The Control of Survival Time of Mice Bearing Methylcholanthrene-induced Fibrosarcomas[1]

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Data have been published which indicate that the rate at which fibrosarcomas appear at the site of the injection of methylcholanthrene into mice (frequency incidence) varies with the litter sequence of a given female (*1*). The susceptibility to chemically induced fibrosarcomas is lowest in mice of the first and second litters and highest in mice of the fifth and sixth litters. The increase of susceptibility in the succeeding litters of a single female mouse has also been shown to be variable, being very small in mice of some stocks and significantly higher in mice of other stocks. Increased susceptibility to fibrosarcomas of later litters is probably not associated with heterosis, since it has been found in mice of inbred strains as well as in mice of an F_1 generation that were obtained by a cross between the inbred Brs subline and the C_{57} blacks as originally determined.

The pBr subline (one of the major sublines of the NHO descent) has been continued for 26 generations by a continuous process of selection for greater resistance to methylcholanthrene-induced fibrosarcomas following the subcutaneous injection of this carcinogen into both parents at 60 days of age. The latent period for the appearance of chemically induced tumors has been increased by such selection from 125 days to 436 days in 22 generations. The pBr methylcholanthrene-treated descent is still being continued.

The pBrunt subline was separated from the pBr-treated series in the F_{17} generation of brother-to-sister matings, at a time when the average latent period for the appearance of induced fibrosarcomas was 363 days. The subline was then continued as an untreated descent. The 2pBrunt subline was separated off in the F_{20} generation, when the average latent period for induced fibrosarcomas was 393 days; this new subline was similarly continued as another untreated descent. Mice of the 2pBrunt subline thus have a greater resistance to the appearance of fibrosarcomas than mice of the pBrunt subline, and this greater resistance is measurable in terms of an increased average latent period (393 days compared to 363 days). After several generations of untreated descent, the offspring of mice of the two descents (the pBrunt and the 2pBrunt) are now being injected with 1 mg of methylcholanthrene dissolved in 0.1 ml of sesame oil at 60 days of age. Unpublished data show that mice of the two descents are different not only in the rate at which fibrosarcomas appear at the site of injection of the methylcholanthrene, but also in survival time (time between the appearance of the chemically induced fibrinosarcomas and the death of the individual).

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TABLE 1

AVERAGE LATENT PERIOD AND AVERAGE SURVIVAL TIME FOR MICE OF THE PBRUNT SUBLINE*

Litter	No. of mice	Latent period	Survival time
1	9	90.3	52.0
2	23	88.2	60.7
3	22	79.7	74.3
4	40	84.3	90.1
1–4	94	84.8	75.5
5	35	68.0	101.1
6	20	85.6	107.0
7	22	82.7	106.6
8	7	85.0	130.0
5–8	84	77.3	106.3
1–8	188	81.4	85.3

* Only mice with latent periods less than 100 days are included.

In this paper data will be given only on the survival time of female mice of the pBrunt subline, which develop induced fibrosarcomas with a latent period of less than 100 days. These data, summarized in Table 1, are presented graphically in Fig. 1. The mice are classed according to the ordinal numbers of the litters to which they belong, beginning with the first and ending with the eighth.

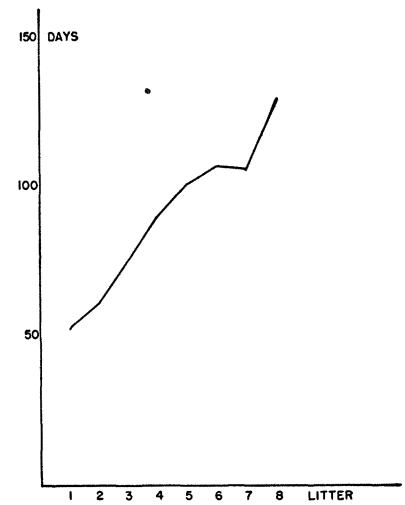

FIG. 1. Litter frequency is given on the base line and the average survival times of mice bearing induced fibrosarcomas are given on the vertical line expressed in days.

An analysis of the data indicates that the survival time of a mouse of the pBrunt descent growing an induced fibrosarcoma increases with the litter frequency of the mother. The group of mice that survive for the shortest length of time (an average of 52.4 days) with the growth of a primary induced fibrosarcoma belong to a first litter. This survival time gradually increases with litter frequency, until, if the mouse belongs to an eighth litter, the average survival time is 130.0 days. After temporary growth, 16 mice belonging to advanced litters have regressed their primary chemically induced fibrosarcomas spontaneously.

Mice of the pBrunt descent growing primary induced fibrosarcomas thus have a greater resistance to the growth of this type of malignant tumor than mice of the C_{57} stock, which under the same conditions survive on the average only 30 to 40 days.

By controlling the ordinal position of the litter in which a mouse has been born, an influence on survival time with growth of a malignant tumor has been determined.

It is difficult to obtain many mice that belong to litters succeeding the eighth one. Later litters should yield interesting data about the growth rates of chemically induced fibrosarcomas, retrogressive potentialities, alterations in latent period, survival time, and perhaps in the histological characteristics, invasiveness, and ability of the tumors to metastasize.

Only a short time ago it was felt that the growth of a malignant tumor was autonomous, that it grows and kills an individual by laws yet unknown. We are now able to determine, to no slight degree, the end results of the growth of a malignant tumor, at least in a carefully controlled experimental animal. Through the construction by genetic principles of a biological system that shows increased resistance to the growth of a malignant tumor, such as obtained in the pBr subline, the fate of the individual can be changed significantly.

Reference

1. STRONG, L. C. *Science*, 1948, **108**, 688.

A Synthetic Diet for the Biological Assay of Thiamine

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Recently these laboratories undertook to determine the thiamine content of rice bran, using the biological method of assay described in the U. S. Pharmacopoeia XIII (*7*). This method is the one most commonly employed in making determinations of thiamine content by the biological procedure.

Twenty weanling rats were placed in cages having wire screen bottoms to prevent access to feces, and were given the basal diet ad libitum. These animals failed to develop polyneuritis in the expected manner. Because of the possibility that autoclaving for the prescribed 5 hr had not destroyed the thiamine naturally present in the yeast and peanut components of the U.S.P. ration, a second batch of each was autoclaved for 7 hr and a new lot of diet satisfactorily prepared. Still the rats failed to develop polyneuritis.

A study was then made of a "synthetic" ration, which was essentially devoid of thiamine and yet contained liberal amounts of all other nutrients known to be required by the rat. It is now recognized that rats maintained on a thiamine-free diet die of inanition without symptoms of polyneuritis (*2–5*), whereas those given small amounts (0.25–0.75 μg per rat per day) of the vitamin develop typical symptoms proportional in severity to the thiamine intake (*1–6*). Ammerman and Waterman (*1*) found that 0.5 μg of thiamine hydrochloride per rat per day, given in conjunction with a vitamin B_1-free diet, produced paralysis in 70% of the animals in 5–8 weeks. Supplee *et al.* (*6*) used 0.75 μg vitamin B_1, 10 μg lactoflavin, and 100 mg autoclaved rice polish concentrate as a supplement to the basal vitamin B_1-free diet, and observed that some animals showed prolonged resistance to development of polyneuritis.

The composition of the synthetic diet used in the present study was:

sucrose	55.5%
vitamin-free casein	27.0%
salts (Hubbell)	3.0%
Crisco	14.0%
vitamin mixture	0.5%.

The vitamin mixture contained, in mg per 0.5 g: riboflavin 0.5, pyridoxine 0.4, niacin 2.0, calcium pantothenate 2.0, *p*-amino benzoic acid 10.0, inositol 200.0, biotin 0.03, choline chloride 300.0, menadione 0.3, α-tocopherol 10.0, folic acid 0.25, ascorbic acid 10.0, and also 1250 U.S.P. units of vitamin A and 125 U.S.P. units of vitamin D.

This thiamine-free synthetic diet was fed ad libitum to four comparable groups of 15 weanling rats each. The animals of group I received no supplement. Those

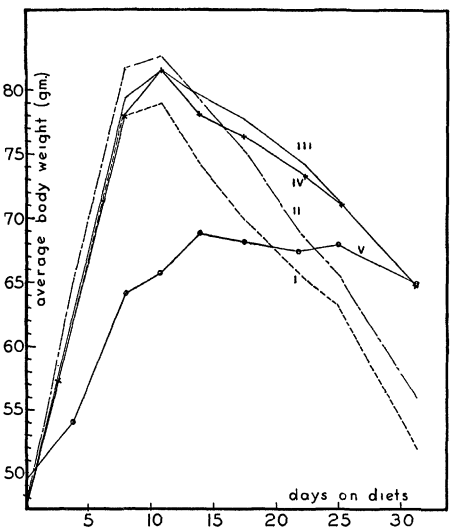

FIG. 1. Body weight during the depletion of groups of rats which eventually developed polyneuritis.

of groups II, III, and IV received, respectively, 0.25, 0.50, and 0.75 μg of thiamine hydrochloride daily, given orally as a solution delivered from a calibrated pipette. A fifth group of 23 rats was given the U.S.P. basal diet. The assays with all five groups were carried out according to U.S.P. procedure (*7*).