# IS IT POSSIBLE TO TRANSMIT OR ACCELERATE THE DEVELOPMENT OF MOUSE LEUKEMIA BY TISSUE EXTRACTS?

*By* J. ENGELBRETH-HOLM, M.D.

DURING the last ten years it has been reported from various laboratories that the injection of certain tissue extracts has been followed by accelerated development of spontaneous leukemia in mice, the disease presenting an increased incidence and (or) an earlier appearance in the experimental animals than in the untreated controls. Although these observations lack satisfactory confirmation, it seems desirable to review them here, and to record some supplementary investigations bearing on the same problem.

In the Year Book of the Carnegie Institute, New York, for 1937, MacDowell and collaborators described experiments in which monthly injections of embryonic tissue extract into mice of the strain C.58 (with a 90 per cent leukemia incidence) were followed by development of the disease in all of the 60 experimental animals at an earlier date than in the controls belonging to the same litters. The results were not reported in detail, nor was it stated whether the test has been repeated. Gorer, who tried to confirm this finding, states merely: "Inoculations of embryonic tissue have had no noticeable effect on either the 'albino' or the 'black' leukemia."

In 1938, Engelbreth-Holm and Frederiksen believed they had transmitted mouse leukemia to young animals of the strain Aka by means of a cell-free extract of leukemic organs from mice of the same strain. The extract was prepared under anaerobic conditions reduced in a cysteine-cobalt-sulphate system as described by Pirie and Holmes. Injection of the extract was followed by the development of leukemia in 8 experiments out of 9, totalling 36 mice out of 179. The tests were carefully controlled in various ways. Thus, *aerobically* prepared extracts showed no effect in 5 experiments including 120 animals. Further, a minimum of 1000 cells was found to be necessary to secure a "take" in the ordinary way; it therefore seemed impossible that the "takes" in these experiments could have been due to presence of sufficient intact cells in the extract, since the latter had been centrifuged twice for fifteen minutes at 3,000 r.p.m. As a most deplorable fault, it must be noted that the extract was not filtered in these tests. Engelbreth-Holm later (in 1942) expressed the view that the findings in these experiments might have been a question of acceleration of spontaneous leukemia rather than of a transmission of the disease.

MacDowell and his collaborators (1939) tried to repeat the observations of Engelbreth-Holm and Frederiksen without success. Still more confusion however, was brought into the matter when, after control injection of the medium used for reduction (a cobalt-sulphate-cysteine solution), MacDowell found leukemia developing in 17 out of 20 mice only twenty-six to thirty-eight days later. Repetition of this experiment gave negative results.

From the University Institute of Pathological Anatomy, Copenhagen.

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Finally, Gorer in 1939 reported a somewhat analogous observation: Inoculation into mice of a "nontaking" sarcomatous tissue appeared to increase the leukemia incidence from 6 to 39 per cent in all animals, and from 2 to 46 per cent in the males. Reinoculation brought about a still greater rise in incidence. Gorer inoculated a sarcoma from an albino strain into a black mouse strain. After repeated inoculations, leukemia developed in 7 out of 10 mice, whereas the spontaneous leukemia incidence was only 6 per cent. Gorer found that the leukemia did not develop until about one year after the inoculations, i.e., probably at the same age at which the disease will appear in untreated mice.

In an attempt to explain the results of our original experiments (Engelbreth-Holm and Frederiksen), new investigations along the same lines were performed during the years 1938–42. Our efforts, however, were no more successful than those of MacDowell. We did not observe any acceleration of the leukemia in mice belonging to the strain Aka after injection of an extract of leukemic organs prepared under anaerobic conditions. Repeated injections of such extracts were made in 25 mice belong to the strain Aka, a total of 17 injections being administered at intervals of two weeks. For control purpose, extracts of normal organs were injected into brothers and sisters of the experimental animals, but no effect was seen in either of the two series.

Likewise, MacDowell's experiments with the cobalt-sulphate-cysteine solution were repeated. Forty-eight Aka-mice were given 1 cc. of the solution; the mice at the beginning of the experiment were one month old, and the injections were administered every two weeks until they died spontaneously. In 23 out of these 48 mice, leukemia developed, but among their 44 untreated brothers and sisters, 23 cases of leukemia were found as well; the treatment, therefore, had had no evident effect.

No more did we succeed in accelerating other tumor types: leukemia and mammary carcinoma of the strain dlb. These experiments, comprising 38 and 15 mice respectively, were equally negative.

The most natural way in which to explain these rather capricious and mostly negative results was to assume that, despite the precautions taken, the spun extract in our 1938 experiments did contain a number of intact cells sufficient to secure "takes"; this explanation still failed to account, however, for the fact that administration of *aerobically* prepared extracts did not give any "takes." In spite of numerous unsuccessful attempts to repeat our 1938 observations, and in spite of the controls having indicated that a few intact cells in the injected substance could not explain the development of leukemia, I initiated one more experiment in order to exclude the possibility that cells when suspended in the cobalt-sulphate-cysteine solution were more capable of "taking" than when suspended in a sodium-chloride solution as in the original control experiments.

Known numbers of leukemic cells, suspended partly in normal saline and partly in a cobalt-cysteine solution, were accordingly injected into series of mice. The result may be seen in table 1.

No explanation was achieved by these experiments, as suspension in the reduction solution had a definite effect in suppressing the "taking" capability of the

cells (a finding which was to be expected beforehand), leaving it, thus, still more improbable that our "takes" or "accelerated cases" in the experiments of 1938 could have been due to the presence of intact cells in the extract solution.

In 1946, however, a paper was published by Silber in Russia, describing investigations which may possibly throw some light on these obscure questions, though it is necessary to await confirmation of the findings before reaching a final opinion. According to Silber, sarcomas induced in mice by 1,2,5,6-dibenzanthracene are transmissible by means of Berkefeld-filtered extracts of tumor itssue which has been treated according to the method introduced by Engelbreth-Holm and Frederiksen (dissection in a closed chamber filled with carbon dioxide, and suspension in a cysteine-cobalt system in order to prevent oxidation).

In 4 out of 5 experiments, Silber had "takes" in a total of 18 out of 114 animals. He makes it clear, however, that the takes occurred only when the substance had been prepared from very young tumors ("incipient" sarcomas), and, further, that the experimental animals required to be "sensitized" by "subcutaneous injection of 0.5 cc. of an oily solution of Dibenzanthracene containing 1 mg. of this substance in one liter of vegetable oil. This injection was administered 1–2 weeks before the test." Without this "sensitization" no takes were seen.

TABLE 1

| Number of leukemic cells | Suspended in saline "Takes"/number of mice | Suspended in cobalt- cysteine solution "Takes"/number of mice |
|---|---|---|
| 1,000,000 | 1/2 | 3/5 |
| 170,000 | 5/5 | 0/5 |
| 30,000 | 2/5 | 0/5 |
| 5,000 | 0/5 | 0/5 |

An observation by Duran-Reynals in fowl sarcomas may also prove of interest in this discussion. Duran-Reynals has pointed out that the virus of fibrosarcomas are detected more frequently at the age of 5 to 10 months than in younger or older fowls.

Whether or not these findings have any bearing on "transmission" of leukemia in mice cannot yet be decided. In our experiments, no attention was paid to the age of the donor animal or to that of the tumor tissue; nor was it possible to pay regard to such changes of character as might have taken place in the inbred mouse strains during the passages.

Attempts have further been made to repeat Gorer's experiments mentioned above. In our experiments, different tumors were inoculated into mice belonging to three different strains in which the tumors used did not take. Inoculation was made subcutaneously and repeated every two weeks (see table 2), one half of each litter being left untreated as controls. The three strains employed were the strain Aka, the strain dlb, and the strain Street. The strain Aka has a spontaneous leukemia incidence of 57 per cent; in the strain dlb (subline of the Little Dba), leukemia will develop in 1 per cent, and mammary carcinoma in about 40 per cent; and in the strain Street, leukemia incidence is 1 per cent, the incidence of mammary carcinoma

being 25 per cent. The transplanted tissues in question were a mammary carcinoma from dlb-mice, a leukemic tissue from Aka-mice, and a squamous cell carcinoma from the strain Aka which had been transferred through several passages (see Engelbreth-Holm 1944).

Further details of the experiment may be seen in table 2. The treatment did not increase or accelerate tumor development, and the results of Gorer's investigation were therefore not confirmed. Unfortunately, however, owing to a fulminating epidemic, all the animals died when 17–19 months old, and it is impossible to decide how many tumors might have developed if the mice had not succumbed prematurely. Nevertheless, since tumors occurring in these strains will generally develop spontaneously from the age of 10 to 12 months, the climax being at about 15 months, it was clear that the treatment did not accelerate tumor development, both the experimental and control animals presenting only a few tumors.

TABLE 2

| Tumor tissue from | Inoculation made into | Number of inoculations | Age of mice at the end of experiment |
|---|---|---|---|
| Mammary carcinoma dlb | 24 Aka-mice | 3 | 17–19 |
| | 53 Street (24 ♂ 29 ♀) | 5 | |
| Leukemic tissue Aka | 55 dlb (34 ♂ 21 ♀) | 6 | 18 |
| | 52 Street (30 ♂ 22 ♀) | 6 | 18–19 |
| Squamous cell carcinoma Aka | 49 dlb (28 ♂ 21 ♀) | 6 | 17–18 |
| | 54 Street (30 ♂ 24 ♀) | 6 | 17 |

The result in each instance was: No effect upon tumor development.

We have thus been unable, with the strains used in these experiments, to repeat Gorer's finding that inoculation of heterologous tumor tissue can bring about an increased leukemia incidence.

## DISCUSSION

A series of experiments is reviewed; although largely supporting each other, they have proved inaccessible to direct reproduction. The various positive investigations originally indicated that development of spontaneous leukemia in inbred mouse strains is accelerated after the injection of embryonic extract or leukemic tissue extract, or after inoculation of heterologous tumor tissue.

MacDowell and his collaborators succeeded in accelerating the leukemia incidence after administration of embryonic tissue extract. Gorer did not succeed in reproducing these experiments, but he gives few details of his negative result to which, indeed, he seems to ascribe but a limited importance.

Englebreth-Holm and Frederiksen thought that they had accelerated the development of leukemia, or transmitted the disease, by means of an anaerobically prepared extract of leukemic tissue. These findings, however, have remained refractory to reproduction in spite of repeated attempts made by MacDowell and ourselves.

MacDowell observed acceleration of leukemia in one experiment by means of a cysteine-containing suspension, but neither he nor we have been able to repeat this observation.

After transplantation of a nontaking tumor tissue, Gorer achieved an accelerated development of leukemia. Despite several attempts to repeat these experiments, however, we have not succeeded in confirming Gorer's results.

How these discrepant findings are to be explained is still obscure. The various control series have been adequate, and no experimental faults which might have produced the positive or negative results have been detected. Most peculiar is the fact that the three positive series of experiments quoted, although mutually different, show one common feature, viz: the injection of rapidly growing tissue or tissue extracts, that is, of homologous or heterologous tumor tissue, and of embryonic tissue extract.

It is a most fascinating thought that in these experiments we may be approaching a factor capable of accelerating tumor development, but undeniably, there must still be a number of factors escaping our control.

Transient changes of disease conditions in the mouse strains used can probably be excluded, to judge from the control series. Certain more recent experiments, however, may possibly throw light on these questions. Silber claims to have transmitted sarcomas in mice by means of cell-free filtrates, using the same technic as we did in our earliest experiments, but he points out that only filtrates from quite young tumors have any effect; and Duran-Reynals has shown that detection of virus in fowl sarcomas most easily will be successful in certain age groups, younger and older animals offering more difficulties.

It is premature to attempt to assess the importance of these experimental results to mouse leukemia, but they seem to call for a re-examination of the relevant problems on a wider basis.

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