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Role of Acetoacetate in Aggravating the Disturbances in the Carbohydrate Metabolism of Scorbutic Animals

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Depletion of vitamin C has been reported by Banerjee & Ghosh (1947) to disturb carbohydrate metabolism. It has also been shown by Attenburger (1936), Shimamura (1938) and Amorelli & D'Ambrosio (1938) that vitamin C is necessary for glycogenesis and that its deficiency in the system brings about a marked depletion in glycogen content of the liver as well as of the muscle. The recent findings of Nath & Chakrabarti (1951) in this laboratory have indicated that repeated injections of acetoacetate cause a marked depletion in the blood vitamin C level of rabbits. The present work was, therefore, undertaken by the authors to study the changes in carbohydrate metabolism following acetoacetate injections in their animals. The present paper describes the increase in blood sugar and rapid depletion in glycogen storage of guinea pigs which were completely denied vitamin C in their diet and received daily acetoacetate in gradually increased amounts. The effect on vitamin C excretion of guinea pigs under different experimental conditions was also studied.

EXPERIMENTAL

A group of forty-eight normal male guinea pigs with an average weight of 464 g. was selected for the pre-experimental period. Each one of them was given green grass and

germinated gram in the morning and the scurvy-producing diet of Banerjee (1945) with slight modification (wheat being substituted for barley) in the evening, and this was continued for 3 days. From the fourth day the green grass and germinated gram, the sources of vitamin C, were discontinued and all the guinea pigs were fed *ad lib.* the scurvy-producing diet for 1 week, supplemented daily with 5 mg. of ascorbic acid given orally, and every third day two drops of a concentrated solution of vitamins A and D (Adexolin; Glaxo Laboratories) were given to each. The weights of the animals were recorded every third day and only those which were found to be gaining in weight were finally selected for the experimental period. The animals thus selected were divided into different groups in such a manner that the average weight of each group was nearly the same. The first group served as control and was kept on the basal diet with a daily oral supplement of 5 mg. ascorbic acid per day to each. The second group was denied vitamin C and was given only the scurvy-producing diet. The animals in the third group were also completely denied vitamin C, but received daily acetoacetate in increasing amounts (175 mg./kg./day given intraperitoneally and increased by 40 mg. every third day so that at the end of the experimental period of 21 days the dose was 415 mg./kg.). All the above three groups were further divided into sub-groups A and B for the observation of blood-sugar values and glycogen storage, respectively. To the fourth group, denied vitamin C, glucose was given daily in a constant dose of 400 mg./kg. (intraperitoneally).

Effect on blood sugar following acetoacetate injections in increasing amounts to guinea pigs completely denied vitamin C

in their diet. The guinea pigs of groups 1A (control), 2A (vitamin C-deficient) and 3A (vitamin C-deficient and receiving acetoacetate) were taken for observations to study the effect of daily administration of acetoacetate, in increasing amounts, on the blood-sugar values of vitamin C-deficient guinea pigs. The blood was taken with the help of a syringe from the heart of the animals. The blood sugar was estimated by the method of Hagedorn & Jensen (1923).

The results are shown in Table 1.

Rapid glycogenolysis in liver and leg muscle of vitamin C-deficient guinea pigs receiving daily acetoacetate injections in increasing amounts. Guinea pigs of groups 1B (control), 2B (vitamin C-deficient), 3B (vitamin C-deficient, given acetoacetate) and 4 (vitamin C-deficient, given glucose) were used to observe the depletion in glycogen content of liver and leg muscle of guinea pigs denied vitamin C and injected with sodium acetoacetate. On the 13th day of the experimental period three guinea pigs from each of the groups 1B, 2B, 3B and 4 were taken to find the glycogen content in liver and in leg muscle. As recommended by Simpson & Macleod (1927), every care was taken to mini-

mize post-mortem glycogenolysis. The animals were anaesthetized by Nembutal prior to their killing. The whole liver was taken out and the left or right sciatic nerve was then severed. The leg muscle (vastus medialis) was cut longitudinally. The longitudinal incision is essential because by so doing little injury is done to the leg-muscle fibres and thus glycogenolysis during the process is checked. The tissue samples were quickly excised, weighed and immersed in hot KOH solution (30% w/v). The glycogen content was estimated according to the method of Pflüger as modified by Good, Kramer & Somogyi (1933). Again, on the twenty-first day of the experiment, the remaining 12 guinea pigs (three from each of the groups 1B, 2B, 3B and 4) were taken and the glycogen stored in their livers and leg muscles estimated as above. In all the above estimations of glycogen storage, the animals were kept fasting for nearly 18 hr. before they were killed. The results are shown in Table 2.

Urinary vitamin C excretion of guinea pigs completely denied vitamin C and injected with increasing amounts of acetoacetate. The urinary excretion of vitamin C was estimated by the method of Roe & Keuther (1943). The

Table 1. *Average blood-sugar values of guinea pigs on normal and vitamin C-deficient diet and on vitamin C-deficient diet with administration of acetoacetate*

Group	No. of guinea pigs	Experimental conditions	Average blood-sugar values (mg./100 ml.)		
			Initial	13th day	21st day
1A	3	Normal (on a basal diet with 5 mg./day of vitamin C)	110.3±0.3	111±0.3	111±0.3
2A	4	Vitamin C denied	111 ±2.3	115±1.9	118±2.5
3A	6	Vitamin C denied and given acetoacetate	111 ±1.7	142±7.0	170±11.9

Table 2. *Effect of repeated acetoacetate administrations on the glycogen content of liver and leg muscle of guinea pigs which were completely denied vitamin C in their diet*

Group	No. of guinea pigs	Experimental conditions	Days of experimental period	Glycogen content (mg./100 g. tissue)	
				Liver	Leg muscle
1B	3	Normal	0	408±8.7	314±12.3
	3	Normal (on a basal diet with 5 mg./day of vitamin C)	21	428±13.5	335±12.2
2B	3	Vitamin C denied	13	118±10.2	260±8.7
	3		21	65±4.1	129±9.0
3B	3	Vitamin C denied; sodium acetoacetate administered	13	54±5.2	167±11.2
	3		21	12±5.9	50±8.0
4	3	Vitamin C denied and given glucose	13	122±5.9	264±5.3
	3		21	69±3.9	132±5.7

Table 3. *Showing urinary vitamin C excretion of vitamin C-deficient guinea pigs as effected by repeated intraperitoneal injections of acetoacetate administered daily in increasing amounts*

Group	No. of animals	Experimental conditions	Average values of urinary vitamin C excretion of guinea pigs (mg./day) after (days of experiment)									
			0	13	14	15	16	17	18	19	20	21
1A and 1B	9	Normal (on a basal diet with 5 mg./day of vitamin C)	1.0	1.43	1.48	1.5	1.55	1.58	1.61	1.63	1.67	1.69
2A and 2B	10	Vitamin C denied	1.0	0.67	0.66	0.62	0.58	0.55	0.52	0.48	0.40	0.38
3A and 3B	12	Vitamin C denied; given acetoacetate	1.1	0.44	0.43	0.41	0.37	0.33	0.28	0.23	0.21	0.18
4	6	Vitamin C denied; given glucose	1.1	0.68	0.67	0.62	0.60	0.55	0.52	0.48	0.41	0.38

Table 4. *Showing the average weights of the animals during the experimental period*

Group	No. of animals	Experimental conditions	Average weights (g.) on different days of experiment							
			0	3	6	9	12	15	18	21
1A and 1B	9	Normal (on a basal diet with 5 mg./day of vitamin C)	450	460	473	478	498	513	520	530
2A and 2B	10	Vitamin C denied	458	462	465	461	470	457	440	381
3A and 3B	12	Vitamin C denied; given acetoacetate	454	452	455	455	458	453	435	374
4	6	Vitamin C denied; given glucose	445	450	455	458	460	450	440	385

24 hr. urine of the animals was collected in flasks in which was kept 0.8 g. of trichloroacetic acid. Before the estimation was made the funnels of the metabolism cages were carefully washed with distilled water and the washings added to the respective flasks. The volume in each flask was then adjusted so that the final concentration of the trichloroacetic acid was 4% (w/v). The results are shown in Table 3.

The average weights of the animals of the respective groups 1, 2, 3 and 4 on different days of the experimental period of 21 days are given in Table 4.

DISCUSSION

As is clear from Table 1, the fasting blood-sugar values of normal and scorbutic guinea pigs did not differ significantly, but after administration of acetoacetate to the vitamin C-deficient guinea pigs there was observed a very appreciable rise in the blood-sugar values of the animals. Again in Table 2 there is seen the depletion in glycogen content of the liver and leg muscle of animals denied vitamin C in their diet, but the intraperitoneal injections of acetoacetate to the guinea pigs denied vitamin C brings about a very rapid depletion in the glycogen storage of both the liver and the leg muscle. Thus, acetoacetate was observed to cause a very significant disturbance in carbohydrate metabolism when administered to animals deficient in vitamin C. Vitamin C seems to be very necessary for glycogenesis, for the animals fed on the basal diet supplemented with vitamin C show an increase in their glycogen content but the complete absence of vitamin C from the diet brings about a depletion in the glycogen storage even though glucose was injected daily in an appreciable amount. Table 3 indicates the marked destruction of vitamin C in the body of the animals receiving repeated acetoacetate injections. Thus, acetoacetate was observed to destroy vitamin C which is known to play an important beneficial role in the carbohydrate metabolism. Banerjee (1944) has observed that in scorbutic animals the insulin content of the pancreas is reduced and the β cells show definite signs of degeneration. Mosenthal & Loughlin (1944) found plasma vitamin C levels of less than 0.8 mg./100 ml. in 43% of more than 100 diabetic children studied. Pfleger & Scholl (1937) and Ergele (1939) demon-

strated a hypovitaminosis C in diabetic patients. Further, it has been shown by Griffith & Eames (1944) that a low excretion of vitamin C is associated with diabetes. Severe depletion of liver glycogen as observed by liver biopsy has been reported by Bondy & Sheldon (1947) in human diabetes. Further, the interesting observation of Nath & Chakrabarti (1951) that repeated injection of acetoacetate causes a marked depletion of vitamin C in the blood of experimental animals, shows how the accumulation of ketone bodies is directly responsible for the development of scorbutic conditions in the animals and then, as shown by us, they bring about a condition of hyperglycaemia and other related symptoms. Thus acetoacetate alone, even without the use of the scurvy-producing diet, brings about very marked disturbances in the carbohydrate metabolism of normal animals and when administered to animals kept on a scurvy-producing diet, the disturbances are observed to be still further considerably aggravated.

SUMMARY

1. Some changes in carbohydrate metabolism as effected by repeated administrations of acetoacetate, in increasing amounts, to guinea pigs denied vitamin C were studied.

2. Repeated intraperitoneal acetoacetate injections administered to guinea pigs kept on a scurvy-producing diet causes a rapid development of hyperglycaemia in the animals.

3. A rapid depletion of glycogen storage of liver and leg muscle is observed in guinea pigs denied vitamin C following the repeated injections of acetoacetate.

4. A low excretion of vitamin C is found in the guinea pigs given acetoacetate, thus showing the destruction of vitamin C by acetoacetate *in vivo*.

5. It has thus been shown how an intermediary fat metabolite (the acetoacetate), which causes such marked disturbances in carbohydrate metabolism, may very well be considered as one of the factors in the aetiology of diabetes.

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