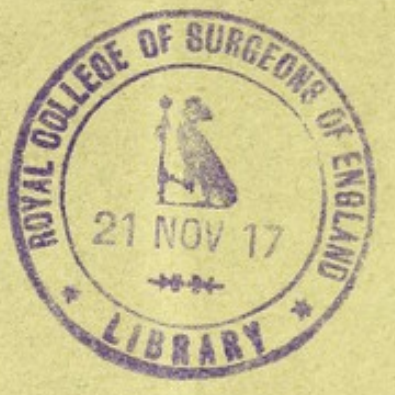
The experimental analysis of the growth of cancer / by E.F. Bashford, J.A. Murray and W.H. Bowen.

[*From the* Proceedings of the Royal Society, B, Vol. 78, 1906.]

The Experimental Analysis of the Growth of Cancer.

By E. F. BASHFORD, M.D.; J. A. MURRAY, M.B., B.Sc.; and W. H. BOWEN, M.S., F.R.C.S.



[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B, Vol. 78]

The Experimental Analysis of the Growth of Cancer.

By E. F. BASHFORD, M.D., J. A MURRAY, M.B., B.Sc., and W. H. BOWEN, M.S., F.R.C.S.

(Communicated by Professor J. Rose Bradford, F.R.S. Received May 30,—Read June 14, 1906.)

In the present paper we shall attempt to analyse the growth of cancer when propagated artificially in mice, mainly on the basis of 25,000 inoculations of Jensen's tumour performed in conjunction with Dr. W. Cramer on behalf of the Imperial Cancer Research Fund; but also with reference to inoculations made with 32 other mouse tumours during the past three years. Although the question of the continuous or interrupted nature of cancerous proliferation is of fundamental importance, both from the standpoint of the ultimate explanation of the nature of the disease, and from the standpoint of its treatment, such an analysis has never been attempted before. It has been assumed that the growth of cancer is vegetative, as inexplicable as any other form of growth, only to be partially understood by an elucidation of the processes by which normal tissues become cancerous. Artificial propagation enabled us to submit this assumption to the test of experiment, and imposed the necessity of determining by direct observation whether propagated cancer exhibited a mode of growth throwing light on the nature of the disease and the apparently continuous proliferation of sporadic tumours. While the experimental propagation of cancer may reveal new facts with a bearing on the nature of the disease it also affords opportunities for rational and empirical therapeutic experiments, and adequate opportunity for controlling the results. These two purposes have been constantly kept in view in our investigations.

When a number of animals are inoculated with a transplantable mouse tumour, all do not develop tumours and the tumours which do develop are not all of the same size after the same interval. In order that propagated cancer might be available for the second of these purposes it was necessary to find out what influence the variable conditions of experiment exerted on the proliferation of the cells. In the course of these preliminary studies facts bearing on the nature of cancer have also been ascertained.

Irregularities in the rate and amount of growth are introduced by (1) Transference from one race of mouse to another even when nearly allied: (2) Transference from young to old mice of the same race or *vice versâ*: (3) Variations in the site of implantation of the cancerous tissue: (4) Variations in the amount of the tissue implanted and in the manner of introducing

it: (5) Variations in the character of the tumour cells themselves. Any one of these factors may cause a very great deviation from the rate and amount of growth observed previous to the subinoculations introducing it, and invalidate the results of experiments of which information as to possible modification of growth was the object. The variations depending on the first four factors mentioned must be eliminated before variations can be referred to the tumour cells themselves.

We have taken the following precautions in studying the fluctuations which we believe depend on differences in the tumour cells.

1. The same race of mice has been used throughout. We have observed differences in the suitability of animals of different colours even among the ordinary English tame mice; and we have avoided the use of those varieties prized by mouse-fanciers. The wild mouse probably offers more uniform conditions than the tame mouse, but a sufficient stock of uniform age is difficult to obtain, keep and supervise. Jensen's tumour rarely yields a number of successful subinoculations in wild mice equal to that obtained in a control batch of tame mice, and this result when once obtained has not been maintained, but is followed by an increasing difficulty of propagation. The experiments in wild mice may be looked on as control observations to those recorded in tame mice.

2. The tame mice used have been of uniform age, and from five to seven weeks old. We showed that young animals provide conditions more favourable for the artificial propagation of cancerous tissue than old animals. This conclusion has been amply confirmed by our later experience, and in one of its aspects also by the work of Ehrlich and Apolant,* who state that the age of the animals is without importance and, especially, that old females are not more suitable than young animals for the propagation of mammary tumours. We have found that the greater suitability of young animals is even more marked than we at first suspected. The inoculation of a tumour into young and old animals respectively may occasionally give similar results in the two cases, or even a less favourable result in young mice, still such results are exceptional in our experience. As a rule, a much higher percentage of tumours develops in young animals, and they attain large dimensions in a shorter time after inoculation. The tumours which have developed most rapidly, *e.g.*, attaining a weight of 1·05 grammes in a mouse of 9 grammes, within five days, and those ultimately attaining the largest dimensions as compared with the size of the host, have always occurred in young mice, although tumours of 7 or 8 grammes also develop rapidly in adult animals. Slow growing tumours, which remain of relatively small dimensions, occur both in

* 'Berl. Klin. Wochenschrift,' No. 28, July, 1905.

old and in young animals. The extent to which the youth of the animals usually favours the continuation of growth after transplantation may be illustrated by the results of 18 series of inoculations, in which portions of the same parent tumours were transplanted simultaneously into young and adult animals respectively; 214 implantations into adult animals three to six months old yielded 62 tumours, or 29 per cent. were successful; 363 implantations into young animals five to seven weeks old gave 172 tumours, or 47 per cent. were successful. This result is by no means an extreme case, either as regards proportion of successes or as regards difference in age of the inoculated animals.

3. When the precautions above indicated are observed, the individual variations in the general suitability of different mice of the same race and age are negligible if implantation be performed in the same site, provided sufficiently large numbers are used. We have preferred the subcutaneous tissue of the back. The attempt to perform collateral series of intra-peritoneal inoculations was abandoned, owing to the frequency with which growth within the peritoneum had occurred secondarily by extension from tissue implanted in the abdominal muscles.

4. We have endeavoured to transplant pieces of healthy-looking tissue of uniform size by means of hypodermic needles, and have obtained more satisfactory results by this method than by breaking tumours down into an emulsion and injecting larger quantities of tissue suspended in physiological salt solution. With certain reservations the rate of development and the size the daughter tumours will attain within 10 days is directly proportionate to the amount of healthy tumour tissue implanted; 0·02 to 0·03 gramme of tissue usually gives larger tumours within a given time interval than 0·005 to 0·01 gramme.

5. When the conditions referred to in the four preceding paragraphs are maintained uniform, fluctuations independent of them appear, and we shall endeavour to show that they are, in all probability, natural features of proliferation. The detailed study of these fluctuations has been undertaken with the tumour which has proved readily capable of transmission during the longest period yet attained, viz., that of Jensen. This tumour has now been propagated for four and a-half years, without permanent alteration in its histological characters or its behaviour. We have obtained success in from 5 to 90 per cent., occasionally even in 100 per cent., of the animals inoculated, the percentages being based on data obtained from those mice which were still alive* 10 days after the inoculations were made. The amount of tissue

* It is our practice to kill from time to time a number of mice during the first 10 days after inoculation, in order to examine the site of implantation. Tumours of transplant-

transplanted in each animal varied between 0·01 and 0·02 gramme.* The pieces were selected from the whole tumour, and hence their behaviour furnishes an estimate of the proliferative energy of its component parts. The use of a restricted number of random fragments is rendered necessary, because it is impossible to transplant the whole of every tumour; the number of animals required of itself limits the investigation.

The method of experimental propagation by implanting minute cellular grafts leads to a progressive subdivision of the parenchyma, and to the distribution over a large number of animals of the descendants of cells previously associated together in one animal. The experimental tumours consist of a parenchyma arranged in alveoli. The study of the early stages after transplantation shows that, at first, single alveoli constitute separate centres of growth, and we may therefore term them the "parent alveoli" of the tumour. Since the cells of different alveoli do not intermingle, the progeny of the discrete growing centres in the transplanted tissue remain separate, and are further separated from one another as the parent alveoli increase in size and bud off daughter alveoli at the surface. When minute portions of such a tumour containing very numerous daughter alveoli are in turn transplanted it is very improbable that any one fragment will contain cells from each parent alveolus, *i.e.*, progeny of all the primary growing centres in the cellular graft which gave rise to the tumour. On the contrary, such a fragment is likely to contain cells closely related to one another, *i.e.*, from only one of the new growing centres of which the tumour is ultimately composed. Thus, in the course of repeated implantation, the tumours obtained come to represent less and less all the constituent cells of any entire tumour in the preceding transplantations made during the long-continued experimental propagation. In order that they should do so it would be necessary to mix homogeneously all the tumours obtained at each series of implantations. The purposes of our investigation were fulfilled by the method of repeated subdivision and isolation. By this method a repeated analysis of the power of growth of small groups of cells and their descendants can be obtained. The limited number of centres of growth represented in any single graft can

able size, 0·75 to 1·5 grammes weight, are rare before 8 to 10 days. As the object of these experiments was to estimate the power of continued growth as distinct from mere transitory proliferation, some such time limit was necessary. Estimates of the percentage of success and of the frequency of the spontaneous cessation of growth in tumours which had established themselves must exclude transitory proliferation of the cells introduced and inflammatory swellings at the site of inoculation.

* The weight of the fragments inoculated into each animal has been arrived at by weighing the mass of tumour used for transplantation and dividing by the number of animals used, *e.g.*, 1 gramme of tumour transplanted into 100 mice gives 0·01 gramme per implantation.

be better appreciated by considering also that they have been obtained as the result of a triple process of selection; firstly, the rapidly growing tumours of a batch have been selected because of the greater powers of growth exhibited; secondly, only the healthy parts were used for transplantation; and thirdly, a further sifting has been effected by the elimination of those cells which degenerate after transplantation. Taken together with the simultaneous reductions of the number of cells continuing growth at each fresh implantation, the repeated implantation of minute cellular grafts renders it practically impossible that any one tumour at the present stage of propagation should still contain cells representing all the growing centres of a tumour even two or three transplantations antecedent to it.

The percentage of tumours developing after transplanting is, however, only one means of measuring the proliferative power of a tumour experimentally.* It is an arbitrary measure selected for its convenience of application. It merely records that, of a number of fragments taken from a tumour, a certain proportion grew and the remainder were absorbed after implantation in fresh animals. It necessarily neglects variations always obtaining in the weights of tumours in every batch. It is obvious that a sporadic tumour may be obtained, or a time may come in the course of the prolonged selection of tissue for implantation in the future continued propagation of Jensen's tumour when all transplantations will yield tumours. Should this ever be so, measuring the energy of growth by the percentage of tumours developing would fail to reveal any fluctuations. The fluctuations in percentage of success which had previously occurred would retain their importance, and a different method of measurement might still reveal fluctuations dependent on the same factors as great as those represented in these experiments by percentages of success varying between 5 and 100 per cent. of the animals used.

We have studied the growth of the tumour in parallel series of experiments at different times. In order to compare the results we have estimated the percentage of success attending the subinoculation of all tumours transplanted. The repeated subdivision of the transplanted tumours results in the separate propagation of many strains, which become increasingly

* The weights which the tumours attain in equal times present great fluctuations as well. In series with a high percentage of success many tumours attain a weight of 1 gramme in the course of 10 days, while series with low percentage of success seldom show tumours of 0·5 gramme weight in the same time. This may be due to the greater number of cells continuing growth in each animal in series of high percentage, and therefore does not necessarily indicate a more rapid rate of proliferation of the individual cells. For this reason we have not been able to use the weight of tissue produced in a given time as a means of comparing proliferative power at different times.

numerous as time goes on, spreading out like the branches of a tree from any tumour selected as a starting point. On the basis of this relation a genealogical tree of all the transplanted tumours has been constructed in which the intervals of time between successive transplantations are also recorded. This result is achieved by measuring the number of days since propagation commenced and marking the respective dates of transplantation, so as to mark off abscissæ; the power of proliferation being measured by marking the percentage of successful implantations as ordinates. The point, determined by these two variables for every tumour transplanted, records its power of proliferation and the date of transplantation.

When the point so obtained from any one tumour at the end of the series is connected by a line with the point similarly obtained for the tumour from which it was derived, and the connections followed backwards through the corresponding points of the preceding transplantations, the absolute duration of propagation and the steps in the lineage of the tumour at the end of the series can be seen at once. As the process of connecting up the points is continued backwards the lines from the points obtained for other strains converge and coalesce till all ultimately unite in the point obtained for the percentage of success attending the primary transplantation of the sporadic growth.

The graphic records accompanying this paper are small portions of a large chart recording in this way the results of all our experiments with Jensen's tumour extending over a period of two and a-half years.

For the purpose of recording the experiments each batch of implantations performed with one tumour is labelled with a number, stating the number of successive transplantations from the beginning of the series. For example, the parent tumour of a batch of implantations belonging to the 40th transplantation has been obtained after 39 successive transferences to fresh mice. To distinguish between several batches, the parent tumours of which have been obtained after the same number of transferences, a letter of the alphabet is added to the number of the transplantation. The genealogy of the various series of implantations is not indicated by this nomenclature, and for this purpose the graphical records, now to be described, have been devised.

As it is important that the exact meaning of this graphical record should be clearly understood, the method by which it is built up may be exemplified by a special case (fig. 1) forming part of another chart (fig. 2).

(1) On the 739th day of propagation, a tumour of Transplantation 45, Series C, was transplanted into 52 mice which were labelled 46 C; 32 mice died in the first 10 days after transplantation. In the remaining 20,

11 tumours developed; *i.e.*, 55 per cent. of the implantations were successful.

(2) On the 751st day of propagation, a second tumour of Transplantation 45, Series C, was transplanted into 30 mice, the experiment being labelled 46 F. No deaths occurred in the succeeding 10 days, and 14 tumours were obtained; *i.e.*, 47 per cent. of the implantations were successful.

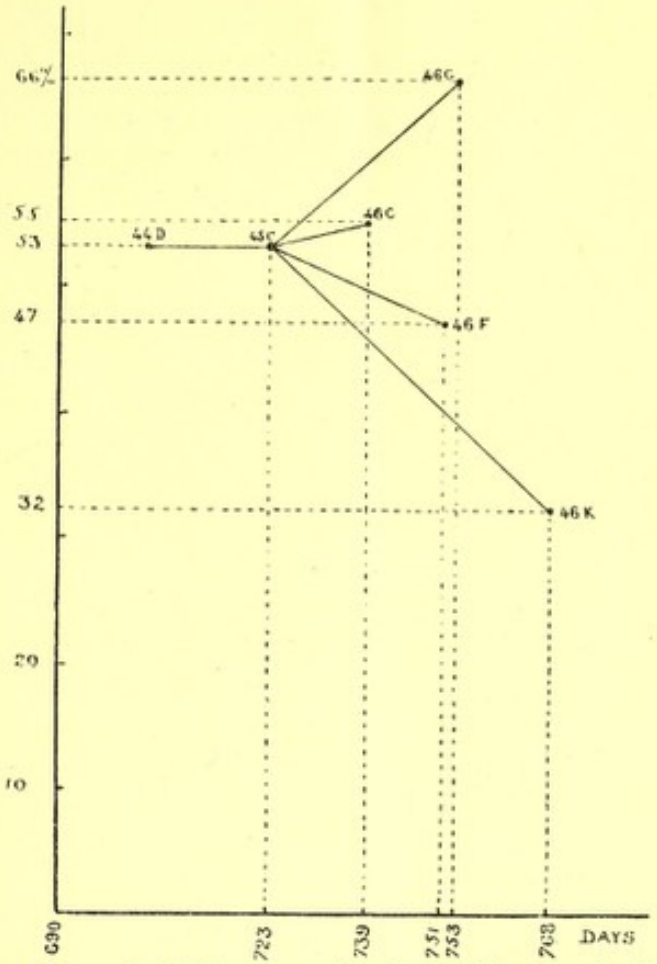


FIG. 1.—Illustrates the method of constructing the graphic records of transplantation experiments (see text).

(3) On the 753rd day of propagation, a third tumour of Transplantation 45, Series C, was transplanted into 40 mice and the experiment labelled 46 G. Eight mice died in the first 10 days following transplantation, and 21 tumours developed in the 32 survivors; *i.e.*, 66 per cent. of the implantations were successful.

(4) On the 768th day of propagation, a fourth tumour of Transplantation 45, Series C, was transplanted into 20 mice, 46 K. One mouse died in the first 10 days and six tumours developed in the remaining 19 mice; *i.e.*, 32 per cent. of the implantations were successful.

The data obtained from these four experiments, viz.:—

	The day of propagation. Abscissa.	The percentage of success. Ordinate.	Name of experiment.
		Per cent.	
1	739	55	46 C.
2	751	47	46 F.
3	753	66	46 G.
4	768	32	46 K.

suffice to determine four points, indicated on the chart by the numbers and letters in the third column.

The four fragments of the *parent* tumour from which the four tumours used for the above experiments (1, 2, 3 and 4) developed were implanted along with 51 other fragments into 55 mice on the 723rd day of propagation, the experiment being labelled 45 C; 23 mice were killed in the first 10 days after transplantation for microscopical examination of the site of implantation; 17 tumours developed in the remaining 32 mice, *i.e.*, 53 per cent. of the implantations were successful. These two numbers, 723 as abscissa marking the date of transplantation, and 53 as ordinate marking the percentage of success of implantations, together fix a fifth point labelled on the chart 45 C. The four points previously obtained represent the results of transplantation experiments on four of these 17 tumours, and to indicate this relation they are each connected with the point labelled 45 C by a straight line.

In the same way a point has been obtained for the parent tumour of 45 C indicated on the chart as 44 D, and similarly for the transplantations antecedent to 44 D and subsequent to 46 G as shown in the larger charts.

The following condensed summary of a number of consecutive experiments will make clear the nature of the results to be recorded in this manner. A tumour of the 39th Transplantation, transplanted into 37 animals, gave tumours in 3 of the 20 animals remaining alive after 10 days (15 per cent.), Transplantation 40, Series I, or shortly 40 I. Of these mice one developed two large tumours weighing together 7·5 grammes in 49 days, when the animal was killed and the tumour transplanted into 24 mice. Tumours developed in 4 of the 20 animals which survived the first 10 days after transplantation (20 per cent.), 41 P; 14 days afterwards one of these tumours weighing 1·3 grammes was transplanted in 66 mice; tumours developed in 7 of the 31 survivors (23 per cent.), 42 L. Of these a tumour, having attained a weight of 4 grammes after 42 days' growth, was transplanted into 45 mice. Tumours developed in 13 of the

43 survivors (30 per cent.), 43 L. One of these, 21 days later, had attained a weight of 3·3 grammes and was transplanted into 27 mice; tumours developed in 8 of the 15 survivors (53 per cent.), 44 D. After 22 days' growth, one of these, 3·7 grammes weight, was transplanted into 55 mice; tumours developed in 17 of the 32 survivors (53 per cent.), 45 C. After 26 days a tumour 1 gramme in weight was transplanted into 40 mice; tumours developed in 21 of the 32 survivors (66 per cent.), 46 G. After 15 days' growth, a tumour weighing 1·3 grammes was transplanted into 40 mice, tumours developing in 26 of the 33 survivors (79 per cent.), 47 H. After 14 days a tumour weighing 1·35 grammes was transplanted into 30 mice, tumours developing in 21 out of 25 survivors (84 per cent.), 48 E. Up to this stage there has been a gradual rise in percentage of success through nine successive transplantations from 15 to 84 per cent. The results of transplanting seven tumours of Series 48 E do not maintain this high percentage. Thus a tumour of nine days' growth, weighing 1·15 grammes, was transplanted into 54 mice, tumours developing in 9 out of 32 survivors (28 per cent.), 49 A. Another, 11 days' growth and 1·7 grammes in weight, was transplanted into 40 mice; tumours developed in 12 out of 38 survivors (33 per cent.), 49 B. A third, also 11 days' growth, weighing 1·4 grammes was transplanted into 31 mice; tumours developed in 10 out of 27 survivors (37 per cent.), 49 C. A fourth, of 15 days' growth, 1·7 grammes in weight, was transplanted into 40 mice; tumours developed in 12 of the 35 survivors (34 per cent.), 49 F. A fifth, of 26 days' growth, 3·2 grammes in weight, was made into an emulsion and injected into six mice. All the mice survived, but no tumours developed, and this experiment is not recorded on the chart below. A sixth, of 27 days' growth, 3·6 grammes in weight, was transplanted into 41 mice and tumours developed in 5 out of 25 survivors (20 per cent.), 49 O. A seventh, after 62 days' growth, weighed 3 grammes. It was transplanted into 40 mice; tumours developed in 20 out of 36 survivors (56 per cent.), 49 X. None of these tumours maintained the high transplantability of the parent growth, although the implantations grew rapidly and were made at intervals of from 9 to 62 days. This sequence in the results has been a constant feature in all the strains propagated, and there is, therefore, reasonable ground for believing that it is a natural feature of growth.

We shall now proceed to a consideration of the graphic records of this series of experiments. In the accompanying chart (fig. 2) the lines joining the points representing the date of transplantation and power of proliferation appear to form a continuous ascending curve rising from 40 I (15 per cent.) through nine successive transplantations to 48 E (84 per cent.).

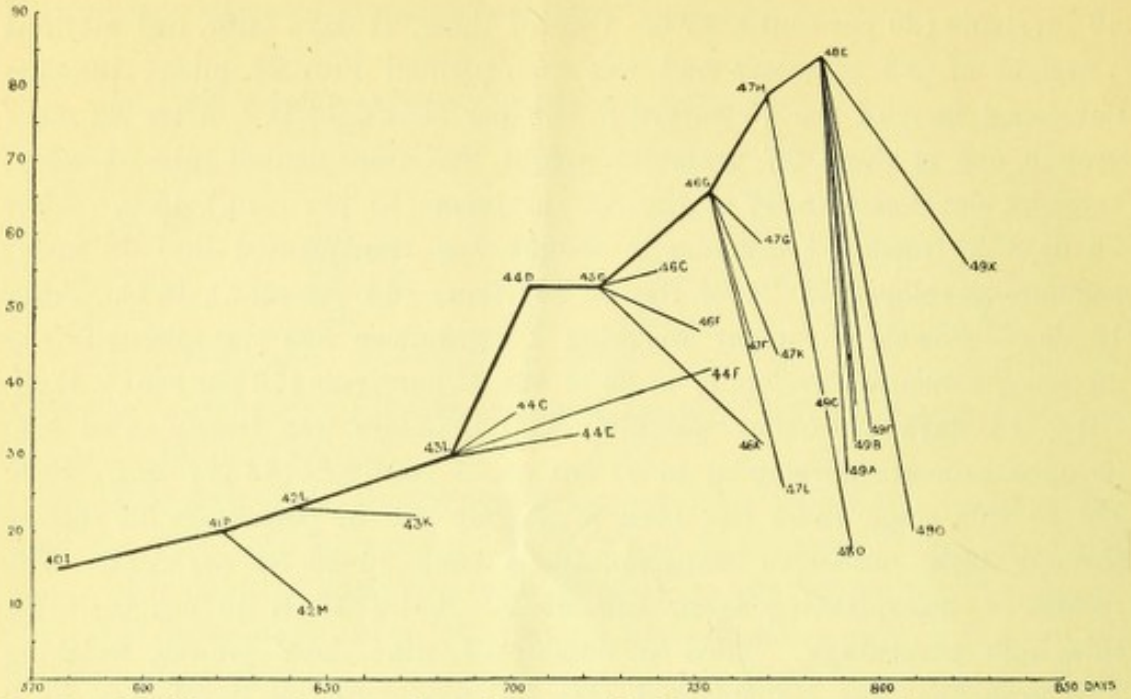


FIG. 2.—Graphic record of steps by which, starting from a tumour giving 15 per cent. of success on transplantation, 40 I, a tumour was obtained after eight subsequent transplantations, giving 84 per cent. of success, 48 E. The success on transplanting some of the other tumours propagated at each step is also recorded by the points at the ends of the lines branching off from the "main stem" of propagation.

When the curve has reached a maximum it falls rapidly in marked contrast to the preceding gradual rise. The seven tumours of 48 E do not all fall to the same level; the degree of the diminution in the success attending subinoculation varies, but the direction of the curve is downwards in all. This sequence of a gradual rise in transplantability followed by a fall has been repeatedly observed during the past two and a-half years. Although in this particular case the fall in percentage of success on transplanting tumours of 48 E is rapid, and attains a minimum at the first essay with many other strains, it has been possible to obtain several estimations on the downward slope of the curve. In such cases, when the tumours of a series following on a maximum are again transplanted, a further diminution in the percentage of success is frequent, if the diminution in transplantability had been slight in the first instance. The accompanying chart (fig. 3) illustrates these points very clearly. It represents the results obtained by transplanting a large proportion of all the tumours of another series, viz., 50 Z, which had indicated 90 per cent. of transplantability. The daughter tumours of this series giving rise to 51 U and 51 T, showed the smallest diminution as compared with 50 Z. From each several tumours have been transplanted, and in each case the diminution in the percentage of

success has continued till a level was reached which other tumours fell to at the first essay. The protocol of this experiment is given in full on p. 211.

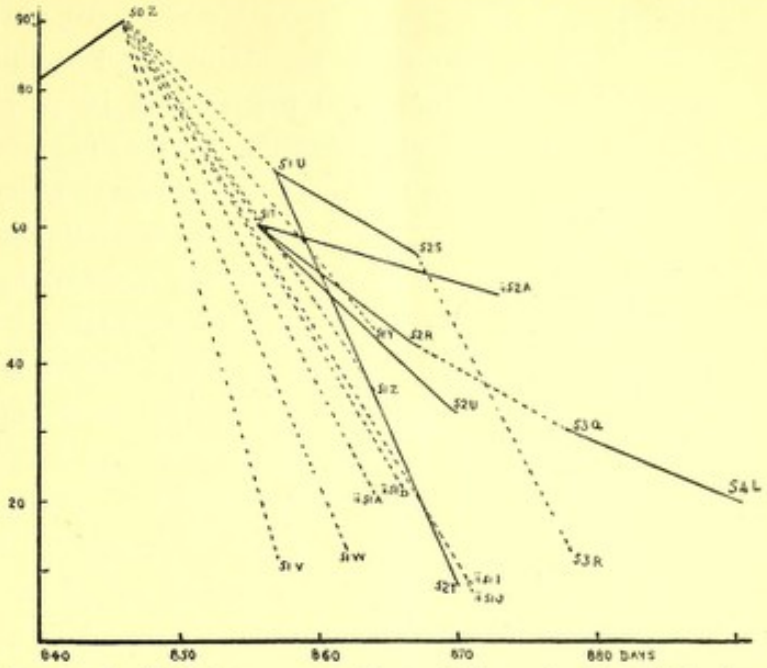


FIG. 3.—Graphic record of further propagation of the majority of the tumours obtained in an experiment (50 Z) in which 90 per cent. of the animals developed tumours. The diminished percentage of success reaches a minimum at the first transplantation in some (51 Y, 51 W); in others after two transplantations (51 U to 52 T and 51 T to 52 U); while in others a third diminution occurs before a minimum is reached (51 U, 52 S, 53 R); and a fourth diminution occurs in the series 51 T, 52 R, 53 Q, 54 L.

The description of the graphic record has so far been confined to illustrating the sequence of events in the tumours in the line giving the highest percentages of success, and their ultimate fate. The other experiments recorded in figs. 1, 2, 4 and 5 must now be considered. The tumours propagated concomitantly with those marking the steps in the ascending curve appear to form the ends of "side-branches" on that curve as a main stem. Some of these "side-branches" also exhibited the upward tendency and duplicated the behaviour of the main stem in that they also rose to a maximum followed by a fall. The others appear to form descending side-branches and to anticipate the ultimate fate of the ascending curve. If the tumours on such descending side-branches be transplanted they may either give a further fall, completely negative results, or gradually increasing percentages of success till they in turn present a maximum followed by a fall.

The most careful attempts to maintain the percentage of success at a high level in the direct line of descent therefore show that the condition leading

to diminished transplantability ultimately affects the descendants of the tumours which had previously escaped it, and hence appeared to constitute an ascending main stem in the graphic record. We have always obtained a rise to a maximum which cannot be maintained, and a subsequent fall which is also not permanent if continued propagation be possible. Up to the present we have encountered no exception to this rule in more than 600 series of inoculations with this tumour, and the rise to a maximum with a subsequent fall has been repeated 50 times in simultaneous series of experiments. If the subsequent behaviour of the descendants of several of the daughter tumours from any one batch of inoculations be followed, successive maxima are seen to arise one after another at short time-intervals. The maximum percentage of success of the experiments as a whole is maintained continuously at a high level between 70 and 90. Each strain, after reaching its maximum, falls and makes way for another which had previously presented a lower percentage, and, after attaining a maximum, in turn falls. A high percentage is thus maintained by successive maxima developing in parallel series of experiments.

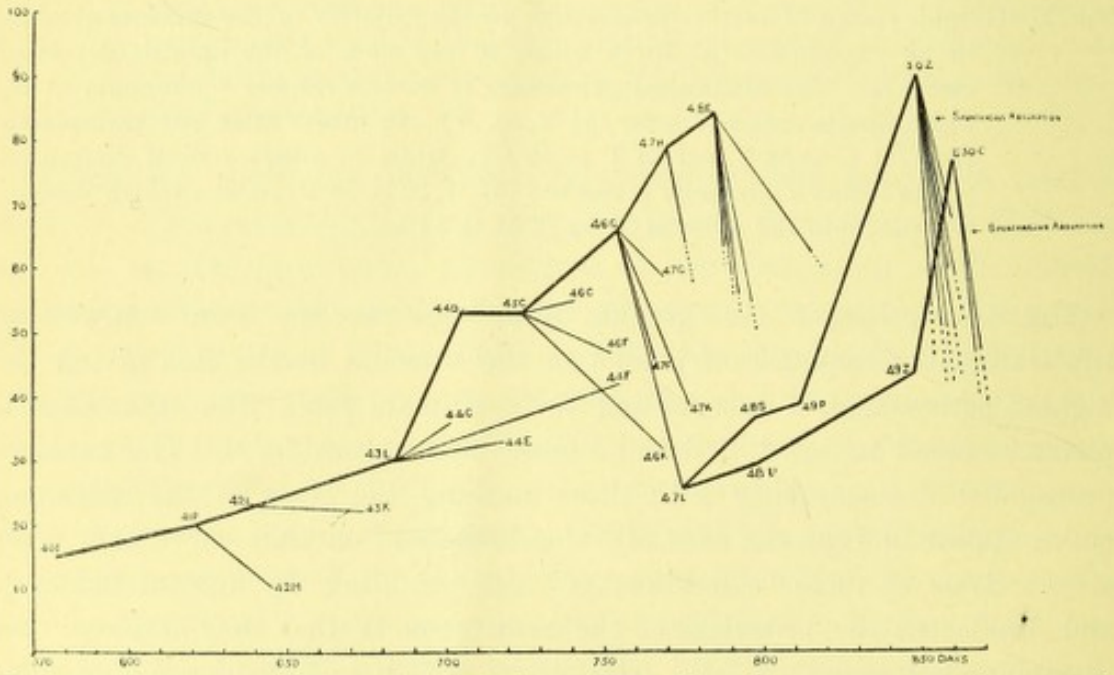


Fig. 4.—Graphic record of results of further propagation of two tumours of an experiment in which 32 per cent. of the animals developed tumours (47 L). Both of these gave an increasingly higher percentage of success till a maximum (50 Z and ii 50 C) followed by a fall was again obtained. The dotted lines are not completed to the point at which they should end, but merely indicate the downward direction of the curve. The details of the fall following the maximum 50 Z are given in the preceding graphic record fig. 3.

The preceding diagram shows clearly the manner in which successive maxima develop. The subsequent behaviour of two tumours of one of the

"descending side-branches" of the "rising main stem" depicted in the earlier chart (fig. 3) is followed through four successive subinoculations till each strain in turn presents a maximum followed by a fall. The same phenomenon is repeated in the experiments recorded in the next chart (fig. 5), where the further results of transplanting two strains derived from 48 E are represented. After giving a low percentage of success, both in turn give a maximum.

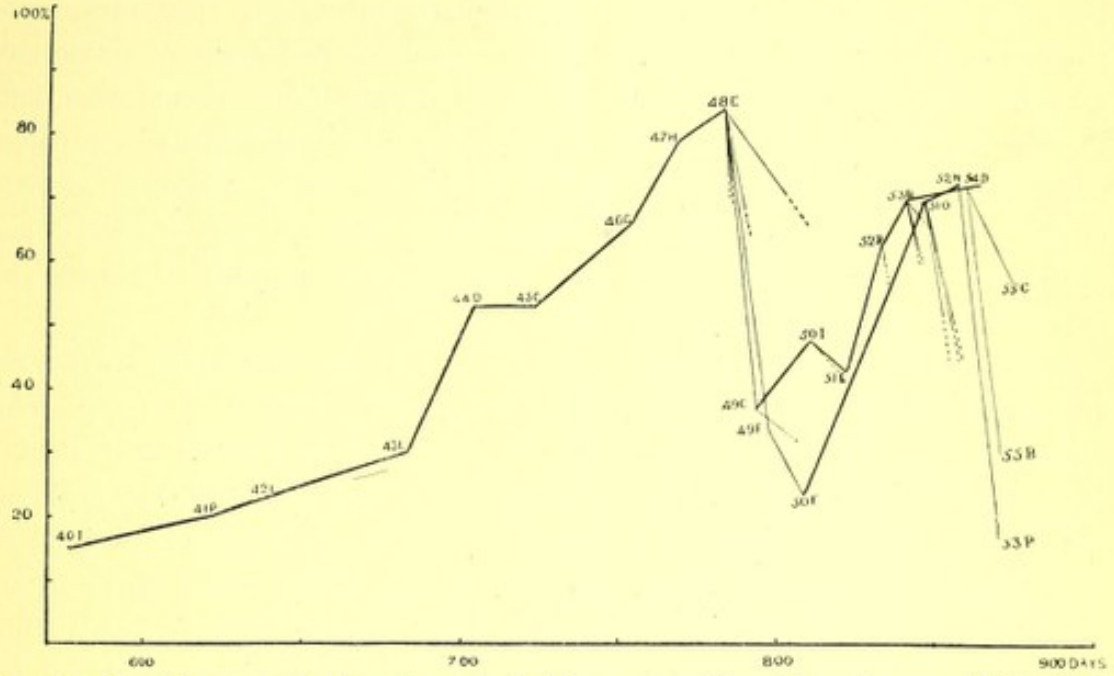


FIG. 5.—Graphic record to show how the further propagation of two tumours (49 C, 49 F) obtained in an experiment with maximal percentage of success (84 per cent. 48 E) gives at first a diminished percentage of success, which, after a varying number of transplantations (in one case six, in the other three), is succeeded by a maximal success after the same interval of time after which the fall is repeated.

When the results of all our experiments are incorporated in one chart it becomes very complex. The orderly sequence of increasing and diminishing percentage of success in individual strains can be followed with difficulty. The confusion, however, is only apparent and indicates how heterogeneous the growth of the tumours viewed as a whole has become. The behaviour of the component parts of this tumour when propagated in a large number of animals represents what may be regarded as occurring simultaneously in different parts of a single tumour, when allowed to grow for a long time in one animal. During the whole course of propagation of Jensen's tumour, after each successful transplantation, the differences in transplantability of the daughter tumours indicate that heterogeneity of this kind develops. Only when, after a number of passages, a tumour is obtained giving the

maximum percentage of success is there any approach to homogeneity in percentage of success on transplantation. After a time any single tumour cannot be regarded as consisting of cells of equal proliferative power. Just as a composite chart of all the strains propagated indicates their very different behaviour at any one date, so in any single tumour at one part growth is proceeding actively, at another growth is proceeding slowly or actually ceases. The same heterogeneity may be postulated for sporadic tumours. In all probability sporadic tumours owe their apparently continuous growth to the simultaneous presence in different areas of numerous growing centres. These mask the effects of concomitant degeneration, and account for the rarity of spontaneous absorption among sporadic as compared with transplanted tumours. The greater frequency of cessation of growth followed by spontaneous absorption in experimental tumours seems to be due to the greater homogeneity resulting from the limited number of centres of growth represented in any one implantation.

The spontaneous absorption of the whole of a transplanted tumour is rare. In the living animal it is preceded by cessation of growth. The tumour apparently remains of the same size for a period of one or two weeks. It gradually diminishes in size, and if examined histologically at this stage, the parenchyma is found to be broken up into small masses and often surrounded by a zone of large phagocytes, external to which there is an overgrowth of sclerosing connective tissue. The process is indistinguishable from what is frequently observed in circumscribed areas in large tumours, and from that which we have described with Dr. W. Cramer* as occurring when tumours disappear under the action of radium. In large tumours in which growth, the cessation of growth and the tendency to absorption show themselves side by side, large cysts are often encountered filled with serum slightly stained with blood. The relation of spontaneous absorption to a definite phase in the fluctuations in transplantability is in our experience a very close one. It occurs most frequently when a high percentage of success has been obtained, and coincides with the time when rapidly growing tumours show a great diminution in the percentage of success on transplantation.

This association with a definite phase in the fluctuations has already been indicated for two strains on chart fig. 4. It is additional evidence that the diminished transplantability is due to a real alteration in the parenchyma cells, inability to establish themselves in new animals coinciding with the spontaneous cessation of growth in an animal in which growth had already

* 'Second Scientific Report of Imperial Cancer Research Fund,' Part II, pp. 59—60.

been established. The following experiment illustrates this association in the clearest manner :—

Transplantation 50, Series iiC.*

Parent tumour. Chocolate and white coloured young male of Transplantation 49, Series Z. Attained a weight 0·65 gramme in 12 days. Very soft consistence. No necrosis. Transplantation was effected into :—

(1) 7 mice 5 days old.
(2) 6 „ 4 „
(3) 10 „ 10 „

(3) were all dead within 10 days. In the remaining 13 mice 10 tumours were evident after 10 days and grew rapidly (77 per cent.). Their subsequent history is as follows :—

No. of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.	Result of transplantation.	Result of microscopical examination.
		grammes.	grammes.			
1	10	5	0·3	No necrosis	51, iiH. No tumours in 10 mice.	Early stages in spontaneous absorption.
2	16	—	Had diminished in size.	... ...	Not transplanted. Preserved entire.	Spontaneous absorption in progress.
3	16	6·22	0·28. Had diminished in size.	Two tumours, both necrotic. Anterior tumour firm and yellowish as if undergoing absorption.	51, iiK. 1 tumour in 9 mice. 11 per cent.	Do.
4	29	7·2	4·12	Diffuse necrosis almost complete, thin layer of healthy tumour immediately subjacent to skin.	51, iiM. 10 tumours in 16 mice. 63 per cent.	
5	31	5·95	3·15	Complete necrosis. Thin layer of healthy tumour on deep surface.	51, iiN. 12 tumours in 19 mice. 63 per cent.	

The tumours, at first growing rapidly, in the other five mice ceased growing two weeks after inoculation, and, after remaining stationary for a few days, diminished rapidly in size, and had disappeared entirely 21 days after inoculation. The five animals and the three which did not develop tumours were then re-inoculated.

The tumours obtained in this experiment form a graduated series. In the

* When the series in one transplantation have arrived at the letter Z, we commence again at A and prefix the numeral "ii."

first tumour (1), inoculated after 10 days' growth, the results were completely negative, no tumours developing. A tumour (2) was preserved entire after 10 days' growth, and showed the histological features of spontaneous absorption, while another (3) transplanted on the same day with the same histological appearance gave 11 per cent. of success. Five tumours (6 to 10) of large size which were not interfered with disappeared spontaneously. Two tumours (4 and 5) continued to increase in size, in each case attaining half the weight of the mouse in which they were growing. They were almost entirely necrotic, but the healthy portions on being transplanted gave 63 per cent. of tumours in each case, both had apparently recovered from the negative phase fatal to those spontaneously absorbed and causing a negative result, or low percentage of success in those transplanted after 10 and 16 days' growth respectively. This one experiment presents all the phenomena, usually only revealed by a study of several consecutive series. The protocol should be compared with that of experiment 50 Z, given on p. 211. The close genealogical relationship of these two experiments so strongly corroborative of each other is shown in the chart, fig. 4, p. 206. If followed backwards, both are seen to arise from tumours of series 47 L, a descending "side-branch" on the "ascending stem" described on an earlier page.

From a review of the observations recorded in the preceding pages we conclude that the proliferation is only apparently continuous. In reality it is made up of a succession of alternating phases of increased and diminished energy of growth.

In the preceding pages we have concerned ourselves solely with estimates of the power of proliferation throughout a long time, although the extent to which cell degeneration goes hand in hand with cell proliferation is remarkable (see two following protocols and table of experiments on pp. 216 and 217). Growth is always accompanied by extensive degeneration of the cells of the tumours. The histological examination of all the tumours propagated has been systematically performed, and shows that, just as all the cells are not equally capable of continuing growth, so all are not histologically identical in any tumour. The histological difference most easily observed is that rapid and complete degeneration which attacks the central areas of the alveoli in which the parenchyma cells are arranged.

Transplantation 50, Series Z.

Parent tumour. Young brown female of Generation 49, Series F. Tumour weighed 5·65 grammes. Mouse alone weighed 9·3 grammes. Two tumours in medial line of back. Anterior practically completely necrotic with thin

healthy layer on deep surface adjacent to muscles. Posterior tumour practically complete necrosis with thin healthy layer under skin. Skin slight early ulceration. The posterior tumour penetrated abdominal wall, pressing on and displacing kidney.

Transplantation into 40 young normal mice, of which two died within 10 days. In the remainder 32 tumours developed as under :—

No. of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.	Result of transplanting.
		grammes.	grammes.		
1	11	10·7	1·45	No necrosis	60 per cent. 51 T.
2	12	10·3	1·3	Early diffuse necrosis ...	68 „ 51 U.
3	12	9·7	0·85	„ „ ...	12 „ 51 V.
4	14	10·0	Too small to weigh.		
5	17	14·8	1·82	Diffuse marked necrosis	13 „ 51 W.
6	18	9·8	1·1	Central necrosis.	
7	19	8·9	0·2	No necrosis.	
8	19	17·5	1·34	Early diffuse necrosis ...	45 „ 51 Y.
9	19	8·45	1·4	„ „ ...	35 „ 51 Z.
10	19	7·66	1·84	„ „ ...	21 „ 51 iiA.
11	20	11·9	0·9	Complete necrosis.	
12	20	13·2	0·3	Central necrosis.	
13	20	9·1	2·4	Necrosis almost complete, therefore difficulty in transplanting	53 „ 51 iiB.
14	20	9·05	0·95	Marked necrosis.	
15	20	19·65	1·05	Marked diffuse necrosis	22 „ 51 iiD.
16	21	10·7	1·1	Complete necrosis ulcerated.	
17	21	9·9	0·7	Diffuse necrosis.	
18	21	15·05	0·55	Practically complete necrosis.	
19	21	8·2	0·9	Early very slight necrosis	28 „ 51 iiF.
20	21	11·35	1·15	Early slight necrosis.....	25 „ 51 iiG.
21	23	10·3	1·3	Marked necrosis.	
22	25	10·1	0·5	Diffuse necrosis.	
23	25	9·0	1·0	„	7 „ 51 iiJ.
24	25	12·8	0·6	Very slight early necrosis	8 „ 51 iiI.

25 to 32.—Nine other tumours ceased growing after three weeks and were ultimately completely absorbed. The mice were then re-inoculated.

Transplantation 51, Series T.

Parent tumour. Young white female of Transplantation 50, Series Z. Tumour weighed 1·45 grammes, mouse alone weighed 10·7. Tumour situated between scapulæ, soft in consistence, very vascular, no hæmorrhage, no necrosis.

Transplanted into 40 normal young mice, of which 10 died within 10 days; in the remainder 18 tumours developed as under:—

Number of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.
		grammes.	grammes.	
1	10	11·8	0·3	No necrosis.
2	11	14·35	0·9	,,
3	14	12·75	0·8	Slight diffuse necrosis.
4	14	8·3	1·1	Very slight necrosis.
5	15	9·2	1·3	Diffuse necrosis.
6	16	6·95	1·16	No necrosis.
7	17	14·6	0·65	Central necrosis.
8	17	12·2	0·2	Slight diffuse necrosis.
9	17	11·85	2·15	Diffuse necrosis.
10	17	16·7	1·0	No necrosis.
11	17	14·8	0·7	Diffuse necrosis.
12	17	13·6	2·25	,,
13	17	13·7	0·8	Slight central necrosis.
14	17	14·2	1·85	Diffuse necrosis.
15	17	8·4	0·9	Marked necrosis.
16	17	8·45	Too small to weigh.	
17	17	15·4	,,	
18	17	13·6	,,	

Of course cells presenting complete degeneration are no longer capable of giving rise to tumours. In fact they are rapidly taken up by phagocytes in the days immediately succeeding transplantation, and it might be concluded that growth was continued by cells which never even tended to degenerate. Parts of the tumours which do not present this central necrosis are not of uniform histological structure. Fig. 6 presents a histological appearance common in these tumours (when preserved in strong Flemming solution) in the portions apparently healthy to the naked eye. Dark and clear areas are seen, the darkly stained portions which usually border on the connective tissue being due to a progressive degenerative process in the cells.

The cells which present this condition in any marked degree degenerate immediately after transplantation, while growth is mainly continued by the clear cells, and it is interesting to note that such degenerating cells form a large proportion of tumours exhibiting early phases in spontaneous absorption.

The effects of eliminating degenerating cells at each transplantation for the series 40 I to 48 E (*vide* p. 202) can be indicated by employing the percentage of success to construct a diagram of the relative proportions of implanted fragments which developed into tumours or were absorbed respectively. The percentage of success in a batch of inoculations is a test of the constitution of the parent tumour. If a series of large squares

represent the series of experiments giving a maximum at 48 E, the constitution of the parent tumours as revealed by the percentage of fragments developing into tumours can be depicted by subdividing each large square

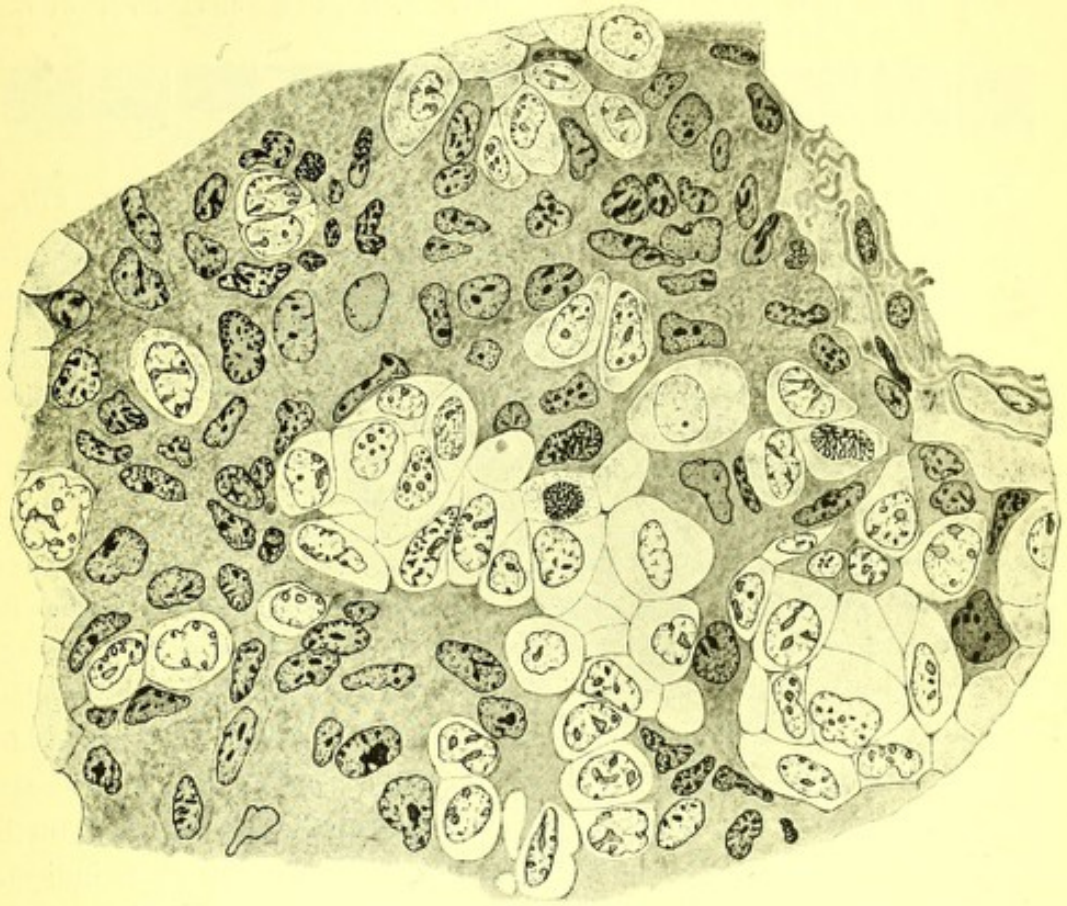


FIG. 6.—Histological differences between cells in a tumour apparently healthy and homogeneous to the naked eye. Islands of clear cells, whose nucleus and protoplasm have little affinity for stains, are surrounded by cells whose nucleus and protoplasm stain intensely. The latter are more numerous on the surface of the tumour alveoli. This differentiation is very frequent in tumours undergoing spontaneous absorption.

into 100 small squares each representing an implanted fragment, and blackening as many as there were implantations which did not yield tumours. In the accompanying diagram (fig. 7) the clear part of each large square represents the percentage of success attending the transplantation of a tumour arising from a single small square in the one before it. We may imagine that the blackened part of each square (fig. 7) represents those implanted fragments of tissue which, healthy at the time of inoculation, are already on the way to degeneration and do so degenerate immediately after transplantation. The continuous diminution in successive subinoculations which this blackened part undergoes, as the number of fragments developing into tumours

increases, would then merely indicate the elimination of degenerating tumour cells by the selection exercised at transplantation and the further elimination occurring in the days immediately following. Tumours are

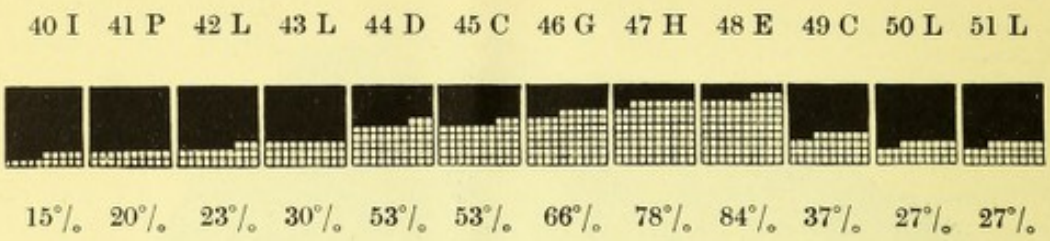


Fig. 7.—Diagram to illustrate the way in which the elimination of degenerating cells by repeated transplantation may result in a progressive increase in the percentage of success in a strain of transplantations. Each large square represents the constitution of the parent tumour of the batch of inoculations whose label is printed above it, as measured by the percentage of success printed below. One hundred inoculations are supposed to be made in every case, and the number of small squares left clear, corresponding to the percentage, shows the number of fragments which developed into tumours.

ultimately obtained free from the original admixture of such doomed cells. They consist entirely of the progeny of those healthy cells (in the first tumour of the series) which were destined to carry on growth. Even the progeny of those healthy cells ultimately enters upon a degenerative phase, as is shown by the sudden reappearance in the diagram (49 C) of a large blackened area when the clear area has attained a maximum. The increased tendency to degeneration reappears over a considerable interval, as a further reduction of the clear area in the diagram at 50 L and 51 L indicates. Thus the tendency to degenerative changes is intercalated in the course of the continued proliferation of the parenchyma cells.

Our methods of propagation and of recording the results enable us to analyse the growth of small groups of cells. So far as the descriptions of experiments published permit us to form an opinion, other investigators have emulsified single tumours, or have emulsified and mixed several tumours, and injected portions of the emulsion. This method maintains a mixture of strains at each inoculation and they have therefore recorded the results as *average* percentages of all subinoculations made after the same number of transplantations, no detailed analysis of the features of growth being attempted. Other authors, therefore, do not give the details of the behaviour of single strains, and we are unable to compare their results with our own. The increase in the percentage of success in our later transplantations as compared with the earlier ones obtained by Jensen himself must not be confounded with a permanent alteration in the character of the cells as the result of the number of transferences from animal to animal. The

highest percentage of success obtained by Jensen* is given as 66 per cent. for single experiments. We recorded in March, 1904,† success in 90 per cent. of the animals used at the third transplantation into English mice. Since then we have repeatedly obtained from 80 per cent. to 100 per cent. of success in individual strains in the manner already described. The variations in percentage of success appear to be quite irregular when recorded in tables giving either the average percentage of success for the successive series of transplantations, or the results of individual experiments in each transplantation (see Table on pp. 216 and 217). The confusion presented led us to study the percentage of success in greater detail in single strains, with the result that the irregularities resolved themselves into the orderly sequences we have described.

The experiments we have already described, and the graphic records pertaining to them, have enabled us to follow the behaviour of single strains in the direct line of descent. The phase of growth brought out by maximal success on transplantation is the same in separate strains if the fluctuations have any meaning at all. In the same way the minimal success represents the opposite phase of growth. In the accompanying graphic record (fig. 8)

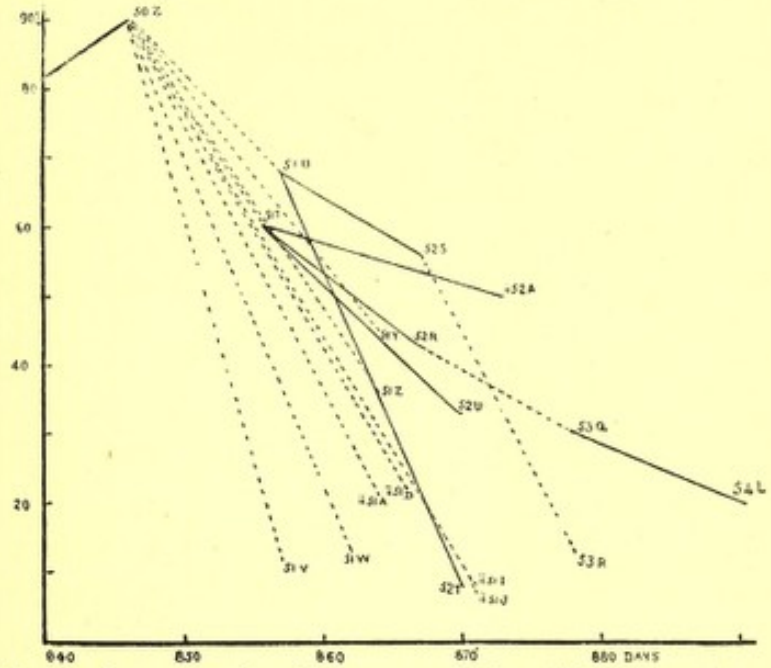


FIG. 8.—Graphic record to show that the same stage of proliferative activity is not always reached after the same number of transplantations. (Repetition of fig. 3.)

the minimum is reached after one transplantation in Experiments 51 V, 51 W, 51 iiA, 51 iiI, and 51 iiJ; after two in Experiments 52 T and 52 U;

* 'Centralblatt f. Bakt.,' vol. 34, 1903.

† 'First Scientific Report of Imperial Cancer Research Fund,' p. 14. *Cf.* also 'Second Scientific Report,' pp. 22 and 54.

after three in Experiment 53 R; and after four in Experiment 54 L. Thus the number of successive transplantations while furnishing a convenient label for experiments does not indicate corresponding stages in all the experiments. The number of times a tumour has been successively transplanted from animal to animal does not give any indication of what its future behaviour on transplantation is likely to be. That is determined

Transplantation.	Average percentage in each transplantation.	Percentage of success													
		A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.	M.	N.
23															
24	5														
25	14·3	15	30	21	7	4	9								
26	22·9	5	16	**8**	—	10	2	**41**	**19**	**36**	**47**	**18**	**12**	**25**	24
27	25·2	29	37	**90**	**17**	**14**	**29**	**25**	16	**9**	—	**17**	—	**43**	**16**
28	15·8	**11**	**10**	—	**40**	20	—	**16**	—	—	**10**	4	—	8	—
29	12·8	13	6	**18**	—	—	5	**15**	**12**	—	**20**	**10**	**17**		
30	15·6	**13**	**10**	**13**	16	**10**	—	**18**	—	**21**	**33**	**7**	—		
31	29·5	**5**	**92**	**23**	**10**	**37**	**30**	**25**	**50**	**50**	**13**	—	**11**	**13**	—
32	29·6	**28**	**44**	**40**	—	**15**	**67**	—	—	**4**	**7**	—	**32**	—	
33	17·5	25	**11**	**4**	**25**	**25**	**17**	**9**	8	—	**20**	—	**38**	4	—
34	14·6	**15**	**15**	**9**	—	**10**	**8**	—	**7**	—	**15**	5	**4**	**16**	**27**
35	19·8	**33**	10	17	**10**	10	—	—	—	22	14	20	**24**	20	31
36	23·5	37	—	—	**24**	**32**	10	**13**	30	**25**	40	40	—	31	**13**
37	24·0	**19**	**8**	**11**	21	—	—	32	31	**28**	**10**	30	45	25	**33**
38	30·0	25	—	**18**	25	100	**75**	**33**	—	—	25	—	—	17	20
39	25·8	50	29	—	—	13	**33**	**17**	9	—	—	**14**	—	—	**11**
40	28·7	63	31	33	22	—	—	—	**13**	**15**	**33**	**21**	12	—	—
41	24·8	31	29	36	**14**	17	**33**	**13**	**17**	21	—	**38**	**11**	**17**	**5**
42	21·7	—	44	29	25	—	**9**	—	**14**	—	—	—	23	**10**	**11**
43	17·8	—	—	16	20	22	**4**	—	5	—	—	**22**	**30**	—	**33**
44	38·8	27	**44**	**36**	**53**	**33**	**42**	63	13	—	—	—	—	—	—
45	32·2	29	**33**	**53**	**13**	33	—	—	—						
46	41·2	—	—	55	—	—	47	66	**46**	**55**	—	**32**	16	33	
47	48·9	23	—	72	47	—	46	59	79	75	**61**	**44**	**26**	62	**73**
48	43·7	—	30	43	**83**	84	—	47	**70**	**50**	—	60	**50**	**50**	**57**
49	40·5	28	33	37	**3**	33	**34**	50	50	29	—	**43**	**33**	—	39
50	41·1	58	**54**	71	55	—	24	**61**	**30**	48	—	—	30	14	40
51	37·5	78	—	7	60	43	43	**64**	**66**	48	40	**18**	**27**	**21**	32
52	36·2	36	63	**66**	74	38	6	33	**10**	7	**5**	**48**	**45**	**22**	**72**
53	39·6	43	70	40	26	48	—	43	38	44	53	—	**56**	**54**	**38**
54	32·2	13	—	64	72	6	11	47	47	60	—	—	20	8	6

The figures in blacker type refer to tumours which

mainly, if not entirely, by its previous behaviour as recorded in the curves, although we are not yet able to predict the immediate results of transplantation in any one case with certainty. In every transplantation series of implantations are obtained with maximal and minimal percentages of success. Series are obtained in later transplantations in which, on a large number of animals, percentages occur as low as in the earlier transplantations and occasionally negative results. They show clearly that the transplantation to which a tumour belongs does not of itself determine its

behaviour. They are as clean experiments as those with high percentage, and must be included in an objective consideration of the energy of growth of the tumour as measured by percentage of success. Recording the results by the average percentage of all the series in each transplantation therefore obscures the behaviour of individual strains and fails to reveal the composite nature of this apparently continuous proliferation.

in each transplantation.

O.	P.	Q.	R.	S.	T.	U.	V.	W.	X.	Y.	Z.	Aii.	Bii.	Cii.	Dii.	Eii.	Fii.	Gii.	Hii.	Iii.	Jii.
—	—	21	33	—	—	50															
40	18	5	28	30	20	14	7	25	25	33											
17	—	9	20	—	13	14	27	—	—	5	30										
—	25																				
—	9	—	—	33																	
3	17	7	—	—	5	52	25	24													
44	7	35	6	31	16	—	8														
32	19	8	8	14	—	35	13														
9	—	67	2	—	33	15	30	—	8												
19	37	10	14	30	24	47	—	—	—	—	33	18	33	—	—	18	33	8	—	28	
21	9	—	33	27	67	—	—	10	—	—	43	27									
40	42	4	50	18	29	15	38														
20	20	36	35	59	40	25	13	15	9	26	41										
39	18	13	9	3	—	—	—	59	20	—	6										
15	6	24																			
13																					
69	61	—	33	34	6	7	46	65													
18	—	50	—	37	67	30	32	22	36	10	71	18	12	23							
20	39	—	63	73	40	74	36	37	56	39	44										
—	15	37	57	6	59	59	15	15	37	53	90	40	14	77	19	31					
70	29	64	—	—	60	68	12	13	19	45	35	21	53	—	22	—	28	25	—	8	7
32	—	33	43	56	8	33	14	80	25	—	12	50	57	42	31	40	—	20	53	7	
47	16	30	13	11	19	63	66	—	—	10	43	8	—	—	—	59	44	80	—	33	15
—	—	—	40	—	—	25															

were necrotic on naked-eye examination.

In the above table the average percentage for each transplantation and the percentage of success for each series are tabulated in numerical and in alphabetical order. Since high percentages were obtained in early transplantations, the apparently progessive increase in the percentage of success in later ones cannot be regarded as indicating a permanent alteration in the powers of growth of the parenchyma cells as a result of the number of passages from animal to animal. The error of such an interpretation is demonstrated by a consideration of the accompanying chart (fig. 9), whicb

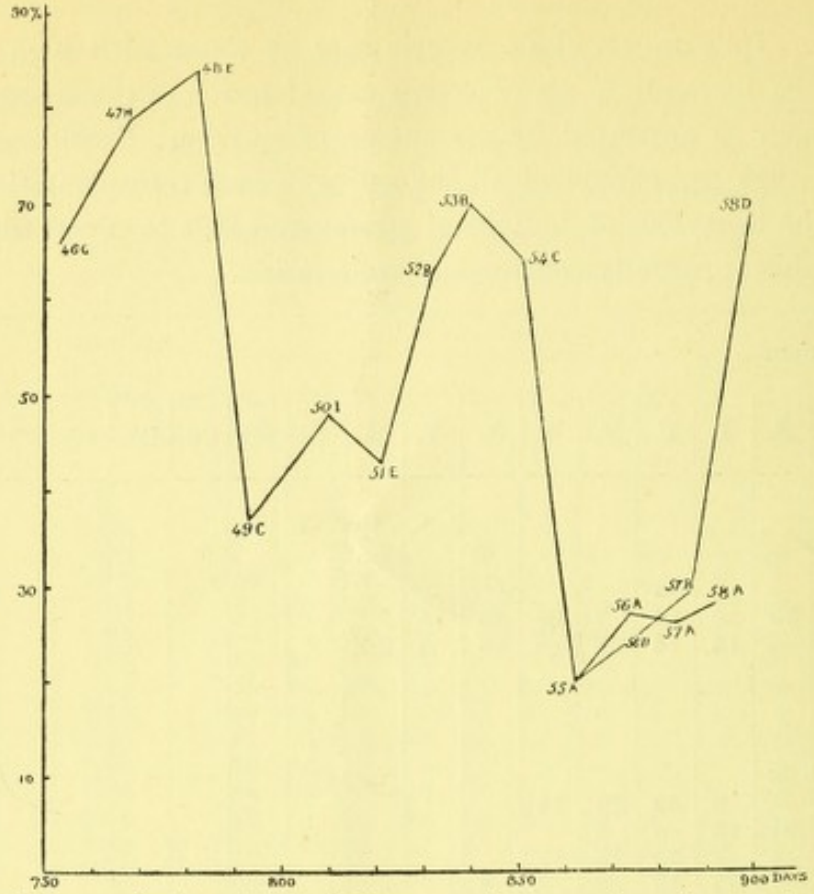


FIG. 9.—Graphic record of a strain of 13 successive transplantations at short intervals The fluctuations already described appear in this series also.

gives the results of rapid passage from animal to animal from the 46th to the 58th transplantations. This series of experiments was rendered possible by the rapid growth of certain tumours arising at each subinoculation. So far from resulting in a progressive increase in transplantability, the 55th transplantation presents the lowest percentage in the whole series. The fluctuations already described for other strains are present here also, when the interval between successive subinoculations is shortened to intervals of 8 to 15 days. Such a curve, representing a succession of rapid transplantations, is a special case like those depicted in the curves* we have already published.

In the preceding pages it is assumed (1) that the conditions have been sufficiently uniform throughout the experiments to exclude fortuitous fluctuations, and (2) that the percentage of success on transplantation furnishes a reliable measure of power of proliferation. If the precautions we have taken warrant these two assumptions, we are entitled to conclude that the fluctuations in proliferative power revealed are natural features of the growth of Jensen's tumour in English mice. They are due to the

* 'Second Scientific Report of Imperial Cancer Research Fund,' Part II, p. 54.

acquisition of renewed powers of growth by the cells when proliferation is becoming exhausted and may actually terminate, resulting in the spontaneous absorption of tumours which had established themselves and grown for a time.

From time to time sporadic mammary tumours have occurred in the mice purchased for these investigations, and with all artificial propagation has been attempted. The resulting proliferation has in no case been equal to that obtained with Jensen's tumour. Thus out of 20 sporadic tumours transplanted the primary implantations have been negative in 9. We select for detailed description the features of the proliferation resulting from the propagation of two sporadic tumours (namely, VII and XIX) which exemplify the behaviour of tumours capable of only limited propagation Both could be transplanted several times from animal to animal. The first was transplanted into 133 mice. One tumour developed in the 12 mice remaining alive after 10 days. It was transplanted after 20 days' growth into 24 mice. One tumour developed in the four mice which remained alive after 10 days. It grew slowly, attaining a diameter of 2 cm. after 184 days' growth, when it in turn was transplanted into 208 mice. Two tumours developed in the 80 mice which survived the first 10 days. They were transplanted after 48 and 66 days' growth respectively. No tumours developed in either case, and the experiment came to an end. The accompanying graphic record (fig. 10) shows the contrast which obtains

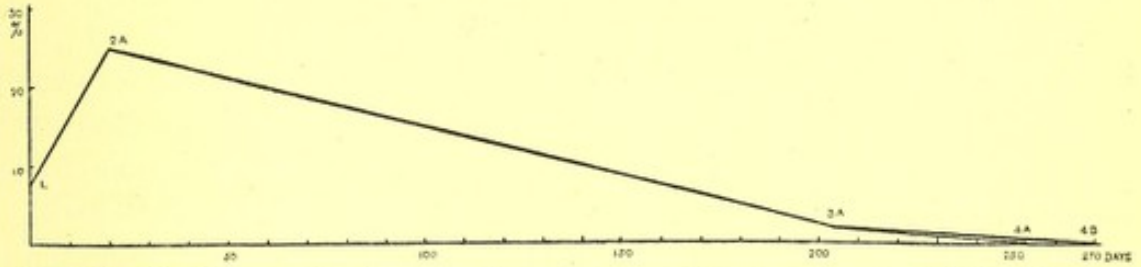


FIG. 10.—Graphic record of propagation of a sporadic mouse tumour VII. Shows a temporary rise (?) in transplantability and extinction of the tumour at the fourth transplantation.

between the artificial propagation of such a tumour and that of Jensen. Another sporadic mammary tumour (XIX) was removed by operation and transplanted in 85 mice. Fifteen tumours developed in 75 mice remaining alive after 10 days. The accompanying graphic record (fig. 11) shows the results of the transplantations.

The ultimate fate of the propagated tumours in these two cases was the same. The interest of the second case lies in the fact that the sporadic tumour recurred rapidly after operation, which was rendered necessary on

three subsequent occasions. The tissue obtained at these operations was transplanted under the same experimental conditions. From the implantations after the first operation fifteen tumours developed in the 75 mice remaining alive 10 days after implantation. Nine of these tumours were used for

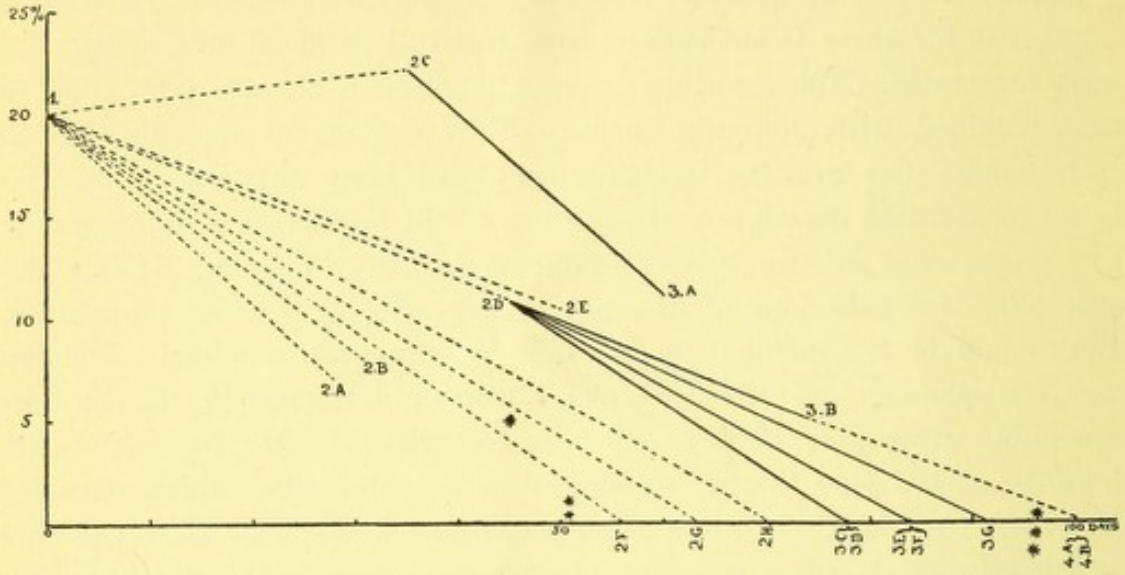


Fig. 11.—Graphic record of propagation of a sporadic mouse tumour XIX. Commencing at 20 per cent. the transplantability gradually diminished through three successive transplantations till negative results were obtained. The tumour was removed by operation in the first instance, and recurred three times. The results of transplanting the material obtained at these three later operations are indicated also on the chart at * 5 per cent., ** 0 per cent., and *** 0 per cent.

transplantation. The other six grew for a time, attaining an approximate weight of 0·5 gramme, then remained stationary, and were ultimately completely absorbed. The histological features of spontaneous absorption were identical with those already alluded to. One tumour developed in the 50 mice alive 10 days after inoculation with the material from the second operation. Negative results were obtained with material from the other two operations, 81 implantations having been made in each instance. When the mouse died three months after the first operation the left pleura and right lung were found filled with metastatic deposits. Thus growth proceeded in the animal primarily affected, and at the same time ceased in the animals inoculated succesfully, either after a transitory proliferation in one animal or in succeeding transplantations. Portions of the growth removed at subsequent operations did not exhibit the same proliferative energy, when transplanted, as those obtained at the first operation. We wish to draw attention to the similarity of behaviour of this sporadic growth at different times with the behaviour of single strains of Jensen's tumour in which cessation of growth

(*cf.* fig. 12) and spontaneous absorption (*cf.* figs. 3 and 4) supervene on a high degree of transplantability and when negative results are obtained either immediately, or by graduated steps, when a tumour of a series giving a high percentage is transplanted.

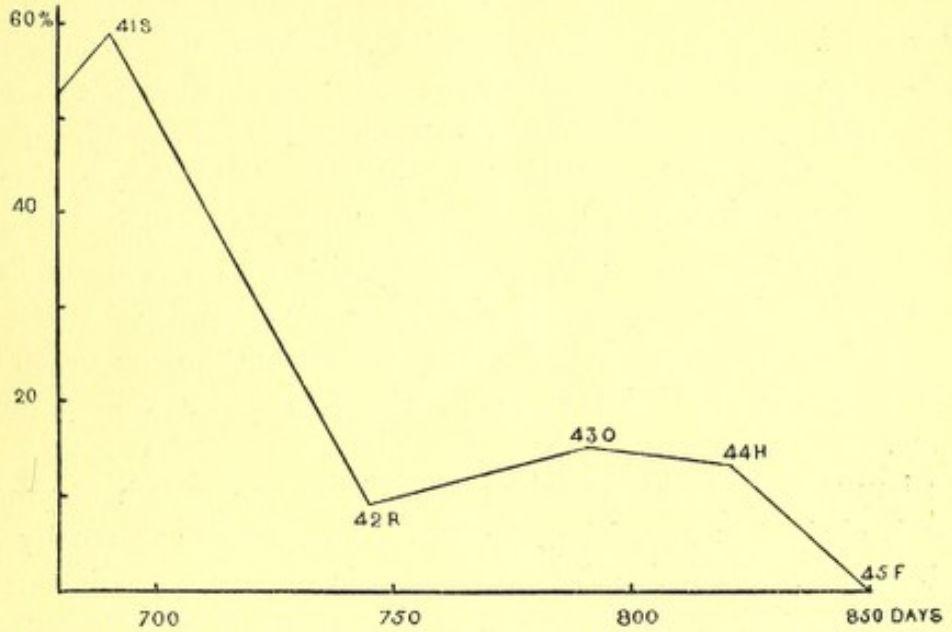


FIG. 12.—Graphic record of propagation of a strain of Jensen's tumour which gradually gave a lower and lower percentage of success till a negative result terminated growth. *Cf.* fig. 10 and fig. 11.

Many tumours of the mouse's mamma give negative results on transplantation, and in this respect resemble the tumours of the other longer-lived mammals. Of those, in which the primary transplantation is successful, the later results often show a gradually diminishing percentage of success till, finally, negative results are obtained, on transplantation. The enormous proliferation obtained with Jensen's tumour is exceptional. Growths, undoubtedly malignant, are not necessarily equally capable of artificial propagation. As our experience of malignant new growths in mice widens, the power which small fragments of tumour possess of establishing themselves in new hosts on successful implantation is found to be rarer than might be expected from the frequency of metastasis formation, to which it is closely related. The factor or the factors actively responsible for the development, the continued growth, and the formation of metastases of the different sporadic tumours in the animals primarily affected are not equally efficacious in ensuring a continuance of proliferation under the similar experimental conditions of artificial propagation. We must therefore conclude that the causative factors have operated with varying intensity, or that additional factors are superadded in some cases.

The behaviour of some strains of Jensen's tumour present a parallel to the other tumours now under consideration. We have already alluded to the negative results sometimes obtained on transplanting Jensen's tumour. In the graphic record (fig. 12) the steps are shown by which a strain of Jensen's tumour, at first giving a high percentage of success, progressively exhibits weaker and weaker powers of proliferation, till finally the tumours obtained gave negative results on transplantation. Such strains are not uncommon; they have frequently been followed to a finish during our experiments. Thus one chapter, as it were, in the life history of Jensen's tumour reproduces the entire life history of other tumours under artificial propagation. These results are difficult to harmonise with the assumption that the apparently continuous proliferation of Jensen's tumour is purely vegetative. Together with the facts of spontaneous absorption they strengthen the conclusion derived from a study of the details of that proliferation, that a cyclical process is involved.

The importance of the preceding analysis of the growth of propagated cancer is obvious in appraising the results of attempts to modify growth experimentally. The experimental conditions whose variations cause irregularities in the success of artificial propagation must be taken account of. In particular, the age of the animals would seem to call for especial attention, because the short duration of the life of a mouse magnifies the effect of the lapse of time involved in procedures for inducing immunity. Specially adapted control experiments must be performed in order to obviate the fallacy which the ageing of the animals introduces. Those fluctuations which cannot be referred to the experimental conditions but are natural features of the proliferation of the tumour cells are an even more urgent reason for caution in interpreting the results of therapeutical experiments. The difficulty or even impossibility of predicting the time at which spontaneous absorption will affect the propagated tumours indicates the necessity for accurate records of their previous history.

In another paper with Dr. Cramer we shall discuss the results we have obtained on re-inoculating mice in which the absorption of well-established tumours had occurred spontaneously and under the action of radium.

Harrison and Sons, Printers in Ordinary to His Majesty, St. Martin's Lane.