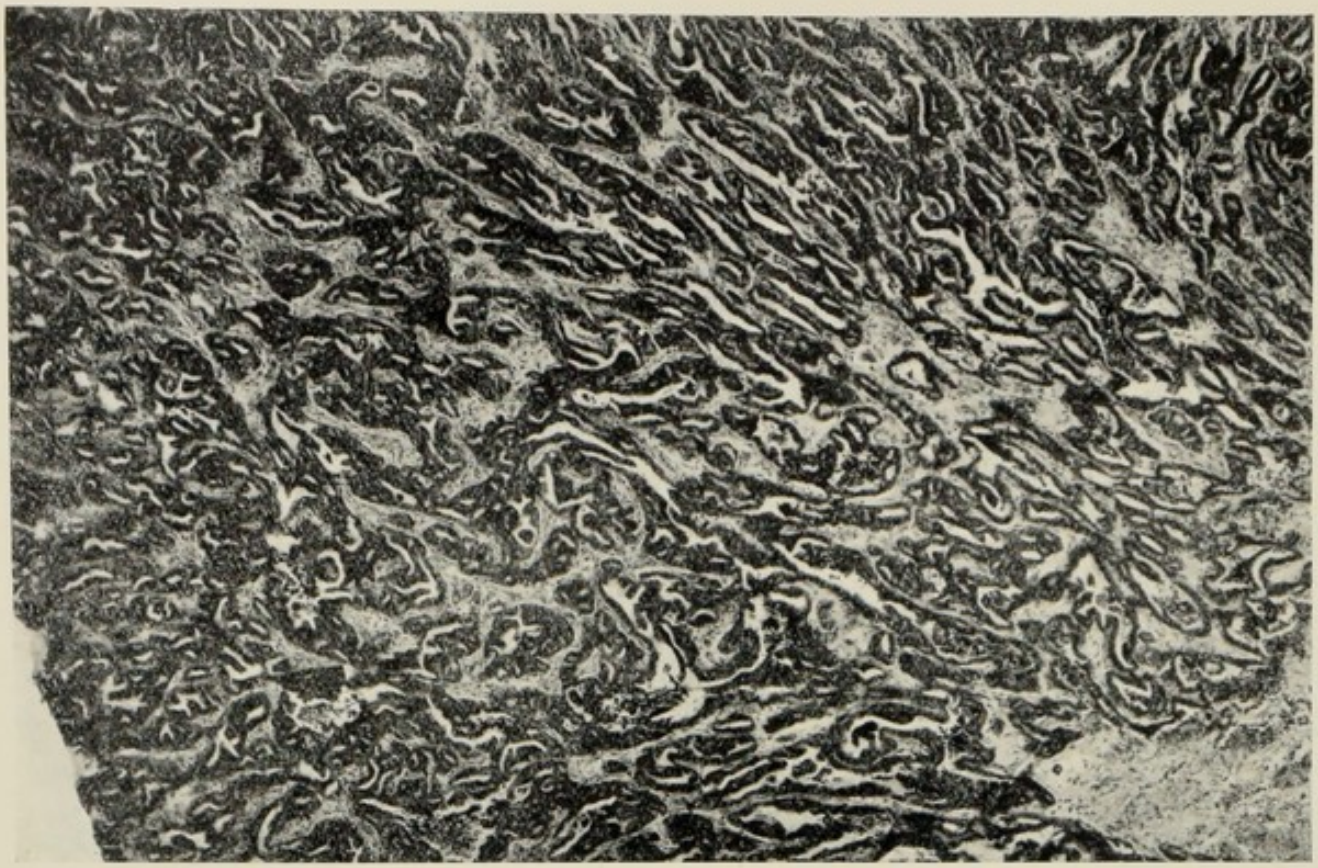
FIG. 57.—37/14 X—15 Z. Tumour (30 days old) from fifth passage of this strain, directly descended from tumour of fig. 56. Polymorph-celled stage of "mixed tumour" with halo-formation, compare figs. 23–26 and 73. Periphery to the left. × $\frac{70}{1}$.



Microphoto, W. Imboden.

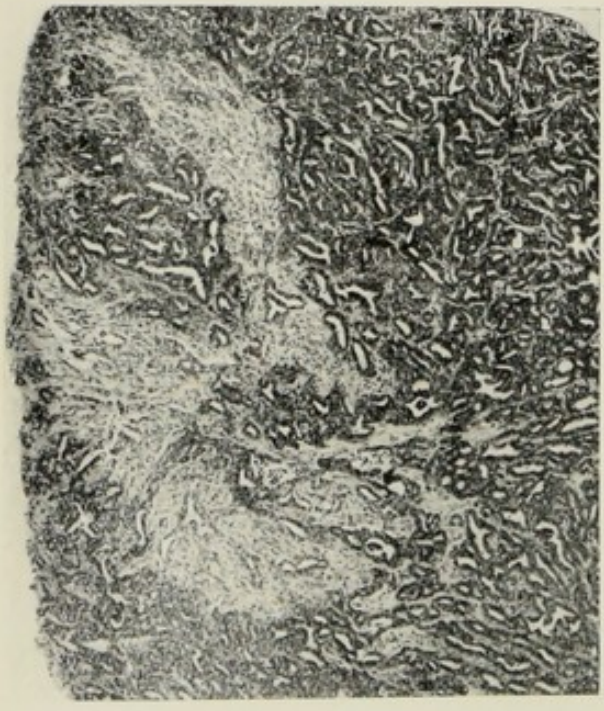
FIG. 58.—37/10_2 K—11_3 N. Tumour (110 days old) from first passage of this strain, derived from tumour of fig. 43. Central part with sclerotic changes. Although the stroma in this tumour is abundant and cellular, it did not become progressively increased in next generations, but the tumours returned to the usual carcinomatous condition. × $\frac{100}{1}$.

Strain from second operation on 37/9 B.



Microphoto, W. Imboden.

FIG. 59.—37/10$_2$ O—11$_2$ Z. Tumour (38 days old) from first passage of this strain, directly descended from tumour of fig. 44. Abundant and slightly cellular stroma. Periphery at the left, necrotic centre at the right. × $\frac{58}{1}$.



Microphoto, W. Imboden.

FIG. 60.—37/10$_2$ O—11$_2$ Z. Same tumour as given above (fig. 59). Localised peripheral area with very cellular spindle-celled interstitial tissue. × $\frac{35}{1}$.



Microphoto, W. Imboden.

FIG. 61.—37/11$_2$ Z—12$_2$ L. Central portion of tumour given in fig. 62. Shows typical picture of sclerosis with accompanying progressive retrocession of carcinoma. Note the scantiness of cells in the sclerotic tissue. × $\frac{45}{1}$.

cellular as compared with that of tumours of other strains. We find no certain evidence in this material, when examined in "early stages," that the introduced stroma cells proliferate.

This strain, parallel to that just mentioned above which showed sarcomatous changes, has been propagated up to the present through 3 subsequent generations without showing any similar alteration.

Strain from the 2nd Operation.

9 B, 2nd operation —— 10_2O (Fig. 44) —— 11_2Z (Fig. 59) —— 12_2L (Fig. 62) —— **13 Y** (Figs. 67 & 68) —— **14 T** (Figs. 70 & 71) —— **15 X** (Fig. 72) —— **16 S.** (Fig. 73)

Propagation of material removed at second operation of 9 B.

Carcinoma up to 12_2L; sarcomatous change in 12_2L–13 Y; in later series mixed tumour stage progressively advancing towards pure sarcoma.

The histology of the mother material (2nd operation of 9 B, giving 10_2O) has already been mentioned. An illustration of a peripheral part is given in fig. 44.

Examination of "Early Stages" of 9 B, *2nd operation, giving* 10_2O.—In four grafts preserved 24 hours after inoculation besides the usual necrosis of the central part of the graft and extensive degeneration of the stroma elements, many of the introduced stroma cells look perfectly healthy, and in a few places isolated mitoses are to be seen.

In 48 hours material, the degeneration of the old stroma is more advanced; at the same time new capillaries are budding in from the surrounding tissue. There are, however, several places where some of the old stroma elements look apparently healthy without visible degenerative changes. No signs of active proliferation can be seen in them.

In 3 days material the number of elements of the old stroma which can be traced as such is very small. The centre is completely necrotic, and the reaction from the host invades the peripheral part and makes it nearly impossible to tell whether or not elements belonging to the old stroma are still alive. The same is the case in 4 days material. We can, therefore, hardly draw any more exact conclusions as to the survival of individual elements of the stroma from the examination of "early stages" of this material than we could from the corresponding stage in the strain from first operation.

In the series 10_2O which was obtained from this material, there were four tumours. Of these, three have been examined carefully, and two of them have been transplanted and examined in early stages. They all show a stroma somewhat more abundant than usual and slightly cellular. In the central part sclerotic changes are found. The general

histological appearance of these tumours is illustrated in fig. 59 which shows a sector of the tumour used to yield series 11_2Z, by which the propagation of the strain is continued. A peripheral part of this same tumour shows in a localised area, a much greater number of large stroma elements : this area is given on the photograph (fig. 60) under a low magnification.

Examination of "Early Stages" of 10_2O *giving* 11_2Z.—In 24 hours material some of the stroma elements still look healthy without degenerative changes. A single mitosis is seen. In 48 hours grafts extensive degeneration of the stroma of the graft has taken place and very few stroma cells are to be seen : there are no signs of proliferation in them, and reaction from the surrounding tissue has commenced. 3 days material was not preserved in this case. 4 days and 5 days material are already vascularised at the periphery, and the elements of the old stroma cannot be followed. There is no certain evidence of survival of the old stroma elements in this material ; but also there is no sign of a reaction especially strong, or different from that to other carcinomatous material.

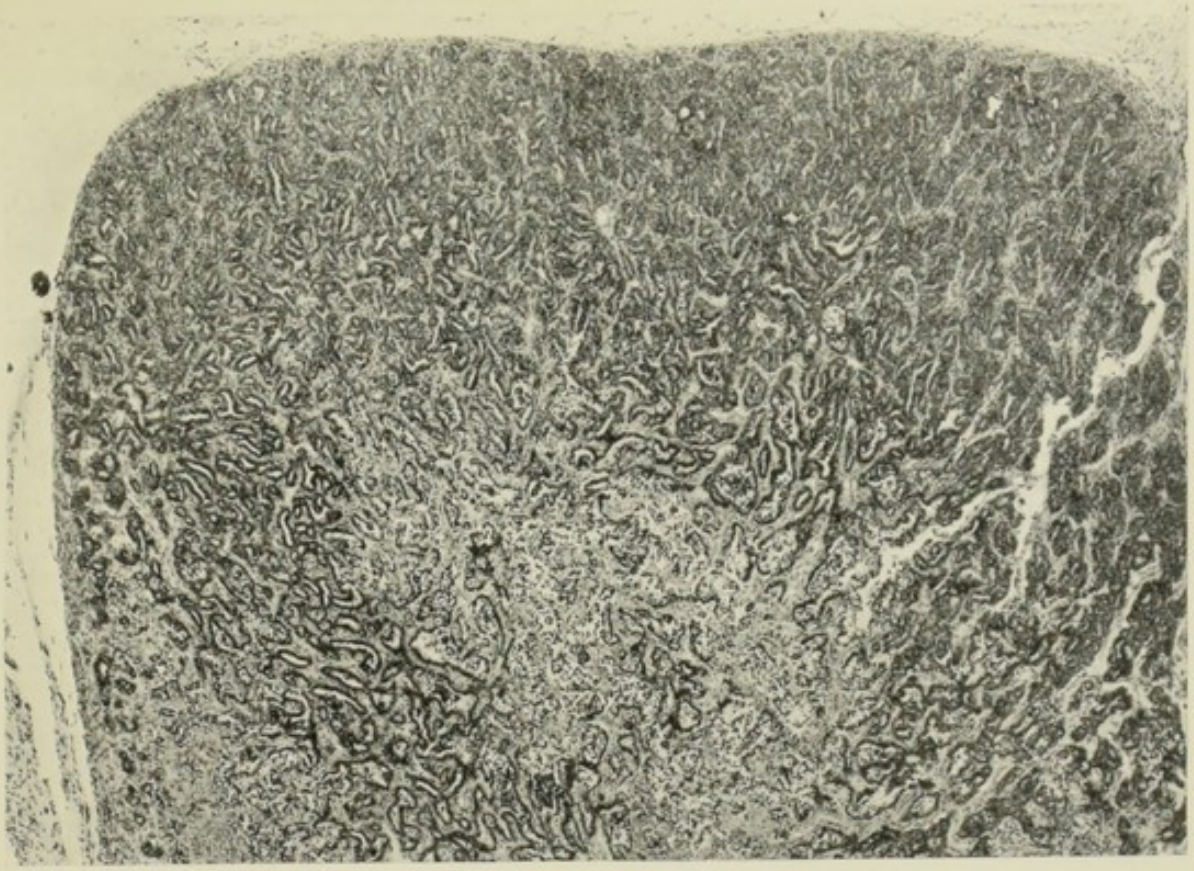
In Series 11_2Z, 7 tumours have been examined from four to six weeks after transplantation, two have been transplanted, giving series 12_2L and 12_2N, and both examined in "early stages."

All these tumours have a more abundant and cellular stroma than usual. As in the tumours of the previous generation, the central parts show marked sclerotic changes with a cellular fibrous tissue. As an illustration of these tumours we give in fig. 62 a low-power view of the tumour used to yield series 12_2L. The tumour is very necrotic and contains only a peripheral zone of living tissue. The stroma is slightly cellular and a little more abundant than usual, in the central parts it is strongly sclerotic ; but, on the whole it does not give an impression of being different from the stroma of most other carcinomatous tumours of this strain.

Fig. 61 shows a central part of the same tumour where it exhibits a typical picture of sclerosis, with slight cellularity of the fibrous tissue. Broad bands of fibrous tissue envelop the carcinomatous alveoli which are reduced to a single layer of cells, while all the rest of the alveolus is necrotic. In other parts of the tumour a cellular granulation-tissue is found on the border of the necrotic mass, demarcating the dead carcinoma cells from the living alveoli (fig. 63). The appearances of this granulation-tissue are exactly like those found in numerous other carcinomatous tumours of strain 37, as illustrated

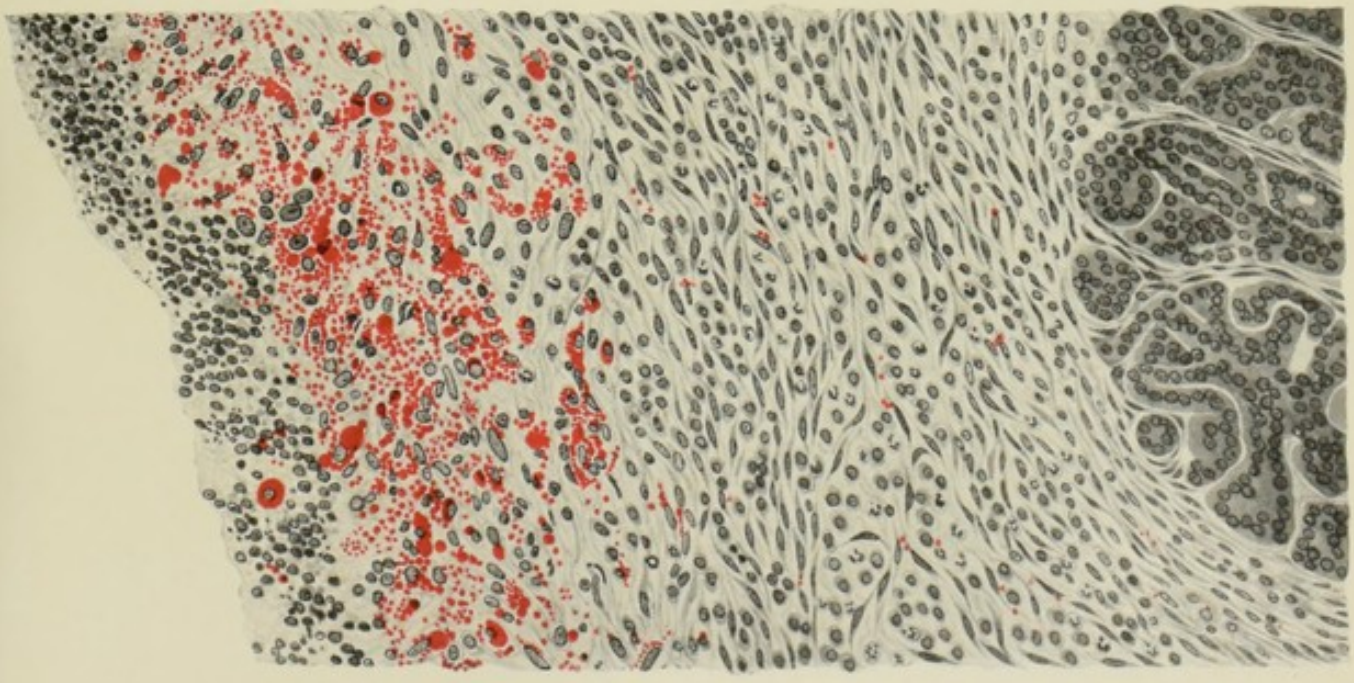
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Strain from second operation on 37/9 B.



Microphoto by W. Imboden.

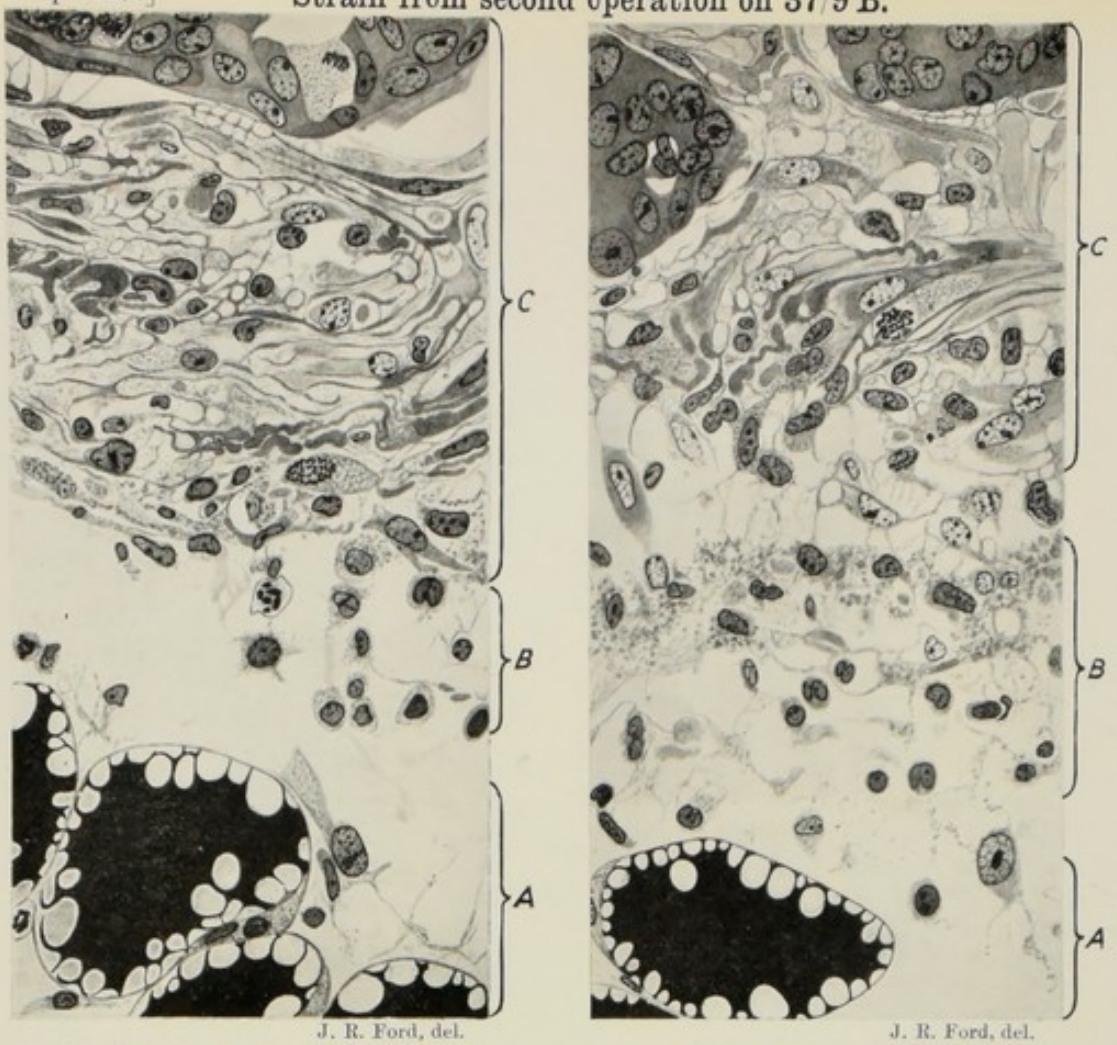
FIG. 62.—37/11$_2$Z—12$_2$L. Tumour (41 days old) from second passage of this strain, directly descended from tumour of fig. 59. The cellularity of the stroma is not more marked than in the direct antecedents (figs. 59 and 44) of this tumour. Zone surrounding centre markedly scelerotic (vide fig. 61). $\times \frac{50}{1}$.



J. R. Ford, del.

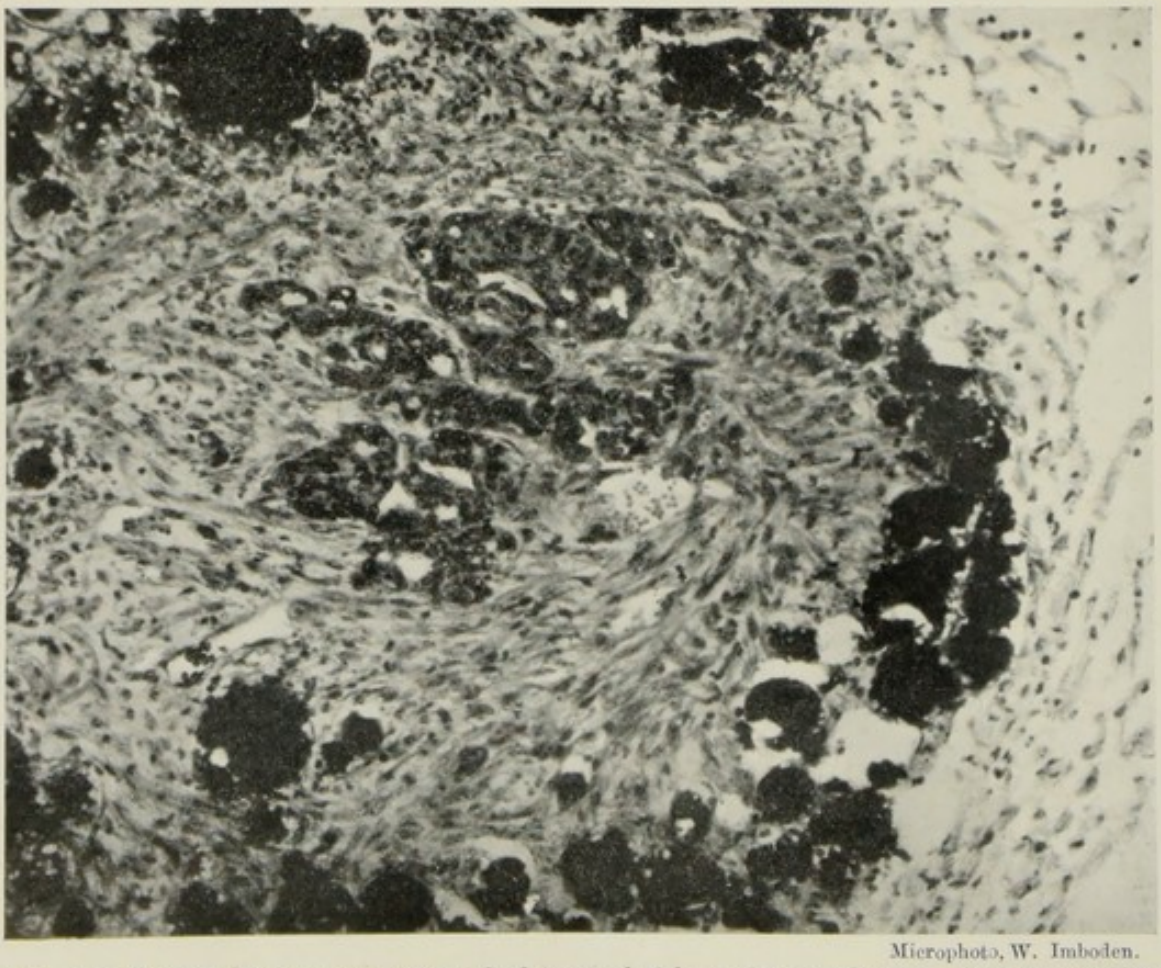
FIG. 63.—37/11$_2$Z—12$_2$L. Same tumour as above. Sector showing cellular granulation tissue surrounding the central necrotic mass (left), adjoining which, cells show marked fatty degeneration (sudan staining). $\times \frac{252}{1}$.

Strain from second operation on 37/9 B.



J. R. Ford, del. J. R. Ford, del.

FIG. 64.—37/11$_2$ Z—12$_2$ L. Same material as shown in figs. 62-63 examined in early stages. Two different sectors of grafts, preserved 24 hours after transplantation. A = tissues of host. B = cleft containing exudate, &c. C = graft containing large number of living connective tissue cells, two of which are dividing mitotically. $\times\frac{500}{1}$.

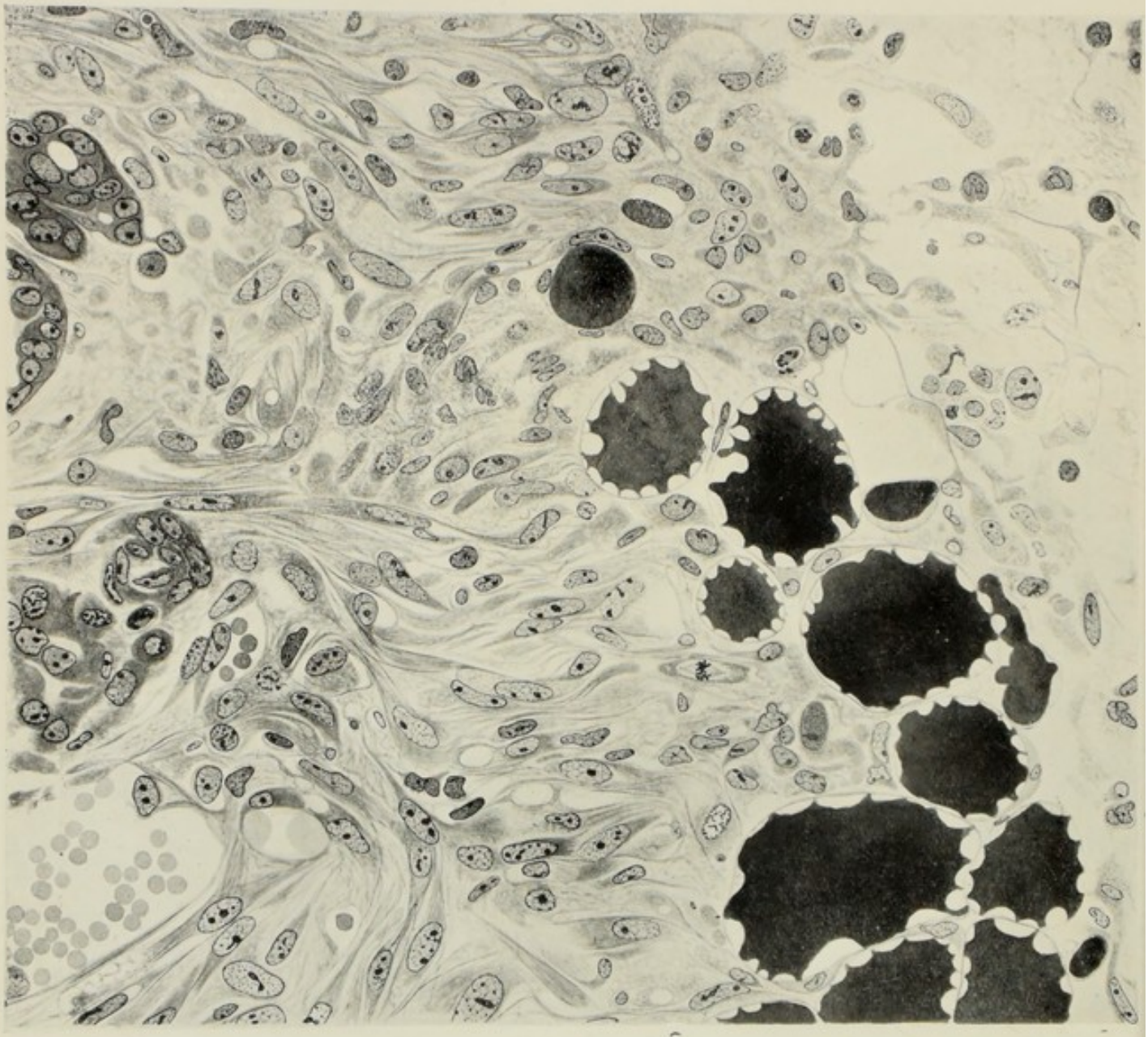


Microphots, W. Imboden.

FIG. 65.—37/11$_2$ Z—12$_2$ L. Same tumour as above. Graft preserved 48 hours after transplantation. Graft demarcated by fat cells of host (black) within which is a broad zone of proliferating large healthy spindle cells, surrounding a central mass of carcinoma. $\times\frac{150}{1}$

To face p. 227.]

Strain from second operation on 37 9 B.



J. R. Ford, del.

FIG. 66.—$37/11_2 Z—12_2 L$. The same graft as shown in fig. 65 (preserved 48 hours after transplantation) Sector of right margin of graft drawn by higher magnification. Right side of figure tissues of host (black masses = fat); left side carcinomatous parenchyma; between them abundant spindle-cell tissue. Compare figs. 64 and 65. $\times \frac{500}{1}$.

in fig. 42. Several mitoses are seen in this tissue. In frozen sections, stained with sudan, we see a zone of large cells loaded with fat surrounding the necrotic mass, as already described above for other carcinomatous tumours : this fatty change appears in other places diffusely throughout the granulation-tissue. From the part of the tumour containing this cellular granulation-tissue and shown in fig. 63, minute fragments were picked out and inoculated for "early stage" examination.

Examination of "Early Stages" of 11_2Z, *giving* 12_2L—In the periphery of the grafts preserved after 24 hours there are as in the previous generations many healthy stroma cells, although the greater number show degenerative changes. As usual all the fibrils are swollen and hyaline. In fig. 64 we have illustrated two different places of this 24 hours material, where mitoses are to be seen in cells which undoubtedly belong to the old stroma. By careful study of the corresponding places in serial sections behind and in front of these dividing cells, we have convinced ourselves that they cannot be explained as cells invading the graft from the host or isolated carcinoma cells.

In 48 hours material we find a somewhat different picture to that of the previous generations. (1) In some places a broad zone of spindle cells surrounds the graft, and at first sight appears to be due to exaggerated fibroblast reaction, much more marked than we have seen it at so early a period in any other tumour. These appearances are shown in fig. 65, a low-power microphotograph of a graft after 48 hours; a portion of this is drawn at a higher magnification and given in fig. 66. (2) The number of healthy-looking cells belonging to the old stroma is greater than in the previous generations, where on the whole, they were very rare after 48 hours. In these grafts we also find cells surviving in the central parts of the graft, where the picture is not so much obscured by the new elements from the host, and for certain of these cells there is no evidence of their degeneration, but on the other hand we cannot prove that they are dividing.

The cellular tissue round the graft suggests a pure reaction from the host. But on examining the grafts more closely, we hardly feel justified in maintaining this view. The graft has been implanted into the fatty tissue, and the fat cells, clearly indicated as black masses (preserved in osmic acid) mark the limit of the tissues of the host. The large cells round the graft are all inside this border line, and there is a striking difference between the extremely cellular tissue immediately surrounding the graft and the tissues which with certainty can be said to belong to the host. The latter show only a very scanty reaction. The general impression of this picture is that there is no conclusive evidence of the cellular tissue round the graft being entirely derived from the surroundings as a reaction. On the other hand, we see in the 24 hours material (fig. 64), that stroma cells are dividing close to the margin of the graft. It is a natural suggestion that these cells dividing in the introduced graft might be the real source for the cellular tissue we find in 48 hours material. Moreover, the further observation of the daughter tumours derived from this same material gives

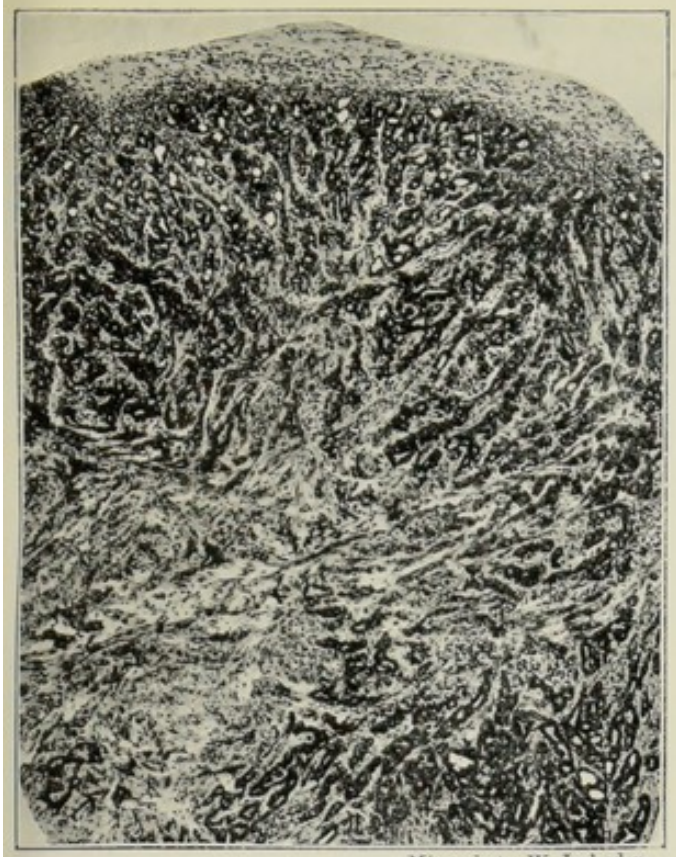
us strong evidence that the latter supposition is the more probable, for, in the next generation there is no doubt that the stroma elements really survive and independently proliferate, as we shall show further on.

The Series 12_2L to which this material gave rise yielded a very low percentage ; only one tumour was obtained out of 17 mice inoculated, *i. e.* 7 per cent. This tumour was operated upon nineteen days after inoculation. As the preliminary examination of frozen sections showed remarkable changes in the stroma, the material removed was inoculated into 40 mice (series 13 Y), and in addition 30 mice were inoculated specially for "early stages."

The removed tumour showed, in about one half of the section, the picture of an ordinary carcinoma with a somewhat increased amount of stroma in the central part. The other half is shown at a low magnification in fig. 67, and the centre again with a higher magnification in fig. 68. Between the carcinoma alveoli there have developed strands of a very cellular tissue consisting of large spindle shaped elements showing numerous mitoses. The carcinomatous alveoli seem to recede from the centre of the graft, so that it consists almost solely of spindle cell tissue with strong sclerotic fibres, intermingled with small necrotic areas.

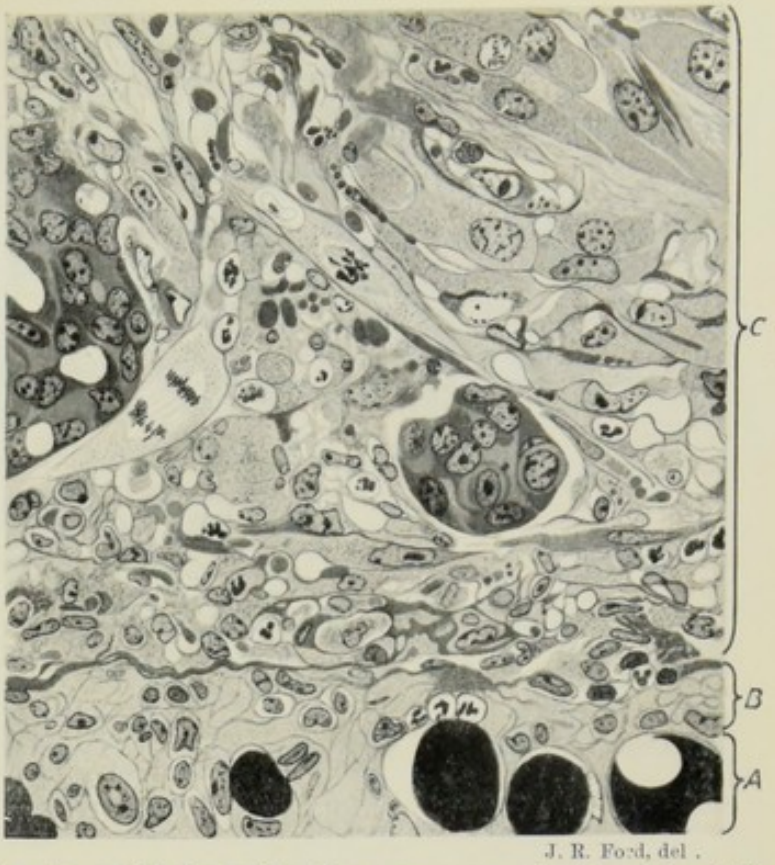
On examining this material in early stages we found that the majority of the large spindle cells remained alive, alongside of the carcinoma cells ; but the fibres of the connective tissue are swollen and degenerated. Fig. 69 shows the appearance of this material 24 hours after inoculation. Numerous mitoses are to be seen in the large cells which have replaced the old stroma. Cells and nuclei of the most extraordinary sizes and shapes are met with, and pathological mitoses are frequently found. In the sarcomatous cells fine fibroglia fibres are present.

The animal, in which a sarcomatous change could first be recognised definitely, was not sacrificed to obtain the material for inoculation. Its tumour was removed by operation, and then we re-inoculated the mouse in the left axilla with a pure carcinomatous tumour (18 E) in order to make quite sure, whether or not peculiarities of the connective tissue of this particular mouse had played a part in the appearance of the sarcoma. The second inoculation gave rise to a tumour and at the same time a huge recurrence developed in the right axilla. The mouse was killed 18 days after the second inoculation. The second tumour had then reached the size of a pea ; microscopically it presents the usual picture of an adeno-carcinoma with extremely scanty



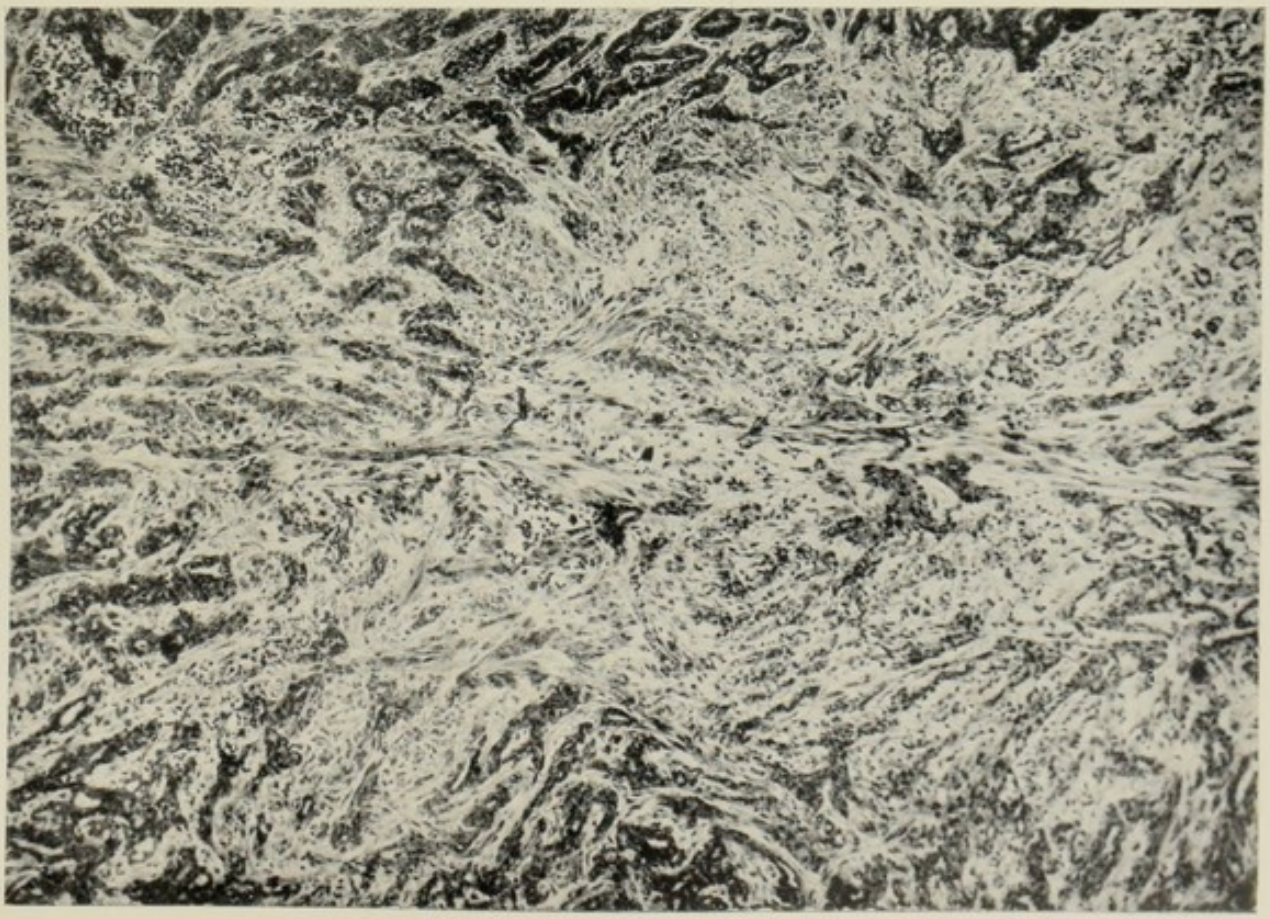
Microphoto, W. Imboden.

FIG. 67.—37/12$_2$ L—13 Y. Tumour (19 days old) from third passage of this strain, directly descended from tumour of fig. 62 (figs. 61–63). Sarcomatous change similar to that in fig. 54. In the centre of the growth strands of large spindle-cells. Periphery to the top of figure. $\times\frac{45}{1}$.



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FIG. 69.—37/12$_2$ L—13 Y. Same tumour as in figs. 68 and 69, examined in early stages. Graft, preserved 24 hours after transplantation. A=tissues of host; B=cleft containing exudate; C=graft. The connective tissue cells of the graft show themselves capable of surviving transplantation and are dividing. Note size of connective tissue cells in this graft as compared with those of carcinomatous tumours (figs. 9 & 10). $\times\frac{500}{1}$.

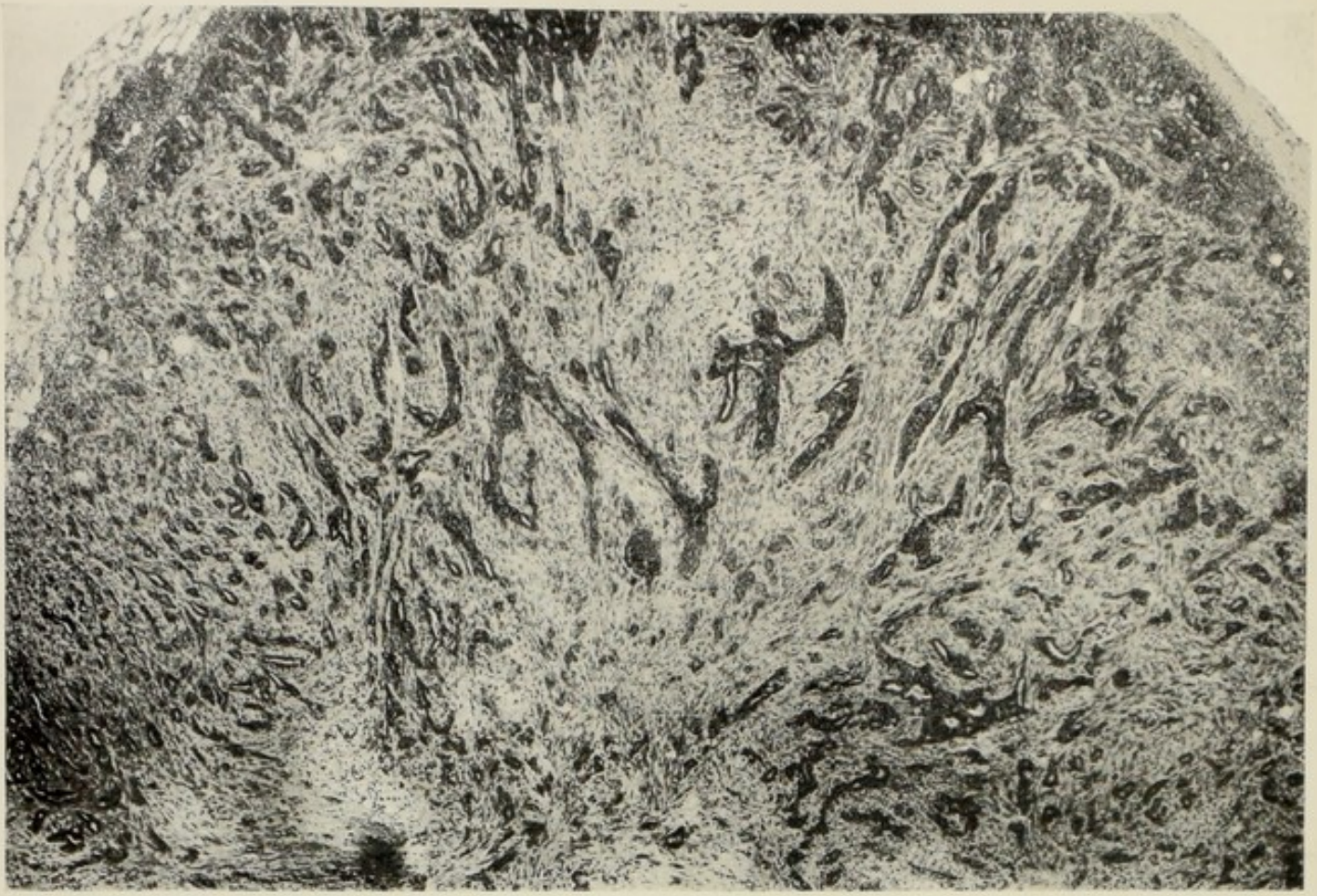


Microphoto, W. Imboden.

FIG. 68.—37/12$_2$ L—13 Y. Central parts of above figure (fig. 67) with higher magnification to show the large spindle cells widely separating the carcinoma-alveoli. Scattered areas of necrosis. $\times\frac{100}{1}$.

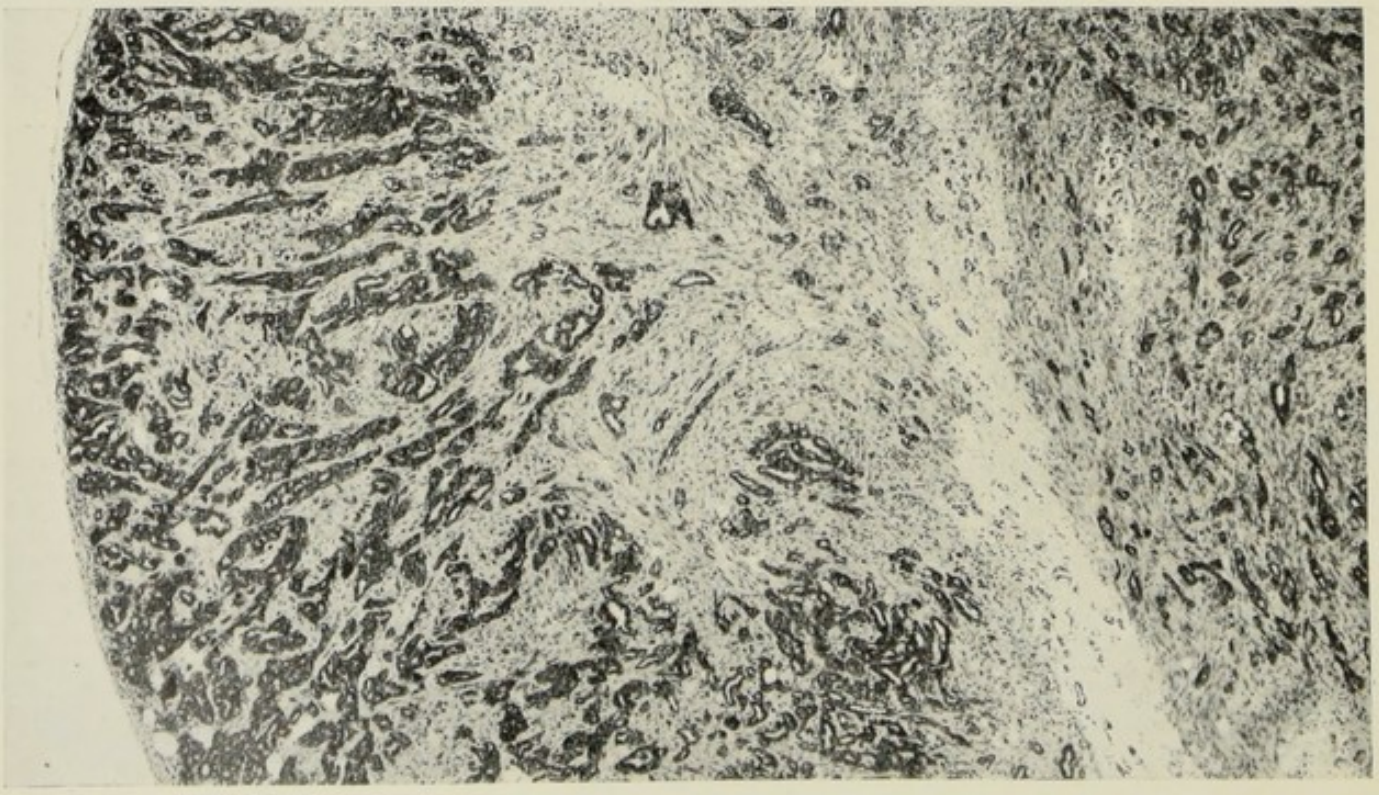
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Strain from second operation on 37/9 B.



Microphoto, W. Imboden.

FIG. 70.—37/13 Y—14 T. Tumour (23 days old) from fourth passage of this strain, directly descended from tumour of figs. 67 & 68. The sarcomatous change has progressed. Broad strands of large spindle-cells separate the carcinomatous alveoli. The change progresses from the centre to the periphery of the growth (upper part of fig.). $\times \frac{55}{1}$.



Microphoto, W. Imboden.

FIG. 71.—37/13 Y—14 T. Another area of the same tumour as above, showing further advance of the process in centre of the growth than in peripheral parts (left). $\times \frac{55}{1}$.

stroma. The reaction between the carcinoma alveoli and round the periphery of the growth does not differ at all from that seen in every normal animal.

In the Series 13 Y, arisen from the above material, there were 16 tumours in 36 mice surviving, *i. e.* a percentage of 40 per cent. Ten tumours have been examined up to the present and four have been transplanted, two of the latter have been examined in early stages.

We have mentioned that the change was pronounced only in one half of the tumour while the other half showed only a slight increase of the connective tissue. The tumour was not minced down and injected with syringe but small fragments of it were introduced through a needle. It is, therefore, hardly surprising to find that the change which was rather localised in the mother-tumour, is progressing quite irregularly in the daughter-tumours. In one tumour used to yield 14 S, the carcinomatous character prevails and only in the central part are long spindle cells like those in 13 Y to be seen. In another mouse we find the tumour consisting of two distinct parts with different histological pictures, the one is a carcinoma with sclerotic stroma showing, however, strands of large spindle cells here and there, the other is a typical mixed tumour with broad bands of spindle cells between the alveoli.

The other daughter-tumours from this series all show more advanced sarcomatous changes. Two parts of the tumour used to yield 14 T are shown in the microphotographs figs. 70 and 71. The strands of large spindle cells between the carcinomatous alveoli have become broader, and increase in breadth towards the centre of the growth, from which the carcinomatous elements have almost disappeared. In this respect the sarcomatous changes show resemblances to the sclerotic process in old carcinomatous tumours.

The subtransplantations from 14 T (Series 15 V and 15 X) all show the development of the sarcomatous tissue progressing, and supplanting the carcinomatous elements by slow degrees. As prototype for this stage fig. 72 shows one of the tumours of 14 T (used to yield 15 V). Along with the spindle cells of the sarcomatous tissue we find a good number of polymorphous elements.

In the next generation (a tumour of Series 15 X yielding Series 16 S has been chosen as prototype) a marked polymorphous celled mixed tumour is found with halo formation round the carcinoma alveoli (fig. 73). With the appearance of halos we have again arrived at that advanced

stage of the process, already described for both our two earlier cases, and also for the development of sarcoma observed in the parallel strain from the first operation of 9 B. A comparison between the strains from the first and second operations of 9 B shows that the process has advanced more rapidly in the first (through 13_2A) than in the second (through 13 Y). In the former, the first stage of the process, characterised by strands of spindle shaped elements between the carcinoma alveoli, lasted only during one generation (about one month), and the typical polymorphous cell stage with halo formation was reached in the second passage, and in the latter only after three months (three passages). Up to the present we have not arrived at the final stage, that of a pure sarcoma, for these two strains.

In examining early stages from this material we obtain the same pictures as shown in fig. 14 from our first case of sarcoma development. *The stroma cells are growing independently in large numbers, showing mitoses already after* 12 *to* 24 *hours.* Their polymorphism is quite extraordinary, giant nuclei, multipolar mitoses, hyperchromatic and hypochromatic nuclei are frequent.

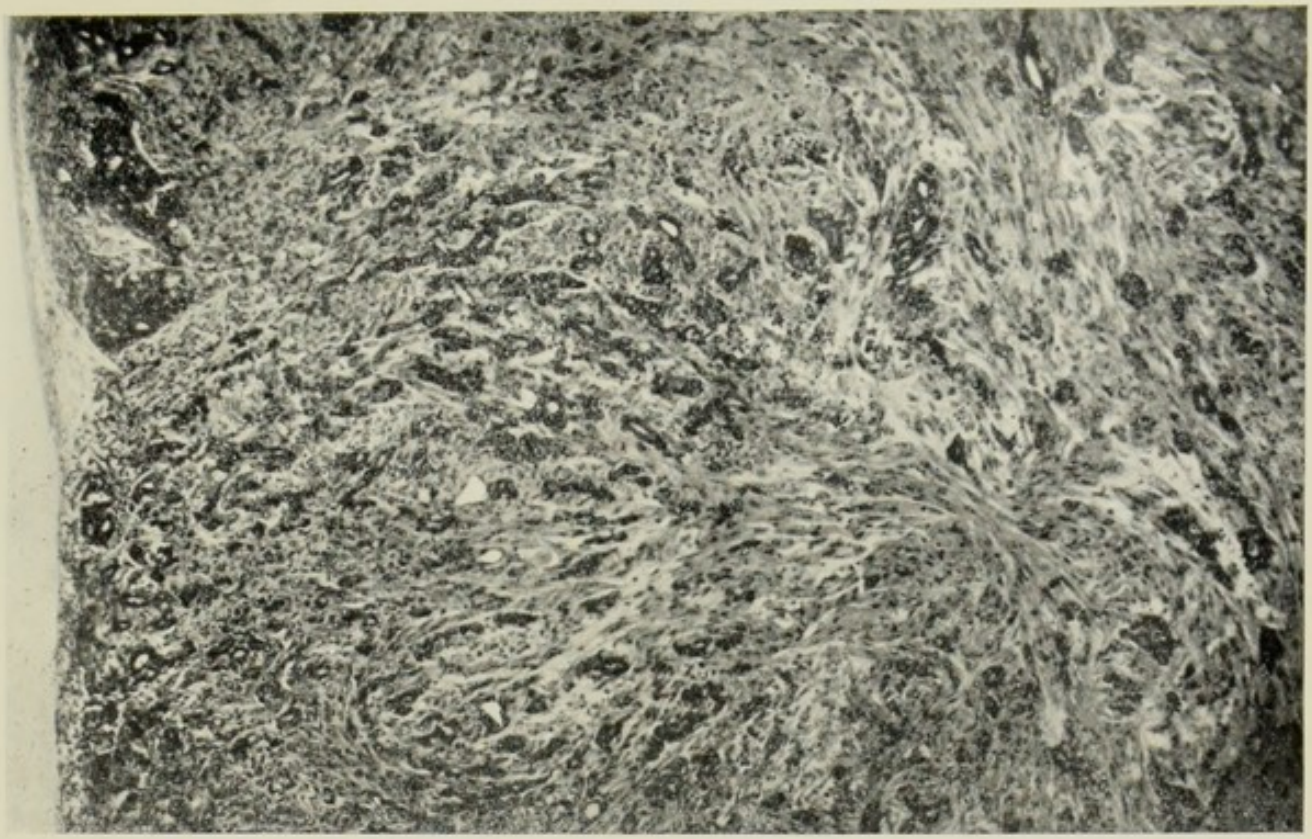
In illustration of the difficulty, already mentioned for our earlier cases, of always easily distinguishing the carcinomatous from the sarcomatous cells, fig. 74 shows part of a mixed tumour. To the left of the figure the acinous parenchyma stains darkly, and passing to the right it is continuous with elongated compressed alveolar masses. The sarcomatous interstitial tissue can be distinguished easily from the darkly stained acinous parenchyma. However, the contours of the lightly stained alveolar masses are not sharp and appear in places as if split up by interdigitating bundles of elongated spindle shaped interstitial cells. Even under high magnification it is frequently impossible to decide from the histological characters whether individual cells belong to the interstitial tissue or to the parenchyma.

Fig. 75 illustrates a similar condition under a higher magnification. The elongation and separation of the peripheral cells of the lightly stained carcinoma alveoli are clearly seen. The elements are in many places practically indistinguishable from sarcoma cells. Apolant apparently refers to this condition in a recent paper to the German Pathological Society at Kiel *. As noted above (p. 196) there can be no question of the origin of the sarcomatous tissue from transformed carcinoma cells. Carcinoma cells may assume altered staining reactions and spindle

* *Loc. cit.* p. 254.

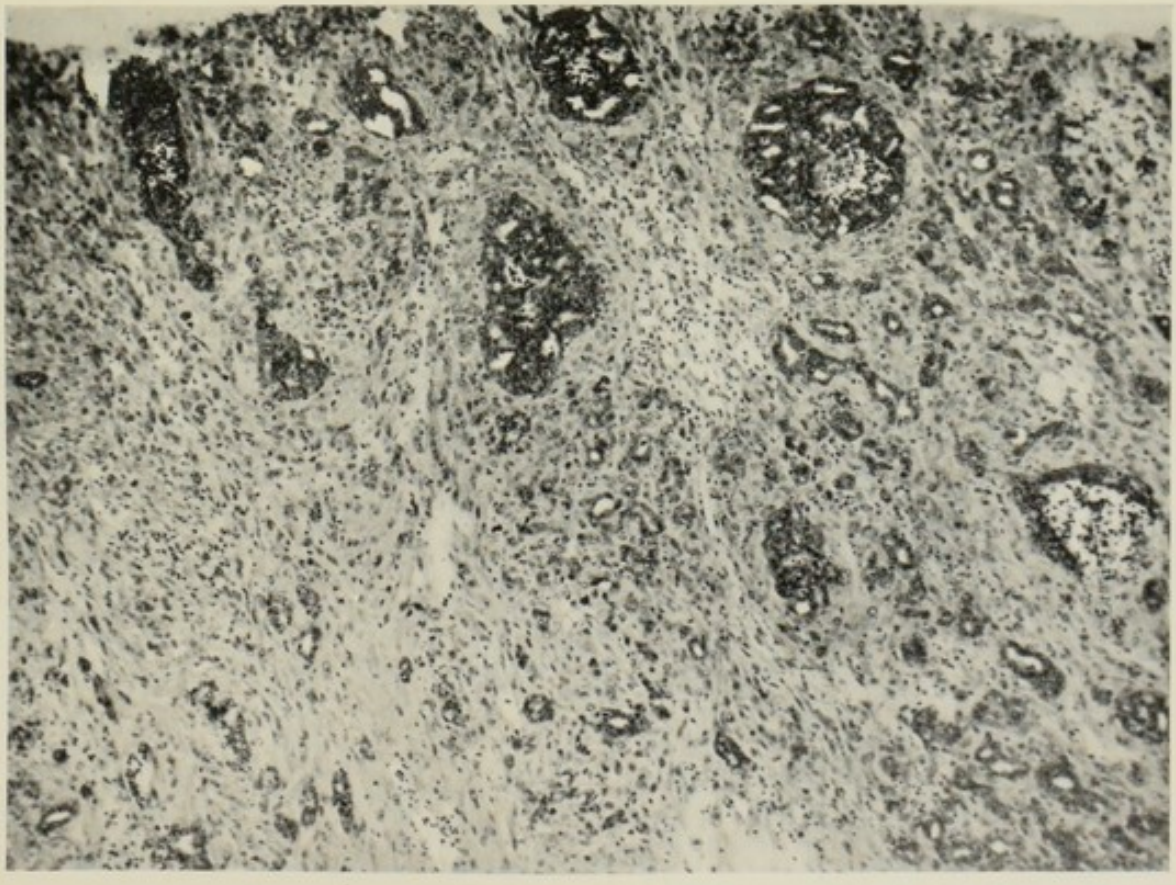
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Strain from second operation on 37/9 B.



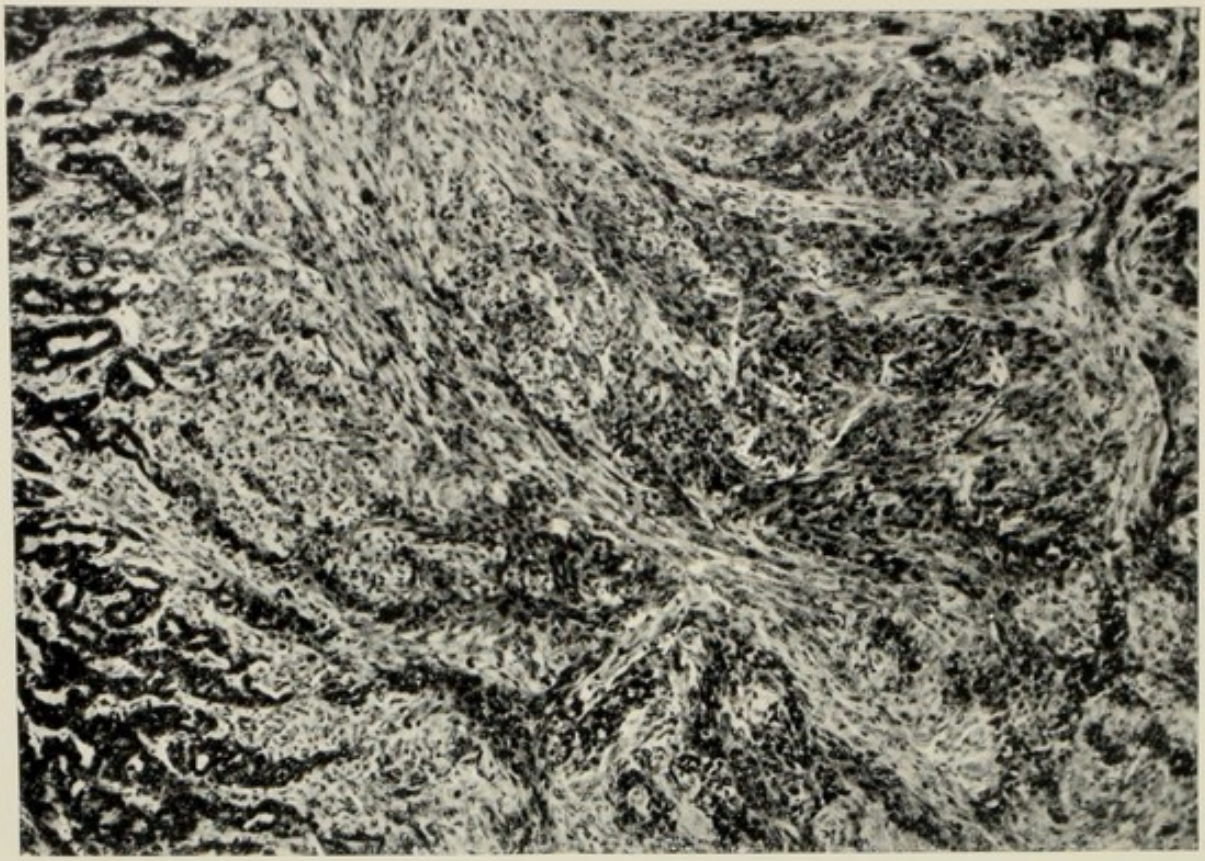
Microphoto, W. Imboden.

FIG. 72.—37/14 T—15 V. Tumour (27 days old) from fifth passage of this strain, directly descended from tumour of figs. 70 & 71. The elements of the interstitial sarcomatous tissue retain still their spindle shape, but polymorphous elements are also found. Periphery to the left. $\times \frac{80}{1}$.



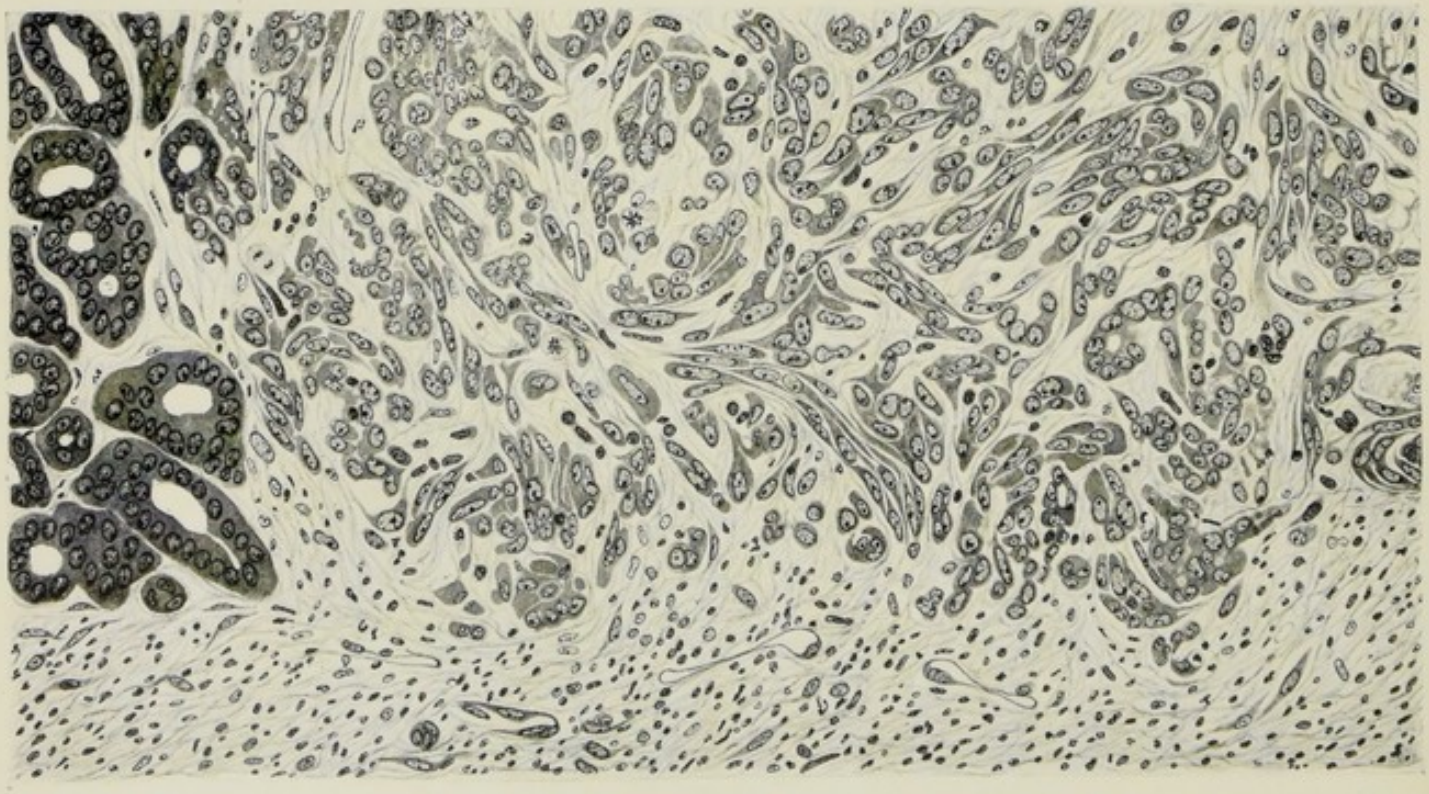
Microphoto, W. Imboden.

FIG. 73.—37/15 X—16 S. Tumour (27 days old) from sixth passage of this strain. Polymorph celled stage with halo-formation, *cf.* figs. 23–26 and fig. 57. The carcinoma-alveoli show commencing central degeneration. Periphery of tumour at top. $\times \frac{80}{1}$.



Microphoto. W. Imboden.

FIG. 74.—37/13 C—14 W. Mixed tumour (24 days old). To the left darkly stained acinous parenchyma, easily distinguished from sarcomatous connective tissue. To the right lightly stained alveolar parenchyma, peripherally split up by sarcomatous tissue. $\times \frac{100}{1}$.



J.R. Ford, del.

Fig. 75.—37/16 Q—17 J. To the left darkly stained acinous parenchyma, to the right the cells of the parenchyma are more lightly stained and present a spindle cell form; in the latter part the parenchyma is more irregularly arranged and also split up by connective tissue. $\times \frac{180}{1}$.

form apart from sarcoma development, and the association of this condition of the parenchyma with late as well as early stages of mixed tumours is not an initial and essential phase in the development of sarcoma.

Tumours in the mixed stage may occasionally give carcinomatous daughter-tumours on further propagation. So long as the sarcomatous change remains localised in the mother-material there is nothing surprising in the fact, because obviously the whole of one graft may be taken from the less altered portions. In other cases this reversion cannot be so explained, *e. g.* in series 16 R the sarcomatous change advanced progressively through three generations and then one of the daughter-tumours showed a reversion to carcinomatous structure, four others showing very advanced sarcomatous changes. The mother-material of these five tumours exhibited a very uniform distribution of the sarcomatous change, large epithelial masses being absent, and it is very unlikely that any such could have been accidentally picked out for inoculation.

Strain from 3rd Operation.

9 B, 3rd operation——10_2Y——11_3J——12_2S——**13_2E——14 Z.**
(Fig. 45) (Fig. 76) (Fig. 77) (Fig. 78) (Fig. 79)

Propagation of material removed at third operation of 9 B.

Carcinoma up to 12_2S; sarcomatous change in 12_2S-13_2E.
Later, mixed-tumour stage.

The tumour removed at the third operation shows a histological picture identical with those seen at the previous operations. A somewhat more abundant and slightly cellular stroma is the most striking feature as shown in the photograph, fig. 45, from a peripheral part of the tumour.

Examination of Early Stages of 9 B *giving* 10_2Y.—The same difficulties are met with as have been mentioned for the material from the earlier operations. A number of the introduced stroma-cells remain alive for the first 24 hours without mitoses being found in them. 48 hours material has not been preserved from this tumour. In the three days material we find the graft vascularised and numerous dividing cells now appear between the carcinomatous alveoli. This material is distinguished from other tumours examined at the same stage by the fact that the proliferating cells appear not only round the surface of the graft, whereby they can be more easily recognised as reaction tissues from the host invading the graft, but also they are far more uniformly distributed in the interior of the graft. Most of them seem to be derived from wandered-in cells, but the possibility remains that a certain number may

be elements from the old stroma. We see numerous cells corresponding in their position to the old stroma-cells, with degenerated collagenous fibrils applied to their surface and sometimes with fatty granules in their protoplasm, and we can hardly believe them to be cells that have wandered in from the host.

In the four days material we meet with a very cellular young proliferating tissue between the carcinomatous alveoli more abundant than we have as yet seen it in the case of other carcinomata at a correspondingly early stage. In reality this four days material suggests a mixed tumour. As to the origin of the interstitial tissue it is hardly possible to bring definite proof either for the one or the other of two possibilities: whether it is to be considered only as a reaction, stronger by far than usual, or whether, besides this reaction which certainly takes place, certain elements of the old stroma survive and join in the formation of the new stroma.

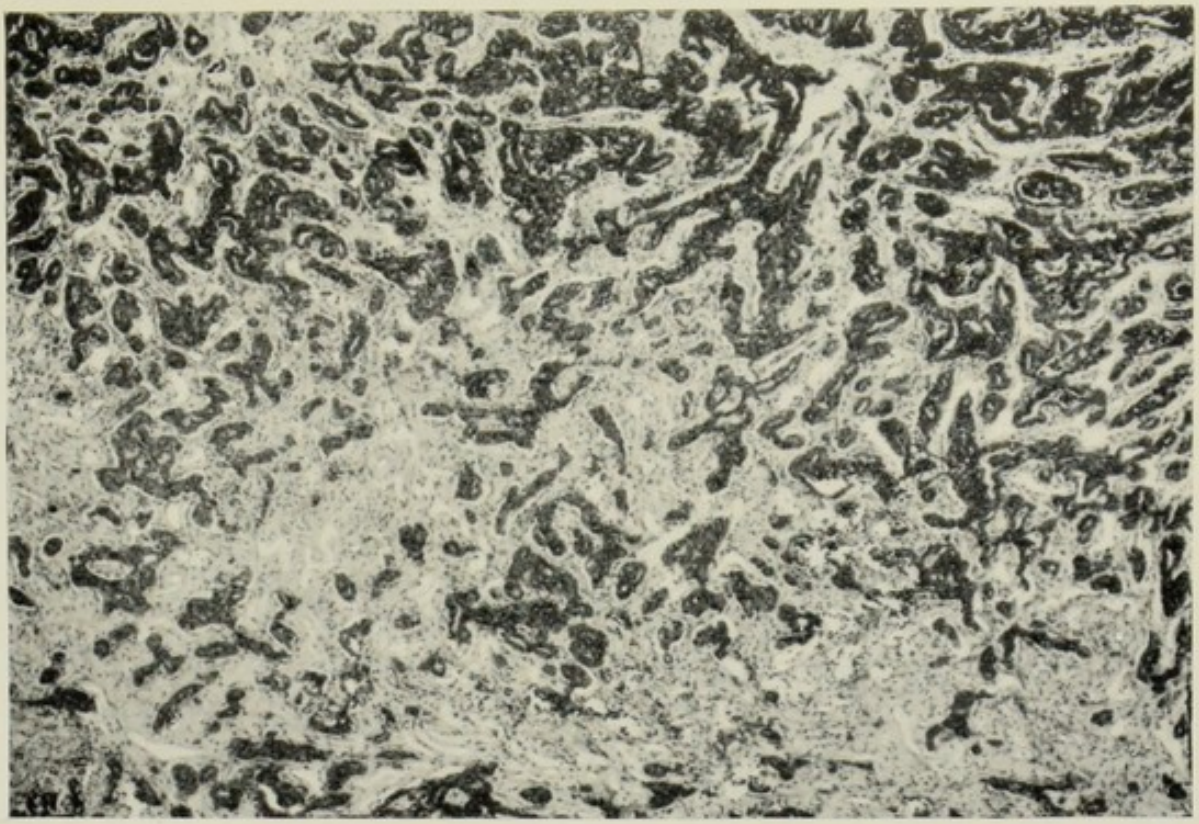
In Series 10_2Y six tumours were obtained. All have been examined and four of them transplanted and examined in early stages. All these tumours show the same characteristic features, viz., a greater abundance of cellular connective-tissue and in the centre of the tumours marked sclerotic changes. To illustrate the tumours of this group we give the most cellular central part of the tumour used to yield Series 11_3J (fig. 76). The abundance and cellularity of the stroma are in this part very striking, but it is nevertheless still easily distinguishable from sarcomatous tissue.

Examination of Early Stages 10_2Y *giving* 11_3J.—After 24 hours the degenerative changes both of parenchyma and of stroma are very marked in the two grafts examined. 48 hours material shows the same degenerative changes more marked still, and only a few parenchyma cells can with certainty be said to be living. In three days material re-vascularisation is in an advanced stage, numerous connective-tissue cells are dividing, and new capillaries appear between the surviving carcinomatous alveoli; here and there a cell can be distinguished crowded with fatty granules while the nucleus still stains well. In four days material the re-organisation is more advanced in the peripheral part; the central part shows necrosis, and close up to it single cells can still be recognised as belonging to the old stroma, mostly crowded with fatty granules.

It is hardly possible to draw any conclusion from this examination of early stages. Most of the old stroma-elements seem to have degenerated and have been replaced by new elements.

In Series 11_3J, only two tumours were obtained in 18 mice, *i. e.* 11 per cent. The histological picture is that of an adeno-carcinoma with a somewhat abundant and cellular stroma in the central part. The photograph (fig. 77) shows a truncated sector from the periphery to the necrotic centre of the only tumour transplanted. In this particular part we see a greater number of large spindle-cells in the interstitial

Strain from third operation of 37/9 B.



Microphoto, W. Imboden.

FIG. 76.—37/10 Y—11_3 J. Tumour (32 days old) from first passage of this strain, directly descended from tumour of fig. 45. Central part of tumour with increased sclerotic and celullar stroma. $\times \frac{60}{1}$.

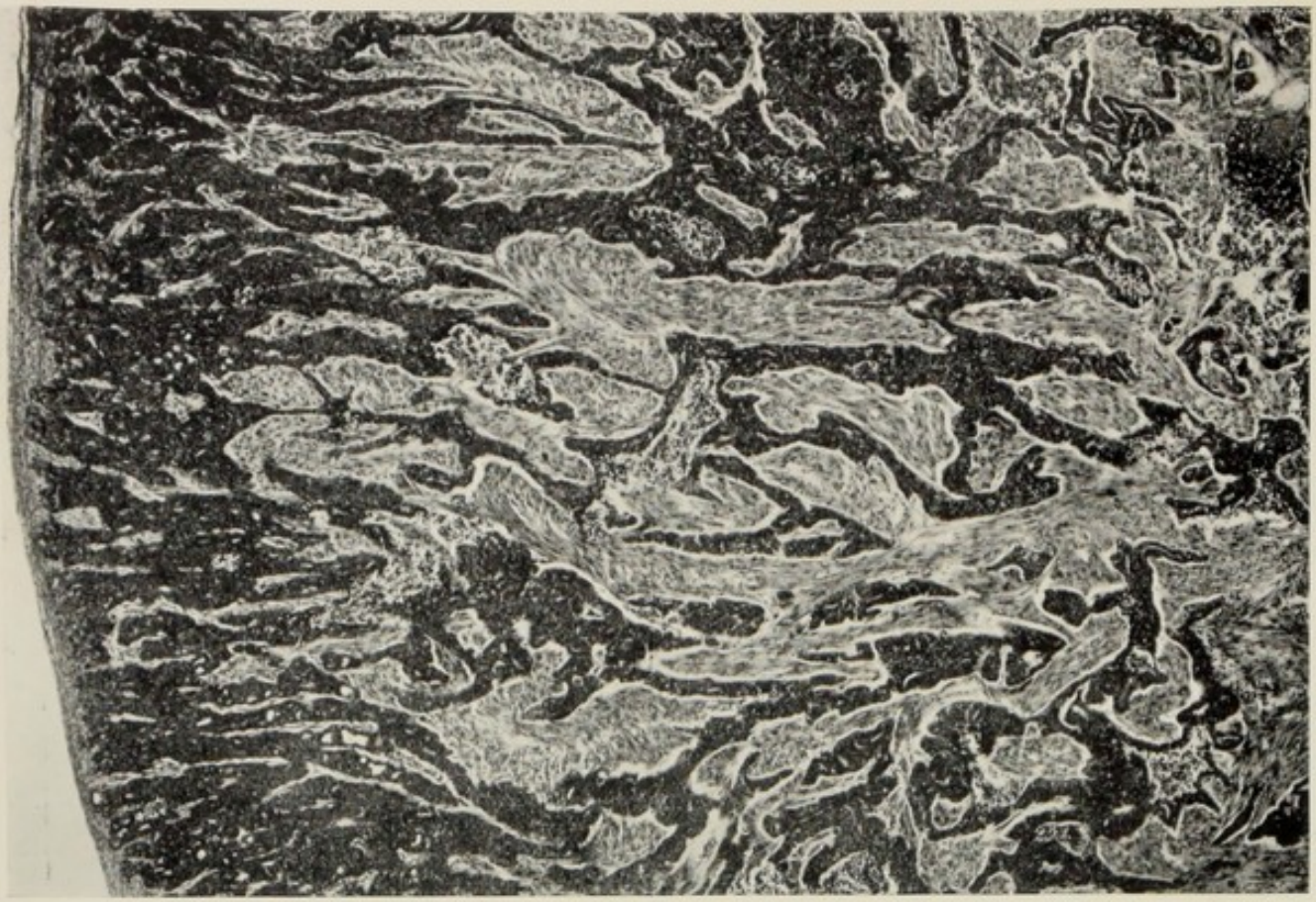


Microphoto, W. Imboden.

FIG. 77.—37/11_3 J—12_2 S. Tumour (27 days old) from second passage of this strain, directly descended from tumour of fig. 76. Sector of growth (periphery left) showing an area with increased amount of stroma consisting of large spindle cells. $\times \frac{70}{1}$.

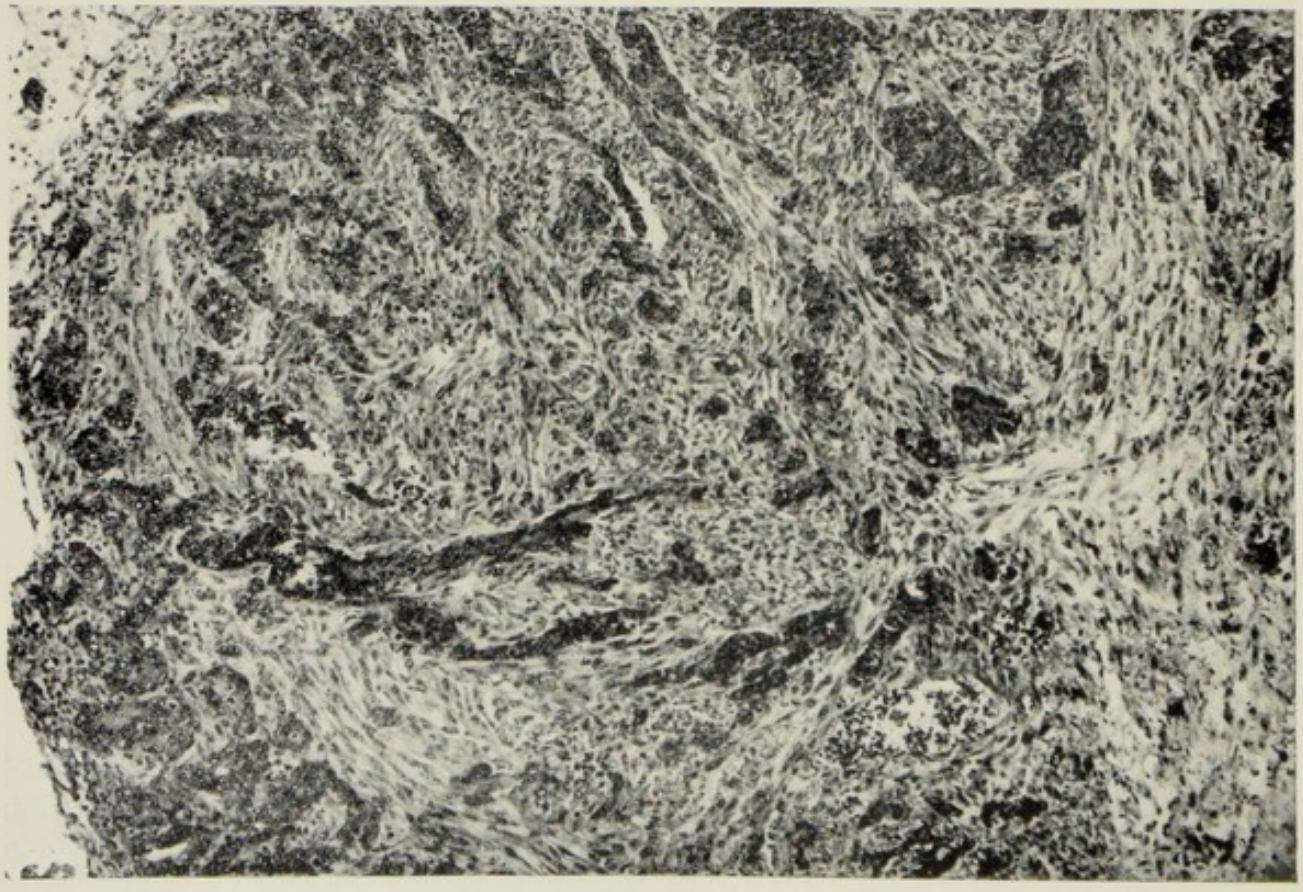
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Strain from third operation of 37/9 B.



Microphoto, W. Imboden.

FIG. 78.—37/12$_2$ S—13$_2$ E. Tumour (45 days old) from third passage of this strain directly descended from tumour of fig. 77. "Mixed tumour" stage. Sarcomatous tissue consisting of large spindle cells. Change further advanced in centre, progressing towards the periphery (left). $\times \frac{50}{1}$.



Microphoto, W. Imboden.

FIG. 79.—37/13$_2$ E—14 Z. Tumour (24 days old) from fourth passage of this strain directly descended from tumour of fig. 78. Later "mixed tumour" stage, showing commencing polymorphism of sarcoma cells. Centre of tumour already almost entirely sarcomatous. *Cf.* corresponding stage in fig. 72. $\times \frac{100}{1}$.

tissue. The changes are quite localised, and the rest of the tumour does not show any noteworthy change.

Examination of Early Stages 11_3J *giving* 12_2S.—The corresponding part of the section to that showing the suspicious stroma was used for early stages, but we fail to recognise any more certain signs of independent growth of the stroma elements, than was the case in the previous tumours. The same difficulties are met with as in other tumours from 9 B; on the one hand we cannot prove definitely that introduced stroma-cells proliferate, on the other hand they do not degenerate completely before the reaction from the host obscures the picture. The material shows on the whole very marked degenerative changes, both of the stroma and of the parenchyma.

The Series 12_2S showed only one tumour out of 13 mice, *i.e.* 7 per cent. The downward tendency of the percentage-curve which the mother-material shows is maintained. Our earlier cases of sarcoma development appeared in series of relatively low percentages ; therefore we anticipated a change, and all the more so because of the suspicious features of the mother-material. Our expectations were more than realised, for the single daughter-tumour exhibits a much more advanced sarcomatous change than the tumours from corresponding stages of the two earlier 9 B strains. As fig. 78 shows, broad strands of large connective tissue elements of pronounced spindle shape separate the carcinomatous alveoli. As in the previous cases, the change seems to start from the centre, where the alteration is most advanced, and from there proceeds towards the periphery of the tumour. In this case also the change is rather localised, being more pronounced in the middle of the elongated tumour while both ends do not show the same degree of alteration. The tumour was transplanted (giving 13_2E) and examined in early stages.

Examination of "Early Stages" of 12_2S, *giving* 13_2E.—The picture is essentially similar to that given in fig. 69. There is no doubt that these connective-tissue elements are capable of further growth. But although as a whole they survive much better and show the reaction of repair more rapidly than the cells of the stroma in the previous generation did, the difference seems to be less a fundamental one than a question of degree. In this sarcomatous material a number of cells show degenerative changes, and only a part of the introduced stroma-cells survive, perhaps even only a small number of individual cells. The reaction from the host is the same as in the carcinomatous tumours ; the budding capillaries and dividing-cells invading the graft from the surroundings place the same difficulties in the way of recognising the elements of the old stroma where they are not actively proliferating shortly after inoculation.

The next generation is shown in fig. 79, a microphotograph from the periphery of a tumour of 13_2E (used to yield 14 Z). The sarco-

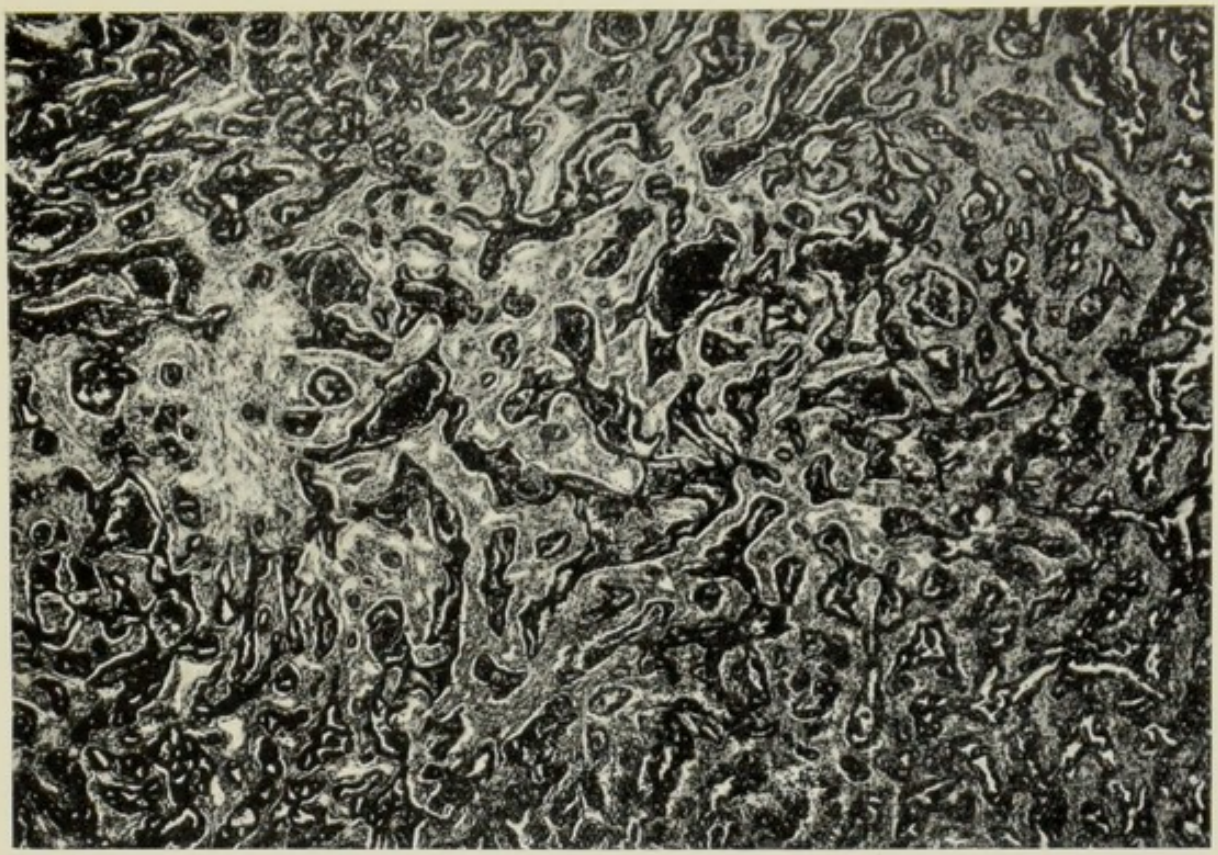
matous change has progressed. The sarcomatous tissue consists mostly of spindle-shaped elements, but a commencing polymorphism is already to be seen. This strain has not been followed any further up to the present. The material examined in early stages shows independent growth of the sarcoma-cells as we have already described it for our earlier cases (*cf.* fig. 14).

The strain through 11_3J and 12_2S which has given the case of sarcoma development, just mentioned, is only one of the strains from the 3rd operation. As will be seen from the genealogical tree, there are other parallel strains, the stroma of which exhibits interesting features, without, however, showing sarcomatous change up to the present. It will not be possible to follow these side-lines in detail without making the description too complicated. We will therefore content ourselves with giving photographs of two tumours from a parallel series of 12_2S, in order to show that the change preceding the sarcomatous alteration, is not necessarily a phenomenon confined to one strain only, while the sister tumours offer quite normal conditions. Figs. 80 and 81 represent two daughter-tumours from Series 11_3H (a sister series to 11_3J, see genealogical tree), both transplanted (giving 12_2T and 12_2Z respectively). In both figures the amount of connective tissue present in the centre of the tumours is much increased, but the elements are very much smaller in size than is the case where we have a pronounced sarcoma development. This fact will be clear when the nuclei in the stroma in fig. 80 are compared with the nuclei in the cellular interstitial tissue of figs. 70, 71, or 78 taken with the same magnification.

In spite of the increased amount of stroma and its cellularity, we hesitate to call these growths mixed tumours. Firstly, the whole appearance of the stroma is that of supporting structures, as we find them in the sclerotic parts of all old tumours of this strain. This is best shown by a higher magnification, as in fig. 81 (100 diam.). We find here a somewhat similar distribution of the stroma elements as in fibro-adenoma mammæ in the human subject. Secondly, in early stages we cannot prove that this stroma is growing after transplantation, in a way which disposes of all doubts. The mere presence of a cellular interstitial tissue in carcinomata is not conclusive evidence of sarcomatous change, and it may be found accompanying various processes, especially those connected with spontaneous absorbtion of tumour and the revascularisation of necrotic areas. Even if we admit that individual cells of this abundant stroma remain alive after transplantation, their power of growth is not above that of normal cells and we are not justified in calling

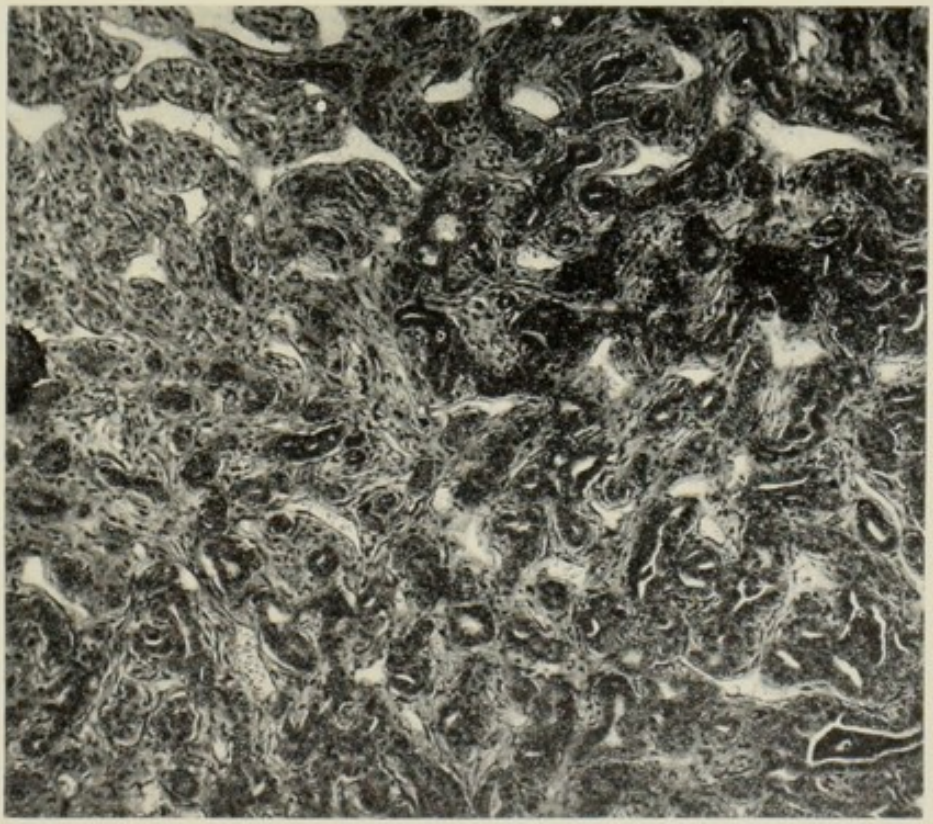
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Strain from third operation of 37/9 B.



Microphoto, W. Imboden.

FIG. 80.—$37/11_3$ H—12_4 Z. Tumour (52 days old) from second passage of this strain descended through a side branch from series $37/10_2$ Y, corresponding to the tumour of fig. 77. Central portion of growth with abundant and cellular stroma. In the descendants of this tumour the stroma changes have not progressed as in the series derived from $37/11_3$ J (figs. 76–79). $\times \frac{55}{1}$.



Microphoto, W. Imboden.

FIG. 81.—$37/11_3$ H—12_2 T. Another tumour (39 days old) from second passage of this strain; same side branch as tumour of fig. 80. Shows the arrangement of the central stroma in concentric bundles round the carcinoma alveoli. Blood vessels dilated. $\times \frac{100}{1}$.

them sarcomatous elements. Only where we find that the elements of this cellular tissue have acquired a higher energy of growth, not only in the same animal, but also after transference into new animals, are we justified in speaking of sarcomatous elements in transplanted tumours.

In the next generation most of the daughter-tumours show the same amount of connective tissue, but do not show any further advance towards sarcoma development. Whether the process will advance here as in other cases can only be known after further propagation.

Strain from the 4th Operation.

9 B, 4th operation——10_3B——11_3P——12_3A——13_2F——**14_2B**——**15_2B**.
(Fig. 46) (Fig. 82) (Fig. 83) (Fig. 84) (Fig. 85)

Propagation of material removed at fourth operation of 9 B.

Carcinoma up to 13_2F; sarcomatous change in 13_2F–14_2B; later progressive advance towards pure sarcoma.

The tumour removed at the 4th operation has already been mentioned. Fig. 46 is a microphotograph of a peripheral part of the tumour *. It shows the same more abundant stroma as mentioned for the other recurrent tumours of 9 B.

Examination of "Early Stages" of 9 B, *4th operation, giving* 10_3B.—In material from this tumour preserved 29 hours after inoculation a great many of the introduced stroma-cells are degenerating, but many still look healthy; there is no sign of proliferation in them.

In 48 hours material old stroma-corpuscles are still found well preserved in many places, without mitoses being found in them. The collagen fibrils are all degenerated and swollen. There is not much reaction to be seen from the host.

In 3 days material the degenerative changes of the stroma have further advanced, only here and there stroma-cells can be seen which seem to be still living. The reaction from the host is still rather poor. The parenchyma is highly necrotic in the graft examined.

In 4 days material the graft is fully vascularised, and numerous young and dividing cells appear between the carcinomatous alveoli. The picture is exactly like that described for the corresponding stage from the tumour removed at the third operation, and the same difficulties of interpretation are met with.

The tumours of the next generation show the same histological picture as the other tumours of this strain, and especially the same

* To facilitate comparison between the tumours of subsequent generations we have photographed corresponding parts of the tumours close to the periphery showing the greatest amount of stroma.

abundance and cellularity of the stroma. Fig. 82 shows a peripheral part of a daughter-tumour of 10_3B (used to yield 11_3P).

Examination of the Early Stages of 10_3B, *giving* 11_3P.—In material preserved 29 hours after inoculation, we see many stroma-cells remarkably well preserved: a single mitosis is seen. Re-vascularisation of the peripheral parts has already started. At 48 hours the graft is re-vascularised, and numerous healthy looking cells appear between the carcinoma alveoli, in part they seem to be elements of the old stroma.

At 3 days an abundant new cellular tissue is found between the carcinoma alveoli, stronger than in other carcinomata at the same stage. A cellular zone surrounds the graft, similar to that in figs. 65 & 66, the spindle cells are often arranged tangentially to the surface of the graft. In the centre of the graft there is slight necrosis, and close to the necrosis surviving stroma-cells are seen. At 4 days the picture is mainly the same as in 3 days material, the reaction from the host being at its height. The centre of the graft is slightly necrotic, cells apparently belonging to the old stroma and still looking healthy can be distinguished.

In the daughter series from this material (11_3P) there were two tumours. Fig. 83 shows a peripheral part of one of them (used to yield 12_3A). The stroma of this tumour is abundant throughout the whole section and shows sclerotic changes; one area in the centre is very cellular, so that the picture might suggest an early stage of sarcoma development, but the cells are smaller than the large elements we have found in the previous cases of sarcoma development.

Examination of Early Stages of 11_3P, giving 12_3A.—In this series the fragments of tumour were implanted under the skin of the back, where the reaction is usually slower than on the flank, especially when the graft has been deposited in the axillary fat-tissue. At 26 hours there is less evidence of degeneration of the stroma in this material than is usually the case. A considerable proportion of the introduced stroma-cells look quite healthy, and mitoses are seen.

At 44 hours there is still little evidence of degeneration, many healthy cells are seen, without, however, showing signs of proliferation. There is very little reaction from the host.

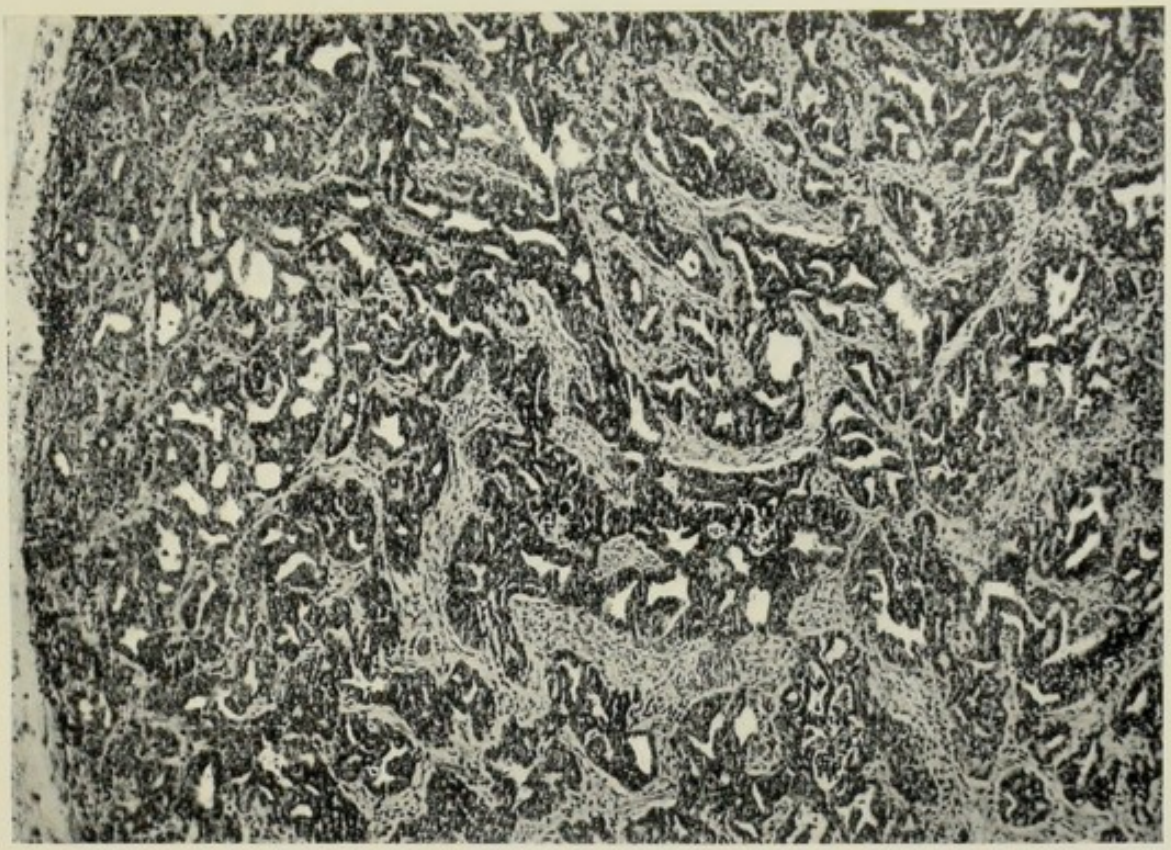
At 3 days there is still very little reaction from the host. Many stroma-cells are seen, evidently more than were introduced with the graft, but there is no evidence of mitosis. Some of them look healthy, while others show degenerative changes, and appear shrunken. The connective tissue-fibres are hyaline and swollen, as usual.

At 4 days the reaction from the host obscures the picture and makes it impossible to distinguish the old stroma-cells any longer in the peripheral parts. Cells belonging to the old stroma can still be seen, mostly showing fatty degeneration, round the necrotic centre of the graft.

In the series 12_3A, arising from this material there were five tumours in 15 mice, *i. e.* 33 per cent. Four of these have been examined. They all show carcinomatous structure with an abundant and cellular stroma,

[*To face* p. 236.

Strain from fourth operation on 37/9 B.



Microphoto, W. Imboden.

FIG. 82.—37/10_3 B—11_3 P. Tumour (23 days old) from first passage of this strain directly descended from tumour of fig. 46. Peripheral part of tumour showing greatest cellularity of stroma. Compare figs. 51, 59, and 76 of corresponding passages of the strains from 1st, 2nd, and 3rd operations. $\times \frac{100}{1}$.

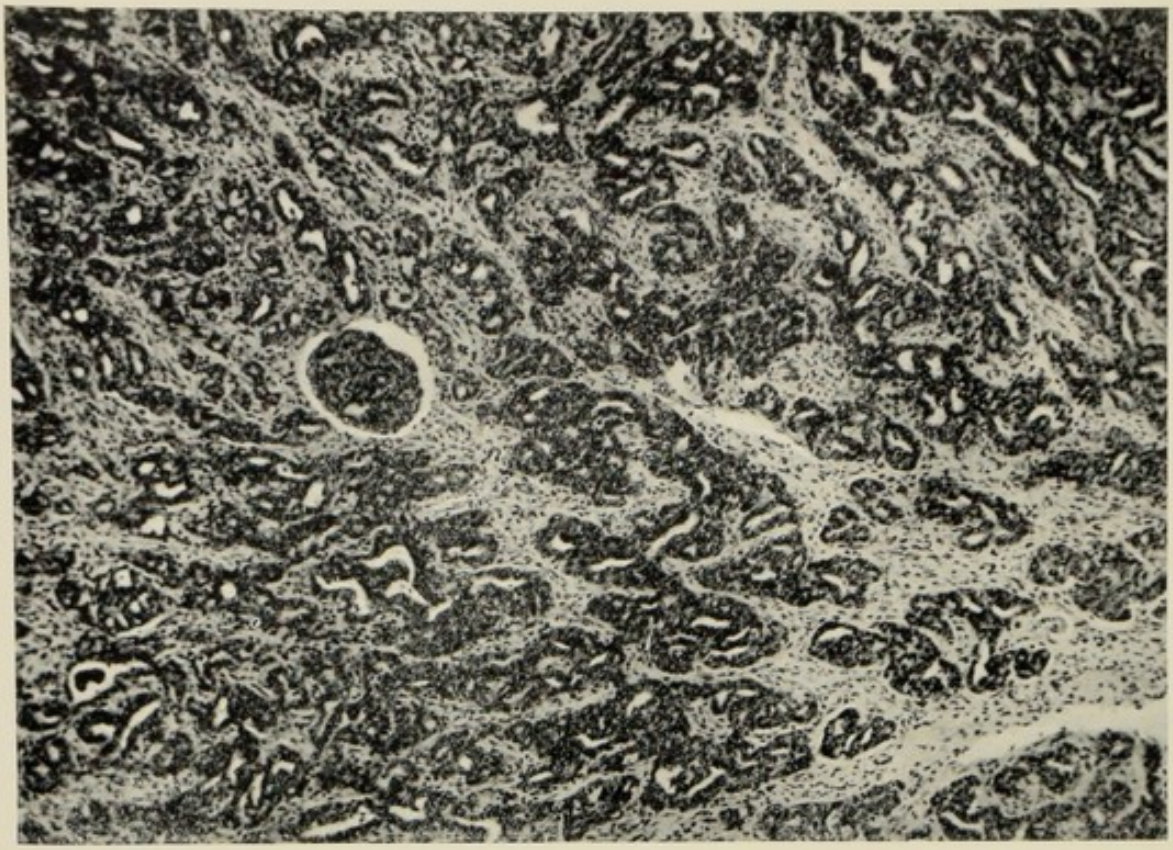


Microphoto, W. Imboden.

FIG. 83.—37/11_3 P—12_3 A. Tumour (28 days old) from second passage of this strain directly descended from tumour of fig. 82. Peripheral part of tumour showing greatest cellularity of stroma. Compare figs. 53, 62, and 77 of corresponding passages of 1st, 2nd, and 3rd strains. $\times \frac{100}{1}$.

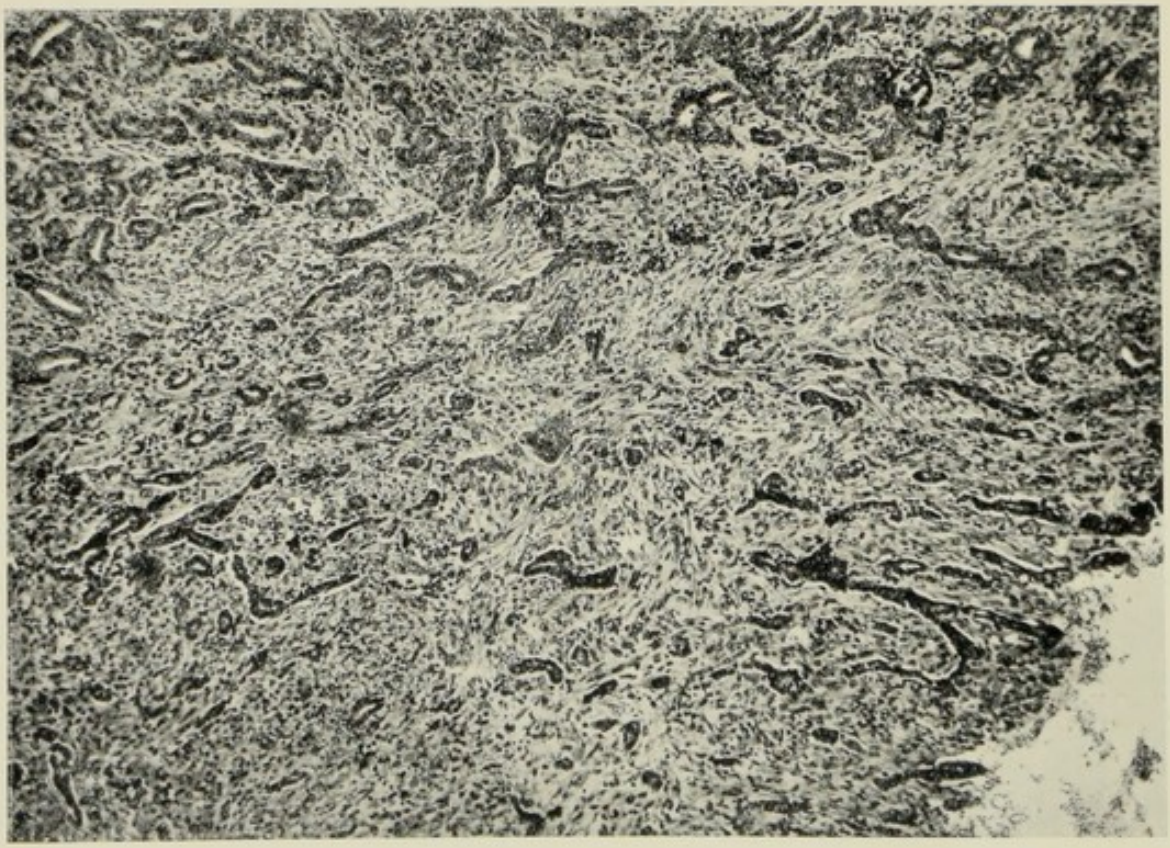
To face p. 237.]

Strain from fourth operation on 37/9 B.



Microphoto, W. Imboden.

FIG. 84.—$37/12_3$ A—13_2 F. Tumour (26 days old) from third passage of this strain directly descended from tumour of fig. 83. Peripheral part of tumour showing greatest cellularity of stroma. $\times \frac{100}{1}$.



Microphoto, W. Imboden.

FIG. 85.—$37/13_2$ F—14_2 B. Tumour (38 days old) from fourth passage of this strain directly descended from tumour of fig. 84. Mixed tumour stage. Compare figs. 54, 67, and 78 of corresponding stages from previous strains. $\times \frac{100}{1}$.

but in none of them can any clear indication be seen of sarcomatous changes like those found in the corresponding stages of the three first strains from 9 B. Fig. 84 shows a peripheral part of one of them (used to yield 13_2F) ; as in all these tumours, the part is chosen which contains the most cellular stroma. This material was not examined in early stages.

In Series 13_2F there were five tumours in 10 mice, *i.e.* 50 per cent. One of these, a rather rapidly growing tumour, was examined 31 days after transplantation and showed the usual picture of carcinoma with a more cellular stroma than usual. 38 days after transplantation another tumour was examined, which, after an initial growth, had remained of the same size for two weeks or had even slightly decreased in size. It shows an enormous increase in the quantity of connective tissue-elements in a localised area near the centre (fig. 85), while the rest of the tumour has less cellular stroma, more like what has been illustrated for the previous generations. There can be no doubt that these changes are of the same kind as those described on previous pages for other strains from the 9 B tumour, and the result of transplantation shows that biologically they are equivalent. A close relation seems to exist between the processes of spontaneous absorption and sarcoma development in this tumour. The two processes are often indistinguishable histologically, and it is only by the biological behaviour of the tissue on further transplantation, that the real differences can be elucidated.

From the next generation only one tumour has been examined and transplanted up to the present. A progressive advance of the sarcomatous change has taken place, and the histological picture of this tumour corresponds to those of figs. 72 and 79 for the parallel strains from the 2nd and 3rd operation.

* * * * *

Three additional strains have been propagated from the later operations on the same tumour of 9 B. Sufficient time has not elapsed to show whether in them also the same progressive changes in the stroma will occur. In the last strain, however, derived from the material obtained when the mouse was killed, one tumour in the third passage (12_3F—13_2Q) exhibits a similar condition of the stroma as shown in figs. 54, 67, 78, & 85. Three other tumours which have been examined from the same series (12_2F) do not exhibit the change.

Summary of 9 B. *Early Stages.*

Summarising the main facts with regard to this "9 B-family," we started with an adeno-carcinomatous tumour with a stroma somewhat more than usually abundant and cellular; it was operated upon six times and recurred after each operation. Each recurrent tumour has been transplanted and has given rise to parallel strains, which have been followed through numerous generations.

The same abundant and cellular stroma is a nearly constant feature of all these parallel strains. While in some strains the characteristics of the stroma remain unaltered, in others the abundance and cellularity of the stroma seem to be increasing in degree in later generations, and when they have been followed through several successive generations, connective tissue elements are seen to start growing independently as sarcomatous cells in five of them.

It is a very interesting fact that there is a difference between prolonging the stay of a tumour in one animal and concomitantly propagating the tumour in a succession of animals. Within the period of time which more than suffices for the process of sarcoma development in a series of subtransplantations, no alteration occurs in the mother-tumour repeatedly operated on in one animal. Thus the successive transference to new hosts seems to be an experimental condition of much greater moment than the duration of the stay in one animal. While sarcoma development takes place in four strains after three passages through new animals, and in a 5th after four passages, no trace of such a development is to be seen in the animal operated on after six recurrences, although the space of time during which the tumour has been growing and recurring in the original 9 B mouse, more than equals that necessary for the change to occur when the tumours are propagated in new animals.

How is this remarkable cellularity of the stroma maintained from generation to generation, and what relation have the sarcomatous elements to the cellular stroma in the previous generations?

Bashford, Murray, and Cramer have demonstrated for other carcinomata with delicate stroma, that the stroma degenerates after transplantation, and is each time replaced by reaction-tissue from the host. We have seen in reviewing the earlier generations of this tumour that there was no indication of this strain of carcinoma behaving differently in this respect from other carcinomata. The only peculiarity

of this tumour seems to be that the new-formed stroma contains more fibroblastic elements than is the case in other transplantable carcinomata.

The greater abundance and cellularity of the stroma in the "9 B family" could, in conformity with the earlier observations, be explained by postulating a stronger "specific stroma reaction" to the parenchyma of these tumours, characterized by a preponderance of the fibroblastic component.

On the other hand, the constancy of the morphological characters of the stroma, both in the several recurrent tumours and in their sub-transplants through successive generations in new animals, suggests the possibility of a transplantation of certain elements of the stroma from generation to generation. And as we have to deal later with connective tissue elements which undoubtedly are transplantable, it must be taken into serious consideration, whether or not stroma cells of earlier generations might have been transferred from generation to generation, before the sarcomatous change was observed.

A careful examination of the changes which occur in this material shortly after transplantation seemed to be the method most likely to help us in arriving at a definite conclusion with regard to this question.

However, the examination in "early stages" of material with abundant stroma, and sometimes with cellular granulation-tissue in the centre, as frequently met with in the slow growing 9 B-tumours, presents much greater difficulties of interpretation than that of other rapidly growing tumours with delicate stroma. There is no doubt that in these tumours also the main bulk of the introduced stroma-cells show marked degenerative changes, and that from about two days after the inoculation onwards the reaction from the host dominates the picture. The question is only whether it is possible from what we see to exclude the possibility of the survival of individual cells from one generation to another.

Degenerative changes in varying degree are a constant feature of all "early stages." Provided that the graft is not quite minute, only consisting of a few groups of cells, we constantly find the centre, including parenchyma and stroma alike, already necrotic after 24 hours. In the periphery of the graft a zone of living cells of varying width usually persists. As has been stated by Jensen, Bashford, Murray, and Cramer the carcinoma cells are much more resistant against the damaging influences accompanying transplantation than are the elements of the stroma. While numerous groups of carcinoma cells look perfectly healthy, and show numerous mitoses, the elements of the stroma as a rule show more or less marked signs of desintegration. We find

all stages of degeneration here, from a few fatty granules in the protoplasm, while the nucleus is still looking healthy, through stages in which the nucleus looks darker, diffusely stained, or shrunken, to stages of undoubted necrosis with karyorhexis and chromatolysis.

The same degenerative changes are also constantly found accompanying transplantation of normal tissues. When we seek for their cause, their similarity to the changes which always occur where the circulation is stopped by complete obstruction of arteries suggests naturally, that in the first instance disturbances of the circulation are the principal cause of this degeneration. As Marchand has pointed out it seems more natural to suppose, that it is the defective supply of oxygen which determines this change, rather than that of other nutritive substances. Besides this principal cause it is probable that difficulties of adaptation to a new soil may account to a certain extent for degenerative changes.

On the whole we have less certain evidence of complete degeneration of all stroma elements introduced during the first days after transplantation in the 9 B strains than in other carcinomata. It is premature to decide whether the difficulty is due to the greater number of connective tissue elements in these tumours compared to the small number which can be observed in other growths, or really due to a greater resistance of the connective tissue elements to injurious influences at the time of transplantation.

The time during which we can study the introduced stroma elements with any certainty is very short. By the end of the second 24 hours after transplantation, the vascularisation of the graft has begun, and numerous young capillaries are seen budding into it. From this moment we are unable to distinguish with certainty between surviving cells of the old stroma and invading cells. Theoretically it ought to be possible, by careful examination of serial sections, to define exactly to which category every cell belongs, but practically this is often impossible. For this reason, to study the survival of the introduced stroma cells we shall have in practice to limit ourselves to the first 36 up to 48 hours, and with regard to what follows after this time, we are forced to content ourselves with hypothetical conclusions of greater or less probability.

It can always be objected that even if a stroma cell survive the first 48 hours until the new vessels are beginning to grow into the graft, we have no certainty that this cell is going to continue its life as a part of the new stroma. To make quite sure with regard to this point it is necessary to prove that the old stroma elements are actively dividing.

But in the great majority of carcinomatous tumours this cannot be proved, at any rate not in the short space of time in which these cells can be distinguished from the angioblasts and fibroblasts formed from the host. Here our methods show their limitations. As long as we do not possess more extensive and detailed observations on transplantation of other forms of connective tissue, and especially different kinds of granulation-tissue, it is hardly possible to settle this question definitely. Conclusions drawn from analogy with other tumours with delicate stroma are rather risky in a branch of research yet so new, where nearly all the details necessary for drawing general conclusions remain to be collected.

The observations published by those who have investigated the transplantability of normal tissues, generally deal with the epithelial rather than with the connective tissues. The vast literature on the subject is reviewed and amplified from personal observations by Marchand in his book on Wound-healing. The instances where the fate of the connective tissues have been carefully followed histologically (Krause, Marchand, Enderlen, Braun) are few, and even then, enough heed has not been paid to differences which may obtain between the transplantation of connective and epithelial tissues in the same individual (homoplastic), as contrasted with similar transference from one individual to another (heteroplastic transplantation). From the surgeon's standpoint, interest centres in homoplastic transplantation; from our standpoint in connection with tumour transplantation, mainly in the heteroplastic.

It is beyond all doubt that normal epithelial tissues can be transplanted, not only to another part of the same individual, but also to other individuals of the same species, and continue their life in the new host. Whether connective tissue elements may follow the same laws and "heal in" in a new situation and continue to live, is much more difficult to prove, seeing that the connective tissue is practically omnipresent, and transplanted connective tissue cannot be so certainly distinguished from elements formed as reaction to injury. It is well known that certain differentiated elements of the connective tissue group, easily distinguishable from the reaction tissues of the host (*e. g.* cartilage), can be transplanted and even for a time proliferate, in another animal. Whether this may be extended to other tissues of the connective tissue group (especially young proliferating cells of granulation-tissue), is less certain. The data available are at present hardly numerous or accurate

enough to definitely settle this question, and our own experiments are not advanced enough to allow a conclusion.

When we take into account the observations made on transplantation of grafts involving the whole thickness of human skin (Krause's graft), there seems to be a certain amount of evidence that normal connective tissue elements (cells and fibres) may be capable of "healing in" and continuing their life in a new place. This is evident not only from a consideration of the final clinical result, but is also proved directly by Krause and Braun by histological examination of the processes following transplantation. It illustrates well the difficulties of this kind of investigation that Enderlen, working in Marchand's laboratory and examining the same process, came to the conclusion that there was very little evidence of transplantation of stroma elements in these grafts, but that the new connective tissue, including the elastic fibres, was formed as reaction-tissue from the host. A part of Enderlen's conclusions are refuted later by Braun's investigations, and Marchand, from his personal observations, also pronounces for the transplantability of normal connective-tissue elements.

From the facts brought out by the study of transplantation of normal tissues there seems, then, hardly any reason for assuming *a priori* that normal connective tissue elements in special cases should be quite incapable of surviving after transplantation. In our mixed tumours, we have to deal with connective tissue elements, which beyond all doubt are transplantable. The question is only when this transplantability has begun. We have started with a tumour, transplanted through many generations and, as a rule, showing a delicate stroma. At this stage we were not able to prove the transplantability of the stroma. Before the sarcomatous elements appear, we have in the "9 B family," a stage when the abundance and cellularity of the stroma is increased, and where we cannot prove with certainty that all stroma elements degenerate before the new angioblasts from the host revascularise the graft. Either we must assume that a certain part of the stroma elements of the graft are transplanted alongside of the carcinoma-cells and join in the formation of the new stroma, or we have in this tumour to deal with a parenchyma which influences the connective tissue of the successive hosts otherwise than in the earlier generations, now producing a more cellular granulation-tissue instead of the old delicate stroma. In the first case it seems to be easier to understand how a sarcomatous tissue with greater power of independent growth can develop by a gradual evolution of the stroma cells already

adapted to several hosts ; in the second case cells from the new-formed granulation-tissue under some influence, inexplicable at present, acquire malignant properties more suddenly.

Certain of the observations described on the previous pages speak rather strongly for the first alternative (*e. g.* the examination of early stages of 12_2L (figs. 64, 65, and 66), but the evidence is not absolutely incontrovertible, as it is almost impossible in these earliest stages of the sarcomatous change to distinguish with absolute certainty between the connective tissue elements of the graft and those entering from the surrounding tissues. Only in later stages where the power of growth of the sarcomatous tissue is much greater than that of the surrounding host-tissues, is it possible to make this distinction in a decisive manner.

But although for the present we prefer to leave the question open, of the transplantability of stroma-elements in the pre-sarcomatous stage of the 9 B tumours, it is nevertheless a matter of subsidiary moment compared with the difference which has been demonstrated between the stroma-elements of the tumours before and after the appearance of sarcoma. The contrast in the behaviour between the connective tissue elements in the carcinomatous and mixed tumours respectively, is of primordial importance. There can be no doubt that the sarcomatous elements possess other biological properties than the stroma-cells in the earlier generations. All the evidence derived from our 9 B strains shows, that the cells which ultimately come to possess sarcomatous properties are the genealogical descendants of stroma-cells, which in antecedent tumours did not exhibit them. The fundamental problem is to elucidate how this biological alteration has come about in the course of propagation.

Other Carcinomatous Strains with peculiar Stroma.

The 9 B family is not the only strain which shows an altered behaviour of the stroma. Two other strains of different parentage (6 O and 9 O) have exhibited the same cellular stroma as a constant feature through numerous generations, and reasoning by analogy we expected to see a sarcoma appear in them. In several successive series of one of these strains we met with such a cellularity of the stroma, that from a merely morphological standpoint, the diagnosis of a mixed tumour seems justified. By using the biological method of transplantation as a test, we see, however, that the stroma elements in

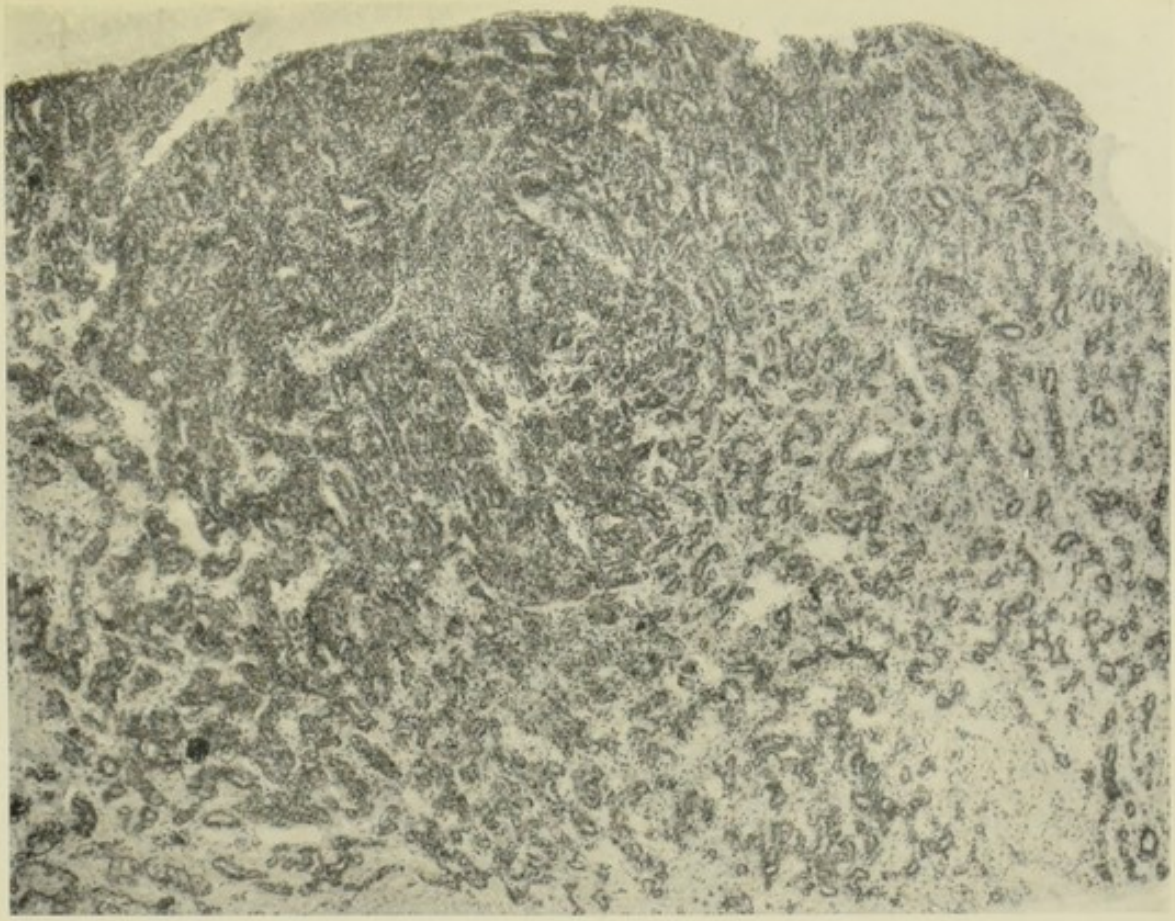
these tumours do not exhibit a power of growth in new animals which can justify our calling them sarcomatous, and the change has not been progressive as yet. After having persisted for five and six generations respectively, this peculiarity of the stroma seems to disappear again and the last generations of both the strains mentioned present the usual stroma with sclerotic changes in the centre.

The mother material for the first of these strains was a very old tumour of series 6 O, which was operated upon twice. The tumour showed on both occasions the microscopical picture of an adenocarcinoma with pronounced sclerotic changes in the centre, and at the same time a certain degree of cellularity of this fibrous tissue, as shown in fig. 86. A peripheral part of the tumour obtained when the mouse was killed is shown in fig. 6.

While the subtransplantations in the first and third strains of this tumour show no especial feature of the stroma, several tumours from the second operation (series 7 Q) present an extremely cellular stroma, and this feature is reproduced in the subtransplants through several generations. Nine of these tumours were transplanted and most of them were examined in early stages. Fig. 88 shows the centre of one of them where the cellularity of the stroma is especially pronounced. The change is equally pronounced in the next generation (figs. 87 & 89) but is not progressive, and the examination of early stages did not give conclusive evidence of continued growth of the stroma elements. This picture persisted in slighter degree for several generations (see genealogical tree), and it is only in the latest generation that the cellularity of the central stroma is less marked and the tumour has resumed more of its normal appearance.

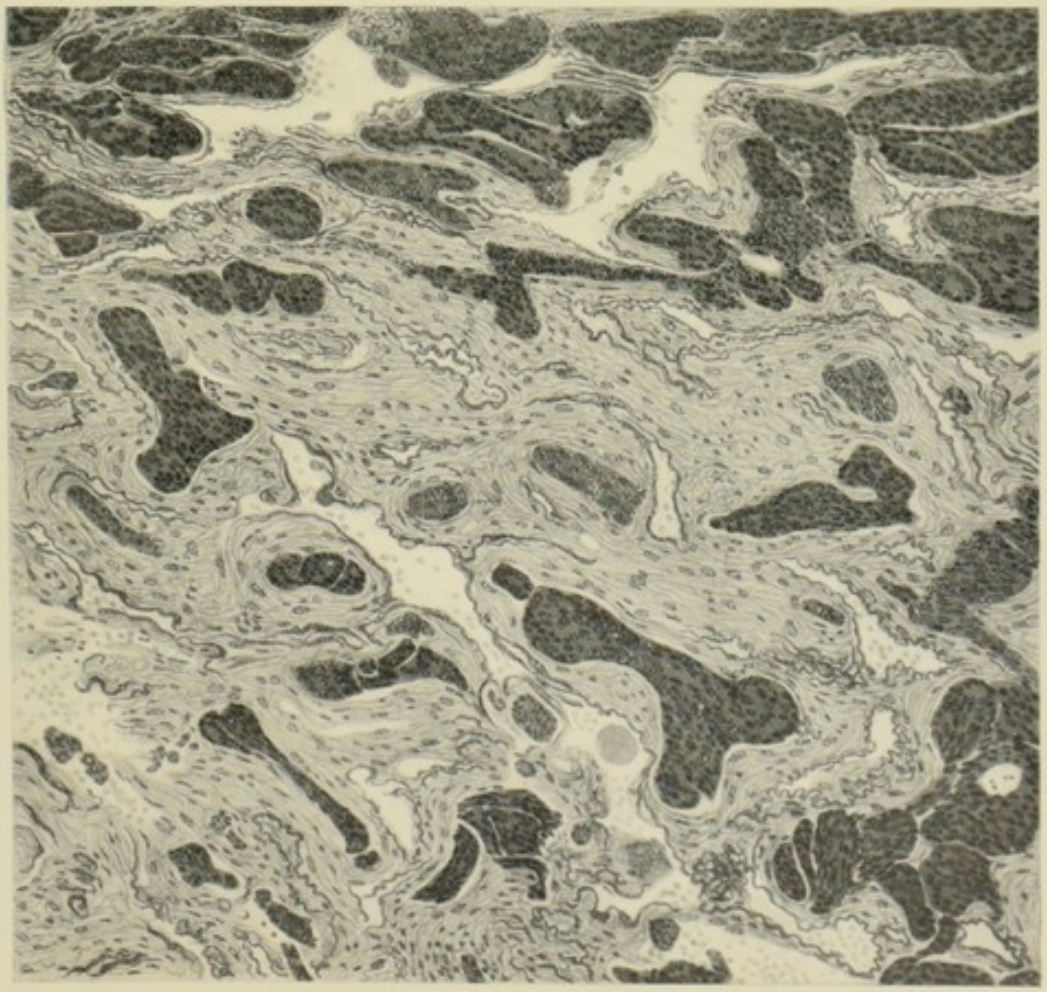
As we cannot prove that stroma-elements survive from generation to generation, we are bound to admit either that our methods are too imperfect to allow a definite conclusion, or that this cellular tissue is formed *de novo* each time as a specific reaction from the host. If the latter be the case, it is very interesting that the carcinoma cells may retain for several generations the property of influencing the connective tissue of the host in this special way, and then later return to the normal condition.

The second case of an extraordinarily cellular stroma as a constant feature through several generations, was found in two parallel strains of another old tumour (9 O), which was operated upon once. To prevent a possible misunderstanding as to the importance of the operation for this change, we must point uot that the behaviour of the stroma in the



Microphoto, W. Imboden.

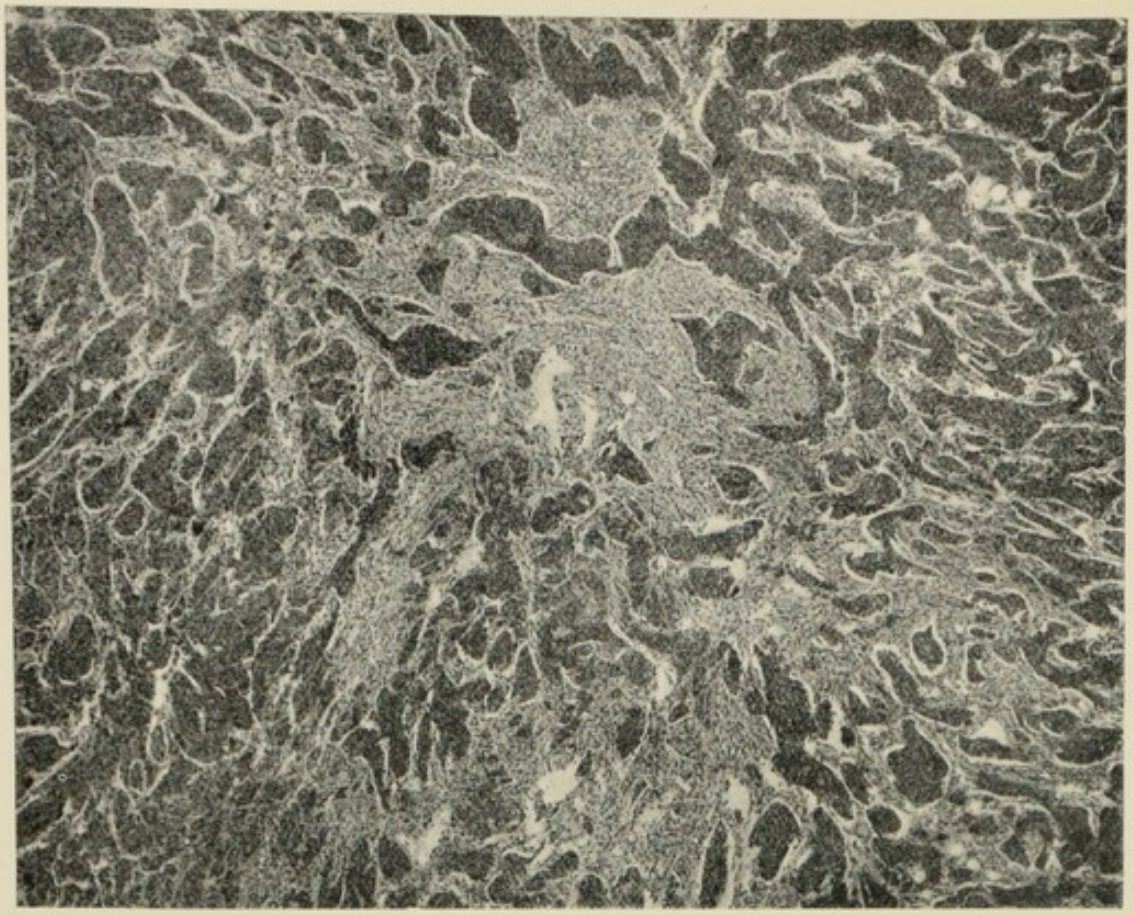
Fig. 86.—37/60, 1st operation—7 P. Tumour (138 days old). Sclerotic and slightly cellular stroma. The descendants of this tumour show the same kind of stroma through several generations, but it has not become sarcomatous. $\times \frac{50}{1}$.



J. R. Ford, del.

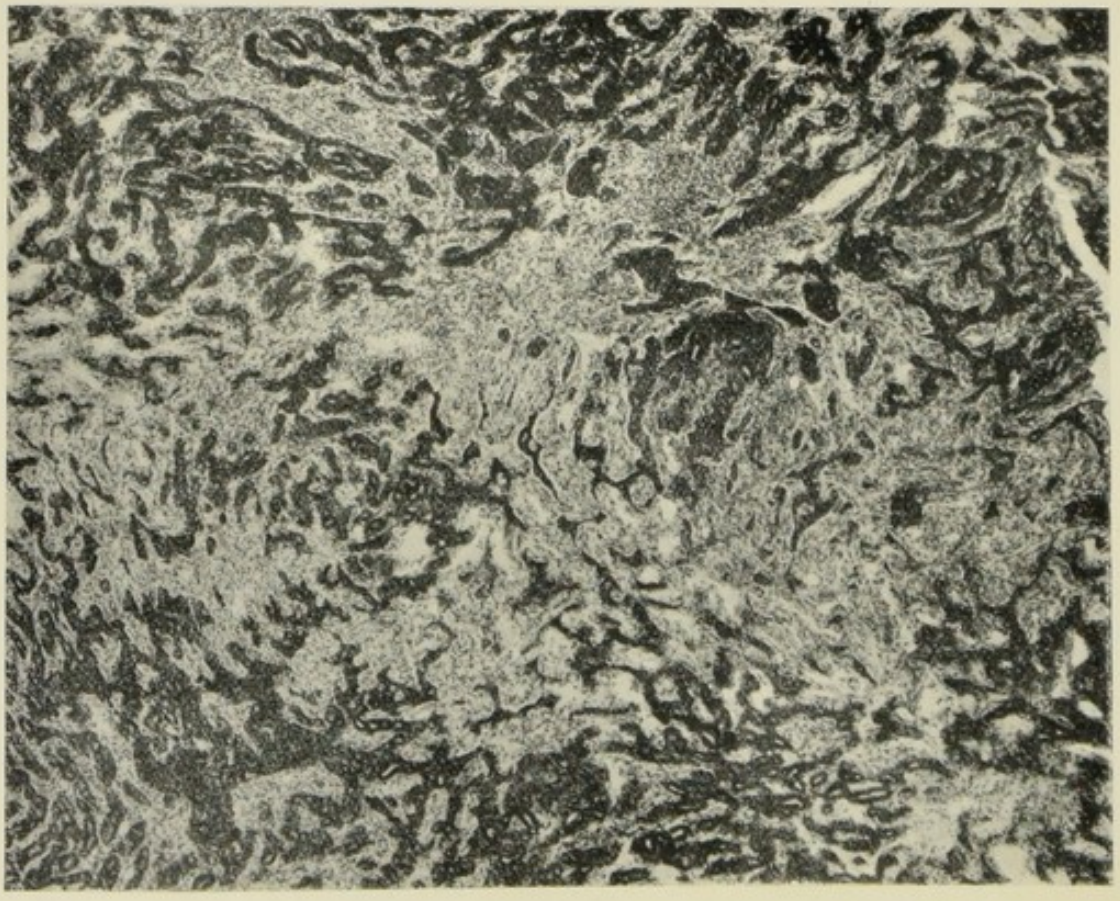
Fig. 87.—37/8$_5$B. Tumour (84 days old) from second passage of strain starting from 2nd operation of 37/60. *Cf.* fig. 86. Shows the arrangement of the sclerotic central stroma round the carcinoma-alveoli. This tumour was not used for transplantation. $\times \frac{124}{1}$.

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Microphoto, W. Imboden.

FIG. 88.—37/7 Q–8$_2$D. Tumour (57 days old) from first passage of strain starting from 2nd operation of 37/60. In spite of the extreme cellularity of the central stroma this tumour on further propagation has not given rise to sarcoma. $\times \frac{50}{1}$.



Microphoto, W. m$_b$oden.

FIG. 89.—37/8$_2$H–9$_2$P. Tumour (55 days old) from second passage of strain starting from 2nd operation of 37/60. Extremely cellular sclerotic stroma which has given place on further propagation to delicate stroma. $\times \frac{55}{1}$.

strain from the recurring tumour is exactly like that in the strain from the original tumour. It is not the operation, as such, which is of importance in the three reported instances (9 B, 6 O, and now 9 O) but rather the fact that we have chosen for our purpose very old slow-growing tumours, in which the sclerotic and other associated changes are most marked.

In the two parallel strains from 9 O, the amount of stroma seems to be slowly increasing in three successive generations ; at the same time the normal appearance of the stroma is retained perfectly, and the cellularity is not excessive. Their parenchyma presents at the same time the greatest variability in its morphological behaviour, as was shown in figs. 7 & 8. After increasing slowly through 4–5 generations the abundance of the stroma has decreased in both these strains. We do not know what may become of these strains by further propagation ; we have only mentioned them at present to show, that a cellular stroma may be a peculiarity maintained through several generations, just as we found it in 9 B preceding the sarcomatous changes, and nevertheless the stroma may revert to the normal condition without proceeding to the development of a sarcoma.

* * * * *

While this paper was going to press, a new (the ninth) case of sarcoma development has been observed in a strain which for 15 generations had retained its carcinomatous characters unaltered (see genealogical tree, top line, series 16 Q). As in other strains the change was preceded by increased cellularity of the stroma, through two generations, indistinguishable from the increased reaction associated with spontaneous absorption.

(6) Biological Features of Several Strains.

Hitherto we have studied mainly the histological features of the tumours derived from the primary growth by propagation, and especially of those strains leading up to sarcoma development. However, it must be admitted that the examination of "early stages" has a bearing not only on the histological side of the question, it also gives insight into the biological properties of the stroma elements.

We shall now review briefly the biological behaviour of some of the strains by analysing their growth as measured by the percentages of

successful inoculations throughout long periods of time, as has been done by Bashford, Murray, and Bowen for other tumours.

All the percentages of success and dates of inoculation are given in the genealogical tree. The method for graphic record is the same as used by Bashford, Murray, and Bowen, and described in detail on a later page in this report. The duration of propagation of the strains transplanted is taken as the abscissa, and the percentage of successes as ordinate.

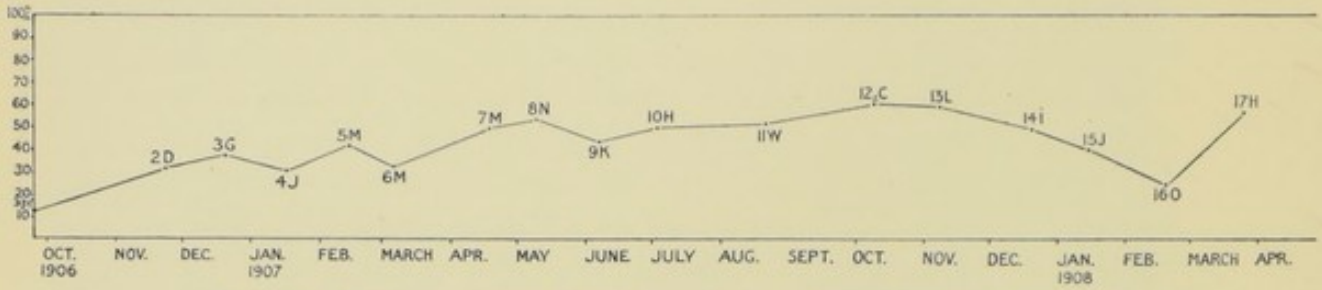


FIG. 90.—Percentage-curve of a pure carcinomatous strain in which sarcoma development has not occurred.

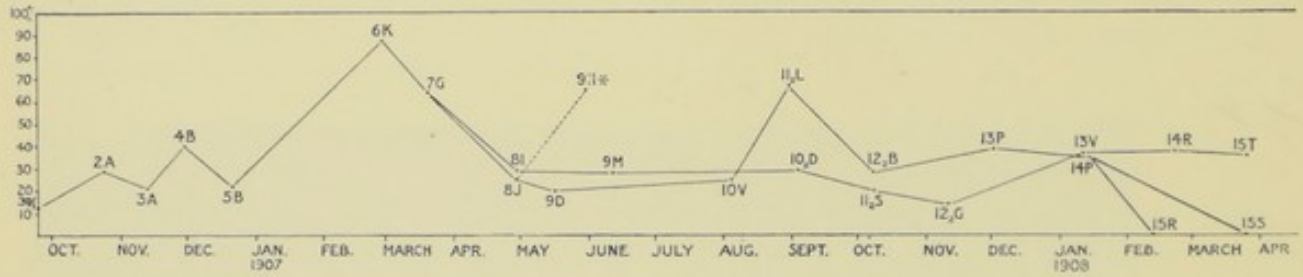


FIG. 91.—Percentage-curve of a strain in which one tumour (*) has shown sarcoma development (second case 9 H, the dotted line, v. curve 4), while two other strains (propagated separately from 7 G onwards) have continued as pure carcinoma. Sarcoma has appeared in the descendants of 15 T after two further passages (16 Q–17 K, ninth case of sarcoma development, see p. 245).

It follows from the consideration of these curves that the carcinomatous tumour in question is rather a slow growing one, as the interval between the transplantations often exceeds two months. Compared with the first transplantation of the primary tumour, the results of transplantation of later generations, as a whole, are better, but the percentages of success are rather low, fluctuating irregularly, the average being rather below than above 50 %. The curves show fluctuations in percentages of successes similar to those recorded by Bashford, Murray, and Bowen for Jensen's tumour; but these fluctuations are in this

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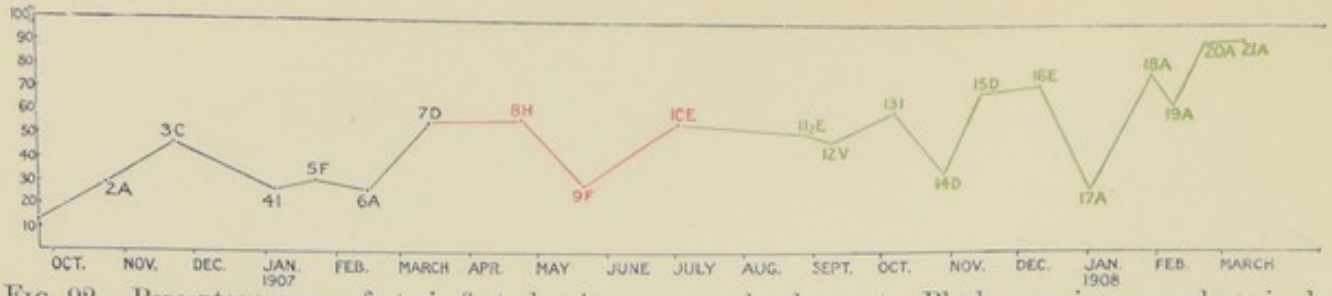


FIG. 92.—Percentage-curve of strain first showing sarcoma development. Black = carcinoma; red = mixed stage; green = pure spindle cell sarcoma. At 9 F passage through carcinoma-immune mice (biological purification, v. text).

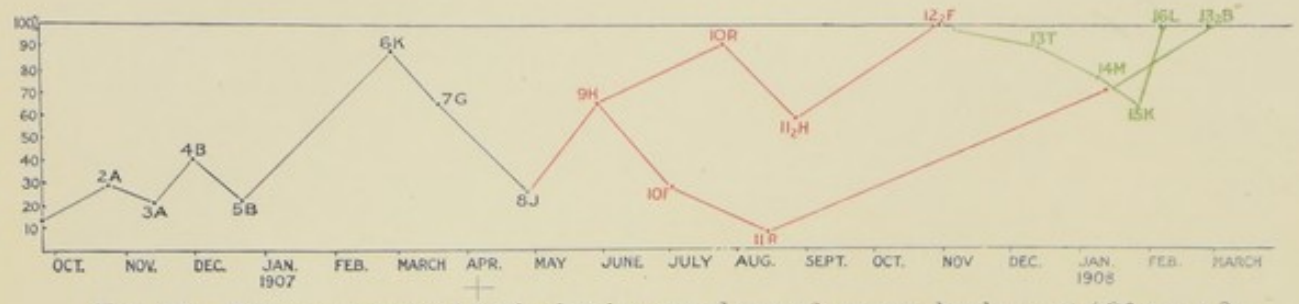


FIG. 93.—Percentage-curve of strain showing second case of sarcoma development (*Cf.* curve 2). Colours as above.

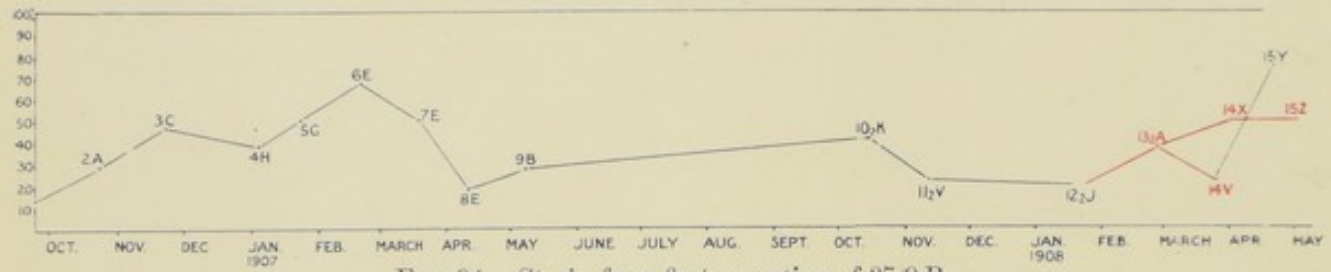


FIG. 94.—Strain from first operation of 37/9 B.

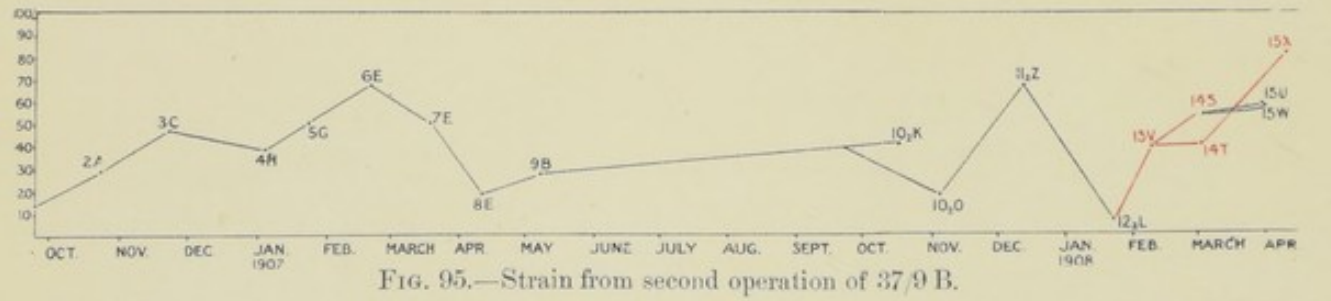


FIG. 95.—Strain from second operation of 37/9 B.

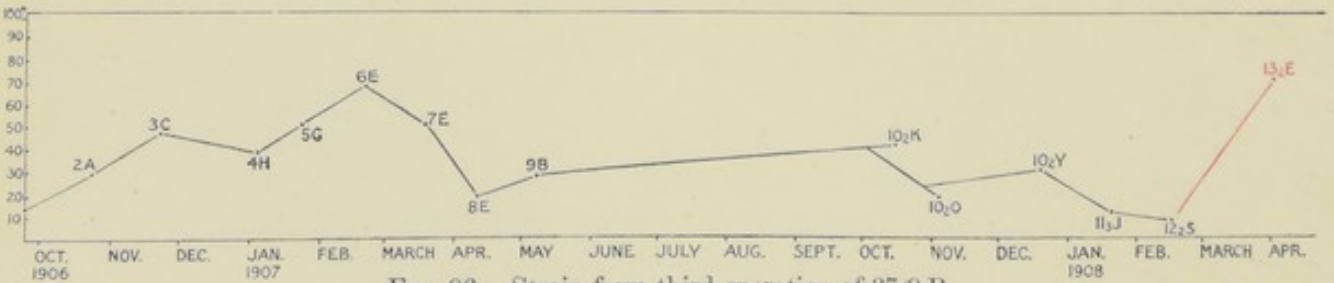


FIG. 96.—Strain from third operation of 37/9 B.

FIGS. 94–96.—Percentage-curves of 3 strains descended from 1st, 2nd and 3rd operations of tumour of 9 B. Red = mixed stage; red line becoming black = reversion to carcinoma.

relatively slow growing tumour much less marked than was the case in Jensen's tumour. Figs. 90 and 91 show strains of this tumour, which have retained the carcinomatous character unchanged during the whole time of propagation up to the present date. In the second of these strains the dotted line indicates a case of sarcoma development (2nd case, followed in fig. 93); at the same time the other strains from the same tumour (of 7 G) continue purely carcinomatous for numerous generations.

Figs. 92–96 show the several strains leading up to sarcoma development. In these curves the same colours are used as in the genealogical tree to distinguish the different histological types of tumours. Black = carcinomatous series; red = mixed tumours; green = pure sarcomata.

In recording in this way the sequence of generations which has given rise to sarcomata, we are struck by a remarkable uniformity in these curves. In almost all our cases the sarcoma makes its appearance in series of low percentage, where the curve is in the hollow of a wave. Figs. 93 and 95 exhibit this feature most markedly, but figs. 94 and 96 also show the same phenomenon. The only exception seems to be the first case (fig. 92); but in this case the simultaneous appearance of four cases out of 7 tumours examined from one series (7 D) renders it probable that the initial change in the process had taken place in the previous generation at the corresponding depression in the curve. On this assumption the relation of the configuration of the percentage curve to sarcoma development would correspond to that of the other cases.

It is difficult to tell at present how much importance should be attached to this phenomenon. We do not possess the details of the observations made by other investigators, and cannot compare their experiences with our own. In series with low percentages spontaneous absorption is as a rule frequent, and this fact might perhaps point to a connection between the processes accompanying spontaneous absorption and those responsible for the development of sarcomatous elements. From the histological observations a corresponding association has been suggested in several cases. We give these curves in the hope of stimulating other investigators in future to give similar attention to these details, when recording the conditions under which sarcoma development occurs.

The curves illustrate also the different length of time in which the tumour has been propagated as carcinoma before the sarcomatous

change occurs. While the first case occurred not quite half a year after the first transplantation of the primary tumour, the last case of the same change has been observed after more than 1½ years propagation of the tumour as carcinoma.

The curves illustrate furthermore the different length of time in which the mixed tumours have persisted as such, before a pure sarcoma was obtained. In fig. 92 the mixed tumour stage lasted only about 3 months (through 3 generations). In this case an attempt at biological purification was made by passage of series 9 F through mice resistant to the carcinomatous strain of this tumour.

In all the other strains the mixed tumour stage persisted much longer. In fig. 93 the purification was completed after an interval of six months in one branch of the curve, and eight months in the other. In both cases the mixed tumour-stage lasted through 4 successive generations. The pure sarcoma which results can apparently be propagated indefinitely without further change.

In the mixed tumours there is a general tendency of the curve to rise during further propagation. In general the pure sarcomatous tumours give a higher percentage than the carcinomatous and mixed strains. The percentages of success are invariably reckoned on the number of *continuously* growing tumours. We have already mentioned that a transitory growth followed by spontaneous absorption is very frequent in the sarcomatous tumours, and the energy of growth does not always correspond to the percentage of continuously growing tumours. Therefore the percentage-curve reveals only a part of the truth with regard to the biological properties of a tumour. We have, on a previous page, tried to represent graphically another side of the same question, viz., that of the rate of growth of these tumours, (fig. 40), and must refer to these records in order to complete the picture of their biological features.

Summary.

During transplantation of a rather slow growing adeno-carcinoma, with a somewhat fibrous stroma, a sarcomatous interstitial tissue has appeared in several separate strains. This has outgrown the carcinomatous elements in subsequent generations, and given rise to a pure spindle-cell sarcoma. In its mature form the sarcomatous tissue seems to have very little resemblance to the connective tissue of the stroma of the carcinomatous tumours. The difference is revealed

in its histological characters but especially in its altered biological behaviour after transplantation.

Before discussing the source of the sarcomatous elements and how this new tumour has arisen, there are certain possible objections which must be dealt with.

(1) The question arises whether these sarcoma-like tumours are true malignant new growths, and not mere infective granulomata, or granulation-tissue round the carcinomatous alveoli.

In examining "early stages" of pure sarcoma we see that the introduced sarcomatous cells remain alive and proliferate: the new tumours are entirely derived from cells introduced without the surrounding tissue taking any part in this process beyond that of supplying the growing cells with vessels and supporting structures. The process corresponds exactly to that which accompanies the transplantation of carcinoma cells, and is entirely different from any reaction round an infective agent.

Metastasis-formation can be followed in all stages, especially in the lungs, and shows the same thing. Each secondary nodule starts from a deposit of living sarcomatous cells as an embolus in a small vessel. The further growth of the secondary nodule takes place only by the proliferation of the cells of the embolus. They first expand the elastic lamina of the vessel, then infiltrate and perforate the wall and spread into the surrounding tissue, without any other reaction from the latter than is caused by any growing cell of a true malignant new growth. This applies both to the mixed tumours in the polymorph-celled stage, where remains of carcinoma are still present, and to the pure spindle-cell sarcomata. The latter have up to the present been transplanted in 16 generations as pure spindle-cell sarcomata without it being possible at any time to find the slightest trace of carcinoma in them.

We hope that these characters of the tumours in question will suffice to meet the criticisms raised by Orth so far as our tumours are concerned. They show that there can be no question either of an infective process, or of a mere formation of granulation tissue round carcinomatous alveoli, but that these new formations are really true malignant new growths, in every respect comparable with the transplantable carcinomata.

We are also in the fortunate position of having a spontaneous transplantable sarcoma of the mouse, to compare with the tumour we have here been studying. This spontaneous sarcoma is described

by Murray on pp. 78–81 of this Report. Consideration of its characters in the spontaneously affected animal and during transplantation, places its sarcomatous nature beyond possibility of doubt. The differentiation of its interstitial substance goes further than in the sarcoma we have obtained experimentally ; but this differentiation to cartilage and osteoid tissue is not present in all strains. The strains which do not show these differentiations are practically indistinguishable histologically from our experimental spindle-cell sarcoma (*cf.* figs. 31 & 34). The continuous and infiltrative growth, recurrence after operation, and formation of secondaries in distant organs, are exactly the same for the spontaneous sarcoma and its sub-transplantations, as we have demonstrated for our experimental sarcomatous growths. The examination of "early stages" shows that in this respect also there are no differences whatever between these tumours.

(2) Are we justified in calling these tumours sarcomata, in the same sense as this term is used in human pathology, implying also a conclusion as to the histogenesis of the tumour ?

Even if the general appearance of the rapidly growing spindle-shaped cells should not allow any conclusions as to their origin, other points permit us to decide with certainty from which elements they have arisen. Under higher magnification we observe in their protoplasm characteristic fine fibrils (Mallory's fibroglia fibres), exactly like those found in all young connective tissue, *cfr. e. g.* the figures given by Maximow*. Other fibrils are found outside the cell, giving all the reactions of collagen. The production of collagenous fibrils and the presence of fibroglia fibres in the cell, prove their origin from fibroblastic elements of the connective-tissue, and justify our calling these tumours true sarcomata.

(3) Is there any probability of these tumours being spontaneous sarcomata, accidentally occurring in one of the transplanted animals, as recently suggested by Sticker ? The possibility of such an event in a single case cannot be denied, but there is no probability whatever that the sarcoma development which interests us here can be explained by such a hypothetical coincidence. Among all the hundreds of sporadic mouse-tumours which have been observed and described up till now, there have been only a few sarcomatous tumours, one of this kind being observed by Jensen (described by Murray, p. 73), one in Ehrlich's Institute, and one, as already mentioned, in the

* Maximow, A. Experimentelle Untersuchungen über die entzündliche Neubildung von Bindegewebe. Ziegler's Beiträge, Supplementheft, 1902.

Imperial Cancer Research laboratory, recorded in detail by Murray on pp. 78–81 of this Report. In each of these cases serious difficulties with regard to further propagation have been met with ; in the first two cases it has not been possible to transplant the tumour ; only in the last case, by transplanting into more than 300 mice, has propagation been obtained. In contrast to the rarity of spontaneous sarcoma, sarcomata arising during transplantation of carcinomata, have been observed repeatedly by different workers, three times in Ehrlich's laboratory, once each by Loeb, by Liepman, and Lewin, and nine times by us in different strains of the same tumour. In contrast to the poor result of transplantation of the sporadic sarcomata, the further propagation of the sarcomatous tumours occurring during transplantation of carcinomata, so far from being difficult, has been remarkably easy, giving rapidly growing tumours with a high percentage of success.

These considerations render it unlikely that the phenomenon can be explained as a chance appearance of a sporadic sarcoma. The study of the commencement of the process, as we have been able to follow it in several instances, shows that there is no question of a fortuitous occurrence. The sarcoma develops as a result of progressive changes in the stroma, and it generally begins in the centre of the tumours. This fact alone disposes altogether of the last-mentioned hypothesis, and proves that the appearance of the change is in some way or other connected with the growth of the existing carcinomatous tumour.

(4) The most important question is whether we can exclude a primarily existing mixed tumour with carcinomatous and sarcomatous components.

This important point has been fully considered by Ehrlich and Apolant, who exclude it, both from the histological examination of the sporadic and the transplanted tumours, and the long period of time which elapsed before the change occurred. Their critics (v. Hansemann, Schlagenhaufer) seem, however, not to consider this evidence strong enough, and maintain that the tumours might have contained sarcomatous elements from the outset. As Bashford pointed out, it is hardly possible to settle this question without following the processes at the site of inoculation step by step.

We have devoted special attention to this question and tried to settle it according to Bashford's suggestion, by systematic examination of the processes at the site of inoculation of early stages.

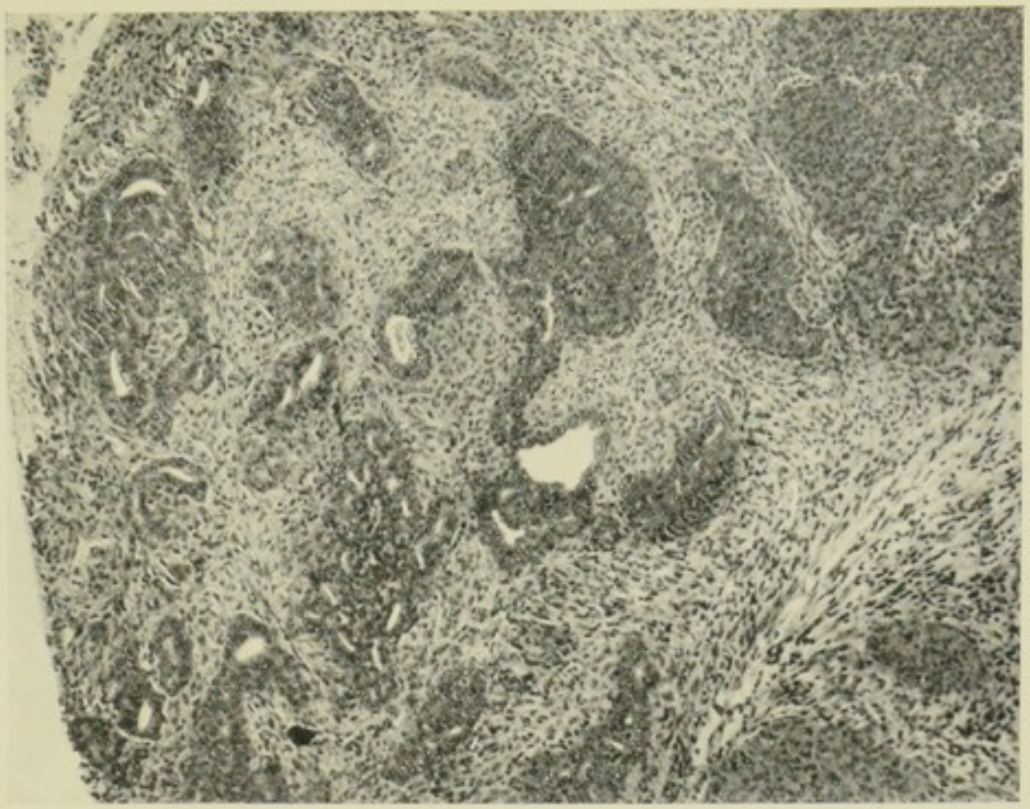
Our investigations are in this respect very complete. The primary

tumour has been examined in the same way, and in addition a tumour of the 4th generation, a long time before any sarcoma development was observed. Moreover, many tumours of the carcinomatous strain have been examined in "early stages," subsequent to the occurrence of sarcomatous changes in other strains. This study, combined with the histological examination of every tumour which has been transplanted, shows that there can be no question of a primary mixed tumour in the usual sense of the word. Apart from the question whether or not elements of the stroma of carcinomatous tumours under certain circumstances may be transplanted along with the carcinoma cells—a question on which we wish to abstain from expressing any definite opinion until more data are available—at the outset there are no signs of a different behaviour from other transplanted carcinomata. Sarcomatous elements, distinguishable either by histological characters or by higher energy of growth, cannot be traced at any stage of propagation, either in the primary tumour or in the later carcinomatous generations, before the described change occurs. The tumours of carcinomatous strains, which have been propagated after the sarcomatous changes had occurred in others, are likewise devoid of sarcomatous elements. Through numerous passages the transplanted tumours show all the characters of typical pure carcinomata with a scanty stroma compared to that of human tumours, and we cannot term these growths mixed tumours unless every carcinomatous growth is to be so called.

We have also studied a transplantable tumour in which the stroma has been transplantable from the primary growth onwards.

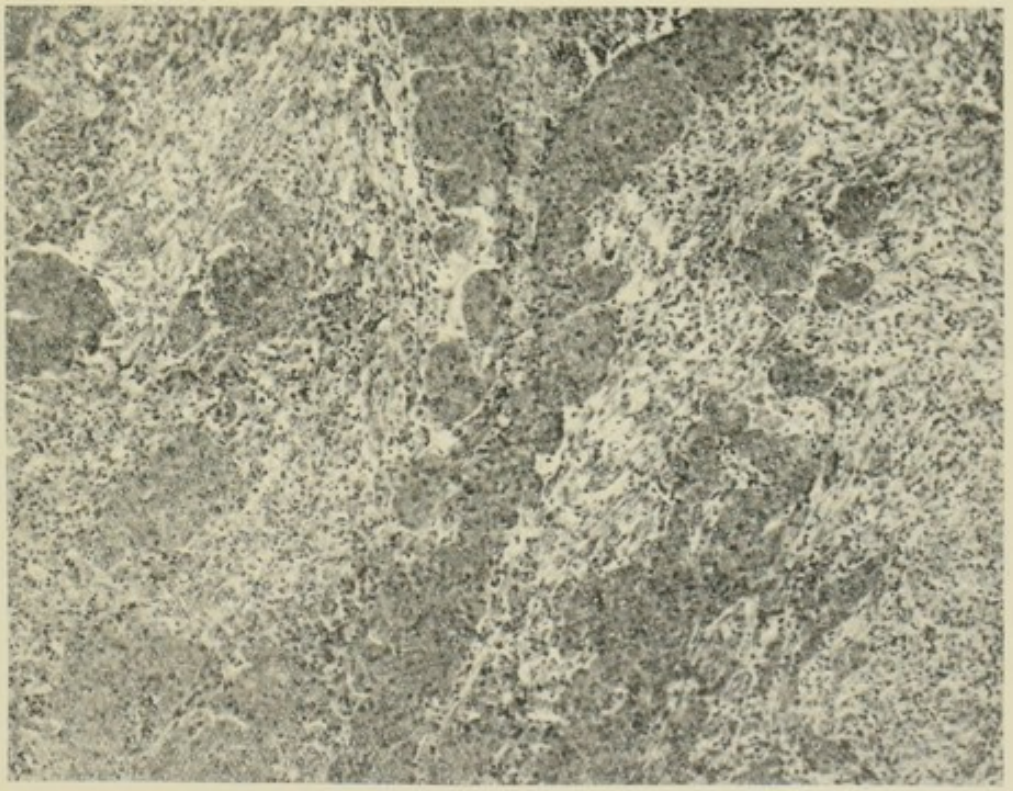
Tumour 129, the primary growth of which has been mentioned by Murray on p. 88, is an instance of such a tumour. The primary growth was of enormous size, weighing more than 10 grams, and evidently very old. Microscopically large parts show a delicate stroma, but in other parts an increased cellularity of the stroma is found, with large spindle cells. The greatest cellularity is found in one particular area at the periphery, which is illustrated in fig. 97. It may perhaps be more than a coincidence that these stroma changes are found in this very old tumour, comparable to the old tumours examined of strain 37. The tumour was inoculated and minute fragments from the parts with cellular stroma examined in early stages. Four tumours of the first generation were examined; they all show an abundant stroma; in two of them the cellularity is so great that the morphological picture is that of a mixed tumour. Fig. 98

[*To face* p. 252.



Microphoto, W. Imboden.

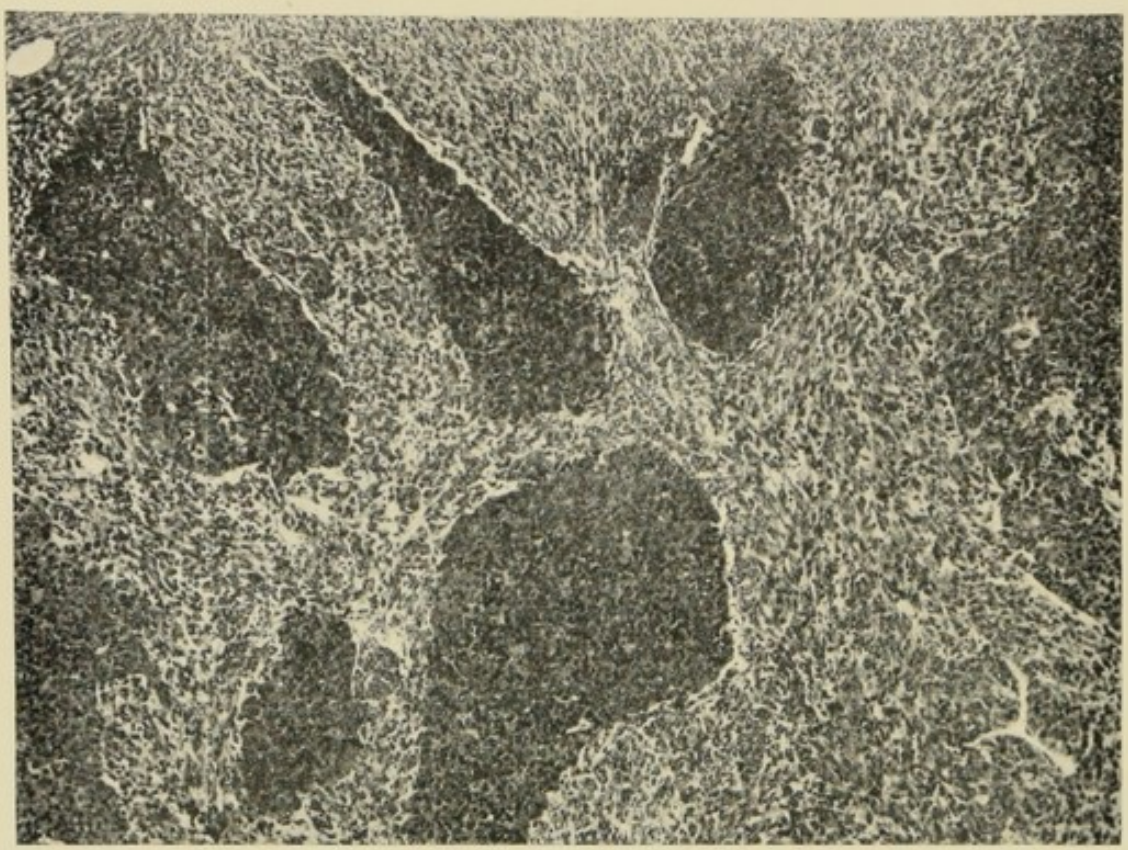
FIG. 97.—$\frac{129}{0}$. Spontaneous mammary adeno-carcinoma. Portion of periphery showing abundant, extremely cellular, stroma, carcinoma sarcomatodes. In other parts of the tumour the stroma is much less abundant. $\times \frac{100}{1}$.



Microphoto, W. Imboden.

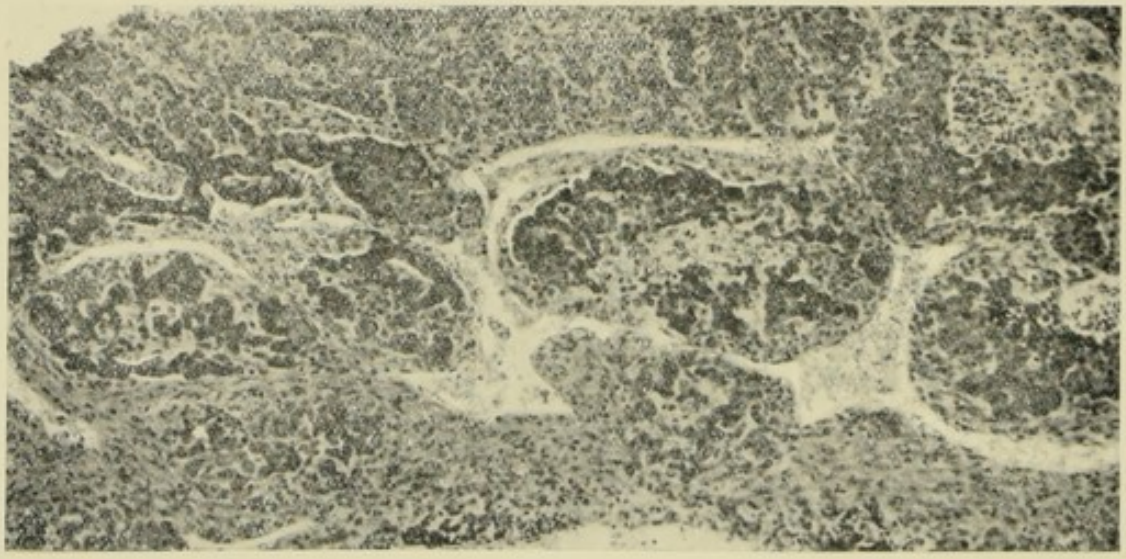
FIG. 98.—$\frac{129}{1}$-2B. Tumour (44 days old) of first generation. Carcinoma-alveoli separated widely by strands of spindle-celled and polymorphous sarcomatous tissue.

To face p. 253.]



Microphoto, W. Imboden.

FIG. 99.—129/2B–3 B. Tumour (29 days old) of 2nd generation directly descended from tumour of fig. 98. Progressive increase of sarcomatous tissue. $\times \frac{100}{1}$.



Microphoto, W. Imboden.

FIG. 100.—129/3 B–4 A. Tumour (25 days old) of 3rd generation directly descended from tumour of fig. 99. Invasion and splitting up of carcinoma-alveoli by polymorphous sarcomatous tissue. The arrangement of the sarcomatous tissue in this tumour recalls the halo-formation seen during development of sarcoma from tumour 37. (*Cf.* figs. 23–26, 57 and 73.) $\times \frac{100}{1}$.

illustrates one of these tumours, viz., the mother material of Series 2 B. In the second generation, from these two tumours we have the same characteristic picture of a mixed tumour, as illustrated in fig. 99 (2 B giving 3 B), while other tumours show the carcinomatous type with only slightly cellular stroma. The same picture is maintained in the third generation, as is shown in fig. 100 (tumour from 3 B, giving 4 A). We observe here a splitting up of the carcinomatous alveoli by connective-tissue elements, and moreover an arrangement of the latter round the parenchyma in a way similar to what we have described as halos in earlier cases of mixed tumours. Tumour 129, which is relatively easily transplantable, has been propagated up to the present only in four generations.

Most of these tumours have been examined in early stages. It is not difficult to prove that stroma elements are capable of surviving after transplantation and of joining in the formation of the new stroma. The process of regeneration takes place in them after transplantation much more rapidly than in the surrounding tissue, but not so rapidly as in the sarcomatous tumours we have studied previously. The fact that the reaction of repair in these stroma cells already starts before the new reaction from the host obscures the picture, allows us in this case to draw conclusions as to their transplantability with certainty *.

In the case of tumour 129 there is no doubt that already in the primary tumour we have to deal with stroma elements with special characters, transplantable, and with enhanced power of growth compared with normal cells. This is not the case in tumour 37. The stroma cells of tumour 37 are apparently from the outset cells with biological properties like normal cells without showing any primary sarcomatous change. If it be supposed that the cells which later give sarcoma originated in the mouse primarily attacked, they must have been propagated for seven to sixteen generations, *i. e.*, for our last cases for more than 1½ years, without showing any particular features before the sarcomatous change occured. The primordial point is the alteration in their biological properties, which is demonstrated most easily by the different behaviour on transplantation before and after the sarcomatous change has taken place. This change occurs in the course of propagation as a secondary process.

* The idea naturally occurs, that even if normal connective-tissue elements possessing the same power of regeneration as the surrounding tissue, may survive transplantation, we should hardly be able to prove this with our present methods.

(5) As to the possibility of the sarcoma cells being derived from the carcinomatous parenchyma, we have already shown that these cells present all the characters of true connective-tissue cells—intracellular fibrils (fibroglia fibrils, Mallory) and collagen fibres between the spindle-shaped elements. As far as we know there exists no analogy for a process of metaplasia which can transform typical epithelial cells, as the cells of the glandular structures of this tumour undoubtedly are, into equally typical connective-tissue cells. The same view was held by Ehrlich and Apolant in their first publications, where they strongly denied a transformation of this kind. In a recent paper read before the German Pathological Society (Kiel, April 23–25, 1908) *, Apolant, however, admits the transformation of carcinoma cells at the periphery of the alveoli into spindle-shaped elements and dissociation of the cells. He does not say whether this means that in his opinion a formation of sarcomatous cells from carcinoma is taking place. This phenomenon, already mentioned by Apolant in an earlier paper †, is evidently the same process as we have described and illustrated in figs. 74–75, and we believe we have shown that it has nothing to do with the origin of the sarcomatous cells. Changes from the acinous to the alveolar condition of the carcinoma cell, accompanied by lighter staining of nucleus and protoplasm in the alveolar parts, have already been mentioned for several of our cases of sarcoma development as adding considerably to the difficulties of distinguishing carcinomatous and sarcomatous tissue from each other in the mixed tumours. The same condition is seen in fig. 74, here accompanied by extraordinary forms and shapes of the lightly stained carcinomatous cells, so that we can hardly distinguish them from the sarcomatous elements. If we had to draw our conclusions from this preparation only, the transformation of epithelial into sarcomatous elements would have to be considered seriously. But in following the series of changes, we see that this picture mostly appears at a later stage of the process a long time after the stroma has become sarcomatous. These considerations show that the bizarre changes in the appearance of the carcinoma cells—interesting as they are—have nothing to do with the first change itself, and do not indicate the origin of sarcoma cells from carcinoma.

We see the process of development of sarcoma taking place from beginning to end in the interstitial tissue, and there is no evidence

* Centralblatt für allgemeine Pathologie, Juni 1908.

† Die epithelialen Geschwülste der Maus, *l. c.*

of a transformation of carcinomatous elements into sarcomatous. The important question is how the sarcomatous elements arise from cells which previously did not exhibit any evidence of malignant properties. We will once more review some facts brought out during these investigations which may have a bearing upon this problem.

(i.) In our tumour 37 we have first a stage in which no indications of sarcomatous changes can be found with our present methods. Neither in the primary tumour nor in the earlier generations of the strains leading up to sarcoma development do we find any stroma elements with peculiar characters. Further, in the numerous strains which have been propagated as pure carcinomata subsequent to the occurrence of the first cases of sarcoma development, we have not been able to perceive any such stroma elements.

(ii.) In the cases in which we were able to follow the strain closely, both before and after the appearance of sarcomatous elements, there is a definite stage before the appearance of the sarcoma in which the stroma of the tumour has become more abundant and cellular. We have discussed the possibilities as to how this cellular stroma arises either by a stronger specific reaction or by transplantation of stroma elements from generation to generation. In examining early stages of tumours in this pre-sarcomatous stage with abundant and cellular stroma, we found in single cases strong evidence of connective-tissue elements being transplantable, before any sarcomatous change shows itself histologically. We have seen the difficulties in the way of deciding when this transplantability of individual stroma elements has appeared for the first time; and the possibility remains that the transplantation of individual stroma elements may go further back than can be proved with our methods. The process has followed a parallel course in five parallel strains derived from the same tumour, and therefore one may argue that it had its inception in the mother-material in which they have their common origin, although the actual sarcomatous change itself takes place later in subsequent propagation.

(iii.) We may propagate tumours showing this abundant and cellular stroma during a long time without observing any real sarcomatous change. Sarcoma development, characterised as we have seen by the appearance of larger connective tissue cells endowed with an increased power of resistance and continued growth after transplantation, is only found in relatively few tumours compared to the enormous number examined. A feature common to these cases is that they have occurred in tumours belonging to series with low percentages of successes, where

the energy of growth of the carcinoma is lower than usual, and in which at the same time spontaneous absorption of tumour frequently occurs. These observations perhaps indicate a connection between the development of sarcoma and a temporary low energy of growth of the carcinoma, or on the other hand a relation to the processes accompanying spontaneous healing of carcinomatous tumours.

(iv.) In all the later cases of sarcoma development we find the first changes beginning in the centre of the carcinomatous tumour, where sclerotic changes are habitually present. These sclerotic changes are a constant feature of old tumours of strain 37 and they are often accompanied by more or less cellularity of the central stroma. The sarcomatous change in the 9 B tumours at its commencement appears as a process of the same kind—but enhanced in degree—as that responsible for the formation of abundant cellular connective-tissue with disappearance of the carcinoma in the sclerotic centre of old tumours.

(v.) When the sarcomatous change has appeared in a tumour, the sarcoma cells have not suddenly acquired the same power of continued growth and the rate of proliferation which they are able to show at a later stage of propagation in the pure sarcomatous tumours. The sarcomatous cells in later stages seem to have reached a higher degree of biological alteration than was possessed by the sarcomatous elements in the first mixed tumours. The altered properties seem to be the result of a process of evolution on the whole progressing from generation to generation, but showing erratic fluctuations.

(vi.) Another series of observations seem to show that the process of transplantation into new animals in itself may be a factor of importance. We have seen the original 9 B tumour recurring six times in the same animal without any change of histological or biological characters. At the same time this tumour, transplanted into new animals, gave rise in five separate strains to sarcoma development, after three passages in four cases and after four in the fifth. The total length of time during which the mother-tumour of these strains grew in the same animal, more than equalled the interval which elapsed before the sarcomatous change occurred in the subtransplantations. It seems accordingly that the duration of growth is not the determining condition so much as other factors connected with transplantation into new animals.

Taking the above mentioned points together, all evidence seems to speak for a gradual process by which apparently normal connective-

tissue cells evolve into sarcomatous elements, endowed with altered biological qualities *. Certain factors which under ordinary conditions are able to call forth an increased proliferation of the connective-tissue cells, seem to play an essential part in this process, viz., those connected with absorption of necrotic material and spontaneous disappearance of tumours. Processes of this kind producing cellular granulation-tissue, or later less cellular sclerotic tissue, are often met with in tumour 37. The cells of granulation-tissue are biologically different from those of sarcoma, and repeated transplantation seems to be a factor of primordial importance in bridging over the gap between them.

If the possibility be admitted that constituent cells of special forms of reaction-tissue may survive transplantation under favourable conditions, it does not seem improbable that survival after repeated subtransplantations into successive hosts, and consequent adaptation to new conditions of existence, may enhance the resistance and powers of growth of such cells, in a manner similar to that suggested by Ehrlich (*cf.* p. 178). Through the intervention of the carcinoma cells a blood supply is provided by the host, and if stroma cells be transplantable, they will benefit from it. To the stimulation of their power of regeneration which follows each transplantation there is added the continuous physiological stimulus exerted by the growing carcinoma cells. Observations on tumours of the 9 B family seem to show that stroma cells may acquire such altered biological characteristics before the histological picture of a "mixed tumour" is produced. What circumstances are immediately and finally responsible for the descendants of these cells ultimately appearing as sarcomatous elements will be made the object of more detailed investigations. Beside the influences of repeated transplantation, certain points in our observations may indicate an interdependence between the energy of growth of the epithelial and connective-tissue components of the tumours. On the one hand, the curves in figs. 92–96, show that the sarcoma development coincides

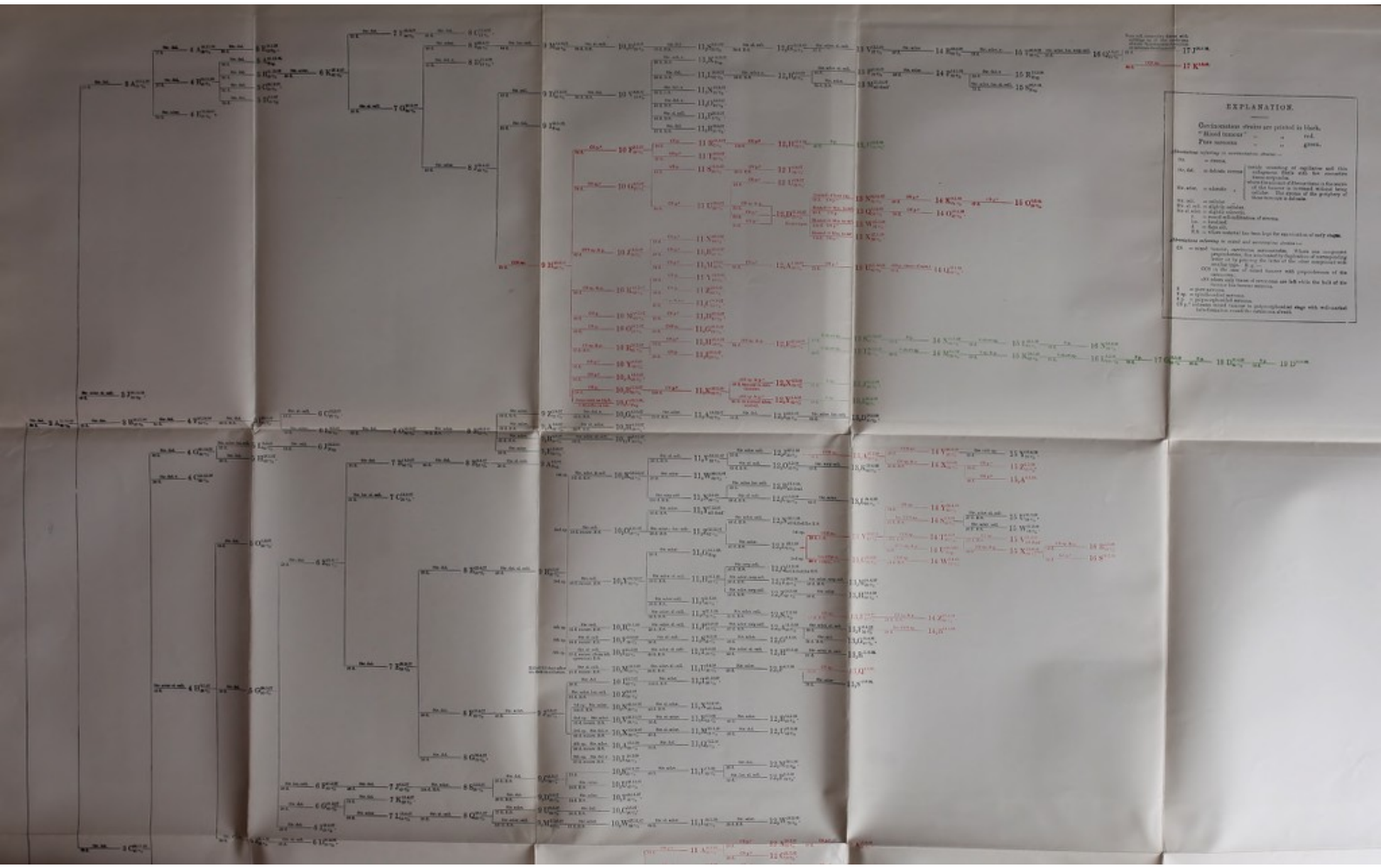
* The altered biological qualities find expression in: (1) the cells are not impaired to the same extent by the damage occurring in the interval between removal from one host and the establishment of connection with the other, (2) the rapidity with which regenerative processes after transplantation take place, (3) the enhanced adaptability to new environment, and (4) accelerated rate and unlimited power of proliferation. These different biological qualities are inherent in the cells and are transmitted by them from one generation to the next. It cannot be doubted that we are justified in speaking of a new race of connective-tissue cells, developed during propagation of a carcinomatous tumour from the elements contained in its stroma.

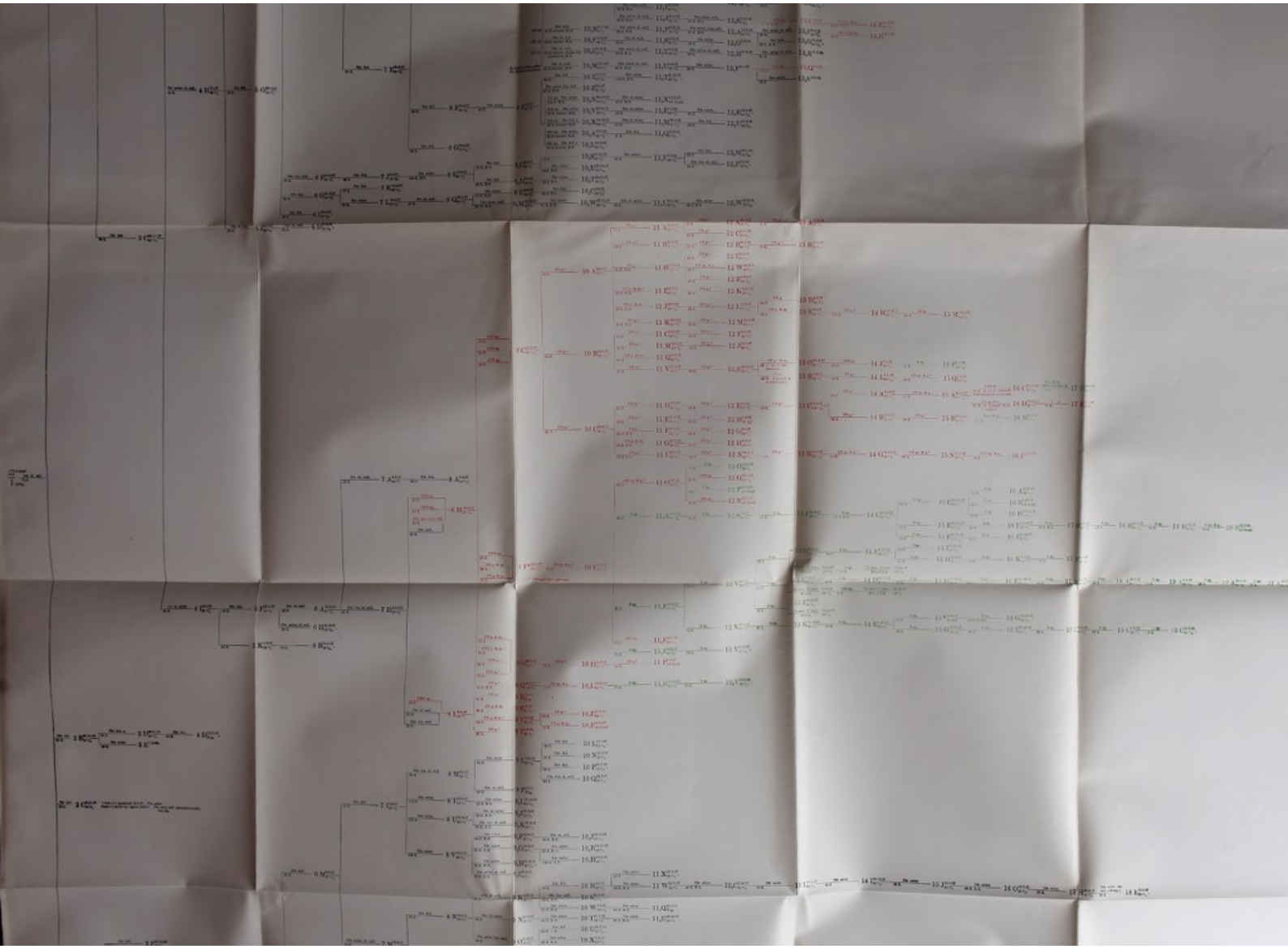
with depression in the proliferation of the carcinomatous tumours. On the other hand, in tumours already well advanced in the mixed stage, the epithelial (carcinomatous) component may again outgrow the connective-tissue (sarcoma) and the picture of an ordinary carcinoma reappear. This secondary suppression of the sarcoma seems to synchronise with a higher energy of growth on the part of the carcinoma. Meantime, however, we consider it inadvisable to enter on a hypothetical discussion, all the more because the phenomena lend themselves to an objective investigation, and further data are required.

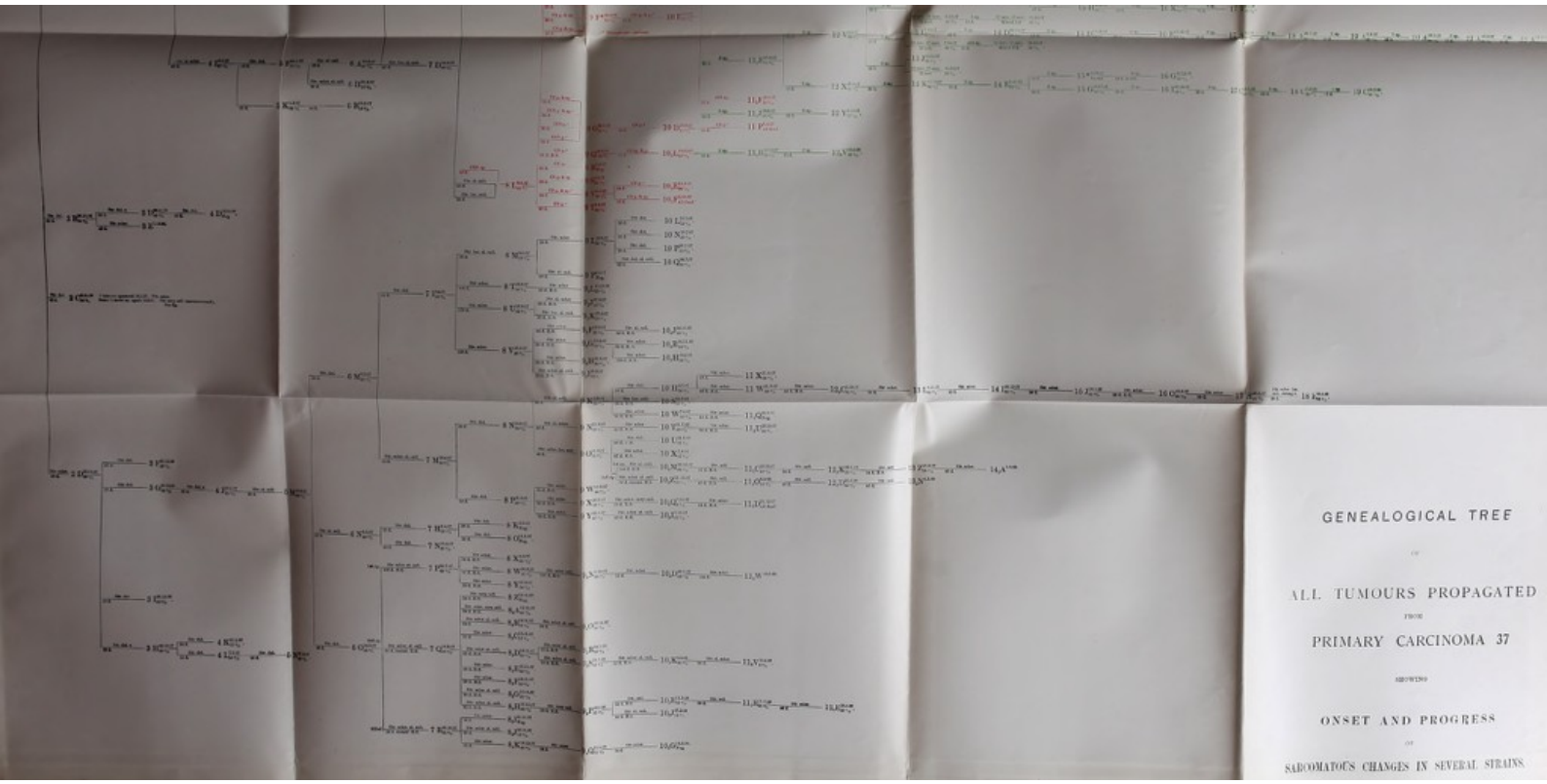
By further propagation the sarcomatous elements acquire, by degrees, increased powers of growth, which allow them at last to outgrow the carcinoma, and to continue as pure sarcomata. This latter part of the sarcoma development seems to be an evolution of a new and stronger strain of cells independent of the organism in which they are growing, and many facts speak for a similar process of evolution playing an important part in the earlier stages. Principles similar to those laid down by Darwin in explaining the origin of species of animals, may also to a certain extent be applied to explain this development of a new kind of connective tissue cells during continued transplantation.

While in our last, best followed cases, there is strong evidence of the importance of continued transplantation in the development of sarcoma, in other cases this factor does not come in at all. In tumour 129 the primary growth already shows stroma cells with sarcomatous properties. Also in our third case (37/2 C) in which changes, probably sarcomatous, have taken place between two operations, another factor must come into play. We have no means of deciding how the sarcomatous component of the spontaneous mixed tumour has arisen. In the 2 C case formative influences exercised by the carcinoma cells on the connective tissue of the host, seem to play the most important part.

When we reflect upon what these two groups of conditions, viz. on the one hand the repeated transplantation, on the other the formative influences exerted by the carcinoma cells, may have in common, there appear to underlie each of them chronic irritative influences similar to those emphasized by Bashford as probable mediate etiological factors in numerous forms of human cancer, at the beginning of this Report. The observations we have recorded on the development of experimental sarcomata seem to help us to an understanding of how chronic irritation may lead to development of malignant new growths. Through the continuous, or intermittent but frequently repeated incitation of the regenerative powers of the cells in some cases a strain of cells may


EXPLANATION.
Carbonaceous strata are printed in black.
" Mixed masses " " " red.
Pure sandstone " " green.





GENEALOGICAL TREE

OF

ALL TUMOURS PROPAGATED

FROM

PRIMARY CARCINOMA 37

SHOWING

ONSET AND PROGRESS

OF

SARCOMATOUS CHANGES IN SEVERAL STRAINS

be evolved, with enhanced power of regeneration and proliferation, no longer subject to the normal regulative forces of the organism. This conception is not formulated on abstract speculations, but on actual observations of all stages of the development of sarcoma under experimental conditions. The importance of the development of sarcomata as described in this paper, lies in the fact that it allows us to attack directly, and by experimental methods, the question of how cells with apparently normal biological properties turn into malignant elements.

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GENERAL RESULTS OF PROPAGATION OF MALIGNANT NEW GROWTHS.

By E. F. BASHFORD, M.D., J. A. MURRAY, M.B., B.Sc., M. HAALAND, and W. H. BOWEN, M.S., F.R.C.S.

(1) Practical Value of Propagation.

The experimental investigation of cancer has now passed from the preliminary stage of having successfully achieved the transference of malignant new growths from one animal to another and elucidated the process of transmission. It now concerns itself with the biology of the cancer cell and the relations of malignant new growths to their hosts during continued propagation. We have now studied the processes of successful transference of seventy distinct tumours, forty of which continue under propagation at the present time. Their investigation has fully confirmed the conclusions advanced in the First and Second Scientific Reports as to the nature and significance of the process. These results have now received general recognition, and need not be detailed beyond repeating that the experimental reproduction of the lesions of cancer in sound animals occurs only under conditions rendering it highly improbable that the disease is usually, or even occasionally, communicated from one living being to another. The experimental communication of cancer is only possible by implantation and continued growth of living cells, and this form of transmission is certainly not the cause of the frequency of cancer, either in human beings or in mice, even if it be conceded that under exceptional circumstances, a possibility for its occurrence is presented.

The attainment of one of the primary objects of the experimental study of cancer permits of conclusions which, while of a negative order, are of great importance. It is of great practical value that the results of experiment re-enforce opinions often expressed for other reasons.

[*To face* p. 262.



FIG. 1.—Exp. $\frac{J}{81G}$. Transplanted alveolar carcinoma (Jensen) killed 25 days after inoculation. Metastases in lungs.

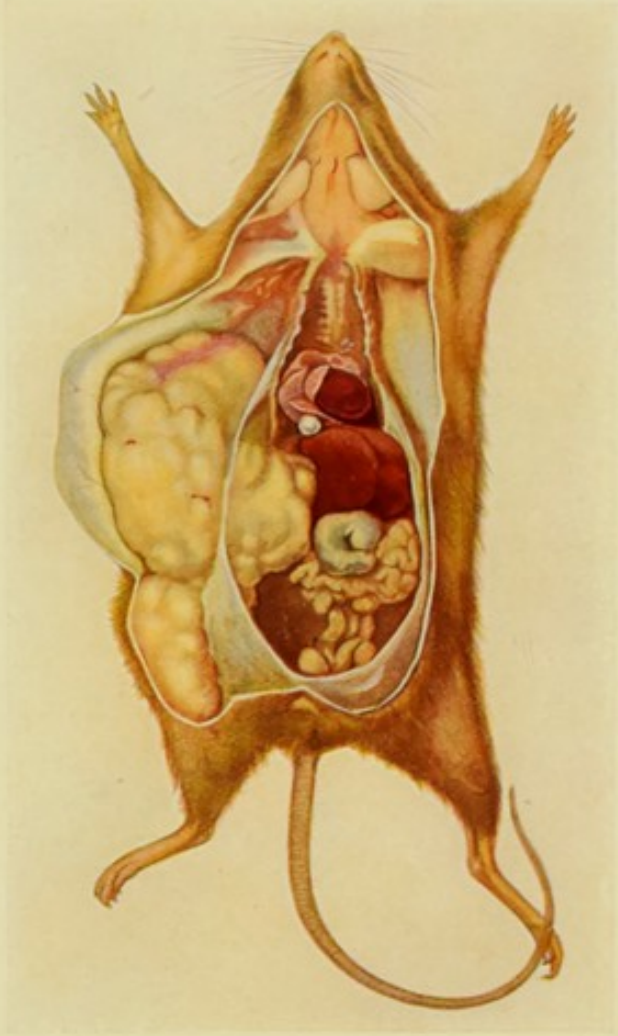


FIG. 2.—Exp. $\frac{32}{5A}$. Transplanted squamous celled carcinoma killed 59 days after inoculation. The growth has penetrated the abdominal wall and fungated into peritoneum. Metastases in lungs.

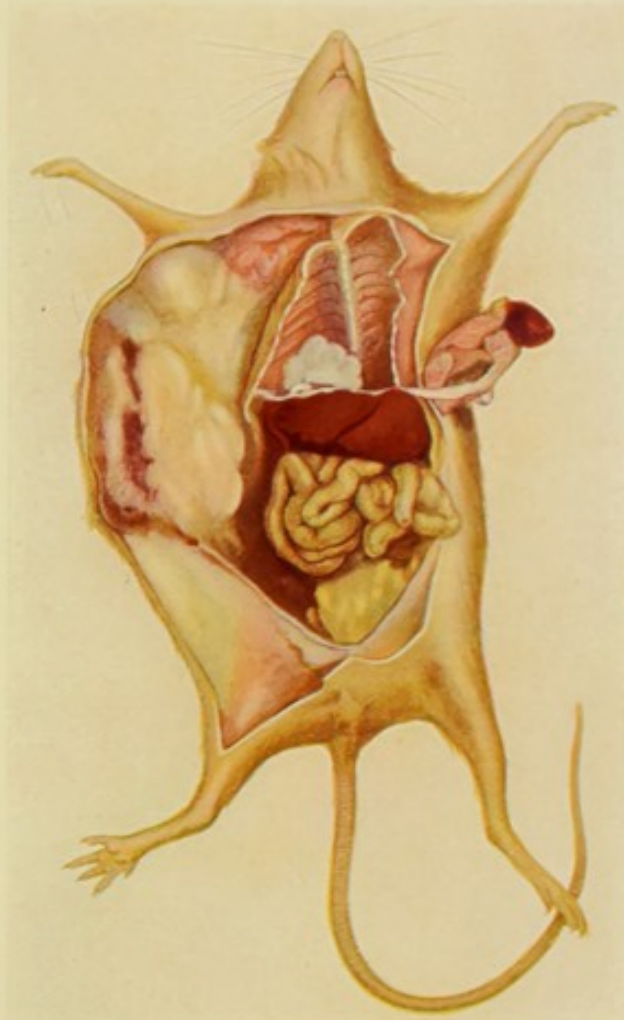


FIG. 3.—Exp. $\frac{37}{17B}$. Transplanted spindle-celled sarcoma killed 80 days after inoculation. Infiltration of thoracic wall and diaphragm. Metastases in lungs.

The public can be assured with greater certainty than hitherto that the presence of some 50,000 persons suffering from cancer in England and Wales does not constitute a direct menace to the health of those near and dear to them or to the health of the population generally. Notwithstanding what unwise enthusiasts may assert from time to time, what was a justifiable cause of public alarm has been removed by experiments on the transference of cancer and the housing of large numbers of cancerous with sound animals over a period of six years, *i. e.*, more than double the span of life in the mouse, the animal on which most of the observations have been made.

Thereafter, the experimental transference of cancer dropped into the position of an item in laboratory routine, a mere preliminary procedure to most of the investigations which it renders possible, and here results of a positive order and of constructive value have already been elicited.

Just as the culture of bacteria is a necessary routine procedure in most bacteriological researches, so is that of the cancer cell in cancer research. But there are fundamental differences between the two. The propagation of cancer is not a mere cultivation of cancer cells, comparable to a culture of bacteria. The cancer cells only grow as a tissue and by becoming part and parcel as it were of a living animal. This greatly complicates the labour involved and the procedure necessary in propagation. The cancer cell will only grow in a satisfactory manner in the living animal, and only in animals of the species from which it was derived. Thus the "*in vitro*" cultures obtained by the bacteriologist are replaced by "*in vivo*" cultures in the case of cancer, and thousands of living mice take the place of thousands of test-tube experiments. The phenomenon of the apparently unlimited propagation of the cancer cell merits study in itself, as a biological phenomenon. Its investigation has clearly revealed features of cancer which are of fundamental importance for understanding the nature of the disease and of spontaneous or natural recovery from it, but which had not been recognised because they are obscured by complications in the course of the disease in man.

Although the observations which follow are of great importance in determining the technique of propagation, and in all questions of immunity, they will be regarded in this paper principally from the standpoint of their bearing on the biology of the cancer cell, and on the analysis of the proliferation which it exhibits during artificial propagation.

(2) Convenience of Propagation in Mice and Rats.

As stated above, over seventy distinct propagable tumours have been studied. In spite of individual variations in the several strains certain features reappear again and again, and we therefore attach to them fundamental importance as essential characters of malignant new growths. Under prolonged propagation tumours retain their distinctive features of histology and biological behaviour, and do not approximate to a common type or pass into one another. Apparent exceptions are presented by tumours such as those described on a previous page, with reference to the experimental production of sarcoma.

The propagation of malignant new growths by transplantation of cancer cells from one animal to another, is entirely distinct from the natural origin or induction of cancer. It has become so familiar that there is a tendency to forget its importance as a biological phenomenon *, and, to detract from the significance of its bearing on cancer in man, by attaching undue significance to its apparent limitation to rodents. In the first place the phenomenon is not limited to rodents. We have been able to propagate through three generations a carcinoma of the mamma in the dog, and when our experiments with rats and mice were as limited as those with dogs, cats, and horses are to-day, we had obtained no better success with them.

Mice have been successfully inoculated with their own sporadic tumours, and the fact is paralleled almost exactly by the accidental implantation of cancer-cells in wounds, made at surgical operations on the human subject. The risk of thus implanting and propagating cancer in tissues previously free from it, is so great in man, that many surgeons have been compelled to draw attention to the necessity for special precautions to avoid it. The same risk has to be guarded against in veterinary practice in operating on animals. Bearing these facts in mind, there can be no doubt that it is false to assert that the artificial propagation of cancer is a phenomenon peculiar to rodents. There is sufficient evidence to justify the contrary proposition, that the artificial propagation of cancer will probably be possible in all the mammals when a sufficient number of attempts have been made, and a suitable

* *Cf.* Roy. Soc. Proc. Jan. 1904, and introduction to Second Scientific Report of the Imperial Cancer Research Fund, part ii, April 1905.

technique has been evolved. The inoculation of a number of young * dogs or of young horses in order merely to duplicate the preliminary results obtained for mice would be a most extensive, and, indeed, an unjustifiable undertaking, not only from the financial side, but from the fact that the satisfactory supervision even of 1000 large animals, *e. g.*, horses, is practically impossible. When our knowledge and methods have improved so that the conditions of success are well defined, it will be advisable to resort to larger animals for the study of definite problems. That progress in this direction is possible, is shown by the fact that hæmorrhagic mouse tumours, which in Ehrlich's experience yielded one success out of 500 inoculations, have been easily transplanted with our methods, and in single series have yielded almost 100 per cent., and that whereas he has transplanted 14 per cent. of his spontaneous tumours successfully, we have transplanted 80 per cent. No deference need therefore be paid to those who criticise the present limitation of experiment on a large scale to small mammals—mice and rats—which can be housed and cared for easily in large numbers. The objections would carry as much weight had we studied inoculation in horses, and the objectors complained that we had not done it in whales.

(3) Experimental Reproduction of the Lesions and Constitutional Accompaniments of Cancer.

At various places in this report isolated references are made to the occurrence of infiltrative growth and metastasis formation by transplanted tumours. No more striking demonstration of the experimental reproduction of spontaneous lesions could be wished for than that given in figs. 47 to 50, pp. 218–220, of spontaneous and of an experimental carcinoma of the intestine, and the figures of metastases given in various places. Metastasis is as much the rule in mice with inoculated cancer when suitable methods are employed, as it is in man or in mice naturally afflicted, and infiltrative growth is also frequent even in subcutaneous transplanted tumours which appear to the naked-eye to be encapsuled (see also figs. 1, 2, and 3). These facts encourage us to undertake the exhaustive study of all the conditions associated

* At the outset of our investigations old animals were selected for inoculation, owing to the association of cancer with senescence, but, contrary to what was anticipated, we found that young animals were more suitable. This observation has received wide confirmation, among others from Flexner and Jobling, Jensen, Borrel, Michaelis, C. Lewin.

with the presence or absence of infiltrative growth and metastasis formation in transplanted cancer, with the assurance that such knowledge will throw light on the corresponding lesions in the human subject.

As regards the transference of cancer by inoculation, it must be obvious to anyone giving even a passing thought to the matter, that when we implant portions of a carcinomatous growth beneath the skin of a mouse by means of a hypodermic needle, the implanted tissue is not deposited in a manner reproducing the normal anatomical relations of tissues to one another, nor are anatomical relations established similar to those obtaining between a small spontaneous malignant new growth and the tissues surrounding it.

The only fact of real moment of which we require to assure ourselves is that the starting-points of our experiments have been malignant new growths and not tumour-formations of a different category, *e. g.*, demonstrably of an infective nature. On preceding pages ample comparative and special evidence has been given that our investigations are made upon true malignant new growths, and for the same reason, in the Second Scientific Report we discarded the infective venereal tumours of dogs as unsuitable for the investigation of cancer. Although the ætiology of these growths has not yet been elucidated completely, notwithstanding recent papers by Ewing and Beebe, Wade and Sticker, there is now and has always been, a general agreement in regard to their infectivity, and the distinction is therefore warranted which we draw between them and the sarcomata proper of the dog for which no such connection between sporadic cases has been made out. Beyond the interesting reference Dr. Seligmann makes to their occurrence in New Guinea, these tumours have not been discussed in this Report. These infective venereal tumours, which histologically are indistinguishable from sarcomata are of great importance, however, and we shall return to them on a future occasion, after the conclusion of further investigations.

The experimental study of cancer is primarily based upon the fact, that carcinoma and sarcoma cells can be implanted into healthy animals, and multiplying in them, can give rise to tumours characterised by the possession of powers of infiltrative growth into surrounding tissues and of wide dissemination by the blood and lymphatic vessels, with subsequent growth in distant organs.

It would have detracted nothing from the importance of being able to propagate the cancer-cell, if all the lesions of the disease, as it develops naturally in man and other mammals, had not been reproduced experimentally. Had the cancer-cell been merely cultivated, we should

still have been placed in a position to study the biological distinction between the normal and the cancerous cell. However, the fact that the lesions of cancer can be reproduced experimentally, places us in an even more favourable position in conducting experiments, since we can also study constitutional conditions comparable to those obtaining in animals spontaneously affected, in the subject of experimental cancer, with the enormous advantage that the parenchyma is the same in many animals. While it was natural that the study of the cancer-cell and of the malignant tumour should precede the study of the organism attacked, no time has been lost in approaching the constitutional problems, as various papers in this report show.

(4) Importance of Methods of Propagation.

The importance of slight variations in details of the experimental methods cannot be too strongly insisted upon, before proceeding to an analysis of the results of experimental propagation.

The age, weight, and race of the animals used, the dose of material inoculated, the site of inoculation, and even the method of inoculation, all introduce factors which may modify the end result profoundly. We have repeatedly drawn attention to these points in previous publications, and more extended experience has further emphasised their importance. In the interests of other investigators who may wish to repeat our observations, and in the hope of advancing the adoption of uniform methods in different laboratories, a detailed description of our technique may profitably be prefaced to the description of the results recorded in this and in the four following papers.

It was of course inevitable at the commencement of the development of the experimental study of cancer, that technical minutiæ should vary from one laboratory to another. It is only necessary to review the methods adopted by various workers in this field of investigation, to realise how varied the procedures have been to which material was subjected during transplantation. Hanau inserted fragments of his squamous-celled carcinoma of the rat into the scrotum of adult animals through a skin incision. Morau transplanted subcutaneously by the same method, but also used syringe injections of an emulsion in normal saline solution. In the main Jensen also injected a saline emulsion of his tumour, a method adopted by Clowes and Gaylord, who, in addition, tried to remove the connective tissues. In a smaller number of experiments Jensen inoculated small intact fragments subcutaneously. The routine

procedure adopted for propagation in the laboratory of the Imperial Cancer Research Fund has been the insertion of small fragments by means of a specially devised hollow needle with a tightly fitting plunger (fig. 1, A & A′). Borrel introduced large fragments subcutaneously through a skin incision at first (adopted also by Hertwig and Poll), but latterly has also used small fragments and the hollow needle, as well as a fine suspension in saline for special purposes. Ehrlich prepares an emulsion without any addition, which is introduced through a skin incision with Pasteur pipettes, in doses of considerable size. Schöne, working in Ehrlich's laboratory, states that 0·3 gram of tumour emulsion was used to induce immunity, but the doses used for ordinary propagation were probably smaller.

Of all these methods the one causing least damage to the tumour cells is that in which small fragments of tumour are introduced intact, either by means of a hollow needle, or through a skin incision. This explains the superiority of the results achieved by it in the transplantation of spontaneous tumours as described in a previous paper. Accurate work can be carried out with it if large numbers of animals are used, but this is frequently a disadvantage, as also may be the narrow range within which the doses must be kept. Reflecting on the discrepancies in the results obtained by different workers and the impossibility of exactly repeating the experiments of others, we became acutely conscious of the necessity for a method which would permit of accurate dosage without serious damage to the cells.

The method now adopted in this laboratory for experiments in which both these requirements must be fulfilled, has proved satisfactory and is as follows :—The tumour having been removed aseptically, is reduced to a uniform emulsion. For tumours of firm consistence this is conveniently effected in the small mincing machine devised by one of us (fig. 4 B). Soft tumours can be reduced to a similar condition by clipping with sharp scissors. In our practice the Pasteur pipette which does not permit of accurate dosage, is replaced by a small graduated syringe. The emulsion is drawn up into an all-glass syringe of 0·5 cc. capacity with a wide nozzle. The syringe (fig. 4 C) is calibrated to 0·05 cc., and in practice half this quantity of material can be accurately delivered. The maximum dose which can be given conveniently is 0·5 cc., but in practice it is seldom advisable to exceed 0·2 cc. A hypodermic needle, such as is used in a serum syringe or slightly wider in the bore, is fitted to the nozzle of the syringe and the injections made in the axillary region, or along the flank, by intro-

[*To face* p. 268.

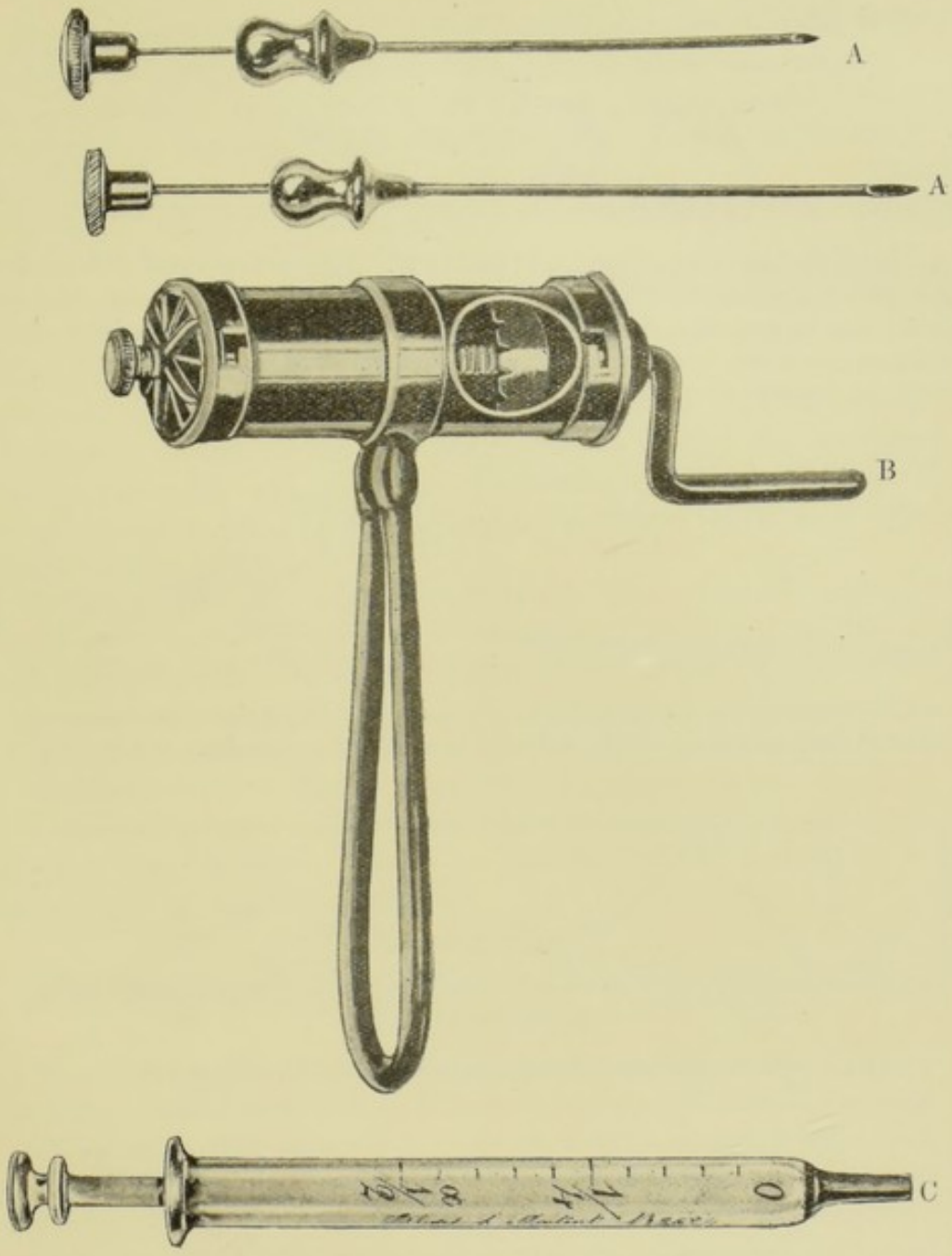


FIG. 4.—A¹. Hollow needle as employed in Imperial Cancer Research Fund laboratory for routine propagation.

A. Ditto as used for early stages.

B. Small mincing machine, latest form devised by Haaland for preparing emulsion of tumours of firm consistence.

C. All-glass syringe of 0·5 c.c. capacity calibrated to 0·05 c.c. for use with A'. The needle A may also be used. All natural size.

To face p. 269.]

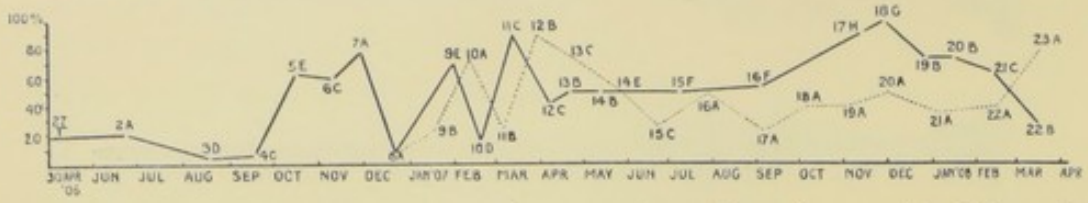


FIG. 5.—Percentage-curve of propagation of tumour 27. After a short period of depression the curve rises rapidly till the 7th generation and then falls. A second and third rise then follow in two strains propagated separately. While one (black line) falls again and then rises slowly to a maximum, the other (dotted line) fluctuates between 35 and 50 % for 9 months, after which it also rises.

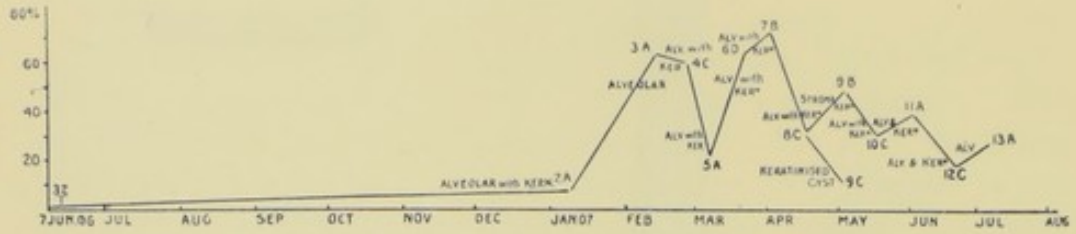


FIG. 6.—Percentage-curve of propagation of tumour 32. Rapid rise in percentage of success at third transference (3 A), followed by a fall and second rise. (*Cf.* p. 162).

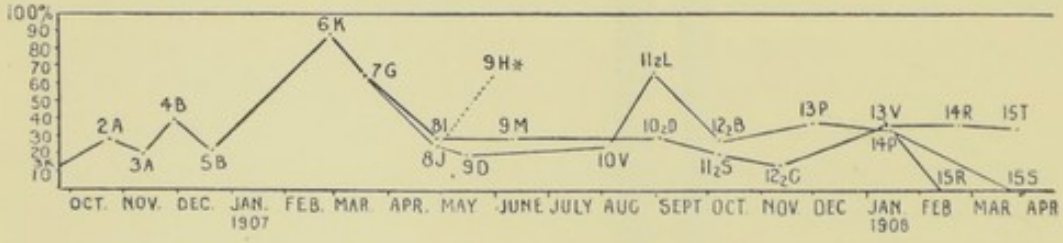


FIG. 7.—Percentage-curve of propagation of tumour 37. *Cf.* p. 246).

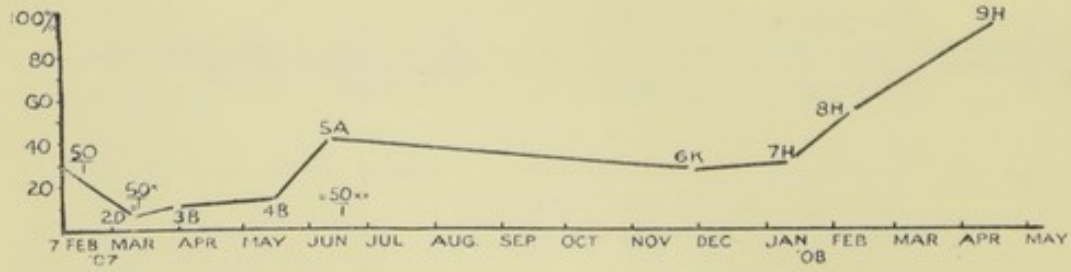


FIG. 8.—Percentage-curve of propagation of tumour 50.

ducing the needle in the groin and ejecting the emulsion during withdrawal. Between each injection the needle is wiped carefully on a pledget of sterile cotton wool moistened with absolute alcohol. It is unnecessary, except in special cases, to epilate the site of insertion, which with advantage is moistened with alcohol.

With this method and the method of small intact grafts introduced with the hollow needle, many thousands of experiments have been performed with a great variety of tumours, the doses of emulsion varying between 0·025 cc. and 0·25 cc. with the syringe, and between 0·03 and 0·005 gr. with the needle.

A consideration of the results of such experiments with tumours of all degrees of transplantability, has convinced us that the constants ascertained in this way vary from one tumour to another, and are of the greatest importance in determining the biological behaviour of each tumour under experiment.

While practically all spontaneous tumours of the mammary region in the mouse* can be induced to grow by inoculating large numbers of young animals with small intact fragments, the number is much more limited, in which inoculation of 0·05 to 0·1 cc. of tumour emulsion gives a successful result. Hence those who have relied on the latter method exclusively for the transplantation of spontaneous tumours, have unconsciously selected a much more one-sided material than is at our disposal, since the method we use has permitted of the propagation of the majority of spontaneous growths.

(5) Increased Adaptability and Biological Alterations of Tumour Cells during Propagation.

The initial transference of a malignant new growth from the spontaneously affected animal to the first series of normal animals (primary transplantation, first generation) is in most cases attended with great technical difficulties. To illustrate the difficulties which have to be overcome, it may be noted, that only 518 tumours resulted from 5791 inoculations made into normal mice with 84 spontaneous malignant new growths, a success of only 11 %. Success varied in individual experiments between 0 % to 30 % of the mice used, and it must be

* As regards the spontaneous tumours inoculated, the proportion of transplantable tumours in our experience is very high. As recorded by Murray and Gierke on earlier pages, the inoculation of 48 consecutive hæmorrhagic mammary carcinomata was successful for 39, or 80 %, and of 71 consecutive mammary tumours only 16 failed to grow.

carefully noted that even the tumours which developed in the series with the highest percentage (exp. $\frac{50}{1}$, fig. 46, p. 105) grew very slowly, and in fact were first palpable as minute nodules 14 days after inoculation. Over two hundred inoculations of a squamous-celled carcinoma were required in order to obtain the single daughter-tumour from which propagation has been continued. In the same way 374 inoculations of a sarcoma were entirely unsuccessful ; in short, the difficulty of starting the propagation of cancer in mice is very great, and for the forms of cancer which are most common in the mouse so great, that Ehrlich assumed they were not propagable at all.

Of the factors affecting the result, the most important in our experience are the age of the inoculated animals (young mice of 6 weeks are most suitable), and the dose, and method of inoculation. It is very significant that the primary transplantation of spontaneous tumours reveals even a more marked variation in susceptibility to the influence of dosage than do tumours in later generations. These characteristics of primary transplantations are, for the most part, due to the failure of the tumour cells to adapt themselves to the altered conditions of existence inseparable from the act of transference from the animal in which the cells first acquired malignant properties to normal animals. The most important consequence is that the cells which survive are much diminished in number compared with the number in the graft introduced. It must be evident, however, that actual death of introduced cells can only be the terminal event of a series progressing gradually through many stages of decreasing damage up to unaltered vitality or perfect adaptation.

With successive transferences following the primary transplantation, the number of cells in each graft which survive and proliferate increases. Whether this is due to elimination of cells less able to adapt themselves to varying conditions, or to the acquisition of greater adaptibility and power of growth and resistance to injury, by cells previously only able to survive with difficulty, is not of any great moment. It is probable that both processes are realised in propagation, and if we incline to ascribe greater importance to the former factor, it is only because on that assumption a greater number and range of facts are easily harmonised and brought under review.

The extent to which increased adaptability alters the facies of the actual experiments can be illustrated in several ways. The percentage-curve of propagation, constructed in the manner described on p. 290 in

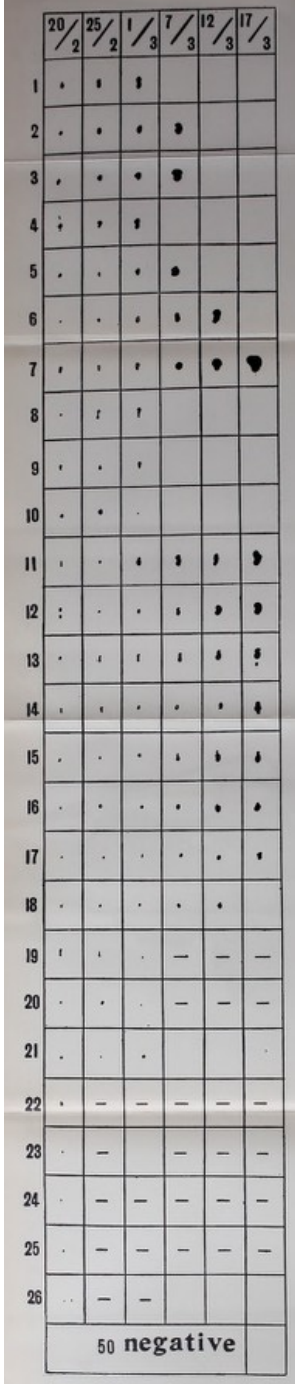


Fig. 9.—Graphic record of exp. $\frac{50}{1}$. 81 mice inoculated 7·2·07 with 0·02 gr. of the primary tumour $\frac{50}{1}$. First charting 13 days later. Cf. fig. 7.

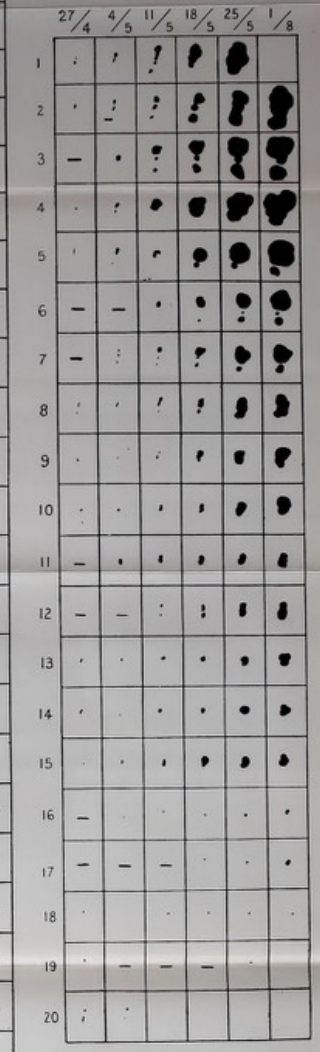


Fig. 10.—Graphic record of exp. 50/9 H. 20 mice inoculated 17.4.08 in right axilla with 0·025 c.c. of an emulsion of a tumour of the 8th generation (8 H, 36 days old). First charting 10 days later. Cf. fig. 6.

the "Experimental Analysis of the Growth of Cancer," shows the phenomenon very clearly. The curve ascends with each transference sometimes rapidly, sometimes more slowly, for three, four, or more generations, as is illustrated for our tumour-strains 27, 32, 37, and 50 in figs. 5, 6, 7, and 8. The same initial rise is shown in the other curves illustrating the development of sarcoma in strain 37 (pp. 246 & 247), not only in the case of the primary tumour but also for each of the sarcoma strains.

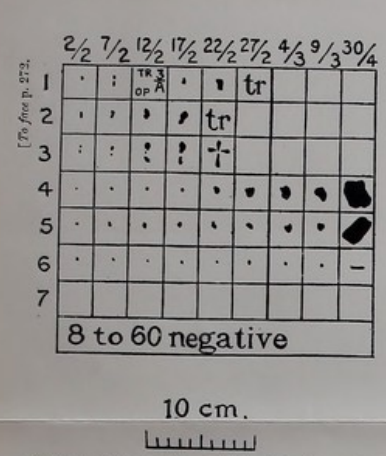
When the rapidity of growth of individual tumours is recorded, the intimate features of the process are more clearly revealed without the obscuring influence of a phase of diminished energy of growth such as immediately follows the primary transplantation in the strains illustrated in figs. 5 & 6 of tumours 27 and 50 *. Fig. 9 shows the rate of growth of the tumours arising from the primary transplantation of tumour 50; fig. 10 shows a later series of the 9th generation, 50/9 H, in which an unusually high percentage of success was obtained. The initial dose of tumour material was practically (0·02 gr., 0·025 cc.) the same in both series, and the charts show, and this is the important fact, that the amount of tumour-tissue produced in the same time is much greater in the later than in the primary transplantation. Figs. 11, 12, and 13 show the same differences for the 2nd, 3rd, and 4th generations of tumour 32. The primary transplantation of this tumour gave four minute growths in 156 mice. The only tumour which grew progressively attained the size of a pea in four months. The tumours of the 2nd generation (2 A, fig. 11) grew much more rapidly, but at a much slower rate than those of the 3rd and 4th generations illustrated in figs. 12 & 13. Figs. 14, 15, 16, & 17 show the difficulty with which propagation was

* The graphic method for recording the growth of tumours is one which is widely employed in different laboratories. The necessity for being able to refer back to the behaviour of tumours at earlier dates speedily convinced many workers that some quickly comprehended record of size of individual tumours in relation to duration of growth was required. The outlining of tumours naturally suggests itself, and it has long been a routine practice, *e. g.*, in Ehrlich's laboratory and at Buffalo, U.S.A., and no doubt elsewhere. In the publication of papers, however, it has not been so widely used, notwithstanding the readiness with which it conveys a clear picture of results to others. Apolant employed it to illustrate the diminution in the size of tumours after exposure to radium. Gaylord and Clowes used it in a paper on the "Spontaneous Cure of Cancer." Haaland employed it to show the different amount of growth produced by the same tumour in mice from different sources, and by different tumours in the same mice. We have further elaborated the method in accordance with the exigencies of our experiments.

continued from primary tumour 72. The primary inoculation gave one progressively growing tumour in 120 mice. The 2nd generation gave 33 % of relatively rapidly growing tumours. The 3rd generation was again very unsuccessful, as was the 4th. A considerable improvement in percentage of successes and rate of growth was not obtained till several generations later. It is unnecessary to multiply instances, by giving similar charts for all the 40 transplantable tumours which are now growing progressively in this Laboratory. Suffice it to state that we have made it the object of special investigation to ascertain if tumours most difficult to propagate possessed restricted or unlimited powers of growth. Tumour 72 (figs. 14–17) illustrates a growth very difficult to propagate and tumour 47 is even more so, but they can be propagated continuously. When once a tumour has been transplanted successfully, the cessation of growth is not an expression of a natural limitation to its amount but reveals insufficient care on the part of the investigator. With slight modifications the figures illustrating the behaviour of the tumours mentioned, might serve for the rest.

The fluctuations in percentage of successes which are a constant feature of all our percentage-curves of later propagation, are accompanied also by corresponding fluctuations in the rapidity of growth of the transplanted tumours, but the parallel is not always very close. While a few slowly growing tumours are usually found in series with high percentage of successes, rapidly growing tumours may, and frequently do, occur in series with very low percentage; but in the aggregate the two characters vary concomitantly. These comparisons can only justly be made between series belonging to the same tumour strain; different strains exhibit as an important biological characteristic, the most diverse combinations of percentage of success and energy of growth of the actual tumours, so that the independence of the two characters is very marked when we pass from one strain to another.

It is probable that secondary biological alterations may accompany adaptation. Since there are natural variations in the rate of growth, an accelerated rate of proliferation may be one result of long continued selection of rapidly growing tumours. The environment may likewise have exercised important influence when a tumour has been propagated for years. One of the earliest results of experimental cancer research was the evidence brought forward by Bashford and Murray and by Michaelis, that the tumours of mice of one country did not grow well, or at all, in the mice of another, although the former observers also showed the rapidity with which the cells could be adapted to their new



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FIG. 11.—Graphic record of exp. 32/2 A. Very slow growth of tumours arising from inoculation of 0·02 gr. of the single tumour of the primary transplantation (32/1, 7 months old) into 62 normal mice on 8.1.07.

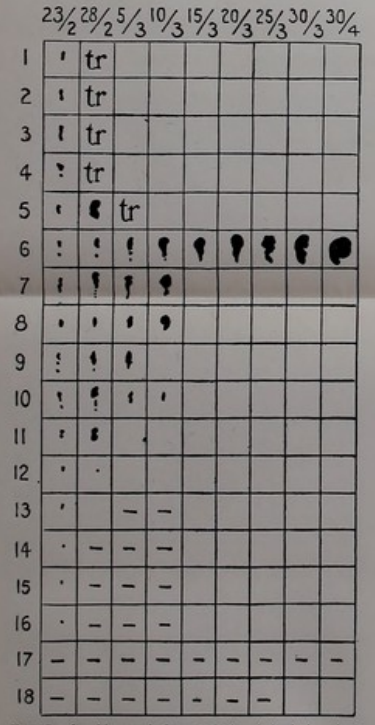


FIG. 12.—Graphic record of exp. 32/3 A. Animals inoculated 12.2.07 with 0.03 gr. each of tumour removed by operation from mouse no. 1 of exp. 32/2 A, fig. 8 (35 days after inoculation).

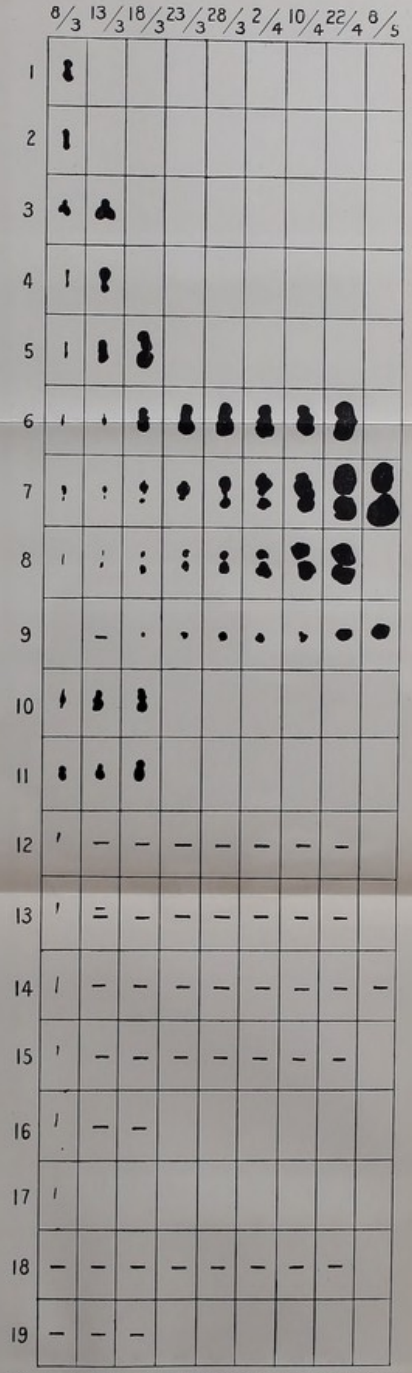


FIG. 13.—Graphic record of exp. 32/4 C. Mice inoculated 26.2.07 in right axilla with 0·025 of tumours from mice nos. 3 and 4 of exp. 32/3 A (13 days after inoculation).

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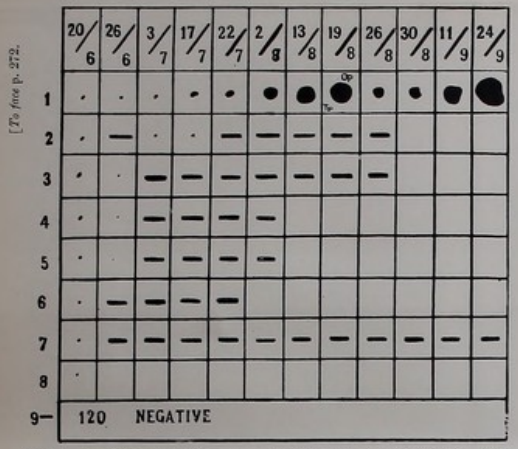


FIG. 14.—Graphic record of primary transplantation of tumour 72, exp. 72/1. Only one tumour was obtained in 120 mice inoculated 1.6.07 with 0·02 gr. of tumour tissue, removed from the spontaneously affected mouse by operation.

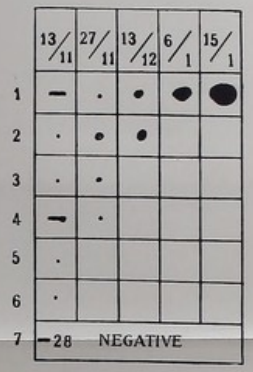


FIG. 16.—Graphic record of exp. 72/3 A. Growth of tumours obtained from inoculation into right axilla of 28 mice, of 0·02 gr. each of tumour obtained from mouse no. 1 of exp. 72/2 A, fig. 12 (58 days after inoculation).

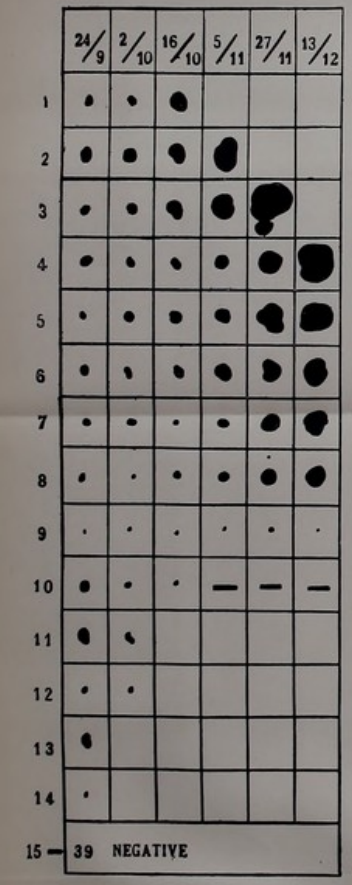
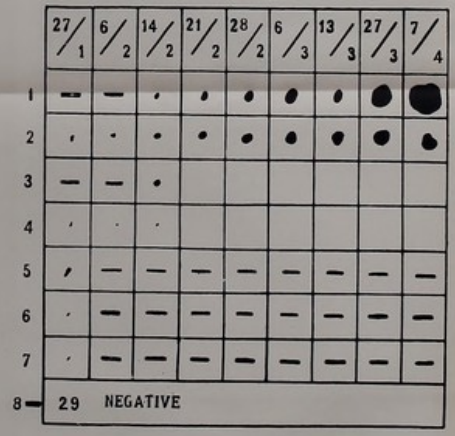


FIG. 15.—Graphic record of exp. 72/2 A. Growth of tumours obtained from inoculation into right axilla of 39 mice of fragments of 0·02 gr. each, of the tumour of mouse no. 1 in exp. 72/1, fig. 11, removed by operation 19.8.07.



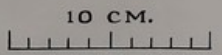


FIG. 17.—Graphic record of exp. 72/4 A. Growth of tumours obtained from inoculation into right axilla of 29 mice, of 0·02 gr. each of tumour no. 1 of exp. 72/3 A.

environment. The phenomenon is probably parallel to that observed for the primary transplantation of sporadic tumours, when a violent change in environment is effected at the time of transference from the animal in which they developed. Further observations in this direction were made by one of us *. Last year, however, the accuracy of all such observations was impugned by Hertwig and Poll on inadequate evidence. We have sent our tumours to a large number of investigators abroad, and Gierke in particular has extensively tested a number of cases, transplanting our tumours into German mice both in London and Berlin, with the result that the objections of Hertwig and Poll have not been sustained.

Theoretically the long continued growth of cells in the soil provided by the mice of one stock or country may handicap them for growth in the different soil provided by mice of another stock or country, or even in the soil to which they were originally accustomed. We have, therefore, repeated our earlier observations on Jensen's tumour on fresh material sent from Denmark, together with some Danish mice. While owing to the limitation of numbers they are not conclusive, the experiments showed that after three years' growth in English mice, Jensen's tumour had not lost its power of growth in Danish mice in which, on the whole, it grew somewhat better than the control Danish tumour although the English tumour grew better in English than in Danish mice. There are, therefore, some experimental indications of adaptation and prolonged propagation leading to other secondary biological alterations. What determines them would appear at first sight to be mainly the long duration of the particular environment rather than the number of successive transferences from animal to animal, but in the preceding paper on development of sarcoma, it has been shown that the experimental transference is also of great moment in determining the progressive alteration which leads to the acquisition of sarcomatous properties. What takes place during artificial propagation may be but an artificial re-production—although perhaps under influences of greater intensity—of what goes on in the animal in which cancer develops naturally.

(6) The Importance of Dose in determining the Rapidity of Increase in size of Tumours.

The size the tumours of any one strain attain in a given time, is in part determined by the proportion of the introduced cells which adapt

* Berliner klin. Wochenschrift, 1907, No. 23.

themselves to the new conditions : *i. e.* by the size of the *effective* initial dose. That this is the case can be demonstrated by experiments in which the initial dose of tumour material varies.

The simplest phenomenon is encountered in the experiments with tumours which grow with a rapidity proportional to the initial dose introduced. A transplantable spindle-cell sarcoma of the rat, for which we are indebted to Professor Jensen, illustrates the subject now under discussion in a diagrammatic manner. In fig. 18 a graphic record is given of an experiment in which two groups of rats were inoculated with different doses of the same material. Rats 1–8 received 0·2 cc. of tumour emulsion in the right axilla, while rats 9–20 received only 1/20th of that amount (0·01 gr.). The size attained by the tumours in the animals inoculated with the larger dose, at the end of ten days, and their subsequent progress, present a striking contrast to those of the tumours arising from the small dose. They are nearly twenty times as large, and the animals succumb much more quickly. It is important to note that the initial percentage of successes is the same in both series, viz. 100 per cent. The table on fig. 18, gives the weights of the tumours produced by large and small doses, in 11 and 15 days.

When experiments with *these doses* are made with other tumours, and especially with transplantable mouse carcinomata, the same result is rarely obtained. Fig. 19 (exp. 32/23 E) records a similar experiment with a transplantable squamous-celled carcinoma (tumour 32). Half the mice (1–14) of this experiment were inoculated with 0·025 cc. of tumour emulsion, and the other half (15–28) with 0·15 cc. of the same material. An initial proliferation took place in all, but although the doses were as 1 : 6, the sizes of the tumours arising from the larger doses are only in a few instances greater than those originating from the small doses, and even then little more than twice as large. The subsequent progress of the tumours in the two series differs in much the same way as in the experiment with the rat sarcoma. While nine of the large dose tumours grew progressively and rapidly, only eight of the small dose series advanced. The remaining six (9–14) disappeared spontaneously in the first half of the experiment, five (24–28) disappeared in the other, and in correspondence with their larger size, more slowly. The difference between the two halves of exp. 32/23 E is due to the differences in initial dose in the two cases. The differences in rapidity of growth between the tumours of the first half of exp. 32/23 E and exp. 32/4 C (fig. 13) can be adequately accounted for by the influence of the same factor ; the effective initial dose is greater in one

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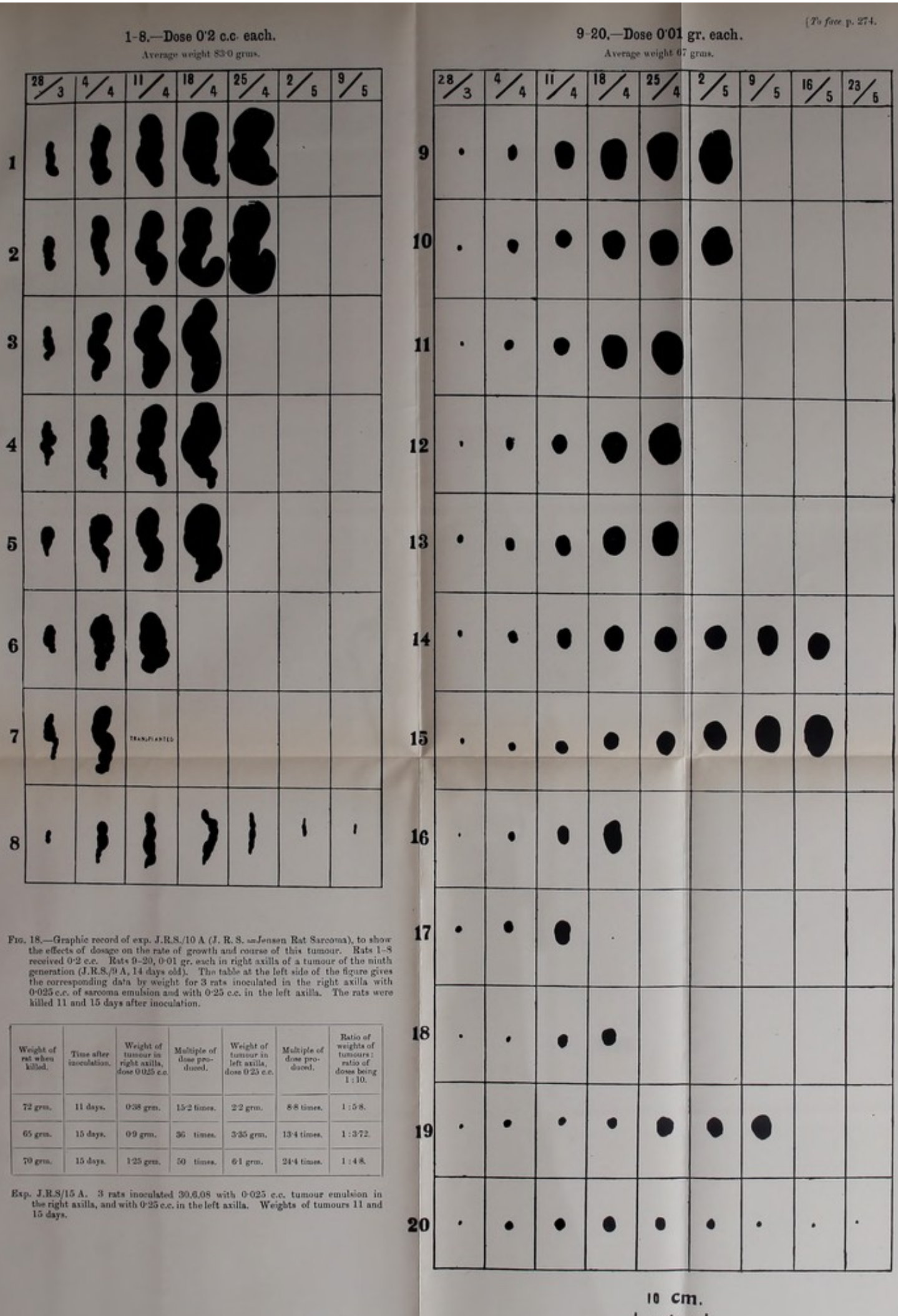


Fig. 18.—Graphic record of exp. J.R.S./10 A (J. R. S. on Jensen Rat Sarcoma), to show the effects of dosage on the rate of growth and course of this tumour. Rats 1–8 received 0·2 c.c. Rats 9–20, 0·01 gr. each in right axilla of a tumour of the ninth generation (J.R.S./9 A, 14 days old). The table at the left side of the figure gives the corresponding data by weight for 3 rats inoculated in the right axilla with 0·025 c.c. of sarcoma emulsion and with 0·25 c.c. in the left axilla. The rats were killed 11 and 15 days after inoculation.

Weight of rat when killed.	Time after inoculation.	Weight of tumour in right axilla, dose 0·025 c.c.	Multiple of dose produced.	Weight of tumour in left axilla, dose 0·25 c.c.	Multiple of dose produced.	Ratio of weights of tumours: ratio of doses being 1:10.
72 grms.	11 days.	0·38 grm.	15·2 times.	2·2 grm.	8·8 times.	1:5·8.
65 grms.	15 days.	0·9 grm.	36 times.	3·35 grm.	13·4 times.	1:3·72.
70 grms.	15 days.	1·25 grm.	50 times.	6·1 grm.	24·4 times.	1:4·8.

Exp. J.R.S/15 A. 3 rats inoculated 30.6.08 with 0·025 c.c. tumour emulsion in the right axilla, and with 0·25 c.c. in the left axilla. Weights of tumours 11 and 15 days.

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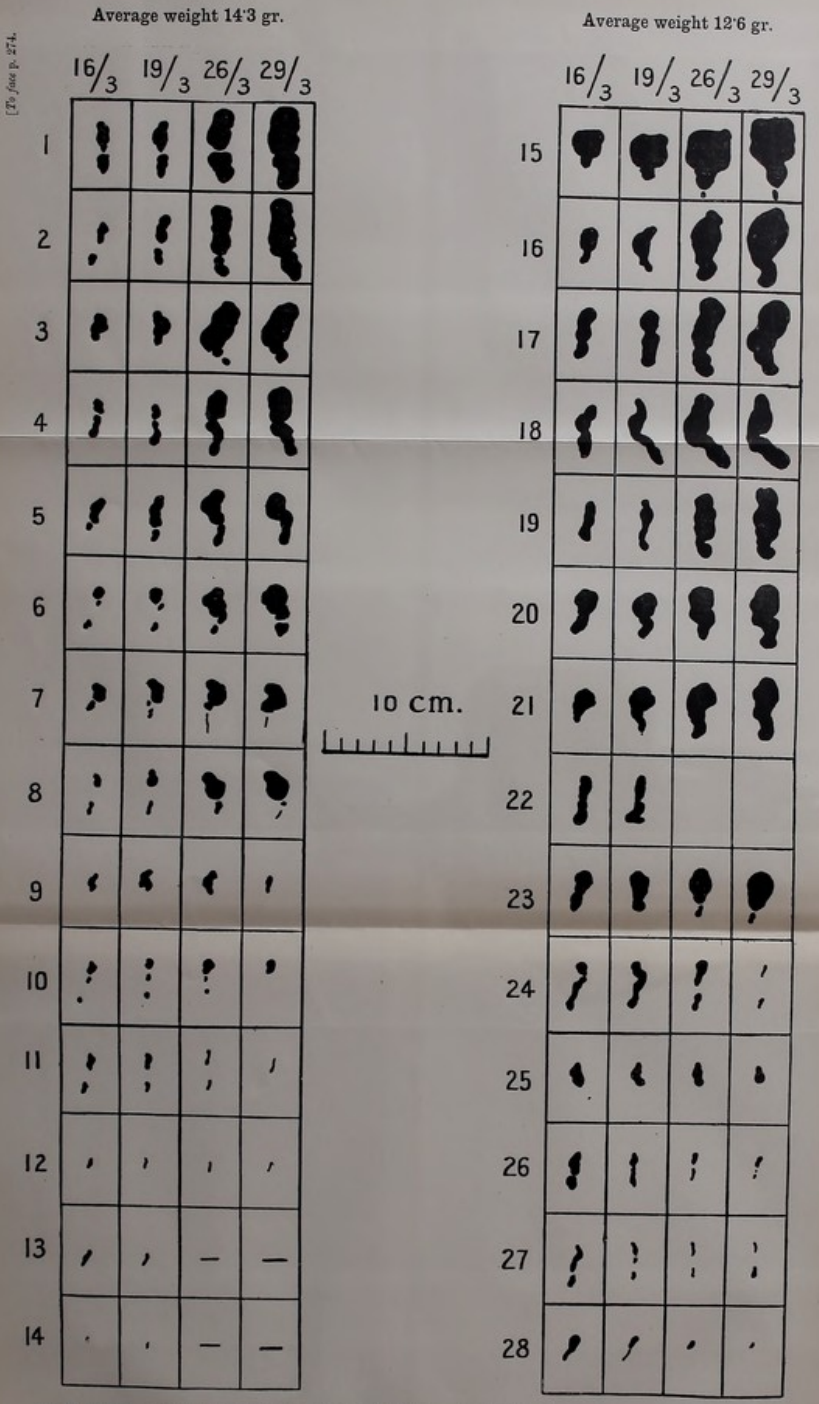


Fig. 19.—Graphic record of exp. 32/23 E to show a similar effect of difference in dose with this mouse tumour to that shown in fig. 18 for Jensen's rat sarcoma. All mice inoculated in right axilla 6.3.08 with emulsion of a tumour of 22nd generation (32/22 B), mice 1–14 with 0·025 c.c., mice 15–28 with 0·15 c.c. Higher percentage and more rapidly growing tumours with the larger dose.

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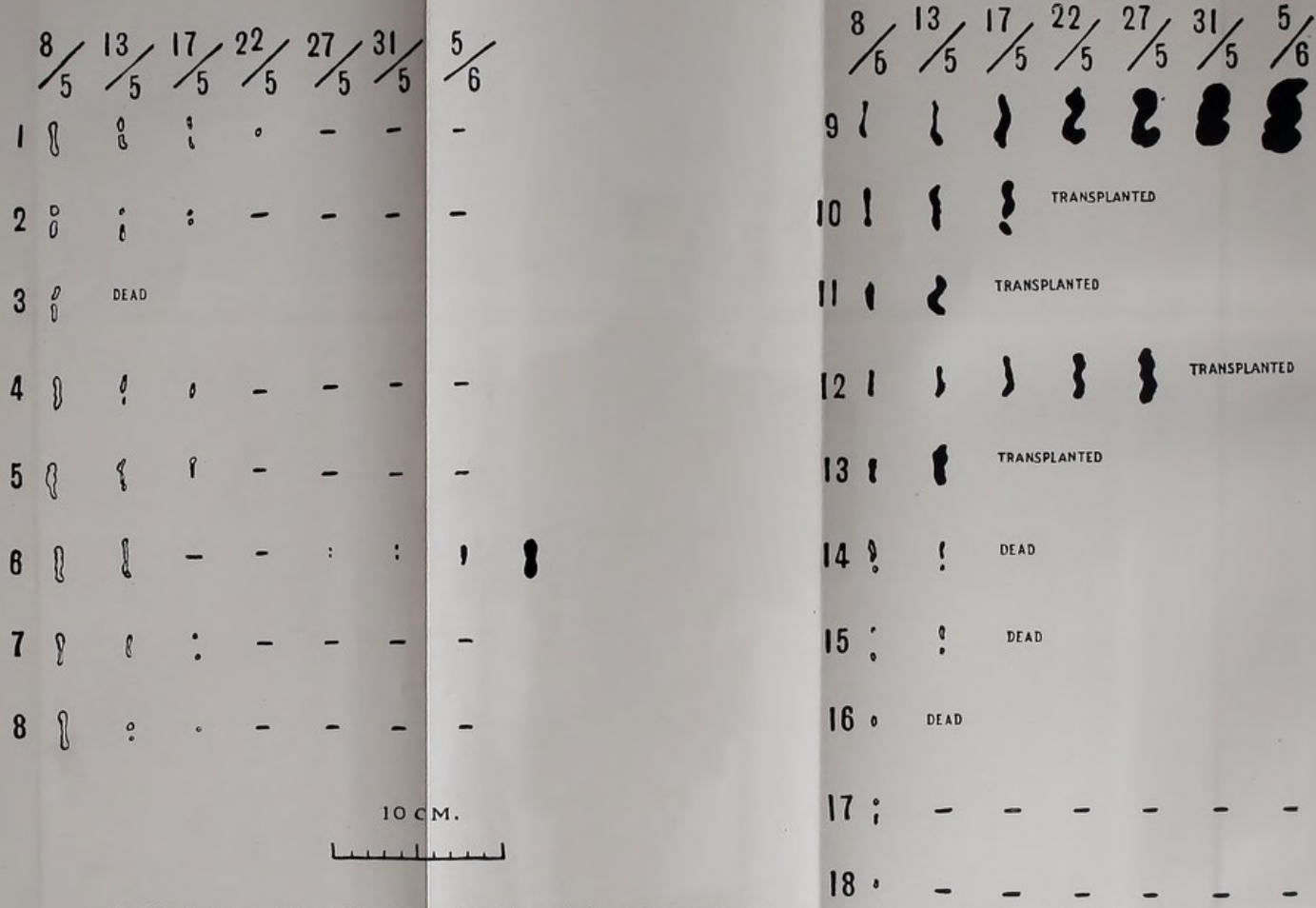


FIG. 20.—Graphic record of exp. 32/8 L. Opposite effect of increase in dose to that shown in fig. 19, on the growth of tumour 32. Mice 1–8 inoculated with 0·1 c.c. Mice 9–18 with 0·05 c.c. of emulsion of a tumour of 7th generation (32/7 E, 22 days old), in right axilla 30.4.07. Complete disappearance of temporary tumours in the mice inoculated with 0·1 c.c., progressive growth of the tumours arising from 0·05 c.c. In mouse no. 6, inoculated with the larger dose, the tumour reappeared and grew progressively.

than in the other, although the amount of tissue introduced was the same in both, and there is no reason to suppose that the contrast between Exp. 32/4 C and Exp. 32/2 A requires a different explanation. In Exp. JRS/10 A (fig. 18), and Exp. 32/23 E (fig. 19), these differences are deliberately introduced. The diminution of the effective dose in Exp. 32/2 A as compared with Exp. 32/4 C is unintentional and due to the still imperfect adaptability of the tumour cells to new hosts.

Similar experiments with many tumours show that the contrast between the results of inoculation of large and small doses are often the reverse of those which we have been considering so far. Fig. 20, Exp. 32/8 L, shows graphically the contrast in the results of inoculation in two series of similar mice, the only difference being that while one lot received 0·1 cc. each of an emulsion of squamous-celled carcinoma, the other received 0·05 cc. of the same material. The figure shows that in both series a considerable proliferation had taken place eight days after inoculation. The subsequent course of the tumours in the two lots, recorded every five days, presents a remarkable contrast. The tumours which developed in the animals inoculated with the smaller dose, mice 9–18, grew rapidly and progressively with the exception of the small nodules in mice 17 and 18. The initial proliferation in the mice which received 0·1 cc. of tumour emulsion, mice 1–8, was not maintained. Sixteen days later no trace of tumour-cells could be felt in any of those which survived. In one (no. 6) a small nodule appeared after a further interval of ten days and grew progressively ; the other mice remained negative. This is the most usual result when mice are inoculated with large and small doses. Small doses grow progressively and well, whereas large doses, even when followed by a more pronounced initial proliferation, give tumours which remain stationary or disappear spontaneously. The natural resistance of the mice cannot be invoked to explain the anomaly. The days immediately after inoculation preceding the revascularisation of the graft, constitute the most vulnerable period in the course of transplanted carcinomata, and the parallelism in the course of events in the two series of exp. 32/8 L, shows that the constitutional characteristics of the mice in them, do not differ essentially.

Figs. 21 & 22, which record similar experiments with Jensen's carcinoma, illustrate the same contrast between the results of inoculating large and small doses at different times. In the experiment shown in fig. 21 three groups of similar mice were inoculated with different doses of the same material (exp. J/99 C). Mice 1–10 received 0·025 cc.,

11–19 0·05 cc., and 20–31 0·1 cc. The sizes attained by the tumours in ten days, and their subsequent rates of growth are progressively greater with increased initial dose (*cf.* exp. JRS/10 A and exp. 32/23 E. Exp. J/102 C (fig. 22) shows a different result. The disposition of the experiment is the same, but while tumours appeared and grew in the mice with the smallest and the largest doses, only one small slow growing tumour was obtained in the group inoculated with the medium dose (0·05 cc., 11–19). These phenomena will be discussed on a later page. They are probably due to the quantitative relations between the dose of tumour and the reaction of the animals, and to concomitant immunization which was not effective in the small dose, was effective and not overcome by the medium dose and overcome by the large dose.

The contrast between the behaviour of tumours 32 and Jensen at different times, with respect to the effects of variations in the dose of material inoculated, can only mean *that the cells are biologically different at different times.* The phenomenon is also seen in similar experiments with most other transplantable tumours. It is important to note that the experimental sarcoma developed during the propagation of tumour 37, exhibits it also, in contrast with Jensen's rat sarcoma, which in our experience does not. At times large doses give a higher percentage of more rapidly growing tumours than small doses, at others the initial proliferation from large doses is followed by spontaneous absorption. The transplantable chondro-osteo-sarcoma (tumour 92) behaves in the same way.

(7) Effect of Concomitant Immunization and of Alternations in Tumour Cells.

The effect of absorption of tumour-tissue concomitantly with the initiation of growth after inoculation is manifested not less clearly, though to a slighter degree, and in another way, in practically every graphic record. This particular effect must be discussed here, because of its important bearing on the interpretation of percentage-curves and graphic records of growth, as an analysis of cancerous proliferation. It will not have escaped notice that in most series of inoculations of which illustrations have been given, a number of animals occur in which temporary proliferation * is followed rapidly by absorption of the tumour nodules. This phenomenon is a feature of all propagable tumours

* This temporary proliferation is quite different from the phenomenon described and figured in detail by Bashford, Murray and Cramer in the Second Scientific Report as natural healing of a large tumour, and recorded independently by Clowes,

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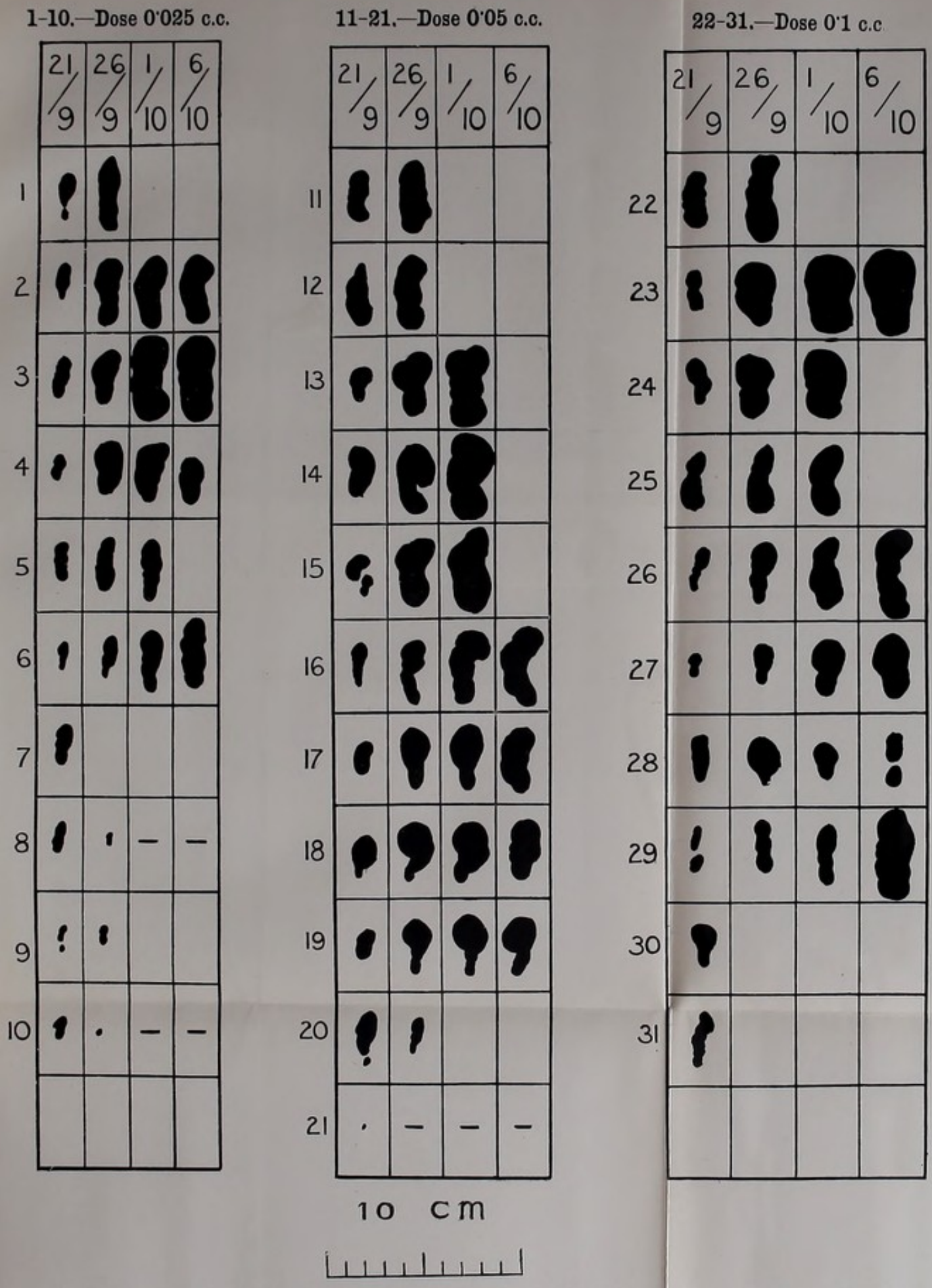


Fig. 21.—Graphic record of exp. J/99 C. All mice inoculated in right axilla, 11.9.07, with 1–10, 0·025 c.c., 11–21, 0·05 c.c., 22–31 0·1 c.c. of emulsion of Jensen carcinoma (tumour of J/98 B, 44 days old).

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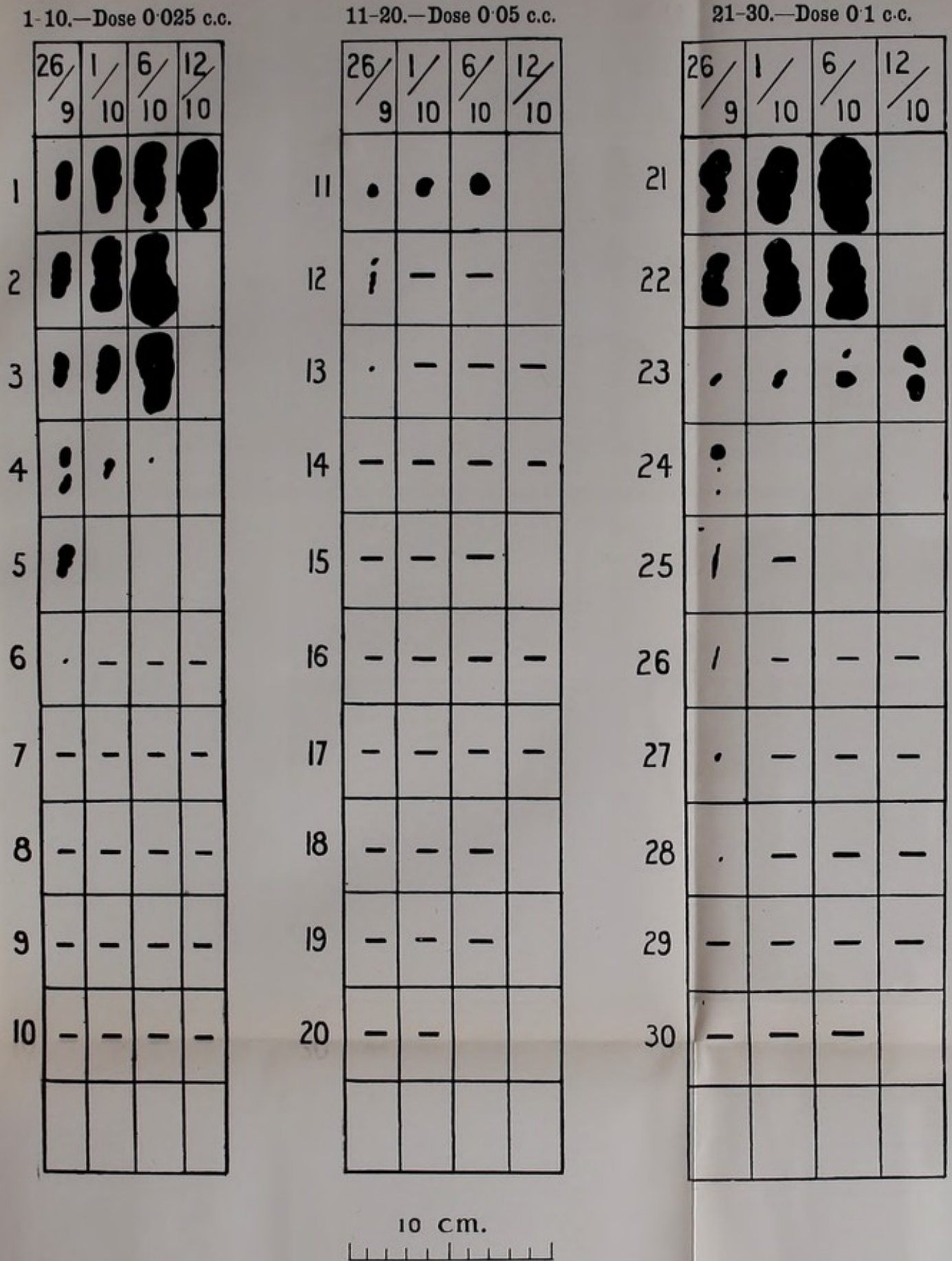


Fig. 22.—Graphic record of exp. J/102 C. All mice inoculated in right axilla 16.9 07, 1–10, ·0250 c.c., 11–20, 0·05 c.c., 21–30, 0·1 c.c. of emulsion of a tumour of 101st generation (J/101 A, 18 days old tumour).

we have studied, and is also seen in the primary transplantations of spontaneous tumours. It is probably not correct to explain this absorption of temporary tumours by assuming natural resistance of the inoculated animals. If the animals were naturally resistant, we should expect proliferation to be inhibited from the start, as it will be shown is the case in animals rendered resistant by artificial means. This is seldom the case, however, the resistance is not pre-existent, but an alteration in the resistance is consequent on the inoculation. The alteration is in all probability brought about by the absorption of tumour material in the days following inoculation. The inoculated tumours which grow progressively, frequently show a retardation of the rate of growth, or even a temporary diminution in size, simultaneously with the commencement of the disappearance of the temporary tumours in the same experiment. This is exhibited in an exaggerated form in Mouse 6, Exp. 32/8 L (fig. 20). It takes place between the 10th and 24th days after inoculation and generally sets in immediately after the 10th day, is very noticeable at the 17th day, and, if the tumours grow progressively, has already passed off by the 24th day, when the tumours are charted for the third time. How is it that in some animals the influence of tissue absorption leads to complete disappearance of temporary tumours, while in others only a temporary retardation of growth occurs? Two factors apparently combine to produce this effect: the intensity of the reaction (due either to peculiarities of the animal, or to a greater or less absorption of tissue), and the condition of the tumour cells which are growing. The second of these two factors, although not yet accurately defined, is probably of equal importance with the first. In effect the tumour-cells which, as pointed out above, are biologically different at different times, present phases in which they are more susceptible to unfavourable conditions, alternating with phases in which they are less susceptible. The discrepancies between the results of inoculating large and small doses of the same tumour-strain at different times, indicates this difference of phase in one way; the fluctuations in percentage of successes indicate it in another, and these temporary fluctuations immediately following inoculation in single inoculated tumours, indicate it in a third. The different behaviour of the tumours arising from larger and smaller doses, and the temporary diminution in size or disappearance after initial proliferation, must be referred to the effects of the absorption of more or less tumour material, inducing an adequate specific resistance of the animals in the one case and not in the other. The nature of the difference between tumours

which support transplantation by large doses and those which do not, is by no means clear. It is not merely a difference in capacity for independent life of the cells; a closer approximation to the "cytotypic" mode of growth in R. Hertwig's terminology. The strength of the specific resistance following absorption of the same quantities of different tumours seems to vary from one strain to another, as does also the susceptibility of the tumour cells to such altered resistance, and it is obvious that differences of this kind will manifest themselves also, by varying susceptibility to dosage.

This process which may be described as a concomitant active immunization, is in part responsible for the low percentage of success attending the primary transplantations of sporadic tumours. The ratio of absorbed tissue in them is higher, and the initial proliferation is less, in conformity with the failure of adaptability. This effect is exaggerated when primary transplantation is carried out with large doses, and is diminished when minute grafts are introduced. Where mice are naturally resistant to the tumour inoculated, concomitant immunization greatly enhances it.

(7) Alternating changes in Tumour-Cells.

Temporary diminution in size also occurs in large tumours, long after the period in which the effects of tumour absorption manifest themselves in the manner described above. This is of much rarer occurrence than the earlier phenomenon, and is seen only when tumours remain under observation for periods of $1\frac{1}{2}$ to $2\frac{1}{2}$ months. It has usually occurred in our experiments simultaneously with the diminution in size which ends in the complete spontaneous absorption of sister tumours of the same series, and was a conspicuous feature of one of the tumours, of which Clowes and Gaylord published a chart in 1906 *. The chart of Exp. $\frac{50}{1}$ at p. 105 shows this phenomenon in two tumours, Nos. 3 and 25. Fig. 23 shows it also in single tumours of Jensen's carcinoma. When the cases which have been observed in a single tumour-strain, are entered with reference to the absolute duration of propagation on a percentage-curve as in fig. 23, they show a remarkable grouping. The process may apparently occur synchronously in three strains of the same tumour, two of which have been propagated separately for several months. Although the rarity of the phenomenon admits a wide entrance for coincidence, the whole character of the process when considered

* On Spontaneous cure of Cancer; Surgery, Gynæcology, and Obstetrics, June 1906, p. 9.

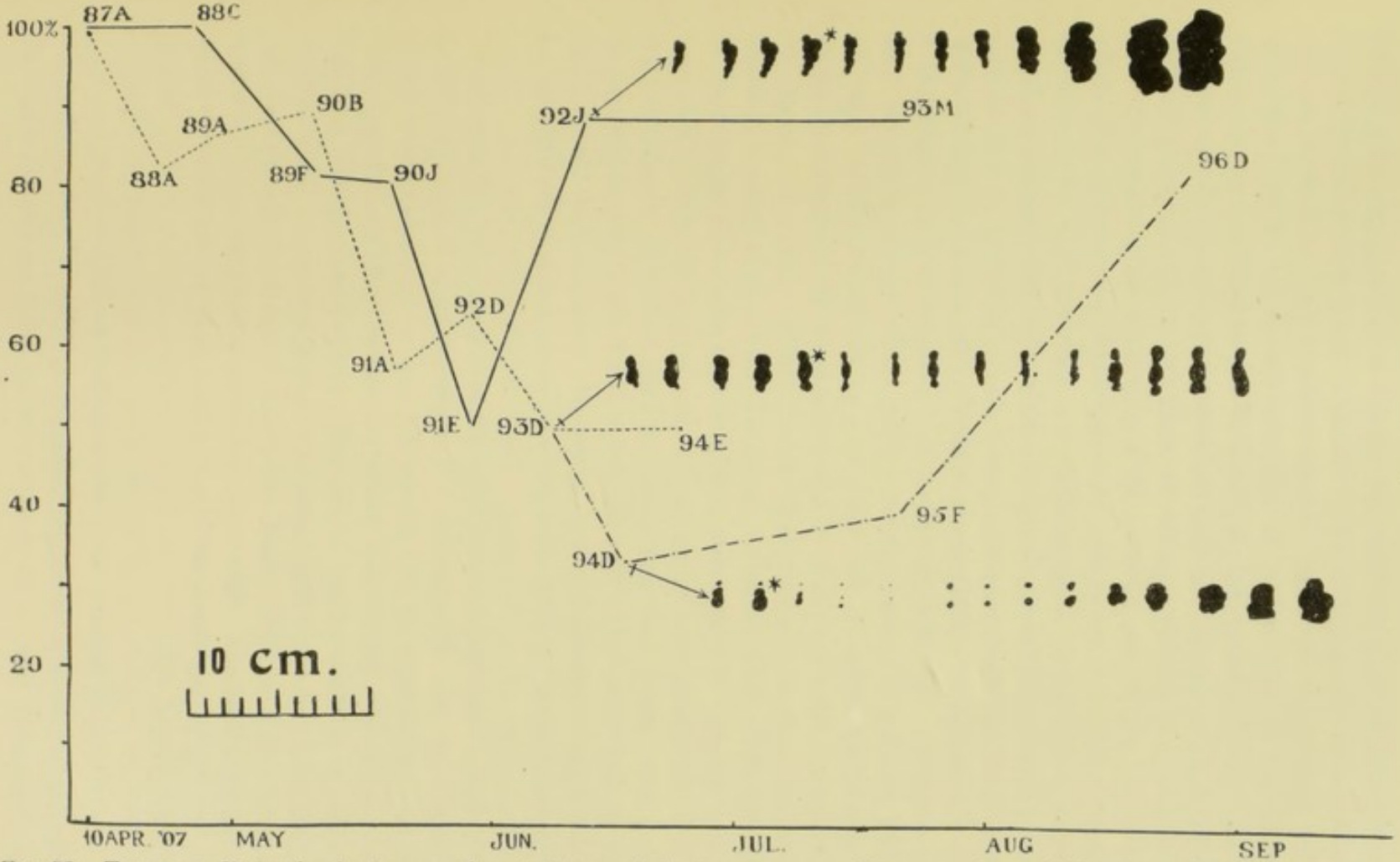


FIG. 23.—Temporary diminution in size occurring simultaneously in three tumours of Jensen carcinoma, belonging to three different series, 92 J ——, 93 D . . . ., and 94 D — · — · —. The silhouettes of the tumours are drawn on the ordinates of the chart representing the dates at which each was made, the first charting being 10 days after inoculation in each instance. The time at which the diminution was first apparent is marked *; renewed growth began in all about the same time.

in conjunction with what has been related above, strongly suggests its dependence on changes of a periodic kind in the parenchyma-cells.

The percentage-curves of separate tumour strains show a general resemblance to each other, in that the curves follow an undulating course. The frequency and amplitude of the undulations vary from one tumour strain to another, so that the general appearance of the curve is characteristic and fairly constant for each strain. The meaning of these differences, which can be seen at a glance on comparing curves for different tumours, is not at once obvious. The rapidity of growth of the tumours is only partly responsible for the differences in frequency. It is obvious that a rapidly growing tumour can be transplanted at much shorter intervals than one which grows slowly, and that in consequence if the percentage of success changes rapidly, the curve will present steep rises and falls. We find, however, that consecutive transferences of a rapidly growing tumour, frequently differ in percentage of success from the preceding series in the same way as the latter differed from its predecessor, so that the phase of increasing or of diminishing percentage of success embraces several successive transferences. The length of time over which the alternating phases extend, seems to be more constant than the number of transplantations, and this point will be referred to again in the succeeding paper on the Analysis of Growth. The conclusion to be drawn from that paper, and the analogous observations on other strains, seems to be that the duration of the alternating phases of increased and diminished energy of growth, is an inherent character of the parenchyma cells, of the same order of permanence as the histological arrangement in which they grow.

The same conclusions may be drawn from the differences in amplitude of the fluctuations. Tumours which habitually give a moderate percentage of success, by no means invariably those which grow most slowly, cannot as a matter of course fluctuate between such wide limits as those which occasionally, or frequently, give a maximal percentage of success. Here again the configuration of the curves is determined only to an inconsiderable extent by the exigencies of the experimental methods. There are biological peculiarities of the tumour cells, as yet of an indefinite kind, which only reveal themselves in this manner.

(8) Bearing upon Cancer in Human Subject.

The variations in percentage of successes, either when a spontaneous or a propagable tumour is transplanted at different times, in rate of

growth of transplanted tumours, in susceptibility to alterations in the dose inoculated and to concomitant immunization, and we may add the facility with which transference can be effected into strange races of mice, all have this in common that they are in great part due to differences in the cells at different times. The evidence permits us to conclude that these differences are due to alternating phases of increased and diminished energy of growth in the cancer cells during proliferation. This alternation in energy of growth, supplies a key to phenomena frequently observed in the progress of malignant disease in man, that the course of a tumour while generally progressive, exhibits periods of amelioration and exacerbation (*cf.* also the silhouette chart of spontaneous tumour 142, p. 103). The persistence of these fluctuations in transplanted tumours, enables us to analyse the phenomenon, and to show that it is determined by a constant feature of malignant cells as such. Our own investigations and those of others have been planned and carried out, for the most part, with the object of illuminating the factors which favour or hinder the growth of cancer-cells in the living body. Propagation has been utilised to a much less extent as a means of throwing light on the nature of cancerous proliferation. The power of continued growth of the cells which becomes manifest in such investigations, has generally been regarded as something incapable of further analysis, their inalienable attribute as particles of living matter, but the artificial propagation of cancer not only permits of a detailed analysis of the conditions favouring and hindering the growth of malignant tumours, but also enables us to further analyse the apparently continuous proliferation itself.

The following paper on the "Experimental Analysis of the Growth of Cancer," gives the details of the evidence for one tumour, viz., Jensen's carcinoma. Its general result has been confirmed by our investigations on other tumours, and by Borrel and Bridré, Hertwig and Poll, Flexner and Jobling, Eloesser, and quite recently by Calkins. Calkins gives a percentage-curve of propagation of the "Brooklyn" strain of mouse carcinoma propagated in the Buffalo laboratory. It shows the same features as those accompanying the present paper, and those we give in the succeeding paper for Jensen's carcinoma. By means of the curve reproduced in fig. 24 from his paper, Calkins* attempts to prove that the fluctuations in percentages of successes (percentage of takes) are independent of the energy of growth of the

* Calkins, G, N.: "The so-called rhythms of growth-energy in mouse cancer." Journal of Experimental Medicine, May 1908.

cancer cells. To measure the latter he has taken the average time in days which the tumours take to kill the mice, in each generation. As we have always recognised (see p. 288), percentage of success can only be regarded as an arbitrary standard by which to measure energy of growth, and in the present paper we have pointed out that in passing from one strain to another the adaptability (percentage of success) changes independently of the energy of growth, but within a single strain there is a very definite although not absolute correspondence between them.

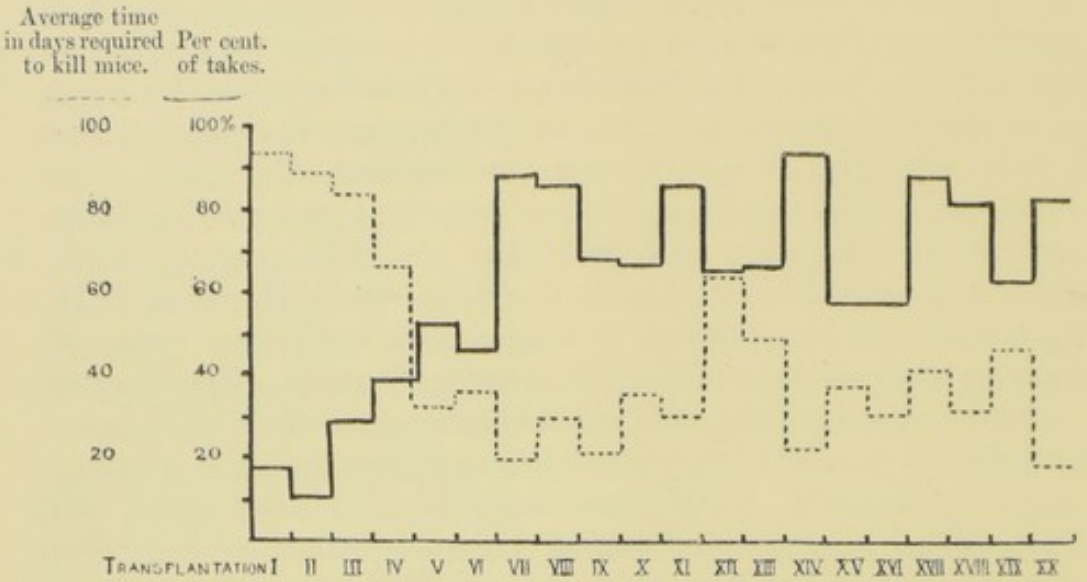


Fig. 24.—Diagram of percentage of takes and energy of growth of "Brooklyn" tumour of Buffalo laboratory. After Calkins.

One of us has attempted to give a more direct measure of energy of growth in the paper on sarcoma development (p. 205 of this Report) by measuring the area of the tumours in silhouette at intervals, and it is also quite possible to obtain an even more objective estimate by killing large numbers of animals with tumours at regular intervals and weighing the amount of tissue produced. Either of these methods gives a more correct estimation of energy of growth than that adopted by Calkins, which is subject to uncontrollable fallacies, since the death of the animals is a secondary result of growth. Whatever method may be employed, the influence of variations in the effective initial dose and of the effects of concomitant immunisation, both of which are entirely ignored by Calkins, affect the apparent energy of growth so profoundly, that the comparison of the results must be undertaken with great caution. Nevertheless the curve constructed by Calkins shows the

same association, for in it the higher the percentage of takes the lower is the interval within which the mice die. We are, therefore, at a loss to understand how Calkins comes to a different conclusion, and do not find either in our own experience or in the data adduced by him, sufficient evidence to warrant the assumption that the fluctuations in percentage of successes, are due to recurring cyclical changes in an associated intracellular parasite. To designate percentages of successes infectivity, is merely to give another name to the facts, and to obscure their significance by introducing a doubtful analogy.

While it is inadvisable at present to speculate on the nature of the mechanism of these fluctuations and their relation to the metabolism of cancer, their ubiquity in practically every strain we have studied, and in most of those studied by others, points in our opinion to their importance. After making every allowance for the effects of technical details on the facies of the curves, the general result seems to be elicited that the amplitude and frequency of the fluctuations are greatest in those tumour-strains which grow most rapidly. It is extremely difficult at present to devise experiments, which will enable us to penetrate with confidence more closely into the nature of the processes producing these effects, but the opinion may be hazarded that when we are able to do so, energy of growth will be found to depend on the rapidity with which these phases succeed each other in the life of the cells, and therewith an important indication will be obtained of the nature of the cellular transformation which takes place in the initiation of cancer, and how it maintains its apparently continuous (vegetative) growth.

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THE EXPERIMENTAL ANALYSIS OF THE GROWTH OF CANCER.

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[Communicated by Professor J. Rose Bradford, F.R.S. Received May 30; Read June 14, 1906.]

In the present paper we shall attempt to analyse the growth of cancer when propagated artificially in mice, mainly on the basis of 25,000 inoculations of Jensen's tumour performed in conjunction with Dr. W. Cramer on behalf of the Imperial Cancer Research Fund; but also with reference to inoculations made with 32 other mouse tumours during the past three years. Although the question of the continuous or interrupted nature of cancerous proliferation is of fundamental importance, both from the standpoint of the ultimate explanation of the nature of the disease, and from the standpoint of its treatment, such an analysis has never been attempted before. It has been assumed that the growth of cancer is vegetative, as inexplicable as any other form of growth, only to be partially understood by an elucidation of the processes by which normal tissues become cancerous. Artificial propagation enabled us to submit this assumption to the test of experiment, and imposed the necessity of determining by direct observation whether propagated cancer exhibited a mode of growth throwing light on the nature of the disease and the apparently continuous proliferation of sporadic tumours. While the experimental propagation of cancer may reveal new facts with a bearing on the nature of the disease it also affords opportunities for rational and empirical therapeutic experiments, and adequate opportunity for controlling the results. These two purposes have been constantly kept in view in our investigations.

When a number of animals are inoculated with a transplantable mouse tumour, all do not develop tumours and the tumours which do develop are not all of the same size after the same interval. In order that propagated cancer might be available for the second of these purposes it was necessary to find out what influence the variable conditions of

experiment exerted on the proliferation of the cells. In the course of these preliminary studies facts bearing on the nature of cancer have also been ascertained.

Irregularities in the rate and amount of growth are introduced by (1) Transference from one race of mouse to another even when nearly allied ; (2) Transference from young to old mice of the same race or *vice versâ* ; (3) Variations in the site of implantation of the cancerous tissue ; (4) Variations in the amount of the tissue implanted and in the manner of introducing it ; (5) Variations in the character of the tumour cells themselves. Any one of these factors may cause a very great deviation from the rate and amount of growth observed previous to the subinoculations introducing it, and invalidate the results of experiments of which information as to possible modification of growth was the object. The variations depending on the first four factors mentioned must be eliminated before variations can be referred to the tumour cells themselves.

We have taken the following precautions in studying the fluctuations which we believe depend on differences in the tumour cells :—

1. The same race of mice has been used throughout. We have observed differences in the suitability of animals of different colours even among the ordinary English tame mice ; and we have avoided the use of those varieties prized by mouse-fanciers. The wild mouse probably offers more uniform conditions than the tame mouse, but a sufficient stock of uniform age is difficult to obtain, keep and supervise. Jensen's tumour rarely yields a number of successful subinoculations in wild mice equal to that obtained in a control batch of tame mice, and this result when once obtained has not been maintained, but is followed by an increasing difficulty of propagation. The experiments in wild mice may be looked on as control observations to those recorded in tame mice.

2. The tame mice used have been of uniform age, and from five to seven weeks old. We showed that young animals provide conditions more favourable for the artificial propagation of cancerous tissue than old animals. This conclusion has been amply confirmed by our later experience, and in one of its aspects also by the work of Ehrlich and Apolant *, who state that the age of the animals is without importance and, especially, that old females are not more suitable than young animals for the propagation of mammary tumours. We have found that the greater suitability of young animals is even more marked than

* 'Berl. Klin. Wochenschrift,' No. 28, July, 1905.

we at first suspected. The inoculation of a tumour into young and old animals respectively may occasionally give similar results in the two cases, or even a less favourable result in young mice, still such results are exceptional in our experience. As a rule, a much higher percentage of tumours develops in young animals, and they attain large dimensions in a shorter time after inoculation. The tumours which have developed most rapidly, *e. g.*, attaining a weight of 1·05 grammes in a mouse of 9 grammes, within five days, and those ultimately attaining the largest dimensions as compared with the size of the host, have always occurred in young mice, although tumours of 7 or 8 grammes also develop rapidly in adult animals. Slow growing tumours, which remain of relatively small dimensions, occur both in old and in young animals. The extent to which the youth of the animals usually favours the continuation of growth after transplantation may be illustrated by the results of 18 series of inoculations, in which portions of the same parent tumours were transplanted simultaneously into young and adult animals respectively ; 214 implantations into adult animals three to six months old yielded 62 tumours, or 29 per cent. were successful ; 363 implantations into young animals five to seven weeks old gave 172 tumours, or 47 per cent. were successful. This result is by no means an extreme case, either as regards proportion of successes or as regards difference in age of the inoculated animals.

3. When the precautions above indicated are observed, the individual variations in the general suitability of different mice of the same race and age are negligible if implantation be performed in the same site, provided sufficiently large numbers are used. We have preferred the subcutaneous tissue of the back. The attempt to perform collateral series of intra-peritoneal inoculations was abandoned, owing to the frequency with which growth within the peritoneum had occurred secondarily by extension from tissue implanted in the abdominal muscles.

4. We have endeavoured to transplant pieces of healthy-looking tissue of uniform size by means of hypodermic needles, and have obtained more satisfactory results by this method than by breaking tumours down into an emulsion and injecting larger quantities of tissue suspended in physiological salt solution. With certain reservations the rate of development and the size the daughter tumours will attain within 10 days is directly proportionate to the amount of healthy tumour tissue implanted ; 0·02 to 0·03 gramme of tissue usually gives larger tumours within a given time interval than 0·005 to 0·01 gramme.

5. When the conditions referred to in the four preceding paragraphs are maintained uniform, fluctuations independent of them appear, and we shall endeavour to show that they are, in all probability, natural features of proliferation. The detailed study of these fluctuations has been undertaken with the tumour which has proved readily capable of transmission during the longest period yet attained, viz., that of Jensen. This tumour has now been propagated for four and a-half years, without permanent alteration in its histological characters or its behaviour. We have obtained success in from 5 to 90 per cent., occasionally even in 100 per cent., of the animals inoculated, the percentages being based on data obtained from those mice which were still alive * 10 days after the inoculations were made. The amount of tissue transplanted in each animal varied between 0·01 and 0·02 gramme †. The pieces were selected from the whole tumour, and hence their behaviour furnishes an estimate of the proliferate energy of its component parts. The use of a restricted number of random fragments is rendered necessary, because it is impossible to transplant the whole of every tumour ; the number of animals required of itself limits the investigation.

The method of experimental propagation by implanting minute cellular grafts leads to a progressive subdivision of the parenchyma, and to the distribution over a large number of animals of the descendants of cells previously associated together in one animal. The experimental tumours consist of a parenchyma arranged in alveoli. The study of the early stages after transplantation shows that, at first, single alveoli constitute separate centres of growth, and we may therefore term them the " parent alveoli " of the tumour. Since the cells of different alveoli do not intermingle, the progeny of the discrete growing centres in the transplanted tissue remain separate, and are further separated from one another as the parent alveoli increase in size and bud off daughter

* It is our practice to kill from time to time a number of mice during the first 10 days after inoculation, in order to examine the site of implantation. Tumours of transplantable size, 0·75 to 1·5 grammes weight, are rare before 8 to 10 days. As the object of these experiments was to estimate the power of continued growth as distinct from mere transitory proliferation, some such time limit was necessary. Estimates of the percentage of success and of the frequency of the spontaneous cessation of growth in tumours which had established themselves must exclude transitory proliferation of the cells introduced and inflammatory swellings at the site of inoculation.

† The weight of the fragments inoculated into each animal has been arrived at by weighing the mass of tumour used for transplantation and dividing by the number of animals used, *e. g.*, 1 gramme of tumour transplanted into 100 mice gives 0·01 gramme per implantation.

alveoli at the surface. When minute portions of such a tumour containing very numerous daughter alveoli are in turn transplanted it is very improbable that any one fragment will contain cells from each parent alveolus, *i. e.*, progeny of all the primary growing centres in the cellular graft which gave rise to the tumour. On the contrary, such a fragment is likely to contain cells closely related to one another, *i. e.*, from only one of the new growing centres of which the tumour is ultimately composed. Thus, in the course of repeated implantation, the tumours obtained come to represent less and less all the constituent cells of any entire tumour in the preceding transplantations made during the long-continued experimental propagation. In order that they should do so it would be necessary to mix homogeneously all the tumours obtained at each series of implantations. The purposes of our investigation were fulfilled by the method of repeated subdivision and isolation. By this method a repeated analysis of the power of growth of small groups of cells and their descendants can be obtained. The limited number of centres of growth represented in any single graft can be better appreciated by considering also that they have been obtained as the result of a triple process of selection : firstly, the rapidly growing tumours of a batch have been selected because of the greater powers of growth exhibited ; secondly, only the healthy parts were used for transplantation ; and thirdly, a further sifting has been effected by the elimination of those cells which degenerate after transplantation. Taken together with the simultaneous reductions of the number of cells continuing growth at each fresh implantation, the repeated implantation of minute cellular grafts renders it practically impossible that any one tumour at the present stage of propagation should still contain cells representing all the growing centres of a tumour even two or three transplantations antecedent to it.

The percentage of tumours developing after transplanting is, however, only one means of measuring the proliferative power of a tumour experimentally *. It is an arbitrary measure selected for its convenience of

* The weights which the tumours attain in equal times present great fluctuations as well. In series with a high percentage of success many tumours attain a weight of 1 gramme in the course of 10 days, while series with low percentage of success seldom show tumours of 0·5 gramme weight in the same time. This may be due to the greater number of cells continuing growth in each animal in series of high percentage, and therefore does not necessarily indicate a more rapid rate of proliferation of the individual cells. For this reason we have not been able to use the weight of tissue produced in a given time as a means of comparing proliferative power at different times.

application. It merely records that, of a number of fragments taken from a tumour, a certain proportion grew and the remainder were absorbed after implantation in fresh animals. It necessarily neglects variations always obtaining in the weights of tumours in every batch. It is obvious that a sporadic tumour may be obtained, or a time may come in the course of the prolonged selection of tissue for implantation in the future continued propagation of Jensen's tumour when all transplantations will yield tumours. Should this ever be so, measuring the energy of growth by the percentage of tumours developing would fail to reveal any fluctuations. The fluctuations in percentage of success which had previously occurred would retain their importance, and a different method of measurement might still reveal fluctuations dependent on the same factors, as great as those represented in these experiments by percentages of success varying between 5 and 100 per cent. of the animals used.

We have studied the growth of the tumour in parallel series of experiments at different times. In order to compare the results we have estimated the percentage of success attending the subinoculation of all tumours transplanted. The repeated subdivision of the transplanted tumours results in the separate propagation of many strains, which become increasingly numerous as time goes on, spreading out like the branches of a tree from any tumour selected as a starting point. On the basis of this relation a genealogical tree of all the transplanted tumours has been constructed in which the intervals of time between successive transplantations are also recorded. This result is achieved by measuring the number of days since propagation commenced and marking the respective dates of transplantation, so as to mark off abscissæ ; the power of proliferation being measured by marking the percentage of successful implantations as ordinates. The point, determined by these two variables for every tumour transplanted, records its power of proliferation and the date of transplantation.

When the point so obtained from any one tumour at the end of the series is connected by a line with the point similarly obtained for the tumour from which it was derived, and the connections followed backwards through the corresponding points of the preceding transplantations, the absolute duration of propagation and the steps in the lineage of the tumour at the end of the series can be seen at once. As the process of connecting up the points is continued backwards the lines from the points obtained for other strains converge and coalesce till all

ultimately unite in the point obtained for the percentage of success attending the primary transplantation of the sporadic growth.

The graphic records accompanying this paper are small portions of a large chart recording in this way the results of all our experiments with Jensen's tumour extending over a period of two and a half years.

For the purpose of recording the experiments each batch of implantations performed with one tumour is labelled with a number, stating the number of successive transplantations from the beginning of the series. For example, the parent tumour of a batch of implantations belonging to the 40th transplantation has been obtained after 39 successive transferences to fresh mice. To distinguish between several batches, the parent tumours of which have been obtained after the same number of transferences, a letter of the alphabet is added to the number of the transplantation. The genealogy of the various series of implantations is not indicated by this nomenclature, and for this purpose the graphical records, now to be described, have been devised.

As it is important that the exact meaning of this graphical record should be clearly understood, the method by which it is built up may be exemplified by a special case (fig. 1) forming part of another chart (fig. 2).

(1) On the 739th day of propagation, a tumour of Transplantation 45, Series C, was transplanted into 52 mice which were labelled 46 C ; 32 mice died in the first 10 days after transplantation. In the remaining 20, 11 tumours developed ; *i. e.*, 55 per cent. of the implantations were successful.

(2) On the 751st day of propagation, a second tumour of Transplantation 45, Series C, was transplanted into 30 mice, the experiment being labelled 46 F. No deaths occurred in the succeeding 10 days, and 14 tumours were obtained ; *i. e.*, 47 per cent. of the implantations were successful.

(3) On the 753rd day of propagation, a third tumour of Transplantation 45, Series C, was transplanted into 40 mice and the experiment labelled 46 G. Eight mice died in the first 10 days following transplantation, and 21 tumours developed in the 32 survivors ; *i. e.*, 66 per cent. of the implantations were successful.

(4) On the 768th day of propagation, a fourth tumour of Transplantation 45, Series C, was transplanted into 20 mice, 46 K. One mouse died in the first 10 days and six tumours developed in the

remaining 19 mice ; *i. e.*, 32 per cent. of the implantations were successful.

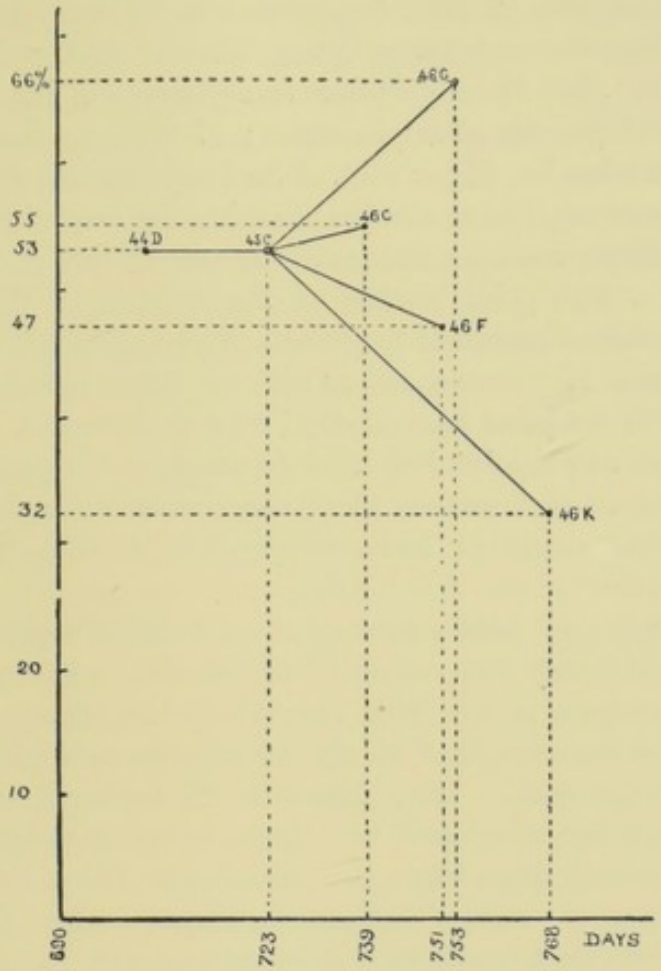


FIG. 1.—Illustrates the method of constructing the graphic records of transplantation experiments (see text).

The data obtained from these four experiments, viz. :—

	The day of propagation. Abscissa.	The percentage of success. Ordinate.	Name of experiment.
		Per cent.	
1....	739	55	46 C.
2....	751	47	46 F.
3....	753	66	46 G.
4....	768	32	46 K.

suffice to determine four points, indicated on the chart by the numbers and letters in the third column.

The four fragments of the *parent* tumour from which the four tumours used for the above experiments (1, 2, 3 and 4) developed were implanted along with 51 other fragments into 55 mice on the 723rd day of propagation, the experiment being labelled 45 C ; 23 mice were killed in the first 10 days after transplantation for microscopical examination of the site of implantation; 17 tumours developed in the remaining 32 mice, *i.e.*, 53 per cent. of the implantations were successful. These two numbers, 723 as abscissa marking the date of transplantation and 53 as ordinate marking the percentage of success of implantations, together fix a fifth point labelled on the chart 45 C. The four points previously obtained represent the results of transplantation experiments on four of these 17 tumours, and to indicate this relation they are each connected with the point labelled 45 C by a straight line.

In the same way a point has been obtained for the parent tumour of 45 C indicated on the chart as 44 D, and similarly for the transplantations antecedent to 44 D and subsequent to 46 G as shown in the larger charts.

The following condensed summary of a number of consecutive experiments will make clear the nature of the results to be recorded in this manner. A tumour of the 39th Transplantation, transplanted into 37 animals, gave tumours in 3 of the 20 animals remaining alive after 10 days (15 per cent.), Transplantation 40, Series I, or shortly 40 I. Of these mice one developed two large tumours weighing together 7·5 grammes in 49 days, when the animal was killed and the tumour transplanted into 24 mice. Tumours developed in 4 of the 20 animals which survived the first 10 days after transplantation (20 per cent.), 41 P ; 14 days afterwards one of these tumours weighing 1·3 grammes was transplanted in 66 mice ; tumours developed in 7 of the 31 survivors (23 per cent.), 42 L. Of these a tumour, having attained a weight of 4 grammes after 42 days' growth, was transplanted into 45 mice. Tumours developed in 13 of the 43 survivors (30 per cent.), 43 L. One of these, 21 days later, had attained a weight of 3·3 grammes and was transplanted into 27 mice ; tumours developed in 8 of the 15 survivors (53 per cent.), 44 D. After 22 days' growth, one of these, 3·7 grammes weight, was transplanted into 55 mice ; tumours developed in 17 of the 32 survivors (53 per cent.), 45 C. After 26 days a tumour 1 gramme in weight was transplanted into 40 mice ; tumours developed in 21 of the 32 survivors (66 per cent.), 46 G. After 15 days' growth, a tumour weighing 1·3 grammes was transplanted into 40 mice, tumours developing in 26 of the 33 survivors (79 per cent.), 47 H. After

14 days a tumour weighing 1·35 grammes was transplanted into 30 mice, tumours developing in 21 out of 25 survivors (84 per cent.), 48 E. Up to this stage there has been a gradual rise in percentage of success through nine successive transplantations from 15 to 84 per cent. The results of transplanting seven tumours of Series 48 E do not maintain this high percentage. Thus a tumour of nine days' growth, weighing 1·15 grammes, was transplanted into 54 mice, tumours developing in 9 out of 32 survivors (28 per cent.), 49 A. Another, 11 days' growth and 1·7 grammes in weight, was transplanted into 40 mice; tumours developed in 12 out of 38 survivors (33 per cent.), 49 B. A third, also 11 days' growth, weighing 1·4 grammes, was transplanted into 31 mice; tumours developed in 10 out of 27 survivors (37 per cent.), 49 C. A fourth, of 15 days' growth, 1·7 grammes in weight, was transplanted into 40 mice; tumours developed in 12 of the 35 survivors (34 per cent.), 49 F. A fifth, of 26 days' growth, 3·2 grammes in weight, was made into an emulsion and injected into six mice. All the mice survived, but no tumours developed, and this experiment is not recorded on the chart below. A sixth, of 27 days' growth, 3·6 grammes in weight, was transplanted into 41 mice, and tumours developed in 5 out of 25 survivors (20 per cent.), 49 O. A seventh, after 62 days' growth, weighed 3 grammes. It was transplanted into 40 mice; tumours developed in 20 out of 36 survivors (56 per cent.), 49 X. None of these tumours maintained the high transplantability of the parent growth, although the implantations grew rapidly and were made at intervals of from 9 to 62 days. This sequence in the results has been a constant feature in all the strains propagated, and there is, therefore, reasonable ground for believing that it is a natural feature of growth.

We shall now proceed to a consideration of the graphic records of this series of experiments. In the accompanying chart (fig. 2) the lines joining the points representing the date of transplantation and power of proliferation appear to form a continuous ascending curve rising from 40 I (15 per cent.) through nine successive transplantations to 48 E (84 per cent.).

When the curve has reached a maximum it falls rapidly in marked contrast to the preceding gradual rise. The seven tumours of 48 E do not all fall to the same level; the degree of the diminution in the success attending subinoculation varies, but the direction of the curve is downwards in all. This sequence of a gradual rise in transplantability followed by a fall has been repeatedly observed during the past two and a-half years. Although in this particular case the fall in percentage of

success on transplanting tumours of 48 E is rapid, and attains a minimum at the first essay with many other strains, it has been possible to obtain several estimations on the downward slope of the curve. In such cases when the tumours of a series following on a maximum are again transplanted, a further diminution in the percentage of success is

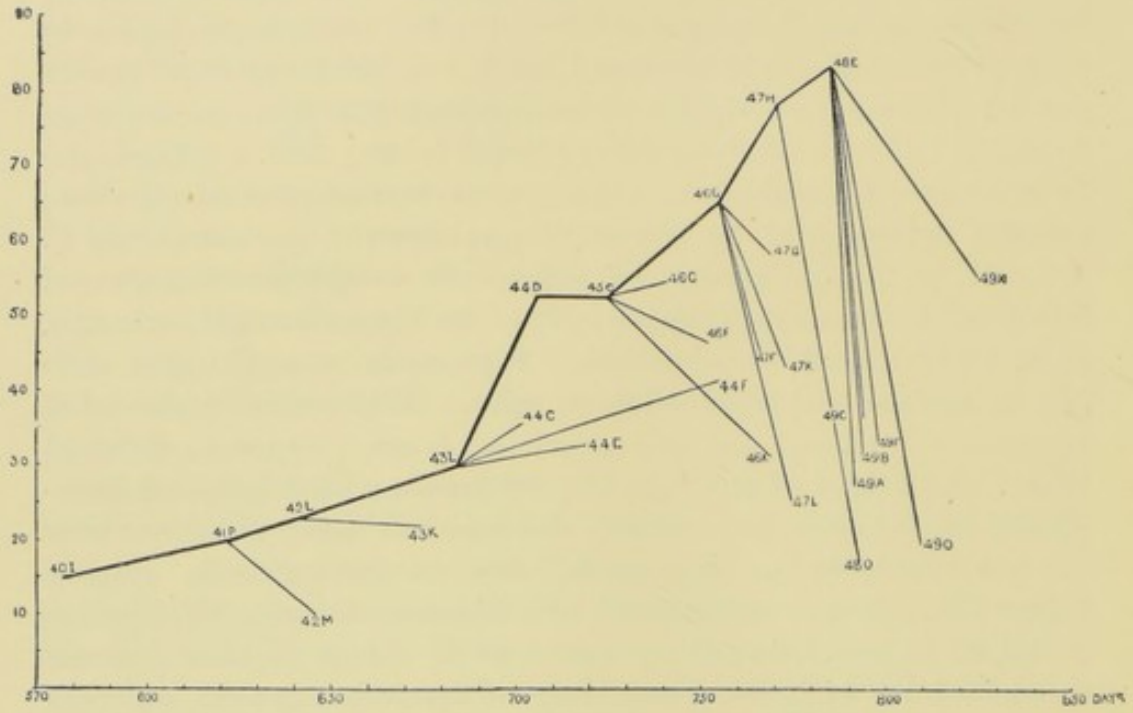


FIG. 2.—Graphic record of steps by which, starting from a tumour giving 15 per cent. of success on transplantation, 40 I, a tumour was obtained after eight subsequent transplantations, giving 84 per cent. of success, 48 E. The success on transplanting some of the other tumours propagated at each step is also recorded by the points at the ends of the lines branching off from the "main stem" of propagation.

frequent, if the diminution in transplantability had been slight in the first instance. The accompanying chart (fig. 3, p. 20) illustrates these points very clearly. It represents the results obtained by transplanting a large proportion of all the tumours of another series, viz., 50 Z, which had indicated 90 per cent. of transplantability. The daughter tumours of this series giving rise to 51 U and 51 T, showed the smallest diminution as compared with 50 Z. From each several tumours have been transplanted, and in each case the diminution in the percentage of success has continued till a level was reached which other tumours fell to at the first essay. The protocol of this experiment is given in full on p. 301.

The description of the graphic record has so far been confined to illustrating the sequence of events in the tumours in the line giving the highest percentages of success, and their ultimate fate. The other experiments recorded in figs. 1, 2, 4 and 5 must now be considered. The tumours propagated concomitantly with those marking the steps in the ascending curve appear to form the ends of "side-branches" on that curve as a main stem. Some of these "side-branches" also exhibited the upward tendency and duplicated the behaviour of the main

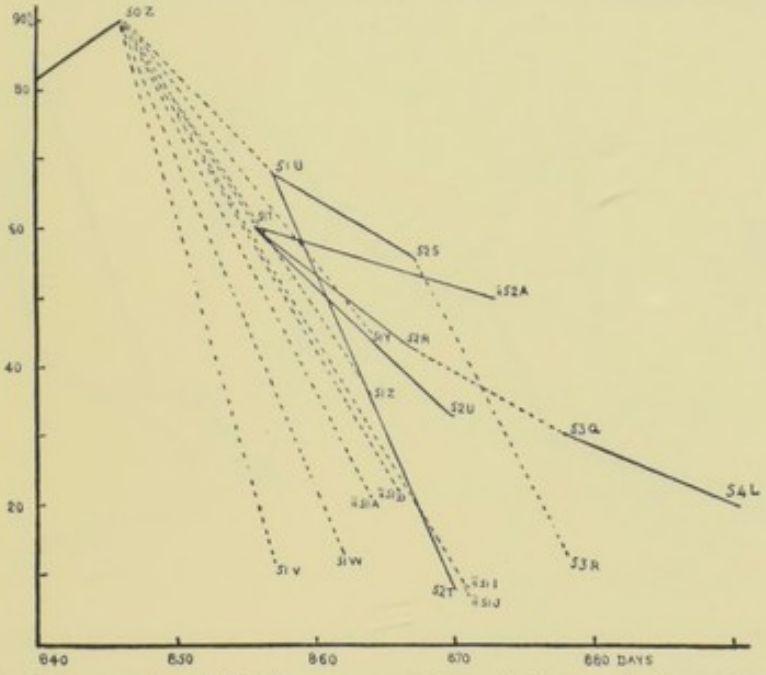


FIG. 3.—Graphic record of further propagation of the majority of the tumours obtained in an experiment (50 Z) in which 90 per cent. of the animals developed tumours. The diminished percentage of success reaches a minimum at the first transplantation in some (51 Y, 51 W); in others after two transplantations (51 U, to 52 T and 51 T to 52 U); while in others a third diminution occurs before a minimum is reached (51 U, 52 S, 53 R); and a fourth diminution occurs in the series 51 T, 52 R, 53 Q, 54 L.

stem in that they also rose to a maximum followed by a fall. The others appear to form descending side-branches and to anticipate the ultimate fate of the ascending curve. If the tumours on such descending side-branches be transplanted they may either give a further fall, completely negative results, or gradually increasing percentages of success till they in turn present a maximum followed by a fall.

The most careful attempts to maintain the percentage of success at a high level in the direct line of descent therefore show that the condition

leading to diminished transplantability ultimately affects the descendants of the tumours which had previously escaped it, and hence appeared to constitute an ascending main stem in the graphic record. We have always obtained a rise to a maximum which cannot be maintained, and a subsequent fall which is also not permanent if continued propagation be possible. Up to the present we have encountered no exception to this rule in more than 600 series of inoculations with this tumour, and the rise to a maximum with a subsequent fall has been repeated 50 times

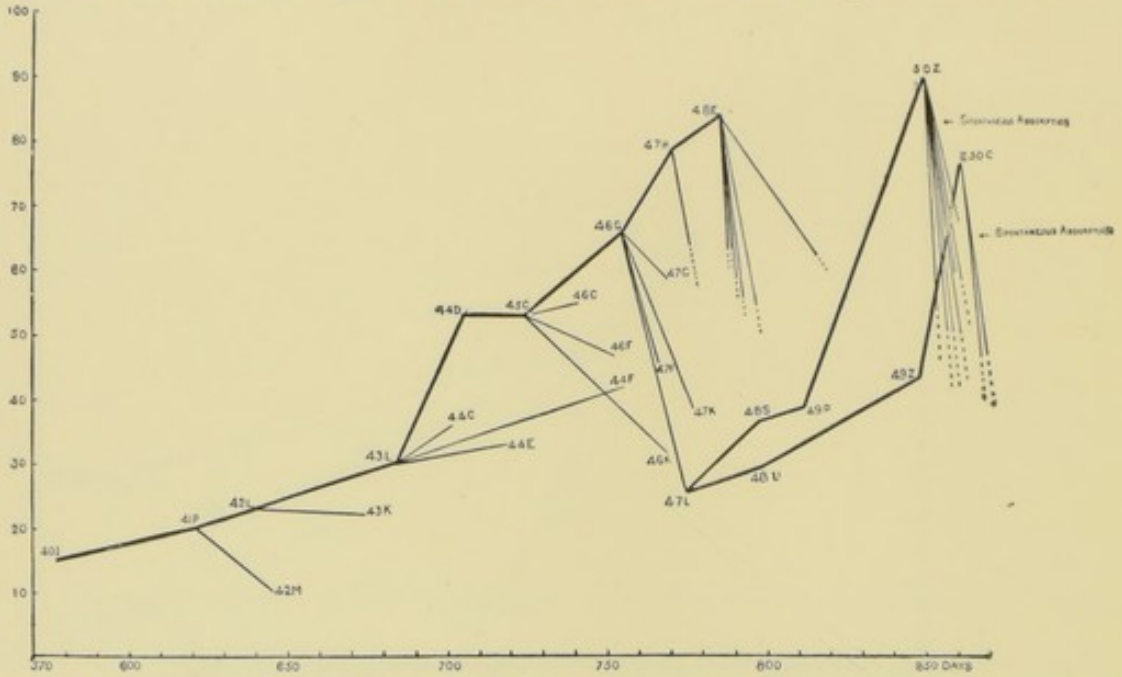


FIG. 4.—Graphic record of results of further propagation of two tumours of an experiment in which 32 per cent. of the animals developed tumours (47 L) Both of these gave an increasingly higher percentage of success till a maximum (50 Z and ii 50 C) followed by a fall was again obtained. The dotted lines are not completed to the point at which they should end, but merely indicate the downward direction of the curve. The details of the fall following the maximum 50 Z are given in the preceding graphic record fig. 3.

in simultaneous series of experiments. If the subsequent behaviour of the descendants of several of the daughter tumours from any one batch of inoculations be followed, successive maxima are seen to arise one after another at short time-intervals. The maximum percentage of success of the experiments as a whole is maintained continuously at a high level between 70 and 90. Each strain, after reaching its maximum, falls and makes way for another which had previously presented a lower percentage, and, after attaining a maximum, in turn falls. A high

percentage is thus maintained by successive maxima developing in parallel series of experiments.

The preceding diagram shows clearly the manner in which successive maxima develop. The subequent behaviour of two tumours of one of the "descending side-branches" of the "rising main stem" depicted in the earlier chart (fig. 3) is followed through four successive subinoculations till each strain in turn presents a maximum followed by a fall. The same phenomenon is repeated in the experiments recorded in the next chart (fig. 5), where the further results of transplanting two strains

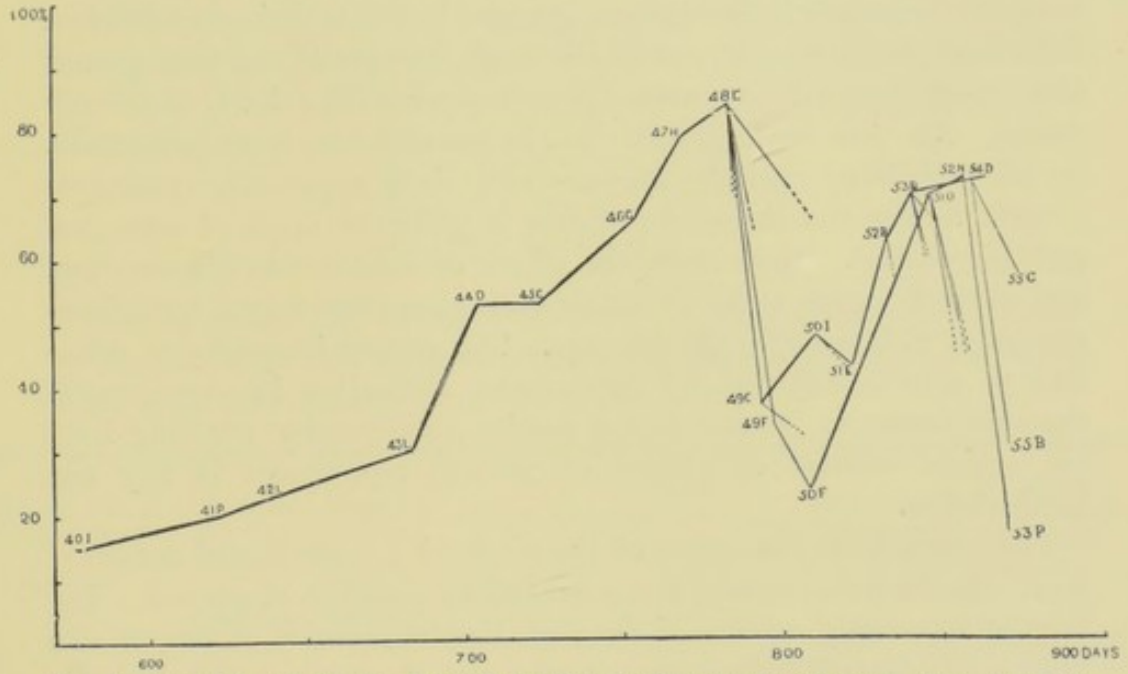


FIG. 5.—Graphic record to show how the further propagation of two tumours (49 C, 49 F) obtained in an experiment with maximal percentage of success (84 per cent. 48 E) gives at first a diminished percentage of success, which, after a varying number of transplantations (in one case six, in the other three), is succeeded by a maximal success after the same interval of time after which the fall is repeated.

derived from 48 E are represented. After giving a low percentage of success, both in turn give a maximum.

When the results of all our experiments are incorporated in one chart it becomes very complex. The orderly sequence of increasing and diminishing percentage of success in individual strains can be followed with difficulty. The confusion, however, is only apparent and indicates how heterogeneous the growth of the tumours viewed as a whole has become. The behaviour of the component parts of this tumour when propagated in a large number of animals represents what may be

regarded as occurring simultaneously in different parts of a single tumour, when allowed to grow for a long time in one animal. During the whole course of propagation of Jensen's tumour, after each successful transplantation, the differences in transplantability of the daughter tumours indicate that heterogeneity of this kind develops. Only when, after a number of passages, a tumour is obtained giving the maximum percentage of success is there any approach to homogeneity in percentage of success on transplantation. After a time any single tumour cannot be regarded as consisting of cells of equal proliferative power. Just as a composite chart of all the strains propagated indicates their very different behaviour at any one date, so in any single tumour at one part growth is proceeding actively, at another growth is proceeding slowly or actually ceases. The same heterogeneity may be postulated for sporadic tumours. In all probability sporadic tumours owe their apparently continuous growth to the simultaneous presence in different areas of numerous growing centres. These mask the effects of concomitant degeneration, and account for the rarity of spontaneous absorption among sporadic as compared with transplanted tumours. The greater frequency of cessation of growth followed by spontaneous absorption in experimental tumours seems to be due to the greater homogeneity resulting from the limited number of centres of growth represented in any one implantation.

The spontaneous absorption of the whole of a transplanted tumour is rare. In the living animal it is preceded by cessation of growth. The tumour apparently remains of the same size for a period of one or two weeks. It gradually diminishes in size, and if examined histologically at this stage, the parenchyma is found to be broken up into small masses and often surrounded by a zone of large phagocytes, external to which there is an overgrowth of sclerosing connective tissue. The process is indistinguishable from what is frequently observed in circumscribed areas in large tumours, and from that which we have described with Dr. W. Cramer * as occurring when tumours disappear under the action of radium. In large tumours in which growth, the cessation of growth and the tendency to absorption show themselves side by side, large cysts are often encountered filled with serum slightly stained with blood. The relation of spontaneous absorption to a definite phase in the fluctuations in transplantability is in our experience a very close one. It occurs most frequently when a high percentage of success has been obtained, and coincides with the time when rapidly growing tumours show a great diminution in the percentage of success on transplantation.

* 'Second Scientific Report of Imperial Cancer Research Fund,' Part II, pp. 59–60.

This association with a definite phase in the fluctuations has already been indicated for two strains on chart fig. 4. It is additional evidence that the diminished transplantability is due to a real alteration in the parenchyma cells, inability to establish themselves in new animals coinciding with the spontaneous cessation of growth in an animal in which growth had already been established. The following experiment illustrates this association in the clearest manner :—

Transplantation 50, Series ii C.*

Parent tumour. Chocolate and white coloured young male of Transplantation 49, Series Z. Attained a weight 0·65 gramme in 12 days. Very soft consistence. No necrosis. Transplantation was effected into :—

(1) 7 mice 5 days old.
(2) 6 „ 4 „
(3) 10 „ 10 „

(3) were all dead within 10 days. In the remaining 13 mice 10 tumours were evident after 10 days and grew rapidly (77 per cent.). Their subsequent history is as follows :—

No. of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.	Result of transplantation.	Result of microscopical examination.
		grammes.	grammes.			
1 ...	10	5	0·3	No necrosis	51, ii H. No tumours in 10 mice.	Early stages in spontaneous absorption.
2 ...	16	—	Had diminished in size.	..... .	Not transplanted. Preserved entire.	Spontaneous absorption in progress.
3 ...	16	6·22	0·28. Had diminished in size.	Two tumours, both necrotic. Anterior tumour firm and yellowish as if undergoing absorption.	51, ii K. 1 tumour in 9 mice. 11 per cent.	Do.
4 ...	29	7·2	4·12	Diffuse necrosis almost complete, thin layer of healthy tumour immediately subjacent to skin.	51, ii M. 10 tumours in 16 mice. 63 per cent.	
5 ...	31	5·95	3·15	Complete necrosis. Thin layer of healthy tumour on deep surface.	51, ii N. 12 tumours in 19 mice. 63 per cent.	

* When the series in one transplantation have arrived at the letter Z, we commence again at A and prefix the numeral "ii."

The tumours, at first growing rapidly, in the other five mice ceased growing two weeks after inoculation, and, after remaining stationary for a few days, diminished rapidly in size, and had disappeared entirely 21 days after inoculation. The five animals and the three which did not develop tumours were then re-inoculated.

The tumours obtained in this experiment form a graduated series. In the first tumour (1), inoculated after 10 days' growth, the results were completely negative, no tumours developing. A tumour (2) was preserved entire after 10 days' growth, and showed the histological features of spontaneous absorption, while another (3) transplanted on the same day with the same histological appearance gave 11 per cent. of success. Five tumours (6 to 10) of large size which were not interfered with disappeared spontaneously. Two tumours (4 and 5) continued to increase in size, in each case attaining half the weight of the mouse in which they were growing. They were almost entirely necrotic, but the healthy portions on being transplanted gave 63 per cent. of tumours in each case, both had apparently recovered from the negative phase fatal to those spontaneously absorbed and causing a negative result, or low percentage of success in those transplanted after 10 and 16 days' growth respectively. This one experiment presents all the phenomena, usually only revealed by a study of several consecutive series. The protocol should be compared with that of experiment 50 Z, given on p. 301. The close genealogical relationship of these two experiments so strongly corroborative of each other is shown in the chart, fig. 4, p. 21. If followed backwards, both are seen to arise from tumours of series 47 L, a descending "side-branch" on the "ascending stem" described on an earlier page.

From a review of the observations recorded in the preceding pages we conclude that the proliferation is only apparently continuous. In reality it is made up of a succession of alternating phases of increased and diminished energy of growth.

In the preceding pages we have concerned ourselves solely with estimates of the power of proliferation throughout a long time, although the extent to which cell degeneration goes hand in hand with cell proliferation is remarkable (see two following protocols and table of experiments on pp. 306 and 307). Growth is always accompanied by extensive degeneration of the cells of the tumours. The histological examination of all the tumours propagated has been systematically performed, and shows that, just as all the cells are not equally capable of continuing growth, so all are not histologically identical in any

tumour. The histological difference most easily observed is that rapid and complete degeneration which attacks the central areas of the alveoli in which the parenchyma cells are arranged.

Transplantation 50, Series Z.

Parent tumour. Young brown female of Generation 49, Series F. Tumour weighed 5·65 grammes. Mouse alone weighed 9·3 grammes. Two tumours in medial line of back. Anterior practically completely necrotic with thin healthy layer on deep surface adjacent to muscles. Posterior tumour practically complete necrosis with thin healthy layer under skin. Skin slight early ulceration. The posterior tumour penetrated abdominal wall, pressing on and displacing kidney.

Transplantation into 40 young normal mice, of which two died within 10 days. In the remainder 32 tumours developed as under :—

No. of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.	Result of transplanting.
		grammes.	grammes.		
1 ...	11	10·7	1·45	No necrosis	60 per cent. 51 T.
2 ...	12	10·3	1·3	Early diffuse necrosis ...	68 „ 51 U.
3 ...	13	9·7	0·85	„ „ ...	12 „ 51 V.
4 ...	14	10·0	Too small to weigh.		
5 ...	17	14·8	1·82	Diffuse marked necrosis	13 „ 51 W.
6 ...	18	9·8	1·1	Central necrosis.	
7 ...	19	8·9	0·2	No necrosis.	
8 ...	19	17·5	1·34	Early diffuse necrosis ...	45 „ 51 Y.
9 ...	19	8·45	1·4	„ „ ...	35 „ 51 Z.
10 ...	19	7·66	1·84	„ „ ...	21 „ 51 iiA.
11 ...	20	11·9	0·9	Complete necrosis.	
12 ..	20	13·2	0·3	Central necrosis.	
13 ...	20	9·1	2·4	Necrosis almost complete, therefore difficulty in transplanting.	53 „ 51 iiB.
14 ...	20	9·05	0·95	Marked necrosis.	
15 ..	20	19·65	1·05	Marked diffuse necrosis	22 „ 51 iiD.
16 ...	21	10·7	1·1	Complete necrosis ulcerated.	
17 ...	21	9·9	0·7	Diffuse necrosis.	
18 ...	21	15·05	0·55	Practically complete necrosis.	
19 ...	21	8·2	0·9	Early very slight necrosis	28 „ 51 iiF.
20 ...	21	11·35	1·15	Early slight necrosis ...	25 „ 51 iiG.
21 ...	23	10·3	1·3	Marked necrosis.	
22 ...	25	10·1	0·5	Diffuse necrosis.	
23 ...	25	9·0	1·0	„ „	7 „ 51 iiJ.
24 ...	25	12·8	0·6	Very slight early necrosis.	8 „ 51 iiI.

25 to 32.—Nine other tumours ceased growing after three weeks and were ultimately completely absorbed. The mice were then re-inoculated.

Transplantation 51, Series T.

Parent tumour. Young white female of Transplantation 50, Series Z. Tumour weighed 1·45 grammes, mouse alone weighed 10·7. Tumour situated between scapulæ, soft in consistence, very vascular, no hæmorrhage, no necrosis.

Transplanted into 40 normal young mice, of which 10 died within 10 days; in the remainder 18 tumours developed as under :—

No. of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.
		grammes.	grammes.	
1	10	11·8	0·3	No necrosis.
2	11	14·35	0·9	,,
3	14	12·75	0·8	Slight diffuse necrosis.
4	14	8·3	1·1	Very slight necrosis.
5	15	9·2	1·3	Diffuse necrosis.
6	16	6·95	1·16	No necrosis.
7	17	14·6	0·65	Central necrosis.
8	17	12·2	0·2	Slight diffuse necrosis.
9	17	11·85	2·15	Diffuse necrosis.
10	17	16·7	1·0	No necrosis.
11	17	14·8	0·7	Diffuse necrosis.
12	17	13·6	2·25	,,
13	17	13·7	0·8	Slight central necrosis.
14	17	14·2	1·85	Diffuse necrosis.
15	17	8·4	0·9	Marked necrosis.
16	17	8·45	Too small to weigh.	
17	17	15·4	,,	
18	17	13·6	,,	

Of course cells presenting complete degeneration are no longer capable of giving rise to tumours. In fact they are rapidly taken up by phagocytes in the days immediately succeeding transplantation, and it might be concluded that growth was continued by cells which never even tended to degenerate. Parts of the tumours which do not present this central necrosis are not of uniform histological structure. Fig. 6 presents a histological appearance common in these tumours (when preserved in strong Flemming solution) in the portions apparently healthy to the naked eye. Dark and clear areas are seen, the darkly stained portions which usually border on the connective tissue being due to a progressive degenerative process in the cells.

The cells which present this condition in any marked degree degenerate immediately after transplantation, while growth is mainly continued by the clear cells, and it is interesting to note that such degenerating cells form a large proportion of tumours exhibiting early phases in spontaneous absorption.

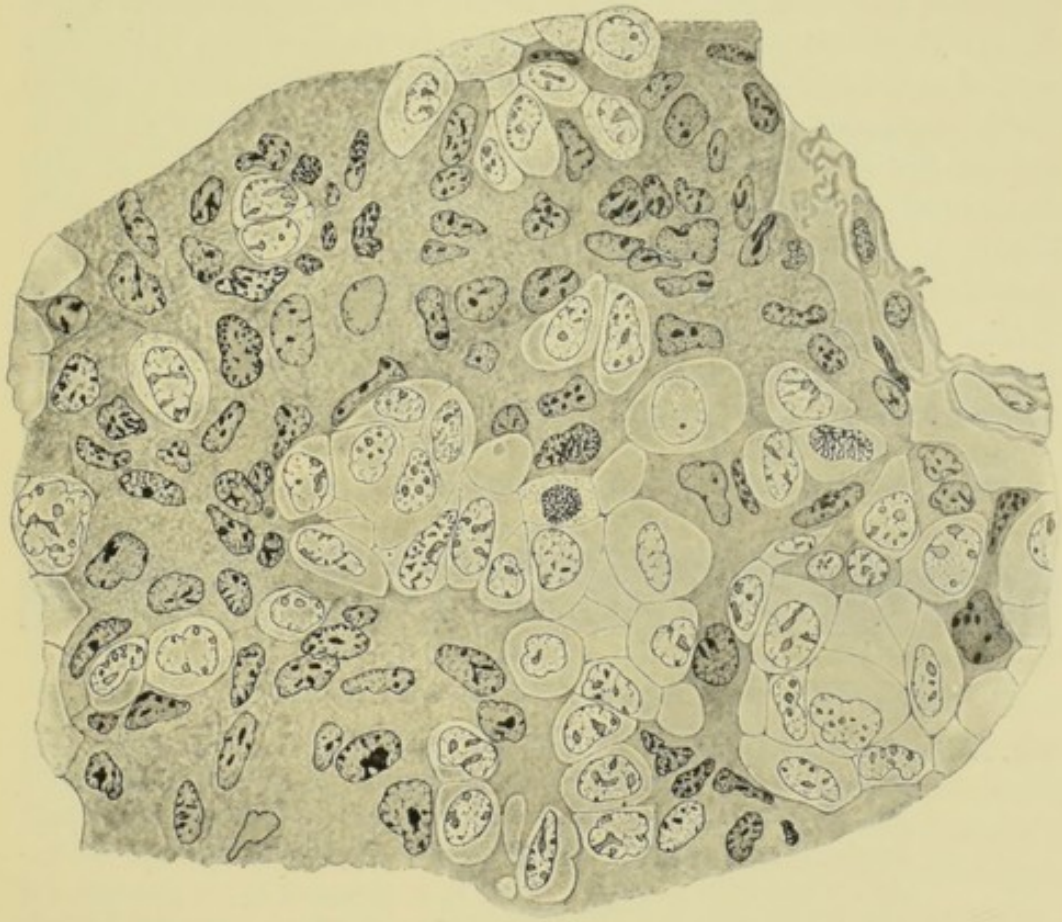


Fig. 6.—Histological differences between cells in a tumour apparently healthy and homogeneous to the naked eye. Islands of clear cells, whose nucleus and protoplasm have little affinity for stains, are surrounded by cells whose nucleus and protoplasm stain intensely. The latter are more numerous on the surface of the tumour alveoli. This differentiation is very frequent in tumours undergoing spontaneous absorption.

The effects of eliminating degenerating cells at each transplantation for the series 40 I to 48 E (*vide* p. 202) can be indicated by employing the percentage of success to construct a diagram of the relative proportions of implanted fragments which developed into tumours or were absorbed respectively. The percentage of success in a batch of inoculations

is a test of the constitution of the parent tumour. If a series of large squares represent the series of experiments giving a maximum at 48 E, the constitution of the parent tumours as revealed by the percentage of fragments developing into tumours can be depicted by subdividing each large square into 100 small squares each representing an implanted fragment, and blackening as many as there were implantations which did not yield tumours. In the accompanying diagram (fig. 7) the clear part of each large square represents the percentage of success attending the transplantation of a tumour arising from a single small square in the one before it. We may imagine that the blackened part of each square (fig. 7) represents those implanted fragments of tissue which, healthy at the time of inoculation, are already on the way to degeneration and do so degenerate immediately after transplantation. The continuous diminution in successive subinoculations which this blackened part undergoes, as the number of fragments developing into tumours increases, would then merely indicate the elimination of degenerating

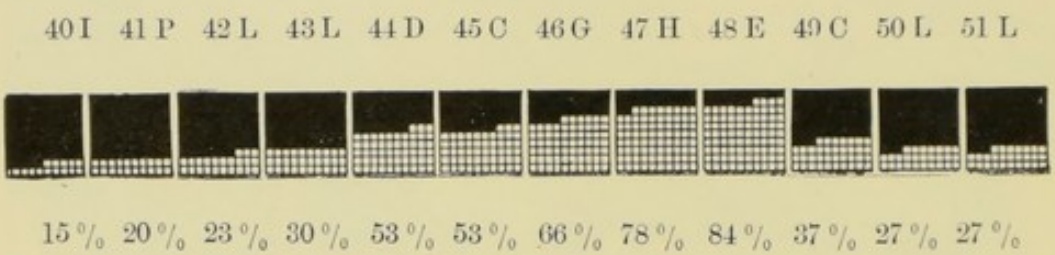


FIG. 7.—Diagram to illustrate the way in which the elimination of degenerating cells by repeated transplantation may result in a progressive increase in the percentage of success in a strain of transplantations. Each large square represents the constitution of the parent tumour of the batch of inoculations whose label is printed above it, as measured by the percentage of success printed below. One hundred inoculations are supposed to be made in every case, and the number of small squares left clear, corresponding to the percentage, shows the number of fragments which developed into tumours.

tumour cells by the selection exercised at transplantation and the further elimination occurring in the days immediately following. Tumours are ultimately obtained free from the original admixture of such doomed cells. They consist entirely of the progeny of those healthy cells (in the first tumour of the series) which were destined to carry on growth. Even the progeny of those healthy cells ultimately enters upon a degenerative phase, as is shown by the sudden reappearance in the diagram (49 C) of a large blackened area when the clear area has

attained a maximum. The increased tendency to degeneration reappears over a considerable interval, as a further reduction of the clear area in the diagram at 50 L and 51 L indicates. Thus the tendency to degenerative changes is intercalated in the course of the continued proliferation of the parenchyma cells.

Our methods of propagation and of recording the results enable us to analyse the growth of small groups of cells. So far as the descriptions of experiments published permit us to form an opinion, other investigators have emulsified single tumours, or have emulsified and mixed several tumours, and injected portions of the emulsion. This method maintains a mixture of strains at each inoculation and they have therefore recorded the results as *average* percentages of all subinoculations made after the same number of transplantations, no detailed analysis of the features of growth being attempted. Other authors, therefore, do not give the details of the behaviour of single strains, and we are unable to compare their results with our own. The increase in the percentage of success in our later transplantations as compared with the earlier ones obtained by Jensen himself must not be confounded with a permanent alteration in the character of the cells as the result of the number of transferences from animal to animal. The highest percentage of success obtained by Jensen * is given as 66 per cent. for single experiments. We recorded in March, 1904 †, success in 90 per cent. of the animals used at the third transplantation into English mice. Since then we have repeatedly obtained from 80 per cent. to 100 per cent. of success in individual strains in the manner already described. The variations in percentage of success appear to be quite irregular when recorded in tables giving either the average percentage of success for the successive series of transplantations, or the results of individual experiments in each transplantation (see Table on pp. 32 and 33). The confusion presented led us to study the percentage of success in greater detail in single strains, with the result that the irregularities resolved themselves into the orderly sequences we have described.

The experiments we have already described, and the graphic records pertaining to them, have enabled us to follow the behaviour of single strains in the direct line of descent. The phase of growth brought out by maximal success on transplantation is the same in separate strains if

* 'Centralblatt f. Bakt.,' vol. 34, 1903.

† 'First Scientific Report of Imperial Cancer Research Fund,' p. 14. *Cf.* also 'Second Scientific Report,' pp. 22 and 54.

Trans-plantation.	Average per-centage in each trans-plantation.	Percentage of suc																							
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
23																									
24	5·0																								
25	11·3	15	30	21	7	4	9																		
26	22·9	5	16	**8**	—	10	2	**41**	**19**	**36**	**47**	**18**	**12**	**25**	24	—	—	21	**33**	—	—	**50**			
27	25·2	29	37	**90**	**17**	**14**	**29**	**25**	16	**9**	—	**17**	—	**43**	**16**	**40**	**18**	5	**28**	**30**	20	**14**	**7**	**25**	**25**
28	15·8	**11**	**10**	—	**40**	20	—	**16**	—	—	**10**	4	—	8	—	17	—	**9**	**20**	—	**13**	**14**	**27**	—	—
29	12·8	13	16	**18**	—	—	5	**15**	**12**	—	**20**	**10**	**17**												
30	15·6	**13**	**10**	**13**	16	**10**	—	**18**	—	**21**	**33**	**7**	—												
31	29·5	**5**	**92**	**23**	**10**	**37**	**30**	**25**	**50**	**50**	**13**	—	**11**	**13**	—	—	**25**								
32	29·6	**23**	**44**	**40**	—	**15**	**67**	—	—	**4**	**7**	—	**32**	—											
33	17·5	25	**11**	**4**	**25**	**25**	**17**	**9**	8	—	**20**	—	**38**	4	—	—	**9**	—	—	33					
34	14·6	**15**	**15**	**9**	—	**10**	**8**	—	**7**	—	**15**	5	**4**	**16**	**27**	**3**	**17**	**7**	—	—	**5**	52	**25**	**24**	
35	19·8	**33**	10	17	**10**	10	—	—	—	22	14	20	**24**	20	31	**44**	**7**	35	6	**31**	**16**	—	**8**		
36	23·5	37	—	—	**24**	**32**	10	**13**	30	**25**	40	40	—	31	**13**	**32**	19	8	8	14	—	35	**13**		
37	24·0	**19**	**8**	**11**	21	—	—	32	31	**28**	**10**	30	45	25	**33**	**9**	—	**67**	**2**	—	**33**	**15**	30	—	**8**
38	30·0	25	—	**18**	25	100	75	**33**	—	—	25	—	—	17	20	19	37	**10**	14	**30**	24	47	—	—	—
39	25·8	50	29	—	—	13	**33**	**17**	9	—	—	**14**	—	—	**11**	**21**	**9**	—	**33**	**27**	67	—	—	**10**	—
40	28·7	63	31	33	22	—	—	—	**13**	**15**	**33**	**21**	12	—	—	40	**42**	**4**	**50**	18	**29**	**15**	**38**		
41	24·8	31	29	36	**14**	17	**33**	**13**	**17**	21	—	**38**	**11**	**17**	**5**	**20**	**20**	**36**	35	**59**	**40**	**25**	**13**	**15**	9
42	21·7	—	44	29	25	—	**9**	—	**14**	—	—	—	23	**10**	**11**	**39**	**18**	**13**	**9**	3	—	—	—	**59**	**20**
43	17·8	—	—	16	20	22	**4**	—	5	—	—	**22**	**30**	—	**33**	**15**	**6**	**24**							
44	38·8	27	**44**	**36**	**53**	**33**	**42**	63	13	—	—	—	—	—	—	13									
45	32·2	29	**33**	**53**	**13**	33	—	—	—																
46	41·2	—	—	55	—	—	47	66	**46**	**55**	—	**32**	16	33											
47	48·9	23	—	72	47	—	46	59	79	75	**61**	**44**	**26**	62	**73**	69	61	—	**33**	**34**	**6**	7	46	65	
48	43·7	—	30	43	**83**	84	—	47	**70**	**50**	—	60	**50**	**50**	**57**	**18**	—	**50**	—	**37**	**67**	**30**	32	**22**	**36**
49	40·5	28	33	37	**3**	33	**34**	50	50	29	—	**43**	**33**	—	39	**20**	**39**	—	**63**	**73**	40	74	36	**37**	**56**
50	41·1	58	**54**	71	55	—	24	**61**	**30**	48	—	—	30	14	40	—	**15**	37	**57**	**6**	59	**59**	15	15	3
51	37·5	78	—	7	60	43	43	**64**	**68**	48	40	**18**	**27**	**21**	32	**40**	29	64	—	—	60	**68**	**12**	30	1
52	36·2	36	63	**66**	74	38	6	33	**10**	7	**5**	**48**	**45**	**22**	**72**	**32**	—	33	43	56	8	33	14	80	2
53	39·6	43	70	40	26	48	—	43	38	44	53	—	**56**	**54**	**38**	47	16	30	13	11	19	63	66	—	—
54	32·2	13	—	64	72	6	11	47	47	60	—	—	20	8	6	—	—		—	40	—	25	—	41	—
55	44·5	—	—	—	—	**58**	**70**	30	36	—	—	33	39	—	16	38	—	**10**	**23**	**42**	—	**32**	**37**	**27**	—
56	48·2	—	—	—	—	47	—	—	**33**	**12**	13	24	20	7	14	33	—	**19**	**24**	—	**54**	**38**	—	—	**50**
57	56·1	—	29	18	**91**	64	35	33	—	—	79	—	**44**	**37**	—	—	**33**	56	**20**	75	7	45	—	56	**85**
58	50·0	28	33	24	69	**14**	—	**15**	55	31	57	**22**	41	53	45	—	**40**	**57**	**38**	**20**	**41**	**50**	**53**	**77**	—
59	48·0	—	12	21	38	—	25	40	—	17	—	**50**	**47**	**53**	—	—	31	59	**50**	—	**43**	100	61	95	**8**
60	49·0	9	—	—	30	20	—	—	7	—	—	16	12	**33**	54	**42**	**63**	47	100	**75**	**84**	84	81	80	**10**
61	62·0	—	—	—	**17**	**7**	—	—	—	—	70	**30**	—	64	—	—	65	**66**	45	**60**	73	20	38	**15**	**71**
62	63·5	—	43	—	—	—	53	11	37	55	36	57	37	54	**100**	**62**	**40**	**66**	**77**	**62**	43	83	**78**	75	**3**
63	60·0	17	—	47	58	**75**	39	74	**20**	**16**	—	75	**25**	**100**	100	100	100	80	—	11	—	**29**	—	90	**100**
64	58·0	18	17	5	84	**70**	43	21	50	44	**70**	50	**50**	83	74	72	90		9	90	**99**	—	**81**	**72**	**50**
65	57·0	28	**72**	5	9	**38**	47	**86**	**44**	**48**	30	60	100	100	66	100	71	**71**	100	10	90	54	27	—	—
66	62·0	72	37	90	63	30	12	57	37	100	56	31	**60**	—	**96**	80	80	25	81			**88**	18	—	3

The figures in blacker type refer to tumours w

The above table gives the percentages of successes for 880 series of transplanta
The figures refer to some 25,000 mice which remained alive 10 days after transpl
reasons, *e.g.*, in controlling experiments on immunity, or where, for other reasons
results or suspicions of septic infection. The figures omitted refer both to series

The average of successes has risen from 5 to 66 per cent in the 66th gener
animals inoculated. Low percentages occur in the latest series as well as in

*** Additional figures not published in the Roy. Soc. Proc. 1906, are

ach transplantation.

Z	Aii	Bii	Cii	Dii	Eii	Fii	Gii	Hii	Iii	Jii	Kii	Lii	Mii	Nii	Oii	Pii	Qii	Rii	Sii	Tii	Uii	Vii	Wii	Xii	Yii	Zii
30																										
33	18	33	—	—	18	33	8	—	28																	
13	27																									
11																										
6																										
71	18	12	23																							
44																										
90	40	14	77	19	31																					
35	21	53	—	22	—	28	25	—	8	7	—	—	63	63	21	40										
12	50	57	42	31	40	—	20	53	7	—	—	—	—	—	—	5	30	6	33	—	—	31				
43	8	—	—	—	59	44	18	—	33	15	—	13	40	66	40	13	25									
13	7	29	39	24	9	18	14	10	—	—	48	27	7	60	27	81	33	42	55	37	30	53	18	53	29	64
00	—	—	—	48	—	66	—	61	53	89																
66	31	94	92	50	76	81	85	31	81	62	82	44	67	70	74	30										
37	95	100	90	82	70	35	41	90	64	72	77	41														
41	58	58	100	—	—	—	50	63	71	82	—	88	60	59												
37	40	63	44																							
35	6	41	36	20	42	29	—	67	88																	
60	59	56	82	25	100	40	41	79	84	78	100	88	50	94	90	83	50	94	82	100						
00	33	83	100	67	94	94	100	41	67	94	87	100	55	94	100	28	—	58								
9	88	88	50	81																						
80	100	17	63																							
—																										
35	75	89	67	100	100	73																				

otic on naked-eye examination.

s tumour in English tame mice from the 23rd to the 66th subtransplantation.
ies are omitted where the methods of inoculation were altered for special
re not comparable, *e. g.*, small number of mice surviving at 10 days, negative
with low percentages of success.
cation). In single series success has been attained in 100 per cent. of the

ack line dividing the figures into an upper and a lower group.

the fluctuations have any meaning at all. In the same way the minimal success represents the opposite phase of growth. In the accompanying graphic record (fig. 8) the minimum is reached after one transplantation

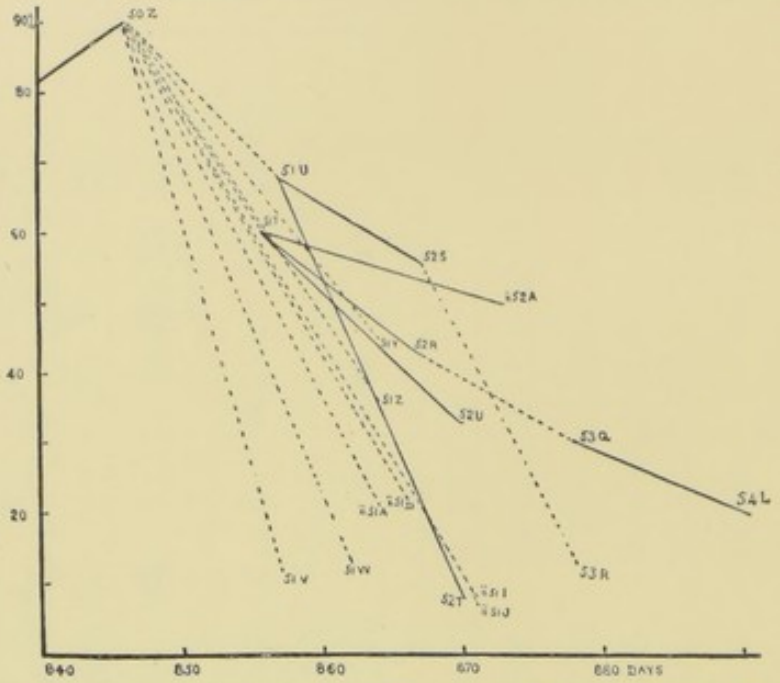


Fig. 8.—Graphic record to show that the same stage of proliferative activity is not always reached after the same number of transplantations. (Repetition of fig. 3.)

in Experiments 51 V, 51 W, 51 iiA, 51 iiI, and 51 iiJ; after two in Experiments 52 T and 52 U; after three in Experiment 53 R; and after four in Experiment 54 L. Thus the number of successive transplantations while furnishing a convenient label for experiments does not indicate corresponding stages in all the experiments. The number of times a tumour has been successively transplanted from animal to animal does not give any indication of what its future behaviour on transplantation is likely to be. That is determined mainly, if not entirely, by its previous behaviour as recorded in the curves, although we are not yet able to predict the immediate results of transplantation in any one case with certainty. In every transplantation, series of implantations are obtained with maximal and minimal percentages of success. Series are obtained in later transplantations in which, on a large number of animals, percentages occur as low as in the earlier transplantations and occasionally negative results. They show clearly that the transplantation to which a tumour belongs does not of itself determine its behaviour. They are as clean experiments as those with high percentage, and must be included

in an objective consideration of the energy of growth of the tumour as measured by percentage of success. Recording the results by the average percentage of all the series in each transplantation therefore obscures the behaviour of individual strains and fails to reveal the composite nature of this apparently continuous proliferation.

In the above table the average percentage for each transplantation and the percentage of success for each series are tabulated in numerical and in alphabetical order. Since high percentages were obtained in

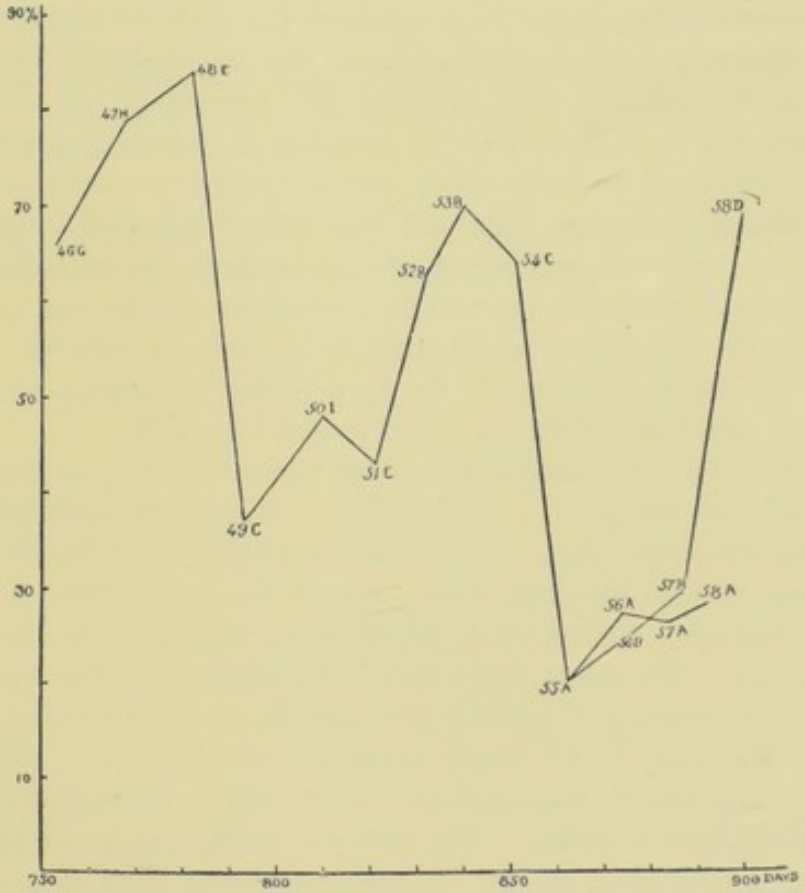


FIG. 9.—Graphic record of a strain of 13 successive transplantations at short intervals. The fluctuations already described appear in this series also.

early transplantations, the apparent progressive increase in the percentage of success in later ones cannot be regarded as indicating a permanent alteration in the powers of growth of the parenchyma cells as a result of the number of passages from animal to animal. The error of such an interpretation is demonstrated by a consideration of the accompanying chart (fig. 9), which gives the results of rapid passage from animal to animal from the 46th to the 58th transplantations.

This series of experiments was rendered possible by the rapid growth of certain tumours arising at each subinoculation. So far from resulting in a progressive increase in transplantability, the 55th transplantation presents the lowest percentage in the whole series. The fluctuations already described for other strains are present here also, when the interval between successive subinoculations is shortened to intervals of 8 to 15 days. Such a curve, representing a succession of rapid transplantations, is a special case like those depicted in the curves * we have already published.

In the preceding pages it is assumed (1) that the conditions have been sufficiently uniform throughout the experiments to exclude fortuitous fluctuations, and (2) that the percentage of success on transplantation furnishes a reliable measure of power of proliferation. If the precautions we have taken warrant these two assumptions, we are entitled to conclude that the fluctuations in proliferative power revealed are natural features of the growth of Jensen's tumour in English mice. They are due to the acquisition of renewed powers of growth by the cells when proliferation is becoming exhausted and may actually terminate, resulting in the spontaneous absorption of tumours which had established themselves and grown for a time.

From time to time sporadic mammary tumours have occurred in the mice purchased for these investigations, and with all artificial propagation has been attempted. The resulting proliferation has in no case been equal to that obtained with Jensen's tumour. Thus out of 20 sporadic tumours transplanted the primary implantations have been negative in 9. We select for detailed description the features of the proliferation resulting from the propagation of two sporadic tumours (namely, VII and XIX) which exemplify the behaviour of tumours capable of only limited propagation. Both could be transplanted several times from animal to animal. The first was transplanted into 133 mice. One tumour developed in the 12 mice remaining alive after 10 days. It was transplanted after 20 days' growth into 24 mice. One tumour developed in the four mice which remained alive after 10 days. It grew slowly, attaining a diameter of 2 cm. after 184 days' growth, when it in turn was transplanted into 208 mice. Two tumours developed in the 80 mice which survived the first 10 days. They were transplanted after 48 and 66 days' growth respectively. No tumours developed in either case, and the experiment came to an end. The accompanying graphic record (fig. 10) shows the contrast which obtains

* Second Scientific Report of Imperial Cancer Research Fund, Part II, p. 54.

between the artificial propagation of such a tumour and that of Jensen. Another sporadic mammary tumour (XIX) was removed by operation and transplanted in 85 mice. Fifteen tumours developed in 75 mice

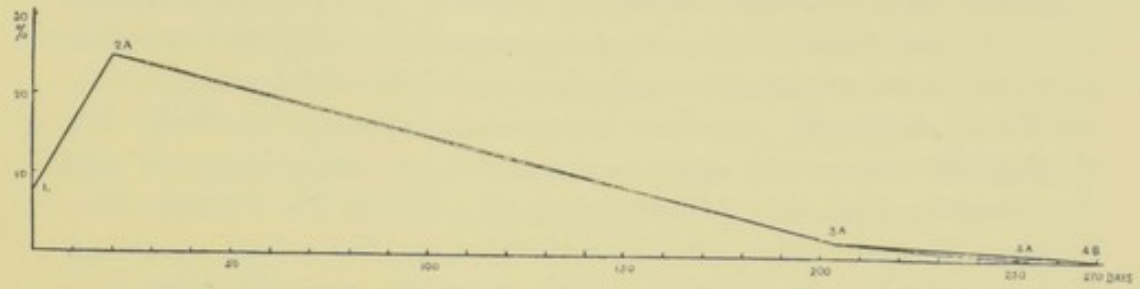
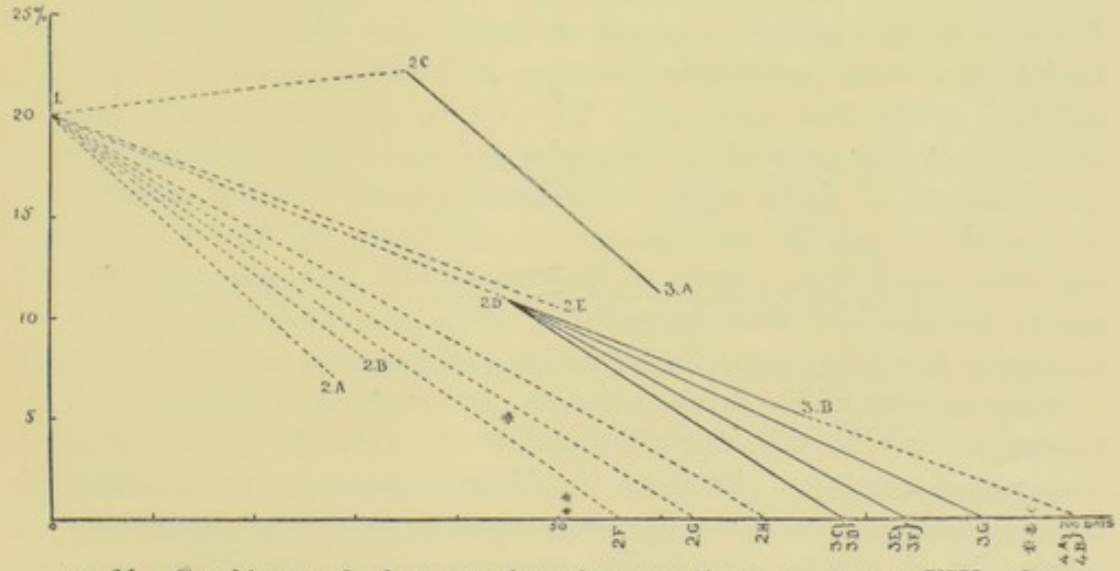


FIG. 10.—Graphic record of propagation of a sporadic mouse tumour VII. Shows a temporary rise (?) in transplantability and extinction of the tumour at the fourth transplantation.

remaining alive after 10 days. The accompanying graphic record (fig. 11) shows the results of the transplantations.



IG. 11.—Graphic record of propagation of a sporadic mouse tumour XIX. Commencing at 20 per cent. the transplantability gradually diminished through three successive transplantations till negative results were obtained. The tumour was removed by operation in the first instance, and recurred three times. The results of transplanting the material obtained at these three later operations are indicated also on the chart at * 5 per cent., ** 0 per cent., and *** 0 per cent.

The ultimate fate of the propagated tumours in these two cases was the same. The interest of the second case lies in the fact that the sporadic tumour recurred rapidly after operation, which was rendered

necessary on three subsequent occasions. The tissue obtained at these operations was transplanted under the same experimental conditions. From the implantations after the first operation fifteen tumours developed in the 75 mice remaining alive 10 days after implantation. Nine of these tumours were used for transplantation. The other six grew for a time, attaining an approximate weight of 0·5 gramme, then remaining stationary, and were ultimately completely absorbed. The histological features of spontaneous absorption were identical with those already alluded to. One tumour developed in the 50 mice alive 10 days after inoculation with the material from the second operation. Negative results were obtained with material from the other two operations, 81 implantations having been made in each instance. When the mouse died three months after the first operation the left pleura and right lung were found filled with metastatic deposits. Thus growth proceeded in the animal primarily affected, and at the same time ceased in the animals inoculated successfully, either after a transitory proliferation in one animal or in succeeding transplantations. Portions of the growth removed at subsequent operations did not exhibit the same proliferative energy, when transplanted, as those obtained at the first operation. We wish to draw attention to the similarity of behaviour of this sporadic growth at different times with the behaviour of single strains of Jensen's tumour in which cessation of growth (*cf.* fig. 12) and spontaneous absorption (*cf.* figs. 3 and 4) supervene on a high degree of transplantability and when negative results are obtained either immediately, or by graduated steps, when a tumour of a series giving a high percentage is transplanted.

Many tumours of the mouse's mamma give negative results on transplantation, and in this respect resemble the tumours of the other longer-lived mammals. Of those, in which the primary transplantation is successful, the later results often show a gradually diminishing percentage of success till, finally, negative results are obtained, on transplantation. The enormous proliferation obtained with Jensen's tumour is exceptional. Growths, undoubtedly malignant, are not necessarily equally capable of artificial propagation. As our experience of malignant new growths in mice widens, the power which small fragments of tumour possess of establishing themselves in new hosts on successful implantations is found to be rarer than might be expected from the frequency of metastasis formation, to which it is closely related. The factor or the factors actively responsible for the development, the continued growth, and the formation of metastases of the

different sporadic tumours in the animals primarily affected are not equally efficacious in ensuring a continuance of proliferation under the similar experimental conditions of artificial propagation. We must therefore conclude that the causative factors have operated with varying intensity, or that additional factors are superadded in some cases.

The behaviour of some strains of Jensen's tumour present a parallel to the other tumours now under consideration. We have already alluded to the negative results sometimes obtained on transplanting Jensen's tumour. In the graphic record (fig. 12) the steps are shown

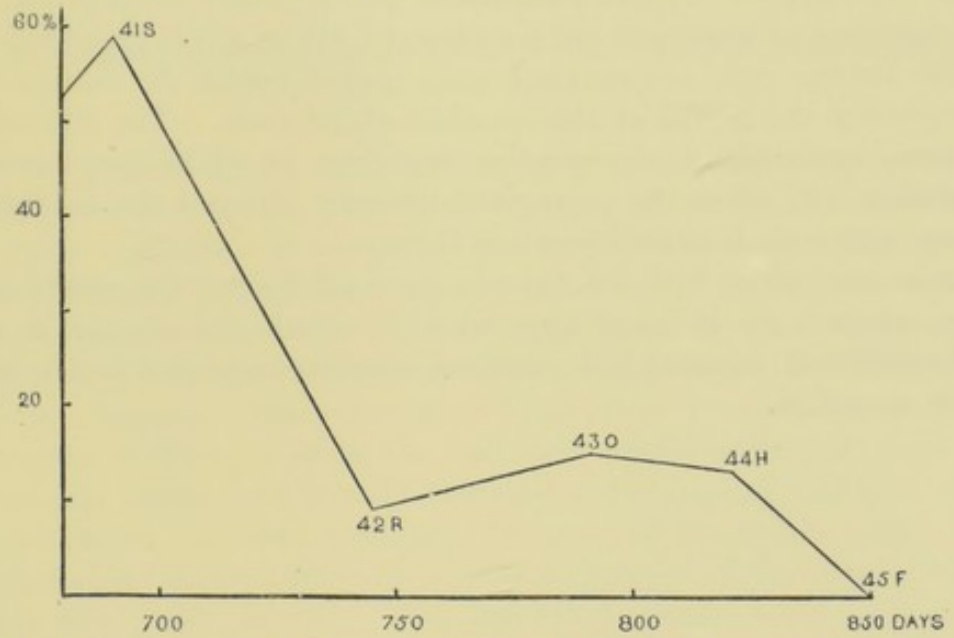


FIG. 12.—Graphic record of propagation of a strain of Jensen's tumour which gradually gave a lower and lower percentage of success till a negative result terminated growth. *Cf.* fig. 10 and fig. 11.

by which a strain of Jensen's tumour, at first giving a high percentage of success, progressively exhibits weaker and weaker powers of proliferation, till finally the tumours obtained gave negative results on transplantation. Such strains are not uncommon; they have frequently been followed to a finish during our experiments. Thus one chapter, as it were, in the life history of Jensen's tumour reproduces the entire life history of other tumours under artificial propagation. These results are difficult to harmonise with the assumption that the apparently continuous proliferation of Jensen's tumour is purely vegetative. Together with the facts of spontaneous absorption they strengthen the conclusion derived from a study of the details of that proliferation, that a cyclical process is involved.

The importance of the preceding analysis of the growth of propagated cancer is obvious in appraising the results of attempts to modify growth experimentally. The experimental conditions whose variations cause irregularities in the success of artificial propagation must be taken account of. In particular, the age of the animals would seem to call for especial attention, because the short duration of the life of a mouse magnifies the effect of the lapse of time involved in procedures for inducing immunity. Specially adapted control experiments must be performed in order to obviate the fallacy which the ageing of the animals introduces. Those fluctuations which cannot be referred to the experimental conditions but are natural features of the proliferation of the tumour cells are an even more urgent reason for caution in interpreting the results of therapeutical experiments. The difficulty or even impossibility of predicting the time at which spontaneous absorption will affect the propagated tumours indicates the necessity for accurate records of their previous history.

In another paper with Dr. Cramer we shall discuss the results we have obtained on re-inoculating mice in which the absorption of well-established tumours had occurred spontaneously and under the action of radium.

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THE NATURAL AND INDUCED RESISTANCE OF MICE TO THE GROWTH OF CANCER.

By E. BASHFORD, M.D., J. A. MURRAY, M.B., and W. CRAMER, Ph.D., D.Sc.

[Communicated by Dr. J. Rose Bradford, F.R.S. Received December 10, 1906; Read January 17, 1907.]

In the present paper we propose to give an account of experiments conducted during the past three years on the means whereby mice may be completely protected against the inoculation of transplantable carcinomata, which grow readily in normal mice. At the same time we shall show that these experiments throw fresh light on the nature of cancer. The Executive Committee and the Pathological Sub-Committee of the Imperial Cancer Research Fund have been informed of the progress of these investigations, and some provisional results have also been laid before the General Committee at the Annual Meetings. The experiments are being continued, but some of the results which have accumulated make it advisable to give an account of the stage at which they have now arrived. They are based mainly on a study of 23 transplantable carcinomata of the mamma of the mouse, and of other malignant new growths which could not be propagated artificially.

In these experiments we have used, for the most part, the growth of Jensen's tumour in normal animals to bring out the changes induced in protected animals and the refractoriness of insusceptible animals; but we shall also employ another tumour (XXVII) of different histology, which grows equally well under artificial propagation. We have employed Jensen's tumour as a standard for the following reasons:— (1) It was the only tumour used to control some of our earlier observations; (2) With proper precautions it gives regularly 85 to 100 per cent. of successful inoculations in a large number of animals; (3) Within ten days the inoculation of 0·01 to 0·02 gramme of tumour tissue gives large easily recognisable tumours, often 1·5 grammes in weight; (4) We have fully demonstrated that it may produce large metastases, that it may extend by the blood or lymphatic streams, and behave

under experimental conditions typically as a malignant new growth; (5) Its rate of growth is not exceeded by any mouse tumour now being propagated; (6) It is in the hands of most investigators throughout the world, who will be able to repeat our observations. It therefore fulfils better than any other tumour the requirements of such a standard.

Historical.

Jensen stated in papers appearing in 1901, 1902, and 1903 * that he had observed the complete disappearance of tumours from mice that had been inoculated successfully. He added, however, that the "results of attempts to cure mice of tumours have hitherto been uncertain; at times they have been positive to an unexpected extent, at other times they have been entirely negative." For this reason he postponed the detailed discussion of his experiments till he had come to a clearer understanding of their significance, and to more reliable results. Referring to the fact that 40 to 50 per cent. of the total inoculations of his tumour had been unsuccessful, Jensen interpreted this to mean that the mice were protected by some natural means. It seemed to him impossible to explain the fact that a tumour which was growing well at the time of its removal from one mouse should be unable to grow in other mice, except on the assumption that the tumour had been transferred to a more refractory group of mice; but he stated, "it appears that the nature of the inoculated tissue is of no little importance for the success attained. So far as it is possible to form an estimate, the results appear less certain when small young tumours are used which are actively growing, and the percentage of successful inoculations is higher if the healthy portions be selected from older and larger tumours." Jensen especially pointed out that mice which had been unsuccessfully inoculated at the first essay were also refractory to subsequent inoculations; but he was careful to point out that it would be a mistake to assert that the second negative result was solely the consequence of natural refractoriness, since it would be wrong to assume that the material inoculated and absorbed in the first instance had not contributed to the exemption subsequently exhibited.

* "Nogle Forsøg med Kraeftsvulster," Lecture, December, 1901; "Forsøg med Musecancer," Lecture, April, 1902, 'Biolog. Selskabs Forhandlinger, Köbenhavn,' 1901–02; "Experimentelie Untersuchungen über Krebs bei Mäusen," Centr. f. Bakt.,' vol. 34, 1903.

Borrel * drew attention to the difficulties attending primary transplantation and recorded suggestive experiments directed to obtaining a polyvalent immune serum against human cancer, on the assumption that distinct sera might be necessary for tumours of different histological types.

In November, 1903, we were able to add the study of Jensen's tumour to that of tumours discovered by ourselves, and we at once began investigations along the lines indicated by Jensen. In our earlier experiments, the successful inoculation of Jensen's tumour fluctuated between 20 and 90 per cent. of the number of animals inoculated.

In a series of papers † we have drawn attention to the greater susceptibility of young than old mice, to the important influence on the success of transplantation, of the character and phase of the tumours inoculated, the dosage, and site of inoculation. In the course of a careful study of the process of the absorption of tumours after exposure to radium, we showed its identity with localised changes in the majority of propagated tumours, changes which were found to have become general in the only case we had then noted, where a tumour of three weeks' growth and of 1 cm. in diameter had actually diminished in size ‡. While recognising the importance of hæmorrhage as an integral part of the process of absorption both when spontaneous and following exposure to radium, we pointed out that the phase of growth of the tumour was also of great and probably decisive importance. These observations harmonised well with Jensen's experience, and tended to explain the irregularities in the disappearance of tumours under his observation. Our position was summarised as follows :—No conclusion has yet been arrived at as to what are the essential and what the subsidiary features in this prolonged proliferation. The variations make it difficult to correlate different observations, and to be sure of their proper interpretation. Efforts to influence growth by immune

* "Epithélioses infectieuses et Epithéliomas," 'Annales de l'Inst. Pasteur,' vol. 11, 1903; Roy. Soc. Proc. vol. 73, 1904.

† First and Second Scientific Reports of Imperial Cancer Research Fund, 1904, 1905; Roy. Soc. Proc. B, vol. 78, 1906.

‡ We recorded the precautions we had taken in searching for tumours undergoing spontaneous absorption. Clowes has taken this criticism of our own procedure as implying that in our opinion his "spontaneous recoveries were ulcerations" ('Brit. Med. Journ.' December 1, 1906). Regression of inflammatory swellings may take place without ulceration, and our remarks apply to our own doubtful "tumours" of less than 14 days' growth, within which time Clowes made no mention of tumours having appeared.

(cytolitic) serum and other means have, in consequence, not yet led to any definite conclusion."

Clowes *, working also with Jensen's tumour, obtained 30 per cent. of success in normal animals. He recorded spontaneous absorption in from 15–20 per cent. of his transplanted tumours. The animals in which spontaneous absorption had occurred were stated to possess a specific anti-body in their serum, which, on injection into animals with transplanted tumours, exerted a curative effect. In only one case out of 20 mice so treated had the beneficial influence of the immune serum been missed. In view of the low percentage of success obtained, the slow development of the tumours, and Jensen's and our own experience, we then expressed scepticism of the curative results obtained by Clowes. In his later papers these results are quoted, but a repetition of the positive results has not been recorded. Evidence is adduced to show that the animals which have recovered spontaneously cannot be successfully inoculated. This is regarded as an action of anti-bodies. The presence of anti-bodies is further supported by experiments in which tumour material had been exposed, in the test-tube, to the action of the serum of spontaneously cured mice. On inoculation, such material gave 12 per cent. of success, while material similarly treated with normal mouse serum gave 31 per cent. These three sets of observations are progressively evidence of a more limited action of the reputed immune sera. The age of the animals used is not stated. In March 1906, Ehrlich † remarks with reference to these experiments : "So berichtet Clowes, dass Mäuse, bei denen Tumoren spontan zur Resorption gelangen, in ihrem Serum carcinomfeindliche Stoffe enthalten. Allerdings lassen sich gegen die Deutung dieser Versuche mancherlei Einwendungen machen." Other papers, in which these and other observations are used as evidence of the infective nature of cancer, have also been published by Clowes, Gaylord, and Baeslack, from the Buffalo Cancer Laboratory, U.S.A.‡

* "Preliminary Communication regarding an Immune Body Capable of Inhibiting the Development of Cancer in Mice," 'Johns Hopkins Hosp. Bull.' April, 1905; "Further Evidence of Immunity against Cancer in Mice after Spontaneous Recovery," Clowes and Baeslack, 'Medical News,' November 18, 1905.

† "Experimentelle Carcinomstudien an Mäusen," 'Arbeiten a. d. Kgl. Inst. f. exp. Therapie,' 1906.

‡ "On Spontaneous Cure of Cancer," 'Surgery, Gynæcology, and Obstetrics,' June 1906; "Incubation of Mouse Tumours," 'Journ. of Exp. Medicine,' August 1906; "A Study of the Influence Exerted by a Variety of Physical and Chemical Forces on the Virulence of Cancer in Mice," 'Brit. Med. Journ.' December 1, 1906; "Evidences that Infected Cages are the Source of Spontaneous Cancer developing among Small Caged Animals," *ibid.*

Michaelis* obtained success in the same proportion as in normal animals, on inoculating mice previously treated with tumour material killed by chloroform and other chemical agents.

Ehrlich† found that from 60 to 90 per cent. of mice inoculated unsuccessfully with spontaneous (especially hæmorrhagic) tumours were refractory on subsequent inoculation with a tumour giving 75 to 100 per cent. of success in normal animals. Further, animals unsuccessfully inoculated with a tumour giving nearly maximal percentage of success in normal mice, were highly refractory to subsequent inoculation. This resistance was manifest whether the second inoculations were made with the same or other tumours (pan-immunity). Animals already successfully inoculated with a quickly growing tumour could not again be inoculated with a rapidly growing tumour (atreptic immunity): the tumour already established withdraws special nutritive substances (substance X) from the circulation, so that the new graft cannot grow. A similar explanation is invoked for the rarity, or small size, of metastais in mice with large transplanted growths. Repeated inoculation of negative mice leads to a very refractory condition in the animals which never develop tumours.

Rats can be successfully inoculated with rapidly growing mouse tumours. Growth proceeds for a time and then ceases (atrepsy), and the animals are then refractory to further inoculation (active immunity).

Summary of the Present Investigation.

After discussing :—

1. The distinction to be drawn between the conditions of origin of a sporadic tumour and the conditions of growth of cancer in mice, and its bearing on experimental investigations.

2. The importance of natural variations in the resistance of mice to inoculation and of inherent variations in the energy of growth of the tumour cells.

We shall adduce evidence that :—

3. Mice in which a growing carcinoma has been spontaneously absorbed may be completely protected against subsequent inoculation of the same growth, and to a lesser extent against other and different

* 'Zeitschr. f. Krebsforschung,' vol. 4, 1906.

† "Experimentelle Carcinomstudien an Mäusen," 'Zeitschr. f. ärtzl. Fortbildung, 1906, and *loc. cit.*

growths. Similarly, protection follows absorption of tumours after exposure to radium.

4. Protection may be induced, or, when naturally present, enhanced by the inoculation of tumour material when no growth has resulted. This protection is specific, and has only been induced in mice by the inoculation of mouse tumours, and not by those of strange species. This specificity has an important bearing on the nature of cancer.

5. Protection can also be induced by the inoculation of normal mouse tissues, and particularly by the inoculation of blood.

We shall discuss the probable nature of the protection conferred ; its action by means of the body fluids, at any rate in part, leading to the death of the cancer cells after short sojourn in the body of protected animals.

(1) The Distinction to be drawn between the Conditions of Origin of a Sporadic Tumour and the Conditions of Growth of Cancer in Mice, and its bearing on Experimental Investigations.

When the energy of growth of the cells of a propagable tumour is great, and a high percentage of all inoculations are successful, the variations in the natural resistance of the mice become of subsidiary importance. On the contrary, they are of great practical importance when the energy of growth is low, and in the primary transplantations of sporadic tumours. As we have shown, the growth of a sporadic tumour is probably to be regarded as heterogeneous. Growth is proceeding rapidly in one part, and slowly or actually ceasing in another. When a sporadic tumour is transplanted into a large number of mice, according to our procedure, it is subdivided into minute fragments, and not made into a general emulsion. Each fragment is separately emulsified before transplantation, and the result of transplantation is that the tumour is distributed over a large number of animals. Success, as a rule, follows only in a small proportion of the inoculations, and it appears probable that it is determined by the segregation of groups of cells of higher energy of growth in mice whose resistance to inoculation is below the average. The energy of growth of the tumour cells and the resistance of the mice together contribute to the positive result. In this way the cells most likely to continue to grow are separated and, by the repetition of the process, a tumour is propagated by transplantation from one series of mice to another. We may

illustrate this by a summary of the transplantation of some sporadic tumours.

We have found that malignant new growths are encountered in mice of all ages taken at random in a proportion of one tumour in 3500 animals. The final results of transplanting 32 spontaneous tumours of the mamma show that 2278 of the mice inoculated survived a sufficient time * to permit of a final estimation, and 72 tumours have developed, *i. e.*, one inoculation in 31·1 is successful, or 3·2 per cent. The conditions for successful transplantation are at least 100 times more frequent in mice than spontaneous tumours.

Fifteen out of 32 sporadic tumours transplanted gave negative results on 1073 inoculations. Many of these tumours were apparently very rapid in their rate of growth in the animal spontaneously affected, and extensive metastasis was present in the lungs after death.

Ehrlich records similar results on primary transplantation of 94 spontaneous tumours: 1504 inoculations were made and 41 tumours were obtained in the inoculated animals (2·8 per cent.). The percentage of success (8·2 per cent.) of the primary transplantation of the tumours (14 in number) which gave positive results at all, was much higher than in our experiments. Apparently our method of primary transplantation by small grafts in a large number of young animals, gives success with tumours which would be completely negative when propagated in the way he has adopted (large doses and smaller number of animals).

The following experiments were undertaken because of the difficulties met with in obtaining a positive result in the primary transplantations of spontaneous tumours. It was of importance to determine whether the spontaneously affected animals were more suitable for transplantation, both of their own tumours (on the analogy of metastasis) and of other spontaneous tumours. The difficulty of transplanting many mouse tumours, especially those with extensive hæmorrhage, has led observers who had failed to transplant them to conclude that those tumours were of low "virulence." We had already succeeded † in three instances where others had failed in transplanting these hæmorrhagic tumours, the daughter tumours being also hæmorrhagic, and had observed that they produced extensive metastasis in the animals primarily affected. We were, therefore, of the opinion that it was wrong to conclude from the difficulty of transplantation that they were of low virulence.

* The results of the primary transplantation of sporadic tumours have been calculated on the number of mice surviving three weeks later.

† Second Scientific Report, 1905, Part II, pp. 19 and 30.

We have prolonged the lives of mice presenting spontaneous tumours by removing the primary growth by operation, wholly or partially. This has permitted us to study their suitability for transplantion, both for their own and for other spontaneous tumours, and to compare it with the suitability of normal animals.

The opportunities for such experiments are rare. Up to the present 13 animals presenting spontaneous tumours have been inoculated with their own and other spontantaneous tumours. The results for 11 of these animals are now available, and are as follows:—

Two mice (XXXIII and XXXIV) could be successfully inoculated with their own tumours, and in case of one of them (XXXIII) three normal animals also developed tumours out of 97 inoculated and surviving three weeks after inoculation. In the case of the second mouse, her tumour gave negative results in 140 normal animals, although successfully inoculated into herself.

A third spontaneous tumour (XXXII) gave four tumours in 156 normal animals, was negative in the mouse spontaneously affected, but positive in another mouse (XXXIII) with spontaneous cancer. A fourth spontaneous tumour (XL) inoculated into 49 normal animals, failed to grow and was likewise negative in two mice with spontaneous cancer (XXXVII and XXXVIII). A fifth spontaneous tumour (XXX) gave one tumour in 72 inoculations into normal animals, but failed to grow in three other mice (XXV, XXVII and XXVIII) spontaneously suffering from cancer, as well as in the mouse herself. Three inoculations were made in each of these four mice.

A mouse which had been repeatedly inoculated unsuccessfully with Jensen's tumour developed a spontaneous growth of different histological type (XXXVIII) seven months later. This tumour, transplanted into 86 normal animals, gave rise to 20 tumours, of which 18, after attaining a diameter of 5 mm. and over, disappeared spontaneously. The mouse in which this tumour had developed remained protected against Jensen's and refractory to four spontaneous tumours.

The experiments are very few in numbers, and only tentative conclusions can at present be drawn from them.

Five spontaneous tumours were transplanted into 11 mice in all, spontaneously affected with cancer. The results above recorded show that transplantion does not reveal conditions exceptionally favourable for growth in them, as compared with normal animals. The animal itself is only exceptionally successfully inoculated.

Bearing in mind the small number of spontaneously affected animals inoculated as compared with the large numbers of normal animals, these experiments show that spontaneously affected animals are not greatly more susceptible to cancerous inoculation than normal animals.* Tumours which do not grow in normal animals rarely grow when transferred to other parts of the animal's own body, and not at all in other spontaneously affected animals.

These facts, together with the development of a tumour in a mouse previously inoculated unsuccessfully with Jensen's tumour, further emphasise the distinction to be drawn between the conditions of origin and the conditions of growth. They also seem to render superfluous any subsidiary assumption of a constitutional condition as necessary in addition to the primary cancerous transformation in the development of spontaneous cancer.†

A clear conception is required of the necessity for interpreting the results of the experiments about to be described as bearing directly on the conditions of growth only and their artificial modification. By inference, they have also important bearings on the nature of cancer; but we desire to enforce caution in anticipating prophylactic or therapeutic actions as likely to follow the use of those measures we have found efficacious in raising the resistance of animals to transplantation.

* The results of the above experiments might, on superficial examination, appear to justify the opposite conclusion to that drawn by us. The tumours of mice XXXIII and XXXIV were transplanted successfully in those mice themselves in single experiments, as against only three successes out of 99 attempts in normal animals in the one case, and with negative results in 176 attempts in the other. Of all the sporadic tumours transplanted into other mice suffering naturally from cancer, XXXII alone grew in a single experiment, giving at the same time only four successes out of 156 attempts in normal mice. It would thus appear that 100 per cent. of success had been attained on three separate occasions when transplantation was effected into mice naturally suffering from cancer, as against very low percentages in normal mice. The results speak for a greater suitability for inoculation on the part of individual mice, but not for a greater suitability on the part of all mice which are naturally the victims of cancer. The conclusion as to individual susceptibility is only justified if the distinction be borne in mind between transplanting a tumour into the mouse in which it grew primarily, and into any mouse afflicted with spontaneous cancer. The two cases are not the same, as pointed out later on. The fact that a mouse is suffering naturally from cancer does not imply a greater suitability for transplantation generally.—*Added Feb.* 8, 1907

† *Cf.*, however, p. 337, Summary.

(2) The Importance of Natural Variations in the Resistance of Mice to Inoculation, and of Inherent Variations in the Energy of Growth of the Tumour Cells.

In previous communications we have directed attention to variations in the susceptibility of individual mice to inoculation with a number of different mammary tumours of the mouse. The natural insusceptibility to inoculation to which we particularly wish to draw attention in the present communication, is that which we have stated was still present after the disturbing influences of race, age, dosage, and fluctuations in the powers of growth of the cancerous cells, have been allowed for in artificial propagation of transplantable tumours. In earlier papers we used the natural resistance of the animals as an indicator of the variations of the power of proliferation of the cancer cells. We shall now conversely use the tumour cells as indicators of the susceptibility of the inoculated animals. In such experiments the number of animals necessary is inversely proportionate to the percentage of successful inoculations attainable. Where the tumour gives a maximal success in normal animals, the number necessary in each experiment can be reduced, and must be correspondingly increased when the percentage of success in normal animals is lower.

Experiments in which less than 60 per cent. of the normal animals develop tumours are open to grave objections, and will not be employed in this paper.

Until we can experimentally determine the development of cancer *de novo*, we are necessarily confined to a study of its growth and the conditions which favour or hinder it when cancerous tissue is inoculated into mice. The differences between mice of different susceptibility are expressed *quantitatively* in differences in the percentages of successful inoculations.

In all these experiments we have to take account of two variable factors of prime importance which are beyond experimental control :—

(1) The varying natural susceptibility of the mice ;

(2) The varying energy of growth of the tumour cells.

These vary independently of each other, and under normal circumstances either alone or in conjunction with one another, they determine the phenomena of growth under artificial propagation. Hence, extensive control experiments are necessary at every step in attempts to modify growth artificially, for a low percentage of success may be due to a refractory condition in the mice used, or to a lower energy of growth

of the tumour cells. In addition, other factors of less importance can introduce variations which are detailed below, and must be excluded.

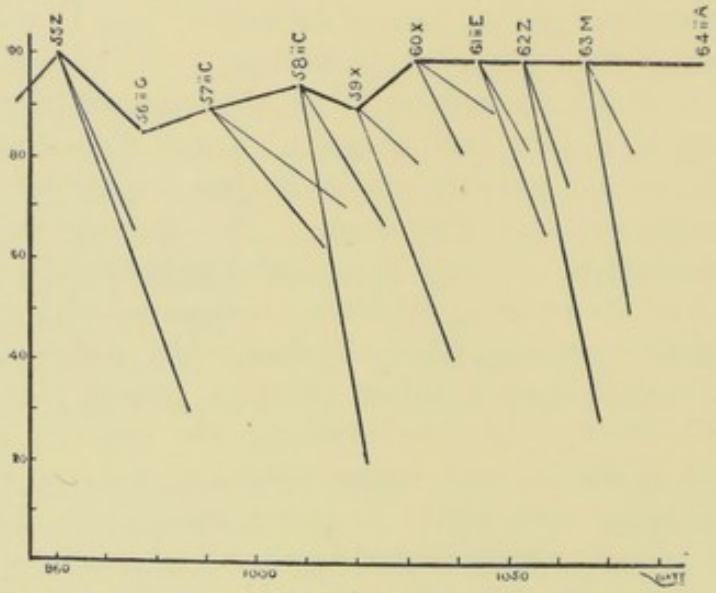
The following precautions have been taken to obtain results which may be legitimately compared. The differences which have been elicited in these experiments are in many cases slight, and dependent on very subtle alterations in the animals. In order to measure them, it has been found advisable to work on the margin of maximum success in transplantation. In order to obtain comparable results under otherwise similar experimental conditions, attention must be paid to (1) the dose of tumour material, (2) the age of the animals, and (3) the site of inoculation. The inoculations must be carried out in such a way that "tumour formation" or "no tumour formation" can be determined by the conditions of the animals for the most part. We have worked mostly with a "minimal tumour-forming" dose, although multiples of this dose have been employed for special purposes. Large doses frequently lead to the development of tumours in animals which are completely protected against, or refractory to, smaller doses. Then the refractory condition, or relative protection, may only be indicated by the smaller size and less rapid growth of the resulting tumours, as compared with normal mice. A cleaner result is obtained when the dose, while sufficient to produce tumours in normal animals within 10 days, fails to lead to any proliferation whatsoever, thereby indicating the presence of abnormal refractoriness in the animals whose resistance is being tested. Our animals not being very highly protected, many of the differences we shall record are only clearly elicited by the use of small doses. The use of large doses obscures them when results are recorded as percentages of successful inoculations. The difference between different sites is best brought out by small doses, and obscured by the use of large doses.

Young mice are more susceptible to inoculation than old animals. The difference in percentage of success may be as great as 60 per cent. between animals of seven weeks, on the one hand, and those of one year old, on the other hand. In the following experiments the control animals have always been chosen of the same size (and therefore of approximately the same age) as the animals whose resistance is tested. This precaution is necessary in all such experiments, because the duration of the preliminary treatment whose effect is under discussion, of itself entails an alteration in the susceptibility of mice whose total duration of life is short.

During the continued propagation of Jensen's tumour in the last

three years, series of inoculations have been obtained repeatedly with maximal percentage of success. The method of repeated subdivision of the parenchyma into the small grafts necessary for the analysis of growth, showed that each such maximum was followed by a rapid and great diminution in the percentage of success, on which again an increase ensued, till a fresh maximum was encountered. The complication which these fluctuations introduce has been in great part avoided in the following way.

By choosing a suitable interval for inoculation of tumours selected from series with from 90 to 100 per cent. of success, and especially by increasing the initial dose introduced into each animal from 0·01 to



Graphic Record of Propagation through ten Passages of a single Strain of Jensen's Carcinoma in which success has not fallen below 85 per cent.

0·05 gramme, we have been able to evade the diminution usually following each maximum for a considerable number of transferences. This result is artificial. It does not indicate an increased "virulence" of the tumour cells, or a continuous growth of uniform energy on their part. It is in complete harmony with the results of the experimental analysis of the growth of cancer as we have already described it.

In the case of other tumours we have obtained the same result in the same way, but most speedily in the case of spontaneous tumour XXVII, which at the third transplantation gave 80 per cent. of successful inoculations, and at the fifth 98 per cent., the success of the primary transplantation being only 20 per cent.

Separate strains of Jensen's tumour have been propagated, as indicated in the accompanying graphic record, through successive transplantations, without the percentage of success in the direct line of propagation having fallen below 85 per cent. This is merely the result of an artificial selection of the tumours obtained at each successive transplantation. It furnishes a special case of the process already described in detail, and receives mention here because of the great convenience of maximum success in inoculation for controlling the experiments with which the present paper deals, and because other observers have not yet succeeded in attaining to this maximum of transplantability in the case of Jensen's tumour. These results, together with the other facts mentioned on a previous page, entirely do away with the statements that Jensen's tumour is not virulent. We have sent our strain of Jensen's tumour to Professor Uhlenhuth and Professor von Dungern. The primary results they have communicated to us show that, in English mice, Professor Uhlenhuth obtained 90 per cent. of success and Professor von Dungern 85 per cent. In German mice the results were 90 per cent. and 50 per cent. respectively.

The slight differences which suffice to determine the success or failure of transplantation are exemplified in the following series of experiments, which were devised to demonstrate the inherent fluctuations of nearly allied strains of the same tumour under the same conditions at each transference. Incidentally the experiments revealed the danger of comparing together inoculations not strictly in the same site.

Two strains of the same tumour were propagated simultaneously on opposite sides of the body of the same mice. The strains used were chosen because of moderate powers of growth, and the fluctuations they had exhibited. On a series of 11 passages, it was found that the axilla was invariably a more suitable site than the dorsal subcutaneous tissue: 59 mice out of 286 developed tumours both back and front, 18 mice had tumours only on the back, and 59 only on the front. The difference between the dorsal and ventral sites is in all probability comparable to that already recorded by us between the dorsal subcutaneous tissue and the peritoneal cavity. The most natural explanation of the difference would seem to be that the connective tissue reaction, which we have shown is specific and without which the grafts cannot grow, is more readily supplied by the connective tissue of the mammary region.

In these experiments we also noted that whenever a tumour of the low percentage strain developed on the back, it almost always had its fellow in the axilla, the converse not holding good.

When the greater suitability of the axilla had been established beyond all possibility of doubt, the experiment was continued through five successive passages in the right and left axilla. The results, as a whole, are more uniform in this series. Out of 95 mice inoculated, 59 developed tumours on both sides, 5 on the right side only, and 11 on the left side only. The two strains, propagated in the right and left axilla respectively, fluctuated practically independeutly of one another, at one time a higher percentage was obtained in the right axilla, at another time in the left. It is to be noted, however, that whenever the strain with the lower percentage for the time being was able to establish itself on its side, the tumour giving a higher percentage always succeeded in establishing itself on the opposite side of that individual mouse, so that a tumour of the strain with lower percentage never occurred singly, each had its fellow on the opposite side in the more numerous tumours of the high percentage strain. Hence the differences in percentage of success between the two strains are considered to be due to fluctuations in the powers of proliferation of the cells of each, and to be independent of the susceptibility or resistance of the mice in which they are, or have been, growing. If the resistance of the mice in the right or left axilla be assumed to be similar, then this resistance really serves merely as a measure of the varying qualities of the tumour cells. It plays the part of a sieve, as it were, which keeps back the cells with less powers of assimilation and growth, and permits those others to pass which can survive the hindrance to their continued existence and multiplication.

(3) Mice in which a Growing Carcinoma has been Spontaneously Absorbed may be Completely Protected against Subsequent Inoculation of the same Growth and to a lesser extent against other and different Growths.

Animals in which tumours have developed and then have been absorbed are highly refractory to further inoculation. This protection may be absolute. This fact is sufficiently evidenced by the following experiments. The animals were re-inoculated in another part of the body after complete disappearance of the first tumour. The control animals were inoculated in the same place as the second experiment of the spontaneously-recovered mice.

PROTOCOL TO ILLUSTRATE ABSOLUTE PROTECTION AFTER SPONTANEOUS ABSORPTION.

A. Experiment 61, ii M (1). Tumour taken from a mouse of transplantation 60, Series V, was transplanted into the right axilla of 20 normal mice. Eight tumours developed in the 12 animals surviving after 10 days (64 per cent.).

(2) The same tumour was also inoculated at the same time into 36 mice which had been previously inoculated successfully in the right axilla, but in whom the tumours evident after 10 days ultimately disappeared. Two small tumours developed in the 33 animals surviving after 10 days (6 per cent.).

B. (1) Experiment 62, ii K. Thirteen days after the inoculation recorded above, 15 of those mice which had presented no tumours were re-inoculated in the left axilla, with tumour of transplantation 61, Series ii N. No tumours developed, while 15 normal animals inoculated with the same tumour presented 13 tumours after 10 days (87 per cent.).

(2) Experiment 62, ii L. The remaining 15 mice of Experiment 61, ii M, were inoculated in the left axilla on the same day with a tumour of transplantation 61, Series ii J. No tumours developed, while 15 normal animals inoculated with the same tumour all presented tumours after 10 days (100 per cent.).

The protected animals were further inoculated unsuccessfully, and were then used to test the properties of the blood.

Protection similar to that afforded against Jensen's tumour by its spontaneous absorption is also manifested by animals in which other spontaneous tumours of different histology have grown for a time, and the tumours been absorbed. Of the tumours on which such observations have been made, XIX was an adeno-carcinoma with cystic spaces and widespread hæmorrhages; XXVII another adeno-carcinoma tending to become alveolar, *i. e.*, approaching the histological type of Jensen's tumour, and XXXVII a malignant adenoma with little tendency to form solid alveoli, while XXXVIII was also an adeno-carcinoma with small acini interspersed with solid alveolar portions.

The animals in which tumours have disappeared after exposure to radium are refractory in the same way and to a similar degree as those in which absorption has occurred spontaneously. This is only what was to be expected in view of the probable identity between the process of absorption in the two cases, as described in a previous paper.

The general conclusion can be drawn that recovery from one form of tumour of the mamma protects against subsequent inoculation with other forms. When protection is absolute in the case of the tumour recovered from, it is not necessarily also absolute for other tumours. There is thus a suggestion of a specificity in the protection. The mice

which have recovered from experimental cancer have undergone a change conferring on them a degree of protection as high as that presented by the most refractory of normal animals. This alteration is not confined to the tissues in the immediate neighbourhood of the spontaneously absorbed tumour ; it must have become general by means of the body fluids.

(4) Protection may be Induced or, when Naturally Present, Enhanced by the Inoculation of Tumour Material when no Growth Results.

We have already pointed out that the animals which have been unsuccessfully inoculated with small fragments of a tumour giving a low or moderate percentage of success may, on re-inoculation, give a positive result in a proportion little inferior to a series of control animals. The negative result in the first instance was thus due not entirely to the inoculation of refractory animals, but largely to the low powers of proliferation of the tumour cells introduced.

Protocol.

Tumour XV. Mouse with a large tumour of the fifth transplantation received from Cologne (Dr. O. Schmidt), together with 10 normal mice.

We effected the sixth transplantation into 10 normal Cologne mice, and 147 normal English mice. In the English mice, three tumours presented themselves in the 61 mice which survived 10 days after inoculation (5 per cent.). Four tumours developed in the 10 Cologne mice, all having survived (40 per cent.).

One month later one of the tumours which had developed in a Cologne mouse was transplanted into 45 English mice and the three Cologne mice which alone had survived out of the six negative animals in the previous experiment. All the three Cologne mice developed tumours, 100 per cent., while only two tumours developed in the 20 English mice surviving after 10 days.

Repeated re-inoculation of the negative animals with small doses of tumour leads to the sifting out of a residuum which may possess a high degree of resistance. As the end result of such a process of elimination by successive inoculation, animals can be obtained in which, while similar control animals give 70 to 80 per cent. of success, only from 10 to 12 per cent. of successful inoculations are obtained. When, however, the small number of animals unsuccessfully inoculated in an experiment with sub-maximal success are re-inoculated, they evince a decided increased refractoriness as compared with normal animals. Some change in the animals following upon the absorption of inoculation

material is not an unimportant factor in the development of this condition, as already suggested by Jensen. That such is the case seems more than probable from the result following, when the initial and subsequent inoculations are made with large doses, more than 0·05 gramme of tumour material. Here the refractory condition is increased in a much shorter series of inoculations, so that already at the third or even at the second inoculation, a diminution of 50 to 60 per cent. may be observed as compared with normal animals. This result confirms Ehrlich's observations on the same subject.

PROTOCOL TO ILLUSTRATE INCREASED RESISTANCE AFTER A PRECEDING NEGATIVE INOCULATION.

Nineteen normal mice were inoculated in the left axilla with 0·04 gramme of a tumour of transplantation 62, Series ii C*, the experiment being labelled "63 S." All the mice survived, but two only presented tumours after 10 days. Thirteen days after this inoculation, 13 of those 17 negative animals were re-inoculated in the right axilla with a tumour of transplantation 63, Series M, the experiment being labelled "64 W." Of the six animals surviving after 10 days, only one presented a tumour (17 per cent.). Of 15 normal mice which had been similarly inoculated among the 11 surviving after 10 days, eight presented tumours (72 per cent.).

Twenty-three normal mice were inoculated in the left axilla with 0·04 gramme of another tumour of transplantation 62, Series ii C, the experiment being labelled "63 T." No tumours developed in the 20 animals surviving after 10 days. Thirteen days after the preceding inoculation, 19 of the mice were re-inoculated in the right axilla with 0·03 gramme of a second tumour of transplantation 63, Series M, the experiment being labelled "64 V." Of the 18 mice surviving after 10 days, five presented tumours (27 per cent.). Fifteen normal mice were similarly inoculated, 12 survived, and 10 presented tumours after 10 days (81 per cent.).

Differences in the size of dose of tumour probably explain the apparently contradictory experience of others who have failed to produce protection in this way, and of our own earlier experiments where the re-inoculation of the negative animals gave a percentage almost as high as in the first instance. The mass of tumour absorbed in the course of three or four negative inoculations with 0·05 gramme is so great that the animals, although never developing tumours, cannot be regarded as comparable with animals in our earlier experiments

* Transplantation 62, ii C, had given 19 tumours in 19 mice (100 per cent.). The low percentage obtained in 63 S, and the negative result in 63 T, the experiments providing the negative mice referred to, is a phenomenon to which we have previously drawn attention (*vide* Roy. Soc. Proc. June 1906).

The tumour tissue inoculated was in what we have designated, for convenience, the negative phase of growth.

which, inoculated the same number of times, have only received relatively insignificant doses (0·01 to 0·02 gramme).

Refractoriness to various spontaneous mouse tumours is apparently reciprocal. Animals which have been unsuccessfully inoculated with large doses of one spontaneous tumour, whether carcinoma or sarcoma, are less suitable for the transplantation of other carcinomata than are normal animals. This fact receives a natural explanation in the results of experiments detailed below on the protection conferred by the injection of normal mouse blood. On the other hand, the inoculation of mice with tumour material from strange species—rat, cat, dog, man—has not induced a refractory state. The protection induced in mice is therefore to be interpreted, not as conferred by properties common to the malignant new growths of different species, but only of the same species, by the absorption of substances common to the tumours and tissues of the mouse.

(5) Protection can also be Induced by the Inoculation of Normal Tissues, and Particularly by the Inoculation of Blood.

Early in 1904 we were enabled, through the courtesy of Professor C. J. Martin, F.R.S., of the Lister Institute, to study the effects of immunising animals, including rabbits, guinea-pigs, rats, and mice against mouse tissues (liver, kidney, testis) and tumour, all of which had been freed from blood by washing out the circulation with normal saline, and ground to a fine powder at the temperature of liquid air. The results were unsatisfactory, apart from the exhibition of a distinct specific hæmolytic action on the erythrocytes of mice on the part of the serum of animals immunised against each of the blood-free tissues and a precipitin reaction with mouse serum. In particular, no satisfactory evidence of a specific action on the living cells of mouse tumours could be obtained either in the test-tube or in the body.

We were led to investigate more fully the effects of the absorption of mouse blood on normal mice, not only because of the difficulty of working with the other tissues, but because of the following considerations : Hæmorrhage is frequent in the process of absorption of tumours after exposure to radium, and during spontaneous absorption. A number of hæmorrhagic tumours successfully transplanted in the first instance were found to be peculiarly liable to spontaneous absorption, and in their case the condition was comparable to what is now well

known to occur in human chorion-epithelioma. In this connection, we are indebted to Dr. J. H. Teacher for drawing our attention, early in 1905, to the frequency of the spontaneous disappearance of the metastatic growths of chorion-epithelioma and their hæmorrhagic character. It was found that an injection of 0·3 to 0·5 c.c. of defibrinated normal mouse blood induced a definite refractory condition, even in young animals. It is not so marked four days after injection as at 10 days, and persists for at least three weeks. The repetition of the blood injection after 10 days does not markedly increase the refractory condition. The animals thus protected do not ultimately develop tumours when observed during a period of three months.

Protocol to Illustrate Protection Conferred by a Preceding Injection of Normal Mouse Blood.

Experiment 61 T. Sixteen mice received an injection of 0·3 c.c. of normal defibrinated mouse blood on the back. Five days later they were inoculated in the right axilla with 0·01 to 0·02 gramme of a tumour of transplantation 60, Series ii P. All the mice survived, and seven presented tumours after 10 days (44 per cent.). At the same time 30 normal mice were inoculated with the same doses of the same tumour, also in the right axilla. Of the 26 animals surviving after 10 deys, 19 presented tumours (73 per cent.). The tumours in normal animals were throughout larger than those in the mice previously treated with blood.

Experiment 62 Q. Twenty-eight mice received 0·3 c.c. normal defibrinated mouse blood on the back. Eleven days later they were inoculated in the right axilla with 0·01 to 0·02 gramme of a tumour of transplantation 61, Series S. Three tumours developed in the 15 animals surviving after 10 days (20 per cent.). At the same time 30 normal animals were inoculated in the same place with the same dose of the same tumour. All survived, and 19 presented tumours after 10 days (66 per cent.). The tumours in treated animals were smaller and grew less rapidly than those in the normal mice.

Experiment 62 Z. Fifteen mice received an injection of normal defibrinated mouse blood. Ten days later they were inoculated in the right axilla with 0·02 gramme of a tumour of transplantation 61, Series ii E. All the mice survived, and 10 presented tumours after 10 days (67 per cent.). At the same time 15 normal animals were inoculated in right axilla in the same way. Ten mice survived, and all presented tumours after 10 days (100 per cent.). The same relation was again observed between the tumours in the two series.

Experiment 61, ii E. Thirty-nine mice received 0·3 c.c of normal defibrinated mouse blood on the back. Seven days later they were inoculated in the right axilla with 0·02 grmmme of a tumour of transplantation 60, Series X. Of the 38 mice surviving after 10 days, 15 presented tumours (39 per cent.). At the same time 20 normal animals were similarly inoculated. All the 15 animals surviving after 10 days presented tumours (100 per cent.). The same difference in the size and rate of growth was observed in the animals treated with blood.

The difficulty of readily obtaining large numbers of young mice artificially protected against carcinoma has been a hindrance to the studies of this kind. We have depended on the selection of animals which had been negative to repeated inoculations, or on collecting mice in which spontaneous absorption had occurred. Both proceedings take up a large amount of time and lead to experiments being conducted under unfavourable conditions, owing to the greater suitability of young animals for growth (and therefore for control experiments). By means of a preceding injection of blood, however, the initial stages of the refractory state can be quickly induced in young mice, and the condition then enhanced by suitable doses of tumour material. When once the initial stages have been passed, there is little difficulty in increasing the refractoriness.

Experiments conducted in this way appear to show that normal mouse serum (0·3 to 0·5 c.c.) free from corpuscles does not confer protection to anything like the same extent, if at all. The blood-corpuscles, suspended in saline after being freed from serum by centrifuging, on the contrary, were nearly as efficacious as full defibrinated blood. The blood was injected into the dorsal subcutaneous tissue over the root of the tail. The inoculations were then made into the axilla, either on one or both sides. The absence of the same degree of protective action after the injection of serum appears to show that it is not due to mere super-addition of protective substances normally present in the blood of the mouse, but rather that the protection results from the active intervention of the living tissues in dealing with the injected corpuscles.

Although young mice are much more susceptible to inoculation, no difference could be discerned between animals treated with the blood of young and old mice respectively.

The blood of other nearly allied animals (rat, rabbit, guinea-pig) called forth no such alteration, so that the action is strictly specific to the mouse.

These effects are best brought out when the inoculations are made with the smallest convenient dose capable of giving rise to maximal percentages of tumours and which lies in our experiments between 1 and 2 centigrammes. When larger doses (5 centigrammes) are employed, the percentage of tumours developing in the treated animals rose, although it did not reach that in control animals, and the tumours which did develop also remained smaller. In one experiment, however, in which massive doses were inoculated, tumours subsequently developed

in the animals in which no growth had appeared within 10 days. Here, apparently, the larger dose of tumour had exhausted the protective property, and subsequently growth was possible. The resistance of animals in which spontaneous absorption has occurred has not been overcome in the same way by increasing the dose of tissue inoculated. Whether this points to a difference in degree only, or a qualitative difference in the protection in the two cases, remains to be determined.

The nature of the action which the body fluids of protected animals have upon tumour tissue in the days immediately following transplantation has been approached in the following manner, after failure to obtain satisfactory results in the test-tube. The failure signifies nothing particular, and may be due either to low degree of protection or imply merely that a complex action takes place in the body, and one we have not reproduced *in vitro*.

A number of animals in which tumours had spontaneously disappeared were inoculated with large doses of tumour tissue, and at the same time twice as many normal animals were inoculated in the same way. After varying intervals the implanted tissue was removed from the protected animals and transferred to young normal animals, and the results compared with those obtainied by re-inoculating the tissue removed from a similar number of the control animals, sufficient of whom were left alive to prove the vitality of the tissues inoculated in the first instance. These experiments are still in progress, but there seems little doubt that, as compared with normal animals, the tumour tissue rapidly dies in those protected by spontaneous absorption.

Summary.

Many apparently contradictory statements as to induced resistance of mice to inoculation may be harmonised when due attention is paid to (1) the susceptibility of young mice and the refractoriness of old mice, (2) to the variations in success which depend on the size of the dose of tumour inoculated, and (3) on the varying qualities of the cells of any one tumour at different times according as they are in the positive or negative phases of growth.

When small doses of tumour, 0·01 to 0·02 gramme, are unsuccessfully inoculated into young and susceptible mice, a second inoculation is often successful ; similar doses will give negative results in a large proportion of adult animals at the first inoculation, and also on subsequent inoculation. The differences between young and old mice and between

the employment of small and large doses explain contradictory statements to the effect that apparently refractory animals of a primary inoculation can or cannot be subsequently inoculated. When large doses of growing tumour are successfully inoculated into young mice, giving nearly 100 per cent. of success, the same proportion is not always obtained in adult animals, although their relative refractoriness can often be overcome by such an increase in dose, provided the tumour tissue is in the positive phase of growth. Young mice which prove refractory to such large doses also prove refractory on subsequent inoculation, as also do adult and old animals.

The nature of the tumour tissue inoculated is of great importance. Where small doses give a maximum success, large doses may also do so, producing very much larger tumours in the same time; on the other hand, the larger doses may be less successful than the small ones, this being explained by the extent to which the simultaneous absorption of tumour tissue hinders growth. The absorption of tumour tissue is inversely proportionate to the number of cells growing in the graft. Whereas a large dose of cells in the positive phase leads to large tumours and no immunity, a large dose of cells in the negative phase * yields very few tumours, permits of much absorption, and gives a high proportion of resistant animals. There is much talk about the "virulent" and "avirulent" nature of different tumours, but the recognition of the fact that the "virulence" of the cells of a single tumour fluctuates between negative and positive phases will explain the contradictory statements of those who have succeeded and those who have failed to induce immunity by the injection of living tissue. It also explains the unexpected positive results obtained in some curative experiments with immune sera and the absence of any effect in others. Great care is necessary in accepting such positive results as "cures" artificially effected, unless spontaneous absorption has been excluded. For example, two tumours out of 12 disappeared after one or two injections of rabbit serum. Spontaneous absorption occurred in the same proportion of the control animals. The injections had no effect on tumours of other series in which no spontaneous absorption took place.

The phenomena discussed in the preceding pages point clearly to the interaction of two independent factors: (1) the variations from mouse to mouse of the susceptibility to (or suitability for) the growth of cancerous grafts; and (2) the variations in the energy of growth of the tumour cells.

* *Cf.* protocol. p. 331, and footnote.

The first variable fluctuates between fairly wide limits in individual animals, but when large numbers of young mice are compared together their resistance acts like a sieve, with unequal meshes, it is true, but, on the whole, of the same average fineness.

Spontaneous tumours vary considerably in the ease with which they can be propagated, *i. e.*, with which they can pass the sieve. These differences have been regarded as depending on differences in "virulence." There is some evidence to show that the sporadic tumours fluctuate in energy of growth just as do the propagated tumours *, and the direct conclusion as to "virulence" from one primary transplantation may be upset when the same spontaneous tumour gives an opposite result, *i. e.*, is again transplanted after recurrence. The term "virulence" is therefore unfortunate, and must be used with so much reservation that it would be better discarded altogether.†

The influence of the individuality, *i. e.*, the sum total of changes due to the past life of the organism, will be to make any mouse different from all others, and these differences will increase the longer the animal lives. The difficulty of obtaining success in the primary transplantation of spontaneous tumours would be accounted for by supposing that the new animals provide an environment to the cancer cells so strange, that they cannot survive the interruption to their nutrition. Their failure to grow does not necessarily imply that they would fail to proliferate in their new hosts if the conditions to which they had been accustomed could be immediately supplied in the experiment. Cells which have lived and become accustomed to the body fluids of one mouse for, say, two years, may easily die or fail to adapt themselves when transferred to the bodies of new animals. The frequency, in our experience, of large metastases in animals spontaneously affected is in marked contrast to the difficulties in obtaining growth in normal animals, and harmonises well with this view. What has been said of the differences in transplantability between spontaneous tumours appears to hold also with reference to separate strains of propagated tumours. If the resistance of the mice be made the criterion for the energy of growth of sporadic tumours, the same standard is equally applicable to the continued propagation of a single tumour. It is

* *Vide* Roy. Soc. Proc., B, vol. 78, 1906, p. 220. Graphic record of transplantation of a sporadic tumour (XIX) which recurred three times after partial excision, p. 311 of this Report.

† *Vide* Sci. Report II, Part 2, p. 40; Ehrlich, 'Berl. Klin. Woch.,' No. 28, 1905, where he also proposes "energy of growth" as an alternative.

impossible to imagine that the differences brought out by our experiments on the fluctuations in energy of growth are due entirely to variable susceptibility of the animals. The simultaneous appearance of numerous spontaneous absorptions, and associated therewith the rapid diminution in the percentage of success in parallel experiments,* can only be referred to fluctuations in the energy of growth of the parenchyma cells. Any other explanation, and especially an explanation by assuming increased resistance of the animals, introduces an arbitrary assumption which further complicates the conceptions.

The resistance of the animals can be altered, and this change has been described as an active immunity. We shall continue to speak of resistance or refractoriness. The absorption of tumours which have grown for a time undoubtedly calls forth this alteration in a high degree. The absorption of inoculated material without obvious tumour formation has similar consequences. Increased resistance results when blood injected subcutaneously is absorbed. The refractory condition induced in this way is not so perfect as that following the absorption of tumour material. It is, however, perfectly definite, and removes any cogency which might attach to the argument that the induction of a refractory condition by absorption of growing tumour or living tumour cells indicates the existence of a hypothetical cancer virus. The evidence we have obtained that the blood of other animals is unable to induce such an alteration in mice, points to the same conclusion. The absorption of the tumours of animals of alien species when inoculated into mice is likewise devoid of effect.

All these actions are curiously limited to the production of an insusceptibility to subsequent transplantation. We have not been able to induce such an alteration in animals with growing inoculated tumours that they should become unsuitable for growth to continue once it has started. It would appear that the negative phase in growth of the tumour cells plays the principal *rôle* when spontaneous absorption occurs, the fall in the energy of growth and the resistance of the mouse together contributing to the final absorption. Once the cellular graft has been vascularised it is in a much stronger position with reference to conditions which may be unfavourable to it than is a graft in the days immediately following transplantation. This is much more vulnerable.

* Roy. Soc. Proc., B, vol. 78, 1906. Graphic record on p. 296, and protocol, pp. 299 and 301.

The biological reactions described in this paper are effective by means of the body fluids. They are analogous to those that are now well known as specific hæmolytic, cytolytic, and precipitin reactions. As pointed out in Second Scientific Report, 1905, pp. 32–33, they are of far greater delicacy, and have only been revealed by using living cells as indicators.

The experiments recorded in this paper give further evidence of the great delicacy and the very narrow range of variations in the soil furnished by the living animal, within which growth can proceed or is inhibited. Growth has only been hindered by the intervention of mouse tumour or normal mouse tissue, to an infinitesimal degree by the normal tissues of the rat, and not at all by those of less nearly-related animals. We therefore feel justified in concluding that these reactions are parallel to those others whereby blood relationship has been established (Uhlenhuth).

When we attempt to formulate an explanation of the nature and mechanism of the changes leading to inhibition of growth, it must be clearly kept in mind that the evidence is almost entirely in the direction of showing an alteration in animals whereby they are unsuitable for or inimical to the establishment of grafts. There is no satisfactory evidence of an induced action on growing vascularised tumours, but only one against newly-introduced grafts. Spontaneous absorption, depending largely on alterations from the side of the parenchyma, is sufficiently frequent to enforce caution in assuming an artificial alteration in the resistance of animals already carrying growing tumours. Complete absorption is necessary to induce absolute refractoriness to subsequent inoculation. Hence the action of various procedures in inhibiting the growth of grafts is not necessarily or even probably due to a direct action on the parenchyma cells.

The connective tissue reaction, which a cancerous graft elicits, is taken advantage of by the cancer cells and acquires characters specific for each sporadic tumour. It is immaterial whether this reaction is protective on the host's part, and only not effective because the slight differences between the tumour cells and normal mouse tissues * do not sufficiently stimulate the protective process. The necessity which exists on the part of the cancer cells for this reaction if they are to continue to grow, renders it possible that a refractory condition may

* Once the tumour cells are dead they excite an energetic phagocytosis. *Cf.* figs. 43–48, 'Second Scientific Report,' Part 2, 1905.

indeed not have anything to do with an action against the cancer cells, but, on the contrary, be due to an alteration of the connective tissue of the host which hinders it from supplying the necessary connective tissue reaction. The possibility must also be entertained that the alteration is directed against the chemotactic influence which may be presumed to be exerted by the cancer cells on the connective tissue of the host. Our own experiments have not yet given satisfactory direct evidence of an anti-toxic or anti-cellular action, but we are far from denying the possibility that such an action may be obtained.

The phagocytosis of formed cellular elements plays an important *rôle* in inducing resistance ; serum is impotent to produce resistance, blood corpuscles do so. The energetic phagocytosis which accompanies the spontaneous absorption of transplanted tumours, and which occurs in absorption after exposure to radium, speaks strongly for the conclusion that the processes are the same in kind when blood or tumour cells, being absorbed, produce resistance. But we are as yet unable to determine the extent to which agencies directed against the tumour cells themselves may assist in determining their early death in protected animals. Other experiments still in progress may be expected to clear up the relative importance of the parts played by the hypothetical inhibition of the specific stroma reaction, or of an equally hypothetical direct lethal action on the tumour cells.

THE NATURE OF RESISTANCE TO THE INOCULATION OF CANCER.

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In view of the fact that the serum of mice rendered resistant to the inoculation of cancer, had not been proved to exhibit a direct toxic action on cancer-cells in the test-tube, nor to possess curative properties when injected into mice bearing tumours, Bashford, Murray and Cramer attempted to elucidate the phenomena of resistance by experiments and direct observations "in vivo." They compared the results of exposing the cancer cells for varying intervals to the action of the tissues and fluids in living animals, in normal and resistant mice respectively. They observed that tumour tissues speedily died when implanted into immune mice. In continuation of these observations the following series of experiments has been made to ascertain if it were possible to find any constant differences between what occurred in cancer grafts implanted into normal and into resistant mice, which could be of service in throwing light upon the manner in which this resistance is produced. The method employed for this purpose has been the systematic examination of the processes at the site of implantation of cancerous grafts into normal and immune animals respectively. This consists in the inoculation of small fragments of tumour which are removed after varying periods, 6, 12, 24 hours, etc., and which are examined histologically after appropriate fixation and staining.

It was through the employment of this method of procedure, that Jensen [1] was able to demonstrate conclusively that the tumour, which developed from inoculation, was produced entirely through an active proliferation of the epithelial cells introduced, and not through any

[1] Centralblatt f. Bact. Bd. xxxiv. 1903.

transformation of the cells of the new host. Bashford and Murray[1], who worked with Jensen's tumour and with numerous other sporadic tumours of the mouse, found by themselves, were able to confirm Jensen's results, and, always employing the method of "early stages," were able to extend our knowledge of the changes which occurred after transplantation of a tumour, to the other constituent of the new growth, namely the stroma. According to them, there took place during the first two and three days after the transplantation an extensive necrosis involving the stroma of the graft, which was later followed by a reaction on the part of the tissues of the new host, and which led to the revascularisation of, and the provision of a new stroma for the graft.

The systematic examination of what occurs at the site of implantation by this method involves a great deal of labour and patience on the part of the investigator before an amount of material can be collected sufficient to base conclusions on. The tumours which are to be studied must be propagated with great care so as to ensure a constant and adequate supply of tumour tissue free from extraneous pathogenic organisms. The growth of the tumours obtained by propagation must be carefully charted from week to week, in order that the past history of any particular tumour may indicate what result may be anticipated on transplanting it. In this way tumours can be selected as likely to give high or low percentages of daughter-tumours, as desired. Where a number of the inoculated mice are sacrificed in the days immediately following implantation, it is also important that the tumours developing in those left alive should be charted with equal care, in order that observations made on "early stages" can be controlled by the later consequences of inoculation ; this is particularly essential when modifications are being sought for as the result of introducing a new experimental factor. For the purpose of this paper upwards of 4500 inoculations have been made and the results carefully followed in the manner described.

The actual technique employed in carrying out the inoculations and the removal of the implanted tissue for examination likewise requires the exercise of great care and patience during its frequent repetition, if comparable results are to be obtained. A detailed description appears desirable, since the technique itself is somewhat difficult until it has been sufficiently practised.

As regards the material to be employed, choose as rapidly growing a

[1] Verhandlung des Komités für Krebsforschung Oktober 1903. British Medical Journal, Dec. 12, 1903. Roy. Soc. Proc. Jan. 1904. First Scientific Report Imperial Cancer Research Fund, 1904. Second Scientific Report, ditto, 1905, etc.

tumour as can be obtained from the strain to be experimented with, about a fortnight old, and cut small portions from the periphery where necrosis has not yet taken place. These slices can be easily broken down with two dissecting needles into small particles about the size of a pin-head, which when drawn into a hollow needle, are ready for inoculation. The needle of an ordinary anti-toxin syringe gives about the right calibre, and, if fitted with a closely ground plunger, will on withdrawing the plunger create enough negative pressure to suck the fragment of tumour up into the needle without damaging the graft to the extent which forcing it there would do. In practice the needles described and figured in this report on page 268 have been employed.

The best site for inoculation is about midway between the groin and the axilla ; this lands the graft in the clean fascial tissue below the cutis, and avoids the extensive adipose tissue which is present in the axillary and groin regions of the mouse, corresponding to the distribution of the mammary gland (fig. 24, p. 84). To prevent the introduction of hair along with the needle, epilate a small area in the groin, and moisten the skin with absolute alcohol ; then plunge the needle through this area, and carry it up under the skin to about the middle of the abdomen, when the graft may be deposited by gently forcing it out of the needle with the plunger. On withdrawing the needle the graft will be left lying at the top of the needle track.

Asepsis must be carefully practised throughout, and should it fail, this will usually be found to be due to some general septic infection of the mouse from which the tumour material was taken. A mouse may appear to be in perfect health, although its tumour contains organisms which, on transference to another animal, will give rise to a virulent infection, and destroy the experiment.

Where a set of immune mice are to be tested, it is usual to inoculate about fifteen, and about the same number of normal mice to serve as a control ; from each series three mice are killed at 24 hours, two, three days, etc.

In removing the graft make a wide snip with the scissors in the tissues of the groin, and reflect up the skin, when the graft will be seen lying on the under surface of the reflected flap, from which it can be easily removed with a pair of sharp pointed scissors. Great care must be taken in dissecting out the graft, and to bring it away still lying in a bed of connective tissue, and thus preserve its true relations. Where the graft has taken successfully, it appears gelatinous and of a greyish tint ; where it has not taken, it is white and opaque.

Osmic acid was the fixative fluid used, made up either according to Flemming's or Borrel's formula, with eventual embedding of the grafts in paraffin. Serial sections through the whole extent of the graft must be employed, as these are absolutely essential for the correct interpretation of many of the microscopic pictures obtained.

One great advantage of using small grafts is that it is possible to get the whole series mounted on one slide, thus saving a great deal of time in staining and mounting, as well as in the microscopical examination. An idea of the labour involved even with these precautions may be formed from the fact that 880 slides of "early stages" have been examined during this enquiry, each slide containing from 50 to 200 serial sections. But the great advantage of small grafts, and this cannot be too strongly emphasised, is that where small portions of tumour are employed, there is not the tremendous necrosis both of parenchyma and stroma, which is so evident in all those cases where large grafts are employed.

It might be thought that by breaking up the tumour into such small fragments, a great deal of damage would be done through crushing of the cells at the surface. This, as will be seen later, is not the case ; the epithelial cells which undergo necrosis are not those situated at the periphery, but those lying in the centre of the graft farthest away from the source of nutrition. The nearer the tumour cells can be kept to the source of nutrition, which is of course at the surface of the graft, the less will be the amount of necrosis, and the truer will be the reaction to the tumour, for of course necrotic tissue has its own reaction. To again emphasise the importance of using small grafts, to know what the true reaction to the tumour really is, it is necessary to eliminate as far as possible the reaction to necrotic material. The reaction to the trauma of introduction of the graft cannot of course be entirely abolished but care in carrying out the grafting will reduce it in amount.

Immune mice, or mice which are resistant to tumour growth can be obtained in several ways as described on preceding pages. Either mice may be taken which have been inoculated unsuccessfully with tumour tissue, or normal mice may be treated with mouse-blood[1] according to Bashford, Cramer and Murray, or with embryo emulsion as was later described by Schöne[2], or by separate tissues as practised in the laboratory of the Imperial Cancer Research Fund. Where the first method has been

[1] British Medical Journal, July 18, 1906. (Annual Report of Imperial Cancer Research Fund.)

[2] Münchener Medizinische Wochenschrift, Dec. 1906.

employed, the attempt has been made to enhance the resistance by re-inoculating for a second and even a third time, with the same tumour, until one has in one's hands a set of negative mice, which will almost certainly be negative to all further inoculation of that tumour. Hertwig and Poll [1] believe that by this process we are only eliminating mice which were naturally resistant, but this will not explain the negative result of reinoculation in those cases in which tumours have grown for a time, and then been absorbed, thus inducing a persistent high degree of immunity in the mice which had exhibited this phenomenon.

The results obtained in mice previously treated with mouse-blood, mouse-embryo emulsion or emulsions of single normal tissues, have been similar to those obtained in mice negative to inoculations of tumour tissue, pointing to the similarity of the immunity in both cases. When using mice which have been immunised with embryo skin emulsion, it is necessary to wait about 20 days before testing their immunity; that is, until a large absorption of the inoculated material has taken place. In one experiment where the mice had been inoculated 14 days previously with 0·05 c.c. embryo-skin, the grafts for the first five days showed growth as in normal animals, although a number of the same mice which were allowed to go on, and were charted at the tenth day after inoculation of the tumour-grafts, showed themselves to be highly immune. This can be explained by the assumption that the immunity only became sufficiently developed about the 20th day, and was able after that date to overcome what growth had already taken place during the first five or six days after introduction of the graft.

The tumour which has been principally employed in these experiments, is tumour 27 of the Imperial Cancer Research Fund; a tumour which is extremely favourable for "early stages" examination, and gives results which are more easily analysed, than is usually the case. The conclusions drawn from the consideration of the microscopical pictures which have been obtained, were first based on examinations of this tumour; but the extension of the investigation to several other tumours of different types, has resulted in the general confirmation of the principles at work in this tumour. It is therefore proposed to give first a detailed account of the results with it, and afterwards to note the points of differences in the various other tumours, and thus avoid the superfluous repetition of histological description, which a detailed account of each tumour employed would involve.

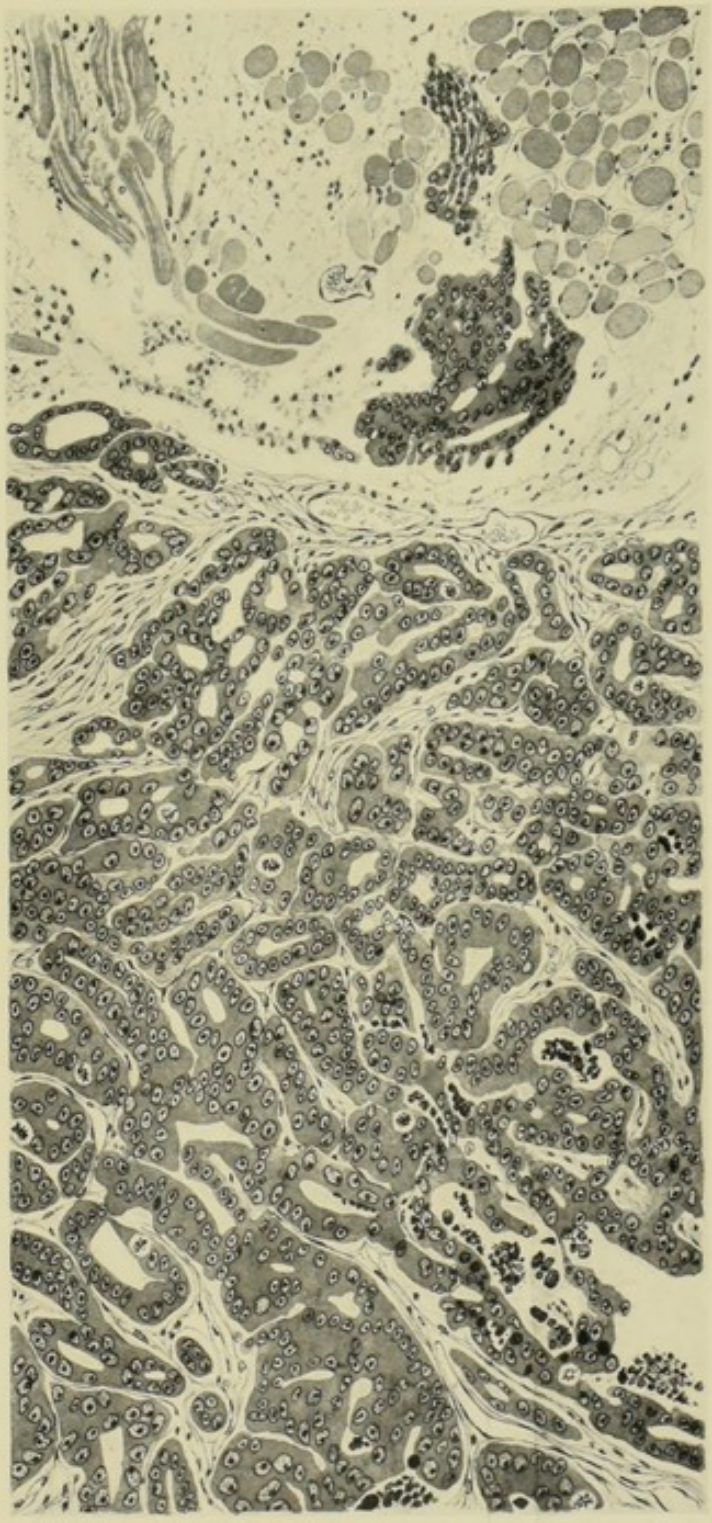
[1] Hertwig and Poll, 'Zur Biologie der Mäusetumoren,' Abhandlungen der kgl. preuss. Akademie der Wissenschaften, Berlin, 1907.

The primary tumour 27 was an adeno-carcinoma of the mamma which was first transplanted about two years ago, and has now reached the 23rd generation, which works out at about a generation a month. This is a comparatively slow rate of transference for a mouse tumour. The percentage of successes on transplantation varies very much ; thus whilst the average percentage of successes during the propagation has only been about 30 per cent., in a few series a much higher percentage has been obtained, 90, and indeed on one occasion 100 per cent. has been seen. This high level has never been maintained for more than one or two generations later, the percentage has fallen, and this fall has been in turn replaced by a second rise. Not all of the strains show this marked fluctuation, one strain has been running between 40 and 45 per cent. for nine generations eventually rising to 80 per cent. in the 23rd generation (*cf.* chart on p. 269).

The rate of growth generally varies directly with the percentage of successes, that is, when the tumour gives a high percentage, the nodule of growth in each mouse is much larger at a given time, than it is in the case of a batch of a low percentage. The largest tumour attained from 0·025 grammes at the end of 10 days, seldom weighs more than 0·6 to 0·75 grammes, whilst in a slowly growing tumour, this size is only reached after about six weeks. The spontaneous disappearance of tumours is not infrequently observed, there are also cases where the growth remains stationary for a long period after the tumour has attained a certain size. As the general percentage of successes on transplantation is low, there are always numerous negative mice, which can have their resistance to growth still further enhanced by reinoculating with a strain of the same tumour which belongs to a series giving at the time a high percentage, in order that the control observations in normal mice as well as those made in resistant mice may be simplified.

The histological picture of tumour 27 is one of the most typical seen amongst the mouse carcinomata ; the cells of the tumour are very large and the arrangement into acini, especially in the earlier stages of the growth, is very characteristic (see fig. 1). As growth proceeds these acini become elongated, and finally drawn out into long canals lined on either side by a regular layer of columnar epithelium. The typical arrangement of a single layer of epithelium forming the wall of the acinus, is present only in a small number of the acini ; the atypical arrangement of several layers of super-imposed cells preponderates more especially in the later stages of growth. Along with this acinous type the alveolar type of growth also occurs, where no lumina are present in

[*To face* p. 346.



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FIG. 1.—27/20 B—21 B.—To illustrate the histology of tumour 27 (25 days old). The tumour consists of compressed and folded acini lined by a single layer, or several layers of large clear cells. Necrosis is commencing in the more centrally placed acini, lower part of the figure. At the surface of the tumour (top of figure) a small process of tumour tissue penetrates between the bundles of muscle fibres adjacent to the growth. $\times \frac{120}{1}$.

the islands of epithelial cells. Both types of growth are present in variable quantity in most of the tumours, but the acinous type preponderates in the more slowly growing representatives.

The stroma in a fully developed tumour is very small in quantity, consisting of delicate strands of connective tissue carrying capillaries between the acini and alveoli. At the periphery of the tumour there is a well defined layer of connective tissue, showing a slight amount of small round-cell infiltration. Growth at the periphery of the tumour takes place partly by expansion of this tissue, but also by infiltration of this spurious capsule. One can see on examination of the growing edge how columns of cubical cells penetrate between the laminæ of the connective tissue, where they develop into acini and alveoli, and thus add to the bulk of the tumour by infiltrating the surrounding tissue.

Corresponding to a poorly developed vascular system, necrosis sets in early, and can be recognised by the naked eye in a ten days old tumour as greyish points scattered throughout the centre. The process extends rapidly until the whole centre of the tumour is occupied by a white caseous mass enclosed by a rim of grey gelatinous healthy tumour tissue. Microscopically the necrosis is seen to start first in the epithelial cells which have been thrown off into the lumina of the acini ; these necrosed epithelial cells accumulate until the acini become distended with necrotic material, and finally the wall of the acinus itself becomes involved. The fusion of adjacent necrotic points leads to the formation of extensive areas of necrosis, scattered through which are a few trabeculæ of connective tissue lined on either side by healthy epithelium.

Macroscopical metastases in the lungs have not as yet been seen in this tumour, although numerous cases have shown extensive infiltration of the abdominal and chest walls. When the tumour is infiltrating muscular tissue, it does so in the form of strands of epithelial cells separating the muscle fibres and leading to their eventual atrophy (see fig. 1).

After this description of the histology of the tumour, attention may now be directed to what occurs on transference of a small nodule to a new host. At the end of 24 hours the host tissues in the immediate neighbourhood of the graft are œdematous, the connective-tissue fibrillæ are more widely separated and the whole tissue is looser. There is no change in the fixed connective-tissue cells, and no increase in their number, but there is an abundant exudate of polymorphonuclear leucocytes and lymphocytes. In many cases the amount of injury to the

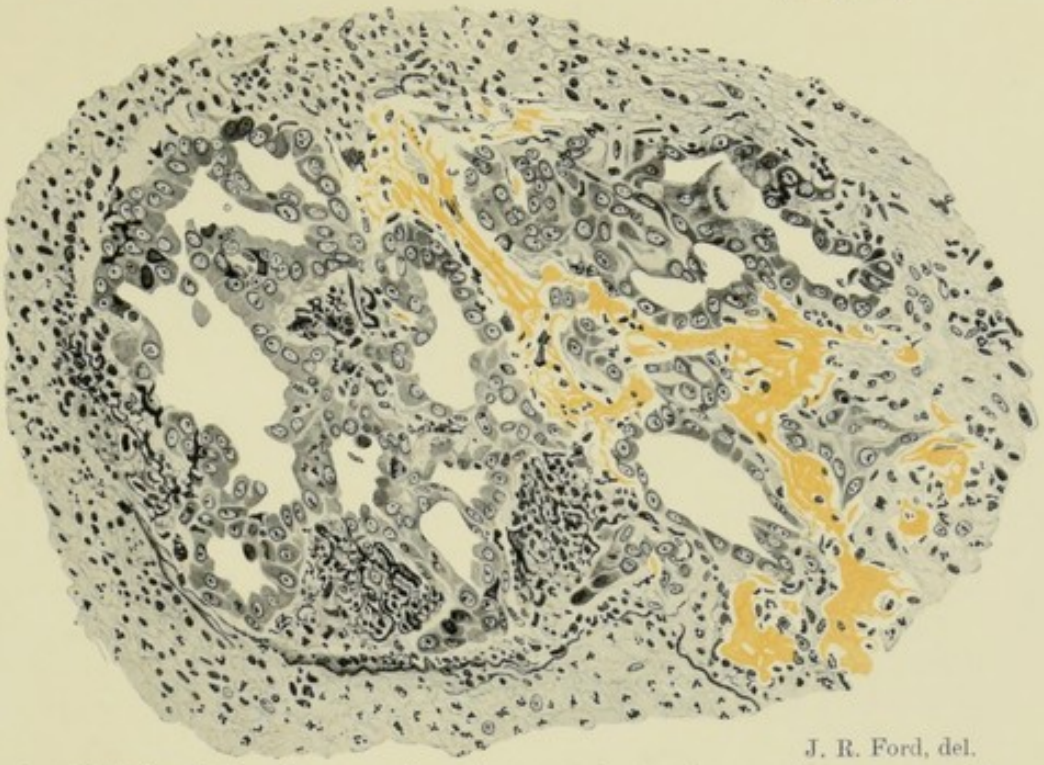
host tissues is slight, but in others there may be seen at some part of the periphery of the graft an area where the tissues are undergoing necrosis, as evidenced by the breaking down of the nuclei of the fibroblasts. The number of cells derived from the blood is less where the amount of injury done to the host tissues is slight, and where there is very little evidence of necrosis in the graft itself. The cleft between the graft and the host tissues is marked by a layer of fibrin, but at some part of its circumference the graft may be directly in contact with the host tissues with no evidence of any inflammatory reaction at all.

As regards the graft itself, even with the smallest grafts there will usually be found some necrosis in the centre, but at the periphery the islets of tumour cells remain uninjured, and the parenchyma can be seen to be dividing actively. The stroma between these islands already shows signs of degeneration ; the collagen fibrils swell up and lose their sharp contours, whilst many of the nuclei become irregular and angular in outline ; the chromatin of these nuclei aggregates, and the whole stains very darkly. A few of the nuclei still retain their normal appearance, although no evidence of division has been observed. Some of the leucocytes have by this time penetrated into the peripheral parts of the graft.

After two days the number of polymorphonuclear leucocytes is diminishing except in cases where there has been much injury to the tissues, or a large quantity of necrosis in the graft. Rarely one may see mitoses in the connective-tissue cells of the host as early as the second day. The degeneration of the stroma of the introduced graft is now more marked (see fig. 2) ; the collagen fibrils are converted into a homogeneous mass, which has a peculiar glassy appearance and which stains faint yellow with Heidenhain's hæmatoxylin. Lying in this faintly stained mass numerous aggregations of darkly staining granules can be seen, the remnants of degenerated stroma cells, whilst other cells can be seen, which have not yet advanced so far in the process of destruction. The barrier of fibrin between the host tissues and the graft is still present, and a large number of polymorphonuclear leucocytes and polyblasts have penetrated into the degenerated areas of the graft. Mitoses are frequent in the tumour cells, and many of them lose their cubical or columnar shape, becoming spindle-shaped and extremely like fibroblasts, and could be mistaken for such, did not the use of serial sections clear up all doubt as to their identity.

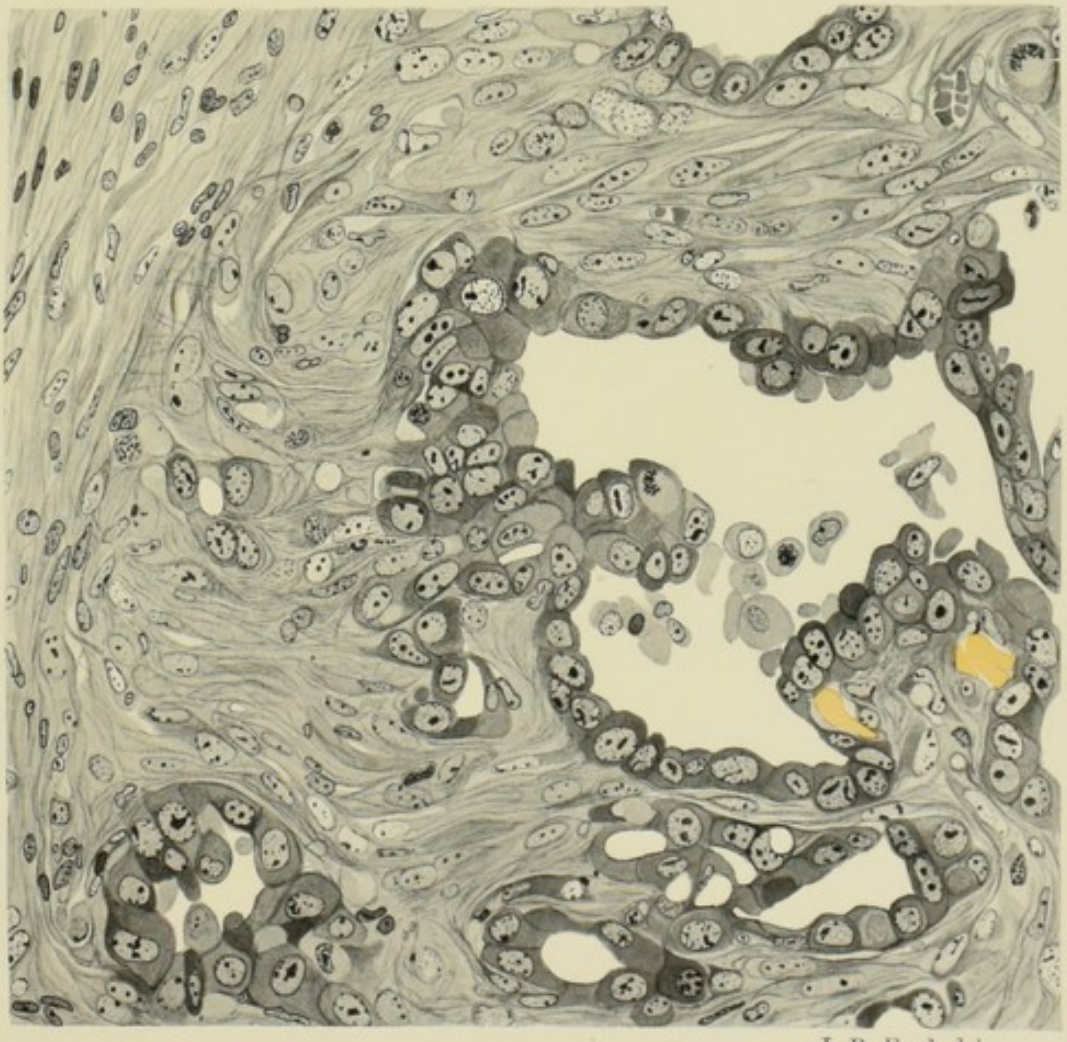
The changes occurring during the first two days are the same in

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FIG. 2.—27/17 H—18 G. Degeneration of stroma in "early stage" of tumour 27. Killed two days after inoculation. Entire graft, separated from host tissues by an interrupted layer of fibrin. Degenerated collagenous fibrils (brown). Small cell infiltration of surrounding tissues and of necrotic areas in the graft. $\times \frac{120}{1}$.



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FIG. 3.—27/17 H—18 G. Formation of new stroma in "early stage" of tumour 27. Killed four days after inoculation. Surface of graft showing ingrowth of new fibroblasts and capillaries. Proliferation of parenchyma with formation of new acini. Vestiges of degenerated introduced stroma (brown). $\times \frac{330}{1}$.

both normal and immune animals, but after this period it is as a rule easily possible to distinguish a graft taken from an immune from one from a normal animal. During the succeeding three or four days the process of re-organisation, as described and figured for Jensen's tumour and for a hæmorrhagic carcinoma in the Second Scientific Report, takes place in normal animals. Briefly this consists in an active division, direct and indirect, of the connective-tissue cells of the host, leading to the development of a cellular reaction-tissue which penetrates between, and applies itself closely to the islets of parenchyma, and clears up as it goes the old degenerated stroma introduced along with the parenchyma. Concomitantly with this, there is a rich development of new blood-vessels from the capillaries of the host, which are carried in with the stroma cells, and lead to the vascularisation of the outer area of the graft during the fourth day.

The process, as it occurs in tumour 27, does not run its course quite so rapidly as in other tumours, but the changes are the same in both. The growing edge of a four day old graft of tumour 27 in a normal mouse is seen in fig. 3. This shows an old dilated acinus from the previous generation, lined mostly by one layer of cells which are dividing actively, and forming at the edge of the acinus solid buds of epithelium which become hollowed out later to form daughter acini. The epithelial cells are distinguished through their being stained a deeper tint than the surrounding mesoblastic tissue of the preparation. Penetrating between and surrounding these acini spindle-shaped fibroblasts are streaming in from the tissues of the host, and along with them the new capillaries. At the part of the graft farthest from the host tissues, portions of the old degenerated stroma are still present. A few polyblast cells can be still found amongst this stroma, but the polymorphonuclear leucocytes have almost entirely disappeared. The further changes which occur lead to the total re-organisation of what is now a new generation of the tumour ; the reaction-tissue becomes converted into the delicate stroma described as characteristic for the tumour, in the account of the histological structure of a fully developed nodule.

In an immunised animal the processes occurring on and after the third day are the following. The necrosis in the centre of the graft extends, until all the graft has degenerated with the exception of the epithelial cells in the immediate neighbourhood of the host tissues ; the fibrinous exudate becomes absorbed, and the number of polymorphonuclear leucocytes decreases. The acinous arrangement is destroyed, and the tumour cells lie as a single layer of cells between the necrotic

centre of the graft and the host tissues. There is not the active proliferation of the host fibroblasts seen normally, nor is there any development of new capillaries. The epithelial cells still continue to divide mitotically.

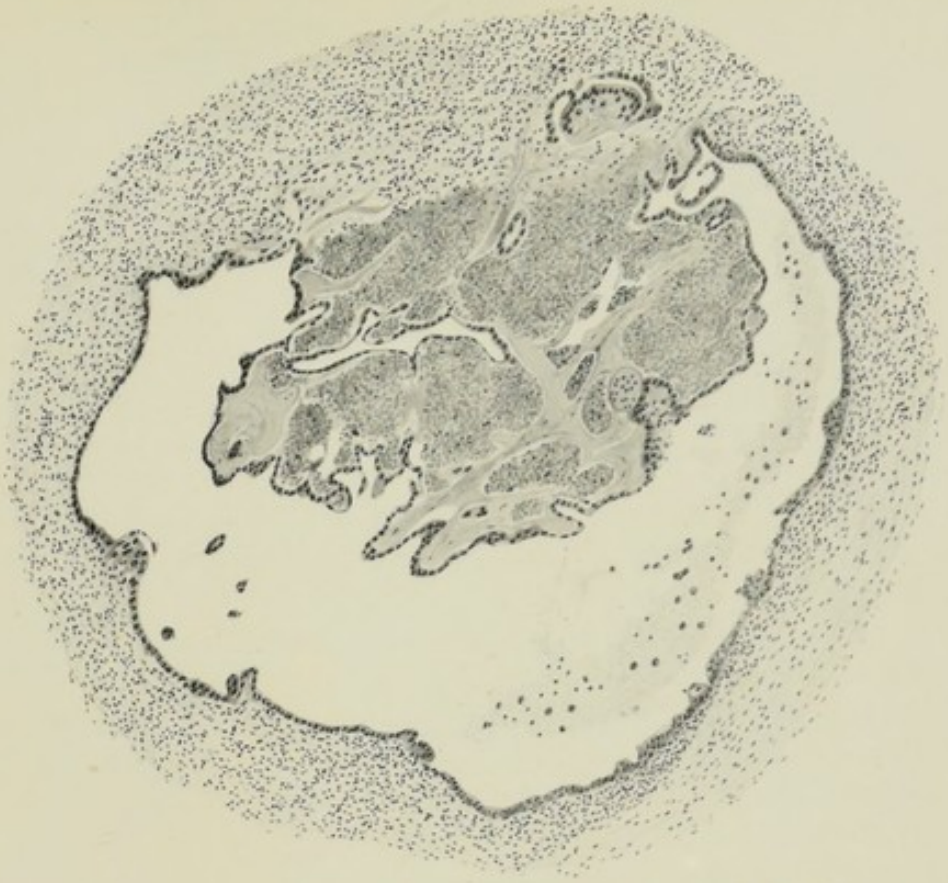
As shrinkage of the necrotic centre of the graft occurs, there is produced a cleft between the graft and the host tissues, and the epithelial cells spread themselves out over this free surface, producing a cystic cavity. Fig. 4 is a section of a five days old graft from an immune mouse, and shows a cystic cavity with fluid contents, scattered through which is the débris of necrotic desquamated epithelium. The main mass of the graft projects at one side into the cavity of the cyst, and is covered on its free surface by healthy epithelium ; the remaining acinous spaces lying in the centre of the necrotic tissue are seen on serial section to be continuous with the main cavity.

The line of contact between the host cells and the pedicle of this dead material does not show anything comparable to the rich development of new tissue seen in the picture of a normal graft at four days. When one examines the outer wall of this space with a high power (fig. 5), the lining membrane is seen to be composed of a layer of columnar epithelium, one or more cells deep and sharply demarcated, as if by a basement membrane, from the host tissues. The cells still exhibit mitoses, but not in the number customary in a growing graft.

The host tissues themselves are more cellular than the normal areolar tissue of the mouse, but this is mainly due to the presence of polyblast cells and polymorphonuclear leucocytes. There is no evident increase in the vascularity of the tissues. The absorption of the necrotic mass takes place very slowly, only after about seven days do the fibroblasts and polyblasts penetrate into it in any number, and about 12 to 14 days is necessary for the whole to be cleared up. This can be easily understood, since there is no rapid development of new capillaries, which could penetrate into the mass, and give an abundant supply of the phagocytes necessary for rapid absorption.

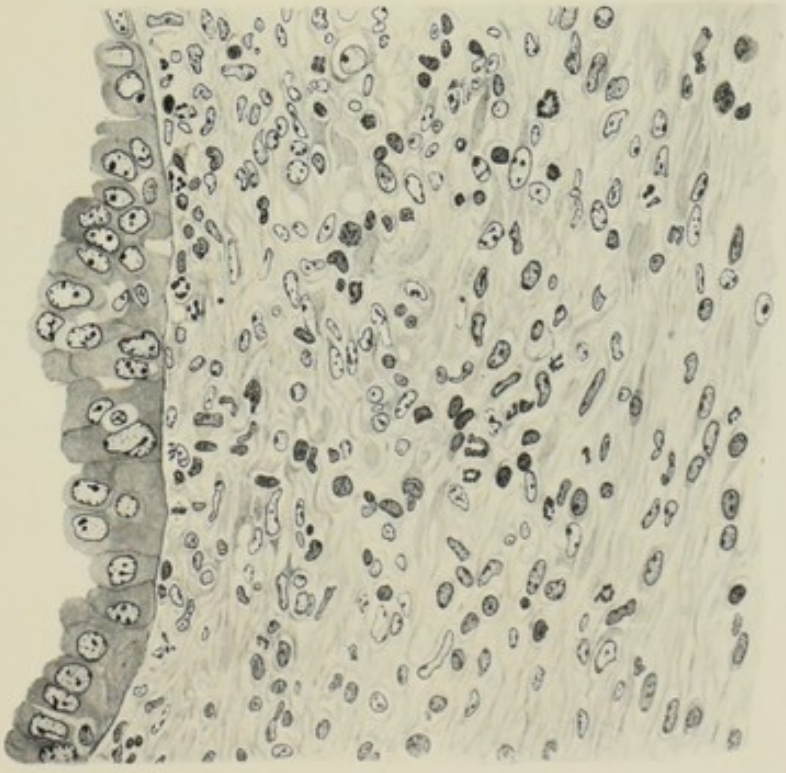
Fig. 6 is taken from a seven day old graft in a mouse previously inoculated with embryo-skin emulsion, and shows the inception of the process which leads to the eventual destruction of all epithelial elements. The fibroblasts of the host connective tissues grow in, and surround the epithelial elements compressing them and eventually producing their atrophy. In the later stages it is usual to find many of the epithelial cells multinucleated, which may be accredited to the gradual paralysis of the protoplasm preventing the completion of the mitoses. About 12 days is necessary for the final extinction of all epithelial elements, but this varies considerably in different animals.

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FIG. 4.—27/16 G—17 D. The whole of a graft in a "27-immune" mouse, preserved 5 days after inoculation. The main mass of the graft is necrotic and has shrunk somewhat. A thin layer of healthy cells remains applied to the host tissues, and another covers the necrotic centre. The cystic space between these two layers contains fluid and degenerated tumour cells. There is no attempt at revascularisation. The tissues of the host contain a large number of polymorphonuclear leucocytes, lymphocytes and polyblasts. $\times \frac{40}{1}$.



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FIG. 5.—27/16 G—17 D. Same preparation. Right side of cyst-wall adjoining host tissues, under higher magnification. The tumour cells form a columnar epithelium sharply demarcated from the very cellular connective tissue of the host. No new formation of large fibroblasts or young capillaries, *cf.* Fig. 3. $\times \frac{220}{1}$.

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FIG. 6.—27/17 H—18 J. The whole of a graft preserved 7 days after inoculation into a mouse rendered resistant by previous injection of skin of mouse embryos, *cf.* fig. 4. The figure shows commencing ingrowth of fibroblasts from the host into the necrotic tumour tissue. $\times \frac{20}{1}$.



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FIG. 7.—Jensen 107 B—108 C. The whole of a graft preserved 6 days after inoculation into a "Jensen-immune" mouse. The graft is transformed into a minute cyst lined by a thick layer of cells many of which are in mitosis. No new formation of young fibroblasts or capillaries in the surrounding adipose tissue of the host. $\times \frac{220}{1}$.

The sequence of changes in the early stages of Jensen's tumour, as occurring in immune mice, has also been studied, using grafts from the same tumour in normal mice as a control. For the description of the changes occurring normally, the reader is referred to the Second Scientific Report, pp. 24–33. It may only be added, that this tumour is more difficult to work with than tumour 27, as the amount of necrosis occurring is very extensive, and several series of experiments have to be done before a set of grafts suitable for a control to an immunity experiment can be obtained.

What usually happens in a resistant mouse is, that by the third day all the graft has degenerated, excepting some scattered alveoli of tumour cells which lie at its periphery and in intimate contact with the host tissues. The cells of these alveoli appear quite healthy, and continue to divide, but not so actively as in a normal mouse, and the amount of tumour tissue is very much less than in the control at the same date. On the fifth day there are in most cases, signs of degeneration in these islets of cells; the nuclear outline becomes angular, many of the nuclei are much smaller and several of the cells contain two or more nuclei. Invasion of the necrotic tissue of the graft by fibroblasts is now well advanced, and large mononuclear phagocytes laden with cell débris and fat are abundant in every field.

The development of acini in the early stages of this tumour is occasionally observed in normal mice, and in one case of a six-day old graft in an immune mouse, the only remnant of tumour left was in the form of a large dilated acinus or small cyst, lined by a layer of cells six or more deep. These cells appeared normal in every respect and mitotic divisions were quite numerous, as will be seen in fig. 7, drawn from this preparation. The whole was embedded in adipose tissue, and was sharply demarcated from the host tissues, in the same manner as was described in grafts of tumour 27 from immune mice at the fifth day. The epithelial elements of a Jensen tumour can be recognised as late as the ninth or tenth day, in the form of large multinucleated masses of protoplasm, the nuclei being much smaller than normal.

Another tumour (tumour 50) belonging to the group of hæmorrhagic adeno-carcinomata was also investigated. A description of the histology and biology of this tumour will be found in the paper by Dr. Gierke[1] (p. 115), as it was largely employed by him in his experiments. In the tumours which were employed for early stages, the alveolar type of

[1] *V.* p. 115 of this Report.

growth has preponderated over the acinous. What occurs in grafts in normal mice is briefly, rapid degeneration of stroma and of the centrally placed tumour-cells proper, until, by the third day, there is left only a mere shell of healthy epithelium at the periphery several layers of cells deep. Vascularisation of this shell of tumour occurs very rapidly, and the number of new capillaries formed exceeds that met with in other tumours. Fig. 8 is taken from a three days old graft of this tumour, and shows clearly how the cells of tumour 50 are enabled to call out an early and extensive reaction on the part of the blood-vessels of the new host. This is quite in keeping with the enormous number of dilated blood-vessels found in these tumours when fully developed. In immune mice, on the third day, the amount of necrosis is the same as in normal mice, but the tumour-cells have not attached themselves to the host-tissues, and with the shrinkage during fixation and embedding, the grafts come to lie in small spaces in the connective-tissue. The outer wall of these spaces is not covered with tumour-cells, as was the case with tumour 27, the cells of this tumour not possessing the degree of "thigmotaxis" exhibited by that tumour. This condition of the grafts of tumour 50 persists until after the 6th or 7th day, when the invasion of fibroblasts from the host takes place, and leads to the compression and atrophy of the tumour elements.

Tumour 32, a propagable squamous-celled carcinoma has also been examined, both in immune and in normal mice. Inoculations were made from acinous and from alveolar tumours; but none showing keratinisation were employed, which was unfortunate, as it would have been of great interest to see what effect, if any, an immune animal had upon the keratinisation. This tumour is very unsuitable for examination of early stages, the cells divide rapidly, the nodule increases in size at a very quick pace, and the amount of stroma developing is so scanty as to render the interpretation of what is occurring difficult or impossible. The stroma development and the vascularisation occur in the same manner as has already been described in more suitable material. In the immune animals the tumour-cells continue to divide, but less actively, for the first four or five days, until there is formed a small growth, devoid of all stroma, and sharply demarcated from the host tissues, which have not undergone any proliferation. Signs of degeneration of the cells in the centre next make their appearance, and rapidly advance to the periphery until, on or about the seventh day, the whole graft is dead; the final stages of absorption and repair are similar to those already described in detail for other tumours.

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FIG. 8.—50/9 A—10 D. Margin of a graft of haemorrhagic mammary carcinoma preserved three days after inoculation into a normal mouse. Exaggerated angioplastic reaction: the loose tissue adjacent to the tumour consists almost entirely of young capillaries. $\times \frac{330}{1}$.



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FIG. 9.—27/19 F—20 E. Tumour in a normal rat, 9 days after inoculation. Cellular stroma, parenchyma consists principally of darkly stained cells lining the acini. Many epithelial cells are dividing. *Cf.* fig. 1. $\times \frac{330}{1}$.

Ehrlich [1] was the first to observe the very interesting phenomenon that mouse-tumours, if transplanted into rats, grew well for about eight days, but that after this period the growth diminished, until finally the whole was absorbed. The occurrence of the same phenomenon was later described also for Jensen's tumour from the Laboratory of the Imperial Cancer Research Fund. Ehrlich claimed that this phenomenon was due to the exceptional virulence of his tumours, and could not be obtained for Jensen's tumour. Rats treated in this manner were further found to be resistant to all subsequent inoculations, a fact which he ascribed to the production of an active immunity in the animals.

When one had completed a long series of observations on what occurred to tumour grafts in immune mice, the investigation was extended to the observation of what occurred in rats immunised against mouse-tumour. A large series of rats was inoculated on the back with 0·1 c.c. of an emulsion of tumour 27. The rats were carefully observed from day to day, but no palpable increase in size of the mass injected could be recorded; instead, there was a gradual diminution until about the 7th or 8th day, when all trace of the injection had disappeared, or it was at least no longer palpable. This, as will be seen later, was not due to the absence of growth, but to the slowness of growth.

Fourteen days after the inoculation of the mouse-tumour, these rats were used for early stages of another 27 tumour, a control in normal rats being made at the same time. On examining the early stages in the control, pictures quite similar to those seen in normal mice were obtained, the rat-tissues proliferated and supplied a new stroma and vascular apparatus for the growing nodule. If any difference existed, the vascularisation ran a quicker course than in mice, and the amount of reaction on the part of the host-cells was greater. The epithelial elements divided actively, the mitoses were normal, and the development of new acini resulted in the formation of a perfect adeno-carcinoma similar to the parent tumour. Fig. 9 is taken from a nine days old graft in a normal rat, and shows how at this date, the tumour-cells were still dividing. It will be seen that many of the tumour-cells have taken up the stain more than others, and are of a much deeper tint. This is, as was shown by Bashford, Murray, and Bowen [2], evidence of impending degeneration. Mitotic figures can also be seen in the stroma of the graft depicted, and, indeed, it was most striking to see the

[1] Carcinom Studien an Mäusen, 1905.

[2] Proc. Royal Society, vol. B, 78.

manner in which these tumour-cells of the mouse were able for a time to make the rat-tissues subservient to their needs. As pointed out above, tumour 27 is a tumour with poor powers of growth—far behind those of Jensen's tumour. Ehrlich's explanation of the transitory growth of mouse-tumours in rats as due to the exceptional virulence of his tumours is shown to be untenable by the present observations, and those previously made with Jensen's and other tumours.

In the rats previously rendered immune, there took place a rapid destruction of all the elements introduced, so that as early as the third day, there only remained a few scattered tumour-cells, all of which showed marked signs of advancing necrosis (fig. 10). On the fourth day no formed elements of the introduced graft could be detected, but there was an active invasion of polyblasts and fibroblasts from the host-tissues, and the late stages of cleaning up of the field ran a very rapid course. They were accompanied by an active proliferation of the host connective-tissue cells over an extensive area. The same effect is obtained in rats when immunised with normal mouse-tissues, but not when rat-tissues are used.

Before passing to a general consideration of the results obtained, attention may first be drawn to the conclusions arrived at from detailed investigation of the changes in normal animals only. As already observed, Jensen, from the examination of early stages, was able to state that the new tumour arose from the epithelial elements introduced. Bashford and Murray later confirmed this result, and were able to add that the new stroma was derived by proliferation of the connective-tissues of the new host, and that the old stroma degenerated and became finally absorbed. The character of the stroma of a propagable tumour, with the exception of those cases where a sarcomatous transformation has occurred, remains constant within narrow limits for the succeeding generations. Bashford, Murray, and Cramer stated that this can only be ascribed to certain properties inherent in the parenchyma-cells and influencing the reacting tissues of the host in a specific manner, for these are the only elements which continue from one generation to another. An alveolar carcinoma continues on transplantation to retain the delicate stroma of the parent tumour; a hæmorrhagic adeno-carcinoma continues to develop large dilated vascular sinuses, and so on.

Ehrlich *, in accepting this specific stroma reaction, likewise ascribes to the tumour-cells specific chemiotactic properties acting on the

* Zeitsch. f. Krebsforschung, Band v. Heft. i. 1907.

[*To face* p. 354.



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FIG. 10.—27/19 J—20 D. Entire graft preserved 3 days after inoculation into a rat previously treated with 0·1 c.c. of emulsion of tumour 27. The whole graft is necrotic, even the superficial parts in contact with the tissues of the rat. A few islands of mouse tumour in the form of collapsed cysts with shrunken cells. Very abundant small cell infiltration of surrounding tissues. Compare fig. 9, graft of tumour 27 in normal rat and fig. 4, a similar graft in an immune mouse. $\times \frac{100}{1}$.

fibroblasts of the new host. From the examination of a transplantable chondroma which very early showed hæmorrhages on transplantation, he came to the conclusion that the cells of this tumour possessed special angiotactic powers, which led to an over-production of capillaries. As mentioned by Gierke on an earlier page, it seems therefore possible to subdivide up the stroma reaction into two components, the fibroblastic, which leads to the development of the true supporting tissue, and the angioplastic, which provides the new blood-vessels.

Fig. 8, from a three day old hæmorrhagic tumour, serves as a good example of the case where the tumour-cells possess to an unusual degree an angioplastic chemiotaxis; whilst the figure of the stroma formation at four days, in tumour 27 (fig. 3), shows a case where the fibroblastic reaction preponderates.

Turning next to the consideration of the processes occurring in grafts in immune animals, the outstanding feature in all of the tumours employed has been the total failure of the new host to supply a vascular stroma. The failure of this re-organisation quite explains all the histological pictures which we have seen in the various tumours in immune mice. In the solid tumours the failure of a vascular supply led to the necrosis of all the graft, excepting only the cells at the periphery, which obtained their nourishment by diffusion from the host-tissues. In tumour 27, where the cells had arranged themselves in two layers, the one adhering to the host-tissues, and the other to the necrotic mass of the graft, the latter layer must have received its nutrition by diffusion across the cystic space intervening between the outer and inner layers. The fact of this tumour being able to grow along the free surface of the host-tissues, does not appear to be of fundamental importance, it is merely the expression of the inherent tendency of the epithelial cells to spread along a free surface. The cells of two other adeno-carcinomata which were tested in immune mice, but which are not given in the preceding description, showed the same power of lining a free surface, although to a lesser degree. In the experiments done by Ribbert upon the transplantation of pieces of normal epidermis, he found that the epithelium introduced, bent round in its growth, and by fusion of the two free edges led to the formation of a small cyst.

Having demonstrated the failure of the stroma reaction in immune animals, the question arises, how is this brought about? The failure of the reaction can be ascribed to one of two factors; either the tissues of the animal have been altered in such a way by the process of immunisation, that they no longer react to the stimulus of the cancer

cell, or else the cancer cell itself becomes robbed of its power of inciting a specific reaction.

A consideration of the specific nature of the stroma reaction to various tumours, which can only be determined by the parenchyma cells themselves, as well as of the specific nature of the phenomena of immunity, as recorded on other pages of this Report, leads one to regard the immunity as being directed against the cancer cell itself, the most probable explanation being, at the present time, that it is directed against the chemiotactic influences exerted by cancer cells on the connective-tissues of the host, as suggested by Bashford, Murray and Cramer, in the preceding paper, p. 340.

Professor Starling's experiments upon the development of the mammæ of virgin rabbits, produced by the inoculation of an extract of embryo-emulsion, have also an indirect bearing upon this question. These inoculations stimulated the secreting cells of the mamma, and led to an increase in the bulk of that organ, which could only take place along with an increase in the vascular supply. Tumour 27 is so typically an adeno-carcinoma of the mamma as to resemble closely at times the structure of the normal lactating organ, and yet it has been found that the previous inoculation of embryo-emulsion prevents the provision of a vascular supply for any subsequent inoculation of this tumour. This may be the expression of a fundamental difference between the normal cells of the mamma, and those of an adeno-carcinoma of the same organ, although the fact that in Starling's experiments we are dealing with an organ already laid down cannot be neglected.

Ehrlich found that a hæmorrhagic chondroma, when inoculated subcutaneously, grew with a white instead of a blue colour under two conditions, either where he used immune mice, or where the vitality of the tumour-cells had been previously injured by heat. Gierke has recorded on p. 115 how hæmorrhagic mammary tumours alternate naturally between hæmorrhagic and non-hæmorrhagic phases, and we have not been able to influence the angioblastic chemiotaxis for them in the way described by Ehrlich for his chondroma, any more than Murray has keratinisation in tumour 32.

It therefore seems justifiable to ascribe the absence of "the specific stroma reaction" in immune animals, to the suppression of the chemiotactic properties of the cancer cells. The suppression of this chemiotactic power robs them at the same time of their powers of assimilation and growth, so that they lie inert when inoculated subcutaneously, and die.

There must be present in the resistant animals, either in the circulating fluids or in the tissues, something which inhibits this chemiotaxis. Owing to the absence of any evidence which would justify an analogy with the antitoxines or anti-bodies to infective organisms and their products, we refrain from the use of these terms. The resistance induced to cancer is in nature *sui generis* and requires to be studied as such. All attempts made by other workers to demonstrate the presence of an active cell poison *in vitro* have been inconclusive as yet. The extensive investigations made on this subject in the Imperial Cancer Research Laboratory have been entirely negative. It is not impossible that the positive results claimed by others are fallacious. That the induced resistance to inoculation is not due to a very active cell poison may be seen from the power which the cancer cells retain, of continuing their proliferation for 7–10 days in this unfavourable medium, provided that they can obtain sufficient nourishment. Further, the cells at the periphery of the graft are those which go on growing, and these are the very cells which are most exposed to the influence of any supposed poison, whereas the cells towards the centre of the graft, which are not so exposed to a free supply of this inimical substance, are the ones which die rapidly because of the interference with their food-supply.

The action of the cyto-toxins and cytolysins, which are produced by the inoculation of epithelial tissues into a strange species, as was shown by von Dungern, is much more powerful than this, leading as it does to a rapid disintegration of the cells tested. The results obtained in the early stages in immune rats as described above, help us in reconciling the differences. In the rats previously treated with 0·1 c.c. of tumour 27, there was a rapid degeneration of the cells of the new graft; this degeneration involved the cells throughout the whole graft, and led, in about three days time, to their total necrosis. If any difference could be detected here in the location of the process, that is, between the cells at the periphery and those centrally placed, it was rather in favour of the latter retaining their vitality for a longer period, but the difference was not marked enough to lay any weight upon it. The rate of degeneration in immune rats was, however, much more rapid than in immune mice, and this can be interpreted as being due to a more active production of the inimical factors in the rat, and, in addition, to qualitatitive differences in their nature when induced in a strange species. Considering that the rats had been immunised with tissues from another species viz., the mouse, this result is only what would have been expected. Further experiments are being made to determine whether the pheno-

mena in the case of rats lend themselves more readily to investigation *in vitro* than in the case of mice.

The influence exerted by the body-fluids in the immune mouse cannot be referred to an active cell-poison, since it allows the implanted cells to continue proliferating for about 8–10 days, but eventually it injures them to such an extent that they can no longer withstand the encroaching fibroblasts of the new host. These latter penetrate between the cells and producing scar tissue instead of the connective tissue scaffolding required by the parenchyma cells, they compress the latter, leading to their final atrophy and degeneration.

The minute details of the final process are similar to those described by Orth [1] in human epitheliomata, by M. B. Schmidt [2], Lubarsch and Gierke [3] in carcinomatous emboli in the lungs, and by Bashford, Murray and Cramer [4], and later by Gaylord and Clowes [5], in spontaneous healing of mouse tumours. The probability is great, therefore, that in all these cases, local or general conditions interfering with the requisite re-organisation of the affected groups of cells, are responsible for the progression of the reactive processes towards encapsulation of the cancer cells, and spontaneous healing.

In conclusion it is necessary to express thanks to the Executive Committee of the Imperial Cancer Research Fund for permission to work in the laboratory, and to Dr. Bashford and his colleagues for their encouragement and assistance, and for the opportunities which have permitted the conduct of this investigation, and my fulfilment of the conditions of tenure of the Georgina McRobert Cancer Research Scholarship at the University of Aberdeen.

[1] Zeitschr. f. Krebsforschung, Band i. Heft. v. 1904–55, § 399.

[2] Die Verbreitungswege der Karzinome. Jena, Fischer, 1903.

[3] Page 131 of this Report.

[4] Discussion on paper on "The Growth of Cancer." Transactions of the Medical Society of London, 1905, *also* Second Scientific Report of the Imperial Cancer Research Fund, 1905.

[5] Surgery, Medicine, and Gynæcology, June 1906.

RESISTANCE AND SUSCEPTIBILITY TO INOCULATED CANCER.

By E. F. BASHFORD, M.D., J. A. MURRAY, M.B., and M. HAALAND.

It has been pointed out repeatedly in the preceding pages and elsewhere, that with the achievement of the experimental propagation of cancer, opportunities are afforded for studying the conditions of growth of malignant tumours. Experimentally produced conditions unfavourable to growth, have received particular attention from practically all investigators of experimental cancer, because of the aspiration to further the rational treatment of malignant disease in man.

In this connection more almost than in any other, the distinction between the continued growth of cancerous cells either in the animal spontaneously attacked, or in normal animals to which they have been transferred experimentally, and the inception of malignant proliferation must be ever present in the mind of the investigator and of the reader. This consideration has dictated the wording of the title of this paper, and although in the sequel, the terms "immunity," "immune mice," etc., will be frequently used, this is done entirely as a matter of convenience, and in no case must be taken to imply a diminished liability to the development of spontaneous cancer, but only such an alteration in the animals, that on introducing intact cancer cells into them, no tumour is produced.

The description of the various procedures by which this effect can be produced, and the precautions which must be observed in carrying out the experiments or in interpreting the results, will involve some repetition of observations already described. This is rendered necessary by the intricacy and delicacy of the processes, and by our desire that others may be enabled to repeat our experiments.

Since the publication of the paper, reprinted at p. 315, on "Natural and Induced Resistance of Mice to the Growth of Cancer," in which the earlier literature is discussed, this subject has been dealt with in communications by Haaland, Borrel, Bridré, Schöne, Lewin, Flexner, and Jobling.

Haaland was able to show, that mice might be resistant to one strain of transplanted cancer and yet susceptible to another, and that the absorption of even considerable quantities of Jensen's carcinoma did not necessarily protect against subsequent inoculation of the strain of Ehrlich's experimental sarcoma with which he worked. Borrel and Bridré showed, confirming earlier observations, that resistance could be induced by the absorption of tumour material without intervening tumour formation. That a certain considerable quantity of tumour tissue was necessary, and that similar results could be achieved by preliminary treatment with normal mouse tissues (liver, spleen, but not testis), and not by tissues of other species. They devoted considerable attention to the results of successive inoculation with the same and other tumours, and came to the conclusion, that where small doses were used, the second inoculation generally gave a result parallel to that of the first inoculation.

In the account of the general features of the propagation of mouse tumours on p. 262, attention is again drawn to the importance of technical minutiæ in modifying the results of experiment. All that is there laid down, applies with increased force to experiments designed to show differences in growth in animals subjected to preliminary treatment. The greatest care must be exercised that the animals used as control in all such experiments should conform closely in race, age, weight, and general health, to those with which they are compared. Further, the material for inoculation must be distributed among the animals as uniformly as possible. To this end, the site of inoculation and the dose of material inoculated must be identical, and the latter must be accurately measured. When inoculation is carried out with the hollow needle and plunger, uniformity of dosage is attained by paying the greatest heed to the equal subdivision of the material and to the choice of the fragments. When the syringe method is used, the tumour emulsion must be as uniform as possible, prepared by sharp cutting instruments and not by crushing. The dosage in the syringe method is by volume, and when this is small, it is important to use the finest hypodermic needle which will transmit the fragments making up the emulsion of tumour.

The quantity of the active substances must be most carefully stated. It is obvious that it would be impossible to compare the protection conferred by preliminary treatment with two separate tissues (*e. g.*, normal and cancerous), if unequal and unmeasured quantities of each were introduced. The interval between the preliminary treatment and the testing inoculation, must be also specified. Its importance is clearly manifest in the experiments given in figs. 13 & 15. There can be little doubt, that if the experiments of different investigators could be minutely recorded in this manner, many of the apparent contradictions would disappear.

It is desirable to consider the importance which should be assigned to fortuitous individual variations in the suitability of the animals for inoculation. The negative results of inoculation are commonly ascribed to natural resistance, and many investigators have made it a serious difficulty in appraising the value of the results of *in vivo* experiments with cancer.

It is probable that more precise conceptions will have to be substituted for the indefinite terms natural resistance or immunity or natural atrepsy, to explain the varying ratio between the number of positive and negative animals after inoculation with different tumour strains. We have previously insisted that, when the necessary precautions are taken and sufficient numbers of animals inoculated, the results of experiments are comparable.

That the negative results of inoculating normal animals is in all probability not due to the presence of anti-bodies naturally in the serum, is shown by the fact, that small doses give better results in the inoculation of spontaneous tumours, than do large doses.

Hertwig and Poll have contended, that the varying distribution of positive and negative animals, in groups of mice which had been subjected to special preliminary treatment, was due entirely to uncontrollable fortuitous fluctuations in natural resistance.

It is easy to prove that the limits within which it is permissible to invoke the influence of natural resistance are very narrow. A number of the tumours propagated in our laboratory grow with great difficulty in normal animals. In consequence large numbers of animals must be inoculated for each experiment, and they have been kept in separate boxes of ten or twelve each. When the numbers of tumours in a long series of mice in such an experiment are taken, it is found that the ratio of positive to negative animals in each box, is nearly constant for any one experiment. When considerable and constant differences are

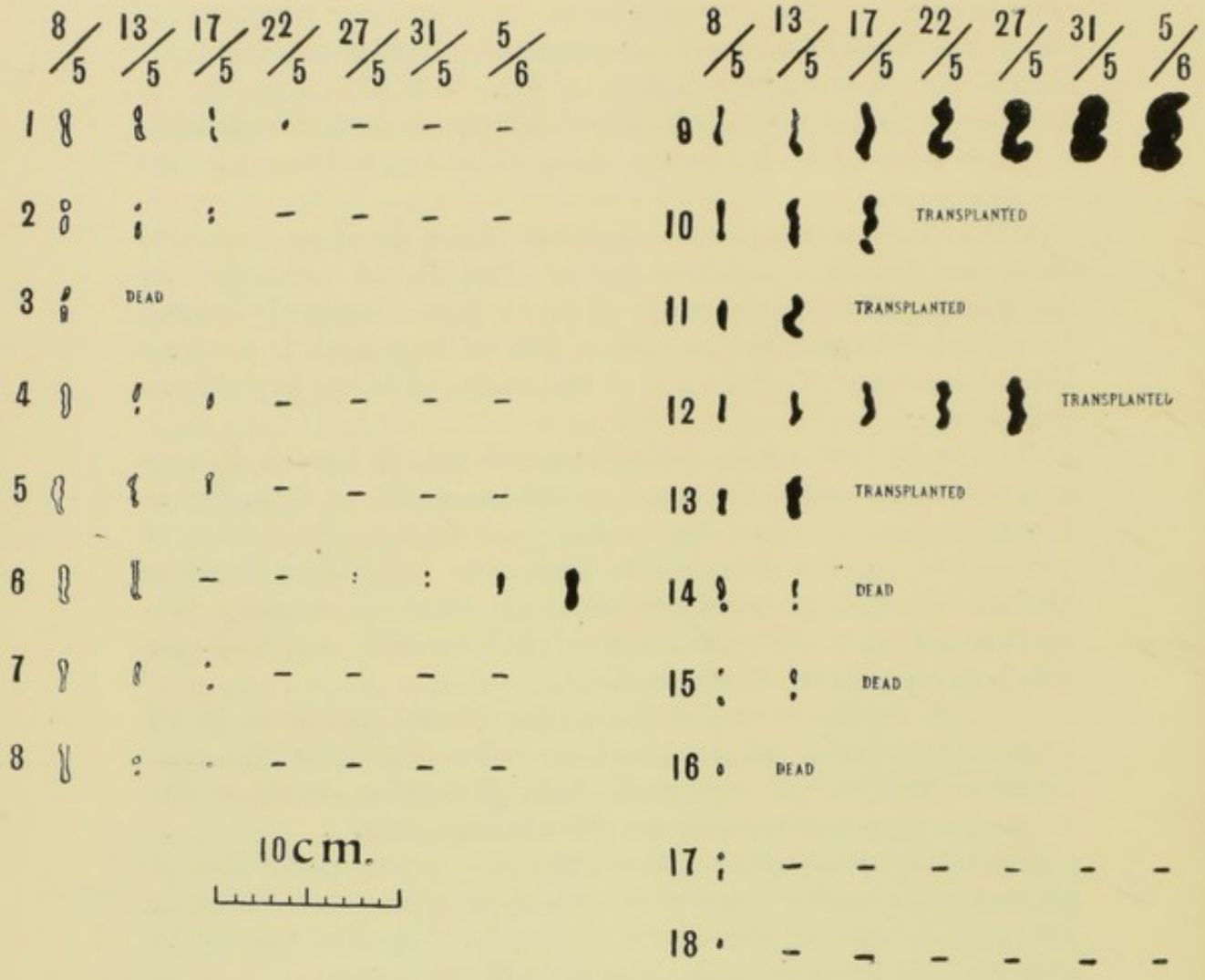


FIG. 1. Exp. 32/8 L.—Contrast between the results of inoculating large and small doses of the same tumour emulsion into normal mice. All mice inoculated in right axilla, 30.iv.07, with tumour emulsion: dose 1–8, 0·1 c.c.; 9–18, 0·05 c.c. Compare figure 2 showing an experiment with the same tumour, in which the contrary effect is produced by increase of dose.

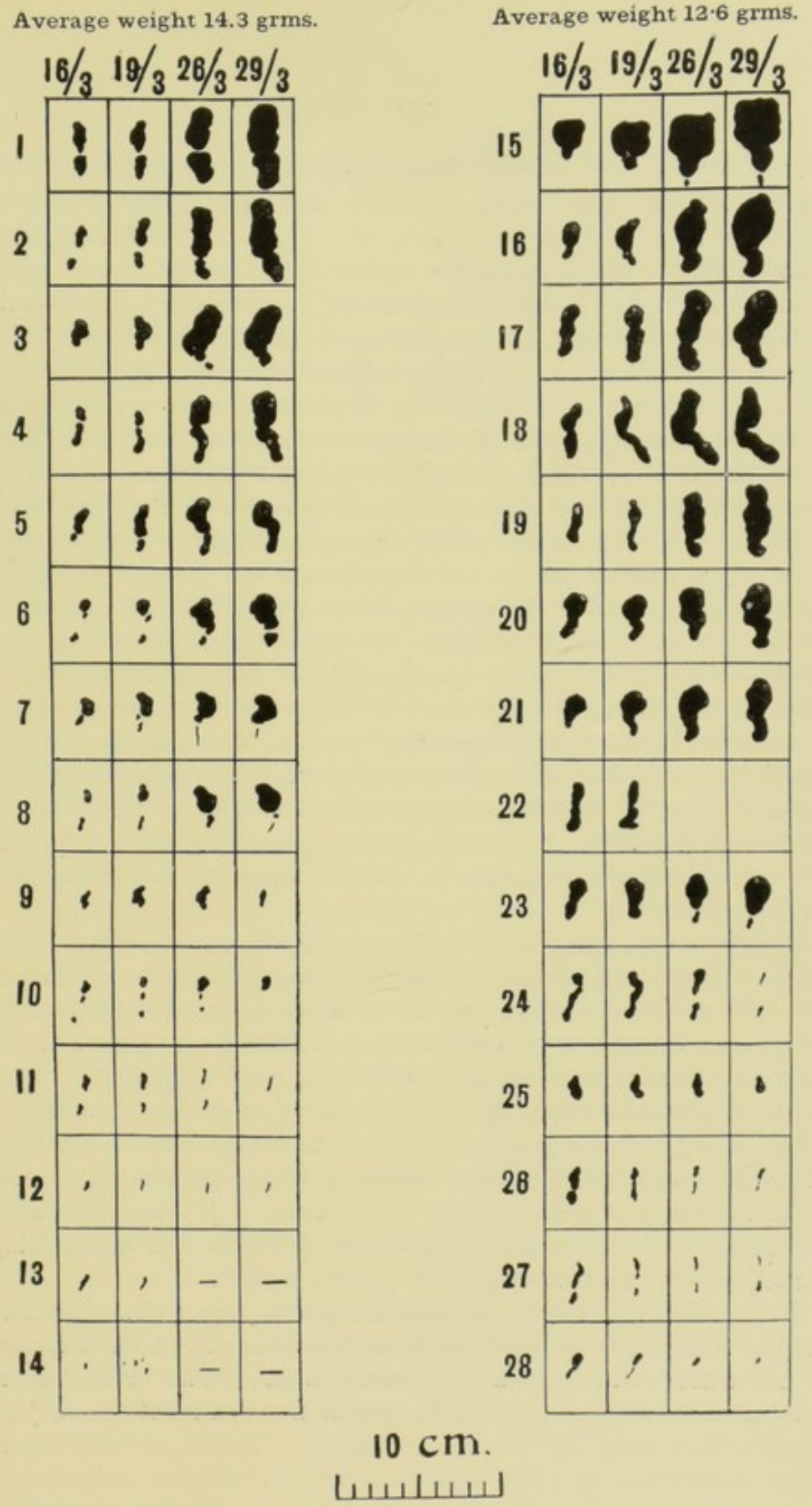


FIG. 2. Exp. 32/23 E.—Higher percentage of success and more rapidly growing tumours, in mice inoculated with a large as compared with a small dose.

All mice inoculated in right axilla, 6.iii.08. Dose: 1–14, 0·025 c.c.; 15–28, 0·15 c.c. of tumour emulsion (syringe method). First charting ten days after inoculation, second charting three days later, and thereafter at intervals of one week.

encountered in the results of inoculating normal as contrasted with treated animals, it is absurd to invoke the intervention of natural resistance.

In order that the necessity for uniformity in dosage may be again strongly emphasised, and its importance for immunity experiments realised, attention may be directed again to two experiments with tumour 32, which show how easily, unless the necessary precautions be taken, the results may be vitiated by this factor when several groups of animals are inoculated with the same material, at the same time. Fig. 1 shows how in Exp. 32/8 L, the tumours arising from 0·1 c.c. of tumour emulsion all disappeared after a preliminary growth, while of those which developed from 0·05 c.c. five out of seven grew progressively. The late development which supervened in one mouse of the first group (No. 6), illustrates an important phenomenon which will be referred to again in connection with successive multiple inoculations (*cf.* p. 386). Fig. 2 on the other hand shows another experiment with the same tumour strain, Exp. 32/23 E, in which large doses of tumour material gave a higher percentage of more quickly growing tumours, than did simultaneous inoculation of similar mice with a small dose. The explanation of this different behaviour at different times is to be sought in alternations of the biological qualities of the tumour cells, as already explained on p. 278, and corresponding alternations in their vulnerability to unfavourable environment.

Equally we must emphasise the importance of accurate dosage when we use tumours or tissues as immunising substances. In our experiments we have used for this purpose doses embracing a wide range. In practice we have found it inadvisable to introduce more than 0·2 c.c. of emulsion of solid tissue or more than 0·5 c.c. of defibrinated blood. When larger doses than these are employed, the risk of secondary infection is greatly increased, with disastrous diminution of the number of animals available. There is a further advantage in selecting a dose of immunising substances which is just sufficient to produce measurable resistance without obscuring its nature, as will appear later in discussing the experiments themselves (*cf.* also pp. 334 & 335).

Further experience has confirmed the general conclusions drawn in the preceding paper by Bashford, Murray, and Cramer, on the induction of resistance to inoculation by various means. The differences of opinion which still exist among the several investigators of this subject, concern themselves with the significance of the results, and the nature of the mechanism by which they are produced. The minutest details

of the conditions under which the effects are produced are therefore of great importance.

In order that the following account of our investigations may be as concise as possible, we shall first state categorically the conclusions we have drawn. The evidence for these conclusions will then be advanced, mostly in the form of graphic records showing the percentage of success and rate of growth of the tumours obtained in the experiments, with short but complete protocols of their disposition. The experiments referred to in this paper, are selected as typical illustrations from a large number recorded in the same manner. In this way much verbal description will be avoided, and at the same time the quality of the evidence will be appreciable to those who have no personal experience of experimental tumours.

(1) The protection following absorption of tumour material is effective only against the inoculation of tumours derived from the same species of animal. Absorption of rat-tumour does not protect against subsequent inoculation with mouse-tumour, and rats treated with mouse-tumour are not resistant to inoculation with transplantable rat-tumour.

(2) The protection which follows absorption of tumour material from the same species as the animal protected, is greatest against subsequent inoculation of the tumour absorbed. It is less against other tumours of similar histogenesis and still less for tumours of different histogenesis.

(3) In the same way preliminary treatment with normal tissues may induce protection against subsequent inoculation, and, as for treatment with tumour-tissue, this also only holds within the same species. Mice cannot be protected against inoculation of mouse-tumours by tissues of the rat, rabbit, guinea-pig, or more widely distinct species.

(4) The degree of protection which can be induced by normal tissues, used separately, is not uniform. In general, in correspondence with their histogenesis, the more nearly related a normal tissue is to a tumour, the greater is the protection resulting from absorption of the normal tissue.

(5) Some absorption of tumour-tissue always occurs after inoculation unless the grafts be very minute. If the amount of this absorption be considerable it may modify, profoundly, the suitability of the animal for a second inoculation at a later date, and may even affect the continued growth of the tumour which arises from the first inoculation itself. In this way we account for the disappearance of small tumours which after growing for ten days after inoculation, then diminish in size. As has already been pointed out, natural resistance, in the sense of a naturally



FIG. 3. EXP. **J.R.S.**/3 C.—Comparison of growth of spindle-celled sarcoma (Jensen's rat sarcoma) in normal rats and in rats treated with mouse and cat sarcoma respectively.

1–10. Normal rats, Control .....
11–15. Rats treated with 0·1 c.c. of mouse sarcoma (37), 18 days previous to inoculation .....
16–26. Rats treated with 0·1 c.c. of cat sarcoma 15 days previous to inoculation .....

All rats inoculated 3.12.07 in right axilla with 0·05 c.c. of emulsion of Jensen's rat sarcoma. First charting 11 days after inoculation and thereafter at intervals of one week.

Treatment with sarcomata of strange species does not induce protection against subsequent inoculation of rats with transplantable rat sarcoma.

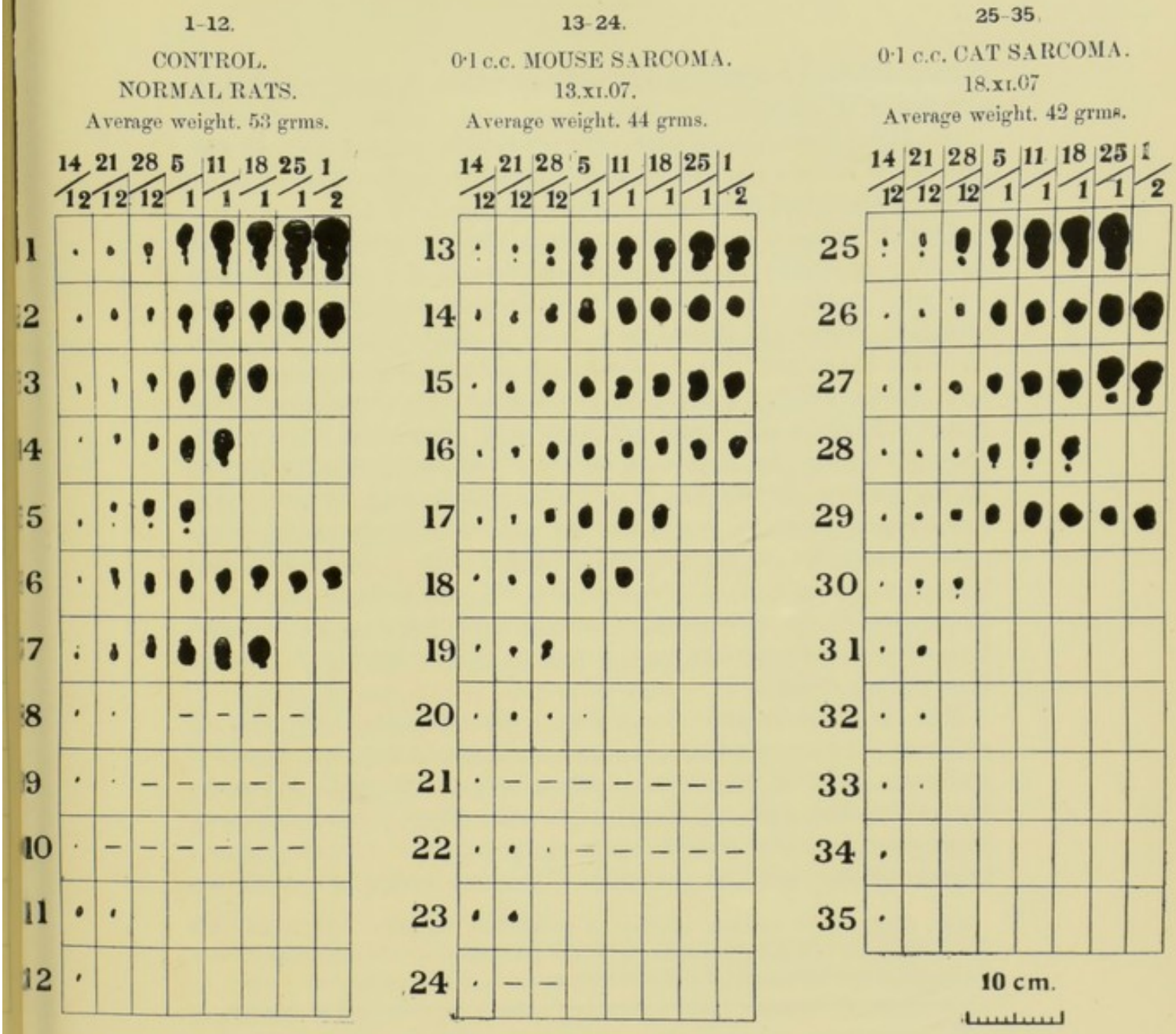


Fig. 4. Exp. **F.R.**/14 B.—Comparison of growth of Flexner's rat carcinoma in normal rats, and in rats treated with mouse and cat sarcoma respectively.

1–12. Normal rats, Control .....

13–24. Rats treated with 0·1 c.c. of mouse sarcoma (37) subcutaneously on the back 19 days previous to inoculation .....

25–35. Rats treated with 0·1 c.c. cat sarcoma subcutaneously on the back, 14 days previous to inoculation .....

} All rats inoculated with 0·02 gram. of rat carcinoma in right axilla. 2.xii.07.

There is no protection induced by this preliminary treatment with tumours of strange species.

present inhibitory mechanism, cannot explain the phenomenon in question in all cases.

(6) These facts (5) indicate that susceptible animals may become resistant to inoculation, and it has been possible to effect this alteration in animals in which tumours are actually growing.

Ehrlich adduced convincing evidence that the absorption of considerable quantities of spontaneous tumours of the mouse, rendered otherwise normal mice resistant to subsequent inoculation of transplantable tumours. Since then Schöne, Borrel and Bridré, and C. Lewin have stated that the absorption of tumours from alien species had a similar effect. Schöne, Borrel and Bridré used human cancerous material, Lewin made cross-experiments with mouse and rat tumours. Figs. 3 & 4 show the disposition and result of analogous experiments. It will be seen (fig. 3) that, as compared with normal, untreated rats, rats treated with mouse or cat sarcoma, are not immune to the subsequent inoculation of rat sarcoma. Similarly fig. 4 shows that rats treated with mouse or cat tumours are not refractory to subsequent inoculation of rat carcinoma. Working quantitatively, we have not been able to produce, or increase, resistance to inoculation with tumours from strange species.

The protection against inoculation resulting from absorption of spontaneous tumour by normal animals is obtained when large doses are used, as, *e. g.*, in the experiment of the accompanying table. When the immunising dose does not exceed 0·1 c.c. the effect is usually not very pronounced, and when inoculation is performed after a longer interval, hypersensibility may be induced. When we compare it with the

Exp. 32/20 C.—Comparison of effect of preliminary treatment with mouse-testis and spontaneous mammary carcinoma (15 days previously) on the suitability of mice for inoculation with squamous-celled carcinoma.

Treatment & dose.	Control.	Spontaneous tumour 0·1 c.c. on back.	Mouse testis 0·1 c.c. on back.
Average weight	11·5 grams.	15 grams.	13·88 grams.
Number of Mice	15	9	8
Number of progressively growing tumours	5	0	3

The mice were inoculated, 22.xi.07, in the right axilla with 0·025 c.c. of tumour emulsion.

immunising effect of similar doses of normal tissues as in later experiments, the conclusion can be drawn with a high degree of probability that the spontaneous tumour is efficacious because of its properties *quâ* mouse tissue rather than *quâ* cancer.

When we pass to the effects of absorption of transplantable tumours on the resistance of mice to subsequent inoculation, we are confronted with the difficulty raised by Hertwig and Poll. These authors held that the preliminary negative inoculation merely selected naturally resistant mice, and therefore, it was to be expected that subsequent inoculations would also be negative.

This difficulty is easily obviated by testing the suitability of negative mice to a second inoculation with the same tumour and also with distinct tumours, particularly such as grow less energetically than the former. It is then found (figs. 5, 6 & 7) that while the protection against the same tumour as was used for the first inoculation is great and frequently absolute, it is seldom absolute against a different tumour strain, even when the latter grows worse in control animals than the tumour against which protection is absolute. In these experiments there is a less degree of protection against new tumours than against that which was absorbed in the first instance. In Ehrlich's experiments the protection induced by different tumours appeared to be general and of equal degree even as between carcinoma and sarcoma ; he therefore gave this general protection the name of pan-immunity. While admitting its existence as a general immunity induced by mouse-tissues, we must recognise that the stronger protection produced by absorption of any one tumour is specific and mainly efficient against itself, as revealed by careful quantitative experiments.

The experiments with normal tissues of the mouse and rat throw light on these phenomena. The first experiments of this kind were those of Bashford, Murray, and Cramer, showing that normal mouse blood protected against subsequent inoculation with Jensen's carcinoma. The blood of alien species (rat, rabbit, guinea-pig, dog) did not produce this effect. Further analysis of the properties of blood showed, that the corpuscles were the active constituent, serum alone did not induce protection. We could find no difference either in the corpuscles or serum of normal and resistant mice. The protection induced by blood is illustrated for Jensen's carcinoma in fig. 8. The graphic record shows the relative immunity of mice treated with mouse-blood, and its absence in mice treated with rabbit's blood. In the experiment illustrated we have inoculated a larger amount of tumour-tissue than the minimal tumour forming dose, in order to illustrate the quantitative relations we have described on p. 334.

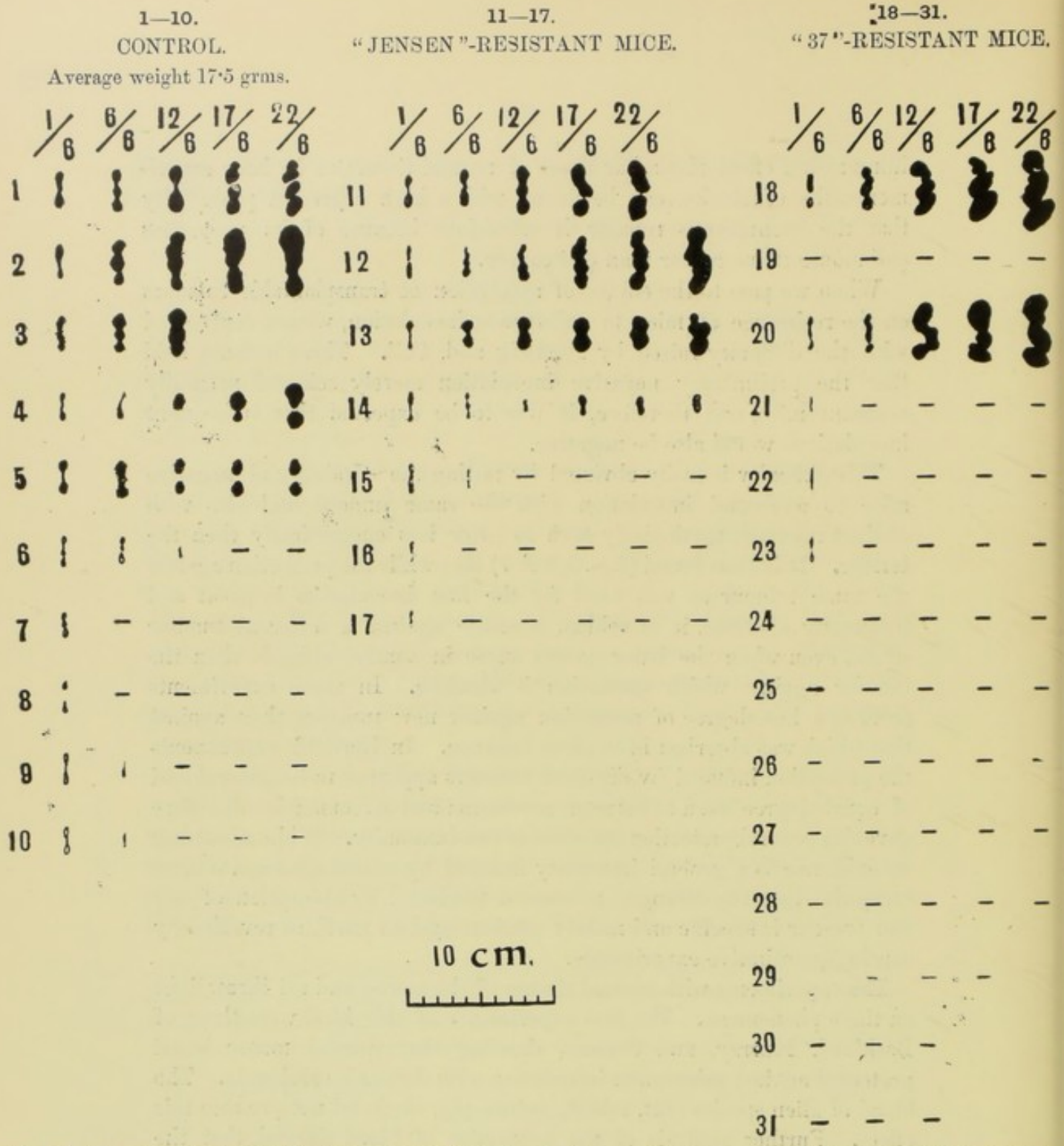


Fig. 5.—Growth of transplantable squamous-celled carcinoma (tumour 32) in normal mice compared with the growth obtained in mice resistant to inoculation with Jensen's carcinoma, and to tumour 37 (adeno-carcinoma).

Exp. 32/9 J.

1–10. Normal mice, Control .....

11–17. Jensen-resistant mice, negative mice of earlier Jensen experiments received a second inoculation of 0·25 c.c. of Jensen carcinoma 4 weeks previous to inoculation .....

18–31. 37-resistant mice .....

All mice inoculated in left axilla with 0·05 c.c. of emulsion of tumour 32, 22.5.07.

Tumour 32 grows nearly as well in Jensen-resistant mice as in normal mice, and only slightly worse in 37-resistant mice.

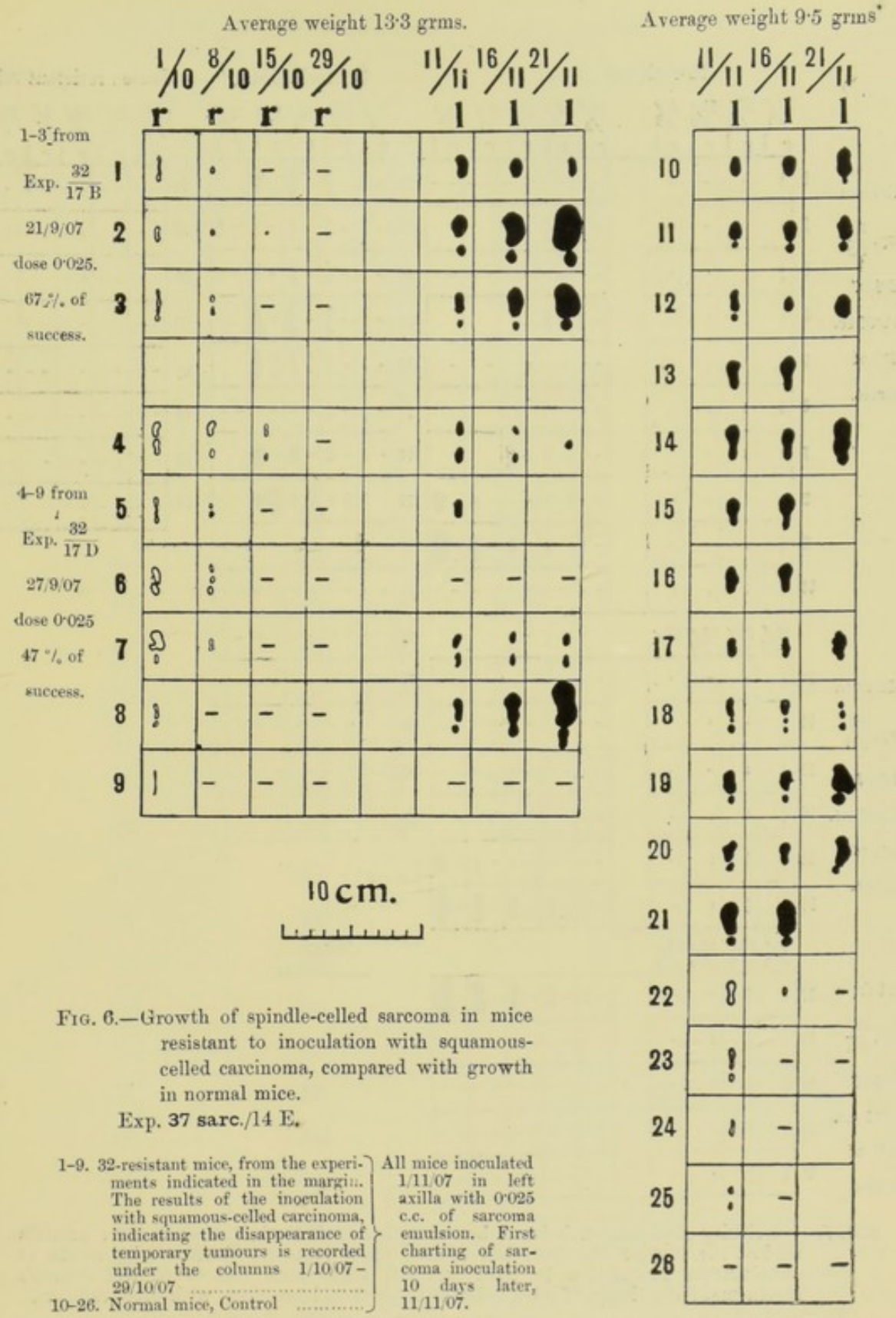


FIG. 6.—Growth of spindle-celled sarcoma in mice resistant to inoculation with squamous-celled carcinoma, compared with growth in normal mice.

Exp. **37 sarc./14 E.**

1-9. 32-resistant mice, from the experiments indicated in the margin. The results of the inoculation with squamous-celled carcinoma, indicating the disappearance of temporary tumours is recorded under the columns 1/10/07–29/10/07 .....

10-26. Normal mice, Control .....

All mice inoculated 1/11/07 in left axilla with 0·025 c.c. of sarcoma emulsion. First charting of sarcoma inoculation 10 days later, 11/11/07.

Spindle-celled sarcoma grows very well in mice in which squamous-celled carcinoma will not grow at all.

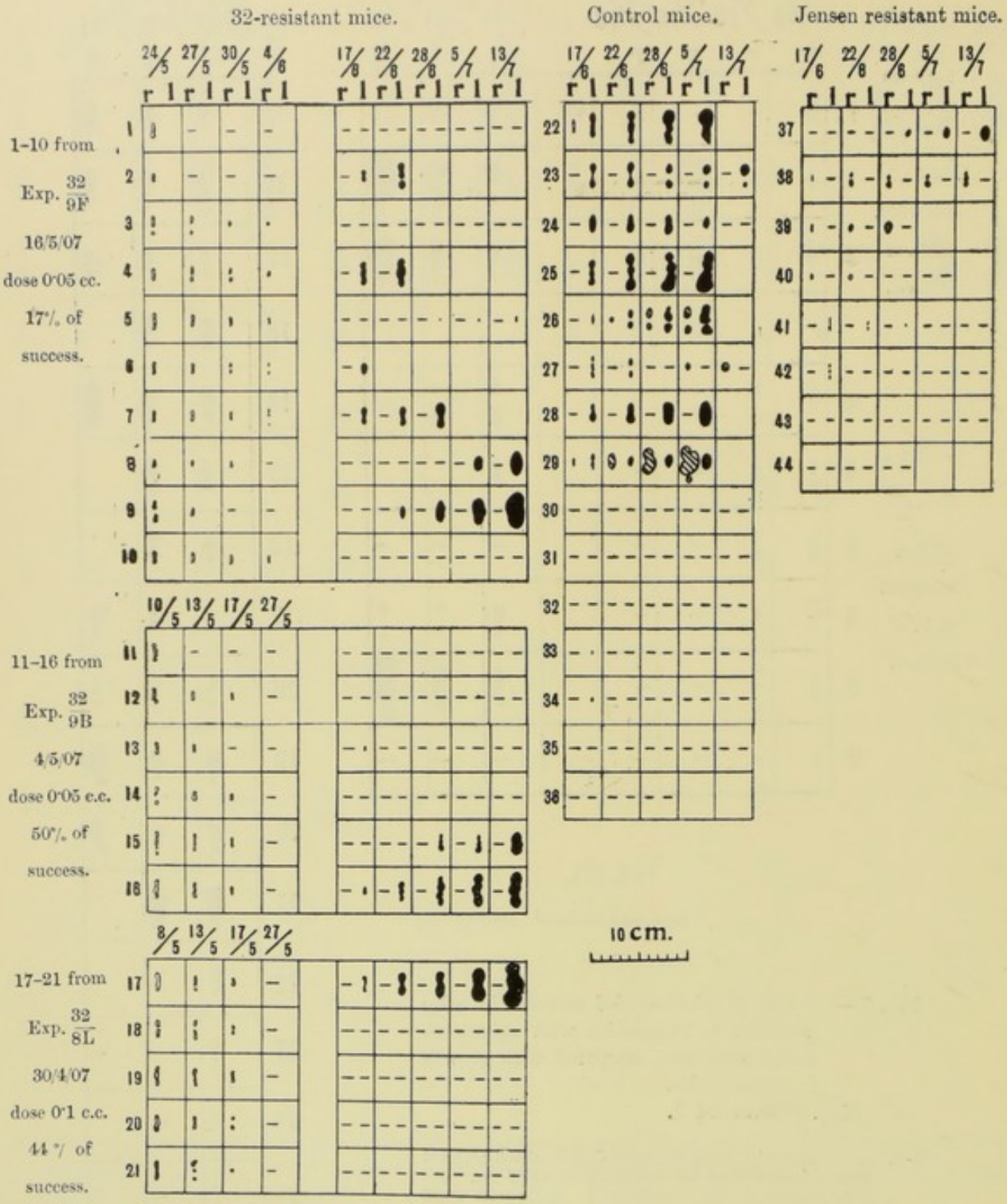


FIG. 7.—Specificity of protection induced by absorption of distinct mouse-tumours; greatest against the tumour absorbed, less against other tumours, illustrated by results of simultaneous inoculation in right and left axilla with tumour 32 and Jensen's carcinoma, respectively, of mice resistant to previous inoculation with these tumours.

1–21. 32-resistant mice .....
37–44 Jensen resistant mice.....
22–36 Normal mice, control of same size .....

All mice inoculated 7.6.07, with 0·025 c.c. of emulsion of tumours 32 in right axilla, and with 0·025 c.c. of emulsion of Jensen's carcinoma in left axilla.

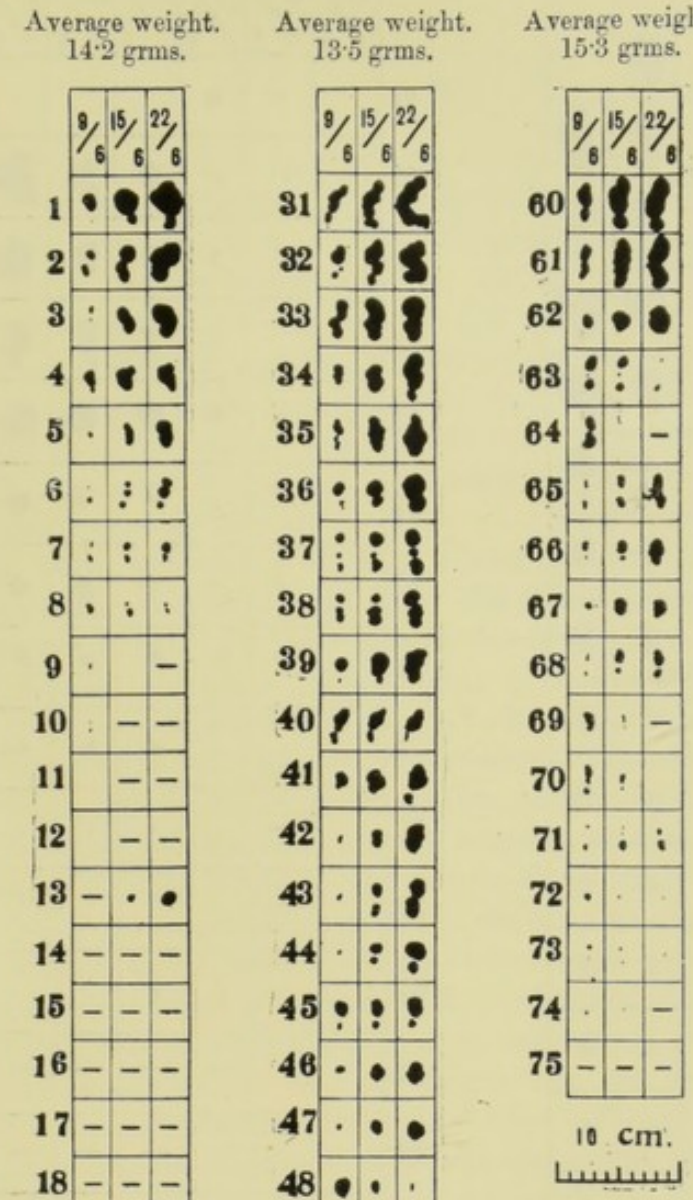


FIG. 8.—Protection against inoculation with Jensen's carcinoma by preliminary treatment with defibrinated mouse-blood and not by defibrinated rabbit-blood.

1–30.—Mice treated with 0·5 c.c. defibrinated mouse-blood subcutaneously on the back, 11 days previous to inoculation .....

60–75.—Mice treated with 0·5 c.c. defibrinated rabbit's blood subcutaneously on the back, 11 days previous to inoculation .....

31–59.—Normal mice, control ...

All mice inoculated 30.5.08 with 0·025 c.c. of Jensen-tumour emulsion in right axilla. First charting ten days later.

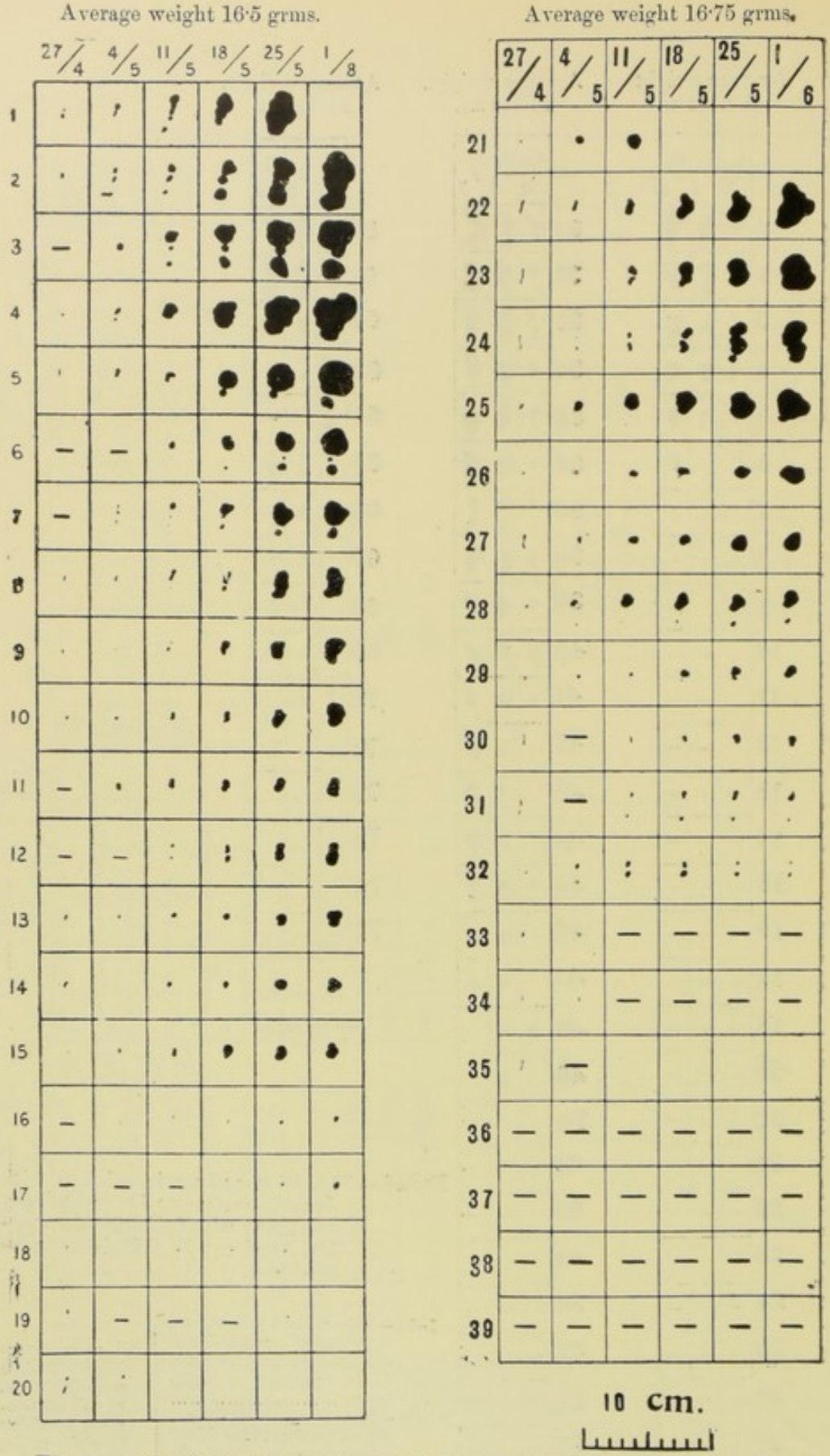


Fig. 9.—Slight degree of protection against inoculation with tumour 50, induced by preliminary treatment with defibrinated blood of the mouse.
Exp. 50/9 H.

1–20. Normal mice, control .....
21–39. Mice treated with 0·3 c.c. of defibrinated mouse-blood, subcutaneously on the back 16 days previous to inoculation .....

All mice inoculated 16.4.08 in right axilla with 0·025 c.c. of emulsion of tumour 50. First charting 11 days later.

We published these fundamental experiments on the immunity which can be induced by normal tissues only with reference to Jensen's tumour, and did not generalise. Other investigators have repeated our observations and confirmed them for different tumours, *e. g.* Lewin for rat sarcoma, Flexner and Jobling also for a rat tumour, while others again, *e. g.* Ehrlich, Schöne, Apolant, were unable to obtain the same degree of protection, and concluded that this was due to the greater virulence of the tumours they studied as compared with Jensen's tumour. As pointed out, the general application of the results we recorded for Jensen's tumour, was not made by us. Fig. 9 reproduces an experiment with tumour 50 which is a hæmorrhagic mammary carcinoma, described by Gierke on page 115. The figure shows that the degree of protection induced by blood, falls in this case behind that given in fig. 8 for Jensen's tumour. Tumour 50 is a tumour which is much more difficult to propagate than is Jensen's tumour, and only on few occasions has it grown so well as in this experiment. The same result is however obtained, even when it is growing badly, as recorded by Gierke on p. 134. In the case of this hæmorrhagic tumour, it is of course possible to conceive that the blood in the tumour-tissue neutralises the effect of the blood previously employed to induce immunity: but in this case the tumour-tissue inoculated, was taken from a tumour in the non-hæmorrhagic phase of growth. We have tested the influence of blood with reference to other tumours, *e. g.* tumours 32 and 27, for both of which it is almost nil. We see therefore in our own experience and in the divergent results obtained by other investigators merely further evidence that the conditions of growth are specific for different tumours, and that the induction of conditions which are unfavourable to the growth of a particular tumour are not necessarily applicable to all tumours.

Schöne showed that protection could be obtained by preliminary injection of emulsion of mouse-embryos. This constituted an important technical advance, since it is perfectly easy to obtain a sterile emulsion of mouse-embryos in large amount, whereas in blood experiments, there is great danger of contamination of the defibrinated blood from single cases of septicæmia in the animals bled, and from the long exposure during its preparation. Schöne did not state the quantitative relations of immunising emulsion and inoculation dose. We have confirmed Schöne's results with our own tumours, and, again applying the analysis we had made of the effects of the constituents of blood, we were able to show that the separate tissues of the mouse-embryo were not equally potent in inducing protection against our transplantable tumours.

Borrel and Bridré also studied the immunising effects of preliminary treatment with single mouse-tissues with careful attention to the doses employed. They obtained protection with liver, spleen, and brain, but not with mouse testis.

The fact that in addition to a number of undoubted mammary carcinomata of very varied histology, we possessed a squamous-celled carcinoma, a spindle-celled sarcoma and an osteo-chondro-sarcoma, all transplantable, as well as a transplantable sarcoma and a carcinoma of the rat, enabled us to vary the disposition of our experiments in an instructive manner. By stripping the skin from nearly full grown mouse-embryos, we are able to immunise mice (1) with skin alone; (2) with the remainder of the embryonic tissues ; furthermore (3) by dissecting out the mamma of pregnant or lactating females the mammary gland alone could be used for preliminary treatment. Fig. 10 illustrates the difference in the result, when mice previously treated with an emulsion of embryo skin, and with emulsion of the remaining tissues of the embryo, are inoculated with squamous-celled carcinoma (tumour 32). In this experiment, while the mice treated with embryo-emulsion (skinless) are only slightly less suitable than normal animals, those treated previously with emulsion of skin are highly resistant. This immunising effect of skin is constant for tumour 32, and is shown in several other graphic records (figs. 11 to 15). Mamma emulsion, however, has very little power of preventing the growth of the squamous-celled carcinoma, see fig. 11, and as fig. 12 shows, may, after a certain interval, lead to quite a contrary effect, viz. hypersensibility. A similar result is sometimes obtained after treatment with skinless embryos, as shown in fig. 13. Against alveolar mammary carcinoma, however, the effect is more pronounced. On the other hand, the immunising effect of skin is not limited to the squamous cell carcinoma alone, but it also protects to a high degree against mammary tumours (*cf.* fig. 14).

Just as normal blood of alien species does not confer immunity against subsequent inoculation of mouse carcinomata or sarcomata, so also with normal tissues of strange species. So delicate are the differences which can be measured by using the survival and proliferation of living cells as indicators, that the distinction between the skin of mouse and rat embryos manifests itself by complete inhibition of growth, and entire absence of this effect, respectively. Fig. 14 shows an experiment in which the susceptibility of six groups of mice treated with separate single tissues of the mouse and rat, is compared with that of normal animals and of each other, by simultaneous inoculation

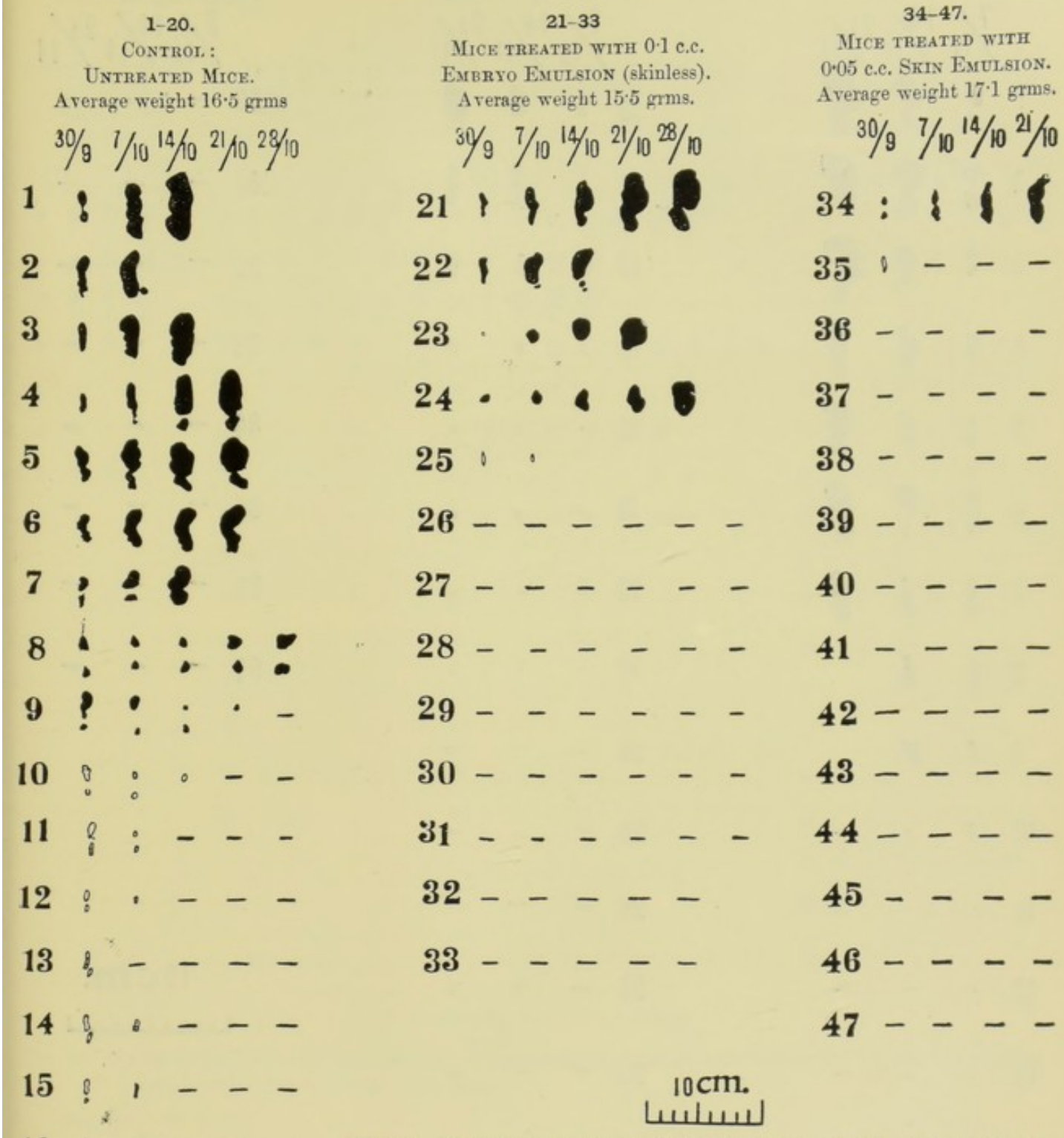


Fig. 10.—Protection against inoculation of squamous-celled carcinoma induced by prelin inary treatment with normal tissues of the mouse.

Exp. 32/17 A.

1–20. Normal mice, control .....

21–33. Mice treated with 0·1 c.c. of emulsion of mouse-embryos from which the skin had been removed subcutaneously on the back, 17 days previous to inoculation .....

34–47. Mice treated with 0·05 c.c. of emulsion of the skin of mouse-embryos subcutaneously on the back, 17 days previously to inoculation .....

All mice inoculated 20.9.07 in right axilla with 0·025 c.c. of emulsion of tumour 32. First charting 10 days later.

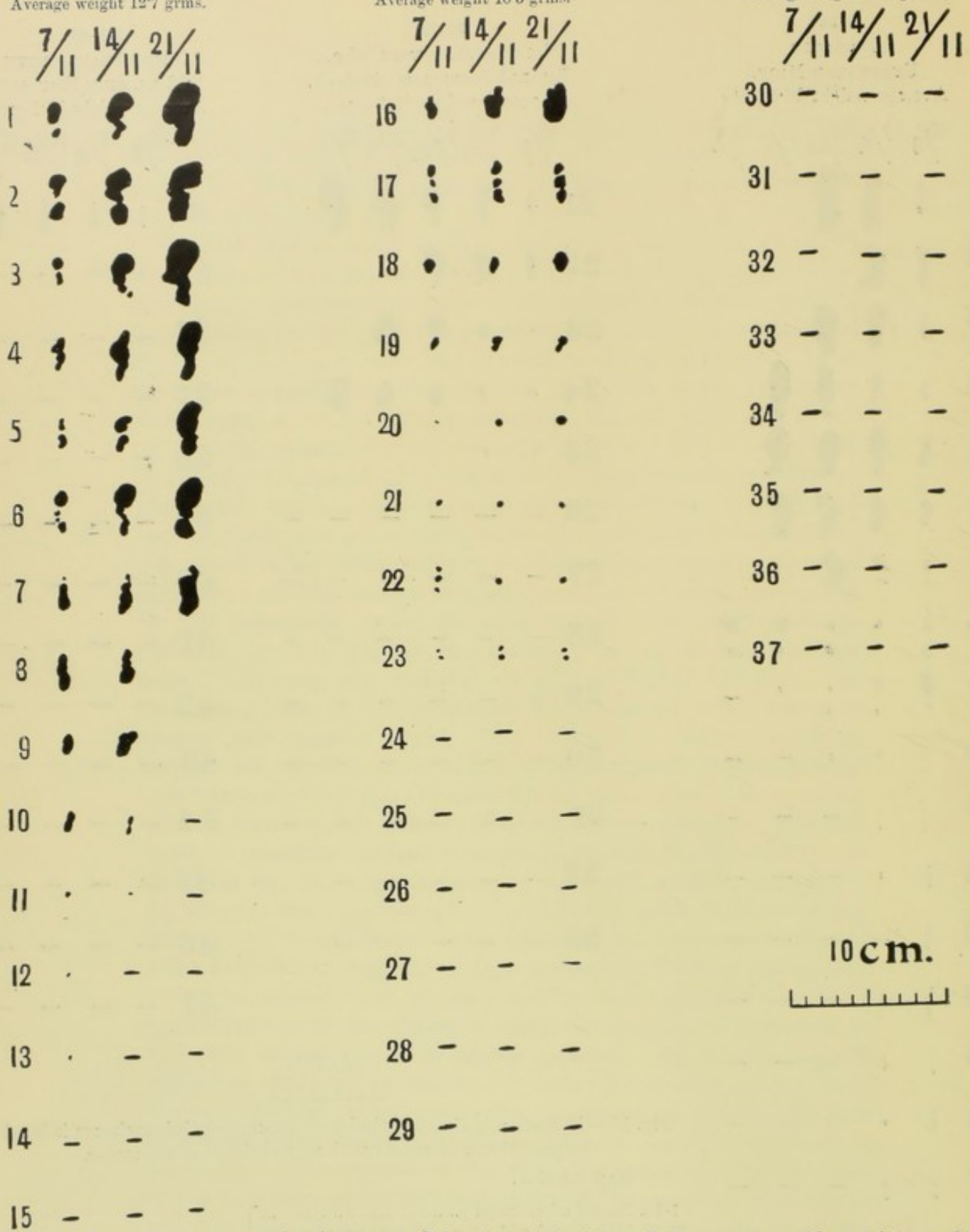


FIG. 11.—Protection against inoculation of squamous-celled carcinoma induced by preliminary treatment with normal tissues of the mouse. Greater protection induced by treatment with skin than with normal mamma although twice as much mamma was injected.

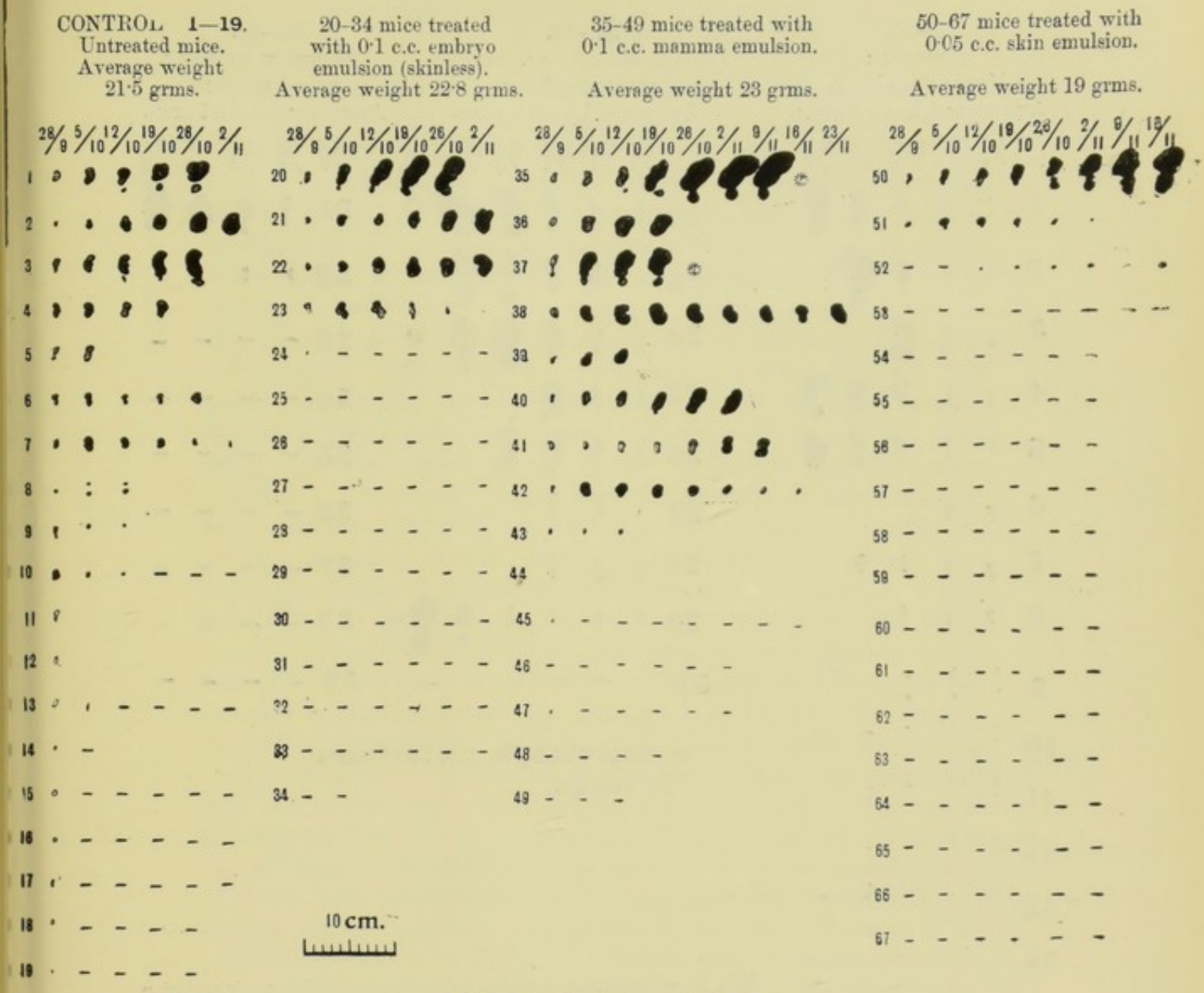
Exp. **32**/14 F.

1–15. Normal mice, control ....

16–29. Mice treated with 0·1 c.c. of emulsion of mamma of the mouse subcutaneously on the back, 20 days previous to inoculation.

30–37. Mice treated with 0·05 c.c. of emulsion of skin of mouse-embryos subcutaneously on the back, 20 days previously to inoculation.

All mice inoculated 28.10.07 in right axilla with 0·02 gram of tumour 32. First charting 10 days later.



* Macroscopic metastases in lungs.

FIG. 12.—Protection against, and hypersensibility to, inoculation of squamous-celled carcinoma induced by preliminary treatment with normal tissues of the mouse.

Exp. 32/16 D.

1–19. Normal mice, control .....
20–34. Mice treated with 0·1 c.c. of emulsion of mouse-embryos from which the skin had been removed, subcutaneously on the back, 15 days previous to inoculation .....
35–49. Mice treated with 0·1 c.c. of emulsion of mamma of the mouse subcutaneously on the back, 16 days previous to inoculation .....
50–67. Mice treated with 0·05 c.c. of emulsion of the skin of mouse-embryos subcutaneously on the back, 15 days previous to inoculation .....

} All mice inoculated 18.9.07 in the right axilla with 0·01–0·02 gram of tumour 32. First charting 10 days later.

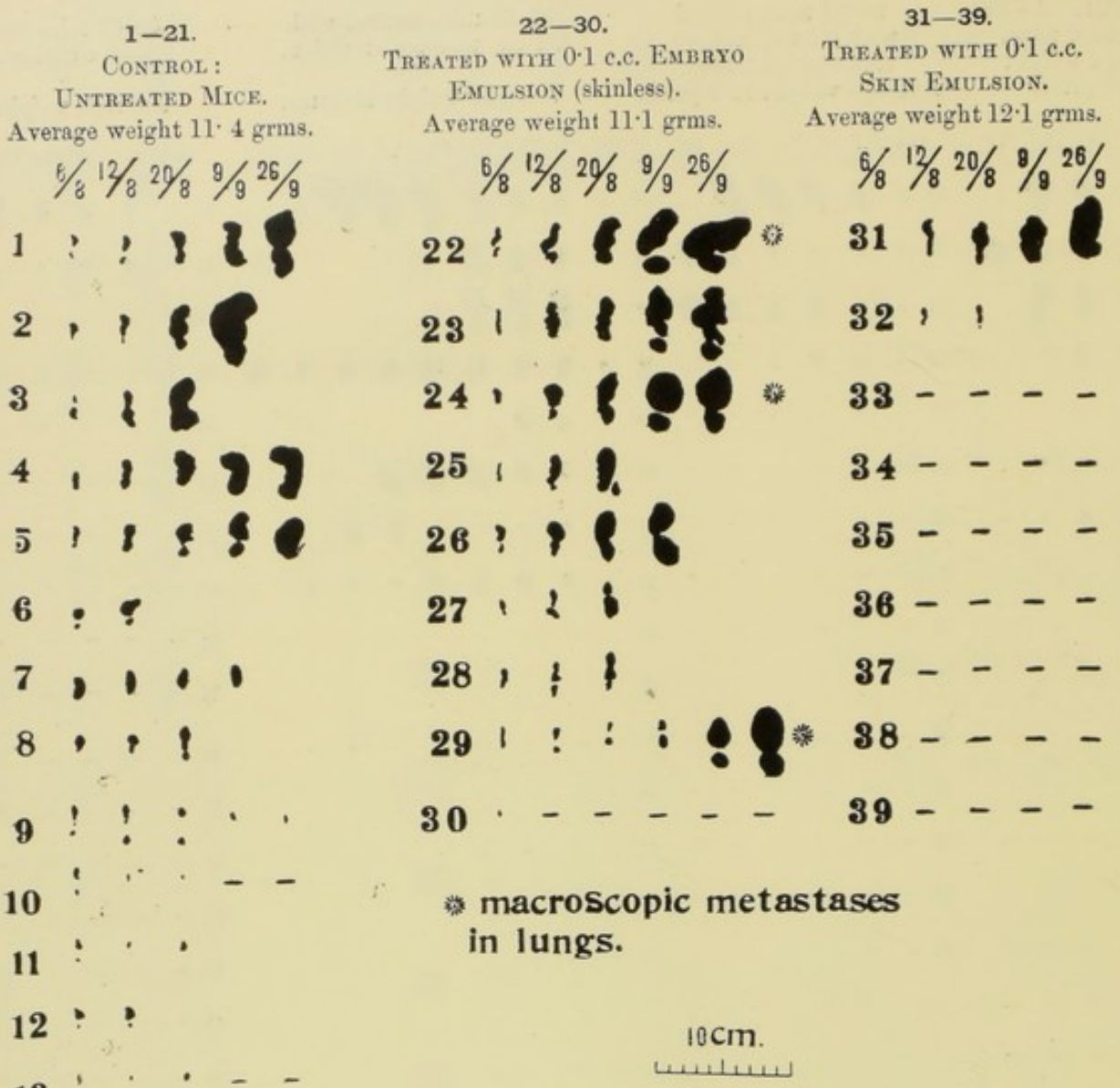


Fig. 13.—Protection against, and hypersensibility to, inoculation with squamous-celled carcinoma, induced by preliminary treatment with normal tissues of the mouse.
Exp. 32/13 F.

1-21. Normal mice, Control .....
22-30. Mice treated with 0·1 c.c. of emulsion of mouse-embryos from which the skin had been removed, subcutaneously on the back, 30 days previous to inoculation .....
31-39. Mice treated with 0·1 c.c. of emulsion of the skin of mouse-embryos subcutaneously on the back, 30 days previous to inoculation .....

All mice inoculated 27.7.07 in the right axilla with 0·025 c.c. of emulsion of tumour 32. First charting 10 days later.

of squamous-celled carcinoma (in the alveolar condition) and of alveolar mammary carcinoma. The immunising injections were made under the skin of the back 18 days before the mice were inoculated. The tumour inoculations were done at one sitting, the squamous-celled carcinoma (tumour 32) being inoculated into the right axillary region, while the alveolar mammary carcinoma (tumour 46) was inoculated symmetrically on the left side. The contrast between the tumours in mice treated with mouse and rat skin respectively, is very striking. In this experiment the effects of the injection of mammary tissue are not so clear. The mammary carcinoma is certainly inhibited to a greater degree than the squamous-celled carcinoma by mouse mamma, but rat mamma seems to have acted still more effectually; the latter effect is however inconstant, and in this experiment probably mainly accidental. The animals treated with the emulsion of mouse embryos from which the skin had been removed, were protected only slightly, whereas the series treated with the corresponding emulsion of rat embryos, instead of exhibiting protection shows an apparent hypersensibility. It is of moment in attempting to make a practical application of this observation, that the inoculation of normal tissues may be followed not only by the induction of conditions unfavourable to growth, but also by such as favour it. Figs. 12 & 13 illustrate this state of affairs for tumour 32 after the inoculation of mouse mamma and skinless embryo emulsion. Fig. 15 illustrates another series of experiments also with this tumour and with a spindle-celled sarcoma, 37. The control experiments show that a relatively poor growth occurred not only in normal mice, but also in all the mice treated with rat and mouse tissues, with the exception of mice 40–46 which had been treated with rat mamma six weeks before inoculation of the tumours. The mice in the adjacent column, mice 47–50, also treated with rat mamma, but only 16 days before the tumour was inoculated, do not show this phenomenon. The interval of time, and the dose of normal tissue used, are of importance in determining whether protection or hypersensibility will follow. Hypersensibility seems to be a less specific phenomenon than resistance. It can apparently be obtained by preliminary treatment with very various substances as the protocols show, and especially by the tissues of strange species. This subject is being pursued further, owing to the indications it gives of the induction of constitutional conditions favourable to growth, and therefore possibly to dissemination, *cf.* mice 22, 24, & 29 of fig. 13.

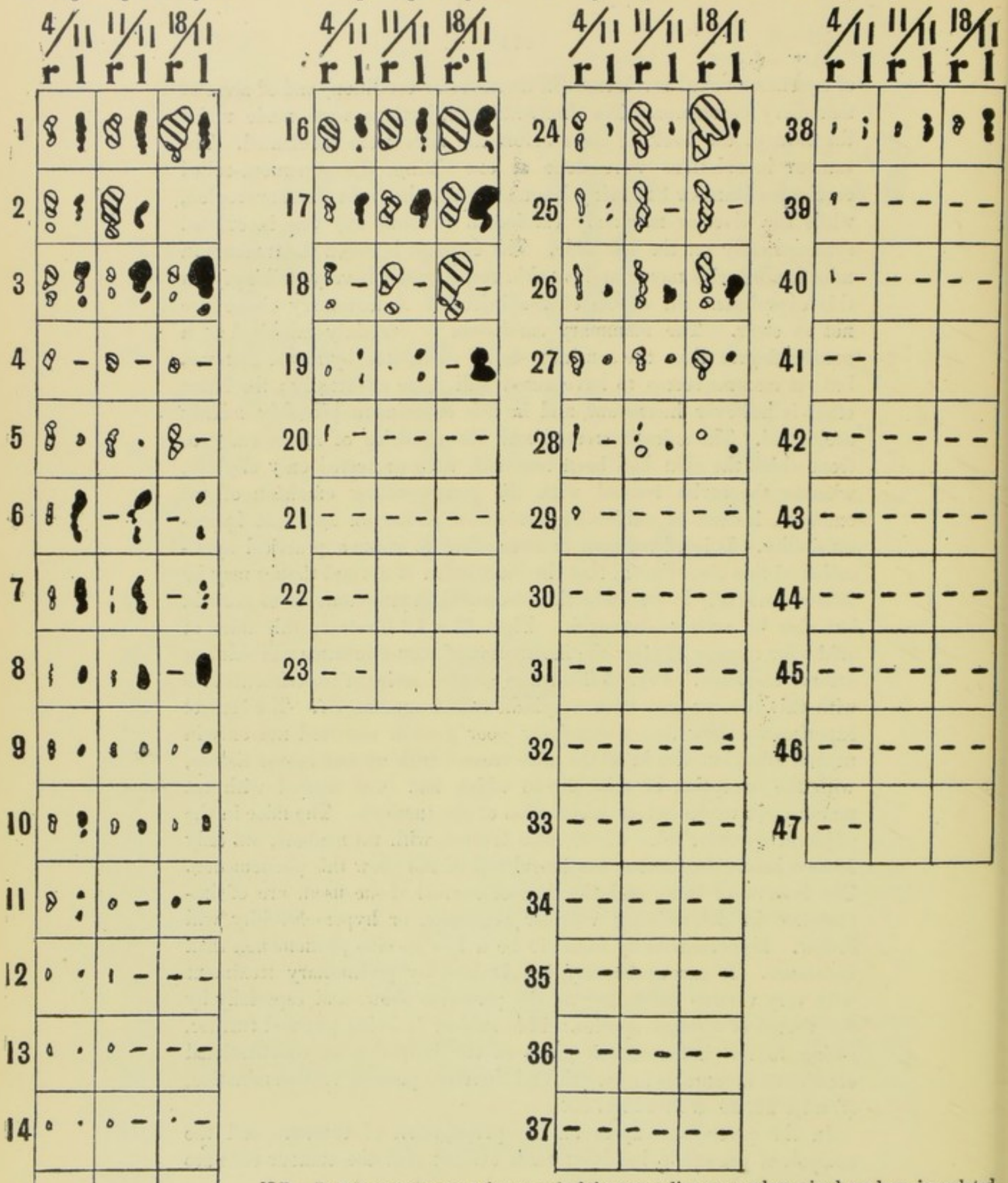
In the preceding papers on the propagation of tumours and the analysis of growth, it has been made evident that the tumour cells are

1—15.
CONTROL:
NORMAL MICE
Average weight 11·3 grms.

16—23.
EMBRYO (skinless).
0·1 c.c. ON BACK.
Average weight 13 grms.

24—37.
MAMMA.
0·1 c.c. ON BACK.
Average weight 12·8 grms.

38—47.
SKIN.
0·05 c.c. ON BACK.
Average weight 10·5 grms.



N.B.—In this experiment as in several of the succeeding ones, when mice have been inoculated in the right and left axilla with different tumours simultaneously, or with the same tumour at different times, the tumours which developed in the right axilla are represented as diagonally striped, and those in the left axilla as full black silhouettes.

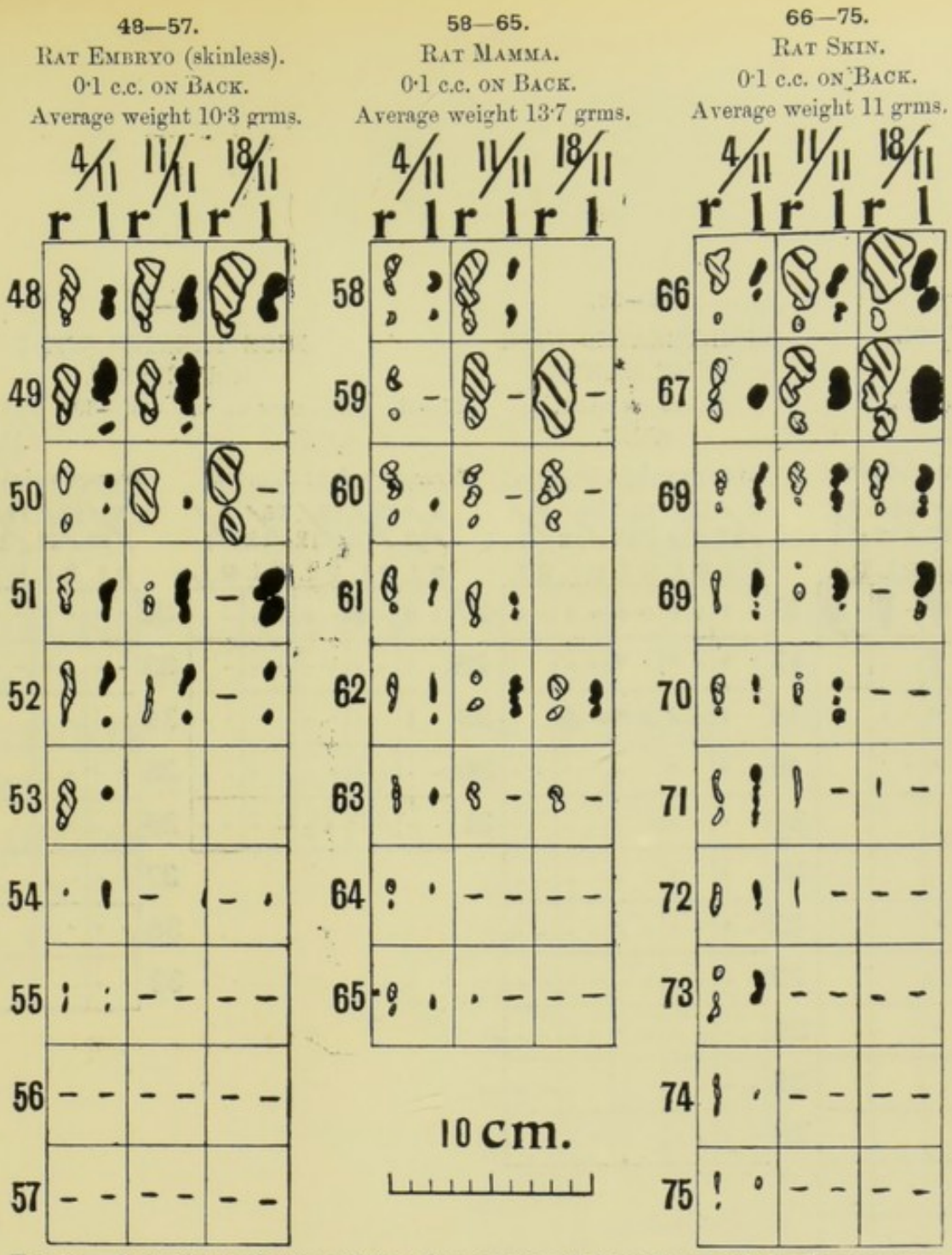


Fig. 14.—Protection against inoculation of squamous-celled carcinoma and alveolar mammary carcinoma induced by preliminary treatment with normal tissues of the mouse, and not by treatment with normal tissues of the rat.

Exp. **32**/18 E: **46**/10 D.

1–15. Normal mice, Control .....
16–47. Mice treated with mouse tissues .....
16–23. Mice treated with 0·1 c.c. of emulsion of mouse embryos from which the skin had been removed, subcutaneously on the back 15 days previous to inoculation .....
24–37. Mice treated with 0·1 c.c. of emulsion of mouse mamma, subcutaneously on the back 15 days previous to inoculation .....
38–47. Mice treated with 0·05 c.c. of emulsion of the skin of mouse embryos, subcutaneously on the back 15 days previous to inoculation .....
48–75. Mice treated with rat tissues .....
48–57. Mice treated with 0·1 c.c. of emulsion of rat embryos from which the skin had been removed, subcutaneously on the back 16 days previous to inoculation .....
58–65. Mice treated with 0·1 c.c. of emulsion of rat mamma, subcutaneously on the back 16 days previous to inoculation .....
66–75. Mice treated with 0·1 c.c. of emulsion of the skin of rat embryos, subcutaneously on the back 16 days previous to inoculation .....

All mice inoculated 24.10.07, in the right axilla with 0·025 c.c. of emulsion of tumour 32 (squamous-celled carcinoma), and in the left axilla with 0·025 c.c. of emulsion of tumour 46 (alveolar mammary carcinoma). First charting 11 days later.

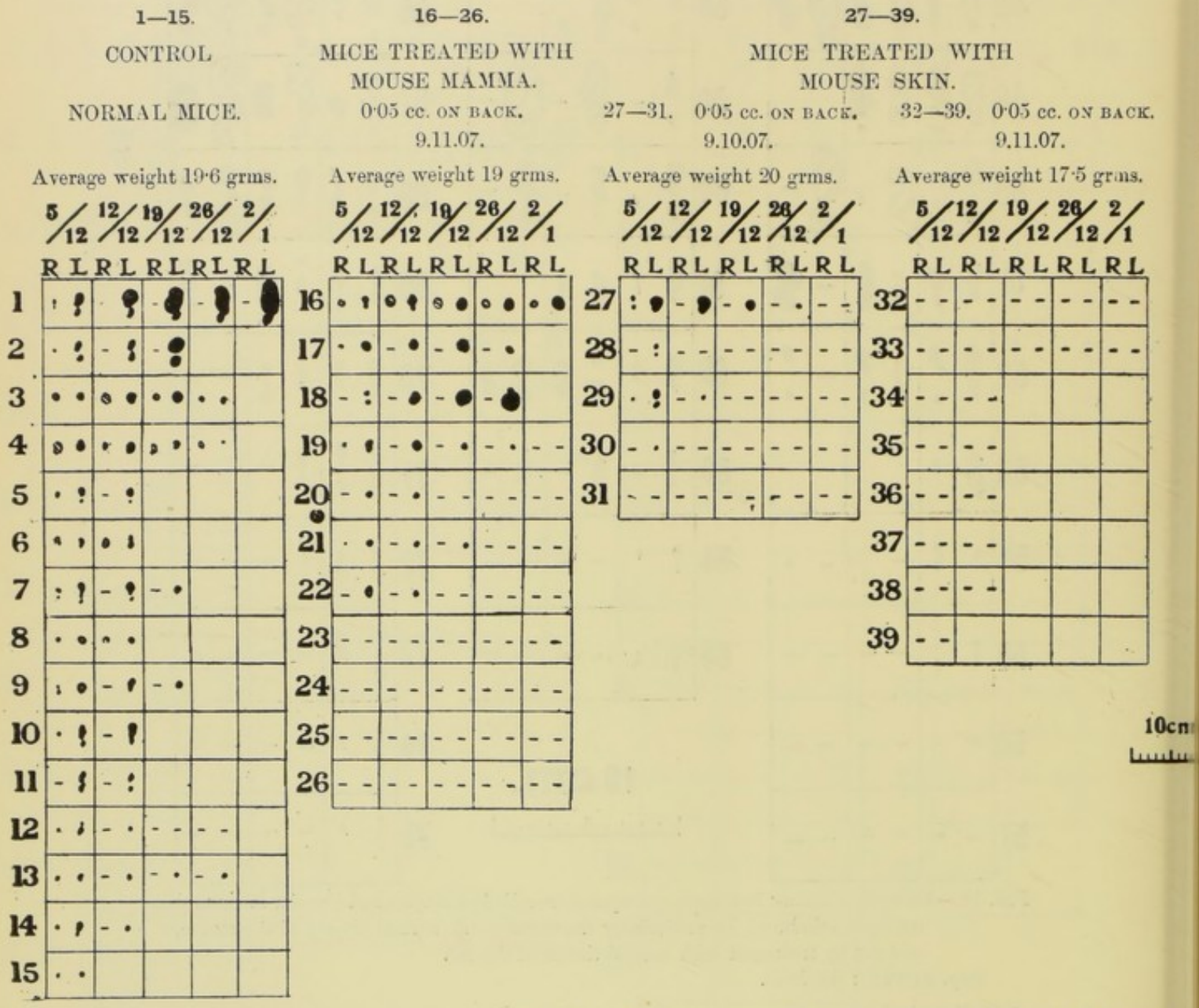


FIG. 15.—Similar experiments to that shown in fig. 14, showing sarcoma induced by preliminary treatment with with rat tissues. In some cases (*e. g.* with mamma)

Exp. 32/18 F : 37/15 G.—All mice inoculated 25.11.07 in the carcinoma) and in the left axilla with 0·01 gr. of

The details of the preliminary treatment are indicated at the

40—50.

MICE TREATED WITH RAT MAMMA.

40—46. 0·1 cc. ON BACK. 8.10.07. Average weight 15 grms.

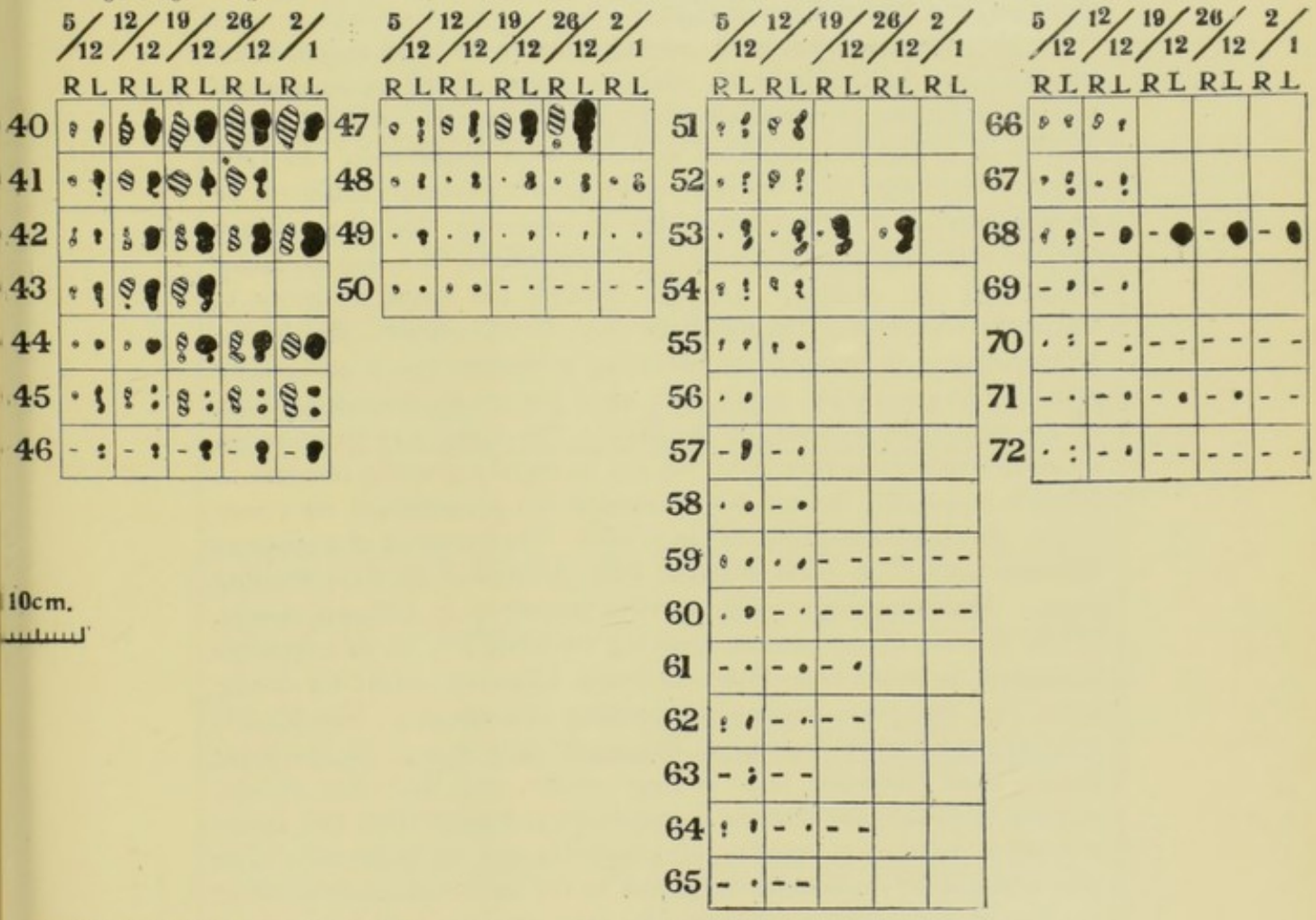
47—50. 0·05 cc. ON BACK. 9.11.07. Average weight 16·9 grms.

51—72.

MICE TREATED WITH RAT SKIN.

51—65. 0·1 cc. ON BACK. 8.10.07. Average weight 17·33 grms.

66—72. 0·05 cc. ON BACK. 9.11.07. Average weight 15 grms.



10cm.

protection against squamous-celled carcinoma and spindle-celled mouse tissues, and the absence of protection after treatment the rat tissues appear to produce hypersensibility.

right axilla with 0·01 gr. of tumour 32 (squamous celled tumour 37 (spindle celled sarcoma).

top of each column.

biologically different at different times, and it has also been indicated that they are probably more vulnerable to unfavourable conditions during the negative phase of growth.

The question naturally arises, what is the effect of a tumour on the animal? Does a successful inoculation protect against a second one? Or does the presence of a tumour modify the animal in the opposite direction, and favour dissemination?

In 1904 we stated that mice bearing tumours could be re-inoculated successfully. In the light of what we know of the occurrence of metastases in man, the positive results, resembling the artificial reproduction of metastases, appeared to us to be of importance. In 1905 Ehrlich stated that mice bearing rapidly growing tumours could not be re-inoculated, and he explained this by assuming that the primary tumour attracted all the food-supply to itself, so that the secondary inoculation was starved. On this Ehrlich has based a theory of "atreptic" immunity for which he has found support also in the temporary growth of rapidly proliferating mouse-tumours in rats, and by assuming in general the rarity, and, when present the microscopic size, of the metastases occurring in the lungs. The temporary proliferation of mouse-tumours in rats is not limited to rapidly growing tumours as Ehrlich supposed. Russell has illustrated the phenomenon for a very slowly growing tumour (No. 27) on p. 353. The formation of metastases has been referred to frequently and fully illustrated on the preceding pages. Metastases occur with varying frequency in different strains. While for one of our slowest growing tumours, No. 27, no naked-eye metastases have yet been found although diligently sought for during more than two years, our rapidly growing tumours, *e. g.*, Nos. 32, 37-sarcoma, and Jensen's carcinoma frequently show them. In the latter strains their frequency is to a large extent, and their size mainly, dependent on the time that the animal lives, and apart from this factor a constant relation to rapidity of growth cannot be made out. The size attained by metastases is subject to the same conditions as affect the size of transplanted tumours. In particular the initial dose is of importance, for it must be self-evident that the pulmonary emboli from which they take their origin are far smaller than any doses used in transplantation. Hence, if the transplanted tumour starts from a large dose and grows rapidly, the death of the animal supervenes before the metastases can have attained macroscopic size. The hindrance to growth exerted by the arterial walls in the lungs, and the initial difficulty of vascularisation, also lengthen the period of growth of emboli, apart from any constitutional effect of the presence of the primary growth.

As regards the re-inoculation of animals already bearing tumours, we shall call them for brevity, positive mice or "positives." The following table gives the general results of homologous re-inoculations for tumours 50, 27, Jensen's mouse carcinoma, 32, Twort's carcinoma, and Jensen's rat sarcoma. These six tumours have been selected as exemplifying the results of re-inoculating tumours with which the average percentage of successes obtained in normal animals varies from 27 to 91 per cent.

General Results of Re-inoculation of Positive Mice and Rats.

50.				27.			
No. of "positives" re-inoculated	38	Control ..	82	No. of "positives" re-inoculated	39	Control ..	64
Positive to 2nd inoculation	25	Positive..	22	Positive to 2nd inoculation	23	Positive..	28
Percentages	66 %	....	27 %	Percentages	59 %	....	44 %
Jensen.				**32.**			
No. of "positives" re-inoculated	68	Control ..	111	No. of "positives" re-inoculated	41	Control ..	113
Positive to 2nd inoculation	26	Positive..	55	Positive to 2nd inoculation	23	Positive..	64
Percentages	39 %	....	50 %	Percentages	56 %	....	56 %
Twort.				**Jensen's Rat Sarcoma.**			
No. of "positives" re-inoculated	39	Control ..	38	No. of "positives" re-inoculated	50	Control ..	83
Positive to 2nd inoculation	26	Positive..	26	Positive to 2nd inoculation	25	Positive..	76
Percentages	66 %	....	66 %	Percentages	50 %	....	91 %

It will be seen that the figures bear out what we stated in 1904, and also illustrate the phenomenon recorded by Ehrlich. The question is merely whether more importance is to be attached to negative than

to positive results obtained in positive mice, or if it is possible to harmonise the apparent contradictions.

The figures show that the result of re-inoculation is not what we should anticipate on the assumption that the positive animals are merely unaltered suitable individuals, selected by the tumour. If this were so, re-inoculation should be successful in a much higher proportion than in the controls. This is realised in the case of tumours 27 and 50 in which re-inoculation is successful in 59 per cent. and 66 per cent., while the controls give only 44 per cent. and 27 per cent. respectively. In the case of tumour 32, the re-inoculation and the controls give the same result, viz. 56 per cent. In the case of Jensen's mouse carcinoma and Jensen's rat sarcoma the re-inoculations are less successful, viz. 39 as compared with 50 per cent. for the mouse carcinoma and 50 as compared with 91 per cent. for the rat sarcoma. We have, therefore, in these experiments, on the one hand the phenomena described by ourselves in the First Scientific Report, and by Hertwig, Gierke, and Borrel, and on the other hand in the case of the Jensen mouse and rat tumours the phenomenon described by Ehrlich as atreptic immunity.

As tumours 27 and 50 are of relatively low energy of growth while Jensen's mouse and rat tumours and tumour 32 grow rapidly, these figures confirm the accuracy of Ehrlich's observations with rapidly growing tumours. The fact that, even in series with the most rapidly growing tumours, re-inoculation may be possible, shows that the negative result of re-inoculation is not determined by the rapidity of growth of the primary tumour, and necessitates a closer analysis of the conditions under which a second inoculation is positive or negative, in different tumour strains. Average percentage results are of little assistance for this purpose. The graphic records of individual experiments in which the doses of the first and second inoculations differ considerably, with tumours in which re-inoculation is not more successful or even less successful than in the controls, elucidate the apparent discrepancies.

Fig. 16 shows the result of re-inoculating with small and large doses the animals of a series in which the first inoculation was made with a small dose. At the first inoculation nine tumours developed in twelve mice inoculated in the right axilla with 0·025 cc. of tumour emulsion (Exp. 32/13 J). These nine positive mice were divided into two lots. The first four were inoculated with the same dose 0·025 cc. in the left axilla. Three tumours developed in four positives, but only two grew well, while the third (Mouse No. 2) remained small. The fourth mouse was negative *from the start*. The second lot were re-inoculated with

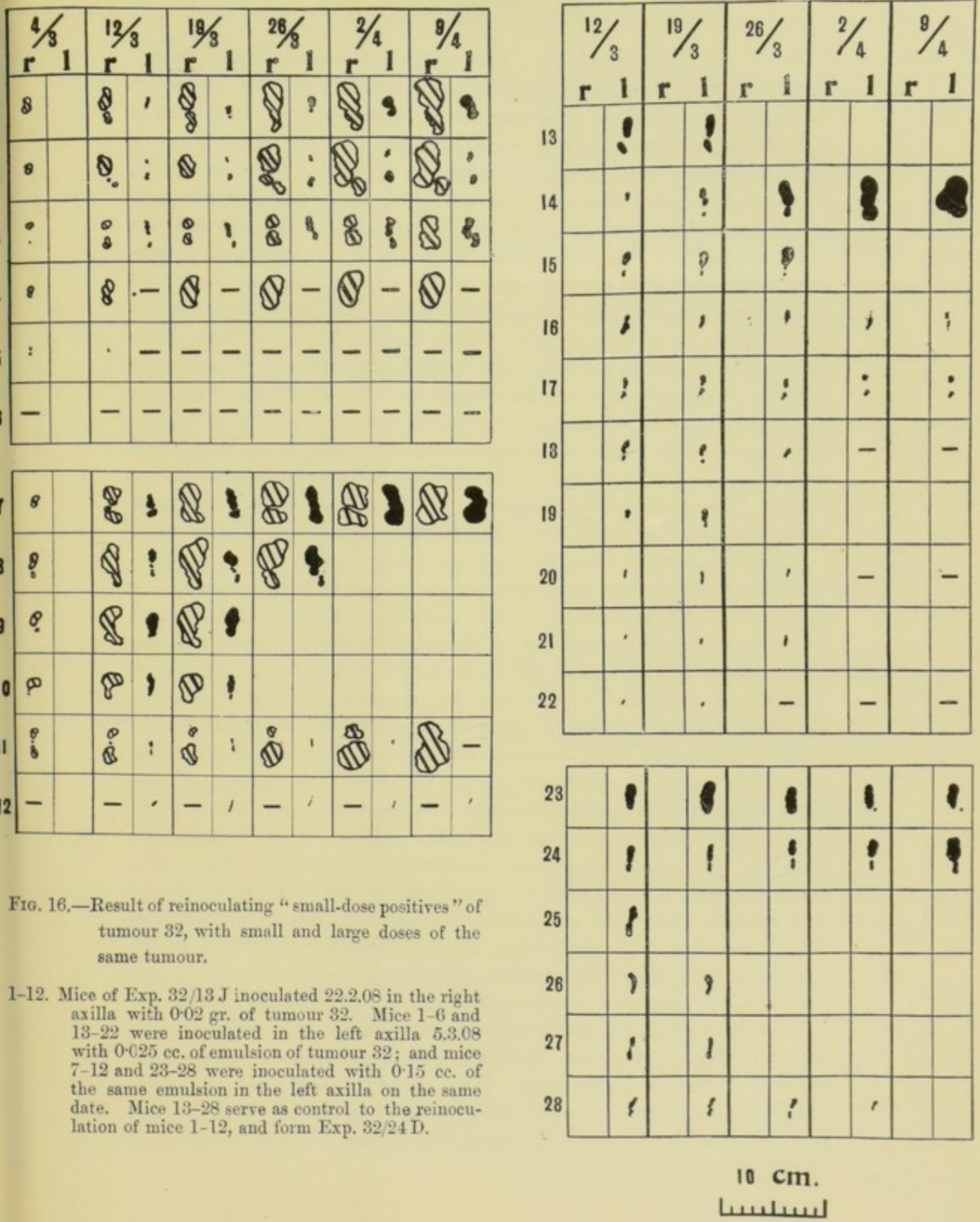


FIG. 16.—Result of reinoculating "small-dose positives" of tumour 32, with small and large doses of the same tumour.

1–12. Mice of Exp. 32/13 J inoculated 22.2.08 in the right axilla with 0·02 gr. of tumour 32. Mice 1–6 and 13–22 were inoculated in the left axilla 5.3.08 with 0·025 cc. of emulsion of tumour 32; and mice 7–12 and 23–28 were inoculated with 0·15 cc. of the same emulsion in the left axilla on the same date. Mice 13–28 serve as control to the reinoculation of mice 1–12, and form Exp. 32/24 D.

0·15 cc. (six times the dose). Four large tumours developed in five positives, and a temporary proliferation occurred in the fifth and sixth. The large dose therefore overcame the resistance and was successful in a way quite incompatible with the assumption that the negative result, or slow growth, could be due to exhaustion of food. When the second inoculation is made with a small dose while the primary inoculation is made in two lots with large and small doses, the result is not less clear and even more conclusive. Fig. 17, Exp. 32/23 E shows the disposition and result. While the small dose gave success in five of ten positives inoculated primarily with small doses ("small-dose-positives"), only three tumours developed on re-inoculating nine "large-dose-positives." These three tumours developed in the mice with largest tumours, and the only one which grew rapidly was found in the mouse with the largest tumour at 10 days. The protection was greatest in the mice with small slowly growing tumours. For this it may be suggested that, as the initial dose was the same (0·15 cc.) more material must have been absorbed in the latter. Concomitant immunisation at the first inoculation is the most natural explanation of the facts—the atreptic explanation is inadequate.

When tumours have attained such a size that the limit of the nutritive capacity of the animals is reached, re-inoculation is either negative or gives small stationary tumours. This result is analogous to that obtained on primary inoculation of mice in ill-health for other reasons, and the phenomenon is illustrated in the succeeding figures of re-inoculation of "large-dose positives" of Jensen's rat sarcoma. It is again referred to from another standpoint in Cramer's paper on gaseous metabolism.

The experiments with Jensen's rat sarcoma show the phenomenon with diagrammatic clearness. Fig. 18 shows the results of re-inoculating animals bearing tumours developed from small and large doses respectively, with large and small doses. Five "small-dose-positives" gave four tumours on re-inoculation with a large dose. The only rat which was negative to re-inoculation was that in which the first tumour grew badly and finally disappeared. When re-inoculation was carried out with small doses, only two tumours developed in six positives. Here again the positive animals are those bearing the largest tumours. Concomitant immunisation not only explains the results, but is the only explanation possible; natural resistance is as inadequate as atrepsy. Fig. 18 also shows the result of re-inoculating six "large-dose-positives" with a large dose. Here again the only rat in which re-inoculation failed practically from the start is that in which the first tumour grew badly. An initial natural resistance is out of the question

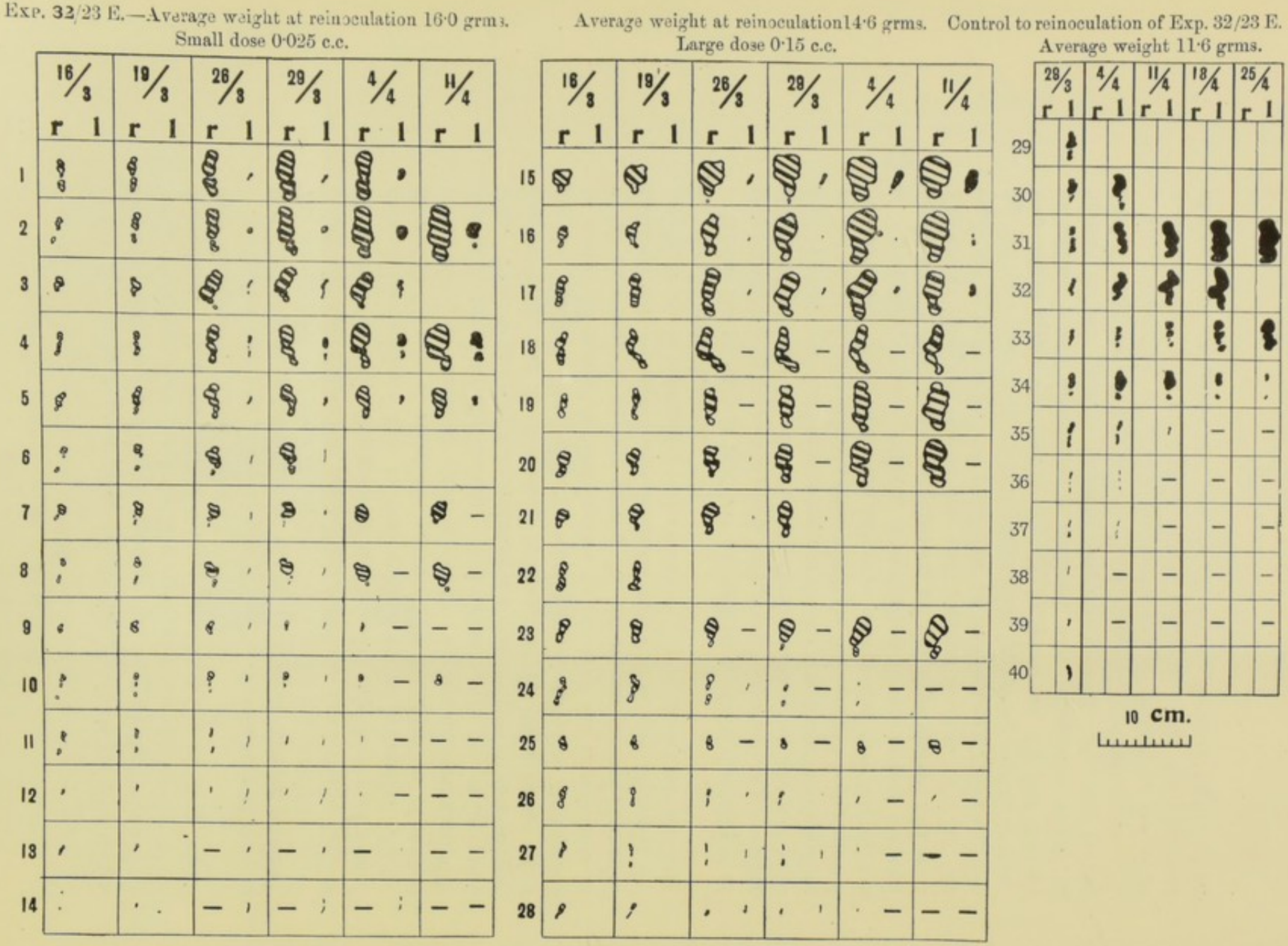


Fig. 17.—Result of reinoculating "small" and "large dose positives" of tumour 32 (positive mice of Exp. 32/23 E), with a small dose of the same tumour (Exp. 32/26 B control).

Mice 1–14 and 15–28 were inoculated in the right 5.3.08 with 0·025 c.c. and 0·15 c.c. of emulsion of tumour 32, respectively; Exp. 32/23 E.
They were reinoculated 19.3.08 (*i. e.* 14 days later) in the left axilla with 0·025 c.c. of emulsion of tumour 32 (of the 25th generation), and at the same time mice 29–40 (normal unused mice) were inoculated with the same dose of the same emulsion as control; Exp. 32/26 B.

as the first tumour grew progressively though slowly for five weeks. The other rats bore such large tumours that they all died within three weeks of re-inoculation, and the late result of re-inoculation cannot be determined. It is probable, however, that at least two of them would have disappeared. (Nos. 4 and 5.)

A negative result on re-inoculation is due to a secondary change in the positive animal following on the absorption of tumour material. The quantitative relations obtaining between the tumour-tissue introduced and the degree of protection resulting, demonstrate, just as effectually in the case of animals bearing tumours as in the case of normal animals, that we have here to deal with an active immunity. As we showed in the case of resistance produced by the injection of normal mouse blood, an immunity which is effective against an inoculation with a certain dose of tumour material, may be overcome by an increased dose. The resistance to re-inoculation is a consequence of concomitant immunisation, and in this sense independent of the presence of a growing tumour. Re-inoculation when positive therefore corresponds to metastasis formation, and enables us to carry out experiments in which an attempt is made to prevent it. If we can prevent successful re-inoculation by any means, in cases where it usually succeeds, the possibility can be entertained that metastasis formation may also similarly be brought under control.

Where the attempt is made to prevent successful re-inoculation of positive animals by the means which have proved efficacious in preventing successful primary inoculation, considerable difficulties are met with (see fig. 19). Schöne has encountered similar difficulties in attempting to immunise positive animals, from which the tumours had been removed by operation. Here again, the factors of dosage and time interval are of cardinal importance, and our investigations in this respect are still proceeding. The mere complication of the experiments makes it difficult to interpret the results, and imposes caution. So far our experiments only show the possibility of intercalating resistance (see fig. 20), and what is of equal importance, in indicating caution to those who might apply the results directly to treatment, the fact that an anomalous result sometimes is obtained, and procedures which in normal animals produce powerful resistance to inoculation, may induce a relative hypersensibility in those bearing transplanted tumours.

The phenomena of cancer resistance and sensibility, so far as at present known, give no support to the conception that the biological characters of malignant new growths are due to the intervention of an

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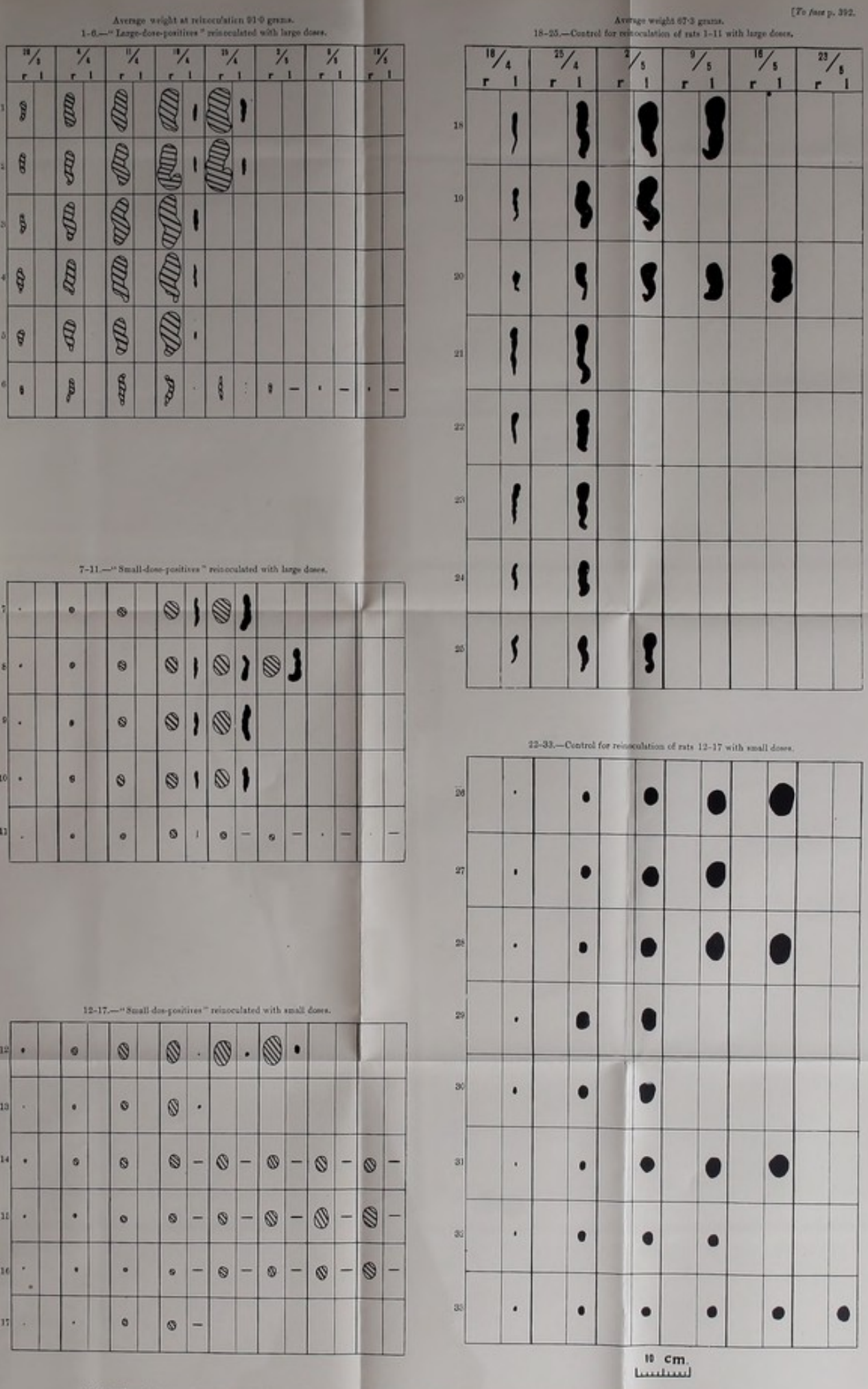


Fig. 18.—Result of reinoculating " large-" and " small dose positives " of Jensen's rat sarcoma with large and small doses of the same tumour. When " large dose positives " are reinoculated with a large dose, the secondary tumours do not grow so rapidly as the controls but the largest secondary tumour is found in the rat with the largest primary tumour. When " small dose positives " are reinoculated with a large dose, the resulting secondary tumours grow as rapidly as the controls, and, here again, best in the rats with the largest primary tumours. When " small dose positives " are reinoculated with small doses secondary tumours develop only in the rats with the largest tumours.

Rats 1–6 and 7–17 were inoculated 18/3/08 in the right axilla with 0·2 c.c., and 0·01 gr. of emulsion of Jensen's rat sarcoma, respectively; exp. J.R.S./10A. Rats 1–11 were reinoculated in the left axilla 7/4/08 with 0·2 c.c. of emulsion of Jensen's rat sarcoma, rats 12–17 were reinoculated in the left axilla with 0·01 gr. of the same material; rats 18–25 and 26–33 were inoculated as control to the reinoculation on the same date with 0·2 c.c. and 0·01 gr. of tumour respectively; exp. J.R.S./11A.

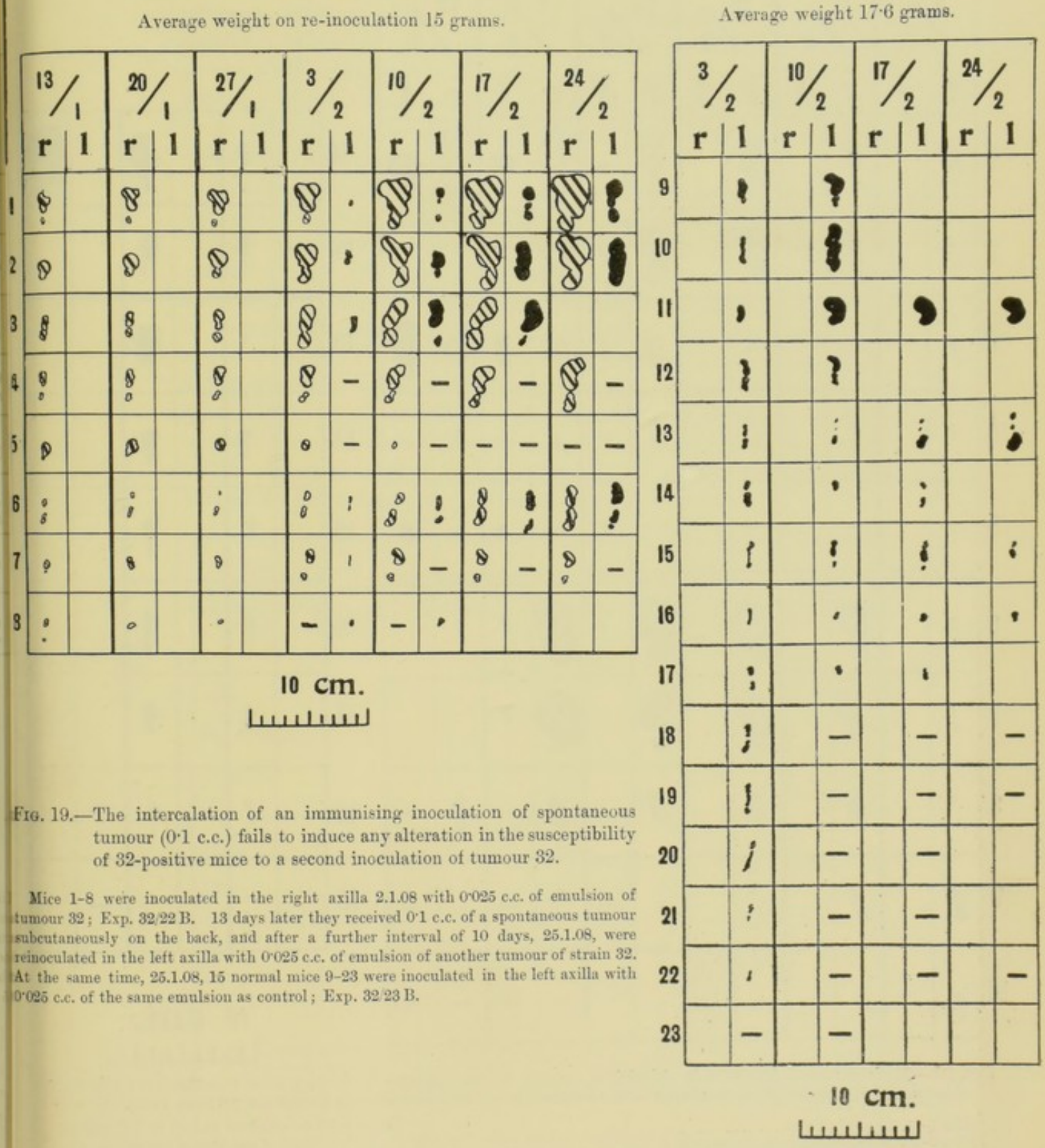


FIG. 19.—The intercalation of an immunising inoculation of spontaneous tumour (0·1 c.c.) fails to induce any alteration in the susceptibility of 32-positive mice to a second inoculation of tumour 32.

Mice 1-8 were inoculated in the right axilla 2.1.08 with 0·025 c.c. of emulsion of tumour 32; Exp. 32/22 B. 13 days later they received 0·1 c.c. of a spontaneous tumour subcutaneously on the back, and after a further interval of 10 days, 25.1.08, were reinoculated in the left axilla with 0·025 c.c. of emulsion of another tumour of strain 32. At the same time, 25.1.08, 15 normal mice 9-23 were inoculated in the left axilla with 0·025 c.c. of the same emulsion as control; Exp. 32/23 B.

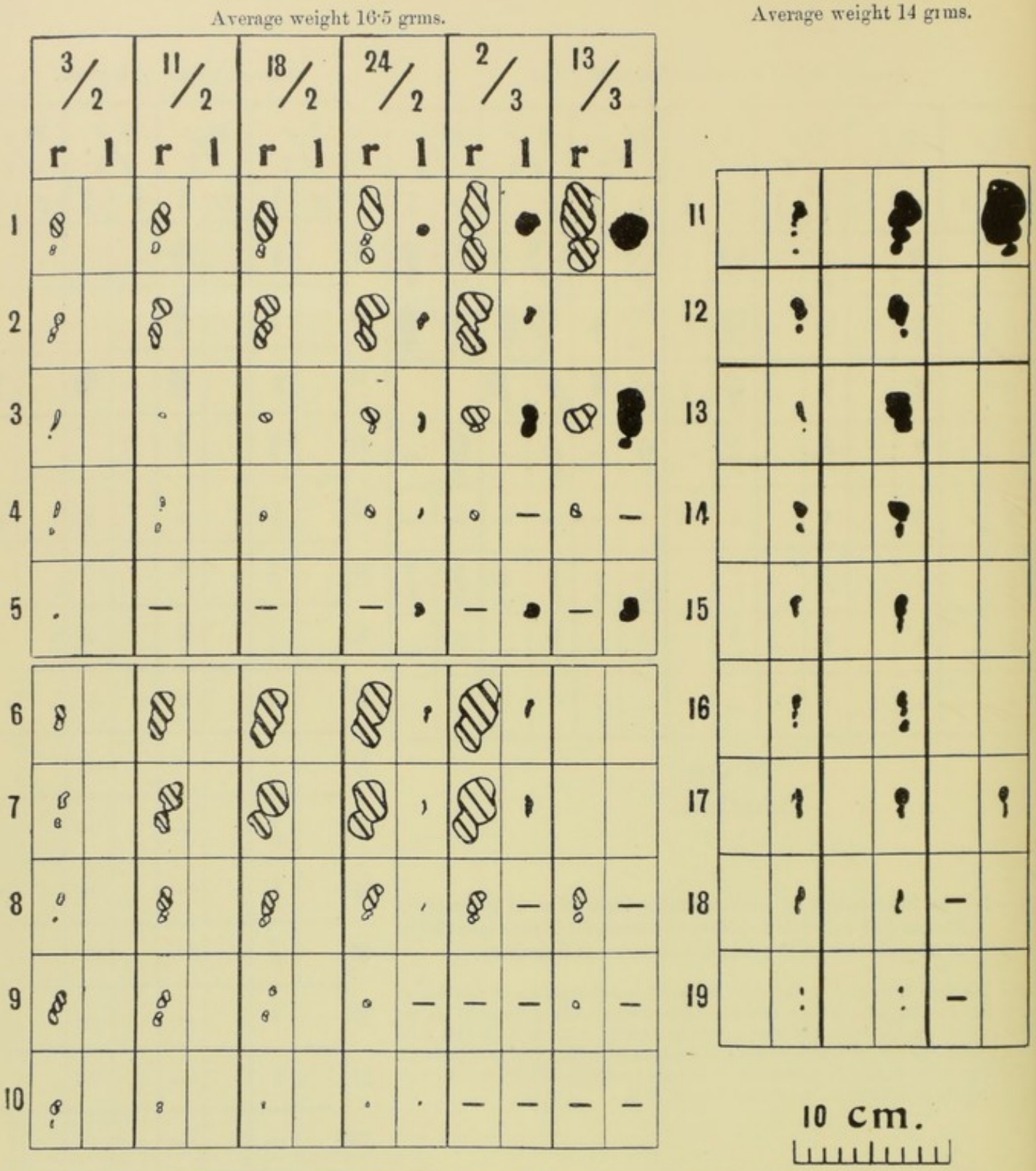


FIG. 20.—Resistance to subsequent inoculation of tumour 32, may apparently develop after tumours have arisen, in consequence of a preliminary treatment with mouse-skin injected immediately before the primary inoculation.

Mice 1– 5. Normal mice .....
Mice 6–10. Mice treated with emulsion of skin of mouse-embryos 2 days previous to inoculation .....

Inoculated 24.1.08 with 0·025 c.c. of emulsion of tumour 32, in right axilla; Exp. 32/17 I.

Mice 1–10 reinoculated 14.2.08 in left axilla with 0·025 c.c. of emulsion of tumour 32; at the same time mice 11–19 were inoculated with the same dose of the same material, as control; Exp. 32/24 C.

infective agent or virus. While in many respects we must regard resistance to inoculation as an active immunity, it is not yet definitely proved what is the exact mechanism of the process. The quantitative relations which subsist between degree of resistance and amount of tissue absorbed, render it probable that active substances in the body fluids play a rôle in producing the results.

It has been mentioned in earlier papers that up to the present it has not been possible to demonstrate directly any anti-bodies in the serum of mice naturally or artificially resistant to cancer. Similarly in Russell's experiments by histological examination of grafts in immune mice, direct evidence of their existence cannot be obtained. Attempts to demonstrate such substances in an indirect way have also given negative results. We have tried whether young, born of mice rendered highly resistant by repeated inoculations, were any more resistant than normal mice of similar age. Ehrlich's experiments on ricin-immunity showed that anti-bodies can be transferred from the mother to the young through the milk. It is also possible that such substances might be transferred by the placental circulation.

Number of times mother was re-inoculated with 0·25 c.c. of Jensen's carcinoma.	Number of young.	Age in weeks.	Result July 6, 1907, ten days after transplantation.		Result July 13, 1907.	
			+	−	+	−
Once	3	3-4	3	0	2	1
Four times.....	4	3	3	1	3	1
Five „	2	5-6	1	1	1	1
Six „	6	2 (still sucking)	6	0		0
Ditto	2	3-4	2	0	2	0
Ditto	4	4-5	2	2	2	2
Total	21	....	17	4	15	5
CONTROL.						
12 small normals as like in size as could be obtained at that time			4	3	4	3

The accompanying table shows the results of a corresponding experiment in the case of cancer. Twenty-one young mice, 2–6 weeks old, bred from mice which were injected repeatedly with large doses (0·25 cc.) of Jensen's tumour, were inoculated with 0·02 gr. each of Jensen's tumour (exp. J. 94 E, 26.6.07). At the same time twelve young normal mice of similar age were inoculated with the same dose as control. The result shows that the young of immune parents are not more resistant than normal animals. The negative result points conclusively to the absence of anti-bodies in the milk, since six young, 2 weeks old, developed rapidly growing tumours, while still sucking a highly immune mother that had been six times inoculated with 0·25 gr. of Jensen's tumour.

The reactions responsible for the resistance which can be induced to the inoculation of malignant new growths, present many new features as compared with the well known reactions to infective organisms and their products. In particular they exhibit a higher degree of specificity, both as regards the species of animal and as regards the individual tissues of a species. At the same time they are of even greater delicacy. It has not been possible to reproduce them in new animals, by passively immunising them with the fluids of resistant mice ; no evidence has been obtained of the natural conveyance of resistance from highly immune mothers to their offspring, and still less has it been possible to reproduce these reactions *in vitro*. The only change we have recognised is an active immunity in the resistant animals themselves, revealed by using living cells as indicators.

Nevertheless the changes which can be induced experimentally are strikingly demonstrated ; they can be elicited at will ; quantitative relations have been shown to exist between the degree of resistance of the animal and the amount of tumour tissue or normal tissue inducing it, as well as between the dose of tumour tissue with which the resistance of an animal is tested. We are at present unable to determine whether the phenomena depend upon actual substances formed by the resistant animal and interacting with substances in the cancer cell, or are manifestations of vital activities less easily defined.

As regards the hope of a practical outcome from these observations, we consider that it is not at present to be sought in the direction of a curative serum which can be employed after the manner of antitoxic sera, since the body fluids appear to be incapable of conveying anything to other animals, nor in the direction of a vaccine which will prevent the development of cancer. In fact we have found that mice which were

highly protected to inoculation, subsequently developed spontaneous tumours of their own (*cf.* p. 322), an observation recently confirmed by Thorel.

An emphatic *caveat* must be entered against the premature application of any of the results to man until such time as they can be extended to animals suffering from spontaneous, as distinct from inoculated, cancer; but the results obtained encourage us to continue the work, in the hope that ultimately it will be possible to control the growth of cancer in man. Before this goal can be reached, we shall require to learn much more of the actual mechanism by which mice can be rendered resistant artificially, and at the same time, of that which is responsible for natural healing. The hope of applying practically the results of the experimental study of cancer must lie in the direction of imitating or reinforcing the processes of natural cure.

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[For papers from the laboratory see complete bibliography, Appendix I.]

REPORT ON A STUDY OF THE VARIATIONS IN THE SECRETION OF HYDROCHLORIC ACID IN THE GASTRIC CONTENTS OF MICE AND RATS, AS COMPARED WITH THE HUMAN SUBJECT, IN CANCER.

By

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and

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The object we had in view in commencing this research was to determine especially, among other constituents, the physiologically active hydrochloric acid in the gastric contents of mice suffering from cancer, as compared with normal mice under identical conditions of feeding and periods of digestion *.

The experiments, of which the following is a brief summary, were commenced in December 1905 and continued up to the present time (June 1908) and have recently been described in detail in a paper now published in the 'Proceedings of the Royal Society' †.

The term *physiologically active hydrochloric acid* is intended to include both the free acid and that combined with proteids and nitrogenous organic bases, since, as originally pointed out by Lüttke ‡ and recently referred to by Willcox, much misconception has arisen in the use of the terms "free" and "combined" as applied to hydrochloric acid in the

* We found that the only chemical work which had been done in connection with mice along these lines was by Dr. Cramer in the Laboratories of the Imperial Cancer Research Fund, and referred to by the author in the 'Biochemisches Centralblatt' (Bd. iv. p. 65, Juni 1905). Dr. Cramer estimated the *total acidity* in the gastric contents of mice inoculated with alveolar carcinoma (Jensen), and found no diminution in this respect on comparing the results with those he obtained with normal mice.

† The paper in the 'Proceedings of the Royal Society' gives full particulars of all the methods employed by us and a discussion of their precise value from the chemical point of view; only a brief résumé of results is possible in this Report, but tables are appended giving detailed *data* of each series of experiments.

‡ Deutsch. Med. Woch. 1891, p. 1325.

gastric contents, and it has been erroneously held that the presence of the free acid is of more importance than the organically combined acid. But inasmuch as the latter may have been free a short time before its estimation, the exclusive determination of "free" hydrochloric acid cannot but lead to fallacious conclusions.

The method which we adopted, after careful consideration, for the estimation of the physiologically active hydrochloric acid, is based on Volhard's volumetric estimation of chlorides by precipitation with excess of standard silver nitrate, in the presence of free nitric acid, and the subsequent determination of the excess of silver nitrate by standard ammonium thiocyanate, using iron-alum as an indicator.

By estimating (*a*) the *total chlorides* and (*b*) the *inorganic chlorides* in an aqueous extract of the gastric contents by the above method, we obtain the *physiologically active hydrochloric acid* $= a-b$. A further determination (*c*) of the *total acidity* with standard sodium hydrate, using phenol-phthalein as an indicator, gives us in addition the *free organic acids* $= [c-(a-b)]$; all of these constituents being expressed in terms of hydrochloric acid.

We did not, at first, see our way to determining these various constituents of the gastric contents in single stomachs (owing to their small weight, frequently not exceeding 0·5 gramme), and we therefore took batches of stomachs, varying in number from 6 to 60. Later, by using $\frac{N}{50}$ or $\frac{N}{100}$ solutions in place of $\frac{N}{10}$ solutions, and by slightly varying the details of the method, we were able to make the estimations in single stomachs, with accuracy and comparative ease.

We made, in the first instance, five series of experiments in which no account was taken of the period of digestion, the mice being simply removed from their cages while feeding.

Under the above conditions, and somewhat to our surprise, an *increase* in the secretion of active hydrochloric acid was indicated in mice with transplanted tumours, as compared with normal mice. Taking the average of all five series, we found that 150 normal mice gave an average of 0·1121 per cent. hydrochloric acid, while in 178 mice with transplanted tumours the average was 0·1752 per cent. (*see* Tables I., VI., VII.).

A second series of five experiments was next instituted on similar lines except that a period of one hour's digestion was selected, the mice being put in empty cages overnight and fed next morning for one hour before removal. We found, again, an average increase of hydrochloric acid in the gastric contents of mice with transplanted tumours as compared with normal mice : thus 144 stomachs from normal mice

gave an average of 0·1488 per cent. hydrochloric acid, 183 stomachs from mice with non-ulcerated tumours gave 0·1627 per cent., and 52 stomachs from mice with ulcerated tumours gave 0·2100 per cent. during one hour's digestion (*see* Tables III., VIII., X.).

A brief preliminary note of these results was published in 'The Lancet' of November 10th, 1906, and the work was referred to by Dr. Bashford in the Fifth Annual Report of the Imperial Cancer Research Fund (June 1907).

We next made a number of experiments to determine, if possible, the usual period of digestion for normal mice; but the results obtained, after periods of half an hour, one hour, and one hour and a half respectively, showed that while, on the whole, more mice attained a maximum secretion of hydrochloric acid at one hour and a half, many attained a maximum at one hour and a few even at half an hour (*see* Tables II., IV., V.).

Similar experiments were carried out with mice with transplanted tumours for the same periods of digestion, and we found practically the same variations during these periods but with a general tendency to increase of hydrochloric acid (*see* Tables IX., XI.).

Summarising all the results obtained in the experiments relating to definite periods of digestion, we found on an average that 245 stomachs from normal mice gave 0·1456 per cent. hydrochloric acid and 290 stomachs from mice with transplanted tumours gave 0·1673 per cent. for periods of digestion of one hour and one hour and a half.

We also examined thirteen single rat stomachs (weighing, with contents, from 2·5 to 10 grammes). Six of these were from normal rats and seven from rats with transplanted tumours (the tumours varying in weight from 0·3 to 15 grammes). The former gave an average of 0·1427 per cent. hydrochloric acid after one hour's digestion, while the latter gave an average of 0·1837 per cent. after the same period (*see* Table XII.).

We also had the special opportunity of examining, between May 1906 and May 1908, 15 single stomachs of mice with spontaneous tumours. These showed an average of 0·1929 per cent. hydrochloric acid during different periods of digestion (*see* Table XIII.).

Hence, comparing the secretion of hydrochloric acid in the stomachs of normal mice and of mice with transplanted tumours during definite and indefinite periods of digestion, we found practically the same variations throughout, but with a general tendency towards *increase* of hydrochloric acid in the case of mice with transplanted tumours.

The total number of experiments made, from which the above averages have been deduced, was about 150, involving from four to six estimations in each experiment.

These results are interesting not only as showing that, chemically, the digestive process in mice is comparable with that in the human subject, but also as confirming, and to some extent explaining, the observations made by Dr. Bashford in 1905 as to the absence of cachexia in mice suffering from cancer. Now, inasmuch as recent extensive statistics, collected by the Imperial Cancer Research Fund from the various London Hospitals, have shown that cachexia is not a constant accompaniment of cancer in man, we might expect to find, in its absence, the same compensating influence as regards increased or undiminished secretion of hydrochloric acid in the stomachs of human beings afflicted with this disease.

As regards this point, however, so far as indicated by the investigations of various scientific workers, the opinion is prevalent that the reverse obtains ; Moore, Palmer, and others having indeed asserted that there is a marked diminution or even an absence of hydrochloric acid in the gastric contents in malignant disease of organs other than the stomach.

These conclusions, however, have been mainly based on estimations of "free" hydrochloric acid only, in the gastric contents, as represented by the fluid withdrawn from the stomach after the administration of "Test Meals" ; but as we have shown, such estimations may easily lead to fallacious conclusions.

We felt that inasmuch as our determinations of physiologically active hydrochloric acid in the gastric contents of mice seemed to be in contradiction to Moore's results as regards "free" hydrochloric acid in human gastric contents, it was obviously necessary to repeat Moore's experiments, and, at the same time, for purposes of comparison, to carry out a parallel series of estimations by the same method that we had employed in our work on mice.

We therefore examined the gastric contents as represented by the fluid withdrawn one hour after administration of test-meals * to 34 patients suffering from cancer, which by the courtesy of the Surgeons to the Cancer, Middlesex, and Westminster Hospitals, we were able to obtain from time to time.

We estimated, among other constituents, the "free" hydrochloric acid by the inversion of methyl acetate, the method employed by Moore

* The Test-Meal in all cases consisted of one pint of tea and a large round of toast it was given in the morning, fasting, and withdrawn one hour afterwards.

and others; and the physiologically active hydrochloric acid by Volhard's method, as already described, which we had used in the experiments with mice-stomachs.

Summarising these experiments we found that our estimations of "free" hydrochloric acid by the methyl acetate method, more or less agreed with those of Moore in his later experiments published in the 'Biochemical Journal' (Vol. i. p. 274), where, as an average of 13 cases of cancer, he found 0·0515 per cent., our average, also for 13 cases, being 0·0407 per cent. Moore, however, had found in 13 previous cases (Roy. Soc. Proc. Ser. B, vol. 76, p. 138) an average of only 0·0039 per cent, and Palmer in 14 cases found an average of 0·0217 per cent. Such discrepancies are not re-assuring, and some explanation may be found in the varying periods of digestion selected by these workers, viz., from 1 to 2 hours, but in our opinion a more probable explanation is afforded by the evanescent character of the so-called "free" hydrochloric acid to which we have referred above.

On the other hand the determination of the physiologically active hydrochloric acid in our 13 cases averaged 0·1626 per cent., and in five of them was above 0·18 per cent. (*see* Table XIV.)

We also found that a considerable difficulty often arose in connection with the withdrawal of test-meals, and that it was a not altogether uncommon practice to add water to assist the withdrawal. Obviously the amounts of hydrochloric acid found in such diluted test-meals and the conclusions deduced from them can be of little or no value. We therefore refrain from publishing the details of these estimations (21 in number).

We cannot, of course, from the comparatively few experiments made by us on the human subject, assert that the secretion of hydrochloric acid in cancer is normal, or above normal, for in a few cases it was decidedly below normal, but we think it has been sufficiently indicated that the whole question of amount of hydrochloric acid in human gastric contents in cancer would repay further investigation, and that it is not justifiable in the absence of more comparable experiments to conclude that it is always greatly diminished in the presence of this disease.

In conclusion, we desire to express our thanks to the Executive Committee of the Imperial Cancer Research Fund, from whom we received a pecuniary grant which rendered possible the completion of this research. To Drs. Bashford and Murray also, we are much indebted for the trouble they have taken in providing us with the necessary material, and for the interest they have shown in the progress in the work.

TABLE I.—Normal Mice.

Age over twelve months. Period of Digestion, uncertain.

Series 1.—December 19th, 1905.

Number of experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)
I. A	6	$\frac{N}{100}$	—	0·3718 grms.	5·5770 grms.	0·3010	0·2345	0·1963	**0·0382**	0·2628

Series 2.—January 4th, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. A	19	$\frac{N}{10}$	—	0·4036 grms.	4·7325 grms.	0·3606	0·2915	0·1074	**0·1841**	0·1765

Series 3.—January 31st, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. A	46	$\frac{N}{10}$	—	0·3239 grms.	6·4785 grms.	0·2676	0·2366	0·1239	**0·1127**	0·1549

Series 4.—March 12th, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. A	45	$\frac{N}{10}$	—	0·4503 grms.	10·1327 grms.	0·1081	0·2305	0·1224	**0·1081**	nil.

Series 5.—May 7th, 1906.

(I)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. A	40	$\frac{N}{10}$	—	0·5101 grms.	10·2025 grms.	0·1610	0·1860	0·0930	**0·0930**	0·0680

TABLE II.—Normal Mice.

Age, over twelve months. Period of Digestion, ½ hour.

Series 12.—February 4th, 1908.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)
II. *a*	6	$\frac{N}{10}$	23·7 grms.	1·4922 grms.	8·9535 grms.	0·1223	0·2038	0·1427	**0·0611**	0·0612
II. *b*	1	$\frac{N}{100}$	19·0 „	1·0190 „	1·0190 „	0·2507	0·2059	0·0269	**0·1790**	0·0717
II. *c*	1	$\frac{N}{100}$	20·0 „	2·0090 „	2·0090 „	0·1907	0·2180	0·0363	**0·1817**	0·0090
II. *d*	1	$\frac{N}{100}$	19·5 „	1·1545 „	1·1545 „	0·1976	0·2292	0·0316	**0·1976**	nil.
II. *e*	1	$\frac{N}{100}$	20·0 „	1·1650 „	1·1650 „	0·3133	0·2350	0·0313	**0·2037**	0·1096

Series 16.—February 11th, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
II. *b*	4	$\frac{N}{10}$	24·2 grms.	0·4637 grms.	1·8550 grms.	0·2853	0·2361	0·0885	**0·1476**	0·1377
II. *c*	2	$\frac{N}{100}$	26·5 „	0·3365 „	1·8730 „	0·2631	0·2290	0·1047	**0·1243**	0·1388
II. *d*	1	$\frac{N}{100}$	25·5 „	1·1370 „	1·1370 „	0·3371	0·2327	0·0562	**0·1765**	0·1606

Age, under three months.——**Series 14.**—February 7th, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
II. *a*	6	$\frac{N}{10}$	10·3 grms.	0·5262 grms.	3·1575 grms.	0·1156	0·3178	0·1011	**0·2167**	nil.
II. *b*	4	$\frac{N}{100}$	7·4 „	0·2380 „	0·9520 „	0·2300	0·2396	0·0958	**0·1438**	0·0862
II. *c*	2	$\frac{N}{100}$	11·5 „	0·4417 „	0·8835 „	0·1653	0·2685	0·0723	**0·1962**	nil.
II. *d*	1	$\frac{N}{100}$	10·0 „	0·7670 „	0·7670 „	0·1903	0·2498	0·0714	**0·1784**	0·0119

TABLE III.—Normal Mice.

Age over twelve months. Period of Digestion, 1 hour.

Series 6.—May 30th, 1906.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of stomach material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)
I. A	32	$\frac{N}{10}$	18·7 grms.	0·4480 grms.	7·1686 grms.	0·2444	0·2494	0·1120	**0·1374**	0·1070

Series 7.—June 21st, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. A	16	$\frac{N}{10}$	—	0·5037 grms.	4·0310 grms.	0·0724	0·4256	0·2264	**0·1992**	nil.

Series 8.—January 15th, 1907.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. A (Contents).	40	$\frac{N}{10}$	—	1·0463 grms.	20·9270 grms.	0·0628	0·1587	0·0399	**0·1221**	nil.
I. A (Walls) ...	40	$\frac{N}{10}$	—	0·2579 „	5·1585 „	0·1415	0·1839	0·0778	**0·1061**	0·0354

Series 9.—February 4th, 1907.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. A	30	$\frac{N}{10}$	—	1·3607 grms.	20·4102 grms.	0·0823	0·1537	0·0224	**0·1313**	nil.

Series 10.—February 28th, 1907.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. A (Contents).	26	$\frac{N}{10}$	—	0·5018 grms.	6·5236 grms.	0·2014	0·2797	0·0615	**0·2182**	nil.
I. A (Walls) ...	26	$\frac{N}{10}$	—	0·2232 „	2·9014 „	0·2516	0·2138	0·0629	**0·1509**	0·1007

TABLE IV.—Normal Mice.

Age under three months. Period of Digestion, 1 hour.

Series 19.—March 4th, 1908.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of stomach material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)
I. *a*	11	$\frac{N}{10}$	10·7 grms.	0·4182 grms.	4·6000 grms.	0·1785	0·2777	0·1091	**0·1686**	0·0099
I. *b*	4	$\frac{N}{50}$	14·5 „	0·7500 „	1·5000 „	—	0·2190	0·0852	**0·1338**	—
I. *b*	4	$\frac{N}{100}$	14·5 „	0·7500 „	1·5000 „	0·1825	0·2190	0·0821	**0·1369**	0·0456
I. *c*	2	$\frac{N}{100}$	10·0 „	0·3790 „	0·7580 „	0·2406	0·2858	0·1204	**0·1654**	0·0392

Series 20.—March 11th, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. *a*	12	$\frac{N}{10}$	11·8 grms.	0·6806 grms.	8·1680 grms.	0·2234	0·2681	0·1675	**0·1005**	0·1229
I. *b*	4	$\frac{N}{50}$	11·5 „	0·5630 „	1·1260 „	—	0·3728	0·1702	**0·2026**	—
I. *b*	4	$\frac{N}{100}$	11·5 „	0·5630 „	1·1260 „	0·3566	0·3647	0·1661	**0·1986**	0·1580
I. *c*	2	$\frac{N}{100}$	11·5 „	0·4250 „	0·8500 „	0·2361	0·4079	0·1234	**0·2845**	nil.

Series 21.—March 17th, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. *a*	14	$\frac{N}{10}$	12·6 grms.	0·8532 grms.	11·9445 grms.	0·0993	0·2215	0·0955	**0·1260**	nil.
I. *b*	4	$\frac{N}{50}$	12·5 „	0·9072 „	1·8145 „	—	0·2313	0·0805	**0·1508**	—
I. *b*	4	$\frac{N}{100}$	12·5 „	0·9072 „	1·8145 „	0·1609	0·2313	0·0805	**0·1508**	0·0101
I. *c*	2	$\frac{N}{100}$	12·5 „	0·9797 „	1·9595 „	0·1257	0·1769	0·0372	**0·1397**	nil.

TABLE V.

Normal Mice.

Age over twelve months. Period of Digestion, 1½ hours.

Series 11.—February 4th, 1908.

Number of experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)
I. *a*	6	$\frac{N}{10}$	22·5 grms.	0·9035 grms.	5·4210 grms.	0·3030	0·2693	0·1178	**0·1515**	0·1515
I. *b*	1	$\frac{N}{100}$	19·5 „	1·3430 „	1·3430 „	0·2038	0·2582	0·0272	**0·2310**	nil.
I. *c*	1	$\frac{N}{100}$	21·0 „	0·6560 „	0·6560 „	0·2782	0·2921	0·0278	**0·2643**	0·0139

Series 15.—February 11th, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I. *a*	6	$\frac{N}{10}$	23·4 grms.	0·7928 grms.	4·7570 grms.	0·1534	0·3069	0·0959	**0·2110**	nil.
I. *b*	4	$\frac{N}{100}$	22·2 „	0·8182 „	3·2730 „	0·2175	0·2565	0·0892	**0·1673**	0·0502
I. *c*	2	$\frac{N}{100}$	22·0 „	0·8942 „	1·7885 „	0·2143	0·2551	0·0867	**0·1684**	0·0459
I. *d*	1	$\frac{N}{100}$	23·0 „	1·1875 „	1·1875 „	0·1537	0·2151	0·0576	**0·1575**	nil.

TABLE V. (*continued*).

Normal Mice.

Age under three months. Period of Digestion, 1½ hours.

Series 13.—February 7th, 1908.

Number of experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)
I. *a*	6	$\frac{N}{10}$	10·7 grms.	0·8356 grms.	5·0135 grms.	0·1456	0·2730	0·0728	**0·2002**	nil.
I. *b*	4	$\frac{N}{100}$	10·0 „	0·4157 „	1·6630 „	0·2085	0·2524	0·0439	**0·2085**	nil.
I. *c*	2	$\frac{N}{100}$	11·0 „	0·6735 „	1·3490 „	0·2164	0·2300	0·0406	**0·1894**	0·0270
I. *d*	1	$\frac{N}{100}$	11·5 „	1·0645 „	1·0645 „	0·1543	0·2572	0·0643	**0·1929**	nil.

Series 18.—February 25th, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
I.	12	$\frac{N}{50}$	10·8 grms.	0·5184 grms.	2·0736 grms.	—	0·2904	0·1144	**0·1760**	—
I.	12	$\frac{N}{100}$	10·8 „	0·5184 „	2·0736 „	0·2640	0·2640	0·0968	**0·1672**	0·0968

TABLE VI.—Mice with Transplanted Tumours (non-ulcerated) [Jensen].

Age over twelve months. Period of Digestion, uncertain.

Series 1.—December 19th, 1905.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)	Average weight of Tumour. (12)
I. B	4	$\frac{N}{100}$	—	0·3989 grms.	3·1916 grms.	0·6175	0·3431	0·1262	**0·2169**	0·4006	2·7 grms.

Series 2.—January 4th, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. B	17	$\frac{N}{10}$	—	0·2060 grms.	2·1890 grms.	0·6503	0·3670	0·2667	**0·1003**	0·5500	—

Series 3.—January 31st, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. B	27	$\frac{N}{10}$	—	0·2842 grms.	3·8377 grms.	0·3234	0·3234	0·1712	**0·1522**	0·1712	—

Age about three months. Period of Digestion, uncertain.

Series 4.—March 12th, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. B	37	$\frac{N}{10}$	—	0·2440 grms.	4·5130 grms.	0·4044	0·3559	0·2426	**0·1133**	0·2911	2·1 grms.

Age over twelve months. Period of Digestion, uncertain.

Series 5.—May 7th, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. B	61	$\frac{N}{10}$	—	0·2536 grms.	7·7345 grms.	0·6418	0·3115	0·0566	**0·2549**	0·3869	—

TABLE VII.—Mice with Transplanted Tumours (ulcerated) [Jensen].

Age over twelve months. Period of Digestion, uncertain.

Series 1.—December 19th, 1905.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)	Average weight of Tumour. (12)
I. c	2	$\frac{N}{100}$	—	0·2440 grms.	0·8135 grms.	0·7627	0·4487	0·2166	**0·2321**	0·5306	0·5 grms.

Series 2.—January 4th, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. c	2	$\frac{N}{100}$	—	0·2852 grms.	1·1410 grms.	0·5545	0·2133	0·0639	**0·1494**	0·4051	—

Series 3.—January 31st, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. c	10	$\frac{N}{10}$	—	0·2559 grms.	1·2795 grms.	0·5135	0·3993	0·2282	**0·1711**	0·3424	—

Age about three months. Period of Digestion, uncertain.

Series 4.—March 12th, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. c	14	$\frac{N}{10}$	—	1·1992 grms.	1·3452 grms.	0·7597	0·3798	0·2713	**0·1085**	0·6512	1·7 grms.

Age over twelve months. Period of Digestion, uncertain.

Series 5.—May 7th, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. c	4	$\frac{N}{10}$	—	0·2098 grms.	0·8393 grms.	0·6088	0·5219	0·3479	**0·1740**	0·4348	—

TABLE VIII.—Mice with transplanted Tumours (non-ulcerated) [Jensen].

Age, over twelve months. Period of digestion, 1 hour.

Series 6.—May 30th, 1906.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)	Average weight of Tumours. (12)
I. B	35	$\frac{N}{10}$	18·0 grms.	0·5091 grms.	8·9104 grms.	0·2622	0·2294	0·0901	**0·1393**	0·1229	1·3190 grms.

Series 7.—June 21st, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. B	15	$\frac{N}{10}$	—	0·4964 grms.	3·7227 grms.	0·0980	0·2942	0·1373	**0·1569**	nil.	1·3259 grms.

Series 8.—January 15th, 1907.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. B (Contents).	50	$\frac{N}{10}$	—	0·9086 grms.	22·7148 grms.	0·0739	0·1992	0·0209	**0·1783**	nil.	—
I. B (Walls) ...	50	$\frac{N}{10}$	—	0·1803 „	4·5080 „	0·2429	0·2186	0·1134	**0·1052**	0·1377	—

Series 9.—February 4th, 1907. [Tumour 27.]

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. B	45	$\frac{N}{10}$	—	1·1530 grms.	25·9175 grms.	0·0817	0·1677	0·0169	**0·1508**	nil.	—

Series 10.—February 28th, 1907.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. B (Contents).	38	$\frac{N}{10}$	—	0·5733 grms.	10·8937 grms.	0·1273	0·2345	0·0201	**0·2144**	nil.	—
I. B (Walls) ...	38	$\frac{N}{10}$	—	0·1796 „	3·4125 „	0·2355	0·1925	0·0535	**0·1390**	0·0965	—

TABLE IX.

Mice with transplanted Tumours.

Age at time of inoculation, under three months. Period of digestion, 1 hour.

Series 25.—May 15th, 1908.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Weight or average weight of mice. (4)	Weight or average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)	Weight or average weight of Tumour. (12)	Period elapsed since transplantation. (13)	Nature of Tumour. (14)
I.	4	$\frac{N}{50}$	12·5 grms.	0·6390 grms.	2·5560 grms.	0·1714	0·2713	0·0857	**0·1856**	nil.	1·2 grms.	34–41 days	27

Series 27.—May 21st, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
I.	1	$\frac{N}{50}$	15·0 grms.	0·9120 grms.	0·9120 grms.	0·3602	0·3002	0·0900	**0·2102**	0·1500	1·0 grms.	17 days.	$\frac{32}{27}$ C.
II.	4	$\frac{N}{50}$	12·5 „	0·5420 „	2·1680 „	0·3199	0·2609	0·1178	**0·1421**	0·1778	1·3 „	14 „	$\frac{32}{27}$ D.
III.	1	$\frac{N}{50}$	21·0 „	0·5560 „	0·5560 „	0·3939	0·4595	0·2953	**0·1642**	0·2297	2·5 „	55 „	$\frac{32}{21}$ G.
IV.	1	$\frac{N}{50}$	14·0 „	1·8810 „	1·8810 „	0·2814	0·2329	0·0776	**0·1553**	0·1261	12·5 „	45 „	$\frac{32}{27}$ B.

TABLE X.

Mice with Transplanted Tumours (ulcerated) [Jensen].

Age over twelve months. Period of Digestion, 1 hour.

Series 6.—May 30th, 1906.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c. c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)	Average weight of Tumours. (12)
I. c.	21	$\frac{N}{10}$	19·0 grms.	0·4133 grms.	4·3395 grms.	0·2869	0·3112	0·0673	**0·2439**	0·0421	1·4800 grms.

Series 7.—June 21st, 1906.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. c.	11	$\frac{N}{10}$	—	0·4894 grms.	2·6917 grms.	0·1084	0·3254	0·1356	**0·1898**	nil.	2·5966 grms.

Series 8.—January 15th, 1907.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
I. c. (Contents) .	20	$\frac{N}{10}$	—	0·8939 grms.	8·9396 grms.	0·1184	0·2164	0·0224	**0·1940**	nil.	—
I. c. (Walls) ...	20	$\frac{N}{10}$	—	0·1480 „	1·4800 „	0·2466	0·2713	0·1357	**0·1357**	0·1109	—

TABLE XI.

Mice with Transplanted Tumours.

Age at time of inoculation, under three months. Period of digestion, $1\frac{1}{2}$ hours.

Series 22.—May 5th, 1908.

Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Average weight of Mice. (4)	Average weight of Stomachs. (5)	Actual weight of Stomach Material extracted by 100 c. c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)	Average weight of Tumour. (12)	Period elapsed since transplantation. (13)	Nature of Tumour. (14)
I.	6	$\frac{N}{50}$	18 grms.	1·2696 grms.	7·6175 grms.	0·0838	0·2156	0·0629	**0·1527**	nil.	3·0 grms.	11–101 days.	No. 32.
II.	6	$\frac{N}{50}$	15 „	1·2831 „	7·6985 „	0·0830	0·2276	0·0593	**0·1683**	nil.	1·3 „	14–47 „	Jensen.

Series 23.—May 6th, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
I.	6	$\frac{N}{50}$	20·3 grms.	0·9431 grms.	5·6590 grms.	0·1161	0·2257	0·0967	**0·1290**	nil.	9·8 grms.	36–125 days.	No. 32.
II.	2	$\frac{N}{50}$	25·0 „	1·2677 „	2·5355 „	0·0720	0·2015	0·0864	**0·1151**	„	1·5 „	71–112 „	„
III.	3	$\frac{N}{50}$	15·6 „	0·6218 „	1·8655 „	0·1174	0·2446	0·1174	**0·1272**	„	4·3 „	36–71 „	„

Series 24.—May 9th, 1908.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
I.	5	$\frac{N}{50}$	20·5 grms.	0·8862 grms.	4·4310 grms.	0·1112	0·2183	0·0947	**0·1236**	nil.	4·4 grms.	53–64 days.	No. 27.
II.	2	$\frac{N}{50}$	23·5 „	0·8245 „	1·6490 „	0·1328	0·2435	0·1217	**0·1218**	0·0110	3·5 „	85 „	„ $\frac{27}{22}$ C.
III.	2	$\frac{N}{50}$	15·0 „	0·7655 „	1·5310 „	0·1311	0·2384	0·1371	**0·1013**	0·0298	2·5 „	35 „	„ $\frac{27}{22}$ C.
IV.	2	$\frac{N}{50}$	21·0 „	0·9145 „	1·8290 „	0·2694	0·2594	0·1064	**0·1530**	0·1164	6·5 „	116 „	Jensen.

TABLE XII.—Rats with Transplanted Tumours.

Age under three months at time of inoculation. Period of Digestion, 1 hour.

Series 26.—May 19th, 1908.

A.—*Positive.*

Number of experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Weights of Rats. (4)	Weights of Stomachs. (5)	Weight of Stomach Material extracted by 100 c.c. water. (6)	Total Acidity. % (7)	Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)	Weights of Tumours. (12)	Period elapsed since transplantation. (13)	Nature of Tumour. (14)
VII. ...	1	$\frac{N}{10}$	130 grms.	10·7735 grms.	10·7735 grms.	0·2880	0·1863	0·0720	**0·1143**	0·1737	4·5 grms.	153 days.	Flexner, 15 B.
XI. ...	1	$\frac{N}{50}$	68 „	3·6875 „	3·6875 „	0·2920	0·3761	0·1485	**0·2276**	0·0644	10·0 „	123 „	„ 16 A.
XII. ..	1	$\frac{N}{50}$	120 „	2·5525 „	2·5525 „	0·3575	0·3718	0·1144	**0·2574**	0·1001	3·5 „	123 „	„ „
XIII. ...	1	$\frac{N}{50}$	116 „	3·0840 „	3·0840 „	0·3432	0·2959	0·1124	**0·1835**	0·1597	14·5 „	106 „	Jensen, 8 A.
XIV. ...	1	$\frac{N}{10}$	106 „	9·8030 „	9·8030 „	0·3165	0·2048	0·0884	**0·1164**	0·2001	0·5 „	106 „	„ „
XVI. ...	1	$\frac{N}{10}$	133 „	8·4710 „	8·4710 „	0·3662	0·2801	0·0754	**0·2047**	0·1615	0·3 „	61 „	„ „
XVII. ...	1	$\frac{N}{50}$	51 „	3·1040 „	3·1040 „	0·2940	0·2704	0·0882	**0·1822**	0·1118	15·0 „	18 „	„ „

B.—*Negative.*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
V. ...	1	$\frac{N}{10}$	155 grms.	9·9500 grms.	9·9500 grms.	0·2751	0·1926	0·0734	**0·1192**	0·1559	No	153 days.	Flexner, 15 B.
VI. ...	1	$\frac{N}{10}$	150 „	11·9285 „	11·9285 „	0·2766	0·2075	0·0999	**0·1076**	0·1690	Tumours	153 „	„ „
VIII. ...	1	$\frac{N}{10}$	124 „	8·4760 „	8·4760 „	0·3445	0·2799	0·1399	**0·1400**	0·2045	had developed,	123 „	„ 16 A.
IX. ...	1	$\frac{N}{10}$	141 „	9·4060 „	9·4060 „	0·3492	0·2813	0·1455	**0·1358**	0·2134	hence	123 „	„ „
X. ...	1	$\frac{N}{10}$	132 „	8·7535 „	8·7535 „	0·3544	0·2502	0·0938	**0·1564**	0·1980	negative.	123 „	„ „
XV. ...	1	$\frac{N}{10}$	117 „	8·7770 „	8·7770 „	0·3743	0·2539	0·0624	**0·1975**	0·1768		106 „	Jensen, 8 A.

TABLE XIII.—Mice with

Age, over twelve months.

Date and Reference Number.	Number of Experiment. (1)	Number of Stomachs extracted. (2)	Strength of Standard Solutions used. (3)	Weight or average weight of Mice. (4)	Weight or average weight of Stomachs. (5)	Actual weight of Stomach material extracted by 100 c.c. water. (6)	Total Acidity. % (7)
May 29th, 1906. 25/0	I B	1	N/100	Adult.	0·9580 grms.	0·9580 grms.	0·3429
Nov. 4th, 1907. 111/0	I B	1	N/100	Adult.	1·4325 grms.	1·4325 grms.	0·1274
Nov. 5th, 1907. 110/0	I B	1	N/100	Adult.	1·5290 grms.	1·5290 grms.	0·2934
Nov. 8th, 1907. 113/0	I B	1	N/100	Adult.	0·9945 grms.	0·9945 grms.	0·1835
Nov. 26th, 1907. 93, 100, 106, 116	I B	4	N/100	23·7 grms.	0·8757 grms.	3·5030 grms.	0·7815
Jan. 18th, 1908. 112/0	I B	1	N/100	26·2 grms.	0·5695 grms.	0·5695 grms.	0·2563
118/0	I C	1	N/100	26·7 „	0·5900 „	0·5900 „	0·2165

Age over twelve months.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Feb. 25th, 1907. 105/0	I B	1	N/100	22·5 grms.	0·7640 grms.	0·7640 grms.	0·2866

Age over twelve months.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
May 18th, 1908. 141/0	I	1	N/100	29·5 grms.	0·4280 grms.	0·4280 grms.	—
126/0	II	1	N/100	32·0 „	1·7860 „	1·7860 „	0·3576
152/0	III	1	N/100	27·0 „	0·7745 „	0·7745 „	0·8954
157/0	IV	1	N/100	29·5 „	1·1985 „	1·1985 „	0·6548

Period of digestion, uncertain.

Total Chlorides. % (8)	Inorganic Chlorides. % (9)	Physiologically active Hydrochloric Acid. % (10)	Organic Acids. % (11)	Weight or average weight of Tumour. (12)	Time under observation. (13)	Nature of Tumour. (14)
0·4115	0·1829	**0·2286**	0·1143	—	7 weeks.	Hæmorrhagic adeno-carcinoma. Septicæmia (terminal.)
0·3121	0·1274	**0·1847**	nil.	—	2 days.	No tumour, abscess.
0·2267	0·0895	**0·1372**	0·1612	6 grms.	2–3 days.	Carcinoma mammæ. Metastases in lungs.
0·2936	0·1284	**0·1652**	0·0183	2–3 grms.	1 day.	Adeno-carcinoma mammæ. Large metastases in lungs.
0·2605	0·0469	**0·2136**	0·5679	5·5 grms.	93–12 weeks. 100–8 „ 106–3 „ 116–1 week.	Adeno-carcinoma mammæ. Large metastases in lungs.
0·1762	0·0801	**0·0961**	0·1692	Own tumour, no recurrence. Transplanted = 4·5 g.	6 weeks. —	Alveolar - carcinoma. No metastases.
0·2320	0·0464	**0·1856**	0·0309	Own tumour = 1·5 g. Transplanted = 7·5 g.	9 „	„

Period of digestion 1½ *hours.*

(8)	(9)	(10)	(11)	(12)	(13)	(14)
0·3105	0·0478	**0·2627**	0·0139	Own tumour recurred after operation. 0·2 grms. Transplanted tumour = 7·5 grms. in 12 weeks.	17 weeks.	Adeno-carcinoma mammæ. Metastases in lung.

Period of digestion 1 *hour.*

(8)	(9)	(10)	(11)	(12)	(13)	(14)
0·4690	0·2558	**0·2132**	—	7·5 grms.	10 weeks.	Adeno-carcinoma.
0·2554	0·0920	**0·1634**	0·0942	8·0 „	18 „	Adeno-carcinoma; metastases in lungs.
0·3534	0·1414	**0·2120**	0·6834	9·0 „	7 „	Adeno-carcinoma.
0·3045	0·1142	**0·1903**	0·4645	12·0 „	7 „	Adeno-carcinoma.

TABLE

TEST-MEALS (*undiluted*).

Number of Test-meal. (1)	Date when received. (2)	Hospital. (3)	Sex of Patient. (4)	Age. (5)	Nature of disease. (6)	Volume of Stomach fluid obtained. (7)	Gunsburg Test. (8)
5	June 21/07	Westminster	F	61	Cancer of Bile-duct	52 cc.	Very marked.
7	Oct. 21/07	Cancer Hospital	„	62	Carcinoma of Breast	21 „	Marked
8	„ 21/07	„ „	„	63	„ „	80 „	Faint
9	„ 22/07	„ „	„	40	Advanced Carcinoma of Cervix.	35 „	Nil
26	March 10/08	„ „	„	59	Recurrent Carcinoma of Breast.	105 „	„
27	„ 13/08	„ „	„	50	Inoperable Carcinoma of Cervix.	115 „	·0693
28	„ 18/08	„ „	„	50	Carcinoma of Cervix.	137 „	Nil
29	„ 27/08	„ „	„	34	Carcinoma of Uterus Inoperable.	60 „	Faint
30	„ 31/08	„ „	M	51	Cancer of Stomach	210 „	„
31	April 7/08	„ „	F	64	Carcinoma Uteri	112 „	0·0803
32	May 15/08	„ „	„	40	Recurrent Carcinoma of Uterus.	65 „	0·0305
33	„ 21/08	„ „	„	62	Carcinoma Mammæ	148 „	Nil
34	„ 28/08	„ „	„	62	Recurrent Carcinoma Mammæ.	140 „	0·0182

NORMAL CASE

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
35	June 2/08	—	M	25	Normal.	90 cc.	0·1314

XIV.

Period of Digestion, 1 *hour*.

Total Acidity to Phenol-phthalëin reckoned in HCl. % (9)	Total Chlorides. % (10)	Inorganic Chlorides. % (11)	HCl. Physio-logically active. % (12)	HCl (free and organically combined). (Mörner Sjöqvist.) % (13)	HCl (free). calculated from velocity of inversion of Methyl acetate. % (14)	Organic Acids reckoned as HCl. % (15)	Organic Acids (free and combined) as HCl. % (16)
0·2737	0·3650	0·0182	**0·3468**	0·3525	0·1940	nil.	0·0036
0·1825	0·2190	0·0547	**0·1643**	—	0·0753	0·0182	0·0182
0·2993	0·2482	0·0255	**0·2227**	0·1240	0·0305	0·0760	—
0·1642	0·2555	0·0730	**0·1825**	—	0·0074	nil.	0·0182
0·0839	0·2208	0·1095	**0·1113**	0·0564	0·0045	„	0·0255
0·1971	0·2774	0·1149	**0·1625**	0·1566	0·0310	0·0346	0·0328
0·0474	0·1022	0·0383	**0·0639**	0·0376	0·0022	nil.	0·0073
0·1095	0·2044	0·0730	**0·1314**	0·1128	0·0072	„	0·0182
0·1606	0·1752	0·0912	**0·0840**	0·0125	0·0036	0·0766	0·1022
0·2117	0·3467	0·1277	**0·2190**	0·2146	0·1076	nil.	0·0328
0·1533	0·3212	0·1606	**0·1606**	0·1630	0·0401	„	0·0876
0·0620	0·1350	0·0620	**0·0730**	0·0673	0·0061	„	0·0182
0·1825	0·2847	0·0931	**0·1916**	0·1848	0·0201	„	0·0328

(*undiluted*).

(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
0·1861	0·3175	0·0839	**0·2336**	0·2068	0·1195	nil.	0.1004

GLYCOGEN AND FAT IN MALIGNANT NEW GROWTHS OF THE MOUSE.

By M. HAALAND.

In the transplantable mouse-tumours we meet with a rapidity of growth which is hardly surpassed by any other known form of cell proliferation. A few centigrams of tissue when introduced into a mouse are in many cases capable of producing 1–2 grms. of solid tumour mass in 10 days, and 4–5 grms. in 2–3 weeks, in mice of 15–20 grms. weight. An exact comparison with the growth of fœtal tissues is hardly possible, because we have no means of ascertaining how many cells form the starting point of proliferation in the transplanted material. The interest of this cell proliferation is not limited to its bearing on the problems of cancer alone, but it is also of the greatest importance for a general biological study of cell-life and cell-metabolism in higher animals.

Of the phases of cell-metabolism that can be studied with histological methods, those characterised by the appearance of glycogen and fat have attracted greatest interest, partly because of the ease with which these chemically sharply-defined substances can be demonstrated. The great amount of glycogen in fœtal tissue suggested long ago its relation to rapid cell growth. Brault brought forward the hypothesis that the presence of glycogen in tumours is in proportion to their rate of growth, and that the estimation of its amount can be used to form a prognosis as to the malignancy of tumours. His results, however, have not been confirmed by Lubarsch, Best, and Gierke. The conclusions of these authors as to the significance of the appearance of glycogen differ. While Lubarsch ascribes great importance to inherited qualities of the cells, Best has shown that tissues normally containing no glycogen, may under inflammatory conditions, contain a great amount. He therefore considers the appearance of glycogen as an inflammatory reaction. Gierke concluded from his observations that it could be

explained as a result of circulatory conditions. Lubarsch has pointed out that the presence of glycogen in different kinds of cells can hardly be explained from a single standpoint.

In this connection we have thought it of some interest to examine the rapidly growing mouse-tumours for the presence of glycogen. In Best's method of staining glycogen in sections by a special carmine solution *, we have an easy and elegant method which, with the employment of different Iodine methods † as a control, offers every surety, so far as histological reactions can give them.

* *Best's Method for staining Glycogen (latest modification).* (Zeitschrift für Mikroskopie, Bd. 23, 1906, p. 319.)

Fix in absolute alcohol and imbed in celloidin.

Prepare the following stock solution :—

Carmine	2 parts
Potassium carbonate . .	1 part
„ chloride . .	5 parts
Aq. destill.	60 „

Boil for a few minutes. Allow to cool and add

Liq. Ammon. Fort. . .	20 parts

The solution is then ready for staining. Keep in the dark. The solution deteriorates after about 2 months in winter, after a few weeks in summer. Filter before use.

---

(1) Stain the celloidin-sections in Delafield's, Böhmer's, or other hæmatoxylin till nuclei are dark (differentiate in acid 70 % alcohol if necessary). Wash in tap water.

(2) Transfer to the following, prepared immediately before use :—

Stock solution	2 parts
Liq. Ammon. Fort. . .	3 „
Methyl alcohol (pure) .	3 „

Stain for 5 minutes.

(3) Transfer the sections directly into following differentiating solution :—

Absolute alcohol . .	80 parts
Methyl alcohol (pure) .	40 „
Aq. destill.	100 „

Differentiate for 1–3–5 minutes till no more red colour is given off.

(4) 80 % Alcohol.

(5) Absolute Alcohol.

(6) Xylol or Toluol.

(7) Canada Balsam.

Nuclei and protoplasm blue ; glycogen granules red.

† Ehrlich's, Barfurth's, and Langhans's method. See Encyklopädie der mikroskopischen Technik 1903, paragraph Glykogen.

The tumours we have had the opportunity of examining in this way are :—

(1) A great many spontaneous mammary adeno-carcinomata of the mouse, collected in the laboratory of the Imperial Cancer Research Fund and described by Murray on previous pages. In all these we failed to find glycogen in the parenchyma.

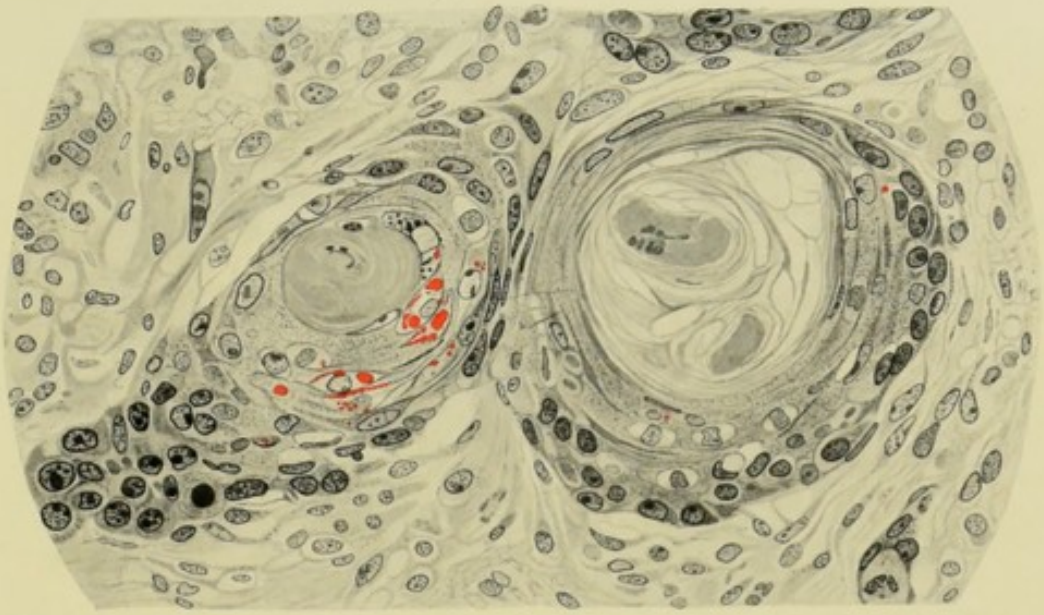
(2) Numerous transplanted tumours of mammary origin of different strains, mentioned in various papers in this Report (strain 27, 37, 39, 50, 46, and Jensen's tumour). Of these the first four have an adeno-carcinomatous structure, 39 and 50 are hæmorrhagic tumours as described by Gierke in this Report, 46 and Jensen's tumours are prototypes of alveolar carcinomata.

In none of these tumours, examined when they are used for transplantation, have we been able to find glycogen in the specific tumour parenchyma with certainty, even in the most rapidly growing tumours. We frequently find granules of glycogen in the leucocytes penetrating between the cancer-cells, and in the surrounding tissue, fatty tissue, neighbouring skin, and hair follicles. A positive result by chemical examination therefore need not necessarily imply that this glycogen is present in the specific tumour parenchyma, and only Best's method, with its distinct staining of both glycogen and nuclei, can show us exactly in which cellular elements it is present.

(3) In the transplanted tumours of a squamous-cell carcinoma (tumour 32), described in detail by Murray in an earlier paper in this Report, glycogen is found in the alveoli showing distinct keratinisation, and there only in the middle layer of cells, corresponding to the upper part of the stratum mucosum in the normal skin (fig. 1). On the other hand, we do not find glycogen in the great bulk of the tumour, *i. e.* in the cells of the undifferentiated alveoli. In this tumour the appearance of glycogen seems to be limited to a certain phase in the differentiation of the squamous epithelium, such as is already known to be exhibited normally by the same layers in skin.

(4) The mixed tumours we have described in the paper on sarcoma development occurring during continued transplantation of carcinoma have been examined. In them we occasionally meet with granules of glycogen in some of the sarcomatous cells of the stroma.

(5) The pure sarcomatous tumours developing from the mixed tumours, and two other transplantable sarcomata of spontaneous origin have been examined, one of mouse (tumour 92, described by Murray on pp. 78–81)



J. R. Ford, del.

FIG. 1.—32/9 B—10 D. Transplantable squamous cell carcinoma (20 days old). To show presence of glycogen in the middle layer of stratified squamous epithelium in a keratinised tumour of this strain. Glycogen could not be demonstrated in the tumours when keratinisation was absent. $\times \frac{350}{1}$.



J. R. Ford, del.

FIG. 2.—37/7Q—8G. Margin of graft of mammary adenocarcinoma, preserved 48 hours after inoculation. Granules of glycogen in the parenchyma-cells and in the cells of the reaction tissue. Adipose tissue of the host at the lower part of the figure. $\times \frac{500}{1}$.

and one of rat (Jensen's rat-sarcoma *). They are all typical spindle-cell sarcomata, and all grow rapidly. In them we sometimes meet with extremely fine granules in the protoplasm, or with a diffuse red staining by the carmine, when at the same time the iodine method does not show the presence of glycogen clearly. In the spontaneous sarcoma of the mouse (tumour 92) a greater amount of glycogen is found in the cells of the areas in which a further differentiation of the interstitial tissue to cartilage and osteoid tissue has taken place. As in the mammary tumours we often find a pronounced glycogenic reaction of the leucocytes. This reaction is most pronounced where these tumours have been inoculated into foreign species—rat sarcoma into mouse, and mouse sarcoma into rat.

(6) In Flexner's carcinoma of the rat an indistinct staining by carmine has been found, while the iodine method has not indubitably shown the presence of glycogen.

The presence of glycogen in the tumours of the mouse and the rat, examined when they are used for transplantation, seems to be limited mainly to a certain phase of differentiation of the squamous-cell carcinoma and to the sarcomatous tumours, especially where a differentiation of the interstitial tissue into cartilage has taken place. In all the tumours of mammary origin we have not succeeded in demonstrating glycogen in the adult tumours, whatever their rapidity of growth may be. However, when we examine grafts of these mammary tumours shortly after transplantation ("early stages"), we are surprised at finding glycogen appearing in quantity in their parenchyma. Fig. 2 shows a graft of mammary carcinoma 37, preserved in absolute alcohol 48 hours after transplantation. Glycogen is here found in great quantity in most acini. It is also found as fine granules in the cells of the reaction-tissue from the host, surrounding the graft. Likewise in "early stages" from sarcomatous tumours, the sarcomatous cells contain distinct granules of glycogen. The appearance of glycogen in "early stages" of tumours which normally do not show this phenomenon, offers an unequalled opportunity for studying its significance experimentally. As pointed out by Lubarsch, it may be doubtful whether the histological demonstration of glycogen has always the same significance, *e.g.*, where it appears, on the one hand in the differentiated areas of squamous-cell epithelium and in cartilage cells, and on the other hand in "early stages" of tumours which normally show no glycogen. The presence

* Jensen, C. O.: Transplantable rotte sarkomer. (Beretning om den danske Cancerkomité's virksomhed, 1905–1907. Copenhagen, 1907.)

of glycogen in adult tumours mainly in the differentiated squamous-cell epithelium, and in sarcomatous cells where differentiation of the interstitial substance has taken place, may point to a causal relation with circulatory conditions as suggested by Gierke. But the fact that glycogen does not appear as a constant phenomenon in adult tumours round the necrotic foci where the circulatory disturbances are amply demonstrated by the presence of fatty changes, shows that, in the majority of cases, a defective circulation is not sufficient to determine the appearance of glycogen. In "early stages," glycogen appears where besides circulatory disturbances active process of repair is going on. This fact accords with Best's observations on the appearance of glycogen under influences of inflammatory character in cells which normally do not contain it. Also an observation of Gierke on the appearance of glycogen in fat cells in guinea-pigs, when a period of abundant feeding followed close upon a period of starvation, may be mentioned in connection with our observations on "early stages."

As to the fat, the staining of frozen sections with Sudan shows us in numerous spontaneous tumours big cells filled with fat, like colostrum-corpuscles in the mamma, and lying free in the lumen of the adeno-carcinomatous acini. In the transplanted tumours, fatty infiltration constantly accompanies the necrotic changes which are so common in these tumours. Both in the centre of single alveoli with beginning necrosis, and where a larger central mass of tumour tissue has become necrotic, the zone of cells immediately surrounding the necrotic mass shows more or less fatty infiltration, and this change, as revealed by sudan-staining, extends more widely in the apparently healthy parenchyma than the other methods of staining entitle us to suppose. Where a zone of granulation tissue is formed round the necrotic part, as is frequently the case in tumour 37, immediately surrounding the necrotic mass a zone of cells loaded with fat is found (fig. 63, p. 226). In other cases the fatty infiltration of this granulation tissue is more diffuse. In the sarcomatous and mixed tumours, we find foci of fatty degeneration scattered diffusely in the tumour tissue, and here also associated with necrosis. It is obvious that this fatty change appears as a result of disturbances of circulation, but this kind of circulatory disturbance is not as a rule connected with the appearance of glycogen.

In "early stages," fatty infiltration is found as an indication of degenerative changes of the cells. In the centre of the graft it precedes the necrotic changes of parenchyma and stroma alike; in the peripheral parts it is found nearly constantly in the stroma cells of

carcinomatous tumours as fine granules stained black or brown with osmic acid. These fatty changes are not present to the same extent in the connective tissue cells of mixed and sarcomatous tumours. This seems to indicate that the chemical constitution of the sarcomatous connective tissue cells has become altered to some extent, so that the protoplasm is less modified by the injurious influences consequent upon transference to a new host, especially by the defective oxidation which undoubtedly is the main cause of the fatty changes appearing in the first 48 hours after transplantation before a new circu'ation is established.

The conclusions to be drawn are that there is no relation between the amount of glycogen which can be demonstrated by histological methods in the cells of transplanted tumours, and their rate of growth, and these results correspond so far to those drawn by Lubarsch, Best, and Gierke in examining human tumours. Glycogen seems to be constantly absent in all the fully developed tumours of mammary origin hitherto examined ; but, although judging from histological investigation, it does not seem to be essential in the normal metabolism of these cells, yet under certain conditions, viz., when they have been transplanted into other animals, glycogen appears in them in quantity. The relative importance of intensive synthetic processes of repair, and of defective oxidation in determining the appearance of glycogen under these conditions remains to be definitely settled by further observations.

In fully developed tumours glycogen is only found in those originating from cells which normally show the same phenomenon, viz., in squamous-cell epithelium in a certain phase of differentiation, and in connective tissue cells, especially where their interstitial tissue shows differentiation into cartilage. It is possible that circulatory conditions play an important part in the appearance of glycogen in these cells. The inconstancy of the presence of glycogen in these tumours perhaps indicates that the whole state of nutrition of the animal, or more particularly of the tumour, may be of importance. Glycogen is frequently met with in the surrounding fat-tissues of tumours containing no glycogen, and very frequently in the leucocytes. This glycogenic reaction of the leucocytes is more accentuated where the reaction has a more inflammatory character, as a consequence of the introduction of hairs, or the presence of pathogenic micro-organisms, and also where the inoculated tissues have come from another species, but it is also found, though in a lesser degree, accompanying the processes at the site of inoculation when tumour-tissue of the same species is transplanted. We have already pointed out that the presence of glycogen in the

leucocytes may be a source of fallacy in quantitative chemical analyses of these tumours.

The appearance of fatty changes is closely connected with disturbances of circulation, but there is no close parallel between the appearance of glycogen and of fat in these tumours. Round the necrotic areas where a fatty degeneration is constant, it is not as a rule accompanied by a glycogenic reaction of the cells.

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THE GASEOUS METABOLISM IN RATS INOCULATED WITH MALIGNANT NEW GROWTHS.

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THIS investigation is the first attempt to study the influence of the growth of a neoplasm on the metabolism of an otherwise normal animal. The experiments were carried out on young white rats into which a quickly growing tumour had been transplanted. The parent tumour was a tumour of the 13th generation of the strain J. R. S.* The transplantations were made into the right axilla on June 10th, 1908, into three young rats belonging to a litter (Litter B) of six rats, born May 1st, 1908. The remaining three rats were kept as controls. Small doses (0·05 c.c.) were used for transplantation. Seven days after the inoculation the tumours could be felt. They then grew rapidly, and soon extended over the whole right side. Notwithstanding the enormous size of the tumours, the weight of which, a month after the inoculation when the animals were killed, amounted to almost a third of the weight of the host, the animals appeared to be quite healthy. The coat looked smooth ; the animals were very active and appeared to be well nourished. The animals were killed on July 8th, before any signs of emaciation appeared. At the necropsy fat was found in the peritoneal cavity of the tumour-animals although not nearly as much as in the control-animals.

A most unexpected result was obtained by comparing the weights of the tumour-animals with the control-animals.

Table I. shows that on June 19th, when the tumours were still quite small, the tumour batch weighed 30 gr. more than the control-animals. On July 1st the three normal rats weighed 50 gr., 50 gr., and 70 gr. respectively, while the tumour rats had a weight of 80 gr., 80 gr., and 90 gr. The differences in weight cannot of course be accounted for solely

* A transplantable spindle-celled sarcoma of the rat for which the Imperial Cancer Research Fund is indebted to Professor C. O. Jensen of Copenhagen.

by the weight of the tumours, since these hardly reached such a weight a week later, as will be be seen from the following figures:—

	Tumour Rat.	Weight of Host.	Tumour.	Weight of Control Rats.
Killed July 8th...	90	70	20	65
,, ...	100	75	25	70
,, ,, ...	110	80	30	80

It will be seen that the growth of the tumours appears to have had a distinctly favourable influence on the growth of the animals in the earlier stages. In the later stages, however, after the tumour had reached a weight $\frac{1}{3}$ or $\frac{1}{4}$ that of the animal which served as a host, the growth of the animals had been retarded.

The method used for the estimation of the gaseous metabolism was the one devised by Haldane and Pembrey. All the precautions recommended by these authors (weighing against dummy tubes and a dummy animal chamber, insertion of control tubes, etc.) were observed. In order to exclude individual variations as much as possible, the gaseous metabolism of the three tumour rats together, and of the three normal rats together, was determined. The temperature of the room in which the estimations were made was fairly constant, and as a rule the tumour batch and the control batch were subjected to the experiment on the same day.

Table I. gives the results of the estimations of the gaseous metabolism of the fasting animals, *i. e.* 18–24 hours after feeding. In every case the experiment lasted 60 minutes, so that the figures given for the CO_2 and H_2O discharged represent the actual amounts found in one hour.

The results show that no essential difference exists between the metabolism of the tumour-rats and the control-rats. The respiratory quotients are the same in both batches, and vary within the limits observed by Pembrey and Spriggs for fasting rats. Taking the individual animal as the unit, the amount of CO_2 discharged and O_2 absorbed is higher in tumour rats than in the normal rats. The more energetic metabolism of the tumour rats is accounted for by the more rapid growth of the organism which served as a host. The growth of the tumour itself

TABLE I.

Date.	Duration of experiment.	Number of rats.	Total Weight.	H_2O discharged in cg.		CO_2 discharged in cg.		O_2 absorbed in cg.		$\frac{CO_2}{O_2}$.	CO_2 per hour and kilo in grammes.	O_2 per hour and kilo in grammes.	Temperature.	
				Found.	Calculated per rat.	Found.	Calculated per rat.	Found.	Calculated per rat.				In	Out
Control.—Three young white rats of Litter B.														
17 VI..	1 hour.	2	95	21	10·5	34	17	32	16	·76	3·52	3·37	14°	16°
19 VI..	,,	3	130	29	9·7	47	15·7	46	15·3	·74	3·61	3·54	17°	20°
1 VII.	,,	3	170	28	9·3	45	15	44	14·7	·74	2·65	2·59	17°	20°
2 VII.	,,	3	170	27	9	50	16·7	53	17·7	·68	2·94	3·12	18°	21°
Three young white rats of Litter B, with tumours, inoculated 10 VI. 08.														
19 VI..	1 hour.	3	160	40	13·3	62	20·7	62	20·7	·73	3·88	3·88	16°	19°
1 VII.	,,	3	235	55	18·3	75	25	78	26	·70	3·15	3·28	18°	20°
2 VII.	,,	3	230	74	24·7	63	21	62	20·7	·73	2·74	2·69	19°	21°

does not appear to have a marked influence on the gaseous metabolism of the fasting animals.

An interesting difference was observed in the metabolism of the animals after a meal of bread and milk. Preliminary observations confirmed those of Pembrey and Spriggs *, that the rise of the respiratory quotient after a meal rich in carbohydrates was due to an increase in the CO_2 excretion, the absorption of O_2 remaining almost the same as in the fasting condition, as the following figures show :—

Respiratory exchange before and after feeding †.

	3 Tumour-rats.		3 Normal rats.	
	CO_2 in cg. per hour.	O_2 in cg. per hour.	CO_2 in cg. per hour.	O_2 in cg. per hour.
Fasting ...	63	62	50	53
Fed.....	90	67	75	54

In order to study more minutely the changes in the CO_2 excretion taking place after a meal, a series of half-hourly estimations of the CO_2 excretion only, were made after the animals had been fed on bread and milk. The results are given in Table II. The experiments were made July 7th.

The results show that the rise in the CO_2 excretion is much more pronounced in the normal rats than in the tumour rats, so much so, that in the third hour after the meal the normal animals excrete more CO_2 than tumour rats, although in the fasting condition the reverse condition obtains. Since the O_2 absorption is not materially affected by the feeding of the animals, the respiratory quotient can be roughly calculated on the basis of our former estimations of the O_2 absorption. It is then found that it rises considerably above unity in the normal animal, and remains above unity for several hours after feeding, while in the tumour animals it hardly exceeds unity and rapidly falls again. It may be noted that the respiratory exchange of the tumour-rats returns sooner to the fasting condition than that of the normal animals.

* Pembrey and Spriggs, Journal of Physiology, vol. 31, 1904, p. 320.

† These estimations were made in the 3rd and 4th hour after feeding.

TABLE II.

Time of experiment.	CO_2 discharged (per half hour) in cg.	CO_2 per hour and per kilo in grs.	Temp.
3 *Tumour-rats.* [Weight 290 g. Fed with 18 g. bread and milk from 8^{45}—9^{15}.]			
9^{40}—10^{10}	54	3·73	20°
11^{25}—11^{55}	45	3·10	20°
12^{52}—1^{22}.....	45	3·10	20°
2^{35}—3^{5}	39	2·69	21°
4^{10}—4^{40}	39	2·69	20°
3 *Normal rats.* [Weight 190 g. Fed with 20 g. bread and milk from 9^{25}—9^{50}.]			
10^{35}—11^{5}	50	5·32	20°
12^{8}—12^{38}	48·5	5·10	20°
1^{35}—1^{5}	38	4·00	21°
3^{20}—3^{50}	30	3·16	20°
4^{50}—5^{20}.....	28·5	3·00	20°

The rise in the CO_2 excretion has been interpreted by Hanriot, and by Pembrey and Spriggs as being the result of chemical processes, which lead to the transformation of carbohydrates into fats, CO_2 being split off. If this interpretation is accepted, it can be concluded from our observations that in the tumour-rats such a storing of the absorbed food-material in the form of fat does not take place at all, or not to the same extent as in normal animals.

It is not difficult to understand why this should be the case. The rapid growth of the tumour-cells is the result of their absorbing more nutritive material than the cells of the organism in which they grow. In the normal animal the mechanism which presides over the assimi-

lation of the food, is regulated in such a way that more nutritive material can be absorbed than is immediately required by the organism. Part of the excess is stored as fat. But this condition is disturbed by the presence of a rapidly growing mass of cells with an enormously increased nutritive capacity. At first, the demands made by the tumour-cells necessitate a larger intake of food and a more rapid absorption of nutritive material, so that at first the organism serving as a host to the tumour grows more rapidly. The increased activity of the digestive organs probably accounts for the condition which we observed some years ago in mice with transplanted tumours, and which has since been independently studied by Copeman and Hake, namely the increased secretion of hydrochloric acid in the gastric juice of these animals. Then, as the tumour goes on growing, the animal, instead of being able to absorb more nutritive material than is wanted immediately, will have to use all the material it can absorb in order to satisfy the growing demands of the tumour, and will not have an opportunity of storing part of the absorbed food as fat. Finally, however, the requirements of the tumour will increase to such an extent that they will overstep the limit within which a physiological adaptation is possible. The organism of the host, unable to absorb sufficient nutritive material, will have to yield nutritive material from its own tissues to the tumour. The animals then become emaciated and die within 1–2 days. The tumour rats of Litter B had not yet reached this stage.

This condition was studied in six young rats of another batch, Litter A, which was inoculated on the same day as Litter B and with the same tumour, but with a bigger dose (0·2 cc.). The tumours grew much more rapidly than those of Litter B. The growth of the animals was retarded, and all the animals died within the third week after the transplantation in an emaciated condition. The gaseous metabolism of these animals although at first quite normal, began to show irregularities after the second week, and abnormally high quotients of the value ·86, and even 1·00 were observed in the fasting animals. These quotients were the result of a diminished O_2 absorption, not of an increased CO_2 excretion. These observations will however not be discussed here in detail. They are mentioned only in order to emphasize that the growth of a tumour in a normal organism leads at first to physiological changes; pathological changes in the general metabolism do not ensue until the physiological resources of the animal have become exhausted. This must be borne in mind in future investigations on the metabolism of cancer if confusion and contradiction are to be avoided.

The effect which a growing tumour produces on a normal organism is a problem of nutrition similar to the growth of a fœtus in a pregnant animal. It cannot be explained by attributing to the cancer cell the formation of pathogenic substances of a hypothetical nature, such as a "cancer ferment" or a "cancer toxin." In a review of recent work on this subject the present position has been summed up as follows * :—
"The experimental investigation of cancer has not produced any evidence in favour of the existence of a specific cancer toxin. But it has shown that cancer affects the organism in which it grows by virtue of the increased nutritive capacity of the cancer cells." Our observations on the gaseous metabolism afford further evidence in favour of this view.

* W. Cramer: 'Some problems of the metabolism of cancer from the standpoint of Experimental Cancer Research' in Von Noorden—Walker Hall: 'Metabolism and Practical Medicine,' vol. iii. p. 824.

APPENDIX I.

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APPENDIX II.

Draft of Scheme for Enquiring into the Nature, Cause, Prevention, and Treatment of Cancer *.

1. GENERAL NATURE OF THE ENQUIRY AND GENERAL ORGANISATION.

Scope.

The term "Cancer" must be taken to include all malignant new growths, and this conception must be extended to similar diseases in animals, both for studying the subject in them, and in relation to the possibility of transmission to man.

Character Constituting Malignancy and Spread.

The investigation must further include the conditions favourable to spread in the body and the other features of malignancy, since these constitute the serious aspect of cancer as distinct from innocent growths. Any change which would prevent the acquisition of malignancy, or which would hinder such spread, would be of very great value.

Statistics as to Incidence. General Causes.

The enquiry should include the compilation of accurate statistics bearing on all the conceivable conditions possibly associated with the incidence of the disease.

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The Possibility of Different Causation in Different Groups; Separation of Special Groups, some possibly Parasitic.

The study of all new growths in order to classify them, and to distinguish the various form which at present may be grouped together as malignant growths is important. It might thus be possible to eliminate some types which may be found to be of definite, and possibly of very diverse parasitic origin. This will constitute an important feature of the work to be done.

Experimental Production of "Cancer."

It will be necessary to attempt to produce new growths in various ways, or to attempt so to modify non-malignant growths that they become malignant.

Bio-chemical and Physiological, possibly including Therapeutic Substances.

Investigations along modern bio-chemical lines, with consideration of the conceivable production of cytolytic, agglutinative, antagonistic, perhaps therapeutic sera, and the possible occurrence and antagonistic nature of internal secretions, etc., will demand very serious attention.

The organisation will consist of :—

(1) A central bureau and laboratory for general direction of the undertaking, for study, research, control of work being done and of the results obtained elsewhere, and for the summarising of results and the interpretation of statistical investigations by those working there.

(2) Associated workers carrying on special parts of the investigation in their own laboratories or hospitals, etc. There would thus be an attempt to utilise so far as possible existing laboratories, and also skilled workers, who occupy other important positions.

(3) Other persons from whom statistics might be collected, etc.

The ultimate greater development of the experimental side of the enquiry being borne in mind, the control work might be begun by classifying all that is grouped under the term malignant disease, and by collecting statistics.

At the outset two or three rooms giving accommodation for the director, one or two good secretarial assistants, and two or three assistants skilled in microscopical work would be required.

At a subsequent date the investigation will in all probability require much more extensive laboratory accommodation, and it will be necessary to obtain some place where animals can be bred and kept, and probably a farm will be required.

Of the skilled assistants, one should if possible be a trained veterinary surgeon who, apart from his special duties in connection with the important veterinary aspects of the research, would at a later date be invaluable in regard to supervision of animals destined for research purposes.

In an enquiry which may ultimately require the keeping of many animals, both of the larger forms, *e. g.*, horses, anthropoid apes, etc., proper attention to the hygiene of the animals would be essential; apart from the experimental importance of being able to exclude previous infection, ultimately a proper farm with efficient means for isolation and perhaps for the breeding of animals would be necessary.

A second assistant should be specially detailed to assist in the bio-chemical researches, and should have had a proper chemical training, if possible; also a training in physiological chemistry. A third assistant should preferably be a man skilled in zoological and general biological enquiry. All assistants should be skilled in microscopical and general histological technique.

At a later date a larger expert staff will probably be found necessary. In the compilation of statistics the services of a trained actuary would be almost essential for a time.

The enquiry must start at selected points and be allowed to develop itself in the hands of those conducting it along those lines which the experience accumulated, will in natural course dictate. The proceedings must therefore be cautious, and conducted on the very widest basis. At present we possess no knowledge which justifies limiting the enquiry in any special direction, but no reasonable line of special enquiry should be discouraged.

The scope at the outset being so very wide, the work should primarily be directed to attempting to define the field of legitimate enquiry, and within the shortest time possible attempting to focus the efforts on a rational research for the causal factor or factors. This limitation may best be obtained within a reasonable period by following out systematically various lines of enquiry at the same time:—Statistical, histological, chemical; pertaining to cancer in various human races, animals, plants (?), and with consideration of all alleged causes—heredity, race, climate, soil, etc., and also the reputed increase of cancer.

2. RECOMMENDATIONS BEARING ON SPECIAL LINES OF ENQUIRY WHICH MAY BE DIVIDED INTO STATISTICAL, EXPERIMENTAL, ETC.

Statistical.

The proper study and interpretation of the returns of the Registrar-General should be augmented by special enquiry directed by the central body, because the special statistics already compiled and commented on, and especially those of the German and Dutch Cancer Committees, and those relating to Massachusetts, have not yet brought to light evidence free from ambiguous interpre-

tation, nor have they the value which presumably would attach to the similar compilation based on the widely divergent races, regions, isolated communities, etc., which would come within the scope of the present enquiry, comprising as it will the whole British Empire.

The compilation of statistics would have to be conducted along the soundest lines, and with regard to all conditions which may be supposed to favour the occurrence of malignant disease in man and animals.

Any scheme drawn up should be submitted to a statistical expert for approval.

A sufficiently extensive statistical enquiry may be expected to lead to the accumulation of facts helping to decide whether any form of disease comprised under the term " malignant " is communicable from one individual to another. It may further be of help in determining the direction enquiries as to the cause or causes are to take.

Owing to the time it will take to get together the mass of important evidence, which is waiting to be collected in different parts of the globe, I would suggest that early steps be taken to organise this collection.

I would divide into two great classes those to be relied upon for the immediate collection of the information sought, viz. those voluntarily assisting, and those who can be enlisted in the service through the different government offices and various local authorities.

It will thus be necessary at an early stage to ascertain how far the Local Government Board, India Office, Colonial Office, etc., town and county councils and other authorities having medical or veterinary officers in their services would be inclined to assist the enquiry.

Those voluntarily assisting would be essentially the staffs of hospitals and general practitioners. A direct appeal to each institution and individual seems desirable in order to direct attention to the special points on which definite information is wanted.

To avoid misapprehension, secure uniformity, intelligibility, and ready classification of the information obtained from such divergent sources as isolated Crown Colonies, Town and County communities, etc., definite questions should be asked. These being intended to elicit from distinct sources, both similar and different kinds of information, it would be necessary to draw up the various subjects of enquiry. In regard to this matter, advice might well be sought from the German Cancer Committee, who have already some experience of it.

In the interval that may elapse before the return of these enquiry forms, arrangements would have to be made for the classification and interpretation of the returns under the combined direction of a trained statistician and a director or other medical official.

Importance of Co-operation in Experimental Work.

In order to obtain the co-operation of skilled workers who would carry on special parts of the investigation at their own laboratories, etc., it would be

necessary to arrange both for grants to them for their expenses, and for honoraria for their work. There are many who might thus be encouraged to assist the enquiry by undertaking special work on suggestions given to them by the central body, *e. g.*, those who wish to use their work for the purpose of a graduation thesis at a University.

Of course, any work of this nature may be performed in any laboratory, the only condition being that the worker is fully qualified to undertake the work proposed, and that any control or suggestion on the part of the central body would be permitted by the chief of the laboratory in which the work is being done. In order to emphasise this side of the enquiry, circulars would have to be directed to all institutions likely to afford facilities for work of this nature, or, to provide such workers. The importance of central control would have to be emphasised, and the necessity for avoiding undue overlapping brought into prominence.

Importance of Study of "Malignancy."

The causes of, or changes which may accompany, or be responsible for, spread in the body must be investigated with especial care. This study may indicate the means by which power to spread may be removed, or may point the way to limitations in other directions. In this connection only the emaciation, secondary anæmia, and leucocytosis have received much attention. The not infrequent occurrence of pigmented moles, warts, etc., in albino and other animals seem to offer favourable conditions for a much more extensive investigation into the occurrence of other conceivable phenomena. Thus, the possibility of converting innocent conditions into malignant growths may be studied, and the importance of investigating the possible influence of internal secretions, by means of the injection, etc., of emulsions, watery and other extracts, fluids obtained under high pressure, or by means of a gelatine filter under high pressure from malignant growths must not be lost sight of. The study by recent methods, of changes in the serum and other fluids in cases where spread is occurring, or where an "innocent" tumour has become malignant, with a view to detecting there the presence of anything of the nature of an immune body, in Ehrlich's sense, must receive attention. It is impossible to express any opinion on the likelihood of being able by these means to ascertain the presence of factors which may cause spread, be concomitant with spread, or have really an antagonistic influence on spread.

Importance of properly controlling all Bio-chemical Enquiries in order to avoid fallacies and all tendency towards their assuming a too speculative nature.

Owing to the extreme complexity of the experimental methods necessary to this line of enquiry, it could only be carried out under the direction of one familiar with the methods, the manifold fallacies likely to present themselves, and the equally numerous control experiments necessary.

Granted due attention be paid to the above sources of error, purely hypothetical considerations must for the present suffice to suggest the following lines of enquiry.

A thorough scrutiny of malignant disease in the light of the knowledge which has been recently acquired in regard to hæmolysis, cytolysis, cytotoxines, immune-bodies, and the mechanism of their action, etc., ought to be undertaken.

The following special lines of enquiry also suggest themselves :—

A search should be made for evidence of anything arising pathologically, or experimentally, in consequence of the prolonged irritation of tissues, or the existence of cancerous conditions. Such evidence might be sought for in the existence of anything in the nature of excessive waste products, modified alkalinity of fluids, autolysines, isolysines, etc., such as it may be assumed might possibly stimulate local or general vegetative activity, or give rise to detectable excess, or deficiency of any constituent of cells, or of fluids. Also any evidence of local or general irritation arising from such causes should be sought.

An endeavour might be made to determine if the chemistry of the normal cell shows any divergence from that of the cancer cell which has developed from it. By means of modern methods applied to the albuminous bodies, ferments, etc., it might be possible to determine any divergence in the nature of the constituents of these cells; the presence or absence of normal or abnormal constituents might be sought for.

Hofmeister has recently pointed out the large number of distinct ferments contained in any one cell of the liver. It would be interesting to ascertain to what extent these ferments are present in the cells of malignant disease of this organ.

An enquiry might be instituted to determine if any stimulation of cells can be made to lead to the production in the latter of manifestations of activity with depression of the special function (secretion, motile phenomena, etc.), *i. e.*, without calling forth what is regarded as the special function of the cells stimulated.

Classification and attempted Experimental Production of "Cancer."

In regard to the attempted experimental production of innocent and malignant new growths in animals, or the possibility of setting up the features of malignancy in benign growths, it may be well to repeat some old experiments, for example, in relation to embolism, etc., where the results obtained may have been vitiated by the conditions under which the experiments were at the time carried out.

In this connection the possible experimental production of "Petroleum cancer" and "Sweeps' cancer" would have to be borne in mind, and experiments carried out on a very wide series of differing species of animals.

The classification of different forms of cancer if properly carried out, might lead to the elimination of some forms which may be found to be due to special parasites (as for example, actinomycosis has been eliminated), and to the separating off of what may be found to be special types, or only seeming cancer. In all investigations of this nature full and detailed histological, chemical, bacterial, or other parasitic investigations must be made and a full history of each case clinically recorded.

The reputed transplantation of cancer into wound margins ought to be specially enquired into, with a view to the important bearing such an occurrence would have on other transplantation experiments. Such transplantation experiments should only be carried out with regard to the special species of animal in which the original growth occurred, and the special tissue in which it seemed to have had its origin. For all experiments of this nature the importance of the assistance of a trained veterinary man is very evident, for it would be especially necessary to exclude the possibility of previous infection.

A systematic study of the effects of persistent irritation of different epithelial surfaces in different species of animals might be found to have important bearings. In this category possibly the study of "Petroleum cancer" and "Sweeps' cancer" might be placed.

Those engaged in post-mortem examinations should be encouraged to search systematically for, and record all evidence of incipient or undetected malignant disease. Such an investigation might throw much light on the internal conditions, *e. g.*, in the alimentary canal, which in themselves may perhaps lie long dormant, and in the end become malignant, in the same way as other conditions on the surface of the body are known to do.

A comparative study of mitosis and vegetative activity would require to be undertaken. Such an investigation would cover not only mitotic and vegetative activity in embryonic and cancer cells, but also that occurring in the cells of benign tumours, and of tissues which have reverted to an embryonic type in processes of repair, and with consideration of that over-production which is an essential feature of repair.

I would respectively submit that any such scheme as the foregoing can only be provisional, and has been drawn up in full consciousness of the difference between drawing up a scheme on paper and putting the same into practice. I do not claim that this hastily drawn up scheme is adequate to cover the whole field of enquiry. In conclusion I would point out that the investigation must necessarily follow the lines which accumulated experience will dictate.

(Signed) E. F. BASHFORD.

Edinburgh,
October 24*th*, 1902.

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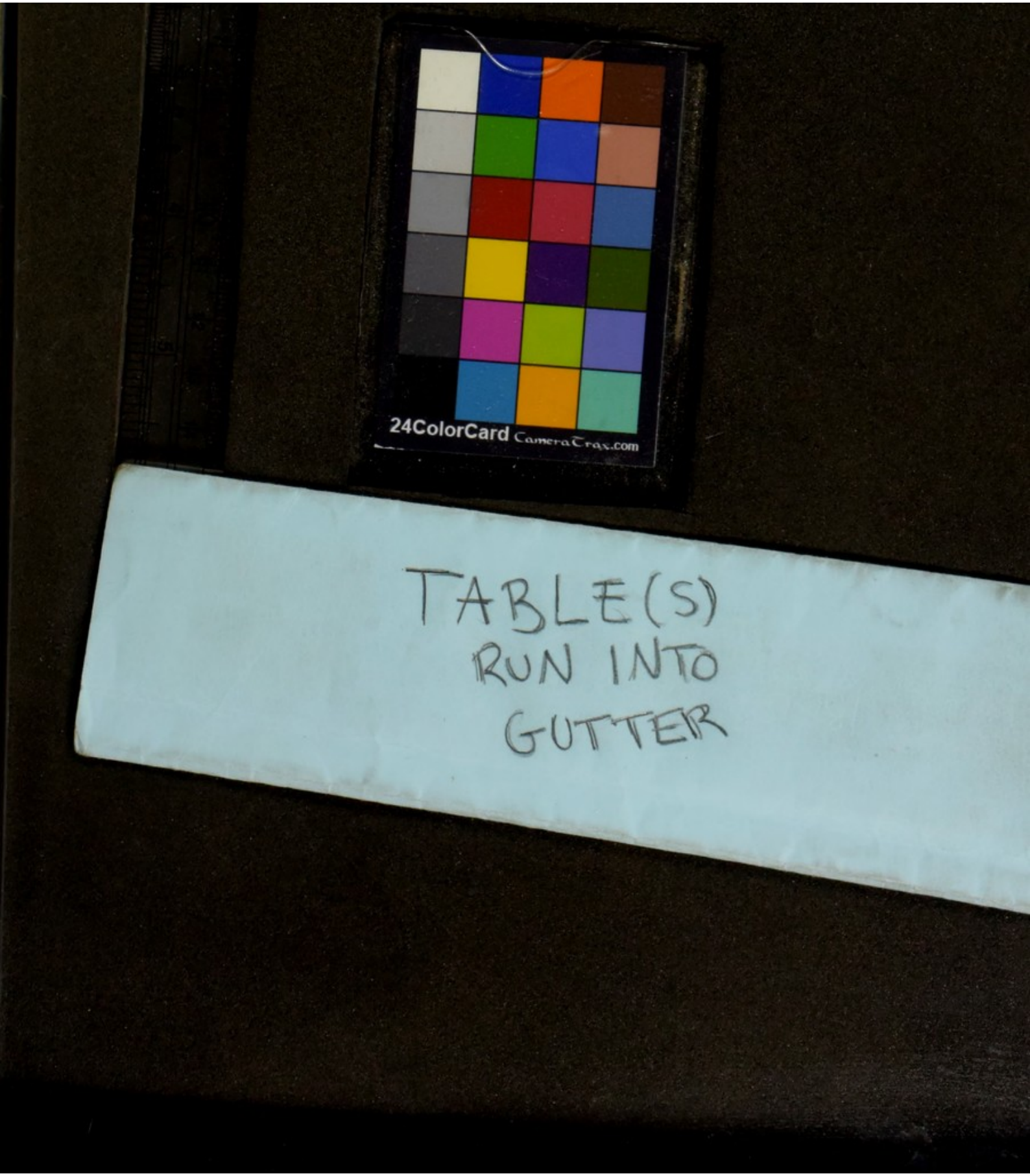
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