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

FIFTH 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.

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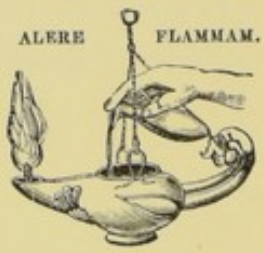

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CONTENTS.

[*Fifth Scientific Report of the Imperial Cancer Research Fund*, 1912.]

THE MANIFESTATION OF ACTIVE RESISTANCE TO THE GROWTH OF IMPLANTED CANCER.

By B. R. G. RUSSELL, M.D.

In the present communication there will be considered certain of the problems which have presented themselves during the investigation of resistance and susceptibility to the inoculation of transplantable tumours. The subject of the immune reactions in animals with spontaneous growths will not be entered into, for this has recently been amply discussed in these Reports by Haaland (28, pp. 79–85). The sequence in which the various points will be raised has been made as far as possible a logical one, and though it has not been possible to carry this out in every case, still it is thought that the arrangement under the following headings will avoid much redundancy of description, and also preserve the continuity of the argument which runs through the whole series of experiments.

The headings adopted are :—

The Reaction of Animals to Tumours of various Types of Growth.
Simultaneous Inoculation of two Tumours of Different Types of Growth.
Can Animals Bearing Progressively Growing Tumours be Protected against Re-inoculation ?
Attempts to Arrest the Development of Progressively Growing Tumours.

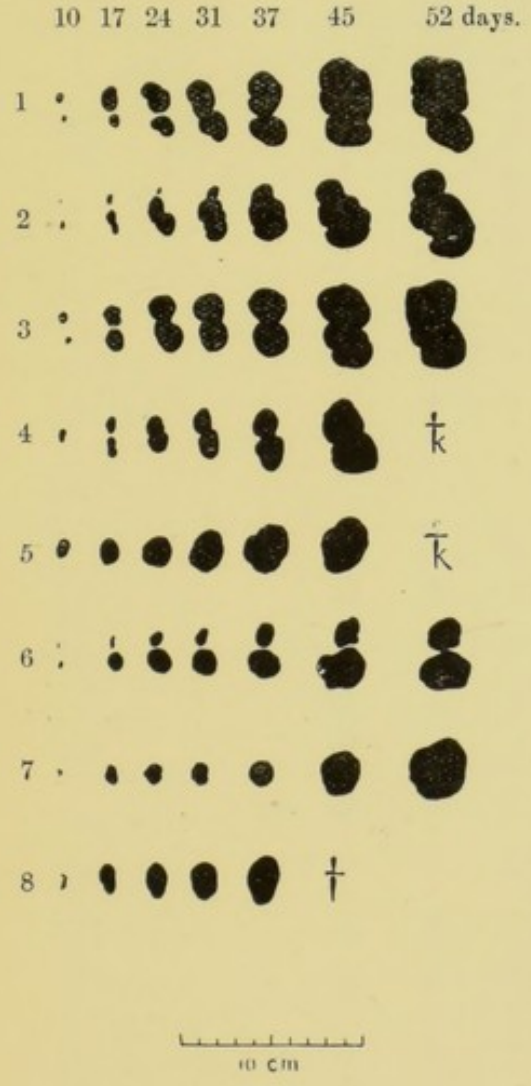
The Reaction of Animals to Tumours of various Types of Growth.

Wide variations in the rate of growth, and in the percentage of success following the inoculation of different tumour-strains have been observed wherever several tumours have been cultivated over an extended period of time. Further, there are also features characteristic of the growth of different tumours, and it is this point which will be more closely followed in the succeeding pages. Citation of all the published articles bearing upon this subject has already appeared in these

Reports, and on this occasion reference need be made only to two figures published in the Fourth Scientific Report (4). On page 208, fig. 69, a chart of tumour-strain **63** is given, where in every animal inoculated a *progressively growing tumour* has developed. On page 195, fig. 61 *a*, a chart of tumour **206** appears, but in this case all the growths which developed finally disappeared spontaneously. Both of these figures are reproduced here (figs. 1 and 2) for the convenience of the reader. These two carcinomatous strains exemplify the possible extremes of type of growth, and the intermediate gradations between them have been actually filled up in practice in this laboratory by the cultivation of a large number of different tumour-strains. There must be one or more factors in constant operation to give the two extreme results cited above, and the understanding of these phenomena has to a considerable extent been advanced by Bashford, Murray, and Haaland (10, 11), since they have shown that the resistance to re-inoculation which a tumour-bearing animal frequently exhibits, is of the nature of an active immunity. This development of an active immunity or rather resistance during the growth of a transplanted tumour (concomitant immunity) is a most important factor in determining the character of growth which a tumour will show, and the following experiments demonstrate this in as clear a manner as can be expected from a biological experiment.

The procedure adopted in the experiments now to be described has been to inoculate in one flank a series of animals with a given tumour-strain, and then to extirpate surgically all the growths after 10–30 days, *i. e.* after intervals long enough to allow the tumours to attain a considerable size, 1–4 grammes. It may here be mentioned that young animals of the same breed, about 6–8 weeks old and weighing 14–18 grammes, have been used throughout. One, two, or three days after extirpation, the animals have been re-inoculated on the other flank with a tumour of the same or of another strain. In this way two readings are obtained ; the result of inoculating a given series of normal animals, and the result of inoculating the same animals after a tumour had been growing in them over a known period. The precise way in which the experiments have been carried out will be rendered clearer by the accompanying charts which portray the tumours first inoculated as black silhouettes, whilst the tumours inoculated after operation, as also the controls to the second inoculation, are given in red. In addition, such data as the date of inoculation, of re-inoculation, of recording the result, the amount of tumour-tissue injected, etc., have also been printed on the charts.

EXP. 63/48 K.—INOCULATED (15.5.11) WITH 0·015 GRMS. IN RIGHT AXILLA. NEEDLE.



EXP. 206/11 C. 15 MICE INOCULATED IN RIGHT AXILLA WITH 0·02 GRM.: NEEDLE (7.1.09).

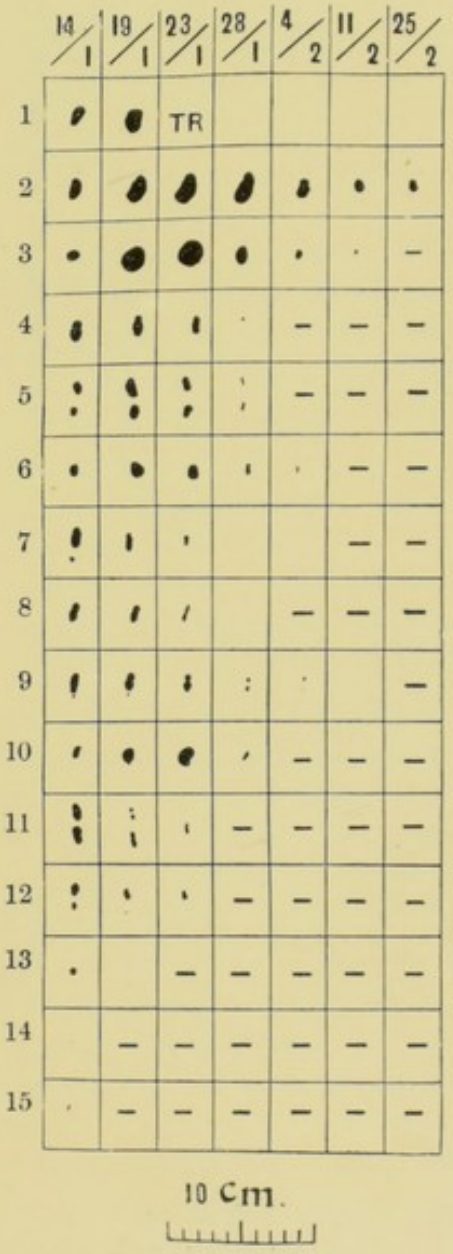


FIG. 1. Exp. 63/48 K.—Chart of growth of strain 63, which shows progressive growth of all tumours.

FIG. 2. Exp. 206/11 C.—Chart showing the temporary character of the growth of tumour 206 in normal mice.

EXP. **63**/52 E. MICE 1–8 INOCULATED IN RIGHT AXILLA, DOSE 0·02 C.C. (31.8.11). THE RESULTING TUMOURS EXCISED (19.9.11), AND THE MICE RE-INOCULATED IN LEFT AXILLA WITH 0·03 C.C. OF **63**/53 C (21.9.11). MICE 9–18: CONTROL TO RE-INOCULATION.

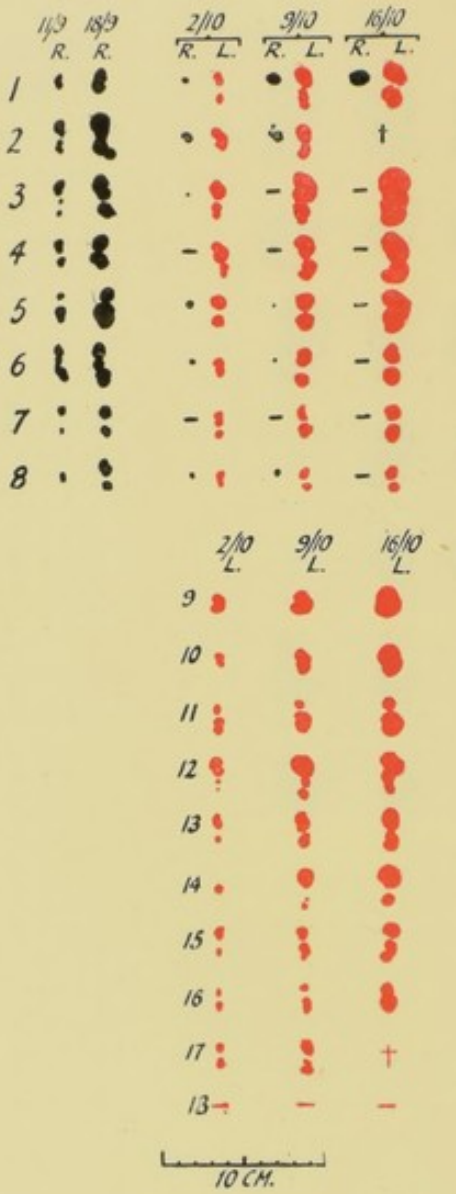


FIG. 3.—The black silhouettes represent the tumours developing from the primary inoculation, and, when present, recurrences after operation. The red silhouettes represent the tumours developing from re-inoculation after operation upon mice 1–8, and also the control growths in normal mice 9–18. The experiment shows that complete or incomplete surgical removal of tumours of strain **63** does not alter the original suitability of mice for growth of this tumour-strain.

A description of the behaviour of a tumour-strain exhibiting one extreme type of growth, viz., a strain giving progressively growing tumours in practically every animal inoculated, will serve as a suitable introduction. Carcinoma **63** is such a strain, and in fig. 3 is given a chart of an experiment where eight mice were inoculated in the right axilla with this tumour, and where all the mice developed growths. The sizes of these tumours at the 11th and 18th days are reproduced to scale in the two left-hand columns in black. On the 19th day all the eight tumours were excised, and two days later the mice were re-inoculated in the left axilla with another growth of the same strain. In two mice only (Nos. 1 and 2), was there any recurrence from incomplete extirpation, and these recurrent tumours are portrayed as black silhouettes in the chart under the column headed R (R=right axilla). The small nodules present in mice Nos. 3, 5, 6 and 8 were certainly not recurrent growths, but were in all probability inflammatory nodules due to the presence of ligatures. The tumours developing from inoculation into the left axilla are charted in red under the column headed L (L=left axilla); in addition ten normal mice, Nos. 9–18, were inoculated with the same material, their growths appearing also in red. This experiment shows clearly that this particular strain (tumour **63**) in no way alters the suitability of mice for subsequent inoculation, in other words, its power of inducing resistance is *nil*.

In this experiment, the circumstance that the excision was followed by recurrence in two of the eight cases, in no way invalidates the reading given, because the result obtained was the same in all cases, but when strains which induce resistance in a certain percentage of cases are to be tested, then it it is absolutely essential that the excision be complete in every case. When the reaction set up by a tumour is not the same in every animal of a series, incomplete excision will lead to recurrence in certain cases but not in others, and the observer will be hampered in the reading of the experiment by the play of an involuntary selection. The experiment with strain **63** was one of the first of the present series, and is the only one where the excision was shown to be incomplete in any member of a series. This result has been obtained by taking precautions during inoculation of the tumour to ensure that the emulsion was deposited within the layers of the subcutaneous fascia. If too sharp a needle be employed for inoculation, the abdominal and thoracic walls are apt to be infiltrated early, rendering subsequent excision arduous and uncertain.

Another strain, carcinoma **T**,* behaves in a manner similar to the preceding. It is a more slowly growing tumour, gives a high percentage of success on transplantation, and only in exceptional series shows much evidence of spontaneous absorption. Fig. 4 depicts a chart where a series of eleven mice bearing this tumour have had their

EXP. **T**/40 C. MICE 1–11 INOCULATED IN RIGHT AXILLA WITH 0·02 C.C. (20.10.11). TUMOURS EXCISED (22.11.11), AND THE ANIMALS RE-INOCULATED IN LEFT AXILLA WITH 0·02 C.C. OF **63**/55 E (24.11.11).

FIG. 4.—The growth of tumour-strain **T** followed by surgical removal does not render mice unsuitable for subsequent inoculation. Tumours of primary inoculation in black, of secondary inoculation in red.

growths excised on the 33rd day, and have been re-inoculated with strain **63** two days later. The eleven animals all remained free from recurrence, and in every case strain **63** gave rise to a rapidly and progressively growing tumour. Carcinoma **T**, therefore, belongs with carcinoma **63** to the group which in no way alters the suitability of

* The material from the primary tumour of this strain was obtained from Dr. F. W. Twort.

mice for subsequent grafting. These two strains have been extensively used for re-inoculation in the following experiments conducted with other strains which alter the suitability for re-inoculation, because they grow regularly in a high percentage, 80–100 per cent., and also because they are what may be called delicate indicators of inoculability. It has just been shown that they themselves do not induce resistance, and, therefore, when they fail to develop in a series of animals this may justly be attributed to pre-existing causes.

A tumour of an intermediate type of growth will be described next, *i. e.* one exhibiting a certain amount of spontaneous absorption. The sarcoma developed from the stroma of carcinoma **100** (34) (sarcoma **100**) grows very irregularly, but always shows a high percentage of spontaneous absorptions. Sometimes it grows at a speed surpassing all other strains, at others comparatively slowly. When this strain is growing well, the phenomenon of spontaneous absorption may be greatly masked, as large tumours are developed within a short period, and the animals live only for a short time after inoculation. Tumours weighing 4 grammes have been observed as early as the twelfth day after inoculation of such a small dose as 0·015 grms. Fig. 5 gives the chart of an experiment carried out with sarcoma **100** when growing rapidly. In this case operative measures had to be taken as early as the 10th day, as the tumour infiltrates muscles and skin very rapidly. The chart has been constructed in the same way as the preceding ones, and requires no special elucidation. It will be seen from the figure that ten days growth of sarcoma **100** lowers the percentage of "takes,' on re-inoculating with strain **63**, from 92 per cent. to 40 per cent. In other words, of ten mice suitable for the growth of strain **63**, six have been rendered unsuitable by sarcoma **100**. The surgical interference cannot be inculpated, as it in no way interfered with the re-inoculability of mice from which tumours of strain **63** and strain **T**, the two previously described, had been excised.

A carcinomatous mouse tumour, strain **199**, exhibits certain peculiarities in its manner of growth, which were described in a communication published two years ago (12). It was then noted that in a series of mice inoculated with this strain, about one-third would show progressively growing tumours, another third would show spontaneous absorption after temporary growth of variable duration, whilst the remaining third would after temporary cessation resume the progressive type of growth. It was also shown that there was an active resistance induced not only in the cases where the tumours had undergone

EXP. 100/58 B. MICE 1-10 INOCULATED IN RIGHT AXILLA WITH 0·02 C.C. (6.11.11). TUMOURS EXCISED (16.11.11), AND MICE RE-INOCULATED ONE DAY LATER WITH 0·03 C.C. OF 63/55 D IN LEFT AXILLA. MICE 11-22: CONTROL TO RE-INOCULATION.

FIG. 5.—Sarcoma 100 renders the majority of mice unsuitable for growth on re-inoculation, only 4 out of 10 mice developing tumours as against 11 out of 12 in the control.

EXP. 199/30 A. ALL MICE INOCULATED IN RIGHT AXILLA WITH 0·02 C.C. (14.6.11).

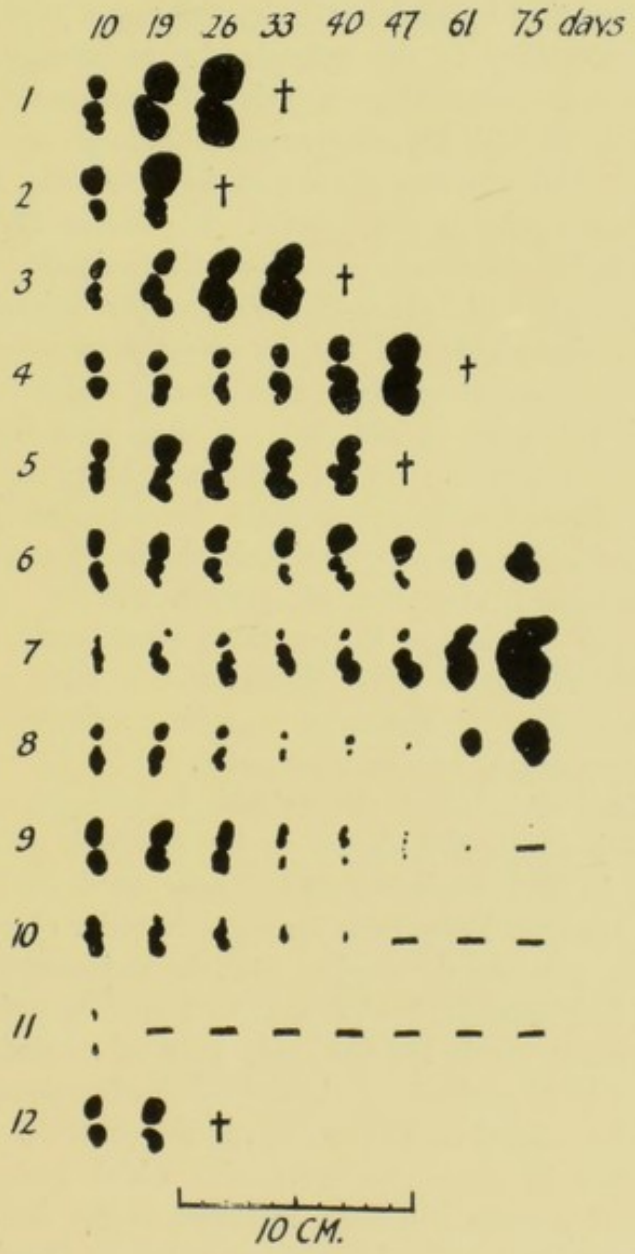


FIG. 6.—Shows various types of growth of the same tumour-parenchyma in one batch of animals.

spontaneous absorption, but also where they had temporarily ceased to enlarge their dimensions. The chart of a comparatively recent series of this strain is shown in fig 6, from which it will be seen that the tumour still continues to manifest the type of growth observed in the earlier generations. An analysis of the reaction set up by it in a series of mice was attempted by excising the growths on a given day, and then testing the suitability of the animals for re-inoculation. Fig. 7 exemplifies an experiment where a series of tumours of strain **199** had been removed on the 16th day, whereupon the animals were re-inoculated with strain **T**. Four tumours developed in nine mice thus treated as against nine in ten mice in the control. Carcinoma **199** thus induces active resistance in rather more than half the animals within a period of sixteen days. It will be noted that the four animals which were found to be re-inoculable did not in every case present the most rapidly growing tumours of **199** ; mice Nos. 5 and 6 each showed a rapidly growing tumour of **199**, and yet they were found to be unsuitable for re-inoculation. On referring back to fig. 6, where the **199** tumours were allowed to complete their development, it will be seen that one cannot in every case predict from the first two chartings alone what the subsequent character of growth will be. In some cases disappearance or diminution in size takes place early, in others it is deferred even for weeks. There is therefore a wide variation in the period at which the consequences of active resistance will become manifest in any member of a series of mice.

This individual variation of the animals is brought out in another experiment with strain **199**, shown in fig. 8, which differs only from the preceding in that carcinoma **63** has been used for re-inoculation. Here the **199** tumours have been arranged in order of size, but it will be seen that the growths developing from the re-inoculation do not fall upon the first four animals, but are scattered over the series of ten mice. In this experiment strain **199** has again induced a considerable degree of resistance, only 40 per cent. of the animals having developed tumours on re-inoculation as against 82 per cent. in the controls.

The discussion of those tumour-strains which present the other extreme type of growth, has been reserved to the last. This group comprises all strains which give rise to tumours growing only transitorily for varying periods, and in almost every case terminating in complete disappearance. The number of transplantable tumours exhibiting this peculiarity is large, but the date at which spontaneous absorption sets in varies widely from series to series, and from animal

EXP. **199**/32 C. MICE 1–9 INOCULATED IN RIGHT AXILLA, DOSE 0·015 C.C. (30.9.11). THE RESULTING TUMOURS EXCISED (16.10.11), AND THE MICE RE-INOCULATED IN LEFT AXILLA WITH 0·02 C.C. OF **T**/40 C (20.10.11). MICE 10–19: CONTROL TO RE-INOCULATION.

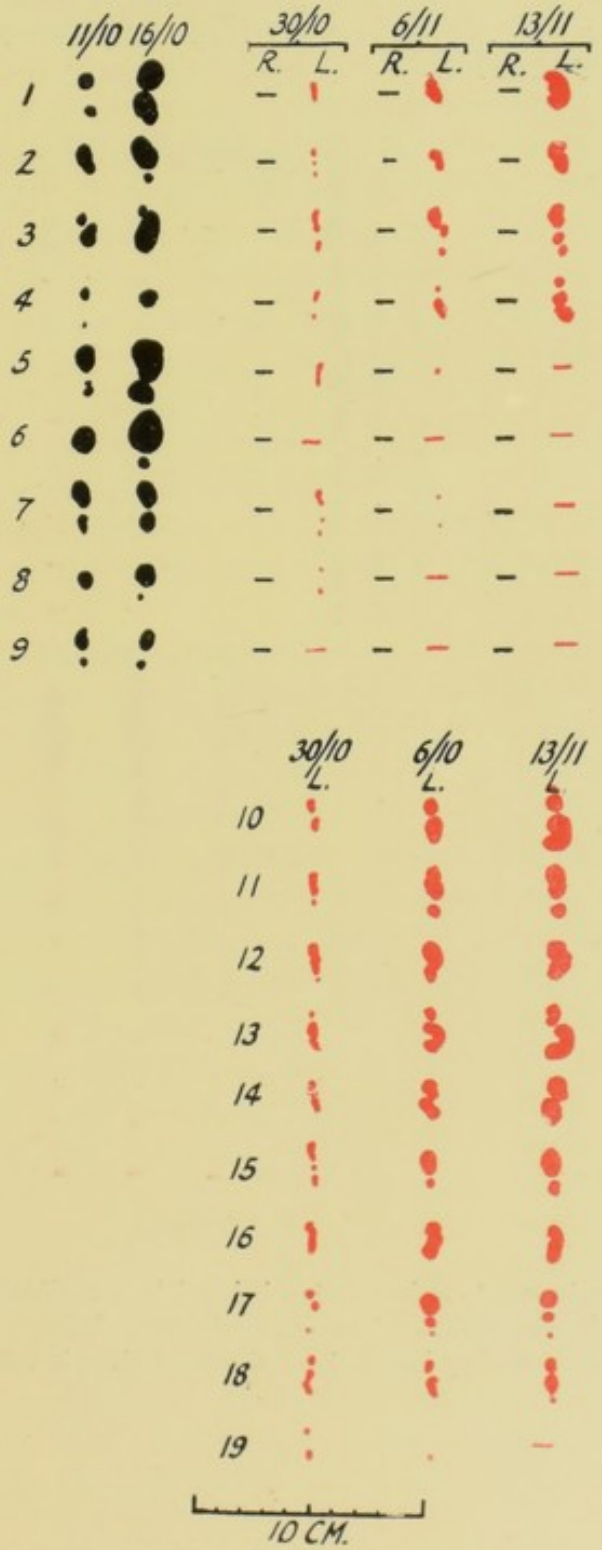


FIG. 7.—Growth of strain **199** induces a considerable percentage of resistance to re-inoculation of strain **T**. Primary inoculation given in black, secondary inoculation and control given in red.

EXP. **199**/33 A. MICE 1-10 INOCULATED IN RIGHT AXILLA, DOSE 0·02 C.C. (16. 10. 11). THE RESULTING TUMOURS EXCISED (3. 11. 10), AND THE MICE RE-INOCULATED IN LEFT AXILLA WITH 0·02 C.C. OF **63**/54 F (6. 11. 11). MICE 11-21: CONTROL TO RE-INOCULATION.

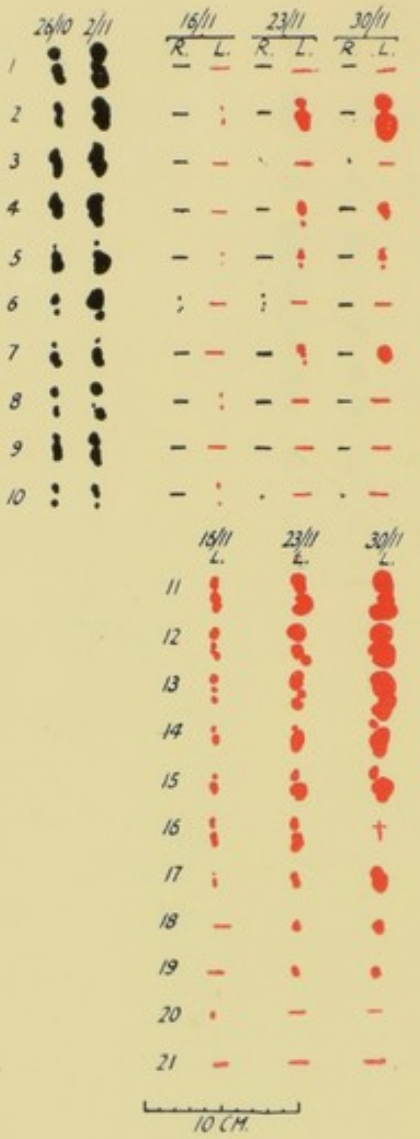


FIG. 8.—Shows resistance to re-inoculation induced in a considerable percentage of cases by the growth of tumour-strain **199**. Primary inoculation given in black, secondary inoculation and control given in red.

to animal, so that the majority of these strains are ill-suited for the investigation of resistance. Tumour-strain **206** is, however, an exception, as it grows in a very high percentage of inoculated animals, and the date of onset of spontaneous absorption is remarkably regular. Several experiments have been made with this carcinomatous strain, but the results obtained have been so decisive, that only one need be illustrated. The result of this experiment is given in fig. 9 where it will be seen that eleven days growth of this strain in eleven mice has been sufficient to render every one of these animals unsuitable for the growth of carcinoma **63**. This concludes the description of the findings with mouse tumours, and indicates how extremely variable in action their parenchymata may be, leading from the case where no resistance is induced, through all gradations to the case where resistance is induced in every animal.

The behaviour of a transplantable rat sarcoma, obtained from Jensen, has also been investigated in a manner similar to that already detailed for various mouse tumours. This strain, **J.R.S.**, is a rapidly growing spindle-cell sarcoma which gives a high percentage of success on transplantation.

Spontaneous healing occurs with great frequency in series of this tumour, and large masses of growth, weighing 10–15 grms., often disappear entirely. The re-inoculability of rats bearing this tumour has been already described and figured in the Third Scientific Report (10, p. 390), where it was concluded that the results obtained on re-inoculation could be explained only by assuming the development of resistance during the growth of the tumour first inoculated. The presence of large rapidly growing neoplasms during the period in which the re-inoculation tumours are developing, presents both an actual and a theoretical complication which it seemed desirable to eliminate. Accordingly the tumours from the first inoculation have been allowed to develop for a certain period, then all have been excised, and a re-inoculation has been made to ascertain what alteration had taken place in the suitability of the soil. Such an experiment is given in fig. 10 which represents the much reduced chart of a series of fourteen rats inoculated with this sarcoma, and shows the size to scale of the growths at the 11th, 18th, and 25th days. The dose of tumour-tissue injected was 0·02 c. c., except in the case of rats Nos. 1, 2, 8, 9, and 11 which received a dose of 0·05 c.c. The rats were divided into two batches on the 27th day ; rats Nos. 1–7 had their tumours excised completely, whilst in rats Nos. 8–14 a small piece of tumour was left behind without interrupting

EXP. **206**/100 B. MICE 1–11 INOCULATED IN RIGHT AXILLA, DOSE 0·03 C.C. (11. 12. 11). TUMOURS EXCISED (22. 12. 11), AND THE MICE RE-INOCULATED IN LEFT AXILLA WITH 0·02 C.C. OF **63**/56 D (23. 12. 11). MICE 12–23 : CONTROL TO RE-INOCULATION.

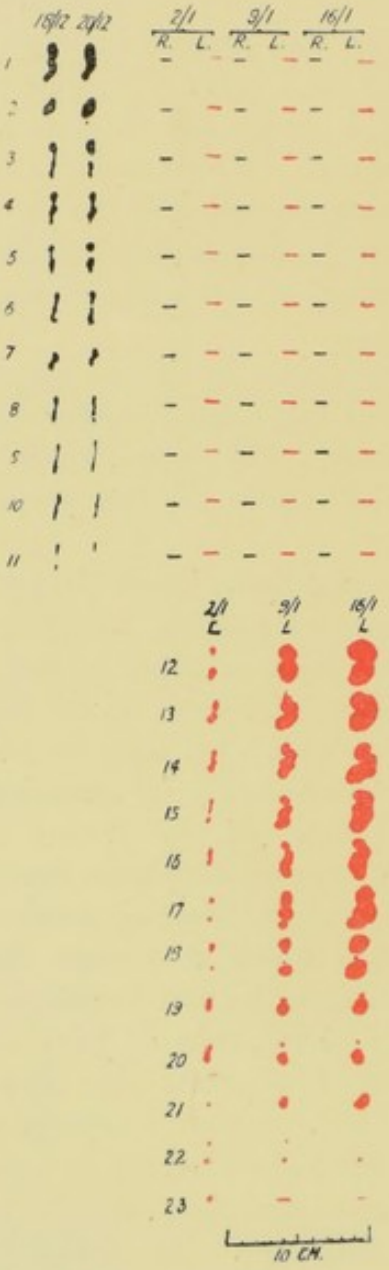


FIG. 9.—Shows the induction of resistance by strain **206** in all mice. Primary inoculation given in black, re-inoculation and control in red.

EXP. **J.R.S.**/68 B RATS 1–14 INOCULATED IN RIGHT AXILLA (31.10.11). TUMOURS EXCISED COMPLETELY (27.11.11), EXCEPT IN RATS 8–14 WHERE A FRAGMENT OF TUMOUR WAS LEFT. RE-INOCULATED IN LEFT AXILLA WITH 0·05 C.C. OF **J.R.S.**/69 B (28.11.11). RATS 15–24: CONTROL TO RE-INOCULATION.

FIG. 10.—Rat sarcoma **J.R.S.** induces a very high degree of concomitant immunity, which renders rats unsuited for re-inoculation. Incomplete surgical excision does not restore their original susceptibility. Primary inoculation and recurrences given in black, re-inoculation and control in red.

its vascular supply. Twenty-four hours later the animals were re-inoculated in the left axilla with 0·05 c. c. of sarcomatous tissue, together with a number of normal rats to serve as controls. The result of re-inoculating the seven whose tumours had been completely extirpated, was that in only one animal did this procedure give rise to a tumour approximating those of the control series in the speed of its growth. In two cases the re-inoculation was entirely negative, whilst in the remaining four only small nodules developed. In six of the seven animals there was thus a considerable degree of resistance developed from the tumour first inoculated. In the seven rats where the excision was intentionally incomplete, almost exactly the same result was obtained. The resistance induced by the partially excised tumour was effectual in reducing not only the amount of growth from the re-inoculation, but also was in five out of seven cases fatal to the fragment of tumour left behind at the operation. Concomitantly induced immunity explains clearly these findings with the **J.R.S.** rat tumour.

These results are contradictory to those obtained by Uhlenhuth, Haendel and Steffenhangen (38, 39), who experimented with the same strain of sarcoma growing in German rats in Berlin. They state that if the whole tumour be excised re-inoculation fails, but if a part be left behind it continues to grow, and re-inoculation is now successful. The chart given in fig. 10 demonstrates clearly that incomplete excision is not the factor determining whether the re-inoculation is to be successful or not. This is determined solely by the reaction taking place between the animal and the tumour first implanted. In rat No. 1 of this chart, it is immaterial whether the operation be complete or incomplete ; the re-inoculation will give rise to a tumour because no immunity has been induced, whilst rat No. 11 cannot be re-inoculated under either condition because a reaction of immunity has been set up by the primarily inoculated tumour. Since these findings do not agree with Uhlenhuth, Haendel and Steffenhangen's it is unnecessary to enter fully into the subsidiary hypotheses which they have created to explain their results. They assume that the tumour parenchyma becomes insusceptible to the forces of immunity, but the systematic attempts made in this laboratory to produce this alteration have failed. By cultivating out a tumour which has grown as an exception in a series of mice immunized previously with embryo-skin emulsion, a strain is not obtained in any way characterised by resistance to the forces of immunity as called into play by previous injection of embryo-skin emulsion.

All of the above experiments have been chosen with the special view of demonstrating how very differently the parenchymata of various tumours of distinctive types behave in regard to the production of immunity, and the detailed description of this class of experiments given above will now be followed by a general discussion upon the interpretation which is to be put upon the results obtained.

On the one hand two tumour-strains have been shown, **63** and **T**, which in the course of their development do not alter the suitability of mice for re-inoculation ; on the other hand a tumour strain has been shown which so alters the animals, that all are refractory to subsequent inoculation. It is apparent that such wide differences can only be attributed to inherent properties of the tumour parenchymata, and the contrast in their behaviour may be defined by stating that the parenchyma of strain **206** induces a resistance which the parenchymata of **63** and **T** fail to do. The terminology of modern immunity studies would label the former an efficient antigen, whereas the latter would be inefficient. In these extreme cases the differences are so wide, and the reactions so marked, that the medium, *i. e.* the inoculated mouse can be regarded as indifferent. When tumours are considered, however, which only induce a resistance in a certain percentage of cases, slight differences in the medium turn the scale for or against the inoculated graft in individual cases.

To take the specific instance of strain **199** : why does this strain induce resistance in 60 per cent., and fail to do so in the remaining 40 per cent ? The parenchyma which has been distributed over 10 mice for example, although of exactly the same quality and quantity throughout, fails to induce resistance in four mice. Again in the extreme cases of strains **63** and **T**, resistance is induced occasionally in a certain number of animals, whilst strain **206** sometimes gives rise to progressively growing tumours in animals exhibiting no reaction of resistance. These variations in the development of resistance in the individuals composing a series must be regarded as the expression of slight differences in the constitution of the animals composing such a series, and whilst in general the reaction is determined by the tumour parenchyma, a slight individual peculiarity is sufficient at times to determine or prevent the development of resistance. Tumour-strains such as **63** and **206** usually mask all individual variations in the animals, but strain **199** and many others bring them out with distinctness.

Murray's studies (31) upon the heredity of cancer have shown that it is possible to breed out families of mice whose members will show an

extremely high incidence of spontaneous cancer, although they are not more suitable for the implantation of a transplantable tumour than mice not specially bred (28). Tyzzer (37) has carried out investigations in mice upon the inheritance of susceptibility to tumour-grafting, and has obtained most interesting results more especially as regards a carcinoma which developed in a Japanese waltzing mouse. He found that this tumour failed to grow in common tame mice, although it took in 100 per cent. in waltzing mice. By crossing waltzing mice and tame mice a susceptible set of mice were obtained, but inter-breeding of members of this first generation of hybrids produced animals totally unsuitable for growth of the waltzing mouse tumour.

Cuénot and Mercier (18) have made the attempt to breed out from one and the same strain of mice, families suitable and unsuitable for the implantation of cancer. They claim to have isolated two families, in one of which a tumour-strain will take in 86 per cent., whereas in the other it will only take in 20 per cent. Should these findings be confirmed, and it seems desirable that they should be repeated and tested with a variety of tumours, they would help greatly to explain the character of growth exhibited by such strains as **199**. It might be possible to isolate families of mice in which this strain produced no resistance, and the inoculation would lead to the development of progressively growing tumours in all cases. A complete alteration in the character of growth of a series of tumours such as might follow implantation into selected animals is no mere hypothesis, for the effect has been actually produced with strain **199**. In one experiment the twelve best growing tumours from a series of sixty mice inoculated with 0·02 c.c. of strain **199**, were extirpated on the 15th day, and the animals re-inoculated six days later, and also twelve normal mice, with another tumour of the same strain. Growth took place for 10–15 days in all the control animals, but except in three cases was succeeded by spontaneous absorption. The re-inoculation of the twelve operated animals gave a totally different picture ; four of them were negative from the start, the other eight showed progressively growing tumours. In four of the twelve selected mice active resistance had been induced, whereas the other eight were animals which failed to give any immune reaction with strain **199**, and the character of growth of the strain was radically altered in consequence. This experiment was simply the selection of eight individuals incapable of giving an immune reaction with carcinoma **199**, from a batch of animals produced by random breeding.

Variations in the power of tumour parenchymata to induce resistance may be made in part responsible for the adaptations which tumours undergo more especially during their earliest transference to new hosts. When a spontaneous growth is transplanted, there is usually a rapid rise in the percentage of success attached to the first three or four *passages*. This rise can scarcely be due to the mere accustoming of the tumour to the act of transplantation, for Haaland has shown that if the inoculation be made into the primarily affected animal which has given rise to the tumour, the result is almost invariably successful. Might it not be possible that the rapid rise in transplantability is due to a greater or smaller loss of the power of the tumour parenchyma to induce resistance? This possibility requires consideration because careful microscopic examination of grafts during the first ten days shows normal growth in nearly every case, even although the tumour-strain only gives in control series an eventual percentage of success of about forty.

The degree of adaptation eventually exhibited varies in different strains, and even in sub-strains of the same tumour. Bashford (4, pp. 208–9) has described and pictured the latter phenomenon for carcinoma **63**, and has shown how one sub-strain has retained to a great extent the power of inducing resistance, whereas the other, that which has been used in the present series of experiments, has almost entirely lost this property. The phrase, natural resistance of animals to tumour inoculation, has been much employed, but it may perhaps be more correct to talk of animals which readily develop an active resistance.

The next question to be discussed is, how do the results obtained by re-inoculation after the first tumour is removed compare with those obtained when this growth is not interfered with? It may be stated at once that the results obtained under the two conditions are exactly identical, and the removing or leaving behind of the tumour first inoculated neither favours nor hinders specifically the development of the second one. That a mouse bearing an implanted growth can be successfully re-inoculated was recorded from this laboratory as early as 1904 (5, pp. 11–15), and more extended researches led to the formulation of the dictum that the better the first tumour grows, the more favourable are the chances of the second inoculation being successful (10, p. 390). The whole question of the re-inoculation of animals bearing tumours and the literature on the subject will be found reviewed in publications from this laboratory in 1908 (9, 10, 23),

1909 (11), and 1910 (12), which also contain an ample criticism of the athreptic hypothesis of Ehrlich in its bearing on this particular point. In the paper by Bashford, Murray, Haaland, and Bowen, the conclusion was drawn that negative results on re-inoculation of an animal already bearing a tumour were due to the development of concomitant immunity. However, mention may be made of an interesting result obtained by Gay (22), who, working with the Flexner-Jobling rat tumour, found that rats bearing this growth could be readily re-inoculated after the 30th day, but not earlier. This he correlated with the late development of metastases, and designated the two periods as "pre-metastatic" and "post-metastatic." The pre-metastatic phase he considers as the period during which the animal possesses reaction products to cancer tissue. Jobling (29) himself, however, could not confirm these findings.

To continue the discussion of the tumours used in the present series of experiments, it has been found that the re-inoculation of mice which have been previously inoculated with strain **206**, is attended with great difficulty. Haaland (26) has shown that even when the re-inoculation be performed as early as the eighth day, the mice are found to be highly resistant. The re-inoculation of mice bearing tumours of strain **199** (12) succeeds only in about 33 per cent. of the animals, and this again corresponds with the findings where the tumours first inoculated have been removed, as shown in figs. 7 and 8. Mice bearing tumours of strain **T** can be readily re-inoculated, as shown in the first half of fig. 14, and as regards the re-inoculability of mice with tumour **63**, the experiment given in fig. 11 shows with what facility this can be accomplished. In this particular experiment the primary inoculation was performed with a large dose of tumour emulsion, and re-inoculation with a smaller dose was carried out 11 days later, when large rapidly growing tumours were already present. Only in one mouse, No. 10, was the re-inoculation absolutely negative, and in this animal the tumour first inoculated grew very slowly after the tenth day. Four weeks after the beginning of the experiment all the mice were killed, and the tumours excised and weighed. The nine growths from the first inoculation weighed 51·2 grms.; the eight from the re-inoculation weighed 6·1 grms.; and in the twelve animals constituting the control to the re-inoculation twelve tumours developed weighing 8·85 grms. The average weight of the two latter series of tumours is 0·76 grms. and 0·74 grms. respectively, so that the large rapidly growing tumours have in no way hindered the development of the re-inoculated tumours.

This result again confirms what was obtained after excision of tumours of this strain, as depicted in fig. 3.

Schöne (36), who performed re-inoculation after excision of the first tumour, found that the mice could now be readily re-inoculated. If one does not err in the reading of his paper—for the statement is not directly made—this result was obtained with a Frankfort tumour strain which exhibits the athreptic phenomenon of Ehrlich. In the absence of

EXP. 63/54 D. MICE 1–10 INOCULATED IN RIGHT AXILLA, DOSE 0·1 C.C. (24. 10. 11). RE-INOCULATED IN LEFT AXILLA WITH 0·05 C.C. OF 63/54 E (4. 11. 11). MICE 11–22: CONTROL TO RE-INOCULATION.

FIG. 11.—Animals bearing large progressively growing tumours are here shown to be highly suitable for the development of tumours on re-inoculation. Primary inoculation in black, re-inoculation and control in red.

confirmation from the same laboratory, judgment must be reserved on Schöne's experiments, and the statement cannot be accepted that by removal of an inoculated tumour a mouse previously refractory can be rendered suitable for re-inoculation. Amongst the many strains of tumours exhibiting all types of growth, which have been tested in this laboratory, none behave in the manner indicated by Schöne, nor does the literature on cancer contain any charts of a tumour-strain behaving

in this way. Apolant (3) has repeated Schöne's procedure, and has encountered considerable resistance to re-inoculation after excision of the first tumour. In the charts accompanying his paper, the large majority of the animals, rats and mice, exhibit a high degree of resistance to re-inoculation. The explanations which Apolant gives to explain these negative results are not very convincing. He attributes part of the result to the operation, which in the experiments described in the present paper has had no direct influence upon the suitability of an animal for re-inoculation. The removal of tumours of strain **63** leaves mice which are suitable for re-inoculation, whereas with strain **206** the opposite result is obtained, so that the operation cannot be a determining factor. The same considerations refute his attribution of the result to the possession of a natural immunity on the part of the animals. Apolant appears to ascribe spontaneous absorption to the presence of a natural immunity, although he concedes the development of active immunity from the absorption of tumour-tissue in those cases where surgical removal may not have been complete. Further, he believed that the natural immunity can be explained by means of Ehrlich's athreptic hypothesis, although the manner of application does not seem to be very logical. It may be recalled that Ehrlich (19), in his exposition of this hypothesis, attributed his failure to obtain tumours on re-inoculation of mice bearing rapidly growing tumours to the withdrawal of necessary food-substances from the blood. Later (20) he restricted this form of immunity in its application to the inhibition of re-inoculation, to the cases where the first inoculated tumours were of maximal virulence, and possessed the highest degree of avidity for food-substances, including special substances absolutely necessary for the tumour-growth. This view seems to have undergone a considerable alteration in the hands of Apolant (1) in his attempt to explain the experiments of Cuénot and Mercier cited above. According to Apolant, Cuénot and Mercier's experiments are best explained by supposing that the several families of mice vary in the amount of necessary specific substances (products of internal secretion) which they produce. Further, he carries this view to its logical conclusion in stating that highly virulent tumour-strains, which grow in every animal, are those which make the least demands (die geringsten Ansprüche) on these food-substances. The contradiction contained in these two applications of the athreptic hypothesis is obvious ; on the one hand, the failure to obtain success from re-inoculation is attributed to the exhaustive or ereptive powers of a highly virulent tumour, and on the other hand, its high percentage

of success is attributed to its slight requirements of this indispensable food-substance. Postulating the induction of active resistance leads to no such contradictions, and all the facts observed harmonize so well with this explanation, that it is the view which can be safely upheld.

Finally, it is thought necessary to discuss briefly the manner of action of the normal tissues, adult or embryonic, and of tumour-tissue, which induce a resistance against subsequent inoculation of tumours. Conceptions of the nature of this action are highly speculative, for the subject is one which has scarcely lent itself to investigation by direct observation. A concrete example taken from fig. 6, mouse No. 9, will serve well as an instance of the problem to be discussed. It will be seen that the tumour in this animal grew for nineteen days and then gradually diminished in size until all tumour-tissue disappeared. During the period of diminution, such an animal is found to be refractory to re-inoculation. Is this refractoriness due primarily to the absorption of tumour-tissue which has been killed, or is the death and absorption of the cells due to a resistance previously elicited by the living cells? The bulk of the evidence is in favour of the latter view. Mechanical disintegration of the tumour-cells not necessarily accompanied by the death of the protoplasm, robs them, as Haaland (27) has shown, of their power of inducing resistance. Again, partial necrosis is a process occurring in all tumours, but only some tumours induce resistance. The date of onset of resistance is usually antecedent to the inception of spontaneous absorption. Haaland (26) found with tumour-strain 206 that as early as the eighth day a very marked degree of resistance was present, whereas the initiation of spontaneous absorption took place at a later date. The percentage incidence of spontaneous absorption in a series of tumours is not the same as the incidence of concomitant immunity as tested by re-inoculation, for in many cases an immunity will be developed sufficiently strong to kill a graft but not an established tumour, although even in this case the immunity will usually retard the growth of the established tumour.

The extensive experiments done by Woglom (40) upon the effect of inoculating an animal with its own tissues are also in favour of the view that the living cell is the producing agent of resistance. He was unable to obtain resistance by auto-inoculation of kidney, spleen, or testes, whereas all these tissues can induce immunity in other mice. Such a fine gradation of difference as that existing between autologous and homologous inoculations can be more readily conceived as elicited by a living cell, rather than by autolytic or other disintegration products of the dead protoplasm.

The failure of many investigators to induce resistance with tumour-tissue which had been killed by various agencies, led Clowes (16) to believe that actual growth must take place before immunity is produced, and that the intervention of living cells or virus is essential. The subsequent discovery that normal blood can induce resistance leads necessarily to a modification of this view, as here growth in the anatomical sense with multiplication of cells does not take place. In all the other tissues, neoplastic or normal, which have been found efficacious in eliciting resistance, growth does take place, but the exception in the case of blood is sufficient to invalidate the statement that without growth no immunity arises. The necessity of the intervention of living cells stills holds good, and the evidence adduced in the preceding paragraphs favours the view that the metabolic activities of these living cells, rather than the processes of death and autolysis, are to be regarded as the means by which immunity is induced.

Simultaneous Inoculation of Two Tumours of Different Types of Growth.

A clear recognition of differences in the behaviour of transplanted tumours, induction of resistance in a high percentage of cases on the one hand, contrasted with practically total absence on the other, led to a study of the influence which a strain of the former type might have upon the growth of a tumour of the latter type, where both tumours were inoculated at the same time. Simultaneous inoculation of two tumours in opposite axillæ has already been carried out. Bashford, Murray, and Cramer (7) inoculated two separate strains of the same tumour in the right and left axilla respectively for five successive *passages*, and found that each tumour-strain varied in its growth quite independently. Bridré (14) also performed the double inoculation of two separate strains, and found that each grew as if it alone had been inoculated.

The strain of carcinoma **63**, which shows no evidence of concomitant immunising powers, but grows progressively in nearly every case, was chosen as the tumour to show any possible influence. The sarcoma developed from the stroma of carcinoma **37** (25), which induces concomitant immunity in a high percentage of cases, was chosen as the tumour to produce the possible influence. Fig. 12 illustrates the result which has been obtained by laying down an experiment on these lines. Mice Nos. 1–12 constitute the control to the growth of tumour **63** when

EXP. 63/49 K & 37/91 B. MICE 1-12 INOCULATED IN R. AXILLA WITH 0·01 C.C. OF 63/49 K (6. 6. 11). MICE 13–24 INOCULATED IN L. AXILLA WITH 0·01 C.C. OF 37/91 B (6. 6. 11). MICE 25–40 INOCULATED SIMULTANEOUSLY IN RIGHT AND LEFT AXILLÆ WITH 0·01 C.C. OF 63/49 K AND 37/91 B RESPECTIVELY.

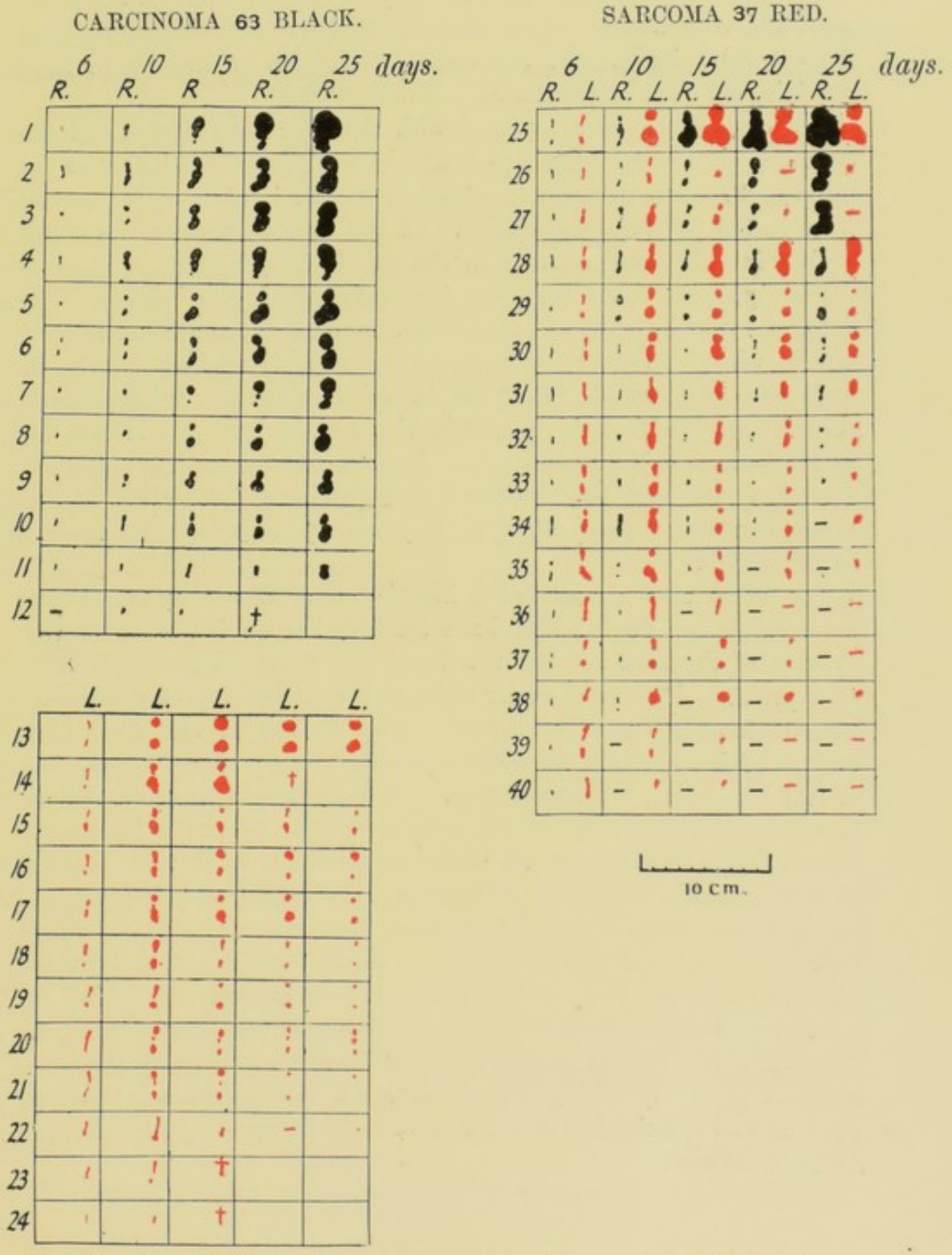


FIG. 12.—Shows that in simultaneous inoculation of two tumours of different types of growth, the progressively growing tumour is markedly affected.

inoculated singly in the right axilla, and mice Nos. 13–24 fulfil the same office for sarcoma **37** when inoculated in the left axilla. Mice Nos. 25–40 were inoculated with each tumour in the right and left axillæ respectively, and it is at once apparent that the growth of the carcinoma given in black has been greatly inhibited. Seven of the sixteen mice were quite negative at the 25th day, four showed small nodules only, and only five developed growths at all comparable to those in the control animals. In the control series, all of the eleven mice surviving 25 days bore tumours.

This experiment was repeated with the same two strains, and the result is given in fig. 13, where it will be seen that the influence of the sarcoma **37** upon the growth of carcinoma **63** is even more marked than in the previous experiment. In the control, tumours developed rapidly in every case, whereas in the double inoculations the carcinomatous growths were very few in number and grew slowly. The effect of a tumour inducing concomitant immunity upon another tumour which, when established, grows progressively, is elicited clearly in both these experiments. It is not intended to convey the impression that the sarcoma has this power in virtue of its being a sarcoma, but rather because it is a tumour which induces concomitant immunisation. The same result has been obtained with carcinoma **206**, which has, in common with sarcoma **37**, a high power of inducing concomitant immunisation.

The effect of a simultaneous inoculation of mouse embryo-tissue upon carcinoma **63** has also been studied, but an inhibition of the growth of the tumours has not been observed. It suggests itself as a perfectly legitimate explanation, that the inefficacy of the simultaneous inoculation of embryonic tissue to inhibit the growth of tumour **63** is due to the later development, it may perhaps be only one or two days later, of the resistance. By using tumour-tissue to induce immunity, the immunity can be brought to bear upon the inoculated tumour-tissue of carcinoma **63**, before the latter has had time to become fully established. This latter circumstance is of considerable importance, and has been demonstrated by extension of these experiments to other tumour-strains. Another carcinoma, strain **91**, has been used, but with this tumour it has not yet been possible to demonstrate any inhibition where simultaneous inoculation with sarcoma **37** has been performed. Apparently this tumour cannot be overtaken in its growth by the concomitant immunity arising from the sarcoma, but continues to develop quite as well in animals where the sarcoma is disappearing as it does in the control series.

EXP. 63/49 L & 37/93 B. MICE 1–12 INOCULATED IN R. AXILLA WITH 0·01 C.C. OF 63/49 L (28.6.11). MICE 13–24 INOCULATED IN L. AXILLA WITH 0·01 C.C. OF 37/93 B (28.6.11). MICE 25–48 INOCULATED SIMULTANEOUSLY IN RIGHT AND LEFT AXILLÆ WITH 0·01 C.C. OF 63/49 L AND 37/93 B RESPECTIVELY.

CARCINOMA 63 BLACK. SARCOMA 37 RED.

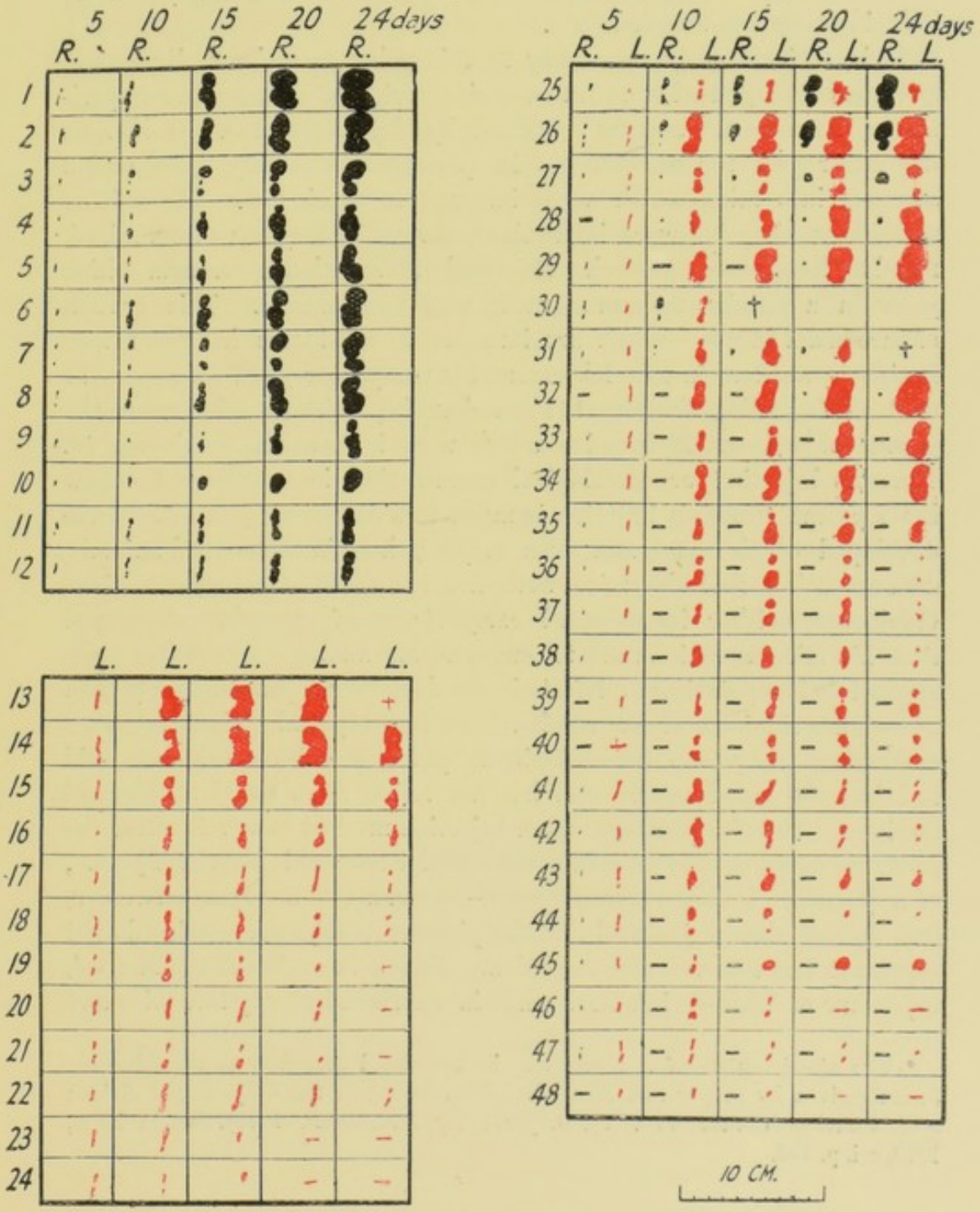


FIG. 13.—The concomitant immunity induced by sarcoma 37 inhibits the development of grafts of carcinoma 63.

The behaviour of tumour **63**, however, demonstrates very clearly a case where the immunity can overtake the early phases of growth and prevent further development. This explanation further accords well with the histological findings in early stages of grafts inoculated in immune animals. Briefly stated, these led to the conclusion that the resistance was directed mainly against the cancer cell's power of inducing a stroma reaction (33).

It is, unfortunately, necessary at this point to make a digression, as these results, based upon an extensive series of comparative experiments, have been severely criticized by Goldmann (24). Goldmann did not repeat the experiments in question, but merely inoculated a sarcoma into mice which were immune to carcinoma. He neither states how his mice were immunized, nor when they were immunized, nor does he give any control. He simply affirms that a sarcoma which grew in a carcinoma-immune was well vascularized. This growth of sarcomata in mice which are immune to carcinoma has been well known to workers in this laboratory for many years, and on page 371 of the Third Scientific Report (10) a chart is published which shows the growth of sarcoma **37** in mice which were immune to carcinoma **32**. Here no injection or histological examination is necessary to show the sarcoma growing in mice immune to a carcinoma ; tumours have developed which equal, and even surpass, those in the control animals, demonstrating that the mice were not resistant to sarcoma. Since Goldmann neither investigated early stages of growth, nor proved that his mice were immune to sarcoma, his findings are rather to be appreciated as pointing to defects in the doctrine of pan-immunity than as serious criticisms of our results. He lays especial weight upon his findings, as they have been obtained by using Chinese ink as a fluid to inject the blood-vessels, and has not relied upon serial histological sections. The disadvantages of an injection method which involves the pumping into a mouse, whose normal vascular content is about 1–1½ c.c.*, of a quantity of fluid varying from 8–10 c.c. are of a serious character, and the criticism is justified that the pictures Goldmann obtains do not represent the real vascular condition. To test the value of the method, injections of Chinese ink were made in a series of susceptible mice and

* The blood-volume of a mouse may be calculated according to the following formula given by Dreyer and Ray. Blood-volume = $\frac{2}{3}$ of the body-weight divided by k ; k having the value 6·70. *Cf.* Dreyer & Ray, Phil. Trans. of Roy. Soc., Series B, 1910, cci. p. 133.

in a series of resistant mice engrafted with a carcinoma, but in making the injection regard was paid to the normal vascular content, and not more than 1 c.c. was injected into the tail vein of the etherised animal. Under these conditions it was not found that the injection was a better method of analysing the development of new capillaries than the serial sectioning of excised grafts, and accordingly Goldmann's main contention falls to the ground. The misconceptions of our work appearing on pages 63 and 66 of his paper, apparently shared by Apolant (2), do not require to be gone into; they have not appeared in the papers written by Da Fano (21), Burgess (15), and Rous (32), who have performed experiments on somewhat similar lines.

The material and methods of investigation employed by Goldmann could not elucidate the mechanism of the resistance exhibited by immunized animals, and his criticisms cannot be accepted as valid. It has been necessary to reaffirm the view that resistance is directed in the first place against the stroma-eliciting powers of the tumour-cells, since it affords a clear explanation of the behaviour shown by two strains of different types of growth when inoculated simultaneously. In the two experiments depicted in figs. 12 and 13, the early resistance induced concomitantly by sarcoma **37** is able to prevent the establishment of grafts of carcinoma **63**, whereas this result is not obtained when resistance develops somewhat later, *e. g.* after the inoculation of embryo-skin, or when the tumour establishes itself very rapidly, as is the case with carcinoma **91**. The inefficiency of the resistance in the two latter cases can be best explained by assuming that there is no immunity present until after the graft has received a stroma and a vascular supply. The experiments to be described in the two succeeding chapters support this conception of the action of resistance in every respect.

Can Animals bearing Progressively Growing Tumours be protected against Re-inoculation?

Procedures such as the inoculation of normal tissues as blood (6), liver, spleen, etc. (13), or of embryonic tissues (35), have been found to be very efficacious in inhibiting the development of inoculated tumours. These results are only obtained when tissues of the same species are used throughout the experiments (7, 10), and the best results are seen when an interval of 10 days for blood and 14–18 days for embryonic tissues (8), is allowed to elapse before the testing of the

degree of resistance against a given tumour-strain is carried out. The efficacy of this preliminary treatment stands out in marked contrast with the disappointing nature of the results hitherto obtained when the attempt is made to produce by the same means the involution of an already established tumour which tends to grow progressively. Why this marked difference should occur under these two conditions could be explained in several ways, all of them open to experimental investigation. The establishment of a progressively growing tumour might so alter the bio-chemical constitution of the animal that it could no longer react to an agent which had hitherto been capable of eliciting an immune reaction. Again it might be postulated that the immune reaction took place, but that the growing tumour was capable of annihilating its action by destroying any supposed "immune forces" which had developed. A third possibility, and as will be seen later this is the most probable, was that resistance was induced, but was incapable of inhibiting the further growth of a tumour which had already established organic connections with the host. The most striking and evident difference between established tumours and mere grafts is that the latter have not yet acquired a stroma with a vascular supply, and it has been already demonstrated that animals, which have been rendered resistant by previous inoculation of embryonic tissue, etc., owe their resistance in large part to powers which enable them to paralyse the cells of a tumour-graft so that they can no longer elicit a stroma-reaction. Fortunately these more or less speculative explanations can now be dispensed with, as it has been found possible to carry out experiments capable of giving a direct answer to the question whether an animal bearing a progressively growing tumour can be induced to give an immune-reaction.

On page 392 of the Third Scientific Report (10) are noted experiments planned to give an answer to this question. Mice bearing tumours were inoculated with either spontaneous growths or with embryonic tissues, and after a suitable interval their resistance to re-inoculation with an easily transplantable tumour was tested. The results obtained were not such as could give a definite anwer to the question, but only allowed the conclusion to be drawn that there existed the possibility of intercalating resistance.

Since these results were published, tumour material has been accumulating, and strains of tumours more suitable for such an experiment have been obtained. The technical difficulties attaching to such an investigation are considerable, for it requires the inoculation of

a large number of mice, some of them on two and three occasions, and the preparation of two or even three control series. The inoculated animals require to be kept under observation for a long period, which necessitates the use of a tumour-strain growing rather slowly, and also in a high percentage. Strains which exhibit the phenomenon of concomitant immunisation are quite unsuitable for testing this point. Carcinoma **T** fulfils all the above conditions, and it is from observations on this tumour that the following conclusions have been arrived at although subsequently the experiments were repeated with another adeno-carcinoma, strain **91**. It is not very easy to follow the course of such a complicated investigation, and the attempt will be made with the help of the accompanying chart given in fig. 14 to render clear an illustrative experiment, whilst for further experiments simply the figures obtained will be cited.

On the 19th January, 1910, 40 mice, weighing from 14–16 grms. each, were inoculated with 0·015 c.c. of **T**/27 C in the right axilla. Twenty-nine of the thirty-five surviving mice developed tumours—83 per. cent. Twenty-five of these tumour-bearing mice were divided into two batches, Nos. 1–13 and Nos. 14–25 on the accompanying chart. When the tumours were twelve days old, batch Nos. 14–25 was inoculated on the back with 0·05 c.c. of mouse carcinoma **J**, and at the same time 15 normal mice were treated in the same way. Strain **J**, the original Jensen strain, at that time gave rise to temporary proliferation only when an emulsion was inoculated by means of a syringe ; the mice in which this temporary growth had taken place became highly refractory, and advantage was taken of this behaviour of the tumour to use it for immunising purposes. Eleven days after this inoculation, and 23 days after the start of the experiment, all these mice were re-inoculated in the left axilla with 0·015 c.c. of **T**/28 F, and, in addition, 12 normal mice to serve as an indicator of the transplantability of series **T**/28 F. The degree of transplantability of this series was found to be 75 per cent.

With regard to the major part of the experiment, it will be seen on referring to the chart that the re-inoculation, depicted in red, was successful in 10 out of 13 mice with tumours, these mice having undergone no further treatment. In mice Nos. 14–25, where an immunising dose had been intercalated, only 3 out of 12 developed tumours on re-inoculation, also shown in red. Here there is on the one hand 77 per cent. of successful re-inoculations in untreated mice, and on the other hand only 25 per cent. in treated mice. The conclusion seems warranted that mice bearing tumours can be rendered refractory to

subsequent inoculation. Whether such mice can be rendered resistant to a graft as easily as normal mice was ascertained by comparing the result in mice Nos. 14–25 with the result in 15 mice which received an inoculation of carcinoma J alone. In the former there were three

EXP. T/27 C. ALL MICE INOCULATED IN RIGHT AXILLA, DOSE 0·015 C.C. (19.1.10). ALL MICE RE-INOCULATED IN LEFT AXILLA, DOSE 0·015 C.C. OF T/28 F (11.2.10). MICE 14–25 WERE INOCULATED WITH 0·05 OF J/154 E, WHICH GREW ONLY TEMPORARILY, AND IS NOT SHOWN IN CHART.

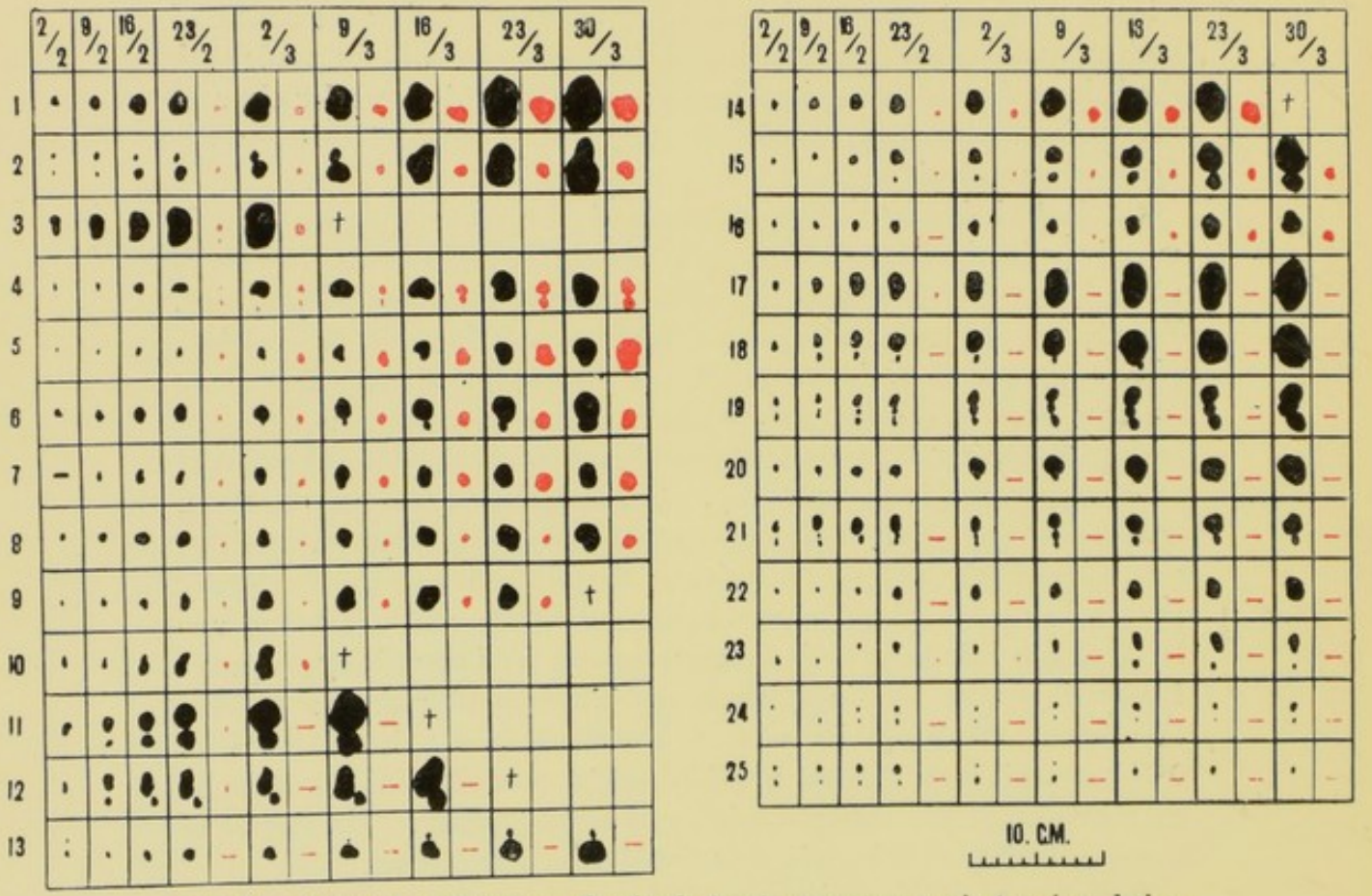


FIG. 14.—Shows immunisation of animals bearing tumours against re-inoculation. Primary inoculation in black, re-inoculation in red.

tumours in 12 mice, in the latter series, not shown in the chart, 1 in 15. Judging from this experiment alone, it would therefore seem that the presence of a growing tumour does to a certain extent lower the degree of resistance following an immunising dose. This point will be returned to later, after the figures obtained from other series carried out on similar lines have been given.

The result of the above experiment may be briefly summarized in percentages in the following way :—

Exp. T/27 C : success of primary inoculation = 83 per cent.

Re-inoculation of Positives.	Re-inoculation of immunised positives.	Re-inoculation of immunised controls.	Control to Re-inoculation.
10 in 13	3 in 12	1 in 15	6 in 8
77 %	25 %	7 %	75 %

Three further experiments were performed with the same strain ; the intervals elapsing between the several inoculations were maintained, but the tissue employed to induce the resistance was varied. The results obtained will be given only in a tabulated form.

Exp. T/27 B : success of primary inoculation = 100 per cent.

The method employed to induce resistance was the inoculation of 0·1 c.c. of mouse embryo emulsion 16 days after the first tumour inoculation. The result obtained was :—

Re-inoculation of Positives.	Re-inoculation of immunised positives.	Inoculation of immunised controls.	Control to Re-inoculation.
8 in 12	7 in 15	5 in 18	10 in 11
66 %	47 %	28 %	91 %

In this experiment the evidence in favour of an immune reaction taking place in mice already bearing tumours was not so marked as in the preceding experiment, and again the degree of immunity elicited by the embryonic tissue in mice without tumours was higher than in the mice with tumours.

Exp. T/28 G : success of primary inoculation = 77 per cent.

Embryo emulsion in a dose of 0·1 c.c. subcutaneously, 14 days after the first tumour inoculation, was again used to induce resistance, and gave the following result :—

Re-inoculation of Positives.	Re-inoculation of immunised positives.	Inoculation of immunised controls.	Control to Re-inoculation.
5 in 10	4 in 16	1 in 16	12 in 18
50 %	25 %	6 %	66 %

This experiment confirmed exactly the result obtained from the preceding one.

Exp. T/26 C: success of primary inoculation = 100 per cent.

Resistance was here induced by inoculating, 13 days later, 0·015 c.c. of carcinoma **206** which invariably gives rise to tumours characterised by temporary proliferation followed by spontaneous healing. The figures obtained are the following:—

Re-inoculation of Positives.	Re-inoculation of immunised positives.	Inoculation of immunised controls.	Control to Re-inoculation.
7 in 12	2 in 13	4 in 10	Absent.
58 %	15 %	40 %	

This was the earliest of the present series of experiments to be undertaken, and a control to the inoculability of the tumour used for re-inoculation was not made. The series differs from the three preceding in giving a higher degree of resistance in the mice bearing tumours than in the mice which had been simply inoculated with the immunising agent employed.

Two experiments were performed with another carcinoma, strain **91**, and gave the following result:—

Exp. **91**/19 B: success of initial inoculation = 48 per cent.

The agent used to induce immunity was 0·1 c.c. of homologous embryo emulsion, inoculated subcutaneously on the fourteenth day.

Re-inoculation of Positives.	Re-inoculation of immunised positives.	Inoculation of immunised controls.	Control to Re-inoculation.
6 in 10	1 in 8	6 in 19	13 in 18
60 %	12 %	31 %	72 %

In the second experiment with this strain, Exp. **91**/20 G, which gave an initial success of 40 per cent. only, and was an exact repetition of the preceding, the figures obtained were:—

Re-inoculation of Positives.	Re-inoculation of immunised positives.	Inoculation of immunised controls.	Control to Re-inoculation.
5 in 8	2 in 6	5 in 17	10 in 14
63 %	33 %	30 %	71 %

The figures from these two latter experiments, although comparable with those obtained when using the carcinoma **T**, are not of quite so

much value. As will have been noticed, strain **91** did not grow in so high a percentage of animals inoculated. For example, out of ten mice inoculated, strain **91** selected four which it found suitable for its continuous growth; results obtained from such a selected material are less convincing than those obtained in cases where this factor of selection is reduced to a minimum, or totally eliminated as it is in tumour **T**, which can grow continuously in every animal of an inoculated series (fig. 4). Again, strain **91** introduces another complicating factor, inasmuch as it frequently disappears spontaneously, and this process, as already demonstrated, is accompanied by a high degree of resistance to re-inoculation on the part of the animal in which it takes place. Strain **T** only rarely shows the phenomenon of spontaneous absorption, and in accordance with this resistance to re-inoculation is regularly absent. When, therefore, any marked degree of variation in the re-inoculability of mice bearing this tumour is obtained after a definite procedure, it may be safely concluded that the alteration is entirely due to the procedure in question. On summing up all the figures obtained in the above six experiments, this change will be seen, as in the following totals :—

Re-inoculation of Positives.	Re-inoculation of immunised positives.	Inoculation of immunised controls.	Control to Re-inoculation.
41 in 65	19 in 70	22 in 95	51 in 69
63 %	27 %	23 %	73 %

Whereas 63 per cent. of mice bearing tumours of these two strains have been shown to be receptive to a second inoculation, this figure is reduced to 27 per cent. when the second inoculation is preceded some 14–16 days by the injection of an immunising dose of tissue. The figures are too large to allow the simple interpretation of the results as the expression of an involuntary selection. The percentages given in columns two and three, which deal with the "immune reaction" in tumour-bearing mice and in normal mice respectively, show that in general mice with tumours can be rendered resistant with almost the same facility as normal mice. This is a point of considerable importance, and its bearing upon the resistance to implanted tumours will be discussed later. Since the resistance is effective against inoculation of a graft, even in those cases where a progressively growing tumour is present, it seemed desirable to test the efficacy of this resistance against the spontaneous grafting of transplanted tumours, which takes place in those cases where metastases develop.

The observations recorded by several workers show that pulmonary metastases, either in the form of intravascular emboli or of nodules invading the lung-tissue, are found in a percentage of cases varying according to the tumour used, and the length of time over which growth has taken place. The minute size of these metastases in many cases is sufficiently accounted for, as Bashford, Murray, and Haaland (10, p. 386) point out, when one takes into consideration the small number of cells from which they develop, the initial difficulty of vascularisation, and the hindrance to growth exerted by the arterial walls in the lung.

Clunet (17) has recently denied the importance of duration of growth upon the development of the metastases to macroscopic size, but has adopted a view similar to Ehrlich's athreptic hypothesis. The tumour which he used for his experiments was an adeno-carcinoma of the mouse mamma, exhibiting a medium rate of growth. Ten to twenty days after inoculation, he attempted total excision of the tumours which developed, but in only six of twenty-four cases was he successful in preventing recurrence. In nine out of eighteen mice with recurrence, the metastases attained macroscopic size ; in six, where no recurrence took place, metastases did not develop. The larger size of the metastases in the operated cases he attributed to the removal of the subcutaneous growth, which for a time allowed the cells disseminated in the viscera to obtain an adequate supply of specific food-substance. In the six animals where the removal of the tumour was not followed by recurrence, failure to develop metastases speaks against the early visceral dissemination. His rejection of the view of duration of growth as being important is scarcely consonant with his findings. Nine cases where recurrence took place but in which no metastases developed, lived on an average 48 days after inoculation ; eight cases (omitting number 20 in which the evidence for vascular dissemination is not clear) where recurrence followed the excision and macroscopic metastases developed, lived on an average 137 days. Clunet's own experiments, therefore, fully support the view that duration of growth is an important factor, both in determining the occurrence of dissemination, and the size which the metastases attain.

Tumour-strain **63** has always exhibited a marked tendency to produce pulmonary metastases, the size of these being entirely dependent upon the length of time over which growth has been allowed to proceed. The formation of pulmonary emboli by this tumour is a comparatively late phenomenon ; early and complete removal of the subcutaneous tumour prevents their formation.

The experiment has been carried out to see whether the induction of resistance after the subcutaneous tumours were established, could prevent the vascularisation of the pulmonary carcinoma emboli. For this purpose a large series of mice were inoculated subcutaneously with 0·05 c.c. of this tumour, one half of the series receiving at the same time 0·05 c.c. of mouse-embryo skin. There were thus obtained thirty mice, some of which had been immunised and some not, but all of which had had a subcutaneous growth of tumour over 8–10 weeks. The lungs of the whole thirty have been examined microscopically in serial sections. Twenty-four of the lungs showed metastases, most of them of macroscopic dimensions, equally distributed over the treated and untreated animals. There was likewise no evidence of a suppression of the stroma reaction in the intra-vascular growths in the immunised mice. The failure to prevent the development of pulmonary nodules in this experiment cannot be accepted as finally demonstrating the impossibility of doing so with the present methods; the very long period over which growth took place in the above case, 8–10 weeks, may well have overrun the duration of effective resistance following a single injection of embryonic skin. Repetition of the experiment will be necessary with a view to prolong effective resistance, perhaps obtainable by repeating at suitable intervals the injection of skin or other resistance-inducing tissue.

Attempts to Arrest the Development of Progressively Growing Tumours.

When carring out the experiments upon the immunisation of mice bearing tumours, described in full in the preceding pages, some cases were noted where the tumour, which had already started growth before the immunisation was carried out, was retarded greatly in its growth or even totally inhibited. On referring back to fig. 14, it will be seen that the tumours of mice Nos. 22–25 developed very slowly as compared with the control tumours. These four animals belonged to a series of twelve which had been immunised after they had developed tumours, and it was thought that this inhibition of growth might well be the expression of the effectiveness of the immunity. In several other series treated in the same way, a similar phenomenon was observed, and systematic experiments were made to ascertain whether the phenomenon was of regular occurrence, or whether it was merely the expression of chance. The question was one of great importance, for if it were possible to obtain regularly such a result, the cure of

transplanted tumours which did not disappear spontaneously would be accomplished. Strains of tumours which grew progressively were tested by subjecting the mice bearing them to the inoculation of embryonic tissue, or of tumour tissue which induces concomitant immunity. The effect of single inoculations of varying amounts of immunising tissue was tested, and also that of the repetition of the immunising dose at various intervals of 7, 10, and 14 days, in the hope that in this way a high degree of immunity might be maintained over a long period.

Such an experiment is illustrated by fig. 15, which shows a series of 42 tumour-bearing mice. Mice Nos. 1–21 received no treatment, and their tumours constitute the control to the mice Nos. 22–42, which, starting at the twelfth day after the tumours were engrafted, received five doses of 0·1 c.c. of embryonic tissue at weekly intervals. It will be seen from the chart that the result is not encouraging; taken over all the control-tumours are larger, but in the treated mice regression of the implanted tumours has not been obtained. No better result has been obtained by extension of the procedure in several other experiments of a like character. The tumour-strain T, which was used for the experiment illustrated, is easily inhibited in its growth where the mouse has been treated previous to the tumour inoculation. Charts published by Murray (30) show how it fails to grow in animals which have received such small doses of embryo-skin as 0·01 c.c. 21 days previously. The facility with which it falls a prey to resistance evoked before grafting, and the resistance which it exhibits after the graft has established vascular relations serve again to indicate what an important part the stroma reaction plays in artificially induced immunity.

Conclusions.

All the questions formulated in the four headings on the opening page have now been discussed, and a survey of the material exposed allows certain conclusions to be drawn.

The first of these is that different tumour parenchymata vary widely in their power of inducing resistance. This is a decision of considerable importance, and will undoubtedly prove of value in reconciling many apparently contradictory observations in the field of immunity. It also emphasises the necessity of examining several tumours of different growth types before drawing general conclusions as to the nature of immunity to transplanted tumours exhibited by any species of animal. The analysis of the findings with these different types of tumours

EXP. T/32 A. ALL MICE INOCULATED IN RIGHT AXILLA WITH 0·015 C.C. (21. 9. 10). MICE 1–21 = CONTROL. MICE 22–42 RECEIVED INJECTIONS OF 0·1 C.C. OF TOTAL EMBRYO EMULSION, 12, 19, 26, 33, AND 40 DAYS AFTER IMPLANTATION OF TUMOUR.

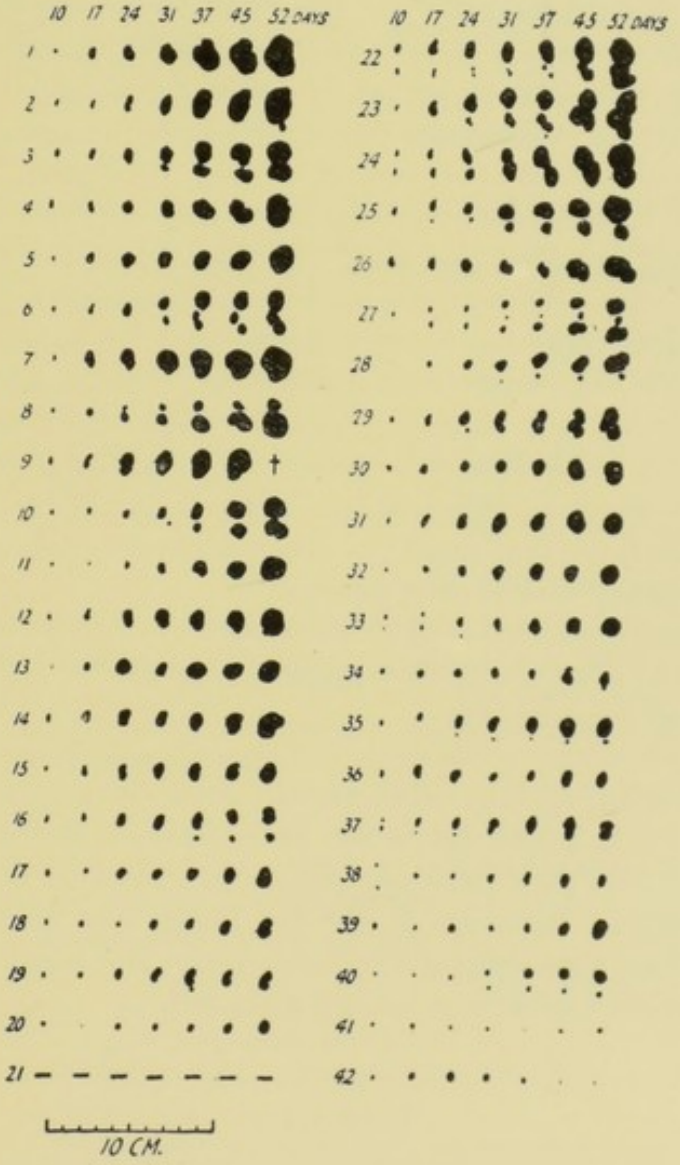


FIG. 15.—Repeated inoculations of embryonic tissues after the establishment of inoculated tumours has only produced a slight retardation in the rate of their growth.

corroborates the view expressed in previous publications from this laboratory, that resistance to re-inoculation, when present, is due entirely to the concomitant development of active immunity, and at the same time builds a good foundation for subsequent experimentation in the domain of immunity.

The quality of the soil exhibited by the inoculated animal has also been shown to have an influence upon the development of resistance, but in this its powers are weaker than those of the tumour.

The wide variations presented by different tumour strains in their power of inducing concomitant immunity may have indirectly a bearing upon the question of the genesis of new growths. It must be remembered, however, that these variations have only been demonstrated in propagated tumours, and were not detected in the spontaneously affected animals. Even if the view be accepted that the degree of power to induce resistance on transplantation is an inherent character of a tumour, it cannot yet be determined that this character is stamped on its cells at the time of their conversion from normal into neoplastic elements. The behaviour of a tumour cell towards a strange organism may only represent an individual character appertaining to the normal cell from which the tumour cell has been derived, and accordingly have no direct bearing upon tumour genesis.

Simultaneous inoculation of a tumour strain which induces resistance rapidly can inhibit or retard the growth of grafts from a strain which tends to grow progressively.

It has also been shown that mice bearing progressively growing tumours can be immunised against re-inoculation, even although this immunity does not as a rule inhibit the further development of the tumour already established. This is in strict accordance with previous findings, where it was shown that resistance is directed in the first place against the stroma-eliciting properties of the cancer-cells, and is accordingly more effective against a graft than against a tumour.

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THE NATURE OF THE IMMUNE REACTION TO TRANSPLANTED CANCER IN THE RAT.

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For many years it had been assumed by most pathologists, and with good reason, that the parenchyma was the characteristic and active moiety of epithelial growths, the stroma representing merely the scaffolding upon which the tumour cells were arranged and through the agency of which they obtained their nourishment. The investigations which had been prosecuted by embryologists upon the early developmental stages of various organisms had proved that the epithelial cell was the chief director of morphological differentiation, but until the time of Jensen and his contemporaries, the lack of suitable material had precluded experimental demonstration in the case of new growths, for the proof of the regulating influence of the neoplastic cell could be advanced only through the observation of small, actively growing tumours removed at successive stages of development and submitted to histological study. This addition to the expedients at the command of the pathologist has settled definitely also the long-argued question whether a tumour grew from its own resources—"*aus sich heraus*"—as Ribbert and many others had always maintained, or whether it spread by the infection of neighbouring cells, with their consequent conversion into elements possessing the power of malignant growth.

Jensen (15) saw the possibilities inherent in the study of the recently implanted graft, and examined small pieces of tumour removed one, two, three, four, or more days after inoculation. He found that after one or two days the fragment was necrotic except for a few small collections of cells, and that although after three or four days it consisted for the greater part of somewhat hyaline connective tissue enclosing the empty spaces left by degenerated parenchyma, living tumour cells could still be found more or less surrounded by the degenerated connective tissue, or in the case of larger grafts, assembled about the periphery. A few

days later most of the dead material had been absorbed, the connective tissue had become even more hyaline, and nearly all of the cells had disappeared from the central part of the graft, while coincidently a number of newly formed blood vessels and fibroblasts could be found at the edge of the fragment penetrating the old stroma, which was meanwhile gradually disappearing. The surviving elements of the parenchyma were in the midst of a lively proliferation, but there was nothing to suggest that their numbers were being increased through the addition of fibroblasts or any other type of cell belonging to the host. There could be no doubt, Jensen concluded, that the transfer of tumours from one animal to another was an instance of real transplantation.

The solution of the question was undertaken at about the same time by Loeb (16), but this author was unable to decide definitely whether the process was one of transplantation or infection, probably because his observations were conducted upon a sarcoma—a tumour-type in which the analysis of early growth is notoriously difficult.

At this point the investigation was taken up by Bashford and Murray (2), who entirely confirmed the findings of Jensen, and published with Cramer (3) in the following year more detailed observations, directing attention both to the connective tissue and the blood vessels. They showed that twenty-four hours after transplantation the introduced stroma, originally delicate and fibrillar, was now less distinct, and that its fibrils had become fused into thicker strands of glassy appearance, in the fissures of which lay many polymorphonuclear leucocytes. The connective tissue cells frequently showed such signs of commencing degeneration as granular protoplasm and pycnotic nuclei. Three days after inoculation, the cleft between the tumour and the tissues of the host was almost obliterated, and wandering cells were distinguishable in the graft itself, while the collagenous fibrils of the stroma had fused into thick glassy bundles, its cells in the meantime having undergone fatty degeneration and chromatolysis. Even though the graft had been well penetrated by connective tissue elements at the end of the three-day period, vascularisation did not set in until the fourth day. Once initiated, however, this process went on with great rapidity, and the young tumour was soon pervaded by a rich anastomosing system of blood capillaries. In still later stages, there was a continuous recession of the old stroma and an orderly progress of vascularisation by extension from the capillaries already formed ; and although on the eighth day the new connective tissue was still very cellular, grafts preserved eleven days after inoculation contained fibrils

of collagen. In the second publication, it was pointed out that the character which the connective tissue and blood vessels would assume in a transplanted tumour was determined, not by the tissues of the host, but by the parenchyma of the growth itself. The vascular arrangement conformed to that of the spontaneous tumour, and as the primary character was preserved during continuous propagation, the authors described the reaction as specific. There was thus presented a close parallel to the phenomena accompanying the development and differentiation of the various tissues and organs of the body as a whole.

In discussing the relations between a tumour and the blood vessels nourishing it, Ehrlich (8) also entertained the idea that its elements exercised a direct action upon the fibroblasts and angioblasts of the host, and that it was essential for the proliferation of the transplanted cells that they should do so. A propagable chondroma under observation in his laboratory showed a predisposition toward early hæmorrhage, because its cells exerted an influence upon angioblasts so energetic as to evoke a profuse outgrowth of capillaries, which underwent secondary sinusoid dilatation and finally ruptured. The power of inciting angioblastic growth was suppressed by intraperitoneal inoculation, exposure to heat, and implantation into immune animals.

Loewenthal and Michaelis (17) described the development of the new tumour from the surviving elements of the graft, and Da Fano (9) confirmed the degeneration of the old stroma and the elaboration of a new one by the host's connective tissue, while Flexner and Jobling (10, p. 40) extended the study of "early stages" to include their rat tumour, in which it was similarly found that the parenchymal cells gave rise to the new growth, while the connective tissue degenerated and was replaced by elements derived from the host.

Gierke (11) concurred with Ehrlich in analysing the stroma reaction into the fibroplastic and angioplastic types, in accordance with the varying extent to which the connective tissues and blood vessels responded to the stimulus of the parenchymal cells. If the two were in equilibrium, well nourished carcinomata with either large or small alveoli would be built up, while a predominance of the fibroplastic influences would exert an inimical effect upon the nourishment of the cell nests, and might explain, in part at least, the central necrosis of epithelial masses in a part or all of a tumour. A preponderance of angioplastic influences would, on the contrary, result in the formation of a small amount of connective tissue only, and of a rich supply of blood vessels such as was found in the hæmorrhagic carcinomata. As Bashford, Murray, and

Cramer (4) had already suggested, the nature of the stroma reaction might play a prominent part in the establishment of tumours after transplantation, a too powerful reaction leading to absorption, and too weak a one to insufficient nourishment and death of the engrafted tissue. It was conceivable, therefore, that the reaction was an essential element in determining individual susceptibility to implantation, and that the continuous growth of a graft was possible only when there was present in the host an adequate power of response to the stimulus eliciting the specific stroma reaction.

The information that had been gained through the observation of early stages of growth in the newly implanted graft, was applied by Russell (19) to an investigation of the refractory condition which follows unsuccessful tumour implantation or treatment with various normal mouse tissues. The findings were in all cases identical, a fact suggesting similarity between the resistance produced by unsuccessful inoculation with tumour and that evolved by the injection of normal tissue. That this one type included also the immunity described by Ehrlich (7) under the name "athreptic," was later demonstrated by the work of Bashford and Russell (5).

The results of Russell's investigations into the nature of resistance were briefly as follows :—The reaction taking place during the first two days about the implanted fragment in immune mice was the same as that in normal animals, but on and after the third day the active penetration by fibroblasts occurring in the latter was absent in refractory mice. As shrinkage took place in the necrotic centre of the graft, a cleft was produced between the tumour and the host's tissues, and along this free surface the cells of the parenchyma were spread out to form the wall of a cystic cavity. The host's tissues themselves were more cellular than the normal areolar tissue of the mouse, but this condition was mainly due to the presence of polyblasts and polymorphonuclear leucocytes. There was no evident increase in vascularity. Absorption of the necrotic mass was accomplished very slowly, for capillaries, fibroblasts, and polyblasts did not penetrate it in any number until after about seven days, and a space of about twelve or fourteen was necessary for the whole graft to be cleared up. The eventual destruction of the epithelial elements was brought about by an ingrowth of fibroblasts which so compressed them as to cause their dissolution. In the later stages of the process it was usual to find many of the epithelial cells multinucleated, a condition which Russell accredited to a gradual paralysis of their protoplasm inhibiting the completion of mitosis.

The outstanding feature in the resistant animals was their failure to supply the characteristic connective tissue and vascular scaffolding, but it was not clear to which one of two factors the absence of the reaction was to be ascribed. Thus it was conceivable that either the tissues had been altered in such a way that they no longer replied to the stimulus of the cancer cell, or that this cell itself had been robbed of the power to incite the specific stroma reaction. It seemed justifiable to explain the failure by the assumption that the malignant cell was deprived of its chemotactic properties, and eventually of its power of assimilation and growth. There must be present either in the circulating fluids or the tissues of resistant animals something able to inhibit chemotaxis ; but this unknown agent could not be a very active cell poison, because the cancer cells retained their proliferative power for from seven to ten days, and because, furthermore, it was those at the periphery of the graft which persisted in their growth, although they were the very ones most exposed to the influence of a hypothetical poison.

Burgess (6) described necrosis of the central part of the graft, degeneration of the stroma, and invasion of the tumour mass by new capillaries and proliferating connective tissue cells, not only in normal mice but in those of a naturally resistant breed as well. In the latter, however, after about a week of active growth, the tumour became surrounded by an inflammatory exudate which impaired its nutrition, and apparently as a part of this reaction there occurred in many of the non-susceptible mice an over-production of fibrils on the part of the more centrally located portions of the new stroma. Peripheral extension ceased, central necrosis at the same time advanced, and ultimately the whole tumour underwent destruction and absorption. The author pointed out that the increased connective tissue reaction in the graft which he had observed in refractory mice, where Russell, on the contrary, had established an absence of reaction, might have ensued because his own mice belonged to very closely related varieties capable of interbreeding freely.

It is hardly necessary, however, to go so far afield for an explanation of the difference. There has been an unfortunate failure of late on the part of several authors to distinguish between mice which are immune at the moment of implantation, so that the tumour never gains a foothold in them, and those in which spontaneous absorption is preceded by a certain period of growth. The histological appearances substantiated for the latter cases by Gaylord and Clowes, Bashford and Murray, and others, vary so widely from those described by Russell in

the case of grafts which have never established themselves as to indicate the existence of a distinct difference between the reactions manifested under the two conditions. Nor is further evidence of this dissimilarity lacking. Da Fano (9) found plasma cells distributed throughout the tissues of mice in which tumours were undergoing spontaneous absorption, as well as in those that had been unsuccessfully inoculated with sporadic growths. But when animals had once become resistant, a second inoculation of an immunising material was powerless to reproduce the reaction.

Russell's investigations have been recently confirmed by Anitschkow (1), and their value and accuracy have, in fact, met with almost universal recognition, although an occasional doubt regarding their validity has found expression, as in the paper of Goldmann (14). The thesis which he defended, and which has been expressed perhaps more clearly in earlier papers (12, 13), was, that the presence of blood vessels within tumours represented the product of a defensive reaction on the part of the host ; the subsidiary assumption was entertained that the vessels were endowed with the power of destroying tumour cells.

The opinion was expressed that the method of removing grafts practised in Bashford's laboratory was suitable only for the investigation of the larger blood vessels, and that in its employment the smaller ones were necessarily destroyed. Still, fig. 18 in the Second Scientific Report and fig. 8 in Russell's article, representing fragments removed in this way, show a large number of capillaries at the margins of the grafts, most of them so fine in calibre as hardly to exceed the diameter of a red blood corpuscle, while capillaries no greater in size can be discovered without difficulty in the figures accompanying the present article. The adequacy of the method cannot, therefore, be gainsaid.

Goldmann urged further against the validity of Russell's conclusions, that spontaneously receding tumours were well provided with blood vessels. There is, however, a fundamental difference between the failure of a graft to establish itself in a new host, and the regression of a tumour which has begun to grow. How great this difference must be is shown by the fact that it absolutely nullifies all efforts to bring about the disappearance of established growths by means of the inoculation of embryo skin, for example, which nevertheless is capable of evolving an almost complete immunity against implantation.

The purpose of the present paper is to extend the investigation of early stages to another species, and to describe in detail the reactions taking place in and about fragments of the Flexner-Jobling adenocarcinoma of the rat, after its transplantation into normal or resistant animals.

Immune rats were obtained by selecting those which had proved themselves resistant to one or two inoculations of the tumour in question, or by subjecting animals to previous treatment with 0·2–0·3 c.c. of an emulsion of rat embryo skin. The rats injected with this latter material were divided into three groups, two of which received no further immunising inoculation, while in the the third, preliminary treatment was repeated 21 days later with the tumour under consideration, to which they were found resistant. The introduction of the grafts destined to be excised for the study of early stages took place in all cases two or three weeks after the last immunising treatment.

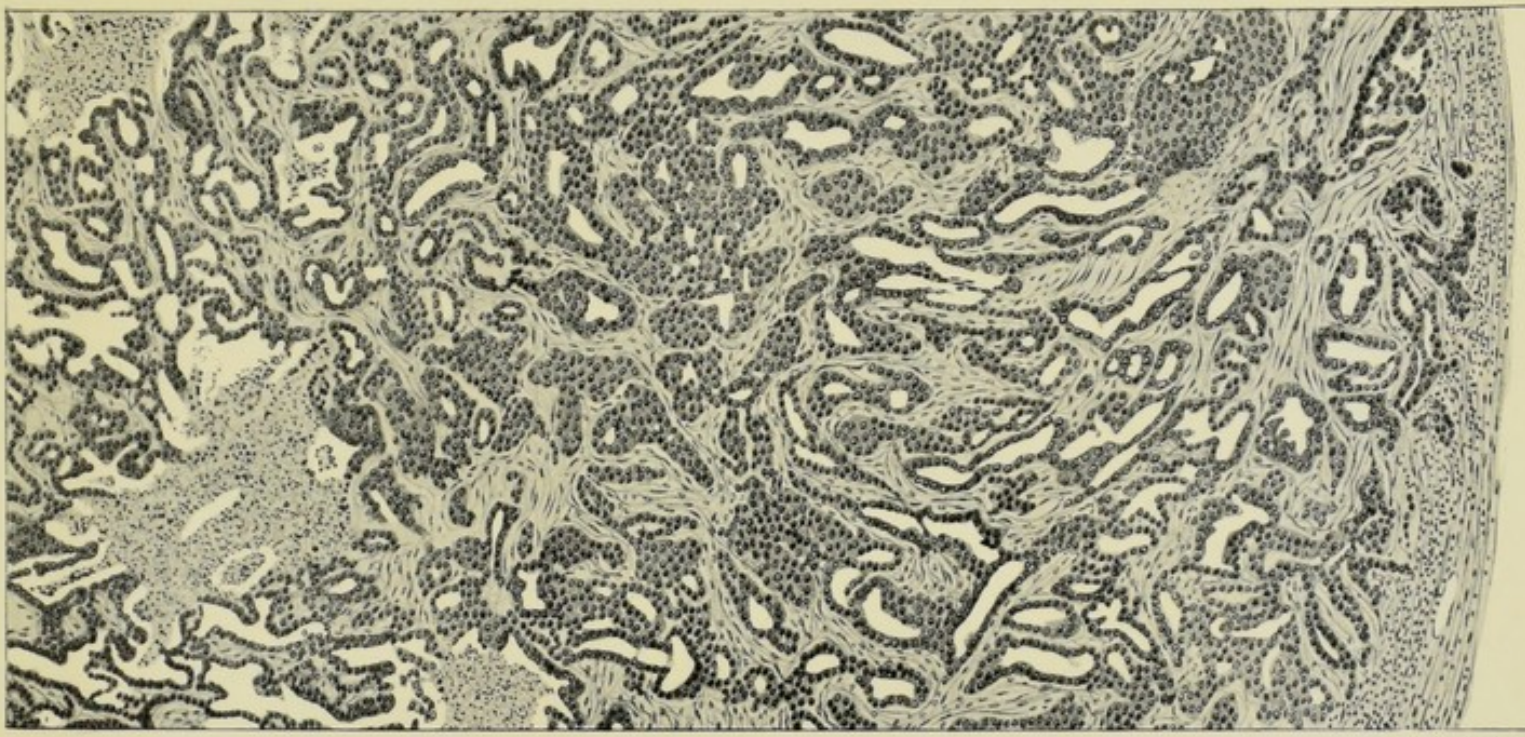
The technique employed throughout the investigation was identical with that which Russell has already described in full. Small fragments, selected from the healthy margin of a growing tumour, were deposited in the subcutaneous tissue of the axilla by means of a hollow needle inserted into an area in the groin previously epilated to prevent the introduction of hairs with its consequent confusion of the histological picture. Grafts introduced in this manner were removed with sharp curved scissors at varying intervals after inoculation, and in the extirpation as much of the neighbouring tissue as it was possible to include was maintained in undisturbed connection with the implanted fragment.

Six series in all were employed, and several grafts, usually three from normal, and an equal number from resistant rats, were procured in every one of the series for each of the stages investigated. The specimens thus obtained were fixed in Borrel's fluid and cut in serial paraffin sections, which were stained in Heidenhain's iron hæmatoxylin. It would be hard to emphasize sufficiently the imperative necessity of serial sections in work of this sort, for only by their aid is it possible to resolve an assortment of complicated phenomena into anything approaching an orderly sequence of events. From the large number of sections available, there were chosen for purposes of illustration those affording the best general idea of the conditions extant at the particular period that was to be described ; hence, all the appearances discussed for any one period may not be found in the corresponding illustration, for the text attempts a description of the state of the average graft.

The tumour utilised for these experiments was an adeno-carcinoma which was discovered in 1906 by Flexner and Jobling in the left seminal vesicle of an adult white rat. An extensive description of the histology of the primary growth, and that of the transplanted tumours belonging to the earlier generations, has been given by the authors (10) just mentioned. Through the kindness of Drs. Flexner and Jobling, the tumour was received at the laboratory of the Imperial Cancer Research Fund, where it has now been in cultivation for about four and a half years. While the yield of tumours attendant upon the inoculation of this growth into English rats varies enormously from one generation to another, it may be said that ten days after implantation from 85 to 100 per cent. of the animals have developed growths of from 0·2 to 0·5 gm. in weight. Many of these nodules, however, begin to recede soon afterward, and 50 per cent. of progressively growing tumours is but seldom realized, while in individual series all the growths may disappear spontaneously. As with the strain retained in America, so in the one grown here, a diagnosis was not readily to be made in the first few generations, although after a short time the growth proved clearly enough to be an adeno-carcinoma. The parenchyma, except at the growing edge, arranges itself in acini, and there is a frank tendency toward the assumption of a papilliferous character, which, however, is always curtailed by the onset of necrosis. The stroma, which in common with other fibrous connective tissue of the rat is significant for its collagenous nature, is well vascularized, and appears fairly cellular by reason of the large number of fibroblasts which are distributed through it. The histology of the adult growth is reproduced in fig. 1.

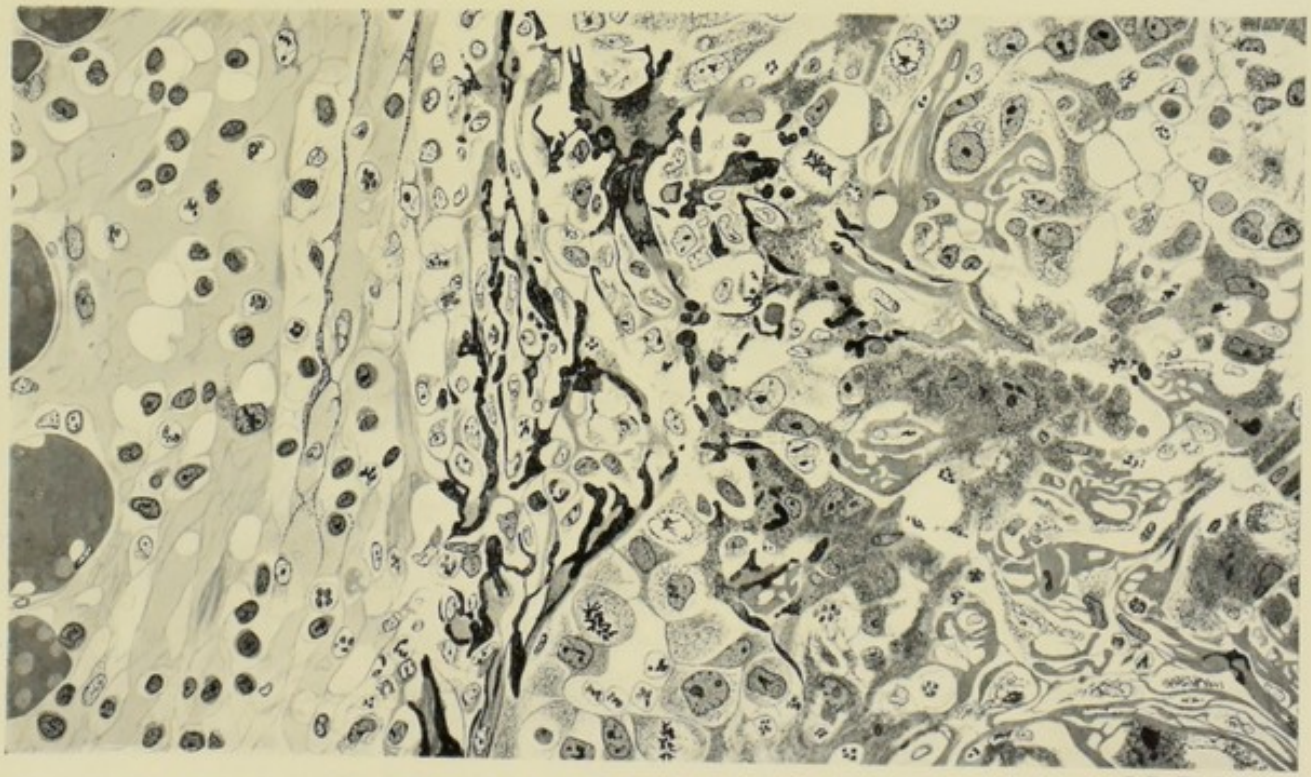
The various phases of the connective tissue elements of the rat have been so fully and so accurately described by Maximow (18) that any further description would be superfluous.

Gratts removed from normal rats twenty-four hours after inoculation exhibit changes of which those set forth in fig. 2 may be regarded as typical. The fragment is infiltrated with polymorphonuclear leucocytes, and in the parenchymal cells toward its centre degenerative changes have already commenced. These elements are shrunken, their protoplasm is filled with tiny granules stained a deep brown or even black by the osmic acid in the fixative, their nuclei have become smaller and irregular, and active proliferation is in progress only among those at the margin. The collagen fibrils of the stroma have fused into thick glassy bundles, and the great majority of the connective



J. R. Ford, del.

Fig. 1.—Adeno-carcinoma of the rat (Flexner-Jobling), 26 days old, 66th generation. (Zenker; Weigert—van Gieson. $\times \frac{87}{1}$.)



J. R. Ford, del.

Fig. 2.— $\frac{\text{FR}}{56\text{A}}$. Graft removed from a normal rat 24 hours after implantation.
(Borrel; iron-alum-hæmatoxylin. $\times \frac{410}{1}$.)

tissue corpuscles show the same phenomena of degeneration as have just been described for the cells of the parenchyma, while capillaries are discoverable only with difficulty because of the destruction in which their endothelial coat has already become involved. These conditions obtain in the stroma of most grafts at this period, but occasionally one may be found in which the connective tissue elements are fairly well preserved, although mitotic figures are not to be found. The implantation, as a rule, is separated from the tissues of the host by a cleft containing leucocytes, and associated with this space there is a fibrinous exudate increasing the isolation from the host's tissues. The connective tissue surrounding the graft is œdematous, infiltrated by polymorphonuclear leucocytes and small round cells, and more richly cellular than normally, although division figures are only rarely discoverable in its fibroblasts. As these elements seem to have increased out of proportion to the number of mitoses present among them, it is impossible to deny the suggestion that they undergo amitotic division, although indisputable evidence in favour of such a process cannot be adduced.

Grafts taken from immune rats after the expiration of the twenty-four hour period present changes similar to those found in fragments excised from normal animals, with perhaps this slight difference, that the emigration of polymorphonuclear leucocytes is not so marked. But this disparity, if indeed it be constant, is too slight to merit more than passing notice.

In pieces of tumour removed from normal rats after forty-five to fifty-four hours, the degenerative changes affecting the parenchyma are at once more advanced and more widely distributed. The protoplasm of many of the cells toward the central region is now represented only by granular fatty débris, while the nuclei are converted into irregular particles of chromatin or are, at the best, shrunken and of irregular dentate outline. Those elements of the parenchyma more happily situated at the margin of the implantation, have, on the other hand, been able to survive, and in some cases even to undergo division. The emigration of polymorphonuclear leucocytes has come to an end, and the greater number of those which have succeeded in entering the graft are to be found gathered about the necrotic parenchyma. The collagen of the stroma is very glassy, most of the connective tissue cells are shrunken, their nuclei pycnotic, while their protoplasm contains granules staining brown or black with osmic acid. The cleft and the fibrin barrier are still present. The space between the implanted fragment and the tissues of the host is the result of shrinkage, which,

taking place during fixation, denotes the absence of any union between the two structures.

While fragments excised from immune rats after forty-five to fifty-four hours are themselves in much the same condition as those removed from normal ones at the end of the same space of time, there is nevertheless some difference in the surrounding tissues. This reveals itself in a more active proliferation of the connective tissue corpuscles in normal animals, and the process may lead occasionally to a penetration of the graft by fibroblasts and capillaries even at this early period, although such an occurrence is unusual before the third day.

The most interesting period in the history of a graft which is establishing itself in a new soil, is the third day. In the majority of tumour fragments removed from normal rats at this time, the fibrinous exudate and the cleft, both formerly separating the graft from its host, have disappeared. The fibroblasts of the surrounding connective tissue have entered into the growth and are engaged in the building of a new stroma, while penetrating capillaries can be discovered at the edge of the young tumour. The outermost cells of the parenchyma are in active mitosis, but the centre of the graft is quite necrotic. In fig. 3, representing the edge of a nodule which was removed seventy hours after implantation, the close connection between the growth and its host can be readily appreciated from the intimate commingling of tumour cells and connective tissue elements, as they are reproduced in the drawing. A new capillary, containing blood, has entered well into the fragment, and is indicated toward the bottom of the illustration near the centre. The hyaline remains of the old stroma, its penetration by polymorphonuclear leucocytes, and the serious involvement of its connective tissue cells are also reproduced, as well as the active mitosis that is in progress among the elements of the parenchyma at the growing edge of the graft ; but the necrotic tumour cells in the centre of the fragment are not included in the picture. The surviving elements, which have hitherto been content merely to sustain life and to proliferate as best they may now often show signs of an attempt to assume an acinous arrangement.

Whether the new framework is derived entirely from the connective tissue of the host, or whether certain cells of the transplanted stroma survive long enough to participate in its construction, is a question difficult of decision. Certain it is that all the elements of the transferred stroma seem to be considerably damaged before the entrance of connective tissue corpuscles from the host ; still the possibility cannot

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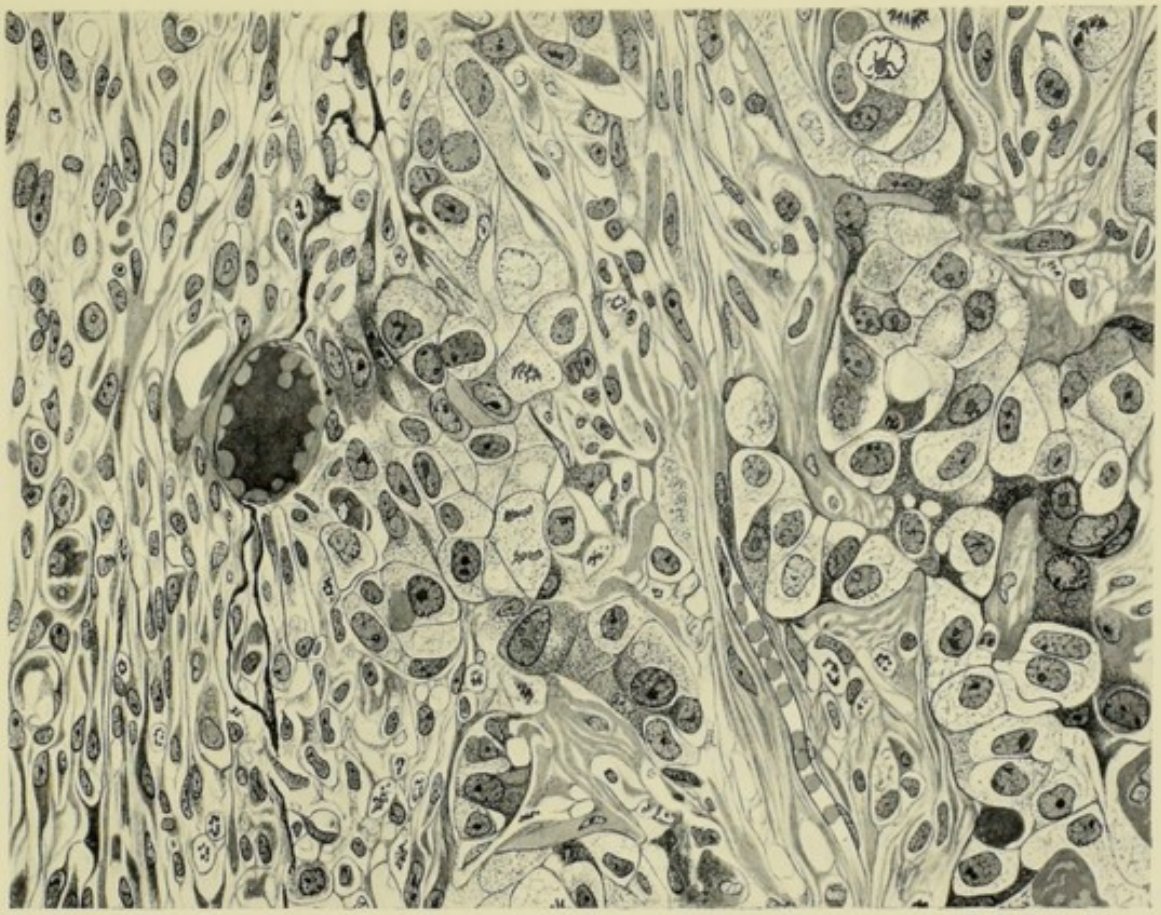

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Fig. 3.— $\frac{FR}{60\,A}$. Graft removed from a normal rat 70 hours after implantation.
(Borrel; iron-alum-hæmatoxylin. $\times \frac{410}{1}$.)

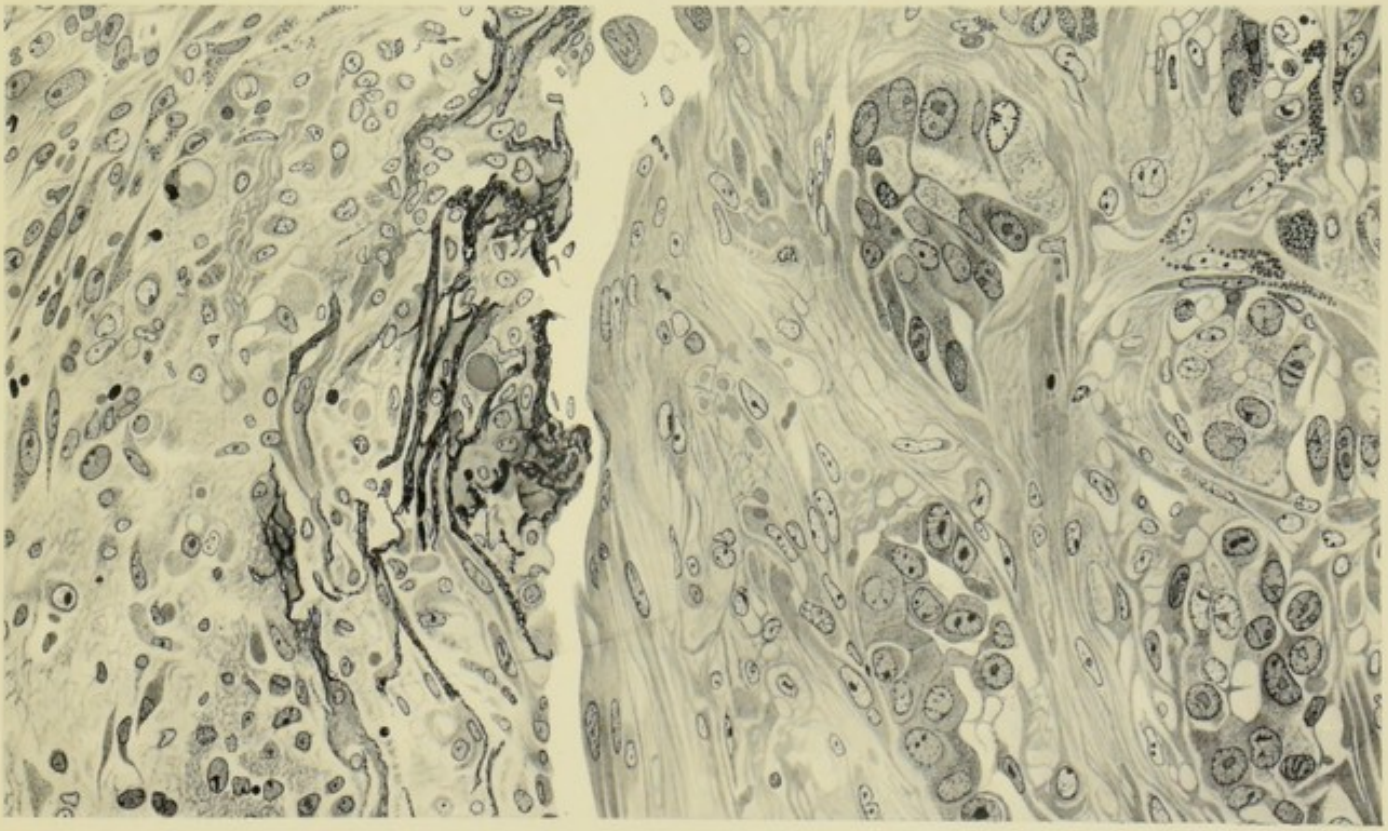

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Fig. 4.— $\frac{FR}{56\,A}$. Graft removed from immune rat 72 hours after implantation.
(Borrel; iron-alum-hæmatoxylin. $\times \frac{410}{1}$.)

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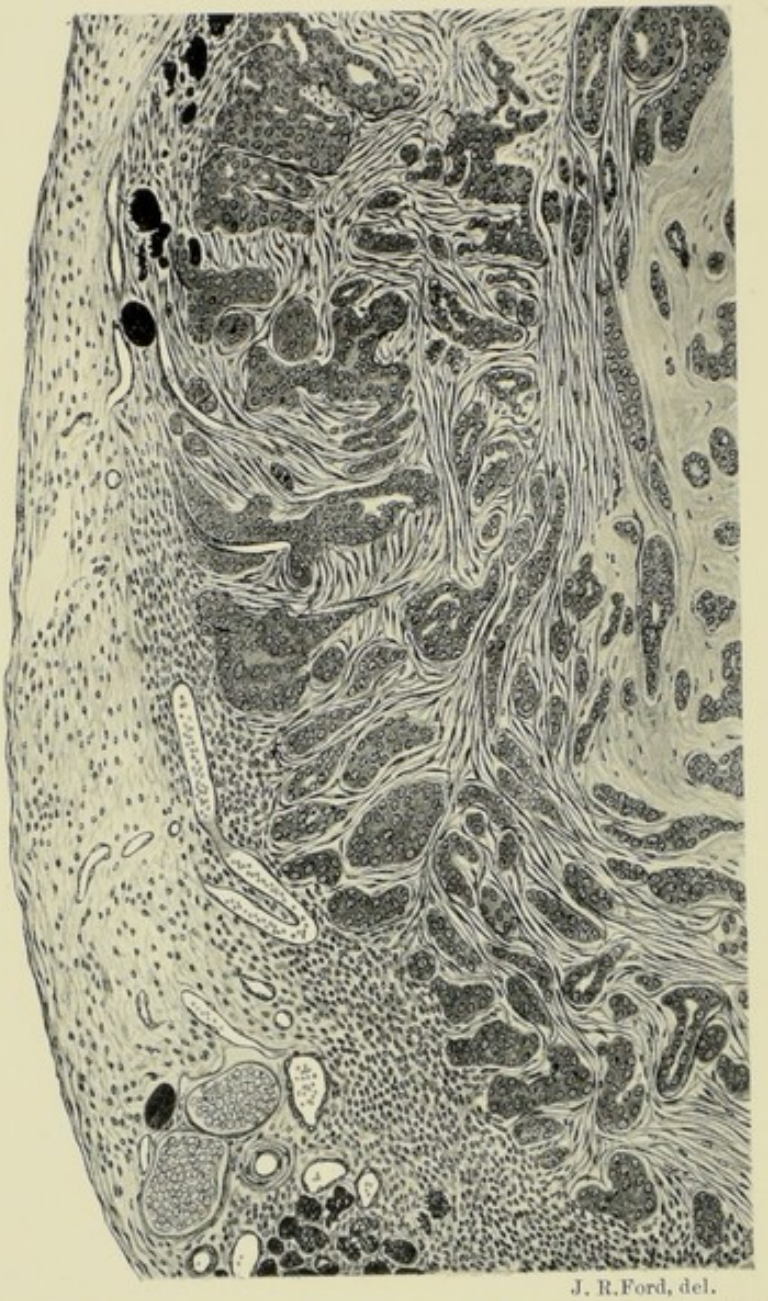

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Fig. 5.— $\frac{FR}{56A}$. Graft removed from a normal rat 5 days after implantation.
(Borrel ; iron-alum-hæmatoxylin. $\times\frac{78}{1}$.)

be eliminated that the damaged cells may in some cases be able to recover and continue their proliferation for a time. Beyond reasonable doubt, however, nearly all of the introduced stroma perishes within the first few days, so that the framework of the new tumour is entirely, or almost entirely, the product of the host. This view coincides with that of Flexner and Jobling, who have expressed the opinion that only the epithelial cells of this growth survive transplantation, that the new tumour is the result of their proliferation, and that its stroma is furnished by the connective tissues of the host.

The contrast between the condition just described and that represented in fig. 4, which reproduces a graft taken from an immune rat seventy-two hours after inoculation, is so striking that there is little need to insist upon it. The outlying cells of the parenchyma are still well preserved at this period, and division figures are not infrequent, but the centre of the graft is entirely necrotic. All traces of acinous arrangement have been lost, and the cells lie either in irregular groups within the lacunæ of the stroma, or else in single layers at the edges of the cleft. The vanquished fragment is shrunken, it remains entirely separated by a space and a barrier of fibrin from the neighbouring connective tissues of the host, and nowhere can there be detected that projection of fibroblasts between the cells of the tumour which invariably occurs in successful grafts. In many cases the fibrin barrier is even more persistent than in the specimen from which the drawing was made.

Figs. 5 and 6 reproduce the condition of the young tumour in normal rats on the fifth day, the latter representing a high power view of the edge of the graft at the junction of the upper and middle thirds of the low power view. Fig. 5 shows the vigorous inrush of capillaries and fibroblasts to form the stroma of the new tumour. The cutting off by the fibroblasts of small cell groups, and the initiation of an acinous arrangement of the elements of these groups, give promise of the fidelity with which the architecture of the tumour will be reproduced. As a contrast to this healthy young connective tissue there has been included at the right hand side of the illustration a portion of the old stroma, in which the hyaline condition of the collagen and the degeneration of the fibroblasts are obvious. Although the part of the parenchyma surrounded by this dead stroma is not very badly damaged, in the more central regions of the graft, outside the limits of the cut, destruction of the parenchymal cells is complete. The cellularity of the host's tissues at the edge of the graft, most marked in the lower portion of the drawing between the sections of nerve and the margin of

the nodule, is partly the result of an intense proliferative activity on the part of the fixed elements of the connective tissue, while a further factor in its production is the presence of cellular infiltration and of a large number of young blood vessels.

Fig. 6 was drawn at a higher magnification to show the process in more detail. The greater number of tumour cells are well preserved, and division figures are not uncommonly discovered among them. In many of the cell groups the elements have arranged themselves in acini, although this has not occurred in the small field of the growth which has been reproduced. The graft is swarming with new capillaries and young fibroblasts, but there is no evidence yet of that abundant production of collagen which is the salient characteristic of the stroma of the tumour under discussion. In many cases the fibroblasts have penetrated as far as the centre of the fragment, and vascularization is further advanced than it is in three day specimens. What remains of the old stroma is found chiefly toward the inner regions of the nodule, in an excessively degenerated condition, the nuclei of its cells pycnotic and its collagen fibres represented by a hyaline homogeneous material which stains somewhat more darkly than was the case in three day grafts.

After the fifth day the graft in a normal animal is the theatre of a progressive and orderly vascularization and formation of stroma. The recently entered fibroblasts and the proliferating parenchyma, in which acinous differentiation is now quite or almost complete, encroach more and more upon the remains of the old connective tissue and upon the necrotic tumour cells in the centre. By the seventh day, the production of collagen having commenced in the new stroma, the implanted fragment may be said to be well on its way toward maturity, although there may still remain in the interior a certain amount of detritus representing the old stroma and those parenchymal cells which were unable to survive transplantation.

Figs. 7 and 8 set forth low and high power views of the graft five days after transplantation into immune rats. As may be appreciated from the former, there is nothing left of the implantation but a cyst containing the products of degeneration, and lined by tumour cells. As the next figure demonstrates, while these marginal elements are still alive, and in a few instances even undergoing mitosis, the specific reaction which would have provided a vascular and connective tissue scaffolding for the epithelial cells and permitted a survival of the engrafted tumour, is entirely in abeyance, and at no point of the

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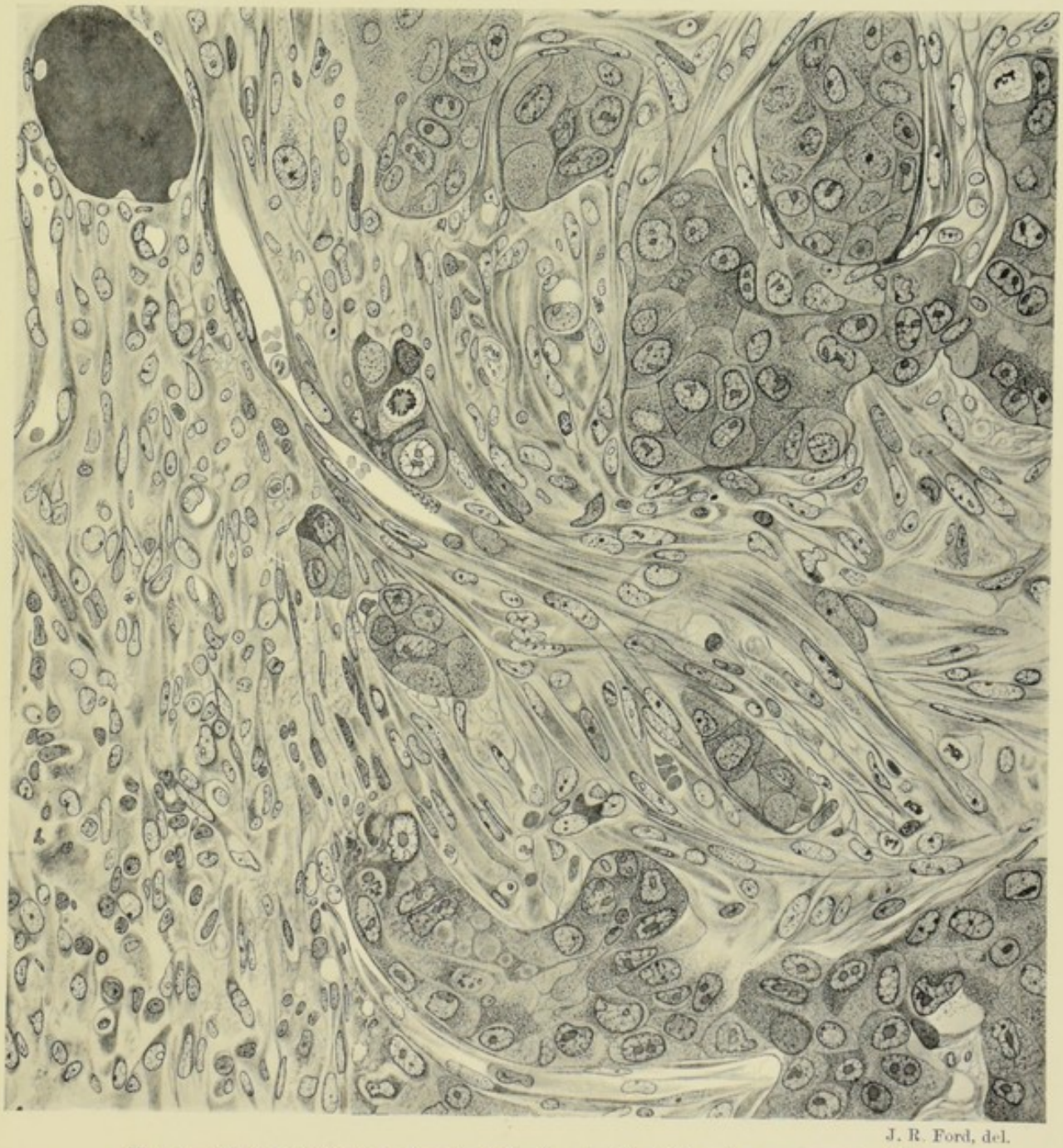

J. R. Ford, del.

Fig. 6.—High power view of part of fig. 5 as indicated by the arrow. ($\times \frac{410}{1}$.)

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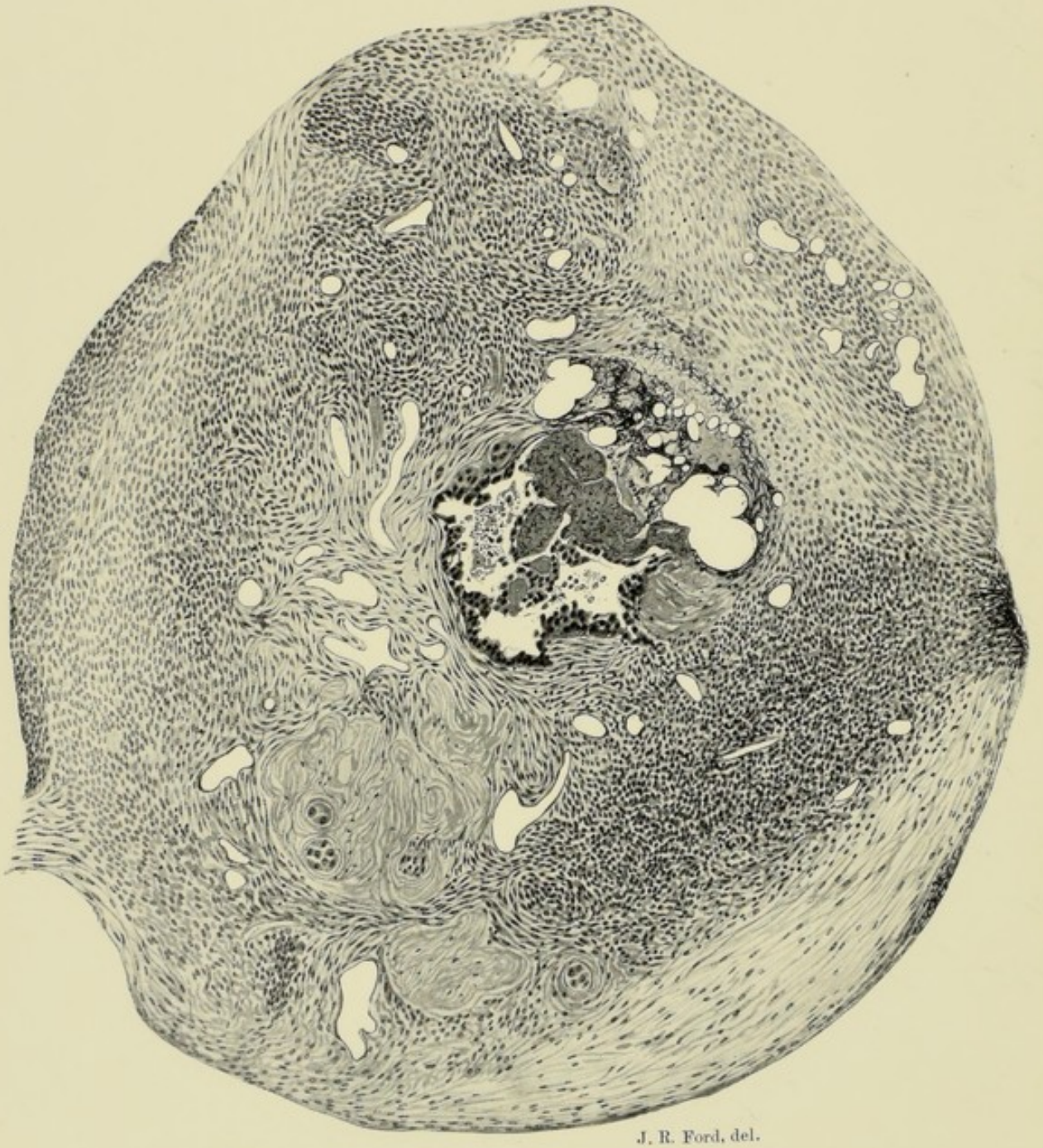

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Fig. 7.—$\frac{\text{FR}}{60\,\text{A}}$. Graft removed from an immune rat 5 days after implantation.
(Borrel ; iron-alum-hæmatoxylin. $\times \frac{78}{1}$.)

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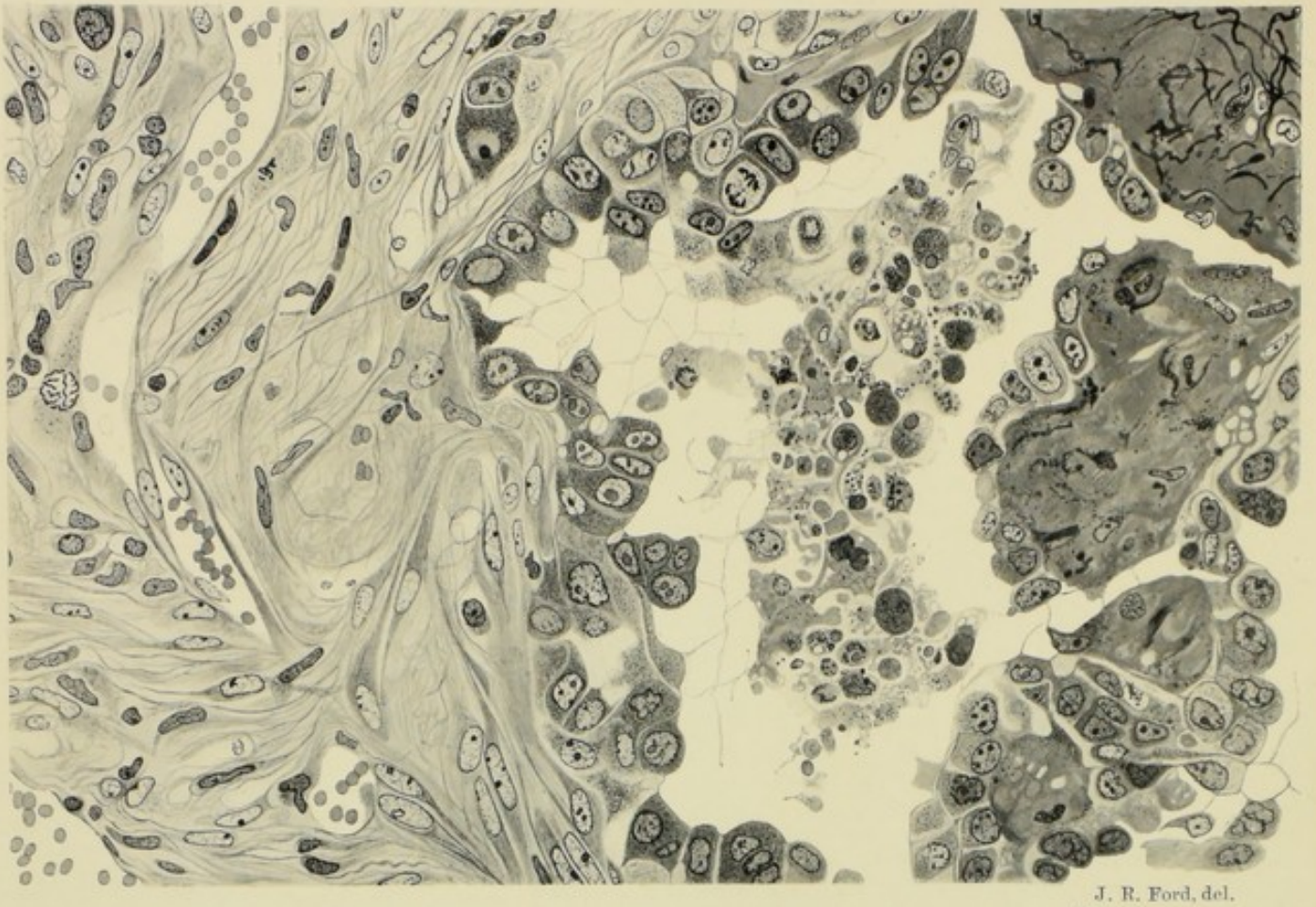

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Fig. 8.—High power view of part of fig. 7 as indicated by the arrow. ($\times \frac{410}{1}$.)

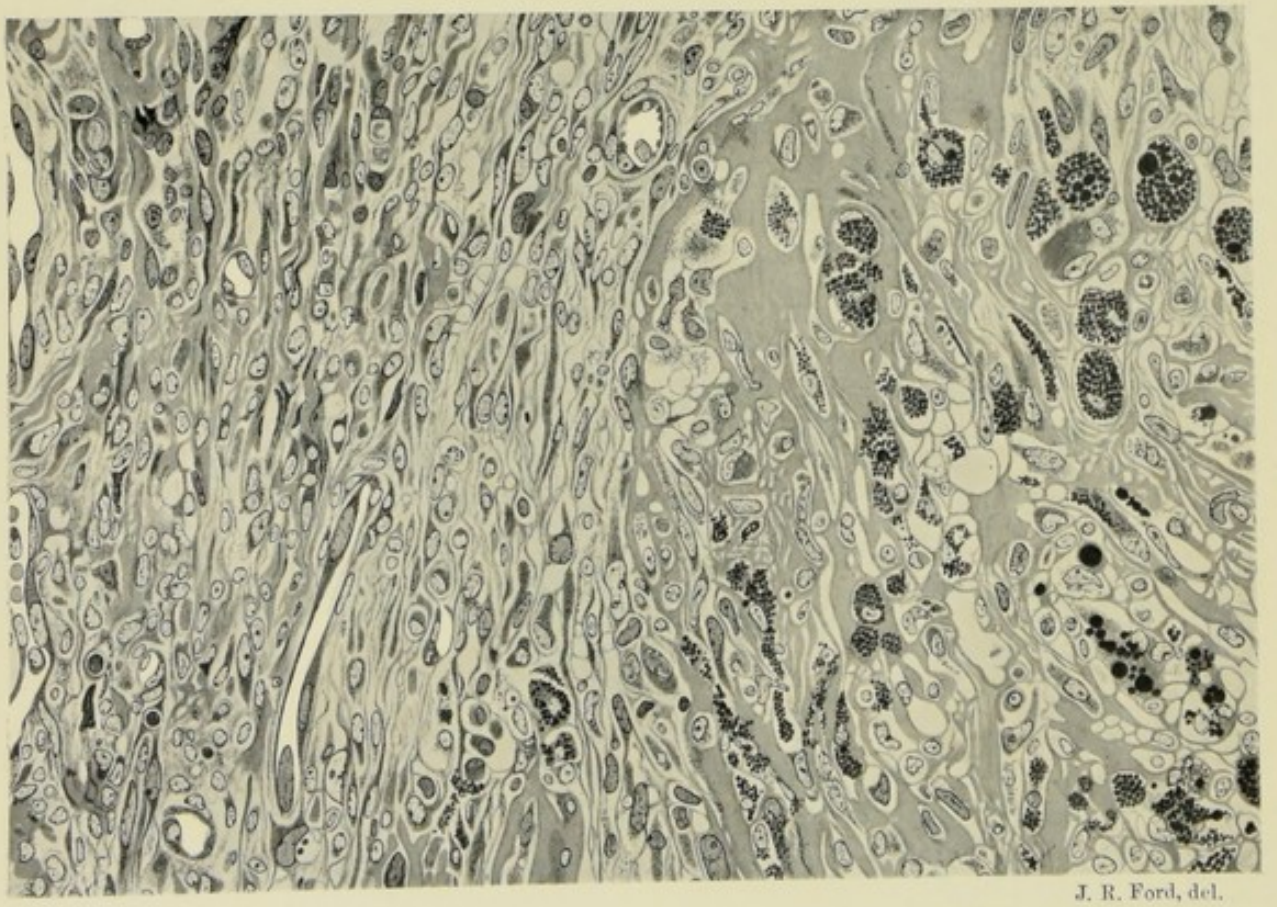

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Fig. 9.—$\frac{FR}{37.4}$. Graft removed from an immune rat 10 days after implantation.

periphery is it possible to demonstrate the entrance of either fibroblast or capillary. Robbed in this way of nourishment, the implanted cells have succumbed to their unfavourable environment, save only those of the outlying layer, which, by virtue of their position, have been able to absorb a certain amount of nourishment from the tissues of the host, and even to proliferate, if only in small degree. But the vitality of these elements themselves is at a low ebb, and in specimens removed a full day earlier it is not difficult to find certain of them with from two to five mitosing nuclei, witnesses—as Russell has indicated for mouse material—of a gradual paralysis of the protoplasm preventing the completion of division. All traces of alveolar arrangement have been lost, and in many cases the fibrinous exudate still encloses the graft, making its isolation even more complete.

Although among the cells bordering the dying graft in immune animals there may occasionally be found a few undergoing division as long after transplantation as eight days, most of them show signs of serious damage at the end of this period. By the tenth (fig. 9), most, if not all, of the epithelial elements have been entirely destroyed. The implant is undergoing penetration by fibroblasts from the host, phagocytosis is in active progression, and the whole process, in short, represents the efforts of the tissues to rid themselves of the defeated tumour fragment.

Conclusions.

(1) The investigation which has been described in the preceding pages leads inevitably to the decision that the phenomena described by Russell as characterizing the immunity of mice to tumour implantation occur also in the case of the resistance offered by rats.

(2) Furthermore, as no difference could be detected between grafts taken from rats treated with embryo skin, and those removed from animals which had undergone a previous unsuccessful inoculation with tumour, it is concluded that resistance is similar in the two cases.

(3) Finally, both of these types are the outcome of a failure on the part of the new host to furnish to the implanted fragment the proper blood vessel supply and connective tissue scaffolding.

The expenses of this investigation were borne in part by the Crocker Cancer Research Fund of the College of Physicians and Surgeons, Columbia University, New York City.

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[*Fifth Scientific Report of the Imperial Cancer Research Fund.*]

A CYTOLOGICAL ANALYSIS OF THE REACTION IN ANIMALS RESISTANT TO IMPLANTED CARCINOMATA *.

By C. DA FANO, M.D. (Milan).

That small experimental animals, rats and mice, can be rendered immune to implanted cancer, is one of the many facts that have been brought to light by the experimental investigation of tumours, and, moreover, is one which has been corroborated by various observers in different laboratories. Attempts to demonstrate an analogy with other forms of immunity, especially with a view to demonstrating antibodies in the blood serum by analysis *in vitro*, have been fruitless, and all that has been accomplished so far, has been to follow the changes occurring during spontaneous healing, and to establish the differences between the connective tissue changes observed in the early stages of growth in grafts from normal and immune animals. These investigations build the foundation for the present series of experiments, which have been designed to elucidate the histological details of these processes.

The connective tissue changes occurring in carcinomata undergoing spontaneous absorption were first studied and compared with those observed in immune animals. The investigation was later extended to a study of the reactions following inoculation of embryo skin, blood, and spontaneous tumour, as well as tumour previously killed by freezing and grinding.

The material and the methods of transplantation were those usually employed in this laboratory, and will be found fully discussed in various papers in previous Reports. Of the special stains utilised, Azur II solution (Grübler), with which very satisfactory results were obtained, may be recommended. As a differentiating fluid, a mixture of nine

* This paper is a somewhat abridged translation of the author's "Zelluläre Analyse der Geschwulstimmunitätsreaktionen" (Zeitschrift für Immunitätsforschung, 1910, Bd. v. Heft 1), and gives the result of work carried out in the laboratory of the Imperial Cancer Research Fund.—E. F. B.

parts of 90 per cent. alcohol with one part of aniline oil was employed, followed by careful washing in 90 per cent. alcohol. The best results were obtained after alcohol fixation. To avoid confusion, the structure and nomenclature of the normal connective tissue elements will be discussed first.

The Loose Connective Tissue of the Mouse, and the Nomenclature of its Elements.

The subcutaneous and intermuscular connective tissues have been especially examined, because they are the usual sites selected for the inoculation of tumours. While a complete review of the literature pertaining to this subject is rendered unnecessary by the recent monographs of Marchand (12) and Maximow (13), the work of the latter author will be discussed fully, as the nomenclature adopted by him has found wide acceptance. He described the following elements in the connective tissue of the rabbit :—(1) Fibroblasts, (2) Wandering cells, (3) Clasmatocytes, (4) Fat cells, (5) Clasmatocyte-like adventitial cells.

By fibroblasts he understands the distinctive connective tissue corpuscles. The wandering cells are small elements analogous to the blood lymphocytes, which in the course of development forsake the blood stream and wander into the tissues.

The clasmatocytes, described first by Ranvier (20), exhibit a denser nuclear network than the fibroblasts, and contain coarse refractile granules in the cytoplasm. Maximow accepts Ranvier's view that they develop from the wandering cells, and may in turn become changed into fibroblasts. Although the clasmatocytes of mammalia are readily distinguished from mast cells by their staining reactions, this distinction does not hold for the amphibia.

The clasmatocyte-like adventitial cells are identical with the elements described by Marchand as adventitial cells, and some of them, according to Maximow, are atypical fibroblasts, while others are clasmatocytes. Marchand's category of adventitial cells certainly includes elements indistinguishable from the wandering cells. Marchand (12) ascribed to the adventitial cells an important part in all inflammatory and reparative processes, and noted their early proliferation under those conditions and their transformation into the following forms :—(1) round cells with varying amounts of protoplasm, and possessing amœboid movement and phagocytic properties, (2) lymphocyte-like cells which form the chief part of the "small cell infiltration," (3) mast

cells, and (4) plasma cells *. To all these various forms of the adventitial cell Marchand applied the name "leucocytoid-cells."

In Maximow's opinion cells from the blood stream also played an important part in reparative inflammations. The clasmatocytes and clasmatocyte-like cells rounded themselves off in the first few hours of an inflammatory process, and became converted into large amœboid cells. In this same period, Maximow found an active emigration from the blood stream of small lymphocytes, which mingled with the pre-existing wandering cells, altered clasmatocytes, and clasmatocyte-like adventitial cells. To all these various cells he gave the name "polyblasts," and as in his opinion plasma cells are simply emigrated and metamorphosed lymphocytes and mononuclear leucocytes, these also were included under the above term. The so-called macrophages he regarded as products of the evolution of polyblasts. The term "polyblast" includes rather a large variety of elements, and Maximow himself later restricted its application by excluding the plasma cell.

It would appear that Maximow's "polyblast" and Marchand's "leucocytoid" cell are interchangeable terms, but with this restriction in view of the respective authors' opinions, that the former are of histogenetic and hæmatogenetic origin, the latter of histogenetic origin only.

The loose connective tissue of the rat was later the subject of a special article by Maximow (14). Two of the histological points in which this animal differs from the rabbit are that the clasmatocytes do not contain specifically staining granules, and that polymorphonuclear leucocytes are present under normal conditions in the connective tissues. In inflammatory reactions the polyblasts take an important part, but plasma cells are never involved in the scar formation.

In a more recent communication, Maximow (15) returned to the classification of the normal connective tissue elements, and gave a review of the rôle played by the various cells in reparative inflammatory processes. He found the connective tissues of all mammalia to contain the following elements :—(1) Fibroblasts, (2) Mast cells, (3) Quiescent wandering cells †, (4) Small amœboid wandering cells,

* In view of the complexity of the question, and the wide differences of opinion on the subject, it has been thought advisable to use in the present article the definition of plasma cells given by Maximow (13). Special attention was paid to the question whether these cells are to be found in the normal subcutaneous or fatty tissues of the mouse. The source of these cells has not been fixed, and the present investigation of the cellular reactions in cancer-immune animals does not decide whether lymphocytes can undergo diapedesis, although it will be seen from the experiments that it is a possibility to be taken into account.

† Quiescent wandering cell is used throughout for "ruhende Wanderzelle" in the original.

(5) Plasma cells, (6) Eosinophile cells, and (7) Fat cells. In inflammation the fibroblasts beget only fibroblasts, whilst the mast cells are destroyed and phagocyted early by the polyblasts. The quiescent wandering cells are the mammalian clasmatocytes of Ranvier, and include Renaut's (21) "cellules rhagiocrines"; in inflammation they become altered into large amœboid cells, the polyblasts, many of which are to be regarded as derivatives of emigrated lymphocytes. The small amœboid wandering cells occur round the blood vessels and in the fatty tissues, and correspond in shape and size with the lymphocytes of the blood, whilst the plasma cells possess all the well-known characters, and are capable of mitotic division.

From this superabundance of names it is difficult to choose those which will carry clearness and conviction. Fibroblasts, mast cells, and fat cells are all well known and well characterized elements. The use of the term "quiescent wandering cells," in place of "clasmatocyte," has certain advantages, but there is little good to be served by replacing the name "lymphocyte" with "small amœboid wandering cell." As regards the plasma cells, it may be stated that these have never been found in the normal connective tissue of the mouse. Although it is difficult to deny the diapedesis of lymphocytes in the formation of connective tissue, and appears logical to classify emigrated lymphocytes with the quiescent wandering cells, still the use of the term "polyblast" for the whole category cannot be recommended, and in the present paper will be replaced by that of "wandering cell." This term will include elements not only of histogenetic, but of hæmatogenetic origin, and is used in the same sense as it was by Bashford, Murray, and Cramer (1). It may be added that the macrophages are excluded from this category, as special observations point rather to their being solely of histogenetic origin. The term "macrophage" does not include all large cells possessing phagocytic powers, but only those having relatively small rounded nuclei with a very fine network of cytoplasm. The finely granular pseudo-eosinophile leucocytes, included by Maximow in the eosinophile cells, will be designated by the older name as polymorphonuclear leucocytes, whilst the coarsely granular eosinophile leucocytes alone will be termed eosinophile cells.

This preliminary discussion forms the preface to a description of the normal connective tissue of the mouse. The two fibroblasts pictured in fig. 1 (Plate 1) exhibit the morphological characters of this cell type, while in fig. 2 (Plate 1) it will be seen that they may resemble, when seen in profile, the endothelial cells. Around blood vessels and nerves, and at the transition between cutis and subcutis, the fibroblasts often

lose their usual outline, and become smaller and irregularly angular. A characteristic quiescent wandering cell, a type of rare occurrence in the mouse, is pictured in fig. 3 (*a*) (Plate 1); but small cells with sharply defined nuclei, such as those shown in fig. 3 (*b*, *c*, *d*, *e*) (Plate 1), have been classified also as quiescent wandering cells. Elements are often encountered resembling both fibroblasts and quiescent wandering cells, *cf.* fig. 2 (*ruh.Wz.*?) (Plate 1).

Lymphocytes (fig. 4, Plate 1) occur in very small numbers in the subcutaneous tissues, and the features of the mast cells are shown in fig. 2 (*Mz.*) (Plate 1).

Polymorphonuclear leucocytes are not infrequent in the normal connective tissue, and the ring-shaped nucleus is a common feature (fig. 2, *pl.Lkc.*) (Plate 1). Eosinophile leucocytes have not been seen. The fat cells (fig. 5) (Plate 1) exhibit no special features apart from a well-marked meshwork in the cytoplasm. Plasma cells, macrophages, and wandering cells are absent.

The Cells of the Stroma of Progressively Growing Tumours.

A. Early Stages.

Bashford, Murray, and Cramer (1) have described in these Reports the changes which occur in the tissues surrounding an implanted graft from two hours up to several days after inoculation. Löwenthal and Michaelis (11), and Russell also (24) have followed in detail the reactions supervening after the grafting of a tumour. With the exception of certain cases of secondary sarcoma development (10), all the authors are agreed that the transferred stroma dies, a conception which the present investigation entirely confirms.

The present research was made especially with carcinoma **27** and a more rapidly growing tumour, carcinoma **63**, which gave essentially the same result. The site of inoculation was examined on the 1st, 2nd, 3rd, etc., days.

The immigration of polymorphonuclear leucocytes into the tumour occurs very early, and in an intense degree. Many of them when stained with Giemsa solution after fixation in Zenker's fluid, show a pseudo-eosinophile granulation. After 4–6 days, eosinophile cells are found in the neighbourhood of capillaries and small hæmorrhages (fig. 6, *eos.Lkc.*) (Plate 1). Under aseptic conditions, the polymorphonuclear leucocytes, which exhibit no phagocytosis, gradually diminish in number and disappear about the fourth day, at a period when the degeneration of the old stroma has also occurred. The majority of them degenerate *in situ*, but a few appear to remain as permanent elements of the new stroma.

With the polymorphonuclear leucocytes, fairly large numbers of lymphocytes appear after 24 hours, but are found especially around the blood vessels and in the fatty tissue at some distance from the grafts. At this period it is easy to distinguish the lymphocytes from the quiescent wandering cells, which have now become more rounded and have acquired a more voluminous cytoplasm, but, during the second day, the lymphocytes undergo changes which render them hardly distinguishable from the quiescent wandering cells. This resemblance is shown well in fig. 40 (*Wz.*) (Plate 4), and as they now correspond exactly to Maximow's polyblasts, or the wandering cells noted by Bashford and Murray, they will be termed simply wandering cells. The number of lymphocytes continues to increase, but in tumours which grow well never becomes excessive.

After the second day, the contour of the fibroblasts becomes more distinct, and they begin to divide mitotically and amitotically. They pass into the graft about the fourth day, as shown on fig. 7 (Plate 1), reproduced from page 348 of the Third Report (24), with which may be compared the figures on page 28 of the second part of the Second Report (1). The central areas of the new stroma are composed entirely of fibroblasts, but in the periphery there are in addition numbers of polymorphonuclear leucocytes, lymphocytes, and quiescent wandering cells. Mitotic division of the last-named class of cells is shown in figs. 31 and 38 (Plate 4). As regards the later stages of stroma development, it may be added that macrophages in small numbers appear about the eighth day, more especially in connection with small hæmorrhages such as have been seen with carcinoma **63**. A few plasma cells appear after the eighth day. To the published descriptions of the development of new blood vessels there is nothing to be added.

B. Fully Developed Tumours.

The same tumour-strains, carcinomata **63** and **27**, were used for studying the stroma of fully developed tumours. The findings are fairly constant, such variations as occur affecting mostly the anatomical arrangement and the quantitative relationships of stroma and parenchyma. Mitoses of fibroblasts like those shown in fig. 8 (*Fbl.*) (Plate 1), are relatively rare.

The quiescent wandering cells are present in large numbers, and especially abundant in the periphery of the tumour (fig. 8, *ruh. Wz.*) (Plate 1), a fact that may be brought into correlation with the continuous production of new stroma. The number of lymphocytes present

(fig. 8, *Lmc.*) (Plate 1), is in excess of that in normal connective tissue, and they are arranged around the newly formed capillaries. Transitional forms between lymphocytes and quiescent wandering cells are also seen, but not in very large numbers.

Mast cells have been found both at the periphery of the tumour and, after 8 days, in the deeper parts of the stroma, but mitotic division has not been detected among them. Small collections of plasma cells, mixed with lymphocytes, are still present in fully developed growths, and some of the small dark areas to the right of fig. 9 (Plate 1) represent such aggregates in a small 11-day old growth of carcinoma **27**, which shows a considerable area of degeneration of the parenchyma in the same situation. Foci of small round cells like these have been repeatedly observed, usually in relation with areas of tumour undergoing spontaneous healing. Macrophages are rarely seen, and giant cells have never been found in well-developed tumours.

Spontaneous Healing.

That transplanted tumours may disappear spontaneously was first noted by Gaylord, Clowes, and Baeslack (7 & 8), who described the histological changes which occur during this process as essentially an overgrowth of connective tissue with a round cell infiltration. A detailed description of the morphological features in this condition will be found in the second volume of these Reports (2).

A re-analysis, more especially of the round cell infiltration, has given the following result for tumour-strains **27**, **32**, **65**, Borrel's tumour **B**, and Jensen's tumour, all carcinomatous growths of the mamma. In nodules of 3–5 mm. in diameter, where the process of absorption is well advanced, the parenchyma assumes the form of small islands of cells, many of which appear healthy or even present mitotic figures (fig. 11, Carc. Z. *a*) (Plate 2). The majority of them, however, are markedly degenerated, sometimes occurring in clumps (fig. 11, *b*) (Plate 2), and show indistinct outlines to their nuclei, vacuoles in their cytoplasm, etc. Tumours which are macroscopically more advanced in this process of healing, show correspondingly more advanced degeneration of the parenchymal cells (fig. 12, Plate 2). This slow degeneration must not be confounded with the necrosis seen in nearly all tumours, and its relation to the development of immunity will be discussed later.

The changes in the elements of the stroma in spontaneously receding tumours are the following. Eosinophile and polymorphonuclear leucocytes do not appear to play anything but an unimportant part in the

process. Where hæmorrhage occurs, the eosinophile leucocytes are especially abundant (fig. 10, Plate 1). The lymphocytes (small quiescent wandering cells) form the main mass of the reaction zone, and extend from the centre of the tumour over a wide area of the surrounding tissue (fig. 11, Plate 2). Structurally they resemble for the greater part small lymphocytes and small mononuclear leucocytes, although larger forms like large mononuclear leucocytes also occur in smaller number.

Wandering cells and quiescent wandering cells exhibit no special increase until the process is almost complete, at a period when the lymphocytes diminish in number. In this late stage pictures are thus obtained indistinguishable from those figured by Maximow (13) in late scar formation. The fibroblasts, which assume an active rôle only towards the end of the process, finally, in conjunction with the wandering cells and quiescent wandering cells, produce the ultimate cicatricial tissue.

The plasma cells, even at an early period, are increased so greatly in number as to present a genuine infiltration (fig. 11, Plate 2), and this is quite characteristic of tumours beginning to show spontaneous healing (fig. 14, Plate 2). They are especially abundant around the vessels (fig. 13, Plate 2), but avoid the immediate neighbourhood of the islands of parenchyma. It is only in the late cicatricial stage that their numbers diminish appreciably, when degenerated forms such as those seen in fig. 13 are observed. The fate of the mast cells in this process is still undetermined, but what are apparently degeneration forms have been seen (fig. 15, Plate 2). Figs. 16 and 18 (Plate 2) depict the types of macrophages seen in spontaneous healing, and fig. 17 (Plate 2) some of the inclusion bodies in these cells. The number of macrophages present varies greatly, but they are especially abundant near the remnants of parenchyma or in the neighbourhood of small hæmorrhages (figs. 11 and 12, Plate 2), and in the latest stages of absorption. The relationship between phagocytosis and the development of immunity will be entered into later.

In addition to the multinuclear macrophages already discussed, fibroblastic giant cells have been met with, the histological characters of which are shown clearly in figs. 19 and 20 (Plate 2). The production of true giant cells from the coalescence of carcinomatous cells has not been observed, except in the regressing carcinomatous moiety of a carcinoma-sarcomatodes (10) (fig. 21, Plate 2).

The presence of hæmorrhages in the healing of tumours was noted by the authors previously cited, but it has not been found a phenomenon of regular occurrence ; in some tumour-strains it occurs frequently in the early stages of absorption, in others less so, while in the later stages of healing it is absent. The new formation of vessels does not take place very early during healing, but is abundant in the later stages of the process, and can be easily followed in serial sections. In most cases the lumina of these vessels are occupied by large numbers of lymphocytes (fig. 22, Plate 2).

It will be seen that the process of spontaneous healing is a complicated one, which can be described in general terms only by some sacrifice of detail. The description just given, taken in conjunction with the circumstance that only living cells can induce immunity to cancer, allows the deduction that the primary phenomenon of spontaneous healing is a special form of degeneration of the parenchymal elements. Where this degeneration has taken place, a reaction tissue is rapidly formed, stimulated, perhaps, by the extravasation of red blood corpuscles and other elements (lymphocytes?). That the tumour cells still possess a certain vitality is evidenced by the occurrence of mitotic figures, but they appear incapable of overcoming the excessive reaction, and the final stage is essentially the organisation of a *caput mortuum*. Plasma cells, macrophages, giant cells, and many of the lymphocytes degenerate *in sitû*, although some of the elements last named appear to remain as permanent constituents of the scar tissue.

The occurrence of spontaneous healing of tumours in man is still the subject of controversy, but the observations of Senger (27), Crosbie (4), Gould (9), Randolph (19), Laurie Watson (29), Rotter (23), Teacher (28), and Risel (22) speak strongly in favour of its occurrence. Local spontaneous healing of parts of human tumours is noted in the papers of Denecke (5), Becher (3), Petersen (18), Schwarz (26) and Orth (16). The changes observed by the above authors are essentially the same as those occurring in mouse tumours, and a similar reaction has been described by Perthes (17) in human tumours which have been treated by X-rays or radium.

During the development of the resistance produced by the absorption of transplanted tumours, characteristic changes were discovered in the stroma as well as in the connective tissue at some distance from the growth. Examination of fragments of subcutaneous tissue, especially fat, taken from the opposite side of the animal in which a fairly large tumour was regressing, revealed the presence of

aggregates of plasma cells (figs. 23 and 24, Plate 2), and their disappearance at a period which would seem to coincide with the onset of immunity. The number of extravasated lymphocytes would appear to be greater under similar conditions. Whether the plasma cells arise where they are found, or metastasize from the healing tumour, is a question difficult to decide.

The Transplantation of Cancer into Immune Mice.

The changes occurring at the site of inoculation in immune mice have been already studied by Russell (24), and the detailed description of these experiments conducted with a number of different tumour-strains appeared in the Third Scientific Report. The main conclusion arrived at was, that in immune animals the cancer cells failed to elicit the specific stroma reaction which they induced in normal animals.

The following series of investigations was undertaken with the view of studying more especially the part played in immune animals by the different classes of cells. When into mice which had proved negative to 0·015 c.c. of carcinoma **27**, and to a repeated inoculation of 0·1 c.c. of the same tumour, small fragments of strain **27** were engrafted, the reactions observed were similar to those described by Russell, as comparison of fig. 25 with fig. 5, p. 350 in Russell's paper (24) will make evident. There are present, at the fourth day, a few fibroblasts in the neighbourhood of the graft, a certain number of quiescent wandering cells, polymorphonuclear leucocytes, lymphocytes and wandering cells, but no reaction comparable to spontaneous healing. The changes taking place in the reaction tissue can be summarised in the following manner :—

(1) The central portion of the graft necroses during the first two days, but the peripheral portion lives for 6–8 days. The tumour cells lose their acinous arrangement, become drawn out, the cytoplasm shows vacuolation, and the nuclei become hypochromatic. Finally they break down and are absorbed. It is probable that this long process of degeneration in immune animals augments the resistance.

(2) Upon the other hand, if the mice do not possess a high degree of resistance, the fragments develop for 4–6 days, and appear somewhat like tumours undergoing spontaneous healing.

(3) The polymorphonuclears which immigrated in the first few hours after transplantation degenerate *in sitû*, and remnants of their deformed nuclei can be seen for 4–6 days.

(4) Macrophages are not present until very late in the process, 10–14 days; their absence in the earlier period can be correlated with the absence of newly developed vessels.

(5) There is practically no lymphocytic infiltration in immune animals, and plasma cells are also absent; a few examples, however, have been seen in cases where the resistance was very weak.

(6) Hæmorrhages are absent, and accordingly eosinophile leucocyte are not seen.

(7) Foreign-body giant cells are rarely seen, and only in the latest stages; epithelial giant cells are frequently formed by coalescence, but the preparations did not carry conviction that amitosis played a part in their formation.

(8) There are no morphological changes in the general fatty and subcutaneous tissues of immune animals during the absorption; all attempts to demonstrate plasma cells at a distance from the site of inoculation have proved negative.

The histological analysis of spontaneous healing leads to the conclusion that in close connection with the development of immunity there is a special local and general tissue reaction; but that the process may be regarded merely as a foreign body reaction, is a view quite untenable, for in the latter the lymphocytic infiltration is never so marked nor, according to Maximow (14), are plasma cells ever found in the foreign body reaction of the rat. Thus the reaction in the fatty and subcutaneous tissues of an animal developing resistance finds no parallel in the encapsulation of a foreign body. The marked leucocytic and plasma-cellular infiltrations, and perhaps also the development of macrophages, play an important part in the development of immunity; the other tissue elements do not appear to have the same significance.

The failure of the stroma reaction in immune animals is from this standpoint of great importance, for the observations now under consideration allow the conclusion that during the development of immunity there is a special change in the connective tissues. If the process of immunisation were merely the exaggeration of natural forces of resistance in the animal, a more marked reaction would be expected. This, however, is not the case, for in immune mice the grafts are treated more as if they were constituent parts of the body—*sit venia verbo*. It

is only in the late stages when the cancer cells have disappeared, that a reaction takes place which is indistinguishable from ordinary healing.

The objection might still be raised that a spontaneously healing tumour is a foreign body in point of size, when compared with the minute dimensions of a tumour graft, and that the peculiar character of the reaction in the former case might be determined solely by the large amount of tissue to be absorbed. Special experiments have therefore been made to decide the validity of such objections.

A. Inoculation of Killed Tumour-cells.

The observations published by Haaland (10 *a*) show that when tumour cells, or other elements capable of inducing immunity, are mechanically disintegrated, they are no longer capable of eliciting resistance ; and as the development of immunity appears to be closely connected with the presence of special elements in the reaction tissue, their absence would be expected after inoculation of killed material. Tumour **63**, frozen and ground, was inoculated into three batches of mice, one group receiving 0·01 c. c. subcutaneously, and two others, 0·1 c. c. The inoculation site and the subcutaneous tissues were examined at intervals between 24 hours and 19 days.

No changes are discoverable in the general subcutaneous tissues, and the collections of plasma cells found in spontaneous absorption are absent.

The process occurring at the inoculation site is the same whether large or small doses of frozen tissue have been introduced, except that in the latter case it terminates sooner. During the first twenty-four hours there is an extreme immigration of polymorphonuclear leucocytes, but the lymphocytes are few in number. As fig. 26 (Plate 3) shows, the polymorphonuclear leucocytes are breaking down after two days, while the fibroblasts have increased in number, contain many mitoses, are more sharply outlined, and form a broad zone between the inoculated material and the normal subcutaneous tissue. A few quiescent wandering cells, lymphocytes, and polymorphonuclear leucocytes are present in this reaction tissue, as well as a few apparently degenerating cells with metachromatic granules (fig. 27, Plate 3). No plasma cells, macrophages, or giant cells are present.

In the succeeding periods, the fibroblasts and the quiescent wandering cells gradually undergo a change indistinguishable from the healing process around a foreign body. Careful search for plasma cells failed

to reveal their presence at any period, excepting only one case in which a firm fibrous nodule of the inoculated material persisted for sixteen days, and had become surrounded by a ring of fibroblasts containing a few plasma cells. The lymphocytes are slightly increased in number in the earlier stages, and later they appear to change into wandering cells and take part in the final cicatrisation. In number they never approach that seen in spontaneous healing.

The characteristic feature of the reaction around killed material is the proliferation of fibroblasts.

B. Inoculation of Spontaneous Carcinomata.

When grafts into normal animals, of a spontaneous adeno-carcinoma (tumour **207**) were examined, it was found that the majority of the epithelial cells were degenerated after five days, and the reaction tissue showed numerous newly formed capillaries containing lymphocytes. By ten days the site of inoculation was scarcely recognizable from the neighbouring tissue. In the case of a spontaneous hæmorrhagic carcinoma (tumour **285**), macrophages appeared at the periphery of a graft as soon as 24 hours, but, after two days, were to be found only around the degenerated red blood corpuscles introduced with the graft. Serial sections of connective tissue remote from the site of inoculation revealed the presence of small aggregates of plasma cells, which were found best 7–9 days after inoculation.

C. Inoculation of Cancer of a Strange Species.

The growth of a mouse tumour for a certain period in rats was first described by Ehrlich (6), and later studied by Russell (24). The latter author also pictured the rapid destruction of a graft of mouse tumour in a rat previously treated with an immunising dose of mouse tissue. The reverse procedure, *i. e.* the inoculation of a rat tumour (Flexner-Jobling adeno-carcinoma) into a mouse, will now be described. When grafts are inoculated into normal mice, the epithelial cells proliferate for 6–7 days, and then degenerate in the manner described in spontaneous healing. The early invasion of polymorphonuclear leucocytes is followed by a later one of lymphocytes, which, at the 7th day, infiltrate a wide zone of tissue around the graft (fig. 28, Plate 3). A limited number of plasma cells lie amongst the lymphocytes. The old stroma introduced with the graft degenerates rapidly, but no new

stroma is elicited by the graft, and the tumour cells rapidly lose their acinous arrangement. In the later stages the macrophages, giant cells, and fibroblasts behave as in spontaneous absorption. Investigation of the subcutaneous tissues of distant areas again reveals the presence of collections of plasma cells (fig. 30, Plate 3), which, however, disappear after 15 days.

The changes observed when the grafts of this rat tumour are implanted into mice inoculated 18 days previously with a fragment (0·005 grms.) of the same growth, are as follows :—

The epithelial cells both at the centre and periphery of the fragment degenerate more rapidly than in normal mice. The polymorphonuclear leucocytes remain longer in the graft than they did in the case of normal mice, and can still be detected at the 4th day around the degenerating parenchyma cells (fig. 29, Plate 3). Lymphocytes invade the graft during the first 24 hours, but their numbers do not increase in the following days, and finally they are converted into large wandering cells.

Although plasma cells have not been seen in the reaction tissue around grafts in these mice, there are a few elements resembling them in many respects (fig. 35, *x*, Plate 4). The cytoplasm of these cells is less abundant, and contains small metachromatic granules, while the particles of nuclear chromatin are not stained so deeply as in the case of plasma cells, and it is probable that these cells are lymphocytes containing azur granules.

About the 2nd day a formation of new capillaries takes place around the graft, but these penetrate into it only after 5–7 days, when the parenchymatous cells are already completely degenerated. Synchronously with this development of blood vessels, macrophages appear in the preparations, following the distribution of the capillaries.

The general appearance of a graft on the 7th day is shown in fig. 29 (Plate 3), in which is depicted the small central area of necrosis, surrounded by a zone of reaction tissue not unlike that in a normal mouse (fig. 28, Plate 3). When examined under a high power, however, this zone is seen to be composed internally of macrophages (fig. 34, *a*, Plate 4), and externally of scar tissue (fig. 34, *b*, Plate 4), while that in a normal mouse (fig. 36, Plate 4), consists largely of lymphocytes and plasma cells.

The fibroblasts show early and rapid proliferation, appearing as groups of distinct enlarged cells, as shown in fig. 32 (Plate 4), drawn from a two-day old preparation. They are especially well marked at sites

where the necrotic parts of the graft border directly on the host's tissues. After the first and second days peculiar cells with red metachromatic granules in their cytoplasm are present (figs. 32 and 33, Plate 4), some of them recalling those seen after the inoculation of killed material (fig. 27, Plate 3), while others have a very different appearance and frequently show mitotic figures (figs. 32, *c* and 33, *c*, Plate 4).

D. Immunisation with Embryo skin.

Since preliminary inoculation of homologous embryo skin renders mice resistant to the implantation of a tumour, an investigation was made of the reaction which occurs around a graft implanted into mice previously treated with this material, and also that surrounding fragments of skin inoculated into normal mice. After the inoculation of embryo skin into normal mice there is an abundant immigration of polymorphonuclear leucocytes and lymphocytes, and at the same time many rounded quiescent wandering cells appear (fig. 37, Plate 4). Not a few of the last named elements exhibit mitotic figures (fig. 38, Plate 4). After 48 hours the fibroblasts begin to divide, and a certain number of the immigrated lymphocytes are transformed into wandering cells (fig. 40, Plate 4). The proliferation of all these elements leads after 5–7 days to the formation of a capsule around the implantation, containing here and there small accumulations of lymphocytes. Of special interest, however, are certain cells with a cytoplasm similar to that of the quiescent wandering cells, some of them provided with a dark, coarsely granular nucleus (fig. 39, *a*, Plate 4), while in others it is pale and irregular (fig. 39, *b*, Plate 4). Plasma cells do not appear until about the 20th day, but thereafter persist until the graft has almost completely disappeared (fig. 42, Plate 4). Fragments of inoculated embryo skin persist for a long time, and can be found after 6–8 weeks, in the form of small cysts containing some macrophages. The reaction around a tumour graft implanted in a mouse treated with mouse embryo skin (0·05 c. c. on two occasions six weeks previously), is the same as that described in mice immunized with tumour tissue. If there be a difference, it lies in a somewhat stronger lymphocytic immigration in the former case. When the first injection of embryo skin is followed by a second, it has been noted that the epidermal cells of the second inoculation die more quickly, the lymphocytic reaction is weaker, and plasma cells are absent.

E. Immunisation with Blood.

In normal mice plasma cells appear as soon as 48 hours after the inoculation of blood, their numbers are increased in the succeeding days, until on the 4th, small collections of plasma cells can be found in every section. Where the inoculation of blood has been repeated, examination fails to reveal the presence of plasma cells after the second inoculation.

Fragments of coagulated blood have also been inoculated into mice immunised with tumour tissue, but here plasma cells could be found, as fig. 43 demonstrates *.

Summary.

A general survey of the experiments shows that the development of immunity is accompanied by a general reaction of the connective tissue throughout the body. The results may best be given by following the changes which each class of cells has undergone under the various conditions of the experiments. It may be noted that an examination of the hæmopoietic system has not been conducted.

(1) *The polymorphonuclear leucocytes.* These are the first to appear at the site of inoculation. Under aseptic conditions, they exert no apparent phagocytic action, but degenerate rapidly *in sitû*. Some of them enlarge, and seem to remain as permanent constituents of the stroma of the new tumour. They do not appear to stand in any determinate relationship with the development of immunity, but behave in the same way under all the conditions examined. Their action is mainly to prepare the soil for other cell types, and to facilitate the absorption of dead cells and necrotic débris. This view accords well with the findings of Schultze (25), who used the indo-phenol blue synthesis as a method of analysing cellular processes.

(2) *The lymphocytes* (small amœboid wandering cells of Maximow, ungranulated leucocytes of Weidenreich (30)). These appear in large numbers around the inoculation site during the development of immunity, and decrease, gradually, in number. Their intimate connection with the development of immunity appears certain, as they are always

* The repetition of this experiment with carcinoma 206 which induces a strong active immunity has failed to confirm this observation. If a fragment of blood-clot be inoculated into mice previously immunised with strain 206, and the subcutaneous tissues examined on the succeeding days, aggregates of plasma cells cannot be detected.

found in abundance after the inoculation of any tissue capable of inducing the resistant condition. They do not reappear, or do so only in small numbers, when such an inoculation is repeated on an immune animal. Killed tissue fails to elicit a lymphocytic infiltration, and this can be correlated with its failure to induce immunity. Variations in the amount of living tissue injected do not appear to have any influence upon the intensity of the lymphocytic reaction. Small groups of lymphocytes in tumours that are growing well, are found only in areas of spontaneous healing. Healthy cells on the one hand, and necrotic masses on the other, call forth no lymphocytic reaction ; it would appear to be determined rather by a special stage of the process of necrobiosis, and it may be supposed that the lymphocytes have been specifically affected, and play a part in diffusing the resistance throughout the body. The rapid accumulation of lymphocytes within 48 hours, and the rarity of examples dividing by mitosis force one to conclude that they are derived by a process of diapedesis from the blood-vessels.

(3) *The plasma cells.* The circumstance that these are also found in areas remote from the site of inoculation during the production of immunity, speaks strongly for their having an intimate relationship with its development. At the same time the wide distribution of these elements throughout the tissues makes it appear that the immune reaction against cancer is a general rather than a local phenomenon. The presence of plasma cells may be looked upon as the morphological expression of a defensive mechanism.

(4) *The macrophages.* These appear at the site of inoculation concurrently with the newly developed vessels, and their phagocytic powers seem to be directed more against partially destroyed cells than against cell detritus.

(5) *The mast cells.* These do not appear to play anything but a passive part in the various processes. The peculiar elements containing metachromatic granules, and pictured in figs. 27, 32 and 33 (Plates 3 & 4), require a few exp.anatory remarks. Some appear to be degenerated mast cells (*cf.* figs. 15 and 27, Plates 2 & 3), but others have more the appearance of large lymphocytes which have taken up the granules of such corpuscles (figs. 32 and 33, Plate 4) ; in favour of the latter view is the actual presence of ingested cell fragments (fig. 33, *c*, Plate 4). It is impossible to decide whether the dividing cells shown in figs. 32, *c* and fig. 33, *a* (Plate 4) are mast cells or lymphocytes.

(6) *The fibroblasts.* These cells do not appear to have a definite

relation to the induction of resistance. Inoculation of grafts of a carcinoma into immune mice has already shown that the carcinoma cells are robbed of their power of inducing the fibroblastic and angioblastic reaction whch is characteristic of their establishment in normal animals. Preliminary inoculation of an animal with killed material does not suppress this reaction to a subsequent inoculation of tumour.

(7) The other connective tissue elements, resting wandering cells, giant cells, etc., do not appear to possess a definite bearing upon the induction of immunity.

Literature.

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EXPLANATION OF THE FIGURES.

ABBREVIATIONS.

Carc. Z. = Carcinoma cells.
Edk. = Endothelial cell nucleus.
Eos.Lkc. = Eosinophile leucocyte.
Erc. = Erythrocyte.
Fbl. = Fibroblast.
L. = Lumen of blood vessel.
Lmc. = Lymphocyte.
Mk. = Macrophage.
Mz. = Mast cell.
P. = Cell débris.
Plz. = Plasma cell.
Pl.Lkc. = Polymorphonuclear leucocyte.
Rz. = Giant cell.
ruh. Wz. = Quiescent wandering cell.
Wz. = Wandering cell.

Alc. = Absolute alcohol.
Z. = Zenker's fluid.
Mbl. = Polychrome methylene blue.
Az. = Azur II solution.
G. = Giemsa-Romanowsky stain.
I.A.H. = Iron-alum hæmatoxylin.

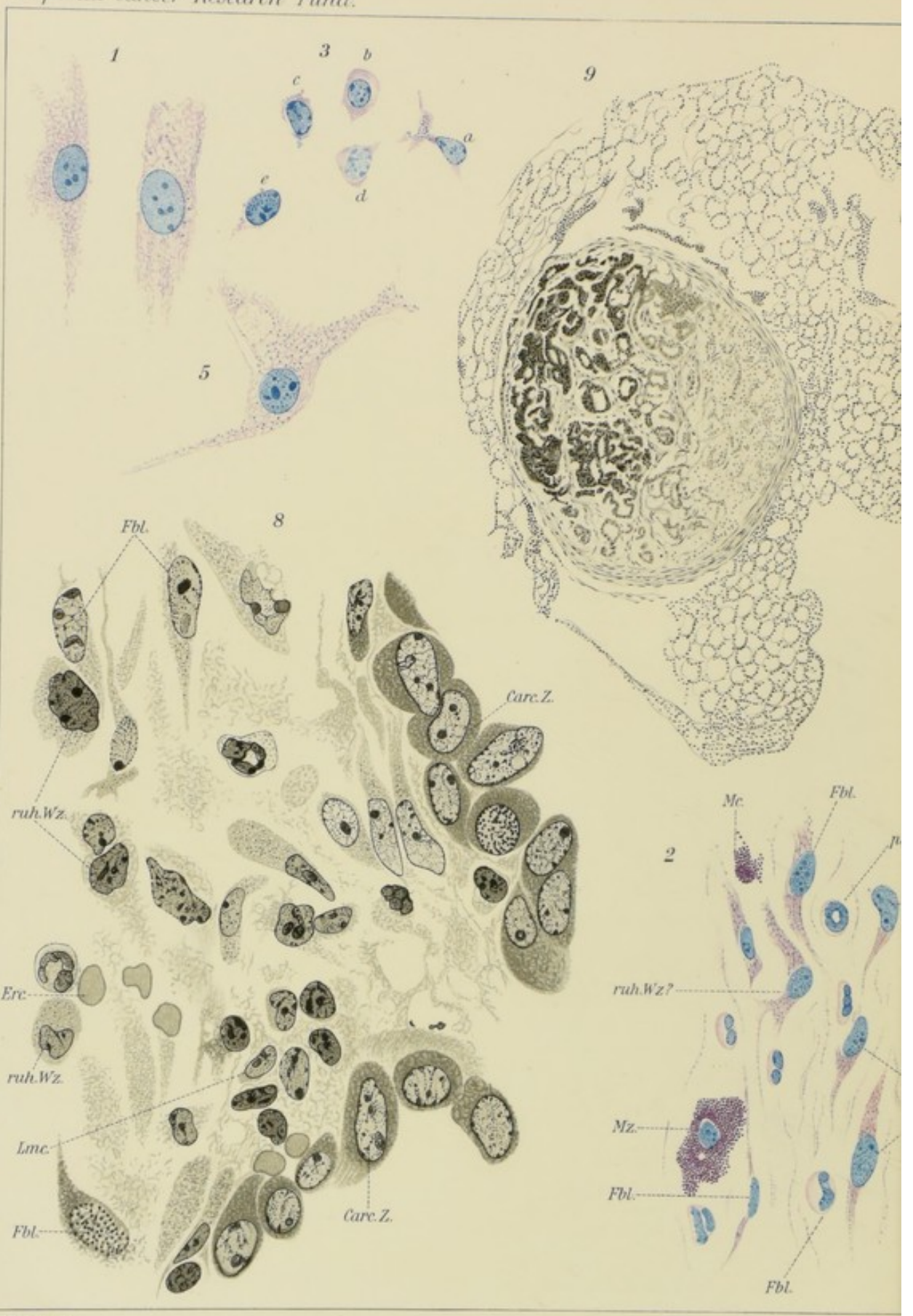
All the figures are taken from cancer cells or connective tissue elements of the mouse or of rat tumour cells in the mouse, and drawn with the Abbé-Zeiss camera lucida.

Plate 1.

Fig. 1. Normal fibroblasts. Alc, Az. $\times \frac{1050}{1}$.
Fig. 2. Normal connective tissue elements. Alc, Az. $\times \frac{700}{1}$.
Fig. 3. Quiescent wandering cells of normal subcutaneous tissue: *a*, with deep-blue staining granules; *b*, *c*, *d*, *e*, without definite granules. Alc, Az. $\times \frac{700}{1}$.
Fig. 4. Lymphocytes from normal subcutaneous tissue. Alc, Az. $\times \frac{700}{1}$.
Fig. 5. Normal fat cell. Alc, Az. $\times \frac{700}{1}$.
Fig. 6. Eosinophile leucocytes, polymorphonuclear leucocytes, and lymphocytes near a small hæmorrhage. 11-day tumour of carcinoma **27**. Z, G. $\times \frac{1050}{1}$.
Fig. 7. Formation of new stroma in "early stage" (4 days) of carcinoma **27**. Borrel, I.A.H. $\times \frac{410}{1}$.
Fig. 8. Connective tissue elements in a small area of spontaneous healing in the capsule of a fully developed carcinoma **65**. Z, Weigert's hæmatox., v. Gieson. $\times \frac{1050}{1}$.
Fig. 9. Low-power view of 11-day graft of carcinoma **27**. Partial degeneration of the parenchyma and corresponding reaction. Alc, Mbl. $\times \frac{37}{1}$.
Fig. 10. Spontaneous healing of carcinoma **65**. Z, G. $\times \frac{700}{1}$.

Plate 2.

Fig. 11. Spontaneous healing of carcinoma **65**/24 B. Alc, Mbl. $\times \frac{250}{1}$.
Fig. 12. Degenerated carcinoma cells surrounded by macrophages; carcinoma **32**/51 A. Alc, Mbl. $\times \frac{1050}{1}$.
Fig. 13. Spontaneous healing of carcinoma **65**/24 B; plasma-cells and lymphocytes around blood vessels. Alc, Mbl. $\times \frac{1050}{1}$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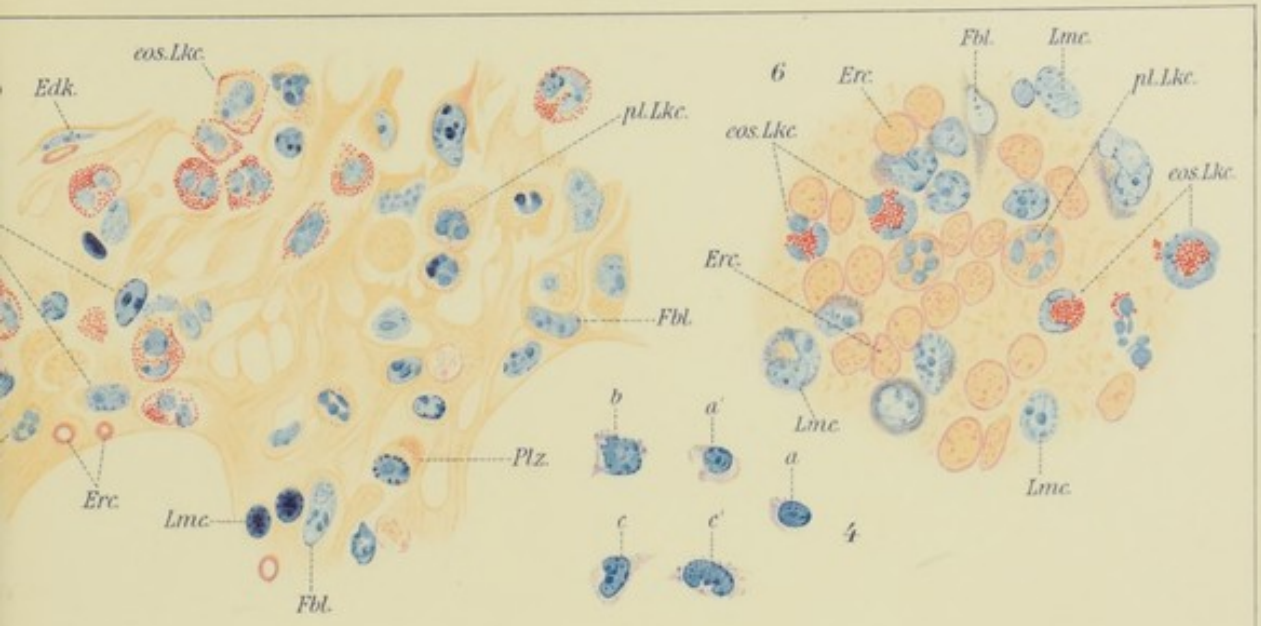

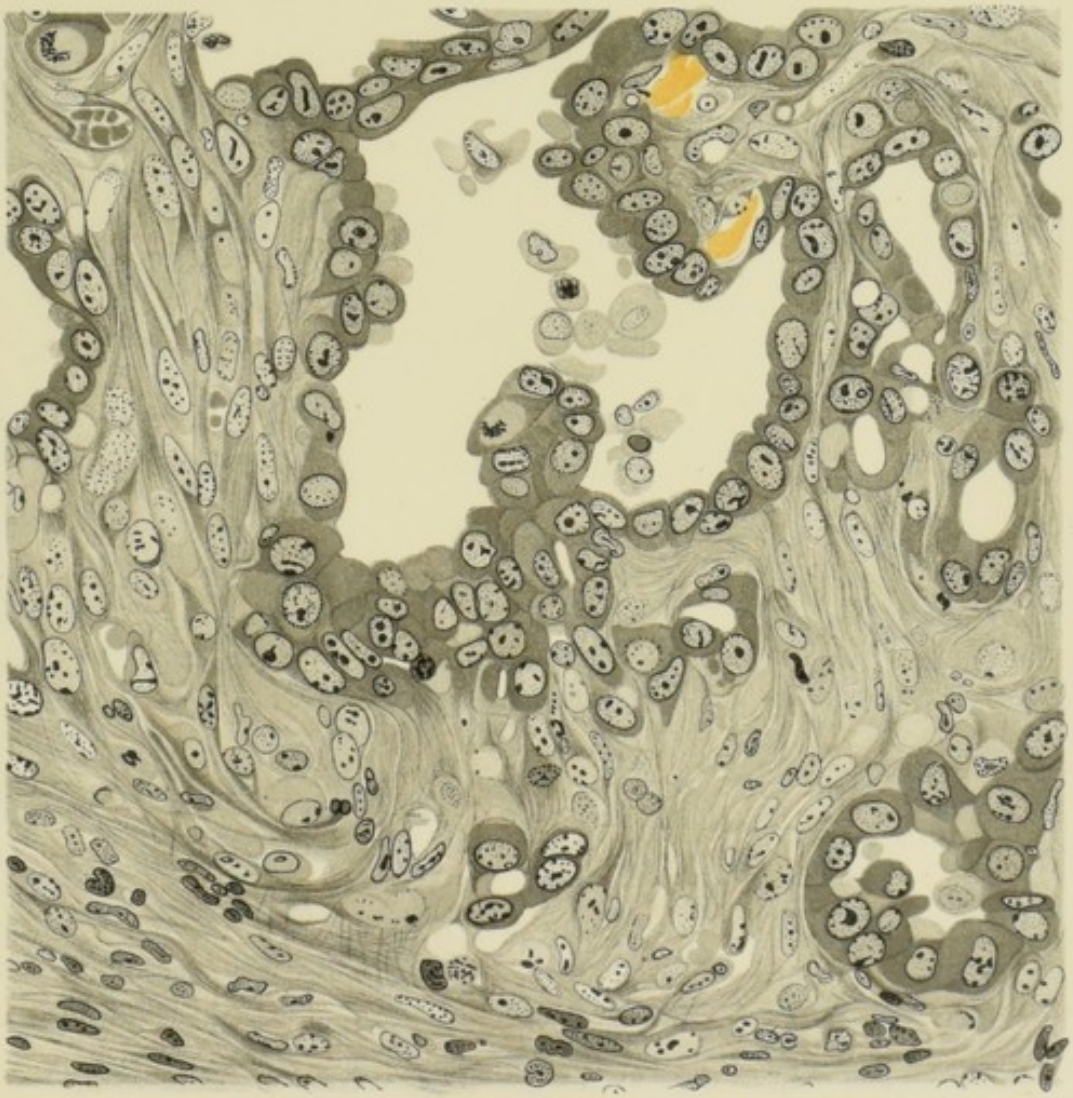

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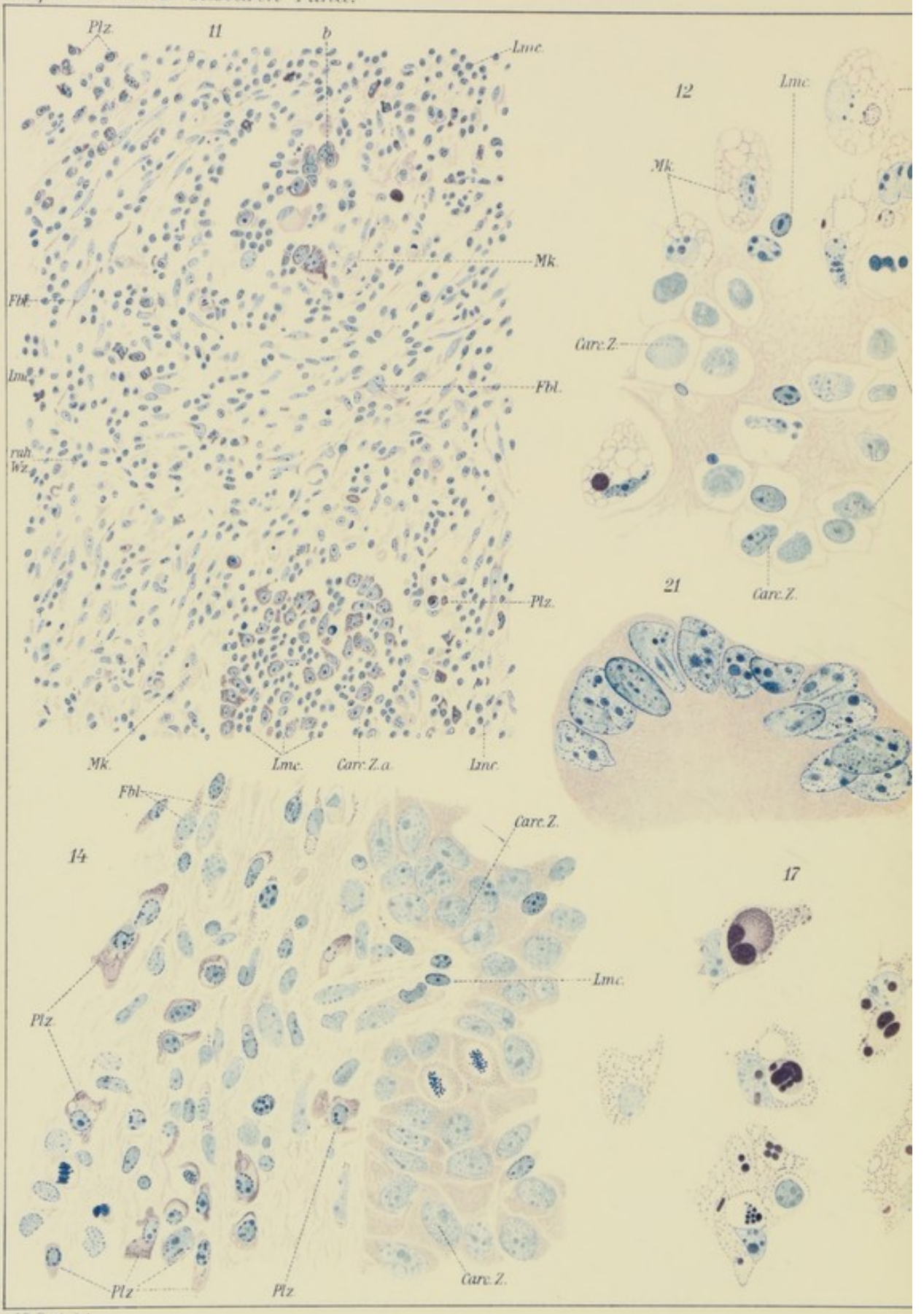
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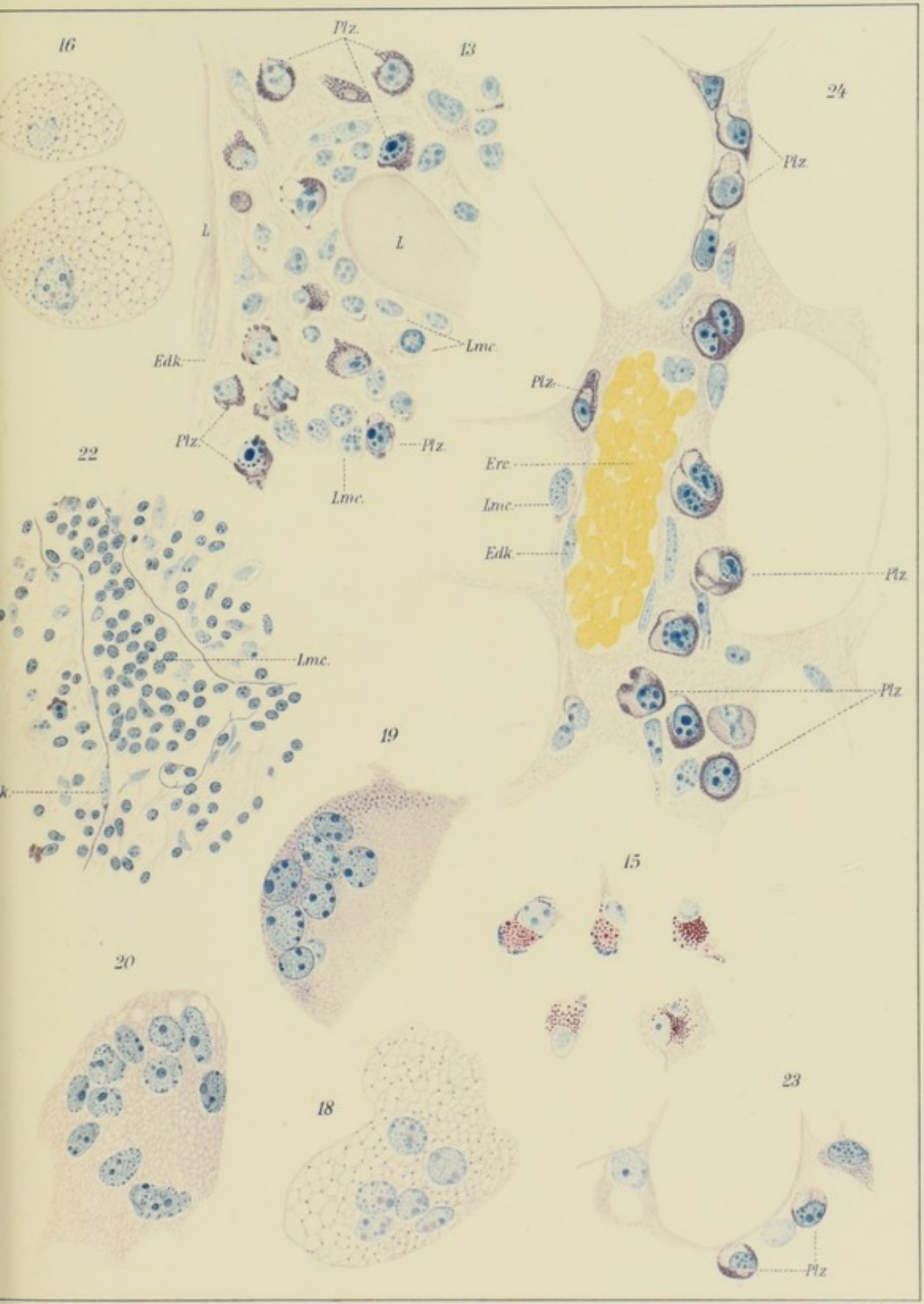

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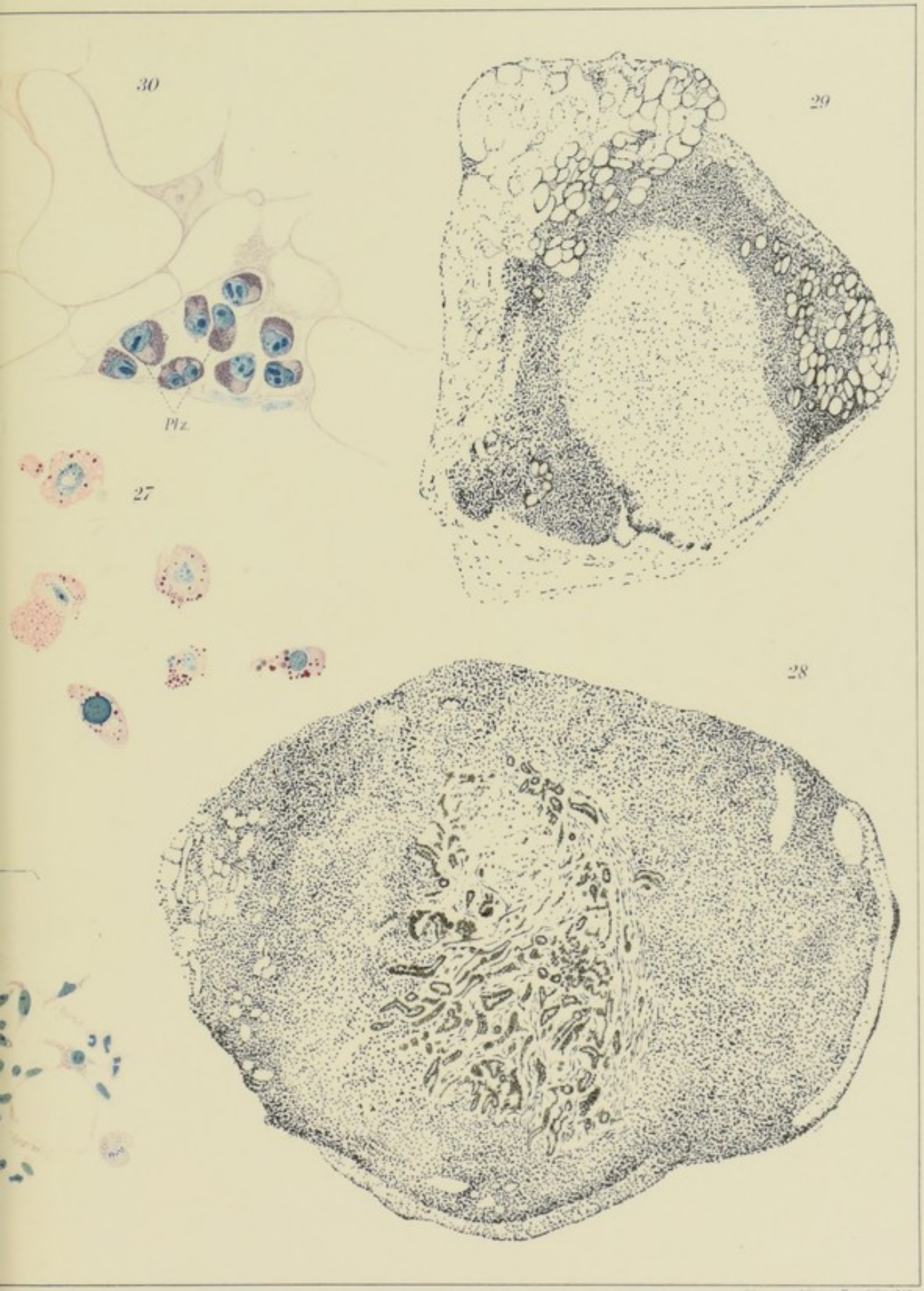
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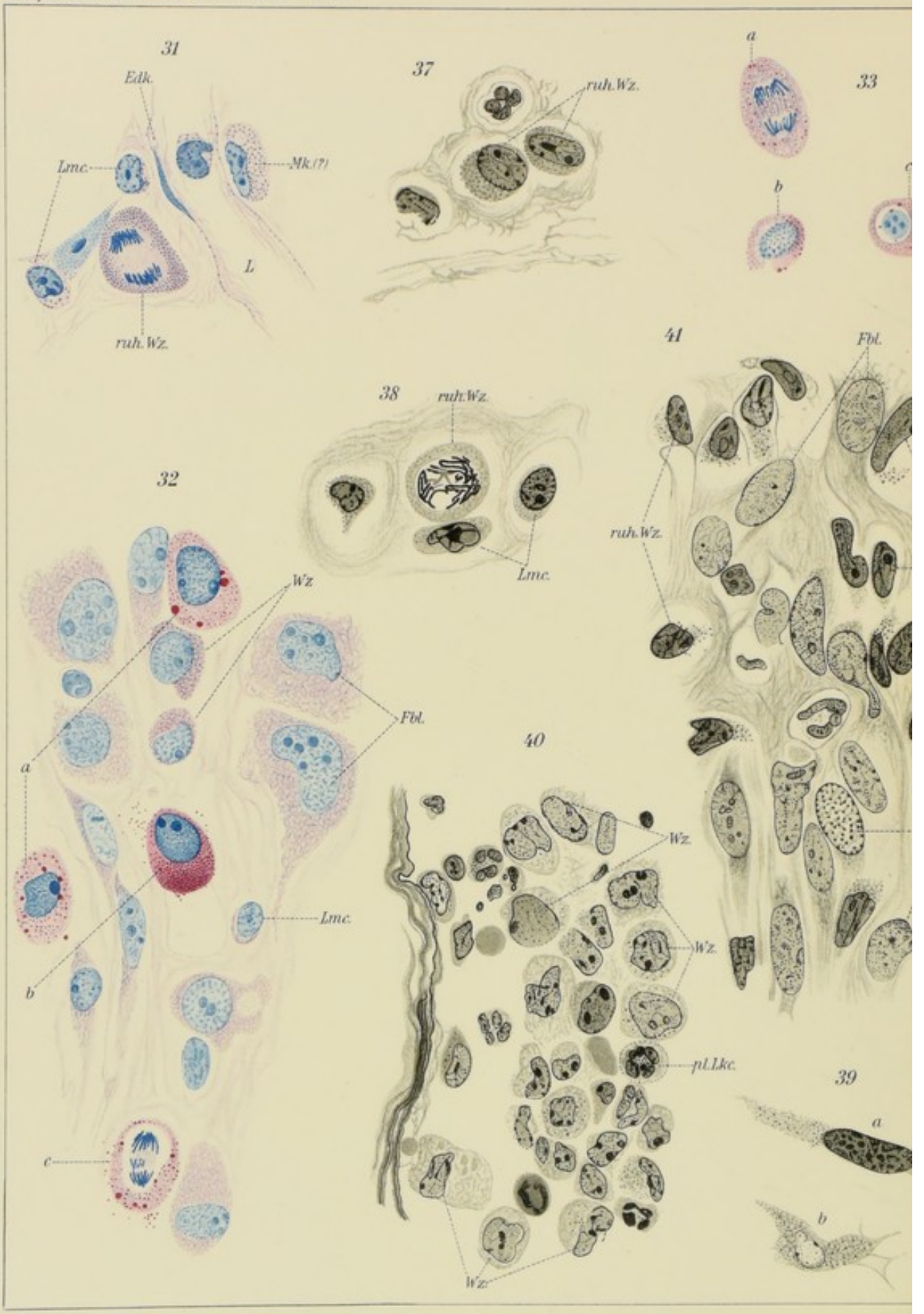
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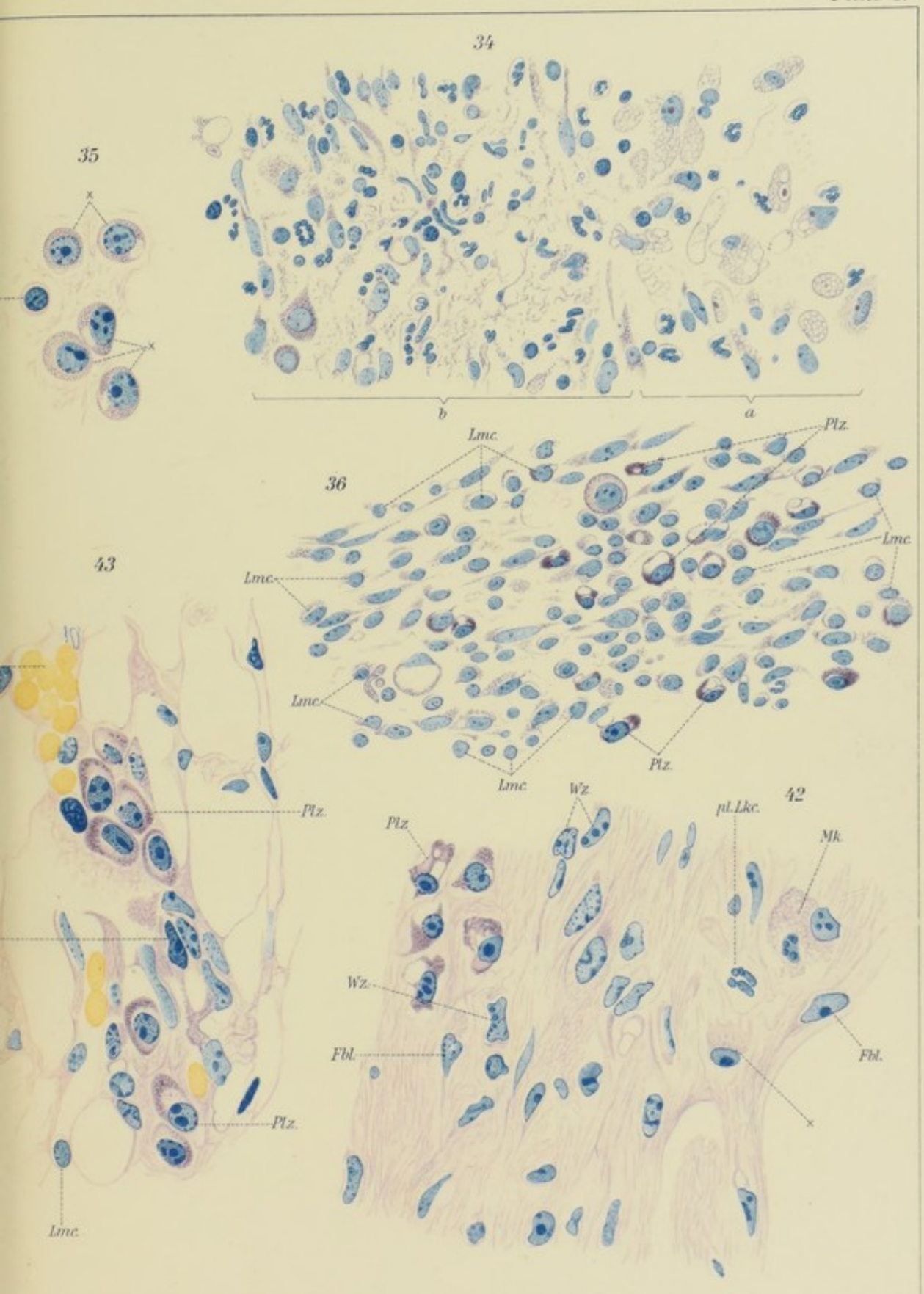

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Fig. 14. Commencing spontaneous healing in carcinoma **B**, showing overgrowth of stroma and collections of plasma-cells. Alc. Mbl. $\times \frac{700}{1}$.

Fig. 15. Degenerated mast cells with red and blue granules. Spontaneous healing in carcinoma **65**/24 B. Alc, Mbl. $\times \frac{1050}{1}$.

Fig. 16. Macrophages. Spontaneous healing of carcinoma **65**/24 C. Alc, Mbl. $\times \frac{1050}{1}$.

Fig. 17. Macrophages which have ingested cell débris. Alc, Mbl. $\times \frac{1050}{1}$.

Fig. 18 Giant cell formed by coalescence of macrophages. Carcinoma **65**/24 C. Alc, Mbl. $\times \frac{1050}{1}$.

Fig. 19. } Foreign-body giant cells. Sarcoma **92**/14 B. Alc, Az. $\times \frac{1050}{1}$.
„ 20. }

Fig. 21. Part of an epithelial giant cell in the mixed stage (carcinoma-sarcomatodes) of **37**/10 A. Alc, Az. $\times \frac{1050}{1}$.

Fig. 22. Newly-formed capillary filled with lymphocytes. Spontaneous healing of carcinoma **65**/24 C. Alc, Mbl. $\times \frac{525}{1}$.

Fig. 23. Spontaneous healing in carcinoma **J**/139 B: three plasma cells in adipose tissue at a distance from the tumour. Alc, Az. $\times \frac{1050}{1}$.

Fig. 24. Same material as fig. 23. Plasma cells in fat and in perivascular space Alc, Az. $\times \frac{1050}{1}$.

Plate 3.

Fig. 25. Early stage of carcinoma **27** in immune mouse four days after inoculation. Note slight amount of reaction. Alc, Az. $\times \frac{700}{1}$.

Fig. 26. Hypertrophy and multiplication of fibroblasts two days after inoculation of carcinoma **63**/25 A, killed by freezing and grinding. *a*, necrotic tissue; *b*, layer of fibroblasts. Alc, Az. $\times \frac{525}{1}$.

Fig. 27. Degenerating mast cells (?). Same material and method as fig. 26. $\times \frac{1050}{1}$.

Fig. 28. Low power view of whole graft of Flexner rat carcinoma in a normal mouse seven days after inoculation. Very extensive and intense lymphocytic reaction. Alc, Az. $\times \frac{37}{1}$.

Fig. 29. 7-day graft of Flexner rat carcinoma in a mouse previously immunised with this tumour. Total degeneration of the parenchyma, reaction tissue consisting mainly of macrophages and polymorphonuclear leucocytes restricted to the immediate neighbourhood of the graft. Alc, Alz. $\times \frac{37}{1}$.

Fig. 30. Group of plasma cells in adipose tissue at a great distance from an 11-day graft of Flexner rat carcinoma in a normal mouse. Alc, Az. $\times \frac{1050}{1}$.

Plate 4.

Fig. 31. Quiescent wandering cell in mitosis. From the capsule of a two-day graft of carcinoma **285**. Alc, Az. $\times \frac{1050}{1}$.

Fig. 32. Large fibroblasts and cells containing metachromatic granules from the capsule of a 2-day graft of Flexner rat carcinoma in a mouse immunised with the same tumour. Z, Mbl. $\times \frac{1050}{1}$.

Fig. 33. Cells with metachromatic granules. *a*, in mitosis; *b*, degenerating; *c*, with ingested polymorphonuclear leucocyte. Z, Mbl. $\times \frac{1050}{1}$.

Fig. 34. Reaction around 7-day graft of Flexner rat carcinoma n Flexner-immune mouse. Two zones are seen: *a*, inner zone of macrophages; *b*, outer zone of polymorphonuclear leucocytes and fibroblasts. Alc, Az. $\times \frac{525}{1}$.

Fig. 35. Cells (x) with Azur granules from the same preparation. $\times \frac{1050}{1}$.

Fig. 36. Elements comprising the reaction tissue around a 2-day graft of Flexner rat carcinoma in a normal mouse; lymphocytes, plasma cells, and a few fibroblasts. Alc, Az. $\times \frac{525}{1}$.

Fig. 37. Quiescent wandering cells in spherical condition. From the reaction around an implantation of mouse embryo skin 24 hours after inoculation. Borrel, I.A.H. $\times \frac{1050}{1}$.

Fig. 38. Enlarged lymphocytes and quiescent wandering cell in mitosis. Same material as in fig. 37. $\times \frac{1050}{1}$.

Fig. 39. Two forms of quiescent wandering cell. Same material as preceding. $\times \frac{1050}{1}$.

Fig. 40. Wandering cells around an implantation of embryo skin, 48 hours after inoculation. Borrel, I, A, H. $\times \frac{1050}{1}$.

Fig. 41. Young scar tissue around an implantation of embryo skin 8 days after inoculation. Borrel, I, A, H. $\times \frac{1050}{1}$.

Fig. 42. Plasma cells from capsule of an implantation of embryo skin 30 days after inoculation. Alc, Az. $\times \frac{1050}{1}$.

Fig. 43. Plasma cells in subcutaneous tissue 4 days after inoculation of defibrinated mouse blood in a mouse previously immunised with carcinoma. Z, Mbl. $\times \frac{1050}{1}$.

[*Fifth Scientific Report of the Imperial Cancer Research Fund*, 1912.]

ON THE IMMUNISING POWER OF THE PLACENTA, BLOOD, EMBRYONIC SKIN, MAMMARY GLAND, AND SPLEEN OF DIFFERENT SPECIES AGAINST CARCINOMA OF THE MOUSE.

By S. HIGUCHI, M.D., Tōkyō.

A new field of experimental and biological investigation bearing upon the nature of cancer was opened up when it was demonstrated that the inoculation of tumour tissue, and also of normal tissue of the same species could induce resistance to the implantation of mouse cancer in mice. There arose at once the questions, whether the resistance induced in these two apparently different ways was identical in its nature or not, and whether the tumours and tissues of alien species were endowed with an equal or similar power to induce resistance. The first question appears to have received a satisfactory answer from the investigations conducted in this laboratory and elsewhere. The properties by virtue of which homologous normal or tumour tissues induce resistance to the implantation of cancer of the same species overlap to a large extent because of the properties they possess *quâ* properties common to the tissues of the species of animal. There are, however, in addition, subtle differences both among the tumours and the normal tissues as regards their powers of inducing resistance. For some this property is evidenced in higher degree than for others, without, however, manifestation of anything incompatible with the views that tumour tissue, *e. g.* of the mouse, is potent *quâ* mouse tissue, and that tumour tissue and normal tissues induce a certain degree of resistance which is common to both, and certain higher degrees which are specific for particular tumours or tissues.

In regard to the second question, it was found that *e. g.* the blood of rabbits and guinea-pigs did not possess the power to protect mice

against the inoculation of mouse cancer, and that if rat blood possessed any such property it was only to a very slight degree. Corresponding observations made with tumours of alien species appeared fully to agree with the observations made with blood and other heterologous normal tissues.

These two sets of observations seemed to go a long way towards excluding the intervention of a virus common either to the new growths of distinct species of animals or even to those of a single species. However, experiments leading other workers to opposite conclusions have not been wanting. The result of their conclusions is, in extreme cases, to assign both to normal tissue and to tumour tissue of widely removed species, a power of inducing in mice resistance to the implantation of mouse cancer equal to that possessed by the tissues and tumours of the mouse. In short, these observations are calculated to establish, on the one hand, a property—a virus is tacitly understood by some—common to all malignant tumours of whatever species, by virtue of which they induce resistance, and, on the other hand, an inability to distinguish the normal and the pathological tissues of different species in respect of this property. Schöne (10), in addition to inducing resistance to the implantation of mouse cancer by means of mouse embryonic tissue, stated, with cautious reservation, that he had obtained a similar result after treating mice with fowl embryos and human mammary cancer. Carl Lewin (7) stated that rat carcinoma protected mice against the inoculation of mouse cancer. Isaac Levin (8) found that skin and spleen of mice protected rats against rat sarcoma, and Apolant (1) made a categorical statement that he was able to induce resistance with alien blood. Moreschi (9) found that the mammary gland of the rat, while efficacious against one mouse carcinoma, was without effect against a second. Lactating guinea-pig mamma was also active but not in so high a degree as mouse mamma.

The present investigation is limited to endeavouring to clear up the contradictory statements as to the action of normal tissues of the same (homologous) species and of alien (heterologous) species when used to induce resistance to the implantation of cancer in mice.

Methods of Immunising and Testing.

I shall first indicate the method I employed, since different results have been recorded by those observers who have investigated the

powers of tissues of alien species to produce immunity or resistance to the inoculation of tumours. It is probable that some of the differences in the results recorded by the various authors are due to variations in method, since uniformity has not always been maintained. The methods are those usually employed in the laboratory of the Imperial Cancer Research Fund and already described in previous Reports. The tumour, after aseptic removal, is reduced to a fine emulsion. Should necrotic portions be present, they are carefully removed, as is also the connective tissue capsule, to insure a uniform emulsion for inoculation. To obtain accuracy and uniformity of dose the emulsion is inoculated by a calibrated glass syringe. Normal tissues, when used in immunity experiments, are emulsified in the same way. Mice are selected as similar as possible in age, condition of nourishment, and weight. Age, body-weight, variety, nutrition, site of injection, the nature and amount of the tissue emulsion as well as the dose of tumour used to test immunity, have all an influence on the result of such experiments.

In testing the immunity, the amount of tumour used should not be large, because of the risk of causing simultaneous immunity : I generally used 0·01 c.c. or 0·02 c.c. In order to induce immunity, I injected 0·1 c.c. of tissue emulsion and 0·5 c.c. of defibrinated blood. As regards the sites of inoculation, solid tissues were deposited in the axilla, and blood was injected under the skin of the back, the puncture being closed with a small clamp to prevent leakage, and absorption assisted by gentle massage for a few minutes. After the lapse of a sufficient number of days the testing inoculation of tumour was made in another site, preferably the axilla. The number of days between the two inoculations varied according to the variety of tissue. For those which were absorbed speedily the interval was short, and long for those absorbed slowly. As shown in fig. 1, the results of the testing inoculation were first recorded on the tenth day, and thereafter every week until the fourth or fifth charting, that is, till the thirty-first or thirty-eighth day after inoculation of the tumour. Those mice which presented a tumour on the last day are regarded as positive, as are also those which, although dying during the course of the observations, presented clear evidence of the development of a tumour. Indurations resembling early tumours were counted negative if absorbed without increase in size, and the mice included with those in which no tumours developed.

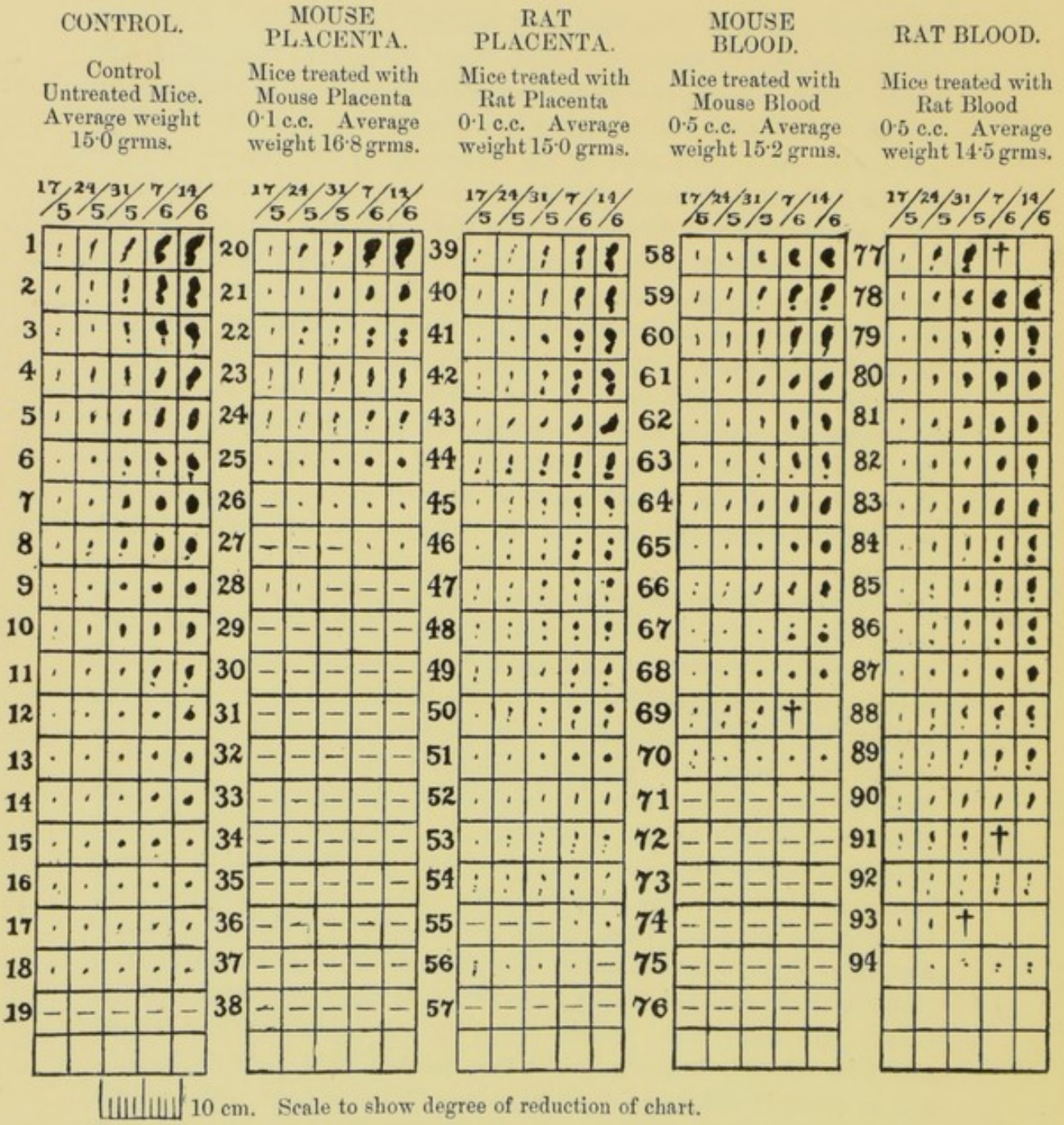

FIG. 1. Shows high degree of immunity induced by preliminary treatment with mouse placenta, less with mouse blood, and absence of immunity after treatment with the corresponding rat tissues.

[Exp. T/29 E.—The mice were treated with placenta 0·1 c.c. and blood 0·5 c.c. on the 26th April, 1910; on the 6th May 0·02 c.c. of carcinoma was transplanted in right axilla, and on May 17th the first charting was made.]

Can the Placenta induce Immunity?

Bashford, Murray, and Cramer (2), Schöne (10), Borrel (3), Bridré (4) and other investigators demonstrated immunity after previous treatment with normal (visceral) tissues, and I extended these observations to include the placenta (Table I. and fig. 1), both homologous and heterologous (rat and guinea-pig). Since the placenta consists of spongy tissue, and contains a large quantity of blood, in the event of immunity following its inoculation it would have to be ascertained whether this result was due to the placental tissue or to the contained blood. For this reason experiments were devised so that blood alone and placenta were injected on the same date into parallel series of mice. On one occasion placental tissue was used from which the blood had been removed by washing in physiological salt solution, and its powers of immunising estimated. Carcinoma **T** was employed to test the resistance, and seven series of experiments were performed. Table I shows that in the control animals the average number of tumours developing in the seven experiments was 64 per cent., while in those immunised with placenta the number was 18 per cent. This result shows that placenta *plus* its contained blood is able to induce a strong degree of resistance. In the mice immunised with blood alone 40 per cent. developed tumours. Although this fact clearly shows that blood induces protection, nevertheless it is very faint as compared with that induced by placenta *plus* blood, the strong immunising power of which is not due solely to the blood contained in it. Moreover, placental tissue washed free from blood by normal saline has also a strong immunising power. However, this experiment was performed only once, and the exact extent of immunising power cannot be estimated from a single experiment. The experiment suffices, however, when taken in conjunction with the others performed without removal of the blood to show that homologous placental tissue possesses the power to induce resistance. This power appears to be less than that possessed by the skin of the embryo, or the mammary gland (*cf.* Tables I., II. & III.).

In corresponding experiments performed with the placenta of rats, 55 per cent. of tumours were obtained, and in the case of guinea-pig placenta 63 per cent., as compared with the 64 per cent. obtained in normal control animals. Therefore, when contrasted with the 18 per cent. of tumours obtained after injection of mouse placenta, the placenta of the guinea-pig seems to have no power to induce immunity against

Table I.—Immunising Power of Placenta and Blood.

Immunising inoculation on back.		Interval between immunising and testing inoculations.	Testing inoculation in right axilla.			Control.			Placenta.										Blood.								
									Mouse.			Mouse-Placenta washed with 0·6 per cent. NaCl-solution.			Rat.			Guinea-pig.			Mouse.			Rat.			Guinea-pig.		
Quantities.			Tumour Series.	Quantities.	Date.	B	C	D	B	C	D	B	C	D	B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
Placenta.	Blood.																												
c.c.	c.c.	Days.		c.c.	1910.			%			%			%			%			%			%			%			%
0·1	0·5	10	T/29 E	0·02	6–5	300/20	18/19	95	320/19	8/19	42	...	...	...	300/20	17/19	90	...	...	...	290/19	13/19	68	290/20	18/18	100			
0·1	0·5	10	T/29 G	0·02	1–5	330/20	13/19	68	325/20	5/20	25	...	...	...	...	...	...	290/20	14/18	78	280/19	9/17	53	310/20	17/18	94			
0·1	0·5	11	T/31 B	0·02	20–6	320/20	8/19	42	315/20	2/20	10	...	...	...	315/20	7/19	37	280/18	7/14	50	290/20	6/19	32	270/19	12/19	63	245/17	14/16	88
...	0·5	10	T/31 D	0·02	28–7	220/20	6/16	38	...	...	...	...	...	...	...	...	...	...	...	...	450/27	5/25	20	460/29	14/27	52	360/22	10/21	48
0·1	...	10	T/30 L	0·02	6–8	220/20	13/18	72	210/17	1/15	7	...	...	...	240/19	7/18	39	175/17	6/11	55									
0·1	...	11	T/30 N	0·02	15–8	210/16	5/13	38	240/17	0/15	0	220/16	2/15	13															
...	0·5	10	T/31 H	0·02	29–8	220/20	15/17	88	...	...	...	...	...	...	...	...	...	...	...	...	250/19	6/17	35	250/19	14/16	88	200/17	10/11	91
Average	...	...	...	...	...	...	78/121	64		16/89	18	...	...	...	...	31/56	55	...	27/43	63	...	39/97	40	...	75/98	77	...	34/48	71
0·1	...	10	32/71 A	0·02	15–8	255/20	6/16	38	245/19	4/17	24																		
0·1	...	10	219/35 C	0·02	18–8	320/20	6/20	30	290/20	0/16	0																		

Remarks:—In the above table column B indicates $\frac{\text{weight of batch of mice}}{\text{number of mice}}$ at time of testing inoculation; column C gives $\frac{\text{no. of tumours}}{\text{no. of mice surviving}}$, and this fraction is expressed as a percentage in column D.

the inoculation of carcinoma of the mouse, and that of the rat very little.

In the above experiments the immunity to carcinoma **T** was already present ten or eleven days after inoculation of the homologous placenta. For a squamous cell carcinoma, tumour **32**, the yield was 38 per cent. in the control and 24 per cent. in mice treated with homologous placenta. Tumour **219**, a sarcoma, gave 30 per cent. in the control but no tumours in sixteen mice after treatment with placenta. It thus appears that homologous placenta immunises not only against various carcinomata, but also against sarcoma.

Have the Tissues of Heterologous Species the Power to Immunise against Mouse Carcinoma?

In view of the inconclusive results of the previously recorded experiments dealing with this question in a rather fragmentary manner, similar investigations were instituted on a large scale with a variety of tissues.

For the sake of comparison placenta, blood, mammary gland, embryo skin, and spleen, of mice, rats, and guinea-pigs were employed for the preliminary treatment of mice. Carcinoma **T** was used to test the effect following the injection of placenta, blood, and spleen. Carcinoma **91** was similarly used in the case of mammary glands and embryo skin, and carcinoma **199** was employed to test the effect of spleen. The dose of tumour was settled at 0·01–0·02 c.c., and that of immunising tissue at 0·5 c.c. for defibrinated blood, and 0·1 c.c. for other tissues. The number of days elapsing between the injection of normal tissue and the testing inoculation of tumour varied from 10–14 days, except that in the case of embryonic skin the interval was increased to 21 days, because it was absorbed more slowly.

Placenta. (Table I.)—The result obtained with carcinoma **T** showed 64 per cent. of tumours in the control, and only 18 per cent. after the injection of mouse placenta, whereas there were 55 per cent. and 63 per cent. of tumours after treatment with rat and guinea-pig placenta respectively. Although the difference in the case of rat placenta may appear to indicate a slight immunity, it is too small to permit of a definite conclusion. Guinea-pig placenta clearly possesses no power to induce immunity.

Blood. (Table I.)—As the experiments were performed at the same time as those with placenta, the same tumour material was used for

testing, and the same control served for both series. Tumours developed in 40 per cent. of the mice which had received a preliminary injection of mouse blood, as against 64 per cent. in the control, while in those which had received an injection of rat or guinea-pig blood there were 77 per cent. and 71 per cent. of tumours respectively. Therefore not only had the blood of these alien species no power to induce immunity, but, on the contrary, its injection rather enhanced the suitability of the mice for inoculation.

Mammary gland. (Table II.)—In the control mice 61 per cent. of tumours developed, but only 15 per cent. in those previously treated with mouse mamma. There was thus an obvious immunity induced. Following treatment with rat mamma, however, 62 per cent. of tumours developed, which indicated an absence of resistance in the treated mice. Where injection of guinea-pig mamma had preceded tumour inoculation, there were 56 per cent. of tumours, a difference too slight to prove definite resistance.

Embryo skin. (Table III.) — In the control series 57 per cent. of tumours developed, but after treatment with mouse skin only 14 per cent., indicating marked immunity. On the contrary the injection of guinea-pig skin induced no immunity, there being 59 per cent. of tumours in the treated animals. After injection of rat skin, however, only 33 per cent. of tumours developed, a circumstance suggesting that a certain degree of immunity had been induced.

Spleen. (Table IV.)—Two carcinoma strains, **199** and **T**, were used to test the immunising power of splenic tissue. The control for **199** gave 88 per cent. of tumours, as against 60 per cent. in the animals treated with mouse spleen. There was 84 per cent. and 80 per cent. after treatment with the spleen of rat and guinea-pig respectively, the differences being too slight to establish the occurrence of immunity. The control for carcinoma **T** yielded 86 per cent. of tumours, and only 21 per cent. in the mice previously treated with mouse spleen. Thus, although the results of control experiments for both tumour strains were the same, there was a stronger degree of immunity induced against one of them. Carcinoma **T** gave 47 per cent. of tumours in mice treated with rat spleen and 78 per cent. in those treated with guinea-pig spleen. Therefore the former had induced some immunity and the latter none. It will be recognised from the fact that mouse spleen had a strong power of immunising against carcinoma **T**, and only a weak one against **199**, that there is a difference in the susceptibility of different carcinomata to resistance.

Table II.—Immunising Power of Mammary Gland.

Immunising inoculation.		Interval between immunising and testing inoculations.	Testing inoculation.				Control.			Mouse mamma.						Rat mamma.						Guinea-pig mamma.					
										Living.			Dead.			Living.			Dead.			Living.			Dead.		
Quantity.	Situation.		Tumour Series.	Quantity.	Situation.	Date.	B	C	D	B	C	D	B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
c.c.		Days.		c.c.		1910.			%			%			%			%			%			%			%
0·1	Back.	11	91/20 D	0·02	R. ax.	24–5	325/20	9/19	47	330/20	5/19	26	...	...	...	330/19	13/19	68									
0·1	„	11	91/20 E	0·02	„	25–5	270/20	10/17	59	...	...	...	255/19	13/19	68	...	...	...	240/19	16/17	94						
0·1	„	11	91/20 F	0·02	„	30–5	300/20	16/20	80	265/16	4/16	25	280/18	10/18	56	...	...	...	...	...	...	290/20	11/20	55	280/20	13/20	65
0·1	R. ax.	11	91/21 B	0·01	L. ax.	28–6	145/9	3/9	33	150/9	0/9	0	...	...	...	145/9	6/9	67									
0·1	„	14	91/22 A	0·02	„	16–7	260/19	14/18	78	230/17	2/15	13	...	...	...	...	...	...	...	...	...	210/16	8/14	57			
0·1	Back.	14	91/21 F	0·02	R. ax.	6–8	220/20	8/17	47	195/15	1/12	8	...	...	...	285/20	9/20	45									
0·1	„	10	91/22 D	0·01	„	26–8	270/20	12/18	67	280/20	2/20	10	...	...	...	270/20	13/18	72									
Average		...	...	...	...	...	...	72/118	61	...	14/91	15	...	23/37	62	...	41/66	62	...	16/17	94	...	19/34	56	...	13/20	65

Table III.—Immunising Power of Embryo Skin.

Immunising inoculation.		Interval between immunising and testing inoculations.	Testing inoculation.				Control.			Mouse embryo skin.			Rat embryo skin.			Guinea-pig embryo skin.		
Quantity.	Situation.		Tumour Series.	Quantity.	Situation.	Date.	B	C	D	B	C	D	B	C	D	B	C	D
c.c.		Days.		c.c.		1910.			%			%			%			%
0·1	Back.	21	91/20 B	0·02	R. ax.	17–5	286/17	4/16	25	297/18	0/18	0	178/16	2/16	13			
0·1	„	21	91/20 E	0·02	„	25–5	285/17	12/17	71	280/16	3/16	19	...	...	...	310/19	8/18	44
0·1	R. ax.	21	91/21 C	0·01	L. ax.	1–7	300/18	11/16	69	260/16	4/16	25	220/14	8/13	62	330/19	16/19	84
0·1	Back.	21	91/22 C	0·02	R. ax.	18–8	175/12	6/9	67	180/15	2/13	15	230/17	4/14	29	240/18	6/14	43
Average ...		...	...	...	...	...	...	33/58	57	...	9/63	14	...	14/43	33	...	30/51	59

Table IV.—Immunising Power of Spleen.

Immunising inoculation on back.		Interval between immunising and testing inoculations.	Testing inoculation in right axilla.			Control.			Mouse spleen.			Rat spleen.			Guinea-pig spleen.		
	Quantity.		Tumour Series.	Quantity.	Date.	B	C	D	B	C	D	B	C	D	B	C	D
	c.c.	Days.		c.c.	1910.			%			%			%			%
	0·1	14	199/16 B	0·02	3–5	320/20	18/20	90	290/20	12/19	63	210/14	8/14	57	315/19	14/19	74
	0·1	14	199/17 B	0·02	11–6	295/20	16/19	84	Died out.			325/16	15/16	94	355/18	15/17	88
	0·1	14	199/17 D	0·01	15–7	250/19	18/18	100	200/16	13/14	93	220/17	14/14	100	290/16	14/15	93
	0·1	14	199/18 D	0·01	6–7	230/19	14/18	78	210/18	5/17	29	Died out.			280/20	14/20	70
Average	...	...	...	...	...	...	66/75	88	...	30/50	60	...	37/44	84	...	57/71	80
	0·1	14	T/30 J	0·01	22–7	220/20	16/19	84	230/17	3/17	18	240/18	8/15	53	250/19	11/18	61
	0·1	14	T/30 I	0·01	13–7	220/19	14/16	88	220/18	4/16	25	205/15	6/15	40	240/19	17/18	94
Average	...	...	...	...	...	...	30/35	86	...	7/33	21	...	14/30	47	...	28/36	78

Comparison of the Immunising Power of Normal Tissues.

The figures given in Table V. show that the number of tumours developing varies in normal healthy animals independently of any preliminary treatment. The lowest percentage in the control mice in the table is 57 and the highest 88 per cent., giving an average of 69 per cent.

Table V.—The Comparison of the Immunising Power of each Animal Tissue.

	Control.		Mouse.		Rat.		Guinea-pig.	
Placenta. (T)	$\frac{78}{121}$	64	$\frac{16}{89}$	18	$\frac{31}{56}$	55	$\frac{27}{43}$	63
Blood. (T)	„	„	$\frac{39}{97}$	40	$\frac{75}{98}$	77	$\frac{34}{48}$	71
Mammary Gland. (91)	$\frac{72}{118}$	61	$\frac{14}{91}$	15	$\frac{41}{66}$	62	$\frac{19}{34}$	56
Embryonic Skin. (91)	$\frac{33}{58}$	57	$\frac{9}{63}$	14	$\frac{14}{43}$	33	$\frac{30}{51}$	59
Spleen. (199)	$\frac{66}{75}$	88	$\frac{30}{50}$	60	$\frac{37}{44}$	84	$\frac{57}{71}$	80
Spleen. (T)	$\frac{30}{35}$	86	$\frac{7}{33}$	21	$\frac{14}{30}$	47	$\frac{28}{36}$	78
Average	$\frac{279}{407}$	69	$\frac{115}{423}$	27	$\frac{214}{338}$	63	$\frac{195}{283}$	69

The employment of a variety of mouse tissues, and of several different carcinomata, demonstrates that homologous tissue has always a strong power of immunising, an average tumour yield of no more than 27 per cent. occurring after such treatment. Moreover, in not one of twenty-seven experiments did the percentage of tumours rise to that in the control animals. The skin of mouse embryos has the strongest immunising power, but it would be unsafe to generalise too freely from this result since the tumours tested differed among themselves. Of the other homologous tissues, the mammary gland, the placenta, the spleen, all show marked immunising powers and the blood least of all.

The heterologous tissues do not act in this way. After the injection of rat tissue the average yield of tumours is about the same as that

in the controls, although occasionally smaller. Hence, while rat tissues do not constantly exhibit that strong power of immunising mice against mouse carcinoma which is characteristic of homologous tissue, still the occasional presence of a weak immunity cannot be denied.

The other heterologous tissues, viz. those of the guinea-pig, are devoid of immunising power, for the percentage of tumours developing after their injection, agrees with that found for the series of controls. Such experiments were performed seventeen times. A yield of tumours greater than that in the control series appeared eight times, and a smaller number occurred nine times. Therefore, although the tissues of the guinea-pig appear occasionally to have a power of immunising, yet this is weak in comparison with rat tissues, and never exceeds the probable experimental error so that they may be said to have no such power.

Do Dead Tissues Possess the Power to Induce Immunity?

The solution of this problem has already been attempted by several authors and most recently by Haaland (6), who destroyed normal and tumour tissue by mechanical disintegration or radium, and found that killed homologous tissues had not only lost their power to induce immunity, but, furthermore, that they elicited a tendency toward the augmentation of tumour growth. Flexner and Jobling (5) had previously stated that rat tumour tissue after being killed by heat produced no immunity against rat carcinoma, but on the contrary made animals more suitable for implantation. It was deemed desirable to compare the effects following the introduction of dead heterologous tissue.

The lactating mammary glands of mice, rats, and guinea-pigs were ground in a mortar immersed in ice and salt mixture until no intact cell could be found on examining a drop of the emulsion under the microscope, the process usually occupying 3 or 4 hours. The turbid semi-fluid mass obtained in this way was injected into the subcutaneous tissue of mice, and the effect tested after a certain number of days in the manner recorded in Table II. Carcinoma **91** yielded positive results in 61 per cent. of the control mice inoculated, and 62 per cent. in those treated with killed mouse mamma, 94 per cent in those treated with killed rat mamma, and 65 per cent. in those treated with killed guinea-pig mamma. Dead heterologous tissues, therefore, have no power to induce immunity, but, on the contrary, the yield of tumours tends to be increased after their introduction.

The Effects of Variations in Dose and Time Interval on the Immunity produced by Emulsions of Internal Organs.

While a preliminary treatment with embryo skin produces a strong immunity to most epithelial tumours irrespective of the tissue of origin, emulsions of internal organs do not exhibit the same uniformity in action. Experiments were performed to determine with accuracy the conditions under which spleen emulsion produced its greatest effect.

Table VI.—Effect of Varying Doses on Immunising Power of Spleen.

Immunising inoculation on back.	Interval between immunising and testing inoculations.	Testing inoculation in right axilla.			Control.			Mouse spleen.			Rat spleen.			Guinea-pig spleen.		
Quantity.		Tumour Series.	Quantity.	Date.	B	C	D	B	C	D	B	C	D	B	C	D
c.c.	Days.		c.c.	1910.			%			%			%			%
0·1	14	199 / 18 B	0·01	6–7	230 / 19	14 / 18	78	210 / 18	5 / 17	29	Died out.			280 / 20	14 / 20	70
0·05	,,	,,	,,	,,	,,	,,	,,	290 / 19	12 / 18	67	145 / 10	7 / 10	70	270 / 20	12 / 17	71
0·02	,,	,,	,,	,,	,,	,,	,,	240 / 16	10 / 15	67	Died out.			230 / 18	11 / 17	65
0·1	14	T / 30 I	0·01	13–7	220 / 19	14 / 16	88	220 / 18	4 / 16	25	205 / 15	6 / 15	40	240 / 19	17 / 18	94
0·05	,,	,,	,,	,,	,,	,,	,,	230 / 18	6 / 14	43	225 / 17	8 / 15	53	240 / 19	13 / 14	93
0·02	,,	,,	,,	,,	,,	,,	,,	227 / 19	4 / 17	23	222 / 18	12 / 16	75	245 / 18	9 / 16	56

Two strains of carcinomata were used: **199** and **T** (see Table VI.). The testing inoculations were made after 14 days, the spleen emulsion having been injected in diminishing doses (0·1 c.c., 0·05 c.c., 0·02 c.c.).

The first experiment with carcinoma **199** gave 78 per cent. of tumours in the control series, 29 per cent. after treatment with 0·1 c.c. of mouse spleen emulsion, and 67 per cent. after treatment with 0·05 c.c. and

0·02 c.c. Only one of the groups treated with rat spleen (0·05 c.c.) survived and gave 70 per cent. of tumours. Those treated with guinea-pig spleen gave 70 per cent. (0·1 c.c.), 71 per cent. (0·05 c.c.), and 65 per cent. (0·02 c.c.) respectively. In this experiment only the

TABLE VII.—EFFECT OF TIME-INTERVAL ON IMMUNISING POWER OF SPLEEN.

Immunising inoculation on back.	Interval between immunising and testing inoculation.	Testing inoculation in right axilla.			Control.			Mouse spleen.			Rat spleen.			Guinea-pig spleen.		
Quantity.		Tumour Series.	Quantity.	Date.	B	C	D	B	C	D	B	C	D	B	C	D
c.c.	Days.		c.c.	1910.			%			%			%			%
0·1	22	199 / 17 D	0·01	15–7	250/19	18/18	100	290/19	14/17	82	220/13	13/13	100	350/19	17/19	89
,,	18	,,	,,	,,	,,	,,	,,	290/18	16/18	89	290/20	20/20	100	300/20	18/19	95
,,	14	,,	,,	,,	,,	,,	,,	200/16	13/14	93	220/17	14/14	100	290/16	14/15	93
,,	10	,,	,,	,,	,,	,,	,,	Died out.			110/10	8/8	100	300/20	18/18	100
0·1	22	T / 30 J	0·01	22–7	220/20	16/19	84	300/19	3/18	17	300/18	11/18	61	290/19	14/18	78
,,	18	,,	,,	,,	,,	,,	,,	250/14	4/14	29	205/14	5/11	45	250/17	12/15	80
,,	14	,,	,,	,,	,,	,,	,,	230/17	3/17	18	240/18	8/15	53	250/19	11/18	61
,,	10	,,	,,	,,	,,	,,	,,	100/9	0/8	0	Died out.			200/17	12/14	86
0·1	24	63 / 48 D	0·02	29–8	300/19	10/18	56	270/18	5/15	33	150/11	4/9	44	260/17	9/18	50
,,	20	,,	,,	,,	,,	,,	,,	290/19	8/19	42	260/16	8/16	50	260/17	10/15	67
,,	16	,,	,,	,,	,,	,,	,,	160/11	2/11	18	Died out.			300/20	9/19	47
,,	12	,,	,,	,,	,,	,,	,,	290/19	1/16	6	300/20	11/20	55	300/20	7/19	37

largest dose of mouse spleen produced a measurable effect on the growth of this tumour (**199**).

The second experiment with carcinoma **T** gave similar results. Of the control animals 88 per cent. developed tumours. In the animals

treated with mouse spleen the percentages of tumours were reduced to 25 per cent. (0·1 c.c.), 43 per cent. (0·05 c.c.), and 23 per cent. (0·02 c.c.), respectively. There was thus a distinct immunity with all three quantities, but the fact that the highest was attained with the smallest dose detracts from its significance. After treatment with rat spleen the results were 40 per cent. (0·1 c.c.), 53 per cent. (0·05 c.c.), and 75 per cent. (0·02 c.c.). After treatment with guinea-pig spleen 94 per cent. (0·1 c.c.), 93 per cent. (0·05 c.c.), and 56 per cent. (0·02 c.c.) were obtained. The spleen of strange species does not give nearly the amount of immunity produced by mouse spleen, and while traces of immunity follow treatment with rat spleen, that of the guinea-pig tends rather to produce hypersensibility. While the quantity of mouse spleen injected is of definite importance, the dose of rat and guinea-pig spleen is without constant effect on the result.

Three series of experiments were performed to determine the duration of the immunity following treatment with 0·1 c.c. of mouse spleen. They were carried out with three different carcinomatous strains, **199**, **T**, and **63**, and the resistance was tested after 10, 14, 18, and 22 days in each experiment. The results are summarised in Table VII. It will suffice to point out that while the immunity after mouse spleen is highest at the 10th and 14th days, it diminishes appreciably after the lapse of 18 and 24 days. Rat and guinea-pig spleen, on the contrary, show no marked immunity at any period, and no constant differences in the result according to the interval which has elapsed.

Summary.

The following conclusions are drawn from the foregoing experiments :—

(1) Mouse placenta produces a strong immunity against transplanted carcinoma and sarcoma in this animal. It is surpassed in efficiency only by embryo skin and by mammary gland. This power is not due to the contained blood, but largely to the proper placental tissue elements.

(2) The strongest immunity against mouse carcinoma is elicited by mouse tissues. The epithelial tissues, skin, mammary gland, and placenta produce a stronger reaction than do the non-epithelial, such as blood and spleen.

(3) Rat tissues appear at times to produce a weak immunity against mouse carcinoma, but this result is not constant, and is always feeble. Guinea-pig tissues are practically incapable of inducing the resistant condition.

(4) Killed tissues not only have no power of calling forth immunity, but seem in some cases actually to induce a condition of hypersensibility, and it is immaterial in the case of killed tissues whether they come from species the same as, or alien to, that furnishing the carcinoma.

(5) With a dose of 0·1 c.c. of mouse spleen, normal mice can be immunised with great constancy. The spleens of other animals are devoid of this power, and do not present constant differences according to the quantity injected.

(6) The immunity following an injection of mouse spleen is strongest 10–12 days following injection, after which it gradually becomes weaker. The effect of injections of the spleens of other species seems to be uninfluenced by the length of the interval between treatment and testing inoculation.

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