

CARBOHYDRATE METABOLISM IN THE ASCORBIC ACID-DEFICIENT GUINEA PIG UNDER NORMAL AND ANOXIC CONDITIONS

By HAZEL C. MURRAY* AND AGNES FAY MORGAN

(From the Department of Home Economics, University of California, Berkeley)

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Although there has been much effort expended in the study of the possible rôle of ascorbic acid in carbohydrate metabolism, this relationship remains obscure.

Sigal and King (1) showed that the glucose tolerance was markedly lowered in a group of guinea pigs deficient in ascorbic acid for 10 days, that the tolerance fell further through the 20 day period during which the deficiency was maintained, and that treatment with ascorbic acid returned the tolerance to normal in 15 days. Altenberger (2) noted that the liver glycogen of scorbutic guinea pigs was lower than that of normal animals. Giroud and Ratsimamanga (3) also found liver and muscle glycogen in ascorbic acid-deficient guinea pigs lowered in direct ratio to the duration of the deficiency and the muscle phosphocreatine decreased. Nair (4) reported decreased glucose tolerance and liver glycogen in scorbutic guinea pigs. Hamne (5) obtained evidence with pair-fed guinea pigs that in chronic scurvy the glycogen content of the liver and muscle was lower than in the normal animal. The liver glycogen was decreased in the early stages of scurvy and the muscle glycogen at a later stage, while the glycogen of the heart was not affected.

Involvement of the adrenal and thyroid glands in the changes incident to ascorbic acid deficiency has been suggested. La Mer and Campbell (6) first reported increased size of the adrenals in scorbutic guinea pigs, and this has been confirmed by several other investigations (7-9). The most recent report is that of Baldwin, Longenecker, and King (10), who found in a series of twenty-five pairs of matched guinea pigs that the average weight of the adrenal glands of the normal animals was 182 mg. and that of ascorbic acid-deficient animals 257 mg. The earlier literature contains many reports of degenerative and congestive changes, including lipid deposition, in the adrenals of scorbutic guinea pigs, but in nearly all of these studies the effect of inanition was not ruled out. MacLean, Sheppard, and McHenry (11) compared the tissues of scorbutic guinea pigs with those of normal animals which had been restricted to the reduced food intake of

* Present address, State College of Washington, Pullman, Washington.

the deficient group and found by microscopic examination no differences in the adrenal glands. Baldwin, Longenecker, and King (10), likewise using paired animals, found increased size of the adrenals of the deficient animals but no changes in their lipid content.

No studies of the effect of ascorbic acid deficiency upon the behavior of animals under anoxia could be found. When Sacerdote (12) subjected normal guinea pigs and rabbits to mixtures of nitrogen and oxygen in which the concentration of the latter was gradually reduced to 3 per cent in 10 hours, there occurred a notable increase in the ascorbic acid level of the blood and a decrease in the ascorbic acid content of the adrenals. No observations were made on the carbohydrate metabolism.

Wertheimer (13) found no change in the blood sugar of guinea pigs, presumably normal, kept for 3 to 11 days at 340 mm. pressure, little change in the liver glycogen, and none in the muscle glycogen.

The response to anoxia of pair-fed normal and scorbutic guinea pigs might be expected to offer some index of the condition of the adrenal glands, since it is now believed (14) that adaptation to anoxic conditions is dependent on the mediation of that gland.

Several reports of abnormal oxygen consumption in the later stages of scurvy have appeared. Mosonyi and Kézdi (15) attributed this to hyperactivity of the thyroid, as did Hamne (5). Spence and Scowan (16), however, found no hyperplasia of the thyroid in acute scurvy and not in all cases of chronic scurvy. Törnblom (17) reported diminished oxygen consumption toward the end of the deficiency state. These contradictory observations made it seem worth while to study the oxygen consumption of pair-fed normal and scorbutic guinea pigs.

This experiment was undertaken to determine the extent of carbohydrate absorption, blood sugar levels, glycogen content, and oxygen consumption of normal and scorbutic guinea pigs under normal atmospheric pressure, and their utilization of carbohydrate under moderate anoxia. Some observations were also made on the weights of adrenal glands of normal and severely deficient animals and on the lipid content of livers and carcasses.

EXPERIMENTAL

Young guinea pigs (350 to 400 gm.) of both sexes were used. They were obtained from the laboratory colony and were paired carefully as to weight, sex, and litter origin. One of each pair was given no ascorbic acid but was allowed to consume the basal diet *ad libitum*. Each day the amount consumed was ascertained and the normal member of the pair allowed only that amount on the succeeding day.

The basal diet was a commercial rabbit food¹ which has been found satisfactory for the stock colony when supplemented with ascorbic acid. In addition to the feed, the animals were each given 5 gm. of wheat germ and 0.5 gm. of gray fish oil (20,000 units of vitamin A per gm.) weekly. The normal animals were given by pipette 10 mg. of ascorbic acid three times a week. All the animals were kept at a temperature of 25–30°.

The animals kept on this diet without ascorbic acid supplements developed the first loss of appetite usually on the 14th day and definite scurvy in 19 to 24 days, as was evidenced by extensive hemorrhages of the fascia of the musculature, particularly of the legs. The deficient animals often lived 35 to 40 days or longer after the ascorbic acid supplement was removed from the diet. The progress of the deficiency varied so that guinea pigs, maintained different lengths of time on the basal diet, presented similar stages of scurvy, as manifested by loss of appetite and of weight, inactivity, soreness of joints, and unkemptness of fur. The attempt was made to institute the final determinations on all the animals at the same stage of the deficiency so far as this was possible. Because of the abrupt loss of weight of the scorbutic animals within a day or two of the onset of the symptoms, the final weight of the normal members of the pairs was usually 50 to 60 gm. the greater.

When the deficient member of the pair was judged to be in an acute but not critical stage of scurvy, an event which usually occurred between the 18th and 27th days on the diet, the carbohydrate utilization technique of Cori and Cori (18) was applied at once to both members of the pair. The animals were allowed to fast for 24 hours, were then given orally 5 ml. of a solution containing 2.5 gm. of glucose, and were sacrificed 6 hours later by injection of 1 ml. of 6 per cent sodium amytal intraperitoneally.

Blood was taken by heart puncture. The liver was weighed, transferred at once to a weighed, glass-stoppered Erlenmeyer flask containing 30 ml. of hot 50 per cent KOH, hydrolyzed by heating in a boiling water bath, cooled, and weighed. The gastrointestinal tract was removed, the carcass weighed, plunged at once into a beaker containing 350 to 400 ml. of boiling KOH, and boiled until hydrolysis was complete.

Glucose absorption was determined by the method of Cori and Cori (18), but only the small intestine was used, since even after fasting 24

¹ Globe A-1 Wonder rabbit pellets, distributed by the Globe Grain and Milling Company, Oakland, California, and made of alfalfa meal, ground barley, ground oats, soy bean meal, wheat shorts, wheat bran, molasses, linseed meal, limestone, strained bone meal, salt, and dried whey. It contained, in per cent, crude protein 15.0, fat 2.5, fiber 19.0, ash 10.0, added salts 1.0, and, in mg. per cent, thiamine 0.28, riboflavin 0.65, carotene 0.58.

hours the large intestine of the guinea pig still contained considerable amounts of organic matter. Trials on several animals showed that no glucose was present in the large intestine.

The blood proteins and the protein in the digestive contents were precipitated by the method of Somogyi (19). The blood sugar and the reducing value of the digestive tract were determined by the use of ceric sulfate, according to the method of Giragossintz, Davidson, and Kirk (20). Glycogen in liver and carcass was determined by the method of Good, Kramer, and Somogyi (21). Titration of the resultant reducing solution was carried out with ceric sulfate, and the reducing value of all titrations expressed as glucose.

In a preliminary experiment five pairs of animals were used to determine any differences as to urinary excretion of sugar. The normal animals excreted 115 mg. of reducing substance during 24 hours without food and the ascorbic acid-deficient animals 106. When 2.5 gm. of glucose were given and the urine collected for 6 hours, the excretion of the groups was 45 and 35, or 180 and 140 mg. of glucose in 24 hours. Since the fasting excretion did not differ markedly from that following sugar feeding, true glycosuria was assumed to be absent and it was concluded that no significant effect could be attributed to the deficiency. Similar comparisons were not made, however, in the anoxia experiment.

For the anoxia experiment the animals were also pair-fed. When the appetite of the scorbutic member of a pair declined, both animals were placed in the low pressure chambers and kept there without food for 24 hours. No acclimatization was attempted, but the reduction in pressure was accomplished gradually. The previous fast and glucose feeding used in the glucose utilization experiments were omitted, as was also the analysis of gastrointestinal contents. The animals were sacrificed upon removal from the chambers and blood sugar, liver glycogen, and body glycogen determined.

The low pressure apparatus² consisted of a series of 2 liter glass jars connected with the evacuating system by copper tubes sealed in the lids. The air inlet and outlet of the evacuating line were provided with a needle valve which allowed regulation of the adequately rapid air flow through the chambers. A calibrated mercury manometer inserted in the evacuating line was used to indicate the pressure maintained, 349 mm. of Hg, corresponding to 20,000 feet altitude (22).

Results

Glycogen and Blood Sugar, after Glucose Ingestion—In Table I are given the results obtained with three groups of animals. The differences be-

² The apparatus was designed by V. V. Herring, Institute of Experimental Biology, University of California, Berkeley.

tween the normal and ascorbic acid-deficient animals were of the same order in all the groups. It is evident that the glucose tolerance of the deficient guinea pigs was lowered since their blood sugar was significantly greater than that of the normals in all cases. The mean glycogen content of the normal animals was in all three groups greater than that of the deficient group. This applied both to the absolute quantity in the liver and carcass and to the proportion per 100 gm. of liver and of carcass. The variability of these values was large, however, and the significance of the difference not evident, except in Group III, the scorbutic members of which

TABLE I

Carbohydrate Levels of Normal and Ascorbic Acid-Deficient Guinea Pigs 6 Hours after Glucose Ingestion

Experimental group No.		No. of animals	Glucose fed after 24 hrs. fast	Glucose of gastrointestinal tract	Liver glycogen	Body glycogen	Blood sugar	Body weight
			gm.	mg.	per cent	mg. per cent	mg. per cent	gm.
I	Normal	5	2.57	15 ± 2	4.44 ± 0.90	383 ± 26	74 ± 4	496 (426-540)
	17-20 days deficient	5	2.57	51 ± 9	3.06 ± 0.52	300 ± 32	101 ± 7	436 (374-490)
II	Normal	9	2.59	17 ± 2	2.81 ± 0.27	379 ± 17	74 ± 4	498 (454-530)
	19-36 days deficient	11	2.59	102 ± 31	2.12 ± 0.23	333 ± 26	112 ± 10	433 (352-470)
III	Normal	9	2.66	58 ± 20	4.07 ± 0.20	362 ± 14	92 ± 6	462 (380-510)
	21-27 days deficient	9	2.66	166 ± 41	2.66 ± 0.33	311 ± 19	162 ± 20	404 (326-450)

were in a more advanced and uniform stage of deficiency than were those of Groups I and II.

The absorption of sugar was reduced in the deficient guinea pigs, as was indicated by the larger reducing value of their gastrointestinal tracts. That absorption was complete in 6 hours in the case of the normal animals was ascertained by allowing twelve normal guinea pigs to fast 24 hours, after which they were sacrificed and the reducing value of the gastrointestinal tract determined. The average content was 17 mg. of glucose, about the same quantity found in the normal animals (Table I) 6 hours after glucose had been given. The difference in absorption between normal and deficient animals was consistent and significant, but not striking when the difference in weight of the animals is considered. Lowered in-

testinal absorption has been observed in various other conditions, in adrenalectomized, fasting, and in thiamine-, riboflavin-, or pantothenic acid-deficient animals.

These differences (Table I) in blood glucose and glycogen levels between normal and scorbutic guinea pigs are in agreement with those described in earlier reports (1-5).

Carbohydrate Metabolism under Anoxia—The differences in carbohydrate utilization between the normal guinea pigs fasting 24 hours at sea level and at 20,000 feet altitude were not as striking as were those found by Evans (23), Lewis *et al.* (14), and others for rats. The blood sugar level was significantly raised, but the liver glycogen was not significantly increased and the carcass glycogen appeared to be significantly decreased (Table II).

TABLE II

Effect of Anoxic Anoxia on Carbohydrate Levels of Fasting Normal and Ascorbic Acid-Deficient Guinea Pigs

Experimental group	No. of animals	Liver glyco-	Body glyco-	Blood sugar	Body weight
		gen	gen		
		<i>mg. per cent</i>	<i>mg. per cent</i>	<i>mg. per cent</i>	<i>gm.</i>
Normal, sea level	6	414 ± 17	307 ± 11	81 ± 4	469 (370-540)
“ 20,000 ft. (349 mm. Hg)	12	433 ± 8	257 ± 17	109 ± 6	441 (400-570)
Deficient, sea level	6	55 ± 4	230 ± 6	84 ± 4	428 (370-500)
“ 20,000 ft. (349 mm. Hg)	12	610 ± 14	308 ± 23	118 ± 9	410 (350-570)

The deficient animals also had increased blood sugar levels under the reduced atmospheric pressure and significantly increased liver glycogen and carcass glycogen. The levels of glycogen deposits attained were, however, in no case comparable with those seen by Lewis *et al.* (14) in normal rats and rabbits and by Wickson and Morgan (24) in rats under similar conditions.

Size of Adrenals—Thirteen pairs of animals which had been pair-fed for 19 to 27 days were used to determine the relative weights of the adrenal glands. The body weights of the normal guinea pigs ranged from 400 to 510 gm. with a mean of 440 ± 6 gm. and those of the deficient animals from 372 to 442 with a mean of 418 ± 2 gm. In all but two of the pairs the weight of the adrenal glands was greater in the deficient than in the normal member, the mean of the former being 294 ± 8 mg. and of the latter 249 ± 7 mg. The weight of the adrenals of the deficient animals was 0.71

mg. per gm. of body weight and of the normal 0.57 mg. This is similar to the observations recorded by others (10).

Fat Content of Livers and Carcasses—Since all the scorbutic guinea pigs lost weight rapidly as soon as the deficiency was established, it was of interest to determine whether this was due chiefly to fat or water loss. As is shown in Table III, total carcass lipids, determined on aliquots of the hydrolysates (25), were nearly the same in all the groups, deficient and normal, whether exposed to anoxia or not. The liver lipids similarly determined were increased in the anoxic groups, but again there was no significant difference between the normal and scorbutic animals. Sundstroem and Michaels (26) found that rats exposed to low pressures developed fatty yellow livers, a condition duplicated in these guinea pigs.

TABLE III

Liver and Carcass Lipid Content of Normal and Ascorbic Acid-Deficient Guinea Pigs

Condition	No. of animals	Body weight	Carcass*		Liver	
			Weight	Lipids	Weight	Lipids
			gm.	per cent	gm.	per cent
Normal, no anoxia.....	5	478	380	11.2 ± 0.4	16.5	8.0 ± 0.8
Deficient, no anoxia.....	4	440	357	12.2 ± 1.1	17.5	7.2 ± 0.5
Normal, exposed to anoxia†.....	12	434	361‡	12.5 ± 0.7	18.1	13.5 ± 0.6
Deficient, exposed to anoxia†.....	12	416	350‡	14.3 ± 0.9	20.0	11.5 ± 0.5

* Body minus gastrointestinal tract and liver.

† 24 hours at 349 mm. of Hg.

‡ Eight animals only used for carcass analysis.

Because it has been shown (27) that scorbutic guinea pigs in these early stages of the deficiency remain in nitrogen equilibrium, it may be conjectured that protein catabolism is not the cause of the abrupt weight loss, and since there was no demonstrable change in fat content of the body the loss may probably be ascribed to dehydration.

Baldwin, Longenecker, and King (10) have also found that in normal and ascorbic acid-deficient pair-fed guinea pigs the gross amounts of lipids in livers and carcasses were alike.

Oxygen Consumption in Ascorbic Acid Deficiency—The oxygen consumption and carbon dioxide production measurements were made by Dr. Max Kleiber,³ with his apparatus designed for metabolism work with small animals (28). Two series of tests were run, the first on animals fed *ad libitum*, the second on pair-fed groups. The animals were fasted 15 hours

³ College of Agriculture, University of California, Davis.

previous to the tests, during which time they were kept in an air-conditioned room at a temperature of 30° with free access to water. The test period was 5 hours in length. Two groups of guinea pigs of seven pairs each were used. The body weights were nearly the same, since the deficiency had been in effect only 15 to 30 days. The oxygen consumption of the normal animals corresponded to the production of 36 calories per day or 106 and 89 calories per kilo. The energy output of the deficient animals was 40 and 38 calories per day per animal or 100 and 92 per kilo. The respiratory quotients were the same, 0.74 to 0.78 for all groups. The calories per day per kilo¹ were 80 ± 3 and 71 ± 2 for the normal groups, and 79 ± 4 and 73 ± 1 for the deficient groups. Thus, there was no difference in energy output between normal and deficient animals. The conclusion may be drawn that the abnormality in carbohydrate metabolism observed in this study was not due to deranged thyroid function. This conclusion was borne out by the histological findings. Microscopic examination of sections of the thyroid of the deficient animals gave no indication of hyperplasia of the epithelium or other abnormality.

DISCUSSION

Under the conditions of this experiment, under normal oxygen tension, ascorbic acid-deficient guinea pigs appeared to be somewhat less able to form glycogen than the pair-fed control animals. This might indicate excess epinephrine secretion in the deficient group, as has been suggested by Banerjee (29), or participation by the ascorbic acid in the process of glycogenesis or in the prevention of glycogenolysis.

Under reduced oxygen tension for 24 hours the fasting normal animals were unable to increase their liver glycogen and actually lost muscle glycogen. But the deficient group under these circumstances definitely increased both liver and body glycogen. This may indicate exhaustion of epinephrine or dampening of its effect (30, 31) in the deficient animals under anoxia and stimulation of glyconeogenesis through mediation of the adrenocortical hormone (14). The normal animals apparently resisted these changes, possibly because ascorbic acid exerts a protective effect upon the secretory activity of both the adrenal medulla and cortex (29). Glycogenesis under sea level conditions was favored by the presence of ascorbic acid but glyconeogenesis under anoxia was prevented thereby. The effect may be direct or through changes in the activity of the adrenal secretions.

A comparison of the fasting metabolism of normal and riboflavin-deficient rats by the same technique indicated lowered blood sugar and liver and body glycogen at ordinary pressure in the deficient animals and little increase in carbohydrate levels under anoxic anoxia (24). The normal rats responded under anoxia with increased blood sugar and liver glyco-

gen. Apparently the mechanisms affecting carbohydrate utilization through riboflavin are different from those affected by ascorbic acid.

SUMMARY

Ascorbic acid-deficient guinea pigs were found to have significantly higher blood sugar and probably significantly lower liver and carcass glycogen than the pair-fed normal animals 6 hours after they were fed glucose following a 24 hour fast. The intestinal absorption of the sugar was less complete in the deficient animals.

When similarly paired but fasting animals were exposed to anoxic anoxia, 349 mm. of Hg for 24 hours, the deficient group was able to maintain or increase blood sugar and glycogen stores more effectively than did the normal. Fasting deficient guinea pigs at ordinary atmospheric pressure had lower carbohydrate stores than did those under anoxia, but this was not true of the normal animals.

The ascorbic acid-deficient guinea pigs were found to have a significantly increased size of adrenals, as compared with their paired controls.

The lipid content of livers and carcasses of the deficient animals was found to be nearly the same as in the normal, whether the animals had been subjected to anoxia or not. The sudden loss of weight in the former is therefore probably due to dehydration. The liver lipid values were increased in both groups after exposure to anoxia.

Oxygen consumption was not altered by the ascorbic acid deficiency, and no changes were seen in the thyroids.

The reduced glycogenesis and increased blood sugar exhibited by ascorbic acid-deficient guinea pigs following glucose feeding accord with the theory that the adrenomedullary mechanism is hyperactive in this deficiency. The increased size of the adrenals, rapid dehydration, and glycogenesis of the deficient animals under anoxia point to similar compensatory hyperactivity of the adrenocortical mechanism.

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