CHANGES IN THE REACTION POTENTIAL OF A TRANS-PLANTABLE TUMOR.*

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PLATE 27.

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INTRODUCTION.

In the present paper a tumor will be described which gave very uniform results in a series of mice of known pedigree for a period of about a year. We may say that the reaction potential existing between the tumor and the individuals inoculated was constant. In the course of routine transplantation of the tumor, there was a sudden change in this hitherto constant reaction potential resulting in at least three new types of transplantable tumors—new types as far as their proliferative vigor and reaction potential (transplantability) are concerned.

It is the purpose of this paper to discuss the bearing of the phenomenon of the change in the reaction potential of the transplantable tumor on the so called genetic theory of transplantation, together with some remarks concerning the problem of tissue specificity and allied topics.

The Tumor.

The tumor which has been employed in the present investigation is the fourth spontaneous neoplasm to arise in a relatively homogeneous dilute brown strain of mice which has been rigidly inbred brother to sister for at least 40 generations. The other three tumors, which were designated dBrA, dBrB, and dBrC, were subjects of extensive experimental studies to determine the influence of the genetic make-up of the tumor and of the host on the fate of transplants.^{1,2} It was found

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¹ Little, C. C., and Strong, L. C., *J. Exp. Zool.*, 1924-25, xli, 93.

² Strong, L. C., *Genetics*, in press.

that all animals of the dilute brown strain were uniformly susceptible to all three tumors. On the other hand, another inbred strain, known as the Bagg albino strain, was found to be uniformly resistant to the same transplantable tissue. Further analysis showed that the first filial generation of mice resulting from crossing the original dilute brown strain with the Bagg albinos was uniformly susceptible to all three tumors. In the second filial generation, two classes of individuals were obtained, the relative percentage of susceptible to non-susceptible individuals being different in each of the three neoplastic tissues employed. These findings were interpreted as indicating that Mendelian factors were involved in the transplantation of malignant tissue.

From these experiments we developed the so called genetic theory of transplantation, which may be stated as follows: susceptibility to a transplantable neoplastic tissue is brought about by a reaction between the host and the tumor cell. The reaction of the host is governed largely by its genetic constitution (derived from the zygote from which it arose) and that of the transplant is somewhat controlled by certain intrinsic or genetic factors.¹

The new tumor arising in an individual of the same dilute brown strain of mice we have designated dBrD, in order to continue the nomenclature previously used. It is a mammary gland carcinoma. A photomicrograph of the tumor is given in Fig. 1.

EXPERIMENTAL.

Experiment 1.—The first experiment consisted in the inoculation of the new tumor, dBrD, into individuals of the inbred dilute brown strain. It was found that they were uniformly susceptible to the transplant (94.00 ± 0.00 — or 100.00 per cent susceptible). At the same time another series of individuals belonging to the Bagg albino strain was inoculated with some of the same transplantable tumor. It was determined that these individuals were uniformly resistant (0.00 ± 104.00 — or 0.00 per cent susceptible). The F_1 generation produced by crossing these two stocks gave individuals all of which grew the transplant (125.00 ± 0.00 — or 100.00 per cent susceptible). It is evident therefore that the new dBrD tumor gave very similar results to the three previous tumors of the same series, dBrA, dBrB, and dBrC. At the present time 112 individuals from the F_2 generation have been inoculated with the transplantable tumor, dBrD, and 16 of these grew the transplant progressively, while 96 proved to be resistant to the same tissue.

In attempting to fit the observed data to Mendelian principles as has already been so successfully done in the case of five transplantable tumors (two by Tyzzer and Little, two by Little and Strong, and one by Strong), it is evident that the behavior of the transplant placed it in the

same class as the three other transplantable tumors derived from the same inbred dilute brown stock of mice. The results can be explained if the assumption be made that for the successful growth of the transplant there must be present within the host at least from six to eight independently inherited genetic units or factors (genes). It is probable that the number of genetic factors is seven. The degree of probability determined by the comparison of the probable error between observation and expectation for six, seven, and eight factors is greater in the case of seven factors than it is for the other two possibilities although the six and the eight factor interpretations are not definitely excluded by the present data.

The degree of significance between the observation on the original dBrD tumor and the expectation in the F_2 generation according to Mendelian principles is given in Table I.

After the collection of the above data, no further genetic studies being contemplated, the tumor was simply continued for some generations in individuals of the F_2 generation. In the course of this routine continuation of the tumor, it was found that one of the transplants grew with remarkable rapidity. In subtransplants from this particular tumor the rapidity of growth continued. This phenomenon had already been encountered in the case of the dBrC tumor.² I had also already determined that the average rate at which a transplant grew within a given constant genetic strain of mice was an index of its intrinsic or genetic make-up. I consequently continued the transplantation of this aberrant type. Two other subtransplants were observed that apparently had deviated from the normal growth rate of the original dBrD tumor. These transplants were also continued.

Experiment 2.—The second experiment consisted in the inoculation of the first aberrant tissue, which received the name dBrDm, into a number of F_2 individuals. It was found that of the 99 individuals inoculated, 56 grew the transplant progressively while 43 proved to be resistant to the same tissue. The data are tabulated in equation 5, Table II.

Experiment 3.—Another series of F_2 individuals was inoculated with the second aberrant tissue, dBrDBI. 122 individuals of this generation were inoculated. Of these, 66 were susceptible and 56 were negative. The data for this neoplastic tissue are given in equation 9, Table II.

Experiment 4.—The third tissue, dBrDBs, was inoculated into a number of F_2 individuals. In this case 124 individuals were employed. 88 of these were sus-

TABLE I.
Comparison of the Observed Data for the F₂ Generation Together with the Expectation for the Six, Seven, and Eight Mendelian Factors. Original dBrD Tumor.

	F ₂ generation.	No. of individuals.	Per cent negatives.	Difference between.
1	Observation.	16.00+;96.00-±2.47	85.71%±2.20	1 and 2 = 3.57%±3.26 or 1.09 × P.E.
2	Expectation 6 factors.	19.96+;92.04-±2.70	82.14%±2.41	1 " 3 = 0.90%±3.11 " 0.03 × P.E.
3	" 7 "	14.95+;97.04-±2.40	86.61%±2.18	1 " 4 = 5.18%±2.85 " 1.81 × P.E.
4	" 8 "	10.20+;101.80-±2.03	90.89%±1.82	

TABLE II.
The Data Obtained for the Three Transplantable Tumors, dBrDm, dBrDBI, and dBrDBs, in the F₂ Generation Together with the Comparison for Similar Data Obtained with the Original dBrD Tumor.

Experiment No.	Name of tumor.	Equation.	F ₂ generation.	No. of individuals.	Per cent negatives.	Difference between equations.
1	Original dBrD	1	Observation.	16.00+; 96.00- ±2.47	85.71% ±2.20	
2	dBrDm	5	"	56.00+; 43.00- ±3.29	43.43% ±3.32	1 and 5 = 42.28% ±3.98 or 10.62 × PE.
		6	Expectation 1 factor.	74.25+; 24.75- ±2.91	25.00% ±2.94	5 " 6 = 18.43% ±4.52 " 4.07 × PE.
		7	" 2 factors.	56.68+; 43.31- ±3.28	43.75% ±3.31	5 " 7 = 0.32% ±4.88 " 0.09 × PE.
		8	" 3 "	41.77+; 57.23- ±3.27	57.80% ±2.88	5 " 8 = 14.37% ±3.80 " 3.78 × PE.
3	dBrDBI	9	Observation.	66.00+; 56.00- ±3.66	45.90% ±3.00	1 " 9 = 39.81% ±3.72 " 10.70 × PE.
		10	Expectation 1 factor.	91.50+; 30.50- ±3.19	25.00% ±2.61	9 " 10 = 20.90% ±3.97 " 5.01 × PE.
		11	" 2 factors.	68.63+; 53.37- ±3.65	43.75% ±2.99	9 " 11 = 2.15% ±4.24 " 0.51 × PE.
		12	" 3 "	51.48+; 70.52- ±3.63	57.80% ±2.98	9 " 12 = 11.90% ±4.22 " 2.82 × PE.
4	dBrDBs	13	Observation.	88.00+; 36.00- ±3.03	29.03% ±2.44	1 " 13 = 56.68% ±3.28 " 17.28 × PE.
		14	Expectation 1 factor.	93.00+; 31.00- ±3.21	25.00% ±2.42	13 " 14 = 4.03% ±3.43 " 1.17 × PE.
		15	" 2 factors.	69.75+; 54.25- ±3.71	43.75% ±3.00	13 " 15 = 14.72% ±3.87 " 3.80 × PE.

ceptible while 36 failed to show progressively growing masses. These data are tabulated in equation 13 of Table II.

It may be well to point out, however, that some of the F_2 individuals were inoculated simultaneously with two of the tissues, so as to compare the tissues in the same soil. When this was done, one of the tumors was placed subcutaneously in the right axilla, while the other one was placed in the left axilla.

The data obtained in Experiments 2, 3, and 4 for these new transplantable tumors, in the F_2 generation, together with the comparison for similar data obtained with the original dBrD tumor (Experiment 1), are given in Table II. The degree of significance, based upon the calculation of the probable error, is also given. For every tumor, the data are given for the estimated nearest Mendelian interpretation or expectation.

The original dBrD tumor failed to grow in 85.71 per cent of the second filial generation individuals. It may be said, therefore, that the tumor is highly specific, or that it possesses a high degree of tissue specificity. Very few F_2 individuals have the necessary make-up for the continued growth of the transplant. From this high state of specificity, there split off during the course of the present experiment new tissues that gave different degrees of specificity (transplantability) in the same series of F_2 individuals; one subtransplant, dBrDm, growing in all but 43.43 per cent of the individuals; another, dBrDBI, giving 45.90 per cent negative reactions; and a third, dBrDBs, growing in all but 29.03 per cent of the F_2 individuals. The degree of specificity for two of the subtransplants, dBrDm 43.43 per cent and dBrDBI 45.90 per cent, is about the same. Experiments are now under way to ascertain if this result is determined by the same genetic background or whether a similar genetic complex is involved.

The degree of probability of difference between the original dBrD and the three subtransplants is significant in every case; that degree of probability difference between the first subtransplant dBrDm and the original dBrD tumor is 10.62 times the probable error; the degree of probability difference between the second subtransplant dBrDBI and the original dBrD tumor is 10.70 times the probable error; while in the case of the third subtransplant, dBrDBs, there is 17.28 times the probable error difference.

Obviously there must have been clear-cut variations from the type reaction which had been investigated for over a year. From a state of high specificity, the transplantable tumor has deviated into other types showing different degrees of specificity. Correlated with this change of tissue specificity there was also obtained an increased growth or proliferative vigor. The data on the growth rates are reserved for publication in the near future.

Photomicrographs of tumors dBrDm and dBrDBs are given in Figs. 2 and 3.

SUMMARY.

A transplantable tumor, known as dBrD, gave very uniform results in a series of mice of known pedigree for a period of about a year.

TABLE III.

Tabulated Synopsis of the Various Transplantable Tumors Used in the Present Experiment, Showing the Probable Number of Independently Segregating Genetic Factors Involved in the Process of Transplantation.

Name of tumor.	No. of genetic factors.	No. of F ₁ individuals used.
Original dBrD.....	7	126
dBrDm.....	2	99
dBrDBl.....	2	122
dBrDBs.....	1	124

We may say that the reaction potential existing between the tumor and individuals inoculated was the same during this first part of the experiment. This tumor will grow in a mouse progressively provided there be present simultaneously at least from six to eight independently segregating genetic factors (genes) in the fundamental make-up of the host derived from the zygote that gave rise to the individual. In the course of routine transplantation of this tumor, there was a sudden change in this hitherto constant reaction potential resulting in at least three types of transplantable tumors—new types as far as their physiological activity and reaction potential are concerned. From a single original transplantable mass, I have therefore obtained four masses that remain true to type at least for some months of continued experimentation. These four types are (1) the original tumor,

dBrD, still giving a six to eight Mendelian factor ratio, (2) dBrDm giving a two factor ratio, (3) dBrDBI showing another two factor ratio, and (4) dBrDBs giving a one factor ratio. The number of factors involved in the transplantability for the several tumors investigated are given in Table III.

GENERAL DISCUSSION.

It is probable that neither the theory of adaptation nor the theory of virulence will explain completely the data outlined in the present paper. These theories are descriptive phrases applied to the tumor mass and apparently enter into the equation after the change or changes have been produced; that is, they are the result of the change and not the cause of the phenomenon.

If the genetic theory for the transplantation of malignant tissue is valid, then it ought to demonstrate a mechanism whereby such a change in the reaction potential of the transplantable tumor, as described in this paper, may be brought about. The change, whatever it is, must of course be either in the host component or in a possible tumor variable. Let us consider first the host. Obviously, if the host's tolerance of the transplantable tumor is dependent to a large extent at least upon its genetic constitution, when a change occurs in the genetic constitution of the host then this change of necessity would alter the reaction potential under consideration. Changes in the genetic constitution of the mice employed, however, cannot explain the present data. The second filial generation individuals which gave the indication of the genetic complex involved, as far as the host was concerned, had been picked out at random and inoculated with one type of transplantable tumor or another. The parents were the same for all the F_2 's, while the number of susceptible grandparents was very small. Sometimes a single mouse was inoculated with two different types of tumors simultaneously. The genetic constitution of the mouse, as far as this experiment on testing its physiological response to two different types of tumors was concerned could not possibly have been different. For these reasons, the host complex during this experiment must have been a constant one, or at least, has not influenced the observed change in the reaction potential.

Several suggestions arise as to how this change in the reaction poten-

tial may be brought about by a change in the tumor component. The most obvious one is that perhaps a new neoplasm has developed out of the host tissue in contact with the malignant tissue inoculated, either as a matter of chance or as a result of some influence exerted by the transplant upon the host's cells in that locality. There are several reasons that appear to throw considerable doubt on this conception. First, the histological appearance of all the subtransplants is very nearly the same as that of the original tissue (Figs. 1 to 3). Second, the F_2 generation is one in which the range of variability among the individuals is enormous. It is extremely difficult to get a spontaneous tumor from any F_2 individual to grow in any other individual. Yet in this experiment the subtransplants grew in an increased number of F_2 individuals. (Spontaneous tumors arising in F_2 individuals have been found to be, without exception, highly specific, whereas the subtransplants encountered in this experiment show low specificities.) Third, all individuals in this experiment were inoculated at 6 weeks of age. The incidence of spontaneous carcinoma in individuals of this very immature age is certainly of rare occurrence. Fourth, the change from high specificity toward a lower degree of specificity is a phenomenon of rather frequent occurrence. I have, however, never encountered any change in the opposite direction, although I have watched and devised experiments to detect such a change if it ever occurred. For these four reasons, primarily, the probability of a new spontaneous tumor arising in the inoculated individual is extremely slight.

Another possibility is that by some fortunate circumstance the tumor was a mixed tumor to begin with, that is that the mass was made up of a mixed aggregate of cells of differing potentialities—the less malignant strains were killed off to the benefit of the more malignant ones. There appear to be pretty good grounds for considering the majority of spontaneous tumors as mixtures, and transplanting them to many diverse soils cannot fail to result in selection. By continued transplantation the less adaptable tissue is weeded out and the tumor gains “virulence” and after a time tends to a more fixed type. This suggestion is tenable but is not satisfactory. Since all the derived transplants were made up of histologically indistinguishable cells, one would have to assume a mixture of at least four histologically identical

cells all possessing different physiological characteristics. The nature and origin of such a mass could only be conjectured—not demonstrated nor proven. There are several difficulties in the way of accepting this interpretation. First, the phenomena of the change in the reaction potential has been encountered several times. In fact I have witnessed it in seven of the nine neoplastic tissues derived from individuals of this inbred dilute brown strain of mice. In every case, this breaking down in the tissue specificity of the tumor mass has occurred after the mass had settled down to a *constant* type for at least several months. Second, at every inoculation the tumor was pulped and pushed through a small trochar into the mice inoculated. The tumor is rather a soft one. There is therefore only a slight possibility of all *four* types of cells clinging together at every inoculation for more than a year—and then all separating from each other within a period of 3 weeks. The number of neoplastic cells that survive after transplantation is certainly very limited, thus militating against the persistence of four distinct types of cells during the extent of the first part of the experiment in which the reaction potential of the mass remained constant. Third, the proliferative vigor of the various types of cells differed considerably. The subtransplants showing a low degree of tissue specificity possess several times as much proliferative vigor as those showing high specificity. The slow growing cells would consequently have been completely swamped in a period of a year. Both Ehrlich³ and Apolant⁴ determined that “synthetic” mixed tumors could *not* be produced by the intermingling of two distinct types of neoplastic tissues, provided the vitality and proliferative energy of the two components differed to any marked degree.

For these several reasons, the probability is that the tumor was not a physiologically mixed tumor at least at the time of the occurrence of the change of the reaction potential, although this conception is not absolutely ruled out by the present data.

Another conception is that this change of the reaction potential existing between the tumor and the host inoculated is brought about by an internal change in the genetic constitution of the tumor cell. This hypothesis of course implies that the tumor cell is controlled to

³ Ehrlich, *Z. Krebsforsch.*, 1907, v, 67.

⁴ Apolant, H., *Z. Krebsforsch.*, 1908, vi, 251.

some extent at least by an internal genetic or biological constitution. Changes within the genetic constitution of living forms, if they are germ cells, are called mutations; if they are somatic cells (occurring especially in plant tissues) the changes are called somatic mutations. The conclusion is reached that somatic mutations may occur within the tumor cell, and that when these mutations do occur, they change the reaction potential and other physiological activities of the tumor cell. The data at hand are not sufficient to determine more in detail the nature of this mutational process. It may be either a change or shifting of a complete chromosome or chromosomes, or a change or changes within a chromosome or chromosomes (genic), or it may be even cytoplasmic in nature. By mutation I merely mean to use the term in its broadest sense; that is, a change or shift within the genetic or biological constitution that results in definitely clear-cut or discernible differences in behavior or structure that are perpetuated by the process of heredity (in this case cell division). It may be well to state however that the data at hand do not necessarily prove the presence of mutation. The conception of mutation does explain the phenomenon at hand—the other suggested explanations are certainly less probable—consequently it is justifiable to accept the conception of mutation in form of hypothesis.

We have, therefore, in the analysis of the data obtained in this experiment, an explanation of the phenomenon of the so called adaptiveness or virulence of the tumor cell. If the genetic change such as a somatic mutation is responsible for the change in the reaction potential of the transplantable tumor cell and other physiological activities, such as the proliferative vigor, then it is highly probable that the real cause of the phenomenon has been determined. A tumor increases its proliferative vigor or manifests an increased adaptiveness as a result of this mutational process. This somatic mutation may occur within a single cell in the neoplastic tissue. Then by the process of selective vitality or adaptiveness of the cells in the mass, the new type of behavior is obtained.

One other point I desire to mention. If one assumes that the spontaneous tumor possesses the same genetic constitution as the host tissue that gave rise to it (and we have every reason to suppose that it does have, since the tumor has arisen by some process or other from

cells derived from the body of the normal individual), then we must conclude, from our present data, that a tumor may deviate from the genetic constitution of the host tissue from which it arose, at least during the process of transplantation.

CONCLUSIONS.

1. Mutations or hereditary genetic changes may occur within the tumor cell at least during the process of transplantation.
2. These mutations may explain the phenomenon of the change in the reaction potential and deviations in the proliferative vigor of the tumor cell during the process of transplantation.
3. The nature of this mutational process is as yet undetermined.
4. The phenomenon of tissue specificity is determined to a large extent at least by the genetic constitution of the neoplastic tissue.
5. The tumor mass may deviate from the genetic constitution of the host tissue that gave rise to it, at least during the process of transplantation.

EXPLANATION OF PLATE 27.

FIG. 1. Photomicrograph of the original tumor, dBrD, used in Experiment 1. The tumor gave a 5.7 Mendelian factor ratio when inoculated into a series of F_2 individuals.

FIG. 2. A tumor transplant (dBrDm) derived from the original tumor, dBrD, given in Fig. 1. dBrDm gave a 2 factor ratio when inoculated into the same series of F_2 individuals used in Experiment 1 (Experiment 2).

FIG. 3. Another tumor transplant (dBrDBs) derived also from the original tumor, dBrD, during the process of transplantation. This tumor shows a 1 factor ratio when inoculated into a similar series of F_2 individuals (Experiment 4).

(Strong: Reaction potential of transplantable tumor.)