XLIII. THE OUTPUT OF CREATINE IN GLYCOSURIA.

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In order to obtain further evidence in support of the view that the appearance of creatine in the urine in animals, where the metabolism of carbohydrate has been interfered with, is actually due to the disturbance of the metabolic activities, and is not merely an error in observation due to the presence of aceto-acetic acid, the present experiments were carried out.

It has already been shown by Cathcart and Orr [1914] that in the case of the administration of phloridzin a creatinuria can exist without a concomitant acidosis. As the "error" in phloridzin glycosuria is generally considered to be renal, it was of some interest to examine the urine in other forms of glycosuria in which the cause is less obvious. The forms of glycosuria selected were (1) that following the injection of adrenaline, and (2) post-anaesthetic glycosuria.

PART I.

ADRENALINE GLYCOSURIA.

Since the discovery by Blum [1901] that the injection of adrenaline caused glycosuria, many confirmatory experiments have been carried out, but there is not yet perfect agreement as regards the mode of action of this substance. Noël Paton [1903] found a break-down of protein and a rise in the output of ammonia after the subcutaneous injection of adrenaline and concluded that the protein metabolism behaved as in ordinary diabetes. Underhill and Clossen [1907] were unable either to detect any increase in the catabolism

of protein or to find any alteration in the distribution of the nitrogenous constituents of the urine in adrenaline glycosuria. They believed that the action of the adrenaline was to stimulate the sugar-storing organs through the sympathetic nerves and thus prevent the storage of glycogen. The ability of the organism to utilise dextrose subcutaneously administered was not seriously impaired by the injection of adrenaline. From the fact that he was able to produce glycosuria with a dose of adrenaline which would produce a vaso-constriction in the tissues, Ritzmann [1909] concluded that the glycosuria was probably due to some sympathetic stimulation with a mechanism similar to that for vaso-constriction. On the other hand Wilenko [1912] thought that his results pointed directly to the glycosuria being due to some interference with the power of the tissues to oxidise the sugar. He found no rise in the respiratory quotient and maintained that sugar injected into an animal suffering from adrenaline glycosuria appeared in quantitative amount in the urine. Lusk [1913] however did not think that the oxidation was interfered with or that the protein metabolism was increased.

The present experiments were carried out on an Airedale bitch weighing 13.2 kilo. She was kept in an ordinary metabolism cage and fed on a fixed diet, in one experiment carbohydrate-rich and in the other carbohydrate-poor. The animal was catheterised each morning at 10 a.m. and the urine mixed with that collected in the receiver of the cage. It was diluted to a constant volume each day. The methods of analysis employed were: total nitrogen, Kjeldahl; ammonia, creatinine and creatine, Folin; urine treated in the way recommended by Graham and Poulton; urea, urease method; sugar, Benedict; and acetone and aceto-acetic acid, Messinger-Huppert. The adrenaline employed was the 1 : 1000 solution of Parke, Davis and Co.

Exp. I. Carbohydrate-rich diet: 100 g. tapioca, 70 g. oatmeal, 40 g. cane sugar, 30 g. dried skimmed milk, and 20 g. margarine.

As soon as the animal was in nitrogenous equilibrium 7 cc. of the adrenaline solution were injected subcutaneously. As a result of this injection the dog became very restless, and had free salivation but no marked dyspnoea. After the initial excitement passed off she lay down on the floor of her cage and remained quietly there for the rest of the day. She only ate a small part of her food this day. The following day, although she still looked somewhat ill, she ate all her food. On the next day at the site of injection which had become indurated a small centre of necrosis appeared. No albumin appeared in the urine either on the day of injection or any following day.

TABLE I. (*Carbohydrate diet.*)

Day	Amount in cc.	Re- action	Specific gravity	T.N. in g.	Urea N.		NH ₃ N		Creatinine N.		Creatine N. in g.	Sugar in g.	Acetone and aceto- acetic acid in g.
					in g.	p. c.	in g.	p. c.	in g.	p. c.			
vi	520	acid	1011	2.85	1.55	54.3	0.143	5.0	0.126	4.4	—	—	—
vii	430	„	1013	2.88	1.52	52.7	0.157	5.5	0.121	4.2	—	—	—
viii	520	„	1011	2.82	1.77	62.8	0.122	4.3	0.129	4.6	—	—	—
ix	1100	„	1011	4.64	2.55	54.9	0.240	5.1	0.159	3.4	0.072	5.85	0.0043
x	1220	„	1008	4.22	2.09	50.0	0.253	6.0	0.177	4.2	0.045	0.61	0.0023
xi	460	„	1018	3.76	1.88	50.0	0.099 (?)	2.6	0.141	3.8	—	—	0.0035
xii	280	„	1018	3.61	1.85	51.2	0.166	4.6	0.114	3.2	0.002	—	—

It will be seen from the table, that as the result of the injection of the adrenaline there is a very definite rise in the output of total nitrogen. This rise is not due to any increase in one particular constituent but to a general increase of all the constituents estimated, as will be noted from the examination of the percentage outputs. As was to be expected, on the day of the injection there was glycosuria and associated with it the appearance of creatine. Although the output of sugar on the day following had fallen to a low level, the output of creatine was still quite marked. That this is a real creatine excretion and not merely apparent, due to the presence of aceto-acetic acid, is clear when the total acetone excreted is considered.

Exp. II. Carbohydrate-poor diet consisting of 70 g. oatmeal, 30 g. dried skimmed milk and 90 g. margarine. The same dose of adrenaline as in the last experiment was given but this time intramuscularly. The results of the injection were practically identical with those in Exp. I. Two days after the injection, in spite of the fact that the injection was into the deeper tissues, a slight necrosis of the skin appeared.

TABLE II. (*Fat diet.*)

Day	Amount in cc.	Re- action	Specific gravity	T.N. in g.	Urea N.		NH ₃ N		Creatinine N.		Creatine N. in g.	Sugar in g.	Acetone and aceto- acetic acid in g.
					in g.	p. c.	in g.	p. c.	in g.	p. c.			
v	1050	amph.	1007	2.18	1.48	67.9	0.259	11.9	0.153	7.0	—	—	—
vi	1130	„	1008	2.39	1.30	54.4	0.290	12.1	0.165	6.9	—	—	0.0044
vii	1600	„	1008	3.58	1.88	52.5	0.672	18.7	0.166	4.6	0.101	0.81	0.0070
viii	1300	alk.	1007	2.60	1.21	46.5	0.852	32.8	0.151	5.8	0.023	—	0.0041
ix	1000	„	1007	2.39	1.43	59.8	0.459	19.2	0.150	6.2	—	—	0.0098
x	1000	amph.	1006	2.42	1.57	64.9	0.381	15.7	0.155	6.4	—	—	0.0059

As the result of the injection there is again quite a definite rise in the output of total nitrogen, but in this instance there is a much greater disturbance in the percentage composition. It will be noted that here there is a very marked rise in the output of ammonia and a definite rise in the excretion of total nitrogen and urea. As was to be expected the output of sugar

is much smaller although there is an increase in the output of creatine as compared with Exp. I. Here again, although there is a slight increase in the output of the total acetone, it is not sufficient to account for the creatine result.

PART II.

POST-ANAESTHETIC GLYCOSURIA.

It is probable that the glycosuria which follows the administration of ether is due to some disturbance of the hepatic metabolic activities. Seelig [1905], although he was unable to prove that glycosuria was a symptom of ether anaesthesia in the human subject, found that in the case of the dog a hyperglycaemia occurred after ether narcosis. As regards the glycosuria he found that it was very transitory, and even did not occur at all if, previous to the administration of the anaesthetic, an intravenous infusion of oxygen was given. He was quite certain that the glycosuria was due to the action of the ether and not to any associated symptoms arising during the narcosis.

King, Moyle and Haupt [1912] came to the conclusion that the glycosuria following ether administration was not due to asphyxia caused by the ether inhalation but was due to the intoxication with ether itself, as intravenous injection of saline saturated with ether also induced glycosuria.

As regards the influence of ether inhalation on the protein metabolism this subject has been fully discussed by Hawk [1908], who, as the result of his own experiments, found that ether anaesthesia was invariably followed by an increase in the excretion of nitrogen.

As in the previous experiments the same two diets were employed, the subject of experiment being an Airedale bitch weighing 11 kilo. On account of the transitory nature of the changes produced by ether anaesthesia, the urine of the day on which the animal was anaesthetised was divided into two parts and separately examined; (a) the urine which was excreted and obtained from the bladder by catheter before the anaesthetic was given, and (b) the urine collected for the rest of the 24-hour period after the anaesthetic.

Exp. III. Carbohydrate-rich diet. After the animal had reached a state of nitrogen equilibrium it was anaesthetised with ether for 90 minutes, from 2.30 p.m. to 4 p.m.

The animal was given a small dose (2–3 grains) of morphia before the ether was administered. Small doses of morphia have no influence on protein metabolism and have but little influence in causing glycosuria. Following the

anaesthetic there was slight vomiting but no other symptom. The animal rapidly recovered.

TABLE III. (*Carbohydrate diet.*)

Day	Amount in cc.	Reaction	Specific gravity	N. in g.					Sugar in g.	Acetone and aceto- acetic acid in g.
				T.N.	Urea	NH ₃	Creatinine	Creatine		
iv	850	acid	1006	2.25	1.43	0.238	0.148	—	—	—
v	1000	„	1006	2.21	1.27	0.248	0.147	—	—	0.0029
vi	1030	„	1005	2.25	1.48	0.260	0.147	—	—	0.0029
vii	220	amph.	1006	0.52	0.31	0.055	0.032	—	—	0.0005
	560	alk.	1014	1.76	1.34	0.045	0.098	0.009	0.45	0.0039
	780			2.28	1.65	0.100	0.130	0.009	0.45	0.0044
viii	800	amph.	1006	2.78	1.97	0.318	0.129	—	—	0.0054
ix	1350	acid	1005	2.29	1.31	0.507	0.138	—	—	—

There is no rise in the output of total nitrogen on the day of narcosis but there is a very slight rise in the output of the following day. There is however a slight rise in the excretion of urea and a very definite fall in the output of ammonia, a slight fall in creatinine, the appearance of a trace of creatine, a slight glycosuria and a slight increase in the output of total acetone. The appearance of the creatine was checked by the diacetyl test.

Exp. IV. A repeat experiment on the carbohydrate-rich diet but without the preliminary dose of morphia was carried out. The administration of the anaesthetic was continued for 150 minutes from 3 p.m. to 5.30 p.m.

TABLE IV. (*Carbohydrate diet.*)

Day	Amount in cc.	Reaction	Specific gravity	N. in g.					Sugar in g.	Acetone and aceto- acetic acid in g.
				T.N.	Urea	NH ₃	Creatinine	Creatine		
v	1000	amph.	1006	2.28	1.44	0.280	0.141	—	—	—
vi	1240	„	1003	2.31	1.58	0.229	0.130	—	—	—
vii	1100	„	1006	2.34	1.51	0.246	0.131	—	—	—
viii	420	„	1004	0.62	0.34	0.068	0.028	—	—	0.0007
	1000	„	1009	1.71	0.97	0.112	0.103	0.011	2.5	0.0087
	1420			2.33	1.31	0.180	0.131	0.011	2.5	0.0094
ix	1120	acid	1003	3.28	2.34	0.389	0.138	—	—	0.0076
x	1220	amph.	1005	2.39	1.50	0.314	0.123	—	—	0.0059

Again there is no rise in the output of total nitrogen on the day of the experiment but quite a marked one on the day following. The fall in the output of ammonia is quite definite although not so marked as in *Exp. III*. There is a slight fall in the output of urea and the excretion of creatinine is not affected. The output of creatine and sugar is much more marked, as

is also total acetone. The diacetyl test was again used to control the appearance of creatine.

Exp. V. Carbohydrate-poor diet. Without previous administration of morphia the animal was anaesthetised for 150 minutes from 2.30 p.m. to 5 p.m. Apart from slight vomiting after the anaesthetic there were no ill effects of the ether.

TABLE V. (*Fat diet.*)

Day	Amount in cc.	Reaction	Specific gravity	N. in g.					Sugar in g.	Acetone and aceto- acetic acid in g.
				T.N.	Urea	NH ₃	Creatinine	Creatine		
iv	800	acid	1011	2.64	1.65	0.179	0.116	—	—	0.0124
v	1060	„	1010	3.12	1.93	0.220	0.125	—	—	0.0113
vi	1000	„	1010	2.80	1.68	0.185	0.125	—	—	0.0097
vii	200	„	1005	0.37	0.23	0.024	0.016	—	—	0.0021
	1210	„	1007	2.54	1.65	0.214	0.106	0.007	—	0.0117
	1410			2.91	1.88	0.238	0.122	0.007	—	0.0138
viii	1000	amph.	1009	3.75	2.59	0.179	0.113	—	—	0.0135

The effect on the output of total nitrogen is again marked on the day following. In this experiment instead of a fall in the excretion of ammonia there is quite a definite rise on the day of anaesthesia. The output of creatinine is unaltered on the day of experiment but there is quite a marked fall on the day following. As regards the output of sugar none was detected. The output of creatine is a mere trace. There was a small but appreciable rise in the excretion of total acetone.

Thus in both sets of experiments, in which the modes of production of the glycosuria are very different, there is associated with the disturbance of the carbohydrate metabolism an output of creatine. There is no question in any of these experiments, with the possible exception of *Exp. V*, of the output of creatine being only an apparent output due to the presence of aceto-acetic acid interfering with the estimation of the preformed creatinine.

CONCLUSIONS.

1. The injection of adrenaline produces a glycosuria associated with which there is an output of creatine.
2. There is also a rise in the output of total nitrogen due to the injection of the adrenaline.
3. Ether anaesthesia is followed by a slight glycosuria and creatinuria.

4. A rise in the output of total nitrogen occurs on the day following the anaesthesia.

5. The output of creatine in these experiments is not "apparent," due to the presence of aceto-acetic acid, but is a real output.

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