The Manifestation of Active Resistance to the Growth of Implanted Cancer.

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(From the Laboratory of the Imperial Cancer Research Fund.)

The current classification of neoplasms has been almost entirely founded upon their structural characters, qualified wherever possible by the knowledge available upon their histogenesis. The researches which have been made, more especially with transplanted tumours of the rodents, have superadded to the recognised morphological differences and resemblances which tumours exhibit, much information upon the more physiological side of their activities, and have shown how variable tumour strains may be in their manner of growth. In the present communication, the individuality of various transplantable tumour strains will be brought out by subjecting them to an analysis with a new factor. This factor will be the quality of the reaction which a strain induces when implanted into a fresh series of animals, and this reaction will be tested by showing how the animals behave to subsequent inoculation of a transplantable tumour. In essence, therefore, the test applied is one for the presence or absence of a soil suitable for the sowing of tumour cells. The completion of this analysis will be followed by the exposition of a series of experiments bearing more especially upon the range of action of the unsuitability of the soil which can be artificially induced.

The propagation during an extended period of over 80 different tumour strains in the laboratories of the Imperial Cancer Research Fund* has provided an extensive material, presenting very different types of growth, and suitable for a comparative analysis. Exactly what is conveyed by the term, type of growth, will be apparent from the two accompanying figures of tumour strains T and 206. Strain T, shown in fig. 1, has given rise to a slowly but progressively growing tumour in 20 out of 21 mice, whereas with strain 206 (fig. 2) all tumours have finally disappeared spontaneously. These two carcinomatous strains exemplify the possible extremes of type of growth, and the intermediate gradations between them have been actually filled up

* Bashford, E. F., "The Behaviour of Tumour-cells during Propagation," 'Fourth Scientific Report of the Imperial Cancer Research Fund,' 1911, p. 131.

Exp. T/32 A. All mice inoculated in right axilla with 0·015 c.c. (21.9.10).

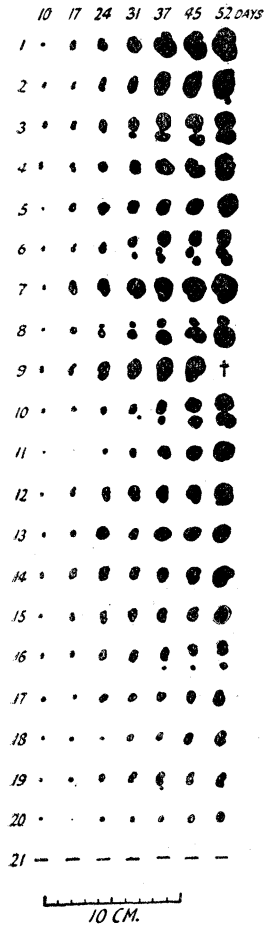

FIG. 1.

Exp. 206/11 C. All mice inoculated in right axilla with 0·02 c.c. (7.1.09).

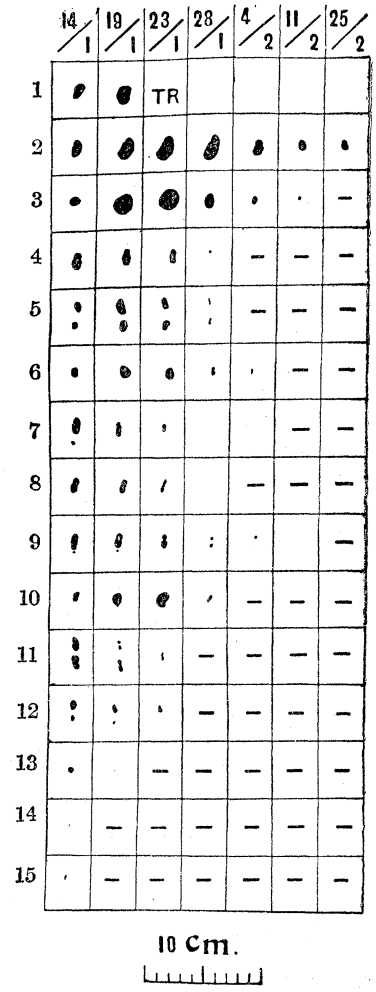

FIG. 2.

Fig. 1.—Chart showing progressive character of the growths developing after implantation of carcinoma T.

Fig. 2.—Chart showing the temporary character of the growth of tumour 206 in normal mice.

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,* 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 resistance during the growth of a transplanted tumour is a most important factor in determining the character of growth which a tumour will show, and the following experiments will demonstrate that this power of rendering a soil unsuitable for further inoculations is a distinctive feature of various tumour strains.

The procedure adopted has been to inoculate in one flank a series of young animals with a given tumour strain, and then to excise all the growths after 10–30 days, *i.e.* after intervals long enough to allow the tumours to attain a considerable size, 1–4 grm. One, two, or three days after extirpation, the animals have been 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 outlined in black, and filled in with dots.

The experiment with strain T, shown in fig. 3, exemplifies the course of such an experiment conducted with a progressively growing tumour strain. Eleven mice bearing this tumour had their growths excised on the 33rd day, and were re-inoculated with carcinoma 63 two days later. The 11 animals all remained free from recurrence, and in every case strain 63 gave rise to a rapidly and progressively growing tumour. When strain 63 is subjected to a similar analysis, it has been found to give a result similar in every respect to that just shown with strain T. These two carcinomata do not affect the suitability of mice for subsequent grafting, and have been much employed in the present series of experiments, where it was desired to obtain a very accurate estimate of the suitability of a series of mice for transplantation.

The discussion will now be directed to a tumour exhibiting the other extreme type of growth, namely, one which gives rise to temporary growths only. 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 to animal, so that the majority of

* Bashford, E. F., Murray, J. A., and Haaland, M., "Resistance and Susceptibility to Inoculated Cancer," 'Third Scientific Report of the Imperial Cancer Research Fund, 1908, p. 359.

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 inoculated in left axilla with 0·02 c.c. of 63/55 E (24.11.11).

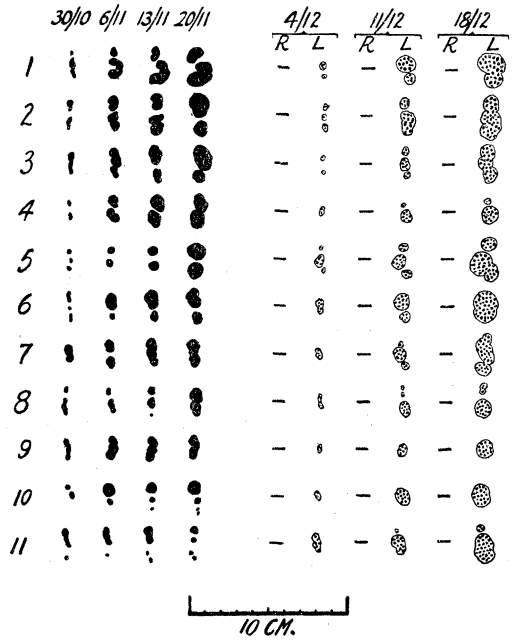

FIG. 3.—The growth of tumour strain T followed by surgical removal does not render mice unsuitable for subsequent inoculation.

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. 4, where it will be seen that 11 days of growth of this strain in 11 mice has been sufficient to render every one of these animals unsuitable for the growth of carcinoma 63.

Between these two extremes, the other transplantable mouse tumours, carcinomata and sarcomata, range themselves according as they render animals in a higher or lower percentage unsuitable for re-inoculation. Several of these strains have been tested, and resistance to re-inoculation has been found in a percentage varying between 30 and 75. One of these carcinomata, strain 199, exhibits certain peculiarities in its manner of growth, which were described in a communication published in these

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 inoculated in left axilla with 0·02 c.c. of 63/56 D (23.12.11). Mice 12—23 : control to re-inoculation.

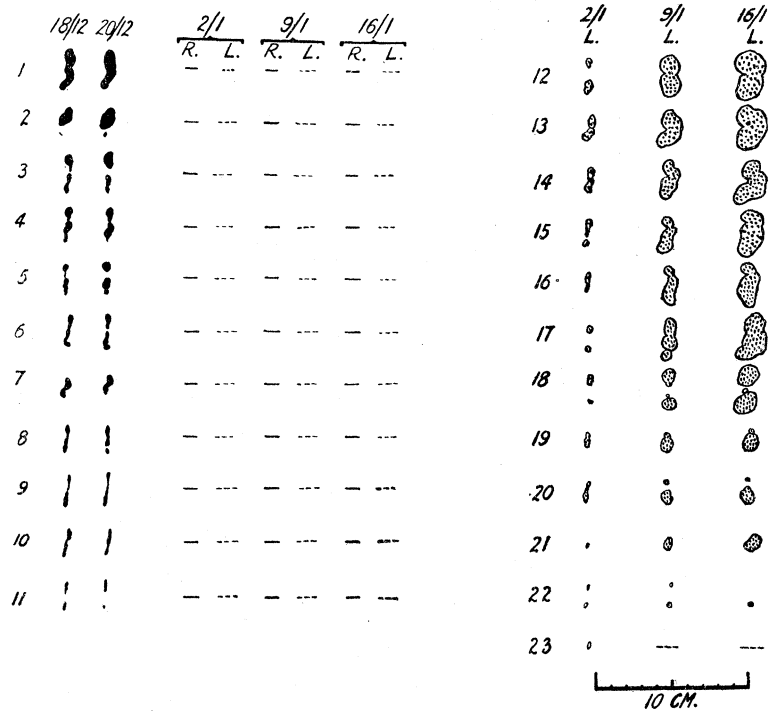

FIG. 4.—Shows the induction of resistance by strain 206 in all mice.

'Proceedings' two years ago.* 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 remainder 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 spontaneous absorption, but also where they had temporarily ceased to enlarge their dimensions. The strain 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

* Bashford, E. F., and Russell, B. R. G., "Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths," 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 298.

then testing the suitability of the animals for re-inoculation. It was found that, in about 60 per cent. of the cases, strain 199 had rendered the animals resistant to re-inoculation, a result which conforms with that previously obtained.

This concludes the description of the findings with mouse tumours, which 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 those 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 grm., often disappear entirely. The re-inoculability of rats bearing this tumour has been already described and figured,* and it was then 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.

In one experiment conducted with this rat sarcoma, the result of re-inoculating seven rats, whose tumours had been completely extirpated, was that in only one animal was a tumour obtained 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 seven other rats from the same series the excision was intentionally incomplete, but the result of re-inoculation was practically the same as in the rats where the excision was complete. These findings do not harmonise with those of Uhlenhuth, Haendel, and Steffenhagen,† who found that incomplete excision left the animals suitable for re-inoculation. In the above series of

* *Loc. cit.*, p. 203.

† Uhlenhuth, Haendel, und Steffenhagen, "Experimentelle Untersuchungen über Rattensarkom," 'Arb. a. d. kais. Gesundheitsamt,' 1911, vol. 36, p. 465.

experiments with rat and mouse tumours, the success or failure of re-inoculation has been found to be determined solely by the nature of the reaction set up by the first implanted tumour.

All of the above experiments have been chosen with the special view of demonstrating how very differently the parenchymata of various tumours of different types behave in regard to the alteration of the suitability of the soil which they induce, 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, of course, apparent that such wide differences can only be attributed to inherent properties of the tumour parenchymata, and the contrast in their behaviour may be drawn 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 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, but strain 199 and many others bring them out with distinctness.

Murray's studies* 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 cancer, although they are not more suitable for the implantation of a transplantable tumour than mice not specially bred.†

Cuénot and Mercier‡ 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.

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. 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 10 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 40. The natural resistance of animals to tumour inoculation is a phrase which has been much employed, but it might perhaps be more correct to speak 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

* Murray, J. A., "Cancerous Ancestry and the Incidence of Cancer in Mice," 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 42.

† Haaland, M., "Spontaneous Cancer in Mice," 'Roy. Soc. Proc.,' B, vol. 83, p. 532.

‡ Cuénot, L., et Mercier, L., "L'hérédité de la sensibilité à la greffe cancéreuse," 'Comptes Rend. de l'Acad. des Sciences,' 1910, vol. 150, p. 1443.

from this laboratory as early as 1904,* 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.† In a 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, and the view was rejected that the negative result was attributable to an exhaustion of specific food-substances (athreptic immunity) by the first tumour. The latter view is still upheld by Apolant,§ but in one of his later papers the accompanying charts show that after removal of the first tumour the majority of the animals, rats and mice, are resistant to re-inoculation, which is scarcely in harmony with the hypothesis he entertains.

The conclusion is inevitable that tumour parenchymata vary widely in the extent to which they alter the suitability of an animal for growth of a subsequently implanted tumour, and that this alteration of the suitability of the animal is due to the development of an active resistance or immunity.

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|| 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¶ also performed the double inoculation of two separate strains, and found that each grew as if it alone had been inoculated.

When, however, double inoculation is carried out with tumours of different types of growth, the two tumours do not grow independently of each other.

* Bashford, E. F., and Murray, J. A., "The Transmissibility of Malignant New Growths from one Animal to Another," 'First Scientific Report of the Imperial Cancer Research Fund,' London, 1904, p. 11.

† *Loc. cit.*, p. 205.

‡ Bashford, E. F., Murray, J. A., Haaland, M., and Bowen, W. H., "General Results of Propagation of Malignant New Growths," 'Third Scientific Report of the Imperial Cancer Research Fund,' London, 1908, p. 262.

§ Apolant, H., "Ueber die Immunität bei Doppelimpfungen von Tumoren," 'Zeitschr. f. Immunitätsforsch.,' 1911, vol. 10, p. 103.

|| Bashford, E. F., Murray, J. A., Cramer, W., "The Natural and Induced Resistance of Mice to the Growth of Cancer," 'Roy. Soc. Proc.,' 1907, B, vol. 79, p. 164.

¶ Bridré, J., "Recherches sur le Cancer Expérimental des Souris," 'Ann. de l'Institut Pasteur,' 1907, vol. 21, p. 760.

It has been found that strain 63 is inhibited in its growth by the simultaneous inoculation of sarcoma 37, a tumour which renders mice resistant to re-inoculation in a high percentage of cases. The percentage of success on inoculation of strain 63 has been lowered from 100 down to 50, and even 25, whilst in addition the rate of growth is greatly retarded as compared with that in normal animals.

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 of resistance after this treatment, and it may be a question of one or two days only. 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.

The behaviour of tumour 63, however, demonstrates very clearly a case where the immunity can overtake the early phases of growth and prevent its continuation. 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.* The experiments next to be described support the above interpretation of the different behaviour of strains 63 and 91.

The treatment of mice by the inoculation of normal tissues of the mouse, as described in these 'Proceedings,'† has been found to prevent the development of tumours implanted 10 to 20 days later, and the resistance evoked by these normal tissues is of the same nature as the resistance evoked by tumour tissue. The efficacy of this preliminary treatment stands out in marked contrast with the disappointing nature of the results hitherto

* Russell, B. R. G., "The Nature of Resistance to the Inoculation of Cancer," 'Third Scientific Report of the Imperial Cancer Research Fund,' London, 1908, p. 341.

† *Loc. cit.*, p. 209.

obtained when the attempt is made to produce, by the same means, the involution of an already established tumour which tends to grow progressively.

By means of a rather complicated experimental procedure it has been found possible to demonstrate that an immune reaction can be evoked in an animal bearing a progressively growing tumour. 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.

On January 19, 1910, 40 mice, weighing from 14 to 16 grm. each, were inoculated with 0·015 c.c. of T/27 C in the right axilla; 29 of the 35 surviving mice developed tumours—83 per cent. Twenty-five of these tumour-bearing mice were divided into two batches, when the tumours were 12 days old, and one batch was inoculated on the back with 0·05 c.c. of mouse carcinoma J, while at the same time 15 normal mice were treated in the same way. Strain J 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 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.

In the batch which did not receive an immunising dose of tumour J, 10 out of 13 mice developed tumours on re-inoculation; whereas, in the batch which received an intercalated dose of tumour J, only 3 out of 12 mice developed tumours on re-inoculation. In the 15 control animals which were inoculated first with carcinoma J, and then tested with T/28 F, only one developed a tumour from the re-inoculation.

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

Expt. 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 77 per cent.	3 in 12 25 per cent.	1 in 15 7 per cent.	6 in 8 75 per cent.

Three further experiments on these lines 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 behaviour of carcinoma 91 was also tested on two separate occasions.

On summing up the total figures obtained in the six experiments the change induced can 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 63 per cent.	19 in 70 27 per cent.	22 in 95 23 per cent.	51 in 69 73 per cent.

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 to 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 2 and 3, 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.

When carrying 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. If this result could be regularly obtained, the cure of transplanted tumours which did not disappear spontaneously would be accomplished. Strains of tumours which grow progressively were tested by subjecting the mice bearing them to the inoculation of embryonic tissue or of tumour tissue, which disappeared spontaneously. The effect of single inoculations of varying amounts of immunising tissue was tested, and also 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.

The result obtained was not encouraging, even although the strains employed fell a ready prey to a resistance evoked before grafting. Taken over all, the tumours grew more slowly than in the control animals, but their regression was not brought about.

The main conclusions to be drawn from the above investigations are :—

1. Tumour parenchymata vary widely in their power of inducing resistance. This factor must not be lost sight of in the prosecution of researches in immunity to transplanted cancer.

2. The individuality of the animal inoculated may contribute to the development of resistance, although not to so marked a degree as the tumour parenchyma.

3. Simultaneous inoculation of a tumour strain which induces no resistance, and a strain which induces resistance, may be followed by marked inhibition of the growth of the former strain.

4. Mice bearing progressively growing tumours can be rendered resistant to re-inoculation, but the tumour first inoculated need not necessarily be affected.

5. Repeated inoculations of tissues, such as mouse embryo skin which renders animals resistant to subsequent inoculation, have not been shown to have a constant effect upon the growth of established tumours.

6. The conclusions drawn in (4) and (5) support the view previously expressed that immunity to cancer is directed mainly against the stroma-eliciting properties of the cancer cell.