nephelometric test is a better indication of the similarity of the antigenic substances, for it registers the total reaction, involving all antigens and all antibodies in the reacting systems.

12043

An Attempt to Produce Hepatic Cirrhosis by a Diet Deficient in Vitamin B Complex.

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Interest in the possible rôle of a vitamin deficiency in the production of cirrhosis of the liver has resulted from (1) the clinical observation that patients with that disease have improved following the administration of a high vitamin diet (Patek[1]), and from (2) the experimental observations that hepatic necrosis developed in rats offered a vitamin deficient diet (György and Goldblatt[2]) and more recently that cirrhosis without necrosis occurred in rabbits on a diet deficient in some factor that is contained in yeast (Rich and Hamilton[3]). The latter investigators believed that a lack of thiamin, riboflavin, or nicotinic acid could be eliminated as the responsible factor. In order to investigate this matter in another species of animal we have administered to the rat one of the diets that Rich and Hamilton found effective for the production of hepatic cirrhosis, but have failed in that animal to secure comparable results.

Procedure. Eighteen apparently normal, male, adult, albino rats of an inbred Wistar strain were selected. The mean body weight was 300 ± 80 g. Twelve of these animals were placed on a diet deficient in the vitamin B complex, while the remaining 6 received B complex in the form of brewers' yeast and served as controls. All animals in groups of three received daily a food bolus consisting of

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1 Patek, A. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 329.

2 György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

3 Rich, A. R., and Hamilton, J. D., *Bull. J. H. Hosp.*, 1940, **66**, 185.

10 g of vitamin-free casein obtained from the Borden Co.; 4 g of "Crisco"; 25 g of dextrin (Baker's N F V white powder); 24 g of boiled rice; 25 g of pulpified raw carrots, and 4 g of salt mixture (Weech, *et al.*[4]). The six control animals received powdered brewers' yeast (Harris) mixed with the bolus of food (5 g of yeast per 3 rats). The weighed amount of food placed in the cage daily was in excess of that eaten; the uneaten portion was weighed and discarded. Each animal was given in addition, every second day by means of a stomach tube, vitamins A, D, and E (0.23 cc of a mixture of 4.4 cc of peanut oil, which contained 50 mg of Merck's alpha tocopherol and 29.2 cc of U.S.P. cod liver oil).

The rats, in groups of 3, were housed in cages, the bottoms of which were made of large-mesh wire screening, so as to prevent refection. The cages were also so constructed and arranged as to prevent the access of food or feces to the animals of another cage. A measured amount of fresh water was supplied daily and the 24-hour intake measured. Body weight was recorded daily.

The 12 animals on the B-complex deficient diet were allowed to live until death occurred spontaneously, except in 3 instances in which it was evident that demise would occur during the next few hours; these were decapitated under light ether anesthesia so as to obtain specimens that would be free of changes due to postmortem autolysis. Four animals were subjected to exploratory laparotomy on the 36th day of the experiment. At death the liver of each rat was removed, weighed and fixed in 10% formalin solution. Sections were stained with hematoxylin eosin, Masson's trichrome connective tissue stain and scarlet red.

Results. The 12 animals on the deficient diet maintained their weight for one week, and then showed a progressive loss that continued until their demise, which occurred, on the average, when 51.2% of the initial body weight had been lost. The control animals gained weight steadily during the first 8 weeks of the experiment, and then the weight tended to remain fairly constant (Fig. 1).

The average food intake of the individual rat on the deficient diet for each of the first 5 days of the experiment was 27 g. This decreased progressively to 10 g during the twelfth week. The controls, in contrast, ingested an average of about 30 g per day during the first 5 days; this decreased to about 26 g by the end of the second week and then remained at that level throughout the rest of the experiment. The daily consumption of water paralleled rather closely that of the food in both groups of animals, decreasing in the

[4] Weech, A. A., Goettsch, E., and Reeves, E. B., *J. Exp. Med.*, 1935, **61**, 299.

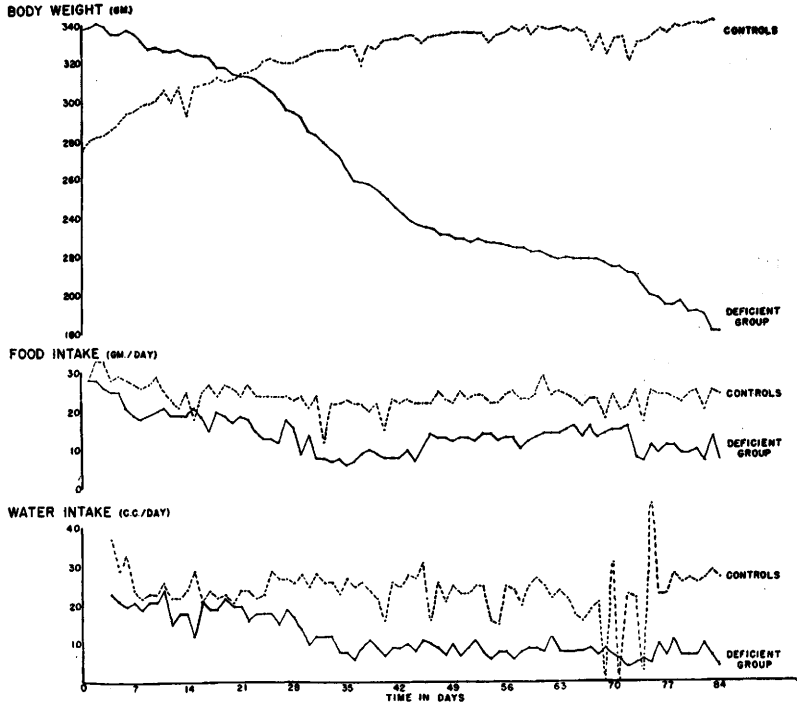

FIG. 1.

The trend in body weight, food and water intake of animals on a B-complex deficient diet (solid lines) and of control animals (dotted lines) receiving brewers' yeast over a period of 12 weeks. Each point on the curves represents the average per animal in a group of 3 per cage. The fluctuations in water intake of the control animals from the 70th to the 77th day are artefacts due to clogging of the glass tubing of the inverted water bottle by air bubbles.

deficient group and being more or less constant in the controls (Fig. 1).

Along with the progressive loss of weight in the deficient group, there occurred a looseness of the skin and a shagginess of the coat of hair, some of which finally fell out in patches, particularly over the haunches. A low grade dermatitis of the margins of the ears and of the inner aspects of the paws and feet appeared. During the terminal stages, the animals had a characteristic "hunched up" appearance. The wasting of the subcutaneous tissues resulted in a prominence of the osseous structures, and the animals appeared to hold their balance with difficulty. Tetanic convulsive movements were frequently observed just prior to demise. No bleeding from the skin or mucous surfaces and no keratinization of the eyelids occurred. (Fig. 2.) The eyegrounds retained their pink color throughout the experiment. The 6 controls had sleek coats and otherwise appeared to be in excellent physical condition. (Fig. 2.)

FIG. 2.

Photograph of animals after 9 weeks on the diet. The animal in the center received the basic diet plus brewers' yeast (control), while the others did not receive the brewers' yeast. The animal on the right is practically moribund as a result of advanced B-complex deficiency while the one on the left is as yet but moderately deficient.

The 4 animals on the deficient diet that were subjected to exploratory laparotomy after 5 weeks, with the intention of removing some hepatic tissue for biopsy, had in each instance a normally appearing liver which made the latter procedure unnecessary. All of the animals on the B-complex deficient diet lived at least 6 weeks except one that died during the 6th week. Two lived 13 weeks, and the remainder either were killed or died within that length of time. Thus most of the animals lived sufficiently long for cirrhosis to develop, were it going to.

Pathologic Findings. All the livers of the animals on the B-complex deficient diet were decreased in size, having an average weight of 4.56 g as compared to an average of 10 g for the controls. The surface of the organ was smooth, glistening and deep red in color. No exaggeration of lobular markings or irregularity of the surface was observed. The livers of the control animals were large, the surfaces smooth and the color light red, suggesting an increased amount of fat.

Microscopically, the sections of the livers of the vitamin-deficient animals showed no evidence of degeneration of the hepatic cells or of proliferation or condensation of connective tissue. The cells were small and did not contain stainable fat. The hepatic cells of the control animals, on the other hand, were large and contained stainable fat in small droplets scattered throughout. These also showed no evidence of hepatic cell necrosis, fibrous tissue proliferation or condensation.

Comment. Rats administered the identical diet on which rabbits have been reported to have developed hepatic cirrhosis failed to do so, though some of the animals took the diet for as long as 13 weeks.

They did, however, exhibit recognized manifestations of B-complex deficiency, suggesting the probability of some other factor being responsible for the hepatic cirrhosis observed in rabbits by Rich and Hamilton or for its absence in our rats.

One possibility was that the carrots and rice, or possibly the peanut oil, contained enough of the unknown cirrhosis-preventing factor to interfere with the development of the disease in rats. Accordingly, another group of 6 rats (body weight, 198 ± 30 g) was placed on a diet similar to the one described except that the boiled rice and raw carrots, also alpha tocopherol in peanut oil, and cod liver oil, were omitted. These animals, younger than those previously used, also failed to develop hepatic lesions. They lost weight rapidly and were dead within 35 days of the onset of the experiment. In this connection it is to be noted that one of the rabbits described by Rich and Hamilton was observed to have cirrhosis after 25 days on the diet.

Another possible explanation of the negative observations in the rat under conditions similar to those reported to produce hepatic cirrhosis in the rabbit may be a species difference; the rat may be less susceptible. György and Goldblatt reported observation of focal or patchy hepatic necrosis in some of a group of rats kept on a vitamin B-complex deficient diet. The lesions, however, could not be produced at will, and they did not occur regularly under identical conditions. The apparently chance occurrence of the lesions raises the question whether they were the result of the experimental procedure instituted or were related to coincidental factors. A case in point is that in which hepatic necrosis in rats was described by Davis, *et al.*,[5] following the administration of sulfanilamide. Similar lesions were found by Machella and Higgins[6] in the livers of rats receiving sulfanilamide, and also in apparently normal animals serving as controls and not receiving the drug. Such lesions have been ascribed by Buchbinder, *et al.*,[7] to an enzootic form of paratyphoid infestation.

In this same category lies the question whether the cirrhotic process in the liver of rabbits noted by Rich and Hamilton was due to infestation with coccidia. Ophuls[8] and also Smetana[9] have

[5] Davis, H. A., Harris, L. C., Jr., and Schmeisser, H. C., *Arch. Path.*, 1938, **25**, 750.

[6] Machella, T. E., and Higgins, G. M., in press.

[7] Buchbinder, L., Hall, L., Wilens, S. L., and Slanetz, C. A., *Am. J. Hygiene*, 1935, **22**, 199.

[8] Ophuls, W., PROC. SOC. EXP. BIOL. AND MED., 1910, **8**, 75.

[9] Smetana, H., *Arch. Path.*, 1933, **15**, 516.

demonstrated that this organism is capable of inciting fibrous tissue proliferation in the perilobular zones in the livers of rabbits. In this connection, furthermore, it is noteworthy that the animals used by Rich and Hamilton were infested with coccidia, and that one of their control animals, which took the diet containing brewers' yeast, but poorly, developed cirrhosis. In agreement with the above is the recent work of Spellberg and Keeton,[10] who reported the development of hepatic cirrhosis in a rabbit on a seemingly adequate diet which contained B-complex as found in cereal foods, and also in a guinea pig on a somewhat similar diet but supplemented with desiccated brewers' yeast.

Summary. Adult male albino rats administered a vitamin B-complex deficient diet over a period of from 6 to 13 weeks failed to develop histologic evidence of hepatic cirrhosis, in spite of the fact that they did develop the usual signs of B-complex deficiency. Data as to food and water intake as well as weight changes are presented.

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Effect of Pantothenic Acid Alone and in Natural Products on Nutritional Achromotrichia in Rats.

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Within the last year much attention has been centered around the rôle of pantothenic acid[1-4] in prevention and cure of nutritional achromotrichia. That pantothenic acid can be identified *in toto* with the anti-graying potency of natural products, such as rice bran, liver and yeast, has been claimed by certain workers[1, 3] and denied by others.[2, 4, 5] Recently György[6] has named biotin (vitamin H) as an additional factor in normal pigmentation, but the evidence presented was not entirely conclusive. Results of work in our laboratory

10 Spellberg, M. A., and Keeton, R. W., *Am. J. Med. Sc.*, 1940, **200**, 688.

1 György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, 1940, **132**, 789.

2 Dimick, M. K., and Lepp, A., *J. Nutrition*, 1940, **20**, 413.

3 Unna, K., and Sampson, W. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 309.

4 Williams, R. R., *Science*, 1940, **92**, 561.

5 Nielsen, E., Oleson, J. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **133**, 167.

6 György, P., and Poling, C. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 773.