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

FOURTH 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.

4

Published by the Authority of the Executive Committee.

LONDON:
PRINTED AND PUBLISHED BY
TAYLOR AND FRANCIS, RED LION COURT, FLEET STREET, E.C.
1911.

Price 7s. 6d.

FOURTH 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.

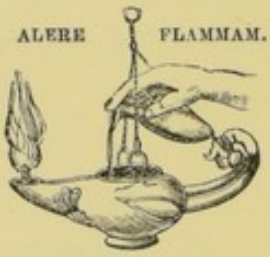
Published by the Authority of the Executive Committee.

LONDON:

PRINTED AND PUBLISHED BY

TAYLOR AND FRANCIS, RED LION COURT, FLEET STREET E.C.

1911.



PRINTED BY TAYLOR AND FRANCIS,
RED LION COURT, FLEET STREET.

THE IMPERIAL CANCER RESEARCH FUND.

PATRON.

HIS MAJESTY THE KING.

PRESIDENT.

THE DUKE OF BEDFORD, K.G.

VICE-PRESIDENTS.

LORD LISTER, O.M., F.R.S.

LORD STRATHCONA AND MOUNT ROYAL, G.C.M.G. RIGHT HON. A. J. BALFOUR, M.P.
THE KNIGHT OF KERRY. SIR J. WERNHER, BART. W. WALDORF ASTOR, Esq.

GENERAL COMMITTEE.

ADELINE, DUCHESS OF BEDFORD.
DR. F. W. ANDREWES.
SIR THOMAS BARLOW, Bart., K.C.V.O.
MRS. BISCHOFFSHEIM.
SIR JOHN ROSE BRADFORD, K.C.M.G., Sec.R.S.
DR. BYROM BRAMWELL, P.R.C.P.E.
SIR HENRY T. BUTLIN, Bart., P.R.C.S.
LADY GWENDOLEN CECIL.
SIR W. WATSON CHEYNE, Bart., C.B., F.R.S.
SIR WILLIAM CHURCH, Bart., K.C.B.
ARCHIBALD COATS, Esq.
DANIEL COATS, Esq.
SIR JAMES COATS.
PETER COATS, Esq.
W. A. COATS, Esq.
Professor J. R. A. DEWAR.
GERARD FIENNES, Esq.
HENRY L. FLORENCE, Esq.
SIR THOMAS GLEN-COATS, Bart.
SIR ALFRED PEARCE-GOULD, K.C.V.O.
SIR HENRY G. HOWSE.
LADY LECONFIELD.
ROBERT LITTLEJOHN, Esq.
HUBERT GORE LLOYD, Esq.
SIR JOHN MCFADYEAN.
DR. SIDNEY MARTIN, F.R.S.
Professor A. E. METTAM.
SIR HENRY MORRIS, Bart.
SIR CHARLES W. MORRISON-BELL, Bart.
Professor ROBERT MUIR, F.R.S.
J. ASHLEY MULLENS, Esq.
LUDWIG NEUMANN, Esq.
S. NEUMANN, Esq.
DR. ARTHUR NEWSHOLME.
Professor A. C. O'SULLIVAN.
EDMUND OWEN, Esq.
H. J. READ, Esq.
LORD ROTHSCHILD, G.C.V.O.
CHARLES D. RUDD, Esq.
HAROLD J. STILES, Esq.
DR. J. F. W. TATHAM.
SIR JOHN TWEEDY.
DR. T. T. WHIPHAM.
JAMES WILCOCK, Esq.
Professor G. SIMS WOODHEAD.
ROBERT H. WOODS, Esq., P.R.C.S.I.

EXECUTIVE COMMITTEE.

SIR WILLIAM CHURCH, Bart., K.C.B., *Chairman*.

DR. F. W. ANDREWES.
SIR THOMAS BARLOW, Bart., K.C.V.O.
SIR JOHN ROSE BRADFORD, K.C.M.G., Sec.R.S.
SIR HENRY T. BUTLIN, Bart., P.R.C.S.
SIR W. WATSON CHEYNE, Bart., C.B., F.R.S.
SIR ALFRED PEARCE GOULD, K.C.V.O.
R. CLEMENT LUCAS, Esq.
SIR JOHN MCFADYEAN.
DR. SIDNEY MARTIN, F.R.S.
SIR HENRY MORRIS, Bart.
DR. ARTHUR NEWSHOLME.
EDMUND OWEN, Esq.
Professor G. SIMS WOODHEAD.

TRUSTEES.

SIR R. DOUGLAS POWELL, Bart., K.C.V.O.
J. ASHLEY MULLENS, Esq.
LUDWIG NEUMANN, Esq.
DR. T. T. WHIPHAM.

HONORARY TREASURER.

SIR HENRY MORRIS, Bart.

GENERAL SUPERINTENDENT AND DIRECTOR OF THE LABORATORY.

DR. E. F. BASHFORD.

SECRETARY.

MR. FREDERIC G. HALLETT.

AUDITOR.

MR. ERNEST R. FRERE.
(Messrs. Prideaux, Frere, Brown, and Co.)

OFFICE AND LABORATORY.

EXAMINATION HALL, VICTORIA EMBANKMENT, LONDON, W.C.

STATISTICAL SUB-COMMITTEE.

DR. JOHN TATHAM, *Chairman.*

SIR FRANCIS LOVELL, C.M.G.
DR. ARTHUR NEWSHOLME.
H. J. READ, Esq.
DR. T. H. CRAIG STEVENSON.
SIR ARTHUR WHITELEGGE, K.C.B.

PATHOLOGICAL SUB-COMMITTEE.

SIR JOHN MCFADYEAN, *Chairman.*
DR. F. W. ANDREWES.
PROF. SIR JOHN ROSE BRADFORD, K.C.M.G.
PROF. G. SIMS WOODHEAD, M.D.

PUBLICATION SUB-COMMITTEE.

DR. SIDNEY MARTIN, F.R.S., *Chairman.*
SIR HENRY T. BUTLIN, Bart., P.R.C.S.
SIR JOHN MCFADYEAN.
PROF. G. SIMS WOODHEAD, M.D.

CONTENTS.

INTRODUCTION.

The Fourth Scientific Report, apart from this introduction, is restricted to three papers. The first, "On Spontaneous Cancer in Mice," by Dr. Haaland, treats of a large number of the additional spontaneous tumours of the mouse observed since the Third Scientific Report was prepared. These tumours consist of carcinomata and sarcomata occurring in a variety of sites other than the mamma, and are considered from clinical, pathological, histological, and experimental standpoints. The animals in which they were found have been submitted to a number of experimental tests in order to elucidate the relation between a tumour and the animal in which it arises. The second paper, "On Cancerous Ancestry and Cancer in Mice," by Dr. Murray, deals with breeding experiments bearing upon heredity, which have been in progress for some six years with mice of known ancestry, and from which a large number of the tumours and mice studied in the first paper have been obtained. The third paper, "On the Behaviour of Tumour-Cells during Propagation," is a general survey of the observations made on all the tumours observed or propagated in the laboratory during the past eight years, and of the bearing of their relative constancy and variability upon the nature of some forms of cancer. All three papers are intimately interdependent as regards the material upon which they are based, and they overlap in so far as each treats more especially of particular problems not excluded from consideration in the

other two. Thus, as in all previous reports, the effort is made to co-ordinate the features of cancer as it occurs naturally with its behaviour under experimental conditions.

New facts are brought forward in support of the view that a malignant new growth arises from local causes in a circumscribed area, and that the relation of each malignant new growth to the affected animal is an individual one, parallel to that obtaining between the organs of the body and the organism as a whole.

Precise evidence is advanced of the existence of hereditary predisposition to the development of spontaneous cancer. It is apparently of the nature of a predisposition of certain tissues to pass into cancerous proliferation, and is not effective by determining an increased suitability of the animals primarily affected for the growth of cancer as tested by transplantation.

Tumour-cells derived from a single primary growth are shown to be liable during extended propagation to variations such as are met with, either singly or in combination, in other primary growths. It is assumed that this demonstration permits of the inference that corresponding variations occurring in the course of the prolonged proliferation of normal cells under the influence of chronic irritation may be responsible for the development of some forms of cancer.

The relations between benign and malignant new growths, and of both to normal tissue have been studied both histologically and experimentally on an extensive material. Among the large number of tumour-strains that have been propagated by *passage* from one batch of mice to another for extended periods, there are included several reproducing very closely the features of normal tissue, either as regards its histology or its limited * power of growth in any one

* In spite of being possessed of a power of only limited growth in any one animal tumours can be maintained in extended propagation by suitably accelerating the rapidity of *passage*. This has not yet been accomplished in the case of normal tissues.

animal after homologous transplantation. Some tumour-strains while retaining almost perfect histological differentiation grow progressively in any one animal, while others, notwithstanding that they are quiet devoid of histological differentiation, possess only a limited power of growth in any one animal. The gaps between the structure of normal tissue and the least differentiated tumours on the one hand, and between the growth of normal tissue, when transplanted, and that of even the most rapidly proliferating tumours on the other, have been filled in by a continuous series of tumour-strains. Some of these approximate to normal tissue both in respect of structure and of power of growth on transplantation, and experiment has brought out still more clearly the pure arbitrariness of the conception of a fundamental difference between benign and malignant new growths.

The demonstration that cancer occurred in practically all races of mankind and throughout the vertebrates even when living in a state of nature, together with the demonstration of the only manner in which cancer can be transferred from one individual to another of the same species, viz. by implanting living cells, proved that it was not due to a common causal parasite. The wide zoological distribution of the disease down to marine fishes showed that it was not a recent acquirement such as might be referred to influences dependent on man's particular forms of civilisation. As has been frequentlypointed out, the age-incidence of cancer in man and animals is, in the absence of communicability, compatible only with the recognition of the intrinsic cellular nature of cancerous proliferation.

The parallel behaviour of normal and cancer tissue both as regards the absence of continued growth, and the nature of the cytotoxic reactions induced, when cancer is transferred from one animal to another of a strange species, proved that cancer had all the properties distinguishing the normal tissues of one species from those of another species.

The fact that transplantable tumours grow in normal animals as well as they do in spontaneously affected animals, is evidence that the latter

do not present a soil for the growth of cancer substantially different from that presented by normal animals. When this fact is contrasted with the almost invariable success of reimplanting into the animal a portion of its own spontaneous tumour, and the almost invariable failure of implantation of any spontaneous tumour into other spontaneously affected animals, the conclusion is arrived at that each tumour is peculiarly and genetically related to the individual in which it arises.

This conclusion, drawn from studying the growth of tumours under the different conditions just enumerated, is supported by the results of elaborate experiments on inducing resistance or immunity to the inoculation of cancer-cells under these different conditions. The features of resistance bearing upon the nature of cancer are briefly as follows. Resistance is induced only by the living cells, either cancerous or normal, of the same species. Under similar conditions the cancerous cells and the normal cells of strange species are both devoid of the power to induce resistance. An animal's own tumour and its own normal tissue are devoid of this power, and the means which prevent the successful inoculation of the tumour of another individual do not prevent the successful inoculation of an animal's own tumour. Tumour-tissue usually induces resistance against itself quite as well as, and, with regard to the phenomenon of spontaneous healing, much more effectively than, any other tumour. Furthermore, animals which have proved resistant to the repeated inoculation of a tumour, have subsequently developed spontaneous tumours showing progressive growth. Thus experimental enquiries into the production of growth by inoculation on the one hand, and its prevention on the other, agree in demonstrating individual relations as obtaining between a tumour and the animal in which it arises. The individuality of tumour-cells will be referred to later.

The individuality of cancer, both as regards the organism attacked and the tumour, would thus appear to have been placed at last beyond all further doubt. Such a relationship has long been maintained in

various forms on the basis of deductions drawn from histological examination of the tissues at the site of the primary lesion, and from the nature of dissemination, but this interpretation of the findings has been as vehemently combated. The combination of the results arrived at by microscopical investigation and experimental study appears to complete the demonstration. A long step has thus been taken in defining the direction in which the future investigation of cancer is alone likely to be profitable.

The conclusions as to the individuality of cancer are supported also by most important new statistical information given in the last Report of the Registrar-General *, of which some account may be given. It will be remembered that the policy pursued in regard to the statistical investigation of cancer has been to supplement the national statistics, and, if possible, to add to their utility by special inquiries, but not to endeavour to overlap or in any way to replace them. This policy has been dictated by the belief that the adequate investigation of most of the statistical features of cancer is best carried out by the Registrar-General's staff, since the data available for such a purpose do not exist outside of the national statistical office. This plan has been made effective by the willingness with which the Medical Superintendents of Statistics, Dr. Tatham and his successor Dr. T. H. C. Stevenson, have collaborated with the Imperial Cancer Research Fund in supplementing and amplifying information calculated to assist purely pathological and experimental investigations. Such collaboration and co-ordination, which does not exist in the case of organisations for the investigation of cancer in other countries, where independent statistical inquiries have been undertaken with the voluntary assistance of the medical profession, has been of the greatest importance in England and Wales by preventing profitless overlapping, and in effecting real advances in the accurate statistical knowledge of the incidence of cancer.

* Seventy-Second Annual Report of the Registrar-General of Births, Deaths, and Marriages in England and Wales (1909). His Majesty's Stationary Office, 1911.

For several reasons it had long been desired * that the reports of the Registrar-General should contain detailed information on the incidence of cancer in different parts of the body, and Dr. Tatham was able to make arrangements for obtaining this information at the outset of these investigations. The application of the law of age-incidence of cancer to short-lived as well as to long-lived animals reinforced the other reasons for obtaining it, and suggested that knowledge would be advanced by more detailed information about the age-incidence of cancer in the several organs of man as distinct from its dependence on the age-distribution of persons †. It was anticipated that the data would be of both biological and statistical value, and the facts published by Dr. Stevenson in the last report of the Registrar-General show that this hope has been fulfilled in several directions.

The new tabulation of the data for the years 1901–09 for England and Wales has permitted of an analysis being made of the figures recording the increase of deaths attributed to cancer, which brings out the fact that the increase during this period is referable to certain anatomical regions and not to others. Thus, for males the main increase falls on the alimentary tract, especially the stomach. The liver and gall-bladder and the skin show no, or only a slight, increase. For females, the increase, although it falls mainly on the alimentary tract (stomach and intestines), affects also the mamma, while the uterus, ovary, liver and gall-bladder, rectum and skin show little or no increase. It is also of importance that the recorded mortality from cancer of the generative organs has not increased at the same rate as that for other organs, and that most of the increases affect the higher age-periods predominantly.

* *Cf.* "Proposed letter to the Registrar-General of England of Births, Deaths, and Marriages on the subject of the increase of Cancer, drawn up by Mr. Butlin, of which the following is a copy." Proceedings of Council, British Medical Journal, Nov. 12, 1887, p. 1070; King, George, & Newsholme, Arthur : On the Alleged Increase of Cancer. Roy. Soc. Proc. vol. 54, p. 209, 1893.

† Bashford, E. F., & Murray, J. A.: The Statistical Investigation of Cancer. Second Scientific Report of the Imperial Cancer Research Fund, Part 1, pp. 3, 24, & 51.

For the first time it is fully demonstrated that it is erroneous to make statements of a disquieting nature about the increase of cancer in general. In conjunction with investigations * into the errors of diagnosis among hospital patients, means are afforded of determining, for parts of the body where the disease appears to be increasing, whether the increase is real or only apparent, and of ascertaining the causal factors peculiar to such parts. While it is evident that several of the differences brought out by the figures can be explained by more accurate diagnosis and by allocation of the seat of the disease from the secondary to the primary situations, as illustrated, *e. g.*, by the relation revealed between cancer of the liver and gall-bladder and the alimentary tract, this may not account fully for certain other features. In particular, the increased incidence of cancer recorded for the mamma in women, and the tongue in men, requires further study and elucidation.

The analysis also shows that the incidence is very unequally distributed among the several situations, indeed, that the whole curve of incidence may be different for different organs. A progressive increase up to the highest age-periods is characteristic of the face, lip, mouth, bladder, urethra, and breast only. The other organs show a distinct diminution in the highest age-periods; but it is not yet possible to determine whether this curve indicates a liability rising to a maximum and followed by a fall, or is merely the result of ascribing deaths to other causes in the case of cancer of internal organs in aged people. The proportion of total deaths ascribed to the ill-defined cause of old age is 65·6 per 1000 deaths from all causes as compared with 65·7 for cancer, and it must be borne in mind that the increases recorded for cancer affect principally the higher age-periods and that the average age of the population is increasing.

* Comparison of the clinical diagnosis with the results of pathological and microscopical examination. *Vide* Second Scientific Report of the Imperial Cancer Research Fund, Part 1, pp. 18–24; Bashford, E. F.: Address on Cancer in Man and Animals. General Meeting of the XVIth International Medical Congress, Buda Pest, 1909, and in 'Lancet,' Sept. 4, 1909, p. 694; Berl. Klin. Woch. 1909, Nos. 36 & 37.

Sufficient has been said to indicate how important are the problems which are solved or revealed by the improvement in the details given in the national statistics.

The study of the occurrence of cancer in mankind, and in domesticated animals in widely separated parts of the globe, has shown that the practice of peculiar customs (involving the application of chronic irritants to particular parts of the body), provokes the disease in situations and organs from which it is absent when these customs do not obtain. It is reasonable to suppose that the frequency of cancer would be diminished, if such practices as the use of the Kangri in Kashmir, chewing betel-nut in India, and eating very hot rice in China, were discontinued. It is also reasonable to assume that the introduction into England of these exotic customs would greatly increase the frequency of cancer in this country.

So definite is the evidence of the *mediate* causation of certain forms of cancer by chronic irritants, that the possibility of variations in the cancer death-rate may be admitted as regards particular organs and regions of the body. The possibility of a variation of the main incidence of cancer in conformity with changes in certain customs may also be admitted.

That irritation is really an important causative factor of cancer is an assumption which at present is justifiable only for certain forms of cancer occurring in particular parts of the body. The knowledge of the irritants to which different species of animals and the individual tissues of the same animal may be susceptible is of very considerable importance. The acquisition of this knowledge will doubtless require extensive study, and it is advisable to commence this investigation in man statistically.

In view of these considerations, and also because of the results of experiment as recorded in this report, it appeared desirable to have data of the incidence of cancer in persons pursuing various occupations and having different habits of life. This matter was discussed with

Dr. Tatham in 1903–04, and again with Dr. Stevenson of the General Register Office, who has explained to the Statistical Sub-Committee that the new arrangements made by the Registrar-General for tabulating deaths, would permit of this information being abstracted. For the purposes of comparison it will be necessary to learn not only the incidence of cancer on particular sites liable to irritation, but also its incidence on all other sites, as well as the frequency of the other causes of death in the occupations considered. This information will be embodied in the next Decennial Supplement to the Reports of the Registrar-General.

Breeding experiments with mice of known ancestry have been in progress for many years, and have been alluded to on several previous occasions, but only now have the data become sufficiently numerous to permit of conclusions being drawn. All due precautions have been taken to avoid errors in the interpretation of the figures. The data show that heredity plays a part in affecting the liability of the mouse mamma to develop cancer. At all age-periods the disease is more frequent when the mother, or either grandmother, or all three, have died from cancer of this organ than in the group in which these ancestors were free from the disease. The maxima occur at the same age-period in both groups, viz. 16–18 months, being respectively 21·6 and 32·1 per cent. of the total number of animals, there being no clear evidence that the maximum incidence of cancer of the mamma has been made to occur at a younger age in mice of recent cancerous ancestry.

Apart from its bearing upon heredity, the obtaining of such mice is most important for furthering the experimental investigation of the genesis, nature, and, should it be necessary, artificial production of cancer, and for attempting to define the reasons for its apparently greater frequency in some geographical areas than in others. It will be obvious that a large field of investigation has been opened up by the segregation of mice into two groups of different liability, and it should be possible to obtain groups of animals of a still higher and

a still lower liability. While it is, at present, impossible to explain how the liability is transmitted, it can be averred with certainty that it does not consist in the inheritance of a soil more suitable for the growth of cancer in general. It can only be inferred with some probability, that it is a local or circumscribed tissue predisposition, in virtue of which the mammary tissue is prone to pass from mere proliferative reaction into continuous or cancerous proliferation *. Further, hereditary predisposition is only one of the factors concerned, for it has been found that chronic inflammatory changes are remarkably frequent in the mammæ of female mice of the laboratory, and other factors still unrecognised may exist. There is a considerable body of evidence to show that the predisposition is not a general one affecting the whole body equally, but that the tendency transmitted affects mainly one organ system, so that groups of animals may ultimately be obtained in which different organ-systems will present a definite predisposition, the other organs of the body not being unduly liable to the development of the disease.

To guard against pessimistic conclusions it is well to point out that the influence of heredity has only been demonstrated by studying stocks in which this factor has been concentrated by careful mating, and that the influence is mainly exerted in the immediate descendants. Such a concentration as can be attained in experimental animals can only occur in the human subject, by hazard, as a coincidence of considerable rarity, and it is probable that the influence of heredity in the general population is manifested as an average predisposition of low general intensity.

In all previous Reports guarded reference has been made to the well-known association of chronic irritation and certain forms of cancer and it has been pointed out that in common with all external

* "Congenital germs" or "embryonic rests" are not referred to here; they do not afford sufficient explanation. The alleged congenital origin of cancer was considered in the Second and Third Scientific Reports of the Imperial Cancer Research Fund and other papers, and dismissed as untenable. *Cf.* Second Scientific Report, p. 31, and pp. 74–81; Third Scientific Report, p. x.

conditions they can only have *mediate* relation to the occurrence of cancer, the essential preliminaries which lie between them and its inception being regarded not as their specific reactions but as manifestations of properties inherent in the cells. The employment of the term "mediate" when drawing attention to the relationship, is due to an effort to elucidate those forms of cancer with which irritation is most constantly associated, without considering other forms in which the particular irritants concerned do not play a part, and due to the fact already frequently emphasized, that these irritants have nothing in common beyond the capacity to excite extended proliferation of tissues (chronic inflammation), and their association with cancer. The varied investigations of the past nine years have added a knowledge of new forms of irritation. It has become more and more evident that irritation, effective in one case, may be, and often is, quite ineffective in another species of animal or even in other individuals of the same species. The experiments recorded in this report throw light both on the nature of predisposition to cancer as alluded to above and also on the long recognised, but inexplicable, relation between chronic irritation and cancer.

For more than eight years numerous varieties of cancer-cells arising from the epithelium of the mamma, have been maintained in proliferation and pertinaciously studied with a view to separating the innate properties from such as are due to environment. Ever since the beginning of these investigations it has been maintained that the mere cultivation of cancer had important, if only indirect, bearings upon its nature and genesis. Thirty-five of the tumour-strains have now been growing for over three years, *i. e.* for longer than a mouse lives, while fifty other strains have been grown for extended periods. The one common feature of all these tumour-strains, which distinguishes them from normal mammalian tissues, is the power of continuous growth which they exhibit when repeatedly transplanted, even in spite of the most divergent structure, and of extremes in the rate of growth varying from an almost explosive rapidity to one much inferior to that of embryonic tissue, as determined by weighing-experiments.

It can be shown that along with a constancy in the behaviour of a tumour-strain there is a variability which is individual. The variations which occur are of the same order of magnitude as those which distinguish other strains comprised in the total of 85 different strains from one another. They are not mainly induced by the environment, but arise spontaneously; otherwise all strains would approach a common type, which they do not. The demonstration of the occurrence of these variations under artificial conditions permits of the inference that they could also occur under natural conditions, and yields objective evidence of the validity of the conclusion that the cancer-cell is a biological modification of the normal cell endowed with many inherent properties of the latter. It has been ascertained that every fresh transplantation effects a disturbance of the cancer-cells. They are thrown into a state of renewed proliferation from which they tend to return to their customary behaviour. The process is as analogous as possible to reactive proliferation when occurring naturally. Attention is drawn to this analogy between the phenomena called forth in non-cancerous animals by chronic irritants leading to extended proliferation (chronic inflammation) occasionally becoming cancerous, and the results of long-continued propagation of cells already cancerous leading to variations in their properties. Just as what is practically a new tumour is seen to develop during propagation from cancerous cells by variation of their characters, so it is inferred cancer may arise by variation of normal cells during the prolonged proliferation which accompanies prolonged or repeated attempts at repair under the influence of chronic irritation. The objection is at once suggested that these variations during prolonged propagation are secondary, and do not necessarily indicate corresponding primary changes as responsible for genesis; but this objection cannot be maintained against the facts that the potentiality for variation has been demonstrated, as has also the tenacity with which the several varieties are adhered to. Hence the features observed under artificial propagation appear to throw suggestive light upon the nature of cancer and its genesis in those

cases in which a prolonged phase of reactive proliferation has intervened between the application of irritants and the development of cancer.

It follows from the argument pursued in the preceding paragraphs that a closer definition of the nature of cancer will involve an analysis of the relation obtaining between the individual developing cancer and the tumour.

This final analysis will be possible only on animals naturally afflicted with the disease, for, as pointed out consistently from the First Annual Report onwards, the genesis and the growth of cancer are distinct phenomena. The study of propagated cancer supplements its observation under natural conditions by investigation under varied artificial conditions, and has only an indirect bearing upon the genesis of the disease. Hence the breeding experiments, to which allusion has already been made, acquire enhanced significance, and are already being, and will continue to be conducted on a much more extensive scale. An adequate supply of animals of differing liability to the disease must be made available for the elucidation of problems some of which are already adumbrated, while past experience makes it likely that others, as yet unsuspected, will arise.

Because of the hope that they may ultimately have therapeutical bearings, another reference may be made to the induction of resistance to the inoculation of cancer and the means which modify the growth of transplanted tumours. Experiments along these lines bear at present, as has often been emphasized, upon the nature, but not upon the prevention, treatment, or cure of cancer; notwithstanding this fact the application of the results to the human subject has been urged and even made in some quarters. In 1906–07 it was pointed out that a high degree of resistance to the transplantation of cancer did not exempt an animal from the spontaneous development of the disease. The importance of the observation was great; had immunity to the natural acquirement of cancer been also obtained, the control of the scourge would have been in sight. In the Third Scientific Report the previous

warning was re-enforced, and it was pointed out that none of the methods which had been found to influence transplanted cancer should be applied to man till they had been tested and found efficacious in the case of animals naturally affected. These warnings are borne out by further experience and inability as yet to modify the growth of cancer in the animal naturally affected is fully described on pp. 79–85. Instead of revealing analogies with infective diseases, and placing similar remedial and preventive measures in the hands of the physician and surgeon, the study of resistance to cancer has, up till now, but brought investigators to the verge of a region filled with problems previously undreamt of. In the solution of these problems, or of more crucial ones not yet reached, lies the best hope of preventive and remedial measures; but the preliminary facts are so new to experimental biology and as yet so imperfectly comprehended, that observers throughout the world are still engaged in discussing what may be their true significance as signposts indicating a road or roads by which the correct advance is to be made. For these reasons it has seemed well not to burden the present Report with an account of the greater proportion of the work on these topics.

The full ground of the experimental investigations carried out during the three years that have elapsed since the Third Scientific Report was issued is not covered by the three papers comprised in this Report. The bibliography appended at the end of this volume shows the extent of the omissions. The limitation has been made with the object of emphasizing the conclusions arrived at by convergent lines of investigation of which the intimate interdependence has been indicated in this introduction, although this interdependence may not be so apparent to readers of the elaborate details given in the following papers who have not followed the gradual building up of the comparative and experimental investigation of cancer during the past decade.

The sum of observations is the result of the organised efforts of the entire official and voluntary staff of the Imperial Cancer Research

Fund. During eight years twenty investigators have had their share, including in particular, Dr. Cramer, who has maintained his interest in the bio-chemical problems, Dr. Haaland, Dr. Murray, Dr. Russell, and Dr. Woglom in arriving at the present conclusions. I desire to acknowledge my indebtedness to each of them on account of their long collaboration in the work, their wide knowledge, their originality of thought, and the independence of their observations. I desire also to acknowledge the valuable work of Mr. W. H. Bowen and Dr. A. G. Wells, the two other official members of the staff.

E. F. BASHFORD.

August, 1911.

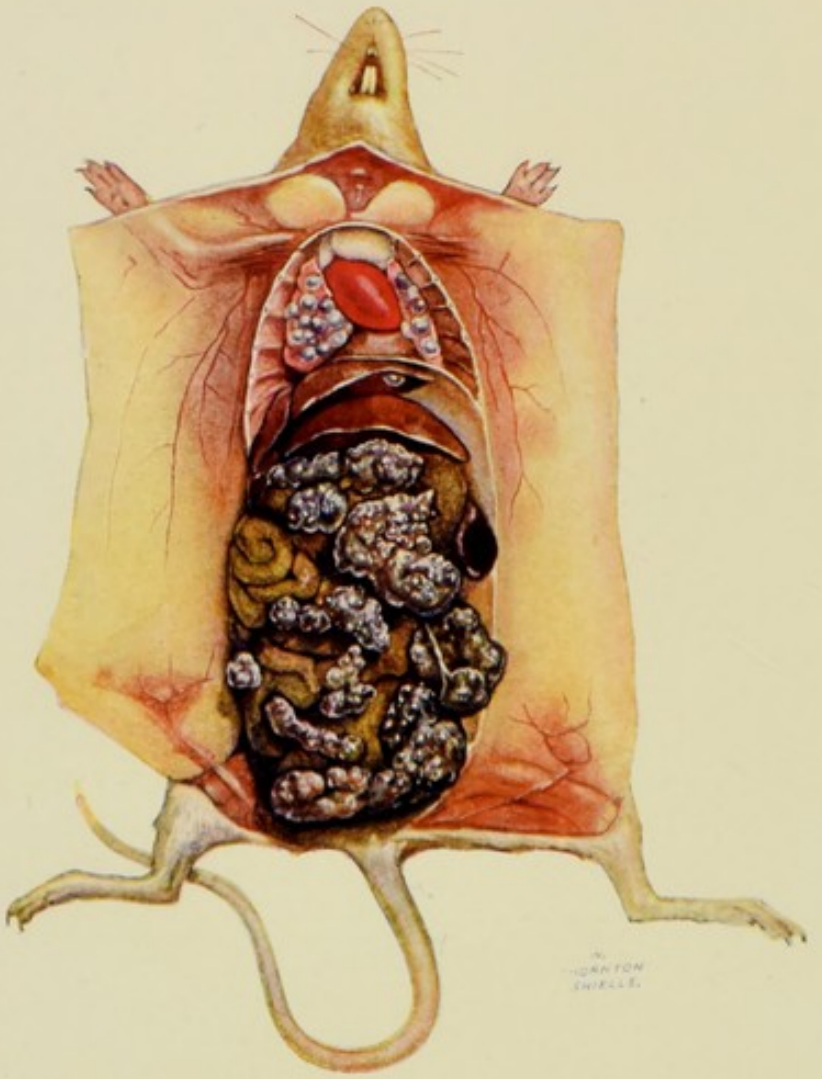


FIG. 39.—Mouse 396. Resu't of intraperitoneal implantation of a large dose (0·50 c.c.) of its own spontaneous tumour (hæmorrhagic adeno-carcinoma of the mamma). Mouse killed 15 weeks after inoculation. Multiple large and highly vascularised masses of tumour in the abdominal cavity attached by pedicles to viscera and parietes. Numerous metastatic nodules in the lungs. Natural size.

[*Fourth Scientific Report of the Imperial Cancer Research Fund*, 1911.]

SPONTANEOUS TUMOURS IN MICE.

By M. HAALAND.

The recognition of the fact that processes analogous to cancer in man exist in the different animals throughout the vertebrate scale, together with the demonstration of the transmissibility of the disease from one animal to another of the same species, cleared the way for the employment of experiment in the investigation of a number of problems hitherto not approachable by direct methods. By means of the material made available the properties of the cancerous cell could be investigated in the most thorough fashion. The study of transplanted cancer in normal animals has, therefore, played a prominent part in recent years in the systematic investigation of cancer. The value of the method is shown by the varied and accurate information as to the biology of the cancer cell, its immunity reactions, etc., which has thus been acquired. This procedure has played a rôle in cancer research similar to that taken by the study of hæmolysis in the investigation of the immunity reactions against infectious diseases. However, it would be a mistake to identify the modern investigation of cancer along biological lines with the study of transplanted cancer alone. With all its convenience for laboratory use, it covers only the growth of cells, already malignant, in normal animals, as has constantly been pointed out by those engaged in these investigations. The conditions under which cancer arises spontaneously are not necessarily approached, and only in certain instances (*e. g.* development of sarcoma) or in an indirect way has this method hitherto had a bearing on the problem of the origin of cancer, viz. how a normal cell becomes a cancer cell. Ideal material

for studying the problem of the origin of cancer is still lacking, and up to the present one is restricted to conclusions deduced from observations on cancer arising spontaneously. One part of the problem which can be approached more directly is the question as to the rôle played by the organism in which cancer arises. Opportunity for approaching this question is, at present, afforded by spontaneous cancers only, and their study continues, therefore, to remain an indispensable complement to the experimental investigation of propagated cancer. So long as we are unable to produce primary cancers at will, the spontaneous cases are the only source of fresh material and of information both as to how the disease arises and, more directly, as to the part played by the spontaneously affected organism.

Among the spontaneous cancers, the tumours of mice continue to offer exceptional facilities for investigation. Apart from the relative ease with which they can be transplanted into normal animals, the facility of obtaining and keeping large numbers of old mice, and the frequency of tumours amongst them, naturally tend to make the mouse the animal which offers the greatest convenience for the investigation of spontaneous cancer also.

To anyone who has followed with an open mind the evidence brought forward during recent years no doubt can exist that the process of malignant growth is essentially the same throughout all the vertebrates. On the other hand, differences exist between the tumours of different species ; in the same way as a normal organ, *e. g.* the skin, offers minor differences, structurally and otherwise, in the different species of animals living under different conditions, so also the tumours of one organ vary greatly from one species to another. But these differences, which have exercised some authors greatly, so far from diminishing the importance of the study of tumours in the different species of animals, on the contrary make it all the more valuable. In this way light is thrown on what is essential in the process and general throughout the different species of animals, and what is due only to special circumstances in the animal chosen for investigation. Furthermore, the infinitely varied conditions under which cancer arises become strikingly obvious, and their influence in modifying the incidence of the disease is elicited.

The present paper is based upon observations and experiments on 300 mice in which cancer had developed spontaneously. These mice have been obtained in the laboratories of the Imperial Cancer Research Fund since the summer of 1908, when the account of the previously observed cases by Dr. Murray was published in the Third Scientific Report, of which the present paper forms the continuation.

The observations will be grouped and considered from two main standpoints, viz. the nature of the tumours and the properties of the mice, and, in addition, attention will be given to the points raised under the subjoined subsidiary headings :—

I. THE TUMOURS.

A.—Histological Part.

(1) *Localisation and Histology.*

When cases of lymphomatous tumours, lung adenomata, and sebaceous adenomata are excluded, there remain 353 primary tumours, of which the true tumour nature can be demonstrated. These tumours were found in 288 mice at the time of first entering them in the laboratory register ; tumours which developed later, or were only found at post-mortem examination, are not included in these figures * : they will be dealt with separately.

* Except in a few cases of rare types of tumours in the internal organs.

The localisation of these 350 primary tumours was as follows :—

A.—Mammary Region.

Front and side of Neck	33
Back of Neck	3
Back between Shoulders	8
Axilla	113
Flank	8
Shoulder	17
Groin	83
Haunch	7
Thigh	3
Vicinity of Vulva	61

B.—Other Sites.

Jaw	3
Face	1
Ear	1
Preputial gland	2
Anus	4
Kidney	2
Ovary	2
Uterus	1
Vertebral column (spinal cord?)	1

All the tumours in the first group were subcutaneous and follow in their distribution most closely that of the mammary apparatus, a feature of these growths which has been pointed out by all earlier observers and especially emphasized by Apolant * and Murray †. It must be considered as satisfactorily proved that the great majority of these tumours originate from the mammary gland, and there is nothing to add to the evidence given by Apolant and Murray in favour of this view. In addition to the mammary tumours the material includes a number of tumours of different structure, some found in the mammary region, and others originating in different organs. The latter are of great interest, as they show that although the mamma is the most frequent site for tumours in mice, it is by no means the only organ which is liable to tumour-formation ; the cells of the different tissues can undergo a transformation into malignant cells in the mouse just as in man. This general liability of the organs of the mouse to carcinoma is of importance. Experimental observations

* Apolant, H.: Die epithelialen Geschwülste der Maus. (Arbeiten aus dem Königlichen Institut für experimentelle Therapie zu Frankfurt am M. Heft 1, 1906.)

† Murray, J. A.: Spontaneous Cancer in the Mouse. Third Scientific Report of the Imperial Cancer Research Fund, 1908.

can now be extended to tumours of other organs than the mamma whenever necessary. Thereby the whole superstructure is laid upon wider foundations. These tumours can be chosen for discussion when wanted instead of the mammary tumours, the true malignant character of which some authors have tried to contest on quite inadequate evidence.

Of the 353 tumours of the present material, 311 presented the structure of mammary adeno-carcinomata ranging from adenomata to solid carcinomata. Twenty-two were squamous-celled carcinomata with marked keratinisation ; of these, 14 arose in the mammary region, 8 entirely outside it (jaw, face, vulva, and anus). Five were sebaceous carcinomata, and of these 3 arose in the mammary region, 2 outside it (anus). The rest of the tumours observed were :—

2 Carcinomata of the preputial gland.
2 Adeno-carcinomata of the kidney (1 a hypernephroma ?).
2 Do. do. ovary.
6 Sarcomata, 4 spindle celled, 1 round celled, 1 polymorphous celled.
1 Spontaneous "mixed" tumour.
1 Melanoma.
1 Fibro-myoma uteri.

(Not included in the above figures are 30 lung adenomata observed in 19 mice and 21 cases of lymphomatous tumours.)

The total number of tumours with a structure different from that of the mammary tumours is 42, of which 25 have with certainty arisen outside the mammary apparatus. When lung tumours and lymphomata are excluded, 90 per cent., roughly speaking, of all the tumours in the present material are adeno-carcinomata of the mamma. This enormous prevalence of tumours of the mamma is one of the striking peculiarities of the mouse tumours and has long been recognised.

Apart from the mammary tumours, the adenomatous nodules in the lungs are the most frequent. However, it has been decided not to include them in the above figures; their exact number can only be ascertained by a systematic examination of the lungs of every mouse in serial sections, and this has not been feasible up to the present. In a large proportion of cases they are undoubtedly tumours, and some show the malignant mode of growth. In other cases their exact nature is uncertain. For similar reasons the lymphomatous tumours have not been included. Their exact category is somewhat doubtful, *i. e.* whether all are tumours or, in some instances, are hyperplastic conditions of the pre-existing lymphoid tissue.

Mammary Tumours.

With regard to the mammary tumours, their histology has been described so often that it is unnecessary to dwell on this subject. For detailed descriptions reference should be made to the papers by Apolant and by Murray, to whose material the present one conforms very closely, and only a few general remarks need be made. These tumours form one large group of growths, in which the ground type is more or less the same, although all gradations of structure occur, from that of an adenoma to that of a solid carcinoma. In studying their histology it is convenient to make a distinction between the general type of the growth and the minor changes occurring in this general type, *e. g.* more or less differentiation into acinous or solid parts. The first often comes out more clearly in the subtransplants of the tumour, and is relatively constant on propagation, while the second is capable of varying from tumour to tumour and from place to place in the same tumour. It is also well to keep in mind that in the spontaneous tumour traces of the whole history of development of the growth may be present. If the growth has not arisen in one step from the normal mammary acini, but has developed more gradually in the manner exhibited in many instances, the different stages of the development may be present in the spontaneous tumour.

The general type varies widely from tumour to tumour, although there can be little doubt that all these growths have originated from the same normal parenchyma. This in itself is a most interesting problem, and any attempt at explaining the nature of the cellular transformation in cancer must fail if it does not account for this extraordinary variety of the types of tumours arising from the normal cells of one organ. What is now known makes it most natural to look upon tumours as individual creations, as the results of spontaneous variations of cells from the normal, giving rise to varieties of cells with enhanced power of growth. This change obviously affects the different functions of the cell in a different degree, and their relative independence may be inferred. The increased power of multiplying itself, which constitutes the malignancy of a cell, is not necessarily accompanied by essential alterations of the other functions of the cell so far as we are able to judge them. Thus tumours are seen in which, histologically, all the normal characteristics of the cell are retained, and where, nevertheless, the clinical behaviour, recurrence after operation, metastasis to other organs, and unlimited propagation in other animals prove their malignant character. Loss of normal histological characters is thus no indispensable condition in order that a cell may become

malignant. The change in the biological behaviour of the cell is the essential feature ; it may or may not be accompanied by changes of other kinds finding expression in altered histological characters.

In most cases, however, an alteration of the normal histological characters is found accompanying malignant growth. This may be partly a secondary change in consequence of the changed rate of growth, viz. an adaptation of the cells to a new mode of life. To a certain extent the histological picture will be influenced by the altered biological character of the cells. A rapidly proliferating tumour more frequently takes the form of an alveolar carcinoma with less differentiated cells, a slowly growing one has more chance of presenting a differentiated picture, time being a necessary factor for differentiation. On the other hand, it is obviously wrong to assume that this interdependence of structure and biological behaviour is a very close one. The histological characters often show a relative independence of the biological properties. Two tumours with the same histological picture may, biologically, be quite different, as can be shown by their clinical course and behaviour on transplantation into other animals. The individual stamp of the altered histological characters, and their constancy on propagation, show that one has to deal with alterations of a kind parallel to those responsible for the altered power of growth. These characters cannot be explained as the result of any regressive kind of process, a return to an undifferentiated state, to embryonic conditions, as so often has been asserted. On the contrary they are obviously progressive specific differentiations which it seems most natural to regard as correlative variations of the cell in other directions. The specificity of these differentiations has been shown by the results of propagation and immunisation.

Minor changes are superposed on the general type, which in the spontaneous tumours practically never occurs pure. Especially noticeable are the changes from acinous to alveolar parts. Two views are held to account for them. Apolant considers these changes from acinous to alveolar structure an expression of advancing malignant transformation in a relatively benign (adenomatous) tumour. On the other hand, Gierke * and Murray showed that in many cases they were expressions of the subsequent differentiation of an originally less differentiated parenchyma—changes from alveolar to acinous structure †—and also adduced evidence for the transition described by Apolant. Both

* Gierke, E.: Die hæmorrhagischen Mäusetumoren. Zeigler's Beiträge, 1908. Third Scientific Report of the Imperial Cancer Research Fund.

† An interpretation also given previously by the writer (Annales de L'Institut Pasteur, no. 3, 1905, p. 172).

cases occur. But whatever their explanation, they cannot be looked upon as very stable changes. The difference between alveolar and acinous structure has not in the mouse tumours the significance which has been attributed to it in human oncology, and does not afford a basis for classification.

In addition to all the primary factors, of which the structure is the outcome, secondary changes in the stroma with œdema and hæmorrhage, to which these tumours are so liable (described by Apolant, Murray, and Gierke), contribute to complicate and vary the histological picture still more. Although these changes to a certain extent undoubtedly are expressions of special biological qualities of the tumour cells, they are not constant features by which the tumours can be classified except in a very rough manner.

The general conclusion is that the variations of the histological picture of the mammary tumours of the mouse are not sufficiently stable to form the basis of a rigid classification. The different forms pass one into another and occur together in the same tumour, so that strictly one tumour may come under different groups. On the other hand, two tumours with the same histological picture may be different biologically. The value of classifying them along the lines of human pathology is therefore very slight. In particular an adenomatous structure does not invariably accompany a benign character. In general such tumours are more or less malignant, as is shown in their clinical behaviour, recurrences after operation, metastases, and power of unlimited propagation in normal animals, and the differences in histological structure do not always correspond to similar differences in biological characters.

The two epithelial structures of the normal mammary gland (acini and ducts) are both more or less clearly reproduced in the mammary tumours, and the tumours may be grouped according as the one or the other preponderates. In the great majority the picture of the mammary acinus is retained with varying clearness ; these are the adenomata and adeno-carcinomata, with all transitions to solid alveolar carcinomata. In another group it is more the structure of the duct which is reproduced, as larger lumina with papillary excrescences ; the cells are often larger and distinctly columnar. These latter are the papilliferous adeno-carcinomata (Apolant's fissure-forming and papilliferous carcinomata) ; they generally form a more sclerotic stroma, without the tendency to secondary hæmorrhagic changes which is characteristic of the delicate stroma of the first group. If there be any biological difference between the two groups it seems to be that the latter, on the

whole, grow relatively more slowly, which is already indicated by the sclerotic stroma, and generally they show a moderate power of growth in normal animals.

In the table appended to this paper the attempt is made to give purely descriptive names to the tumours, following the example of Murray, without entering upon any detailed classification. The individual tumours will be found entered in the same sequence as that in which they came under observation. The figures throughout the text refer to the index number of the mouse in the table. In a tabulation of this kind it is only possible to pay attention to the general character of the tumours ; the cases to which more interest are attached will be found described in the text.

Tumours outside Mamma.

The relatively rarer tumours arising outside the mammary apparatus will be described briefly. In general it can be said that all the tissues of the mouse are capable of giving rise to tumours. To the cases previously observed by a number of investigators several new ones can be added. The unusual types of tumour are melanoma, carcinoma of the preputial gland, fibro-myoma of uterus, adeno-carcinoma of the kidney, hypernephroma (?), and a peculiar adeno-carcinoma of the ovary. There are several cases of squamous-cell epitheliomata of the mucous membrane of the mouth, another from the surface of the face, others of vulva, of anus, and of the skin of the body ; furthermore, sebaceous carcinomata of anus and of the skin (mamma?). Several sarcomata occur, one of them being an osteo-sarcoma of the thigh and another a sarcomatous tumour of the spinal cord.

Of all the tumours in the present material only six have arisen in males : two tumours of the preputial gland, one sarcoma of the thigh, one squamous cell carcinoma of the face and two adeno-carcinomata of the kidney (one a hypernephroma ?). All the other tumours were found in females. The lung adenomata occur in males as well as in females, but no exact figures can be given for the present material. Tyzzer * also states that they occur in both sexes.

Melanoma.

Mouse 265, a black female of the breeding experiments of the laboratory †, had both ears slit, when young, for identification. When the mouse was 14 months old, a small melanotic tumour measuring ca. 5 × 5 mm. was found on the right ear at the

* Tyzzer, E. E.: A Series of Twenty Spontaneous Tumours in Mice. Fourth Report of the Caroline Brewer Croft Fund Cancer Commission. Boston, 1907.

† The breeding experiments of the laboratory are described in the following paper by Murray (p. 114).

apex of the slit. The tumour was of firm consistency, its colour deep black; in one place it was slightly ulcerated with an adherent scab. The skin was adherent to the tumour. The histology of the tumour is shown in fig. 1 at a low magnification. The tumour consists of large cells filled with dark brown pigment-granules, some of them round cells, others spindle-shaped; they are apparently connective tissue elements. Between the pigmented cells there are only vessels and scanty fibres.

The growth was partially removed and transplanted into normal mice. The fragment left behind, in order that recurrence might take place, increased a little in size during the first 2-3 weeks, but then remained stationary for 10 weeks. It was then again partially removed; the mouse was grafted with fragments of its own tumour in four different places, viz., both axillæ, and both groins. The minute fragment that was left behind at the operation diminished gradually in size and finally disappeared, so that when the mouse was killed 16 weeks after the second operation there was nothing left of the original tumour. On the right side of the neck an enlarged lymph gland was found, measuring 6 × 4 mm., and entirely black. This gland was used for transplantation and not examined. The four points of inoculation were still visible after death, as black spots: on examination it was found that the pigmented cells of the grafts had remained alive, without however showing signs of active growth. No metastases were seen in the lungs.

The tumour was transplanted into normal mice on three occasions, altogether into 168 mice of which 145 survived after four weeks, with entirely negative results.

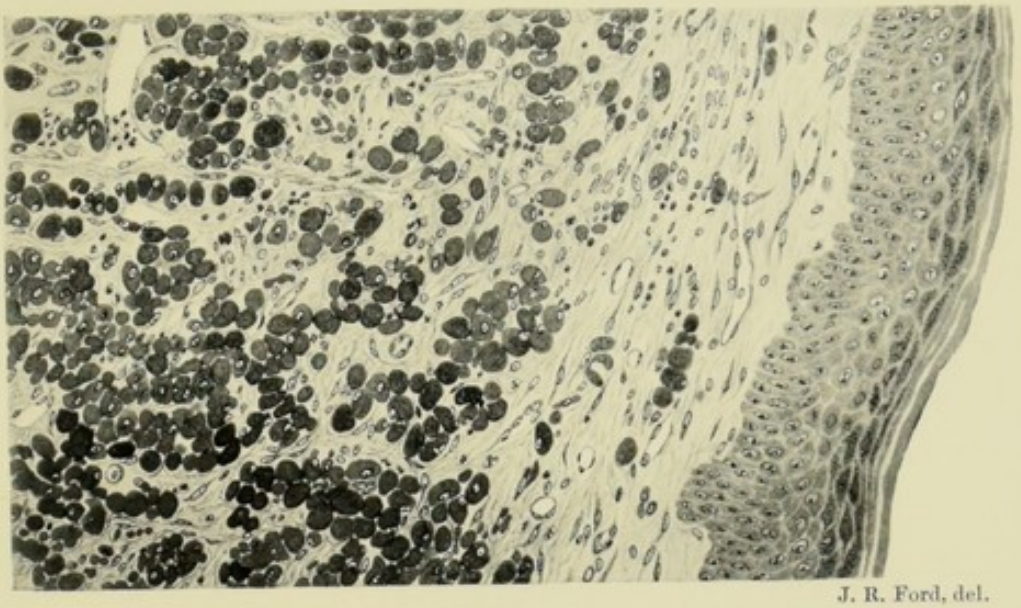
Adeno-carcinoma of the Kidney.

In a 22 months old male of the breeding experiments (Mouse 504), a firm white tumour as large as a small walnut was found at the post-mortem examination, replacing the left kidney. A shell of kidney tissue was found on the external side of the tumour; the suprarenal gland was found on the top of the tumour and presented the normal appearances. Microscopically the tumour is an adeno-carcinoma, consisting of tubes and acini, the cells of which resemble closely those of the renal canaliculi from which the tumour seems to have originated. In some parts of the tumour solid cylinders and alveoli are found. The histological picture is shown in fig. 2. The tumour infiltrates and compresses the kidney tissue, which shows extensive degenerative changes. The tumour has invaded the vascular system as shown by the fact that in a large vein at the hilus of the kidney an embolic mass of tumour is found, probably a continuous extension of tumour from the periphery. No metastases were found in the lungs or other organs by naked-eye examination. As the mouse had died before the tumour was found, transplantation was not attempted.

Hypernephroma (?).

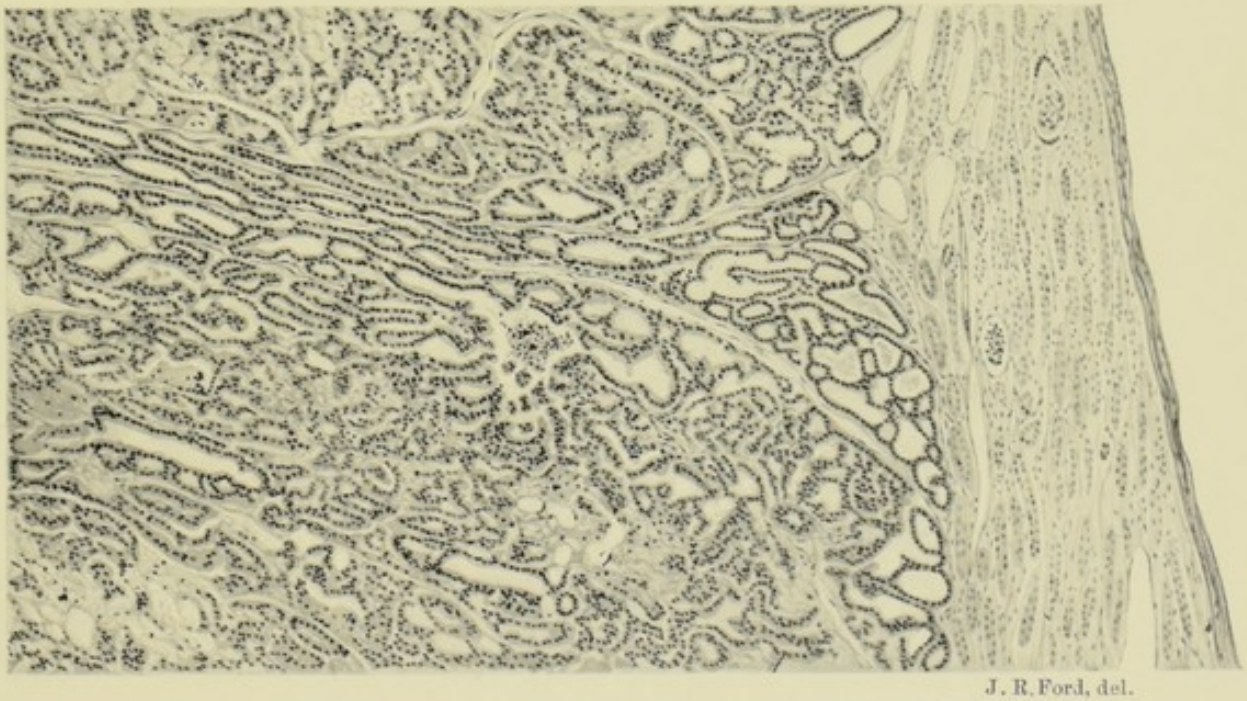
In another 22 months old male of the breeding experiments (Mouse 328) a brownish vascular growth, as large as a pea, was found to occupy the lower pole of the left kidney. When examined microscopically the tumour is seen to have infiltrated the kidney tissue which is also compressed by the growth. The tumour consists of large cells with a large amount of lightly stained protoplasm, heaped together with an indistinct alveolar or columnar arrangement; the stroma is very scanty, consisting mainly of capillaries. Figure 3 shows the histological picture. It is difficult to draw any conclusion as to the cells from which the tumour has taken its origin, from elements of the kidney or from those of the

[*To face* p. 10.



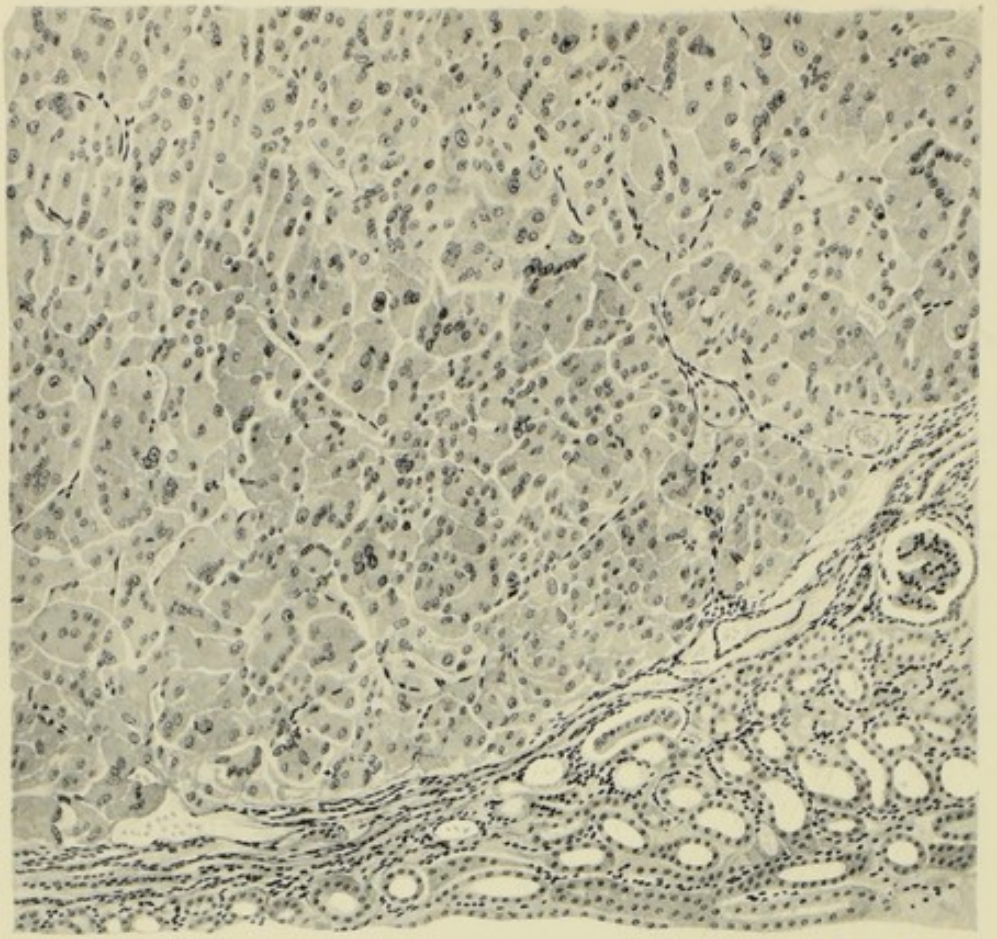
J. R. Ford, del.

FIG. 1.—Mouse 265. Melanotic tumour. Part of growth adjoining the skin. Large round cells filled with dark brown granules are seen diffusely infiltrating the subcutaneous tissue. $\times \frac{166}{1}$.



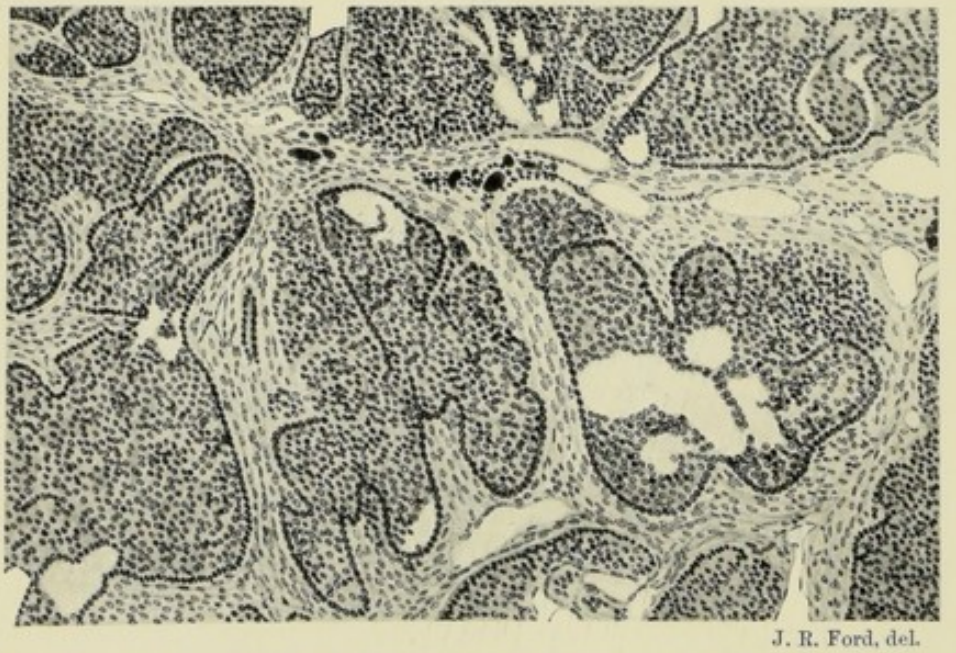
J. R. Ford, del.

FIG. 2.—Mouse 504. Adenocarcinoma of the kidney. Section from peripheral part of the growth. To the right a shell of compressed renal cortex. The resemblance of the tubuli of the tumour to renal canaliculi is very close; in parts solid alveoli of the same cells are found. $\times \frac{87}{1}$.



J. R. Ford, del.

FIG. 3.—Mouse 328. Hypernephroma (?) Peripheral part of the tumour, with normal kidney at the bottom of figure. The tumour-cells contain a large amount of protoplasm, the limits of the individual cells are not sharp; they show an indistinct alveolar or columnar arrangement. The stroma is very scanty consisting mainly of blood-vessels. $\times \frac{130}{1}$.



J. R. Ford, del.

FIG. 4.—Mouse 70. Adeno-carcinoma of the ovary. Large alveoli filled with epithelial cells, the peripheral layer being distinctly columnar. Irregular spaces are seen in the alveoli; the arrangement resembles that of the normal ovarian follicles. $\times \frac{87}{1}$.

suprarenal gland. From the general impression obtained from the appearance of the cells of the tumour, they might have originated from the suprarenal gland. This case would thus correspond to the cases of malignant hypernephroma in the human subject, originating from an aberrant bud of suprarenal gland in the lower pole of the kidney. Tumours of this kind in the mouse have been described by Tyzzer * who regards them as hypernephromata. On the other hand, the picture resembles in many points that of the alveolar parts of the adeno-carcinoma of the kidney described above, and it is possible that it has originated from the renal epithelium. In the lungs a small nodule was observed, which, on microscopical examination, was found to be a lung adenoma; no metastases were found.

The tumour was inoculated into 40 mice, of which 24 survived after 4 weeks, with entirely negative results.

Adeno-carcinoma of the Ovary.

In Mouse 70, the primary tumour of which was a sarcoma which had developed in a scar, a tumour of the size of a hazel nut was found in the left ovary at the post-mortem examination. Fig. 4 shows the histological structure of this growth; it consists of large alveoli filled with epithelial cells resembling those of the follicles. The peripheral layer consists of a low columnar epithelium, the rest of round cells. The resemblance of some of these alveoli to the normal follicles is enhanced by spaces filled with serous fluid (*cf.* fig. 4). No metastases were found. The tumour was transplanted into 120 normal mice; small nodules developed in 4 mice, but all disappeared spontaneously.

In Mouse 274 a tumour as large as a pea was found in the right ovary at the post-mortem examination. The mouse had suffered from a mammary adeno-carcinoma which had been operated upon and had recurred in the scar; at the same time multiple nodules were found in all the mammæ. The tumour in the ovary is a papiliferous adeno-carcinoma and may be a primary ovarian tumour of the type described by Jobling†; on the other hand, the difference in structure from that of the original mammary tumour is not great enough to exclude the possibility that the ovarian tumour might be a metastatic nodule.

Fibro-myoma of the Uterus.

Mouse 334, a 21 months old female of the breeding experiments with an adeno-carcinoma of the mamma, presented at the post-mortem examination a firm white tumour as large as a bean in the upper part of the left horn of the uterus. On microscopical examination the tumour is found to be situated in the wall of the uterus and to consist of interlacing bundles of unstriped muscle and cellular fibrous tissue, similar to the structure of fibro-myoma uteri in the human subject, only somewhat more cellular (fig. 5). The uterine cavity is distended by the tumour, but the mucous membrane is normal. In one corner of the tumour glandular acini of unknown origin are seen.

Doubtful tumour-formations have been found in the uterus in two cases (383, 415), apparently on the basis of an organised retained placenta. They form large nodules, consisting of dilated vessels separated by a cellular connective tissue, and here and

* Tyzzer, E. E.: A series of Spontaneous Tumours in Mice. Fifth Report of the Caroline Brewer Croft Fund Cancer Commission. Boston, 1909.

† Jobling, J. W.: Spontaneous Tumours of the Mouse. Monographs of the Rockefeller Institute. No. 1. New York, 1910.

there islands of epithelial-looking cells. One of the cases was bilateral, the other unilateral, and had apparently extended through the uterine-wall. In another case (406) uterine glands of normal appearance were found all through the uterine wall and as small cysts under the serosa. A similar case has been described by Tyzzer.

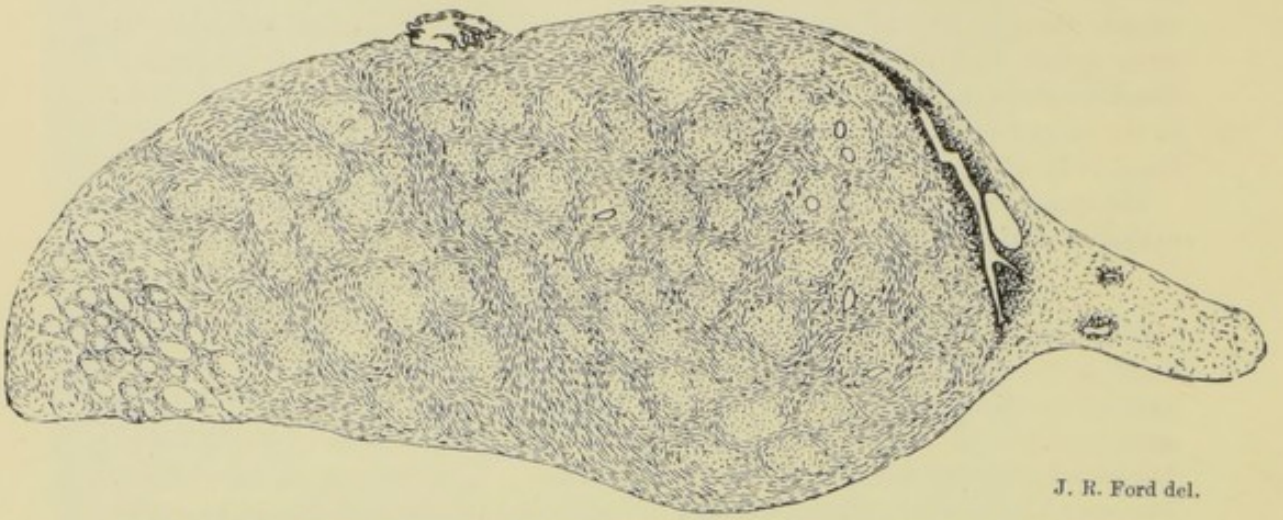


FIG. 5.—Mouse 334. Section of uterus with large fibro-myoma in wall. Uterine cavity on the right, mass of glands of unknown origin on the left; Fallopian tube above. $\times \frac{13}{1}$.

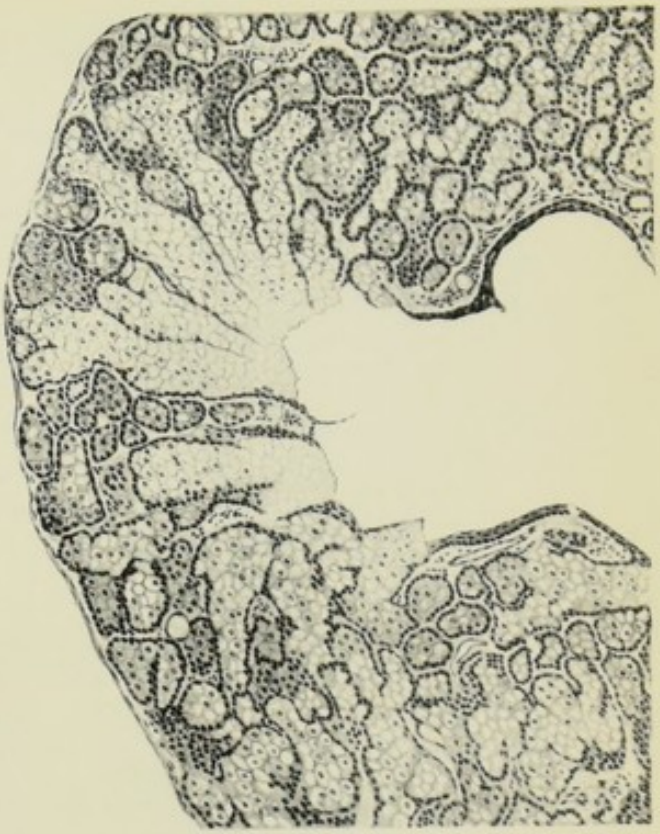
Adeno-carcinoma of the Preputial Gland.

In an old male from an outside breeder (mouse 297) a sausage-shaped swelling of firm consistence was found on the side of the penis, corresponding to the site of the preputial gland. It measured 15 × 5 mm. when found, and grew in 10 days to about double the size. It was partially removed by operation; the fragment left behind grew in 7 weeks to the size of the original tumour and infiltrated the abdominal wall. The mouse was killed 7 weeks after the operation. There were no naked-eye metastases in the lungs. The tumour on both occasions consisted of a lobulated mass, containing inspissated fatty secretion, surrounded by a shell of healthy tissue. Histologically the picture is exactly that of the normal preputial gland, so similar that the two pictures are hardly distinguishable (figs. 6 & 7). The differentiation into fatty masses in the tumour is the same as that of the normal gland. In spite of thus being without distinctive histological differences from normal gland, it exhibits typical malignant growth. In this respect the tumour recalls the behaviour of certain adenomatous tumours of the mamma of mice, which also may retain in all essentials the features of the normal gland and still show themselves as typical malignant growths by their recurrence after operation,

[*To face* p. 12.

J. R. Ford, del.

FIG. 6.—Mouse 297, male. Adeno-carcinoma of the preputial gland, showing the general histological features of the tumour and its close resemblance to the normal gland (fig. 7), in spite of its definitely malignant character. The propagated tumours retain the same histological picture. $\times \frac{87}{1}$.



J. R. Ford, del.

FIG. 7.—Normal preputial gland from adult male mouse to compare with the tumour in fig. 6. The larger ducts of the gland are lined with cells resembling squamous epithelium. $\times \frac{87}{1}$.

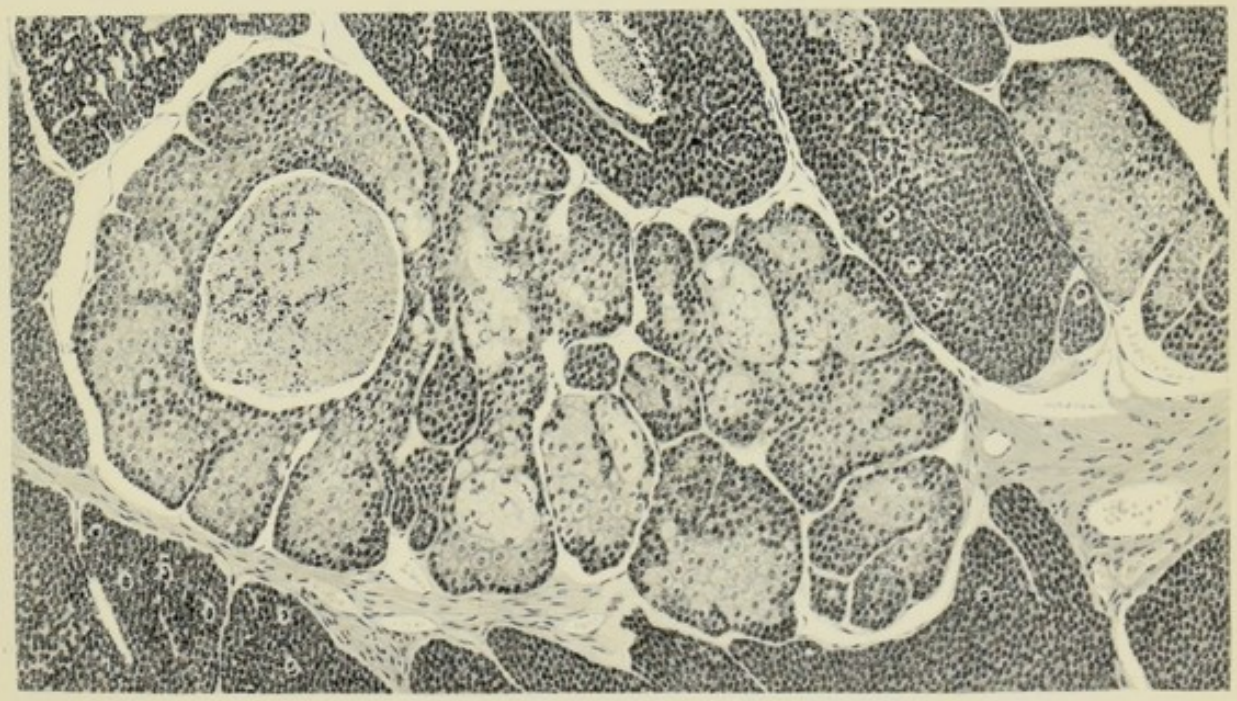


FIG. 8.—Mouse 292. Sebaceous adeno-carcinoma. Spontaneous tumour in left groin, showing the same typical differentiation as the cells of a sebaceous gland. *Cf.* with this the preputial tumour (fig. 6). $\times \frac{135}{1}$.

formation of metastases, and capacity of being propagated. Certain thyroid tumours in the human subject may be quoted as other instances of the retention of normal histological structure being compatible with malignancy.

This tumour was transplanted successfully into normal mice, and has been propagated during 11 months through six generations, although with considerable difficulty as compared with other tumours. The percentage of successful inoculations has always been very low, the tumours appear late, grow slowly, and nearly all tumours show a tendency to disappear spontaneously after 3–6 weeks growth. In later generations the tumours show a tendency to grow for a longer time, 8–10 weeks, before absorption sets in. During propagation the tumour has retained all its original characters, especially its histological similarity with the normal gland and the specific differentiation into fatty sebaceous matter.

Another tumour of the same kind was found in a black and white male of unknown age from an outside breeder (Mouse 466) as a tumour on the right side of the penis and of the size of a hazel nut. The tumour was removed by operation; but the mouse died under the anæsthetic. Histologically, the tumour resembles closely the first case as illustrated in fig. 6. There were no metastases. The tumour was transplanted into 100 normal mice. In about 10 per cent. of the inoculated mice it grew for 4–5 weeks, reaching a size varying from a small shot up to that of a large pea in one animal. The growth was only temporary, followed by spontaneous absorption. The only daughter tumour that was transplanted showed on histological examination differentiation into typical squamous epithelium (kerato-hyalin granules in places and formation of squames). This differentiation is interesting as it throws a certain amount of light on the question of metaplasia, and will be referred to later under sebaceous carcinoma. The tumour is now in the third generation; biologically it behaves exactly as tumour 297.

Sebaceous Adeno-carcinoma.

Beside the small superficial adenomata of the sebaceous gland, described by Murray, a malignant tumour of this type has been described by Tyzzer, who succeeded in propagating it. In the present material there are several of these tumours originating in different localities, and of somewhat different histological structure; two of them have been successfully propagated. The tumour of the preputial gland just described is closely related to this group.

Mouse 378 had a nodule on the dorsal margin of the anus, which, histologically, presented itself as an adeno-carcinoma of sebaceous gland with its typical arrangement and differentiation. Like the preputial gland tumour, this tumour also shows but little deviation of its cells from the normal histological type. Biologically, however, it presented marked malignant characters. Recurrence followed upon an attempt at removal and the recurrent tumour possessed infiltrative and destructive

growth. The anal ring was destroyed and replaced by an open ulcerated sloughing surface of tumour. The death of the mouse occurred after a second attempt at removing the tumour, which had then reached a considerable size (that of a large hazel nut). It had infiltrated the rectum, vagina, and the skin of the neighbourhood. No naked-eye metastases were found. The tumour was transplanted into 36 mice, but the material proved to be septic and ulcerated out. At the post-mortem examination of the spontaneously affected mouse, a squamous-cell carcinoma of the jaw was found which had previously escaped observation; it will be referred to later.

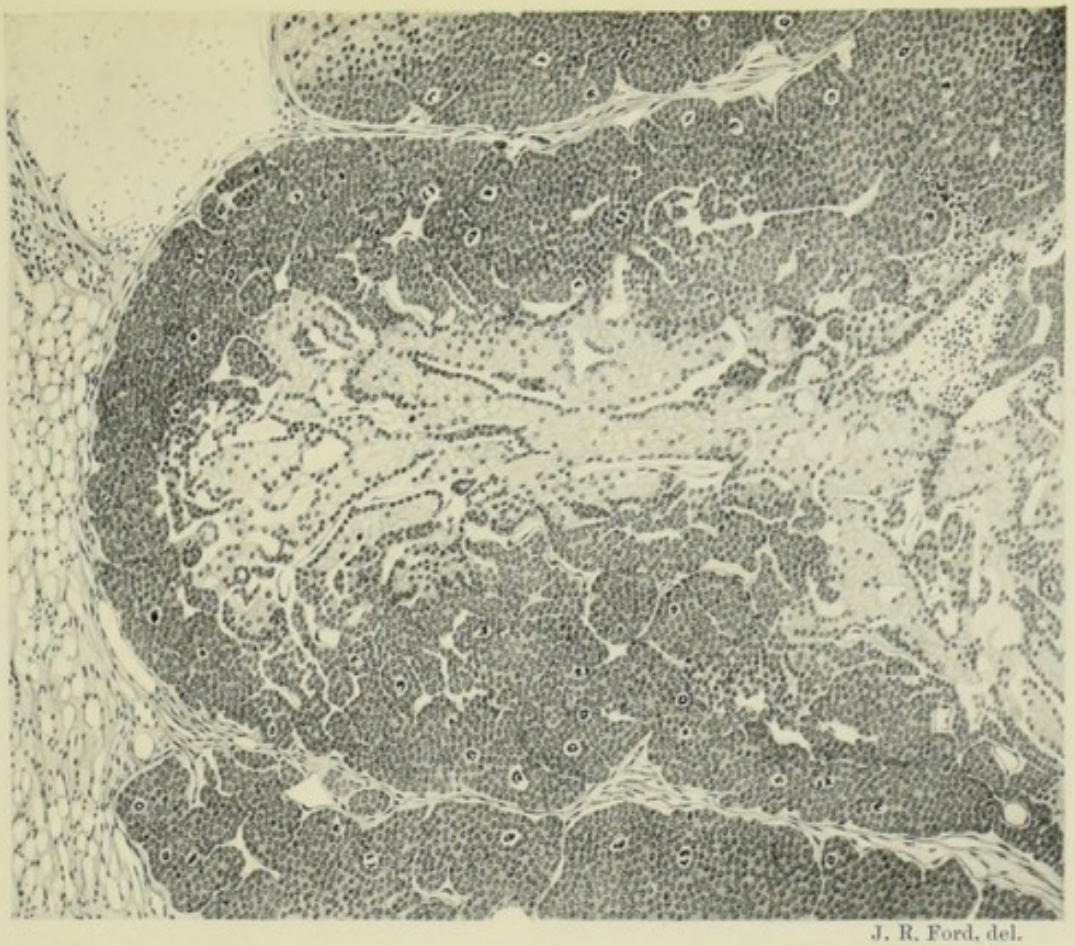
Mouse 333 presented in addition to a mammary adeno-carcinoma in one axilla, a verrucous growth of the dorsal margin of the anus, which histologically is a mixture of a squamous celled wart and a sebaceous adenoma. The tumour recurred after incomplete operation; it did not, however, reach any great size. Metastases were not observed.

In these cases there was no doubt as to the place of origin of the tumour, which in both cases has been the sebaceous glands of the anus. Other similar tumours have arisen from the sebaceous glands of the skin. To the cases of sebaceous adenomata described by Murray, three new similar cases can be added.

It is more difficult to decide where sebaceous tumours have arisen, when they appear in the regions where mammary tumours are most frequent, and situated in the subcutaneous tissue, apparently without connection with the skin.

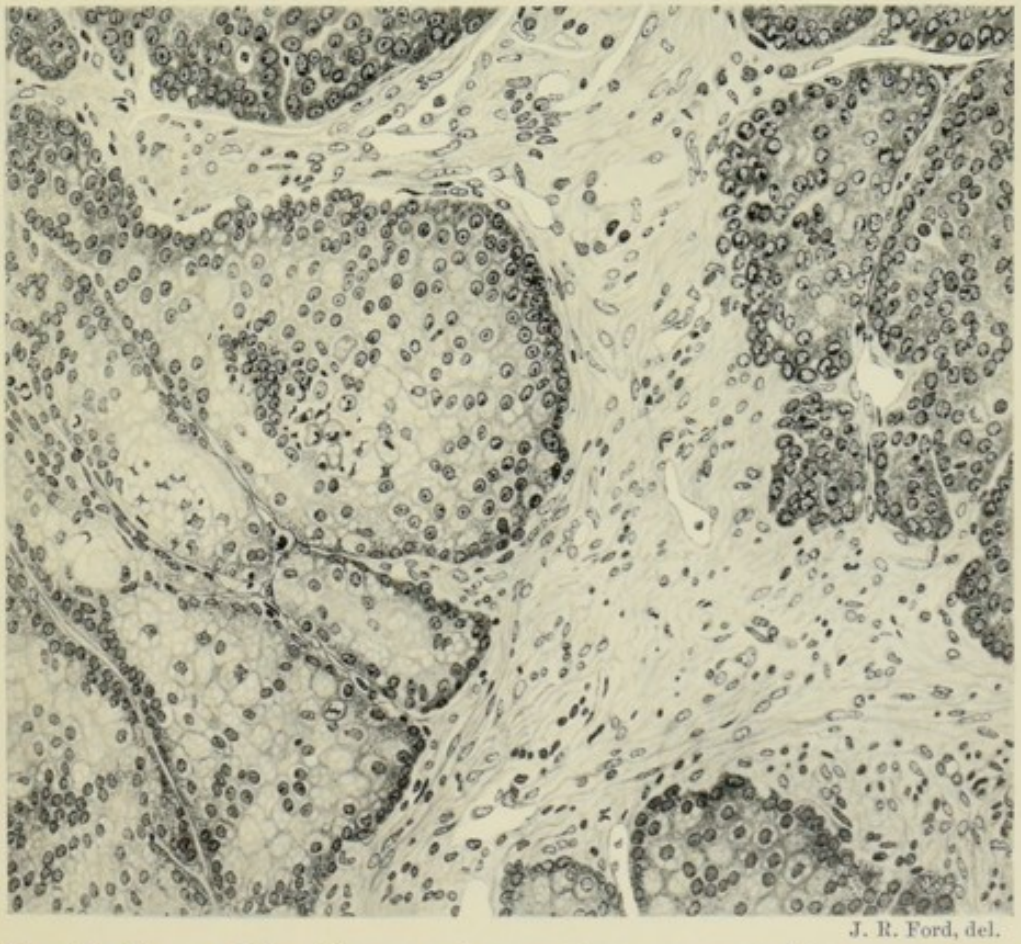
Tumour 239 originated subcutaneously in the axillary region in a mouse which had a mammary adeno-carcinoma in the other axilla, but the differentiation into sebaceous cells throughout the whole tumour was so marked, and the glandular arrangement so typical, that it would seem most natural to regard it as having originated from the sebaceous apparatus of the skin. This tumour is shown in figs. 9 and 10. The same was the case in mouse 292 whose tumour is illustrated in fig. 8. This mouse had also a mammary tumour in addition to the sebaceous one. The question of origin becomes more difficult where the tumours in all their details correspond to the usual mammary tumours in acinous structure, secretion, etc., and show sebaceous differentiation only in limited areas (tumour 427). In these cases it is difficult to avoid the conclusion that the differentiation into sebaceous cells is a property which may lie latent, and occasionally come to development in cells which in all other respects show all the characters of cells of the mammary gland. It is not so surprising that this is so when the near relation of mamma to sebaceous glands is kept in mind. Another differentiation, viz. into more or less typical squamous epithelium is found frequently in the subtransplants of one of these sebaceous tumours (tumour 292). In no other respect does it show any essential difference from the other sebaceous tumour (tumour 239) in which this differentiation has not been

[*To face* p. 14.



J. R. Ford, del.

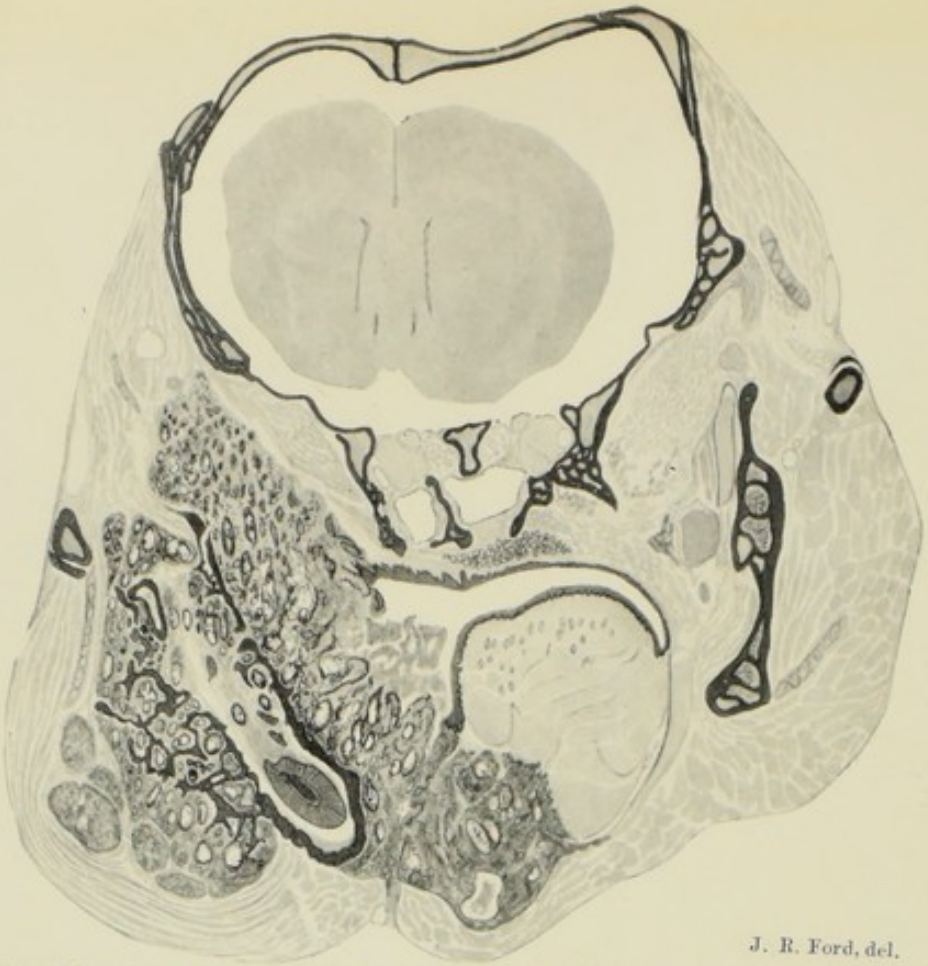
FIG. 9.—Tumour 239. Sebaceous adeno-carcinoma. A peripheral lobe of a propagated tumour of the 6th generation to show the typical differentiation into sebaceous cells in the centre of the lobe and the undifferentiated condition of the peripheral alveoli. $\times \frac{83}{1}$.



J. R. Ford, del.

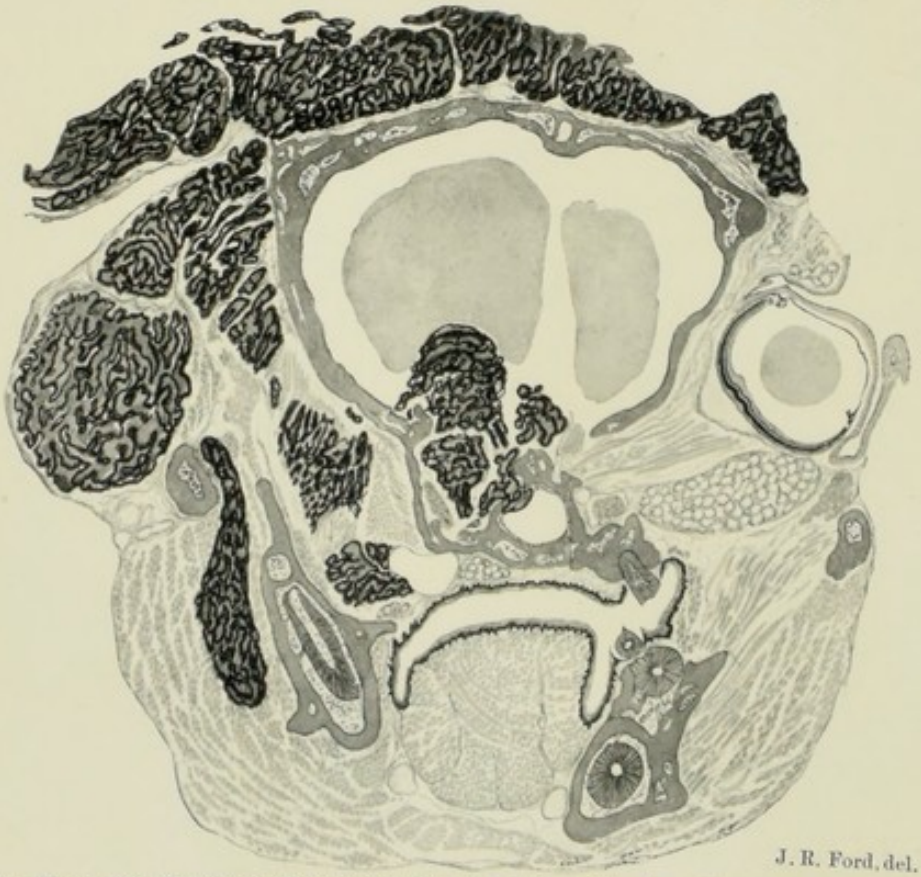
FIG. 10.—Tumour 239. Sebaceous adeno-carcinoma, propagated tumour of the 2nd generation. The same typical differentiation with a high power. $\times \frac{230}{1}$.

To face p. 15.]



J. R. Ford, del.

FIG. 11.—Mouse 378. Squamous-celled carcinoma of the jaw. Transverse section through the head. The growth has infiltrated the muscles of the jaw and disintegrated the bone; the tongue is pressed over to the right; malignant ulcer between tongue and palate. $\times \frac{8}{1}$.



J. R. Ford, del.

FIG. 12.—Mouse 486. Squamous-cell carcinoma from the surface of the face. Transverse section through the head. The left eye has been destroyed and the orbit completely filled with tumour. The growth has formed a cap over the skull, penetrated into the nasal and cranial cavities, and infiltrated all the muscles of one side of the face; a prolongation of tumour has extended down into the masseter muscle. $\times \frac{8}{1}$.

found. A similar instance has been already referred to in describing the second of the preputial carcinomata. The sudden appearance of squamous epithelium in a subtransplant of the preputial tumour, as in the sebaceous tumour, shows the near relation of these different kinds of epithelium to each other, and practically proves that epithelial cells which ordinarily differentiate into fatty masses may still possess a latent power of squamous differentiation that may be manifested under special conditions. Additional evidence of this kind of metaplasia in mouse tumours will be given later.

Keratinising Tumours *.

Twenty-two of the tumours were found to exhibit keratinisation in larger or smaller areas. These tumours fall into several groups.

Three (378, 395, and 467) were squamous-cell carcinomata of the mucous membrane of the mouth cavity, identical with the cases described from Borrel's laboratory in 1905 †. In all three the growth seemed to have originated from the mucous membrane of the inside of the cheek or on the lower jaw, and had infiltrated the jaw muscles and invaded and disintegrated the bone (fig. 11). In one case the lymph glands of the neck were involved.

One similar tumour, strongly keratinising, was found, in another mouse (486), to have penetrated into the nasal and cranial cavity, where the brain was invaded, to have destroyed one eye, which was completely filled out with tumour, and to have formed a cap of tumour tissue over the side of the skull (fig. 12). In this case the tumour seemed to have started from the surface of the head, but the primary site cannot be decided exactly. This tumour has been propagated successfully, and is at present in the 3rd generation.

Three tumours (348, 477, and 481) are superficial verrucous growths. Tumour 348 was a verrucous growth of the vulva, which recurred once after incomplete removal, but did not recur after a second removal. No metastases were found in this case. Tumour 481 was situated at the anus; 477 on the left flank; both were squamous-celled verrucæ. A somewhat similar case of mixed squamous and sebaceous carcinoma of the vulva has already been mentioned (333).

Four tumours (217, 266, 273, and 487) exhibit a peculiar structure which places them in a group by themselves. They consist of long cylinders of cells radiating from the centre of the tumour. The central part of the cylinder is entirely keratinised, the peripheral part ends in a small acinus, often somewhat dilated as shown in fig. 13. They are identical with the tumour described in 1905 from Borrel's laboratory as "tumeur molluscoide." They have all ‡ been found in the mammary region, but their

* Throughout cancroid is used as a short term for keratinising squamous-cell carcinoma.

† Annales de L'Institut Pasteur, 1905, no. 3.

‡ In Borrel's case the tumour was situated near the middle line over the ensiform process.

structure is so characteristic and differs so considerably from that of all other mammary tumours, that there is some probability for the view that their origin is to be looked for somewhere else than in the mammary gland. The long, straight, completely keratinising alveoli, with the bulbous arrangement at the ends, lead one to think of hair bulbs, and the original assumption was that they originated from cells of the hair follicle. An oblique section through the periphery of these tumours has a good deal of resemblance to a mammary adeno-carcinoma; but a closer study reveals the peculiar structure of the acini, with desquamated cells, on the whole different from mammary tumours. The tumours of this group have been all negative on transplantation into, altogether, more than 1000 mice. In their own mice, however, they exhibit typical infiltrative and destructive growth, in one case with metastases to the mediastinal areolar tissue.

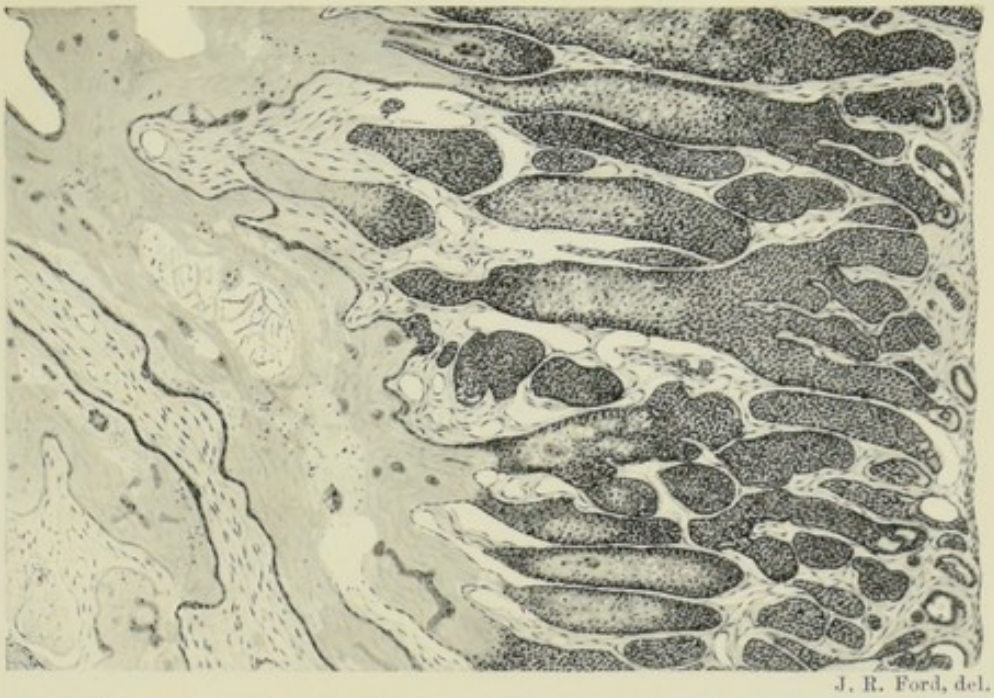
One tumour (438) is a typical squamous-cell carcinoma of the skin of the chest, developed as a superficial ulcerating growth, undoubtedly from the skin itself, or from the mamilla.

Four tumours (349, 357, 405, and 412) are typical squamous-celled alveolar carcinomata in the mammary region. They have developed subcutaneously, and there is no means of deciding the tissue of origin exactly. They all show abundant typical keratinisation in solid alveoli, with formation of kerato-hyalin granules, flattening of the cells and transformation into typical squames. Two of them have been successfully transplanted; in the subtransplants keratinisation is in some tumours prominent, in others nearly or totally absent.

Six other tumours (164, 184, 229, 282, 286, and 347) belong to the group of "adeno-cancroids," cases of which were described by Murray in the Third Scientific Report. 286 is the type of such a tumour. The structure is markedly adenomatous and adeno-carcinomatous, with no special feature to distinguish it from other adeno-carcinomata, except that foci of keratinisation occur scattered all through the tumour, in intimate connection with the acini of the tumour.

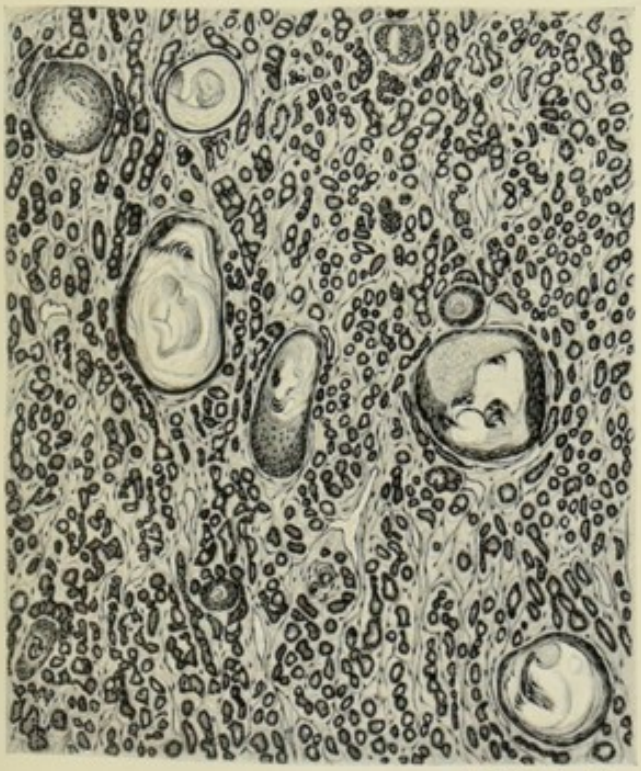
Fig. 14 illustrates the primary tumour 286. It shows a marked adenomatous, in some parts more adeno-carcinomatous picture, with larger alveoli exhibiting typical keratisation scattered among the acini. In the sub-transplants keratinisation has not been prominent until in a tumour of the third generation the picture shown in fig. 15 was obtained. In this case there cannot exist any doubt as to these distinct differentiations being manifested by one and the same epithelium. There seems to be sufficient evidence to show that the tumours are mammary carcinomata, only the power of typical differentiation into squamous cells has been retained or regained. This tendency to keratinisation is an inherent character of the cells of this tumour; it may lie latent for a time and then suddenly appear; it may recur from time to time; it may appear once and never be seen again. An interesting case of this latter kind is furnished by tumour 282. This is a typical mammary adeno-carcinoma, in which no trace of keratinisation was found either in the primary tumour or in its numerous sub-transplants, but in the tumour developed after grafting the mouse itself with its own tumour-cells keratinisation was found scattered all through the tumour, although there can be no doubt of the graft examined or that it is the same tumour as the primary one. In later generations this differentiation has not been observed, although traces of atypical keratinisation have appeared now and then in the sub-transplants. On the whole, keratinisation tends to disappear in later generations with better adaptation and more rapid growth of the tumours.

[*To face* p. 16.



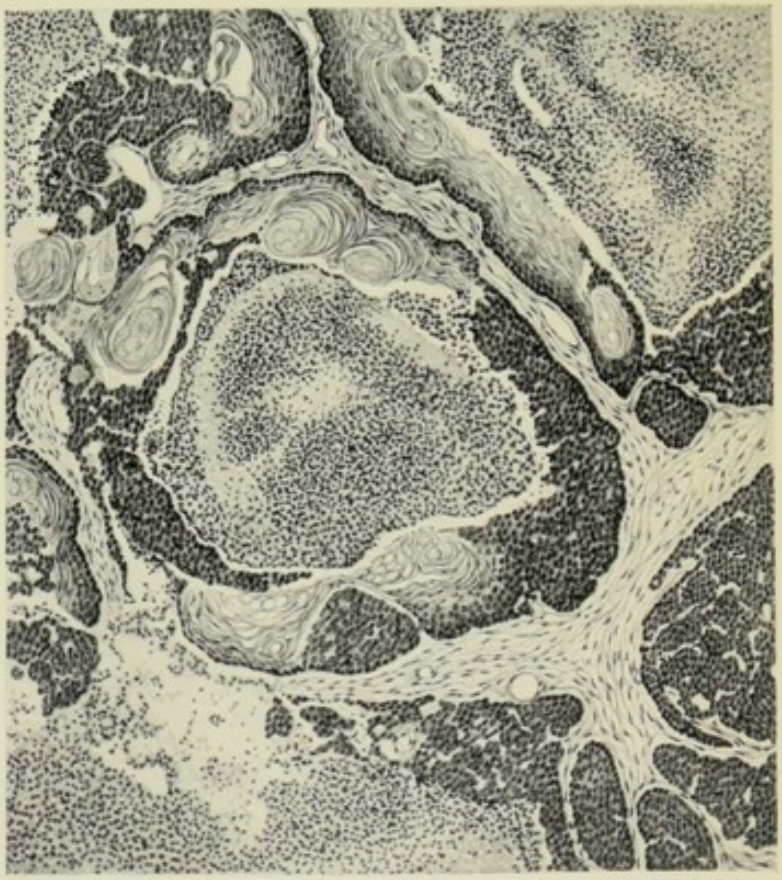
J. R. Ford, del.

FIG. 13.—Mouse 273. Peripheral part of strongly keratinising tumour showing the long radiating cylinders of parenchyma with complete keratinisation in their central part, ending in acinus-like formations close to the periphery. $\times \frac{52}{1}$.



J. R. Ford, del.

FIG. 14.—Mouse 286. Adenocancroid. Adenomatous structure of the primary tumour interspersed with keratinising alveoli. $\times \frac{62}{1}$.



J. R. Ford, del.

FIG. 15.—Tumour 286 in its 3rd generation. Alveoli showing foci of keratinisation; the centre is necrotic. $\times \frac{83}{1}$.

The occurrence of squamous-celled cysts in the normal mamma may throw light on the origin of these keratinising tumours. On examining serial sections of the apparently normal mamma of mice, in which cancer had arisen spontaneously, and also of old normal mice, small cysts have been found on several occasions lined with squamous epithelium and filled with squames (fig. 18). These cysts are found subcutaneously, imbedded in the mamma, in some cases at a considerable distance from the nipple ; they seem to arise from mammary ducts in which the epithelium has become metaplastic. The epithelium lining the cysts appears in most cases to be normal squamous epithelium and does not show any evidence of growth; in other cases several mitoses are found. In one case with multiple hypertrophic nodules of the mamma minute keratinised foci have been found scattered among the acini of the hypertrophic nodules, and in this case it is obvious that the terminal acini of the mamma may have given rise to such keratinised foci. The finding of such keratinised cysts in the mamma makes it easier to understand how keratinising tumours can arise in this organ.

Lung Tumours.

Thirty nodules occurring in 19 mice in the lungs have been observed and examined in this material. The greatest number of them are small adenomatous nodules, usually multiple, 2, 3, or more in the lungs of one animal, and do not exhibit signs of active growth. In other cases they show active growth, invading the surrounding lung tissue and available lumina (*e. g.* bronchi and larger vessels) ; in some of these cases they have all the characters of malignant new growths. The lung adenomata will be referred to again in some detail on a later page (p. 45). In one case a squamous-celled tumour similar to those described by Tyzzer * was found in a mouse which had a spontaneous sarcoma in the axilla (336).

Sarcomata.

Several cases of primary sarcoma have been found. Three (336, 460, and 476) are typical spindle-celled sarcomata. One arose deep among the pectoral muscles, two others subcutaneously in the middle of the back, slightly behind the line between the two axillæ. Epithelial elements have been found only in the periphery of one of them, and then only as included normal acini of mamma. All three were transplanted, and all showed a similar behaviour on transplantation. They grew in 20–60 per cent. of the inoculated animals for the first 3–4 weeks, then rapidly underwent spontaneous absorption. The

* Tyzzer, E. E.: A series of Spontaneous Tumours in Mice. Fifth Report of the Caroline Brewer Croft Fund Cancer Commission. Boston, 1909.

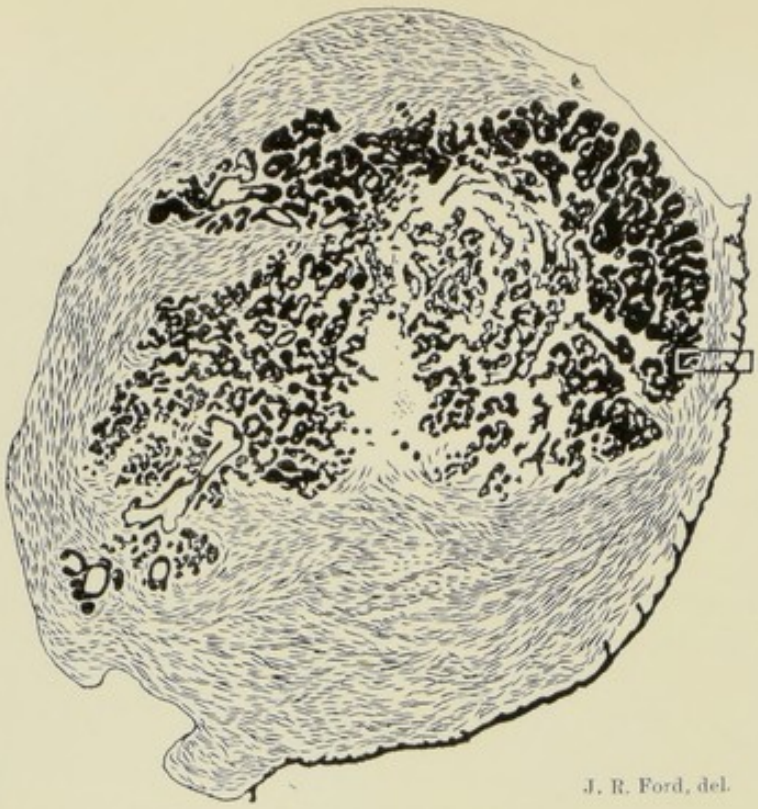
first of the three was further propagated by transplanting during this period of temporary growth; as for the others not enough attention was paid to the tumours during this period, and attempts at propagation when spontaneous absorption had started were not followed by success.

Mouse 70 was inoculated, with negative result, in the right axilla with a sporadic tumour when about 6 weeks' old. Five months later a thickening was observed in the fatty tissue on the back of the neck; it was under observation for another 5 months, and was then removed by operation. It was found to consist of fatty tissue of normal appearance (lipoma). The mouse remained under observation, and a year later, when the mouse was about 24 months of age, a tumour measuring 17 × 10 mm. was found under the scar of the old operation. At the same time another tumour was felt in the abdomen (adeno-carcinoma of the ovary, *cf.* p. 11). The mouse was killed. The tumour on the back presented the appearances of a polymorphous-celled sarcomatous tumour consisting of spindle-shaped and round cells mingled here and there with larger elements and even giant cells. No micro-organisms were found. In the right axilla there was found a secondary deposit; there were no metastases visible to the naked eye in the lungs. The tumour was transplanted, but died out after one passage.

Tumour 219 was found in a male, as a large growth of the size of a walnut, embedded among the muscles of the thigh. The mouse was operated upon, but the tumour was too large for complete operation. A rapidly growing recurrence developed within the first week, and the mouse was killed. The tumour was very soft, whitish, and of the consistence of brain substance. Histologically it consists of round cells with large nuclei, heaped together without any definite arrangement. The connective tissue is very scanty, practically limited to the blood-vessels. Numerous islands of osteoid tissue are found throughout the tumour, indicating that it may be classified as an osteosarcoma of the thigh. It was transplanted into 800 mice, of which 415 survived four weeks, and among them 15 tumours developed. The further propagation has met with no difficulty; the tumour grows in a varying, not maximal percentage, from 20 to 70 per cent., mostly ca. 50 per cent. Its initial rate of growth is fairly rapid, but not equal to other transplantable sarcomata; spontaneous absorption is met with far less frequently than in these latter. The propagated tumours are very soft round-celled sarcomata. Differentiation into osteoid tissue has not been observed in them.

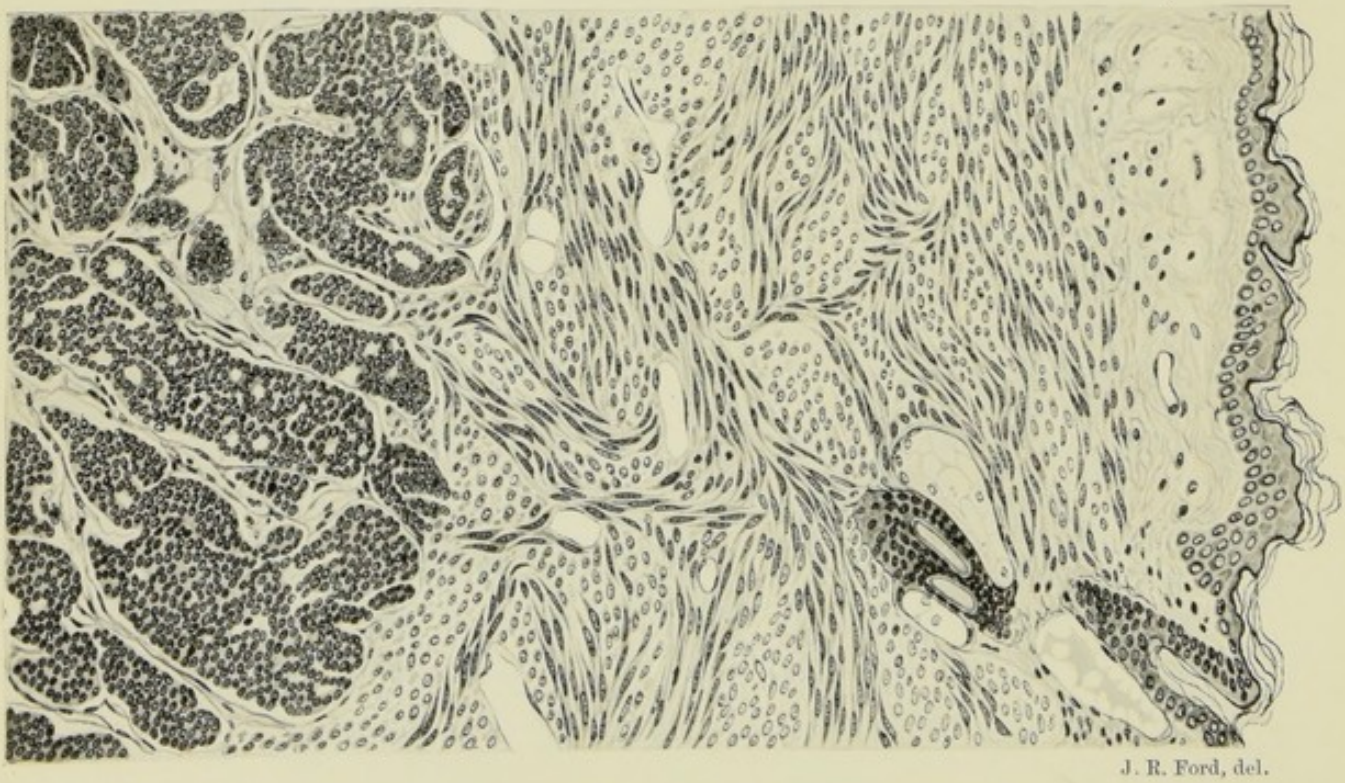
In mouse 431, a female of the breeding-experiments, 28 months old, a hæmorrhagic adeno-carcinoma of the mamma, as large as a small hazel-nut, was found on the right haunch. The tumour was operated upon and no recurrence took place during 7½ weeks, *i. e.* till the death of the animal. Five weeks after the operation it was found that the hind legs were completely paralysed. No tumour could be felt. The mouse was killed 2½ weeks later. The spinal column was apparently destroyed between the lumbar and dorsal region, but no tumour could be recognised by naked eye examination. In serial sections of the affected parts a sarcomatous tumour is found in the central part of the spinal cord, infiltrating and compressing the nervous substance and in part filling out the vertebral canal and disintegrating the bone. The cells of the tumour are large, of round or polymorphous shape with large nuclei. Numerous mitoses are seen. The place of origin of the tumour cannot be definitely decided. The tumour runs for a considerable distance along the central canal of

To face p. 19.]



J. R. Ford, del.

Fig. 16.—Mouse 469. Sarcoma development in a spontaneous tumour. Semi-schematic figure representing a section across the whole of the primary tumour. The black masses are the carcinomatous alveoli, the peripheral zone of delicate spindles represents the sarcoma. The spaces left clear in the centre were occupied by sclerotic connective tissue. $\times \frac{10}{1}$.



J. R. Ford, del.

Fig. 17.—Mouse 469. Represents the marked portion of the above figure, at a higher magnification. To the left the carcinoma, to the right the skin, between the sarcoma infiltrating the subcutis.

the spinal cord, and it has the appearance as if it might have originated here, and only later have grown outwards. No naked eye metastases were found in the lungs. A squamous-cell cyst was present in one of the mammæ.

An interesting case of sarcoma development was observed in a spontaneous tumour.

Mouse 469, a female, from an outside breeder, was brought into the laboratory with a small firm tumour in the left axilla, only little larger than a hemp-seed. The tumour remained stationary for 10 weeks, at the end of which time it was exactly the same size as when the mouse was entered on the laboratory register. It then started to grow rapidly; at the end of the 11th week it had more than doubled its size (5×5 mm.), and at the end of the 12th week it measured 8×10 mm. The tumour was then removed by operation, and was found to consist of a central carcinomatous part and a peripheral broad band of spindle-cell sarcoma (figs. 16 & 17). The carcinomatous part consisted of a central part with very sclerotic stroma, corresponding in size to the original tumour, and a younger part adjoining the sarcomatous zone; both parts showed the picture of an adeno-carcinoma mixed with pure alveolar cancer. Both the peripheral zone of carcinoma and the sarcoma contained numerous mitoses. The tumour was inoculated into 48 normal mice; at the same time the mouse itself was inoculated with a fragment of the central part of the tumour in the left groin and with a fragment of the peripheral part in the right groin. Both grafts grew, that from the peripheral part considerably more rapidly than the other from the central part. Both consisted of pure sarcoma. The tumour grew also in the normal mice, and in all the subtransplants examined up to the present only sarcoma has been found. The sarcoma developed in association with a carcinomatous nodule of long standing, which had been under observation for 10 weeks without any interference whatever. This seems to be a case of sarcoma developed under conditions closely allied to those under which development of sarcoma is observed in subtransplants of tumour 100, described by Russell *.

Lymphomata.

In 21 mice tumours have been found consisting of lymphoid tissue. In some cases the tumours were localised, and some had the appearances of being true primary growths to be classed as lympho-sarcomata. In other cases the condition seems to be that of a general hyperplasia of the

* Russell, B. R. G.: Sarcoma development occurring during the propagation of a Hæmorrhagic Adeno-carcinoma of the Mamma of the Mouse. Journal of Pathology and Bacteriology, 1910, vol. xiv.

lymphatic tissue practically wherever it exists normally. There is no decisive evidence of the true tumour-nature of this condition, except that in some instances the cells are not strictly confined within the capsule of the lymphatic gland, but invade and infiltrate the surrounding tissues. These tumours could not be successfully transplanted into normal mice, and attempts at demonstrating growth by inoculation into the same spontaneously affected mouse have not given any result, as the mice have died too early after the inoculation. Two of these cases were accompanied by typical leucæmia; in two further cases a considerable increase in number of the white blood-corpuscles was found in the blood at the moment of death. A similar case is reported by Jobling.

A. *Lymphomata as more or less localised conditions.*

Mouse 243, a female mouse, from an outside breeder, with an alveolar mammary carcinoma on the right side of vulva. The tumour was removed by operation and was without recurrence for 5 weeks, *i. e.* until the mouse died. At the post-mortem examination a large bilateral growth was found on the site of the thymus, filling out the upper part of the thorax. Microscopically it consists of typical lymphomatous tissue; no Hassal's corpuscles are to be found. A small secondary nodule was found in the lower lobe of the right lung. The other organs were apparently normal. The blood was not examined.

Mouse 345, a 15 months old female, of the breeding experiments, with a large gland in neck, in front of trachea (17×9 mm.). The tumour was removed by operation and had not recurred during 13 weeks, *i. e.*, till the mouse was killed. Large glands were found in the mediastium, in the mesentery and retroperitoneally. The spleen was enlarged. Large nodules were present in the lungs. The external glands were not enlarged. The blood was not examined. The tumour was transplanted into 30 mice with negative results.

Mouse 408, a 17 months old female, of the breeding experiments. When found dead the spleen was very large, there was a large white mass in the right lobe of the liver, enlarged retroperitoneal glands, and a smaller gland in the mediastinum. Histological examination shows lymphoid tissue of fairly typical appearance. The external glands were not enlarged. Fragments of the tumours were transplanted into 39 mice, of which 29 survived after four weeks with negative results.

Mouse 422, a 27 months old female, of the breeding experiments, had a diffuse growth in the left axilla, involving the skin, mamma, and glands. Microscopically it consists of lymphomatous tissue. The general appearances of the lesion suggest that one has here to deal with an infection, but no micro-organism has been detected. The tumour was inoculated into 40 mice, of which 35 survived, with negative results.

In 6 other mice localised lymphomatous tumours were found, in the kidney (439), in the mesentery and retroperitoneally (361, 423, and 489), in the thymus and the mediastinum (310 and 407). The exact nature of these tumours cannot be decided; in some instances there is decidedly evidence of sarcomatous tumours consisting mainly of small and round cells, but these conditions cannot be distinguished with certainty from hyperplasia on the one hand and infectious processes on the other.

B. *Lymphomata as generalised conditions.*

Mouse 255 was a 10 months old female, of the breeding experiments, with multiple swellings of all external glands. The mouse was killed and the tumours transplanted. Large mesenteric and retroperitoneal glands were found. There were no secondaries visible to the naked eye in the lungs, kidneys, or the liver. The blood was not examined. The results of transplantation in 108 mice, of which 90 survived after 4 weeks, were all negative. In this case, as in most of the following, there is no evidence of one of the tumours being the primary focus and the others metastatic. Their mode of occurrence and pathological anatomy cannot be harmonized with the views of Tyzzer, who regards multiple lymphomata as metastatic from one primary focus.

Mouse 350, a 14 months old female, of the breeding experiment. All external glands in the neck, axilla, and groin were found enlarged. The mediastinal, retroperitoneal, and mesenteric glands equally were enlarged and firm. One mass of retroperitoneal glands measured $2\frac{1}{2} \times 1$ cm. The spleen was very large ($3{\cdot}5 \times 1{\cdot}1$ cm.), firm, with whitish hyaline points, like sago spleen. The lymphoid tissue of the intestine was hypertrophied, and protruded on the peritoneal surface as small tumour nodules. The thymus was very much enlarged and of firm consistence ; it covered the whole heart and most of both lungs.

The whole mouse was cut in serial sections. The tumours consist of lymphoid tissue of apparently normal structure; it is confined to the glands and other organs where lymphoid tissue normally exists and does not perforate the capsule and infiltrate the surrounding tissues.

The blood was examined 15.xi.09. The white corpuscles were greatly increased in number, but they were not exactly enumerated. The picture was that of a lymphatic leucæmia.

23.xi. On the examination of the blood from the tip of the tail, after some massage of the tail, 331,000 white corpuscles in cmm. were counted, and on 24.xi. (immediately after death) in the blood from the heart there were 109,500 white corpuscles, and 2,050,000 red corpuscles in cmm.

Transplantation was attempted intravenously (with blood, into 14 mice), intraperitoneally and subcutaneously with spleen, thymus, and gland into altogether 101 mice, of which 86 survived after 4 weeks. The blood of a large number of these animals was examined when they showed sign of illness, but it failed to give the leucæmic picture. Three of the mice injected intravenously and one injected intraperitoneally, examined 2-3 months after inoculation, had multiple nodules in their lungs, which were found to be due to the bacillus of pseudo-tuberculosis murium, probably an accidental infection. Apart from these, no lesions were found which could be interpreted as positive results of inoculation. The organs of the spontaneously affected mouse were examined for pseudo-tuberculosis or other infection, but with negative results.

Mouse 382, a 19 months old female, from the breeding experiments, with enlarged glands in both axillæ, presented at the post-mortem examination large glands in the mediastinum, in the mesentery, and the retroperitoneal space, which formed soft white masses filling out the pelvis. The thymus and spleen were not enlarged.

Mouse 387, an 11 months old female, of the breeding experiments, and a daughter of the previous case, mouse 350, presented the following condition :—All external glands were enlarged (four in neck, two in each axilla, one in each groin). The mouse was

killed in order to obtain material for transplantation. A very large mass of glands was found in radix mesenterii, obviously formed by fusion of several glands, and others retroperitoneally in the deeper part of pelvis. The spleen was very large (4×1·1 cm.). The thymus was not much enlarged, the glands of the mediastinum appeared not to be enlarged. The liver and lungs were apparently normal.

Examined microscopically the tumours consist of lymphoid tissue without any marked histological difference from that of the germinal centres of the normal lymph gland. The lymphoid tissue contains numerous mitoses. In some places it is not strictly confined to the gland, but appears on the outside of the capsule infiltrating the surrounding fatty tissue.

The blood from the tip of the tail examined on the 14.ii.10 showed 655,000 white corpuscles in cmm., and on the 19.ii.10 (when the mouse was killed) 410,000 white corpuscles and 520,000 red corpuscles. Microscopically, in smear preparations, the great majority of cells consist of large mononucleated cells with faintly granular protoplasma without any distinct granula. Nucleated red blood-corpuscles are seen (Giemsa's and Ehrlich's triacid stains).

The tumours were transplanted intravenously and intraperitoneally into 112 mice, of which 89 remained alive for more than four weeks. The result was entirely negative.

Mice 456 and 458 were females, from different outside breeders, with adeno-carcinomata of the mamma at the side of the vulva. The first developed a general lymphomatous condition three weeks after entrance into the laboratory register, and after the removal of the mammary tumour; the second presented such a condition as well as a mammary tumour when it was first observed. In both cases all the external glands were enlarged, the thymus was of an enormons size; the spleen was firm, very large, with mottled surface, and contained greyish nodules; the liver was large and firm, with whitish patches; the mesenteric and retroperitoneal glands were very much enlarged.

On microscopical examination the tumours in both cases were found to consist of round cells with large nuclei of the same appearances as the lymphoid cells in normal germinal centres; they contain numerous mitoses. In both cases these cells have penetrated the capsule of the gland and infiltrate the surrounding fatty tissue; here also numerous dividing cells are found.

In mouse 458 the white blood-corpuscles were counted 10 days before death, the result was 3595 pr. cmm. Examined at the time of death the blood of both mice showed a considerable increase in the number of the white corpuscles, mainly of large mononuclear cells.

A further case of generalised lymphoma was found in mouse 438, which had developed a cancroid on the flank. Furthermore, lymphomatous tumours were found in two females of the breeding experiments. In mouse 463 (17 months' old), the changes were more marked in the internal glands, in the mediastinum and thymus, mesenterium and retroperitoneal space, while in mouse 498 (15 months' old) the changes were chiefly confined to the external glands, with only one mesenteric gland enlarged, spleen and liver apparently normal.

Two other lymphomata, 210 and 494, are described on a later page (pp. 47–48).

It is remarkable that all the cases of lymphoma of this material have been found in females. As a rule the breeders keep far more females than males, but this does not apply to the same extent to the breeding experiments of the laboratory, in

which an almost equal number of males have been kept up till advanced age. Of the total of 19 cases of lymphomata 12 were found in mice from the breeding experiments, and there is at present no explanation why they all occurred in females. Tyzzer, however, has found two similar cases in males.

(2) *Multiplicity and Development of New Primary Tumours.*

The number of mice in which two or more tumours are found simultaneously is striking. Apolant found multiple mammary tumours in 38 cases out of 221 mice, *i. e.* ca. 12 per cent. Of the 119 cases previously recorded from this laboratory by Murray, multiplicity occurred in 18, *i. e.* 15 per cent. In the present material this proportion is slightly higher, 49 cases of multiple tumours out of 288 spontaneously attacked mice, *i. e.* 17 per cent. In this number lymphomata and lung adenomata are not included.

Both Tyzzer and Jobling have included lung adenomata in their totals, for which reason it is difficult to compare their figures with those mentioned above. In the 26 mice observed by Jobling, multiple tumours were found 10 times, *i. e.* 38·4 per cent. For Tyzzer's material of 70 mice 11 (*i. e.* over 15 per cent.) had two or more primary tumours which differed in type from one another. In addition the tumours of the lungs were multiple in several cases and there were double ovarian tumours in two cases.

Of the mice with multiple tumours in the present material :

41 (*i. e.* 14 per cent.)	had 2	tumours.
6	3	,,
2	4	,,

In several mice multiple nodules occurred throughout the whole mammary apparatus. These cases will be described separately.

The main question is whether this multiplicity is real, caused by the development of several independent primary foci, or only apparent and to be explained as dissemination, *i. e.* as secondary nodules derived from one primary focus. Only when the tumours show different histological structure is there no difficulty in concluding as to their multiplicity of origin. Nevertheless, for certain multiple tumours it can be inferred, with great probability, that we have to deal with a number of distinct primary tumours arising independently, even in the absence of distinct histological difference between them. This is the most likely assumption, where they seem to have developed simultaneously, are of approximately the same size, and are situated in different mammæ wide apart from each other. In other cases where the tumours differ considerably in size, it is difficult to exclude the possibility of the smaller tumour being a secondary nodule from the larger primary focus. Such a relation between the two nodules has been proved in single instances, *e. g.*, in mouse 331, in which a large tumour was present in the left axilla and a

small one in the right axilla. The smaller nodule was found surrounded by typical lymphoid tissue showing that it had developed in the axillary gland as a metastasis from the larger tumour in the opposite axilla. The great majority of the multiple tumours under consideration occur in the mammæ themselves, and there is no evidence of a metastatic origin. The general impression left by their localisations, simultaneous occurrence, and histological characteristics is, that they developed independently and are manifestations of a multicentric origin.

Subsequent development of New Tumours.

The difficulty of deciding whether one has to deal with multiple primary tumours or metastases becomes much greater in the case of nodules which appear later in a mouse in which a spontaneous tumour has already arisen and been removed surgically. Murray observed that where mice were kept under observation for relatively long periods, new tumours occasionally developed in parts of the mammary apparatus remote from that occupied by the tumour first observed. He left the question open whether these tumours were to be considered metastases or new tumours. In the present material a relatively great number of mice have lived for a considerable time under observation, and among them new tumour nodules have developed with remarkable frequency. When lung adenomata and lymphomata are excluded, 106 new tumours were observed in 77 out of 288 mice bearing spontaneous tumours, *i. e.* in about 27 per cent. It must be taken into consideration that of this total number a large proportion died shortly after the primary growth was placed on record, and thus afforded no opportunity for judging how many tumours would have developed had they remained longer under observation. When the mice are excluded which have not been more than four weeks under observation before death occurred, there remain 209 mice of which 73, *i. e.* about 35 per cent. have developed new tumours at a date subsequent to the observation of the primary growth.

When one comes to inquire how these new nodules are to be considered, whether as metastases from the primary focus or as a multicentric development of new tumours, it is obvious that each case has to be examined carefully and judged on its merits. The problem is much the same as in multiple primary tumours, only the appearance of a new nodule in a later stage of the disease makes a metastatic origin much more likely than where the two tumours seem to have originated about the same time. As mentioned for multiple tumours, arising simultaneously, when a fundamental histological difference

exists between the primary and the secondary tumour, the circumstances are clear, and multicentricity is proved. In a fair number this is the case, as shown on a later page. In the large majority, however, both the primary and secondary nodules belong to the common type of adeno-carcinoma, and the differences in structure between them are not great enough to allow of any definite conclusion. In these cases it seems natural first to keep a metastatic origin in mind until evidence to the contrary is produced. In certain instances the metastatic origin can be proved, *e.g.*, when the new nodule has appeared in a lymph gland or in organs where cells having the character of those of the tumour parenchyma do not exist primarily. All the cases in which there is any reason for assuming metastatic origin have been discarded from consideration in this connection, and also all in which a new nodule may, with any probability, be explained as extension or recurrence of the primarily existing tumour. When these cases are eliminated there remains a large number which may be either secondary nodules or new primary tumours. Certain features of the new nodules attract attention. Their distribution shows a remarkable resemblance to that of the primary tumours. If they were metastatic they should be expected to occur more especially in certain places, *e. g.*, first of all near and around the primary tumour and in the same mamma, or in the regional lymph glands or their neighbourhood. This, however, is not the case ; they do not generally develop in lymph glands. Neither are they distributed uniformly over the subcutaneous tissues of the body. They occur only in anatomical sites which correspond to other mammæ, and on microscopical examination are almost always found embedded in mammary tissue. If they are secondary tumours we have to deal with a remarkable tendency of these tumours to form metastases in other mammary glands. This would be a restriction for which there is no parallel from any other animal, and for which there appears to be no anatomical reason. It seems difficult to understand why a tumour of the cervical mamma should give a metastasis in the mamma beside the vulva, with exemption of all lymphatic glands and all intervening tissue. Nevertheless, the localisation near the vulva is a very frequent one. There are also other points which do not seem to support the theory of metastatic origin. The structure of the fresh nodules is essentially identical with that of young primary tumours, and an adenomatous structure of the smaller nodules is likely to attract special attention, when the first tumour has been an alveolar carcinoma or an adeno-carcinoma. They often occur a long time (4–5 months) after the successful removal of the primary

TABLE 1.—*Development of new Tumours close to the Vulva, the primary Tumour being situated in a Mamma remote from the Vulva.*

Spontaneous mouse.	Length of observation.	Primary tumour.		Results of operation.	Development of new tumours.			
							Time interval.	
		No.	Site.		No.	Site.	After entrance.	After operation.
155	22 w.	1	l. axilla.	Recurred 10 weeks after op.	1	r. of vulva.	10 w.	5 w.
179	5 w.	1	r. axilla.	Not op.	1	l. of vulva.	5 w.	
191	32 w.	1	r. axilla.	Not op. before after appearance of new tumour.	2	r. of vulva l. of vulva.	8 w. 28 w.	
211	28 w.	1	middle of neck.	Recurred 21 w. after op.	1	r. of vulva.	..	14 w.
218	13 w.	2	l. shoulder. base of l. ear.	No rec. (12 w.).	1	l. of vulva.	..	9 w. 3 w.
223	24 w.	1	r. axilla.	No rec. (24 w.).	1	l. of vulva	..	
270	22 w.	2	r. of neck. r. axilla.	Rec. 9 weeks after op.	1	r. of vulva.	..	9 w.
317	28 w.	1	l. groin.	No rec. (28 w.).	2	l. axilla. r. of vulva.	.. ..	12 w. 22 w.
324	15 w.	2 nod.	l. axilla.	No rec. (12 w.).	2	r. shoulder. l. of vulva.	.. ..	4 w. 7 w.
335	12 w.	1	r. groin.	No rec. (10 w.).	1	l. of vulva.	..	3 w.
353	36 w.	1	l. haunch.	No rec. (35 w.).	1	l. of vulva.	..	22 w.
355	22 w.	1	r. groin.	No rec. (19 w.).	1	r. of vulva.	..	16 w.
361	18 w.	2	r. shoulder. l. groin.	No rec. (15 w.).	1	r. of vulva.	..	7 w.
364	11½ w.	1	l. groin.	No rec. (10 w.).	3	r. groin. r. of neck. l. of vulva.	.. .. ..	2 w. 9 w. 8 w.
370	37 w.	1	l. axilla.	Rec. (?) 26 weeks after op.	1	r. of vulva.	..	2 w.
386	14 w.	2	l. axilla. r. of vulva.	No rec. (9 w.).	1	l. of vulva.	12 w.	
400	9 w.	1	l. axilla.	No rec. (7 w.).	1	r. of vulva.	..	1 w.
405	8 w.	3	back of neck. l. axilla. l. groin.	.....	1	r. of vulva.	4 w.	
413	13 w.	1	l. axilla.	Rec. (9 w.).	2	l. of neck. l. of vulva.	..	8 w.
424	7 w.	1	r. of neck.	No rec. (6 w.).	1	l. of vulva.	..	6 w.
427	5½ w.	2	l. axilla. l. of vulva.	Rec. (3 w.).	1	r. of vulva.	..	2 w.
430	24 w.	1	r. axilla.	No rec. (18 w.).	2	l. axilla. l. of vulva.	..	18 w.

tumour, of which there has been no recurrence. These points taken together, viz. origin always in mammary tissue, no lymphatic tissue round the beginning nodule, but the lymph gland found free from growth ; same predilection for certain sites as the primary tumours ; occurrence in a mamma far remote from the primary tumour ; and same prevalence of adenomatous structure as in young primary tumours, rather point towards the majority of these new nodules being new primary tumours, although now and again a metastatic origin cannot be excluded.

For a number of cases there seems to be fairly strong evidence in support of the view that the new nodules, even if corresponding in structure to the primary tumour, must be considered as new primary tumours. As such instances may be regarded the frequent cases in which the new tumour has developed on the side of the vulva where there is no lymph gland, and where it is difficult to see any special reason why metastases should be more frequent than in other parts of the body. Table 1 shows the cases of this kind. Other cases in which the evidence in favour of multicentric origin is fairly strong, are those in which new tumours develop in widely removed mammæ, a long time, often 4–5 months, after the primary tumour has been successfully removed and has not recurred in the interval. Table 2 shows cases of this kind.

For the other cases of development of new tumours entered on the

TABLE 2.—*Development of New Tumours after long interval without recurrence at the Primary Site.*

Spontaneous mouse.	Length of observation.	Primary tumour.		Result of operation.	Development of new tumours.		
		No.	Site.		No.	Site.	Interval after operation.
207	37 w.	1	l. axilla.	No recurrence until death of animal (36 w.).	2	r. axilla. l. of neck.	25 w. 26 w.
240	21 w.	3	l. of neck. l. axilla. l. of vulva.	„ (20 w.).	1	r. groin.	14 w.
257	33 w.	1	l. groin.	„ (27 w.).	1	l. axilla.	32 w.
261	16 w.	1	r. axilla.	„ (15 w.).	1	l. axilla.	15 w.
313	40 w.	1	l. of neck.	„ (35 w.).	2	r. groin. r. axilla.	28 w. 35 w.
363	21 w.	1	r. of neck.	„ (19 w.).	1	l. groin.	14 w.

large table appended to this paper the evidence is not conclusive either way. From what has been said, it seems natural to infer that a large number of them can be also considered as genuine new primary tumours.

The several tumours occurring simultaneously in one animal, or developing subsequently, most frequently show the same histological structure, viz. that of the common adeno-carcinoma with subsidiary differences. In some cases, however, different histological types are met with.

In reviewing multiple tumours in the human subject, v. Hansemann * groups them under three headings :—

(1) Simultaneous occurrence of quite different benign or malignant tumours.
(2) Pluricentric occurrence of same type of tumour in the same organ.
(3) Simultaneous occurrence of cancer of same type in several organs.

It is preferable to subdivide v. Hansemann's group (1) because of the occurrence of definitely different types of tumours in the mamma of the mouse, for which the possibility exists that they have originated from the same parenchyma.

1. Different types of tumour in different organs.
2. Different types of tumour in the same organ. 1. and 2. correspond to v. Hansemann's group (1).
3. Same type of tumour occurring in distinctly separated places of the same organ. (v. Hansemann's group (2).)
4. Same type of tumour in different organs. (v. Hansemann's group (3).)

In the first group the cases observed are :—

1. Sebaceous carcinoma of anus with cancroid of jaw. (378.)
2. Adeno-carcinoma of the mamma with fibro-myoma uteri. (334.)
3. Cystic adeno-carcinoma of mamma with sebaceous and squamous-celled carcinoma of anus. (333.)
4. Hæmorrhagic adeno-carcinoma of mamma with liver angioma. (393.)
5. Sarcoma (subcutaneous) with adeno-carcinoma of ovary. (70.)
6. Sarcoma (subcutaneous) with squamous-celled tumour in the lungs (336).
7. Hæmorrhagic adeno-carcinoma of mamma with sarcoma of the spinal cord (431).

When lung adenomata and lymphomata are included, these cases become more frequent. Thus we have :—

7 cases of hæmorrhagic adeno-carcinoma of mamma with lung adenoma.
2 cases of cancroids (subcutaneous) with lung adenoma.
1 adeno-cancroid of mamma with lung adenoma.
3 sebaceous carcinomas with lung adenoma.
1 hypernephroma with lung adenoma.

Examination has not yet been made expressly for the lung adenomata, and there is reason to believe that their frequency in reality is a good deal greater.

* v. Hansemann, D. : 'Das gleichzeitige Vorkommen verschiedenartiger Geschwülste bei derselben Person.' Zeitschrift für Krebsforschung, 1904, Bd. 1, H. 4.

Of combinations with lymphomatous tumours there are seven cases of mammary adeno-carcinoma with lymphomata in mediastinal, retro-peritoneal or external glands.

Three cases of hæmorrhagic adeno-carcinoma were combined with sebaceous adenomata.

In the second group where tumours occurring in the same organ system differ in structure, there are:—

3 cases of hæmorrhagic adeno-carcinoma with sebaceous carcinoma in the mamma. (239, 292, 427.)

2 cases of hæmorrhagic adeno-carcinoma with cancroid. (329 and 405.)

The difficulty here is to decide whether these tumours really belong to the same system, or ought not rather to be entered under the first group. This point has been already discussed while reviewing the histology of these tumours. It seems as if the parenchyma of the mammary apparatus, in certain cases, is capable of giving rise to tumours of different histological type, according to the tendency to differentiation of the cells, either with retention of the mammary ground type or with differentiation into keratinised epithelium or into sebaceous gland-like structures.

The third group is the most numerous by far. Of the 103 new tumours which have been observed, the histological structure of 91 corresponds in the main to the primary tumour, when smaller and not stable histological differences are ignored.

The fourth group has only one representative in this material, if the ovarian tumour in mouse 274 be accepted as a primary tumour; in this case we have adeno-carcinoma of ovary combined with a mammary adeno-carcinoma.

To recapitulate the data on multiple tumours, when primary multiplicity and the appearance of new nodules are reckoned together, 122 mice with multiple tumours have been observed in a total of 288 tumour mice. The total number of additional tumours observed in these 122 mice has been 162. Even if allowance be made for a certain number of these tumours being of metastatic origin, a possibility which cannot be excluded, the previous considerations lead to the conviction that the great majority must be considered as of pluricentric origin.

The frequency of multiple tumours in the mouse is thus very great, and seems to be at variance with the facts in human pathology. However, multiple tumours are by no means rare in the human subject (vide v. Hansemann and R. Williams *). When the data are analysed and the mammary tumours are excluded, the frequency of multiple tumours of other types in mice is very much as in man. It is only the mammary tumours which seem to form an exception in this respect †. Several factors may contribute to this result. One of these is that the mouse has five pairs of mammary glands, spread out over a large surface of its body, whereas in man there is only one pair

* Williams, W. Roger: The Natural History of Cancer. London, 1908.

† In addition to lung adenomata and lymphomata.

packed together in a relatively compact organ. Multicentric development of tumours in the mamma will be much less easily detected under the circumstances obtaining in man than in the mouse, and will more easily be taken for recurrence or extension of the primarily existing tumour. Although the significance of the differences between man and mouse is thus diminished, nevertheless the frequency of multiple tumours in the mamma is striking and must be kept in mind as a peculiar feature of mouse tumours which calls for a fuller explanation.

In order to account for the frequency of multicentric development of tumours a general systemic change might be supposed, or, an increased liability of a special organ system. The fact that, apart from the mammary tumours, multicentricity is relatively rare, not more frequent than can be explained by coincidence, shows that it can hardly be accounted for on the first supposition. The data accumulated point towards an increased liability of the whole mammary system of the mouse, either as an inherited condition, or as the result of a general diseased condition with diffuse changes in the mamma leading to development of cancer. It will be necessary to exclude the second of these possibilities before the first can be accepted.

It is interesting to note that, in spite of this greater liability to spontaneous cancer-development of their own cells, these same mice, as will be shown later on, do not possess any increased disposition for the growth of other tumours.

(3) *Histological Changes in the Mammæ of Old Female Mice.*

The mammæ of a large number of old mice, cancerous and non-cancerous, have been examined histologically in serial sections, in order to investigate whether changes might be found which could account for the multicentric origin of the tumours. The histology of the mamma varies greatly in different normal animals according to age and physiological conditions, and it is therefore necessary to allow somewhat wide limits to what is to be regarded as normal, and to consider carefully the influences of pregnancy and lactation.

(a) *Mamma of Mice which had already developed Tumours.*

The normal condition of the mamma in aged mice after the time of physiological activity is over, appears to be a more or less atrophic condition, accompanied by sclerotic changes in the connective tissue. The groups of normal acini seen in the resting mamma of the adult are scarce ; the ducts are frequently dilated and surrounded by a very sclerotic tissue, often appearing as hyaline zones round the ducts.

Sclerotic processes in the arteries are also seen. It cannot be decided whether the more marked sclerosis is to be considered a merely senile change or whether it is the result of pathological processes.

Pathological changes are very frequently met with, both of the interstitial tissue and of the epithelium. The first are more or less of an inflammatory character, the latter either cystic or hypertrophic changes. These interstitial and epithelial changes are often combined.

Changes in the interstitial tissue of a chronic inflammatory kind are very frequent, either diffuse or strictly localised. In some cases the inflammatory character is most marked with localised foci of lymphocytes, plasma cells, and phagocytes ; in others the strictly inflammatory character is missing, and the change consists mainly in an increased cellularity of the connective tissue, accompanied by sclerosis. Apart from inflammatory nodules, small accumulations of lymphocytes are frequently found in the perivascular tissue or round the ducts (fig. 24) ; they are probably to be considered as a normal phenomenon, like the small lymphatic nodules found in the lung (*e. g.* of the mouse, the guinea-pig, and the rabbit).

Dilatation of ducts and formation of cysts is frequently seen. The epithelium lining the wall of these cysts is in most cases that of the normal duct or acinus but flattened out by the pressure of the contents ; in the cyst are clumps of inspissated secretion and desquamated cells ; real concretions with concentric and striated structure are seen very frequently. In some cases a squamous-cell epithelium is found lining the cysts, as has previously been mentioned, and such cysts are full of typical squames (see fig. 18) *.

Mouse 418 yields an instance of cystic transformation of the mamma with subsequent development of tumours accompanied by pronounced inflammatory changes in the connective tissue advancing to sclerosis. The mouse was brought into the laboratory from an outside breeder with three minute nodules on the right flank. They grew extremely slowly for 13 weeks. The mouse was, in the meantime, inoculated with a rapidly growing transplanted tumour, and had to be killed. The pathological changes were mainly situated in the right axillary mamma in which multiple nodules were found all over the organ. Fig. 19 shows one of the smaller nodules. It consists of a number of small epithelial cysts embedded in a sclerotic mass of connective tissue. This sclerotic tissue is very cellular and contains numerous small foci of inflammation. Along with lymphocytes these foci contain a great number of large phagocytes crammed with blood-pigment and other débris. The cysts contain clumps of secretion and concretions.

In the main the epithelium of the cysts is still normal, but in one part of the nodule the epithelial cells have become larger and drawn out (goblet-shaped), and the walls

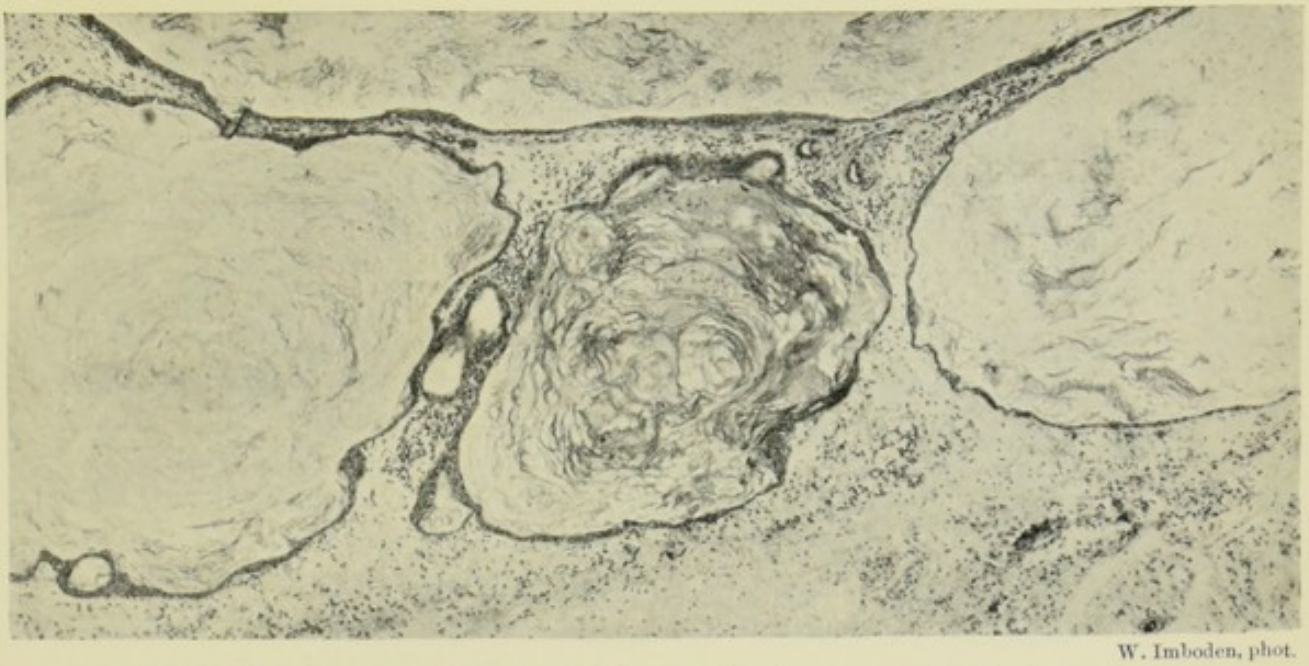
* In addition to epithelial cysts, others are found of a different origin, *e. g.* developed round a cysticercus.

present papilliform excresences. In the larger nodules this is much more pronounced and the picture is that of a cystic and papilliferous adeno-carcinoma with sclerotic stroma, on the whole somewhat cellular, and containing here and there foci of inflammation. In the lumina of the cysts there are found peculiar structures surrounded by a layer of broken down leucocytes. They suggest, at first, parasitic inclusions, but from their concentric and radiated structure they are probably to be regarded as concretions. The nature of the nucleus of these concretions was not ascertained; it is remarkable that they should be surrounded by broken down leucocytes, which are hardly ever found round the concretions usual in such cysts.

In this case tumour growth is already recognisable in a diffusely diseased mamma in which marked inflammatory changes are seen in the interstitial tissue. The interstitial changes are far more pronounced than would be the case were they merely the reactions round a beginning carcinoma, and they are also of a quite different type. The clinical history speaks against the possibility that they might indicate spontaneous absorption of tumour. The most likely explanation of the mass of phagocytes full of blood-pigment and débris is that some acute process with hæmorrhage and destruction of tissue has taken place on the spot. But at the stage to which the process has advanced there is no longer any sign of a causative agent, in particular no micro-organisms have been found. The process happens to have been observed in an intermediate stage; some agent has set up inflammatory changes all over the mamma, this agent itself is gone and only the localised inflammatory foci testify to where it once acted. In this diseased mamma true tumour proliferation has started, and the type of the future tumour is already definitely indicated: that of a cystic and papilliferous adeno-carcinoma with sclerotic stroma, one of the best defined types of mammary tumours of the mouse. Whether any relation exists between the inflammation in the connective tissue and the epithelial proliferation and, if any, what kind of relation one cannot tell. The process chanced to come under observation at this stage, because the mouse was killed for other reasons, while its tumours still were in a very early stage. If the process had been allowed to go on we should, instead of multiple separate nodules, have found in all probability one large tumour with smaller "secondary" nodules in its periphery, and probably no trace of diseased mammary tissue would then have been discovered. The inflammatory character would have been lost and only a slight cellularity and sclerosis would have remained in the adjacent connective tissue.

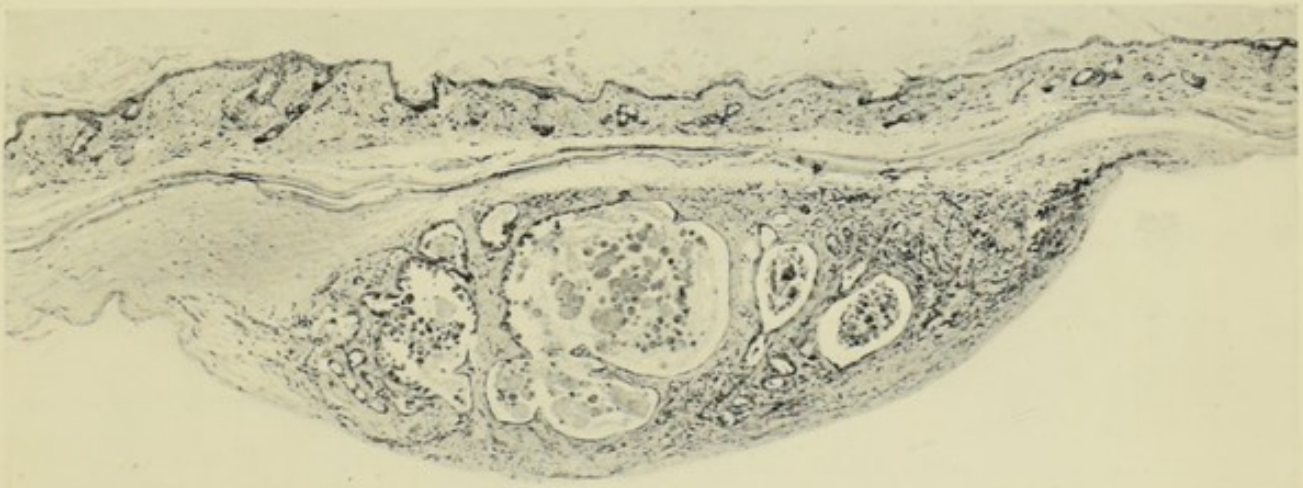
In addition to cystic changes, generalised or circumscribed hypertrophic conditions of the mammary epithelium occur frequently. A general hypertrophic condition mostly indicates physiological activity following on pregnancy, and has no interest for the present purpose. Now and then such hypertrophic changes are found where the mouse is beyond the age when a physiological activity of the mamma is to be expected, and when the mouse has been kept isolated for 5–6 months without a male. In one instance of this kind pathological changes of the uterus and ovaries were found, which might be brought into relation with the changes in the mamma; in other cases there were no such factors. The connective tissue of the mamma in these cases of general hypertrophy shows an increased cellularity, and this

[*To face* p. 32.



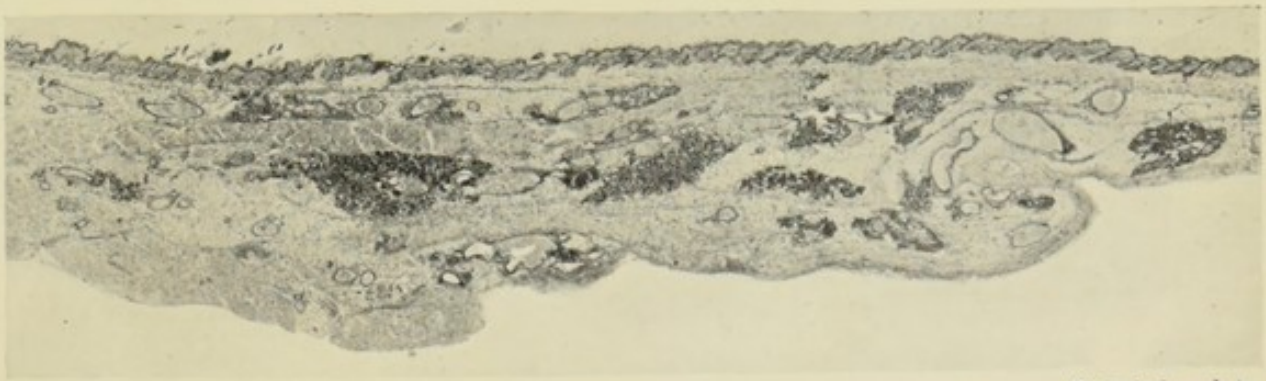
W. Imboden, phot.

FIG. 18.—Mouse 230. Squamous-celled cysts in the mamma. In the squamous epithelium of the central cyst several mitoses are found. $\times \frac{60}{1}$.



W. Imboden, phot.

FIG. 19.—Mouse 418. One of the smaller nodules from the right axillary mamma. In a sclerotic mass of connective tissue several small epithelial cysts are seen, containing concretions. The epithelium in the cysts to the left show curious changes, the cells are goblet-shaped and hypertrophied. Note inflammation in the sclerotic connective tissue. The normal skin at the top of figure. $\times \frac{60}{1}$.



W. Imboden, phot.

FIG. 20.—Mouse 430. Left axillary mamma cut without separation from the skin. Numerous separate hypertrophic nodules are seen throughout the whole gland, in each case in connection with its duct. $\times \frac{12}{1}$.

fact indicates that the condition is no longer a physiological one, but some diffuse pathological change. Mouse 210 (*cf.* p. 47) is a typical instance of this kind.

Nodular Hypertrophy of the Mammary Epithelium.

The hypertrophic condition, when circumscribed, has a special interest because of the similarity of the lesions to early tumour formations. All degrees of the process are found from a slight increase in the number of the normal acini forming one lobe, to definite hypertrophic nodules. On the other hand, all gradations exist between such hypertrophic nodules and true tumours, and changes are often found where it is impossible to decide between the alternatives of a hypertrophic nodule or a commencing tumour. These changes in the gland epithelium are generally accompanied by a more cellular connective tissue. They are very often multiple, and then occur either in the same mamma or in several mammæ from the same individual. The demarcation of such an incipient nodule from the rest of the mamma is often very indefinite, and it may be impossible to tell where the pathological acini begin and the normal ones end.

Fig. 20 illustrates a case of this kind in which all the mammary glands show such nodular hypertrophy. In another part of the same mamma is found a typical adenoma, and in another mamma of the same individual an adeno-carcinoma.

The frequent association of all stages of nodular hypertrophy of the mamma with the presence in the same gland of what are already true tumours makes it very probable that the hypertrophic nodules have to do with the development of tumours, the relations being either such that they are local hypertrophies of the gland, which in a large number of cases form the basis from which the tumour proliferation starts, or that they are already tumour formations from the beginning. Apolant first observed these hypertrophic nodules; he considers them rather as local hypertrophies than as tumours. He remarks, however, that a sharp distinction between both forms is impossible, as a complete encapsulation is only required to give the picture of an adenoma with all its features.

As these nodules have been found mostly in mice with cancer in another mamma, it must first be investigated whether they can be secondary nodules. The crucial test that they are not, is afforded by the examination in serial sections, which shows that they all have their normal connection with the mammary ducts. Furthermore, through

staining of the elastic fibres, it is possible to show ducts in the interior of the nodule, surrounded by an elastic coat which proves them to be long pre-existent on this site, and the relations are such as are never found in metastatic nodules. Therefore they are really hypertrophic parts of the mammary gland, situated in their proper anatomical position, and the adenomatous formations into which they develop are true tumours developing multicentrically. The fact of this nodular hypertrophy being found in spontaneously affected mice in which fresh tumour nodules developed is another point in favour of the view already discussed, that such fresh nodules may be multiple primary tumours.

The details are given below of some of the more interesting cases in which this nodular hypertrophy was observed.

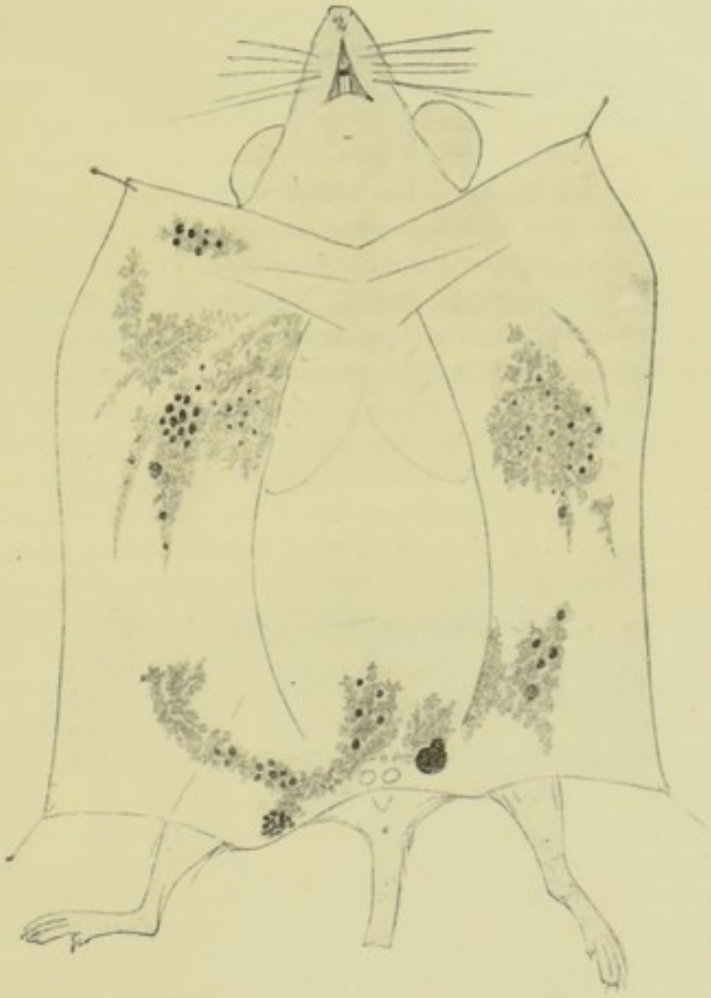
Mouse 430, a female, came from the breeding-experiments of the laboratory. Of the previous history it may be noted that the mouse had littered only once previous to the development of the tumour. When the mouse was fifteen months' old, a small adeno-carcinoma was found on the right shoulder. It was operated upon, and a recurrence appeared six weeks later under the front part of the scar. It was again operated upon, and remained without recurrence eighteen weeks, *i. e.*, until the mouse was killed. At the autopsy multiple minute nodules, just visible to the naked eye and of a brownish colour, were found in all the mammæ. In addition a tumour as large as a pea was present on the left side of the vulva. In the lungs there were three small nodules, which on microscopical examination were found to be lung adenomata. One of them measured 2×3 mm., and had the characters of a real tumour, the others were small hypertrophic nodules and did not show signs of active growth.

All the mammæ were cut in serial sections, the axillary mamma on both sides without separation from the skin. Figure 20 shows a section through the mamma in the left axilla. The whole mamma is full of small hypertrophic nodules, which can be shown to be entirely independent of each other and in each case retain the normal connection with the corresponding mammary duct. There can thus be no doubt that these nodules arose *in sitû* from parts of the mammary gland. The nodules are not encapsulated, their delimitations from the normal mamma being indistinct. The connective tissue is more cellular than normal; it contains here and there accumulations of lymphocytes, partly resembling minute lymph nodes in the perivascular tissue and round the ducts (*cf.* fig. 24) partly showing inflammatory character. Whether the inflammatory reaction in this case is primary or secondary to the changes in the epithelium cannot be decided.

The epithelium of the acini in most of the hypertrophic nodules has the normal appearance. The cells do not show signs of active growth and no mitoses are found. They contain a great amount of brownish pigment, like blood-pigment, giving them a resemblance to phagocytic cells. In some acini nearly every cell is full of this pigment.

In this animal these changes are present in the mammæ from both axillæ and both groins, including the mamma near the vulva. In the left axillary mamma shown on the photograph (fig. 20) there are upwards of a dozen separate nodules. Are these

nodules to be considered a hypertrophic condition of the mamma or are they already incipient tumours? For the larger nodules (not illustrated in the photograph) there can be no doubt; they form regular adenomatous growths, in which the epithelium, here and there, is irregular. In them several mitoses in the epithelial cells indicate that active growth is taking place. As the nodule grows it becomes sharply limited, apparently encapsulated. A nodule of this kind is found in the left axilla; furthermore, in the mamma to the left of the vulva a tumour as large as a pea with



J. R. Ford del.

Fig. 21.—Mouse 490. Multiple minute hypertrophic nodules in the mammæ, reflected with the skin. The structure of one of the nodules in the right axillary mamma is shown in fig. 22. The figure also illustrates the zone free from mammary gland (*cf.* Third Sci. Report, fig. 24, p. 84), employed for autologous inoculation (*cfr.* p. 56).

adeno-carcinomatous structure is formed. For the smaller nodules direct evidence of active growth is missing.

Mouse 490, an old female from an outside breeder, of unknown age, came in pregnant, and with a large hæmorrhagic tumour to the left of the vulva. The tumour was removed by operation. The mouse littered three days later, 3 young surviving for a few days only. The tumour remained apparently without recurrence for

10 weeks, when the mouse died. At the autopsy all the mammæ were found more voluminous than usual, containing numerous minute nodules, varying in size from a pin-point to one the size of a hemp-seed, in the case of a nodule on the right of the vulva (*cf.* fig. 21).

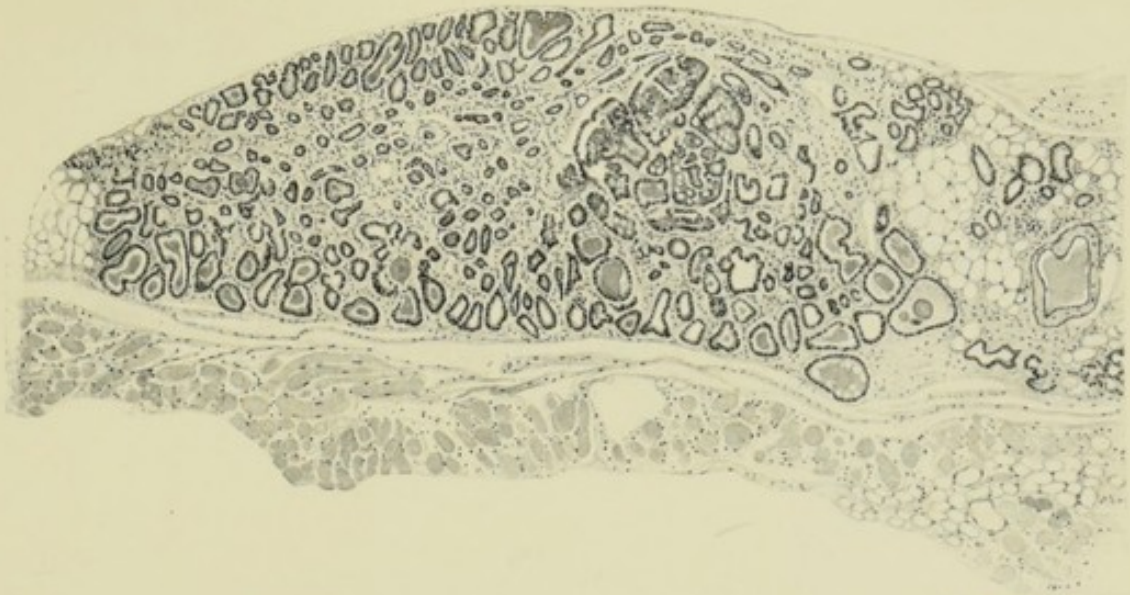
A small nodule (3×3 mm.) was found to the left of the vulva, on the site of the original tumour. The mammæ were dissected out and examined in serial sections. The mammary gland is, on the whole, slightly hypertrophic as if physiologically active, its acini dilated, often containing secretion and in some parts concretions. The nodules observed with the naked eye correspond to localised hypertrophic parts, of which one is illustrated in fig. 22. The stroma is slightly cellular; here and there small foci of inflammation are found, for which no likely cause could be ascertained. In most of these nodules there is no evidence of active growth, but in some of them atypical forms of acini with signs of pressure on the surroundings are seen, as in fig. 22, indicating that one no longer has to deal with a simple hypertrophy.

Mouse 462, a female of the breeding-experiments, *had been kept isolated since young and had never been with a male.* When the mouse was 11 months old a small tumour was observed on the right side of the vulva. The tumour was removed by operation; histologically it showed mainly adenomatous structure. Thirteen weeks later a small recurrence was felt at the same place; it presented again the histological picture of an adenoma. The mouse was then killed, and the mammary tissue from both axillæ and both groins dissected out and fixed in Zenker's fluid. Three minute hyaline nodules could just be discerned by the naked eye in the mamma of the right axilla and right groin.

Microscopical examination in serial sections of the different mammæ shows in the main the same picture. In all mammæ there is found nodular hypertrophy in a very early stage, the nodules being minute with considerable increase of the cellularity of their stroma. In some of them the epithelium is definitely atypical with several layers of cells and even solid acini (fig. 23). A few mitoses were seen. Adjacent to the hypertrophic nodules, widened ducts are prominent with some sclerosis (also with some increase of elastic tissue). The interstitial tissue of the mamma is, in parts, slightly cellular and sclerotic; here and there an infiltration of round cells along the nerves and scattered foci of inflammation occur.

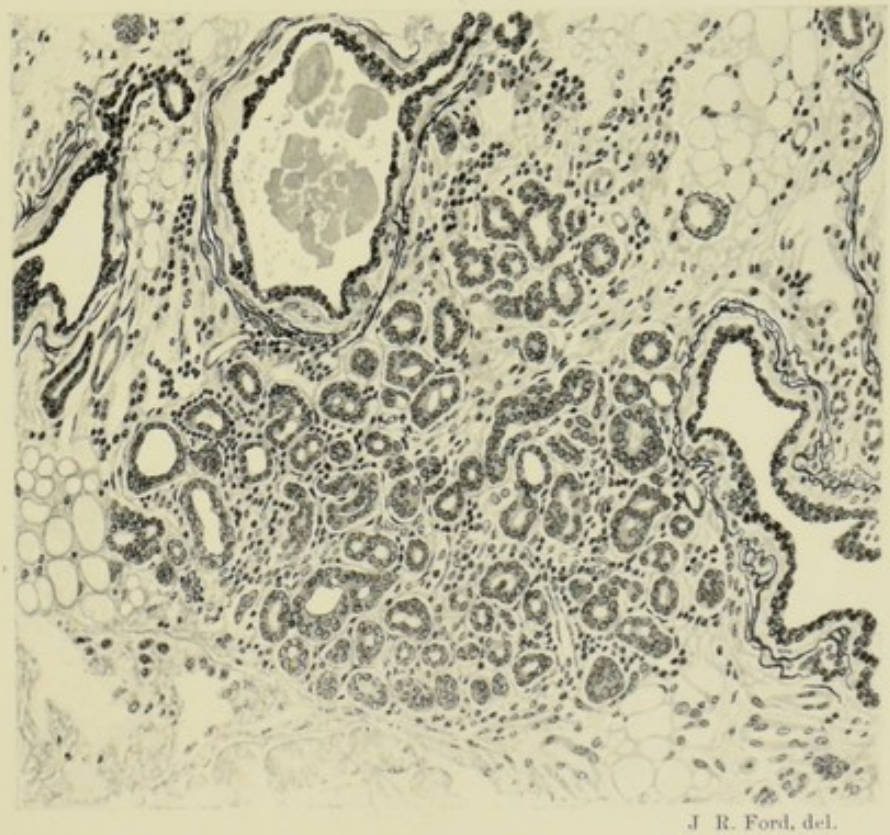
Mouse 370, a female of the breeding-experiments, *had been kept isolated since young and had never been with a male.* When the mouse was 16 months old, a tumour as large as a hazel-nut was found on the left shoulder. The tumour—a hæmorrhagic adenocarcinoma—was removed by operation; at the same time 0·25 c.c. of mouse-embryo-skin was injected on the back for immunising purposes. A new tumour developed on the right side of the vulva, two weeks after the operation; it grew only to the size of a pea, and then remained almost stationary for about 15 weeks, when it was removed by operation. There was no recurrence from this second operation up till the death of the mouse, 18 weeks later (35 weeks after the first operation). Recurrence did not follow the first operation for 26 weeks, when a small nodule was found on the primary site, the left shoulder. Five weeks later (31 weeks after the first operation) a small nodule was observed on the left side of the neck, and again three weeks later a small nodule on the right side of the neck and when the mouse was killed also two nodules in the right axilla. None of these nodules were in the lymph glands, but they were embedded in the mammary tissue, while the lymph glands were found free. There were numerous small nodules on the surface of both lungs; on microscopic examination they are found to be secondary nodules of remarkably

[*To face* p. 36.



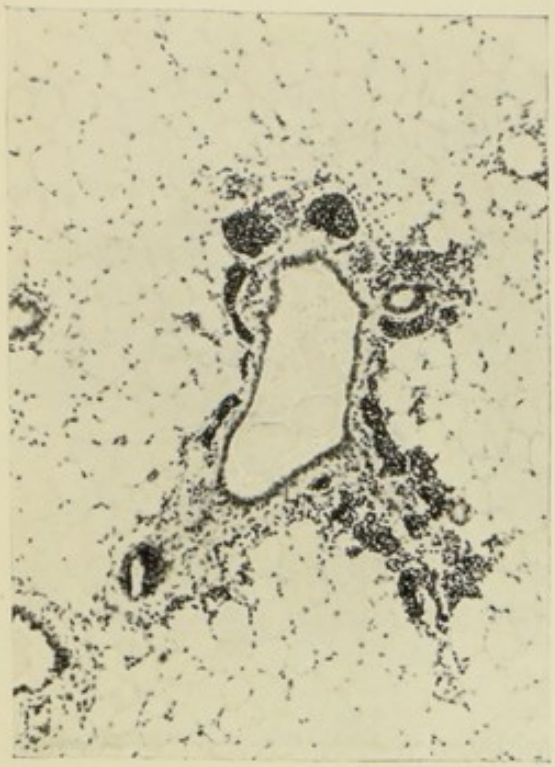
J. R. Ford, del.

FIG. 22.—Mouse 490. One of the nodules shown in fig. 21 at a higher magnification. The nodule consists of mammary acini, on the whole of fairly normal appearance, only increased in number with increased cellularity of the stroma. In the lumina concretions are seen. In one place the acini have assumed atypical form with signs of pressure on the surroundings: no other sign of active growth. $\times \frac{56}{1}$.



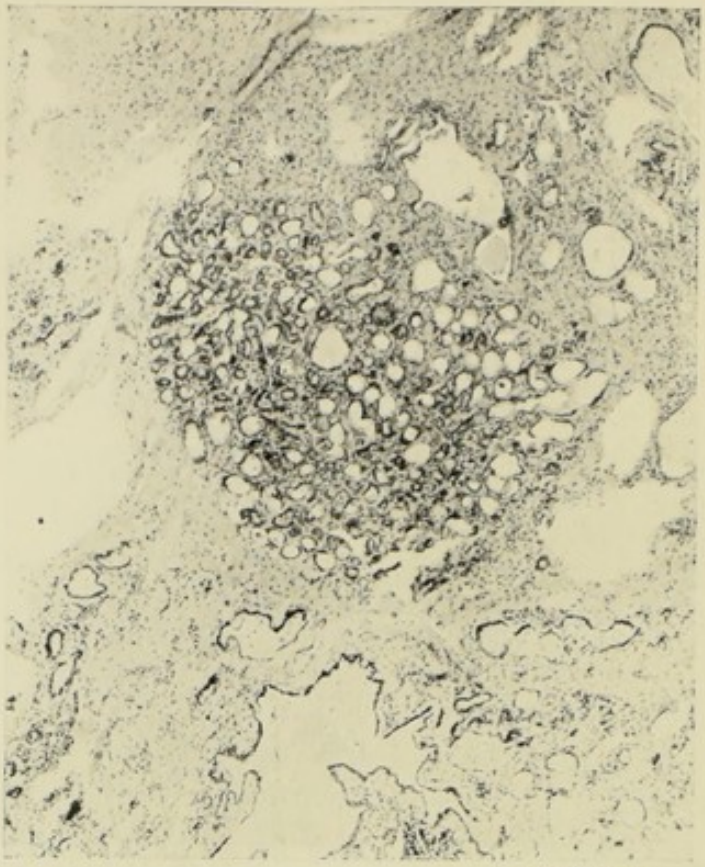
J. R. Ford, del.

FIG. 23.—Mouse 462. One of the small hypertrophic nodules in the mamma, cut through its largest diameter. The epithelium of the acini is distinctly atypical, the cells are larger and more crowded together than in a normal acinus, so that several layers of cells are found lining some acini (not oblique sections). A few mitoses are found. $\times \frac{166}{1}$



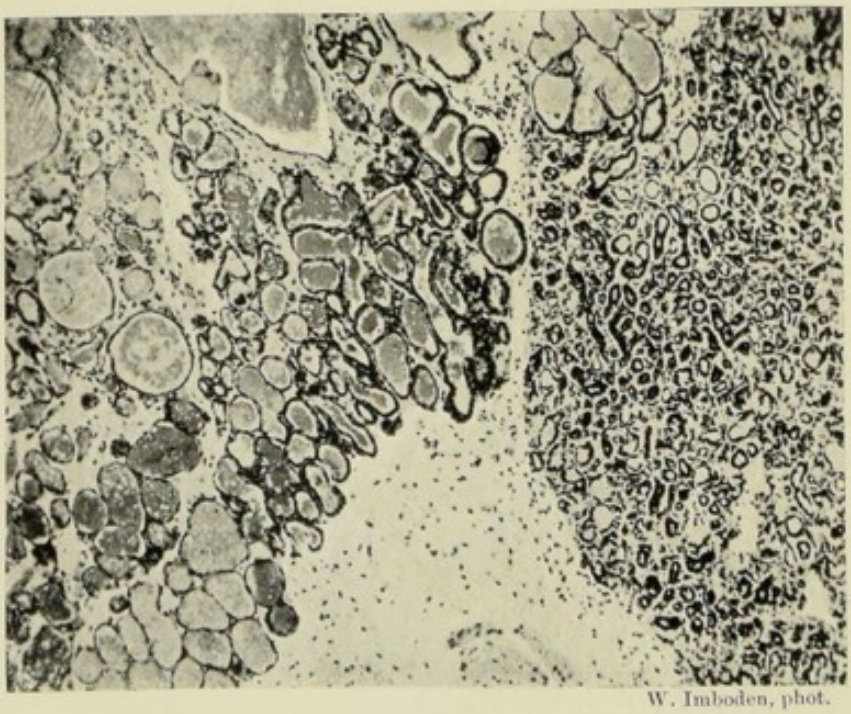
W. Imboden, phot.

FIG. 24.—Mouse 430. Mammary duct with circumscribed accumulations of small round cells. $\times \frac{100}{1}$.



W. Imboden, phot.

FIG. 25.—Mouse 370. Hypertrophic nodule in the left inguinal mamma, surrounded by sclerotic connective tissue with dilated ducts. $\times \frac{60}{1}$.



W. Imboden, phot.

FIG. 26.—Mouse 406. Small adenomatous nodule from left axilla, not sharply delimitated from the rest of the mamma. The mamma (to the left and at the top of fig.) seems to be in a state of physiological activity, as the acini are filled with secretion, whereas the cells of the adenoma (to the right) do not seem to have responded to the same stimulus. $\times \frac{100}{1}$.

adenomatous structure; some of them intravascular emboli, others invading the lung tissue. Apart from these metastatic nodules, one lung adenoma is found.

The mamma from both axillæ and both groins is hypertrophic in all places, in some places more diffusely, in others locally. The connective tissue is on the whole remarkably cellular and sclerotic. One of the hypertrophic nodules from the left groin is shown in the photograph (fig. 25), which also shows the sclerosis of the surrounding tissue.

In this case the several tumours on the right side of the neck, in the right axilla, have all the appearances of being new primary tumours and not metastases from the original tumour.

Mouse 406, a female from the breeding-experiments, when 24 months old, showed a small tumour, as large as a pea, in the left groin. The tumour remained practically stationary during the four weeks the mouse was under observation. When the mouse was killed the uterus was found to be enlarged and examination revealed epithelial cysts with the appearance of normal uterine glands throughout the uterine wall and under the serosa. The right ovary was enlarged and hæmorrhagic. The mammæ were more turgescent than usual; they were examined from both axillæ and both groins. The primary tumour in the left groin is an adeno-carcinoma with small acini. In the mamma of the left axilla there is a small adenomatous nodule with cellular stroma, not sharply delimited from the mammary tissue (fig. 26). The rest of the mamma is somewhat diffusely hypertrophic and resembles lactating mamma. In this case it is remarkable, that, although the rest of the mamma is in an active state—the acini being filled with milk—the adenomatous part does not seem to respond to the stimulus which led to this secretory activity. In another case, however, the cells of an adenomatous tumour are seen to respond by secreting in the same way as the neighbouring lactating mamma.

(b) *Mamma of Non-cancerous Old Mice.*

From what has been shown both the cystic condition of the mamma and the nodular hypertrophy seem to have close relations to the development of real tumours. Apart from the epithelial changes the inflammatory and sclerotic processes in the connective tissue of several of these mammæ are striking. In many cases the appearances suggest that the development of tumour has taken place in a diffusely diseased organ. The question arises whether one can get any nearer to the cause of these lesions.

It is obvious that for an investigation of this kind the process must be examined at as early a stage as possible. The cases just described may be early enough as far as tumour development is concerned, in fact very often one cannot tell whether the development of a real tumour has begun or not, but they are obviously too advanced for the investigation of the possible causes of the nodular hypertrophy or of the changes in the connective tissue.

For this purpose a number of non-cancerous old mice have been examined. They have been chosen of different breeds, all fairly advanced in age, and from batches of mice among which cases of spontaneous cancer have been observed.

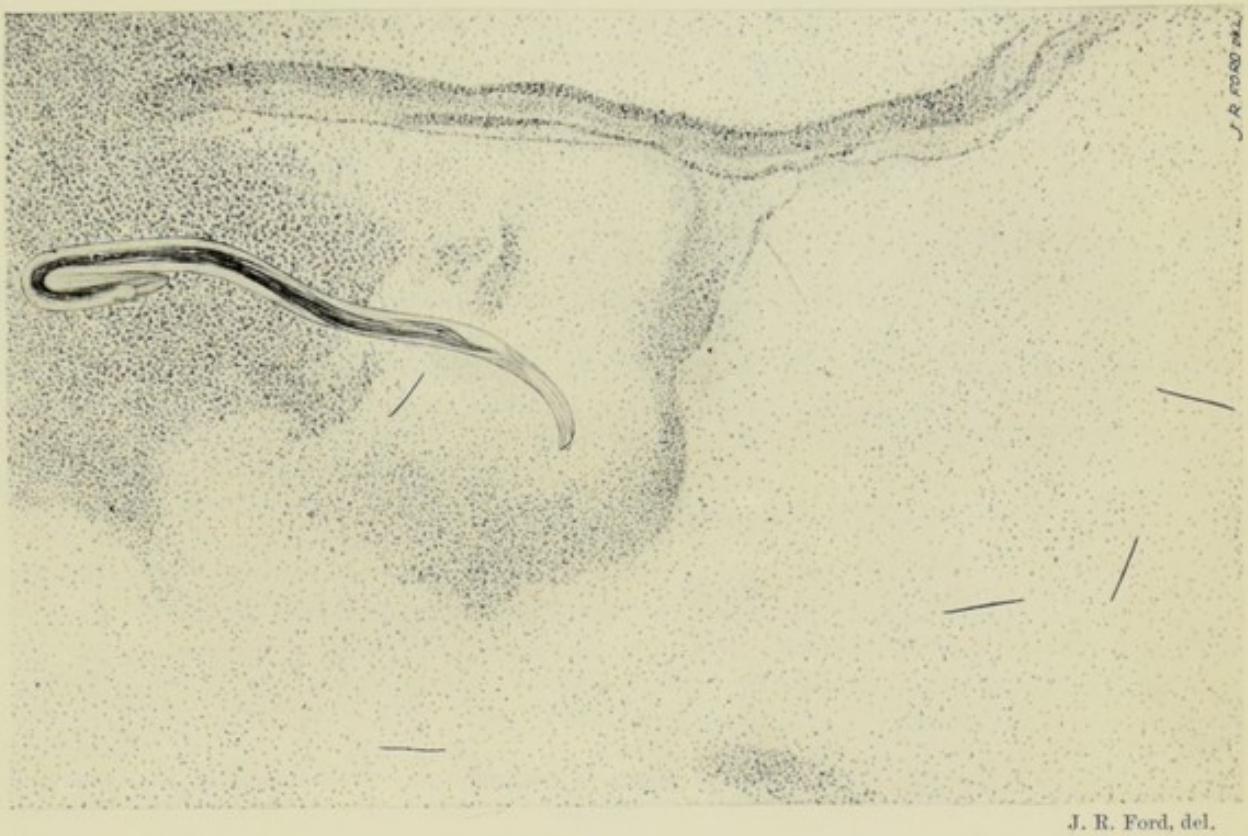
The general result has been the finding of marked inflammatory changes of the connective tissue in a large number of such old mice kept in the laboratory. Sometimes the inflammation is quite acute and localised, most often it appears as streaks of infiltration of round cells following the vessels, and to a less extent the mammary ducts and nerves, and is accompanied by sclerosis of the connective tissue. The search for an explanation of the interstitial changes has led to the finding of one of the possible factors, which up to the present seems to have passed almost unobserved, viz., the presence of nematodes in the subcutaneous tissue of old mice. Borrel first observed nematodes in a vessel of the lung in a case of generalised lymphoma *. Quite recently † he has stated that he has found them in cysts of the mamma and in young tumours and in their surroundings, also in the mediastinal glands of cancerous mice. He considers them the possible carriers of a cancer-virus.

In the material which has been examined, nematodes have been found in the subcutaneous tissue in a large proportion of old normal mice from different breeders. They may be seen with a pocket lens as tiny grey specks either free in the glassy subcutaneous tissue or coiled up in a small greyish nodule, but in most cases they are only found by the method described below, or more occasionally by examining the mamma in serial sections. When present they are mostly few in number and discoverable often only after prolonged careful search, but as many as half a dozen or more may be present in the connective tissue of one mamma. They have also been found in the vessels of the lungs and once in the pleura. The worms observed up to the present (upwards of 50) have all been females containing large numbers of embryos or eggs, whereas males have not hitherto been observed in the subcutaneous tissue. In a great number of cases the large embryos are fully developed and when liberated by pressure on the cover glass, they are able to move about freely. That the liberation of the embryos may take place in the subcutaneous tissue is shown by the fact that healthy looking embryos have been found there,

* Illustrated in a paper from Borrel's laboratory by the writer in the 'Annales de l'Institut Pasteur,' 1905, no. 3.

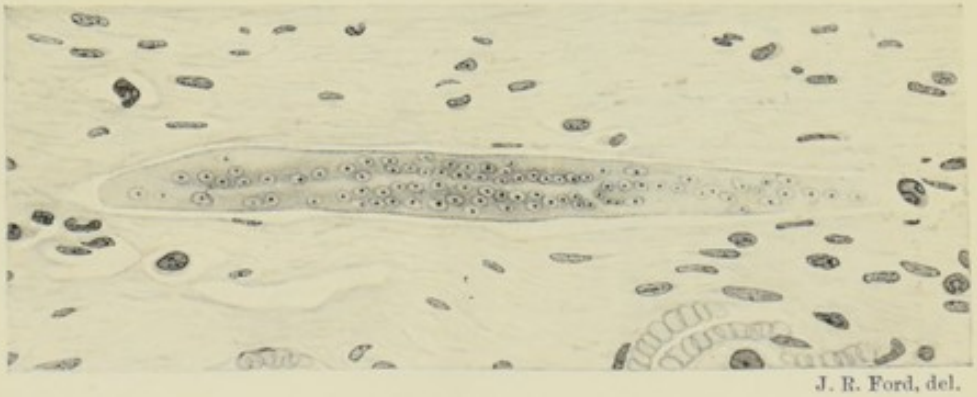
† 'Annales de l'Institut Pasteur,' no. 10, 1910.

[*To face* p. 38.



J. R. Ford, del.

FIG. 27.—Old normal female mouse. Subcutaneous tissue stretched out and stained (*v.* p. 39). A nematode is seen containing large embryos; five extruded embryos are seen in the subcutaneous tissue at a considerable distance from the mother worm. $\times \frac{25}{1}$.



J. R. Ford, del.

FIG. 28.—Represents an oblique section of a nematode embryo free in the interstitial tissue of the mamma of old normal female mouse [(6) p. 40], at a high magnification. $\times \frac{525}{1}$.

as illustrated in figs. 27 & 28. As will be seen from the latter figure, the embryo has not yet got any chitinous envelope. The absence of distinctive histological features makes it very difficult to follow their fate in the tissues.

It is astonishing how difficult it is to find the embryos in the tissues in stained sections. Their presence can best be demonstrated by the following method:—The subcutaneous tissue from axilla to groin is carefully dissected out and stretched on a large slide, covered by a cover-glass, after addition of a drop of saline solution, and examined by a low power lens. If nematodes are present, the slide is fixed (*e. g.*, in alcohol 70 per cent. with 5 per cent. acetic acid after Boveri) and stained, *e. g.*, in borax carmine with differentiation in acid alcohol. In this way numerous embryos can be found which have made their way into the subcutaneous tissue to a great distance from the mother-worm. Fig. 27 represents a preparation obtained by this method; in one field five embryos are seen.

The fact that only fertilised females are found in the subcutaneous tissue, makes one look for another place where males are also to be found and where the fertilisation takes place. Up to the present one has not succeeded in finding males. In the large intestine of the mouse other nematodes are found, both males and females. This nematode of the intestine is probably *Oxyuris tetraptera* *, characterised by four flanges on the side of the head end; the anus is at some distance from the posterior extremity. The worms in the subcutaneous tissue show some different morphological characters; flanges cannot be seen on the worms in the tissues, and the anus is terminal. Hence it must be concluded that in all probability they are two different worms. Up to the present the worm in the tissues has not been found in the intestines.

Whatever may be the exact life-history of these nematodes, it is natural to associate their presence with some of the local or diffuse inflammatory changes in the connective tissue of the mamma, for which, as yet, there is no other adequate explanation. It is evident that the nematodes are capable of setting up intense inflammatory processes. The site where they have rested for some time (encapsulated?) is lined by a zone of leucocytes, and other signs of inflammation follow their path. This occurs whilst they are living; when they lie dead in the tissues a violent reaction with leucocytes and round cells and phagocytosis is set up, and the nematode soon becomes unrecognisable. The mixture of acute and chronic inflammation in the interstitial tissue with sclerotic changes is quite characteristic, and in the majority of cases where this picture has been found it has been possible to demonstrate the round worm. It must be pointed out that

* Mr. A. E. Shipley, F.R.S., and Dr. Leiper kindly identified the species for us. The worm in the subcutaneous tissue cannot with certainty be identified with any of the forms described; in general characters and mode of occurrence it resembles most closely *Ollulanus tricuspis* (Leuckart, Menschliche Parasiten II, p. 102).

the change is essentially one of the loose connective tissue, and the mammary gland is involved only indirectly and only in so far as it is embedded on all sides in this tissue.

A summary is given below of some cases in which these nematodes have been found in normal old mice along with the results of examining their mammæ in serial sections.

(1) Old female from breeder R. The mammæ were slightly orange-coloured. On microscopical examination they all showed marked increased cellularity of the connective tissue in the form of small inflammatory foci and streaks of infiltration following the ducts and vessels (fig. 29). The mammary parenchyma did not exhibit any marked pathological changes. A keratinised cyst was found in one of the mammæ; it appeared to have arisen in connection with one of the smaller ducts. In one place a sarcocystis on its way to the muscles was found inside a blood-vessel.

A dead nematode was found in one place coiled up in a subcutaneous nodule, surrounded by a broad inflammatory zone, mostly consisting of leucocytes. No other causes of inflammation could be detected, in particular no micro-organisms were found.

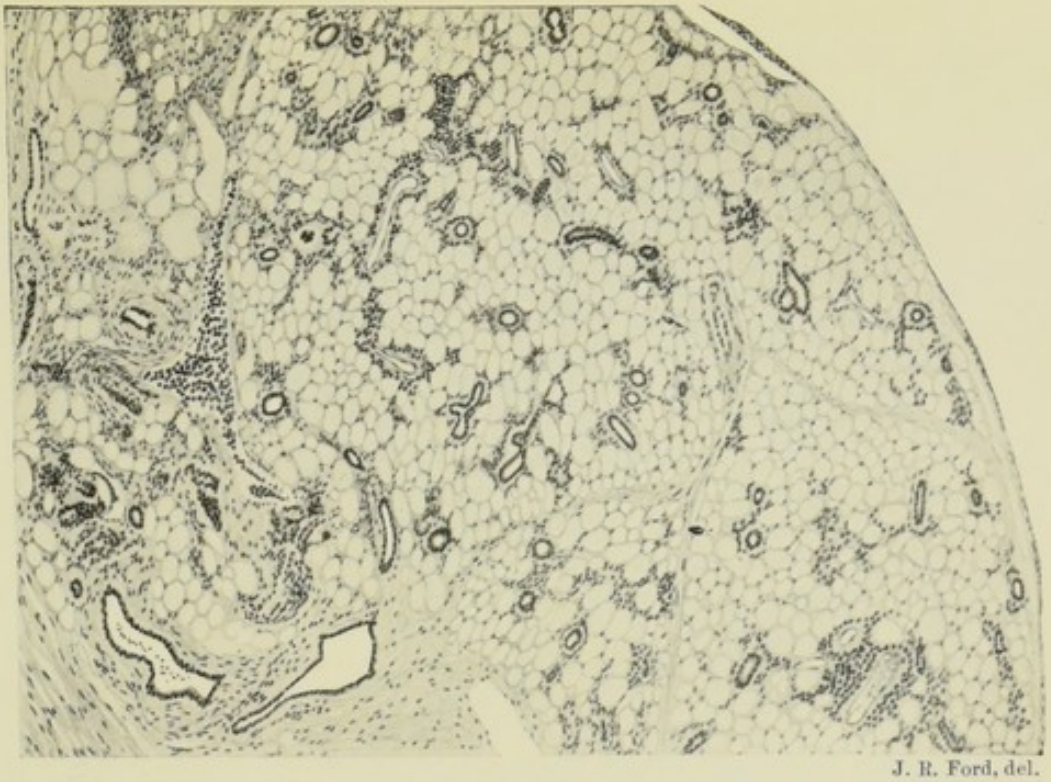
(2–4) Three other old females from same breeder. In all three cases nematodes could be found in the subcutaneous tissue with the pocket lens; in one case only after prolonged search. In all of them the mamma offered in the main the same picture; the parenchyma was somewhat atrophic, the connective tissue sclerotic with scattered foci of inflammation and infiltration of round cells along ducts and vessels; here and there a more diffuse cellularity was seen. The only cause that could be ascertained for these changes was the numerous nematodes that were found, in one case as many as half a dozen in one mamma; some dead and surrounded by strong reaction, others without a trace of reaction as an indication that they had been moving about up to the moment of fixation. The lungs of these cases show numerous inflammatory foci; in one a small lung adenoma is found, illustrated in fig. 31.

(5) Old female from breeder B. The mammæ were atrophic with sclerotic interstitial tissue especially developed around the ducts and the small vessels. In some parts there was marked cellularity of the intramuscular tissue. A dead nematode surrounded by a broad zone of reaction was seen in one mamma; in another a living worm was present between the foci of inflammation.

(6) Another old female from the same breeder. The mammary parenchyma was atrophic, the ducts surrounded by sclerotic tissue. The interstitial tissue showed scattered foci of inflammation and was more cellular than usual. Nematodes were found in all mammæ. Fig. 30 shows a worm in close vicinity to some mammary acini; there is no reaction round the place where the worm was situated when it was fixed, but at the bottom of the figure there are streaks of inflammation which may be followed through other sections up to the worm. In this case embryos have been found free in the subcutaneous tissue (see fig. 28). In the vessels of the lungs nematodes were found in three places; the changes induced are illustrated in fig. 32.

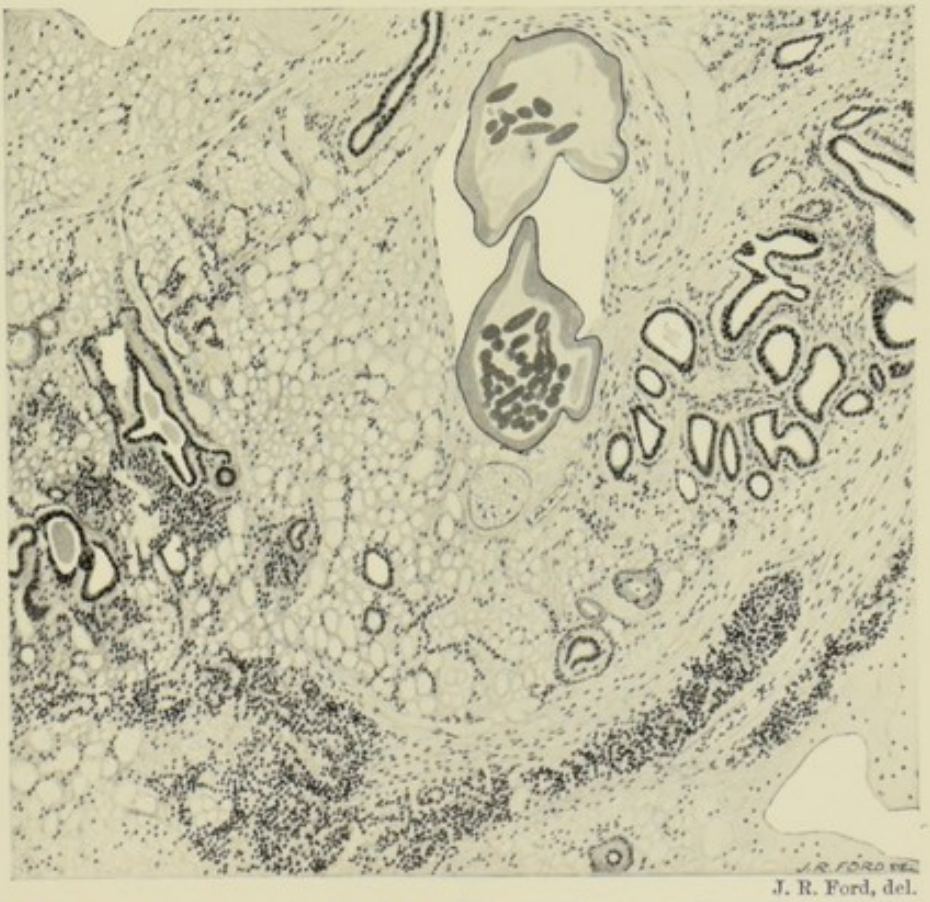
(7) A 25 months old female of the breeding-experiments, which had been kept isolated since quite young, and had never been with a male. All mammæ showed a remarkable sclerosis and cellularity of the connective tissue, with dilatation of

[*To face* p. 40.



J. R. Ford, del.

FIG. 29.—Mamma of old normal female mouse [(1), p. 40], embedded in fatty tissue. Shows diffuse inflammatory changes with infiltration of round cells along vessels and mammary acini, forming larger accumulations locally. In another part of the subcutaneous tissue of this mouse a nematode was found, surrounded by a reactive zone of leucocytes and lymphocytes. $\times \frac{87}{1}$.



J. R. Ford, del.

FIG. 30.—Mamma of old normal female mouse [(6), p. 40]. Shows a nematode in close vicinity to some mammary acini, with streaks of inflammation in the lower part of the figure, which can be followed through other sections up to the worm. $\times \frac{130}{1}$.

ducts and *nodular hypertrophy of the gland*. In one place a nematode was found containing large embryos; its intestine was full of blood.

In addition to the cases just mentioned in which the actual presence of nematodes in the subcutaneous tissue could be demonstrated, a dozen normal old mice were carefully examined and their mammæ cut in serial sections, in which no nematodes were discovered. Only in two or three of these, however, can the mamma be said to present a fully normal picture. In all the others streaks of inflammation and sclerosis by far in excess of what one would expect to find in a merely senile mamma are found in the interstitial tissue. In three of them hypertrophic nodules with cellular stroma are found. In another dozen mice the subcutaneous tissue was examined in the way described above, but the mammæ were not cut. In eight of them, nematodes were present in the subcutaneous tissue. On the whole 32 normal female mice were examined, in half of which the round worm was successfully demonstrated in the tissues *. In about two-thirds of these cases, *Oxyuris tetraptera* was found in the intestine, mostly few in number, sometimes only two or three, in several cases in large numbers, but no nematodes of exactly the same appearance as those in the subcutaneous tissue were found in the intestines.

The frequency of interstitial changes—inflammation and sclerosis—in the mammæ of old normal mice of the material of the laboratory is striking. There is evidence that the presence of nematodes in the subcutaneous tissue may be made responsible, at least to a large extent, for such interstitial changes. In several cases these changes coexist with typical nodular hypertrophy, and this association suggests that the different pathological changes, inflammatory processes, sclerosis and cellularity of the connective tissue, and the nodular hypertrophy of the epithelium may be linked together and referred to a common cause, the presence of nematodes in the subcutaneous tissue. Hitherto only old mice have been studied and the finding of nematodes with subsequent inflammatory changes all refer to them. The evidence that similar processes may be going on in young and adult animals is at present scanty, and limited to the occasional findings of nematodes in one case in the subcutaneous tissue and in another in the lungs of mice with transplanted cancer. However, there can hardly be any doubt that this process may go on either once or repeatedly in the connective tissue of younger mice, as has been seen in older ones †.

* In addition half a dozen males were examined; the worms were found in them in the same proportion as in females. The mammæ consist in males of a few short ducts embedded in fatty tissue or in hibernating gland; no mammary acini are seen.

† Until the life-history of the round worm is ascertained and the mode of infection known, there are a number of points that cannot be satisfactorily cleared up with regard to their rôle in the life of the mouse. The purpose of the present paper is intentionally restricted to the inflammatory reactions associated with their presence.

Inflammatory changes in the mamma associated with the presence of nematodes are found also in several tumour mice of the present material. It must be confessed, however, that as yet only a very small number have been investigated thoroughly for this purpose.

Mouse 506, an old female from an outside breeder, came in with a hard flat tumour in the left axilla and a smaller cystic nodule on the left side of the neck. The tumour in the left axilla felt as if composed of several smaller nodules. The tumour was not operated upon, but the mouse was killed four weeks after admission. The tumour proved to be a papilliferous and cystic adeno-carcinoma with sclerotic stroma (a structure corresponding to that of tumour 418, *vide* p. 31); the nodule on the left of the neck was only a cystic lymph gland. The right axillary and both inguinal mammæ were excised. In all places the gland, embedded in fatty tissue, presented an atrophic appearance; the connective tissue was here and there somewhat cellular, in the form of streaks of infiltration following small vessels or mammary ducts; a few foci of more recent inflammation were seen. In all parts of the mamma nematodes were found free in the subcutaneous tissue, in some places two or three together. In two places embryos were seen free in the tissues.

Mouse 501, from an outside breeder, came in with two growths, one hæmorrhagic adenocarcinoma on the right of the neck and another in the left groin. Both were removed by operation, and remained without recurrence nine weeks, when the mouse was killed. Small points were seen on the surface of the lungs, but no naked eye metastases.

The mammæ were examined from both axillæ and both groins. On the whole the gland is atrophic, with small *hypertrophic nodules* in three places. The interstitial tissue shows sclerotic strands with increased cellularity and small inflammatory foci. In two places a nematode is found *inside a vessel.*

Mouse 479, from an outside breeder, came in with a hæmorrhagic mammary adeno-carcinoma on the left shoulder. The tumour was removed by operation, and remained without recurrence until the death of the mouse, sixteen weeks later. No metastases visible to the naked eye were found in the lungs.

The interstitial tissue of the mammæ was firmer than usual. On microscopical examination it is extremely sclerotic and cellular, with streaks of inflammation. The mammary gland contains a few hypertrophic nodules. The ducts in some places are embedded in large masses of sclerotic tissue in which groups of compressed epithelial cells (acini ?) are seen scattered about. In one place a nematode is found in the interstitial tissue.

(c) *Possible causes of the Nodular Hypertrophy.*

It is very probable that the nodular hypertrophic changes in a large number of cases form the basis on which tumours develop. They can hardly represent a developmental malformation, since they have never been found in the large numbers of young mice which have come under observation. They have only been found in mice of advanced age in an organ in which other pathological conditions are

very frequent, and this fact renders it more likely that they are changes acquired during life.

If they are acquired changes a hypertrophic condition of the mammary gland might well be thought to have a close relation to influences of a physiological order. In fact it has been assumed that the frequency of mammary tumours in mice could be brought into relation with the physiological demands made upon the mamma through a continued condition of pregnancy and lactation *. This point is, however, open to experimental investigation, and it can be proved that the physiological factor is not the all important one. The records of the breeding-experiments of the laboratory give the following data with regard to the previous history of the cases which have developed spontaneous cancer.

Of 74 mice which had developed mammary tumours, and which were under observation from birth, only 33 are recorded to have littered previously to the development of the tumour, while no litter is recorded for 41. Analysing this latter figure, there is conclusive evidence that 20 have been completely isolated since quite young, and have never been with a male ; the other 21 have not been so strictly isolated, but may at some time or other have been in a cage along with a male; none of them have been found to have littered. Of the 33 having littered previously to development of the tumour :—

1 litter is recorded in		11	cases.
2 litters are	„	7	„
3 „	„	7	„
4 „	„	5	„
5 „	„	2	„
7 „	„	1	„

The figures show that it is hardly likely that excessive physiological demands made upon the mamma play the determining rôle in the development of tumours of this organ, but that other factors must be looked for. The same applies to the condition of nodular hypertrophy. Among the mice of which the previous history is known, the most pronounced cases of this hypertrophy have occurred: twice in mice which have been kept isolated from an early age, and have never been with a male (370, 462) ; once in a mouse which, although the isolation has not been so complete, has never littered (453) ; a fourth has had only one litter previous to development of tumour (430), and a fifth has had two litters (406). These facts do not afford any evidence of the

* Ehrlich & Apolant, Beobachtungen über maligne Mäusetumoren, Berl. klin. Woch. 1905, No. 28; Apolant, ' Die epithelialen Geschwülste der Maus,' 1906.

hypertrophic nodules being caused by the wear and tear of physiological activity of the gland with associated injuries and imperfect involution. The age of these mice at death was for mouse 462 (fig. 23) 15 months, for mouse 430 (fig. 20) 21 months, and for mice 370 (fig. 25) and 406 (fig. 26) slightly above 2 years.

In the absence of other likely causes, the suggestion gains in strength that this hypertrophic condition of the mammary epithelium, and the tumours arising on this basis, may be brought into relation with the inflammatory changes in the interstitial tissue so frequently found, and for which at least one likely cause can be adduced. A direct proof of this association cannot be brought forward at present, and can hardly be given by histological methods alone. Nematodes obviously occur commonly in normal mice, probably far more so than tumours do. If a relation exists, this relation is a mediate one, and to be classed with that of other irritants, analogous, *e. g.* to that of syphilis and keratosis linguæ to cancer of this organ. The fact that in this case the irritant may be a nematode or that the inflammation elicited by it may be a determining factor, adds yet another instance to the large number of irritants of very different kinds having mediate relation to the development of cancer. Numerous analogies exist of the association of malignant new growths with such chronic irritants, animate or inanimate, having themselves nothing in common. The possibility exists that the interstitial changes may be the eliciting stimulus, just as the interstitial changes called forth by the tubercle bacillus in lupus is the primary cause leading up to a prolonged pathological proliferation in the covering epithelium of which the end result may sometimes be lupus cancer.

Whether this working hypothesis be tenable or not, it is open to experimental test, and for the present, in the absence of a better explanation, it makes some of the peculiar features of mouse tumours more comprehensible. One of these is the enormous frequency of mammary tumours compared to tumours of other organs in the mouse. The mammary gland is on all sides embedded in the loose connective tissue in which inflammatory changes are so frequent, and is therefore specially exposed to injuries of this kind. Although undoubtedly the nematodes and their embryos are able to wander about in all tissues *, the loose subcutaneous tissue is the place where they have been found most frequently. Another peculiar feature of mouse mammary tumours is their frequent multiplicity in different mammæ of the same animal. This feature, also, will be more easily understood with the recognition

* Embryos have been found, *e. g.* in the vessels of the preputial gland of a male mouse.

of the fact that the tumours arise on the basis of a diffusely diseased organ.

There are, furthermore, two other peculiar forms of tumour formations in mice to which similar considerations apply, the primary tumours of the lung and the multiple lymphomata.

(d) *Association of Inflammation and Adenomatous Tumours of the Lung.*

The adenomatous tumours of the lung vie with the mammary tumours in frequency. A point in common with the mammary tumours is that they are very often multiple. The nature of these formations is by no means clear. Like the nodular hypertrophy of the mamma, there are very often found in the lungs nodules as to which it is doubtful whether they ought to be considered as hypertrophic conditions or as tumours. Fig. 31 shows a nodule of this kind. In addition to the hypertrophy of the epithelium, a most prominent feature is the remarkable increase of elastic fibres in the walls of the alveoli. These latter are lined with a cubical epithelium ; they have retained their normal size, and there are no signs of active growth of the epithelium (no mitoses, nor evidence of formation of new alveoli with pressure on the surroundings). Other nodules exhibit the characters of tumours ; they show signs of active growth, and the epithelium invades the lumina of the bronchi and the surrounding lung tissue. In these undoubted tumour-nodules the increase of elastic tissue is either absent or confined to the central portion.

The increase of elastic tissue in the smaller nodules before they begin to exhibit active growth is very characteristic ; it cannot be explained as a condensation of lung tissue, and it must be assumed that a new formation of elastic fibres has apparently taken place. It suggests a kind of sclerotic process, and shows that the nodule has been there for some time. The exact relation of the elastic tissue to the epithelium is not clear. It may be that the increase of elastic tissue is elicited as a specific "stroma reaction" to the hypertrophic epithelium, but the possibility also exists that it may indicate an older process in the interstitial tissue to which the hypertrophy of the epithelium is secondary. The associated pleural thickening noted by Tyzzer supports the latter view. These nodules have only been found in old animals, wherefore it is not natural to suppose that they can be considered as developmental anomalies.

When lungs of a number of old normal mice are examined, the frequency of inflammatory processes is striking. Apart from pneumonic

foci and inflammations due to different kinds of micro-organisms, *e. g.* pseudo-tuberculosis muris, localised inflammatory foci are often found along the vessels and bronchi and irregularly in the lung tissue, for which it is not possible to discover any bacterial cause. Up to the present, apart from the subcutaneous tissue, the lungs are the only place where nematodes have been found in any number in the tissues of the mouse. The nematodes are found in the vessels, often in the centre of such an inflammatory nodule. Fig. 32 illustrates the pathological changes which may be caused by the presence of nematodes. A kind of infarct has been formed in the corresponding part of the lung, but no necrosis of lung tissue has taken place. The alveoli of the lung have collapsed; their epithelium is more or less cubical, but otherwise intact. An intense infiltration of lymphocytic elements has taken place in the periphery and endarteritis is present.

It cannot be definitely proved what becomes of these peculiar infarcts, but it is possible that the adenomatous nodules with increased amount of elastic tissue may arise from them. If this be the case the frequency of lung adenomata and their multiplicity in the same animal are made more comprehensible. The origin of tumours on the basis of these formations would be in line with what has been discussed for the mammary tumours arising on the basis of hypertrophic nodules and with the origin of tumours after chronic inflammation or other chronic irritation in general *.

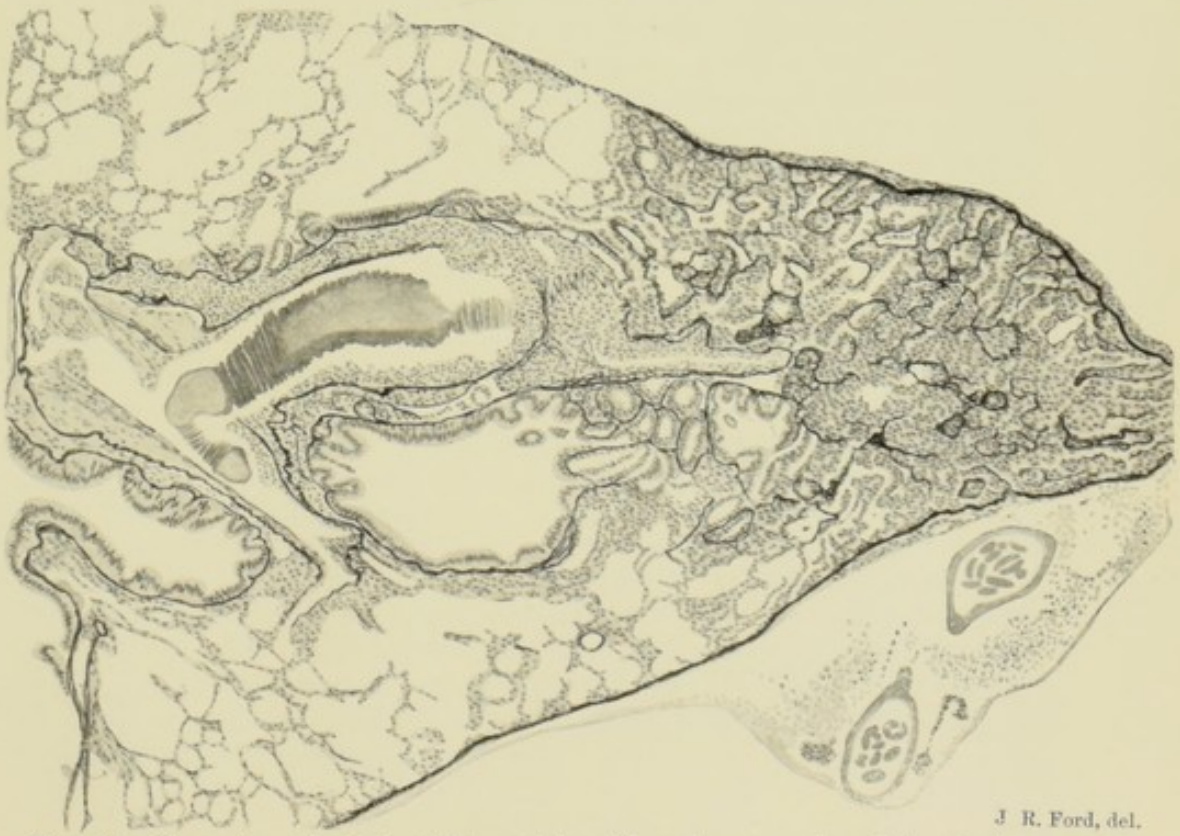
(e) *Association of Inflammation and Lymphomata.*

Another somewhat peculiar tumour formation in mice is the generalised lymphoma so often found in the mice of the present material. It is not improbable that this condition may be brought into relation to similar causes, indirectly or directly. An object found in a vessel of the lung in a case of lymphoma from Borrel's laboratory, and illustrated in the 'Annales de l'Institut Pasteur,' No. 3, 1905 (pl. ix. fig. 5), is undoubtedly a nematode. Our investigation of Borrel's material was for some time directed towards the presence of such nematodes in the glands. The result was, however, negative. Later Borrel and Gorescu have succeeded in finding nematodes in two further cases of this kind. In the present material attention has only been paid to the presence of nematodes in cases of lymphomata, after they had been found so frequently as the cause of inflammatory changes in

* The association of chronic inflammatory processes with primary tumours of the lung has also been noted by Tyzzer. The occasional occurrence of a disintegrated worm is considered by him as a coincidence.

J. R. Ford, del.

FIG. 31.—Adenomatous nodule of lung. Old normal female mouse (*v.* p. 40). Shows the hypertrophy of the epithelium with remarkable increase of the elastic framework of the nodule. The shape and size of the normal alveoli is retained; in the lumina catarrhal cells with carbon particles; otherwise numerous inflammatory nodules throughout the lungs; nematodes present in the subcutaneous tissue. $\times \frac{133}{1}$.



J. R. Ford, del.

FIG. 32.—Mouse 6, p. 40. Infarct-like nodule of lung in connection with a dead nematode in the corresponding branch of the pulmonary artery. No necrosis has taken place, the alveoli of the lung have collapsed, their epithelium is more or less cubical. A live nematode is seen just outside the nodule, imbedded in pleural exudate. $\times \frac{78}{1}$.

the subcutaneous tissue, and only two cases have been examined. In these two cases typical inflammation associated with the presence of nematodes in the subcutaneous tissue has been found combined with a generalised lymphomatous condition, and there may be some reason for assuming some relation between these changes. The frequent hyperplasia of the mediastinal glands and of the thymus seems easier to understand when nematodes can be demonstrated in the mediastinal areolar tissue and in the vessels of the lungs in different places as in the old normal mouse, from which fig. 32 has been drawn.

The two cases of lymphoma in which nematodes have been found are the following :—

Mouse 210, an old female, was brought into the laboratory from an outside breeder with a small swelling under one of the inguinal nipples. This was excised, and was found to be a cyst full of milky secretion, but no tumour. The mouse remained 8 months under observation, looking somewhat ill, with staring coat. During the greater part of this time a general diseased condition of all mammæ was apparent ; at the same time the symptoms of a general lymphomatosis appeared. Small nodules were found in both axillary and inguinal mammæ, besides swollen external lymph glands in the neck, axilla, and groin. At the autopsy there were found lymphomatous tumours in the mediastinum and retroperitoneally, in addition to the external ones. The thymus was enormously enlarged ; there were several nodules in the lungs. The liver and spleen were enlarged and riddled with greyish nodules ; a few of the same nodules were found in the kidneys. The mammæ were full of small milk cysts, and appeared somewhat more voluminous than usual. Microscopically the internal tumours consist of lymphomatous tissue ; the mamma is diffusely hypertrophic, in addition to containing a number of small cysts ; the connective tissue is everywhere cellular, and shows chronic inflammatory changes. No other definite cause for this can be found than that indicated by the finding of a nematode full of embryos in the connective tissue. The staining of the parasite and its embryos leaves no doubt that it was perfectly alive at the moment of fixation. There is no sign of reaction round the worm itself, from which fact it may be inferred that the worm had been moving about.

The lungs of this case are interesting. Scattered all over them there are foci of round cells, of which it is difficult to decide in each case whether they represent inflammatory reactions or dissemination of a lymphomatous tumour. They are mostly perivascular or peribronchial, but are also found intravascular or diffusely infiltrating the lung tissue. In some of these nodules there is some evidence that they are real tumours, *e. g.* in the case of intravascular nodules having perforated the elastic coat of the vessel and invaded the surrounding lung tissue. In addition to these lymphoid nodules, four lung adenomata are found, the largest of which measures 3×3 mm. Two of them are intimately mixed up with lymphoid (inflammatory ?) nodules. In two places of this lung living nematodes are found intravascular, in both cases females containing large embryos. The vessels in which they were found showed in both cases considerable extravascular accumulation of lymphoid cells. The large mass on the site of the thymus consists of round and irregularly shaped cells, among which a number of multinuclear giant cells are observed.

In this case it is natural to associate the presence of living worms and their wanderings with the pronounced inflammatory changes of the connective tissue throughout all the mammæ and again to associate these indirectly with the diffuse hypertrophic and cystic condition of the epithelium. It is also natural to associate this factor with the hyperplastic conditions of the lymphatic apparatus and the tumours arising on this basis, if tumours they are; furthermore with the multiple adenomatous nodules in the lungs. In all probability the process is one of long standing, extending over far more than the eight months the mouse was observed in the laboratory.

The other case is less interesting, being complicated with a septic process.

Mouse 494 was a female of unknown age from an outside breeder. It was brought in with enlarged glands in the neck, and shortly afterwards the lymph glands in both axillæ became enlarged. The glands of the neck grew and formed a tumour the size of a walnut when the mouse was killed nine weeks later; the glands in the axilla remained about stationary.

At the post-mortem examination the packet of glands in the neck contained pus, as an explanation for which an abscess was found round the lower jaw. The large glands in both axillæ presented the picture of hypertrophy of lymphoid tissue. The thymus was very much enlarged and of firm consistence; the same held for one of the mediastinal glands. There were no nodules visible to the naked eye in the lungs. The spleen was very much enlarged, measuring ca. $5 \times 1\frac{1}{2}$ cm., with rounded edges and of firm consistence; the cut surface presented a mottled appearance. The liver was slightly enlarged, with rounded edges.

The mammary glands were pigmented, being brownish in colour. Examined in serial sections the mammary gland itself was somewhat atrophic, with distended ducts surrounded by sclerotic tissue. Foci of inflammation in the connective tissue were found scattered all over the gland. As a cause for these latter changes several nematodes with embryos were found. There is no means of deciding what was the cause of the lymphomatous condition; the abscess of the jaw, with purulent foci in the glands of the neck, seemed to have been a secondary complication.

Apart from the possible association with inflammatory changes, it is of interest to note that in three different groups of tumours of the mouse (of the mamma, of the lung, and of the lymphatic gland) a kind of intermediate stage seems to exist in which the parenchyma shows hypertrophic or hyperplastic changes before the development of a real tumour can be proved to have commenced. It cannot be decided whether this state is to be considered a separate pathological condition or whether it already is the first step of tumour formation. The interest of this condition lies in the fact that it suggests that the malignant transformation of the tissues may take place by degrees and not necessarily in one step from the normal cell to the fully developed cancer cell.

B.—Biological Part.

(4) *Clinical Study. Results of Operation. Spontaneous Absorption. Metastases.*

(a) *Results of Operation.*

Most of the tumours have been removed as soon and as completely as possible. The technique of the operation is that described by Murray. It is obvious that an operation on a mammary tumour in the mouse can never be complete in the same sense as in the human subject; the distribution of the mamma over a large part of the body makes it impossible to remove the whole gland as in man. Furthermore, because of the relatively large size of the tumour as compared with the size of the mouse and the difficulty of covering the wound, one is usually forced to keep fairly close to the tumour. Only with reservations are the results of operation comparable with similar data from the human subject. On the other hand the apparently encapsulated state of the tumour suggests that it should be easy to remove it without recurrence, but this, however, is not the case, as is shown by Murray. The frequency with which recurrence supervenes after apparently complete extirpation of what to the naked eye are encapsulated tumours is striking, and invalidates all statements asserting their benignancy. As in man a complete removal of all tumour cells is necessary to prevent recurrence, and early removal has a far greater chance of being successful. There seems also to be some difference between the different kinds of tumours. The sebaceous adenomata and certain verrucous epitheliomata of the skin can be mentioned as benign, in the sense that they have not recurred after incomplete removal; the melanotic tumour behaved similarly. The adenomatous tumours of the mamma, however, if they have reached any size, show about the same frequency of recurrence as other more alveolar tumours, and their recurrence is obviously only a question of whether cells are left and not of any benignancy of the cells.

When the question is one of freedom from recurrence, it is obvious that the length of time the animal remains alive after operation is of prime importance, and must be stated. The great majority of recurrences (more than 75 per cent.) have developed inside the first eight weeks after the operation. It is therefore preferable to disregard as negative the cases in which the mouse has died without recurrence and has not remained alive upwards of eight weeks after the

operation. Of 174 such cases 80 (=46 per cent.) have been without recurrence, while 96 (=54 per cent.) have exhibited recurrence. In this number tumours of all sizes have been included, most of which have been removed fairly early. These figures correspond closely with those given by Murray: out of 48 animals operated upon, recurrence took place in 23.

Of 96 recurrences there have been observed in

2nd week . . .	12	
3rd „ . . .	8	
4th „ . . .	19	= 64 = $\frac{2}{3}$ of all cases
5th „ . . .	12	
6th „ . . .	13	
7th „ . . .	6	
8th „ . . .	5	
9th „ . . .	8	
10th „ . . .	5	
11th „ . . .	2	= 32.
12th „ . . .	1	
13th „ . . .	1	
21st „ . . .	2	
25th „ . . .	1	
36th „ . . .	1	

Two-thirds of all recurrences are thus observed before the end of the 6th week; one-fourth more between the 6th–10th week, and only isolated cases later. It may be an open question whether these late recurrences are all real recurrences and not, in some cases, newly developing tumours. The frequency with which new tumours develop in different mammæ of these mice, certainly suggests that a number of the tumours occurring in the neighbourhood of the primary tumour need not necessarily be due to cells left behind by the operation, but may be new foci of tumour development in a diseased mamma. There are, however, no means of deciding this point definitely. On the other hand, there can be no doubt of it being perfectly possible that tumour cells can remain in a resting condition for a long time in the mouse as in man. This happens, now and then, when one grafts the mouse with its own tumour, as will be shown later on.

A control to the effects of operation is afforded by a number of mice which have not been operated upon, and in which the disease has

To face p. 51.

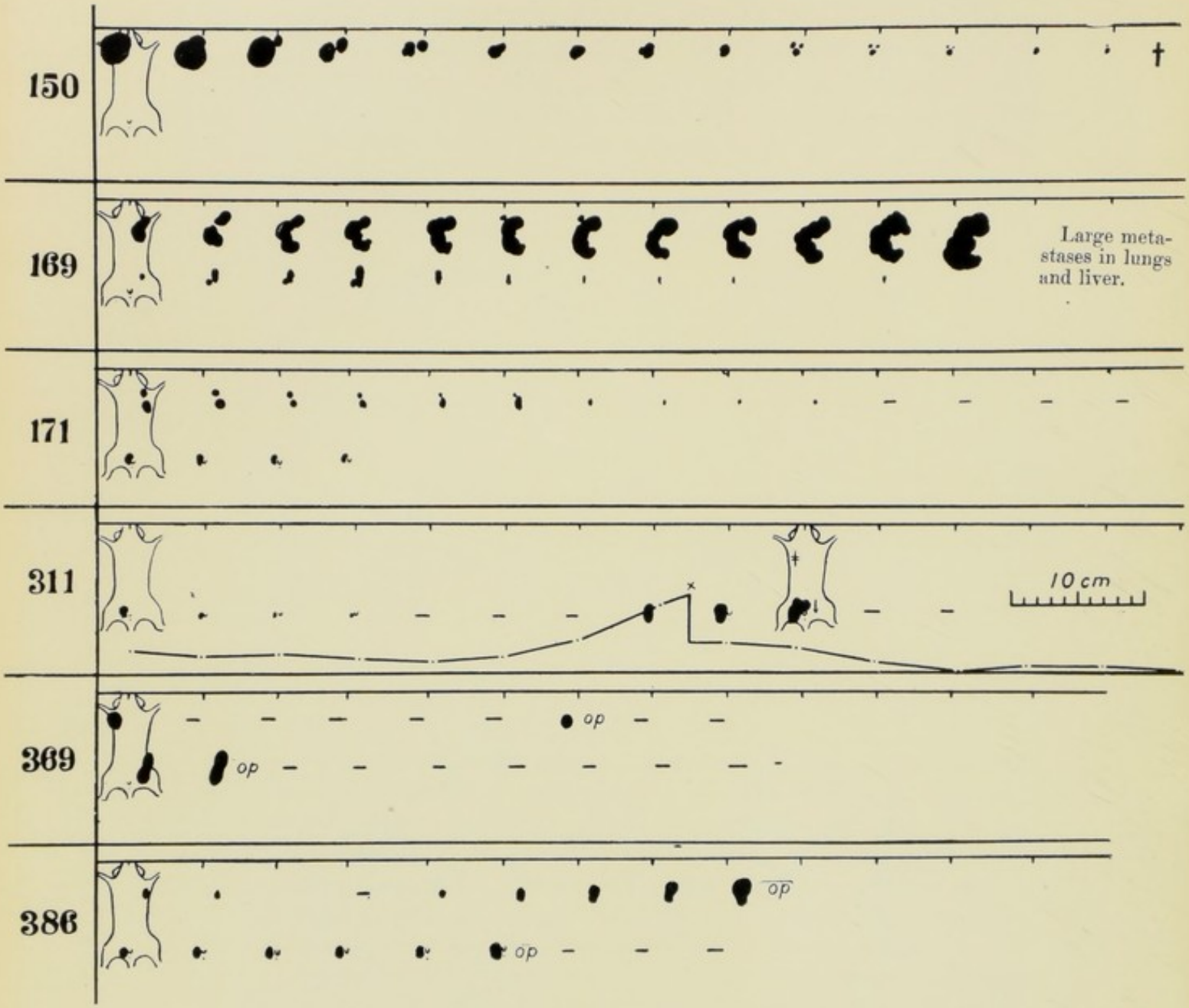


FIG. 33.—Graphic record of the clinical course of the spontaneous mammary tumours in five mice in which absorption of spontaneous tumours was observed. The black silhouettes represent the tumours as charted at intervals of a week, natural size and reduced to scale. In mouse 171 three tumour-like nodules were completely absorbed and there are no means of deciding their exact nature; in the other 5 mice the true tumour-nature of the growths was ascertained by microscopical examination. The tumour of mouse 150 is illustrated in figs. 34, 35, and 36. In mice 311, 369, and 386 the absorption was only temporary, followed by renewed growth. Note the absorption of the inguinal tumour in mouse 169, while the axillary tumour is growing progressively and large metastases are developing in lungs and liver.

followed its natural progressive course. In 23 unoperated cases the average duration of life has been about 6 weeks after the tumour has come under observation. The duration of life varied from 3 to 13 weeks. The cases which have not lived three weeks in the laboratory have been discarded from consideration. On the other hand, of 141 cases operated on, the length of observation has averaged fifteen weeks. In this number all kinds of operated cases are included, also the numerous cases in which grafting with the mouse's own tumour and subsequent rapid tumour growth has led to the premature death of the animal.

Although these figures are thus by no means calculated to show the effects of operation in the best light, as preservation of life has not been the chief consideration, they nevertheless show clearly the advantages of operation in prolonging the life of the animal. The total length of a mouse's life is seldom more than 100 weeks, and the nine weeks won on the average by the operation thus represents nearly one-tenth of the whole span of life.

(b) *Spontaneous absorption in sporadic tumours.*

Murray mentions spontaneous absorption as occurring locally in small areas of some tumours; also that temporary arrest of growth is frequently observed, and in some cases an actual diminution in size is seen. A striking case of a very substantial diminution in size has been observed in this material, and others in which the tumour did actually disappear for several weeks.

The first case (mouse 150, fig. 33) came in with a hæmorrhagic tumour on the right side of the neck, the size of a large walnut. After about a week the tumour began to ulcerate and diminish in size, then sloughed and formed a scab. Three weeks after entrance the size was that of a hazel nut, and sloughing continued for 5–6 weeks. After this time three small nodules, ranging in size from a hemp seed to a pea, were felt; they gradually diminished in size until, when the mouse died, thirteen weeks after entrance, all that was left of the tumour was about as large as a hemp seed. Microscopically the nodule still contains a fair amount of "healthy looking" tumour-tissue (fig. 36), which, however, does not show signs of active growth, and is surrounded by a very sclerotic connective tissue. In the periphery of the nodule and in several places scattered all through it, are great numbers of large phagocytic cells filled with brownish granules or with vacuolated protoplasm like the phagocytes observed in spontaneous absorption of transplanted tumours. These large cells appear both outside the carcinomatous alveoli as a zone of

reaction tissue, and also within the alveolus itself, replacing by degrees the carcinoma cells. Fig. 34 illustrates the enormous sclerosis with hyaline degeneration of the connective tissue and the accumulation of phagocytes round the periphery of the tumour. Fig. 35, drawn at a high magnification, shows a more central part of the tumour in which the substitution of the carcinoma cells in the alveoli by phagocytes has taken place ; the connective tissue here, also, is very sclerotic.

The resemblance of this picture to that found accompanying absorption of transplanted tumours, lies chiefly in the accumulation of phagocytes with sclerosis of the connective tissue, whereas the infiltration of small round cells commonly found in the more rapid spontaneous absorption of transplanted tumours is less marked. Whether the process would have ended with complete disappearance of the tumour cannot be stated with certainty. It is possible that it would have disappeared, but had the mouse not died at such an opportune moment, the not uncommon position would have arisen that a swelling, having all the outer appearances of a tumour, disappears spontaneously, and has to be discarded because of the absence of incontrovertible evidence of its true nature (*cfr.* fig. 33, mouse 171).

A somewhat similar case is show in fig. 33 (mouse 169). When first observed there were two tumours, a large one in the left axilla and a small nodule in the left groin. Both increased in size during the following weeks, but whereas the former continued to grow till the death of the animal, the inguinal nodule grew only for four weeks (it measured then 15×7 mm.) and then diminished in size until it could be hardly felt on the death of the animal. At the autopsy large metastases were found in the lungs and in the liver, in addition to the enormous tumour in the left axilla. On microscopical examination of the just palpable tumour in the left groin a small adeno-carcinomatous nodule and scattered acini were found in a very sclerotic tissue, here and there containing numbers of phagocytes ; in one place also with a strong infiltration of round cells. The picture is, on the whole, similar to that illustrated in figs. 34 and 35. It is of interest that in this case absorption of one tumour has taken place at the same time as another spontaneous tumour has been growing continuously in the same animal, and large lung and liver metastases have developed. The "healing" must in this case be referred to local causes and not to any general change of the soil offered by the animal.

Another case of this kind was still more striking, as the process advanced to complete disappearance of the tumour (mouse 311,

[*To face* p. 52.

J. R. Ford, del.

FIG. 34.—Mouse 150. *Spontaneous absorption.* Peripheral part of the tumour with accumulation of phagocytes. Enormous sclerosis and hyaline degeneration of the stroma, between which the parenchyma is compressed and atrophic. × $\frac{190}{1}$.

J. R. Ford, del.

FIG. 35.—Mouse 150. *Spontaneous absorption.* Another part of the spontaneous tumour, in which sclerosis of the interstitial tissue is prominent with the appearance of phagocytes in the carcinomatous alveoli. × $\frac{550}{1}$.

fig. 33). The mouse came in with a tumour, measuring 10 × 7 mm., on the right side of the vulva. The tumour gradually decreased in size until four weeks after entrance it could no longer be felt, and then for another three weeks there was nothing to be found on the original site. In this case it would have been impossible to state the exact nature of the growth had it not been for the fact that the tumour, after apparently complete disappearance for three weeks, came back again on the same spot and, after growing rapidly, was operated upon; the malignant nature of the growth was ascertained not only by histological examination, but also by its recurrence after operation, its growth after being grafted in another place of the same mouse, and the formation of large metastases in the lungs as found at death. Two similar cases (369, 386) are illustrated in fig. 33 showing the chartings of the clinical course of the above tumours. In spontaneous tumours, when "healing" takes place, the evidence at present speaks rather for the importance of local conditions, probably arising in the tumour cells themselves, than for the intervention of general constitutional changes.

(c) *Metastases.*

If any additional evidence as to the malignancy of these tumours of the mouse besides what has been shown about their tendency to recur after operation, etc. be required, it is afforded by their forming metastases in distant organs. The longer the mouse survives with its tumour, the better are the chances of finding metastases at death. The best opportunity for observing metastases is when the tumour has been operated upon and has recurred, and the mouse has been kept alive for a considerable time. As observed by all writers on this subject, the lungs are the site of predilection for metastases in mouse tumours. This holds good for the mammary tumours especially, whereas in squamous cell carcinomata of the mouth, the lymphatic glands of the neck have been found attacked, and the lungs have been found free.

In the present material, nodules in the lungs clearly visible to the naked eye were found in 103 out of 273 cases = 38 per cent. The exact number of the metastases which were too small to be detected by the naked eye was not investigated. In the cases where there was any doubt as to the nature of the nodules found, microscopical examination was made. In this way a number of lymphomatous nodules, inflammatory foci, and primary adenomata of the lung have been excluded, while

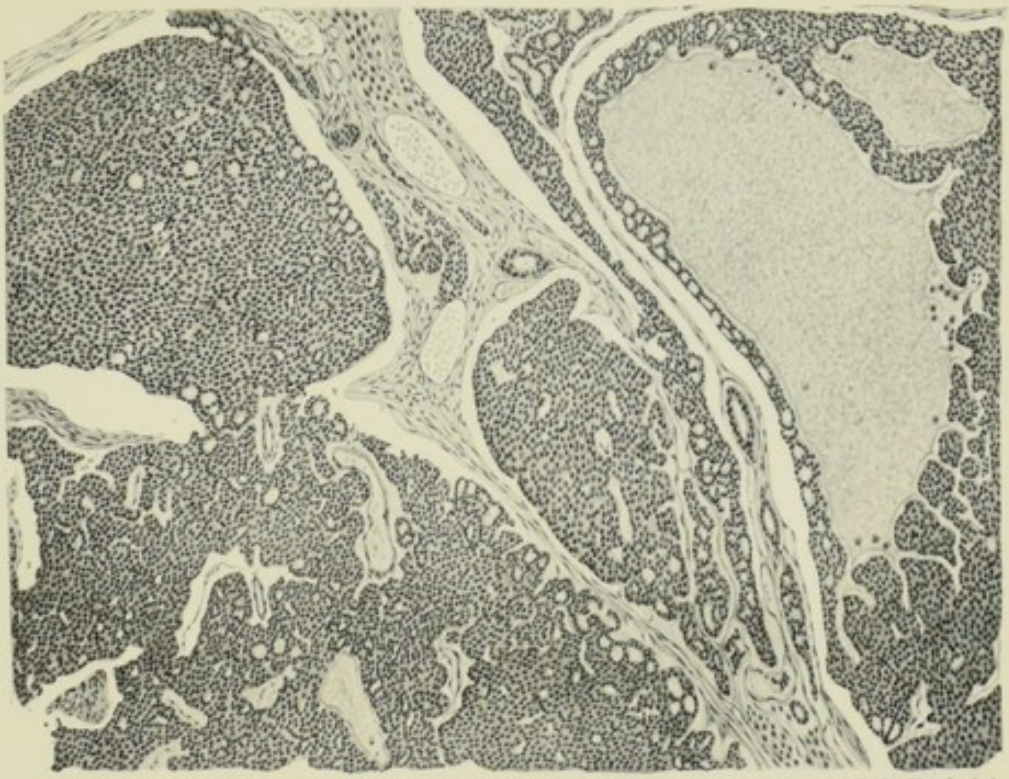
some cases of microscopical true metastases, not detectable with the naked eye have also been found.

Lung adenomata cannot be distinguished from small metastatic nodules without microscopical examination. It is possible that a certain number of what are classed as metastases would be shown by a systematic histological examination of all cases to be lung adenomata, while previous experience shows that a number of metastases would be discovered where they were not suspected by naked eye examination.

Compared with the frequency of secondary nodules in the lungs, metastases are only rarely found in other internal organs. Of these the liver has been found four times to contain large nodules (169, 296, 317, 469), the kidneys once (296), ovaries once (274, metastasis or primary tumour ?). Metastatic nodules have been observed on the peritoneum (200, 296, 322), in the retroperitoneal tissue on the site of the adrenal (369), and under the diaphragm (469). In 273 (cancroid of the mammary region) a secondary nodule was found in the posterior mediastinum, while the lungs were free ; in mouse 314 the tumour cells of a lung metastasis had grown through the vessels into the heart, and floated free in the blood stream. In fig. 37 is shown a small nodule of carcinoma found in the spleen of mouse 426, cut for other reasons. It must be noted that a systematic examination for metastases in these organs has not been made, apart from a naked eye inspection at the post-mortem examination, verified in all cases of tumour-like nodules by microscopical examination. The same applies to the lymph glands, in which metastases have been found in four cases, melanoma (265), cancroid of the jaw (395), and two mammary carcinomata (331 and 369), but, on the whole, they are relatively rare where mammary tumours are concerned. To a certain extent this may be due to the smallness of these organs, and to the fact that the only way of investigating them systematically, viz., by microscopical examination in serial sections, was not applied. Murray has shown that where this examination has been carried out, lymphatic metastases may be found where they are not expected.

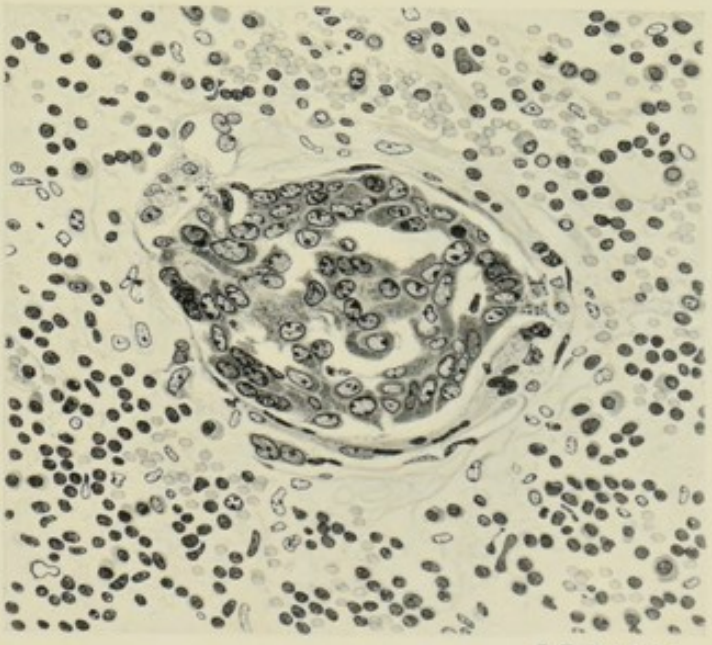
On the whole, however, the mammary tumours do not seem to disseminate in the lymph glands so frequently as in the lungs. As long as the course of the lymphatics in the mouse is not known a suggestion can hardly be made how this peculiarity can be accounted for. The sclerotic processes so frequently found in the connective tissue of the mamma and around it, suggest, however, that previous to the development of the tumour the lymphatics may be occluded and that this perhaps may have to do with the rarity of transport of cancer through the lymph vessels in mice. A similar suggestion might be made to account, to a certain extent, for the frequent secondary changes in the mammary tumours with lymph stasis and œdema of the connective tissues. Such changes would be easier to understand if the lymphatics were more or less obliterated.

[*To face* p. 54.



J. R. Ford, del.

FIG. 36.—Mouse 150. Part of the spontaneous tumour which still shows a healthy parenchyma. The picture is that of an adeno-carcinoma with alveolar and acinous parts, and hemorrhagic changes in the stroma. $\times \frac{83}{1}$.



J. R. Ford, del.

FIG. 37.—Mouse 426. Microscopic metastasis in the spleen. $\times \frac{340}{1}$.

(5) *Experimental Study. Behaviour of the Tumours on Transplantation.*

In the Third Scientific Report Murray showed on a very large material how the spontaneous tumours of the mouse, especially those of the usual hæmorrhagic type, behaved on transplantation into young normal animals. It was shown that the great majority of tumours could be transplanted, granted a suitable method of inoculation and the use of a large number of animals. There was no reason why this special line of investigation should be continued on the same scale. Therefore the indiscriminate transplantation of all tumours was discontinued and attention directed to special problems. Immediately after removal all tumours have been examined histologically in frozen sections, and only those which showed interesting histological features have been propagated in normal animals (squamous-celled and sebaceous carcinomata, and, in general, carcinomata of other organs than the mamma, and sarcomata, lymphomata, etc.). When the ordinary mammary adenocarcinomata have been transplanted, it has been in order to investigate special problems such as the behaviour of the cells when implanted into the same mouse, or in other spontaneously attacked animals, as compared with normal mice. The observations thus made may be considered under the following heads :—

(*a*) Inoculations into the same mouse in which the tumour originated (autologous or autoplastic implantation).
(*b*) Inoculations into other spontaneously attacked mice.
(*c*) Inoculations into normal mice, young and old.

(a) *Autologous Implantations.*

Only few experiments of this kind have been described previously. L. Loeb * describes a case in which a mammary tumour of a rat was re-implanted into the same animal with positive result, and into two other rats with negative results, and also the case of a tumour of a dog re-implanted into the same animal and into three other dogs. From the laboratory of the Imperial Cancer Research Fund † are described implantations of spontaneously attacked mice with their own tumours with two successes out of four inoculations, and into other spontaneously affected mice with only one success out of six inoculations ; three of the tumours employed were tested in normal mice, giving eight tumours in 325 inoculated mice. The distinction was

* Loeb, L. : 'On Transplantation of Tumours,' Journal of Med. Research, 1901. *Ibid.* 1902.

† Bashford, E. F., Murray, J. A., & Cramer, W. : 'The Natural and Induced Resistance,' etc. Proc. Roy. Soc. vol. lxxix. 1907.

emphasized between transplanting the tumour into the mouse in which it grew primarily and into any other mouse afflicted with cancer. The provisional results of 17 autoplastic implantations of the present series, of which 16 were positive, were presented before the Royal Society of Medicine *, 16th November, 1909. At about the same time Apolant† published the results of autoplastic implantations into 8 mice, of which 6 were positive. He does not state how long the mice considered as negative were kept under observation.

The method employed has been to remove surgically the spontaneous tumour as completely as possible, and immediately afterwards to introduce small fragments from different parts of the tumour, amounting altogether to ca. 0·02–0·03 c.cm., into another locality of the same mouse remote from that of the primary tumour, at a site where the fate of the graft could be easily followed. New nodules frequently develop wherever mammary tissue occurs. Therefore a zone in the flanks, midway between the axilla and the groin, has been preferred, a place which, as anatomical dissection shows, is free from mamma (*cf.* fig. 21), and where new primary nodules are not likely to develop.

When the mice are excluded which have died too early after inoculation to allow a definite conclusion as to the result, 56 mice are left, in which 64 autoplastic implantations have been performed. Fifty-nine of these were performed subcutaneously into 51 mice, 53 with small doses in the way already stated, and 6 with large doses (0·15–0·50) and 5 intraperitoneally with large doses (0·40–0·80 c.c.). Of the 59 subcutaneous implantations, 8 were double inoculations into the same animal in order to test either the effects of different doses of the same tumour, or the growth-power of two separate tumours of the same animal, or, in the case of development of sarcoma in spontaneous tumour 469, the growth-power of different parts of the same tumour. The tumours employed ‡ and the results obtained are :—

51 Adeno-carcinomata of mamma	46 subcut.	44+, 1−, 1?
	5 intraperit.	5+
2 Adeno-cancroids	subcut.	2+
5 Squamous cell carcinomata	„	5+
1 Sebaceous carcinoma	„	1+
3 Spindle-celled sarcomata	„	3+
1 Melanoma	„	1?

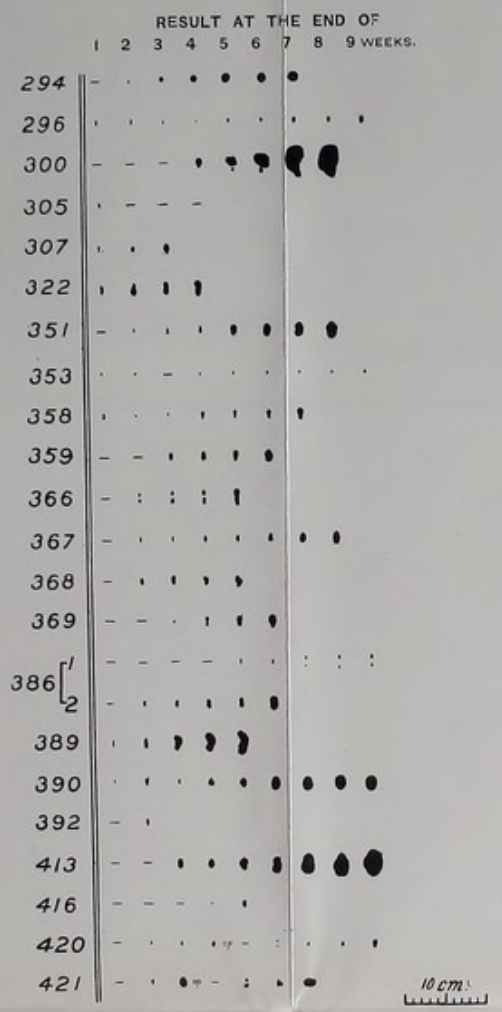
* 'Lancet,' 27th November, 1909, vol. ii. p. 1588.

† Apolant, H.: 'Ueber die biologisch wichtigen Ergebnisse der experimentellen Krebsforschung.' Sammelreferat. Zeitschrift für Allg. Physiologie, Bd. ix. 1909.

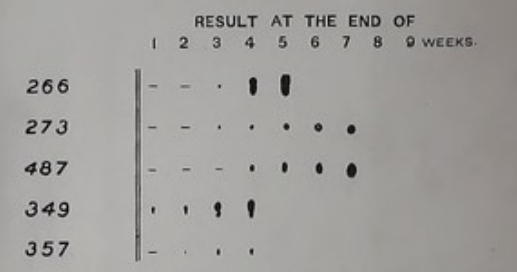
‡ In addition three superficial verrucous squamous-cell growths have been inoculated with negative result; the material was infected and ulcerated out in two cases, and has, therefore, not been counted.

To face p. 57.]

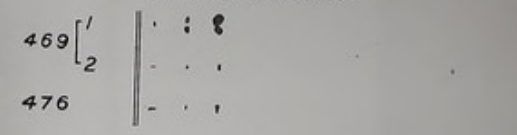
A. *Autoplastic implantation of mammary adeno-carcinomata, small doses subcutaneously.*



B. *Autoplastic implantation of squamous-celled carcinomata, small doses subcutaneously.*



C. *Autoplastic implantation of spindle-celled sarcomata, small doses subcutaneously.*

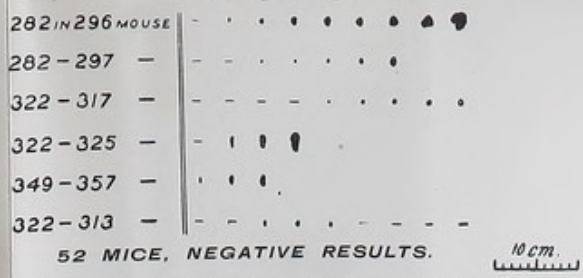


D. *Autoplastic implantation of mammary adeno carcinomata, large doses subcutaneously.*

E. *Autoplastic implantation of mammary adeno-carcinomata, large doses intraperitoneally.*

The silhouettes represent the tumours found at the necropsy in the peritoneum. The distance from the double line indicates the length of the interval between implantation and death. Mouse 396 is shown entire in the coloured frontispiece.

F. *Homoplastic implantation.*



Results of grafting spontaneously affected mice with small doses of *other* spontaneous mammary tumours. Number of spontaneous tumours implanted in first column. Of 58 such experiments only 5 were successful.

FIG. 38.—Results of implanting spontaneous tumours into their own (A–E) and other (F) spontaneously affected mice. The index number of the mouse and its tumour in the first column. The experiments on individual mice have been aligned so that the dates of operation and implantation fall on the double line and from this the growth is recorded at intervals of a week. To compare with the identical results of autoplastic implantation in mice previously treated with mouse embryo-skin, fig. 44, p. 82.

Autoplastic implantation. { A. Mammary adenocarc. / B. Squamous-celled carc. } small doses (0·02–0·03 gr.) subcutaneously. / { C. Spindle-celled sarcoms. }

Autoplastic implantation. { D. Mammary adenocarc.; subcutaneously / E. Mammary adenocarc.; intraperitoneally } large doses (0·15–0·80 cc.).

Homoplastic implantation. { F. Mammary adenocarc.; small doses (0·02–0·03 gr.) subcutaneously.

Out of 59 epithelial tumours of varied histology, 57 have grown on transplantation of the cells into the same spontaneously affected animal. Only two cases have been negative; of these one should not be counted, for the mouse was ill practically the whole time it was under observation, and died four weeks after the inoculation. The other negative case occurred in a mouse surviving for 26 weeks after inoculation without exhibiting any growth at the point of inoculation. Of non-epithelial tumours there are 4, three spindle-cell sarcomata positive on re-inoculation, the fourth a melanotic tumour of peculiar structure and of very low power of growth; no evident increase was noted of the grafts re-inoculated simultaneously in four different places into the mouse in which it had arisen, although the tumour-cells remained alive for four months.

In the cases where the grafting has been carried out subcutaneously, the period elapsing between implantation and the appearance of a palpable nodule showing increase in size is as follows (*cf.* fig. 38) :—

End of 1st week 6.
„ 2nd „ 23.
„ 3rd „ 13.
„ 4th „ 8 (in two of these cases the inoculated tumour remained stationary for a long time—in one case for eleven weeks—then grew slowly).
„ 5th „ 3.
„ 6th „ 1 (large dose).
„ 7th „ 1 (ditto).

On the whole the rapidity of growth of the graft corresponds to the rate of growth of the primary tumour, and the same individual characteristics are retained in the histological picture. Two different tumours of the same mouse may exhibit different powers of growth when re-transplanted into the mouse itself. Sometimes, however, it is surprising how rapidly a graft from an apparently stationary hæmorrhagic tumour may grow. In the great majority of cases the graft is already developing into a new tumour 2–3 weeks after inoculation into the same spontaneously affected mouse. This is on the whole considerably sooner than spontaneous tumours develop when first transplanted into normal mice, and the subsequent rate of growth is much more rapid than that observed in normal animals. This result is most easily accounted for by a larger number of cells surviving transplantation, and the circumstance of their being more in concord with their surroundings.

It is possible by inoculating minute doses somewhat to retard the development. Post-operative recurrences may be supposed to have started from minute traces left behind, perhaps even from single cells. In such cases the recurrrence may be considerably slower than the graftings made with known but minute doses into the same animal. On the other hand, where large doses are injected (0·3–0·5 gr.) a corresponding rapidity of development is not always observed, for in some cases after an initial increase in bulk, most of the injected material is absorbed and the growth is delayed. In one of these cases after injection of 0·15 c.c. the injected material seemed to be completely absorbed, and the growth did not start until seven weeks after inoculation. In this and other similar cases in which the absorption of a great amount of the animal's own tumour-tissue had taken place, no increased resistance has been observed towards a second inoculation with small dose. In five cases in which large doses were injected intraperitoneally, tumour nodules were obtained all over the peritoneum showing rapid growth and causing the death of the animal in 4–9 weeks (fig. 39, frontispiece).

By histological examination it is found that the subcutaneous graft of an animal's own tumour behaves very much as corresponding grafts do in normal mice. Perhaps a larger number of cells remain alive, but the centre becomes necrotic and the reaction is on the whole the same as in young normal mice where the inoculation is going to be positive.

The general result is that autoplastic inoculation of a spontaneously attacked mouse with her own tumour is nearly always successful*, when special tumours of very low power of growth, such as the melanoma mentioned, are excepted. A suitable technique, aseptic conditions, and, above all, the lapse of a sufficient period of time after inoculation before an opinion of the result is formed, are indispensable. The growth of the mouse's own tumour has been repeatedly observed to be evident only after 5–7 weeks, and it is therefore obvious that statements about negative autologous inoculations to be valid, must also contain the number of weeks the animal remained alive after the operation.

(b) *Inoculations into other Spontaneously Affected Animals.*

Of the tumours which have been re-implanted into the same mice in which they have arisen, a certain number have also been tested in other spontaneously attacked mice. The method of implantation, dose, and site were exactly the same as mentioned for the autologous implantations.

* The success attending autoplastic implantation is not limited to tumours of the mouse. It affords a method for obtaining fresh material wherever difficulties arise in transferring tumours to normal animals, especially valuable in the case of larger animals and in ulcerated tumours, as practised in this laboratory.

In all, 85 such homologous implantations have been carried out with five successes. A certain number of these cancer-mice, however, have been inoculated more than once at different times, or have received injections of normal tissue. For the purpose of ascertaining to what extent spontaneous tumours are able to grow in other spontaneously attacked mice, it is necessary to discard all these cases, and only reckon those in which no other interference has preceded the inoculation of the spontaneous tumour. Operation on the spontaneous tumour and grafting the mouse with its own tumour-cells may be excepted, as this procedure has been seen to have no adverse influence on the subsequent inoculation of the spontaneous tumour of another mouse. In the case of several implantations of spontaneous tumours into the same mouse at different times, only the first has been reckoned ; two implantations, if performed simultaneously, have, however, been reckoned separately. The attempt has not been made to draw any distinction between the cases in which the primary tumour has been successfully removed (without recurrence), and those in which it was growing at the time of inoculation, or had recurred later.

With these restrictions 58 implantations remain to be counted, and of these 5 have been successful. These 58 inoculations have been carried out on 48 spontaneously attacked mice, 10 being double simultaneous inoculations with different tumours. Fifty-one have been done with small doses (0·02–0·03), and 7 with large doses (0·3–0·8 c.c.). All the successful inoculations were obtained by inoculating small doses by means of the needle and plunger. In the cases in which a large dose was absorbed, no immunising effect was manifest against a subsequent grafting of the mouse with its own spontaneous tumour.

The tumours employed, and the details of the results obtained, as compared with the results in normal mice, will be seen from table 3 (p. 60, *cf.* also fig. 38 F).

On the one hand there is a marked contrast between the results of autologous inoculation and homologous inoculation into other spontaneously affected animals. The experimental factors in these two groups of mice have been fairly comparable, and if there be a difference, it is that in the autologous inoculations there has been no selection of cases, but all inoculated mice have been reckoned together, those previously treated and those untreated ; whereas, for the homologous inoculations, only the previously untreated mice have been selected in order to obtain as favourable conditions for growth as possible. On the other hand there is little difference between the results of inoculations into spontaneously affected animals and normals. It must be kept in mind that

TABLE 3.

Transplantation	into mouse itself.	into other spontaneously affected mice.	into Normals.	
			Young.	Old.
Cancroid 266	+	$\frac{0}{2}$	$\frac{0}{349}$	
„ 273	+	$\frac{0}{1}$	$\frac{0}{337}$	
„ 349	+	$\frac{1}{7}$	$\frac{5}{180}$	
„ 357	+	$\frac{0}{4}$	$\frac{1}{59}$	
Sebaceous carcinoma 292	+	$\frac{0}{3}$	$\frac{4}{42}$	
Adeno-carcinoma. Mamma. 282	+	$\frac{2}{4}$*	$\frac{4}{26}$	$\frac{0}{12}$
„ „ 322	+	$\frac{2}{7}$	$\frac{8}{52}$	
„ „ 334	+	$\frac{0}{5}$	$\frac{0}{16}$	$\frac{0}{16}$
„ „ 338	+	$\frac{0}{5}$	$\frac{2}{60}$	$\frac{0}{15}$
„ „ 351	+	$\frac{0}{1}$	$\frac{0}{35}$	
„ „ 353	+	$\frac{0}{1}$	$\frac{3}{35}$	
5 other adeno-carcinomata . 5	+	$\frac{0}{10}$	Not tested in normals.	
Spindle cell sarcoma 460	Not transplanted; mouse killed.	$\frac{0}{8}$	$\frac{16}{130}$	$\frac{0}{15}$
TOTAL	$\frac{16}{16}$	$\frac{5}{58}$		

* $\frac{2}{20}$ when all inoculations are reckoned.

spontaneously affected animals can only be imperfectly compared to normal mice, and it would be venturesome to emphasize too much the relatively small differences obtained in these two groups. The general result is, that whereas autologous inoculations are practically always positive, inoculations into other spontaneously affected mice give a positive result in only 8·6 per cent., which is not very different from the results obtained with a great variety of these tumours in thousands of normal mice, *v.* p. 64. The experiments show that when spontaneously affected animals suffer from cancer, it is because of other factors than an enhanced suitability of the soil for growth of cancer cells in general.

These experiments allow some conclusions to be drawn both upon

questions connected with transplantation in general, and on certain aspects of the problems of cancer. First, they illustrate very well the differences obtaining between autoplastic and homoplastic transplantation. Re-introduced into the organism to which they belong, *i. e.* into the soil to which the cells are accustomed, they find all conditions for continued existence and growth. Quite different are the conditions in other individuals of the same species, of the same age and sex. By no other means can the existence of individual biological differences between animals of the same species be proved more clearly and the specificity of the medium which each animal represents, be demonstrated more strikingly. The experiments show the advantages which will be gained by the surgeon in employing tissues from the same individual in all transplantations, and that it is unreasonable to expect an equally good result with tissues from other individuals.

Besides these general conclusions as to transplantation, the experiments have a more direct bearing on certain problems of cancer. They demonstrate very strikingly a well known fact, viz. the danger to the individual operated on, if cancer cells are disseminated in the wound, and the overwhelming probability that recurrence will follow upon such dissemination. On the other hand, they show the relatively insignificant danger these cells present for other individuals, even when introduced under most favourable conditions. This last point has a direct bearing on one of the subsidiary theories of cancer origin, viz. that implantation of cancer cells from one individual to another plays an important part in spreading the disease. Even if no other proofs existed to show that cancer always arises *de novo* in the organism attacked, the consideration of the facts elicited by these experiments shows the improbability of the transference of cancer-cells from one individual to another having any etiological significance.

Among the inoculations of spontaneous tumours into other spontaneouslv affected animals there are two groups of experiments worthy of special attention. The one group of experiments is designed to decide whether a mouse carrying a tumour of a special type, *e. g.*, a highly keratinised cancroid, is more susceptible to implantation of another tumour of the same kind than mice spontaneously affected with a tumour of another kind, *e. g.* the common mammary tumour.

The experiments available are four: three of them have been negative in the sense that the special tumour (highly keratinising cancroid) has not grown in four mice carrying other spontaneous tumours of the same type, nor in three other mice with adeno-carcinomata, whereas in each case the tumours have grown in their own spontaneously

affected mouse. In the fourth, a cancroid (349), giving five tumours in 180 normal mice, has been inoculated into another cancroid-mouse (357*) and six other spontaneously affected mice; it has grown in the former but not in any of the latter. Only one positive result is thus obtained in four experiments. This number is, in itself, too small to allow conclusions to be drawn with certainty, but taken together with the numerous inoculations of mammary tumours into other mice with mammary tumours, there does not seem to be any evidence of better conditions obtaining in mice with the same kind of tumour as compared with mice with other spontaneous tumours.

In the other group of experiments, grafts have been exchanged between two animals carrying spontaneous tumours, and each animal has been grafted with its own tumour, and the tumours tested in normals at the same time. Experiments of this kind, when made with tumours exhibiting different powers of growth in normal mice of known age, are calculated to throw some light on hypotheses assuming cancer growth to be due to a differential of avidity between tumour-cells and body-cells. The "avidity" of cells can only be tested in an imperfect and indirect way; the "avidity" of tumour cells by their behaviour on transplantation into normal animals, and the "avidity" of body cells by comparison of the suitability of the animal with that of normal animals of the same age as a soil for tumour growth. Among these experiments two cancroids have been tested by inoculation into about 1,000 young normal mice, of which 686 have lived more than four weeks. In no animal has there been any evidence of growth taking place. In this case it is difficult to assume an increased avidity on the part of the tumour cells. Each of these tumours has also been transplanted into its own mouse, and has here given rise to a progressively growing tumour. According to the hypothesis in question we should expect that the body cells of the mice which allowed tumour-cells of such low "avidity" to grow, possess a still lower degree of "avidity" and, therefore, that these mice should be more susceptible to transplantation of other tumours. This is not the case. By exchanging grafts between the two animals in which these cancroids have arisen, no growth is obtained from either of the homologous inoculations, and the mice are not only refractory against tumours of low avidity, but when tested with another sporadic tumour (282), which gave four tumours in 26 normal young mice, proved themselves to be completely refractory to this tumour of presumably higher avidity. In another of these experiments grafts of two adeno-carcinomata of the mamma have been exchanged. Transplanted into

* Inoculation of the tumour 357 gave one tumour in 57 normal mice.

normal mice, they show a different power of growth ; one (353) has grown in normals, giving three tumours in 35 mice ; the other (351) has not grown in 35 mice. Both have grown in their own but not in each other's original host. If a lower avidity of the body cells of the spontaneously affected mouse be the deciding factor for the growth of the less avid tumour in its own mouse, how is it that the more avid tumour does not grow in this mouse ?

These experiments show that the conditions for tumour growth are far more complex and specific than is allowed for by the supposition of a mere differential of avidities. In fact the cells of each sporadic tumour are adapted for a specific medium, and only in this medium are they able to unfold their real powers of assimilation and growth. The conditions for which two histologically indistinguishable tumours are adapted, are so different, that they can be only very rarely exchanged without the malignant mode of growth ceasing altogether. The susceptibility of tumour-cells to changes in the race of mice is well known. The importance of the influence of the individuality in affecting the success of transplantation has been pointed out earlier from this laboratory *. Attention has also been drawn previously † to a similar specificity of the conditions for growth, which under certain conditions may be exhibited by transplantable tumours. Two different transplantable tumours, a carcinoma and a sarcoma, each adapted to a different strain of mice, were mixed together and injected into mice of both strains. The cells of each tumour showed in this case an elective power for that strain to which they were adapted, so that in each of the two groups of mice only those tumour-cells grew which were previously adapted to this special strain. In the transplantations of sporadic tumours the same phenomenon is met with, even more pronounced. The conditions of growth are to all intents and purposes specific for each individual sporadic tumour.

While the conditions for *progressive* growth of the cells of a sporadic tumour are relatively seldom found in other spontaneously attacked animals, a transitory growth lasting a few weeks is not so rarely observed. This phenomenon of preliminary growth is the same as that which is so frequently seen in transplanted tumours, and suggests that concomitant immunisation has to do with the final negative result. The reaction set up on the introduction of the cells into the body and the

* Bashford, E. F., Murray, J. A., & Cramer, W.: 'The Natural and Induced Resistance,' etc. Proc. Roy. Soc. vol. lxxix. 1907.

† Haaland, M.: 'Beobachtungen über natürliche Geschwulstresistenz bei Mäusen.' (Berliner klin. Wochenschrift, 1907, no. 23.)

susceptibility of the cancer cells to such reaction largely determine what has been called the "conditions of growth." The preceding experiments show that tumour cells from another individual either excite a more energetic reaction or are far more susceptible to such a reaction than are the cells actually belonging to the same individual. The latter either do not set up any immunity reaction on their re-inoculation in the body, or, if such a reaction is set up, its effects have not been made manifest on the growth of cells belonging to the same organism.

(c) *Inoculations into normal mice, young and old.*

The tumours show all degrees of transplantability on inoculation into young normal mice. Certain cancroids with advanced keratinisation have not been successfully transplanted, in spite of repeated attempts at transplantation into more than 1,500 young animals. Other tumours have been easily transplanted, the percentage of successful inoculations in some tumours reaching 25 per cent. As far as possible, only the continuously growing tumours have been counted. Some tumours show a peculiar curve of growth, growing for a certain time and then disappearing spontaneously; when transplanted during the phase of active growth they can be propagated indefinitely. For this group it has been necessary to count the preliminary growing tumours. This latter form of growth is identical with that exhibited by certain strains of experimental sarcoma. It is found in three cases of spontaneous sarcoma (336, 460, and 476) and it is a special feature of one of the mammary carcinomata (206) and of a preputial carcinoma (297). Such tumours offer special advantages for studying spontaneous absorption and the conditions under which it takes place, to which attention has been drawn elsewhere *.

In all 73 tumours have been transplanted into 7706 mice, of which 5519 have survived four weeks. 307 tumours have been obtained, *i. e.* an average result of ca. 5·6 per cent. The tumours inoculated have been:—

35 mammary carcinomata and adeno-carcinomata.
15 keratinising tumours.
4 sebaceous carcinomata.
2 preputial carcinomata.
5 sarcomata.

---

* Haaland, M.: 'Om organismens reaktioner mod pathologisk cellevekst.' Norsk Magazin for lægevidenskaben, 1909.

1 carcinoma sarcomatodes.
6 lymphomata.
1 melanoma.
1 hypernephroma.
1 ovarian carcinoma.
1 lung adenoma.

Of mammary adeno-carcinomata there have been transplanted * :—

1. Adeno-carcinoma and solid carcinoma.

Successfully.

	Spontaneous tumour.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.	
	152	80	52	1	
	174	30	25	1	
	189	100	80	5	
	198	100	60	1	
	199	40	27	1	
	200	80	71	9	
	235	44	44	4	
	238	108	99	13	
	241	60	43	6	Non-hæmorrhagic adeno-carcinoma with mainly adenomatous structure.
	267	60	40	10	
	338	96	80	2	
	353	40	35	3	
	421	60	47	11	Cellular tissue between the acini.
	493	36	32	1	
By itself stands	206	40	35	20	Preliminary growth followed by spontaneous absorption; the propagated tumours exhibit the same characters.
	Total ..	974	770	88	

* In the following tables the primary transplantation only has been considered. The results of further propagation are briefly indicated for each tumour in the large table appended to this paper; for some tumours it has already been referred to along with the histological description.

Unsuccessfully.

Spontaneous tumours.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.	
157	20	14	2	Underwent spontaneous absorption.
202	20	15	0	
209	33	19	0	
232	30	27	0	
242	40	31	0	
243	40	34	0	
247	40	30	0	
262	40	36	0	
298	26	14	0	
334	40	40	0	
351	40	35	0	
Total	369	295	2	(Spontaneous absorption.)

2. Cystic and papilliferous adeno-carcinoma.

Successfully.

Spontaneous tumour.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.
155	200	132	4
173	110	80	10
230	82	74	8
284	48	36	3
Total	440	322	25

Unsuccessfully.

Spontaneous tumours.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.	
185	40	34	2	Underwent spontaneous absorption.
274	50	34	0	
278	40	30	0	
288	30	14	0	
Total	160	112	2	(Spontaneous absorption.)

A cyst-adenoma with mucoid stroma :—

Spontaneous tumour.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.
397	30	24	0

For twenty-one tumours the attempt at transplantation has been successful *, for fourteen the result has been negative. It must be noted that these latter tumours have been inoculated into a relatively small number of mice, which partly accounts for the negative result. Taken together, 35 mammary tumours have been inoculated into 1973 mice, of which 1523 have survived after four weeks. In all 117 tumours have developed †, *i. e.*, a total percentage of about 8.

Of keratinising tumours there have been transplanted :—

A. Strongly keratinising tumours.

Spontaneous tumours.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.	
217	509	353	0	
266	437	349	0	
273	508	337	0	
487	140	120	1	Underwent spontaneous absorption.
Total	1594	1159	1	(Spontaneous absorption.)

* Cases in which the growth was followed by spontaneous absorption are also included.

† Twenty-four of these, however, have only grown temporarily with subsequent spontaneous absorption.

B. Typical squamous-cell carcinomata.

Spontaneous tumours.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.
349, 357, 405 — Subcutaneous tumours in the mammary region.	200	180	5
	60	59	1
	50	45	0
438 — Ulcerated superficial growth on the flank.	40	35	0
395 Ulcerated tumour of the jaw.	80	72	0
486 Ulcerated tumour of the face.	48	30	3
Total	478	421	9

C. Adeno-cancroids.

Spontaneous tumours.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.
164	300	240	1
184	80	40	3
282	29	26	4
286	297	211	24
322	102	52	8
Total	808	569	40

To summarise, fifteen keratinising tumours have been inoculated into 2880 mice, of which 2149 have survived after four weeks with a positive result in 49, *i. e.* 2·2 per cent. The low percentage is due to the entirely negative result of more than 1100 inoculations with the tumours of one particular group, in which the tendency of the cells to

differentiation is very marked. When this group is excepted, the rest of the keratinising tumours show, on the whole, about the same degree of transplantability as the mammary adeno-carcinomata.

Three sebaceous carcinomata and a mammary tumour with doubtful differentiation into sebaceous cells were transplanted. The result has been that in 460 mice, of which 307 survived after four weeks, 35 tumours have developed, *i. e.* ca. 11 per cent. Tumour 239 has been propagated for 16 months through 14 generations ; tumour 292 for 10 months in 11 generations; both have retained their typical differentiation into sebaceous cells. Tumour 356 has attained 3 generations in 9 months; its subtransplants have not retained this differentiation.

Spontaneous tumours.		No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.
239	In the mammary region.	120	103	18
292	In the mammary region.	264	133	16
378	Ulcerated tumour from the anal margin.	36	35	0
356	Mammary tumour with differentiation into sebaceous cells (?)	40	36	1
	Total	460	307	35

Two carcinomata of the preputial gland have been transplanted into 254 mice, of which 197 survived after four weeks. In 37 preliminary growth has taken place, followed after 4–8 weeks by spontaneous absorption. For the results of further propagation see p. 13.

Spontaneous tumours.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.	
297	152	111	31	Preliminary growth followed by spontaneous absorption.
466	102	86	6	Preliminary growth followed by spontaneous absorption.
Total	254	197	37	,, ,,

Sarcomata.

Six sarcomata have been transplanted into 1364 mice, of which 846 remained alive more than four weeks. In 68, *i. e.* ca. 8 per cent., tumours have developed. In all the spindle-cell sarcomata the growth has been only preliminary, and has been followed after 4–6 weeks by spontaneous absorption. When transplanted before the spontaneous absorption has commenced, the tumours can be propagated indefinitely. Tumour 336 has been propagated for more than twelve generations; all its subtransplants retain the same mode of growth. Tumour 219, although undoubtedly a sarcoma, differs from the other sarcomata in that it grows progressively, exhibiting the mode of growth of most carcinomatous tumours.

Spontaneous tumours.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.	
Spindle cell sarcomata.				
336	140	100	18	Growth followed by spontaneous absorption.
460	young 159	130	16	
	old 18	15	0	
476	60	56	6	
Polymorphous cell sarcoma.				
70	135	100	1	
Spontaneous "mixed" tumour.				
469	48	30	12	Only sarcomatous component grew (*v.* p. 19).
Round cell sarcoma.				
219	804	415	15	*v.* p. 18.
Total	1364	846	68	

Three tumours of peculiar histological structure have been transplanted with entirely negative results: a melanoma (265) into 121 mice, of which 100 survived; a hypernephroma (?) (328) into 40 mice, of which 24 survived; an adeno-carcinoma of the ovary (70) into 120 mice, of which about 80 survived. A lung-adenoma (160) has been transplanted into 40 mice, of which 30 survived, also with an entirely negative result.

Of lymphomata there have been transplanted :—

Spontaneous tumours.	No. of mice inoculated.	No. of mice surviving 4 weeks.	Tumours obtained.	
255	108	90	0	
345	30	20	0	
350 (leucæmia)	101	86	0	Inoculated subcutaneously, intraperitoneally, and intravenously with leucæmic blood, glands, thymus, and spleen.
387 (leucæmia)	112	89	0	
408	39	29	0	
422	40	34	0	
Total	430	348	0	

In all six lymphomata have been transplanted with entirely negative result into 430 mice, of which 348 survived after four weeks.

II. THE SPONTANEOUSLY ATTACKED MOUSE.

(6) *The Spontaneously Attacked Mouse as Soil for Tumour Growth.*

The experiments previously recorded have shown, that whereas the spontaneously attacked animal offers a much more favourable soil for the growth of its own tumour-cells, it is very doubtful whether it offers any better conditions for growth of other spontaneous tumours than do normal animals. The question of the suitability of the soil afforded by cancer-mice to the growth of tumours is a most important one. In order to study this question more closely, the behaviour to different transplantable tumours of 46 mice in which cancer had developed naturally (cancer-mice) has been investigated.

The only previous experiments of this kind are those of Murray, recorded in the Third Scientific Report. In one experiment he obtained growth of a squamous-cell carcinoma in two out of six spontaneous mice, whereas in sixteen normal controls no lasting growth was observed. In another experiment, growth followed on inoculation of the same squamous-cell carcinoma in six out of nine spontaneous mice, whereas in the controls four out of fifteen developed tumours. Murray concluded

that this transplantable tumour adapted itself, on the whole, more readily to the conditions present in animals spontaneously suffering from cancer than to the conditions in animals in which cancer had not spontaneously arisen ; at the same time, however, he pointed out that spontaneously affected animals are not strictly comparable with non-cancerous subjects of similar age.

The following experiments, five in number, with 46 spontaneously affected mice, have been undertaken with four different tumours, representing different types of growth, two of them being tumours which usually grow progressively in a very high percentage, the one rapidly, but the other slowly ; the third is a rapidly growing tumour in which spontaneous absorption follows in a large number of tumours, and the fourth with which two experiments were carried out, shows the peculiarity that nearly all the tumours are spontaneously absorbed after a rapid transitory growth. It has been shown earlier * that this mode of growth is due to immunisation of the animal through its own tumour The latter tumour is of special interest in this connection, as the question of the nature of the soil of the animals is most intimately bound up with the question of the immunity reactions of the animal inoculated.

The method has been, after removal of the spontaneous tumour, to inoculate a small dose (either 0·01 or 0·02 c.c.) of the transplantable tumour into the other axilla. The great difficulty in experiments of this kind is to find controls as comparable as possible to the cancer mice. To overcome this difficulty cancerous mice from the breeding-experiments of the laboratory have been used, and in this case it has been possible to obtain as controls the sisters and other near relatives of the cancerous mice, of known age, and identical with them, and kept under exactly the same conditions.

The results of these experiments are seen from the charts (figs. 40–43).

(1) Exp. 1 (fig. 40) is done with carcinoma 63, a rapidly growing carcinoma which usually grows progressively in a high percentage. Expressed in figures the result is, in young normals six tumours in ten mice, *i. e.* $\left(\frac{6}{10}\right)$, in old normals, $\frac{5}{10}$; in old non-cancerous females of the breeding experiments of exactly the same ages as the cancerous mice, $\frac{2}{10}$ (plus three positive cases which died too early to settle definitely whether the tumour would have grown progressively) ; and in cancer mice from the same breeding-experiments $\frac{4}{7}$.

* *Loc. cit.* pp. 63 & 64.

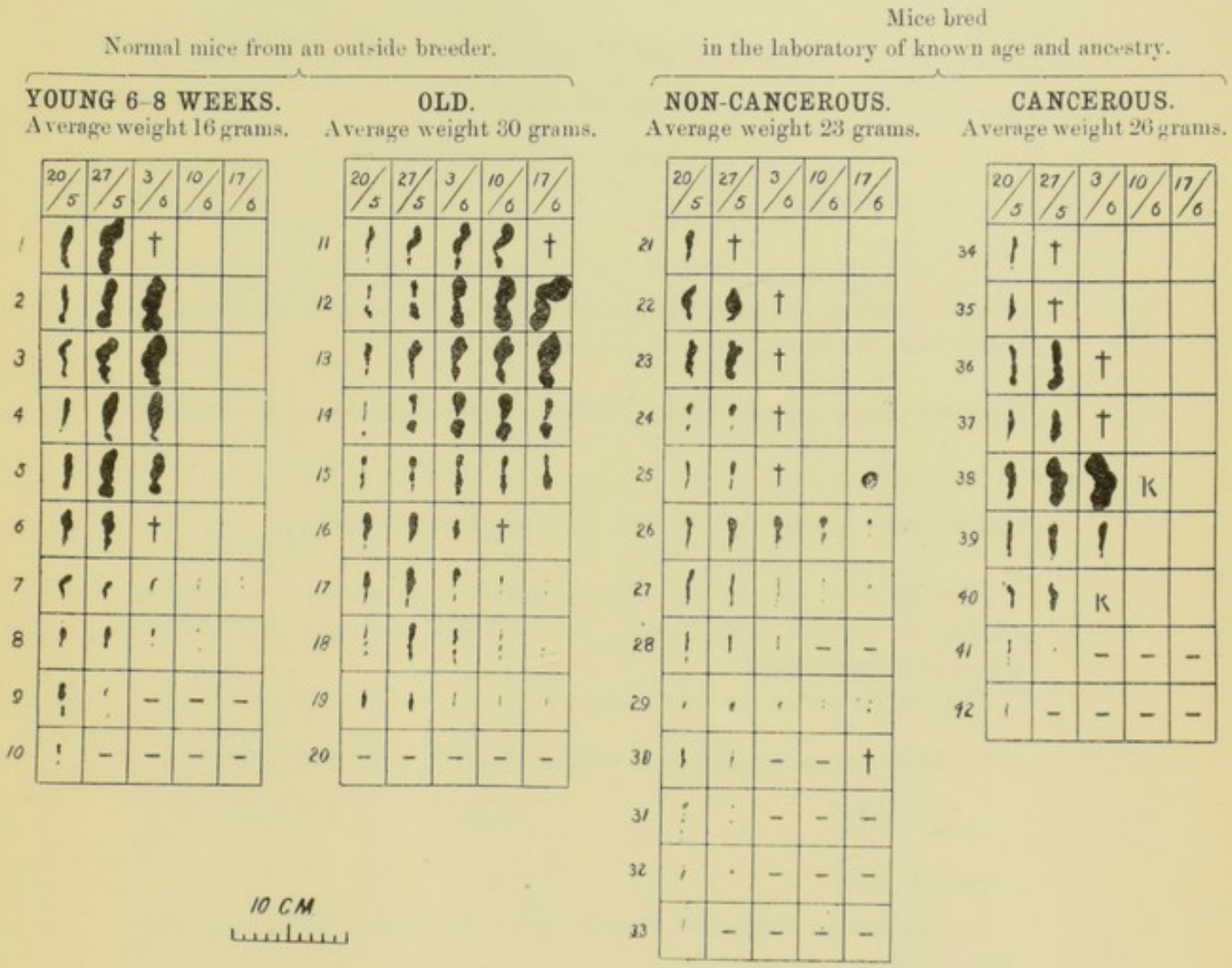


FIG. 40.—Suitability for the growth of transplanted cancer of mice in which cancer has developed spontaneously and has been removed by operation as compared with non-cancerous mice.

All mice inoculated 10.5.10 in the right axilla with 0·02 c.c. of carcinoma 63. (Exp. 63/45 B). Mice 1–33 controls (see headings of columns); 34–42 cancerous mice. **k**=killed and transplanted; †=died.

(2) In another experiment (fig. 41) 0·01 c.c. of a slowly growing carcinoma (carcinoma T) giving progressively growing tumours in a high percentage has been inoculated. The results are as follows :—

Controls young normals $\frac{24}{29}$.

„ old normal females $\frac{17}{23}$.

„ old males of breeding-exp. $\frac{11}{15}$.

♀ Cancer mice $\frac{6}{9}$.

(3) In a third experiment (fig. 42) 0·01 c.c. of a rapidly growing carcinoma which generally shows a fair amount of spontaneous absorption (carc. 199) has been used with the following result :—

Controls young normals $\frac{11}{19}$ progressively and 8 spontaneous absorptions.

„ old „ $\frac{24}{36}$ „ 12 „ „

Cancer mice $\frac{9}{12}$ progressively and 3 spontaneous absorptions.

(4 & 5) In a fourth and fifth experiment a tumour was used in which, in normal mice, nearly all tumours undergo spontaneous absorption (carc. 206).

The results are in the first series :—

Controls young normals (av. weight 11 gr.) . $\frac{1^*}{13}$ + 5 spont. abs.

„ adult „ („ 20 gr.) . $\frac{1^*}{11}$ + 3 large spont. abs.

Cancer mice . . . . . $\frac{0}{11}$

In the cancer mice one tumour grew for three weeks and then disappeared.

In the second series with the same tumour (fig. 43) :—

Controls young normals (av. weight 17 gr.) . . . $\frac{1^*}{12}$

„ old „ („ 27 gr.) . . . $\frac{1^*}{10}$

Cancer mice (av. weight 26·5 gr.) . . . . . $\frac{0}{9}$

In the cancer mice two tumours grew for ca. 14 days.

* Growing progressively up till the death of the animal.

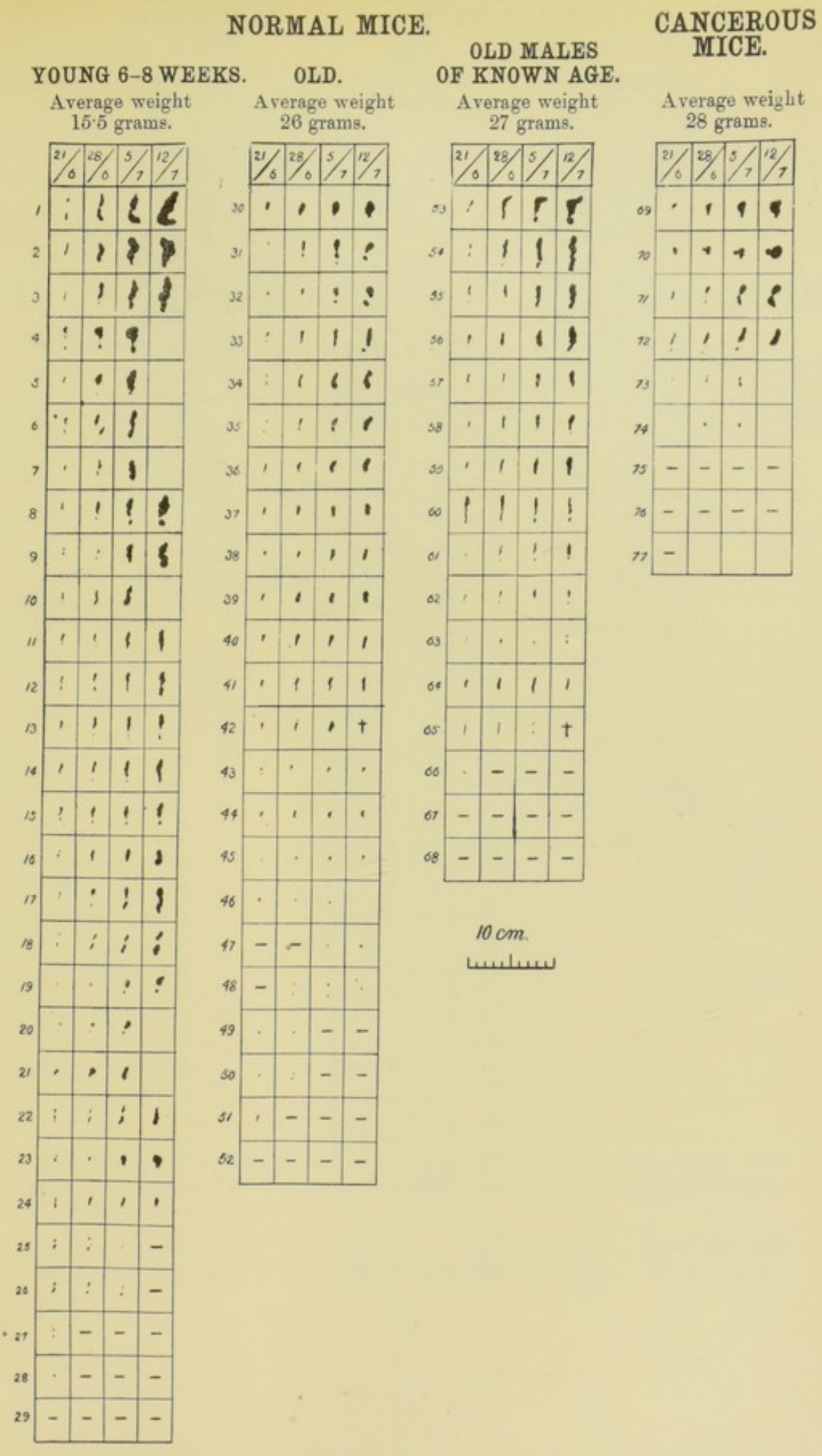


FIG. 41.—Suitability for the growth of transplanted cancer of mice in which cancer has developed spontaneously and has been removed by operation as compared with non-cancerous mice.

All mice inoculated 11.6.10 in the right axilla with 0·01 c.c. of carcinoma T. (Exp. T/29 K). Mice 1–68 controls (see headings of columns); 69–77 cancerous mice.

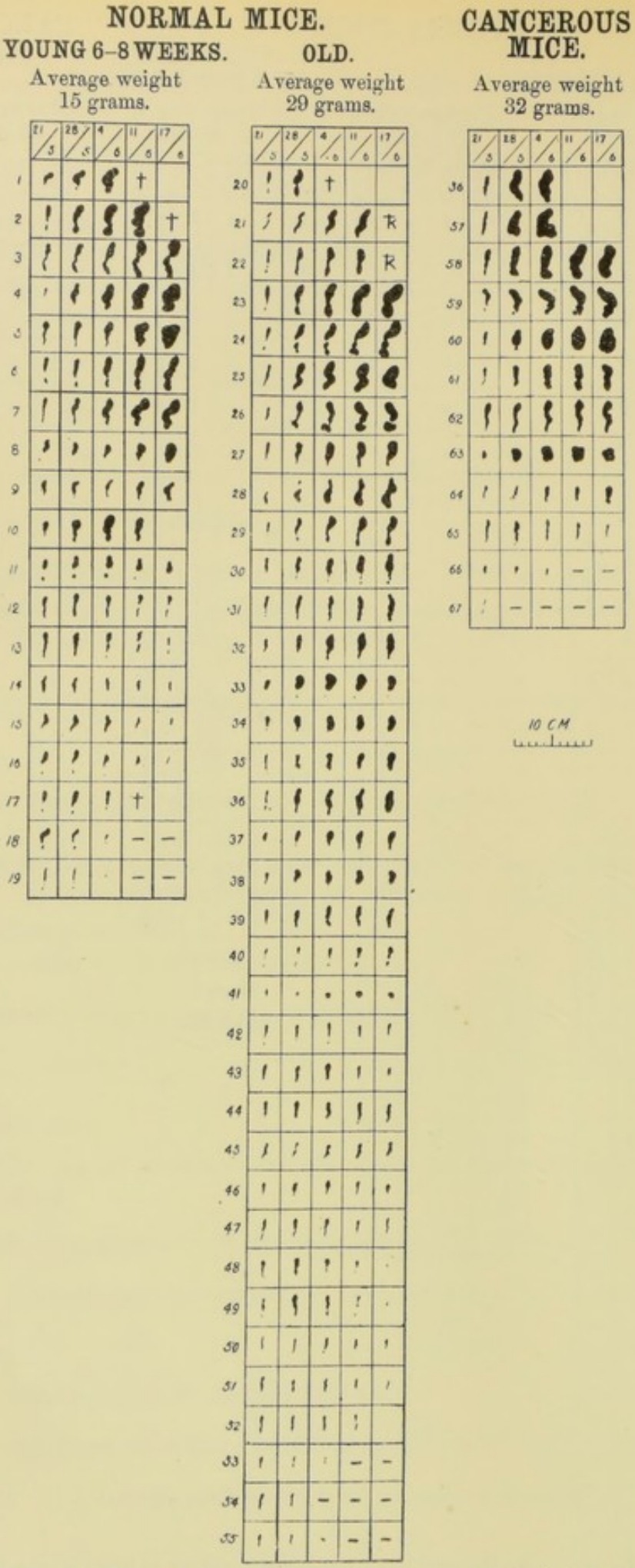


FIG. 42.—Suitability for the growth of transplanted cancer of mice in which cancer has developed spontaneously and has been removed by operation as compared with non-cancerous mice.

All mice inoculated 11.5.10 in the right axilla with 0·01 c.c. of carcinoma 199. (Exp. 199/16 C). Mice 1–55 controls (see headings of columns); 53–67 cancerous mice.

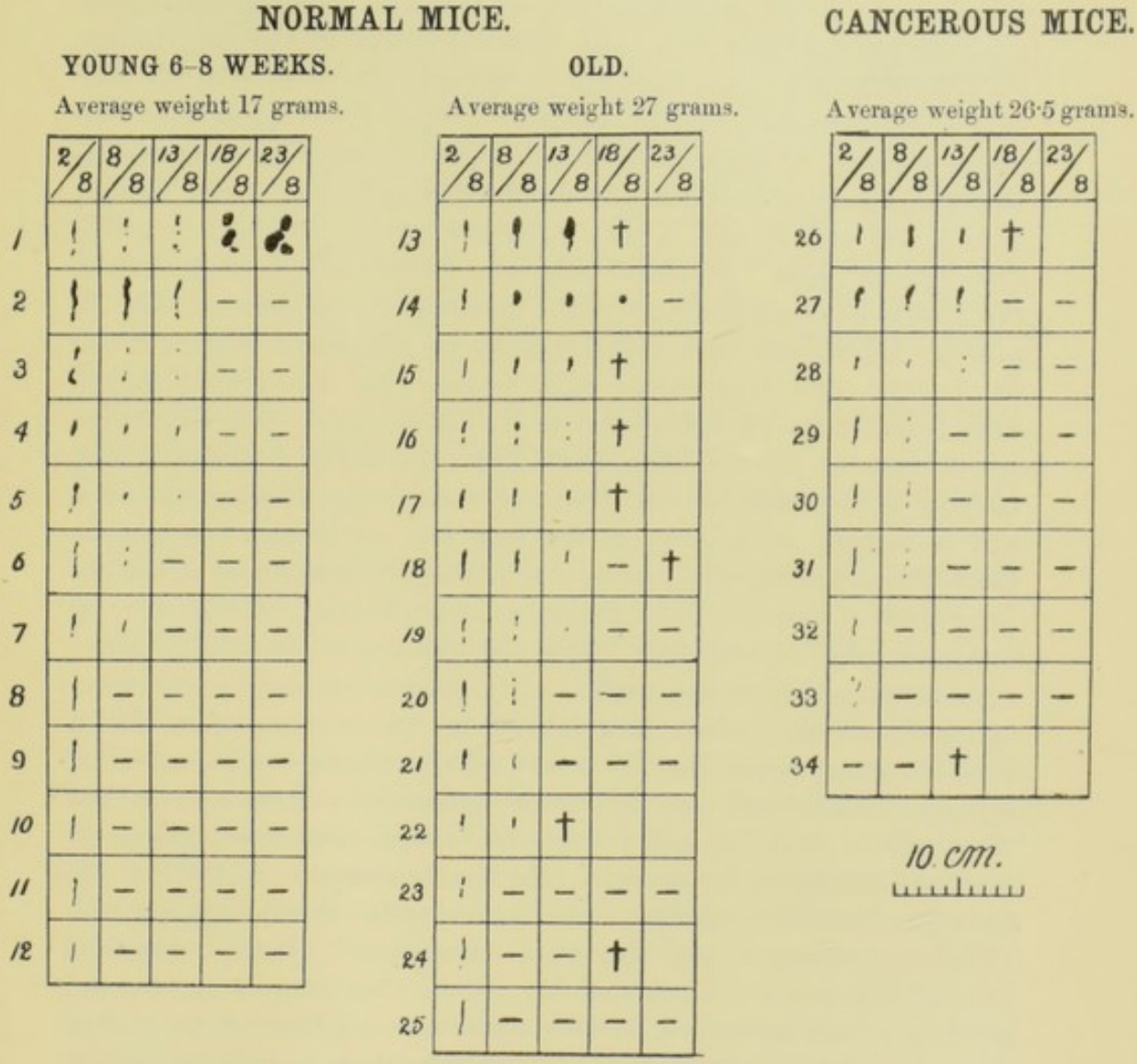


FIG. 43.—Suitability for the growth of transplanted cancer of mice in which cancer has developed spontaneously and has been removed by operation as compared with non-cancerous mice.

All mice inoculated 26.7.10 in the right axilla with 0·025 c.c. of carcinoma 206. (Exp. 206/61 B). Mice 1–25 controls (see headings of columns); 26–34 cancerous mice.

On the whole these experiments have failed to elicit any striking difference in suitability between spontaneously affected and normal animals. As in all transplantations of tumours young animals show a greater susceptibility than older ones. In most of the experiments the transplanted tumours have grown better in the young controls than in the spontaneously affected animals. When these cancer mice are compared with old mice of the same age, there seems in some experiments with certain tumours to be a difference in favour of the former. This is the case in exp. 1 and 3, particularly in the last, in which carc. 199 was used. A similar difference seems to be brought out in the results of implantation of some spontaneous tumours (*v.* table 3, p. 60). Hardly any such difference can be discovered in exp. 2, and in the last two experiments with tumour 206 the growth in the spontaneous mice was behind that in the old normals. The differences which exist in the two first-mentioned experiments are small, and, keeping in view the fact that the conditions in spontaneously affected and in normal mice, even at the best, are not strictly comparable, there does not seem to be evidence of an essentially enhanced suitability for growth of cancer cells in general in spontaneously affected mice as compared with normal mice. This conclusion seems to stand in direct opposition to the perfect conditions of growth offered by the spontaneously affected mouse to grafts of its own tumour cells. But it must be remembered that these are cells belonging to this individual organism, fitted for the special conditions present, and the behaviour of these cells in their own soil does not afford any evidence of any general increased suitability for growth. A general enhanced suitability is, on the other hand, directly disproved by the results of implantation both of other spontaneous and of propagated tumours. If this conclusion be accepted, then it follows that the reason why tumours develop and grow in certain animals is to be sought less in general constitutional anomalies than in local conditions which allow a cell to develop malignant characters and, by virtue of them, to grow in an otherwise normal individual. In other words, it is in the conditions of origin of cancer that cancer mice differ from normal animals, and not in the conditions of growth. Thus the experiments recorded yield a further confirmation of the conclusion that the conditions of origin and the conditions of growth are different and distinct *.

* Bashford, E. F., & Murray, J. A.: 'The Significance of the Zoological Distribution,' etc. Proc. Roy. Soc. vol. lxxiii. 1904. Ehrlich & Apolant, *loc. cit.* p. 43.

(7) *Means of inducing Resistance to Transplantation of Cancer, tested in Spontaneously Affected Mice.*

The investigation of transplanted cancer has shown that a state of increased resistance to inoculation of cancer can be induced in normal mice by treatment with living mouse tissues, both cancerous and normal. In this case the cancer, which is subsequently inoculated, has originated in another individual of the same species. In the sporadic cases, on the other hand, the tumour cells grow in the organism to which they originally belong. It is, therefore, of some interest to test the methods which render normal animals resistant, in spontaneously attacked animals, as to their efficiency against cells belonging to the same organism.

The efficiency of any method of inducing resistance in spontaneously attacked animals could be detected in several ways:—

(1) By preventing local recurrence of the spontaneous tumour after operation.

(2) By preventing dissemination and appearance of metastatic nodules.

(3) By preventing development of new primary tumours.

In each of these cases there is the difficulty of deciding the moment at which the new nodule established itself. Experience with transplantable tumours shows that the immunity in cancer is mainly effective against the graft establishing itself, and far less effective against an already established tumour. To avoid this difficulty, and to make the conditions in spontaneously attacked mice conform as nearly as possible to those obtaining in inoculated normal animals, recourse has been had to artificial metastases, *i. e.* to grafting the mouse in another place with its own tumour-cells. Prevention of successful grafting of the mouse with its own tumour-cells is thus the fourth means by which an increased resistance could be detected, and the only one which is strictly comparable to that by which resistance against implantation of propagated tumours is demonstrated.

The proportion in which local recurrence after operation, metastases and development of new nodules occur, has been shown previously, as has also the success of grafting with the mouse's own tumour-cells in mice not previously treated (*vide* pp. 24, 50, 53, and 56). Special control animals as comparable as possible to the treated mice, have been chosen by selecting a number of spontaneously attacked mice, obtained and observed during the same period, and operated upon in the same way

under conditions as similar as possible to those obtaining in the treated mice. As mentioned earlier, where there is a question of the frequency of local recurrence, the length of time the mouse has remained alive after operation is a most important point, and only cases which have died without recurrence later than eight weeks after operation have been counted as negative.

			Local recurrence after operation.	New tumours developed.	Metastases in lungs.	Successful grafting with own tumours.
Spontaneously attacked mice.	Treated with skin.	Tumour removed..	$\frac{7}{12}$	$\frac{4}{12}$	$\frac{6}{12}$	$\frac{5}{6}$
		Tumour present ..	$\frac{9}{13}$	$\frac{5}{13}$	$\frac{9}{13}$	$\frac{13}{13}$
		Total	$\frac{16}{25}$	$\frac{9}{25}$	$\frac{15}{25}$	$\frac{18}{19}$
	Not treated controls		$\frac{21}{41}$	$\frac{21}{31}$	$\frac{14}{31}$	$\frac{3}{3}$

The bottom line of the table shows the results obtained in 31 such control cases (see also fig. 45). Upon these 31 mice 41 operations have been performed: 20 have been without recurrence up to the death of the animals (observation from 8 weeks to 36 weeks), while after 21 operations recurrences have taken place after 3–21 weeks. Twenty-one new tumour nodules have been observed to develop in different parts of the mammary apparatus of these 31 animals: the difficulty of deciding whether they are in each case to be considered new primary tumours, or subcutaneous metastases, has already been discussed. In 14 of the 31 animals, secondary growths, visible to the naked eye, were found in the lungs. Three of the control animals were inoculated with their own tumours, all 3 successfully.

The treated animals will next be considered. Of the means by which increased resistance to transplantation of cancer can be induced in normal animals, two have been tested in spontaneously attacked animals: one method is the inoculation of a tumour very liable to spontaneous absorption (carc. 206), the other inoculation of mouse embryo skin. Both methods have given the same result, and, as a larger number of cases have been treated in the latter way, the results obtained by it will first be

considered. The embryonic skin has been injected on the back in a dose of 0·10 c.c. The results in 25 spontaneously affected cases thus treated are given in the table above *. On these 25 mice, 25 operations have been performed, 16 of which have been followed by local recurrence after about the same interval as in the controls (2–21 weeks). Nine new tumours have been observed in these 25 mice. Metastases, visible to the naked eye, have been found in 14, and also in another case on microscopical examination, making a total of 15 in 25. Of 19 inoculations of the treated animals, each with its own tumour-cells, 18 have been attended with success (fig. 44). Twelve of these successful autoplastic implantations have been performed between two and three weeks after the treatment with skin, *i. e.* at a time when the immunity is most marked in normal animals after the same treatment.

To compare the figures obtained in the animals treated with skin with those of the controls, it is necessary to take into account some small differences between the two groups. First, the conditions for complete operation have been slightly less favourable in the treated animals, especially in those treated while the tumour has been still present. In their case the tumour has been allowed to grow for two to three weeks after the treatment with skin, before operation was performed. A higher ratio of recurrence is met with in the treated animals; this can be explained by the less favourable circumstances of the operation. Secondly the controls have, on the whole, lived longer after operation than the treated animals, the life of most of which has been shortened by the successful grafting of their own tumour. This factor may account for the smaller number of new tumours appearing in the treated animals as compared with the controls. Thirdly the greater ratio of metastases found in the treated animals is accounted for by the greater number of recurrences and the successful graftings with their own tumours. When the correction for these differences in the conditions obtaining between the two groups of mice is made, the results are very much the same in both sets.

Thus an influence of previous treatment with mouse-embryo skin has not been demonstrated either on recurrence after operation, appearance of new tumours, metastasis-formation, or even on grafting with the mouse's own tumour-cells. The difference from the results obtained by a similar treatment of normal mice, inducing in them a resistance against implantation of tumours, seems to be a fundamental one.

* In addition, 8 further cases treated with skin (0·10 and 0·25 c.c.) have given in the main the same results; they have not been entered in the table.

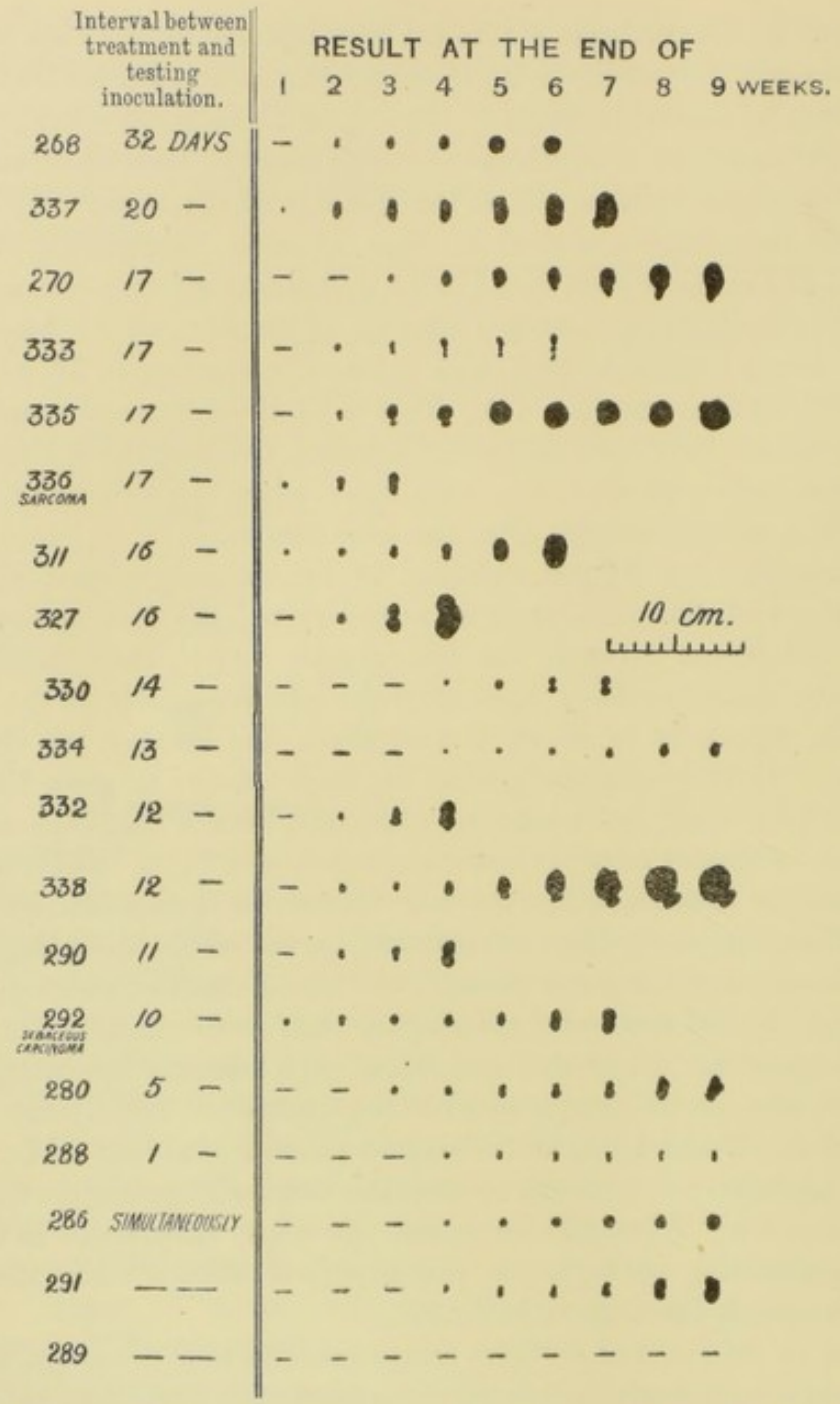


FIG. 44.—Failure of antecedent treatment with skin to inhibit grafting of spontaneously affected mice with their own tumours.

Index number of the spontaneously affected mice in the first column. All were treated with 0·10 c.c. mouse-embryo skin emulsion subcutaneously. The interval between treatment and the testing autologous inoculation (following immediately after removal of the tumour) is given in the second column. The experiments on the individual mice have been aligned so that the dates of operation and inoculation fall on the double line and from this the growth of the grafts is recorded at intervals of a week (*cf.* fig. 38, A–C, p. 57).

[*To face* p. 82.

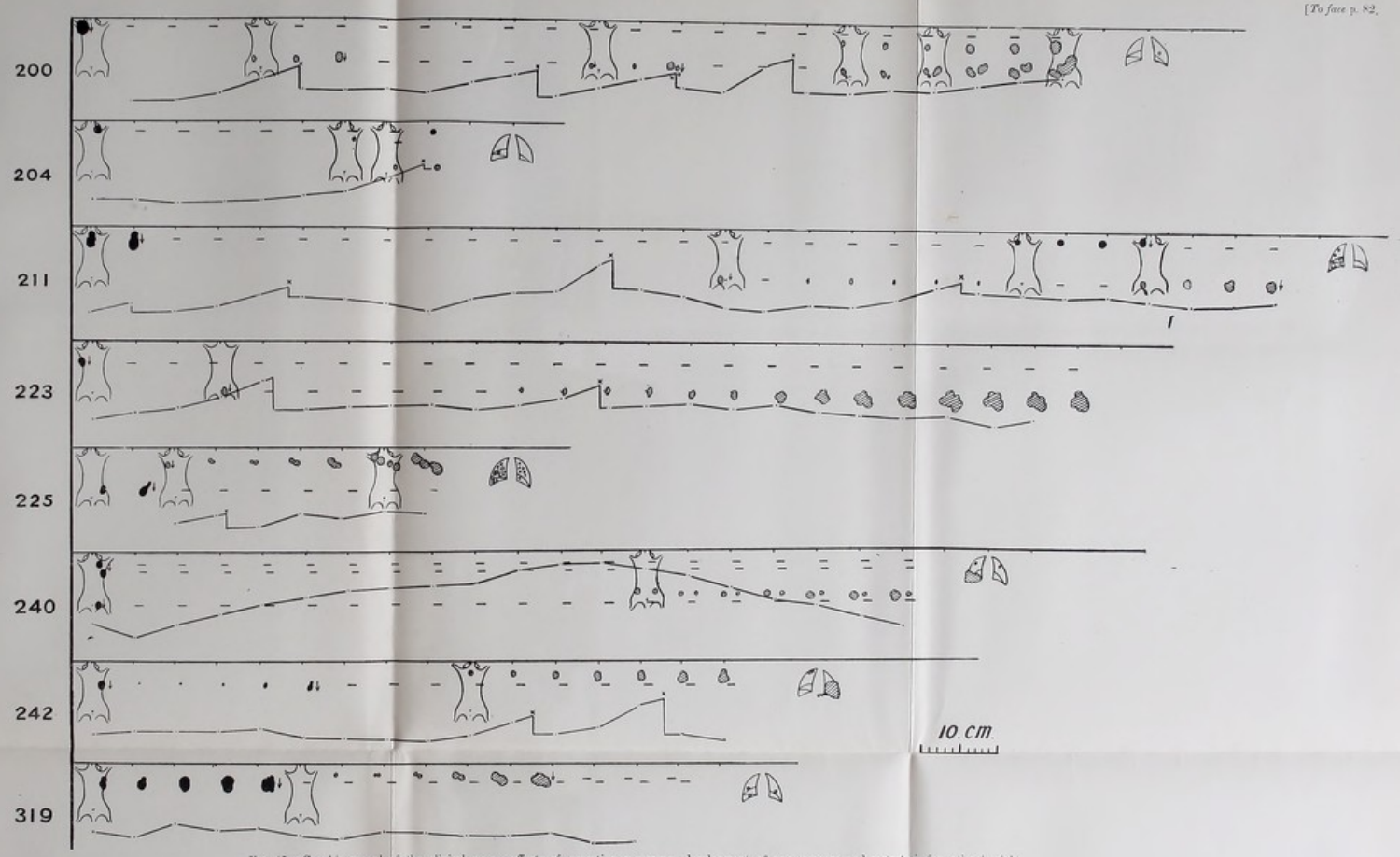


Fig. 45.—Graphic record of the clinical course, effects of operation, recurrence, development of new tumours, and metastasis-formation in eight mice with mammary adeno-carcinomata. These mice may be regarded as controls for the experiments in figs. 46 and 47, not having been treated previously. The size and site of the tumours are represented to scale in silhouettes or outline. The primary tumour found at entrance to the laboratory and its recurrences are entirely black; new nodules observed subsequently in different sites and metastases are striped. Metastases in the lungs are indicated diagramatically on an enlarged scale in the cases in which they were observed. Operation is indicated by an arrow (↓) behind the tumour operated upon, litters by a cross (×); the black line below the record is the weight curve.

To face p. 83.]

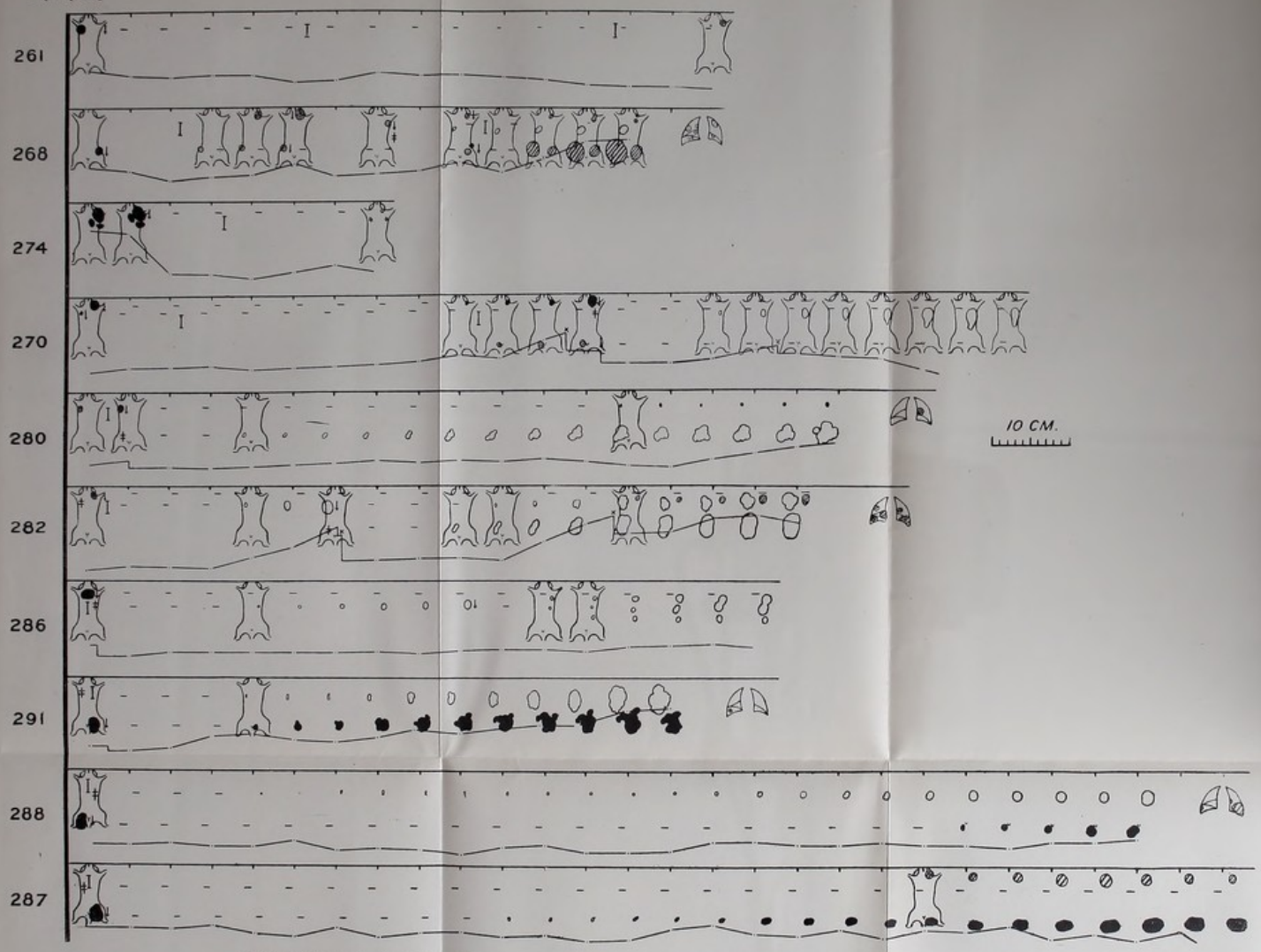


FIG. 46.—Failure of antecedent treatment with mouse-embryo-skin to prevent recurrences, development of new tumours, metastasis-formation, or successful grafting with the mouse's own tumour cells in spontaneously affected mice after removal of their tumours.

Graphic record as in figs. 45 and 47, of ten spontaneously affected mice which have been inoculated with 0·10 cc. of mouse-embryo-skin in tumour free interval. The conventions are the same as in figs. 45 and 47; the time of the inoculation of skin is indicated by (I); operation by (†); the site where implantation of own tumour has been effected by (‡). Primary tumours and their recurrences entirely black; new nodules and metastases striped; the tumours developing as the results of successful graftings with own tumour are represented by outlines.

The same applies to the results of inoculation with a spontaneously absorbing tumour (carcinoma 206). In one experiment 11 cancer mice have been inoculated with 0·02–0·03 gr. of carcinoma 206 ; the tumour has grown for one to four weeks in 4 of them, and has then been spontaneously absorbed, in the other 7 mice absorption followed without evidence of growth. In the 11 mice thus treated, recurrences after excision of the spontaneous tumour have occurred in 7, new tumours have developed in 6, and metastases have been found in the lungs at death in 4. Another experiment of the same kind is afforded by 8 cancer mice inoculated with the same carcinoma 206 (0·025 c.c.) and charted in fig. 43, 3rd column. Five of these mice have been operated upon, and in 2 the operation has been followed by recurrence. In 3 mice new tumours have developed, and in 3 metastases have been found in the lungs at death ; one was grafted with its own tumour with a positive result. There is thus so far no evidence of any influence on the spontaneous tumour, of a previous inoculation of carcinoma 206, followed by temporary growth and spontaneous absorption, which in normal animals induces a marked immunity.

When inquiry is made why the methods, which are efficacious in normal animals, totally fail to produce any similar effect in spontaneously affected mice against their own tumours, two main possibilities have to be considered. There is first the possibility that the same reaction is not set up in spontaneously affected mice as in normal mice, either because of the age of the animal or some other constitutional differences, or because of the presence of the tumour. Secondly there is the possibility that the immunity reaction may be induced, but is not effective against cells belonging to the same organism.

As to the question whether the age may hinder the induction of immunity, this can be excluded as it has been possible to demonstrate that the same immunity reaction takes place in old mice. The same argument applies to a hypothetical constitutional difference between spontaneously affected and normal mice. Twelve cancer mice treated with skin have been inoculated with sporadic tumour 282 with a negative result, although the inoculation of this tumour in 4 not previously treated cancer mice gave 2 successes and in normal mice gave 4 tumours in 26 mice. The absorption after a preliminary growth of carcinoma 206 and other tumours can with great probability be referred to a concomitant immunisation against the cells of the tumour. Cancer mice are capable of checking the growth of these tumours and bringing them to absorption just as normal mice are. The conclusion according to present

evidence seems warranted that the reaction is exactly the same towards strange cells as in normal mice.

The presence of a tumour may undoubtedly hinder the induction of the immunity reaction, as is seen in the difficulty of immunising animals bearing experimental tumours against a second inoculation, as shown in the Third Scientific Report of the Imperial Cancer Research Fund. If this were the only difficulty, it ought to be possible to overcome it by removing the tumour. Attention has been paid to this point, and in the table on p. 80 the treated mice are divided into two groups, the one having been treated after the tumour has been removed by operation (*cf.* fig. 46), the other while still bearing their tumours (*cf.* fig. 47). Up to the present it has not been possible to demonstrate any great difference between the two groups; even where the animals are treated with skin in the "tumour-free" period, an immunity against recurrence, metastasis, etc. has not yet been obtained. But it may be pointed out that even where the main tumour has been removed minute traces of tumour may be left behind as the recurrences and metastases show. At present it cannot be determined how small a quantity of tumour is necessary in order to impair the induction of immunity, and therefore it is preferable to suspend judgment until more data are available. The methods, efficient against transplantation of cancer when applied to an animal while it carries its spontaneous tumour, are without effect either on recurrence, metastasis, development of new tumours, or on the grafting of the mouse with its own tumour-cells. The similar results obtained by treatment with skin in the tumour-free interval seem to afford evidence that, in addition to the difficulty of inducing immunity, caused by the presence of a tumour, there is still another difficulty to be reckoned with in the case of the spontaneous tumours, viz. that of immunising against cells belonging to the same organism, where they are at home with the conditions for continued existence and growth. Thus the second possibility referred to seems the more likely, viz. that a resistance, although effective against strange cells, is without corresponding action upon the cells of the same organism. In all other forms of cell-immunity the same difficulty is met with.

The nearest approach to autoplastic transplantation under natural conditions, although it differs in important points from the transplantation of tumour-cells, is the embedding of the fertilised egg on the uterine mucosa. It may be asked whether the reaction set up by inoculation of skin has any influence on this process. Ten pregnant females have been isolated and, after having littered, they have been inoculated with 0·25 c.c. skin on the back. Three weeks later, at which

[To face p. 84.

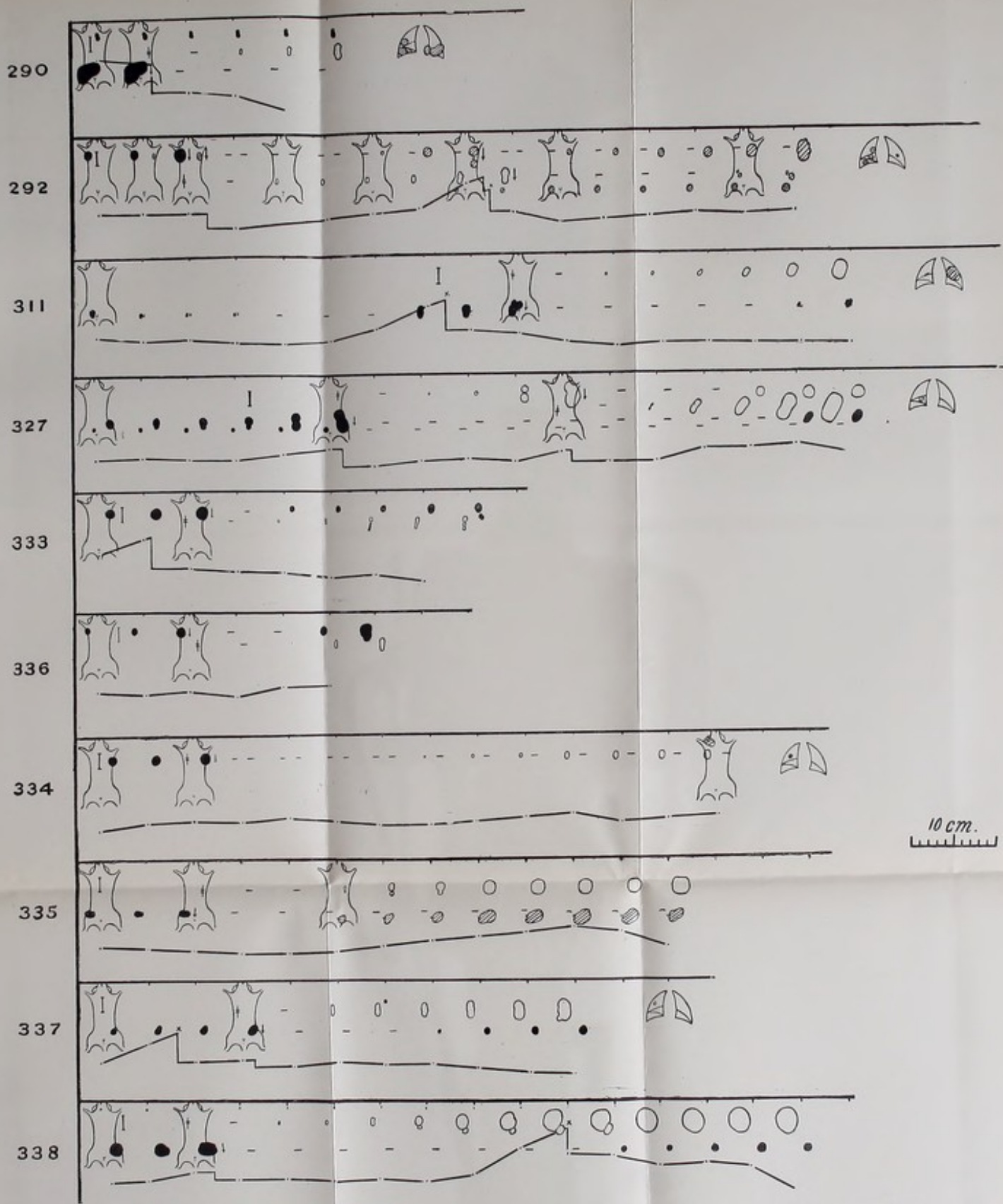


FIG. 47.—Failure of antecedent treatment with mouse-embryo-skin to prevent recurrences, development of new tumours, metastasis-formation, or successful grafting with the mouse's own tumour-cells in spontaneously affected mice while bearing their tumours.

Graphic record, as in figs. 45 and 46, of ten spontaneously affected mice which have been inoculated with 0·10 cc. of mouse-embryo-skin; the time of this inoculation is indicated by (I). Operation is indicated by an arrow (↓); the site where implantation of own tumour has been effected by (±). As in preceding and following figs. the primary tumours and their recurrences are kept entirely black, new nodules and metastases striped; the tumours developing as the results of the autoplastic implantations are represented by outlines. Other conventions as in figs. 45 and 46.

time the immunity against transplantation of cancer would be most marked, a male mouse has been put with each female. Pregnancy has followed immediately in 9 of the 10 cases, and birth has taken place from 21–27 days after the male has been placed in the cage. Further, animals treated with repeated inoculation of large doses of tumour and highly protected against transplanted cancer, breed as freely as do normal animals. The immunity effective against transplantation of malignant cells has thus no influence on the processes connected with the embedding of the egg.

Concluding Remarks.

New and additional evidence has been adduced that a general liability of the different organs of the mouse to the development of tumours exists. That they are real malignant growths is evidenced by their clinical course, their recurrence after operation, the formation of metastases, and the results of transplantation. Cancer in the mouse is essentially the same process as in man.

There are, however, certain characteristic features of its incidence in the mouse which attract attention and demand an explanation. The enormous preponderance of the tumours of special organs, in particular the frequency of growths of the mamma, constitutes one of them. The frequency of their multicentric development stands out as a further characteristic feature of tumours of the mamma in this animal. The same applies to the adenomatous nodules in the lungs, which are also remarkably frequent and most often multiple, and equally, although to a less extent, to the lymphomatous tumours. The first question is, can a cause, local or constitutional, be found to account for these peculiarities?

It has been generally assumed that the conditions of life of the mouse, in particular, factors connected with the physiological demands made upon the mamma through the constantly repeated pregnancies and lactation would suffice to account for the frequency of the mammary tumours. Put to the test by examining the previous history of a number of mice spontaneously attacked by cancer, which have been observed since birth in the laboratory, no evidence is obtained of these physiological factors playing any part. On the other hand, on examining the mammary gland in old normal mice, pathological changes of an inflammatory character are found to be very frequent. These changes have been entirely overlooked, up to the present, in the mammary gland of this animal. Along with these interstitial changes followed by sclerosis, hypertrophic changes of the

glandular epithelium are frequent. In some cases the evidence favours the view that the epithelial changes are intimately connected with interstitial lesions, in others the connection cannot be definitely established. In a large number of instances the hypertrophic condition of the epithelium appears to be the basis on which tumours develop. The suggestion is made that the interstitial lesions may play an essential part as a mediate cause of the epithelial changes. An explanation of the interstitial changes described may be found in nematodes living in the interstitial tissue where their embryos are liberated. They have been shown to be capable of causing considerable inflammatory reaction, and many of the histological pictures of inflammation and sclerosis, so often present in the mamma of old mice, can with great probability be referred to their presence. The hypothesis is put forward that the frequent and multicentric inflammation throughout the subcutaneous interstitial tissue in which the mamma is embedded may explain the frequency and multicentricity of the epithelial hypertrophic changes and the tumours arising on them. Similar considerations apply to the tumours of the lung and perhaps also to the lymphomatous conditions.

The next question is, are there any means of defining the relative parts played by local causes and by constitutional anomalies of the animal in the development of cancer?

Experiments show that growth nearly always follows when a fragment of a spontaneous tumour is re-introduced into the same animal in which the tumour has arisen, whereas the result is positive only in a very small percentage of other spontaneously attacked and normal animals. A general or constitutional idiosyncrasy favourable to the growth of its own cancer seems to be present in the spontaneously attacked animal, but it only applies to cells of its own body and not to cancer-cells in general. On investigating spontaneously affected cancer mice from the standpoint of the soil they offer for the growth of tumour-cells which have originated in other individuals, one finds that the conditions then do not differ much from those obtaining in normal animals. The same inference can be drawn from the results obtained when cancer mice are tested as to their suitability for the growth of propagated tumours, and the result compared with that obtained in normal mice under conditions as similar as possible. Regarded from the standpoint of the organism the comparison demonstrates that there is no evidence of general constitutional changes in the spontaneously attacked animals, greatly favourable to the growth of cancer-cells in general, which can explain the appearance of a cancer. The results of autologous

implantations are to be explained by the fact that the cells introduced belong to the same organism, where they are at home with food and environment, and because they can be re-introduced either without setting up any inimical reaction, or if a reaction directed against them is set up, being in congenial surroundings, they are indifferent to it. Regarded from the standpoint of the cancer-cell, its biological difference from the normal is the all important factor which permits it to grow in an otherwise normal organism.

The third question is: Do reactions similar to those observed in normal mice against propagated tumours take place in spontaneously attacked mice, and if they do, are they efficient against their own tumour? Cancer mice are capable of bringing transplanted tumours to absorption in the same way as do normal mice, and there is no reason to assume that the reactions leading to absorption differ essentially in spontaneously attacked mice and in normal mice, when the tumour-cells introduced are derived from another individual. In some rare instances there is evidence that a process of spontaneous absorption or healing may take place of spontaneous tumours, but it is very rare, and the evidence points rather to the importance of local than to general or constitutional changes. When attempts are made to induce increased resistance in spontaneously attacked mice, by the same means which are successful in normal mice against implantation of cancer, they seem to be totally ineffective against the implantation of the spontaneous tumour in the mouse in which it has arisen. There seems to be an insensibility of the cell in its own animal to reactions which are effective against strange cells in the same surroundings.

The outstanding result of this inquiry is the production of evidence of possible local causes of origin on the one hand, and the absence of general constitutional changes favourable for growth on the other. The importance of local factors for other forms of cancer has long been recognised and has been drawn attention to repeatedly in previous publications of the Imperial Cancer Research Fund. As knowledge of them increases, their many-sided nature appears more and more clearly. Whether they be animate or inanimate, bilharzia, nematodes, growth of other cancer-cells as in the development of sarcoma in mouse 469, or mechanical, chemical, and actinic influences, the ultimate result of their action may be the same: the creation of new cell-strains with continuous power of growth. On the one hand this process of cancerous transformation shows a close association with chronic irritative changes, on the other it has many points in common with what is known as spontaneous variation giving rise to sports in animals or plants. The part played by the chronic irritant is

obviously a mediate one, either in that it produces the altered conditions under which the first departure of the cell from the normal may take place, or that it gives spontaneously occurring sports of cells opportunity of multiplying and by degrees adapting themselves to a new mode of life, similar to what is observed in propagated tumours. This hypothesis rests on the assumption that the malignant transformation of the tissues may take place by stages, for which there now seems to be ample evidence both from the facts obtained from observation of spontaneous cancers and from those obtained in propagated tumours. The nature of this process is still unknown, but it is possible that it is being elucidated indirectly by the study of the variations which, as recorded in the third paper of this Report, have been demonstrated to occur in cancer-cells during their continued propagation. The hope of being able to investigate this problem by more direct methods lies in the possibility of being able to reproduce experimentally the conditions under which it may take place.

Literature.

Apolant, H.: Die epithelialen Geschwülste der Maus. Arbeiten aus dem königlichen Institut für experimentelle Therapie zu Frankfurt am M., 1906, Heft 1.

——: Ueber die biologisch wichtigen Ergebnisse der experimentellen Krebsforschung. Sammelreferat. Zeitschrift für allgem. Physiologie, 1909, s. 63, Bd. ix.

Bashford, E. F., & Murray, J. A.: The Significance of the Zoological Distribution, etc Proc. Roy. Soc., Ser. B, 1904, vol. 73.

——, ——, & Cramer, W.: The Natural and Induced Resistance of Mice to the Growth of Cancer. Proc. of the Royal Society, Ser. B, 1907, vol. 79. Third Scientific Report of the Imperial Cancer Research Fund.

Borrel, A.: Infection vermineuse et spirochètes chez souris cancereuses. Comptes rendus de la Societé de Biologie, 1905, p. 770.

——: Parasitisme et tumeurs. Annales de l'Institut Pasteur, 1910, vol. 24, p. 778.

Ehrlich, P.: Experimentelle Carcinomstudien an Mäusen. Arbeiten aus dem königlichen Institut für experimentelle Therapie zu Frankfurt am M., 1906, Heft 1.

——— Ueber die Genese des Carcinoms. Verhandl. der deutschen pathologischen Gesellschaft, 1908.

—— & Apolant, H.: Beobachtungen über maligne Mäusetumoren. Berliner klin. Wochenschrift, 1905, no. 28.

Gierke, E.: Die hæmorrhagischen Mäusetumoren. Ziegler's Beiträge, 1908, Bd. 43, Third Scientific Report of the Imperial Cancer Research Fund.

Jobling, J. W.: Multiple Tumours in Mice. Proc. of the Soc. for Exp. Biology and Medicine, New York, 1908, no. 1.

——: Spontaneous Tumours of the Mouse. Monographs on medical and allied subjects published by the Rockefeller Institute for medical research, no. 1, New York, 1910.

Haaland, M.: Les Tumeurs de la Souris. Annales de l'Institut Pasteur, 1905, vol. 21, p. 129.

HAALAND, M.: Beobachtungen über natürliche Geschwulstresistenz bei Mäusen. Berliner klin. Wochenschrift, 1907, no. 23.

——: Autoplastic and Homoplastic Transplantation of Tumours in Mice. Trans. Path. Sect. Roy. Soc. of Med., Nov. 16th, 1909. Lancet, 1909, vol. ii. p. 1588.

——: Means of Inducing Resistance to the Transplantation of Cancer tested in spontaneously affected Mice. Proc. of the Path. Soc. of Great Britian & Ireland, Jan. 1910. Journal of Pathology, 1910, vol. 14, p. 407.

——: Om Organismens reaktioner mod pathologisk cellevekst. Norsk Magazin for lægevidenskaben, 1909, no. 11.

v. HANSEMANN, D.: Das gleichzeitige Vorkommen verschiedenartiger Geschwülste bei derselben Person. Zeitschrift für Krebsforschung, 1904, Bd. 1, Heft 4.

LOEB, L.: On Transplantation of Tumours. Journal of Medical Research, 1901.

——: Further Investigations in Transplantation of Tumours. Ibid., 1902.

MURRAY, J. A.: Spontaneous Cancer in Mice, Histology, Metastasis, Transplantability and the Relations of Malignant New Growths to Spontaneously Affected Animals. Third Scientific Report of the Imperial Cancer Research Fund. London, 1908.

RUSSELL, B. R. G.: Sarcoma Development occurring during the Propagation of a Hæmorrhagic Adeno-carcinoma of the Mamma of the Mouse. Journal of Pathology and Bacteriology, 1910, p. 344, vol. xiv.

TYZZER, E. E.: A series of Twenty Spontaneous Tumours in Mice. Fourth report of the Caroline Brewer Croft Fund Cancer Commission of the Harvard Medical School. Boston, 1907.

——: A Series of Spontaneous Tumours in Mice. Fifth report of the Caroline Brewer Croft Fund Cancer Commission. Boston, 1909.

WILLIAMS, W. ROGER: The Natural History of Cancer. London, 1908.

Explanation of Abbreviations in the following Table of Spontaneous Tumours of the Mouse, giving histology, clinical course, metastasis, and results of transplantation: for earlier tumours vide *Second and Third Scientific Reports.*

abs. = absorption.
alv. = alveolar.
carc. = carcinoma.
cell. = cellular.
cyst. = cystic.
diff. = differentiation.
ex. = examined.
gen. = generations.
hæm. (hæmorrh.) = hæmorrhagic.
l. = left.
m. = months.
nod. = nodules.
op. = operations.
pap. = papilliferous.
part. = partial.
r. = right.
scler. = sclerotic.
sl. = slightly.
sp. (spont.) = spontaneous.
stat. = stationary.
str. = stroma.
trab. = trabecular.
w. = weeks.
× = multiple.
\+ = Recurrence, or metastases present.
− = Recurrence, or metastases not observed.

Other abbreviations will be easily understood from the context.

TABLE OF SPONTANEOUS TUMOURS OF THE MOUSE, GIVING HISTOLOGY

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
										No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.	Number of operations.	Tumours.	Interval till death.	Tumours.	Interval till recurrence.
70	ca. 7 m.	1½ year.	1	On the back between shoulders.	Lipoma.	...	.....	.....	1	1	1 year.	...	.....
						1	On the back in the scar of operation.	Polymorphous cell sarcoma.	.....	...	.....	...	.....
						1	Left ovary.	Adenocarc.	.....	...	.....	...	.....
150	...	13 w.	1	R. of neck.	Alv. carc. & adenocarc. Spont. absorption.	...	.....	.....	Not op.	...	.....	...	.....
152	...	7 w.	1	R. of vulva.	Alv. carc. with lumina.	...	.....	.....	Not op.	...	.....	...	.....
153	...	10 w.	1	R. axilla.	Alv. carc. with lumina.	...	.....	.....	Not op.	...	.....	...	.....
154	..	14 w	1	R. groin.	Alv. carc. with lumina.	...	.....	.....	1	1	5 w.	...	.....
						1	L. axilla.	Adenocarc.					
155	...	22 w.	1	L. axilla.	Cyst. and papilliferous adenocarc. (fissure-forming carc.).	...	.....	.....	1	...	.....	1	5 w.
						1	R. of vulva.	Cyst. adenocarc.	1	...	.....	1	8 w.
156	...	2 w.	2	R. groin.	Cyst. & adenom. adenocarc. rich stroma.	...	.....	.....	Not op.	...	.....	...	.....
				L. of vulva.	Cyst. adenocarc.								
157	...	7 w.	2	R. groin.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
				L. of vulva.	Alv. carc. & adenocarc.								
158	...	11 w.	2	L. of neck.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
				L. groin.	Hæmorrh. adenocarc.								
160	...	Killed.	×	Lung.	Adenomata.	...	.....	.....	.....	...	.....	...	.....
161	...	3 w.	1	R. of neck.	Hæmorrh. adenocarc.	...	.....	.....	Not op	...	.....	...	.....
162	...	21 w.	1	R. axilla.	Alv. carc. scler. stroma.	...	.....	.....	.....	...	.....	...	.....
						1	L. of neck.	Hæmorrh. adenocarc.					
163	...	4 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
164	...	11 w.	1	L. of neck.	Adenocarc. with keratinisation (adeno-cancroid).	...	.....	.....	Not op.	...	.....	...	.....
165	...	3 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
167	...	1 w.	1	L. groin.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	..	.....	...	.....
168	...	10 w.	1	L. shoulder.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
169	...	12 w.	2	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
				L. groin.	Adenocarc. (sp. abs.).								
172	...	Few days	1	L. groin.	Cyst. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
173	...	3 w.	3	R. shoulder.	Cyst. adenocarc. & alv. carc.	...	.....	.....	Not op.	...	.....	...	.....
				L. shoulder.	Hæmorrh. adenocarc.								
				L. of vulva.	Cyst. & pap. adenocarc. with scler. stroma.	...	.....	.....	.....	...	.....	...	.....
174	...	1 day.	2	L. axilla.	Alv. carc.	...	.....	.....	Not op.	...	.....	...	.....
				L. groin.	Alv. carc. with lumina.								
175	...	15 w.	1	L. of neck.	Hæmorrh. adenocarc.	...	.....	.....	1	1	7 w.	...	.....
						1	L. axilla.	Adenoma sl. œdematous stroma.					
176	...	5 w.	1	L. shoulder.	Adenocarc.	...	.....	.....	.....	...	.....	...	.....
						1	L. of anus.	Sebaceous adenoma.					
177	...	4 w.	1	R. of vulva.	Adenocarc. with small acini, sl. hæm.	...	.....	.....	Not op.	...	.....	...	.....
178	...	10 w.	2	R. groin.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
				L. of vulva.	Adenom. & adenocarc.								
179	..	5 w.	1	R. axilla.	Hæmorrh. adenom. & adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
						1	L. of vulva.	Hæmorrh. adenocarc.					
181	...	2 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
						1	R. axilla.	Adenom. adenocarc. with œdem. stroma.					
182	...	5 w.	1	R. of anus.	Sebaceous adenoma.								
183	...	2 w.	1	L. axilla.	Hæmorrh. adenocarc. & alv. carc.	...	.....	.....	Not op.	...	.....	...	.....
						1	R. axilla.	Cyst. adenocarc.					
184	...	2 w.	1	R. of neck.	Alv. carc. with traces of keratinisation.	...	.....	.....	1	...	.....	1	3 w.

Spontaneously attacked mouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously attacked mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
70	—											
	.....	.....	...	.....	.....	.....	.....	135	100	1	Died out in 3rd gen.	
	.....	.....	...	.....	.....	.....	.....	120	80	0		
150	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Tumour steadily diminishing in size.
152	—	.....	...	.....	.....	.....	.....	80	52	1	22 gen. in 29 months.	Spont. absorption with sclerosis & phagocytes (*cf.* figs. 33, 34, 35, & 36).
153	—											
154	—											
155	—	.....	...	.....	.....	.....	.....	200	132	4	11 gen. in 31 months.	
156	—											
157	+ lungs.	.....	...	.....	.....	.....	.....	20	14	(2)	Only prelim. growth followed by absorption.	
158	—											
160	.....	.....	...	.....	.....	.....	.....	40	30	0		
161	—											
162	—	.....	...	.....	.....	.....	.....	50	30	0		
163	—											
164	—	.....	...	.....	.....	.....	.....	300	240	1	Died out in 3rd generation.	
165	—											
167	+ large, lungs.											
168	+ large, lungs.											
169	+ lungs & liver (large)	+	...	.....	.....	.....	.....	.....	.....	.....	.....	Tum. in l. groin first growing, then continually diminishing in size; at the same time the tumour in l. ax. grew progressively and large metastases developed in liver and lungs (*cf.* fig. 33).
172	—											
173	— ? aortic gland.											
	.....	.....	...	.....	.....	.....	.....	110	80	10	17 gen. in 29 months.	
174	—		...	.....	.....	.....	.....	30	25	1	Not propagated.	
175	—											
176	—											
177	—											
178	—											
179	—											
181	—											
182												
183	—											
184	+ lungs.	.....	...	.....	.....	.....	.....	80	40	3	Not propagated.	

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
									Number of operations.	No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.		Tumours.	Interval till death.	Tumours.	Interval till recurrence.
185	...	1 w.	1	R. axilla.	Cyst. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
188	...	15 w.	1	R. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	6 w.
189	...	5 w.	1	L. axilla.	Alv. adenocarc.	...	.....	.....	1	1	5 w.	...	.....
190	...	2 w.	1	L. shoulder.	Alv. carc.	...	.....	.....	Not op.	...	.....	...	.....
191	...	32 w.	1	R. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	4 w.
						2	R. of vulva. L. of vulva.	Cyst. adenocarcinoma. Ad. adenocarc. str. cell.	1	1	20 w.		
192	...	10 w.	4	R. of neck.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	3 w.
				R. axilla.	Adenocarc. & alv. carc.	...	.....	.....	1	1	8 w.		
				L. groin.	Hæmorrh. adenocarc.	...	.....	.....	1	1	7 w.		
				L. of vulva.	Alv. adenocarc.	...	.....	.....	1	...	.....	1	3 w.
193	...	13 w.	2	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	7 w.
				L. groin.	Alv. carc.	...	.....	.....	1	...	.....	1	7 w.
						1	R. axilla.	Hæmorrh. adenocarc. & carc.					
194	...	killed.	1	L. groin.	Alv. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
196	...	15 w.	1	R. axilla.	Cyst. & pap. adenocarcinoma.	...	.....	.....	1	1	14 w.	...	.....
197	...	24 w.	1	L. axilla.	Alv. carc. with lumina (large cells).	...	.....	.....	1	1	23 w.	...	... ..
						1	Stomach.	Keratinised cysts.					
198	...	6 w.	2	L. shoulder.	Hæmorrh. adenocarc.	1	L. groin.	Adenocarc. small acini.	.....	...	.....	...	.....
				L. of vulva.	Alv. carc. & adenocarc.								
199	...	17 w.	1	R. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	15 w.	...	.....
200	...	25 w.	1	R. side of neck.	Hæmorrh. adenocarc.	...	.....	.....	1	1	24 w.	...	.....
						2	R. groin.	Trab. adenocarc.	2	...	.....	1	6 w.
							R. axilla.	Alv. & trab. carc.	.....	...	.....	1	4 w.
201	...	10 w.	1	L. axilla.	Alv. adenocarc.	...	.....	.....	1	1	8 w.	...	.....
202	...	17 w.	1	R. groin.	Hæmorrh. adenocarc.	...	.....	.....	3	...	.....	1	3 w.
						2	Lung.	Adenomota.	.....	...	.....	2	4 w.
203	...	10 w.	1	Middle of neck.	Hæmorrh. adenocarc. (large cells).	...	.....	.....	1	1	10 w.	.	.....
204	...	8 w.	1	L. of neck.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	8 w.
						2	L. axilla. L. groin.	Cyst. adenocarc. str. abundant.					
205	...	23 w.	1	L. axilla.	Adenom. & hæmorrh. adenocarc.	...	.....	.....	1	1	22 w.	...	.....
206	...	6 w.	1	R. of neck.	Hæmorrh. adenocarc.	...	.....	.....	1	1	10 w.	...	.....
						3	R. groin. L. axilla. L. of neck.	Alv. carc. Adenocarc. & alv. carc. Hæmorrh. adenocarc.	1	...	.....	1	3 w.
207	...	37 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	36 w.
						2	R. axilla. L. side of neck	Pap. adenocarc.					
209	11 m.	8 w.	1	L. groin.	Alv. & hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	6 w.
211	16 m.	28 w.	1	Middle of neck.	Adenom. & hæmorrh. adenocarc. (one focus of keratinisation).	...	.....	.....	1	...	.....	1	21 w.
						1	R. of vulva.	Adenocarc.	1	...	.....	1	10 w.
213	...	27 w.	2	R. groin.	Hæmorrh. adenocarc. (loc. cell. str.).	...	.....	.....	1	1	26 w.	...	.....
						1	L. axilla.	Hæmorrh. adenocarc.	1	...	.....	1	10 w.
214	...	15 w.	1	L. of vulva.	Alv. & hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	10 w.
						1	L. of neck.	Adenoma.					
216	...	5 w.	1	L. of vulva.	Alv. & hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	3 w.
217	...	9 w.	1	R. axilla.	*Cancroid.*	...	.....	.....	2	...	.....	1	1 w.
						1	Lung.	Adenoma.	(part).			1	2 w.
218	...	13 w.	2	L. shoulder.	Hæmorrh. adenocarc.	...	.....	.....	1	1	12 w.	...	.....
				Basis of l. ear.	Hæmorrh. adenocarc.	...	.....	.....	1	1	9 w.		
						1	L. of vulva.	Hæmorrh. adenocarc.					
						3	Lung.	Adenomata.					
219 ♂	...	2 w.	1	R. thigh.	*Round-cell sarcoma. Osteosarcoma.*	...	.....	.....	1 (part).	...	.....	1	2 w.

Sta-sly ked se.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously affected mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
5	.....	Small, lungs.	...	.....	.....	.....	.....	40	34	(2)	Not propagated.	Grew for 6 weeks, then spontaneous absorption.
8	+ lungs.											
9	+ lungs.	.....	...	.....	.....	.....	.....	100	80	5	9 gen. in 18 months.	
0	—											
1	—	.....	...	.....	.....	— 206		.				
2	—											
3	—											
4	—											
6	—											
7	.....	+ lungs intravasc.										
8	.....	+	...	.....	.....	.....	.....	100	60	1	Not propagated.	Grew for 6 weeks, then spontaneous absorption.
9	—	.....	...	.....	.....	.....	.....	40	27	1	20 gen. in 18 months	
0	Large, intraperit.	Smal lungs.	...	.....	.....	.....	.....	80	71	9	7 gen. in 9 months.	
1	—	—										
2	—	—	...	.....	.....	.....	.....	20	15	0		
3	—											
4	+ lungs.											
5	—	— ?	...	.....	.....	+* 206						
6	+ lungs.	.....	...	.....	.....	.....		40	35	20*	57 gen. in 20 months.	* Only prelim. growth followed by spont. absorption.
7	+ lungs.	.....	...	.....	.....	— 206						
9	.....	—	...	.....	.....	.....	.....	33	19	0	....	3 litters recorded before development of tum.
1	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	4 litters recorded.
3	.....	—	...	.....	.....	— 206						
4	.....	—	...	.....	.....	— 206						
6	—											
7	.....	—	...	.....	.....	.....	.....	509	353	0		
8	.....	—	...	.....	.....	— 206						
9 ♂	—	.....	...	.....	.....	.....	.....	804	415	15	31 gen. in 19 months.	

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
										No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.	Number of operations.	Tumours.	Interval till death.	Tumours.	Interval till recurrence.
221	...	17 w.	1	R. axilla.	Hæmorrh. adenocarc. cyst. (+ nod. of granulation tissue).	...	.....	.....	1	1	16 w.	...	.....
						1	Lung.	Adenoma.					
223	...	24 w.	1	R. axilla.	Alv. hæmorrh. adenocarc.	...	.....	.....	1	1	24 w.	...	.....
						1	L. of vulva.	Cyst. hæmorrh. adenocarc.	1		.....	1	7 w.
224	...	12 w.	1	L. groin.	Alv. hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	6 w.
						2	R. axilla.	Alv. carc. & hæmorrh. adenocarc.	1	...	.....	1	10 w.
							L. shoulder.	Alv. carc.					
225	11 m.	9 w.	1	R. haunch.	Hæmorrh. adenocarc.	...	.....	.....	1	1	8 w.	...	.....
						2	R. shoulder.	Alv. hæmorrh. adenocarc.	1	...	.....	1	1 w.
							L. axilla.	Hæmorrh. adenocarc.					
226	20 m.	7 w.	2	R. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	7 w.	...	.....
				R. groin.	Alv. carc. & adenocarc.	...	.....	.....	1	...	.....	1	2 w.
						1	L. of neck.	Adenocarc.	1	1	5 w.		
227	...	16 w.	2	R. of vulva.	Hæmorrh. adenocarc. & adenoma.	...	.....	.....	1	...	.....	1	7 w.
				L. of vulva.	Hæmorrh. cyst.								
						2	R. axilla.	Ulcerated out.					
							L. axilla.	Adenom. & carc. alv.					
229	18 m.	2 w.	1	R. of vulva.	*Adenocancroid.* Scattered foci of kerat.	...	.....	.....	Not op.	...	.....	...	.....
230	17 m.	9 w.	1	L. groin.	Pap. adenocarc. scirrh.	...	.....	.....	1	...	.....	1	6 w.
						3	Lung.	Adenomata.					
231	...	4 w.	2	L. flank.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	2 w.
				L. of vulva.	Alv. hæmorrh. adenocarc.	...	.....	.....	1	1	3 w.		
232	...	5 w.	2	R. flank.	Alv. hæmorrh. adenocarc.	...	.....	.....	1	1	5 w.	...	.....
				L. groin.	Alv. hæmorrh. adenocarc.	...	.....	.....	1	1	5 w.		
234	...	19 w.	1 (2nod.)	R. axilla.	Pap. adenocarc. necr.	...	.....	.....	1	1	19 w.	...	.....
235	...	5 w.	2	Front of l. foreleg.	Hæmorrh. adenocarc.	...	.....	.....	1	1	4 w.	...	.....
				Behind l. shoulder.	Cyst. adenocarc.	...	.....	.....	1	1	4 w.		
						1	R. shoulder.	Adenocarc. & alv. carc.					
236	...	26 w.	1	L. axilla.	Alv. hæmorrh. carc.	...	.....	.....	1	...	.....	1	9 w.
237	...	4 w.	2	R. groin.	Hæmorrh. adenocarc. & adenoma.	...	.....	.....	1	1	4 w.	...	.....
				L. groin.	Hæmorrh. adenoma, str. abundant.	...	.....	.....	1	1	4 w.		
238	...	24 w.	1	R. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	24 w.	...	.....
239	...	8 w.	2	R. axilla.	Hæmorrh. adenocarc. & alv. carc.	...	.....	.....	1	...	.....	1	8 w.
				L. of vulva.	Hæmorrh. adenocarc.	1	R. groin.	*Sebaceous adenocarc.*	1	..	.....	1	6 w.
									1	1	5 w.	...	.....
240	...	21 w.	3	L. supraclav.	Adenocarc.	...	.....	.....	3	3	20 w.	...	.....
				L. axilla.	Hæmorrh. adenocarc.								
				L. of vulva.	Cyst. adenom.								
						1	R. groin.	Hæmorrh. adenocarc. loc. str. cell.					
241	...	5 w.	1	R. axilla.	Alv. carc. c. necr. str. scler. (loc. cell).	...	.....	.....	1 (part).	...	.....	1	3 w.
242	...	15 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	10 w.	...	.....
						1	Between shoulders.	Hæmorrh. adenocarc.					
243	...	5 w.	1	R. of vulva.	Adenocarc. & alv. carc.	...	.....	.....	1	1	5 w.	...	.....
						1	Mediastinum.	Lymphoma.					
244	13 m.	23 w.	1	R. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	6 w.
245	18 m.	4 w.	1	R. supraclav.	Pap. adenocarc. scler.	...	.....	.....	1	1	3 w.	...	.....
246	...	8 w.	1	L. shoulder.	Hæmorrh. adenocarc.	...	.....	.....	1	1	7 w.	...	.....

ontaneously acked ouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously affected mice.	into normal mice				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
221	.....	+	...	.....	.....	— 206						
223	.....	—										
224	—	—	...	.....	.....	— 206						
225	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	5 litters recorded.
226	Large, lungs.	.....	...	.....	.....	— 206	.....	.....	.....	.....	.....	No record of partus.
227	+ lungs.	.....	...	.....	.....	— 206						
229	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	3 litters.
230	.....	+ ?	...	.....	.....	— 206	.....	82	74	8	7 gen. in 17 months.	Keratinised cysts in both ax. mamma.
231	—											4 litters.
232	—											
234	.....	—	...	.....	.....	.....	.....	30	27	0		
235	—	.....	...	.....	.....	.....	.....	44	44	4		
236	Large, lungs.											
237	Large, lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Adenomatous struct. biol. malign.
238	Large, lungs.	.....	...	.....	.....	.....	.....	108	99	13	16 gen. in 16 months.	
239	—	—										
....	.....	.....	...	.....	.....	.....	.....	120	103	18	14 gen. in 16 months.	Sebaceous alv. carc.
240	Large, lungs.											
241	—	.....	...	.....	.....	.....	.....	60	43	6	15 gen. in 17 months.	
242	Large, lungs.	.....	...	.....	.....	.....	.....	40	31	0		
43	+ lungs.	.....	...	.....	.....	.....	.....	40	34	0		
44	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	No record of littering.
45	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	4 litters.
46	—											

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
									Number of operations.	No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.		Tumours.	Interval till Death.	Tumours.	Interval Till recurrence.
247	...	9 w.	1	R. of neck.	Alv. & hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	5 w.
248	...	11 w.	1	L. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	1	11 w.	...	.....
249	...	14½ w.	1	L. of neck.	Alv. & hæmorrh. adenocarc.	...	.....	.....	2	...	.....	1 1	7 w. 4 w.
250	...	10 w.	1	R. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	5 w.
251	15 m.	2 w.	1	R. groin.	Alv. & hæmorrh. adenocarc.	1	L. axilla.	Alv. carc.	...	...	.....	...	.....
253	...	26 w.	1	R. groin.	Cyst. & pap. adenocarc.	...	.....	.....	1	1	25 w.	...	.....
254	10 m.	9 w.	1	R. groin.	Hæmorrh. adenoma.	...	.....	.....	1	1	9 w.	...	.....
255	10 m.	1 w.	×	glands.	*Lymphoma.*	...	.....	.....	...	...	.....	...	.....
256	12 m.	28 w.	1	L. axilla.	Cyst. & pap. adenocarc.	...	.....	.....	1	1	26 w.	...	.....
257	18 m.	33 w.	1	L. groin.	Hæmorrh. adenocarc.	...	.....	.....	2	1	27 w.	1	5 w.
						2	R. axilla. L. axilla.	Hæmorrh. adenoma. Adenoma.	1	1	22 w.		
259	...	2 days.	1	L. of vulva.	Alv. carc.	...	.....	.....	...	...	.....	...	.....
260	...	7 w.	1	L. axilla.	Adenocarc. sl. hæmorrh.	...	.....	.....	1	1	6 w.	...	.....
261	...	16 w.	1	R. axilla.	Adenocarc.	...	.....	.....	1	1	15 w.	...	.....
						1	L. axilla.	Hæmorrh. adenoma.					
262	6 m.	3 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	4 w.
265	14 m.	30 w.	1	R. ear.	*Melanoma.*	...	.....	.....	2 (part).	1	16 w.	1	1 w.
266	...	12 w.	1	R. axilla.	*Cancroid.*	...	.....	.....	2 (part).	...	.....	1 1	2 w. 3 w.
267	14 m.	11 w.	1	R. thigh.	Adenom. adenocarc. (small acini).	...	.....	.....	1	1	10 w.	...	.....
268	8 m.	13 w.	1	L. hip.	Cyst. & hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	9 w.
						2	R. hip. Left of neck.	Hæmorrh. adenocarc. Hæmorrh. adenocarc.	... ...	... ...	....	1 3	6 w. 2 w
270	12 m.	22 w.	2	R. of neck. R. axilla.	Hæmorrh. adenoma. Hæmorrh. adenocarc. (cyst).	... 1	..... R. of vulva.	..... Cyst. hæmorrh. adenocarc.	2 1	1 1	10 w. 10 w.	1	9 w.
271	...	27 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	27 w.	...	.....
						3	Lung.	Adenomata.					
273	...	14 w.	1	R. groin.	*Cancroid.*	...	.....	.....	3 (part).	...	.....	3	2 w.
274	...	8 w.	1 (3nod.)	L. axilla.	Cyst. adenocarc. str. scler.	... × 1	..... Nodules in Ovary (primary ?).	..... all mammæ. Adenocarc.	1	...	.....	1	6 w.
275	...	2 w.	1	L. groin. L. of vulva.	Adenocarc. str. cell.	...	.....	.....	Not op.	...	.....	...	.....
277	13 m.	1 day.	1	L. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
278	...	27 w.	1	R. haunch.	Alv. carc. & adenocarc. str. scler.	...	.....	.....	1	1	27 w.	...	.....
279	...	2 days.	1	R. flank.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
280	...	19 w.	1	R. axilla.	Alv. adenocarc.	...	.....	.....	1	...	.....	1	12 w.
281	14 m.	2 days.	1	L. axilla.	Trab. adenocarc. & alv. carc.	...	.....	.....	...	...	.....	...	.....
282	...	17 w.	1	L. of neck.	*Alv. carc. & adenocarc. Keratinisation.*	...	.....	.....	2	1	17 w.	1	4 w.
						1	L. axilla.	Hæmorrh. adenocarc.	...	...	.....	...	.....

ponta-neously tacked mouse.	Metastases.		Result of grafting the spont. affected mouse.				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spon-taneously attacked mice.	into normal mice.				
	Macro-scopic	Micro-scopic.	Pos. or neg.	Interval before growth recorded.	Other spon-taneous tumours.	Propa-gated tumours.		No. of mice inocul.	No. of mice sur-viving.	No. of tumours.	Results of further propagation.	
247	—	.....	...	.....	.....	.....	.....	40	30	0		
248	—	—										
249	+ lungs.											
250	—	—										
251	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	2 litters previously to development of tumour.
253	—	—	...	.....	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
254	—	—	...	.....	.....	.....	.....	.....	.....	.....	.....	Kept isolated from males since birth.
255	.....	.....	...	.....	.....	.....	.....	108	90	0		
256	—	—	...	.....	−282	.....	.....	.....	.....	.....	.....	1 litter previously to development of tumour. Inoc. with skin.
257	Large, lungs.	.....	...	.....	−282	.....	.....	.....	.....	.....	.....	1 litter previously to development of tumour. Inoc. with skin.
259	+ lungs.											
260	Lungs, *full.*											
261	—	—	...	.....	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
262	Lungs, full.	.....	...	.....	.....	.....	.....	40	36	0	.....	Kept isolated since quite young.
265	*Glands of neck* +.	.....	−*	16 w.	.....	.....	.....	168	145	0		* No growth, but tum. cells remained alive.
266	—	—	+	3 w.	−273	.....	$\frac{0}{2}$	437	349	0		
267	Large, lungs.	.....	...	.....	.....	.....	.....	60	40	10	6 gen. in 12 months.	Isolated since quite young. Inoc. with skin.
268	Large, lungs.	.....	+	2 w.	.....	.....	.....	.....	.....	.....	.....	No record of litter previous to development of tumour. Inoc. with skin.
270	—	—	+	3 w.	−282	.....	.....	.....	.....	.....	.....	2 litters previously to development of tumour. Inoc. with skin.
271	—	—	...	.....	−282	.....	.....	.....	.....	.....	.....	Inoc. with skin.
273	.....	+ Media-stinum.	+	2 w.	— 266, 282	.....	$\frac{0}{1}$	508	337	0		
274	? + ovary.	lungs —.	...	.....	.....	.....	.....	50	34	0	.....	Inoc. with skin.
275	—											
277	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	1 litter previously to development of tumour.
278	—	—	...	.....	.....	.....	.....	40	30		.....	Inoc. with skin.
279	—											
280	+ lung.	.....	+	3 w.	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
281	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Kept isolated since quite young.
282	Large, lungs.	+	+	3 w.	.....	.....	$\frac{2}{20}$	29	26	4	9 gen. in 10 months.	Inoc. with skin.
	.....	.....	+	4 w.	.....	.....	.....	12	12	0	In old normals of breed exp.	

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
									Number of operations.	No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.		Tumours.	Interval till death.	Tumours.	Interval till recurrence.
284	...	4 w.	1	R. groin.	Pap. adenocarc. +1 sebaceous adenom.	...	.....	.....	1	1	4 w.	...	.....
285	...	7 w.	1	R. haunch.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	4 w.
286	...	16 w.	1	On the back between shoulders.	*Adenocancroid.*	...	.....	.....	1	1	16 w.	...	.....
						2	Lung.	Adenomata.					
288	...	25 w.	1	R. of vulva.	Pap. adenocarc. scler.	...	.....	.....	1	...	.....	1	21 w.
289	...	26 w.	1	L. of vulva.	Hæmorrh. adenocarc. & cyst. adenom.	...	.....	.....	1	...	.....	1	9 w.
						1	L. of neck	Hæmorrh. adenocarc.					
290	...	5½ w.	2	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	4 w.	...	.....
				R. groin.	Alv. hæmorrh. adenocarc.								
291	17 m.	14 w.	1	L. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	3 w.
292	...	14 w.	2	R. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	13 w.	...	.....
				L. axilla.	*Sebaceous carc.*	...	.....	.....	3	...	.....	1	4 w.
						1	R. haunch.	Adenocarc. & alv. carc.				1	2 w.
												1	5 w.
293	...	1 w.	1	R. of neck.	Adenocarc. necr.	...	.....	.....	Not op.	...	.....	...	.....
294	...	7 w.	1	R. groin.	Alv. hæmorrh. & carc.	...	.....	.....	1	1	7 w.	...	.....
295	15 m.	35 w.	1	R. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	1	34 w.	...	.....
						3	Lung.	Adenomata.					
296	...	16 w.	3	R. shoulder.	Cyst. hæmorrh. adenocarc.	...	.....	.....	1	1	13 w.	...	.....
				L. axilla.	Cyst. hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	8 w.
				L. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	2	...	.....	1	3 w.
												1	5 w.
297 ♂	...	8 w.	1	Preputial gland.	*Adenocarc. of preputial gland.*	...	.....	.....	1 (part).	...	.....	1	4 w.
298	...	Killed.	1	R. axilla.	Hæmorrh. adenocarc.	...	.....	.....	Killed.	...	.....	...	.....
300	...	9 w.	1	R. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	4 w.
301	...	2 w.	1	R. groin.	Adenocarc.	...	.....	.....	.....	...	.....	...	.....
302	...	2 w.	1	L. of vulva.	Cyst. hæmorrh. adenocarc.	...	.....	.....	.....	...	.....	...	.....
303	...	3 w.	1	L. axilla.	Hæmorrh. adenocarc. (hæm. cyst).	...	.....	.....	.....	...	.....	...	.....
304	...	4 w.	2	R. axilla.	Hæmorrh. adenocarc.	..	.....	.....	.....	...	.....	...	.....
				R. of vulva.									
305	14 m.	6 w.	1	L. of vulva.	Cyst adenocarc. & alv. carc.	...	.....	.....	.....	...	.....	...	.....
306	18 m.	1 w.	1	R. of vulva.	Adenoma.	...	.....	.....	.....	...	.....	...	.....
307	...	3 w.	2	Back of neck.	Hæmorrh. adenocarc.	...	.....	.....	.....	...	.....	...	.....
				R. axilla.	Adenom. & hæmorrh. adenocarc.								
309	...	1 w.	2	R. axilla.	Trab. carc. c. lumina & cyst with cell. granulation tissue.	...	.....	.....	.....	...	.....	...	.....
				L. groin.	Hæmorrh. adenocarc.								
310	...	6 w.	1	L. of neck.	Hæmorrh. adenocarc.	...	Large lymphomatous tumour in mediastinum with perivasc. growth into the lungs.		Not op.	...	.....	...	.....
311	...	16 w.	1	R. of vulva.	Hæmorrh. adenocarc.	..	.....	.....	1	...	.....	1	6 w.
312	...	1 day.	2	L. axilla.	Alv. carc.	...	.....	.....	.....	...	.....	...	.....
				R. groin.	Hæmorrh. adenocarc. phagocytes.								

...onta-neously ...acked ...ouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously attacked mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
284	–	.....	...	.....	.....	.....	.....	48	36	3	4 gen. in 11 months.	Inoc. with skin.
285	–	–	...	.....	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
286	–	.....	+	4 w.	– 266, 273, 282.	.....	.....	297	211	24	8 gen. in 12 months.	Inoc. with skin.
288	Large, lung.	.....	+	4 w.	– 282	.....	.....	30	14	0	.....	Inoc. own tumour, stat. 11 w., then slow growth. Inoc. with skin.
289	.....	+ (intravasc.).	–	26 w.	– 282	.....	.....	.....	.....	.....	.....	Inoc. with skin.
290	*Large*, lungs.	.....	+	2 w.	– 282	.....	.....	.....	.....	.....	.....	Inoc. with skin.
291	Small, lungs.	.....	+	3 w.	– 282	.....	.....	.....	.....	.....	.....	Isolated from males since quite young. Inoc. with skin.
292	Lungs, large.	.....	+	1 w.	– 282	.....	$\frac{0}{7}$	264	133	16	11 gen. in 10 months.	Inoc. with skin.
293	+ lungs.											
294	–	.....	+	2 w.	– 282							
295	.....	–	...	.....	– 282, 292	.....	.....	.....	.....	.....	.....	1 litter previously to development of tumour.
296	Lungs, liver, kidney, and intraperit.	+	+	5 w.	+ 282							
297	?	.....	...	.....	+ 282 – 300	.....	.....	152	111	31*	6 gen. in 11 months.	* Nearly all spont. disapp., *cf.* p. 13.
298	–	.....	...	.....	...	.....	.....	26	14	0		
300	Large, lungs.	.....	+	3 w.	– 292							
301	–											
302	–											
303	–											
304	–											
305	–	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	No record of litter prev. to develop. of tum.
306	.....	–	...	.....	.....	.....	.....	.....	.....	.....	.....	No record of litter prev. to develop. of tum.
307	Large, lungs.	.....	+	2 w.								
309	–											
310	.....	–										
311	Large, lungs.	.....	+	2 w.	.....	.....	.....	.....	.....	.....	.....	Spont. disapp. of spont. tum.; reapp. after 3 weeks, *cf.* fig. 33. Inoc. with skin.
312	–											

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
										No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.	Number of operations.	Tumours.	Interval till death.	Tumours.	Interval till recurrence.
313	14 m.	40 w.	1	L. of neck.	Adenom. & hæmorrh. adenocarc.	...	.....	.....	1	1	35 w.	...	.....
						2	R. groin.	Hæmorrh. cyst adenoma.	1	1	4 w.	...	.....
							R. axilla.	Adenoma.					
						3	Lung.	Adenomata.					
314	13 m.	5 w.	1	R. shoulder.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
315	...	Few days	1	R. groin.	Adenocarc. & alv. carc.	...	.....	.....	.....	...	.....	...	.....
317	...	28 w.	1	L. groin.	Adenom. & hæmorrh. adenocarc.	...	.....	.....	.....	1	25 w.	...	.....
						3	L. axilla.	Hæmorrh. adenocarc.	2	...	.....	...	.....
							L. thigh.	Hæmorrh. adenocarc.					
							R. of vulva.	Cyst & pap. adenocarc. scler.					
318	...	1 day.	2	L. axilla.	Cyst adenocarc. & fissure form. carc.	...	.....	.....	.....	...	.....	...	.....
				R. of vulva.	Alv. adenocarc. & carc. scler.								
319	...	14 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	9 w.	...	.....
						1	Front of r. foreleg.	Alv. adenocarc. & carc. hæmorrh.					
320	...	2 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
321	18 m.	3 w.	1	L. axilla.	Hæmorrh. adenocarc.	..	.....	.....	Not op.	...	.....	...	.....
322	18 m.	7 w.	1	Back between shoulders.	Alv. adenocarc. & carc. keratinisat.?	...	.....	.....	1	...	.....	1	3 w.
						1	R. haunch.	Hæmorrh. adenocarc.					
323	...	3 w.	1	R. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
324	...	15 w.	1 (2 nod.)	L. axilla.	Adenom. adenocarc.	...	.....	.....	1	1	12 w.	...	.....
						2	R. shoulder.	Cyst adenocarc.					
							L. of vulva.	Alv. carc. & adenocarc.					
325	10 m.	5 w.	1	L. groin.	Adenocarc. (small acini) & alv. carc.	...	.....	.....	.....	...	.....	...	.....
326	...	4 w.	1	R. axilla (stat.).	Alv. hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
327	...	16 w.	2	L. groin.	Alv. hæmorrh. adenocarc. & alv. carc.	...	.....	.....	2	...	.....	1	10 w.
				R. of vulva.	Cyst adenocarc. (large cells).	...	.....	.....	.....	...	.....	1	4 w.
328	21 m.	Killed.	1	L. kidney.	*Hypernephroma.*	...	.....	.....	.....	...	.....	...	.....
						2	Lung.	Adenomata.					
329	...	8 w.	1	R. of neck.	*Squamous cell carc.?*	..	.....	.....	1	1	7 w.	...	.....
						2	R. axilla.	Hæmorrh. adenocarc.					
							R. groin.	Hæmorrh. adenocarc.					
330	...	10 w.	1	L. groin.	Alv. hæmorrh. adenocarc.	...	.....	.....	1	1	7 w.	...	.....
						1	Lung.	Lung adenoma.					
331	...	2 w.	2	L. axilla.	Cyst & alv. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
				R. axilla.	Met. in lymph gland. Alv. adenocarc.								
332	...	6 w.	1	L. groin.	Adenom. & adenocarc.	...	.....	.....	1	...	.....	1	2 w.
333	...	7 w.	1	L. axilla.	Cyst. adenocarc. & trab. carc. scler.	...	.....	.....	1	...	.....	1	2 w.
						1	Anus polyp.	*Seb. & squam. carc.*					
334	17 m.	14 w.	1	L. axilla.	Ac. trab. & alv.	...	.....	.....	1	1	12 w.	...	.....
						1	Front of r. foreleg.	Adenocarc.					
						1	L. uterus.	*Fibro-myoma.*					
335	...	12 w.	1	R. groin.	Adenom. & alv. adenocarc.	...	.....	.....	1	1	10 w.	...	.....
						1	L. of vulva.	Alv. adenocarc.					
336	...	6 w.	1	R. axilla.	*Spindle-celled sarcoma.*	...	.....	.....	1	...	.....	1	2 w.
						1	Lung.	*Squamous-cell tumour.*					

...ntaneously attacked mouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously attacked mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
313	—	—	...	.....	— 322 — 311, 327	+63	.....	.....	.....	....	.....	Kept isolated from males since quite young.
	.....	.....	...	.....								
314	+	+ in lungs & free in heart.	...	.....	.....	.....	.....	.....	.....	.....	.....	1 litter prev. to devel. of tumour.
315	.....	—										
317	Large, lungs & liver.	.....	...	.....	+ 322							
	.....	.....	...	.....	— 327							
318	? +											
319	Small, lungs.	.....	...	.....	— 322, 327, 334							
320	—											
321	.....	—	...	.....	.....	.....	.....	.....	.....	.....	.....	Never littered, kept isolated from males since quite young.
322	Large, lungs.	+	+	1 w.	.....	.....	.....	102	52	8	7 gen. in 8 months.	No record of litter. Inoc. with skin.
323	+ lungs.											
324	+ lungs.	.....	...	.....	— 322, 327, 334							
325	—	—	...	.....	+ 322	.....	.....	.....	.....	.....	.....	No record of litter.
326	—	.....	...	.....	— 322							
327	+ lungs.	.....	+	2 w.	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
	.....	.....	+									
328	—	—	...	.....	.....	.....	.....	40	24	0		
329	+ lungs.	.....	...	.....	— 322	.....	.....	.....	.....	.....	.....	Inoc. with skin.
330	.....	—	+	4 w.	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
331	Lungs full, *ax. gland.*											
332	+ lungs, periton. (extension).	.....	+	2 w.	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
333	—	—	+	2 w.	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
334	+ lungs.	.....	+	4 w.	.....	.....	0 8	16	16 16	0 0	Young normal mice. Old females of breed. exp.	No record of litter. Inoc. with skin.
335	—	.....	+	2 w.	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
336	—	—	+	2 w.	.....	.....	.....	140	100	18*	11 gen. in 7 months.	*Temporary growth, followed by spont. abs. Inoc. w. skin.

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of operation.				
										No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.	Number of operations.	Tumours.	Interval till death.	Tumours.	Interval till recurrence.
337	...	10 w.	1	L. groin.	Pap. adenocarc. scler.	...	.....	.....	1	...	.....	1	4 w.
338	...	15 w.	1	L. groin.	Alv. hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	9 w.
339	...	4 w.	1	L. groin.	Adenocarc. with small acini.	...	.....	.....	Not op.	...	.....	...	.....
340	...	1 w.	1	R. foreleg.	Alv. adenocarc. & fissure form. carc.	...	.....	.....	Not op.	...	.....	...	.....
			1	L. flank.	Seb. adenoma.								
341	...	2 w.	2	R. axilla.	Cyst & pap. adenocarc. scler.	...	.....	.....	Not op.	...	.....	...	.....
				L. groin.	Adenocarc. & alv. carc. large cells.								
342	...	1 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
343	21 m.	9 w.	1	L. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	4 w.
344	15 m.	14 w.	1	R. of vulva.	Adenom. & alv. adenocarc.	...	.....	.....	1	...	.....	1	8 w.
345	16 m.	15 w.	...	Glands of neck.	*Lymphoma.*	...	.....	.....	1	1	13 w.	...	.....
346	22 m.	2 w.	1	R. of neck.	Cyst adenom. & adenocarc.	1	L. axilla.	Cyst & alv. adenocarc.	.....	...	.....	...	.....
						2	Lung.	Adenomata.					
347	17 m.	4 w.	1	L. groin.	Adenocarc. (small keratinis. areas)?	...	.....	.....	1	1	4 w.	...	.....
348	18 m.	22 w.	1	Reg. of anus, polyp.	*Squamous-cell wart.*	...	.....	.....	1 part. 1 compl.	1	9 w.	1	3 w.
349	12 m.	14 w.	1	L. of neck.	*Squamous-celled carc.*	...	.....	.....	2	...	.....	2	4 w.
350	14 m.	1 w.	×	Glands.	*Lymphoma c. leucæmia.*	...	.....	.....	.....	...	.....	...	.....
351	...	11 w.	1	L. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	1	10 w.	...	.....
353	...	36 w.	1	L. haunch.	Hæmorrh. adenocarc.	...	.....	.....	1	1	35 w.	...	.....
						1	L. of vulva.	Adenocarc.	1	1	11 w.		
354	12 m.	2 w.	3	R. axilla, l. groin.	Alv. hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
				L. axilla.	Alv. carc.								
355	22 m.	21 w.	1	R. groin.	Adenom. & adenocarc.	...	.....	.....	1	1	19 w.	..	.....
						1	R. of vulva.						
356	13 m.	22 w.	1	R. axilla.	Alv. adenocarc. & carc. (light areas).	...	.....	.....	2	...	.....	1	6 w.
						1	L. groin.	Adenocarc; loc. diff. into sebaceous cells (?).	.....	...	.....	1	5 w.
357	23 m.	4 w.	1	L. groin.	*Squamous-cell carc.*	...	.....	.....	1	...	.....	1	2 w.
						1	Lung.	Adenoma.					
358	...	13½ w.	1	Back between shoulders.	Alv. adenocarc.	...	.....	.....	1	1	14 w.	...	.....
359	...	6 w.	1	L. axilla.	Adenom. & hæmorrh. adenocarc., in part stroma abundant.	...	.....	.....	1	...	.....	1	4 w.

ponta-sously tacked ouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously attacked mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
337	+ lungs.	.....	+	1 w.	.....	.....	.....	.....	.....	.....	.....	Inoc. with skin.
338	—	... ..	+	2 w.	.....	.....	$\frac{0}{5}$	76	60 15	2 0	Young normal mice. In old females of breed. exp.	Inoc. with skin.
339	Lungs (small), extension to perit.	+ intravasc.										
340	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Mamma exam. r. ax.; scler. connective tiss.
341	.....	—										
342	Large, lungs.											
343	+ lungs.	.....	...	.....	— 334, 338	.....	.....	....	.....	.....	.....	3 litters prev. to devel. of tumour.
344	—	.....	...	.....	— 334, 338	.....	.....	.....	.....	.....	.....	2 litters prev. to devel. of tumour.
345	Large, masses in lung & spleen.	.....	...	.....	— 334, 338	.....	.....	30	20	0		
346	.....	—	...	.....	.....	.....	.....	.....	.....	.....	.....	7 litters prev. to devel. of tumour.
347	—	.....	...	.....	— 334, 338	.....	.....	.....	.....	.....	.....	No record of litter.
348	—	.....	...	.....	— 334, 338	.....	.....	.....	.....	.....	.....	1 litter prev. to devel. of tumour.
349	Large, lungs.	.....	+	1 w.	— 357	.....	$\frac{1}{7}$	200	180	5	6 gen. in 5 months.	Never littered, isolated from males since quite young.
350	Int. glands, thymus, spleen.	.....	...	.....	.....	.....	.....	101	86	0	.....	Transplantation effected with blood intraven.: spleen, thymus, & gland intraperit. & subcut.
351	Large, lungs full.	.....	+	3 w.	— 353	.....	$\frac{0}{1}$	40	35	0		
353	—	.....	+	4 w.	— 351	— 199	$\frac{0}{1}$	40	35	3	.....	Grafting of own tum. pos. after 4 w., then stationary 6 w., followed by very slow growth.
354	—	.....	...	.....	...	.....	.....	.....	.....	.....	.....	2 litters prev. to dev. of tumour.
355	.....	—	...	.....	— 357, 349	.....	.....	.....	.....	.....	.....	No rec. of litter.
356	—	.....	...	.....	— 357, 349	.....	.....	40	36	1	3 gen. in 9 months.	No rec. of litter.
357	.....	—	+	2 w.	+ 349	.....	$\frac{0}{5}$	60	59	1?	.....	1 litter prev. to dev. of tumour.
358	—	.....	+ *	3 w.	— 349, 357							
			+ †	5 w.								
359	—	—	+	3 w.	.....	.....	.....	30	25	3		

* Small dose. † Large dose.

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
									Number of operations.	No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.		Tumours.	Interval till death.	Tumours.	Interval till recurrence.
360	17 m.	15 w.	1	L. groin.	Alv. adenocarc.	...	.....	.....	1	1	14 w.	...	.....
361	25 m.	18 w.	2	R. shoulder.	Adenom.	.....	.....	.....	2	2	15 w.	...	.....
				L. groin.	Adenom. adenocarc.	1	R. of vulva.	.....					
						1	Retroperit.	Lymphoma.					
362	24 m.	10 w.	1	R. flank.	Alv. carc. & adenocarc.	...	.....	.....	1	1	7 w.	...	.....
						4	R. haunch.	Cyst. adenocarc.	1	1	7 w.		
							R. groin.	Adenocarc.	1	1	7 w.		
							L. groin.	Adenom.					
							L. axilla.	Adenocarc. with small acini.					
363	14 m.	21 w.	1	R. of neck.	Hæmorrh. adenocarc.	...	.....	.....	1	1	19 w.	...	.....
						1	L. groin.	Adenocarc. with small acini.					
364	19 m.	11½ w.	1	L. groin.	Alv. hæmorrh. adenocarc. loc. str. cell. abund.	...	.....	.....	1	1	10 w.	...	.....
						3	L. of neck.	Adenom.					
							R. groin.	Adenom.					
							L. of vulva.	Adenocarc. & alv. carc.					
365	...	1 w.	1	L. of vulva.	Adenocarc. small alv.	...	.....	.....	.....	...	.....	...	.....
366	...	11 w.	2	R. axilla.	Cyst. hæmorrh. adenocarc.	...	.....	.....	2	1	9 w.	1	9 w.
				R. groin.	Alv. adenocarc. & carc.	...	.....	.....	.....	...	.....	...	.....
367	...	20 w.	1	R. axilla.	Hæmorrh. adenocarc.	...	.....	.....	2	1	9 w.	1	6 w.
368	...	15 w.	1	L. of vulva.	Cyst. adenocarc.	...	.....	.....	2	1	11 w.	...	.....
							L. of neck.	Hæmorrh. adenocarc.		1	6 w.		
369	...	19 w.	2	R. axilla.	Adenom.	...	.....	.....	2	...	.....	1	13 w.
												1	6 w.
				L. groin.	Cyst. adenocarc. pap. scler.		.....	.....	.....	...	.....	1	11 w.
370	16 m.	37 w.	1	L. axilla.	Alv. adenocarc.	...	.....	.....	1	...	.....	1	26 w.
						2	R. of vulva; stat. for 14 w.	Cyst. & pap. adenocarc. str. scler.	1	1	18 w.		
							R. axilla.						
371	16 m.	22 w.	1	R. groin.	Adenocarc. small acini.	...	.....	.....	1	1	19 w.	...	.....
372	...	1 w.	1 (3nod.)	L. axilla.	Alv. adenocarc. & carc. (diff. into sebaceous cells ?).	..	.....	.....	.....	...	.....	...	.....
373	...	3 w.	1	Back, betw. shoulders.	Alv. carc. & adenocarc. (diff. into sebaceous cells ?).	...	.....	.....	.....	...	.....	...	.....
374	...	24 w.	1	L. groin.	Hæmorrh. adenocarc.	...	.....	.....	1	1	5 w.	...	.....
375	...	3½ w.	1	L. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	.....	...	.....	...	.....
377	...	24 w.	1	L. shoulder.	Hæmorrh. adenocarc.	...	.....	.....	2	1	23 w.	...	.....
										1	7 w.		
378	...	14 w.	1	Margin anus.	*Sebaceous carc.*	...	.....	.....	1 (part).	...	.....	1	1 w.
						1	L. lower jaw.	*Cancroid.*					
380	21 m.	4 w.	1	L. groin.	Cyst. adenocarc. (large cells).	...	.....	.....	.....	...	.....	...	.....
382	19 m.	1 w.	×	Glands & peritoneum.	*Lymphoma.*	...	.....	.....	.....	...	.....	...	.....
383	22 m.	15 w.	1	Back of neck.	Hæmorrh. adenocarc.	...	....	.....	1	1	15 w.	...	.....
						1	Uterus, one side.	Retained placenta ?					
385	15 m.	7 w.	2	R. flank.	Hæmorrh. adenocarc. & alv. carc.		.....	.....	1	1	7 w.	...	.....
				L. supraclav.	Hæmorrh. adenocarc. & alv. carc.								

Spontaneously attacked mouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously attacked mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
360	—	.....	...	.....	— 349	.....	.....	.....	.....	.....	.....	1 litter prev. to dev. of tumour.
361	—	.....	...	.....	— 380 †	.....	.....	.....	.....	.....	.....	2 litters prev. to dev. of tumour.
362	Large, lung.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	4 litters prev. to dev. of tumour.
363	.....	—	...	.....	— 349	.....	.....	.....	.....	.....	.....	Never littered, kept with sister.
364	—	.....	...	.....	— 349	.....	.....	.....	.....	.....	.....	Never littered, kept with sister.
365	—											
366	.....	—	+ *	2 w.	— 369							
	.....	.....	+ †	3 w.								
367	+ lungs.	.....	+ *	2 w.								
			+ †	3 w.								
368	—	.....	+ †	3 w.	.....	.....	.....	.....	.....	.....	.....	No induced resistance from absorption of large dose of own tum. when tested later with small dose.
			+ *	2 w.								
369	Lungs. Retroperit. glands.	.....	+ *	4 w.	— 366	.....	.....	.....	.....	.....	.....	Tum. in r. ax. disappeared spontan. (dry gangrene), but recurred after 5 weeks.
			+ †	3 w.								
370	+ lungs.	+	...	.....	.....	— 63	.....	.....	.....	.....	.....	Never littered. Inoc. with skin. Two sisters, kept together.
371	—	.....	...	.....	.....	+ 63	.....	.....	.....	.....	.....	Never littered. Inoc. with skin.
372	—											
373	Lungs, full											
374	— ?	.....	...	.....	.....	+ 199						
375	.....	—										
377	+ lungs.	.....	+ †	6 w.	.....	+ 199						
378	—	.....	...	.....	.....	.....	.....	36	35	0	.....	Sepsis in some of inoc. mice.
380	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Never littered, kept with sister.
382	Int. glands.											
383	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	5 litters prev. to dev. of tumour.
385	Large, ungs.	.....	...	.....	— 388 †	.....	.....	.....	.....	.....	.....	No rec. of litter.

* Small dose. † Large dose.

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
									Number of operations.	No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.		Tumours.	Interval till death.	Tumours.	Interval till recurrence.
386	18 m.	14 w.	2	L. axilla (sp. abs.).	Alv. hæmorrh. adenocarc.	...	.....	.....	1	1	6 w.	...	.....
				R. of vulva (stat.).	Adenom., hæmorrh. adenocarc.	...	.....	.....	1	1	9 w.	...	.....
						1	L. of vulva (12 w. after entr.).	Hæmorrh. adenocarc.					
387	11 m.	1 w.	×	Glands.	*Lymphoma c. leucæmia.*	...	.....	.....	.....	...	.....	...	.....
388	11 m.	1 w.	1	L. groin.	Adenocarc. & alv. carc. scler.	...	.....	.....	.....	...	.....	...	.....
389	11 m.	7 w.	2	L. groin.	Adenom. & adenocarc.	...	.....	.....	1	...	.....	1	5 w.
				R. groin.	Hæmorrh. adenocarc. & alv. carc.	...	.....	.....	1	1	7 w.		
390	9 m.	13 w.	4	R. supraclav.	Hæmorrh. adenocarc.	...	.....	.....	1	1	9 w.	...	.....
				L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	4 w.
				R. axilla.	Alv. carc.	...	.....	.....	1	...	.....	1	5 w.
				L. groin.	Alv. adenocarc. & carc.	...	.....	.....	1	...	.....	1	9 w.
						1	L. of vulva.	Hæmorrh. adenocarc.	.....	...	.....	1	4 w.
391	...	6 w.	1	L. groin.	Hæmorrh. adenocarc.	...	.....	.....	.....	...	.....	...	.....
392	...	6 w.	1	L. groin.	Hæmorrh. adenocarc.	2	Lung.	Adenomata.	.....	...	.....	...	.....
393	20 m.	6 w.	1	L. groin.	Hæmorrh. adonocarc.	...	.....	.....	1	1	6 w.	...	.....
						1	L. axilla.	Adenoma.					
						1	Liver.	Angioma.					
394	...	2 w.	1	R. axilla.	Not ex.	...	.....	.....	.....	...	.....	...	.....
395	23 m.	1 day.	1	L. lower jaw.	*Cancroid.*	...	.....	.....	.....	...	.....	...	.....
396	16 m.	5 w.	1	R. groin.	Hæmorrh. adenocarc.	...	.....	.....	1	1	5 w.	...	.....
397	23 m.	13 w.	1	R. thigh.	Cystadenoma, mucoid str.	...	.....	.....	1	1	13 w.	...	.....
398	...	4 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	4 w.	...	.....
399	...	5 w.	3	L. axilla.	Adenocarc. & alv. carc.	...	.....	.....	2	2	5 w.	...	.....
				R. groin.									
				R. of vulva.									
400	15 m.	9 w.	1	L. axilla.	Adenocarc. sl. hæmorrh.	...	.....	.....	1	1	7 w.	...	.....
						1	R. of vulva.	*Adenocarc., foci of keratinisation.*					
						1	Lung.	Adenoma.					
401	...	6½ w	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	6½ w.	...	.....
403	...	8 w.	2	L. axilla.	Adenocarc. & abscess (absorp. ?).	...	.....	.....	1	1	8 w.	...	.....
				R. of vulva.	Adenocarc.								
404	16 m.	9 w.	1	R. of vulva.	Hæmorrh. adenocarc.	2	B. of neck.	Alv. hæmorrh. adenocarc.	Not op.	...	.....	...	.....
							L. axilla.						
405	24 m.	8 w.	3	Back of neck.	*Alv. adenocarc. & carc. with foci of keratin.*	...	.....	.....	.....	1	5 w.	...	.....
				L. axilla.	*Cancroid.*		.....	.....	1	1	5 w.		
				L. groin.	Cyst. adenocarc. (large cells).		.....	.....	.....	1	4 w.		
						1	R. of vulva.	Adenocarc., small ac.	.....	1	3 w.		

ponta-neously attacked mouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously attacked mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
386	Large, lungs.	.....	+ vulv. tum.	5 w.	— 388 †	.....	.....	.....	.....	.....	.....	3 litters. Mouse inoc. 0·6 spor. tum. 388 4 w. before grafting with own tum.; + after 5 w. Sec. tum. inoc. into mouse itself 7 w. after 388, + after 2 w. Tum. in l. ax. showed spont. retrogression for 4 w. followed by rapid growth. *Cf.* fig. 33.
	.....	.....	+ ax. tum.	2 w.								
387	Int. glands, spleen.	.....	...	.....	.....	.....	.....	112	89	0	.....	No record of litter.
388	—	.....	...	.....	.....	.....	$\frac{0}{4}$ †	.....	.....	.....	.....	No record of litter.
389	Lungs full.	.....	+	1 w.	— 390 †	.....	.....	.....	.....	.....	.....	Mouse inoc. with 0·5 spor. tum. 390 14 days prev. to inoc. with own tum. Tum. 390 rapidly absorb.; own tum. rapid growth.
390	+ lungs.	.....	+	2 w.	— 389 †	.....	.....	.....	.....	.....	.....	Never littered, kept with sister.
391	—	.....	...	.....	— 388 †							
392	.....	—	+	2 w.	— 388 †							
393	—	.....	+ †	7 w.	.....	.....	.....	.....	.....	.....	.....	Never littered, kept with sister.
394	Lungs full.											
395	Glands of neck.	.....	...	.....	.....	.....	.....	80	72	0	.....	Sepsis in some of inoc. mice.
396	+	.....	+ ‡	Killed. 5 w.	.....	.....	.....	.....	.....	.....	.....	No record of litter, kept with brother which died 3 months old.
397	.....	+	...	.....	.....	+ 63	.....	30	24	0	.....	Never littered, kept with sisters.
398	+ lungs.	.....	+ ‡	Killed. 4 w.								
399	—	.....	+ ‡	Killed. 5 w.								
400	.....	—	...	.....	.....	.....	.....	.....	.....	.....	.....	3 litters before development of tumour.
401	—	...	+ ‡	Killed. 6 w.								
403	—	...	+ ‡	Killed. 9 w.								
404	—	...	...	.....	.....	.....	.....	.....	.....	.....	.....	4 litters before development of tumour.
405	.....	? Met. or lung ad.	...	.....	.....	+ 63	.....	50	45	0	.....	1 litter before development of tumour.

* Small dose. † Large dose. ‡ Intraperit. inoc.

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
									Number of operations.	No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.		Tumours.	Interval till death.	Tumours.	Interval till recurrence.
406	24 m.	3 w.	1	L. groin.	Adenocarc., small ac.	1	L. axilla. Uterus.	Adenom., adenocarc. Epithelial cysts throughout the wall.	Not op.	...	.....	...	.....
407	18 m.	8 w.	1	L. axilla.	Hæmorrh. adenocarc.	... 1	..... Mediastinum.	..... *Lymphoma.*	1	1	7 w.	...	.....
408	17 m.	Found dead.	×	Peritoneum.	*Lymphomata.*	...	.....	.....	.....	...	.....	...	.....
411	...	1 day.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	.....	...	.....	...	.....
412	...	3 w.	1	R. groin.	*Cancroid.*	...	.....	.....	.....	...	.....	...	.....
413	...	13 w.	1	L. axilla.	Hæmorrh. adenocarc.	... 2	..... L. supraclav. L. of vulva.	..... Pap. cyst. adenocarc & alv. carc. Alv. carc.	1	...	.....	1	9 w.
415	...	10 w.	1	R. groin.	Hæmorrh. adenocarc.	2	Uteri, both sides.	Retained placenta?	Not op.	...	.....	...	.....
416	...	14 w.	1	L. axilla.	Hæmorrh. adenocarc.	... 2	..... Lung.	..... Adenomata.	1	1	13 w.	...	.....
418	...	13½ w.	×	R. axilla.	Cyst. adenocarc. pap. with scler. & cell. stroma.	...	.....	.....	Not op.	...	.....	...	.....
419	17 m.	6 w.	1	L. of vulva.	Alv. carc. & hæmorrh. adenocarc.	...	.....	.....	1	1	8 w.	...	.....
420	...	13 w.	1	L. axilla.	Adenocarc. loc. cell. str. like mixed tumour.	... 1	..... Lung.	..... Adenoma.	1	1	13 w.	...	.....
421	...	8 w.	1	Back of neck.	Adenocarc., cell. tissue between acini (ep.?).	...	.....	.....	2	1	8 w.	1	2 w.
422	27 m.	5 w.	1	L. flank.	*Lymphoma?*	...	.....	.....	.....	...	.....	...	.....
423	...	12 w.	1	L. of vulva.	Hæmorrh. adenocarc.	... Mesentery.	..... Lymphoid tumour.	.....	1	1	12 w.	...	.....
424	17 m.	7 w.	1	R. superclav.	Alv. hæmorrh. adenocarc.	... 1	..... L. of vulva.	..... Hæmorrh. adenocarc.	1	1	6 w.	...	.....
425	18 m.	5 w.	1	R. of vulva.	Adenocarc., small ac.	...	.....	.....	1	...	.....	1	3 w.
426	...	11 w.	1	L. groin.	Hæmorrh. adenocarc. cyst.	...	.....	.....	1	1	9 w.	...	.....
427	12 m.	5½ w.	2	L. axilla.	*Alv. carc. with lumina.* In parts sebaceous cells; str. well dev.	...	.....	.....	.....	...	.....	1	3 w.
				L. of vulva.	Alv. hæmorrh. adenocarc.	... 1	..... R. of vulva.	..... Adenom. & adenocarc.	....	1 1	5 w. 4 w.		
428	...	Killed.	1	L. groin.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
429	...	13 w.	1	L. groin.	Hæmorrh. adenocarc. & alv. carc.	...	.....	.....	1	...	.....	1	5 w.
430	15 m.	24 w.	1	R. axilla.	Adenoma & adenocarc.	... 2 3	..... L. axilla. L. of vulva. Lung.	..... Adenoma. Adenoma. Adenomata.	2	1	18 w.	1	6 w.
431	28 m.	8 w.	1	R. haunch.	Adenom. & hæmorrh. adenocarc.	... 1	..... Spinal cord (column ?).	..... *Sarcoma.*	1	...	8 w.	...	.....
432	6 m.	33 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	33 w.	...	.....
433	...	10 w.	1	L. groin.	Alv. carc. with lumina.	...	.....	.....	1	1	10 w.	...	.....
434	...	15 w.	1	L. axilla.	Cyst. hæmorrh. adenocarc.	...	.....	.....	2	2	15 w.	...	.....
				L. groin.	Cyst. hæmorrh. adenocarc.								
435	...	15 w.	1	L. axilla.	Adenom. & hæmorrh. adenocarc.	...	.....	.....	1	1	11 w.	...	.....

...onta-...ously ...acked ...ouse.	Metastases		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously affected mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
406	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	2 litt. bef. dev. of tum. Mamma in both ax. & gr., hypertroph. & with dil. ac., adenomatous nod., fig. 26.
407	.....	—	...	.....	.....	−63	.....	.....	.....	.....	.....	No record of partns.
408	—	.....	...	.....	.....	.....	.....	39	29	0		
411	—											
412	.....	—										
413	— (?)	.....	+	2 w.	.....	.....	.....	.....	.....	.....	.....	Splenectomised and reinoc. with own spleen.
415	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Splenectomised and reinoc. with own spleen.
416	.....	—	+	4 w.	.....	+199	.....	.....	.....	.....	.....	Mammæ ex.; atrophic.
418	+ lungs, small.	.....	...	.....	.....	+199	.....	.....	.....	.....	.....	Multiple nod. in r. ax. mamma, *cfr.* fig. 19 & p. 31.
419	+ lungs, small.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	2 litters.
420	.....	—	+	2 w.	.....	+199						
421	+ lungs.	.....	+	2 w.	.....	−199	.....	60	47	11	7 gen. in 7 months.	Diffuse growth of epith. cells, picture resembling mixed tum.
422	.....	.....	...	.....	.....	.....	.....	40	34	0	.....	*c.* p. 20.
423	+ small, lungs.	.....	...	.....	.....	−199						
424	+ lungs.	.....	...	.....	.....	−63	.....	.....	.....	.....	.....	No litter, kept with sister.
425	+ lungs.	.....	...	.....	.....	+63	.....	.....	.....	.....	.....	No litter, kept with sister.
426	+ lung & spleen.	.....	...	.....	.....	+199						
427	—	.....	...	.....	.....	+63	.....	.....	.....	.....	.....	No litter; isolated since quite young.
428	—											
429	—	.....	...	.....	.....	+199						
430	.....	—	...	.....	.....	−T.	.....	.....	.....	.....	.....	1 litter before devel. of tum. Mamma ex. Nod. hypertrophy, *cfr.* p. 34 and fig. 20.
431	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	3 litters before devel. of tum. Mamma ex. Keratinised cyst, hyalin halos round l. ax. ducts.
432	Alive.	.....	...	.....	.....	+199	.....	.....	.....	.....	.....	Mouse neg. of sarc. 100.
433	.....	—	...	.....	.....	+199						
434	+ lungs.	+	...	.....	.....	+T.						
435	—	.....	...	.....	.....	+T.						

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
										No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.	Number of operations.	Tumours.	Interval till death.	Tumours.	Interval till recurrence.
436	...	6 w.	1	R. axilla.	Cyst. adenocarc. pap. & alv. carc. (keratin. ?).	...	.....	.....	1	1	5 w.	...	.....
438	...	10 w.	1	L. flank.	*Cancroid.*	...	..... Ext. glands. Spleen. Liver.	..... *Lymphomata.*	1	1	8 w.	...	.....
439	20 m.	9 w.	1	Between shoulders.	Cyst. adenocarc. dil. vessels.	... 1	..... Kidney.	..... Sarcomatous tumour (lymphoma ?).	1	...	.....	1	2 w.
440	...	16 w.	1	L. of neck.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	7
441	18 m.	4 w.	1	L. axilla.	Alv. & trab. carc. with lumina.	...	.....	.....	1	1	3 w.	...	.....
442	...	5 w.	1	R. shoulder.	Adenom. & cyst. adenocarc.	...	.....	.....	1	1	5 w.	...	.....
443	...	28 w.	1	R. shoulder.	Hæmorrh. adenocarc.	... 1	..... Base of left ear	..... Adenocarc.	1 1	1 1	28 w. 6 w.	...	.....
444	...	10 w.	1	L. axilla.	Pap. adenoc. str. scler.	...	.....	.....	1	1	7½ w.	...	.....
445	...	9 w.	1	R. of vulva.	Adenom. adenocarc.	...	.....	.....	1	1	9 w.		
446	...	28 w.	1	R. axilla.	Cyst. hæmorrh. adenocarc.	...	.....	.....	1	1	28 w.	...	.....
449	16 m.	8 w.	1	R. axilla.	Alv. adenocarc. & hæmorrh. adenom.	... 1	..... L. groin.	..... Hæmorrh. adenocarc.	1	1	8 w.	...	.....
450	...	27 w.	1	R. axilla.	Alv. carc. & hæmorrh. adenocarc.	...	.....	.....	1	1	27 w.	...	.....
451	...	4 w.	1	R. axilla.	Cyst. adenocarc., secretion in acini.	...	.....	.....	Not op.	...	.....	...	.....
452	16 m.	Killed.	1	R. of vulva.	Alv. & trab. carc.	...	.....	.....	Not op.	...	.....	...	.....
453	15 m	12 w.	1	R. axilla.	Adenocarc.	... 1	..... L. groin.	..... Adenocarc. & alv. carc. (keratinisation ?).	1	1	12 w.	...	.....
456	...	4 w.	1	L. of vulva.	Adenocarc. & alv. carc.	...	..... Multiple swellings of all ext. glands, thymus, liver, and spleen.	.....	1	1	4 w.	...	.....
457	...	25 w.	1	L. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	1	1	18 w.	...	.....
458	...	2 w.	× 1	Glands. L. of vulva.	*Lymphoma.* Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
460	30 m.	Killed.	1	Middle of back.	*Spindle-cell sarcoma.*	...	.....	.....	Not op.	...	.....	...	.....
462	11 m.	16 w.	1	R. of vulva.	Adenocarc. with small acini.	...	.....	.....	1	...	.....	1	13 w.
463	17 m.	3 w.	×	Glands.	*Lymphoma.*	...	.....	.....	Not op.	...	.....	...	.....
464	...	Killed.	1	R. axilla.	Cyst. hæmorrh. adenocarc. & alv. carc.	...	.....	.....	Not op.	...	.....	...	.....
466 ♂	...	Some days.	1	R. of penis.	*Adenocarc. of preputial gland.*	...	.....	.....	.....	...	.....	...	.....
467	27 m.	Few days.	1	R. of jaw.	*Cancroid.*	...	.....	.....	Not op.	...	.....	...	.....
468	15 m.	4 w.	1	L. of vulva.	Retic. hæmorrh. carc.	...	.....	.....	.....	...	.....	...	.....
469	...	15 w.	1	L. axilla.	*Adenocarc. surrounded by sp. cell-sarcoma.*	...	.....	.....	.....	...	.....	...	.....
470	...	13 w.	2	R. groin. L. groin.	Hæmorrh. adenocarc. Hæmorrh. adenocarc.	 1	.... L. of neck.	.... Hæmorrh. adenocarc. with small acini.	1 1	1 1	13 w. 13 w.	...	.....

Spontaneously attacked mouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously affected mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
436	+ lungs.	.....	...	.....	.....	+ T.						
438	—	.....	—	Septic.	.....	+ T.	.....	40	35	0		
439	—	.....	...	.....	.....	+ T.	.....	.....	.....	.....	.....	No litter; kept isolated.
440	+ lungs.	.....	...	.....	.....	+ T.						
441	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	No litter recorded.
442	—	.....	...	.....	.....	+ T.						
443	Alive.	.....	...	.....	−460							
444	—	.....	...	.....	−460							
445	+ lungs (large).	.....	...	.....	−460							
446	Alive.	.....	...	.....	−460							
449	+ lungs (large).	.....	...	.....	−460	.....	.....	.....	.....	.....	.....	1 litter recorded.
450	Alive.	.....	...	.....	−460							
451	+ lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Mamma ex.; lactating. Same condition in tumour acini.
452	? —	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	No litter. Small hypertroph. nod. in mamma with sl. cell. stroma, partly scler.
453	+ large, lungs.	.....	...	.....	−466	.....	.....	20	19	0	.....	No litter.
456	All glands, liver, and spleen.											
457	Alive.	.....	...	.....	−460							
458	All glands, spleen, & liv.											
460	—	.....	...	.....	.....	.....	$\frac{0}{8}$	159 18	130 15	16* 0	Not propagated.	* Growth followed by absorption.
462	—	.....	...	.....	−460	.....	.....	.....	.....	.....	.....	No litter; kept with sister. Mamma ex. Nod. hypertrophy. *Cfr.* fig. 23.
463	Thymus, mesent., glands, & liv											
464	+ large, lungs.											
466	—	.....	..	.....	.....	.....	$\frac{0}{1}$	102	86	6*	3 gen. in 5 months.	* Only preliminary growth, followed by spont. absorption.
467	? lungs.											
468	? lungs.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	No lit., kept with sister.
469	+ lung & liver, mesentery & under diaphragm.*	.....	+ +	1 w. 2 w.	.....	−206	.....	48	30	12	6 gen. in 2½ months.	Tum. stationary for 10 weeks, then growth, 2 weeks later op., mixed tumour. *Cfr.* p. 19, figs. 16 & 17.
470	—	.....	...	.....	.....	−206						

* All metastases sarcomatous.

Spontaneously attacked mouse.	Age when tumour observed.	Length of observation after tumour observed.	Primary Tumour.			Development of new Tumours.			Results of Operation.				
										No recurrence.		Recurrence.	
			No.	Site.	Histology.	No.	Site.	Histology.	Number of observations.	Tumours.	Interval till death.	Tumours.	Interval till recurrence.
471	...	23 w.	2	R. groin. L. flank.	Hæmorrh. adenocarc.	...	.....	.....	1 Not op.	1	22 w.	...	.....
473	...	7 w.	1	L. groin.	Fissure-form. adeno-carc. & alv. carc.	...	.....	.....	1	...	.....	1	4 w.
474	...	5 w.	2	R. of vulva. L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1	4 w.
475	...	4 w.	1	L. axilla.	Alv. carc. with scler. str.	...	.....	.....	Not op.	...	.....	...	.....
476	...	4 w.	1	Middle of back.	*Spindle-cell sarcoma.*	1	Lung.	Adenoma.	1	...	.....	1	3 w.
477	...	7 w.	1	L. flank.	*Verrucous squamous-cell carc.*	...	.....	.....	1 (part).	...	.....	1	5 w.
479	...	16 w.	1	L. axilla.	Hæmorrh. adenocarc.	...	.....	.....	1	1	16 w.	...	.....
481	...	7 w.	1	Anus.	*Verrucous squamous-cell carc.*	...	.....	.....	1 (part).	...	.....	1	4 w.
482	...	22 w.	1	R. groin.	Hæmorrh. adenocarc.	...	.....	.....	1	1	17 w.	...	.....
483	...	22 w.	1	R. haunch.	Adenocarc. & carc.	...	.....	.....	1	1	20 w.	...	.....
484	...	1 w.	1	R. & l. groin.	Adenocarc. & alv. carc. with lumina.	...	.....	.....	Not op.	...	.....	...	.....
485	9 m.	11 w.	1	R. of neck.	Adenocarc., mostly small acini.	...	.....	.....	1	1	9 w.	...	.....
486 ♂	7½ m.	Killed.	1	L. of face.	*Cancroid.*	...	.....	.....	Not op.	...	.....	...	.....
487	28 m.	9 w.	1	R. groin.	*Cancroid.*	...	.....	.....	1	1	6 w.	...	.....
488	15 m.	2 w.	1	L. shoulder.	Adenocarc. with small acini.	...	.....	.....	Not op.	...	.....	...	.....
489	...	20 w.	2	R. axilla. L. axilla.	Hæmorrh. adenocarc. Hæmorrh. adenocarc.	 1	.... L. base of ear. Mesentery.	.... Hæmorrh. adenocarc. with small acini. Lymphomata.	1 1 1	1 1 1	18 w. 16 w. 14 w.	... ...	....
490	...	10 w.	1	L. groin.	Hæmorrh. adenocarc.	...	.....	.....	1	...	.....	1?	10 w.
491	...	1 w.	1	L. axilla.	Cyst. & pap. adenocarc. str. scler.	...	.....	.....	.....	...	.....	...	.....
493	...	13 w.	1	Base of clitoris.	Alv. adenocarc. scler. str.	...	.....	.....	1	...	.....	1	3 w.
494	...	9 w.	×	Glands.	*Lymphoma.*	...	.....	.....	Not op.	...	.....	...	.....
495	...	8 w.	1	L. of vulva.	Adenocarc., alv., & small acini.	...	.....	.....	1	...	.....	1	6 w.
496	...	2 w.	1	R. of vulva.	Hæmorrh. adenocarc.	...	.....	.....	Not op.	...	.....	...	.....
497	...	17 w.	1	R. axilla.	Hæmorrh. adenocarc.	...	Small cysts	on stomach.	1	1	15 w.	...	.....
498	15 m.	5 w.	×	Ext. glands.	*Lymphoma.*	...	.....	.....	Not op.	...	.....	...	.....
499	..	13 w.	1	L. of neck.	Adenocarc., large cells, hem., diff. into squamous ep.?	... 3	..... R. shoulder. L. axilla. L. groin.	..... Hæmorrh. adenocarc. Hæmorrh. adenocarc. & alv. carc.	1	1	13 w.	...	.....

Spontaneously attacked mouse.	Metastases.		Result of grafting the spont. affected mouse				Results of transplantation of Tumour					Remarks.
			with own tumour.		with strange tumour.		into other spontaneously affected mice.	into normal mice.				
	Macroscopic.	Microscopic.	Pos. or neg.	Interval before growth recorded.	Other spontaneous tumours.	Propagated tumours.		No. of mice inocul.	No. of mice surviving.	No. of tumours.	Results of further propagation.	
471	Alive.	.....	...	.....	.....	—206						
473	+small, lungs.	.....	...	.....	.....	—206						
474	—	.....	...	.....	.....	—206						
475	+lungs.											
476	.....	—	+	2 w.	.....	.....	.....	60	56	6*	Not propagated.	* Growth followed by absorption.
477	.....	—	—	6 w.	.....	.....	.....	.....	.....	.....	.....	Grafting with own tum. —, septic material ?
479	—	.....	...	.....	.....	—206	.....	.....	.....	.....	.....	Mamma ex.; connect. tissue very scler., streaks of inflammat.; small nod. hypertrop.; nematodes present.
481	—	.....	—	7 w.	.....	.....	.....	.....	.....	.....	.....	Grafting with own tum. —, septic material, ulcerated out.
482	Alive.											
483	Large, lungs.											
484	—											
485	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	3 litters. Mamma ex. Small hyp. nodule r. ax.; connect. tissue inflamm. changes.
486 ♂	—	.....	...	.....	.....	.....	.....	48	30	3	3 gen. in 4 months.	
487	—	.....	+	4 w.	.....	.....	.....	140	120	1*	.....	* Underwent spont. absorption.
488	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	No litter; kept with female.
489	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Nod. hypertrophy of mamma.
490	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Nod. hypertrophy of all mammæ. *Cfr.* figs. 21 & 20.
491	—											
493	—	.....	...	.....	.....	.....	.....	36	32	1	Died out in 2nd gen.	Mamma ex.
494	Large thymus, mediast. gland enormous spleen & liver.	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Mamma ex. Nematodes in interstitial tissue.
495	—	.....	...	.....	.....	.....	.....	.....	.....	.....	.....	Mamma ex. Atrophy & sclerosis.
496	—											
497	—											
498	One large mesent. gland.											
499	+lungs.											

[*Fourth Scientific Report of the Imperial Cancer Research Fund*, 1911.]

CANCEROUS ANCESTRY AND THE INCIDENCE OF CANCER IN MICE.

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

With the discovery and recognition of the occurrence of true malignant new growths in mice the vexed question of the importance of an hereditary factor influencing the incidence of cancer was seen to be amenable to experimental investigation *. The length of life in the human subject, no less than the uncontrollable conditions of human mating, so limits the opportunities for assembling accurate data that the opportunities afforded by short-lived animals were welcomed and have already formed the subject of several communications. The results, however, are not so conclusive as could be desired, mainly as a consequence of the small numbers of animals and the limited duration of the observations. The published investigations have acquired almost a stereotyped form and give little more information than is contained in the statement that a number of tumours have occurred in a restricted period in a certain number or family of mice all ultimately bred from one original pair. The relative proportion of the two sexes, the ages attained, both by the cancerous and by those that remained free from the disease, and the incidence of cancer as regards the earlier less in-bred or later more in-bred animals are omitted and probably not recorded. Thus Thorel † obtained 14 tumours in one year from a stock consisting of 60 mice (sexes ?) at the beginning of the year. There is no record of breeding during the year nor of the ages attained by the cancerous and non-cancerous animals. Tyzzer ‡ investigated the frequency of cancer in three families of mice obtained in each case by breeding from a mouse suffering from spontaneous cancer. In the first family, consisting of 24 offspring, three developed papillary cyst-adenomata

* Bashford, E. F.: The Problems of Cancer. Brit. Med. Journal, July 18th, 1903; Second Scientific Report, Part I. pp. 5 & 50; Third Scientific Report, pp. xi.–xiv.

† Thorel, Ch.: Kasuistisches zum Kapitel der sog. Mäusekarkinome. Verh. der Deutsch. Path. Gesellsch. zu Kiel, 1908.

‡ Tyzzer, E. E.: A series of Spontaneous Tumours in Mice with Observations on the Influence of Heredity on the Frequency of their Occurrence. Journ. Med. Research, 1907, xvii. p. 155, and Fifth Report of Cancer Commission of Harvard University, 1909.

of the lung. The ages attained were not great, in none more than fourteen months. In the second family of 29 offspring of a female mouse with an inguinal lymphoma, one developed lymphoma and one a papillary cyst-adenoma of the lung, Five died under six months of age and only seven attained the age of one year. The third family consisted of 98 offspring obtained by in-breeding from a male which never developed a tumour crossed with a female in which there was a large cystadenoma of the lung. Thirty-three of the offspring died at ages less than 6 months, sixty-five reached maturity, and of these three were still alive at the time the paper was written. Twenty (32 per cent.) of the sixty-two presented tumours. Seventeen presented primary cyst-adenomata of the lung, and in one of these there was also a subcutaneous squamous-cell carcinoma, in one an ovarian tumour, and in a third a subcutaneous hæmorrhagic carcinoma. In one of the three remaining tumour-mice there was a giant-cell sarcoma of the orbit and in the other two, double papillary cyst-adenomata of the ovaries. Tyzzer therefore concludes that heredity may be a factor in the development of primary tumours. Classified according as the parents did or did not develop tumours, there were sixty-eight offspring of non-cancerous parents with nine tumours and twenty-nine offspring of parents, one of which was cancerous with eleven tumours. Males and females are not distinguished. Corrected for early death of the mice in the two groups the ratios are :—in the non-cancerous, nine tumours in forty mice more than six months old ; in the cancerous group eleven tumours in twenty-four mice. Tyzzer recognises the dangerous smallness of the figures, but doubts the utility of a more elaborate statistical enquiry.

It would be unfair to minimise the value of Tyzzer's careful work, which has involved a great expenditure of time and energy. It is necessary at the same time to point out that the tumours whose occurrence he records are mainly of types of which the malignancy is not very pronounced. The cyst-adenomata of the lung, which comprise the majority of his tumours, have not yet been successfully transplanted, and in many the evidence of continued growth is so slight that it is hazardous to regard them in all cases as malignant. The multiple lymphomata also which take second place in frequency in his material, although they include new formations undoubtedly malignant, cannot be separated satisfactorily from conditions which have more the character of diffuse hyperplasias. It is to be noted also that while cyst-adenomata of the lung occur with equal frequency in males and females, multiple lymphomata are much more common in females than in males

in his material and, as has been shown in the preceding paper, are restricted to the female sex in the material of this laboratory. A serious source of error is hereby introduced when the frequency of tumours as a whole is reckoned on males and females together.

From every standpoint it is desirable that the presumed influence of heredity should be investigated on a material embracing a considerable number of cases of the mammary and other carcinomata which have been so thoroughly investigated histologically and biologically, and that the crude data should be examined with every precaution suggested by statistical experience.

Breeding experiments with cancerous mice have been in progress in the laboratory of the Imperial Cancer Research Fund for the last five years. The purpose of these experiments, kept steadily in view from the start, has been the collection of data sufficiently abundant and accurate to determine whether an enhanced liability to cancer is transmitted from parents to offspring. In a preliminary note * in 1909 a short account was given of the manner in which these experiments have been conducted, but it is desirable again to emphasise the nature of the material and the precautions which are necessary in devising and superintending such inquiries. All the mice concerned in the experiments are descended ultimately from animals known to have suffered from cancer. It is futile to take animals of unknown source and assume that they are of non-cancerous stock. The offspring of cancerous animals, when obtained, are numbered and entered in a special index giving date of birth, father and mother, sex, and identification marks. When new spontaneous cases of cancer in mice bred outside the laboratory are obtained, males bred in the laboratory from cancerous stock are mated with them, and as time goes on the process is repeated, so that a large number of animals with a composite known ancestry is obtained. This continual introduction of fresh cancerous stock to the parentage was decided on to eliminate, as far as possible, the influence of other indeterminate peculiarities which might conceivably influence the incidence of cancer indirectly in the nearly related animals derived by inbreeding from a single pair. For the same reason the animals were kept together and fed in a uniform manner in one room in large cages cleaned regularly, so that the environment has been as uniform as it is possible to make it. The young animals are also mated together, both nearly related and unrelated animals. Each

* Bashford, E. F., & Murray, J. A.: The Incidence of Cancer of the Mamma in Female Mice of Known Age. Roy. Soc. Proc. B. vol. 81, 1909, p. 310.

fruitful mating is recorded in the index, and in this way nearly 1,600 animals have been bred. The two sexes contribute approximately equal numbers to this total, and 562 females have lived for 6 months or more and form the materials of the present paper. Card-pedigrees have been constructed from the index giving the ancestry of each mouse as far back as it is known, distinguishing between cancerous and non-cancerous ancestors, and recording the age at death of the non-cancerous ancestors. The dates of birth and death of the mouse are also entered on the card, and when cancer develops, the date when the growth was first observed. For the purposes of the investigation the cards are arranged and distributed in a great variety of ways, so as to show the frequency of cancerous mice in groups of any desired character. The incidence of the disease is so dependent on the age and sex of the animals that, in order to get comparable groups, only mice of the same sex and approximately the same age may be reckoned together. The preponderance of carcinoma of the mamma in female mice in this material has led to the restriction of the inquiry to females for the present. They have been arranged in age-periods of three months' duration, this being the shortest interval which gives reasonably large figures in each group. When the material becomes more abundant shorter age-periods may be adopted.

From the pathological side the data are nearly perfect. All the animals which did not present a tumour during life were carefully examined for tumours after death, and it is scarcely possible that any growth of considerable size has escaped being examined microscopically and recorded. The mice in which tumours were discovered during life have been kept under observation till death. They have been examined microscopically in every case, the majority by Haaland (see the table at the end of the preceding paper). Only those which were undoubtedly malignant are reckoned in the tables and ancestries.

The results presented in this paper must still be regarded as provisional. The numbers in the various sub-divisions are still small, and the observations are being continued. The provisional results are given in the accompanying tables and charts, and it is desirable to describe briefly the manner in which they have been compiled.

In the first place the figures refer to females only. The number of cases of malignant new growths in males bred in the laboratory is so small that a statistical study of their frequency could give no usefu result, nor do the males which developed new growths appear in the ancestry of the females at present under consideration.

The tables show the ratio which deaths from cancer, and more

especially cancer of the mamma, bear to deaths from all causes at each of seven three-monthly age-periods for female mice over a number of years. The age-period in which mice dying of diseases other than cancer are entered is given by the age at death. The mice which have died of cancer are entered in the age-period embracing their ages at the time the existence of a tumour was discovered, and not on that embracing their ages at death *. This has been chosen as the nearest approximation instead of the actual age at death which varies with the exigencies of other experiments having no direct connection with the statistical studies, and also because in some instances operative cure was obtained and the animal died, from other causes, many months after the operation. In order to include cancerous mice still living, and so increase the volume of the data, it was necessary to increase the non-cancerous totals by the inclusion of living non-cancerous mice. These have been included in the age-periods embracing their ages, on a selected day (in the case of the present tables October 24th, 1910). As the dead outnumber the living by four to one, the approximation attained by this inclusion of living animals in what is essentially a tabulation of causes of death will not in all probability be greatly altered by the subsequent fate of these animals.

TABLE I. (24th October, 1910). Distribution of female mice discriminating between cancer of the mamma and cancer of other organs.

Age (months)	0–3	–6	–9	–12	–15	– 18	–21	–24	over 24	Total.
No tumour.										
Living	..	..	16	7	7	9	16	9	21	85
Dead	..	..	79	85	63	56	41	37	24	385
Tumour Mice.										
Organs other than mamma ..	..	..	..	1	2	2	2	1	3	11
Mamma	..	..	5	11	16	26	10	8	5	81
Total	..	..	100	104	88	93	69	55	53	562
Per cent. of cancer of mamma ..	..	..	**5·0**	**10·6**	**18·2**	**28·0**	**14·5**	**14·0**	**9·4**	**14·4**

Table I. gives the distribution in this way of all the female mice, discriminating between cancer of the mamma and cancer of other

* In the earlier paper one mouse was recorded as exactly 6 months old at the development of the tumour. Actually the tumour was discovered at the age of 6 months and 7 days, and the mouse is therefore entered in the 6–9 months age-period in the present tables.

organs. It shows a rapidly increasing proportion of deaths from cancer commencing after six months is passed, and attaining a maximum in the three-monthly period ending at 18 months. In the succeeding periods the frequency diminishes, till in mice over 24 months old the frequency is barely twice that found in mice under 9 months old. Similar figures for the human female give a corresponding curve.

ENGLAND AND WALES.

Percentages of Deaths of Females, 1901–1908 inclusive, distributed as in Table I.

	-5	5-	10-	15-	20-	25-	35-	45-	55-	65-	75-	85 & over.
Cancer Mamma. / 100 total deaths at each age-period.						0·4	2·1	3·5	2·7	1·8	1·1	0·8
Cancer of *all organs.* / 100 total deaths at each age-period.						3·5	11·0	18·2	17·9	22·2	6·5	2·9

This table gives the result of a similar calculation of the data contained in the Reports of the Registrar-General for England and Wales on the causes of death in the years 1901–1908. The ratios of deaths from cancer of all organs and also of the mamma alone to the deaths from all causes show the same general increase to a maximum with a subsequent decline in the three highest decennial age-periods.

Annual *death-rates* are calculated on the number of persons living at each age-period, and are therefore not comparable with the ratios of this table or of the other tables referring to mice in the present paper. The *death-rate* shows a progressive increase in the mortality from cancer of all organs up to the highest age-period, and the death-rate from cancer of the mamma increases progressively after the period 45–55, at which the ratio is highest in the table. The data for mice are not sufficiently numerous to make the calculation of death-rates useful.

Tables II. and III. were made after a preliminary distribution of the mice in groups according as the first cancerous ancestor occurred in the 1st, 2nd, 3rd ..... ascending generation had shown that the majority of the cases of cancer occurred in mice of which either the mother, one or other grandmother, or all three developed cancer.

Table II. shows the proportions in which mice of recent cancerous ancestry, in this limited sense, died of cancer at the different age-periods. Table III. is to be compared with Table II., and gives the same distribution for the remaining mice in which the cancerous ancestors are more remote. In these tables also, as in Table I., living non-cancerous mice are included in the age-group to which they had

attained on 24th October, 1910. The curves in fig. 1 show the differences between the percentage of deaths from cancer in the two groups at successive age-periods. The two percentage curves differ very little at the early and at the final periods, but diverge in the middle periods.

TABLE II. (24th October, 1910). Female Mice of recent cancerous Ancestry. (Mother, one or both grandmothers, or all three cancerous.)

Age (months)	0–3	–6	–9	–12	–15	–18	–21	–24	over 24	Total.
No tumour.										
Living	..	..	9	7	6	8	7	4	6	47
Dead	..	..	49	48	39	28	22	20	18	224
Tumour Mice.										
Organs other than mamma	..	..	..	1	2	2	1	..	1	7
Mamma	..	..	4	7	15	18	10	5	3	62
Total	..	..	62	63	62	56	40	29	28	340
Per cent.	..	..	6·5	11·1	24·2	32·1	25·0	17·2	10·7	18·2

TABLE III. (24th October, 1910). Female Mice of remote cancerous Ancestry. (No cancer in mother or grandmothers.)

Age (months)	0–3	–6	–9	–12	–15	–18	–21	–24	over 24	Total.
No tumour.										
Living	..	..	7	..	1	2	9	5	15	39
Dead	..	..	30	37	24	28	19	17	6	161
Tumour Mice.										
Organs other than mamma	..	..	..	..	..	..	1	1	2	4
Mamma	..	..	1	4	1	8	0	3	2	19
Total	..	..	38	41	26	38	29	26	25	223
Per cent.	..	..	2·6	9·8	3·8	21·6	0·0	11·5	8·0	8·6

The difficulty of assigning a just significance to these differences arises from the smallness of the groups, and that it is not justifiable to take larger groups (*i. e.* wider age-periods) is shown by the variations between the 3-monthly age-periods of Table I. Two other curves, however (fig. 2),

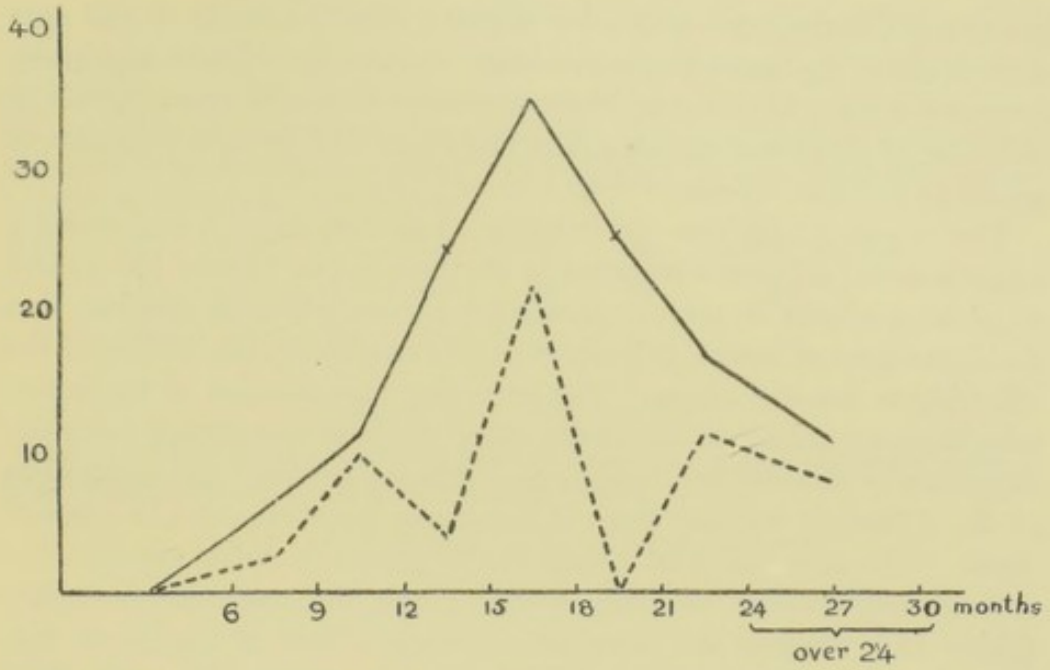


FIG. 1.—Percentage of deaths from mammary carcinoma to deaths from all causes at successive 3-monthly age-periods in female mice of recently cancerous ancestry (mother, grandmothers) ——, compared with the same ratio in female mice having more remote cancerous ancestry (mother and grandmothers non-cancerous) - - - - -.

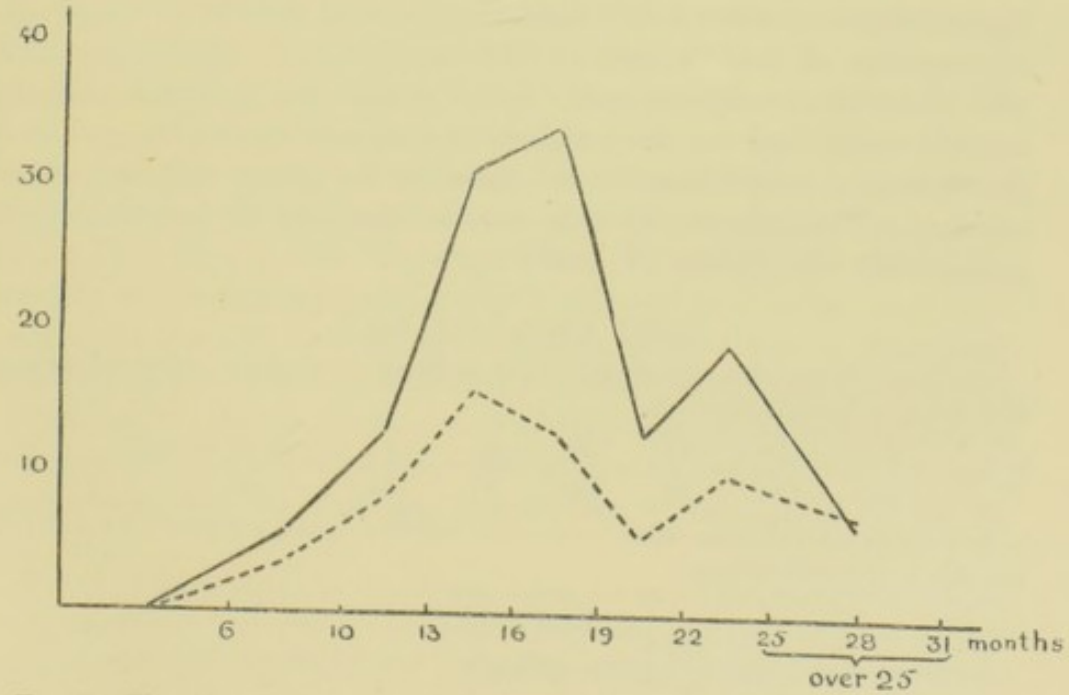


FIG. 2.—The same comparison as in fig. 1 with different limits to the 3-monthly age-periods (2nd period 4 months). As in fig. 1 the frequency of the deaths from cancer in the cancerous group exceeds that of the non-cancerous group at all periods except the last.

constructed in the same way with slightly different limits to the age-periods show the same features, except that in the highest age-group (over 25 months in this case) the percentage of deaths from cancer in the mice of cancerous ancestry falls just below that in the non-cancerous group (1 in 15 as compared with 1 in 14).

The results of the two distributions agree very closely, and strongly suggest a real difference inherent in the data, but in view of the limited numbers available it may be premature to conclude with certainty that the occurrence of cancer in a recent ancestor enhances the liability of the offspring to develop cancer. The following consideration of the factors relegating an animal to one or the other of these two groups (ancestry cancerous or ancestry non-cancerous) enhances, however, the importance of the difference between them in the frequency of cancer as a cause of death. The occurrence of cancer in the mother or either grandmother suffices to place the offspring in the group with cancerous ancestry (Table II.) whether the remaining ancestors died of cancer or not. Therefore the cancerous group includes many mice with only slight hereditary taint. On the other hand, the group with non-cancerous ancestors (Table III.) contains a number of animals whose mother and both grandmothers died early, before attaining an age at which cancer contributes an appreciable quota to the total mortality. It is in the highest degree probable that if these ancestors had lived to advanced age a proportion of them would have developed cancer. Hence, the mice with non-cancerous ancestry are a mixed group—they comprise a certain number which, but for the accident of the early death of parent and grandparents, would have to be added to the group with cancerous ancestry. The influence of this consideration can be appraised in a general way from Tables IV. and V.

TABLE IV. *a*. (*Cf.* fig. 3.)

Ancestors of non-cancerous group. Ages at death of mothers and grandmothers distributed in seven age-periods.

0–9	–12	–15	–18	–21	–24	over 24 months.
207	126	89	130	77	39	58

In these figures the same mice occur over and over again, sometimes as mothers, sometimes as grandmothers.

TABLE IV. *b*.

Ancestors of non-cancerous group, each parent mouse reckoned once only.

0–9	–12	–15	–18	–21	–24	over 24 months.
32	29	16	17	13	12	13

TABLES V. *a* & *b*. (*Cf.* fig. 3.)

Corresponding tables for ancestors of the recently cancerous group. In this group a complication is introduced by the presence, as ancestors, of cancerous mice of unknown age bred outside the laboratory. They have been distributed between the different age-periods in the same proportions as the 91 tumours of the mamma and other organs in laboratory-bred mice given in Table I.

V. *a*.	0–9	–12	–15	–18	–21	–24	over 24 months.
	175	190	117	243	90	99	58
V. *b*.	22	27	25	32	11	14	12

TABLE VI. (*Cf.* fig. 4.)

Ages at death of the ancestors distributed in Table IV. *a* further analysed according as the offspring did (*a*), or did not (*b*), develop cancer.

a. *Ancestors of Tumour Mice.*

0–9	–12	–15	–18	–21	–24	over 24 months.
26	19	15	10	9	5	3

b. *Ancestors of Tumour-free Mice.*

0–9	–12	–15	–18	–21	–24	over 24 months.
181	107	74	120	68	34	55

In these tables the mothers and grandmothers of the mice in the two groups are distributed into seven age-periods as before, according to their age at death, or if living at 24th October, 1910. In consequence of close inbreeding and the number of offspring from each female, the same mice occur over and over again, but of course each mouse always in the same age-period. The tables show that a higher proportion of the ancestors of the non-cancerous stock fall in the earlier age-periods (Table IV.) than of the cancerous (Table V. and fig. 3). When the ancestry of the non-cancerous group is analysed in the same way and a comparison is made between the ancestors of the mice of this group which developed cancer spontaneously (tumour mice) and the ancestors of those which died free from cancer, there is again a distinct difference. The ancestors of the tumour-mice died in greater proportion in the early age-periods, than those of the tumour-free of this group, Table VI. (fig. 4).

Hence, if there could be eliminated from the non-cancerous group those mice which are included in it because of the early death of their female parents and grandparents, the reduction would transfer tumour mice from the non-cancerous group to that with recent cancerous heredity in greater proportion than mice which did not develop cancer, and the

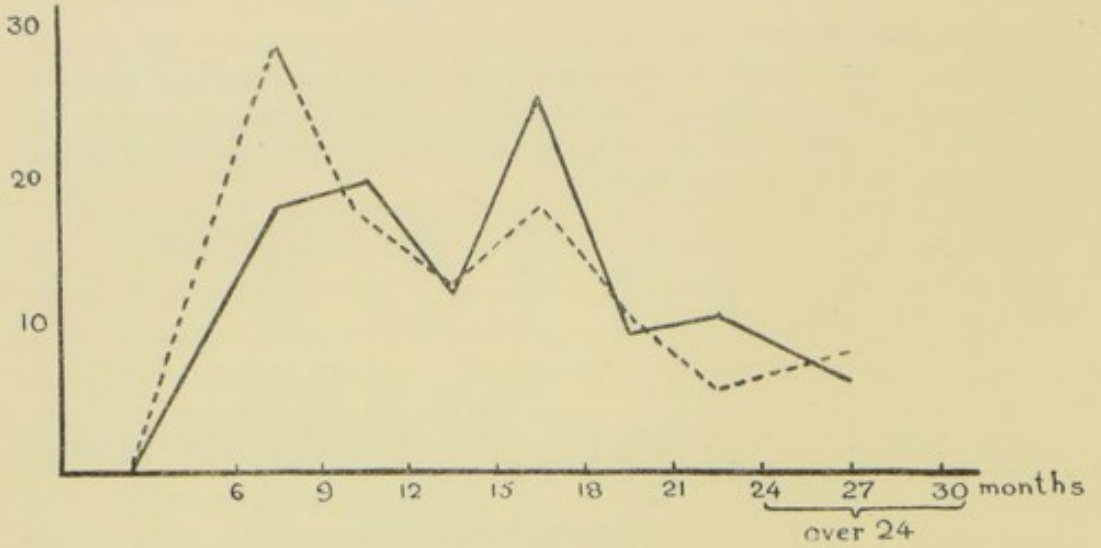


FIG. 3.—Percentage distribution of the ages at death of the mothers and grandmothers of the cancerous group ——— as compared with that of the non-cancerous - - - - - . The curves show that the ancestors of the non-cancerous group died early in higher proportion than those of the cancerous (*cf.* Tables IV. *a*, V. *a*).

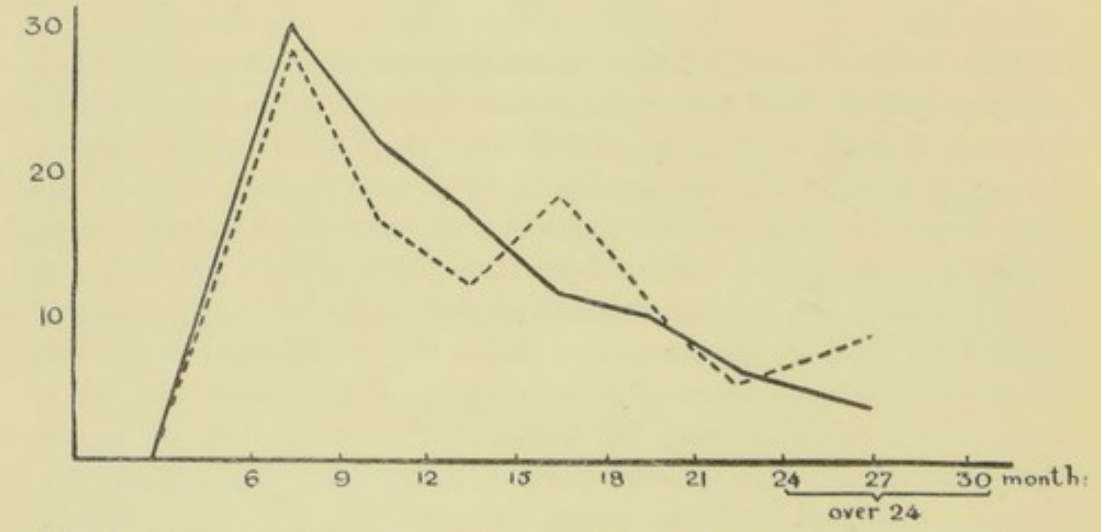


FIG. 4.—Percentage distribution of the ages at death of the mothers and grandmothers of the cancer mice ——— of the non-cancerous group as compared with that of the corresponding ancestors of the non-cancerous members - - - - - of the same group. Early death of these ancestors is more frequent in the case of the mice which developed spontaneous cancer than of those which remained free from it (*cf* Table VI.).

differences between the two groups would be increased. In fact, the difference between the two groups is a minimum difference, and it should be possible by continued selective mating to breed two strains of mice with a still greater difference in their liability to cancer.

In addition to the mammary carcinomata which alone are present in this material in sufficient numbers to repay statistical treatment, a smaller number of tumours of other organs have been obtained. These comprise seven lymphomata, four squamous-cell carcinomata (cancroids), and one spindle-celled sarcoma. One of the mammary carcinomata was accompanied by lymphoma.

The lymphomata are distributed between the 3-monthly age-periods as follows, each being entered under the age-period attained when the tumour was first observed (Table VII.).

TABLE VII.

Lymphoma (24th Oct., 1910). Female Mice.

0–3	–6	–9	–12	–15	–18	–21	–24	over 24 months.
			1	1	3	2		1 combined with carcinoma.

Three of these cases have occurred in mice with remote (earlier than grandparents) cancerous ancestry; in the remaining five, cancer had occurred either in the mother or in one or both grandmothers. In one of these the tumour in the mother was also a lymphoma. The pedigrees of this mouse and those of other two lymphomata, given in fig. 5, show their ancestry as far back as they are known. Up to the present the whole eight cases are descended ultimately from two spontaneously affected mice, mouse $\frac{50}{0}$ and mouse $\frac{84}{0}$. In both of these only mammary carcinoma was found at death. The tumour-free descendants of these mice, however, are very numerous, and no conclusion is warranted which would attribute importance to the restriction of lymphoma to their offspring.

One of the cancroids occurred in a male aged 8 months, the grandson of a spontaneously affected female (mammary carcinoma) bred outside the laboratory.

The remaining three cancroids occurred in three sisters born in one litter. The parents and grandparents died without developing cancer, as is shown in the pedigree given in fig. 6. Two were more than 24 months old when the tumours were discovered, the third had reached the age of 23 months.

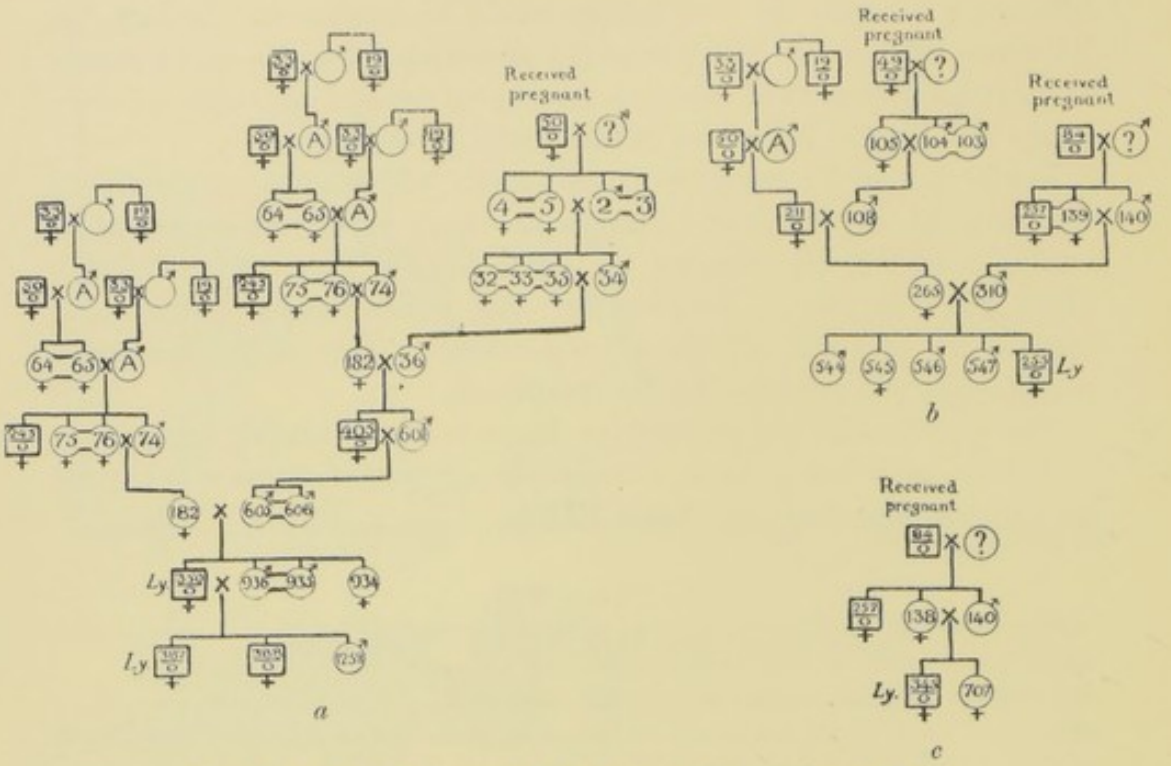


FIG. 5 *a*, *b*, *c*.—Pedigrees of four mice with lymphoma (*Ly.*).

Tumour-free mice in circles, tumour-mice in square cartouches with the index number written as a fraction denominator zero: *e. g.*, $\frac{32}{\varphi}$ carcinoma of mamma. Where the tumour was found to be lymphoma the square cartouche is preceded by *Ly. e. g.*, *Ly.* $\frac{387}{\varphi}$.

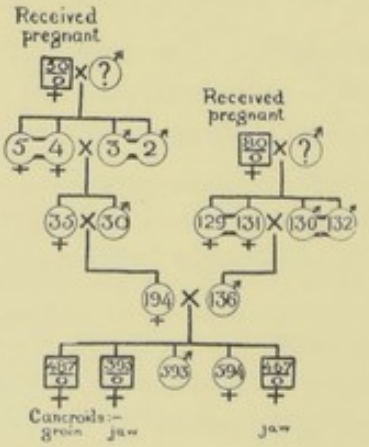


FIG. 6.—Pedigree of three mice which developed squamous-cell carcinoma.

The spindle-celled sarcoma also developed in a very old mouse (30 months) of remote cancerous ancestry.

In conclusion, it is well to consider the importance of these results in the light of the comparative pathology of cancer and with regard to the new and additional evidence brought forward in the preceding paper as to the importance of local as distinct from constitutional causes of new growths. Investigations of the most diverse kind suggest strongly that the actual initation of cancer is in many forms of the disease a terminal phase of a long-continued process of localised chronic irritation. When it can be shown that a large number of individuals subject a particular tissue of their bodies to peculiar forms of continuous stimulation, as in paraffin workers, chimney sweeps, the inhabitants of Kashmir, and betel-nut chewers, cancer supervenes in that tissue in an enhanced proportion as compared with similar groups where this factor is absent. Even in mice in whose ancestry cancer is absent, cancer may arise in consequence of such irritation, particularly when they attain extreme old age in large numbers, and a diminished predisposition to the disease would merely effect a diminution in the number of individuals attacked as compared with a corresponding number of individuals with inherited liability similarly irritated. The phenomena of the experimental production of sarcoma by transformation of the stroma of transplanted tumours of a particular strain, in nearly all animals, however, indicate that the diminished predisposition is never likely to result in a complete absence of cancer. Conversely, it should be possible by shielding individuals even of highly susceptible stock from chronic irritation of specific tissues to diminish considerably the incidence of cancer of these tissues among them.

It would be premature to discuss the nature of the enhanced liability to cancer or its mode of transmission. This is obviously a subject for experimental study when two races of mice of still more widely differing liability have been bred. Other investigations have shown that a constitutional condition favouring the *growth* of cancer and accounting for its incidence is not present in mice which suffer spontaneously from the disease. The determining factors are those which *initiate* cancerous proliferation ; and we may conclude with a high degree of probability that the predisposing condition which is transmitted is some peculiarity of the cells of the tissue in which cancer develops, of such a kind that under long-continued irritation the regenerative and proliferative changes which accompany the inception of the disease are more prone to occur, or take place with greater intensity. The present observations

harmonize with the conclusion drawn from other lines of work, that cancer always arises *de novo* in the organism attacked by a transformation of the ordinary tissue elements, and lend no support to the view that groups of cells, isolated anatomically and physiologically from the rest of the organism from an early period, form the structural basis of the development of malignant new growths.

In the preceding pages the crude data of the breeding-experiments have been examined principally from the standpoint of their pathological and clerical accuracy. The application of the mathematical laws of error permits an estimate in another manner of the validity of the conclusions drawn from the crude data. These methods compare the observed differences with the differences in the frequency of cancer which might exist between mice of recently and remotely cancerous ancestry as a consequence of the mere chances of sampling, even if the liability to the disease were the same in the two groups, by means of "standard deviations of sampling" or "standard errors."

Certain reservations are necessary in applying these methods, the most important being the necessity for caution when the observations are limited in number. It is only when the number of observations is great that these methods can be applied with confidence, and, as is evident from the tables, this is not the case in some of the groups.

The method consists in determining the standard deviations of sampling or standard errors of the proportions of cancerous mice in the several groups, and from these calculating the standard errors of the differences. If the observed differences exceed considerably their standard errors they cannot be referred to the chances of sampling, and are probably significant.

In the first place it is to be noted (Tables II. & III.) that the percentage of cancerous offspring amongst the progeny of cancerous ancestry is greater than that amongst the offspring of non-cancerous ancestry in *every* age-group. If there were no real differentiation between the two strains, we would expect the percentage in one group to exceed and fall short of the percentage in the same age-period in the other group with equal frequency; there being seven age-groups, the actual result would only occur by chance as often as seven heads would occur in a toss of seven coins, *i. e.*, once in 2^7, or 128 trials. This alone is enough to make it almost certain that there is a genuine differentiation.

The actual percentages amongst all the offspring are :—

Ancestry cancerous ..... 18·2 per cent.
Ancestry non-cancerous ..... 8·6 do.

these percentages being based on 340 and 222 cases respectively. Such a comparison might, however, be misleading, as the probability of development of cancer varies with age, and the age-distributions are not the same in the two cases. The difficulty may be overcome by working out corrected percentages based on the age-distribution of all mice as a standard ; reducing the numbers for the 562 mice to the corresponding proportions per thousand the result is :—

Age.	Number in standard population.	Number of cancerous mice in 1000 all ages:	
		Ancestry non-cancerous.	Ancestry cancerous.
– 9	178	4·6	11·6
–12	185	18·1	20·5
–15	157	6·0	38·0
–18	165	35·6	53·0
–21	123	00·0	30·7
–24	98	11·3	16·9
24–	94	7·5	10·1
Total	1000	83·1	180·8
Equivalent percentages		8·3	18·1
Difference		9·8	

It will be seen that the correction to the standard population has altered very little, but has slightly emphasised the difference, which is now 9·8 instead of 9·6 per cent. The standard error of this difference is 2·96. The difference is 3·3 times the standard error, and the chance of its occurring as a mere fluctuation of random sampling only about 1 in 1000.

The following are the differences for the separate age-classes, with their standard and probable errors :—

Age.	Difference.	Standard error.	Probable error * =0·6745 of standard error.
- 9	3·9	4·49	3·03
-12	1·3	6·18	4·17
-15	20·4	9·02	6·08
-18	10·5	7·98	5·38
-21	25·0	8·59	5·79
-24	5·7	9·51	6·41
24-	2·7	8·03	5·42

* The probable error is the fluctuation of sampling that will be as often exceeded as not.

It will be seen that four of the seven differences exceed their probable errors, but the only differences that do so at all considerably are those for the three central age-groups. The difference between the two groups is almost certainly significant, *i. e.*, not due to mere fluctuations of sampling.

From these observations the conclusion is drawn that female mice in whose ancestry cancer of the mamma has occurred not farther back than the grandmothers are distinctly more liable to develop the disease spontaneously in this organ than those in whose ancestry cancer is more remote. The increased liability is probably of the nature of a predisposition of one particular tissue or organ system to undergo cancerous transformation under the wear and tear of life. The differences between the two groups are apparent at all ages, and the age of maximum incidence does not appear to have been lowered in the predisposed group. The magnitude of the difference is such that it cannot be accounted for by the chances of random sampling.

Literature.

Bashford, E. F.: The Problems of Cancer. Brit. Med. Journal, July 18th, 1903; Second Scientific Report, Part I. pp. 5 & 50; Third Scientific Report, pp. xi–xiv.

Bashford, E. F., & Murray, J. A.: The Incidence of Cancer of the Mamma in Female Mice of Known Age. Roy. Soc. Proc. B. vol. 81, 1909, p. 310.

Haaland, M.: Spontaneous Cancer in Mice. This Report, p. 1.

Thorel, Ch.: Kasuistisches zum Kapitel der sog. Mäusekarzinome. Verh. der Deutsch. Path. Gesellsch. 1908, p. 59.

Tyzzer, E. E.: A Series of Spontaneous Tumours in Mice, with Observations on the Influence of Heredity on the Frequency of their Occurrence. Journ. Med. Research, 1907, xvii. p. 155, and Fifth Report of Cancer Commission of Harvard University, 1909.

[*Fourth Scientific Report of the Imperial Cancer Research Fund*, 1911.]

THE BEHAVIOUR OF TUMOUR-CELLS DURING PROPAGATION.

By E. F. BASHFORD, M.D.

In the First, Second and Third Scientific Reports the phenomena of the propagation of tumours were described and discussed in general terms. It is now proposed to revert to this subject and to discuss its bearings upon the nature of cancer in a broader manner than has been attempted hitherto, for the extended experience of the intervening years has yielded an accumulation of data bearing on this subject which it is desirable to pass under review.

After the demonstration that the transference of a tumour meant merely the continued growth of the tumour-cells of one animal in another of the same species, the relative importance of the cells transferred and of the soil into which they were implanted, became the objective of much experimentation which continues to the present day, and has developed most extensively in the direction of the study of induced resistance or immunity. The question of natural and induced resistance is, in the main, outside the scope of this paper and will not be considered except in so far as it is germane to other questions.

Years of work have been devoted to distinguishing the innate qualities of the cancer-cell from such modifications in its histological and biological behaviour as might conceivably be due, partially or solely, to its environment. Apart from the study of the relative importance, in general, of soil and cancer-cell, much technical work had also to be done on the influence of dose, of site of inoculation, on the use of intact fragments of tumour *versus* emulsions (with or without addition of diluents), on the age of animals used, and on the rapidity of *passage*. The possibility of effecting modifications in the cancer-cell by enhancing or diminishing the suitability of the animals inoculated had to be ascertained. The experimental reproduction of the lesions of cancer had to be established before the histological and biological phenomena of growth as manifested during prolonged artificial propagation could be submitted to even so much as a preliminary analysis and appraisement.

At the outset no opinion could be formed as to how long propagation

could be continued, or what duration of propagation would be necessary to enable conclusions to be drawn from phases in the life-history of a tumour-strain: even the duration of the life of a mouse was not accurately known. Now, having some evidence on these points, it seems legitimate to attempt a survey of the data accumulated upon the attributes of the tumour-cell, and their bearing upon the nature of cancer.

In the following pages these data will be considered, so far as the unavoidable overlapping permits, from two main standpoints, viz. the permanency or variability of histological structure, and the permanency or variability of all those other properties of the cancer-cell which have been accessible to experiment and which are called the phenomena of growth, or for brevity, biological. The data will be grouped under the following headings and sub-headings :—

THE SCOPE OF THE INVESTIGATION.

To illustrate the wide basis upon which the behaviour of tumours has been studied, and to justify the critical attitude towards other investigators with whose conclusions those advanced in this paper are at variance more or less acute, it is necessary to dwell, in a manner deliberately avoided in previous reports, upon the large number and varied nature of the malignant new growths of the mouse investigated in this laboratory.

A very large number of mice bearing spontaneous tumours have been examined, and of them over 650 which bore true malignant new growths have been observed continuously till death, the details of the clinical course of the tumours and the pathological findings having been recorded with the great care exemplified in the first paper in this report and other papers in preceding Reports. A large proportion of these mice were obtained from the breeding experiments of the laboratory and of them the family history is accurately known, as recorded by Murray in the preceding paper. As pointed out by Haaland the primary tumours comprise malignant new growths as varied in histogenesis as are the malignant new growths of man. Of the tumours propagated those of the mamma form the majority and exhibit all gradations from pure adenoma to pure carcinoma; in addition there are a large number of adeno-cancroids and several mixed tumours (carcinoma-sarcomatodes) of the mamma. The propagated tumours also include several carcinomata of different histogenesis, *e.g.* of squamous epithelial origin, from the preputial gland, and from sebaceous glands, together with a number of round-cell, spindle-cell, chondro- and osteo-sarcomata. This extensive material furnishes in its mere diversity a wide comparative basis for conclusions on the morphological behaviour or histological problems of tumours. From the biological or experimental aspect it is equally extensive, since 85 propagated tumours have been grown for extended periods and of them 61 are still retained in propagation, because of the features of interest they present. Thirty-five tumours have been growing for longer than a mouse lives, *i. e.* for over three years.

The prolonged propagation of these tumour-strains is really the observation under experimental conditions of the life-history of cancer by histological and biological methods. Whereas histological methods have been applied uniformly to all tumours, the extent to which the biological analysis has been carried out has varied from time to time and from one strain to another in accordance with the particular problems which required study.

The total material available for review has necessitated the observation of more than 200,000 animals. Adequate material for histological examination has been preserved and examined from every tumour used to continue propagation from one set of mice to another, and in addition many sister tumours have been examined both before and after propagation was carried forward to a new set of mice. For more than eight years the weight of each tumour and the weight of each animal when inoculated and when killed for transplantation of its tumour, have been determined. The many thousands of data thus recorded and tabulated, furnish a reliable estimate of the rates of growth of the several tumour-strains, because the animals have all been inoculated with measured doses. The progress of the transplanted tumours has been followed by means of systematic chartings of the sizes of the tumours at regular intervals, appropriate modifications in the general routine having been adopted in keeping with the varying rates of growth and other characteristic features in the biological behaviour of the several tumour-strains. Examples of these charts will be given and also of the percentage curves based upon them. The complete clinical and pathological history of the spontaneous tumours, together with the corresponding records of each propagated tumour-strain obtained from them, exemplifies the value attached to the observation of the growth of cancer during periods relatively and absolutely more prolonged than is possible in the case of the human subject. Taken together the combined records furnish a complete life-history of each tumour and yield a sequence of pictures of the morphology and biology of each tumour-strain, justifying comparisons between observations of the most varied character made on tumours of divergent histogenesis at the same and at widely separated periods of time.

The Relative Importance of Technique and of the Primary Qualities of the Tumour.

The artificial transference of cancer was so difficult and so large a problem in itself, that it claimed the work of many generations of investigators before it received a satisfactory solution, and that only within recent years. Its solution was achieved by overcoming many obstacles, some of which could be foreseen, whilst others were quite unanticipated and revealed themselves only as progress was made. Once the way had been shown by which a measure of success could be attained, it was natural and legitimate to strive to improve upon it, in

the hope that the result of attempts to transfer cancer could be predicted with certainty for any single spontaneous or propagated tumour, the end aimed at being the development of tumours in all animals inoculated. This subsidiary problem is in essence nothing more than a mere technical convenience for the extension of experimental methods. The concentration of investigators upon its solution was often effected, however, at the cost of neglecting matters of equal if not of more real moment in their direct bearings upon the nature of cancer.

The technique employed for transplantation could not in the early stages of its evolution be other than haphazard. The methods employed by investigators in different parts of the world for transferring spontaneous tumours, and maintaining the tumour-strains obtained from them under propagation, ranged in principle between two extremes as regards the treatment of the tumour tissue inoculated. On the one hand the material was subdivided into the greatest possible number of minute intact fragments capable of measurement, and distributed over as large a number of mice as possible. On the other hand the purpose was to give the biggest possible dose of tumour to each animal by reducing the tumour to an emulsion, and then subdividing it among a small number of animals. To increase the number of animals that could be inoculated with a large dose, several tumours were sometimes added together and a mixture of these inoculated.

A very large number of spontaneous tumours was transplanted by one or other of these methods or by their variants. Tumour-strains yielding a uniformly high or a maximal percentage of successful inoculations from the outset of transplantation have been obtained by both methods. But whilst the first method permits of practically all mammary tumours being transplanted if a sufficiently large number of mice are employed, as illustrated by papers already published *, the second method does not, but effects a very strict elimination of those tumours which are unable to withstand it. When a tumour-strain giving a uniformly high percentage of "takes" and of high rate of growth was obtained by the second method, the success has been ascribed to the consequences of technique. An augmentation of the rapidity of growth or "virulence" † observed after the first *passage*, and a progressive loss of differentiation were ascribed by Ehrlich and Apolant to the selection of the most rapidly growing tumours in each successive

* *Cf.* Third Scientific Report of the Imperial Cancer Research Fund, etc.

† *Vide* pp. 192–203 of this Report.

generation and to forcing the rapidity of *passage*. The possibility that the primary qualities of the tumours might be mainly or solely responsible for the results obtained, because they had permitted of the employment of a special technique, was not given full consideration. In this instance concentration on a merely technical detail, combined with the attainment of the object in view, caused the primary qualities of the tumours to be assigned to the influence of the technique. This standpoint narrowed both the technique and the basis for deduction and discussion of the nature of cancer and of the lines along which the possibilities of treatment might be sought.

Both methods of inoculation have been extensively employed in this laboratory. In the light of the wide experience gained, it can be asserted that the technique which consists in the employment of large doses of tumour emulsion and rapid *passage* was responsible for the selection of certain kinds of primary tumours which survived the procedure, and not for the induction of a marked change in their rate of growth. At the same time this technique was also responsible for secondary changes due to active immunization being missed, and therefore for phenomena due to active immunity induced in the animals by the large doses inoculated, being interpreted as due to an artificial increase of the avidity of the tumour-cells ("atrepsia"). This disregard of the primordial properties of the tumour-cells, together with neglect of the modifications induced in the animals, led to several distinct phenomena being confounded. Failure to reproduce the lesions of dissemination was also added to these other consequences and may have been due to the tumours or the technique, but was probably due to both factors (*cf.* footnote on page 187). This combination of circumstances led to the deductions upon which Ehrlich's atreptic theory of cancer and cancer immunity was based. This theory will be discussed later (*v.* p. 187).

Except in a number of papers issued from this laboratory, the technical aspect of propagation has received very little consideration in so far as it affects the biological behaviour of tumours. It is of great importance, however, in interpreting the crude facts. Just as histological study shows that each tumour-strain has individual peculiarities of parenchyma and stroma *, so also the careful examination of the

* Bashford, E. F., Murray, J. A., and Cramer, W.: Source of the Constituent Elements of New Growths obtained by Artificial Propagation. Second Scientific Report of the Imperial Cancer Research Fund, Part ii. pp. 24–29.
——: Stroma is a Specific Reaction on the part of the Host. *Ibidem*, pp. 30–33.

details of propagation brings out the fact that each strain reacts in its own way to the physical intervention of transplantation. Optimum conditions of transplantation are only arrived at for the different strains after trial and error more or less prolonged. The majority of tumours give the best results by the method of implanting minute intact fragments with the hollow needle. Some can only be propagated continuously by this method while others give consistently better results when larger doses of fine tumour emulsion are inoculated with the syringe. The interval between successive transplantations also exercises a profound influence on the result. While some tumours can only be propagated by rapid passage, for others this is impossible. The influence of too rapidly repeated *passage* is particularly noticeable in the frequent experience that rapid *passage* may lead to a temporary exhaustion of the tumour-cells from which they only recover after prolonged sojourn in one animal. It is not always the most rapidly growing tumours which can be transplanted at the shortest intervals, and a permanent improvement in the rate of growth and percentage of "takes" may merely indicate that the optimum interval of grafting has been adopted either deliberately or accidentally.

The subjoined summary (p. 140) of the results of continuous propagation of an extremely varied series of tumours shows a remarkable general constancy of carcinomata and sarcomata over long periods. The morphological variations recorded are slight and with few exceptions only serve to emphasize this striking fact. The apparent loss of acinous structure in the later generations of many adeno-carcinomata of the mamma in spite of its seemingly fundamental character is not necessarily evidence of a real change. In some tumours (*e. g.* Tumours 63 & 91) examination at a short interval after transplantation reveals a purely alveolar condition which gradually gives place to the typical acinous condition as the tumours grow older, and may be completely replaced by perfect acini in very old tumours, *cf.* figs. 13, 14, 15, 16, 28 & 29.

In the Third Scientific Report and earlier papers the apparent increase in the rapidity of growth of transplanted tumours which is frequent after the initial difficulties of propagation have been overcome, was shown to be capable of explanation by an increase in the number of cells which survive the injury of transference, augmenting the effective initial dose, *cf.* fig. 63, p. 202. When this occurs the time between successive transferences can often be diminished and thereafter progressively younger tumours can be, and often are, transplanted and examined microscopically. It is necessary therefore to examine *old*

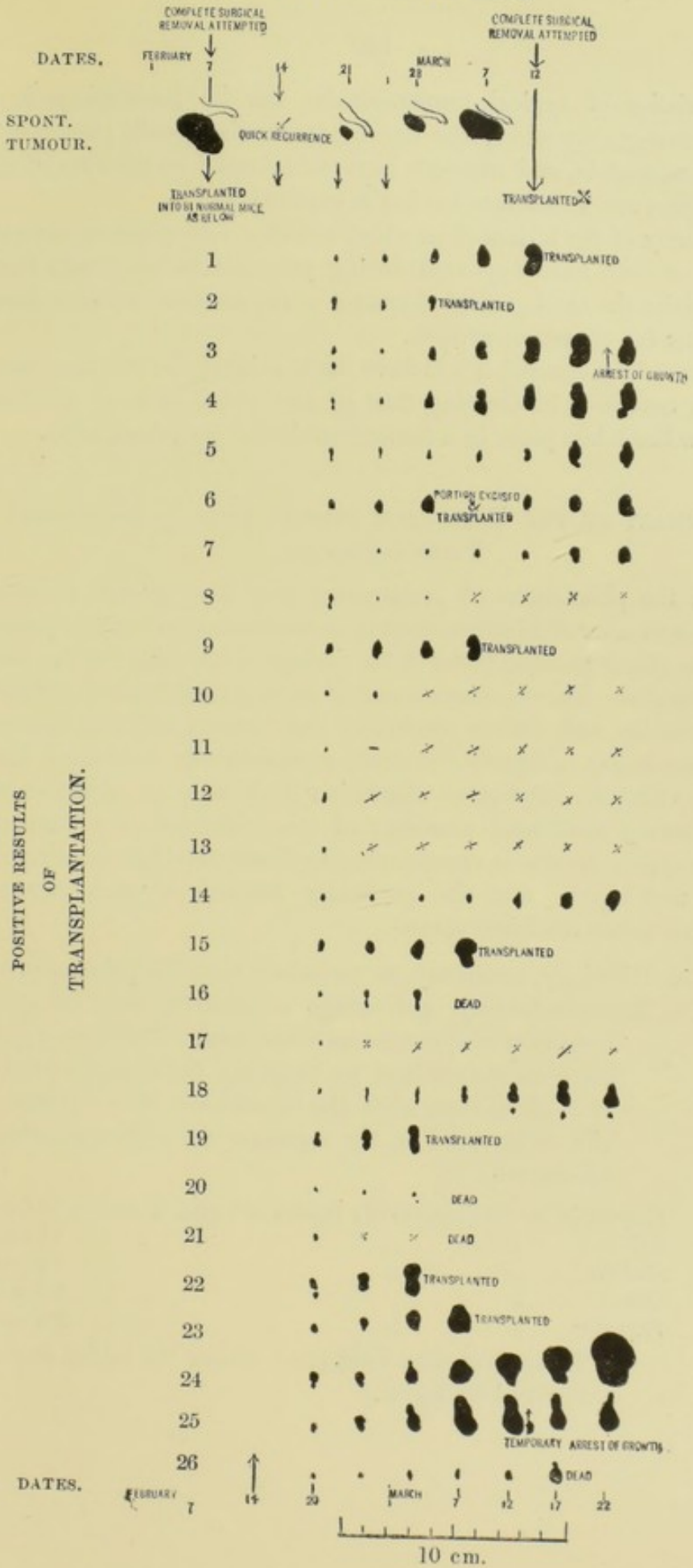
tumours in all cases where normal histological differentiations have been present in the primary or earlier transplanted tumours, and are absent in the younger tumours used for transplantation in its later continuation. When this is done a number of tumours conform to the paradigm afforded by Tumour 91, *cf.* figs. 13, 14, 15, 16, 28 & 29. For example, adeno-carcinomata, primarily hæmorrhagic, may then reproduce the original hæmorrhagic character absent in the period immediately following transplantation. The same is true of some squamous-cell carcinomata ; but, in the case of Tumour 32 (an adeno-cancroid), it is now only rarely that a tumour is found to live so long in one animal that the squamous and acinous conditions reappear, most of the tumours examined being pure alveolar carcinomata. The differences in histological structure between the spontaneous tumour and early transplanted tumours on the one hand, and the later transplanted tumours on the other, are not therefore necessarily final evidence of actual changes in the structural character of a tumour parenchyma in every case in which they occur.

Analogous considerations apply to the increase in rate of growth and percentage of success recorded in the charts and percentage curves. When it is possible to compare the rate of growth of a spontaneous tumour in the primarily affected animal with its behaviour in normal animals at the first transference there is always a great falling off in the strange hosts, *cf.* figs. 1, 2–8 & fig. 40, p. 165. A subsequent improvement may then appear in the earliest subtransplantations, as a recovery from the condition of depression produced by the transference to an unsuitable environment. Parallel with this recovery the percentage of "takes" generally rises, *cf.* figs. 2–8. The interpretation of this improvement as necessarily signifying a rise in "virulence" was discussed and dismissed in the Third Scientific Report.

The general conclusion must be drawn that the cells of many tumour parenchymata preserve unaltered in the most persistent manner the majority of the characters with which they are endowed from the earliest period at which they come under observation. The several tumour-strains do not approximate to a common condition either in structure, in rate of growth, or percentage of "takes." While a number of strains could be selected as forming a group homogeneous in naked eye structure and in histology, this similarity is found to be primary and already associated with the spontaneous tumours : it is not a secondary convergence from an initial heterogeneous condition. The conclusion seems to be warranted, that the relative permanency of the distinctive

Fig. 1.

SPONTANEOUS CARCINOMA AND RESULTS OF TRANSPLANTING IT INTO 81 NORMAL MICE.



characteristics of typical tumour-strains has its foundations in the cellular changes by which the non-cancerous tissue-cells pass over into the cancerous state, and suggests how radical must be the alterations of which cancerous transformation is the expression.

The rarity of the instances in which striking biological or structural changes or both have appeared during propagation serve still further to emphasize the fundamental character of the essential cellular changes responsible for cancerous growth.

The preceding discussion will have made evident the extreme caution which is necessary in deciding that in any given case an undoubted alteration has taken place in a tumour-strain during propagation.

Summary of the Data from certain typical propagated Tumour-strains.

When the phenomena of propagation over long periods are studied in a large number of tumours, certain sequences are met with repeatedly, and these give a peculiar facies to the biological behaviour of the several tumour-strains. The sequences occur in varying combination in different tumour-strains, and, before discussing their nature and significance, it will be necessary to describe in detail a considerable number of typical strains, so that the subsequent discussion shall have an objective basis. The following condensed summary of the behaviour of a number of separate typical strains is accompanied by illustrative figures, percentage curves, and charts, and the particular features of each strain are considered under the headings :—

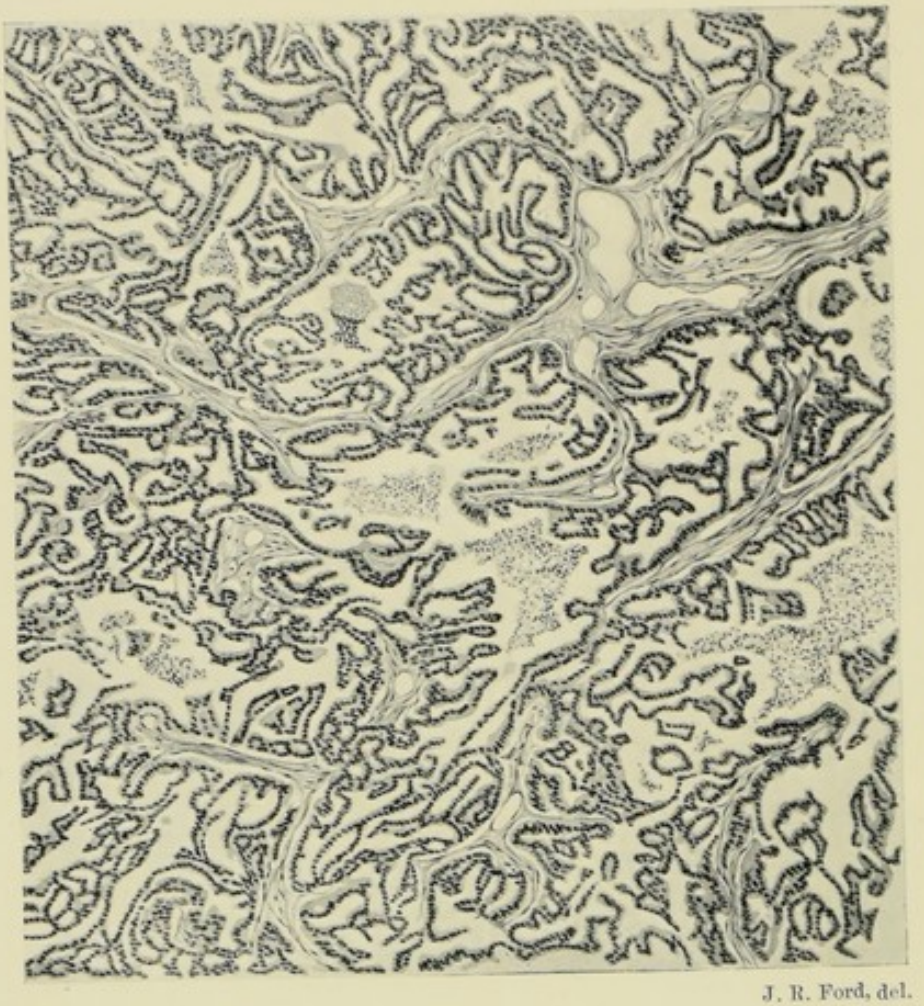
(*a*) Histology, constancy, or variations from the primary growth.

(*b*) Transplantability, percentage of success, rate of growth, frequency of spontaneous absorption. The rate of growth has been determined by weighing the tumours produced in a given time after the inoculation of a known dose. The terms used in the summary are to be understood as follows :—

Very rapid	the development of a tumour of 1 grm. or more in 10 days.				
Rapid	,,	,,	,,	,,	14–16 days.
Medium	,,	,,	,,	,,	3 weeks.
Slow	,,	,,	,,	,,	4–6 weeks.
Very slow	,,	,,	,,	,,	2–4 months.

These estimates hold good where the initial dose does not exceed 0·02 grm.

To face p. 141.]



J. R. Ford, del.

FIG. 9. 72/9 A—10 A. Papilliferous cystic mammary adeno-carcinoma of very slow growth and constant in structure. Tumour 58 days old. $\times \frac{87}{1}$.

GROUP I.—*Strains with only Minor Alterations from Characters of Primary Growth.*

In the first group a number of strains will be considered in which the characteristics of the primary growth have been maintained with only minor alterations.

Tumour-strain 27. Fissure-forming adeno-carcinoma of mamma, alveolar in places; stroma usually delicate.—Two strains propagated separately for 48 generations are indistinguishable histologically from each other and from the primary growth. Propagation has been continued for 5 years. After the initial difficulties of propagation had been overcome the tumour grew well although slowly, with usually less than 50 per cent. of success. For the last 3 years it has usually given over 50 per cent., occasionally reaching 90–100 per cent., but it may still fall to 40 per cent. or less. Rate of growth is slow, about 28 days to produce 1 grm. Spontaneous absorption occurs, but is rare.

Tumour-strain 72. Papilliferous cystic adeno-carcinoma of the mamma, with alveolar areas, stroma rich in collagen.—Histology unaltered. Percentage of success is very low. Growth is very slow (1 grm. in 2–5 months), faster since 8th generation. 15 generations in $3\frac{3}{4}$ years, *cf.* fig. 9.

Tumour-strain 155. Mammary papilliferous cystic adeno-carcinoma; stroma sclerotic.—Histology unaltered, stroma very sclerotic in old tumours. Success of transplantation is low, varying from 10–40 per cent. Growth is very slow (1 grm. in $2\frac{1}{2}$–5 months), 12 generations having been attained in 2 years 10 months.

Tumour-strain 173. Mammary papilliferous adeno-carcinoma; stroma rich in collagen.—Histology unaltered during propagation. Success of transplantation, which was low in the first 3 generations, rose at the 4th and has been maintained between 40 and 70 per cent. Growth is slow (1 grm. in 2–6 months), 18 generations in 2 years 9 months.

Tumour-strain 39. Hæmorrhagic mammary adeno-carcinoma.—Histology has remained unaltered for 4 years 5 months. The percentage of success has remained low throughout, generally under 30 per cent. The rate of growth is medium (1 grm. in 24 days) and spontaneous absorption is common throughout.

Tumour-strain 46. Hæmorrhagic mammary adeno-carcinoma.—Histology fairly constant, the stroma is at times merely œdematous without hæmorrhages, and for a time was very cellular and abundant. The success of transplantation varies from 5–100 per cent. Growth is rapid (1 grm. in 10–17 days), 86 generations in 4 years 2 months. Spontaneous absorption occasionally seen, not frequent.

FIGS. 2–8.—Curves of the percentage of "takes" of typical tumour strains, to show primary depression following transplantation to normal mice and the slow or almost immediate recovery from it. Constancy or variation of histology and rate of growth are indicated in the descriptions under each figure.

HISTOLOGY CONSTANT: GROWTH VERY SLOW (1 GRM. IN 2–5 MONTHS). GROWTH FASTER AFTER 8TH GENERATION.

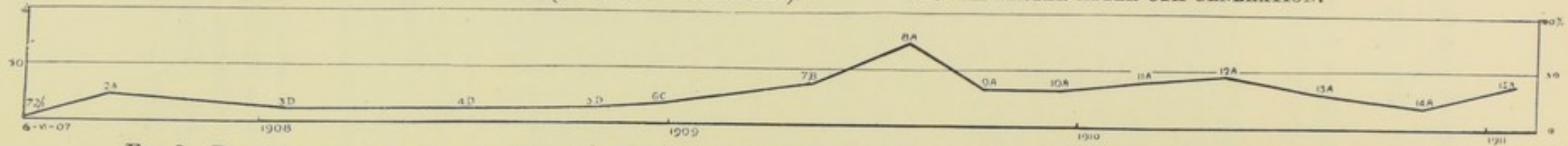


FIG. 2.—Percentage curve of propagation of tumour 72. Practically no alteration in proportion of "takes" and very little in rate of growth. Histology constant (papilliferous cystic adeno-carcinoma). *Cf.* figures 3, 4 and 5.

HISTOLOGY CONSTANT: GROWTH SLOW.

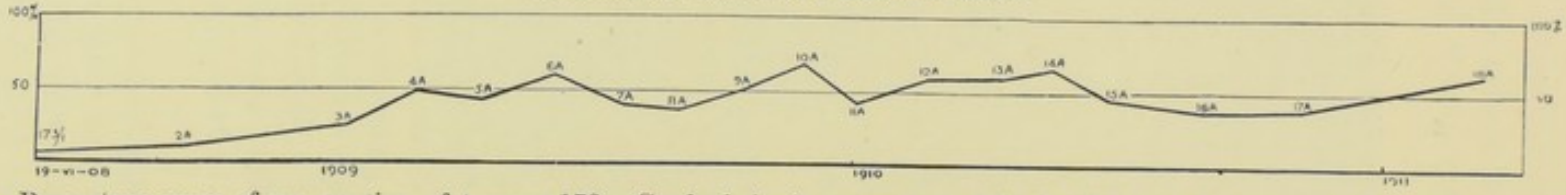


FIG. 3.—Percentage curve of propagation of tumour 173. Gradual rise in percentage of "takes" up to 4th generation, thereafter constant with slight fluctuations. Histology and rate of growth unaltered (papilliferous cystic adeno-carcinoma). *Cf.* figures 2, 3 and 5.

HISTOLOGY CONSTANT: GROWTH VERY SLOW (1 GRM. IN 2½–5 MONTHS).

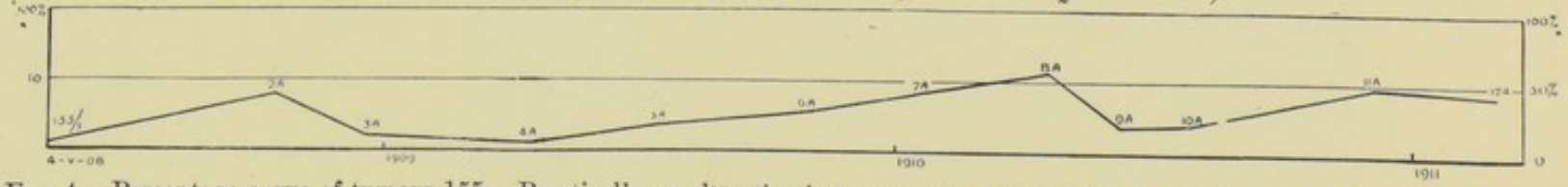


FIG. 4.—Percentage curve of tumour 155. Practically no alteration in proportion of "takes" and rate of growth. Histology constant (papilliferous adeno-carcinoma). *Cf.* figures 2, 3 and 5.

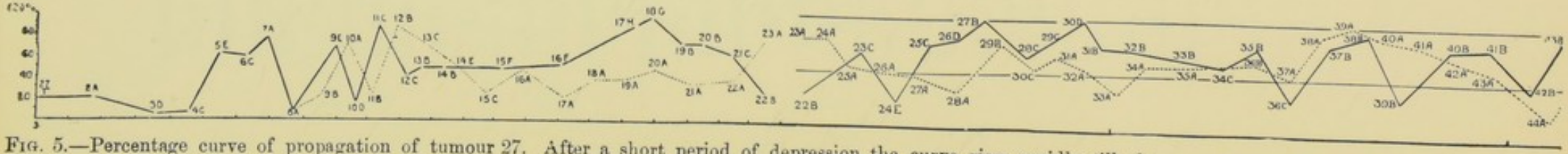


FIG. 5.—Percentage curve of propagation of tumour 27. After a short period of depression the curve rises rapidly till the 7th generation and then falls at the 8th, from which two strains have been propagated separately for 4½ years. The close parallelism in the behaviour of the two strains is exemplified by the parallel rises and falls of the two curves. The rate of growth has remained unaltered as has also the histology (fissure forming adeno-carcinoma). *Cf.* figures

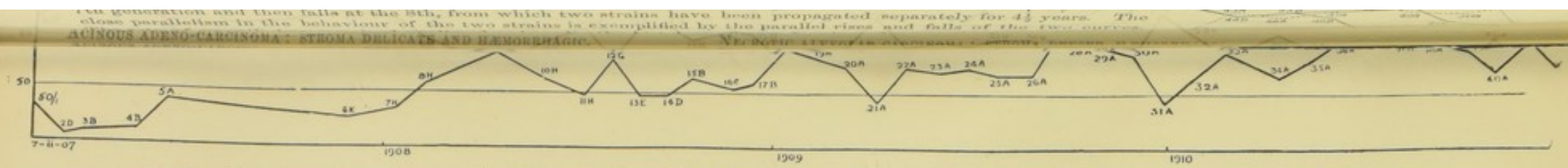


FIG. 6.—Percentage curve of propagation of tumour 50. After a short period of depression the curve rises at the 5th generation and continues to do so till the 9th. Thereafter it fluctuates above 50 per cent.: in later generations the fluctuations become less marked. After the 12th generation the primary histological features (hæmorrhagic adeno-carcinoma) gave place to a more solid structure with necrosis.

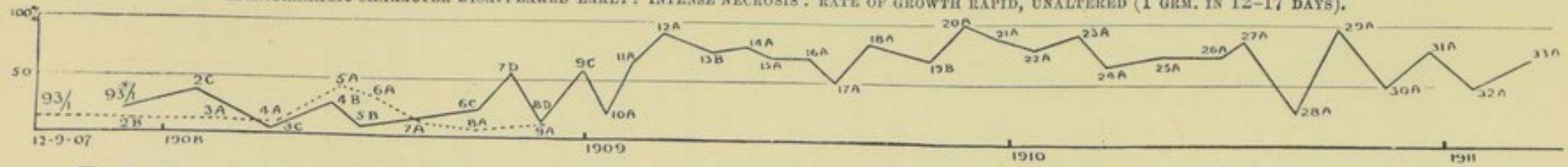


FIG. 7.—Percentage curve of propagation of tumour 93. Two strains were originally propagated from the primary tumour and its recurrence after operation. They behaved in a parallel manner both as regards histology and growth. Primary histological features (hæmorrhagic adeno-carcinoma) disappeared about 18th generation coincident with rise in percentage of "takes." Rate of growth unaltered.

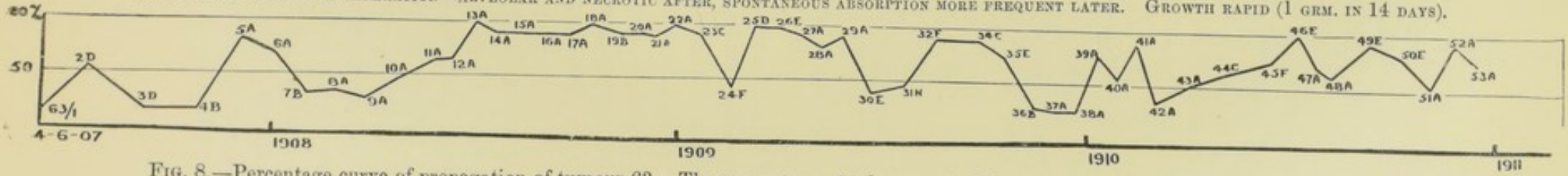


FIG. 8.—Percentage curve of propagation of tumour 63. The percentage of takes rose to 60 per cent. at the second passage, after a fall it rose to 85 per cent. at the 5th generation. After a second fall the curve remains high with fluctuations in the strain shown. A second strain maintained after the 33rd generation maintains a uniformly high percentage 95–100. The primary histological features (hæmorrhagic adeno-carcinoma) have become modified by loss of the hæmorrhagic character, although the adenomatous structure is still retained. The rate of growth has always been rapid, but the two strains differ in this respect and in their susceptibility to concomitant immunisation; while one strain grows progressively the other is liable to spontaneous healing.

Tumour-strain 47. Very hæmorrhagic, acinous and alveolar mammary carcinoma.—Hæmorrhagic character is occasionally absent, but reappears. Light and dark tracts of parenchyma present in the primary growth reappeared in the 31st generation. It is questionable whether these light and dark areas stand in relation to the later mentioned peculiar degeneration. A six-months' old tumour of the 4th generation showed a small circumscribed area of keratinisation. Success in transplantation fluctuates above and below 50 per cent.: 46th generation attained in 4 years 3 months. Growth is medium (1 grm. in 21 days). Spontaneous disappearance is the rule. A peculiar degenerative change occurred in the parenchyma from the 40th generation. This degeneration involved the parenchyma over very wide areas, and whilst nothing abnormal was detectable by the naked eye, changes of a very pronounced character were observed microscopically involving the greater part of the section. The tumour-cells in these areas were characterised by their small darkly stained nuclei, by the great reduction in the amount of their cytoplasm and frequently by their irregular outline. This latter irregularity was most frequently produced by the protoplasm of the body of the cell being drawn out into several radiating arms, which, by coming into contact with the processes from neighbouring cancer-cells, gave rise to a network recalling rather the arrangement of reticular tissue as met with in lymphatic glands. The spaces created by this peculiar arrangement of the parenchyma were filled with œdematous fluid. Over other areas no cell-outlines could be detected, and the nuclei were packed closely together. The cells were small, arranged in alveoli, the nuclei dark and occasionally pyknotic. There was no gross necrosis or hæmorrhage, but the tumours gave either very low percentages of success or negative results on transplantation.

Tumour-strain 58. Hæmorrhagic mammary adeno-carcinoma.—Histology practically unaltered, occasionally the stroma is more cellular and the hæmorrhagic character is absent. Success of transplantation is low and variable. Growth is variable in speed (1 grm. in 18–24 days), and spontaneous absorption is frequent. In 58 the constancy of histology is in marked contrast to the inconstancy of growth.

Tumour-strain 85. Very acinous hæmorrhagic mammary carcinoma.—Histology unaltered, the earlier generations were more alveolar than the later. Stroma delicate with very large blood-sinuses. Success of transplantation fluctuates between 40 and 80 per cent. Growth is slow (1 grm. in 30–35 days). Duration of propagation 3 years 7 months.

Tumour-strain 152. Alveolar mammary carcinoma with lumina.—Histology unaltered during propagation. Success of transplantation varies between 20 and 40 per cent. with one rise to 70 per cent. at the 13th generation. Growth is fairly slow (1 grm. in 3 to 4 weeks), 23 generations having been attained in 2 years 10 months.

Tumour-strain 200. Mammary alveolar carcinoma with lumina.—Histology unaltered, except for a slight increase in density of the stroma. Grows very slowly (1 grm. in 2 months), 12 generations in 24 months.

Tumour-strain 206. Mammary alveolar carcinoma, alveoli small, arranged in trabeculæ.—Histology unaltered. Initial success of transplantation is high (usually 100 per cent.), but nearly all the tumours disappear spontaneously. Growth is very rapid (1 grm. or more in 10 days), after which the tumours rapidly diminish in size, 81 generations in 30 months.

Tumour-strain 230. Papillary adeno-carcinoma of mamma, stroma sclerotic (scirrhus).—Histology unaltered. Success of transplantation has remained low, 5–40 per cent. Growth is very slow, 9 generations in 27 months. The last 2 or 3 generations have not been quite so papilliferous, although the ground character, *i. e.* the formation of fissures remains.

Tumour-strain 138. Mammary adeno-carcinoma with very small alveoli.—Histology unaltered. Success of transplantation low and constant, 20–40 per cent. Growth is slow and usually shows the following remarkable peculiarity. After transplantation the new tumours grow progressively for about 2 to 4 weeks till a weight of 0·5–1·0 gram is attained. The size then remains almost unchanged (sometimes getting larger and then smaller) for 1–3 months, after which the tumours grow slowly and progressively till the death of the animal. 16 generations in $2\frac{5}{6}$ years, *cf.* fig. 10.

Tumour-strain 219. Medullary sarcoma of gluteal region of a male mouse (small area of osteoid substance in primary tumour).—Histology unaltered. Success of transplantation rose slightly at 10th generation but fell again. There has been a general rise in percentage since 20th generation. Growth is fairly rapid (1 grm. in 2–3 weeks), 47 generations in 29 months, but spontaneous absorption is common.

Tumour-strain 336. Spindle-cell sarcoma of subcutaneous tissue.—Histology unaltered. Success of transplantation at first low, has later become higher. Growth slow at first, rose with increase of percentage of

success, but spontaneous absorption became more common ; gives about 1 grm. in 2–3 weeks.

Tumour-strain 239. Sebaceous adeno-carcinoma in the axillary region of a female mouse.—Histology in propagated tumours shows varying amounts of sebaceous differentiation, not tending to disappear. Success

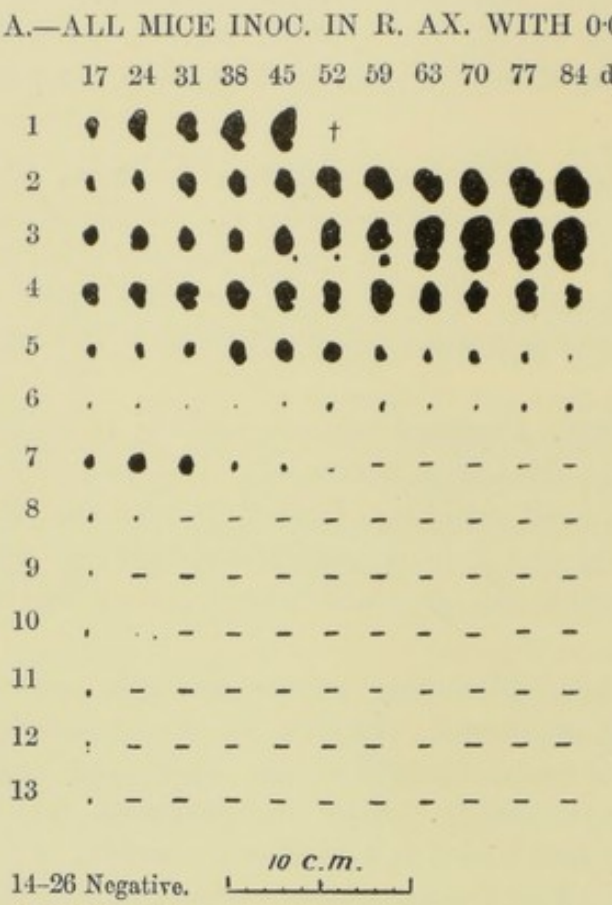
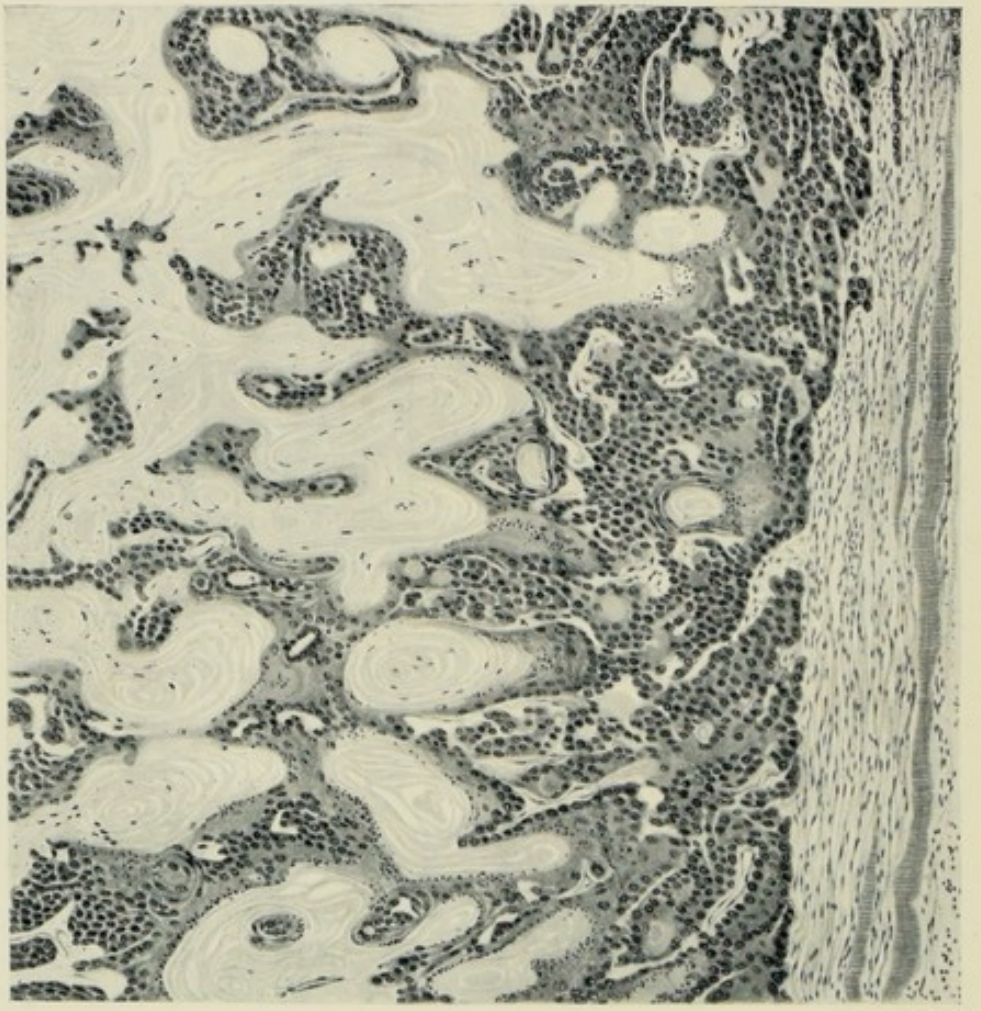


Fig. 10. Exp. 138/12 A.—Chart showing mode of growth of tumour 138 in the 12th generation. After an initial rapid growth the tumours remain stationary for a long period before growth sets in again. Spontaneous absorption may occur during the quiescent period.

of transplantation improved at 2nd generation and has remained constant till the last 2 or 3 generations, which have grown very badly. Growth is fairly slow, 1 grm. in 3–5 weeks, 20 generations in 25 months.

Tumour-strain 297. Preputial adeno-carcinoma, showing structure of normal preputial gland.—Histology unaltered. Lowest possible success of transplantation. Growth extremely slow, about half a gramme in 3 months, and practically all tumours disappear spontaneously ; 12 generations in 21 months.

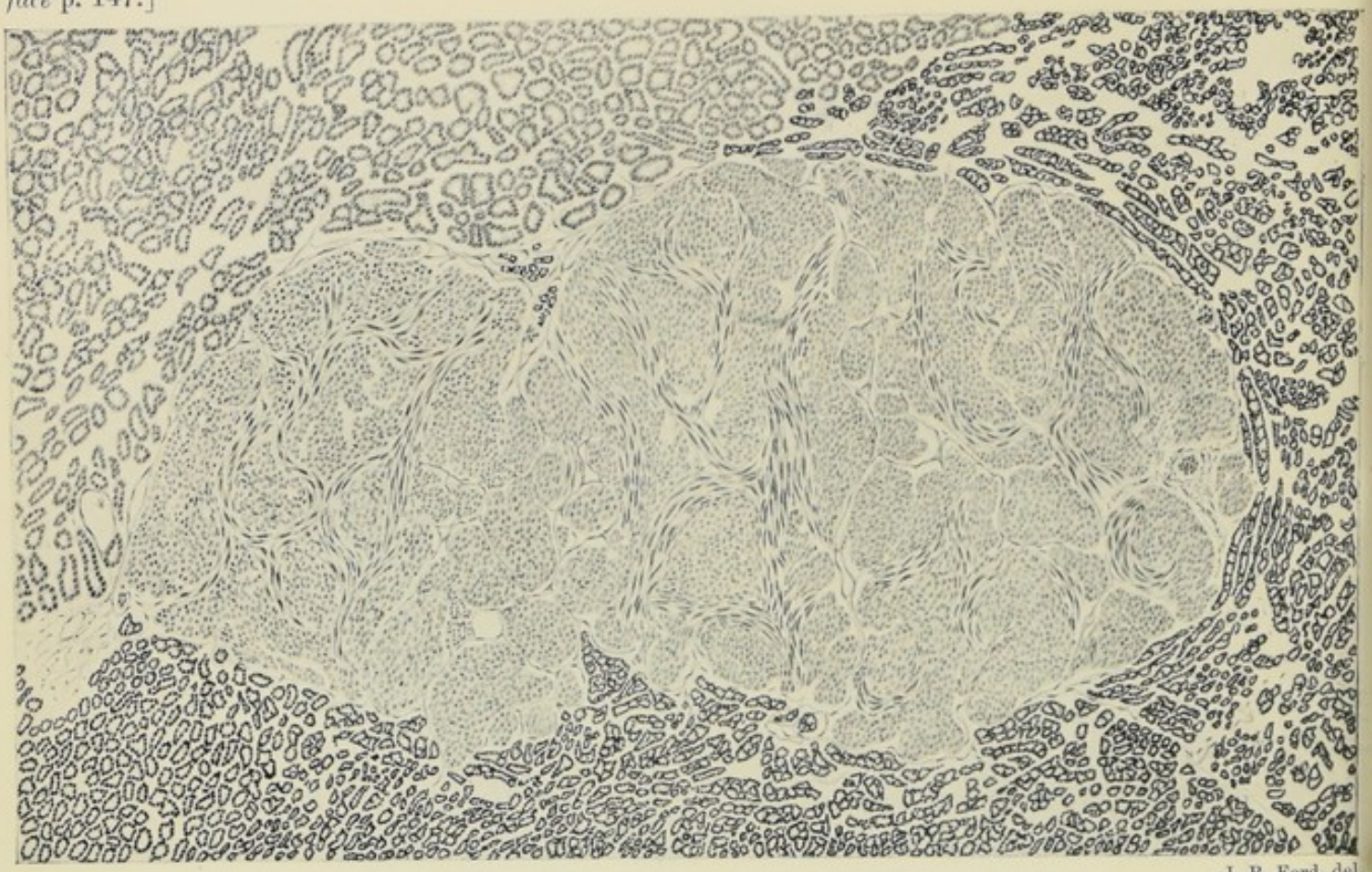
To face p. 147]



J. R. Ford. del.

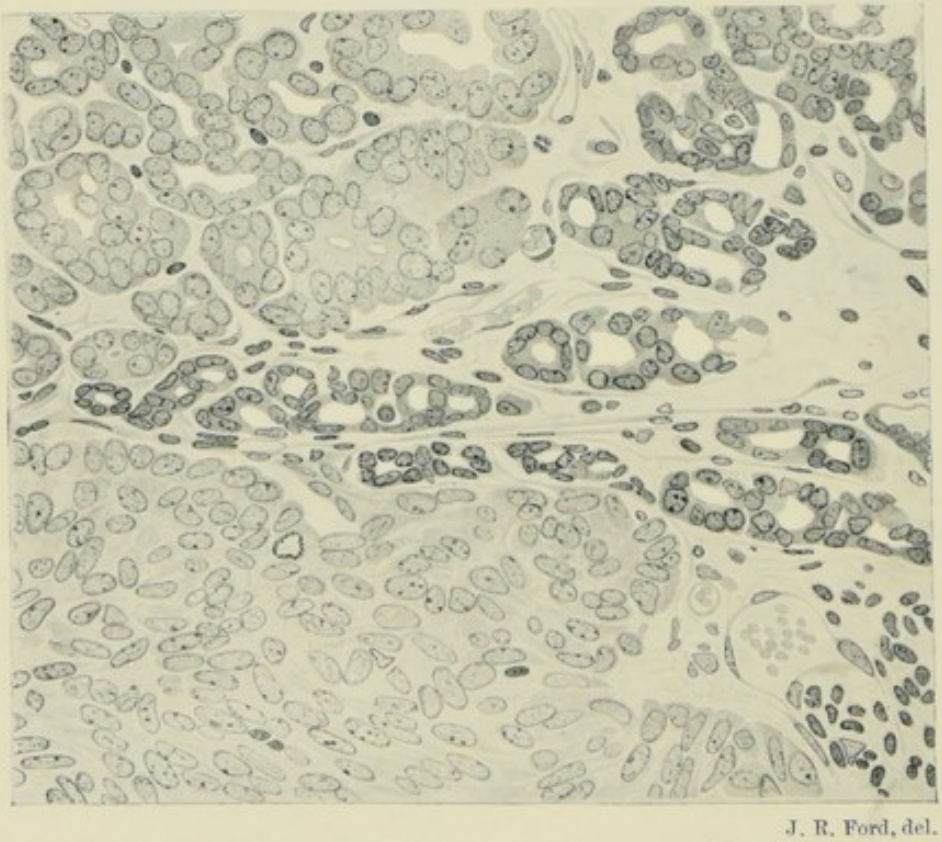
FIG. 11. 486/7 B—8 B. Twenty-six days old tumour of 7th generation. Typical complete keratinisation of the tumour which had the structure of a thick walled cyst filled with masses of keratin. The thin shell of growing cells contains epithelial perles. $\times \frac{87}{1}$. *Cf.* figs. 40, 49, & 50.

To face p. 147.]



J. R. Ford, del.

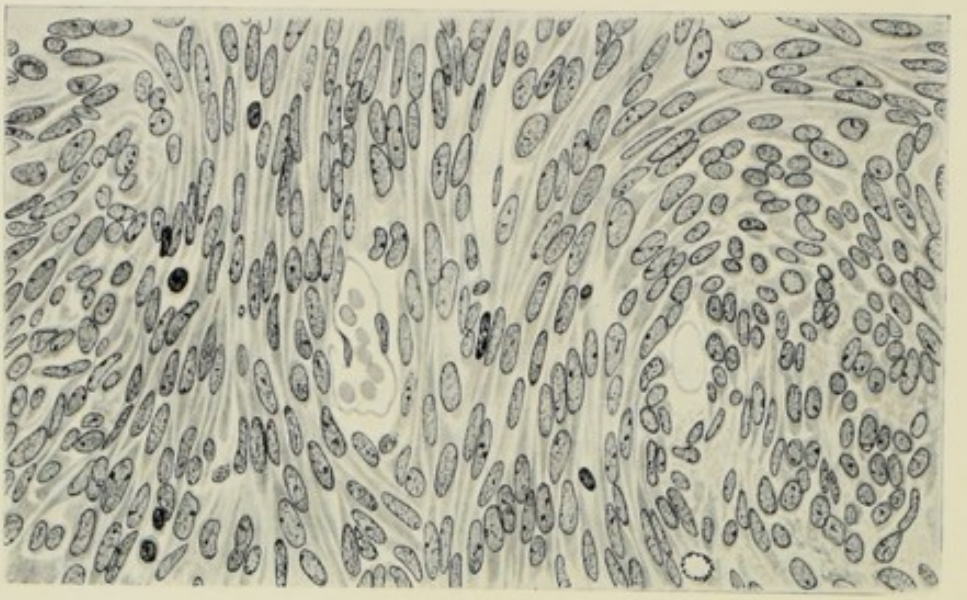
FIG. 13. 91/19 E—20 G. Large and small acinous, and spindle-cell alveolar areas in a tumour 52 days old. $\times \frac{58}{1}$.



J. R. Ford, del.

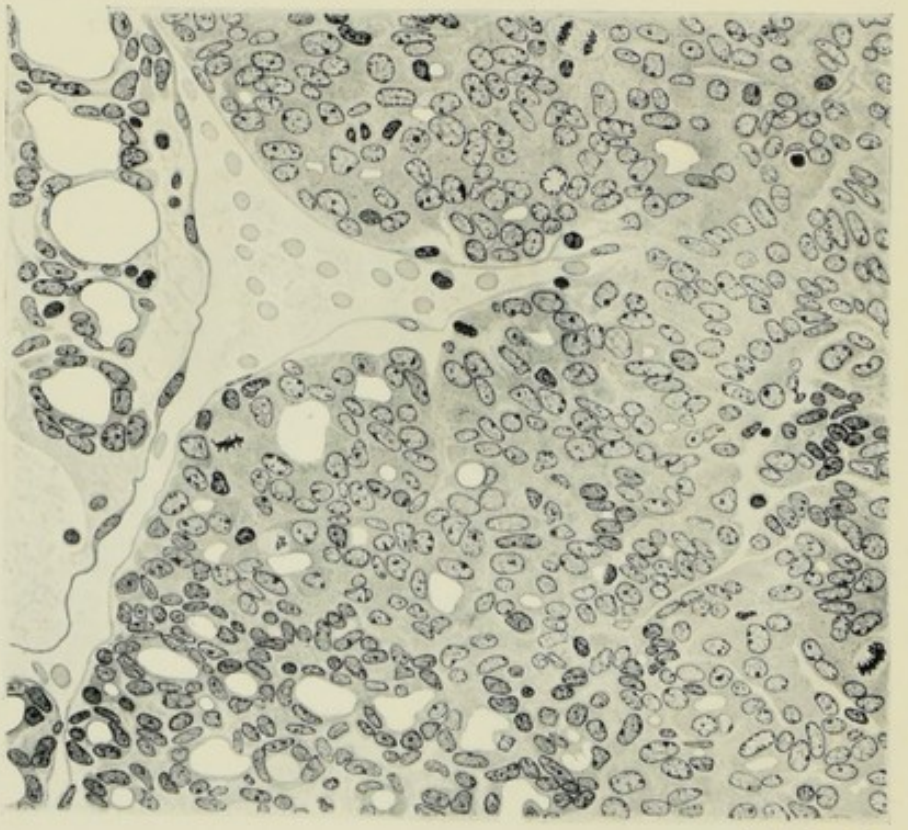
FIG 14. 91/19 E—20 G. High-power view of junction of large and small acinous and spindle-cell alveolar areas of the same tumour as fig. 13. $\times \frac{410}{1}$.

To face p. 147.]



J. R. Ford, del.

FIG. 15. 91/19 E—20 G. High-power view of central part of spindle-cell alveolar area. In spite of their resemblance to sarcoma cells these spindle-cell masses on transplantation give tumours which show acinous differentiation after a period of solid alveolar growth. × $\frac{410}{1}$.



J. R. Ford, del.

FIG. 16. 91/3 A—14 A. Transition from the alveolar to the acinous condition in a tumour 38 days old. Mitotic division is still proceeding rapidly in the solid part of the tumour. × $\frac{410}{1}$.

Tumour-strain 466. Preputial alveolar carcinoma with sebaceous differentiation and keratinisation.—Histology unaltered. Very low percentage of success and very slow growth: 5 generations in 8 months.

Tumour-strain 486. Squamous-cell carcinoma of orbital-region, strongly keratinised.—Histology unaltered. Low percentage of success and slow growth; all the tumours of the earliest generations have disappeared spontaneously, but several tumours of the 8th and 9th passage have grown progressively: 10 generations in 10 months, *cf.* figs. 11 and 12.

GROUP II.—*Strains with Definite Alterations in Structure or Behaviour.*

The second group differs only slightly from the first, and comprises a number of strains in which definite alterations in structure or behaviour have appeared and persisted for an extended period, after which the tumours have reverted to the earlier condition.

The changes may best be considered as an exaggeration of the minor fluctuations seen in some strains of Group I., and differing from them mainly in degree.

Tumour-strain 62. Hæmorrhagic adeno-carcinoma of mamma with very perfect acini.—At the 12th generation the parenchyma became more alveolar, but the hæmorrhagic character of the stroma was retained. The success of transplantation was low until the 11th generation. The 12th generation showed a sudden rise followed by a relapse lasting for several generations, followed again by recovery. Growth is slow (1 grm. in 4–7 weeks), 27 generations in 3 years 9 months, and spontaneous absorption is very common.

Tumour-strain 91. Hæmorrhagic mammary adeno-carcinoma, very acinous.—The transplanted tumours are largely alveolar when young, and frequently show areas where the cells are arranged in bundles and are spindle-shaped as in sarcoma. The transplantation of areas showing only spindle-shaped cells leads to the development of tumours ultimately of a purely adenomatous character. Old tumours are very acinous like the primary growth. Success of transplantation 20–40 per cent. up to 10th generation. A sudden rise to 70 per cent. then occurred and has been maintained. Growth is of medium speed (1 grm. in 21 days), but this is much more rapid when transplantation is effected with medium doses—0·02 or 0·03 grms. by syringe; the tumours are then much more alveolar, and will even surpass a weight of 1 grm. in 10 days, *cf.* figs. 13, 14, 15, 16, 17, 18.

Tumour-strain 107. Hæmorrhagic alveolar and acinous mammary adeno-carcinoma.—The only variation has been in the histology. The

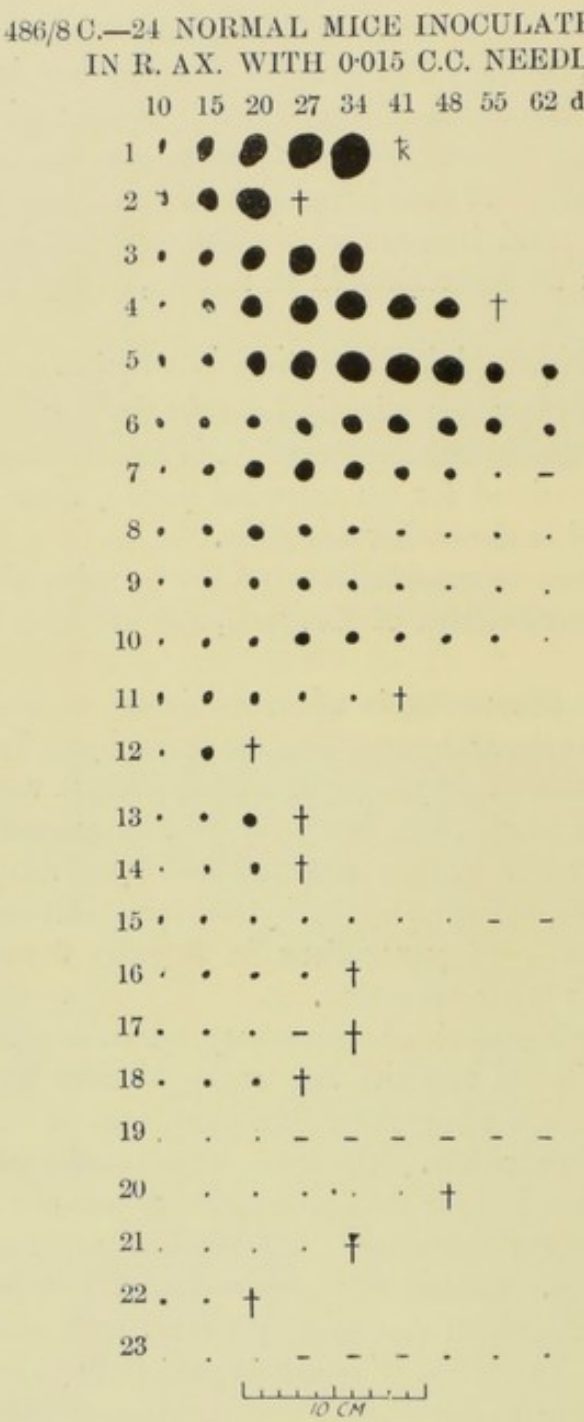


Fig. 12. Exp. 486/8 C.—Chart showing mode of growth ot tumour 486 in 8th generation. After growing to a considerable size all the tumours if left to themselves disappear spontaneously after complete differentiation into epithelial squames.

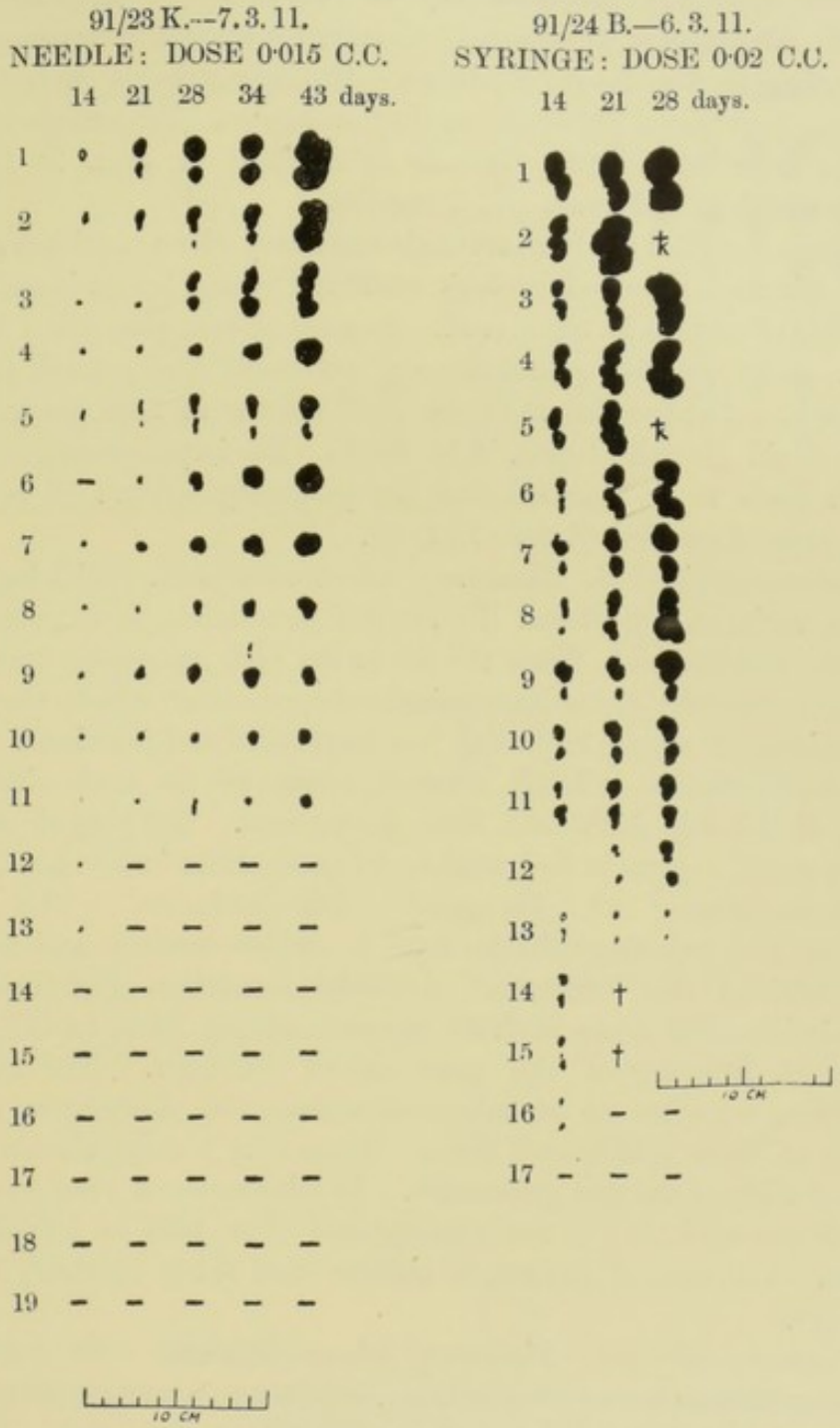


Fig. 17. Exp. 91/23 K.—Chart showing mode of growth of tumour 91 (23rd generation) when inoculated in small dose.

Fig. 18. Exp. 91/24 B.—Chart showing much more rapid growth of tumour 91 (24th generation) when inoculated in large dose of emulsion with syringe.

acinous character preponderated in the first 5–6 generations, after which the tumours were largely alveolar for several generations, finally returning to the acinous condition. The hæmorrhagic character of the stroma is retained although varying in degree. Percentage of success varies from 40 to 80. Growth is medium (1 grm. in 24 days) : 30 generations in 3 years and 5 months.

Tumour-strain 117. Hæmorrhagic mammary adeno-carcinoma. Alveoli with minute lumina.—Histology unaltered except for an increase in the number of lumina in the alveoli. Success of transplantation 10–20 per cent. up to the 11th generation and extremely slow growth (1 grm. in 60 days). Sudden rise to 60 per cent. success at 12th generation and more rapid growth (1 grm. in 35 days). The improvement has latterly given place to slow growth and low percentage again : 18 generations in 3 years 4 months, *cf.* figs. 19 & 20.

Tumour-strain 146. Mammary adeno-carcinoma.—Histology shows minor variations, unaltered till the 4th generation, then pure alveolar for two generations. From the 6th to the 14th generation many of the parenchyma-cells have been spindle-shaped, after which the original appearance of alveoli and acini has reappearad and remained. Success of transplantation is fairly constant, about 40 per cent. with marked rises at the 6th, 11th, and 16th generations. The rate of growth is fairly rapid, 1 grm. in 2–4 weeks : 34 generations in 3 years.

Tumour-strain 129. Mammary adeno-carcinoma. — The primary tumour was indistinguishable from a carcino-sarcoma and the strain has retained this structure. Artificial separation and implantation of spindle-celled areas in fresh normal animals leads to the development of tumours of the same mixed structure. The tumour is therefore regarded as an adeno-carcinoma, the parenchyma of which easily assumes spindle-cell form. Success of transplantation reached 50 per cent. at the 2nd generation. In later series it rises occasionally to 100 per cent., but is not constant and often falls to 30 per cent. or lower. The rate of growth is medium and fairly constant, 1 grm. in 20 days.

Tumour-strain 267. Mammary adeno-carcinoma with small acini ; stroma œdematous and hæmorrhagic.—Acinous character maintained till 6th generation, after which it became much more alveolar. From the 6th to the 13th generation the tumours grew faster, giving a gramme in 18 days, and the success of transplantation was higher. In the 13th and 14th generations the tumours again grew more slowly (1 grm. in 4 weeks) and in a lower percentage : 14 generations in 23 months.

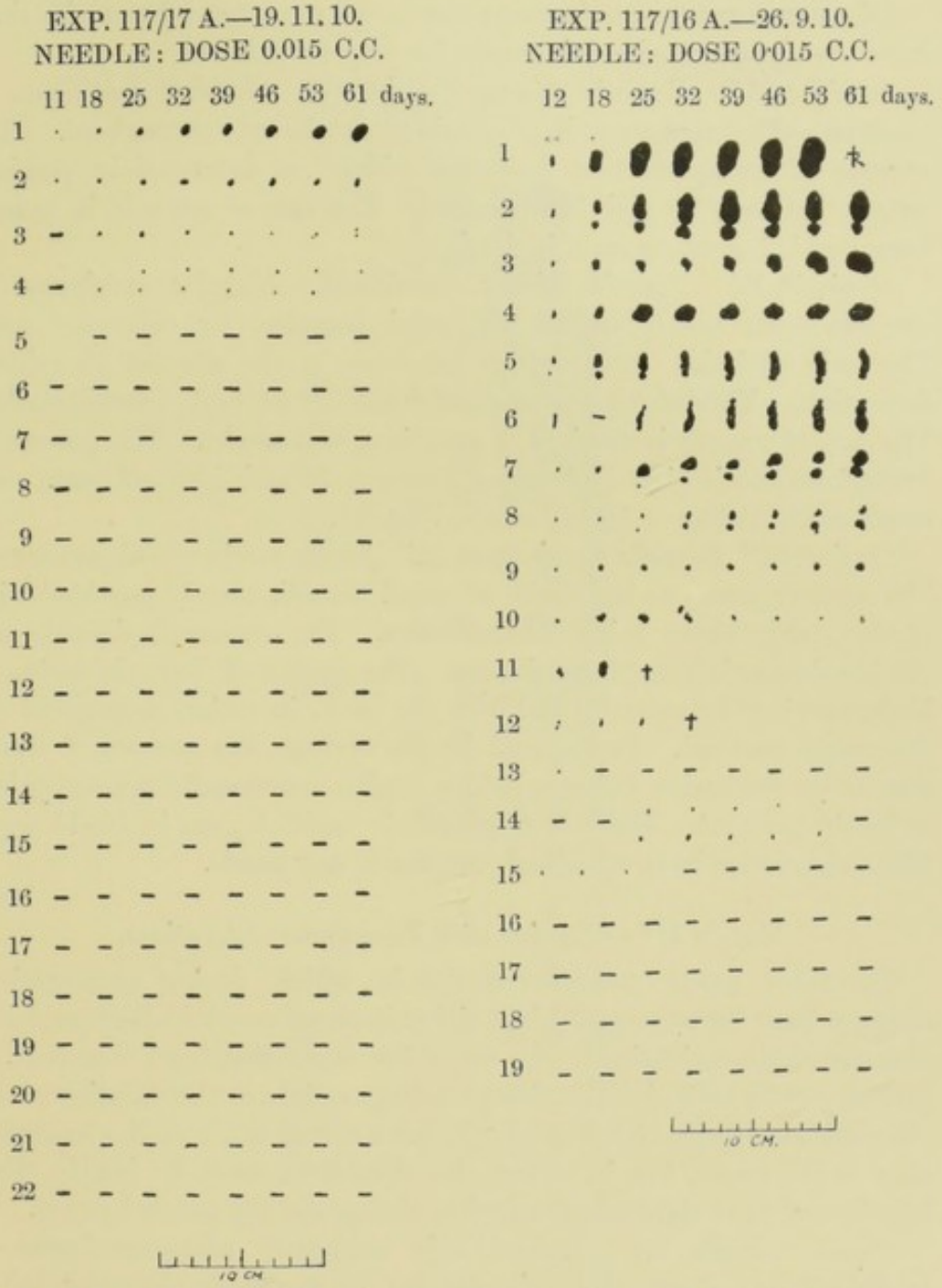


Fig. 19. Exp. 117/17 A—Chart showing extremely slow growth and low percentage of "takes" characteristic of tumour 117 in the earlier and latest generation.

Fig. 20. Exp. 117/16 A.—Chart showing rapid growth in the series (16th generation) of tumour 117, immediately preceding the series shown in fig. 19. Mouse number one provided the material with which series 117/17 A was laid down.

Of several mouse carcinomata obtained from other laboratories three have been propagated continuously for long periods:—

Strain "J." Jensen's Mouse Carcinoma. Alveolar carcinoma of mamma.—The structure has remained unaltered throughout. The success of transplantation fluctuates widely, as described in previous papers, between 10 and 100 per cent. The rate of growth is usually very rapid, 1 grm. or more in 10 days.

Strain "T." Twort's Mouse Carcinoma. Alveolar carcinoma of mamma with small lumina and acini bounded by columnar cells. The only variation in structure has been in the amount of acinous formation. Yielded 90 per cent. of "takes" at first transplantation. The rate of growth is medium, 1 grm. in 2 to 3 weeks. The success of transplantation is high and varies between 70 and 100 per cent., very occasionally as low as 50 per cent, *cf.* fig. 21.

Strain "B." Borrel's Carcinoma "B." Adeno-carcinoma of mamma.—The tumour grows as trabeculæ of small alveoli, usually provided with lumina, large areas being adenomatous. The stroma is delicate and the blood-vessels sometimes dilated. The success of transplantation is high, varying between 70 and 100 per cent., in series propagated by the needle method. Propagated by the syringe, the success of transplantation fluctuates between wider limits, occasionally falling as low as 30–40 per cent. The rate of growth is rapid, 1 grm. in 10–15 days. These characters have remained constant in our hands.

Group III.—*Strains with Permanent Alterations.*

The third group embraces strains in which, during propagation, changes have become established either in structure or biology, or both, and have been maintained. In many the separation from Group II. is probably quite artificial, the change being so slight or the duration of the observations comparatively so short, that a return to the earlier condition may easily occur, but in others the alterations seem to justify their interpretation as significant enduring changes in the parenchymata.

Tumour-strain 65. Hæmorrhagic mammary adeno-carcinoma.—Histology has shown little change, the hæmorrhagic character being well retained. Success of transplantation was poor until the 13th generation, after which 100 per cent. was obtained and maintained with occasional drops to as low as 30 per cent. Growth is rapid (1 grm. in 10–17 days). Spontaneous absorption occurs in nearly one-half of the tumours; 52 generations in 43 months, after which it was allowed to die out.

EXP. T/30 G.—MICE 1–19 INOC. IN R. AX. WITH 0·015 C.C.: NEEDLE. (27. 6. 10).

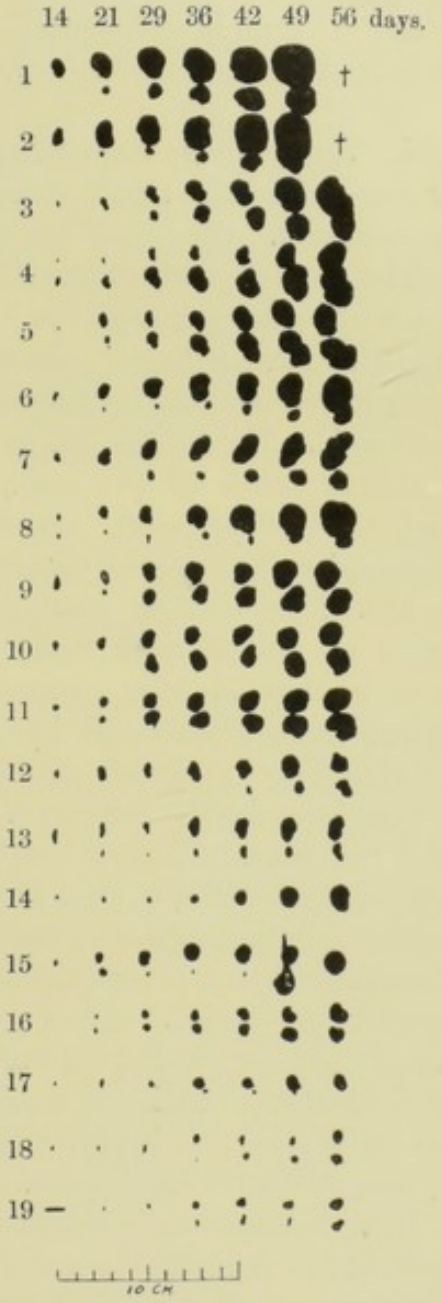


FIG. 21. Exp. T/30 G.—Chart showing slow but progressive growth of tumour "T" in all animals.

Tumour-strain 83. Hæmorrhagic mammary adeno-carcinoma.—Hæmorrhagic character persisted till 20th generation, after which the tumour grew as an adeno-carcinoma without distinctive peculiarities. Success of transplantation fluctuated throughout between 20–90 per cent. Growth is slow (1 grm. in 3–4 weeks), spontaneous absorption rare ; 45 generations in 42 months, after which the tumour was not propagated.

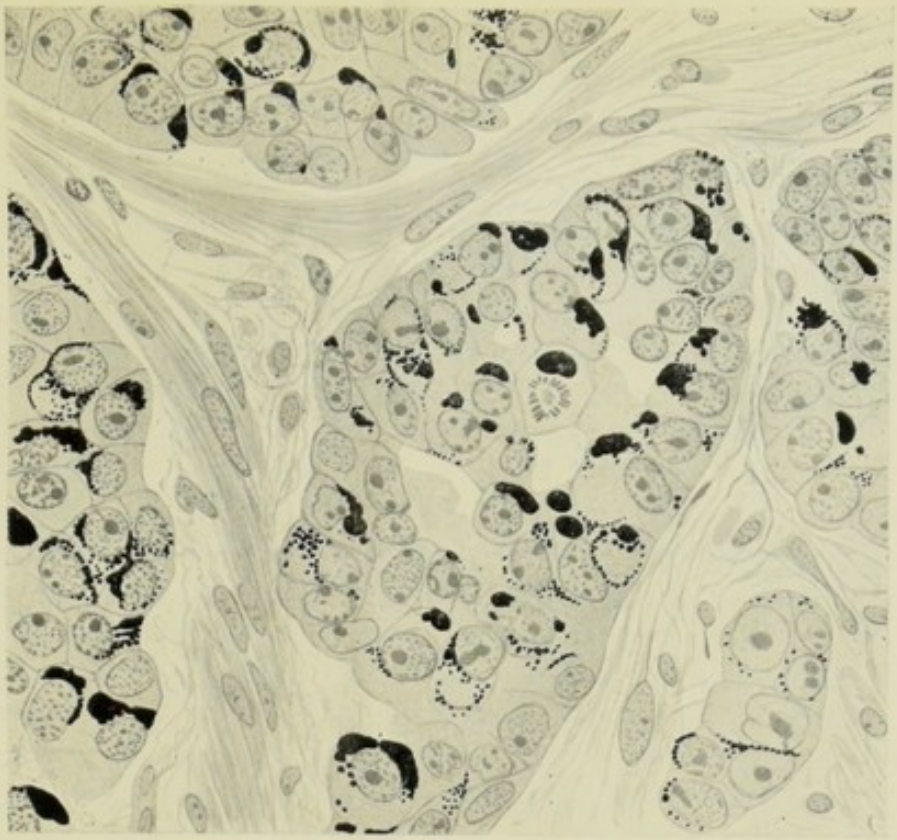
Tumour-strain 94. Very acinous hæmorrhagic adeno-carcinoma of mamma.—In later generations the tumours became less hæmorrhagic and the parenchyma alveolar. Success of transplantation has remained at the same general level throughout (under 50 per cent.). Growth is slow, 1 grm. in 3–5 weeks ; 33 generations in 3 years 4 months.

Tumour-strain 104. Hæmorrhagic acinous adeno-carcinoma of mamma.—In later generations the parenchyma is more alveolar, the stroma less hæmorrhagic. Up to the 6th generation the success of transplantation remained below 50 per cent., afterwards fluctuating between 50 per cent. and 80 per cent. Growth is medium (1 grm. in 24 days) ; 28 generations in 3 years 5 months.

Tumour-strain 113. Hæmorrhagic mammary adeno-carcinoma.—Parenchyma consists of alveoli with many lumina. The acinous character is not so marked in later generations, the tumours are necrotic, and the hæmorrhagic character has given place to a more abundant stroma. Has always produced glycogen in large quantities. Growth is slow (1 grm. in 28 days). Success of transplantation reached 70 per cent. in the 2nd generation, and has fluctuated slowly since ; 28 generations in 3 years 4 months, *cf.* figs. 22 & 23.

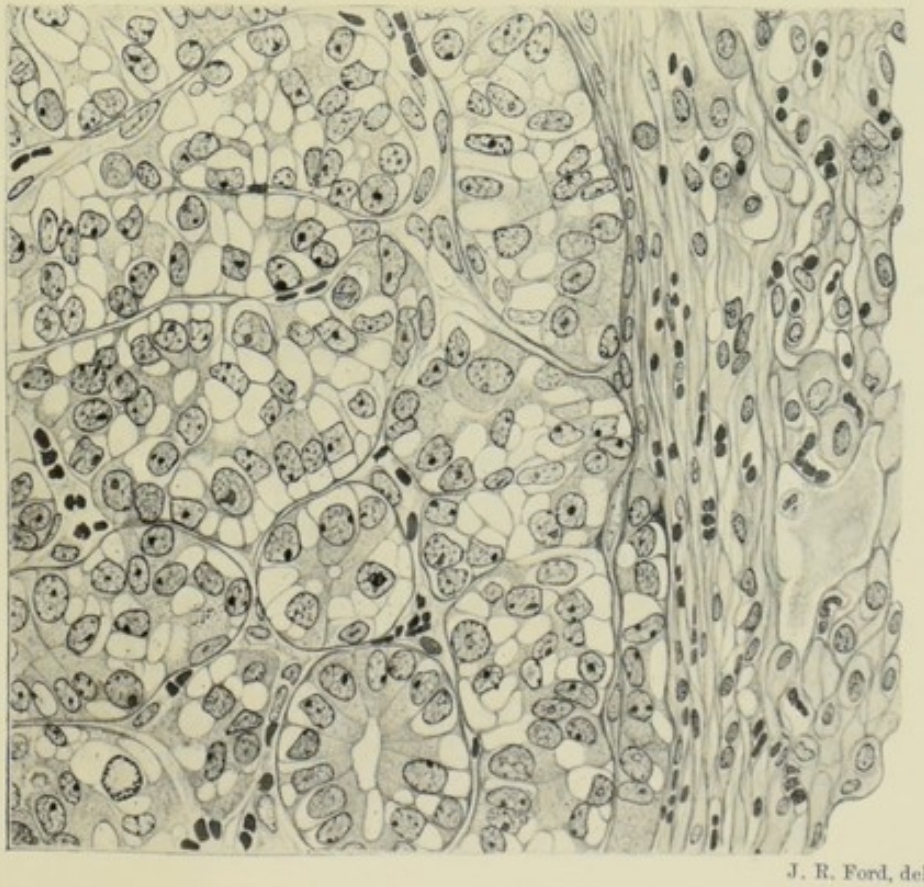
Tumour-strain 121. Hæmorrhagic mammary adeno-carcinoma.—In later generations the parenchyma became alveolar and necrotic, but the hæmorrhagic character is retained. Success of transplantation rose from 30 per cent. to 60 per cent. at the 6th *passage*. The curve thereafter shows wide fluctuations, but keeps a high level. Growth was slow at first(1 grm. in 30 days), but became slightly more rapid (1 grm. in 21 days) later ; 30 generations in $3\frac{1}{6}$ years.

Tumour-strain 128. Hæmorrhagic mammary adeno-carcinoma.—Histology unaltered 1st to 8th generation ; 9–13th generation tumours more acinous ; later more alveolar. The hæmorrhagic character is generally retained. Success of transplantation below 25 per cent. till 12th generation, thereafter fluctuating above and below 50 per cent. Growth is slow (1 grm. in 30 days), and has not increased ; 24 generations in $3\frac{1}{6}$ years.



J. R. Ford, del.

Fig. 22. 113/25 B, 9 days. Transplanted tumour of 25th generation of glycogen-forming mammary carcinoma showing droplets of glycogen in dividing and resting cells. Best's carmine stain. × $\frac{700}{1}$.



J. R. Ford, del.

Fig. 23. 113/0. *Cf.* fig. 22. Pulmonary metastasis in spontaneously affected mouse. The cells show large clear vacuoles identical with those in the transplanted tumours which can be shown to contain glycogen. Fixation in osmic acid.

Tumour-strain 238. Hæmorrhagic mammary adeno-carcinoma with small acini.—Histology very constant, slightly less acinous since 20th generation. Success of transplantation and rate of growth very low till 6th generation, after which the tumours grew more rapidly—1 grm in 12–20 days, and in a higher percentage. Series transplanted by syringe method show more spontaneous absorption than those with the needle ; 26 generations in 26 months.

Tumour-strain 282. Hæmorrhagic alveolar mammary carcinoma with lumina. An autoplastic transplantation in the spontaneously affected mouse showed keratinisation, but this has not reappeared. There was a great improvement in the success of transplantation at the 4th generation, from the 4th to 12th generations it fluctuated between 20 per cent and 80 per cent. Nearly all the later series show extreme spontaneous absorption. Grew more rapidly at the 4th generation and later, but varies considerably ; produces 1 grm. in 3–6 weeks. Macroscopic metastases are the rule where tumours have grown progressively for 2 months ; 17 generations in 22 months.

Tumour-strain 284. Papillary adeno-carcinoma of mamma.—Histology has remained unaltered, except that the tumours are rather more alveolar. Success of transplantation was low at first, but has risen in later generations ; the slow rate of growth (1 grm. in 5–7 weeks) is unchanged ; 8 generations in 22 months.

Tumour-strain 322. Hæmorrhagic alveolar mammary carcinoma with small foci of keratinisation.—Keratinisation has not appeared in the transplanted tumours, and the hæmorrhagic character has been absent since the 8th generation. Growth is slow and success of transplantation low. The speed of growth (1 grm. in 3–4 weeks) remains unaltered but the success of transplantation is higher although irregular, and spontaneous absorption is more frequent.

Tumour-strain 421. Mammary adeno-carcinoma, with cellular tissue between the acini, probably diffusely growing epithelial cells.—This histological picture was retained for three generations, and then the tumour became more alveolar with nothing peculiar in the stroma. Growth most rapid in 4th generation, 50 per cent. success, no further improvement ; 8 generations in 10 months. Tumours take from 2–5 weeks to attain a weight of 1 grm.

Tumour-strain 32. Adeno-cancroid of mamma.—The primary tumour showed acinous and keratinised areas. Up to the 9th generation keratinisation was present and gradually increasing in amount. Thereafter it gradually diminished and gave place to a pure alveolar

condition ; in one series of the 12th generation with acini. This also disappeared, and the tumour has since (82 generations) grown as a pure alveolar carcinoma with no differentiation except in a very old tumour

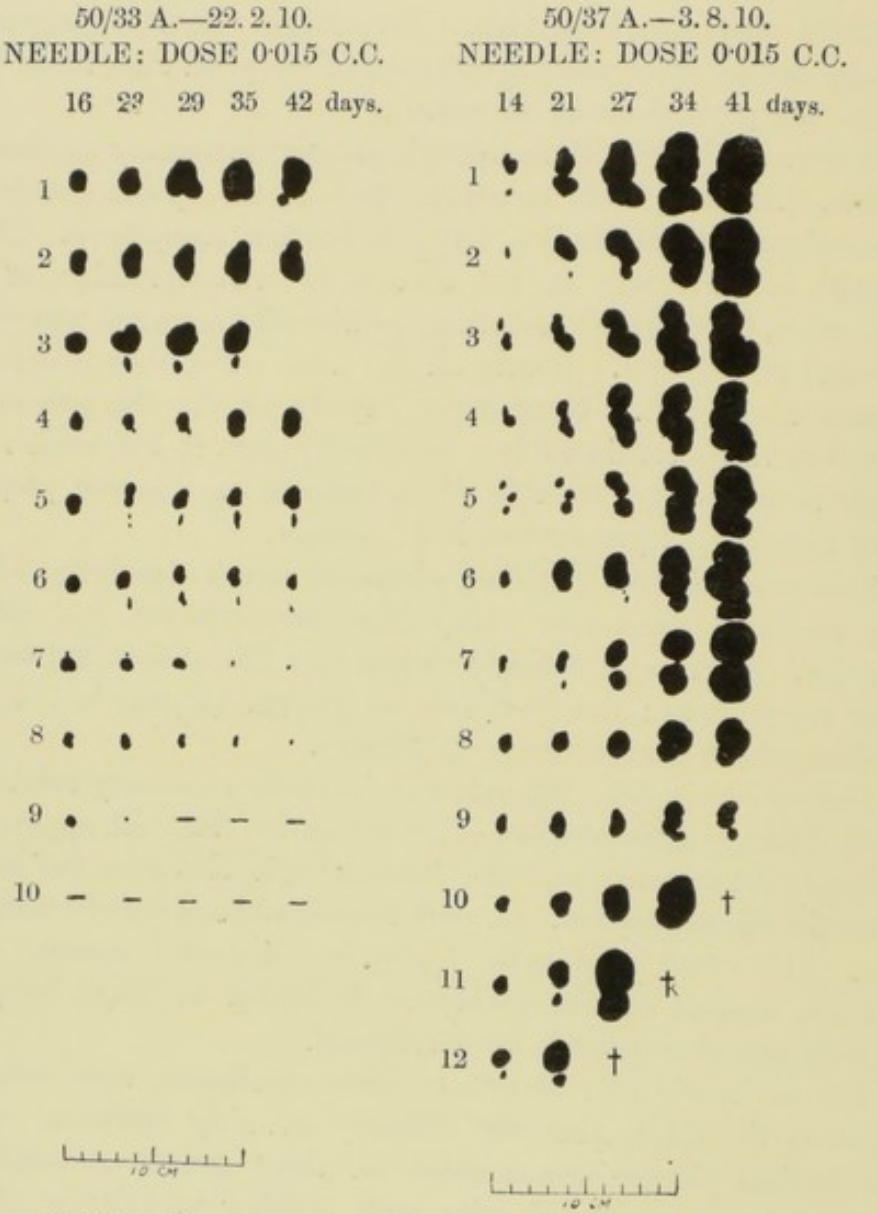
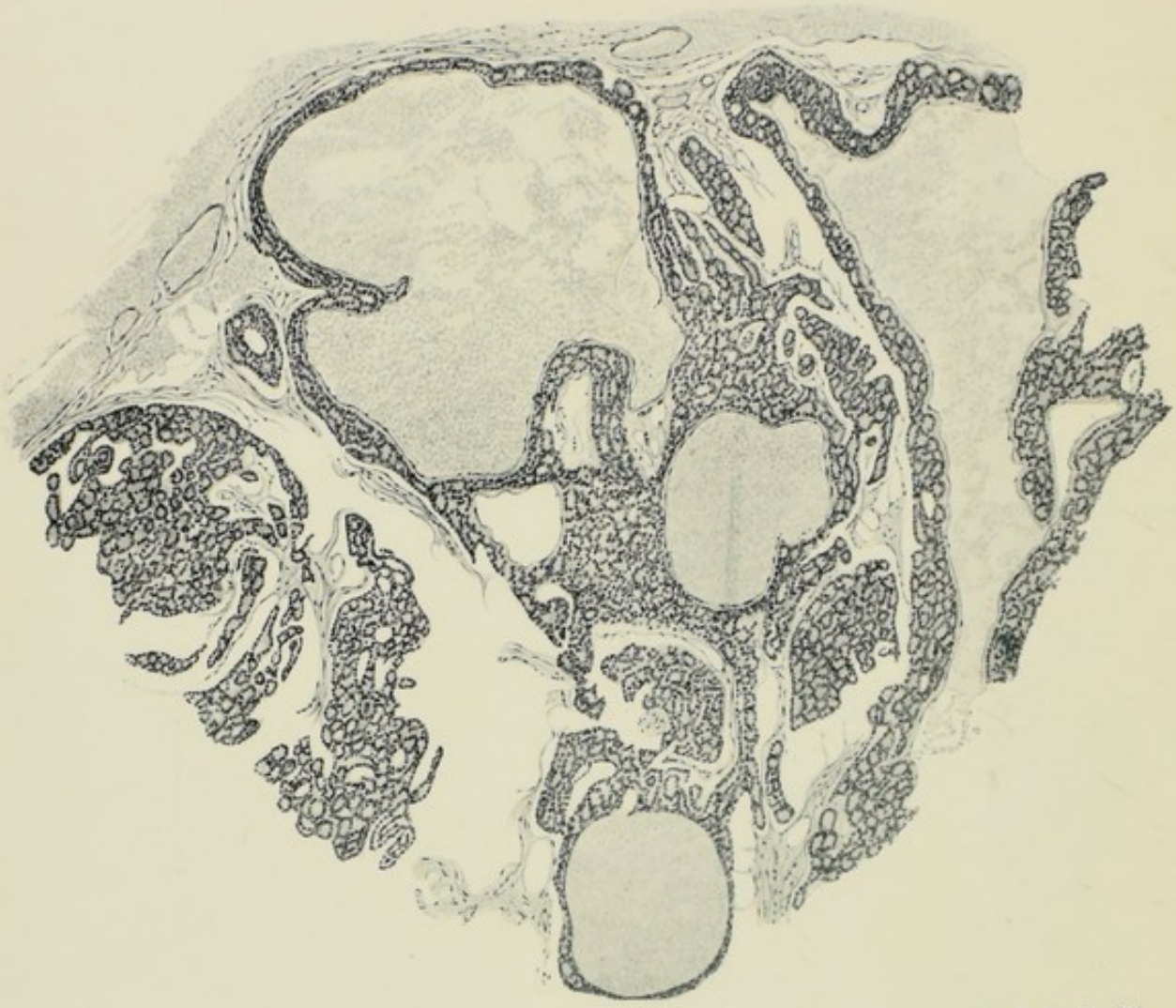


Fig. 26. Exp. 50/33 A.—Chart showing growth of tumour 50 in an experiment (33rd generation) with 50 per cent. of progressively growing tumours.

Fig. 27. Exp. 50/37 A.—Chart showing growth of tumour 50 in 37th generation with 100 per cent. of progressively growing tumours.

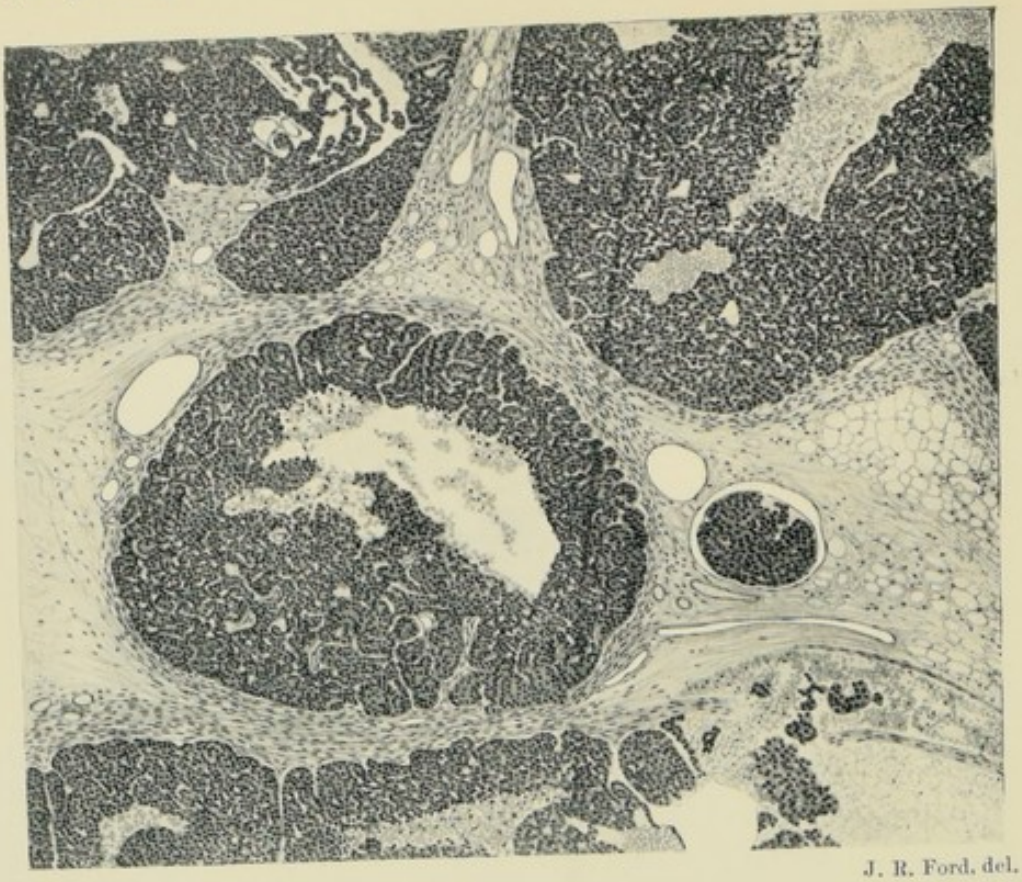
(8 months) of the 56th generation, which showed areas of keratinisation. The success of transplantation reached 80 per cent. at the 4th generation, but there have always been much spontaneous absorption and great

To face p. 157.]



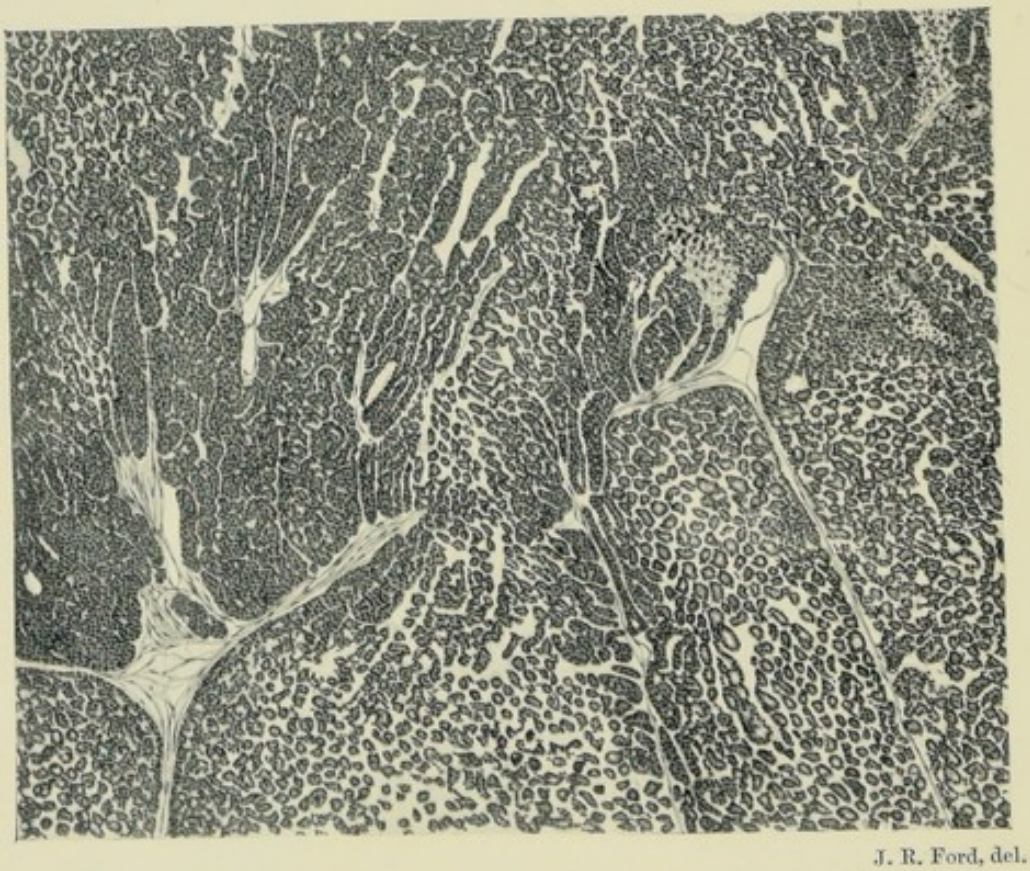
J. R. Ford, del.

FIG. 24.—Mouse. Tumour $\frac{50}{0}$. Transplantable spontaneous hæmorrhagic tumour. $\times \frac{45}{1}$.



J. R. Ford, del.

FIG. 25. 50/29 A—30 A. Twenty-six days old tumour of 29th generation. Large alveoli with atypical attempts at acinus formation and central necrosis. *Cf.* with primary tumour, fig. 24. × $\frac{56}{1}$.



J. R. Ford, del.

FIG. 28. 63/22 B. Forty-two days old tumour of 22nd generation. Acinous differentiation is nearly complete throughout this tumour, while tumours less than 4 weeks old are almost completely solid. × $\frac{56}{1}$.

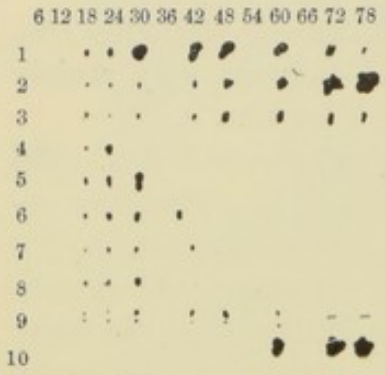
fluctuations, 10–100 per cent. The keratinised tumours of the 1st nine generations grew slowly, the undifferentiated tumours very rapidly (1 grm. in 10 days); 82 generations in 4 years 6 months.

Tumour-strain 50. Hæmorrhagic mammary adeno-carcinoma.—The majority of the transplanted tumours of the first 12 generations reproduced the acinous and hæmorrhagic character of the primary; a smaller number showed a more alveolar type of structure with absence of hæmorrhagic character. Subsequent to the 12th *passage,* the latter type of growth has become predominant, although there still occur tumours reproducing the earlier structure of the strain, especially where the tumours have developed more slowly. The success of transplantation does not now vary so markedly as it did previously, but fluctuates within narrower boundaries situated at a higher level. The speed of growth is medium (1 grm. in 20 days); 43 generations in 4 years, *cf.* figs. 24, 25, 26, 27.

Tumour-strain 63. Hæmorrhagic mammary adeno-carcinoma.—The tumours of the first five *passages* retained the hæmorrhagic character present in the primary, but the acinous differentiation of the parenchyma was not so complete, and greater areas of the field were occupied by alveolar masses. In the subsequent generations the hæmorrhagic character ceased to be a feature, but the arrangement of the parenchyma showed little or no change from the tumours of the early generations. The percentage of success rose at the 2nd passage to 60 per cent., and at the 5th to 85 per cent., followed by a large fall in each case. After the 9th generation the percentage rose steadily until 100 per cent. was attained at the 13th. Since that period the tumour has, with an occasional relapse, given a consistently high percentage of success. Two strains have been maintained separate since the 33rd generation, and differ in this respect, that one shows much spontaneous absorption, whilst the other (depicted on chart, fig. 31) does not show any, and maintains a uniformly high percentage (95–100). Growth is rapid (1 grm. in 14 days); 57 generations in $3\frac{3}{4}$ years, *cf.* figs. 28, 29, 30, 31.

Tumour-strain 93. Hæmorrhagic alveolar carcinoma of mamma.—The hæmorrhagic character disappeared in later generations, and the alveoli underwent very intense necrosis. Up to 10th generation success of transplantation fluctuated between 0 and 50 per cent. A temporary cessation of growth often occurs after a period (10–17 days) of rapid initial growth. Complete disappearance or renewed growth follows after this in varying proportions. The initial speed of growth is great (1 grm. in 14–16 days); 33 generations in $3\frac{1}{3}$ years, fig. 32.

EXP. 63/1.—ALL MICE INOCULATED IN RIGHT AXILLA WITH NEEDLE: DOSE 0·02 GRM. OF SPONTANEOUS CARCINOMA ON 4.6.07.



11–118 negative.

EXP. 63/19 C.—ALL MICE INOCULATED IN RIGHT AXILLA WITH DOSE 0·02 GRM. ON 13. 9. 08.

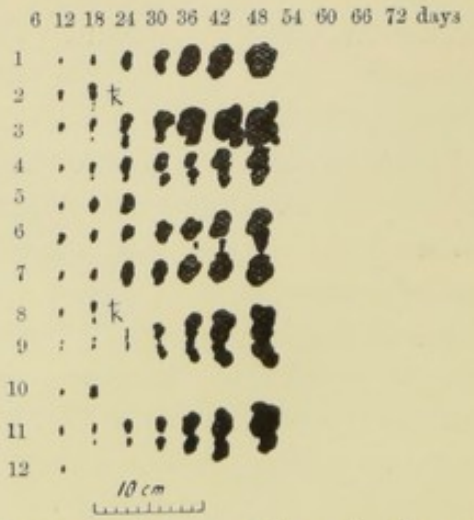
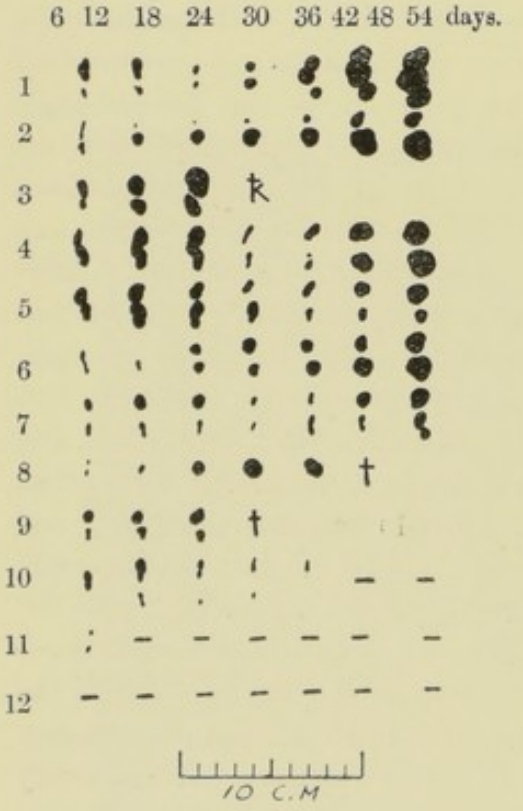


FIG. 29.—Chart showing difference in rate of growth of tumour 63 at the 1st and 19th generations.

EXP. 63/55 A.—ALL MICE INOCULATED IN R. AXILLA WITH DOSE 0·03 GRM. SYRINGE. 30. 1. 11.



EXP. 63/48 K.—INOCULATED IN R. AXILLA WITH DOSE 0·015 GRM. NEEDLE. 15. 5. 11

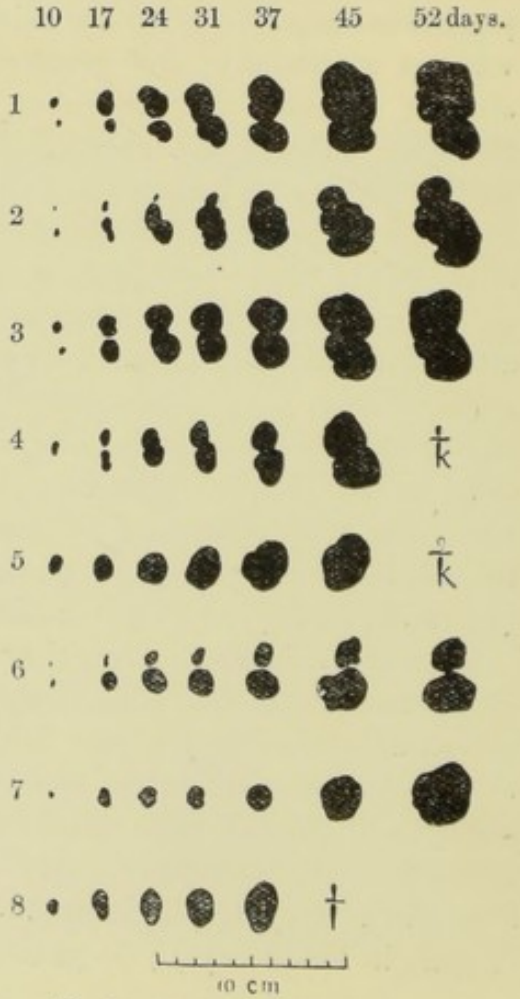


FIG. 30. Exp. 63/55 A.—Chart, for comparison with fig. 29, of growth of the strain of tumour 63 which shows much spontaneous absorption.

FIG. 31. Exp. 63/48 K.—Chart of growth of strain of tumour 63 which shows no, or very little, spontaneous absorption.

Tumour-strain 199. Hæmorrhagic mammary adeno-carcinoma, alveolar in parts.—The alveolar structure preponderates after the 6th generation, the hæmorrhagic character is not so pronounced, and the

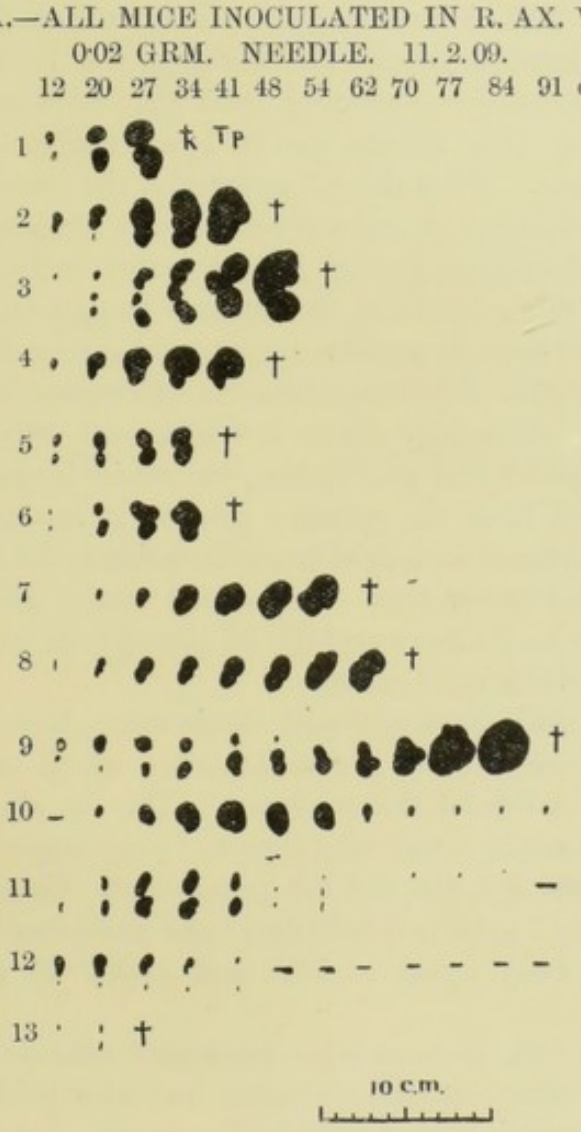


Fig. 32. Exp. 93/11 A.—Chart showing mode of growth of tumour 93. The initial rapid growth may be followed by a temporary halt or by spontaneous absorption. In mouse 9 of this experiment one tumour disappeared while the other grew progressively after a transient quiescent period.

stroma is sclerotic. Success of transplantation rose at 7th and 8th generations. Later the tumour has been propagated by syringe, and although the initial success is high, usually 100 per cent. (0·5–1 grm. in 10 days), there is so much spontaneous absorption that percentage

curves cannot be relied on. This tumour while inducing absolute protection against its own re-inoculation is very insusceptible to resistance induced otherwise. Growth is very rapid; 27 generations in 3½ years.

Tumour-strain 286. Adeno-cancroid.—Very acinous structure with multiple foci of keratinisation. The histology was extremely variable up to the 8th generation, changing from pure adenoma to solid trabecular carcinoma. The stroma was of hæmorrhagic type in the 1st and 6th generations. Since the 8th generation the tumours have been alveolar, with lumina and occasional acini and a hæmorrhagic stroma. Success of transplantation, good at 1st generation, fell very low till the 6th. Since the 8th generation the tumour has grown fairly rapidly, taking from 15–30 days to produce 1 grm., and giving 30–60 per cent.

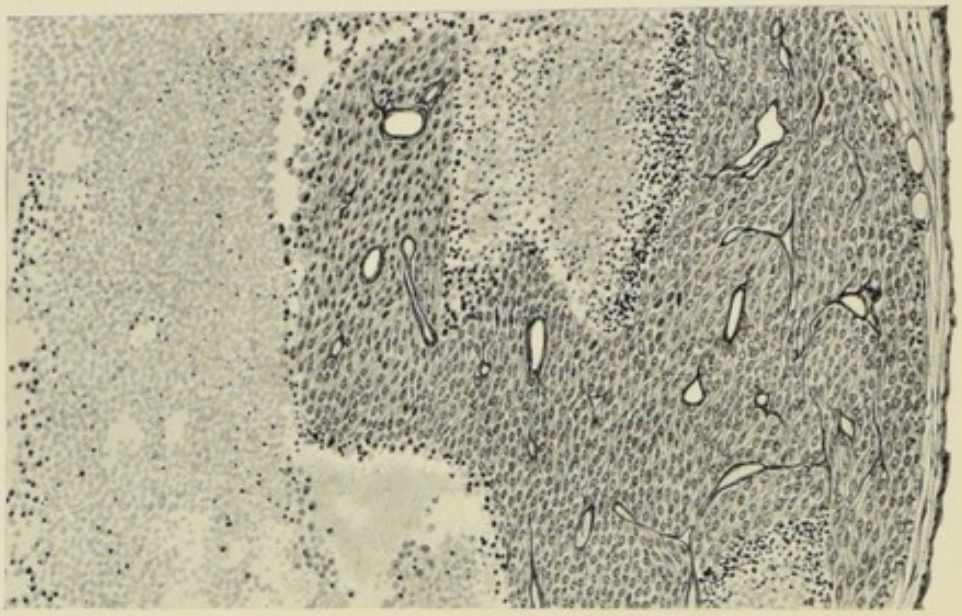
Tumour-strain 297. Alveolar sebaceous carcinoma, with some foci of keratinisation.—Histology shows less sebaceous differentiation and more keratinisation in later generations, but otherwise unaltered. Two strains propagated from the primary growth at separate operations. Both show an increased success of transplantation at the 4th generation, later rising to 70–100 per cent. The second strain, giving 1 grm. in from 60–90 days, grows more rapidly, 22 generations in 20 months as against 13 generations in 21 months.

Tumour-strain 349. Squamous-cell carcinoma.—Keratinisation diminished progressively till 9th generation, after which only clear areas of prickle cells indicated the tendency. This is also diminishing. Growth in 1st generation was very slow and percentage of success low, improvement at the 3rd, 4th, and 6th generations. Great improvement in rate of growth (1 grm. in 15–30 days) and percentage at 7th generation, but fell away again at 14th and 15th; 15 generations in 16 months.

Tumour-strain 356. Hæmorrhagic mammary adeno-carcinoma, with minute areas of sebaceous differentiation and also prickle cells.—No sebaceous differentiation or keratinisation in the daughter tumours. Success of transplantation varies from 5–60 per cent. Growth is of medium speed (1 grm. in 3–5 weeks), occasionally fast, but no improvement in later series. Very little spontaneous absorption; 8 generations in 11 months.

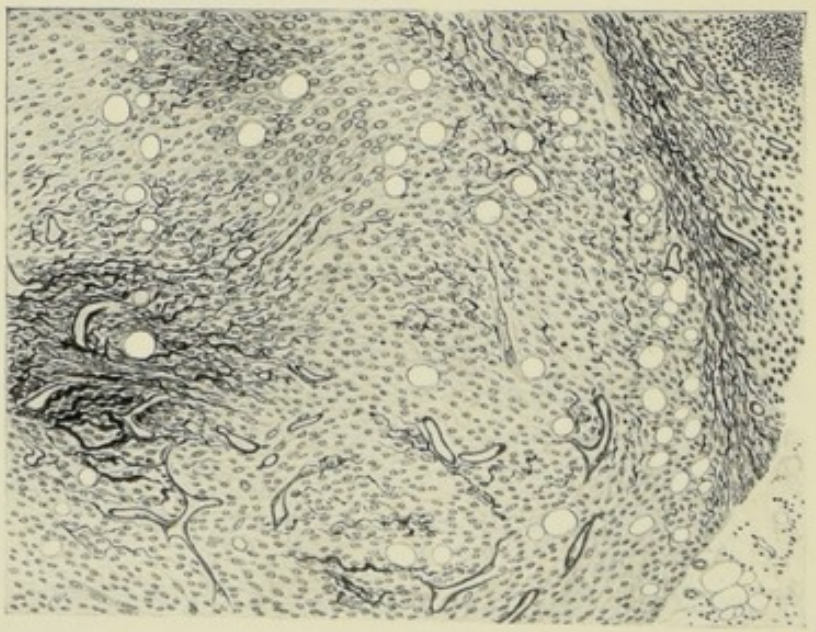
Tumour-strain 92. Osteoid chondro-sarcoma.—The primary tumour was a spindle-cell sarcoma with abundant collagen formation. It recurred after surgical removal as a tumour of complex structure consisting of soft spindle-celled tissue containing large osteoid masses and

To face p. 161.]



J. R. Ford, del.

FIG. 33. 92/35 A—36 A, 38 days. Soft necrotic strain in which very little collagen is produced. Central necrosis with hæmorrhages sets in early and is generally extensive. The majority of the tumours disappear spontaneously after an initial rapid proliferation. Stained in hæmatoxylin; counterstained in Ammonium-pierate rubin. *Cf.* fig. 34. $\times \frac{87}{1}$.



J. R. Ford, del.

FIG. 34. 92/30 B—31 B, 36 days. Collagenous sister strain of propagation. The tumours grow rather more slowly but more often progressively than those of the first strain of this tumour, *cf.* fig. 33. Magnification and staining the same as in fig. 33.

small nodules of cartilage. For the first 8 generations osteoid tissue occurred in the transplanted tumours in considerable masses and, more rarely, nodules of hyaline cartilage. From the 8th generation two lines

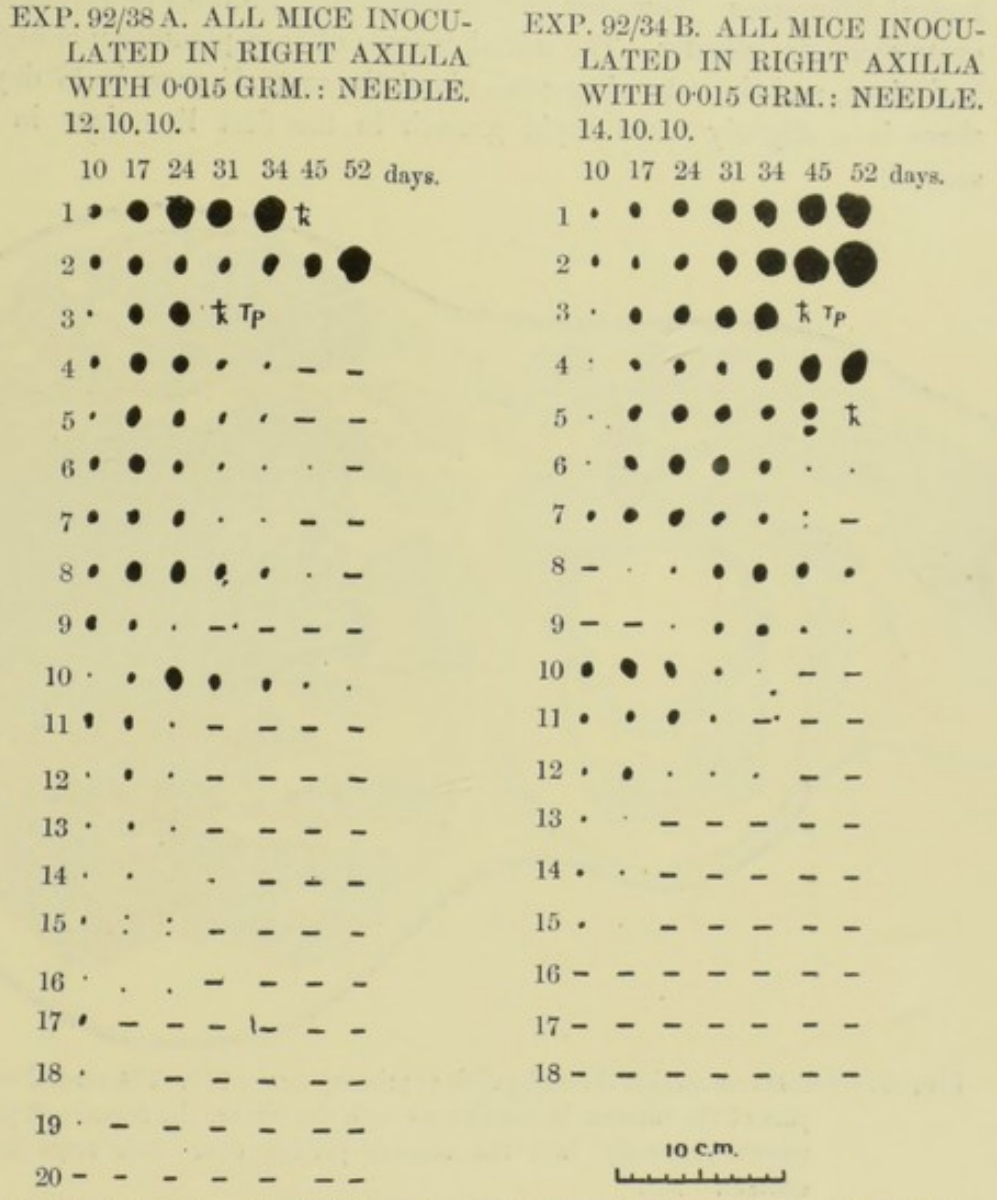


Fig. 35. Exp. 92/38 A.—Chart showing mode of growth of the 1st or soft necrotic strain of tumour 92.

Fig. 36. Exp. 92/34 B.—Chart showing mode of growth of the 2nd or firm collagenous strain of tumour 92.

have been propagated separately in continuous series. The first line produces tumours of soft consistence with large hæmorrhages and extensive central necrosis. There is little formation of collagen even in old tumours. Spontaneous absorption is the rule, and generally sets in after 20–24 days' growth (*cf.* figs. 33 & 35). The second line produces firm tumours

with abundant collagen-formation, which show spontaneous absorption less frequently, and at a later date, than the first, *cf.* figs. 34 & 36. These differences in mode of growth are not so strictly marked as the histological differences. Together they separate the two lines of propagation quite characteristically. The initial success of transplantation is high in both lines, and the rate of growth about the same (1 grm. in 17 days); there is a slightly more rapid growth in the first line than in the second.

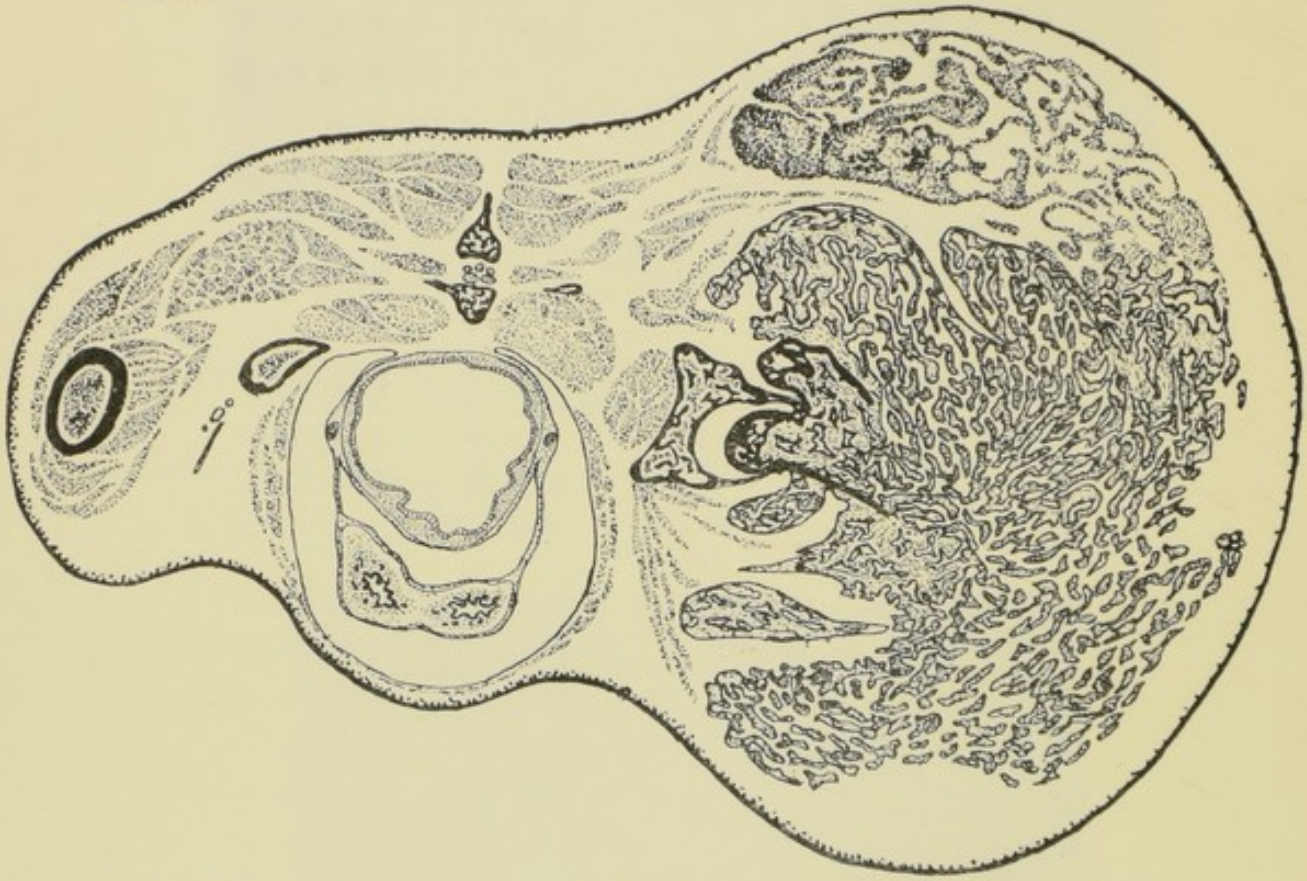
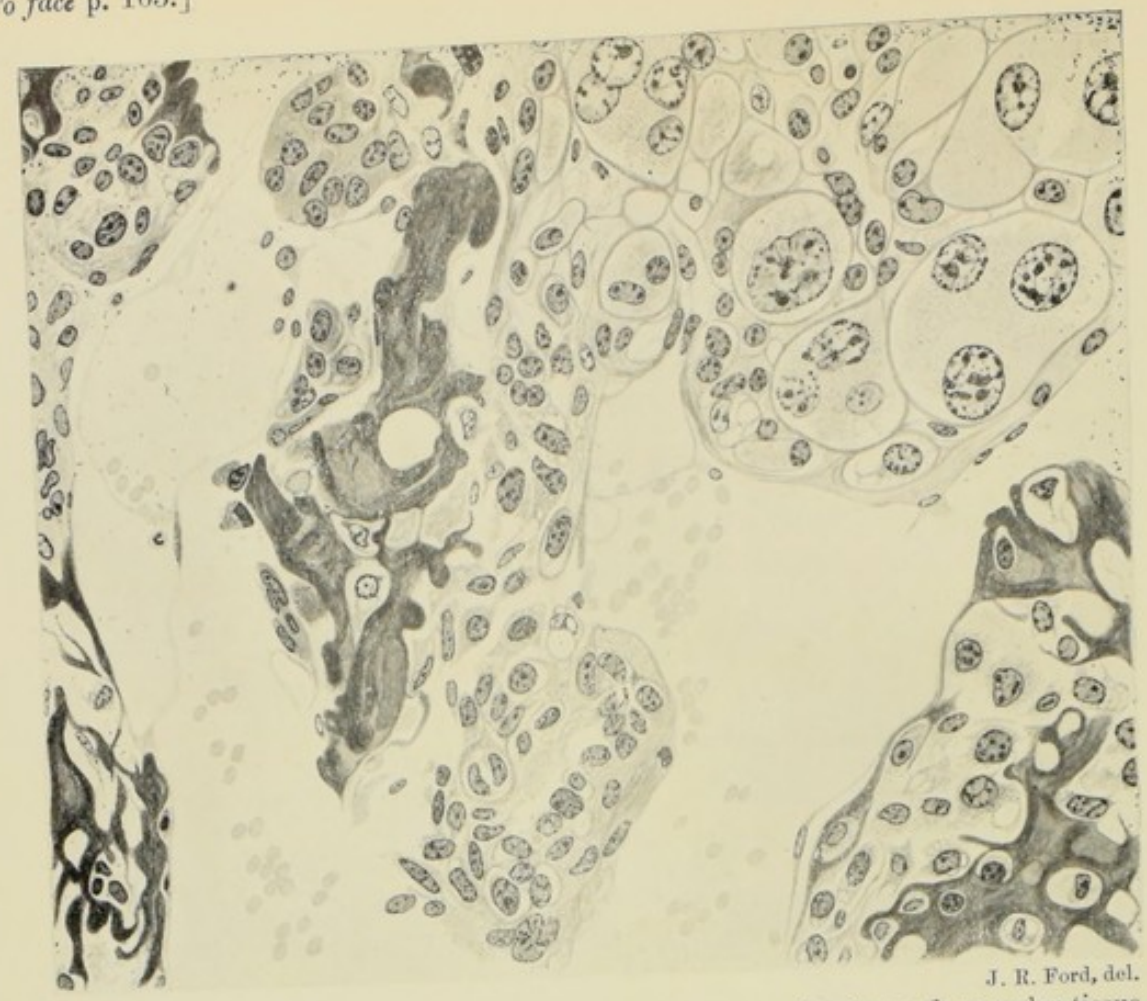


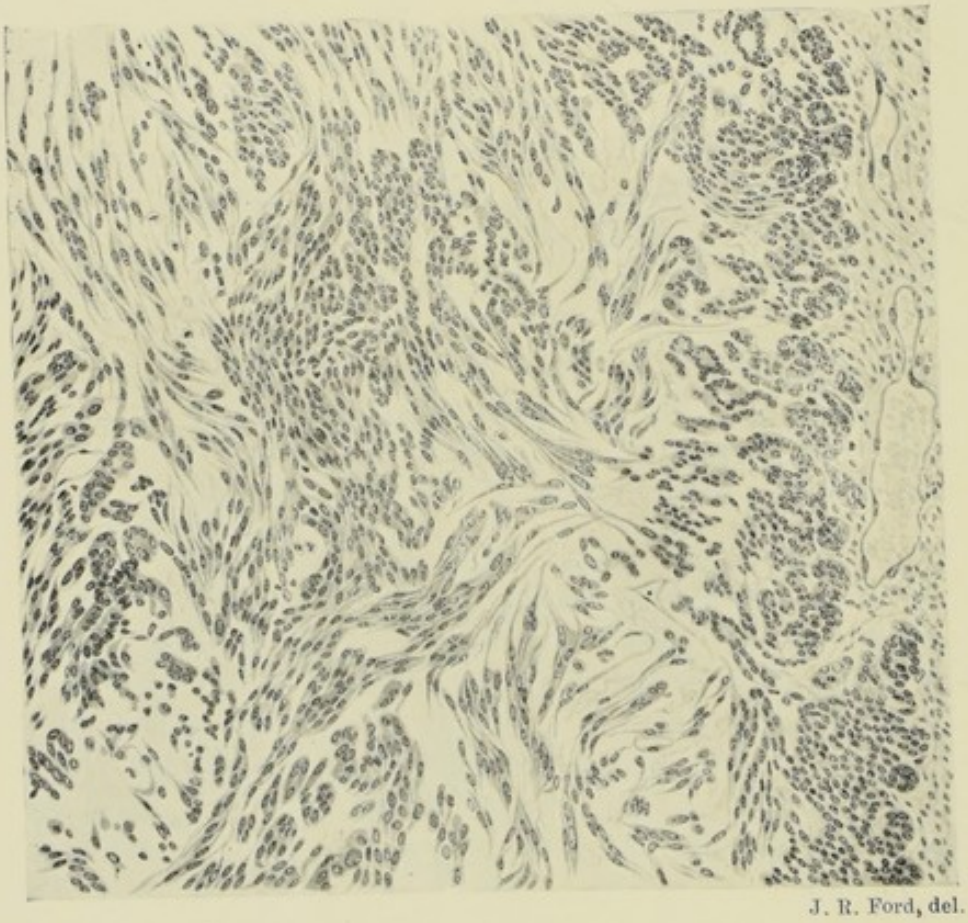
FIG. 37.—Transverse section through pelvis of primary mouse 513. The ventral osseous part of the tumour is continuous with the femur, the dorsal soft portion passes gradually into the osseous portion over their large area of contact. $\times \frac{4}{1}$.

Tumour-strain 513.—Osteo-sarcoma of the femur. The primary tumour consisted of two parts of equal size, a proximal, very hard, bony portion growing from the femur in the region of the great trochanter, and a dorsal cap of soft, very vascular tissue containing isolated bone-spicules at its junction with the first, *cf.* fig. 38. Transplantation was successful with fragments from both parts, and the tumours developing from the hard portion were also osseous or osteoid and very hard. The



J. R. Ford, del.

FIG. 38. 513/0. Osteo-sarcoma of femur; primary tumour, showing soft vascular tissue containing giant-cells and spicules of bone (dark). $\times \frac{410}{1}$.



J. R. Ford, del.

FIG. 39. 37/34 P—35 P. Spindle-cell carcinomatous strain of tumour 37. In old tumours there is a more or less complete return to the acinous condition in this strain. $\times \frac{166}{1}$.

tumours from the soft portion, which alone has proved capable of continued propagation, were likewise soft and vascular at first, but later showed osteoid nodules. The period required for the development of this differentiation seems to be getting longer. Success of transplantation is low, 5–30 per cent., and the rate of growth is slow to medium, 1 grm. in 3–4 weeks. Spontaneous absorption is common (*cf.* figs. 37 & 38).

GROUP IV.—*Strains producing Sarcomata.*

Four tumour-strains have undergone a change whereby the connective-tissue stroma elements have been transformed into sarcoma cells. In two the change supervened during transplantation (Tumours 37 and 100). In the other two (Tumours 469 and 568) the change took place in the primarily affected animal.

Tumour-strain 37. Adeno-carcinoma of mamma.—The primary tumour contained acinous and alveolar areas. Both reappear in the transplanted tumours, sometimes combined in one tumour, as in the primary growth, sometimes separately, so that one tumour is entirely alveolar. From two tumours of the primary transplantation separate strains have been propagated continuously. All the cases of sarcoma development have appeared in the descendants of one of these (2 A Strain). The descendants of the other (2 D Strain) have not shown it, except temporarily in one tumour (20 Z), which reverted to pure carcinoma at the next transplantation. From this strain (2 D), however, a line of tumours has been propagated in which the carcinomatous parenchyma is broken up into isolated spindle-cells (*cf.* fig. 39), and the tumours closely resemble sarcoma histologically. Their rate of growth and percentage of success are unaltered, and old tumours show a return to the condition of adeno-carcinoma with small acini. The sarcomatous change in the stroma of the transplanted tumours, leading through a mixed-tumour stage to pure sarcoma, has occurred four times in all in descendants of series 2 A. The first transformation occurred in the 8th and 9th generations at about the same time, when propagation had lasted about five months. The mixed-tumour stage lasted for 3 and 4 generations respectively. A third development occurred in the 17th generation, which resulted in pure sarcoma at the 22nd generation. The fourth and fifth developments occurred in two of six series, all derived from one transplanted tumour at successive operations and recurrences. Two of these series were propagated as pure carcinoma for more than a year without showing any change.

A third series became mixed, and then pure sarcoma at the 13th and 21st generations, while a duplicate of this line, derived from it after the mixed-tumour condition had appeared, maintained this mixed character for nearly two years, and then reverted to pure carcinoma. The same reversion supervened in a fourth line after more than two years' growth as a mixed tumour, no pure sarcoma being separated. A fifth line grew in duplicate as mixed tumour for 15 months, when it was allowed to die out. In the sixth line propagated in duplicate pure sarcoma supervened in both after propagation as mixed tumours for 9 months and 18 months respectively.

The carcinoma strains of this tumour grow very slowly, with a low or moderate success of transplantation. The mixed tumours are even more difficult to propagate, while the pure sarcomata grow very rapidly (1–1½ grms. in 10 days), with an initial success of transplantation of 100 per cent. and very frequent spontaneous absorption.

Tumour-strain 100. Hæmorrhagic mammary adeno-carcinoma.—The hæmorrhagic character and the acinous structure were reproduced in the earlier generations; later (20–47th generations) the parenchyma was alveolar, surrounding a large cystic cavity filled with clear fluid. This degenerative change masks the original hæmorrhagic character of the tumour. Sarcoma development was first observed in an old tumour of the 10th generation. Further study showed that tumours propagated quickly (less than 5 weeks continued growth) remained pure carcinomata, while sarcomatous change supervened in the stroma of tumours which had grown progressively for more than 8 weeks. Once initiated, the sarcoma rapidly overgrows and destroys the carcinoma component, pure sarcoma resulting in one or at most two generations. The rate of growth of the pure carcinoma is slow (1 grm. in 30 days) (*cf.* fig. 40), and the percentage of success low (20–60 per cent.). The sarcomata have a rapid initial proliferation in nearly all the animals inoculated, followed soon by complete spontaneous absorption. Apart from the development of sarcoma, the tumour belongs to Group I., showing no change. There is no reason to suppose that the power to induce sarcoma development is a new acquisition; it was probably present in the spontaneous tumour, but the conditions under which sarcoma develops in this strain were not fulfilled in the earlier generations, or in the primary growth.

Tumour-strain 469. Adeno-carcinoma of mamma surrounded by a mantle of spindle-cell sarcoma.—The primary tumour when discovered was a minute firm nodule 2–3 mm. in diameter. It remained the

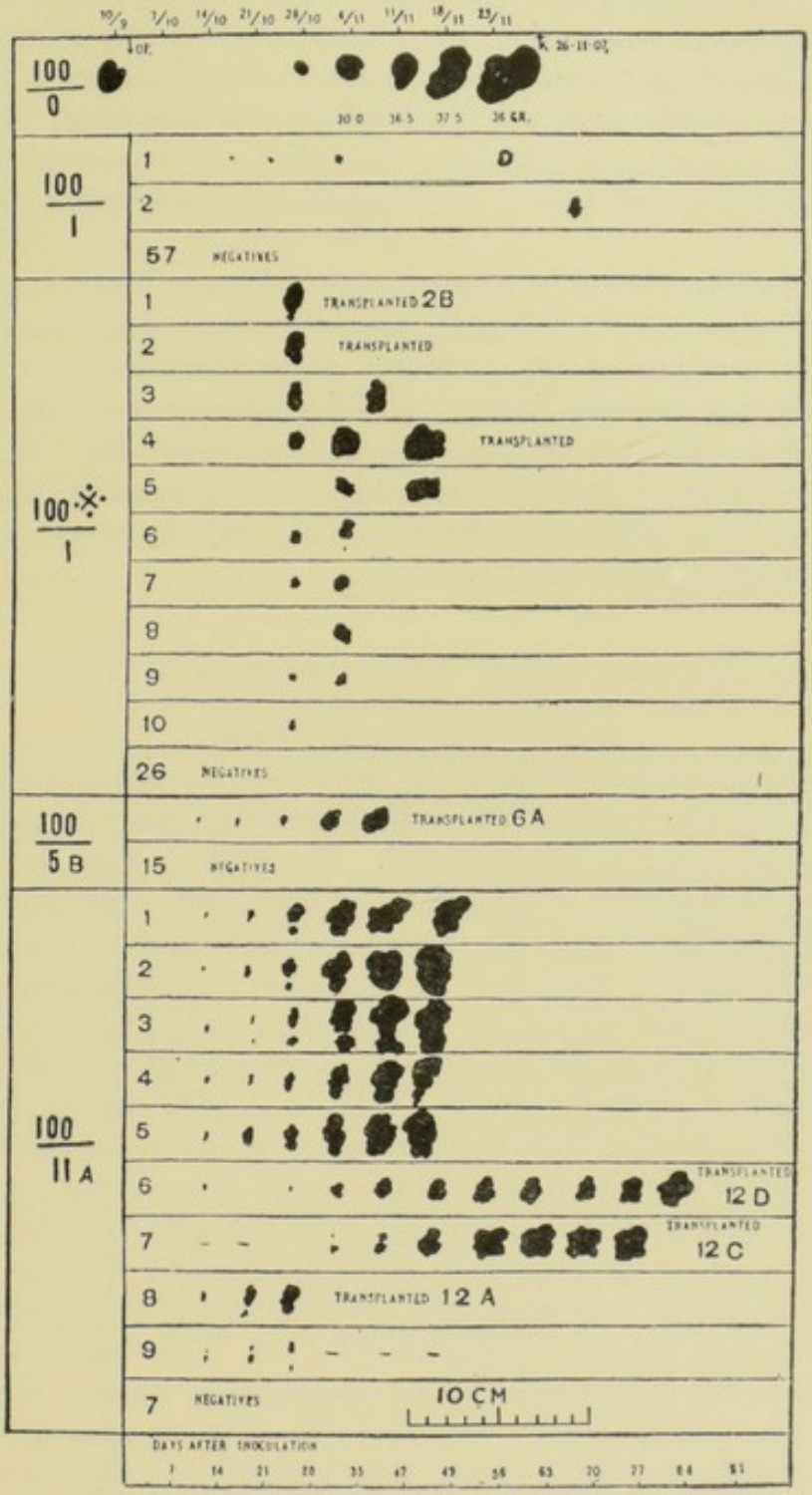


FIG. 40.—Chart showing number of tumours obtained in different generations, and the speed of growth compared with recurrence of primary tumour. $\times \frac{1}{6}$.

same size for 10 weeks, and then began to grow rapidly, reaching a size of 8 × 10 mm. in two weeks, when it was excised and exhibited the structure described above. Transplantation into the mouse itself and into normal animals gave only pure spindle-cell sarcomata, and it has continued to grow as such in later generations. The success of transplantation is low, and there is much spontaneous absorption. Growth is slow.

Tumour-strain 568.—The primary growth showed two components: a central polymorphous and spindle-celled sarcomatous tissue, and a peripheral part having the structure of papillary adeno-carcinoma. Transplantation into the mouse primarily affected and into normal animals gave rise only to pure spindle-cell sarcomata. In normal animals the tumours grow slowly; a high initial percentage of success is followed by spontaneous absorption in the majority of the tumours. Four generations in six months.

CONCLUSIONS FROM THE HISTOLOGICAL DATA.

I. The Minor Alterations.

In drawing conclusions from the behaviour of cancer during propagation, the oft-repeated distinction between spontaneous and transplanted cancer must be borne in mind. The latter is made to grow in a strange environment which, one can infer with great probability, is usually less favourable to the tumour than its natural surroundings in the animal originally nourishing it. A clue to one of the properties of the cancer-cell was elicited by the demonstration, that the tumours accustomed to grow in the mice of one country could be made to grow in the mice of another country. When a Danish tumour was first transferred to English mice, the percentage of successful inoculations fell to a remarkable extent, but in subsequent *passages* the percentage rose until equal to or even above that in Danish mice, according to the care with which it was possible to select the portions of tissue used *. These observations were confirmed and amplified † by the adaptation of other foreign tumours to English mice. A similar adaptation takes place after the transference of a spontaneous tumour to normal mice. Once propagation is established, the cells are adapted to their new environment, which can be maintained practically constant by growing them in large numbers of young normal mice of the same

* First Scientific Report of the Imperial Cancer Research Fund, p. 14, 1904.

† Second Scientific Report of the Imperial Cancer Research Fund, Part ii. pp. 22–24.

breed. When growing in a living animal the tumour-cells are protected from outside influences, but it has been shown that every fresh transplantation effects a disturbance of the cancer-cells. After a period of rapid proliferation analogous to that in tissues subjected to chronic irritation, the cells *tend* to become quiescent and to return to a differentiated condition. This fact is well illustrated by the histological behaviour of strain 91 and others, which present a solid or alveolar arrangement for a varying interval after transplantation, the characteristic differentiation supervening later. Thus the cancer-cells during propagation are thrown repeatedly into a condition as analogous as it is possible to attain under experiment to that reactive proliferation to which their normal ancestors were subjected under the influence of chronic irritation, where it preceded the cancerous change. It is from this standpoint that the characters exhibited by tumour-cells during propagation will be considered, and their relative constancy and variability interpreted.

Polymorphism of Tumour-cells derived from one organ.

When first touching, from the standpoint of experimental and comparative experience, upon the importance of the variations in the histological and biological behaviour of the tumours of one organ, viz. the mamma, both as naturally occurring in spontaneous tumours and also during artificial propagation *, one pointed out that the interrelation of these variations cannot be elucidated by a study of the isolated stages presented in combination by sporadic tumours, but must be studied for themselves, and that this study must be carried out on propagated tumours during long periods, and be controlled by comparing propagated tumours of different primary origin. It was also pointed out that the variations found in one and the same tumour-strain at different times were so great, that pathologists ignorant of the life-history of the tumours would regard them as of different histogenesis †.

The polymorphism of the parenchyma of the spontaneous mammary tumours of the mouse has been described in detail by Apolant ‡,

* Trans. Med. Soc. London, p. 219; Second Scientific Report of the Imperial Cancer Research Fund, part ii. pp. 10, 13–14, 19, 56.

† Interpretation of the Differences and Variations in Transplanted Tumours of one Organ. Second Scientific Report of the Imperial Cancer Research Fund, Part ii. p. 52, 1905.

‡ Apolant, H.: Die epithelialen Geschwülste der Maus. Arbeiten aus dem Königlichen Institut für experimentelle Therapie zu Frankfurt am M., Heft 1, 1906.

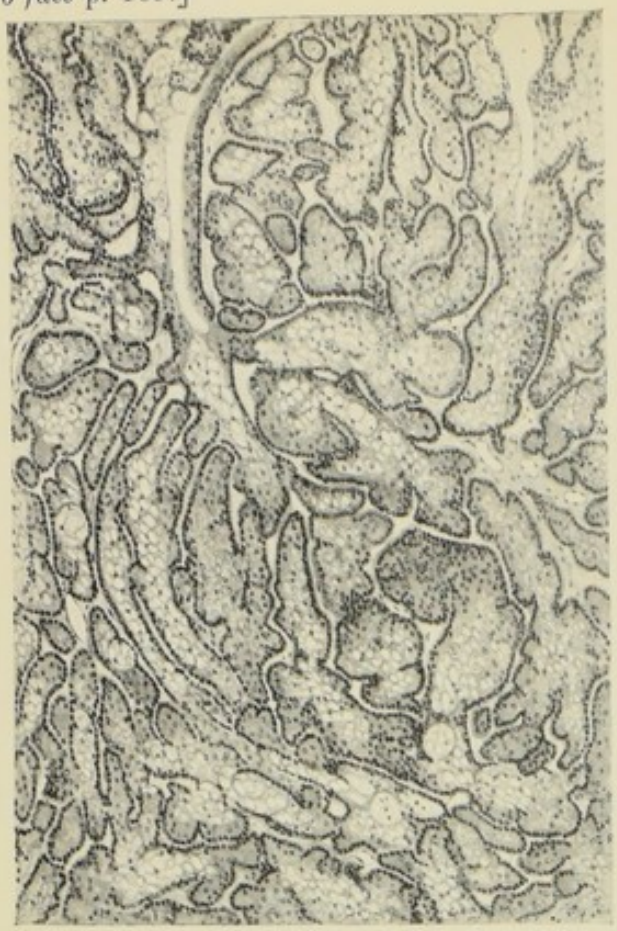
Gierke *, and Murray †, and it is sufficient to repeat that these tumours show all gradations in structure from pure adenomata to pure alveolar, or solid, carcinomata, either combined in one single tumour or separately in tumours presenting one histological picture throughout. The great variety of the parenchymata all derived from the mammary epithelium is significant, and has given rise to lively discussion. The passage of one form into another is evident from the histological examination in some tumours, and led Apolant to the conclusion that all tumours arose primarily as adenomata and, progressively losing their differentiation, passed through an adeno-carcinomatous stage to the condition of pure alveolar carcinoma, in other words, in the majority of cases benign tumours were converted into malignant tumours by this transformation. In some cases he stated that the solid alveolar condition might be primary and arise in one step from the non-cancerous epithelium. Haaland in the first paper in this report has adduced clear evidence for both these modes of origin. He lays special stress on the conditions of multiple nodular hypertrophy and multiple adenoma as transitional or early cancerous stages, both of which are, as he has shown, frequently met with in the mammæ of old female mice not the subject of cancer, as well as those already cancerous. Murray, in the Third Scientific Report, when discussing the transformations met with in fully developed spontaneous tumours, illustrated the transition from the acinous to the alveolar condition and the reverse, where alveolar areas differentiate out into adenomatous structure.

The transformations are met with in transplanted tumours under conditions much more convenient for exact study. In order to test the constancy of the histological characters continuous propagation has been mostly relied upon. The *passage* may have been rapid from one set of mice to another, so that the tumour-cells were kept in a continuous state of disturbance and for prolonged periods never had an opportunity to exhibit differentiation. *Passage* may have been made slowly and opportunity given for recovery from the disturbance of transplantation and for differentiation to take place. Yet another modification has been employed, that of continuous propagation in parallel series by large doses of emulsion and by small intact fragments

* Gierke, E.: The Hæmorrhagic Mammary Tumours of Mice, with Results of Research into Susceptibility and Resistance to Inoculation. Third Scientific Report of the Imperial Cancer Research Fund and Ziegler's Beiträge, 1908.

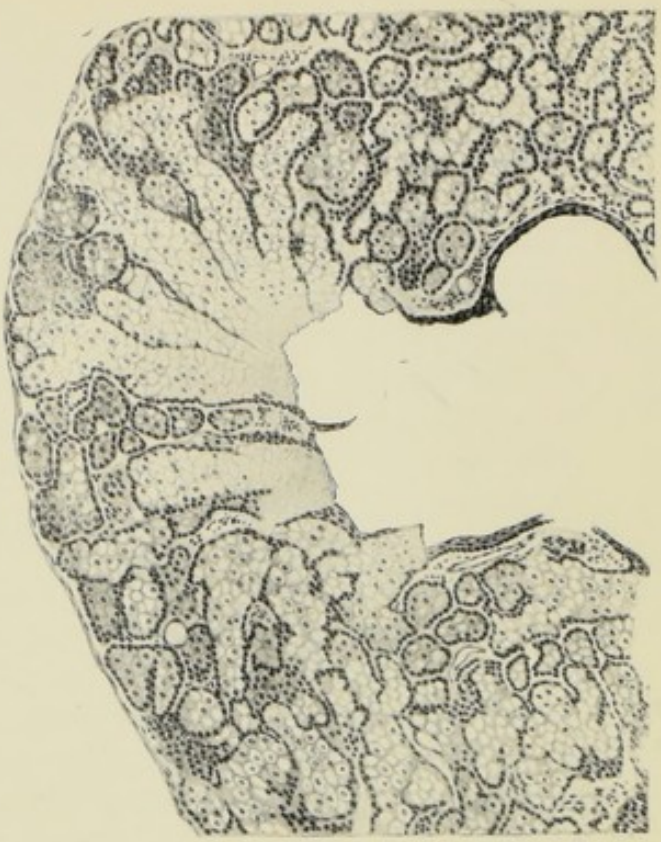
† Murray, J. A.: Spontaneous Cancer in the Mouse; Histology, Metastasis, Transplantability, and the Relations of Malignant New Growths to Spontaneously Affected Animals. Third Scientific Report of the Imperial Cancer Research Fund.

To face p. 169.]



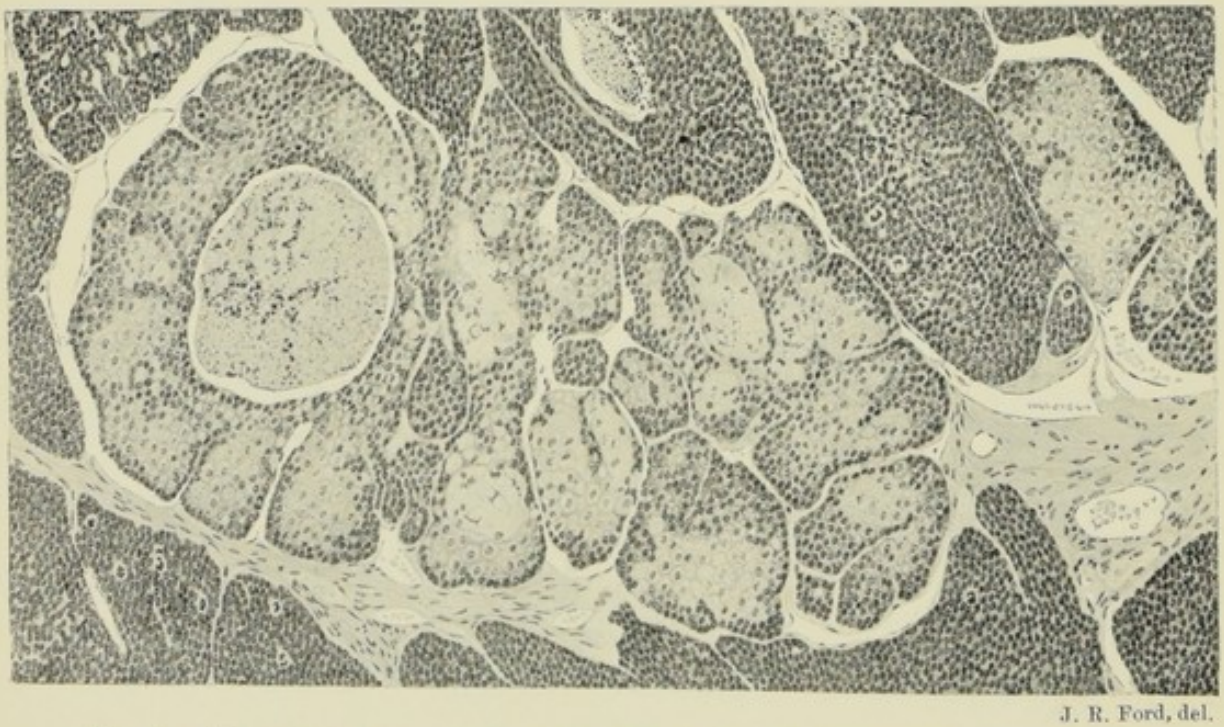
J. R. Ford, del.

FIG. 41.—Mouse 297, male. Adeno-carcinoma of the preputial gland, showing the general histological features of the tumour and its close resemblance to the normal gland (fig. 42), in spite of its definitely malignant character. The propagated tumours retain the same histological picture. $\times \frac{87}{1}$.



J. R. Ford, del.

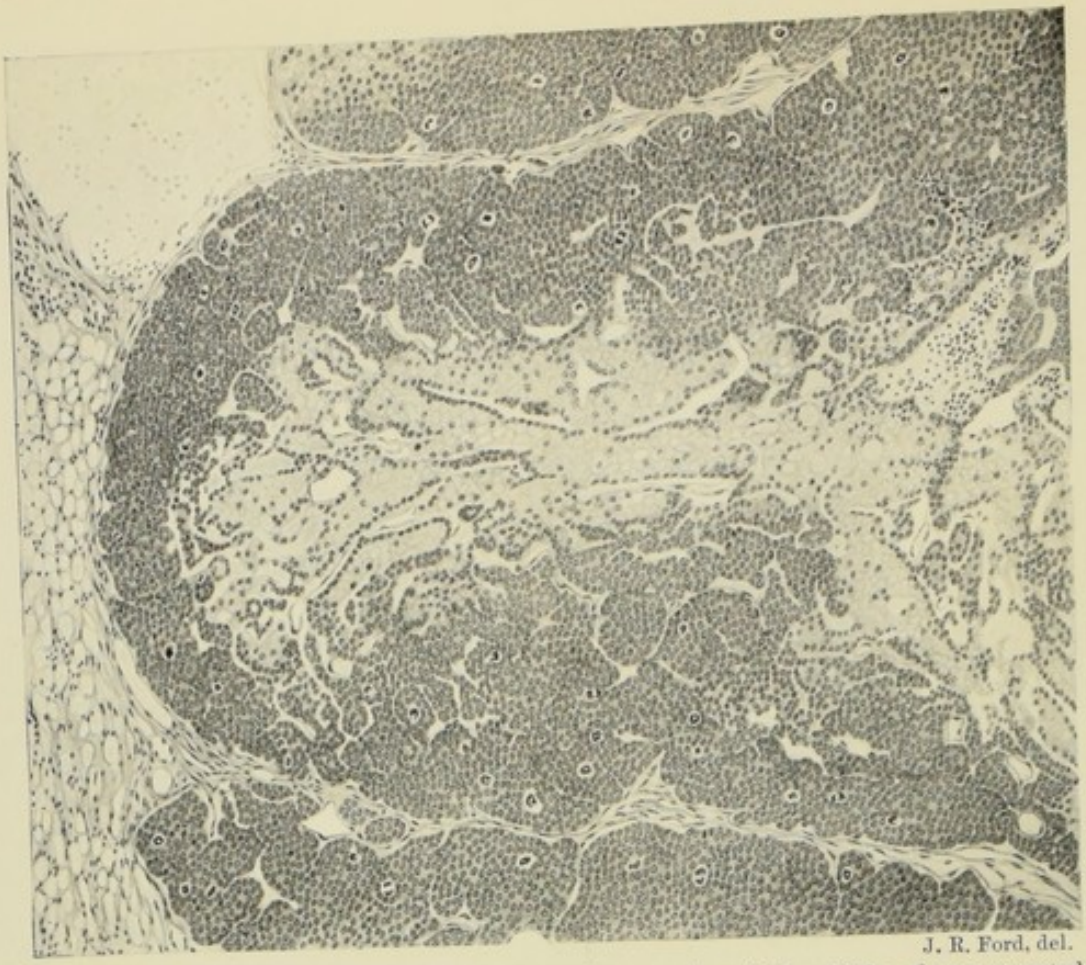
FIG. 42.—Normal preputial gland from adult male mouse to compare with the tumour in fig. 41. The larger ducts of the gland are lined with cells resembling squamous epithelium. $\times \frac{87}{1}$.



J. R. Ford, del.

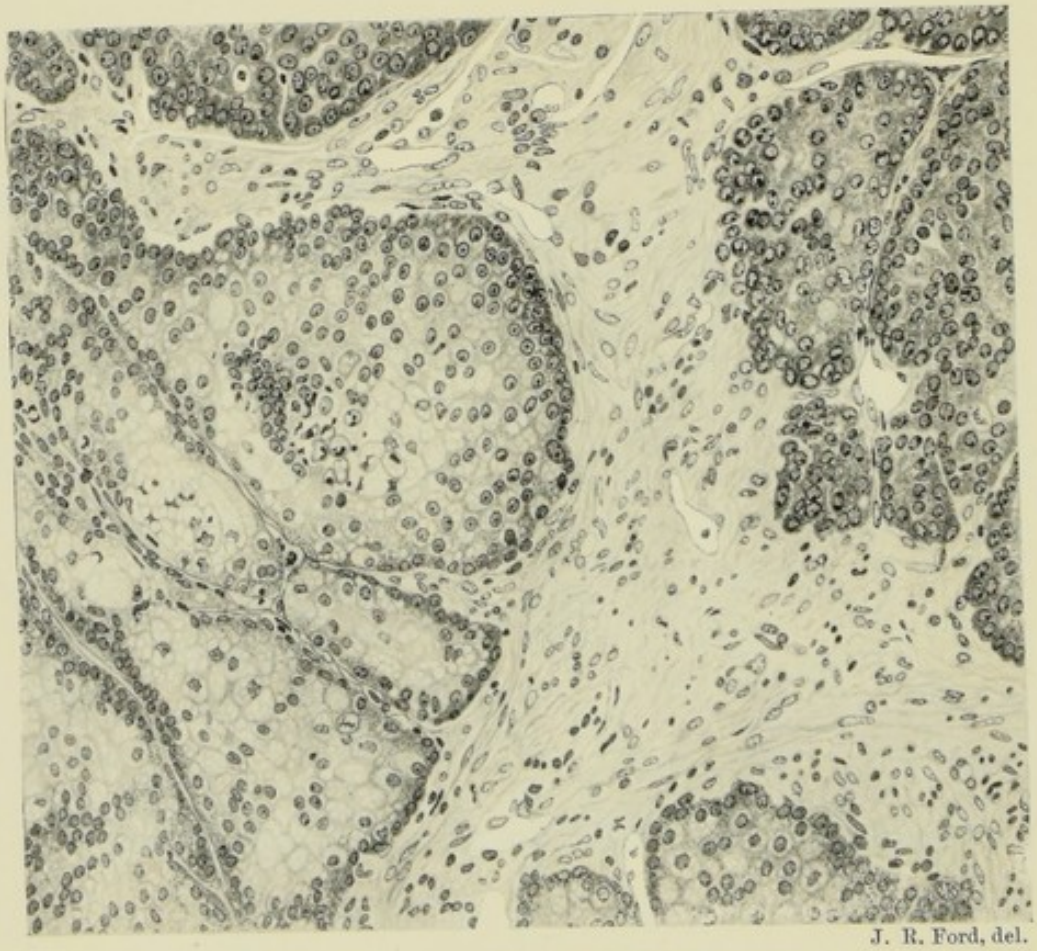
FIG. 43.—Mouse 292. Sebaceous adeno-carcinoma. Spontaneous tumour in left groin showing the same typical differentiation as the cells of a sebaceous gland. *Cf.* with this the preputial tumour (fig. 41). $\times \frac{135}{1}$.

To face p. 169.]



J. R. Ford, del.

FIG. 44.—Tumour 239. Sebaceous adeno-carcinoma. A peripheral lobe of a propagated tumour of the 6th generation to show the typical differentiation into sebaceous cells in the centre of the lobe and the undifferentiated condition of the peripheral alveoli. $\times \frac{83}{1}$.



J. R. Ford, del.

FIG. 45.—Tumour 239. Sebaceous adeno-carcinoma, propagated tumour of the 2nd generation. The same typical differentiation with a high power. $\times \frac{230}{1}$.

respectively. Apart from continuous propagation in a straight line, propagation has been maintained in parallel strains which have taken origin in the spontaneous tumour at different times. The mouse has been kept alive by relieving it of its tumour and transplantation made early in its clinical course and at death, and then either from the recurrent tumour or its metastases. As described by Haaland*, an animal may be inoculated with its own tumour so as to yield a fresh source of material at a later date. Two or more parallel strains have been maintained for a number of other tumours either dating back to the first transplantation or not far removed from it, with the same or varying doses and intervals for the respective strains. Efforts have been made to segregate the component elements of tumours presenting interesting histological variations. Such tumours have been examined microscopically before transplantation by the method described by Haaland† and those portions showing deviations from the normal structure of the tumour have been isolated and transplanted. It is unnecessary to discuss each tumour in detail according to the method employed.

In the discussion on the effects of transplantation, it has already been pointed out that the loss of differentiation in later generations of transplanted tumours may be merely apparent, and due to examination of young rapidly growing tumours, while older tumours of the same series may be well differentiated. Differentiation when present in transplanted tumours, is attained in the vast majority of adeno-carcinomata after an initial period of loss of differentiation more or less prolonged. In these cases loss of differentiation does not indicate any fundamental alteration of the parenchyma, but is merely a passing effect of the interruption of nutrition and reorganisation inseparable from the process of transplantation, and the subsequent revascularisation of the tumour. The extent to which a complete reorganisation takes place after grafting varies in different tumour-strains. In those tumours in which a well-developed stroma is present, the new vessels follow the preformed channels left by the pre-existing vessels which have degenerated. In other tumours with delicate stroma, and in which complete necrosis of the central part of the graft occurs in all except a few layers of superficial cells, the new vessels develop independently of the lines presented by the old and a complete rearrangement takes place. It is particularly

* This Report, p. 58.

† Haaland, M.: Contributions to the Study of the Development of Sarcoma under Experimental Conditions. Third Scientific Report of the Imperial Cancer Research Fund, p. 185.

in such tumours that the transient solid condition precedes differentiation and may indeed endure for considerable periods. This factor of the time necessary for a tumour to differentiate after transplantation has not received consideration from Ehrlich and Apolant in the statements they have made as to loss of differentiation by adenomatous growths. Because the essence of their technique is to push the rapidity of *passage* to the utmost, it is obvious that few tumours would have time to differentiate. Adami * quite correctly summarises Ehrlich and Apolant's observation: "Thus, Ehrlich and Apolant have noted that an adenocarcinoma of the mouse which in the course of numerous transplantations had taken on a more and more atypical and cancerous appearance, in some later passages tended to revert to a less atypical and more adenomatous appearance." In the light of the preceding criticism it will be apparent that their evidence is insufficient to prove a progressive loss of differentiation in this case. The reappearance of differentiation was ascribed by Apolant † to the influence of partial immunity upon the tumour-cells. Gierke's ‡ and Murray's § observations have demonstrated that the influence of partial immunity is the reverse, it hinders differentiation by inducing a tardy stroma reaction, and the reappearance of acini in Ehrlich and Apolant's case may have been due to a comparison of an old tumour which had grown slowly and for a longer time than the control tumours which speedily killed the mice bearing them.

If regard be had to all the transplanted tumours of a strain, and evidence of differentiation in single tumours be held to prove the capabilities residing in all the parenchyma cells, there would remain few tumours in which it would be necessary to assume either the loss of qualities present in the primary growth or the acquisition of new properties. In a certain number of strains, however, the retardation of differentiation is so prolonged that the structural facies of the whole strain differs considerably in later generations from that in the earlier. As instances of this change strain 32, an adeno-cancroid in the primary animal, forms a striking instance. Keratinisation was a marked feature

* Adami, J. G.: The Principles of Pathology, 2nd ed. 1910, p. 709.

† Ueber experimentell erzeugten Rückschlag. Münch. Med. Woch. 1907, no. 35.

‡ Gierke, E.: Die hæmorrhagischen Mäusetumoren. Ziegler's Beiträge, 1908. *Vide* also Third Scientific Report of the Imperial Cancer Research Fund.

§ Murray, J. A.: Die Beziehungen zwischen Geschwulstresistenz und histologischem Bau transplantierter Mäusetumoren. Berl. Klin. Woch. 1909, no. 33.

of the early generations up to the 10th–12th. Thereafter, up to the present (80th generation), all the tumours examined, whatever their ages, have been pure alveolar carcinomata, except for minor small foci of acinous structure discovered at long intervals, and in the case of one very old tumour of the 54th generation, microscopical foci of keratinisation. The capacity for differentiation must be regarded as latent in this tumour, although the majority of growths are completely undifferentiated. Tumour-strain 91, on the other hand, presents the intermediate condition of an adenomatous tumour which passes through a transient alveolar stage after transplantation before differentiation reappears, and leads naturally to a more or less papiliferous structure (fig. 9) which has been retained unaltered for years in the tumour-strains in which the histological structure is constant, *e. g.* strains 27, 72, 155. A curious variant is shown in some pure alveolar tumours such as Jensen's carcinoma. As was shown in the Second Scientific Report, small acini lined by a single layer of columnar epithelium frequently appear in "early stages" examined within the first week after transplantation. They furnish the only indications of differentiation in this tumour and have never been seen in older tumours.

Anaplasia.

It follows from the preceding discussion of the relation of acinous to alveolar structure in mouse mammary carcinomata, that in many instances these histological pictures, apparently so distinct, are merely "growth forms"—transient expressions of the growth-energy and growth-conditions of extremely plastic parenchymata. Great importance was laid by von Hansemann upon the circumstance that, in the human subject, metastases were less differentiated than the primary growth. The reverse has also been shown to occur. These apparent contradictions at once become understandable from the point of view developed here. The least differentiated metastases may often be those of most recent date, but although undifferentiated they need not be so for the reason given by v. Hansemann, viz. that being the latest offshoots of the primary growth, therefore they are more anaplastic. They will often be undifferentiated because they have not had time to differentiate. The doctrine of anaplasia as a progressive irreversible change in cancer-cells loses its objective basis, and must be regarded as a matter of subsidiary importance.

Metaplasia.

Another aspect of the growth of cancer which the examination of spontaneous tumours alone had been incapable of bringing to a definite solution, is that embraced in the phenomena of metaplasia. Here again the tumours of the mouse have furnished a material whose detailed study has supplied data which elucidate the place which must be assigned to metaplasia in the biology of malignant new growths. Metaplasia originally signified any transformation of tissue into another form, and particularly the derivation of the cells of carcinoma from pre-existing connective tissue-cells. To-day it is the term applied to the occurrence of histological differentiation in sites where the conclusion cannot be avoided that the cells presenting the aberrant differentiation must have been derived from cells normally only capable of another differentiation. It is applied, *e. g.*, to the occurrence of keratinisation in columnar and cubical glandular epithelium and also to the occurrence of osseous or osteoid or even cartilaginous structures where normally only collagenous connective tissue is found. These two cases are the best known forms of metaplasia. *Per se* the condition is quite independent of malignant growth. It is known to occur in association with inflammatory processes as well as in isolated instances as a normal post-natal transformation. The transition from the primitive condition of all epithelial structures in the embryo to the adult types, although remotely analogous, is not described as metaplasia, the term being generally reserved for transformations occurring after the fully developed adult differentiations have been established. It is because such aberrant histological transformations, although rare, are mostly observed in connection with malignant new growths that their discussion has become involved with the discussion of cancer. The artificial propagation over considerable periods of time of a number of growths presenting metaplastic differentiations has given an answer to the conundrum hitherto insoluble: of what differentiation are the metaplastic cells capable if they continue to proliferate? The evidence is quite clear that the occurrence of one differentiation does not preclude the later development of another. The parenchyma of these tumours (adeno-cancroids, osteo-chondro-sarcomata, adeno-carcinomata with spindle-shaped epithelium) is throughout capable of either differentiation over long intervals of time and presents one or the other for reasons independent of the conditions of growth. Hence it is unnecessary and inadequate to assume embryonic displacements, heterotopias, to account for heterologous differentiations in

tumours, *e. g.* keratinisation in adeno-carcinomata of the mamma; Ribbert's * assumption of an anomalous developmental leaning towards an aberrant differentiation is equally unnecessary. The intercalation of a long or short period of absence of differentiation is probably erroneously regarded as "loss" of differentiation, and should be interpreted as a period of "latency" of differentiation. No other assumption is compatible with the reappearance of differentiations after the repeated subdivision of a tumour parenchyma which results from continued propagation over long periods. After a few transferences the whole of the cells of a tumour growing in one animal must consist of the descendents of a single cell in a not very remote preceding generation †, and the reappearance of two distinct differentiations in later propagation, in the same form and association as were encountered in the primary tumour, can only accord with the assumption of a homogeneous parenchyma with dual properties of differentiation. These conclusions can be drawn for metaplasia of epithelium on the basis of the large number of adeno-cancroids of the mamma studied from 1904 onwards ‡; examples of adeno-cancroids are found in the summaries of strains 32, 47, 282, 286. Besides occurring in mice, such adeno-cancroids have also been found in the mamma of rats and rabbits. Sebaceous differentiation and keratinisation are shown by strains 239, 297, 292, 466. Sarcomata with diverse differentiations are seen in strains 92, 219, and 513 in which bone, cartilage, and collagenous tissue have appeared at various times. The stroma of many adeno-carcinomata of the breast in the dog exhibits the formation of cartilage and bone. These tumours are not for this reason mixed tumours. In one case where transplantation was effected through three generations only the epithelial cells grew. This seems to demonstrate that the connective tissue of the dog is very liable to such metaplastic changes, just as the connective tissue of the rat or of the mouse is liable to the changes characteristic for these animals.

Relation between Structure and Malignancy (growth).

The majority of the propagable tumour-strains exhibit marked deviations from the normal histological structure of the tissue from which they have arisen, but it is not an invariable rule that these deviations progress further during continued propagation. Therefore complete

* Ribbert, Hugo: Das Wesen der Krankheit, pp. 102–128. Bonn, 1909.

† Bashford, E. F., Murray, J. A., Bowen, W. H.: The Experimental Analysis of the Growth of Cancer. Proc. Roy. Soc. 1906, Series B, vol. 78, p. 196.

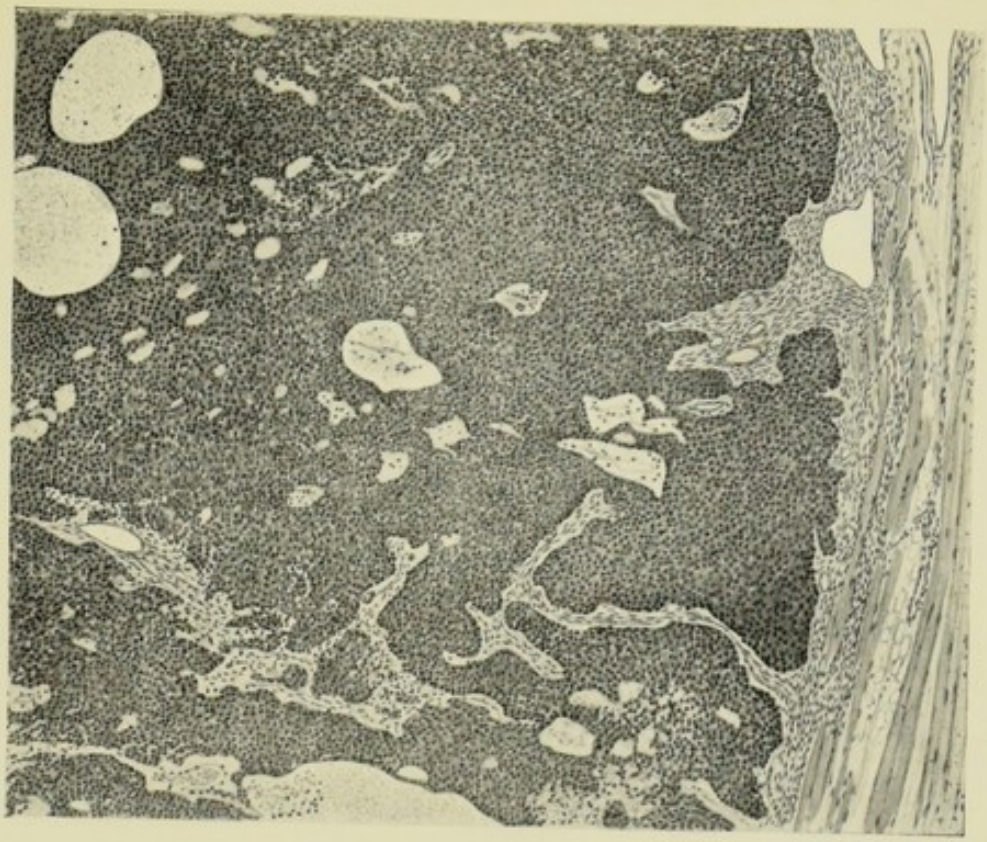
‡ *Cf.* Second Scientific Report of the Imperial Cancer Research Fund, Part ii. p. 20.

loss or latency of differentiation is not necessary to permit of unlimited growth, indeed the purely glandular structure of several adenocarcinomata of the mamma and the structure of several papilliferous cyst-adenomata of the mamma (*cf.* fig. 9, p. 141) afford evidence that the capability for propagation is not necessarily dependent upon, nor accompanied by, marked changes of histological structure. Besides adenomata of the mamma this capability for propagation is exhibited by other tumour-strains exhibiting a high or almost complete degree of differentiation, *e. g.* squamous-cell carcinoma (*cf.* fig. 11, p. 147), and adenomata of the sebaceous and preputial glands which retain their characteristic differentiations and secretory functions, and remind one strongly of certain malignant tumours of the thyroid in man and animals (*cf.* figs. 41, 42, 43, 44, 45). It has thus been demonstrated that tumours, which under the microscope are with difficulty distinguishable from the normal mother-tissue from which they arose, are also capable of unlimited propagation and this fact is of the very highest theoretical importance in showing the independence of biological behaviour (malignancy) and structure.

Thus so far as retention of normal histological structure was a criterion of innocence it loses in significance, and at the present time only the biological properties of some tumour tissues, viz. their power of continued growth—capacity for unlimited propagation—distinguishes them from the corresponding normal tissues. Notwithstanding the tens of thousands of transplantations of normal tissues made in this laboratory, a tumour capable of progressive growth in any one animal or of propagation in series of animals has not been obtained in a single case, so that for the present there remains this sharp gulf between tumour and normal tissue.

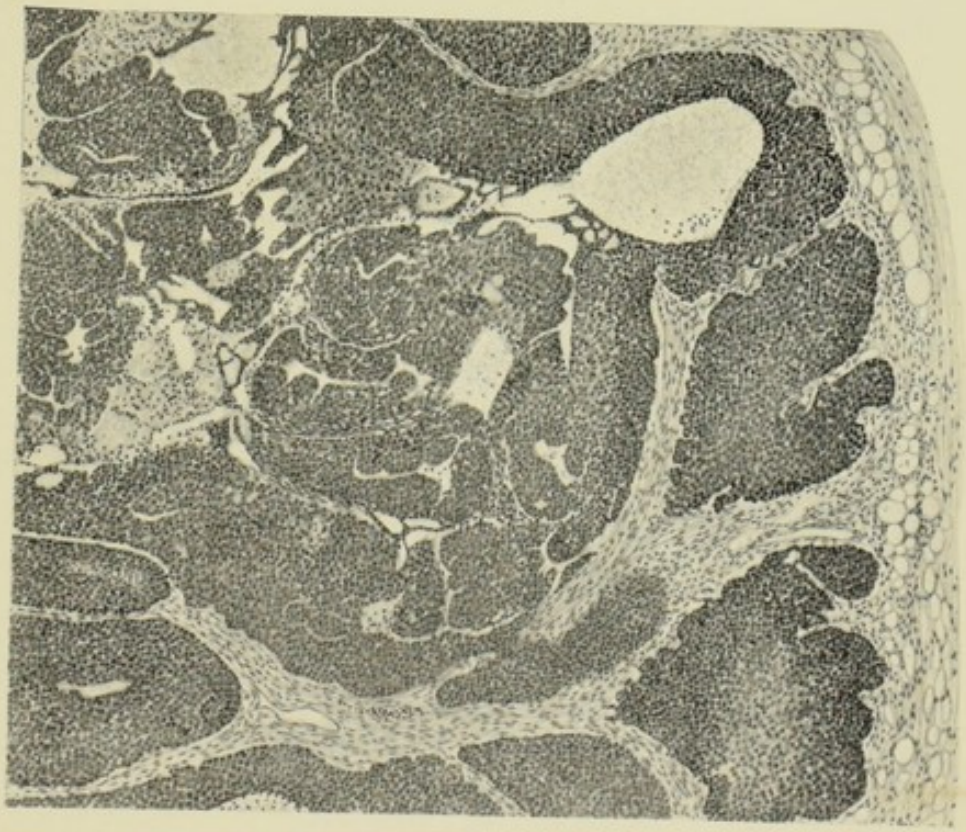
The independence of structure and biological behaviour is, however, illustrated in another manner. As a rule the undifferentiated tumours grow more rapidly than the highly differentiated, but the differentiated may exhibit as high a proportion of "takes" as the undifferentiated. Not all undifferentiated tumours yield a high proportion of "takes" or rapidly growing tumours, as illustrated, for example, by the contrast between strains 206 and 117 (*cf.* figs. 46, 47). Tumours with marked tendency to acinus formation may give a high proportion of "takes," with a higher proportion of rapidly and progressively growing tumours than quite undifferentiated ones. In tumours so highly differentiated as 27, 72, 173, spontaneous absorption is rare while it is the rule for 206, which is quite undifferentiated.

There is this technical point to be borne in mind when estimating the



J. R. Ford, del.

FIG. 46. 206/16 D—17 D. Ten days old tumour of 16th generation. Rapidly growing solid tumour, *cf.* fig. 47. $\times \frac{56}{1}$.



J. R. Ford, del.

FIG. 47. 117/16 A—17 A. Fifty-five days old tumour of 16th generation. Slowly growing solid tumour for comparison with tumour 206, fig. 46, which has the same structure but grows rapidly. $\times \frac{56}{1}$.

comparative frequency at the outset and after a prolonged period of propagation of differentiated and undifferentiated tumours, namely, that the technique and procedure are such as select the rapidly growing and therefore less differentiated daughter tumours, those showing the opposite behaviour being usually neglected except in strains where it is the characteristic feature. Limitations are set to the selection of the slowest growing tumours by at least two factors which may obtain separately or in combination. First, the amount of tissue produced must ultimately be sufficient to inoculate as large a number of animals as is compatible with maintaining the propagation of a strain "taking" only in a low proportion. If the tumours grow only temporarily, the greatest care may not suffice to obtain the necessary amount of material for transplantation before concomitant immunisation has damaged the tumour-cells. Both these factors obtain in enhanced degree in the attempt to obtain progressive growth from normal tissues. It might appear that the difficulty was theoretical only and could be overcome by inoculating larger numbers of animals so as to obtain a greater amount of material ; this is not the case, however, for as contrasted with tumour-tissue which yields usually more, normal tissue yields less each time transplantation is repeated. Examples of tumours occur, however, such as 466, which give for four or five generations less and less material at each *passage*, and are only saved from extermination by the accumulating adaptation to the new environment.

The whole question of the nomenclature of new growths is raised by the conclusions drawn from experiment. It is made obvious that a histogenetic classification although rational, does not always yield a reliable indication of the relative malignancy of new growths. A substitute for it is, however, not suggested ; nevertheless, one must point out that since our histological classification of tissues is purely artificial, still more so is the histogenetic classification of malignant new growths since it is made without any reference to their only common property, viz. continued power of growth, and also conveys no information as to their relative rates of growth, or degrees of malignancy.

Thus a clinical conundrum has been cleared up. The surgeon and the pathologist are familiar with the contradictory behaviour of malignant new growths which are reasonably supposed to be similar on histological grounds; how they present variations in malignancy, and how, even with uniform care in operative procedure, uniform results are rarely obtained. Propagation has revealed that biological behaviour is neither always indicated by, nor parallel with histological structure, and is often

independent of it. Histological structure was almost the only, at any rate often the most trusted criterion from which clinical behaviour—diagnosis and prognosis—was inferred; it still will remain so, but now there will necessarily be more reluctance than in the past to describe a growth as benign merely because it differs little from the normal in structure. Since experiment has bridged over the gap between the most undifferentiated malignant new growths and normal tissue by a continuous series of growths with increasing differentiation, it is demonstrated that malignant growth is not necessarily associated with loss of differentiation, and retention of what is practically complete histological differentiation is compatible with malignancy, and no sure indication of benignancy.

II. The Permanent Alterations.

Whereas the changes described in the preceding pages follow each other rapidly, and as in the case of the transition from alveolar structure to adenomatous, generally complete the cycle in one animal, the variations now to be discussed are characterised by their apparent inde-

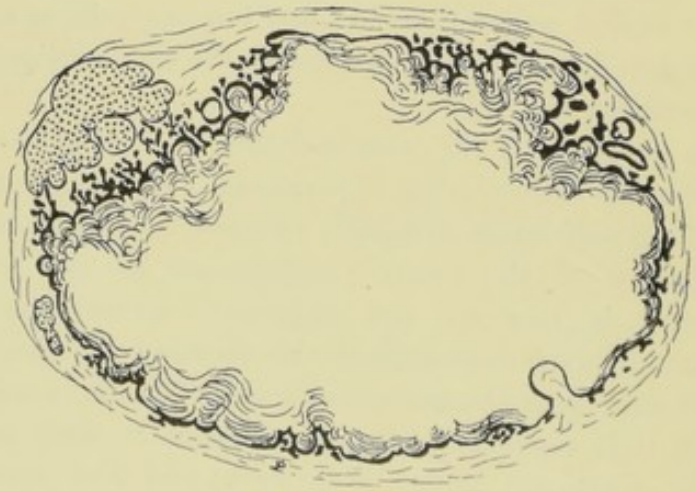
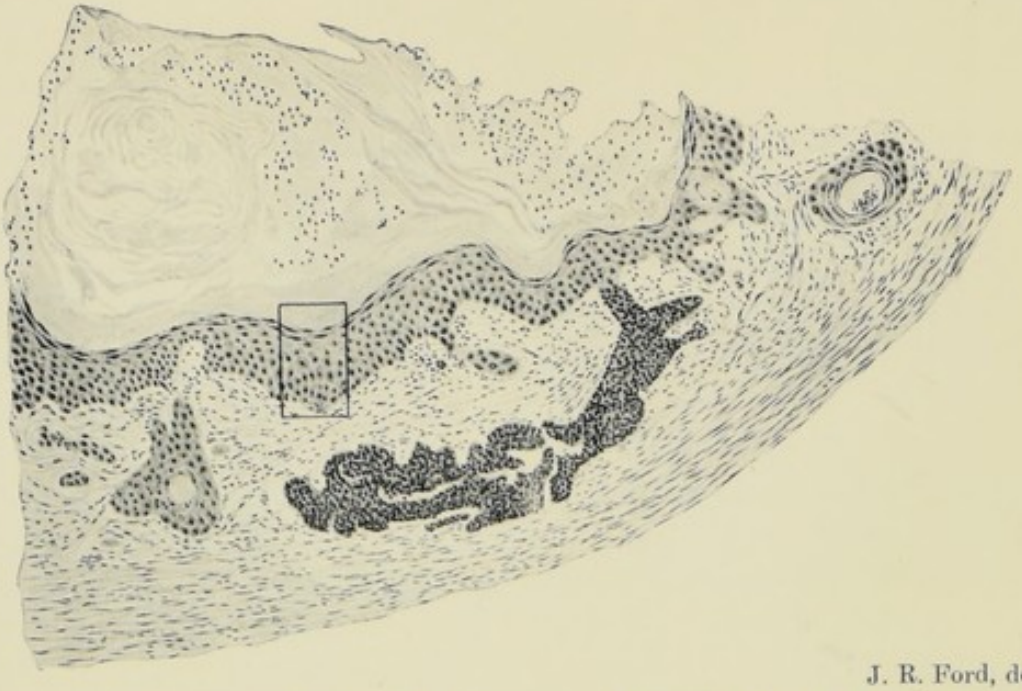


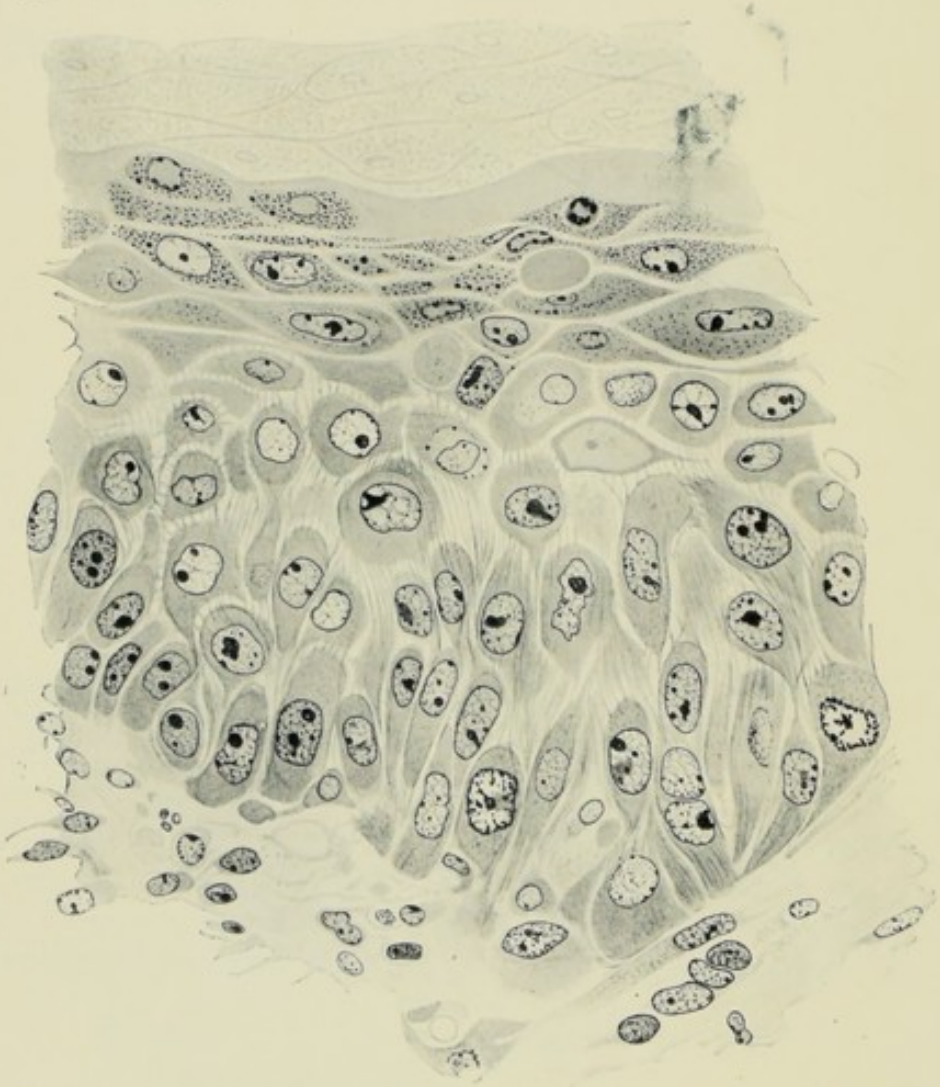
Fig. 48. 32/8 G–9 C.—Schematic figure of whole tumour of 8th generation, consisting of an epithelial cyst filled with keratin scales. A small alveolar area at the left side. $\times \frac{10}{1}$. *Cf.* fig. 11.

pendence of tranference from one animal to another. A number of hæmorrhagic spontaneous tumours have lost this character after a longer or shorter course of propagation. The original disparity between the fibroblastic and vascular components of their stroma becomes less,



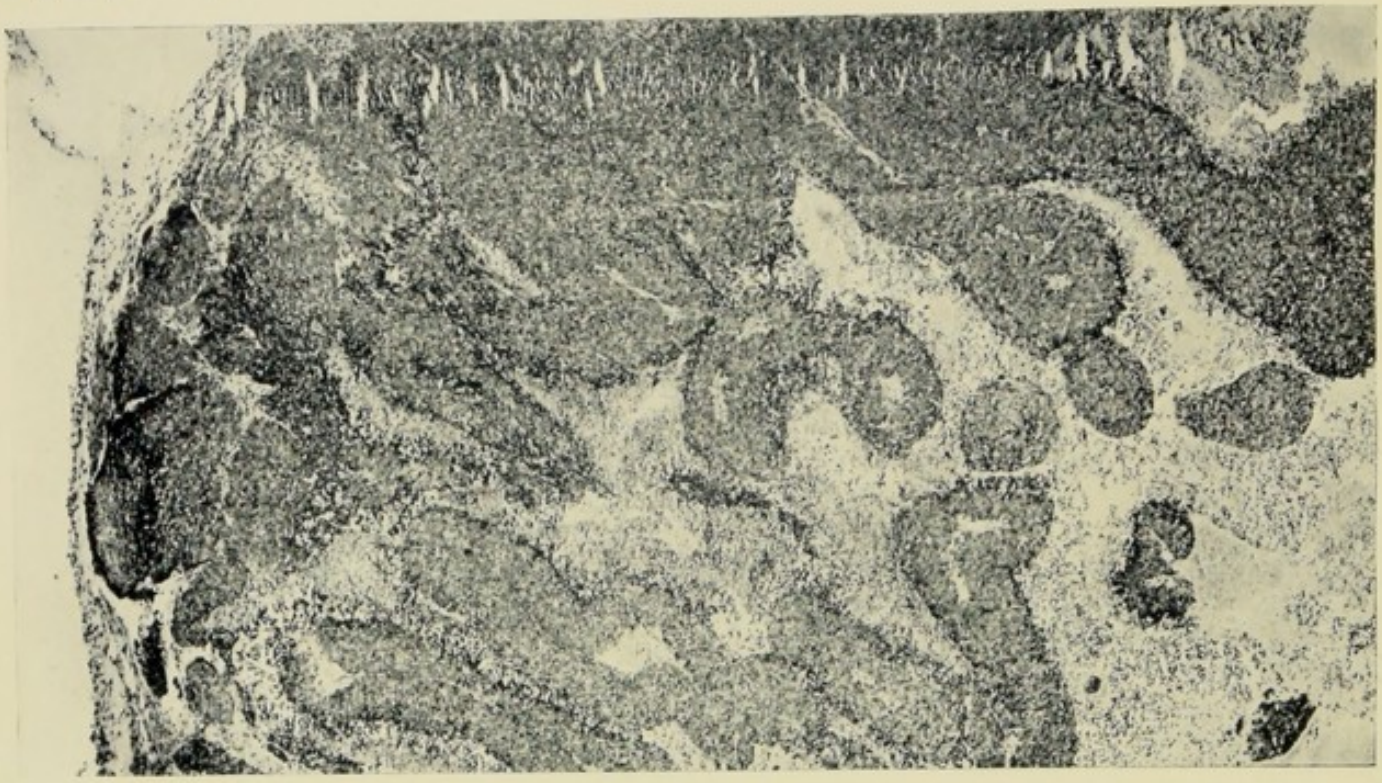
J. R. Ford, del.

FIG. 49. 32/8 G—9 C. Tumour of 8th generation, left side of fig. 48 at median magnification. Perfect reproduction of structure of stratified squamous epithelium in the wall of the epithelial cyst, *cf.* fig. 50. $\times \frac{60}{1}$.



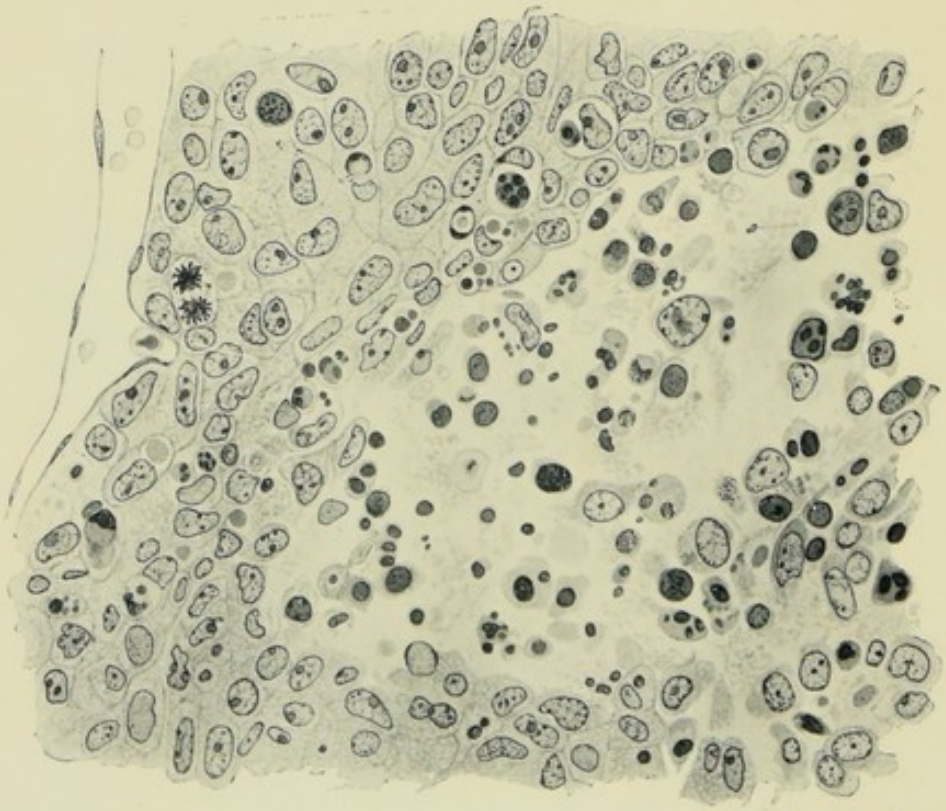
J. R. Ford, del.

FIG. 50. 32/8 G—9 C. High-power view of area marked in fig. 49. Malpighian layer, prickle cells, kerato-hyalin granules and keratinisation as in normal skin. $\times \frac{750}{1}$.



Microphoto, W. Imboden

FIG. 51. 32/20 C—21 B. Alveolar tumour of 20th generation, showing appearance of medullary carcinoma and extensive necrosis; surface of tumour to left side. $\times \frac{50}{1}$.



J. R. Ford, del.

FIG. 52. 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. $\times \frac{500}{1}$.

the connective tissue becomes more abundant, and the vessels being better supported do not dilate into wide tortuous sinuses. When, as is usually the case, this alteration of the stroma proceeds *pari passu* with changes in the parenchyma such that the alveolar condition does not give place to an acinous formation, and the central parts of the alveoli become necrotic, the tumours are no longer recognisable as related to the primary growth. Tumour-strains 50, 93, &c. (p. 152), are examples of this change. Loss of the characteristic differentiation has led to a similar apparently permanent change in certain carcinomata showing keratinisation. Tumour-strains 282, 292, 322, 349, 356, come under this description and strain 32

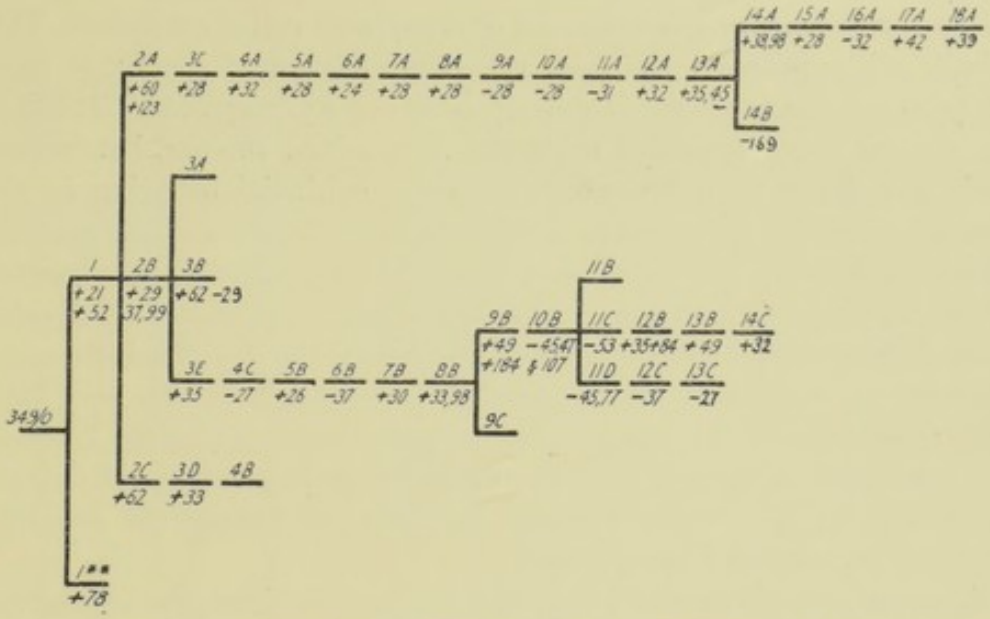


Fig. 53.—Diagram showing the distribution of keratinisation (+) in propagated tumours in various generations (numbers followed by letters above the short horizontal lines, *e. g.* 2 A, 8 B, 14 C) and at various ages (numbers below horizontal lines preceded by + or − indicate the age in days of the tumours examined). The tendency to keratinisation is becoming less pronounced, and in later generations is manifested by the presence of minute areas of prickle cells which are reckoned as +.

now shows so little tendency to differentiate that it also may be included here (*cf.* figs. 48, 49, 50, 51, 52). But, even in their case, caution must be exercised in drawing the conclusion that a permanent loss of differentiation has occurred. After a complete absence for many generations widespread keratinisation may reappear, many tumours of corresponding age being without it, both in previous generations and at the time of its reappearance. The accompanying genealogical tree (fig. 53) of strain 349

indicates the diminution in keratinisation when the minutest traces of its occurrence are reckoned as positive.

Still more striking evidence of the variability of tumour-cells under propagation is afforded by strains in which duplicate lines of propagation present differences extending over long periods. Thus in tumour-strain 37, in addition to a number of adeno-carcinomatous sub-strains differing only slightly from each other and from the primary growth, a sub-strain (fig. 39) has been propagated separately for more than two years, and during that time has presented the appearance of interlacing bundles of spindle-cells like a sarcoma throughout *. The tumours are carcinomata in all other respects, and very old tumours show abundant acinus-formation and disappearance of the spindle-celled condition. This spindle-cell appearance has been a constant feature of strain 129 from the primary tumour onwards during three years propagation. It led to the tumour being regarded originally as a mixed tumour, but further study has shown that the spindle-cells are epithelial behaving in the manner described, but capable of acinous differentiation. This spindle-celled condition is met with as a temporary modification in tumour-strain 91 (*vide* fig. 13) in which small areas of modified alveolar parenchyma consist of closely packed interlacing bundles of spindle-cells. Attempts to separate a strain growing permanently in this way have failed with tumour 91, the daughter tumours reproducing the alveolar and adenomatous structure of the ordinary propagated growths. Its occurrence as a more permanent variation of tumour 37 acquires therefore an enhanced importance.

A second tumour in which two sub-strains with divergent characters have been propagated, is the osteo-chondro-sarcoma 92. After the fourth generation cartilage and osteoid tissue disappeared from the transplanted tumours, and the growths were soft consisting of closely packed spindle-cells with very little interstitial collagenous tissue and large necrotic areas. These appearances are maintained up to the present in the daughter tumours. A second sub-strain originating from an old sclerotic tumour of the 8th generation differs from the parent or main sub-strain in several particulars. The tumours of the second sub-strain are firm, strongly collagenous, and show little or no necrosis, even when of large size (fig. 34). The rate of growth is much the same in both sub-strains, the initial proliferation is somewhat more rapid in the first, or soft, sub-strain, but spontaneous absorption sets in sooner, and is complete earlier than in the second firmer collagenous sub-strain

* Haaland, M.: Contributions to the Study of the Development of Sarcoma under Experimental Conditions. Third Scientific Report, p. 230, fig. 75.

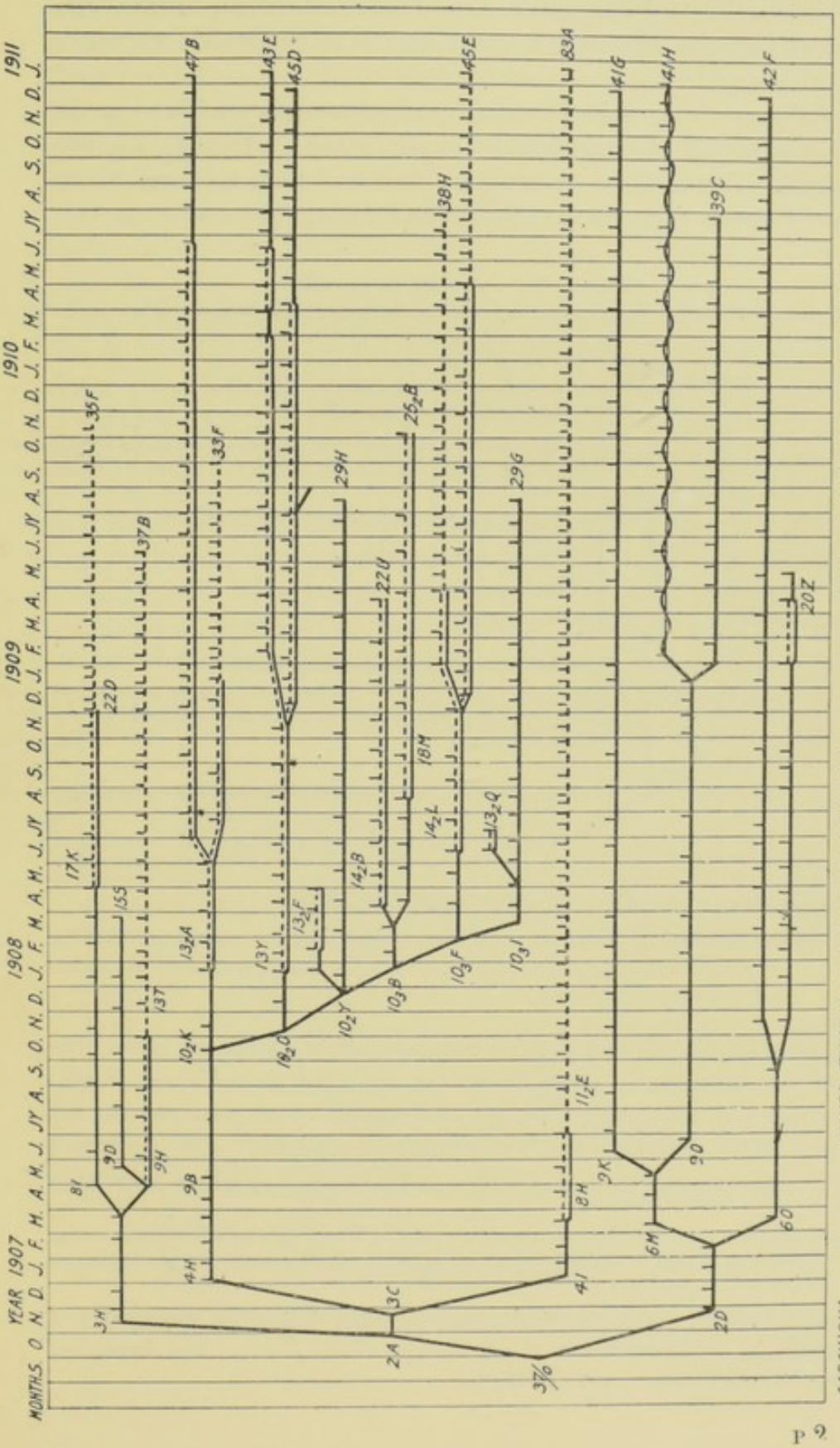


Fig. 54.—General chart of propagation of tumour 37 showing pure carcinomatous, mixed, sarcomatous and spindle-cell carcinomatous strains of this tumour.

(figs. 35, 36). These differences have now been maintained for more than two years and the histological distinction is as marked in old as in young tumours.

In the case of the spindle-celled carcinoma-strain of 37 as in the fibrous strain of 92, the modification seems to have taken place within one animal. The modified parenchyma seems to have appeared in one step, and thereafter has remained unaltered during propagation.

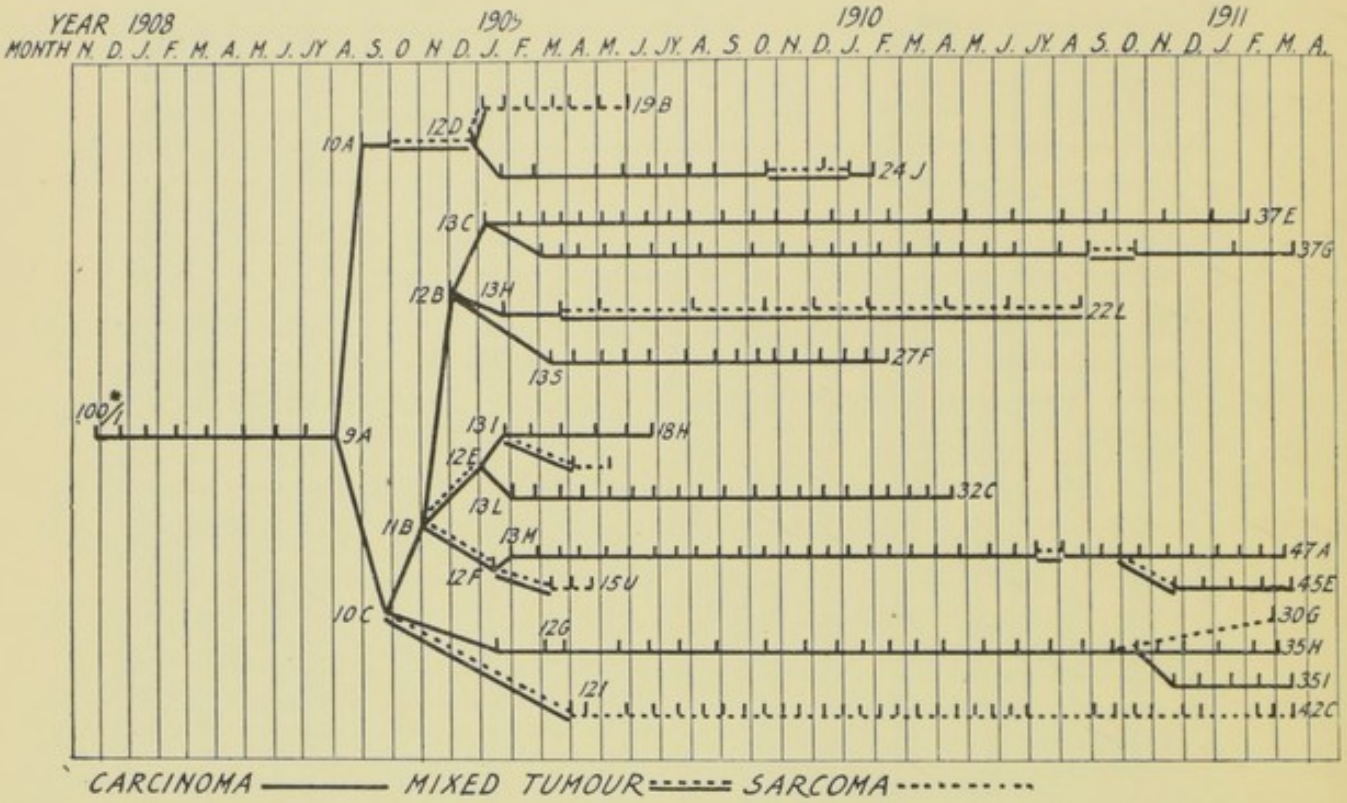


FIG. 55.—General chart of propagation of tumour 100 showing pure carcinomatous, mixed, and carcinomatous strains. Note the short duration of the mixed tumour stage in this case.

The appearance of sarcoma in the connective-tissue of some propagated carcinomata is probably to be regarded as evidence of still more striking specific properties of cancer-cells. The phenomenon has been very fully described for two tumour-strains, 37 * and 100 †, and from the standpoint of cell variability it is interesting to call to mind that

* Haaland, M.: Contributions to the Study of the Development of Sarcoma under Experimental Conditions. Third Scientific Report of the Imperial Cancer Research Fund.

† Russell, B. R. G.: Sarcoma Development occurring during the Propagation of a Hæmorrhagic Adeno-carcinoma of the Mamma of the Mouse. The Journal of Pathology and Bacteriology, vol. xiv. (1910).

not only spindle-cell, but also polymorphous-cell sarcoma has appeared under the influence of the same parenchyma. The carcinomata which induce sarcomatous transformation of their stroma are of rare occurrence. The change is of the nature of a peculiar stroma reaction induced by the parenchyma, and the power to induce it is to be regarded as an attribute of the parenchyma, in connection with which it occurs. In the case of strain 37 the accompanying chart (fig. 54) shows how irregularly the phenomenon supervenes in the various lines propagated, and that the power to induce sarcoma development, if not now lost, is latent in all the parallel strains derived from this tumour. On the other hand, strain 100, of which a corresponding chart (fig. 55) is also given, still maintains its power to induce sarcoma development after sufficiently prolonged growth in any mouse. It is important to note that tumours 37 and 100 are still being propagated as pure carcinomata, because all other investigators who have observed sarcoma development have failed to maintain the carcinomatous component in propagation after the appearance of sarcoma. The cases described by Ehrlich and Apolant, Loeb, Lewin, Liepman, and more recently, Clunet, have been entirely replaced by sarcoma *. The same fate has overtaken two primarily mixed tumours in the material of this laboratory. These gave pure sarcoma on first transplantation and even on auto-inoculation, and it is probable that the carcinomatous components in these two cases were

* Ehrlich, P. & Apolant, H.: Beobachtungen über maligne Mäusetumoren. Berliner klin. Wochenschrift, 1905, no. 28.

——, ——, & Haaland, M.: Experimentelle Beiträge zur Geschwulstlehre. *Ibidem*, 1906, no. 2.

Lewin, C.: Experimentelle Beiträge zur Morphologie und Biologie bösartiger Geschwülste bei Ratten und Mäusen. Zeitschrift f. Krebsforschung, Band vi. Heft 2, 1908.

Liepmann: Münchener med. Wochenschrift, 1907, no. 27.

Loeb, L.: 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. University of Pennsylvania Medical Bulletin, July 1906.

——: Ueber Sarkomentwicklung bei einem drüsenartigen Mäusetumor. Berl. klin. Wochenschrift, 1906, no. 24.

——: Ueber Entwicklung eines Sarkoms nach Transplantation eines Carcinoms. Deutche 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.

Clunet, J.: Recherches expérimentales sur les Tumeurs malignes. Paris, 1910, p. 53.

characterised by too low an energy of growth to compete with the rapidly proliferating sarcoma cells in the primarily affected, and in normal animals. Further investigation of fresh cases must show whether the power to induce sarcoma development is a transitory attribute of certain carcinomatous parenchymata or whether it is a passing phase in some and a permanent property in others.

CONCLUSIONS FROM THE BIOLOGICAL DATA.

Historical: on Growth of Cancer.

The amount of growth under artificial propagation raises questions of the greatest theoretical importance, and so also does the duration of growth. While Virchow made the memorable advance in knowledge embodied in the derivation of all cells, including those of malignant new growths from pre-existing cells, he did not go quite far enough in regard to the histogenesis of cancer. It became customary to speak of the *limitless* proliferation of malignant new growths only after Virchow's conception that they (especially carcinoma) were derived from connective-tissue cells, had been displaced by Thiersch *, Waldeyer †, and others who adduced evidence that carcinoma was derived directly from pre-existing epithelium, and that a recurrence after operation signified its imperfect removal. A last remnant of the influence of the old humoral pathology had led Virchow to postulate an erroneous histogenesis; it likewise led him to miss the significance of the mere growth of cancer, for he supposed that metastases could arise by metamorphosis of the tissue proper to organs in which they occurred, and not because of the continued growth of strange cells after being transported to new sites. Waldeyer's purely morphological studies led him to revolutionary biological conceptions, still embodied to-day in the general employment of the phrase "the limitless growth" of cancer. But this phrase could only remain a mere figure of speech so long as the transference of cancer from one individual to another was unaccomplished, and its continuous propagation a thing undreamt of. Waldeyer's conceptions were embodied in such phrases as the following:—"Von jedem ‡ einmal gebildeten Krebszapfen kann aber wieder eine neue

* Thiersch: "Die epithelialkrebs, namentlich der Haut." Leipzig, 1865.

† Waldeyer, W.: "Die Entwickelung der Carcinome," Virchow's Archiv. Bd. xli. p. 470, 1867, and *ibid.* Bd. lv. pp. 67–159, 1872.

——: "Uber den Krebs," Volkmann's Sammlung Klinischer Vorträge, no. 33, p. 177, 1872.

Wucherung ausgehen, so dass *jeder frisch enstandene Krebsheerd später aus sich selber zu wachsen im Stande ist*, ein Fundamentalsatz für die Lehre vom Carcinom. Die Wichtigkeit dieses Verhaltens leuchtet alsbald ein, wenn wir bedenken, wie einmal dadurch dass schnelle Wachstum vieler Krebse, dass ja in *unmessbaren Progressionen* weiter geht erklärt wird, zweitens aber auch sich ergiebt, dass auf dieser Weise *jeder Krebsheerd und sei er auch, noch so klein, eine gewisse Selbständigkeit seinem Mutterboden gegenüber gewinnt*, und dann wie eine Art Parasit im Körper weiter wächst. Die Selbständigkeit der Entwickelung von einem gegebenen Anfang aus, geht erstaunlich weit." Elsewhere *, Waldeyer uses the phrases—" Wir haben beim Carcinom eine ganz schrankenlose unregelmässige Epithelbildung vor uns," and " schrankenloses Wachstum des Krebses." Nevertheless, the context makes it always perfectly clear that Waldeyer contemplated the growth of cancer only from the standpoint of its behaviour towards the other tissues of the individual. In England, so long ago as 1865, Charles H. Moore †, and later also his colleague Campbell de Morgan ‡, upheld, on the basis of observations made at the Middlesex Hospital, the view that cancer arises in a circumscribed area, and that growth or recurrence after operation results only from the cancer-cells confined within that area. The most skilful defender of this view has been Ribbert, who showed that the area within which malignant new growths arise is in the majority of cases minute and strictly circumscribed, and contended that even the minutest of such areas grow from their own resources only. An important modification was thereby made in Waldeyer's conception of the progressive transformation of an entire organ either through its general liability §, or in consequence of increase by appositional growth. Nevertheless, Ribbert and all other investigators still had their horizon sharply limited by the restriction set definitely and irremovably to the duration and the amount of the growth of cancer by the death of the individual. Although for the past 35 years pathologists have spoken of the limitless growth of cancer, their statements had reference to a growth *limited* as regards duration, and

* Ueber den Krebs, Volkmann's Sammlung Klinischer Vorträge, no. 33, p. 185, 1872.

† Moore, Charles H.: The Antecedents of Cancer. British Medical Journal, August 12 and August 26, 1865, pp. 164 & 201, also p. 473.

——: On the Influence of Inadequate Operations on the Theory of Cancer. Medico-Chirurgical Trans. London, 2nd series, vol. 32, 1867, p. 245.

‡ Morgan, Campbell de: Discussion on Cancer. Trans. Path. Soc. of London, vol. xxv. 1874, pp. 287 & 387.

§ *Vide* pages xvi and 132 of this Report.

inseparately bound up with the life of the individual whose death terminated it. This limitation remained till recent years, and the reality of its influence is reflected in all the hypotheses which were advanced to explain it *. A comparison between Virchow's standpoint and what we know to-day of the unlimited propagation of malignant new growths with retention of their characters, is, in a way, a measure of the advance in knowledge during the past seventy years. The experimental transplantation and the continuous propagation of cancer first fully demonstrated both that all the lesions of the disease followed upon the growth of cells originally confined to the small particle of tissue inoculated, and also that the amount and the duration of growth are not conditioned in the same way as the growth of organisms and their individual organs. The independence of the cancer-cell of its parent organism described by Waldeyer, was also first fully demonstrated by the results of transplanting and propagating it in other individuals.

The continuity of growth maintained by implanting carcinoma from mouse to mouse, apart from its bearing upon cancer, raises questions which go to the root of several fundamental problems of vertebrate biology, yet this phenomenon remains entirely neglected by biologists and has received scant or only speculative consideration from those engaged in the study of malignant new growths. The continuity of growth of cancer, as revealed by continued propagation, is its most important and only constant attribute, and an explanation of it would reach the kernel of the problem.

Of the earlier investigators, Morau † regarded his inoculations in mice as equivalent to infection, as did also Velich ‡ in the case of a rat sarcoma, while Hanau § gave but cursory attention to the manner and significance of transference and propagated his rat tumour for three generations only. Leo Loeb ‖ in considering the alternative explanations of infection or transplantation as responsible for transferring a sarcoma from one rat to another, stated that transplantation would imply immortality of the tumour analagous to what is commonly

* *Vide* Hypotheses explanatory of the Nature and Origin of Cancer. Second Scientific Report of the Imperial Cancer Research Fund, Part ii. p. 69.

† Morau: Recherches expérimentales sur la transmissibilité de certains néoplasmes. Archives de Médicine expérimentale, 1894, p. 677.

‡ Velich: Wiener Med. Blätter, 1898, Bd. xxi. pp. 711 & 729.

§ Hanau: Erfolgreiche experimentelle Uebertragung von Carcinom. Fortschritte der Medizin, vol. 7, 1889.

‖ Loeb, L.: On Transplantation of Tumours. Journal of Medical Research, vol. vi. pp. 36 & 37, 1901.

assumed in the conception of the continuity of the germ-cells. Owing to the difficulty of obtaining unequivocal results with sarcoma, especially in the case of the rat, Loeb did not demonstrate satisfactorily the occurrence of transplantation to the exclusion of infection. Even when discussing the tranference of his tumour from the standpoint of transplantation, Loeb ambiguously refers to "a complicated process," "we probably transplant connective-tissue cells plus the tumour-producing factor which ultimately is a chemical, physical, or physico-chemical agency, whether the intermediary agency be a micro-organism or not *." Within twenty months—a small fraction of the duration of the life of the rat—Loeb's tumour-strain ceased to be capable of propagation, owing to increasing secondary infection, and thus gave no evidence of the total amount or total duration of growth. Jensen †, more fortunate both in his material and in the animal, having satisfied himself that his tumour was transferred from mouse to mouse by actual transplantation of living cells, refrained from discussing its wider significance, and devoted himself to what at the time were the more pressing problems, although he maintained his tumour in propagation. Borrel ‡ being concerned with the etiology of cancer in mice, and holding that transplantation had nothing to do with it, did not maintain his original tumours in propagation, although, at a later date, he followed the details of the prolonged propagation of his Strain "B."

Deductions based upon actual observations of the amount and duration of growth were first made after Jensen's tumour was added in 1903 to other carcinomata of the mouse, discovered in this laboratory. At the time when Jensen's conclusions as to the nature of transference were confirmed and extended to other tumours of this laboratory, his original strain had already been growing for what was then thought to be the normal duration of life of a mouse, viz. 2–3 years; but no opinion could be safely vouchsafed how much longer propagation could be continued,

* Loeb, L.: Investigations in the Transplantation of Tumours. Journal of Medical Research, vol. viii. p. 60, 1902.

† Jensen, C. O.: Experimentelle Undersögelser over Kräft hos mus. Copenhagen, 1903.

——: Experimentelle Untersuchungen über Krebs bei Mäusen. Centralblatt für Bakteriologie, Bd. xxxiv. 1903.

——: Biolog. Selskabs Forhandlinger, 1901–02, pp. 6 & 20.

——: Nogle Forsög med kräftsvulster. Hospitalstidende, no. 19, 1902.

‡ Borrel, A.: Epithélioses infectieuses et epithéliomas. Annales de l'Institut Pasteur, t. xi. 1903.

——: Le Problème du cancer. Bulletin de l'Institut Pasteur, t. iv. nos. 12–15, 1907.

and even the duration of the life of a mouse was not accurately known. Nevertheless, the early facts permitted of some tentative conclusions, bearing upon the nature, the amount, and duration of growth *. The masses of tissue produced were shown to be out of all proportion to the limitations set to the size of the mouse. This phenomenon was stated to be unparalled in the mammalia or vertebrates and comparable to the continuity of species. These conclusions were extended and amplified later †, contrasts being drawn with the phenomena of growth in the higher animals. In addition to the amount of growth, attention was also directed to its duration exceeding the normal limits of life in the mouse, and it was pointed out that if the propagated tumours were suddenly to cease growing our conceptions would be as suddenly modified, for, although the enormous amount of growth already produced would still require explanation, its natural termination would at once bring it into line with other forms of growth in the higher animals. There was, however, at that time, no evidence of the cessation of growth, and no means of determining whether it might not still be continuing fifty or hundreds of years hence. The enormous power of growth was recognised as responsible for all the clinical and pathological features of cancer as embraced under "malignancy." Its unlimited nature, it was stated, had been revealed by propagation and indicated that an explanation of the amount of growth sufficing to kill the individual, might be an inadequate expression of the powers of cell-division residing in malignant tissue. Much emphasis was laid upon the opportunities the propagation of cancer afforded for a study of the mere phenomena of growth, and of the variations it exhibited biologically and morphologically. In the past the opportunities of observation had not extended beyond the stage at which a tumour had arrived, but propagation gave opportunity for following up the behaviour of a tumour-strain for prolonged intervals and observing whether it exhibited phases of importance in explaining its nature.

The only other authors who, from personal experience, have concerned themselves with the extraordinary biological phenomena of

* Bashford, E. F., and Murray, J. A.: The Significance of the Zoological Distribution, the Nature of the Mitoses, and the Transmissability of Cancer. Roy. Soc. Proc. Jan. 1904, vol. 73.

Bashford, E. F.: The Growth of Cancer. March 13th, 1905. Trans. Medical Soc. of London, vol. xxviii. 1905.

† Bashford, E. F., Murray J. A., Cramer, W.: The Growth of Cancer under Natural and Experimental Conditions. Second Scientific Report of the Imperial Cancer Research Fund, Part ii. 1905.

the mere amount and the duration of the growth of cancer under propagation, are Ehrlich and Apolant *, who, subsequent to the appearance of the Second Scientific Report, adopted a physical instead of a biological analogy. They agreed with the foregoing valuation of the importance of propagation, and also pointed out that it would be wrong to regard it merely as a means of obtaining material. It will be necessary to refer at greater length, to other of their statements and deductions, because of the hypotheses they have formulated both of the origin, nature, and growth of cancer, and of the consequences of propagation by the method they employed.

Ehrlich's Atreptic Theory of the Growth of Cancer †.

It is necessary to consider a hypothesis formulated by Ehrlich to explain cancer on the basis of its experimental study in mice. This hypothesis attempts an explanation, in the first place, of certain of Ehrlich's experimental results, viz. (1) of the contrast obtaining between the difficulty of transplanting a primary tumour to normal mice, and the ease with which propagation may be maintained subsequently ; (2) of the failure to reproduce the lesions of dissemination (metastases) ‡ ; (3) of the failure to re-inoculate mice once they bore large rapidly growing tumours ; and (4) of Ehrlich's demonstration of the temporary growth of mouse-tumours in rats. In the second place the

* Ehrlich, P., & Apolant, H.: Beobachtungen über maligne Mäusetumoren. Berl. klin. Wochenschrift, July 1905.

† Ehrlich, P.: Experimentelle Karzinomstudien an Mäusen. Zeitschrift für ärztliche Fortbildung iii. Jahrgang, no. 7, April 1906.

—— : Experimentelle Karzinomstudien an Mäusen. Arbeiten aus dem Kgl. Institut f. exp. Therapie, Heft 1, pp. 77–102, 1906.

—— : Experimentelle Karzinomstudien an Mäusetumoren. Zeitschrift für Krebsforschung, Bd. v. Heft 1 & 2, pp. 59–80. Abhandlung der Internationalen Konferenz für Krebsforschung, Heidelberg, Sept. 25-27, 1906.

—— & Apolant, H.: Ueber die Genese des Carcinoms. Verhandl. deutschen Path. Gesellsch. Zwölfte Tagung, Kiel, 1908.

‡ The development of metastases varies from one tumour-strain to another in a manner not always parallel with the rapidity of growth, and sometimes dependent on the technique of inoculation and the duration of growth of the primary tumour and sometimes not. Although the whole question of dissemination is still the object of study, it may be pointed out that of the tumours depicted on the chart of relative rates of growth (*vide* p. 199) naked eye mestastases are more frequent in strains with powers of growth above the average (*i.e.* above the level "49" on the chart), than below it, notwithstanding that the more rapid is growth the earlier the mice die, and therefore the opportunity for metastases to attain a large size will be relatively less frequent than in strains of slower growth.

hypothesis was held to be applicable to man as explaining the increasing frequency of cancer as age advances. The points raised by (2), (3), and (4) will not be discussed, since they have been already considered in a number of papers in the Third Scientific Report, and in later publications *.

In referring to the application to cancer of Ehrlich's side-chain theory, it is hoped that the non-employment of the elaborate vocabulary in which this theory is clothed will not be misunderstood. Hitherto its employment has not been found necessary in discussing the experimental investigation of cancer which affords conditions where, if the premisses were valid, the assumption of a differential of avidities would make it incumbent to employ the language in which the side-chain theory is couched.

The side-chain theory as applied to cancer, if stripped of its special technical terminology, amounts simply to assuming that the cells of some malignant new growths take up food-stuffs more rapidly and more energetically than do normal tissues. By virtue of this property such tumours can be transplanted to fresh hosts ; once established and growing in these, such tumours can prevent a secondary inoculation or metastases from growing by virtue of the rate with which food-stuffs are withdrawn. A subsidiary assumption is made that this starvation of a natural or artificial metastasis may be effected by deprivation of special food-stuffs. Great importance is attached to the assumption that it is possible to increase artificially the avidity of the tumour-cells for food-stuffs ; in other words, to increase the rapidity of cell-division.

Following the practice observed in the Second Scientific Report, where all the more important theories of cancer were diagrammatically depicted, Ehrlichs's hypothesis, formulated since then, may also be reduced to a diagram without suggesting that he would approve it as correctly reproducing all the details of his elaborate argument.

A number of lines of equal length may represent, as in the

* Bashford, E. F., Murray, J. A., & Haaland, M. Ergebnisse der Experimentellen Krebsforschung. Zeitschrift für Immunitätsforschung, Bd. i. Heft 4, 1909.
—— : Address on Cancer in Man and Animals. Séances Générales. XVI International Medical Congress, Budapest, 1909. Compte-Rendu, volume général, pp. 186–227. *Vide* also Berl. klin. Woch. 1909, Nos. 36 & 37, and 'The Lancet,' Sept. 4, 1909.
—— : The Etiology and Pathogeny of Malignant New Growths, especially of Cancer. Ninth International Veterinary Congress at the Hague, Sept. 1909.
—— & Russell, B. R. G. : Further Evidence of the Homogeneity of the Resistance to the Implantation of Malignant New Growths. Roy. Soc. Proc. 1910, Series B, vol. 82.

accompanying diagram, the normal avidity of the body. Departures from the normal may be depicted by other lines exceeding or falling short of one passing through the tops of those representing the normal. Thus in the accompanying diagrams (*a*) and (*a*^{1}) will represent the normal and (*b*) will represent the simplest departure from it, namely,

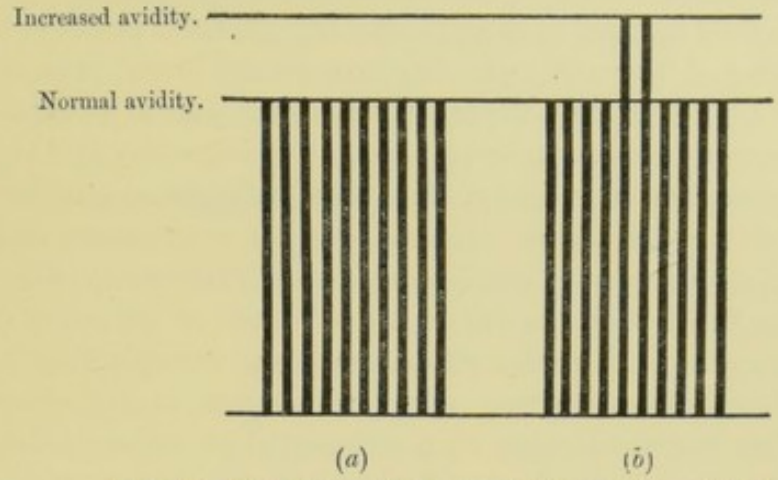


Fig. 56.—Diagrammatic representation of Albrecht's hypothesis.

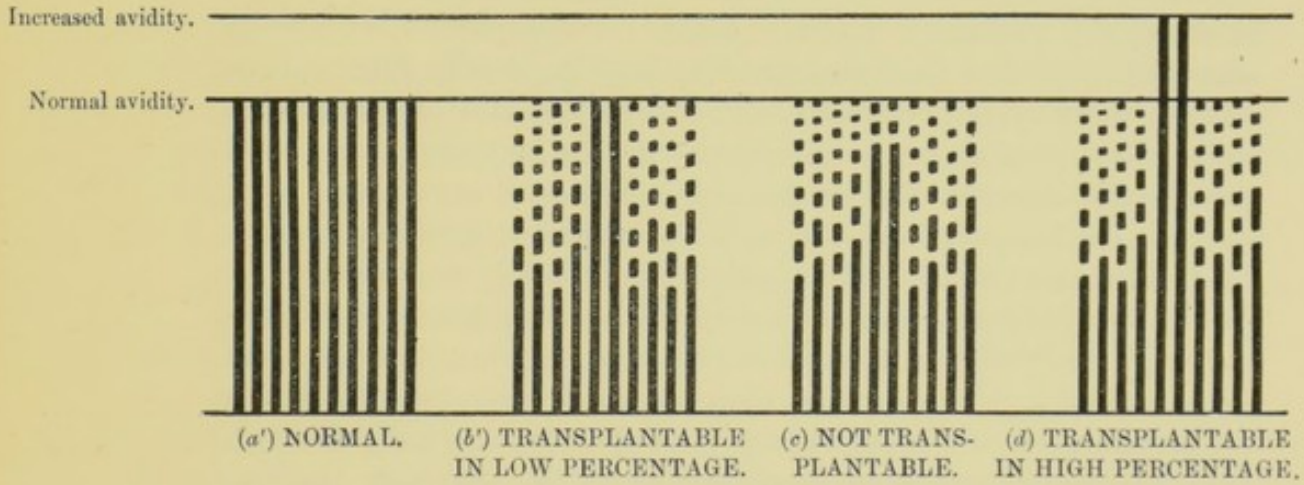


Fig. 57.—Diagrammatic representation of Ehrlich's hypothesis as deduced by him from his transplantation experiments on mice.

an increased avidity; but this assumption requires no further consideration, since, if it were true, then on the basis of Ehrlich's hypothesis every tumour should be easily transplanted into normal animals. This is not the case, and Ehrlich dismisses this assumption, which was put forward by that distinguished pathologist, the late Professor E. Albrecht. Instead of assuming retention of normal avidity by the body, and increased avidity by the tumour-cells, Ehrlich assumes that as a rule the body-cells lose in avidity with increasing age (*b*^{1}), (*c*),

(*d*), while the tumour-cells retain the normal (b^1) or do not lose it in a degree equal to that lost by the body (*c*). In some cases it is assumed that Albrecht's view (*b*) may hold, or that while the body-cells lose in avidity, the tumour-cells may exceed the normal (*d*), and it is further assumed that this exaggerated avidity, when not present at the first transference to normal animals from the animal in which the growth arose, can be artificially induced by a particular experimental procedure, viz. artificial selection of the rapidly growing tumours and forced *passage*.

This hypothesis assigns a very important etiological part to a constitutional change ensuing with advance in years. The ingenuity and the simplicity of the conception are striking. However, sufficient account has not been taken of the differences existing between spontaneous and propagated cancer, as consistently emphasized from this laboratory, since the hypothesis was based upon the unfavourable results of removing a spontaneous tumour from its native environment and transplanting it into normal animals, without giving due consideration to any other possible factor than the assumption of a differential of cell-avidities. At the time when the hypothesis was formulated, the investigations conducted in this laboratory had already fully demonstrated that the hæmorrhagic mammary tumours could be transplanted with ease, whereas Ehrlich had concluded that they were practically untransplantable and therefore of low avidity. The transplantation of spontaneous tumours had been shown to give better results in young than in old animals, and, therefore the peculiar age-incidence of cancer was bound up with the inception and not with the continued growth of cancer. The fact that old animals did not yield a soil uniformly more suitable for growth than did young animals, led as a matter of course to an investigation of the quality of the soil which mice suffering naturally from cancer offered for the growth of their own and other tumours, and to the investigation of the most important question of individuality*, which has been discussed on previous pages of this report by Haaland (*vide* pp. 55–58 and 79–85). This investigation having yielded results bearing upon Ehrlich's hypothesis, references to it have already been made by Murray † and Haaland ‡, but a brief allusion to the subject will

* Bashford, E. F., Murray, J. A., & Cramer, W.: The Natural and Induced Resistance of Mice to the Growth of Cancer. Roy. Soc. Proc. B, vol. 79. p. 184.

† Murray, J. A.: Spontaneous Cancer in the Mouse; Histology, Metastasis, Transplantability, and the Relations of Malignant New Growths to Spontaneously Affected Animals. Third Scientific Report of the Imperial Cancer Research Fund, pp. 69.

‡ Haaland, M.: Behaviour of Tumours on Transplantation. This Report, *vide* p. 62.

not be out of place here. A glance at the diagram given on p. 189 will show how it is possible to contrive experimental tests of the hypothesis, by ascertaining how tumours behave in animals of normal avidity, and in animals in which departures from it are assumed to have occurred because they have developed cancer. The following table, embodying

	Mice with Spontaneous Cancer.			Normal Mice.
	A	B		
Spontaneous Tumour A	+	−		− 0/35
„ „ B	−	+		+ 3/35
	C	D	E	
„ „ C	+	−		− } 0/1000
„ „ D	−	+		− }
„ „ E	−	−	+	+ 4/26

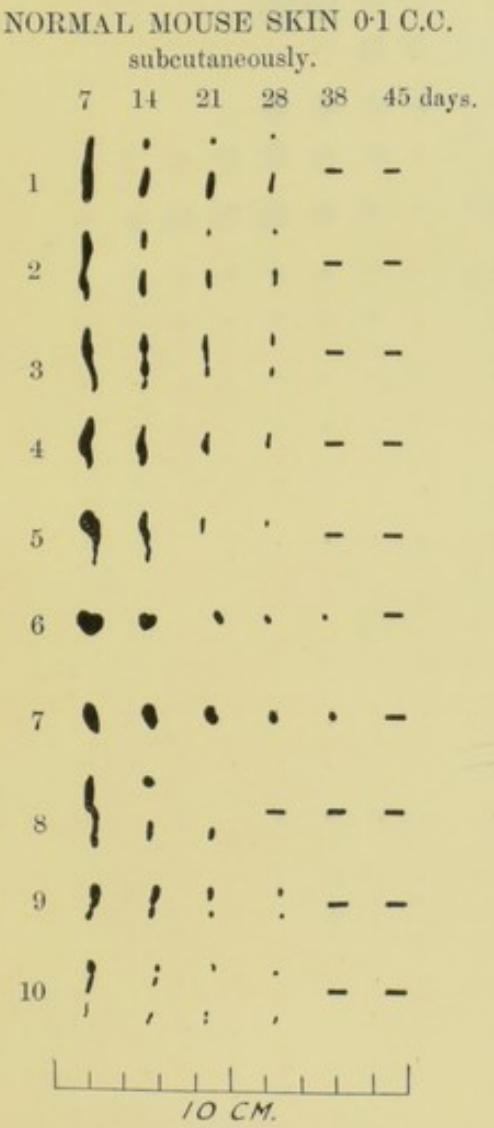
some of Haaland's experiments with five mice and their spontaneous tumours, summarises the kind of results obtained (*vide* p. 62–63). In the first experiment, tumour A, when tested in normal mice, does not grow, therefore the avidity of mouse A is below normal. Tumour B grows in normal mice, therefore it must have a greater avidity than tumour A, and a still greater avidity than the body-cells of mouse A ; but tumour B will not grow in mouse A. It follows that the hypothesis is inadequate to explain the failure of transplantation in this case. In the second experiment, two mice, C and D, have been picked out whose tumours would not grow when tested on a thousand animals of normal avidity ; it follows that tumours C and D were growing in mice C and D in spite of an avidity very much below the normal, and also that mice C and D had an avidity even lower in the scale. Nevertheless, a tumour E, which had so high an avidity that it grew easily in mice of normal avidity, was not able to grow in mice C and D, of which the avidity has been proved to be so low. This is a *reductio ad absurdum*. It follows from these, and from many similar or slightly different experiments, that Ehrlich's atreptic hypothesis is inadequate to explain the growth of cancer either when transplanted into a fresh host, or when growing in the animal in which it arose, and it may be inferred

is equally inadequate to explain the cause and nature of cancer. Without denying that cancer-cells may have *inter se* a different avidity for food-stuffs, the explanation of the development, nature, and prevention of cancer is not to be sought along the lines indicated by Ehrlich's atreptic hypothesis. Other deductions of great importance were drawn by Ehrlich from his experimental studies. In particular he was led to conclude that it was possible to augment the avidity of tumour-cells progressively to a maximum by forcing the rapidity of *passage* to the utmost. This view, and also Ehrlich's views on the duration and amount of growth, will be considered below in the course of the following description of the investigations made here.

The Amount and Duration of Growth.

As stated above, 85 different tumour-strains have been propagated; of these 35 have been propagated for over three years, *i. e.* for longer than a mouse lives. From time to time the propagation of tumour-strains has been stopped intentionally, when they were obviously duplicating the features of other strains, or for other reasons have ceased to be worth further expenditure of energy and money. Just as a thorough mastery of the technique demonstrated that practically all mammary tumours of the mouse could be transplanted, so also it has shown that, once so much is achieved, every tumour can be maintained in continuous propagation, if the technique be carefully adapted to the biological behaviour of each tumour. The histological features of the more interesting of the tumour-strains have been described in detail, and as the summary given on preceding pages illustrates, they are of very varied and widely divergent histology. Whilst the majority exhibit marked deviations from the normal histological structure of the tissues from which they have arisen, others do not do so. It must be borne in mind that the preponderance of undifferentiated tumour-strains corresponds to a similar preponderance in the total number of the primary growths from which propagation has been started, and therefore does not indicate an advance to uniformity of structure. Of highly differentiated tumours propagation has been continued for a prolonged time of squamous-cell carcinoma, of several adenomata of the mamma, and of sebaceous and preputial glands, and there is no reason to assume that their propagation cannot be continued so long as may be wished. As indicated on pages lix and 174, a highly important conclusion is thus permitted; unlimited propagation is

a property inherent in tumours even though they be with difficulty distinguishable from the tissue in which they arose. Thus has been bridged over the extreme contrast between the structure and biological behaviour of the least differentiated new growths and normal tissue. The behaviour



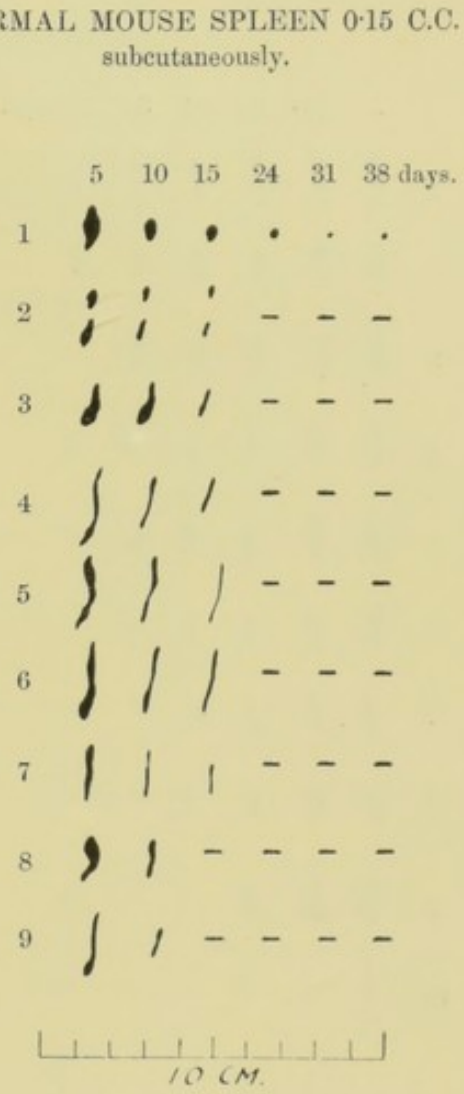


Fig. 58.—Chart showing temporary growth of normal mouse skin in normal mice.
Fig. 59.—Chart showing temporary growth of normal mouse spleen in normal mice.

of normal tissue on transplantation has been referred to already (p. 174) and it will suffice to give here a few figures (58, 59, 60, 61, 61 *a*) illustrating a similarity between the growth of normal tissue and tumour tissue whether highly differentiated or quite undifferentiated. It will be noted that growth soon tends to cease in the case of both, and that both

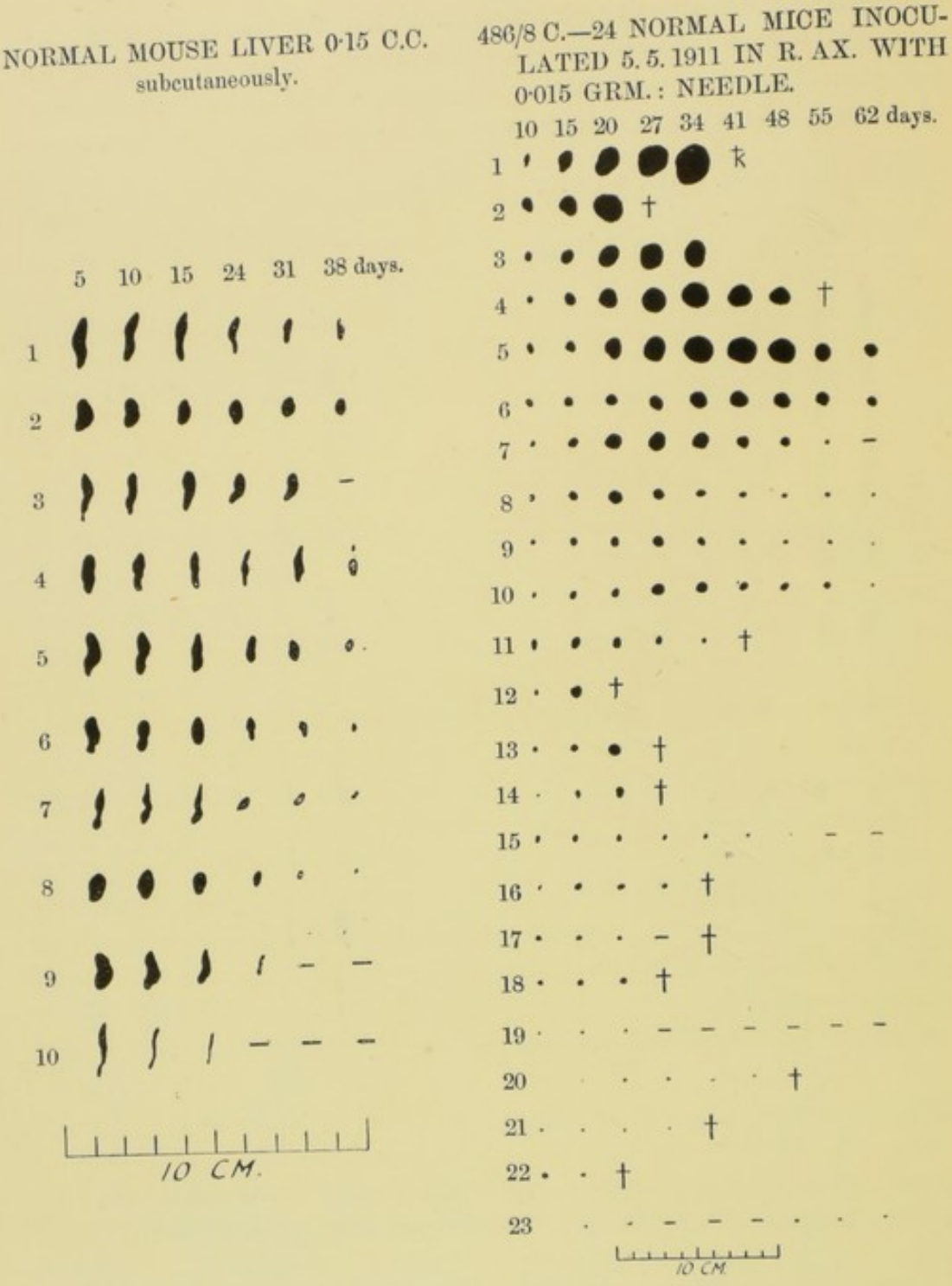


Fig. 60. Exp. 486/8 C.—Chart showing temporary growth of normal mouse liver in normal mice. A certain amount of the increase in size is due to cellular infiltration by host-tissue of the graft.

Fig. 61. Exp. 486/8 C.—Chart showing temporary character of the growth of the highly differentiated tumour 486 in normal mice.

EXP. 206/11 C. 15 MICE INOCULATED IN RIGHT AXILLA WITH 0·02 GRM.: NEEDLE. 7.1.09.

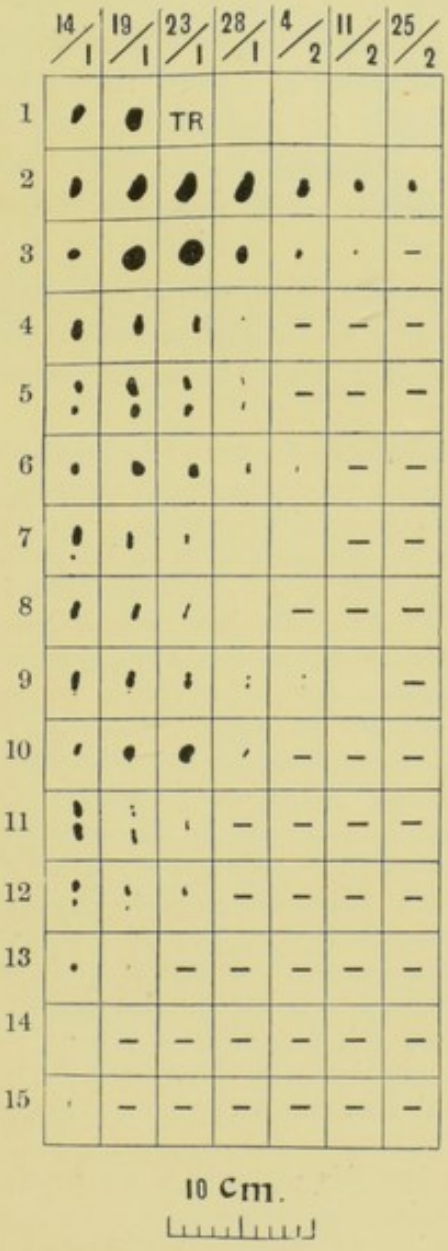


FIG. 61 *a*. Exp. 206/11 C.—Chart showing the temporary character of the growth of the completely undifferentiated tumour 206 in normal mice.

present in this respect a marked contrast to the best growing tumours. Normal tissue differs from the latter both in the amount and in the duration (continuity) of growth. Highly differentiated tumour tissues also exhibit a limited amount of growth in any one animal, but in series of animals growth can be continued indefinitely. Thus while some of the features of the growth of highly differentiated tumours present a close parallel to the behaviour of normal tissues when transplanted, the capacity of such tumours for unlimited propagation allies them with the rapidly growing undifferentiated or differentiated malignant neoplasms by the one character which separates these fundamentally from normal tissues.

In assuming that sarcoma development represents a natural termination to the proliferation of propagated carcinoma, Ehrlich and Apolant * have elevated a technical difficulty to an unwarranted importance. It

* Their views are set forth as follows:—"As long as the transplantability of carcinoma was not established, the existence of a malignant tumour was absolutely inseparable from the life of the individual bearing the growth. The proof of transplantability demonstrates for the cancer-cells an independence from the organism attacked similar to that possessed by the germ-cells, which in contrast to the purely somatic cells are possessed of potential immortality. Now it is highly interesting that this immortality, which at first must be assumed to be present potentially, need not necessarily endure even as a matter of fact in the case of the most virulent carcinomata; indeed limits are set which we can completely compass in our vision, to the existence of a transplantable carcinoma strain. The duration of life varied for the three carcinomata, which were ultimately ousted by sarcomata between 1 and 3 years, the periods represented by the development of the spontaneous tumours not being included. The limitation as regards time is thus much more narrow than is that set to the amount of proliferation, since the latter surpasses all our powers of comprehension if every possible transplantation could be made. Whether similar limitations are set to the growth of the sarcomata, either by spontaneous diminution in virulence, or through fundamental alteration in their structure, which is very improbable, it is impossible at present to say in the absence of all data." (Apolant, Die experimentelle Erforschung der Geschwülste, pp. 455–456, in Kolle and Wassermann's Handbuch der pathogenen Mikroorganismen, 1ster Ergänz, Bd. Heft 2, Jena, 1906.

After the publication from this laboratory of the description of a case in which the carcinoma was retained in propagation, Ehrlich and Apolant somewhat modified their view which, following a description of the value of prolonged propagation, is given as follows, with especial relation to sarcoma development:—"Consequently, in this way the first great fundamental question which meets us face to face is that of the history of a carcinoma-strain. What do we know about the origin of these transplantable tumours in the animal in which they arose, about their further development and their ultimate fate in the inoculated animal? These far-reaching questions resolve themselves into a number of special questions, the histological aspects of which I shall discuss" (Apolant, Ueber die Genese des

has been shown elsewhere (*vide* pp. 163, 179–181) that the carcinomatous component of tumours giving sarcoma may be propagated indefinitely, and when this is not possible the reasons for failure are technical and not due to a failure of the powers of the carcinoma cells after they have elicited the sarcomatous change in the surrounding stroma.

The number of carcinoma strains propagated is now very greatly in excess of those alluded to in the foregoing footnote. Their study has shown that sarcoma development is not the rule but the exception. Further, it has also been shown that the technique employed by Ehrlich and Apolant, viz. forced *passage*, instead of leading up to it, may be the most effective means of preventing it. The rapidity of proliferation is also not the essential factor, since sarcoma development has been observed in strains with a low proportion of "takes" and of slow growth.

The Rate of Growth.

The amount of growth varies, and is intimately bound up with, and dependent upon, the rate of growth and the susceptibility of the tumour-cells to concomitant immunization * ; but the property of continuous proliferation is the one constant and peculiar character of the cells of new growths. In all other respects they exhibit the most diverse variations producing the manifold biological and clinical types of cancer. Of the subsidiary characters which are of importance in separating these types from one another, apart from

---

Carcinoms, (*a*) Histologischer Teil. *loc. cit.* p. 3, 1908). . . . . "Compared with the total number of propagated carcinoma-strains, the number of cases of sarcoma development is so high, that the view, however improbable it appears at the first moment, cannot be quite dismissed, that a certain regular law underlies the process, and that perhaps the development of sarcoma represents the termination of one and every carcinoma ; to be sure we must not conceive of the individual carcinoma as a growth, in terms of the non-transplantable tumours to which a natural end is set by the death of the organism, but in terms of the tumour-strain of which the existence is independent of the life of the individual organism. Especially the circumstance that sarcoma development may occur after a carcinoma has been propagated for several years makes it possible that in the case of other less virulent strains still greater intervals of time are necessary for the occurrence of the metamorphosis. Should it happen that notwithstanding tens of years of propagation of a strain sarcoma development does not take place, the fact would indicate that potential immortality must be ascribed to the carcinoma cells as it is to the germ cells or to the unicellular organisms, and as it is also according to our present experience ascribed to the sarcoma cell. Actually, a spontaneous dying-out, *i. e.* not due to secondary causes such as infection, sarcoma development, etc., of carcinoma strains which have been propagated for long, has never been observed by us."

* See p. 194. Diminished power of growth.

the percentage of "takes," the rate of growth is that most amenable to objective study and most suitable for making comparisons between them. This comparison is only partially allowable in the case of spontaneous tumours growing in the primarily affected animal. Although we can exclude, from the results of experiments, the assumption that spontaneously affected animals afford a soil peculiarly suited to the growth of cancerous cells generally, the perfect adaptation which a malignant new growth exhibits to the tissues and fluids of the organism in which it arose, permits it to unfold an energy of proliferation in its original host partly to be ascribed to the suitability of the conditions of growth, and therefore only in part available as a measure of its powers of growth. When comparisons are made on the basis of the results obtained in the uniform soil afforded by normal mice, the growth obtained furnishes the necessary common denominator for such an estimation, and it need cause no surprise that tumours which in the spontaneously affected animal grew with great rapidity and produced extensive secondary growths, frequently give insignificant evidence of their powers in new hosts. A comparison between the rates of proliferation under these different conditions is only allowable after the preliminary stage of adaptation has been passed, and propagation has been continued with fairly constant results for some time. When these precautions are observed the propagated tumours form a graduated series ranging from the most rapidly proliferating, to others, characterized by a slowness of growth just sufficing to raise them above the level of complete stagnation. The accompanying simple diagram illustrates this serial arrangement graphically (fig. 62). It has been constructed on the basis of the average time each strain requires to produce a weight of one gram from the inoculation of 0·02–0·03 gram, the data having been obtained by weighing the tumours obtained during many years at known intervals after inoculation. The rapidity of growth is inversely proportional to the length of this interval, and the values marked off on the vertical lines ascribed to the several tumour-strains have been obtained by multiplying the reciprocal of the number of days by a constant factor :

e. g. Rate of Growth of Tumour-Strain 32 $\dfrac{1}{10\ (\text{days to produce 1 gram})} \times 1000 = 100.$

The less rapidly growing tumour-strains produce less and less, in a graduated series, from the same dose, viz. 0·02–0·03 till a low limit is reached at which not even so much as 0·5 gram is produced in nine months. Of course such comparisons are in many cases arbitrary,

because no account is taken of the amount of necrosis, the varying production of connective tissue stroma, &c. By weighing mouse embryos at different periods of gestation it has been possible to estimate the rate of growth after the embryo has attained a weight corresponding with the 0·02–0·03 gram of tumour-tissue inoculated. The result is indicated on the chart, from which it is evident that, while some tumours may approximate to the rate of growth of embryonic tissue, the majority fall far below it. Thus, further biological evidence of the inadequacy of the

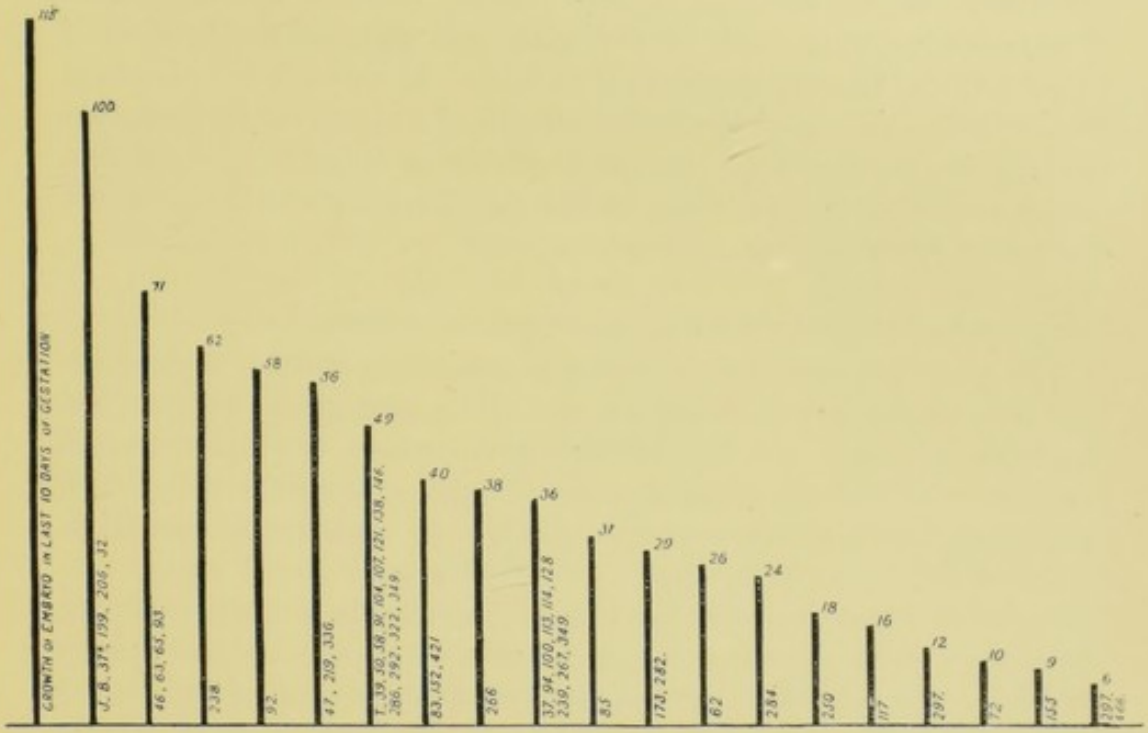


FIG. 62.—Diagrammatic comparison of rate of growth of various tumour-strains on the basis of the number of days required to produce 1 grm. of tissue from a measured dose (0·2–0·3 grm.) inoculated. The rate of growth of embryonic tissue is also indicated on the basis that the mouse embryo weighs 0·02–0·03 grm. at the 11th day of gestation and at birth 1–1·5 grm

assumption that tumours are tissues (embryonic rests) that have retained or reverted to an embryonic condition is added to that previously given. It becomes obvious that tumours may possess the power of continuous growth in spite of having a proliferative energy greatly less than that of embryonic tissue, and from this fact it also follows that the rate with which the tumour-cells take up food-stuffs, *i. e.* their avidity, while intimately bound up with their rate of proliferation, is not the essential determining factor in endowing them with powers of continuous growth or malignancy.

The result of this comparison of a number of tumour-strains, more than half of which have been propagated for more than three years, demonstrates the tenacity with which each strain adheres to its own characteristic rate of growth. The strains at the top of the scale attained their position after the earliest generations were passed. There has not been a gradual passage from the lowest to the highest rungs of the ladder. The initial difficulties of propagation once overcome, the tumour takes its place in the scale and maintains it. The difference between the most rapidly growing tumours and the slowest is primary, inherent, and for the most part also constant. Were it otherwise, the tumour-strains would advance in an orderly series from the bottom to the top of the ladder till all had attained to the maximum rapidity of growth. The consequence of this not being the general rule brings us face to face with one of the matters upon which experiment has not yet yielded clear evidence.

Is an Artificial Acceleration of the Rate of Growth possible?

The question resolves itself simply into deciding whether the cancer-cell is so plastic that the manifestations of its properties are dominated by its environment and moulded by it, or is relatively so stable that it tends to maintain its primary properties with minor alterations which are more readily ascribed to induced than to spontaneous variations. Now, whilst it is obvious that a progressive acceleration of the rate of growth is not the rule, nevertheless, as already pointed out *, the possibility of an acceleration of the rate of growth occurring must be considered as possible until the true significance of the fluctuations † in the rate of growth and percentage of "takes" of many strains (*cf.* figs 2–8, pp. 142–3) has been ascertained. From what has been said above and on earlier pages, evidence of its general occurrence during the continued propagation of mouse tumours is unconvincing. From a clinical standpoint this subject is of great importance. Variations and alterations in the rate of growth of malignant new growths in man are familiar, but the same difficulty has been encountered in resolving them into ordered sequence as in arranging the histological pictures until experiment supplied the key to their connection with one another. It is not easy to decide between those who absolutely deny the possibility

* Bashford, E. F.: The Application of Experiment to the Study of Cancer. Science Progress, July 1907, *vide* p. 17.

† Bashford, E. F., Murray, J. A., & Bowen, W. H.: The Experimental Analysis of the Growth of Cancer. Roy. Soc. Proc. B, vol. 78, 1906.

of an acceleration of growth (Ribbert), and those who as vehemently postulate its occurrence. The matter from the experimental point of view is neither quite so simple as Ribbert's speculations nor as Ehrlich's observations have led Adami to state it. Adami may be quoted again; he writes—referring to Ribbert:—"Apart from mere general considerations, one fact alone, acquired, it is true, since his work was published, demonstrates the incorrectness of this dictum. We refer to Ehrlich's demonstration that, by passage through mice, mouse cancer can be rendered more and more malignant" *. Ehrlich originally claimed that a "distinct and usually a maximum increase in virulence is to be noted during continually repeated inoculation in the case of the majority of the tumours," † and added, "The fact that we have in our possession tumours so tumultuous in their growth, which exceeds all limitations, is not a blind accident, but the consequence of a definite plan laid down by one of us (Ehrlich), and which has been carried out by the use of a great number of animals, and by transplanting as rapidly as possible with the greatest care and trouble. It was our aim to increase the malignity of the tumour-cells to the maximum by continued systematic *passage* from animal to animal on the analogy of bacteriological technique" ‡. In more recent papers Ehrlich admits that this cannot be attained for all tumour-strains. Therefore some of his results are in accordance with the experience of this laboratory as described above; and there is only a minor question as to how the results are to be interpreted, and, in our experience, when a change in the rate of growth has apparently taken place, it is difficult to exclude its being due to the other factors already discussed, and not solely, if at all, to a true acceleration in the rate of cell division.

Doubts may be aroused as to whether the tumour-strains investigated justify the rapidity of their growth being compared with that of Ehrlich's tumours. When the rates of growth of the several tumour-strains are compared, it is seen that a number of them produce within ten days, from a dose of 0·02–0·03 of a gram, a mass of tissue of not less than one gram in weight. This is an under-estimation of the rate of growth of some strains, since several produce even two grammes from

* Adami, J. G.: 'Principles of Pathology,' Oxford, vol. i. p. 674, sec. edit. (1910).

† Ehrlich, P., & Apolant, H.: Beobachtungen über maligne Mäusetumoren. Berl. Klin. Woch. 1905, no. 25.

‡ Apolant, H., & Ehrlich, P.: Experimentelle Beiträge zur Geschwulstlehre. Berl. Klin. Woch. 1906, no. 6.

the dose stated, while for others the output at ten days can be increased in proportion as the dose inoculated is increased. Thus, several of the tumour-strains studied rival those described by Ehrlich and Apolant, who have not more nearly defined the dose they inoculated beyond leaving it to be inferred that it conformed to what they state for the transplantation of spontaneous tumours, of which they write "in our laboratory . . . . . we always employ large quantities of inoculation material *." In the absence, both of any statement to the contrary, and of one giving information as to the exact doses inoculated, it may be assumed that this quotation describes the practice followed in continuing propagation, and, therefore also, that the doses inoculated

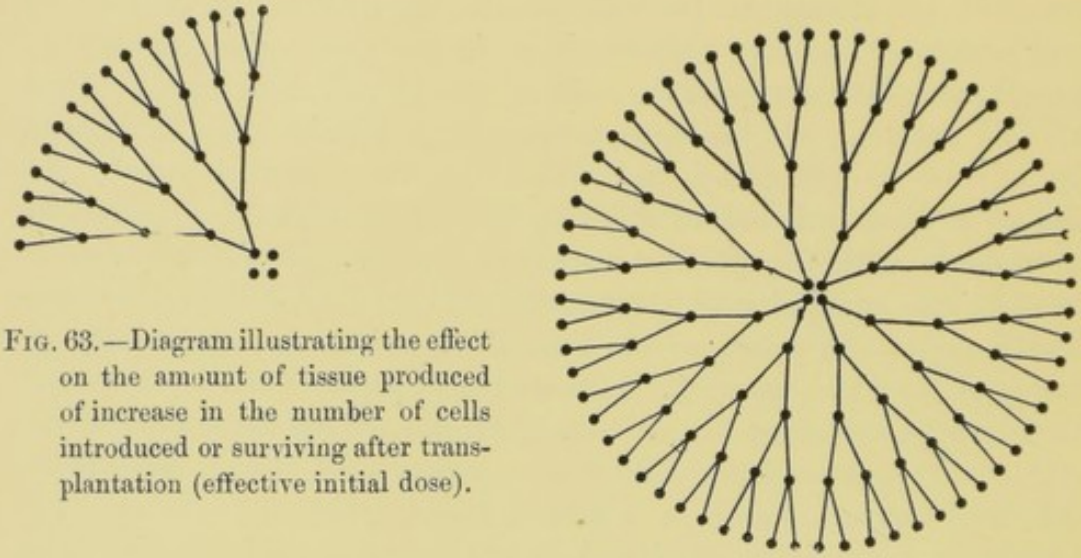


FIG. 63.—Diagram illustrating the effect on the amount of tissue produced of increase in the number of cells introduced or surviving after transplantation (effective initial dose).

by us were not greater but probably less than those employed by Ehrlich and Apolant. If this be so, then several of our tumour-strains equal, if they do not exceed, the rate of growth of those tumours which these authors describe as attaining "the size of an almond in eight days" † and yield tumours "such as have neither been seen in practice ‡ nor in experiment." "In this respect the most favourable results are exhibited in strain 7, which is certainly the most virulent of all carcinomata hitherto observed either in experiments or at the bedside. For a long time the proportion of successful inoculations has varied between 80 and 100 per cent. The energy of growth is so great that in 8 days the tumours reach a weight of 2 grammes, in 2 weeks

* Ehrlich, P.: Experimentelle Karzinomstudien an Mäusen. Zeitschr. f. ärztliche Fortbildung. iii. Jahrgang. Apr. 1906. *Vide* page 206, col. 1.

† *Ibidem*, page 207, col. 1.

‡ *Ibidem*, page 207, col. 1.

3 grammes, and in 3 weeks 5 grammes. After two months growth tumours are by no means rare, which, after removal from the mouse, weigh not less than the latter*." The importance of the dose inoculated in determining the size which the resulting tumours attain in a given time has often been referred to in earlier papers, but the accompanying diagram (fig. 63) will serve to emphasize its importance under conditions where the arithmetical factor is alone assumed to be of moment.

Prolongation of the duration of Growth in any one Animal.

Among the tumour-strains cultivated there are a considerable number which have been maintained in propagation only by rapid *passage* from one set of mice to another. This procedure has been necessary because the tumours exhibit transitory growth only, in any one animal. Strain 206 typifies this behaviour for carcinoma, and 92 for sarcoma. It is exhibited most strikingly by strains possessing a high initial energy of growth in the days immediately following transplantation, but is also a prominent feature in some strains with a high degree of differentiation, *e. g.* strain 486 a squamous-cell carcinoma. The phenomenon is due to the co-operation of several factors, of which a special susceptibility of the tumour-cells and concomitant immunisation of the newly inoculated animals are the most important. As already pointed out, tumours vary both in the readiness with which they induce immunity and also in their susceptibility to its influence. A combination of the two properties leads to a group of tumours which exhibit the phenomenon of temporary growth only, simulating, at any rate superficially, that obtained by transplanting normal tissues (*cf.* figs. 60, 61). For some tumour-strains the phenomenon is exhibited whether the dose inoculated be small or large. In others it is absent if small doses are inoculated, but is induced by the inoculation of large doses. In the latter case it is possible with care to accustom the cells of some tumours to grow better after frequent repetition of this method of transplantation, and then by reverting to inoculation by small doses to make the cells return to their antecedent susceptibility when inoculated in large doses. Still more interesting has been the result of endeavouring to propagate parallel strains of the same tumour. In the case of strain 92, an osteoid chondro-sarcoma, two lines have been propagated separately in continuous series. The initial success of transplantation is high in both lines (see figs. 64, 65) and the rate of

* Ehrlich, P., & Apolant, H.: Beobachtungen über maligne Mäusetumoren. Berl. Klin. Woch. 1905, no. 28.

growth about the same, but there is a slightly more rapid growth, although for a shorter period, in the first line than in the second. Similar evidence of a prolongation of the period of growth or of insusceptibility to concomitant immunisation is afforded by some

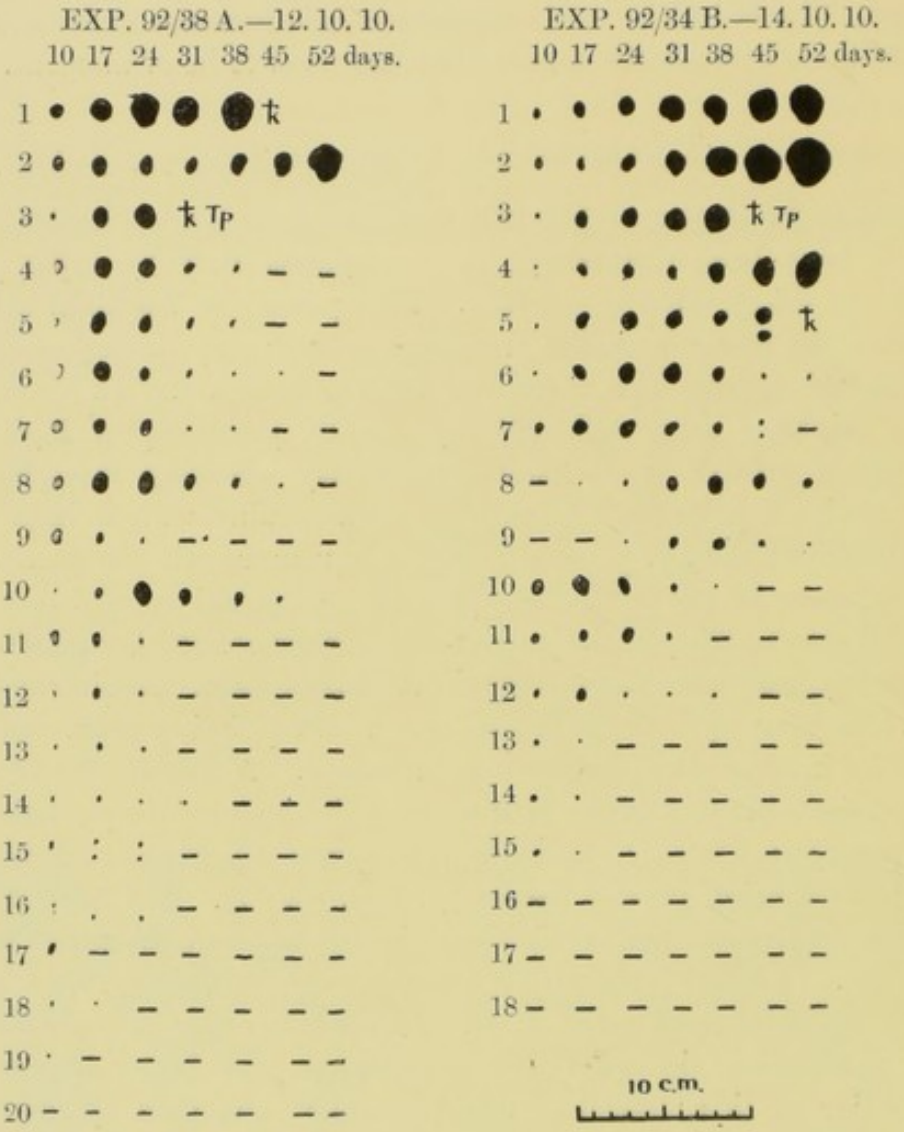


Fig. 64. Exp. 92/38 A.—Chart showing mode of growth of the 1st or soft necrotic strain of tumour 92.

Fig. 65. Exp. 92/34 B.—Chart showing mode of growth of the 2nd or firm collagenous strain of tumour 92.

other strains, *e. g.* 63 (*cf.* figs. 30, 31), and therefore it would appear, that it is possible experimentally to obtain during the continuous propagation of a tumour cells of different powers of growth.

Diminished Power of Growth.

There remains for consideration one other departure from the original behaviour. Some tumours show throughout the whole course of propagation, as an important frequent occurrence, a temporary growth only, followed by diminution in size and total disappearance. This phenomenon of spontaneous healing or spontaneous absorption is met with in the great majority of all transplanted tumours, in some as a rare or isolated occurrence ; but in others it is the rule, and constitutes a persistent biological characteristic of these tumours for long periods. The mechanism of this process is fairly well understood, and it is now certain that the concurrence of at least two distinct factors is necessary. The reaction set up in the new host by the newly grafted tumour-cells provides the new vascular and supporting tissues and occurs in all tumour transplantations. In addition there may be set up a state of active resistance to further grafting of living tumour-cells, which condition in some cases is sufficient to retard or suppress the further growth of the primary graft. The second factor is much more elusive, and cannot be more closely defined at present than as a condition of susceptibility of the parenchyma cells to this resistant condition, an instability of their nutritive balance so that the inimical condition turns the scale against them.

While the first of these two factcrs can be demonstrated to operate in a very high percentage of cases where tumour-cells from strains showing spontaneous cure are grafted into a new normal animal, the second or parenchyma factor varies from one tumour to another, and constitutes a well-defined biological characteristic of these strains. This condition is present in an exaggerated degree in tumour 206, in many transplantable sarcomata, and in some highly differentiated tumours, and has been referred to already (fig. 65 *a*).

An interesting variant of the process is presented by tumours 93 and 138 (figs. 66 & 67). After an initial period of rapid growth lasting from 2 to 4 weeks, the tumours remain stationary for a time. In tumour 138 (fig. 66) this period lasts on an average for two months, and the tumours then begin to grow progressively. In tumour 93 (fig. 67) diminution in size usually occurs during the resting period, and a certain number of the tumours disappear completely. The resting-period is shorter than in tumour 138, and the subsequent period of progressive growth is shorter in consequence of the more rapid growth of the tumours. This phenomenon of an initial rapid growth followed by a pause of considerable duration in which the tumours diminish in size and then grow

EXP. 206/11 C.—7. 1. 09 DOSE 0·02 GR. RT. AX.

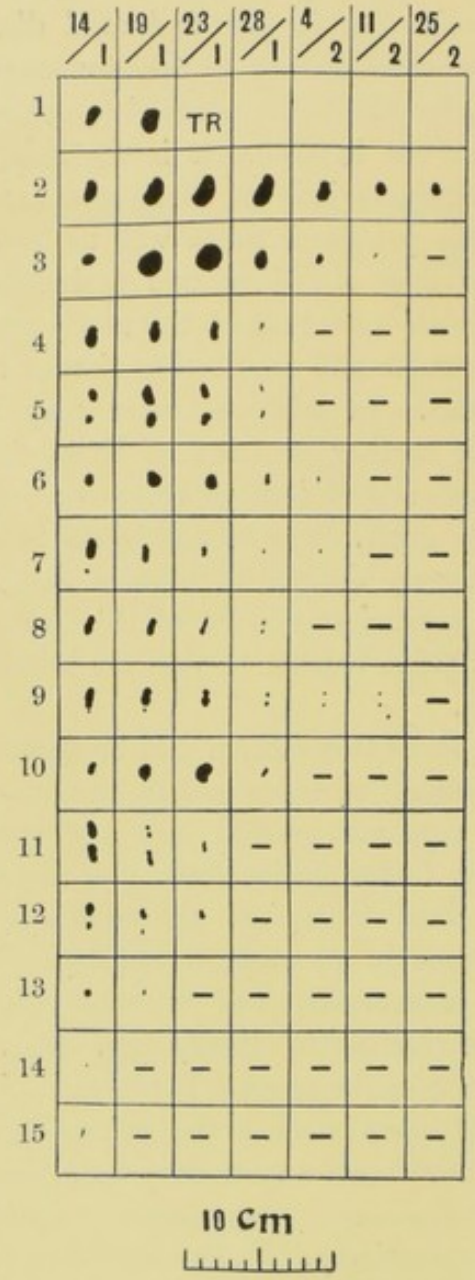


Fig. 65 *a*. Exp. 206/11 C.—Chart showing the temporary character of the growth of the completely undifferentiated tumour 206 in normal mice.

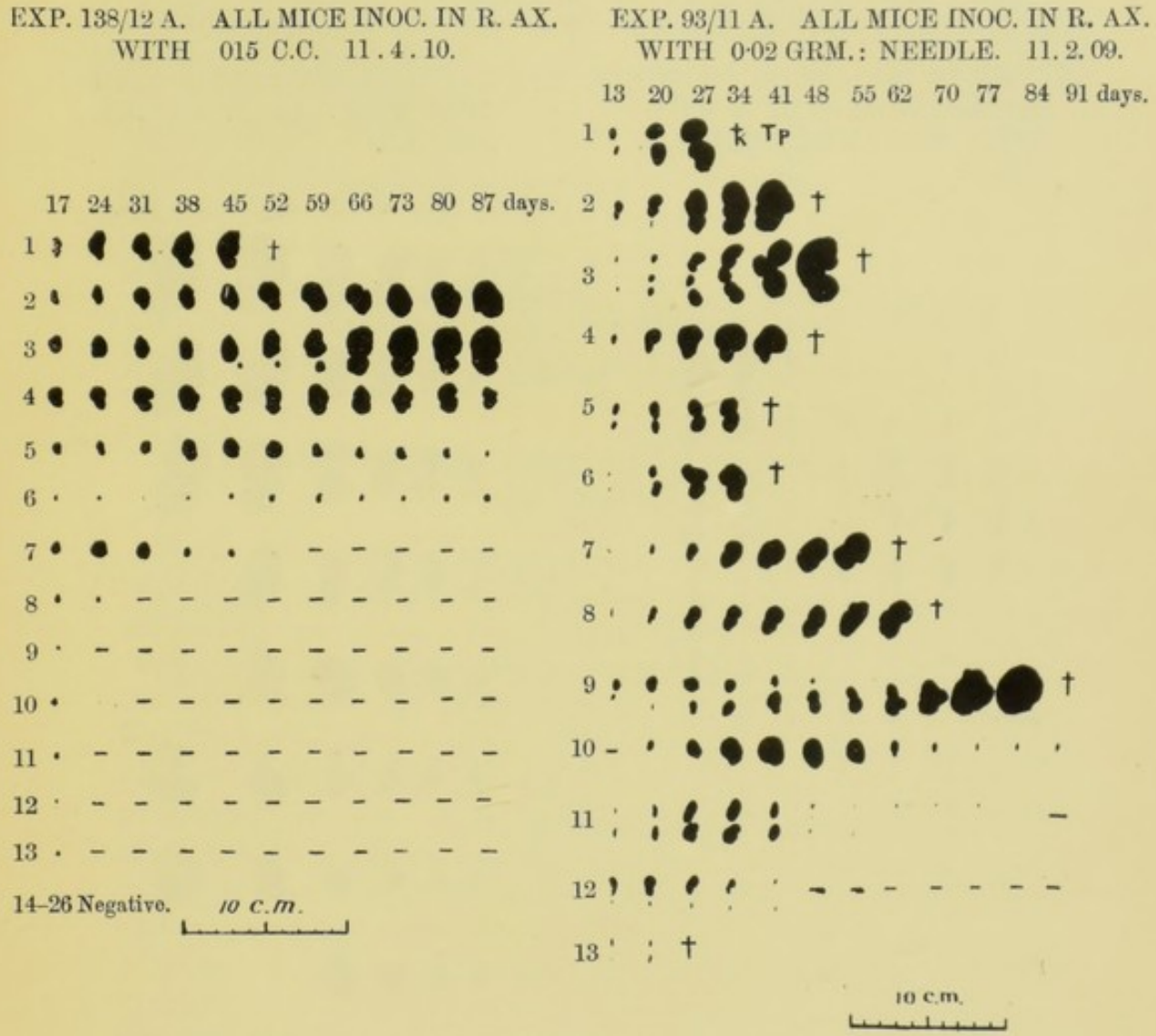
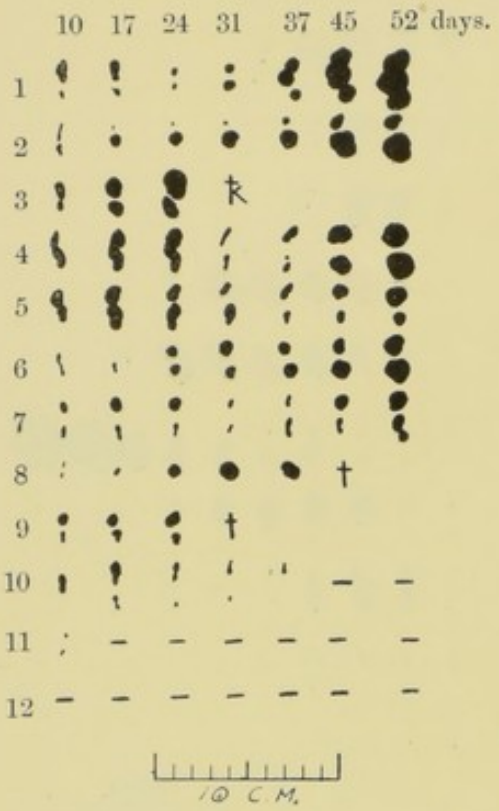


Fig. 66. Exp. 138/12 A.—Chart showing mode of growth of tumour 138 in the 12th generation. After an initial rapid growth the tumours remain stationary for a long period before growth sets in again. Spontaneous absorption may occur during the quiescent period.

Fig. 67. Exp. 93/11 A.—Chart showing mode of growth of tumour 93. The initial rapid growth may be followed by a temporary halt or by spontaneous absorption. In mouse 9 of this experiment one tumour disappeared, while the other grew progressively after a transient quiescent period.

progressively again, is frequent in strain 199*, and is seen in other strains as an isolated occurrence. This is only what was to be expected from the universality in varying degree of the reactive factor in its production.

EXP. 63/55 A. ALL MICE INOCULATED IN R. AXILLA WITH DOSE 0·03 GRMS. SYRINGE. 30.1.11.



63/48 K. INOCULATED 15.5.11 WITH 0·015 GRMS. IN R. AXILLA. NEEDLE.

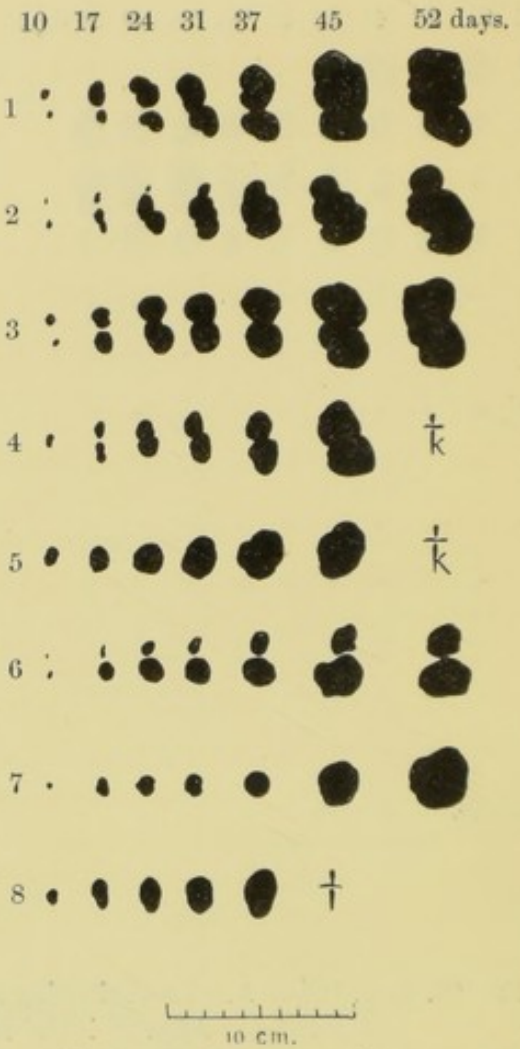


FIG. 68. Exp. 63/55 A.—Chart, for comparison with fig. 69, of growth of the strain of tumour 63, which shows much spontaneous absorption.

FIG. 69. Exp. 63/48 K.—Chart of growth of strain of tumour 63, which shows progressive growth of all tumours.

Its occurrence as a constant character in some tumour-strains is the most striking evidence of the importance of the parenchyma factor, viz. that

* Bashford, E. F. and Russell, B. R. G.: Further Evidence of the Homogeneity of Resistance to the Implantation of Malignant New Growths. Roy. Soc. Proc. 1910, ser. B, vol. 82, p. 301.

temporary or persistent instability which renders the tumour-cells susceptible to the active resistance induced by concomitant immunisation.

Tumours 47, 199, 238, 282, 322, have shown this condition in greater degree in the later generations, although many tumours had grown progressively in the first period of propagation. Deserving of notice is the separation of a strain of "63" presenting this feature in contrast to the progressive growth of another parallel strain of the same tumour (figs. 68, 69). The occurrence of a diminution in power of growth, apart from its bearing upon variability awakens hopes that the mechanism of spontaneous healing will be ultimately analysed, and the nature of the altered condition of the cell leading to natural cure both in propagated and in spontaneous cancer ascertained.

BEARING OF CELL VARIABILITY ON SOME FORMS OF CANCER.

The account of the behaviour of tumour-cells during continued propagation given on the preceding pages demonstrates that cancer-cells can undergo variations. The demonstration of the occurrence of these variations permits of the inference that they could also occur under natural conditions, and yields evidence of the validity of the conclusion that the cancer-cell is a variant of the normal cell endowed with many inherent properties of the latter. That the variations occurring during propagation are not mainly induced by the environment, but arise spontaneously, is proved by the fact that the tumour-strains derived originally from one and the same parenchyma, viz. the mammary epithelium, do not approach to a common type during prolonged propagation. It has been shown that propagable tumours can be adapted to growth in the strange breeds of mice existing in foreign countries, and that similarly a spontaneous tumour can be adapted to growth in normal mice. Hence it may be inferred that of the variations assumed to occur in the mammary epithelium of the mouse, some are capable of that adaptation permitting of their continued growth in the animal in which they arose. However, great caution is necessary before drawing any such general conclusion as to the nature of cancer, and such a general application of the results recorded is not made. They can only be applied with important reservations to explain the nature and genesis of cancer, and then also only to those forms of the disease supervening in tissues which have long been subjected to the consequences of chronic irritation.

It has been shown that every fresh transplantation effects a

disturbance of the cancer-cells. They are thrown into a state of active proliferation after which differentiation tends to reappear. This is well illustrated by the behaviour of strains in which a solid or alveolar condition supervenes regularly on transplantation, and ultimately the differentiations characteristic of the primary tumours reappear. The cancer-cells during propagation are thrown into a condition analogous to that reactive proliferation to which their normal ancestors were subjected where chronic irritation has preceded the onset of cancerous change. The morphological and biological changes exhibited by tumour-cells during prolonged propagation are analogous to, and as a rule indistinguishable from those characterising the many different varieties of the primary tumours derived from a single parenchyma, and often occur associated in one and the same spontaneous tumour. It is inferred that the variability which tumour-cells exhibit during propagation indicates that their pre-cancerous ancestors first deviated from the normal by virtue of the same property, opportunity for such variations being more especially provided by the prolonged phase of reactive proliferation elicited by chronic irritation. Of the variations arising, it is assumed that some are in the direction of the acquirement of powers of continuous growth, as is one of the variations observed under experimental conditions.

The variations of the cancer-cell described in the preceding pages are morphological and biological, and are similar to those occurring side by side in inexplicable and apparent disorder in many primary growths. By propagation they have been segregated and their relationships made clear. When a morphological variation has occurred it makes a striking impression upon the observer, and tempts him to regard it as the development of a new malignant growth out of one already pre-existing, in short as the evolution of a new cancer. But from all that has been written above it is evident that the morphological characters of tumour-cells are, to borrow a zoological term, only "correlated characteristics" of cells capable of continuous growth. They are the accompaniments but not the essentials of cancer, and cannot be shown to be more than outward signs of other variations taking place in the cell-contents. Although they have occurred secondarily during propagation, they are in all cases (from latency or loss of differentiation, dual-differentiation, assumption of spindle-shape by epithelial cells, to the production of sarcoma) duplications of what has been described in the primary growths themselves, other than those from which the tumour-strains showing the variations have been derived. Were it possible to mix together all the daughter tumours

propagated in a vast number of mice from a spontaneous or mother tumour, one would again have the confused histological picture of a parent spontaneous tumour perfectly reproduced upon a magnified scale, but with a knowledge of how it had been obtained. Thus under prolonged propagation the tumour-cell has not exhibited any histological or biological variations of which it was incapable in its original host, and it can be inferred that it behaves in this respect in a parallel manner whether it be in the strange environment of propagation or in the natural environment in which it developed. Considering the difference in the conditions of life and growth in the two cases, the remarkable thing is not that the tumour-cells vary during artificial propagation, but that they vary so little. The tenacity with which the histological characters of several varieties of tumours, all derived from the mammary epithelium, are adhered to, shows the justice of speaking of them as true varieties of a considerable degree of permanency.

Of the biological alterations much the same may be written about their relative constancy and variability, and similar conclusions may be drawn. If an accelerated rate of cell-division has not been satisfactorily demonstrated to occur as a variation, it must not be assumed that an immutability in this respect is postulated any more than of the morphological characters. The constancy with which the several tumour-groups retain their respective powers of growth (percentage of "takes" and rates of growth) is very striking. There is no such thing as advance to a common type with maximum powers of growth. The property common to all tumour-strains is power of continuous growth in successive series of mice whether the tumours are capable of progressive or only of transient growth in individual series of animals. When a tumour is capable of only transient growth in any single animal it behaves in this respect like normal tissue upon transplantation. In the case of such tumour-tissue, however, it has been shown that a power of more prolonged growth may come to be exhibited. It remains to be seen whether it can be elicited in full degree.

The differences between spontaneous and propagated cancer must again be called to mind. No matter how greatly the 85 propagated tumours differ among themselves in their rates of growth and powers of progressive growth when implanted into normal mice, each primary tumour was possessed of the power of progressive growth in the animal to which it was natural. The differences to which so much attention has been devoted have only been revealed by putting all to the common test of how they behaved in normal mice. The following considerations must also be weighed in appraising the importance of the relationship between

chronic irritation and the inception of cancer. The respective parts played by the connective tissue and epithelium requires renewed study. In the human subject the matter has been considered too exclusively from the side of the connective tissue or from that of the epithelium. In the experiments considered above reference has only been possible to the epithelium because the connective tissue component, being supplied afresh at each transplantation, has not been maintained in the repeated state of regeneration possible for the epithelium. The result shows that it is certainly wrong to regard the development of cancer in man solely from the standpoint of a primary change in the connective tissue, but it may also be wrong to go too far in the other direction and consider it solely from the standpoint of a primary change in the epithelium. The cancer-cell when retained in propagation receives a new stroma at each transplantation, and the contiguity of such proliferating connective tissue may well supply conditions analogous to those obtaining where epithelium and connective tissue are together reacting to chronic irritation. The interdependence of the parenchyma and stroma has been shown in a variety of ways : in the specific nature of the fibroblastic and angioblastic reactions which result in supplying a fresh connective tissue and vascular scaffolding at each fresh transplantation, in the fact that immunised animals do not react in this specific way, and in the changed relations obtaining during natural healing both in spontaneous and in propagated cancer. For these reasons and others a re-investigation of the parts played by connective tissue and epithelium in parts subject to prolonged chronic irritation appears desirable, especially with the object of throwing light upon the relations obtaining between chronic irritation, nodular hypertrophy, adenoma, and carcinoma. Their relations to one another are by no means clear when they occur combined in one organ. From a review of the whole evidence it appears necessary to admit that while hypertrophy, adenoma and carcinoma, represent different stages of variation, there are two possible combinations in which they may occur. They may represent primary variations, each arising as a single stage of departure from the normal, or be separate stages in a sequence of events passing by accumulating variation into one another in the course of time. If propagation permits of any conclusion, it is that, although they may, yet they do not necessarily represent stages in an advancing sequence of events from mere hypertrophy through adenoma to the least differentiated carcinoma, but that they indicate different degrees of cellular variations of which some may be relatively permanent and constitute constant features of a propagable malignant new growth.

Literature.

Adami, J. G.: Principles of Pathology, Oxford, vol. i. p. 674, sec. edit. (1910).

Apolant, H.: Die epithelialen Geschwülste der Maus. Arbeiten aus dem königlichen Institut für experimentelle Therapie zu Frankfurt am M., Heft 1, 1906.

——: Ueber experimentell erzeugten Rückschlag, etc. Munich Med. Woch. 1907, no. 35.

—— & Ehrlich, P.: Experimentelle Beiträge zur Geschwulstlehre. Berl. Klin. Woch. 1906, no. 6.

—— ——: Ueber die Genese des Carcinoms. Verhandlungen der Deutschen Pathologischen Gesellschaft. Zwölfte Tagung Kiel, 1908.

——, ——, & Haaland, M.: Experimentelle Beiträge zur Geschwulstlehre. Berliner klin. Wochenschrift, 1906, no. 2.

Bashford, E. F.: Address on Cancer in Man and Animals. Séances Générales. XVI International Medical Congress, Budapest, 1909. Compte-Rendu, volume général, pp. 186–227. *Vide* also Berl. klin. Woch. 1909, Nos. 36 & 37, and 'The Lancet,' Sept. 4, 1909.

——: The Application of Experiment to the Study of Cancer. Science Progress, July 1907, *vide* p. 17.

——: The Growth of Cancer. March 13, 1905. Trans. Med. Soc. of London, vol. xxviii. 1905.

—— & Murray, J. A.: The Significance of the Zoological Distribution, the Nature of the Mitoses, and the Transmissibility of Cancer. Roy. Soc. Proc. Jan. 1904, vol. 73.

—— & Russell, B. R. G.: Further Evidence of the Homogeneity to the Resistance to the Implantation of Malignant New Growths. Roy. Soc. Proc. 1910, Series B, vol. 82, p. 301.

——: The Etiology and Pathogeny of Malignant New Growths, especially of Cancer. Ninth International Veterinary Congress at the Hague, Sept. 1909.

——, Murray, J. A., & Bowen, W. H.: The Experimental Analysis of the Growth of Cancer. Proc. Roy. Soc. 1906, Series B, vol. 78, p. 196.

——, ——, & Cramer, W.: The Growth of Cancer under Natural and Experimental Conditions. Second Scientific Report of the Imperial Cancer Research Fund, Part ii. 1905.

——, ——, ——: Source of the Constituent Elements of New Growths obtained by Artificial Propagation. Second Scientific Report of the Imperial Cancer Research Fund. Part ii. pp. 24–29, 1905.

——, ——, ——: Stroma is a Specific Reaction on the part of the Host. *Ibidem.*, pp. 30–33.

——, ——, ——: Interpretation of the Differences and Variations in Transplanted Tumours of one Organ. Second Scientific Report of the Imperial Cancer Research Fund, Part ii. p. 52, 1905.

——, ——, ——: The Natural and Induced Resistance of Mice to the Growth of Cancer. Roy. Soc. Proc. Series B, vol. 79, p. 184.

——, ——, & Haaland, M.: Ergebnisse der Experimentellen Krebsforschung. Zeitschrift für Immunitätsforschung, Bd. i. Heft 4, 1909.

Borrel, A.: Epithélioses infectieuses et epithéliomas. Annales de l'Institut Pasteur, t. xvii. 1903.

——: Le Problème du cancer. Bulletin de l'Institut Pasteur, t. v. nos. 12–15, 1907.

Clunet, J.: Recherches expérimentales sur les Tumeurs malignes. Paris, 1910, p. 53.

Ehrlich, P.: Experimentelle Karzinomstudien an Mäusen. Arbeiten aus dem kgl. Institut f. exp. Therapie, Heft 1, 1906, pp. 77–102.

——: Experimentelle Karzinomstudien an Mäusen. Zeitschrift für ärztliche Fortbildung, 1906, iii. 205.

Ehrlich, P.: Experimentelle Studien an Mäusetumoren. Abhandlung der Internationalen Konferenz fur Krebsforschung, Heidelberg u. Frankfurt a. M., Sept. 25–27, 1906. Zeitschrift für Krebsforschung, 1907, Bd. v. Heft 1 & 2, pp. 59–80.

——, & Apolant, H.: Beobachtungen über maligne Mäusetumoren. Berl. klin. Wochenschrift, 1905, nos. 25 & 28.

Gierke, E.: Die hæmorrhagischen Mäusetumoren, Ziegler's Beiträge, 1908.

——: The Hæmorrhagic Mammary Tumours of Mice, with Results of Research into Susceptibility and Resistance to Inoculation. Third Scientific Report of the Imperial Cancer Research Fund.

——: Contributions to the Study of the Development of Sarcoma under Experimental Conditions. Third Scientific Report of the Imperial Cancer Research Fund, p. 185.

Hanau: Erfolgreiche experimentelle Uebertragung von Carcinom. Fortschritte der Medizin, 1889, vol. vii.

Jensen, C. O.: Experimentelle Undersögelser over Kräft hos mus. Copenhagen, 1903.

——: Experimentelle Untersuchungen über Krebs bei Mäusen. Centralblatt für Bakteriologie etc., 1903, Bd. xxxiv.

——: Biolog. Selskabs Forhandlinger, 1901–02, pp. 6 & 20.

——: Nogle Forsög. med Kräfstvulster. Hospitalstidende, 1902, no. 19.

Lewin, C.: Experimentelle Beiträge zur Morphologie und Biologie bösartiger Geschwülste bei Ratten und Mausen. Zeitschrift f. Krebsforschung, 1908, Band vi. Heft. 2.

Loeb, L.: 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. University of Pennsylvania Medical Bulletin, July 1906.

——: American Association for Cancer Research, 1st Meeting, 15th Nov., 1907. Extract in the Journal of the Amer. Med. Association, Jan. 4, 1908.

——: Further Investigations in the Transplantation of Tumours. Journal of Medical Research, 1902, vol. viii. p. 60.

——: On Transplantation of Tumours. Journal of Medical Research, 1901, vol. vi. pp. 36 & 37.

——: Ueber Entwicklung eines Sarkoms nach Transplantation eines Carcinoms. Deutche med. Wochenschrift, 1908, no. 1.

——: Ueber Sarkomentwicklung bei einem drüsenartigen Mäusetumor. Berl. klin. Wochenschrift, 1906, no. 24.

Liepman: Münchener med. Wochenschrift, 1907, no. 27.

Moore, C. H.: The Antecedents of Cancer. British Medical Journal, Aug. 12 and Aug. 26, 1865, pp. 164 & 201, also p. 473.

Morau: Recherches expérimentales sur la transmissibilité de certains néoplasmes. Archives de Médecine expérimentale, 1894, p. 677.

Morgan, C. de: Discussion on Cancer. Trans. Path. Soc. of London, 1874, vol. xxv. pp. 287 & 387.

Murray, J. A.: Die Beziehungen zwischen Geschwulstresistenz und histologischem Bau transplantierter Mäusetumoren. Berl. klin. Woch. 1909, No. 33.

——: Spontaneous Cancer in the Mouse. Histology, Metastasis, Transplantability, and the Relations of Malignant New Growths to Spontaneously Affected Animals. Third Scientific Report of the Imperial Cancer Research Fund.

Ribbert, H.: Das Wesen der Krankheit. Bonn, 1909.

Russell, B. R. G.: Sarcoma Development occurring during the Propagation of a Hæmorrhagic Adeno-carcinoma of the Mamma of the Mouse. The Journal of Pathology and Bacteriology, 1910, vol. xiv.

Thiersch, C.: "Die Epithelialkrebs, namentlich der Haut." Leipzig, 1865.

Velich: Wiener Med. Blätter, 1898, Bd. xxi. pp. 711 & 729.

Waldeyer, W.: "Die Entwickelung der Carcinoma," Virchow's Archiv. Bd. xli. 1867, and *ibid.* Bd. lv. 1872.

——: "Ueber den Krebs," Volkmann's Sammlung Klinischer Vorträge, No. 33, 1872.

APPENDIX.

BIBLIOGRAPHY OF ALL COMMUNICATIONS FROM THE LABORATORY OF THE IMPERIAL CANCER RESEARCH FUND.

1903.

The Problems of Cancer. By E. F. Bashford, M.D. British Medical Journal, July 18, 1903.

First Annual Report of the Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London *.
Vide Lancet, 1903, ii. 418, & Br. Med. Journ. 1903, ii. 317, 1558.

Remarks in 'Verhandlungen des Komités für Krebsforschung,' October 1903.
Vide also British Medical Journal, December 12, 1903.

1904.

The Significance of the Zoological Distribution, the Nature of the Mitoses, and the Transmissibility of Cancer. By E. F. Bashford, M.D., and J. A. Murray, M.B., B.Sc. Roy. Soc. Proc. Jan 12, 1904.

The Zoological Distribution of Cancer. By E. F. Bashford, M.D., and J. A. Murray, M.B., B.Sc. First Scientific Report on the Investigations of the Cancer Research Fund, March 1904.

The Transmissibility of Malignant New Growths from one Animal to another. *Ibidem.*

Comparative Cytological Characters of Malignant New Growths. *Ibidem.*

Certain Biological Aspects in the General Pathology of Malignant New Growths. By J. A. Murray, M.B., B.Sc. British Association for the Advancement of Science, Section D, Cambridge, 1904.

* The Annual Reports are business reports presented to the General Committee at the end of each financial year, and must be distinguished from the Scientific Reports. The Annual Reports contain a short summary of the work for each year.

Second Annual Report of the Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London.
Vide Lancet, 1904, ii. 164, & Br. Med. Journ. 1904, ii. 146.

The Comparative Study of Cancer. By E. F. Bashford, M.D. The Sanitary Institute Congress at Glasgow. Journal of the Sanitary Institute, vol. xxv. 1904.

1905.

The Growth of Cancer. By E. F. Bashford, M.D. Discussion on Spontaneous Healing. Transactions of the Medical Society of London, vol. xxviii. 1905.

Ueber die Ætiologie des Krebses. Von E. F. Bashford, M.D. Die Medizinische Klinik, No. 22, 1905.

Limitations to the Statistical Investigation of Cancer. By E. F. Bashford, M.D., and J. A. Murray, M.B., B.Sc. Second Scientific Report of the Imperial Cancer Research Fund.—Part I. The Statistical Investigation of Cancer. March 31, 1905.

Bearing of Biological and Experimental Investigations on Statistics of Cancer. *Ibidem.*

Fallacies inherent in a Cancer Census. *Ibidem.*

Uniformity in Hospital Statistics. *Ibidem.*

Recognition of Cancer. *Ibidem.*

Importance of Microscopical Investigation. *Ibidem.*

Comparison of the Clinical Manifestations with the Results of Microscopical Examination. *Ibidem.*

Distribution of Carcinoma and Sarcoma in the Body at different Age-Periods. *Ibidem.*

Analysis of the Clinical Symptoms of Malignant New Growths which could not be recognised as such during life. *Ibidem.*

Age Incidence of Cancer. *Ibidem.*

Discrepancy between the recorded Cancer Death-rate in Ireland and in Great Britain. *Ibidem.*

Ethnological Distribution of Cancer. *Ibidem.*

Bearing of the provisional Results upon the reputed Increase of Cancer. *Ibidem.*

Bearing of Statistical Inquiries on the Investigation of Cancer. *Ibidem.*

Calculation of Death-rate from uncorrected Data. *Ibidem. Appendix.*

Transplantation of Malignant New Growths. Second Scientific Report of the Imperial Cancer Research Fund.—Part 2. The Growth of Cancer under Natural and Experimental Conditions. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and W. Cramer, Ph.D., D.Sc. April 30, 1905.

Method of Transplantation. *Ibidem.*, Part ii.

General Results of Transplanting Sporadic Tumours of apparently similar Nature. *Ibidem.*, Part ii.

The Growth of Cancer under Natural and Experimental Conditions. April 30, 1905.

Source of the Constituent Elements of New Growths obtained by Artificial Propagation. *Ibidem.*, Part ii.

Stroma is a Specific Reaction on the part of the Host. *Ibidem.*, Part ii.

Comparison between the Transmission of an Infective Granuloma of the Dog and Carcinoma of the Mouse. *Ibidem.*, Part ii.

Infiltrative Extension and Metastasis Formation by Transplanted Malignant New Growths. *Ibidem.*, Part ii.

Absence of a Specific Cachexia in Mice inoculated with Malignant New Growths. *Ibidem.*, Part ii.

Parallel between Propagated and Sporadic Malignant New Growths. *Ibidem.*, Part ii.

Variations in the Rate of Growth and in the Results of Transplantation. *Ibidem.*, Part ii.

Relative Importance of Cells introduced and of Soil. *Ibidem.*, Part ii.

Interpretation of the Differences and Variations in Transplanted Tumours of one Organ. *Ibidem.*, Part ii.

Action of Radium on Transplanted Mouse Tumours and its Relation to the Spontaneous Arrest of their Growth. *Ibidem.*, Part ii.

Hypotheses explanatory of the Nature and Origin of Cancer reviewed in the light of the Results of Experimental Study. *Ibidem.*, Part ii.

The Positive Results of Experimental Study. *Ibidem.*, Part ii.

Third Annual Report of the Imperial Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London.
Vide Lancet, 1905, ii. 104 & Br. Med. Journ. 1905, ii. 96.

Einige Ergebnisse der experimentellen Krebsforschung. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and W. Cramer, Ph.D., D.Sc. Berliner Klinische Wochenschrift, No. 46, 1905.

1906.

Einige Bemerkungen zur Methodik der experimentellen Krebsforschung. By E. F. Bashford, M.D. *Ibidem.*, No. 16, 1906.

On the Occurrence of Heterotypical Mitoses in Cancer. By E. F. Bashford, M.D., and J. A. Murray, M.B., B.Sc. Royal Society Proceedings, B, vol. 77, 1906.

Are the Problems of Cancer Insoluble? By E. F. Bashford, M.D. Lancet and British Medical Journal, Dec. 9, 1906.

Experimental Analysis of the Growth of Cancer. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and W. H. Bowen, M.S., F.R.C.S. Royal Society Proceedings, B. vol. 78, May 1906.

L'État actuel de la question du Cancer. By E. F. Bashford, M.D. Revue Scientifique, Juin 2 et 9, 1906.

Illustrations of Propagated Cancer. By E. F. Bashford, M.D. British Medical Journal, May 26, 1906.

Imperial Cancer Research Fund, London. By J. A. Murray, M.B., B.Sc. La Science au XX^me^ Siècle, Nov, 1906.

Fourth Annual Report of the Imperial Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London.
Vide Lancet, 1906, ii. 314, & Br. Med. Journ. 1906, ii. 207.

The Investigations of the Imperial Cancer Research Fund. By E. F. Bashford, M.D. British Medical Association, Annual Meeting, Toronto, August 1906. British Medical Journal, Dec. 1, 1906.

Address at Opening Ceremony of the First International Cancer Conference. Heidelberg, Frankfurt a/M., Sept. 25–27, 1906. Abhandlung der Internationalen Conferenz für Krebsforschung Heidelberg u. Frankfurt a/M., Zeitschrift für Krebsforschung, 1907, Bd. v. Heft 1 & 2.

Morau and Jensen's Tumour. The Lancet, Dec. 1st, 1906, p. 1544.

Zahl und Grössenverhältnisse der Chromosomen bei *Lepidosiren paradoxa*, Fitz. By J. A. Murray, M.B., B.Sc. Anatomischer Anzeiger, Band xxix. 1906.

Quelles épreuves scientifiques a-t-on aujourdhui de la nature parasitaire des néoplasies, spécialement du Cancer? By E. F. Bashford, M.D. XV. Congrès International de Médecine, Lisbon, 1906.

Classification des Sarcomes. By E. F. Bashford, M.D. *Ibidem.*

1907.

Real and Apparent Differences in the Incidence of Cancer. By E. F. Bashford, M.D. Transactions of the Epidemiological Society of London, Jan, 18, 1907.

Carcinoma Mammæ in the Mouse. By E. F. Bashford, M.D., and J. A. Murray, M.B., B.Sc. Lancet, March 23, 1907.

The Natural and Induced Resistance of Mice to the Growth of Cancer. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and W. Cramer, Ph.D., D.Sc. Royal Society Proceedings, B. vol. 79, 1907.

The Application of Experiment to the Study of Cancer. By E. F. Bashford, M.D. Science Progress, July 1907.

Die Experimentelle Analyse des Carcinomwachstums. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and W. H. Bowen, M.S., F.R.C.S. Zeitschrift für Krebsforschung, 1907.

Fifth Annual Report of the Imperial Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London.
Vide Lancet, 1907, ii. 44, & Br. Med. Journ. 1907, ii. 26.

Nyere undersögelser over krœft. By M. Haaland. Tidsskrift for Den norske laegeforening, Nos. 22–23, 1907.

Ergebnisse der Experimentellen Krebsforschung. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and M. Haaland.—I. Ein trans. plantables Platten-epithelcarcinom der Maus.—II. Entwicklung eines Sarkoms während fortgesetzter Carcinomtransplantationen. Berliner klinische Wochenschrift, Nos. 38, 39, 1907.

1908.

The Induction of Specific Resistance and of General Enhanced Susceptibility to Inoculation of Carcinoma and Sarcoma in Rats and Mice. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and M. Haaland. Proceedings of the Pathological Society of Great Britain and Ireland. Journal of Pathology, vol. xii. 1908.

The Processes at the Site of Inoculation in Normal Mice and in Mice Resistant to Carcinoma. By B. R. G. Russell, M.B., Ch.B. *Ibidem.*

The Clinical Behaviour of Spontaneous Tumours in Mice. By J. A. Murray, M.B., B.Sc. *Ibidem.*

A Transplantable Squamous-Celled Carcinoma. By J. A. Murray, M.B., B.Sc. and M. Haaland. *Ibidem.*

Development of a Spindle-Celled Sarcoma during Propagation of an Adeno-Carcinoma of a Mouse. By M. Haaland. *Ibidem.*

The Occurence of Glycogen in Mouse Tumours. By M. Haaland. *Ibidem.*

Die Hämorrhagischen Mäusetumoren mit Untersuchungen über Geschwulst-resistenz und -disposition bei Mäusen. By E. Gierke, M.D. Ziegler's Beiträge zur pathologischen Anatomie, 1908.

Sixth Annual Report of the Imperial Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London.
Vide Lancet, 1908, ii. 177, & Br. Med. Journ. 1908, ii. 162.

Liquid Air and Cancer. By E. F. Bashford, M.D. Lancet, Feb. 1, 1908, p. 394.

The Ethnological Distribution of Cancer. By E. F. Bashford, M.D. Third Scientific Report of the Imperial Cancer Research Fund, 1908. Taylor & Francis, Red Lion Court, Fleet Street, London.

The Occurrence of New Growths among the Natives of New Guinea. By C. G. Seligmann, M.D., M.R.C.P. *Ibidem.*

The Zoological Distribution of Cancer. By J. A. Murray, M.B., B.Sc. *Ibidem.*

The Occurrence of Heterotypical Mitoses in Cancer (*reprinted*). *Ibidem.*

Spontaneous Cancer in Mice, Histology, Metastasis, Transplantability and the Relations of Malignant New Growths to Spontaneously Affected Animals. By J. A. Murray, M.B. B.Sc. *Ibidem.*

The Hæmorrhagic Mammary Tumours of Mice, with Results of Research into Susceptibility and Resistance to Inoculation. By E. Gierke, M.D. *Ibidem.*

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. *Ibidem.*

A Transplantable Squamous-celled Carcinoma of the Mouse. By J. A. Murray, M.B., B.Sc. *Ibidem.*

Contributions to the Study of the Development of Sarcoma under Experimental Conditions. By M. Haaland. *Ibidem.*

General Results of the 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. *Ibidem.*

The Experimental Analysis of the Growth of Cancer. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and W. H. Bowen, M.S., F.R.C.S. *Ibidem.* (*Reprinted.*) *Ibidem.*

The Natural and Induced Resistance of Mice to the Growth of Cancer. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and W. Cramer, Ph.D., D.Sc. *Ibidem.* (*Reprinted.*) *Ibidem.*

The Nature of Resistance to the Inoculation of Cancer. By B. R. G. Russell, M.B., Ch.B. *Ibidem.*

Resistance and Susceptibility to Inoculated Cancer. By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and M. Haaland. *Ibidem.*

Report on a Study of the Variations of Hydrochloric Acid in the Gastric Contents of Mice and Rats, as compared with the Human Subject in Cancer. By S. Monckton Copeman, M.D. Cantab., F.R.S., and J. Wilson Hake, Ph.D., F.I.C., F.C.S. *Ibidem.*

Glycogen and Fat in Malignant New Growths of the Mouse. By M. Haaland. *Ibidem.*

The Gaseous Metabolism in Rats inoculated with Malignant New Growths. By W. Cramer, Ph.D., D.Sc.

Advance in knowledge of Cancer. By E. F. Bashford, M.D. 'Nature,' Dec. 31, 1908.

1909.

Heredity in Cancer. By E. F. Bashford, M.D. Proceedings of the Royal Society of Medicine, Jan. 1909. Also published separately: The Influence of Heredity on Disease, with special reference to Tuberculosis, Cancer, and Diseases of the Nervous System, a Discussion. W. S. Church, W. R. Gowers, A. Latham, and E. F. Bashford. Longmans, Green & Co. 1909.

Ergebnisse der Experimentellen Krebsforschung. By E. F. Bashford, M.D., M. Haaland, M.D., and J. A. Murray, M.D., B.Sc.

i. Ueber das Wachstum überimpfter Tumoren.

ii. Wachstumshemmende und -förderende Einflüsse. Zeitschrift für Immunitätforschung und experimentelle Therapie. Bd. i. Heft. 4, 1909.

On the occurrence of Cancer in mice of known age. By E. F. Bashford, M.D., and J. A. Murray, M.D., B.Sc. Royal Society Proceedings B. vol. 81, p. 310.

Zur Kenntnis der peptolytischen Fermente verschiedenartiger Krebse und anderer Tumorarten. II. Mittheilung. By Professor E. Abderhalden, A. H. Koelker, and F. Medigreceanu, M.D. Hoppen-Seyler's Zeitschrift für Physiologische Chemie, Bd. 62, 1909 *.

Seventh Annual Report of the Imperial Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London.
Vide Lancet, 1909, ii. 169, & Br. Med. Journ. 1909, ii. 151.

Die Beziehungen zwischen Geschwulstresistenz und histologischen Bau transplantierter Mäuse-tumoren. By J. A. Murray, M.D., B.Sc. Berl. klin. Wochenschr., No. 33, 1909.

Address on Cancer in Man and Animals. By E. F. Bashford, M.D. Before a General Meeting of the XVI. International Congress of Medicine. Buda Pest, Sept. 3rd, 1909. Published in the Transactions of the Congress, in the London 'Lancet,' Sept. 4th, 1909; The New York Medical Record, Sept. 4th, 1909; and in German in the Berl. klin. Wochenschr., Sept. 6th, 1909.

The Etiology and Pathogeny of Malignant New Growths, especially of Cancer. By E. F. Bashford, M.D. Trans. of the IXth International Veterinary Congress, Sept. 1909.

Autoplastic and Homoplastic Transplantation of Tumours in Mice. By M. Haaland. Trans. Path. Sect. Roy. Soc. of Med., Nov. 16th, 1909.

Dissemination of Carcinoma by the Blood Stream in Animals. By J. A. Murray. Trans. Path. Sect. Roy. Soc. of Med., Nov. 16th, 1909.

Experimental Production of Sarcoma in Mice. By B. R. G. Russell, M.D. Trans. Path. Sect. Roy. Soc. of Med., Nov. 16th, 1909.

Om Organismens reaktioner mod pathalogisk cellevekst. By M. Haaland, M.D. Norsk Magazin for lægevidenskaben. No. 11, 1909.

1910.

The Contrast in the Reactions to the Implantation of Cancer after the Inoculation of Living and Mechanically Disintegrated Cells. By M. Haaland, M.D. Proc. Roy. Soc. B, vol. 82, 1910.

Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths. By E. F. Bashford, M.D., and B. R. G. Russell, M.D. Proc. Roy. Soc. B, vol. 82, 1910. 'Lancet.'

On the Relative Sizes of the Organs of Rats and Mice bearing Malignant New Growths. By F. Medigreceanu, M.D. Proc. Roy. Soc. B, vol. 82, 1910.

* In collaboration with the Physiological Institute of the Royal Veterinary College, Berlin.

Sarcoma Development occurring during the Propagation of a hæmorrhagic Adeno-carcinoma of the Mamma of the Mouse. By B. R. G. Russell, M.D. Journal of Pathology and Bacteriology, vol. xiv. 1910.

Zelluläre Analyse der Geschwulstimmunitätsreaktionen. By C. De Fano, M.D. Zeitschrift für Immunitätsforschung und experimentelle Therapie, 1910.

Resistance in Mice against Transplanted Cancer by Auto-inoculation of the Spleen. By W. H. Woglom, M.D. Jour. of Exp. Med., vol. xii. No. 1, 1910.

Ergebnisse eines Fütterungsversuches bei Ratten, die überimpfe Tumoren trugen. By F. Medigreceanu, M.D. Berl. klin. Wochenschr. No. 17, 1910.

Contributions to the Bio-chemistry of Growth.—The Total Nitrogen Metabolism of Rats bearing Malignant New Growths. By W. Cramer, Ph.D., D.Sc., and H. Pringle, M.D. Proc. Roy. Soc. B, vol. 82, 1910 *.

Cyclical Structural Changes in transplanted Tumours. By J. A. Murray, M.D. Proc. of the Path. Soc. of Great Britain & Ireland, Jan. 1910.

Means of Inducing Resistance to the Transplantation of Cancer tested in spontaneously affected Mice. By M. Haaland, M.D. Proc. of the Path. Soc. of Great Britain & Ireland, Jan. 1910.

Sarcoma development during the Propagation of a hæmorrhagic Adeno-carcinoma of the Mouse. By B. R. G. Russell, M.D. Proc. of the Path. Soc. of Great Britain & Ireland, Jan. 1910.

Den experimentelle pathologis betydning for studiet af almindelig biologiske fenomener. By M. Haaland, M.D. Norsk Magazin for laegevidenskaben, No. 7, 1910.

Contributions to the Bio-chemistry of Growth.—Distribution of Nitrogenous Substances in Tumour and Somatic Tissues. By W. Cramer, Ph.D., D.Sc., and H. Pringle, M.D. Proc. Roy. Soc. B, vol. 82, 1910 †.

The Immunity Reaction to Cancer. By E. F. Bashford, M.D. Proc. Roy. Soc. of Medicine, March 1910.

Ueber die Grössenverhältnisse einiger der wichtigsten Organe bei tumortragenden Mäusen und Ratten. By F. Medigreceanu, M.D. Berl. klin. Wochenschr., No. 13, 1910.

On the absence of Spirochætes in Mouse Tumours. By J. McIntosh, M.D. Centralb. f. Bakteriologie, Parasititenkunde u. Infektionskrankheiten, 1910 †.

Zur Kenntnis der peptolytischen Fermente verschiedener Krebse und anderer Tumorarten. By Professor E. Abderhalden and F. Medigreceanu, M.D. Hoppe-Seyler's Zeitschrift für Physiologische Chemie, 1910 †.

* In collaboration with the Department of Physiological Chemistry, University of Edinburgh.

† In collaboration with the Bacteriological Department, London Hospital.

Zur Kenntnis der peptolytischen Fermente verschiedener Krebse und anderer Tumorarten.—III. Mitteilung. By Professor E. Abderhalden and F. Medigreceanu, M.D. Hoppe-Seyler's Zeitschrift für Physiologische Chemie, vol. 66, 1910 *.

Eighth Annual Report of the Imperial Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London.
Vide Lancet, 1910, ii. 265, & Br. Med. Journ. 1910, ii. 205.

Cancer in Domesticated Animals. By J. A. Murray, M.D., B.Sc. Annual Conference of Veterinary Officers of Health, Glasgow. 'Veterinary News,' September 1910.

Address to the Delegates at Opening Ceremony of Second International Cancer Conference, Paris, 1910. By E. F. Bashford, M.D. Travaux de la deuxième Conférence internationale pour l'Étude du Cancer, Paris, Oct. 1–5, 1910. Felix Alcan, Paris, 1911.

1911.

Spontaneous Cancer in Mice. By M. Haaland, M.D. Proc. Roy. Soc. B, vol. 83, 1911.

Cancerous Ancestry and the Incidence of Cancer in Mice. By J. A. Murray, M.D., B.Sc. Proc. Roy. Soc. B, vol. 84, 1911.

The Obligation imposed upon the General Practitioner by the Development of the Experimental Investigation of Cancer. By E. F. Bashford, M.D. 'Practitioner,' March 1911.

Report on Cancer Research (International Conference, Paris 1910). Eyre & Spottiswoode, 1911.

Ninth Annual Report of the Imperial Cancer Research Fund. Taylor & Francis, Red Lion Court, Fleet Street, London.
Vide Lancet, 1911, ii. 315, & Br. Med. Journ. 1911, ii. 171.

Mice Immunised subcutaneously are resistent to the implantation of Cancer in Internal Organs. By W. H. Woglom, M.D. 'The Lancet,' July 8, 1911.

Neue Beiträge zur Theorie der Individualität des Krebses. By W. H. Woglom, M.D. Zeitschrift für Immunitätsforschung, Bd. xi. 1911.

Spontaneous Tumours in Mice. By M. Haaland, M.D. Fourth Scientific Report of the Imperial Cancer Research Fund, 1911. Taylor & Francis, Red Lion Court, Fleet, London.

Cancerous Ancestry and the Incidence of Cancer in Mice. By J. A. Murray, M.D., B.Sc. *Ibidem.*

The Behaviour of Tumour-cells during Propagation. By E. F. Bashford, M.D. *Ibidem.*

* In collaboration with the Physiological Institute of the Royal Veterinary College, Berlin.