NUCLEIN METABOLISM IN A DOG WITH ECK'S FISTULA.

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All nitrogenous substances completely metabolized in the organism of mammals are removed by the urine in form of urea. Every other nitrogenous constituent of the urine is either a substance which has for one reason or another escaped its final transformation, or has not been metabolized by the organism at all. Under metabolism here, of course, is understood a set of chemical reactions which result, first, in maintenance of the integrity of the body's tissue and, second, in supplying it with calories sufficient to perform all the functions of the organism.

The quantity of nitrogen metabolized by the organism for the purpose of energy production is regulated primarily by the intake of nitrogenous food-stuffs, and by the quantity of reserve protein deposited in the organism. The quantity of nitrogen metabolized as a result of tissue deterioration for the purpose of maintaining its integrity is determined by the mass of the cells in the organism, by the amount of work which the tissues are called upon to perform, and by the intensity of the effort employed by the organism in performing the work. Thus, work of short duration, but of such intensity as would occasion a state of fatigue, brings about greater tissue deterioration than prolonged work of lower intensity.

Under normal conditions there exists a definite proportion between the two phases of nitrogenous metabolism, namely, between metabolism of nitrogenous food-stuffs of reserve protein on the one hand, and metabolism of cellular protein on the other. This proportion changes with the change of the plane of nutrition of the organism and with change of the quantity of protein in the food. However, conditions are conceivable in which it is made possible that the organism may be supplied with a sufficient amount of

energy by the sacrifice of the tissue integrity, or conditions may exist in which the tissue integrity is maintained by an unusually high consumption of reserve protein. Thus it is important to be able to trace separately the two phases of nitrogenous metabolism.

Analysis of the intake of food stuffs, a record of heat production, estimation of carbon dioxide and of urea removed by the organism furnish the data required to follow energy production. But one is confronted with considerable difficulty when he attempts to obtain information regarding the condition of the cellular elements of the tissues. This is due to the fact that deteriorated tissue elements are utilized for the purpose of metabolism in the same manner as the protein of the food or as the reserve protein. Their nitrogen is removed in the form of urea. Some components of the cellular elements, however, have a chemical composition distinctly different from that of the reserve protein. These substances, previous to their transformation into urea, undergo a cycle of chemical reactions which is different from that of the reserve protein; the intermediate products of their metabolism differ from those of the latter, and it is very probable that the mechanisms regulating the transformation of the two groups of substances are independent of one another. Indeed, in the light of this assumption one can readily explain the fact that transformation of special components of tissue elements varies considerably in different species, although transformation of simple reserve-protein occurs in an identical manner, and results in the same production of energy.

Among such components of tissue elements special interest is attached to the nucleins, since they constitute the principal substance of the nuclei of all cells. The attempt has been repeatedly made to ascertain the degree of tissue deterioration by analysis of the nuclear derivatives in the urine, the substances of special interest being uric and phosphoric acids. It has been established in recent years that uric acid is formed in the organism through the oxidation of purin bases, which are the principal components of nucleic acids. But uric acid is metabolized further in the organism of mammals, that is, it is oxidized and is finally removed from the organism in the form of urea. Uric acid detected in the urine represents only a fraction of the acid formed in the organism, namely, that part

which has escaped further oxidation. Hence satisfactory information regarding cellular disintegration cannot be gained by the investigation of uric acid output under normal conditions; but the results of uric acid analysis can be made valuable for the solution of the problem, if conditions are found under which the further transformation of uric acid can be prevented.

Clinical observations, and the studies of Pawlow, Nencki, Hahn and Massen especially, have suggested the probability that the liver is the principal organ concerned in the oxidation of uric acid. On the other hand, recent work of Weiner, Burian, Jones and Schittenhelmn have shown that many other animal tissues possess the same power. It must be borne in mind that the Russian investigators were not engaged in the study of purin metabolism especially, and their observations were made at a time when uric acid formation was not yet clearly understood. The facts make a revision of the older work on purin metabolism very desirable.

The present investigation was carried out on a dog in which an anastomosis between the portal vein and the inferior vena cava had been successfully performed by one of us (Sweet). The purposes of investigation were: (1) To ascertain whether or not the output of uric acid was above normal, if the dog was maintaining nitrogenous equilibrium, and was keeping its original weight. (2) To ascertain whether or not the output of uric acid was increased markedly after administration of nuclein or of its derivatives (nucleoproteid of the mammary gland of the cow, nucleic acid of fish sperm, adenin sulphate, and thymin were employed). (3) To ascertain whether the animal possessed the power to metabolize thymin. (4) To ascertain whether or not the output of uric acid was increased on a diet poor in protein material, although containing carbohydrates and fat in quantities sufficient to supply the organism with the required amount of calories. (5) To ascertain whether or not the output of uric acid was increased during starvation.

The present communication represents the results of experiments performed on one dog, and cannot, therefore, serve as a basis for broad generalization. The work is communicated in its present incomplete state, because accidental circumstances render its im-

mediate completion unpracticable; the results already obtained are believed to be of interest.

Methods.—The urine was collected in three-day periods, the dog being catheterized at the beginning and at the end of each period. The receptacle contained a quantity of 10 per cent. solution of sulphuric acid to make up with the urine a solution of about 2 per cent. of sulphuric acid. No other preservative was added.

The nitrogen estimations were made by the Kjeldahl-Gunning method. The phosphorus was estimated gravimetrically, the urine being evaporated and fused with a mixture of potassium carbonate and sodium nitrate. Uric acid was estimated by the Leube-Salkowski method, ammoniacal silver chloride being used instead of the nitrate.

Elimination of Uric Acid.—Information regarding uric acid elimination by dogs with Eck's fistula is limited to the observations of Pawlow, Nencki, Hahn and Massen. Their dogs were fed on a mixed diet, no special attention being paid to the quantity of purin bodies in the food-stuffs, and no special care being exercised in collecting the daily quantities of urine. Moreover, since the dogs used by the Russian investigators developed the typical nervous attacks described by Pawlow, without further evidence the objection might be urged that the high uric acid output was occasioned by deterioration of the nuclear elements of the liver, or was secondary to the convulsive attacks.

In the present work the experiment was not begun until the nutrition of the dog after operation was so much improved that it maintained nitrogenous equilibrium. Furthermore, the dog was fed on cracker dust, plasmon, lard, and salt, to eliminate all purin in the diet, and had been on this diet for several weeks prior to the beginning of our records. It is, therefore, obvious that all the uric acid found in the urine must have originated in disintegration of tissue. The output of uric acid in this experiment was considerably higher than that of a normal dog.

Feeding of Nucleoprotein.—On two successive days the dog received, instead of plasmon, twenty-five grams of nucleoprotein of the mammary gland of the cow. As seen from the table, a slight increase in the uric acid output occurred, but as the nucleoprotein

FEMALE MONGREL SPANIEL—OPERATION, MARCH 5, 1906.

Date.	Dog Received.	Weight.	Total N-Intake.	Total N-Output.	N in Urine.	N in Faeces.	Total Uric Acid.	Ingested P_2O_5	P_2O_5 in Faeces.	P_2O_5 in Urine.	Amount of Urine.	Weight of Dry Faeces.
Apr. 27–28–29.	75.0 g. Plasmon, 300.0 g. Cracker dust, ca. 35.0 g. Lard.	9250	12.891	14.140 g.	12.909	1.231 g.	0.240 g.	3.9045 g.	—	2.826 g.	1500	38.0 g.
30–May 1–2.	75.0 g. Plasmon, 300.0 g. Cracker dust, ca. 35.0 g. Lard.	9400	"	14.867	12.18	2.687	0.492	"	1.3278 g.	2.254	2020	88.0
3–4–5.	25 g. Plasmon, 300 g. Cracker dust + 50 g. Nucleoproteid.	9250		14.226	12.78	1.446	0.663		—	2.157	1700	45.0
6–7–8.	Plasmon, Cracker dust, Lard.	9250	"	12.559	11.2	1.359	0.230	"	—	2.113	1000	43.0
*16–17–18.	" + 1 g. Adenin sulphate.	9250		12.118	10.69	2.028	0.620		—	2.317	2190	65.0
19–20–21.	Plasmon, Cracker dust, Lard.	9150	"	14.631	13.8	0.831	1.050	"	—	3.156	3000	43.0
22–23–24.	" + 10 g. Nucleic acid.	—		15.13	13.37	1.766	1.301		2.519	3.947	3420	68.0
25–26–27.	Plasmon, Cracker dust, Lard.	9200	"	13.75	12.53	1.22	0.681	"	—	2.632	2710	58.0
28–29–30.	" " "	—	"	13.804	12.64	1.164	0.721	"	—	2.644	2560	38.0
31–June 1–2.	" + 3 g. Thymin.	9200		12.512	11.22	1.292	0.707		—	2.408	3005	45.0
3–4–5.	Plasmon, Cracker dust, Lard.	9300	"	14.106	11.986	2.12	0.644	"	—	2.596	3020	58.0
6–7–8.	" + 6 g. Thymin.	9350		12.874	11.708	1.166	0.755		—	2.940	4425	43.0
9–10–11.	Plasmon, Cracker dust, Lard.	9400	"	12.602	11.401	1.201	0.796	"	—	2.517	2800	38.0
12–13–14.	" " "	9550	"	13.542	11.835	1.617	0.840	"	—	2.690	2720	58.0
15–16–17.	" " "	9650	"	14.068	12.513	1.555	0.797	"	—	2.382	2800	60.0
18–19–20.	" " "	9600	"	14.079	12.699	1.380	0.773	"	0.9322	2.467	2650	48.0
21–22–23.	195 g. Cane sugar, 225 g. Cracker dust, Lard as above.	9700	3.042	9.467	8.808	0.659	0.947	0.774	—	1.846	2800	28.0
24–25–26.	195 g. Cane sugar, 225 g. Cracker dust, Lard as above.	—	"	7.638	7.140	0.498	0.972	"	—	1.622	3260	23.0
27–28–29.	195 g. Cane sugar, 225 g. Cracker dust, Lard as above.	10350	"	12.428	8.708	3.720	1.025	"	—	1.902	3590	285.0 †
30–July 1–2.	195 g. Cane sugar, 225 g. Cracker dust, Lard as above.	11200	"	10.824	7.382	3.442	0.857	"	—	1.294	2700	422.0 †
3–4–5.	Starvation.	8700	0.0	7.344	7.344	No faeces.	0.906	None.	0.0	1.518	3200	0.0
6–7–8.	"	8350	0.0	6.65	6.65	No faeces.	0.746	None.	0.0	1.390	1450	0.0

75.0 g. Plasmon (3 days) = 8.835 g. N.; = 2.8725 g. P_2O_5.
300.0 g. Cracker dust (3 days) = 4.056 g. N.; = 1.032 g. P_2O_5.

* May 9–15 not worked out, owing to accidental loss of urine.
† Faeces does not dry thoroughly, owing to large amount of sugar contained.

employed in the experiment had been extracted with alcohol and dried, its absorption and assimilation were perhaps somewhat unsatisfactory. Few experiments in which dogs have been fed on pure nucleoproteins are recorded, but the feeding of organs rich in nucleoproteins and purins in the experiments which have been reported have failed to cause any marked increase in the elimination of uric acid.

Feeding of Adenin.—One gram of adenin sulphate was administered the first day of the experiment. All earlier experiments in which dogs were fed on purin bases failed to show any increase in uric acid output. Only Minkowski[1] records an increased elimination following xanthin or hypoxanthin feeding, but he failed to obtain an increase with adenin. In the present experiment a slight increase in the uric acid output was noticed during the period of feeding; it was more marked in the following period. It is difficult to determine from this one experiment whether the rise was actually occasioned by the administration of adenin or occurred accidentally. Observations are recorded in which increase of uric acid appeared a few days after the administration of nuclear material (Camerer[2]). The increase in uric acid elimination during the period of adenin feeding, and during the following period was 0.3668 gram, an amount equal to 44.27 per cent. of the administered adenin. There was also noted an apparent retention of nitrogen during the period of the experiment, and an increase in the period next following, while during the latter period an increase in the uric acid elimination and diuresis were noted. All these findings may be explained by the action of adenin on the kidneys.

Feeding with Nucleic Acid.—The dog received in addition to its usual food ten grams of the sodium salt of nucleic acid of fish sperm. There was a marked increase of uric acid, an increase of phosphoric acid elimination and increased diuresis. The increase of nitrogen elimination did not exceed 0.8 gram. The nitrogen of this increase apparently had its origin in the absorbed nucleic acid, and corresponded to about 4.8 grams of nucleic acid. In reality, however, part of the increase in nitrogen elimination was occasioned by the

[1] *Arch. f. exper. Path. u. Pharm.*, 1898, xli, 375.

[2] *Zeitsch. f. Biol.*, 1896, xxxiii, 139; 1897, xxxviii, 206.

higher diuresis. According to the analysis of Levene and Mandel,[3] the nucleic acid employed in this experiment contained not more than 0.4 gram of purin bodies. The increase of uric acid elimination was about 0.2000 gram, which represents about 40 per cent. of the purin ingested with the nucleic acid.

In normal dogs an increase in the output of uric acid after nucleic acid feeding is recorded by Minkowski.

Feeding of Thymin.—Feeding experiments with pyrimidin bases have been performed by Stendel[4] with the purpose of studying the synthetic formation of uric acid in the living organism. Administration of thymin remained without influence on the output of uric acid. Stendel's efforts to recover the substance in the urine were unsuccessful.

In the present experiment, the dog received six grams of thymin, which was added to his food. No increase in the total output of nitrogen was noted; there was rather a slight nitrogen retention. On the other hand, a marked diuresis occurred, and the urine contained about 13.5 grams of the ingested thymin.

In order to obtain thymin, one liter of the urine was rendered acid by means of nitric acid and treated for pyrimidin bases by the Kossel-Jones method. The substance obtained in this manner was recrystallized out of very dilute sulphuric acid. A free crystalline base showing all the properties and the appearance of thymin was obtained. The substance had the following composition:

0.1372 grams gave 27.4 c.c. of nitrogen (over 50 per cent. potassium hydroxide) at $t^\circ = 32.0^\circ$ C. and $p = 756$ mm.

For $C_4H_6N_2O_2$:

Calculated $N = 22.22$ per cent.
Found $N = 22.96$ per cent.

Thus the greater part of the ingested thymin is eliminated by the kidneys; but it is difficult to form an opinion as to the extent of the decomposition in the organism, since it is possible that only part of the thymin had been absorbed, while another part may have suffered decomposition in the intestinal tract, through the action of bacteria.

[3] *Zeitsch. f. physiol. Chem.*, 1906, l, 1.
[4] *Ibid.*, 1901, xxxii, 285.

Since it had been observed that the oxidation of thymin in the organism of the dog employed in this experiment was greatly impaired, an attempt was made to discover thymin in the urine obtained during the purin free periods, and also during the periods following the nucleic acid feeding. This was done with a view to establish the extent of nucleic acid decomposition in the organism. It was expected that thymin would be found in the urine, if considerable quantities of it were formed in the organism. From the experience of one of us (Levene[5]) with autolysis of animal organs, and also from that of Jones[6] and of Reh,[7] it is known that on disintegration of tissues, pyrimidin bases are formed. Nevertheless, neither during the period of nuclein free diet nor during that of the nucleic acid feeding, did the urine show any traces of thymin. One is, therefore, led to the conclusion that in the living organism nucleic acid either does not suffer complete disintegration, or disintegrates slowly, the small quantities of thymin thus formed being further decomposed.

Diet with a Low Protein Content.—The object of these experiments was to obtain further information regarding the factors which regulate the output of endogenous uric acid. Our present knowledge of endogenous uric acid is based on a very limited number of observations made on men. On the normal dog the study of nuclein metabolism has not been possible, since the intermediate products of decomposition of these substances are very readily transformed by the animal into urea. However, a dog with Eck's fistula possesses a lower degree of oxidizing power for purin bases than even man. Thus, it is seen, after feeding adenin that nearly 45 per cent. of the substance was eliminated with the urine in form of uric acid. Burian and Schur[8] found in men, after administration of hypoxanthin, a figure approaching this, and one less than half so high after feeding other purin bodies. It is believed by most observers that the output of endogenous uric acid remains practically constant and is totally independent of the quantity and of the quality of the food taken.

[5] *Zeitsch. f. physiol. Chem.*, 1901, xxxii, 546; 1903, xxxvii, 521; 1904, xli, 402.
[6] *Ibid.*, 1904, xlii, 35.
[7] *Hofmeister's Beiträge*, 1903, iii, 569–573.
[8] *Pflüger's Arch.*, 1900, lxxx, 342.

True, Burian and Schur,[9] the principal advocates of this view, considered it necessary to point out that extreme changes in the quantity or in the character of the food may alter the output of endogenous uric acid. Excessive intake of food, which taxes the gastrointestinal glands beyond normal, may result in an increased uric acid elimination, while on the other hand, starvation results in a decreased uric acid output, since it lowers the intensity of general metabolism. Folin[10] much more emphatically expresses the view that the output of uric acid is not invariably the same for an individual, as Burian and Schur[11] claim.

Folin arrives at this conclusion: "When the total amount of protein metabolism is greatly reduced, the absolute quantity of uric acid is diminished, but not nearly in proportion to the diminution in the total nitrogen, and the percentage of uric acid nitrogen in terms of the total nitrogen is, therefore, much increased." L. B. Mendel[12] states, "When the total amount of protein metabolism is greatly reduced, the endogenous output of uric acid is diminished, though this is not the case within ordinary ranges of diet."

A reduction of the intake of albuminous food-stuff should occasion for a time an increased disintegration of the body proteins, and with it a greater destruction of the cellular elements of the tissues. This, in its turn, should result in a greater formation of uric acid. Therefore, it seemed to us probable that the diminished uric acid output, under the conditions indicated by Folin, Mendel, and others, was caused, not by diminished formation, but by more complete combustion of the purin bodies. *A priori,* it might be expected that the organism utilized more completely nitrogenous substances of non-protein nature, when there was a lack of protein in the food. If this assumption were correct, one should find under these conditions an increased elimination of uric acid when the power of the organism to consume purin bodies is diminished. In our experiments, indeed, the diminution of the protein intake and its substitution by fat and carbohydrate (so that the intake of

[9] *Loc. cit.*

[10] *Amer. Jour. of Physiol.,* 1905, xiii, 87.

[11] *Loc. cit.*

[12] Harvey Lecture, *Journal of the American Med. Ass.,* 1906, XLVI, 843, 944.

calories was not altered) was followed by an increase in the uric acid elimination. It was expected that the organism would, on a continued low protein diet, finally adjust itself to the condition and preserve the integrity of the remaining cellular elements. In our dog, the uric acid elimination suffered a marked fall during the fourth low protein period.

Starvation.—In order to understand fully the influence of diet on the output of endogenous uric acid it was considered necessary to ascertain the influence of starvation on uric acid elimination. It was originally planned to have the starvation period precede the period of low protein diet. Since fasting might prove fatal to the dog, it was thought safer to change the order of the experiments. During the first starvation period, the uric acid elimination showed a rise, as compared with the preceding period. The output, however, was not as high as during the early period of low protein diet. Comparing the starvation periods with the periods of diminished protein diet, one gains the impression that the uric acid output was higher during the latter experiment. One must, however, bear in mind that this experiment preceded starvation and the continued low protein diet caused a considerable diminution in the mass of the cellular elements of the body. On the other hand, it is also possible that the absence of activity of the digestive glands is the cause of the lower output of uric acid during starvation.

The Period Following Starvation.—After the second starvation period the animal received the usual diet, consisting of cracker dust, plasmon, and lard. It ate all the food given, but on the next day refused to eat, and later developed the typical symptoms described by Pawlow. Once, typical epileptic convulsions were observed by the writers. The animal remained for forty-eight hours without food, and showed a tendency to recover. It refused the usual food—plasmon, etc.—and also milk, and was then given boiled meat which it ate ravenously, apparently improving. However, after eighteen hours, it again relapsed into the nervous condition described, and was found dead on the morning of the fifth day after the end of the starvation period.

The autopsy showed a condition of extreme emaciation. Aside from cachexia, the organs of the body presented a normal appear-

ance. The liver was possibly of smaller size than usual, but showed no cirrhotic or other lesions. The veins of the abdominal viscera were filled with an injection mass, the vena cava having been tied above the diaphragm, and the site of the anastomosis was minutely dissected; no collateral branches, which sometimes carry portal blood around the ligature about the portal vein, were found. The opening of the fistula was large and perfectly formed.

On the Diuretic Action of Nuclein Derivatives.—A review of the tables clearly shows that a more or less pronounced diuresis follows the administration of nuclear material. The tables of Burian and Schur,[13] and those of Minkowski indicate the occurrence of this phenomenon. In the experiments of these investigators, increased diuresis followed only the administration of nucleins, or nuclein containing tissues, but did not follow the administration of the free purin bases. In our experiments diuresis was less pronounced after the administration of adenin. It is worthy of note, however, that the administration of thymin caused the most pronounced diuresis. As is well known, the methylated dioxypurins possess a much higher diuretic action than other purin derivatives, and it seems probable that the methylated dioxypyrimidin also possesses a high diuretic action. This question, however, can be answered only after further study.

[13] *Loc. cit.*