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Tests for physiological differences in transplantable tumors.

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The purpose of this paper is to present a method whereby two neoplasms, that are histologically identical, can be shown, nevertheless, to be different in their physiological reactions. This experiment was begun June, 1920, and is still being continued. Enough data have been accumulated, however, to warrant a preliminary report.

The tumors employed are two adenocarcinomas of the mammary gland, that arose spontaneously in two female mice of a closely inbred strain, the second one arising some three weeks after the first. The mice have been rigidly inbred, brother to sister matings, for about eleven years. One would expect, in that time, that the strain must have become homozygous in all, or nearly all, genetic factors that no doubt underlie morphological and physiological characters. This conclusion is warranted by evidence obtained from implanting bits of the two tumors into mice of this strain. The trochar method of implanting the neoplastic tissue has been employed throughout the experiment. In this preliminary experiment both tumors grew in 100 per cent. of all mice inoculated, irrespective of whether the two tumors were inoculated into the same mouse or into separate individuals. In case the two were inoculated into the same animal, the first (dBrA) was always inoculated into the right axilla, the second (dBrB) into the left axilla. The growth curves for each were charted from weekly observations. There was apparently no effect of one tumor upon the other when growing in the same mouse, either in percentage of indications or rate of growth. They remained entirely distinct. The question naturally arose, "Are the two tumors actually identical?"

The complexity of the genetic factor system that evidently underlies susceptibility to transplantable tumors makes it highly improbable that two *identical* tumors could arise independently within three weeks of each other, even in the same relatively homozygous strain of mice.

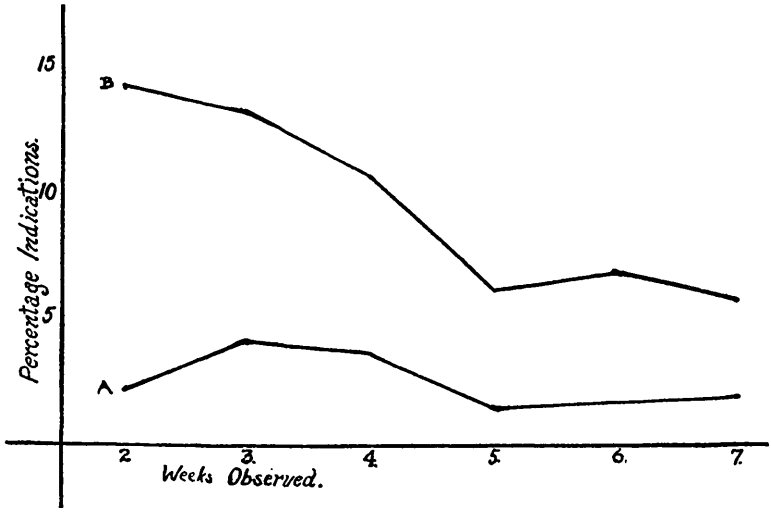
With the hope of discovering a strain of mice that would be susceptible to the dBrA tumor and not to the dBrB, our first choice was the wild house mouse, collected in several localities near the laboratory at Cold Spring Harbor, N. Y.

The first and foremost consideration in an experiment with transplantable tumors is the selection of the strain of mice to be used. Race has been recognized by many investigators in this field as a factor underlying tumor susceptibility and yet it is the one most often ignored. Several investigators have explained their fluctuating results as due to variations in the tumor cell whereas the same results can be explained by assuming fluctuations, although slight, in the strain of mice employed. This last explanation, certain to apply in most cases, is the only one acceptable to present-day geneticists without positive histological proof of tumor-cell modifiability. Racial homozygosity can only be obtained by rigid inbreeding, brother to sister matings, for several generations. The time element necessary to produce mammals genetically and biologically uniform is therefore too great for the patience and resources of most investigators.

As before stated the wild house mouse has been used in this experiment. If collected in the same locality one can be sure of the stock for several reasons (1) mice very seldom migrate, they usually remain in the same building and no doubt sometimes breed in the same nest in which they were born. This is evidenced by the fact that slight variations in coat color tend to be restricted to the same corner of a building, etc. (2) The fact that relatively fewer adult males than females are present, thus necessitating close inbreeding. This inequality of the sexes is brought about possibly by conflicts between individuals of the male sex. Dr. Sewall Wright has pointed out that the fewer the males employed for breeding the quicker homozygosity in the strain is produced. An added precaution was taken in determining whether there is any difference in the two tumors. Both tumors were inoculated into the same mouse, the dBrA tumor into the right axilla, the dBrB tumor into the left. By this method we have eliminated the possibility of even a slight variation in the race employed by comparing the two tumors in the same soil. If there is any difference in the tumor cell we should be able to determine it, since the soil in each case is identical.

Beginning with the second week after inoculation, weekly observations were made by means of palpation. If there were any indications of the tumor, the relative size of the mass was charted, as accurately as possible, on coördinate paper. Six observations were made for each inoculation; it having been shown by previous experiments that if any indication of growth was going to develop it would appear in that period.

Not one mouse in a total of 160 employed for this report ever grew either tumor progressively for the entire period although several indications appeared as shown in the chart (Fig. 1).



Percentage Indications.
Weeks Observed.

FIG. 1.

It will be noted that the dBrB tumor gave the greater percentage indications throughout the experiment.

According to the binomial theorem, the possibility of all six points of the one curve being above the corresponding six points of the other is as 63 : 1, thus indicating that the two curves are significantly different, any odds above 27 : 1 being mathematically significant. There is a mathematically significant difference between the per cent. of indications of the two tumors when the results are massed, this being 8.6 times its probable error. The analysis of the observations by successive weeks shows that the

first three week periods differ significantly in the two tumors, the difference in each case being more than three times its probable error. The slight rise in the curves in the last two periods is not real but due to the dying off of some negative individuals during an epidemic. The last three points approach mathematical significance and possibly will be significantly different when more numbers are obtained. Since both curves are approaching zero there would be convergence. For that reason the difference between corresponding points would not be as great as between points at the other end of the curve.

We are, therefore, led to the conclusion that the two histologically identical tumors possess different physiological reactions. Wherein lies this difference? This cannot be explained by fluctuations in the tumor cell. Within insignificant variation limits, the two tumors have retained their own reaction potentiality throughout the whole experiment.

Physiological differences of tumors of the same general type may, therefore, be independent of *histological* differences. There are two remaining explanations, either (*a*) cytological or (*b*) genetic. The cytological explanation involves fluctuations from the normal type of mitosis, amitosis, etc. Such explanations are not wholly acceptable to the investigators who approach the tumor problem from another angle.

We are led to the conclusion that it is in the genetic constitution of the individual that we are to look for the underlying causes that undoubtedly determine susceptibility to transplantable tumor tissue.

It has long been recognized that tumor cells are not distinct from normal cells. They only differ in their ability to grow indefinitely. Since normal tissues are, to a large degree, dependent upon the genetic complex of the individual, may we not also look for the causes underlying susceptibility to transplantable tumor as being similarly correlated with genetic factors?

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