pasture *et al.*[1] The inoculation was done by gently rubbing a small piece of infected membrane over the center of the chorioallantoic membrane after it had fallen away from the shell. The opening in the shell was closed by sealing a glass cover slip with a petrolatum-paraffin mixture. All 45 eggs were inoculated and divided into 3 groups of 15 each. Group 1 served as the control; 0.25 cc of saline containing 100 Oxford units of penicillin was placed upon the inoculated membrane of each embryo in Group 2 one hour after inoculation and every 24 hours thereafter for 4 days; 0.25 cc of saline containing 0.1 mg of patulin was placed upon the membrane of each embryo in Group 3 one hour after inoculation and every 24 hours thereafter for 4 days. At the end of 5 days the membranes from the live embryos were removed for gross and microscopic study and for subsequent inoculation of 10-day-old chicks; 0.5 cc samples of fluid from immediately under the membrane of 10 embryos receiving penicillin were collected for assay of the penicillin present.

Results. The membranes from all of the control embryos and from all of those receiving penicillin showed the typical lesions of fowl pox on gross as well as on microscopic examination. Ten 10-day-old chicks inoculated with material from the control membranes as well as ten 10-day-old chicks inoculated with material from the penicillin-treated membranes developed typical fowl pox lesions in 3-5 days.

Assay of the embryonic fluid from 10 penicillin-treated embryos showed a concentration of 20 Oxford units per cc, which is approximately twice what one would expect if uniform distribution occurred throughout the embryo and if there was no destruction of the penicillin by the embryo.

Only 5 of the 15 embryos receiving patulin survived the 5-day treatment. The membranes from this group were necrotic in the center but tissue around the margin showed typical inclusion bodies in and hyperplasia of the epithelium.

Summary. Neither penicillin nor patulin have any inhibitory effect upon the infection of fowl pox of the chorioallantoic membrane of the developing chick embryo.

[1] Goodpasture, E. W., Buddingh, G. J., Richardson, L., and Anderson, K., *Am. J. Hygiene*, 1935, **21**, 319.

I am indebted to Dr. M. T. Bush for the penicillin and patulin as well as for the assay of penicillin.

14760

Thiamine Utilization of Rats Maintained on Diets Containing Dextri-Maltose.

GLADYS A. EMERSON AND H. G. OBERMEYER.

From the Merck Institute for Therapeutic Research and the Research Laboratories of Merck & Co., Inc., Rahway, N.J.

Najjar and Holt[1] maintained human subjects for a period of 18 months on a purified diet similar to rations employed in nutrition studies with rats. The experiment was designed to establish the minimum thiamine requirement of man. Dextri-maltose served as a source of carbohydrate in the ration. The percentage employed was not given, but in all probability it comprised 50 to 60% of the diet. Despite the fact that the authors stated that repeated examination of the ration failed to show any trace of thiamine, the minute quantities of this vitamin required for the maintenance of health of their subjects suggested that (1) the diet carried appreciable thiamine or (2) the particular carbohydrate had a favorable influence on intestinal "synthesis" of thiamine. The latter possibility was investigated by Najjar and Holt who

[1] Najjar, V. A., and Holt, L. E., Jr., *J. A. M. A.*, 1943, **123**, 683.

found that free thiamine was present in the feces of a symptom-free subject (receiving no thiamine) and disappeared following the administration of succinylsulfathiazole. These findings were taken as evidence of bacterial synthesis of thiamine in the intestinal tract. Although a single diet was employed the statement was made that the biosynthesis of thiamine was affected by diet and that dietary factors other than thiamine might explain paradoxes in the incidence of beri-beri.

The experiments herein reported were undertaken in an effort to study the response of rats to thiamine-deficient diets containing dextri-maltose. Two types of diets were employed; the one in which the so-called "heat stable" members of the vitamin B complex were supplied in autoclaved yeast and the other in which these vitamins were fed in crystalline form. The tests were carried out by both the prophylactic and curative methods.

The diets employed were as follows:

	Diet				
	52	53	54	55	20
	g per 100 g				
Casein (Labco)	18	18	18	18	—
Casein (technical)	—	—	—	—	16
Dextri-maltose	53	—	68	—	—
Sucrose	—	53	—	68	—
Cornstarch	—	—	—	—	60
Autoclaved brewer's yeast	15	15	—	—	9
Salts (U.S.P. No. 1)	4	4	4	4	4
Hydrogenated vegetable oil	8	8	8	8	8
Cod liver oil	2	2	2	2	2
Non-autoclaved dried brewer's yeast	—	—	—	—	0.2

Dried brewer's yeast was spread out in layers one-quarter inch deep and autoclaved at 15 lb pressure for 5 hours at its natural pH.

Diets 54 and 55 were supplemented with 2 mg of riboflavin, 1 mg of pyridoxine, 10 mg calcium pantothenate, 10 mg nicotinamide, 5 mg inositol, and 100 mg choline chloride per 100 g. This supplement was considered ample to cover the requirement of growing rats for these factors.

Thiamine Content of Dietary Constituents (Thiochrome Method). The diets and the carbohydrates and autoclaved yeast contained therein were analyzed for thiamine by the thiochrome method employing the procedure of Hennessy.[2] Dextri-maltose (Mead Johnson) was purchased in the open market and as the containers bore no lot numbers it was impossible to tell whether different batches were used. Three samples purchased at bimonthly intervals were analyzed. The thiamine values obtained for the different lots were in good agreement. All samples of dextri-maltose contained about 0.9 γ per g whereas the sucrose was practically devoid of thiamine (0.06 γ per g) (Table I). The autoclaved yeast employed in the diets was free of thiamine. Consequently the diets containing dextri-maltose carried 0.5 γ thiamine per g and those with sucrose only traces.

TABLE I.
Thiamine Content of Dietary Constituents.

	Thiamin (by thiochrome method) μg/g
Dextri-maltose (1)	.92
" (2)	.93
" (3)	.90
Sucrose	.06
Autoclaved yeast	.00
Diet 52 (dextri-maltose autoclaved yeast)	.50
Diet 53 (sucrose autoclaved yeast)	.00
Diet 54 (dextri-maltose crystalline vitamins)	.52
Diet 55 (sucrose crystalline vitamins)	.08

Prophylactic and Curative Studies with rats. In the prophylactic tests female rats were started at weaning on the above diets. Controls were dosed daily with 40 γ of thiamine. The results as shown in Fig. 1 demonstrate that dextri-maltose carries appreciable amounts of biologically available thiamine. The animals receiving the ration containing dextri-maltose and autoclaved yeast (Curve 1) attained maximum weights which were only 20% below those of the controls dosed with optimal thiamine (Curve 2). On the other hand, the rats maintained on the identical diet but with sucrose as the carbohydrate, succumbed to thiamine deficiency after an initial gain of 20 g (Curve 3). The controls receiving thiamine (Curve 4) grew at a satisfactory rate.

[2] Hennessy, D. J., *Cereal Chem. Bull.*, 1942, **2**, 25.

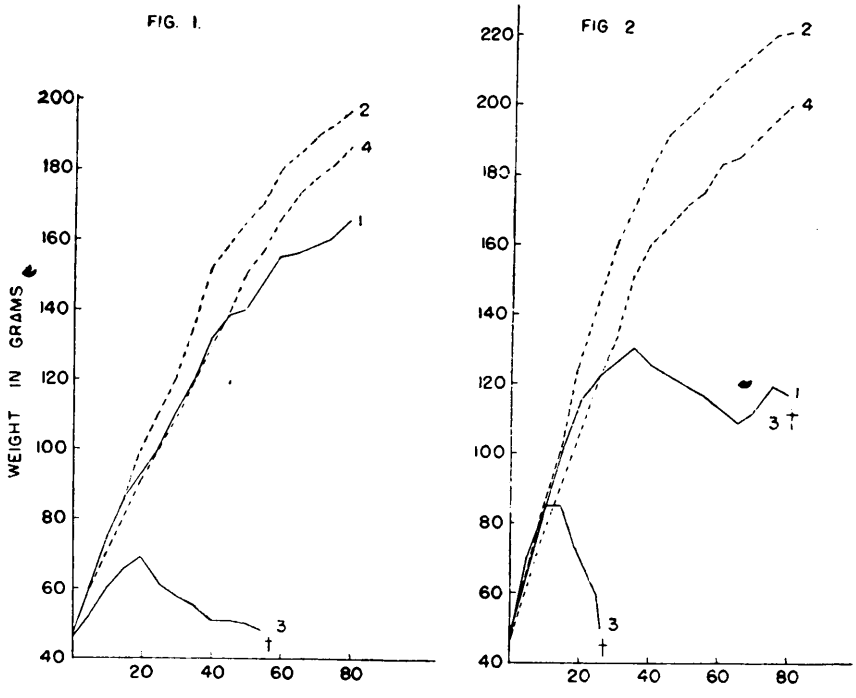

FIG. 1.

Comparison of growth responses with dextri-maltose and sucrose of female rats (5 in each group) maintained on rations containing autoclaved yeast.

Curve 1 Diet 52 (dextri-maltose)
" 2 " 52 + 40 μg thiamine daily
" 3 " 53 (sucrose)
" 4 " 53 + 40 μg thiamine daily

FIG. 2.

Comparison of growth responses with dextri-maltose and sucrose of female rats (5 in each group) maintained on rations containing crystalline vitamins.

Curve 1 Diet 54 (dextri-maltose)
" 2 " 54 + 40 μg thiamine daily
" 3 " 55 (sucrose)
" 4 " 55 + 40 μg thiamine daily

A second prophylactic test comparing dextri-maltose and sucrose was carried out with rats maintained on a diet in which the B vitamins other than thiamine were supplied in crystalline form (Fig. 2). In this experiment the presence of thiamine in dextri-maltose was demonstrated again by the fact that the growth response with dextri-maltose (Curve 1) was superior to that with sucrose (Curve 3). Whereas the rats maintained on the sucrose diet succumbed rapidly (within 26 days) to thiamine deficiency, the animals receiving dextri-maltose reached a plateau in weight during the second month and 3 out of 5 died within the ensuing 60 days. The two remaining rats in this group survived for 120 days at which time the experiment was discontinued. The growth of the controls is shown in Curves 2 and 4.

The curative tests were carried out with two groups of rats depleted of their stores of thiamine by the feeding of a diet (20) employed routinely in the production of thiamine deficiency. At the beginning of the curative test one group had lost 15 to 20 g from their peak weights and the other had declined 20 to 30 g from the maximum. The rats were segregated into groups of like weight losses and weight averages. The controls were allowed to remain on diet 20 and the remaining animals were changed to diets 52 and 54 respectively. As can be seen from Fig. 3, the rats

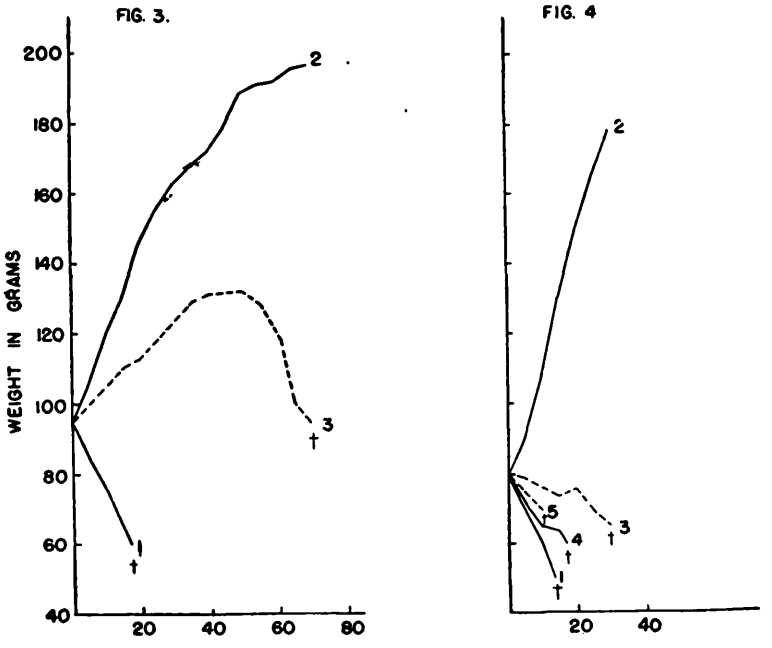

FIG. 3.

Growth responses of thiamine-depleted female rats (5 in each group) to dextri-maltose-containing rations.

Curve 1 Diet 20 (controls)
" 2 " 52 (autoclaved yeast)
" 3 " 54 (crystalline vitamins)

FIG. 4.

Growth responses of thiamine-depleted female rats (5 in each group) to dextri-maltose- or sucrose-containing rations.

Curve 1 Diet 20 (controls)
" 2 " 52 (dextri-maltose, autoclaved yeast)
" 3 " 54 (" crystalline vitamins)
" 4 " 53 (sucrose, autoclaved yeast)
" 5 " 55 " crystalline vitamins)

were rescued from impending polyneuritis and death (Curve 1) by changing from the thiamine-deficient standard diet to the dextri-maltose-containing rations (Curves 2 and 3). However, the animals receiving crystalline vitamins failed to make continuous gains in weight, developed polyneuritis and succumbed within 70 days (Curve 3). The rats receiving autoclaved yeast made continuous gains and were in excellent condition at the termination of the test (Curve 2).

In the second curative test employing rats which had lost 20 to 30 g in weight, the animals were segregated into 5 groups as indicated in Fig. 4. Only the animals receiving the ration containing dextri-maltose and autoclaved yeast (Curve 2) responded with a rapid increment in growth. The average weight decline for the rats receiving the dextri-maltose ration with crystalline supplements (Curve 3) was not as great as for the animals continued on diet 20 (Curve 1) or on the 2 sucrose-containing rations (Curves 4 and 5). Furthermore, the survival time of the rats receiving dextri-maltose was extended from 10 to 20 days beyond that of the other groups. The fact that the weight response did not equal that observed in the previous experiment could be attributed to the debilitated condition of the animals resulting from the longer depletion period.

The superior growth response observed in both prophylactic and curative tests with the diet containing dextri-maltose and autoclaved yeast cannot be ascribed to its thiamine content alone as the ration containing no auto-

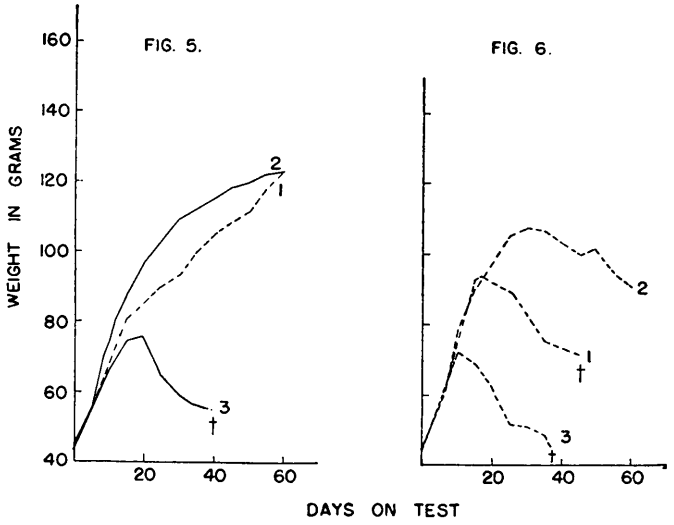

FIG. 5.

Comparison of growth responses with dextri-maltose and sucrose of equal thiamine content of female rats (5 in each group) maintained on rations containing autoclaved yeast.

Curve 1 Diet 53 (sucrose), 0.5 μg thiamine/g added
" 2 " 52 (dextri-maltose)
" 3 " 53 (sucrose)

FIG. 6.

Comparison of growth responses with dextri-maltose and sucrose of equal thiamine content of female rats (5 in each group) maintained on diets containing crystalline vitamins.

Curve 1 Diet 55 (sucrose), 0.5 μg thiamine/g added.
" 2 " 54 (dextri-maltose)
" 3 " 55 (sucrose)

claved yeast gave essentially the same thiamine value by the thiochrome test. Furthermore, the autoclaved yeast *per se* could not explain the differing responses as the same autoclaved yeast was present in the sucrose ration and in diet 20. The possibility existed that degradation products present in the autoclaved yeast were utilized in conjunction with the small quantities of thiamine supplied. In order to test this possibility thiamine was added to the sucrose diet 53 in the concentration present in the dextri-maltose diet 52, namely, 0.5 γ per g. As a check the sucrose ration without the autoclaved yeast (Diet 55) was supplemented in a like manner. The results of these tests are shown in Figs. 5 and 6. As seen from Fig. 5, the rats receiving diet 53 plus 0.5 γ thiamine per g (Curve 1) made weight gains approaching those observed with Diet 52 (Curve 2). Rats receiving diet 53 without added thiamine (Curve 3) succumbed to thiamine deficiency after making maximal weight gains of about 30 g.

On the other hand, rats fed the sucrose diet without autoclaved yeast, but containing 0.5 γ of thiamine per g (Fig. 6, Curve 1), failed to gain at the same rate as did the animals receiving the same intake of thiamine but with dextri-maltose as the source of carbohydrate (Curve 2).

These experiments demonstrate that the growth obtained with small amounts of thiamine (0.5 γ per g) is augmented in the presence of autoclaved yeast which in itself is free of thiamine detectable by the thiochrome method.

Discussion. Dextri-maltose, the carbohydrate employed in the experiments of Najjar and Holt, was found to contain 0.9 γ of thiamine per g as determined by the thiochrome method. The presence of thiamine in dextri-maltose was confirmed by prophylactic and curative tests with rats. However, assays with 2 types of thiamine-low diets, one with autoclaved yeast and the other with the crystalline components comprising the "heat stable" frac-

tion of the vitamin B complex gave divergent results. When thiamine was added to the diet containing sucrose and autoclaved yeast in the same concentration as present in dextri-maltose, the growth response in rats almost equaled that observed with the diet containing dextri-maltose and autoclaved yeast. On the other hand, rats receiving the sucrose-containing ration with crystalline vitamins with added thiamine did not show weight gains comparable to those obtained on the dextri-maltose diet with crystalline vitamins. In this connection it is of interest to note that McIntire and co-workers[3] observed that a sulfite-treated liver extract had a growth stimulating effect in rats maintained on a purified ration sub-optimal in thiamine.

The fact that the growth response to the small amounts of thiamine in dextri-maltose is significantly enhanced by autoclaved yeast led to an investigation of the cleavage products contained in autoclaved yeast and in dextri-maltose. "Resynthesis" experiments with baker's yeast showed that autoclaved yeast carried 4 γ per g of pyrimidine and 7 γ per g of thiazole. This represents about 30% of the original pyrimidine and 50% of the original thiazole moieties of the thiamine present in the yeast before autoclaving. Dextri-maltose contained no pyrimidine, but 0.3 γ of thiazole per g. It appears that these, or other cleavage products, are utilized by the rat in conjunction with small amounts of thiamine. This speculation suggests further research along these lines.

The growth responses obtained in prophylactic and curative tests with diets containing autoclaved yeast are not in agreement with the results obtained by the thiochrome method. From the results obtained by the thiochrome method, identical growth responses were to be expected in rats maintained on diets containing autoclaved yeast or crystalline vitamins. The significant differences observed with the two types of diets in the animal experiments demonstrate that the thiochrome method may not measure all biologically available thiamine.

Summary. Commercial dextri-maltose contains 0.9 γ thiamine per g by the thiochrome assay. Prophylactic and curative tests with rats have demonstrated that the thiamine present in dextri-maltose-containing diets is utilized by the rat. Rats maintained on rations containing autoclaved yeast showed weight increments in excess of those observed with a diet containing the "heat stable" B factors in crystalline form, although both diets contained 0.5 γ thiamine per g by the thiochrome method. Animals receiving diets containing sucrose and autoclaved yeast, but with thiamine added to the same level (0.5 γ per g) present in dextri-maltose, made essentially the same weight gains in prophylactic tests as did rats fed the ration containing dextri-maltose. However, when the autoclaved yeast was replaced by crystalline vitamins, the animals receiving dextri-maltose grew at a rate in excess of that of rats fed sucrose although the thiamine level of the two diets was the same (0.5 γ per g). The results of these experiments indicate that autoclaved yeast apparently carries factor(s) which augment the effect of small amounts of thiamine in the rat. Thus, the thiochrome method may not measure all thiamine which, according to these bioassays, was available to the rat.

[3] McIntire, J. M., Henderson, L. H., Schweigert, B. S., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 98.