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

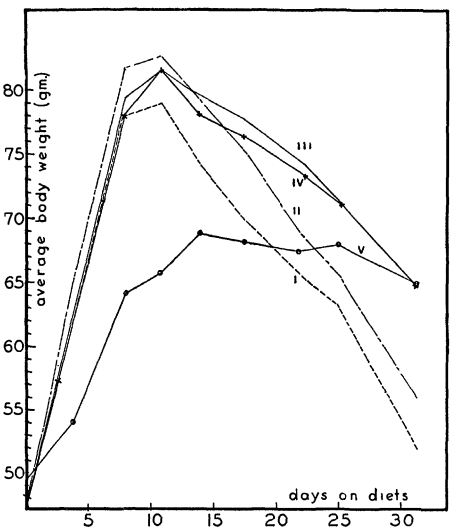

FIG. 1. Body weight during the depletion of groups of rats which eventually developed polyneuritis.

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*).

TABLE 1

SUMMARY OF RESULTS FROM FEEDING TRIALS WITH RATS IN A COMPARISON OF FIVE DIETS FOR THE BIOLOGICAL ASSAY OF THIAMINE

Group No. and diet	No. rats	No. rats that died without polyneuritis	Rats that developed polyneuritis		Avg No. days for development of polyneuritis	No. rats that died before completion of first assay	No. rats that failed to develop polyneuritis 2nd time	No. rats that completed one or more assay periods	No. satisfactory* assays	Percent of rats giving satisfactory results
			No.	Percent						
I Synthetic	15	5	10	67	33.5	7	0	3	3	20
II Synthetic 0.25 μg B_1	14	2	12	86	34.6	4	0	8	6	44
III Synthetic 0.5 μg B_1	14	0	14	100	37.1	1	0	13	11	79
IV Synthetic 0.75 μg B_1	15	0	15	100	44.2	1	2	12	10	67
V U.S.P.	23	0	18	78	46.1	2	3	13	10	43

* Results judged satisfactory according to the U.S.P. method—i.e., when the curative period was not less than 5 days and not more than 15 days.

During the depletion period all rats were weighed twice weekly and observed closely for symptoms of polyneuritis. Animals were considered polyneuritic and suitable for test feeding if they exhibited convulsions, paralysis, or cartwheel motion, or rolled on their backs when twirled by the tail. Rice bran and ground whole corn were used as assay materials. Each test dose was estimated to contain 6 μg (2 I.U.) thiamine hydrochloride on the basis of results from chemical analyses by the thiochrome method. During the test period all rats were weighed daily. The rats of groups I, III, IV, and V were used in successive feeding trials until most had died. Some in group II were killed at the end of the first test period in connection with another study, so that a similar record on them is not available.

The relative rates of growth during the depletion period of the rats in the several groups that developed polyneuritis are shown in Fig. 1. The maximum average weight of group V was considerably less than that of the other four groups; this may indicate that the U.S.P. basal diet contains less than optimum amounts of some nutrient or nutrients essential to rat growth. Calculation of the thiamine content of the U.S.P. diet on the basis of chemical analyses of one batch of ingredients gave a value of 4.95 μg per 100 g, indicating that thiamine was not the limiting factor.

Data pertinent to the several assay groups are summarized in Tables 1 and 2. The animals of group I showed the most sudden and rapid loss of weight. In the three rats giving satisfactory assay results, the symptoms of polyneuritis were severe, the curative periods were short, and complete cures were not obtained in all cases. The response of the animals of group II was intermediate between that of group I and the three remaining groups. The symptoms of polyneuritis were severe and the curative periods were short. In group III the rats showed a gradual decline in weight, the symptoms of polyneuritis were clear-cut and specific, and recovery followed promptly after administration of the Reference Standard or the test food. Most animals in this group served in three assay periods, although the results were not always satisfactory and the length of successive curative periods tended to diminish. The rats of group IV, like those of group III, showed a gradual decline in weight but the individuals of this group showed a wider range (36–62 days) in the time elapsing before the onset of polyneuritis than those of group III (31–43 days) and many assay results were unsatisfactory because of prolonged curative periods. The animals of group V also showed a wide range (38–71 days) in the time elapsing before the symptoms of polyneuritis first appeared, and also showed a tendency toward prolonged curative periods.

On the basis of these findings it would seem that the synthetic diet described, supplemented daily with 0.5 μg thiamine hydrochloride administered to the rats orally, is more suitable for the biological assay of vitamin B_1 than the U.S.P. diet. On this synthetic diet, 100% of the animals developed polyneuritis and it was possible to carry a high percentage through several assay periods.

The results from the feeding trials with rice bran and whole corn indicate that the thiamine content determined

TABLE 2

SATISFACTORY ASSAY RESULTS FOR RICE BRAN AND WHOLE CORN

Rat No.	Duration of curative period in days									
	Assay period No. 1		Assay period No. 2		Assay period No. 3		Assay period No. 2		Assay period No. 3	
	2 I.U. B_1	Rice bran	2 I.U. B_1	Rice bran	2 I.U. B_1	Rice bran	2 I.U. B_1	Corn	2 I.U. B_1	Corn
Group I:										
1	9	5								
2	9	10					8	6		
3	7	5								
Avg	8.3	6.7					8.0	6.0		
Group II:										
1			6	10						
2	8	15								
3	8	6								
4			7	6						
5	8	7								
6			5	9						
Avg	8.0	9.3	6.0	8.3						
Group III:										
1	7	9	6	8					7	6
2			8	8						
3									5	5
4	12	12					6	9		
5	9	11							9	7
6	8	6								
7	9	8	5	6					6	7
8	12	10								
9	9	15					8	7		
10	14	7					5	5		
11					6	7				
Avg	10.0	9.8	6.3	7.3	6.0	7.0	6.3	7.0	6.8	6.3
Group IV:										
1	10	7								
2	10	12								
3	7	8					14	11		
4	11	13								
5							9	8		
6	12	8								
7	10	10								
8	11	14					15	15		
9							9	10		
10	15	10								
Avg	10.8	10.3					11.8	11.0		
Group V:										
1	13	9					9	12		
2	15	14								
3	12	12					9	9		
4	10	13								
5							7	7		
6	14	10					10	7		
7	14	6					6	5		
8	9	12					11	13		
9	9	10								
10							8	13		
Avg	12.0	10.8					8.6	9.6		

by chemical analysis closely approximated that shown by biological assay.

References

1. AMMERMAN, M. and WATERMAN, R. E. *J. Nutrition*, 1935, **10**, 25.
2. CHICK, H. *Biochem. J.*, 1927, **21**, 698.
3. HEYROTH, F. F. *Bull. basic sci. Res.*, 1932, **4**, 1.
4. SANDELS, M. R. *J. Nutrition*, 1930, **2**, 409.
5. SMITH, M. I. *U. S. Publ. Hlth Rep.*, 1930, **45**, 116.
6. SUPPLEE, G. C., and BENDER, R. C. *Ind. eng. Chem., anal. Ed.*, 1938, **10**, 636.
7. *The Pharmacopoeia of the United States*, 1947, **13**, 703.