XIX. THE RELATIONSHIP OF CREATINURIA TO CHANGES IN THE SUGAR CONTENT OF THE BLOOD.

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Previous Work and Object of present Research.

An increase in the normal sugar content of the blood has long been known to be an associated phenomenon of certain pathological conditions, especially pancreatic diabetes or the glycosuria artificially produced by the injection of certain drugs, e.g. uranium. Of recent years it has been shown that a diminution in the normal sugar content, or a hypoglycaemia, may follow the injection of certain other drugs, which cause considerable hepatic disturbance accompanied by the excretion of creatine. Some previous work by the writer [MacAdam, 1913] in corroboration of the hypothesis put forward by Cathcart [1909] that carbohydrates are intimately associated with the metabolism of creatine may explain the onset of this creatinuria.

Frank and Isaac [1911] by the administration of phosphorus to rabbits produced a distinct hypoglycaemia along with marked liver necrosis. The injection of hydrazine sulphate, which has a specific action in causing hepatic necrosis [Wells, 1908], leads to changes in the percentage of sugar in the blood, as shown by Underhill [1911; Underhill and Fine, 1911] both in dogs and rabbits. As the result of numerous experiments on dogs he found that a dose of 50 mgrms. per kilo. invariably produced an appreciable hypoglycaemia, although considerable variations in the degree of diminution of the sugar content occurred. His experiments with rabbits were not so conclusive. On the administration of hydrazine in doses of 50 mgrms. per kilo. a constant decrease in the sugar content was not obtained; for, although a hypoglycaemia of varying amount was found to occur in the

majority of the experiments, in others the blood sugar remained unaltered. On the administration of 100 mgrms. per kilo. the animals died within 24 hours, and Underhill concludes "From this diversity of results it is obvious that the rabbit cannot be relied on invariably to exhibit hypoglycaemia after hydrazine injection."

In these experiments the relationship of hypoglycaemia to creatinuria was not considered, and no attempt was made by the administration of intermediate doses to allow for the idiosyncrasy of individual rabbits. The object of the following experiments has been to try to determine experimentally whether this irregular occurrence of hypoglycaemia in rabbits depends upon the dose of hydrazine injected, and if it is in any way associated with the simultaneous occurrence of creatine in the urine.

Experiments and Methods of Analysis.

The rabbit was kept in a small cage over a funnel leading to a vessel in which the urine was collected. 5 cc. of 5 % H_2SO_4 were placed in this vessel previous to collection to prevent alkaline changes in the urine. At first a difficulty was experienced in obtaining the urine creatine-free during the control days. By means of a carbohydrate-rich diet this was in most instances surmounted or at least so diminished that the amount of creatine still present was negligible. In a few cases persistent creatinuria was found to be dependent upon pregnancy, and the rabbits were accordingly discarded. No attempt was made to obtain the quantitative amounts of creatine and creatinine excreted, partly on account of unequal quantities of urine being passed during each 24 hours, partly because such absolute figures have no direct bearing on the object of the present research. *Percentage* results are dealt with in Table I, the relationship of the excretion of creatine to total creatinine being thereby shown.

Control estimations of the sugar content were first made from 7–10 days previous to the injection of a solution of hydrazine sulphate (2.5 %). This was done without doing any injury to the vascular system of the animal, for after some practice no difficulty was experienced in obtaining from 20–30 cc. of blood from the auricular vein. The blood was dropped directly into a 50 cc. flask containing 5 cc. of a 2 % solution of potassium oxalate, and by the addition of the requisite amount of distilled water from a burette to bring the total amount of fluid up to 50 cc., the quantity of blood obtained was accurately determined. In two of the experiments recorded where the rabbits after the administration of hydrazine became comatose, it was found

impossible to obtain blood from the auricular vein, and hence it was drawn directly from the jugular vein and heart. The sugar content was estimated by the method of Allihn in terms of CuO.

The accuracy of the technique was tested in the case of a dog used for the purpose of another research [MacAdam, 1913] which became comatose within 24 hours after the injection of 90 mgrms. per kilo. of hydrazine. The dog was killed and from its jugular vein two quantities of blood, 32.2 cc. and 60 cc., were obtained, the sugar content of which was found to be 0.064 % and 0.068 % respectively. The sugar content of the normal dog is constant within narrow limits and averages 0.1 %, so that it is interesting to note that the above estimations corroborate the work of Underhill [1911], who found a sugar content of 0.05 % in the blood of a dog one day after the injection of 100 mgrms. hydrazine per kilo. of body weight.

In the initial experiments, doses of 50 mgrms. per kilo. body weight were administered, and 48 hours later blood was withdrawn, as above mentioned, for sugar estimation, while daily estimations of the urinary excretion of preformed creatinine and creatine were carried out.

If no symptoms were exhibited, and no creatinuria resulted, the same rabbit was injected again but with a larger dose in order to try just to exceed the tolerance of the animal's tissues to the poison. The varying doses used in each experiment are recorded in the protocols.

Since the animals, which showed metabolic disturbance, refused all food after the injection, two experiments (X and XI) with the rabbits fasting for two days were carried out in order to eliminate the factor of starvation from the results.

TABLE I.

(The days of the control period in each experiment during which an attempt was being made to obtain the urine creatine-free are indicated by Arabic numerals, while the Roman figures refer to the days following the injection of the hydrazine.)

I. Rabbit A. Weight 3.20 kilos.

Day	Total Creatinine	Preformed C.	Creatine as Creatinine	% Creatine of Total Creatinine	% Sugar Content of blood	Remarks
	mgrms. in 100 cc. urine					
1	127.4	127.4	—	—	0.114	
2	101.5	98.3	3.2	3.1	—	
	Injection of 50 mgrms. Hydrazine sulph. per kilo.					Animal showed no meta-
II	87.0	87.0	—	—	0.108	bolic disturbance.

TABLE I (*continued*)II. *Rabbit B.* Weight 2.73 kilos.

Day	Total Creatinine mgrms. in 100 cc. urine	Preformed C. mgrms. in 100 cc. urine	Creatine as Creatinine mgrms. in 100 cc. urine	% Creatine of Total Creatinine	% Sugar Content of blood	Remarks
1	71.0	65.5	5.5	7.4	0.095	
2	107.7	90.0	17.7	16.4	—	
3	—	—	—	—	—	
4	81.5	81.5	—	0.0	—	
	Injection of 50 mgrms. Hydrazine sulph. per kilo.					
I	72.5	74.1	—	—	—	Nothing abnormal was detected in the rabbit's condition after injection, food being taken as usual.
II	124.5	121.5	3.0	2.4	0.102	
III	110	115.0	—	—	—	

III. *Rabbit B.* Weight 2.47 kilos.

1	126.5	126.5	—	—	0.087	
2	107.5	98.5	9.0	8.3	—	
3	94.3	94.3	—	0.0	—	
	Injection of 75 mgrms. Hydrazine sulph. per kilo.					
I	139.5	146.0	—	—	—	No metabolic disturbance noted.
II	124.5	126.5	—	—	0.107	
III	85.7	82.6	3.1	3.6	—	

IV. *Rabbit C.* Weight 1.77 kilos.

1	206.5	204.3	2.2	1.0	0.101	
2	87.3	87.3	—	—	—	
3	132.1	134.8	—	—	—	
	Injection of 75 mgrms. Hydrazine sulph. per kilo.					
I	142.1 ¹	135.0	7.1	5.0	—	Rabbit died within 12 hours of injection—no sugar estimation.

¹ Urine drawn from bladder post-mortem.V. *Rabbit D.* Weight 2.32 kilos.

1	165.0	139.0	26.0	15.7	0.098	
2	—	—	—	—	—	
3	158.5	158.5	—	—	—	
4	72.0	73.5	—	—	—	
5	107.5	107.5	—	—	—	
	Injection of 75 mgrms. Hydrazine sulph. per kilo.					
II	158.5	62.8	95.7	60.3	0.047	Rabbit showed considerable malaise, refusing all food after injection. Blood could not be obtained from auricular vein, and was taken from the jugular vein.

TABLE I (continued)

VI. *Rabbit A.* Weight 2·82 kilos.

Day	Total Creatinine	Preformed C.	Creatine as Creatinine	% Creatine of Total Creatinine	% Sugar Content of blood	Remarks
	mgrms. in 100 cc. urine					
I	155·7	155·7	—	—	0·098	
2	128·4	125·2	3·2	2·4	—	
3	99·8	101·2	—	—	—	
	Injection of 75 mgrms. Hydrazine sulph. per kilo.					
II	112·5	112·5	—	—	0·110	
III	154·0	156·5	—	—	—	No effects produced.

VII. *Rabbit A.* Weight 2·96 kilos.

I	171·0	162·0	9·0	5·2	—	
2	—	—	—	—	—	
3	62·5	64·5	—	—	—	
4	149·0	144·0	5·0	3·3	—	
	Injection of 100 mgrms. Hydrazine sulph. per kilo.					
II	217·0	135·0	82·0	37·8	0·150	
III	279·0	208·0	71·0	25·4	—	The rabbit from the first day after injection showed considerable malaise. Blood was obtained with difficulty from the auricular vein, only 19·7 cc. being obtained.

VIII. *Rabbit B.* Weight 2·87 kilos.

I	159·6	148·7	10·9	6·8	—	
2	—	—	—	—	—	
3	78·3	78·3	—	—	—	
4	122·1	122·1	—	—	—	
	Injection of 90 mgrms. Hydrazine sulph. per kilo.					
II	172·9	114·2	58·7	33·9	0·060	
III	96·3	68·4	27·9	20·9	—	The usual symptoms of metabolic disturbance. Rabbit recovered, but was not used for further experiment.

IX. *Rabbit F.* Weight 1·70 kilos.

I	137·3	137·3	—	—	0·09	
2	106·0	103·8	2·2	2·0	—	
3	143·4	145·2	—	0·0	—	
	Injection of 75 mgrms. Hydrazine sulph. per kilo.					
I	43·6	39·1	4·5	10·3	0·094	Rabbit found comatose 24 hours after injection: 45 cc. of urine were removed from the bladder—acid in reaction. The blood was obtained directly from the heart.

TABLE I (*continued*)

X. <i>Rabbit G.</i> Weight 2.14 kilos.						
Day	Total Creatinine	Preformed C.	Creatine as Creatinine	% Creatine of Total Creatinine	% Sugar Content of blood	Remarks
	mgrms. in 100 cc. urine					
1	66.5	63.5	3.0	4.5	0.104	
2	130.0	130.0	—	—	—	
3	—	—	—	—	—	
4	106.5	102.6	3.9	3.6	—	
I	Fasting experiment					
II	187.4	143.5	43.9	23.4	0.140	After 2 days' starvation.
III	124.2	101.6	22.6	18.2	—	
XI. <i>Rabbit K.</i> Weight 1.91 kilos.						
1	126.0	128.2	—	—	0.092	
2	137.0	137.0	—	—	—	
I	Fasting experiment					
II	132.5	95.7	36.8	27.7	0.116	After 2 days' starvation.
III	87.2	59.6	27.6	31.6	—	
XII. <i>Rabbit G.</i> Weight 2.0 kilos.						
1	124.5	115.5	9.0	7.2	0.095	
2	97.4	99.2	—	—	—	
3	131.4	128.0	3.4	2.5	—	
	Injection of 75 mgrms. Hydrazine sulph. per kilo.					
II	92.8	58.8	34.0	36.6	—	Rabbit showed usual symptoms of considerable malaise.
III	128.5	95.0	33.5	26.1	0.071	
XIII. <i>Rabbit M.</i> Weight 2.54 kilos.						
4	102.5	74.3	28.2	27.5	0.105	
5	144.5	135.0	9.5	6.5	—	
6	58.6	54.3	4.3	7.3	—	
	Injection of 50 mgrms. Hydrazine sulph. per kilo.					
II	108.0	84.5	23.5	21.7	0.114	Animal aborted after withdrawal of blood.
XIV. <i>Rabbit K.</i> Weight 1.7 kilos.						
1	—	—	—	—	0.101	
2	134.0	136.0	—	—	—	
3	98.4	98.4	—	—	—	
	Injection of 90 mgrms. Hydrazine sulph. per kilo.					
II	89.4	56.4	33.0	37.0	0.042	Rabbit was found comatose on morning of 2nd day, and 18.3 cc. of blood removed from the jugular vein. Urine was obtained directly from the bladder—acid in reaction

Summary of Results.

In the four experiments (I, II, III, VI) where the hydrazine administered was insufficient to produce any obvious metabolic disturbance no creatine was excreted, while the sugar content of the blood, as compared with the control estimation previously made, was within normal limits, when due allowance has been made for experimental error.

Of the six experiments, where creatinuria followed hydrazine injection, four (V, VIII, XII, XIV) showed a condition of hypoglycaemia; one (VII) a state of hyperglycaemia, while in the remaining one (IX) the sugar content remained unchanged. In this last experiment it may be noted that the sugar estimation was carried out on blood obtained directly from the heart 24 hours after the administration of the hydrazine; while the percentage $\frac{\text{creatinine}}{\text{total creatinine}}$ was small, viz. 10%, when compared with the figures obtained in the "hypoglycaemia" experiments. Indeed it was not much higher than the percentage occasionally recorded during the control days.

In Experiment XIII, where the rabbit aborted after the withdrawal of the second quantity of blood, and where pregnancy had not been previously noticed, there is little difference between the two estimations although there is a distinct increase in the creatine percentage of the total creatinine.

In the two fasting experiments, the rabbit of Experiment X showed quite a marked hyperglycaemia, while in XI the sugar content of the blood showed a slight increase when compared with the control estimation. In both a marked creatinuria resulted.

In the remaining experiment (IV) Rabbit C died within 12 hours. No blood was obtained, while the urine drawn from the bladder post-mortem showed a trace of creatine. This was the only rabbit which exhibited such toxic symptoms after a dose of 75 mgrms. per kilo.

While this work was in progress Graham and Poulton [1913] published a communication in which it was suggested that an important fallacy underlies the supposed creatinuria occurring in diabetes and in conditions associated with acidosis, owing to the presence of aceto-acetic acid which tends to lower the value of the preformed creatinine estimations. In order to eliminate this error in the foregoing results, several experiments were performed with special reference to the possible presence of aceto-acetic acid. A positive result was never obtained with the ferric chloride test either before or after the injection of hydrazine. Owing to the extremely unsatisfactory nature of this test in the detection of minute amounts of this acid, if any suspicious

coloration was given with the ferric chloride solution the method recommended by Graham and Poulton was used. No trace of aceto-acetic acid was detected in any of the urines, while creatine was still found to be present in measureable amounts.

Discussion of Results.

The four experiments, in which no alteration in the sugar content of the blood, and no creatinuria, followed the injection of hydrazine, are interesting in relation to the previous work carried out by Underhill [1911].

In the present series of experiments the control estimations, carried out previous to the administration of hydrazine, show a reasonably constant sugar content of the blood, the average being approximately 0.10 per cent., thus corroborating previous investigations; while the divergence in the sugar estimations after injection appears to be dependent on the differences in susceptibility of the rabbits to the amount of the drug injected. Thus Rabbit A was used in Experiments I and VI, and Rabbit B in II and III, when 50 and 75 mgrms. per kilo. body weight were respectively administered with no resulting abnormal symptoms or creatinuria, while a dose of 75 mgrms. per kilo. had such a remarkably toxic effect on Rabbit C that it died within 12 hours of injection. After an interval of approximately one month, a larger dose was administered, 100 mgrms. per kilo. to Rabbit A (Experiment VII) and 90 mgrms. per kilo. to Rabbit B (Experiment VIII), with the result that both showed a distinct alteration in the percentage of their blood sugar, associated with the production of creatinuria. In all Underhill's experiments in which the blood sugar content was estimated, only 50 mgrms. per kilo. were administered, a dose which, in the case of the rabbits used in the present research, was never found to produce any alteration in sugar percentage. In the two other experiments he records, in which a dose of 100 mgrms. per kilo. was used, the rabbits died within five and twenty-four hours respectively, no sugar estimation being carried out. Hence it would appear that the rabbit can be relied upon to exhibit a constant hypoglycaemia after the administration of the amount of hydrazine requisite for each individual animal.

In the six experiments in which distinct creatinuria followed hydrazine injection, and in which no obvious extraneous factors could be detected, the sugar estimations show evidence of a well-marked associated hypoglycaemia. If the relationship of creatine to the total creatinine is considered, it is seen that after hydrazine injection the lowest percentage of creatine is 20.9,

the highest being 60.3 %, whereas during the three control days previous to injection the highest figure recorded, even in the case of those rabbits which were not obtained creatine-free, is 7.2 %. The only exception was Experiment II, in which on the third day previous to injection a percentage of 16.4 was recorded. However, before administration of the drug, no creatine was obtained, while after a dose of 50 mgrms. per kilo. the relationship of creatine to total creatinine remained within what were found to be normal limits. In the two fasting experiments the $\frac{\text{creatine}}{\text{total creatinine}}$ ratio ranged from 18.2 % to 31.6 %—figures quite in accordance with previous results.

As regards the sugar content of the blood in those four experiments where the occurrence of hypoglycaemia and creatinuria were associated, before injection the figures ranged from 0.087 %–0.101 %, while after injection they lay between 0.042 % and 0.071 %, there being thus always a distinct decrease in sugar percentage beyond the limits of experimental error.

Of the two experiments in which creatinuria was not associated with the simultaneous occurrence of hypoglycaemia, no appreciable alteration in the sugar percentage figure was found in Experiment IX; while in Experiment VII, Rabbit A, in which two months previously no change in the sugar content had been produced by doses of half and three-fourths of the amount administered in the subsequent experiment, a marked hyperglycaemia resulted. To account for this contradictory result, no very satisfactory explanation can be offered. The rabbit certainly showed considerable malaise and symptomatic disturbance very shortly after receiving the injection. Considerable difficulty was experienced in obtaining the requisite amount of blood from the auricular vein, and this may have been partly due to a concentration of the blood. This possibility would in itself tend to raise the percentage figures of the sugar content; and in this connection it may also be mentioned that owing to a similar difficulty in obtaining sufficient blood from the veins in Experiment IX it was drawn directly from the heart.

It is thus evident that after the administration of a suitable dose of hydrazine a hypoglycaemia results which is invariably associated with creatinuria, while if no change in the sugar content is produced, creatine is not found in the urine. It has also to be noted that only those rabbits which showed this combined hypoglycaemia and creatinuria exhibited any marked symptoms of metabolic disturbance, the animals being quite dull and listless and refusing all food and drink.

Of the factors which may lead to this alteration from normal metabolism, that of starvation can be eliminated on considering the results of the two fasting experiments (X and XI). Neither of the rabbits showed any reduction in the percentage of blood sugar. On the contrary, Experiment XI showed a slight increase, while in X a distinct hyperglycaemia resulted. In both cases, as already mentioned, there was a marked excretion of creatine.

Thus, although the results of the experiments here recorded point to the intimate association of carbohydrates with the metabolism of creatine, yet the exact nature of this relationship or even its degree of importance is still uncertain. It has been shown that the creatinuria following hydrazine injection is accompanied by a condition of hypoglycaemia, while it is already known that the creatinuria of diabetes and phloridzin poisoning is associated with hyperglycaemia. On the other hand, no such constant change in the sugar content of the blood is found to accompany the creatinuria induced by fasting. Furthermore, some experiments by Underhill and Rand [1910] show that "The perverted creatine metabolism as well as the metabolism of the other nitrogenous constituents of the urine tend rapidly to resume the normal on the rectal administration of dextrose, without necessarily exerting any influence on the pathological condition of the patient."

Nor do the various attempted explanations of the influence of carbohydrates on the excretion of creatine carry us very far. There is no definite evidence in favour of the hypothesis that they are essential for the conversion of creatine into creatinine or other as yet unknown substances, or for its oxidation and excretion as urea. No deductions in this connection can be drawn from previous work on the subject [MacAdam, 1913] because the creatinuria of these experiments was associated with an increased excretion of urea. So many factors enter into urea excretion, that it is impossible to eliminate them satisfactorily; and analogies drawn from *in vitro* experiments to the changes occurring *in vivo* are, in regard to the present problem, essentially untrustworthy.

That an adequate supply of carbohydrates inhibits muscle disintegration and consequently prevents the excretion of creatine is merely a special phase of the more general hypothesis suggested by Mendel and Rose [1911] that "the tissue cells may not functionate properly when the normal amount of carbohydrate food is wanting, and in this case the elimination of creatine would be analogous to the production of the acetone bodies which is also inhibited by the administration of carbohydrates." Again, Krause [1913] supports the idea that creatine may be an end product of metabolism, arguing

that creatinuria may sometimes be not so much a deviation from normal metabolism as an indication that our conceptions of normal metabolism, based as they have been on somewhat limited observations, may not be comprehensive enough. Recent work on the creatinuria of childhood [Rose, 1911; Folin and Denis, 1913] and on its irregular occurrence during the menstrual cycle of normally healthy women [MacAdam, 1914] supports this plea. The excretion of creatine may be merely one of the earliest manifestations of an upsetting of carbohydrate equilibrium, and may occur before a slight exaggeration of an otherwise normal process has become a definitely pathological phenomenon.

CONCLUSIONS.

1. After hydrazine injection in rabbits, (a) if no alteration in the sugar content of the blood occurs, no marked creatinuria results; (b) but the excretion of creatine is associated with a condition of hypoglycaemia.
2. This creatinuria is not dependent on the starvation factor *per se*, for in the two fasting experiments the excretion of creatine was associated with an increased sugar content of the blood.
3. Creatinuria appears to be produced on the least disturbance of the equilibrium of carbohydrate metabolism, of which it is one of the earliest and most sensitive manifestations.

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