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A Study of the Effect of Alloxan Diabetes on the Riboflavin Requirements of Young Rats.*

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Clinical as well as experimental evidence has at various times been interpreted to indicate that various members of the vitamins of the B complex may be useful in the control of diabetes mellitus or that the vitamin requirements of the diabetic animal are abnormally high. It would not be surprising if this were true in view of the well known functions of thiamine, riboflavin and niacin in the metabolism of carbohydrates.[1-3] Recent evidence implicates pantothenic acid[4] and biotin[5-8] in carbohydrate metabolism as well, and choline might be important indirectly in glucose metabolism through its effect upon liver function.

However, all of the clinical data[9-17] may be criticized on the basis that non-diabetic dietary controls were not available for study. The mere fact that the diabetic patient responds favorably to the administration of a vitamin does not necessarily indicate anything abnormal in the requirements or function of the vitamin but may simply reflect an inadequacy of the dietary intake as in any other individual. The experimental evidence is also unsatisfactory. Several authors[15,18-22] have reported changes in the glucose tolerance, the blood sugar level, and the effectiveness of insulin when various vitamins were given to diabetic animals, but again the dietary control has been usually inadequate.

In a previous study from this laboratory[23] the thiamine requirement of the alloxan diabetic rat was found to be approximately the same as the non-diabetic controls and appeared to be related, as in the normal animal,

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1 Baldwin, E., Dynamic Aspects of Biochemistry, The Macmillan Company, New York, 1947.

2 Sumner, J. B., and Somers, G. F., Chemistry and Methods of Enzymes, New York, Academic Press, Inc., 1947.

3 Soskin, S., and Levine, R., Carbohydrate Metabolism, Chicago, University of Chicago Press, 1946.

4 Pilgrim, F. J., Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **145**, 237.

5 Lichstein, H. C., and Umbreit, W. W., *J. Biol. Chem.*, 1947, **170**, 329.

6 Ochoa, S., Mehler, A., Blanchard, M. L., Jukes, T. H., Hoffmann, C. E., and Regan, M., *J. Biol. Chem.*, 1947, **170**, 413.

7 Summerson, W. H., Lee, J. M., and Pattridge, C. W. H., *Science*, 1944, **100**, 250.

8 Lardy, H. A., Potter, R. L., and Elvehjem, C. A., *J. Biol. Chem.*, 1947, **169**, 451.

9 Vorhaus, M. G., Williams, R. R., and Waterman, R. E., *J.A.M.A.*, 1935, **105**, 1580.

10 Sciclounoff, F., *Rev. Med. Suisse Rom.*, 1940, **60**, 1243.

11 Mosonyi, J., and Aszodi, Z., *Klin. Woch.*, 1938, **17**, 337.

12 Sydenstricker, V. P., Geeslin, L. E., and Weaver, J. W., *J.A.M.A.*, 1939, **113**, 2137.

13 Neuwahl, F. J., *Lancet*, 1943, **2**, 348.

14 Gottlebe, P., *Zeit. f. Klin. Med.*, 1938, **133**, 739.

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

16 Schroeder, H., *Z. f. Ges. Exp. Med.*, 1937, **101**, 373.

17 Stepp, W., Schroeder, H., and Altenburger, E., *Klin. Woch.*, 1935, **14**, 933.

18 Mosonyi, J., and Aszodi, Z., *Klin. Woch.*, 1938, **17**, 337.

19 Aszodi, Z., and Mosonyi, J., *Klin. Woch.*, 1937, **16**, 1214.

20 Martin, R., *Z. Physiol. Chem.*, 1937, **248**, 242.

21 Tislowitz, R., *Klin. Woch.*, 1937, **16**, 226.

22 Magyar, I., *Zeit. f. Ges. Exp. Med.*, 1938, **104**, 495.

23 Lowry, P. T., and Hegsted, D. M., *J. Lab. Clin. Med.*, 1945, **30**, 839.

TABLE I.
Comparison of the Rate of Development of Riboflavin Deficiency in Diabetic and Non-diabetic Rats.

Group	Loss of maximum wt in 10 wk, %	Time to reduce food intake to 50%, weeks	Time before wt loss began, days
Diabetic	20.8 ± 1.5*	8.2 ± 0.77*	33.3 ± 3.74*
Non-diabetic	20.9 ± 5.7	8.0 ± 1.0	32.0 ± 3.8

* Mean ± standard deviation.

with the amount of glucose metabolised.

The present study represents an attempt to determine whether the riboflavin requirements of the rat are affected by alloxan diabetes. This appeared worthy of study since (a) the proportion of fat, protein and carbohydrate metabolised is changed during diabetes and this may affect the riboflavin requirements,[21-26] (b) the development of cataracts is common in the diabetic rat[27-28] and in riboflavin deficient rats in some laboratories,[29-31] and (c) the marked diuresis during the course of alloxan diabetes might influence the rate of excretion of the water soluble vitamins especially after the parenteral administration of relatively large amounts of these vitamins.

Experimental. Twenty-six young female rats weighing between 90 and 110 g were divided into two main groups. The first group of 14 animals was rendered severely diabetic by the intravenous administration of 50 mg alloxan monohydrate per kilo body weight. Five of these animals were used as diabetic controls and were given an ordinary purified diet.[32] The remainder of the diabetic rats received the same diet except that the riboflavin supplement was omitted. Twelve non-diabetic rats on the same riboflavin deficient diet were used as controls.

All rats were kept in individual cages and food and water given *ad libitum*. Daily records of weight, food, and water intake were kept and 3-day urine samples were collected through the whole experimental period and analyzed for glucose, using the method of Somogyi.[33]

After the animals had become deficient, as indicated by their weight, they were injected with therapeutic doses of riboflavin. The growth response, change in food and water intake and urinary sugar, and excretion of riboflavin in the urine were studied. Urinary riboflavin was determined by a slight modification of the fluorometric method of Ferrebee[34] using the permanganate, sodium hydrosulfite procedure.

When the animals again began to lose weight after the therapeutic test, they were all injected with another dose of riboflavin. Three tests were thus obtained with each animal. The first dose of 160 mcg of riboflavin per 100 g body weight was given on the 70th day of the experiment. The second dose of 80 mcg per 100 g body weight was given 3 weeks later and the final dose of 40 mcg was given 16 days after the second. The urinary riboflavin during the 6 days prior to riboflavin injections ranged from 2.2 to 3.2 mcg per day per rat in both the diabetic and the non-diabetic rats.

Results. Table I indicates the data obtained during the depletion period. As may

24 Unna, K., Singher, H. O., Kensler, C. J., Taylor, H. C., and Rhoads, C. P., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 254.

25 Sarett, H. P., Klein, J. R., and Perlzweig, W. A., *J. Nutrition*, 1942, **24**, 295.

26 Mannering, G. J., Lipton, M. A., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 100.

27 Bailey, C. C., Bailey, O. T., and Leech, R. S., *New Eng. J. Med.*, 1944, **230**, 533.

28 Foglia, V. G., and Cramer, F. K., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 218.

29 O'Brien, C. S., *Arch. Ophthal.*, 1932, **8**, 880.

30 Baum, H. M., Michaelree, J. F., and Brown, E. B., *Science*, 1942, **95**, 24.

31 Bessey, O. A., and Wolbach, S. B., *J. Exp. Med.*, 1939, **69**, 1.

32 Riggs, T. R., and Hegsted, D. M., *J. Biol. Chem.*, 1948, **172**, 539.

33 Somogyi, M., *J. Lab. Clin. Med.*, 1941, **26**, 1220.

34 Ferrebee, J. W., *J. Clin. Invest.*, 1940, **19**, 251.

TABLE II.
Comparison of the Response of Riboflavin Deficient Diabetic and Non-diabetic Animals to Various Levels of Riboflavin.

Riboflavin injected mcg/100 g body wt	Response period, days	Gain Coefficient		Wt Gain		Increase in food intake		Increase in water intake		Increase in riboflavin excretion	
		Diabetic	Control	Diabetic %	Control %	Diabetic %	Control %	Diabetic %	Control %	Diabetic mcg	Control mcg
160	1	3.46	2.78	19.4	23.9	205	131*	452	148*	20	17
	2-5					229	208	518	156	16	25
80	1	2.55	2.13	11.8	20.5*	81	83	366	159*	7	10
	2-5					33	84*	88	107	11	11
40	1	1.05	1.11	3.4	7.6	59	54	211	149	8	6

* Significant difference (probability 0.05 or less).

be seen, the diabetic and non-diabetic animals developed the deficiency in the same period of time as indicated by the 3 criteria used, *i.e.*, loss of weight in 10 weeks, and the time required to show a loss in weight and reduction of food intake to 50% of the original intake.

The therapeutic responses were difficult to compare because of the fact that the diabetic rats, whether deficient or not, showed a reduced rate of gain in comparison to the non-diabetic animals. In order to obviate this difficulty to the best of our ability, we have compared the diabetic and non-diabetic rats not to each other but to themselves prior to the development of the deficiency, or as the percent response caused by the dose of riboflavin. In Table II the "gain coefficient" is the ratio of the daily gain during the first 5 days after the injection of riboflavin to the daily gain of the animal during the first 21 days of the study before riboflavin became a limiting factor. As may be seen, the diabetic animals showed only slightly larger relative gains in 2 of the test periods, but in no instance were they significantly different from the non-diabetic animals. The percentage increase in weight was not significantly different at the 160 and 40 mcg levels of riboflavin, but at 80 mcg the non-diabetic rats gave significantly greater gains.

Because of the immediate large increase in food and water intake following the administration of riboflavin to the diabetic animals, it appeared desirable to study the response during the first day and the subsequent 4 days as shown in Table II. The increase in food and water intake was greater in the diabetic group than in the non-diabetic group in several periods although in one period the control group showed a significantly greater food intake and somewhat larger water intake. It is doubtful if this is of real significance since as the deficiency develops and appetite fails, the urinary sugar of diabetic animals falls to low and nearly normal levels. When riboflavin is given the animals revert to their prior state in which sugar and water output are much above normal levels.

The increase in riboflavin excretion in the

urine above the level prior to riboflavin injection was in no instance significantly different in the diabetic and non-diabetic groups. It seems apparent that the large urinary volume in the diabetic animals do not "wash out" riboflavin to a significant degree, if at all. Attempts to find a significant correlation between the amount of riboflavin retained and food intake, water intake, gain after dosage with riboflavin, or loss of body weight during the depletion period were unsuccessful.

All diabetic animals including those which received riboflavin developed cataracts after 55 to 65 days while none of the non-diabetic riboflavin-deficient animals developed cataracts during the 110 days of the study.

Conclusion. Studies upon the rate of development of riboflavin deficiency and the response to therapeutic doses of riboflavin, after the development of the deficiency syndrome, failed to show any significant difference in the riboflavin requirement of the alloxan diabetic and the non-diabetic rat. Similarly, riboflavin deficiency was apparently without effect upon the development of cataracts in the diabetic animals.

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Intratesticular Versus Subcutaneous Transplantation of Brown-Pearce Tumor in New Zealand Whites.

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Published data[1] on the susceptibility of various rabbit breeds to the Brown-Pearce tumor failed to include the breed most readily procurable on the open market, namely, the New Zealand White. During the past several years 108 New Zealand white male rabbits have been used as controls or stock animals in experiments with the Brown-Pearce tumor, 42 being inoculated intratesticularly and 66 subcutaneously.

Materials and methods. The 66 animals inoculated subcutaneously belonged to 16 different groups distributed throughout the year as follows: January 24 (1), March 6 (2), March 20 (3), May 22 (5), June 23 (8), June 24 (8), July 24 (9), August 11 (4), September 6 (10), October 9 (8), October 31 (3), November 15 (2), December 13 (1), and December 30 (2).

The 42 animals inoculated intratesticularly belonged to 16 groups distributed as follows: January 24 (1), February 28 (6), June 9 (1), June 23 (1), July 17 (1), August 3 (4), August 30 (2), September 16 (1), September 24 (3), October 16 (2), November 9 (3), November 20 (1), November 22 (3).

The 7 animals comprising the two groups inoculated subcutaneously August 11 and October 31 were 3 months of age, others inoculated subcutaneously and all of those inoculated intratesticularly were animals of 4-12 months of age. Animals inoculated subcutaneously and those inoculated intratesticularly were obtained in almost equal proportions from the same breeders or dealers in Alabama, Louisiana, Illinois, Virginia and Pennsylvania.

As in the earlier studies,[2] a metastatic focus was defined as the presence of one or more tumor nodules at necropsy in any of the

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1 Casey, Albert E., *Am. J. Cancer*, 1937, **21**, 446.

2 Casey, Albert E., *Am. J. Cancer*, 1934, **21**, 760; 1936, **26**, 276.