in tolerance between various species of animals and also among the individuals of the same species. The observation that single blood transfusions in dogs in a state of extreme depression of the prothrombin level were of only temporary benefit, is paralleled by similar clinical findings[11, 8] and calls for particular care in avoiding excessively high dosage.

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Comparative Studies of the Effect of Thiamine Deficiency in Diabetic and Non-Diabetic Rats.

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Certain workers[1, 2, 3] have claimed amelioration of human diabetes with the use of thiamine, particularly when given in combination with other vitamins. Martin[4] reported intensification of diabetes in depancreatized dogs on a vitamin B-free diet, with remarkable improvement in carbohydrate tolerance when thiamine and flavin were given. On the other hand, in normal rats, Lepkovsky, Wood and Evans[5] found the glucose tolerance impaired only in the later stages of deficiency and McIntyre and Burke[6] noted very little change in glucose tolerance, either with deficiency or excess of vitamin B.

The present study was undertaken to determine the effect of deprivation of thiamine on the diabetic condition of depancreatized rats and to ascertain whether or not diabetic animals, when deprived of thiamine, develop signs of deficiency more readily than those without diabetes. It was hoped that the results obtained might elucidate the possible rôle of vitamin B, and specifically of thiamine, in the peripheral neuritis encountered in patients with severe, uncontrolled diabetes.[7]

1 Vorhaus, M. G., Williams, R. R., and Waterman, R. E., *Am. J. Digest. Dis. and Nutrition*, 1935, **2**, 541.

2 Labbe, M., Nepveux, F., and Gringoire, J. D., *Bull. Acad. de Med.*, 1933, **109**, 689.

3 Mosonye, J., and Aszodi, Z., *Klin. Wchnschr.*, 1938, **17**, 337.

4 Martin, R. W., *Z. Physiol. Chem.*, 1937, **248**, 242.

5 Lepkovsky, S., Wood, C., and Evans, H. M., *J. Biol. Chem.*, 1930, **87**, 239.

6 McIntyre, A. R., and Burke, J. C., *J. Pharm. and Exp. Therap.*, 1938, **64**, 465.

7 Joslin, E. P., Root, H. F., White, P., and Marble, A., *Treatment of Diabetes Mellitus*, 7th Edition, Philadelphia, Lea & Febiger, 1940, 254-257.

This neuropathy clinically seems comparable to the peripheral neuritis in known vitamin B deficiency associated with beriberi, alcoholism, pregnancy and gastro-intestinal disturbances. In this connection, Meiklejohn,[8, 9] citing the findings of certain recent investigators,[10, 11] concludes that polyneuritis results not from a deficiency of thiamine, but from a lack of other factors present in whole yeast. He states that the only certain consequence of pure thiamine lack is a disturbance of carbohydrate metabolism, resulting in impaired glucose tolerance and the accumulation of lactic and pyruvic acids in the tissues.

Methods. Male white rats of the Yale strain, from 3 to 10 months old, were used. All had been depancreatized at the age of 27 to 37 days.[12] Diabetes was proven in all rats by glucose tolerance tests, although not all animals excreted sugar on the ordinary dog chow diet. The rats were placed in individual metabolism cages. Food, water, urine and urinary sugar were measured daily and the animals were weighed twice weekly.

Prior to the metabolism study, all diabetic animals were maintained on a stock dog chow* diet, with a daily supplement of 5 g of raw beef pancreas. At the start of the experiment they were placed on a vitamin B-free ration consisting of sucrose 54%, casein (Labco, vitamin-free) 35%, Crisco (Primex) 5%, cod liver oil 2%, and Phillips & Hart salt mixture No. 1[13] 4%. The available carbohydrate of this diet is 75%.

Glucose tolerance tests were done under narcosis with sodium pentobarbital after a fast of 17 hours. Blood from the tail for determination of sugar content was taken fasting and at intervals of ½, 1, 2, 3, and 5 hours after the intraperitoneal injection of 0.35 g of glucose per 100 g body weight in the form of 10% solution. The sugar estimations were done by the Folin-Malmros method.[14]

Fourteen diabetic and 5 non-diabetic rats were maintained for 3 to 4 weeks on the diet outlined above together with 25 γ of thiamine,

8 Meiklejohn, A. P., *New Eng. J. Med.*, 1940, **223**, 265.

9 Meiklejohn, A. P., *New Eng. J. Med.*, 1941, **224**, 420.

10 Williams, R. D., Mason, H. L., Wilder, R. M., and Smith, B. F., *Arch. Int. Med.*, 1940, **66**, 785.

11 Elsom, K. O'S., Lukens, F. D. W., Montgomery, E. H., and Jonas, L., *J. Clin. Invest.*, 1940, **19**, 153.

12 Shapiro, R., and Pincus, G., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 416.

* Pratt's, stated by the manufacturers to contain crude protein 22%, crude fat 3%, crude fibre 5%, carbohydrate 61% and nitrogen-free extract 56%.

13 Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

14 Folin, O., and Malmros, H., *J. Biol. Chem.*, 1929, **83**, 115.

20 γ of riboflavin, 200 mg of autoclaved rice polish concentrate and 50 mg of choline chloride daily. At the end of this period control glucose tolerance tests were carried out. Following this, the rats were deprived of thiamine while continuing to receive the other supplements daily as before.

Results. Anorexia, lassitude and weight loss appeared at about 10 days after deprivation of thiamine. The acute stage of deficiency ("polyneuritis") characterized chiefly by paralysis of the hind legs, inability to walk and (if untreated) death within several hours, was reached on the average at about the 40th day of thiamine deprivation. With the diabetic animals this averaged 39 days and with the non-diabetic rats, 45 days. If, however, one non-diabetic rat which carried on for 65 days be excluded, the average length of time for this group was 41 days. Furthermore, that the diabetic animals were not in as good general condition as the non-diabetic rats at the start of the experiment is shown by the fact that the average weight of the diabetic rats was 301 g as compared with that of 405 g for the non-diabetics, the ages of which were comparable. No significant correlation between the severity of diabetes and the time of development of signs of deficiency was apparent.

At the beginning of the period of deprivation, 9 of the 14 diabetic rats were excreting sugar in the urine. As anorexia developed and the food intake became less and less, glycosuria gradually diminished. Glucose tolerance tests done just before the period of deprivation and at approximately 10-day intervals until the development of polyneuritis, showed no consistent change. The tests performed on the 14 diabetic rats after approximately 3 weeks of deprivation showed an impairment of tolerance in 5 animals, improvement in 3 and no significant change in 6. No significant change in tolerance was noted among the 5 non-diabetic rats studied after 3 weeks of deprivation. Two diabetic and two non-diabetic rats, with which it was possible to carry out glucose tolerance tests at the time of acute polyneuritis, showed impairment.

When signs of marked deficiency had definitely developed, each animal not sacrificed was given 100 γ of thiamine orally or subcutaneously daily for 10 days. Improvement began in 4 to 8 hours and recovery from weakness and anorexia took place within 2 or 3 days of therapy. The animals gained weight rapidly. After 10 days the thiamine allowance was reduced to the original amount of 25 γ daily for 2 or 3 weeks. Then to 5 of the animals, thiamine in excess (200 γ daily) was given for 10 days. Glucose tolerance tests carried out before and after the administration of 200 γ of thiamine

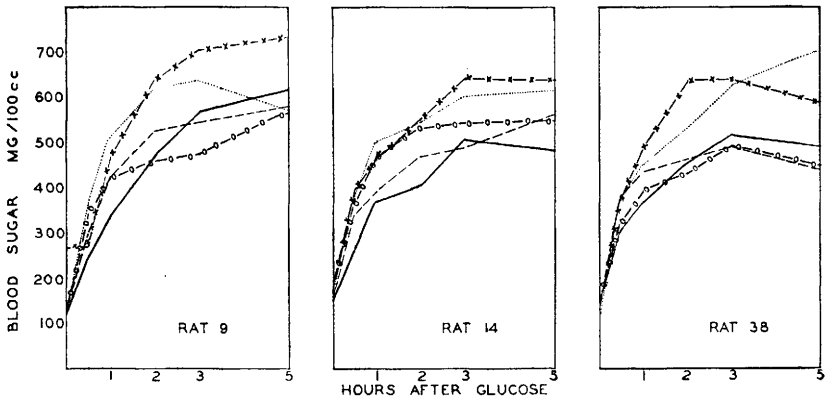

FIG. 1.

Improved Glucose Tolerance in 3 of 5 Diabetic Rats Given Excess Thiamine Following Period of Deprivation.

——— Control glucose tolerance curve.
..... G.T. curve after 23 days deprivation of thiamine.
–o–o–o G.T. curve during recovery period, after 6 days 100 γ thiamine daily.
–x–x–x G.T. curve after return to maintenance dose of 25 γ thiamine daily for 2-3 weeks.
– – – – G.T. curve after 10 days resumption of excess thiamine, 200 γ daily.

daily showed a significant improvement in tolerance in 3 of the animals when compared with that following the period of 25 γ daily. (See Fig. 1.)

Conclusions. 1. No significant difference was observed in the length of time required by diabetic and non-diabetic rats on a thiamine-free diet to develop signs of marked deficiency (39 as compared with 41 to 45 days respectively). Following treatment with thiamine, the diabetic rats recovered as quickly as did the non-diabetic animals. 2. During the period of deprivation, the glycosuria of the diabetic rats diminished, presumably because of the lowered food intake. However, urinary sugar tended to reappear at the final stage of deficiency at the time of "polyneuritis". Carbohydrate tolerance in both the diabetic and non-diabetic rats, as judged by glucose tolerance tests, showed little change until signs of marked deficiency appeared, when there was impairment of tolerance. 3. Following deprivation, a thiamine intake 4 to 8 times the maintenance dosage appeared to improve carbohydrate tolerance.