CXXXVIII. STUDIES IN PURINE METABOLISM.

IV. THE NUCLEAR-PLASMIC RATIO IN DOGS IN CARBOHYDRATE AND PROTEIN FEEDING, AND IN STARVATION.

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IN Part I of this series [1926] it was shown that the nuclear-plasmic ratio of rats is slightly lowered on death from total inanition, whence it was concluded that protein reserves do not exist in the cytoplasm of rats. In this paper similar measurements made on the liver and skeletal muscles of dogs under various conditions of alimentation will be described.

Whilst the theoretical and historical aspects of the question of protein reserve were considered in the above-mentioned paper (Part I), as certain other researches have since come to my notice, I shall commence with their discussion.

Terroine and co-workers have, in a series of papers on inanition, expressed their views on the protein reserve question. Thus Terroine, Feuerbach and Brenckmann [1923] considered that, as the percentage protein content of mice and small birds increases during inanition, protein reserves cannot exist in these animals. In 1926, Terroine, Trautmann and Schneider express the opinion that, if protein reserves do exist, they must be very small in comparison with carbohydrate and fat reserves. Since, however, they found an increase in the total nitrogen content of chicken liver and muscle after protein feeding, they conclude that a limited storage of proteins may exist.

A number of other authors such as Friske [1909] and Junkersdorf [1921] have observed that the nitrogen content of various organs rises in protein feeding as compared to carbohydrate feeding, and have hence drawn the conclusion that this is due to protein storage.

The experiments conducted in this paper show, however, that the premises upon which such arguments are based are misleading, since no analysis is made of the repartition of this nitrogen between nucleus and cytoplasm. This question will be discussed more fully later.

Rubner [1911] describes an experiment of Thomas in which, in a man maintained on a carbohydrate diet after a period of protein feeding, nitrogen elimination falls for nine days, and this Lusk [1924] explains as being due to the gradual solution of "deposit protein," which may amount to 3 % of total

proteins. A very detailed study of nitrogen hunger in man has been published by Smith [1926], whence it appears that, whilst uric acid-nitrogen excretion is practically constant throughout the period of nitrogen hunger, total nitrogen falls constantly during the experimental period of over three weeks; whilst at first sight this research would appear to support the view that protein storage exists, we shall adduce evidence in a later part of this paper that the results of this author might be interpreted otherwise.

Finally, Tichmeneff [1914] and Cahn-Bronner [1914; Berg and Cahn-Bronner, 1914], on the basis of metabolism experiments, conclude that the liver may serve as a storehouse of protein matter.

It is our opinion that metabolism experiments alone, unsupplemented by direct analyses, cannot yield decisive results in such cases. In particular, the question of the fate of retained nitrogen cannot be answered merely by drawing up a nitrogen balance sheet, whilst the evidence on which protein storage in mammals is accepted is extremely slender.

In order to throw further light on the above question, analyses have now been made with the object of determining the total nitrogen, protein and purine-nitrogen contents and the nuclear-plasmic ratio of the liver and skeletal muscles of dogs on a carbohydrate and a protein diet, and in inanition. Should any protein be stored in the cytoplasm of the cells of the above tissues, one would expect the ratio of nuclear to cytoplasmic nitrogen to fall, since we have no reason to believe that reserve protein is a nucleoprotein. Similarly, if in hunger reserve protein is used up, the nuclear-plasmic ratio should rise accordingly.

EXPERIMENTAL.

A series of dogs was fed for three weeks on oatmeal and potatoes—a diet which contains sufficient protein to cover nitrogen catabolism, but which is mainly carbohydrate. The dogs were then killed by injection of chloroform into the heart, the liver and about 100 g. of mixed skeletal muscle being then removed. The liver, after expression of blood and washing, was weighed and minced; from the well mixed mince, 50 g. were taken for hydrolysis and 20 g. were dried to constant weight. In certain cases a further 20 g. portion was extracted with hot alcohol and dried. Portions of the powdered residue were then weighed out, and nitrogen determined by the Kjeldahl method. In this way the percentage of protein nitrogen could be determined, as distinct from total nitrogen, which was determined from the hydrolysate of the 50 g., as described in a previous paper [Truszkowski, 1926]. Purine-nitrogen was determined by the method of Krüger and Schittenhelm [1905] and the nuclear-plasmic ratio was calculated as $N_P/N_T - N_P$.

Skeletal muscle was taken from all four limbs and from various parts of the body; as far as possible fat and sinews were removed, and the remainder minced and treated as for liver, except that protein-nitrogen was not separately determined.

The results obtained are shown in the first parts of Tables I and II. Table I gives the values found for liver. The weight of this organ varies from 151 to 320 g., this depending largely on the weight of the dog examined. Percentage dry substance varies from 20.7 to 25.9, average 24.4. The lowest nitrogen content is 1.898 %, and the highest 2.800 %; for dry weight from 7.33 to 11.46 %. Protein-nitrogen, as determined for dogs 1, 4 and 5 is almost identical with total nitrogen in the first case, and 10 and 5 % higher in the other two cases respectively, indicating an extraction of non-nitrogenous constituents, probably lipins, by the alcohol. Purine-nitrogen varies from 76.4 to 93.3, average 86.7 mg. % live weight and from 325.5 to 396.8, average 355.5 mg. % dry weight. The nuclear-plasmic ratio varies from 32.71 to 47.26—a maximum deviation of about 20 % above and below the average of 40.53×10^{-3} .

Table I. *Liver in carbohydrate and protein feeding, and in starvation.*

No.	Weight of liver g.	% of dry substance	% N live weight	% N dry weight	% Protein-N live weight	% Protein-N dry weight	Purine-N mg. % live weight	Purine-N mg. % dry weight	Nuclear-plasmic ratio $\times 10^{-3}$	Nature of feeding
1 ♂	240	25.6	2.055	8.03	1.979	8.097	89.9	350.9	45.69	3 weeks carbohydrate
2 ♀	267	25.9	1.898	7.33	—	—	84.3	325.5	46.46	3 " "
3 ♂	329	25.4	2.837	11.16	—	—	89.9	353.6	32.71	3 " "
4 ♂	151	25.2	2.800	11.14	—	12.30	—	—	3	3 " "
5 ♂	231	20.7	2.372	11.46	—	11.93	76.4	369.2	33.30	3 " "
6 ♂	320	23.5	2.066	8.79	—	—	93.3	396.8	47.26	3 " "
Mean	—	24.4	2.335	9.65	—	—	86.7	355.5	40.53	
7 ♀	225	24.3	3.311	13.62	—	—	124.4	511.8	39.02	3 weeks meat
8 ♂	280	29.5	3.334	11.31	—	—	—	—	3	3 " "
9 ♂	325	22.7	2.847	12.54	—	—	128.3	565.2	47.19	10 " "
10 ♂	383	26.5	4.489	16.94	3.303	13.62	144.7	546.2	33.33	12 " " *
11 ♀	840	24.0	2.926	12.19	—	—	114.5	476.9	40.70	12 " "
Mean	—	25.4	3.341	13.32	—	—	127.5	525.0	39.69	
12 ♂	141	25.2	4.064	16.11	3.929	16.20	167.6	663.7	43.02	14 days inanition
13 ♂	200	24.3	3.181	13.09	—	—	123.8	509.3	40.49	23 " "
14 ♂	86	26.5	3.931	14.84	—	—	182.9	690.2	48.79	35 " "
Mean	—	25.35	3.725	14.68	—	—	158.1	621.1	44.10	

* Dog No. 10 was killed by strychnine.

A second series of dogs was fed for periods of from 3 to 12 weeks on boiled horseflesh, and the same values determined as before (Tables I and II, second portions). Here we may note that the weights found for the liver are on the whole greater, although the dogs were about the same size. The percentage dry substance is also slightly greater, total nitrogen is 43 and 38 % higher, and purine nitrogen 47 and 41 % higher for wet and dry weights respectively. As a result the nuclear-plasmic ratio shows a slight increase of from 38.58 to 39.69×10^{-3} . One dog of this series was killed by strychnine (No. 10), and this dog shows exceptionally high values for total nitrogen, although protein-nitrogen gives figures comparable with the total nitrogen found in the other four cases. These high values may be partly due to loss of glycogen from the liver, and partly to the presence of extractive non-protein-nitrogen.

Three dogs were subjected to inanition, water being allowed freely. Two were taken for analysis immediately upon death from hunger, and one was killed whilst in the last stages of exhaustion. The duration of life under these conditions depended upon the room temperature, which was, however, never above 10°, and upon the initial state of nourishment. Here the percentage dry substance in the liver (Table I, Part III) is almost identical with that in meat feeding, total nitrogen is 10 % higher and purine-nitrogen about 20 % higher. Protein-nitrogen, as determined for dog No. 12, is almost identical with total nitrogen. The nuclear-plasmic ratio is about 10 % above that found in carbohydrate and protein feeding, pointing to a greater depletion of non-purine than of purine constituents of the liver.

Table II shows, on the whole, a similar state of affairs in muscle to that in liver, under the same conditions.

Table II. *Skeletal muscle in carbohydrate and protein feeding, and in starvation.*

No.	% solid sub-stance	% N live weight	% N dry weight	Purine-N mg. % live weight	Purine-N mg. % dry weight	Nuclear-plasmic ratio $\times 10^{-3}$	Nature of feeding
1 ♂	21.1	2.398	11.36	47.1	223.2	20.03	3 weeks carbohydrate
2 ♀	24.5	2.575	10.51	58.0	236.9	23.07	3 " "
3 ♂	21.3	2.742	12.88	58.2	273.6	21.70	3 " "
4 ♂	21.8	2.531	11.61	55.1	252.8	22.26	3 " "
5 ♂	18.0	2.310	12.83	49.2	273.3	21.76	3 " "
6 ♂	20.3	2.527	12.47	57.4	283.4	23.25	3 " "
Mean	21.2	2.514	11.88	54.2	256.2	22.01	
7 ♀	26.3	3.181	12.09	72.1	274.0	23.18	3 weeks meat
8 ♂	22.3	3.087	13.85	84.3	377.9	28.06	3 " "
9 ♂	24.8	3.357	13.54	87.7	353.8	26.84	10 " "
10 ♂	23.8	3.931	16.52	81.7	343.4	21.23	12 " "
11 ♀	24.0	3.480	14.50	77.3	322.1	22.64	12 " "
Mean	24.2	3.407	14.10	80.6	334.2	24.28	
12 ♂	18.8	3.174	16.88	—	—	—	14 days hunger
13 ♂	24.3	3.527	14.52	83.68	344.3	24.30	23 " "
14 ♂	19.5	3.249	16.66	65.66	336.7	20.63	35 " "
Mean	20.8	3.317	16.02	74.67	340.5	23.02	

In meat feeding, dry substance is 14 % higher than in carbohydrate feeding, total nitrogen is 36 and 19 %, and purine-nitrogen 49 and 30.4 % higher for wet and dry weights respectively. Here, too, the nuclear-plasmic ratio is higher by 10 %, greater variations existing between the various values obtained in the meat than in the carbohydrate series, namely, from 20 to 23 in the latter and from 21 to 28 in the former. This difference is due to variable total nitrogen contents in the meat series, from 3.087 to 3.931 %. In hunger, the percentage solid substance falls to slightly below the carbohydrate level, with a corresponding drop in total nitrogen content, as a result of which the latter value for dry muscle is the highest of all obtained, corresponding to practically pure protein. The purine content is slightly lower than in meat feeding, and the nuclear-plasmic ratio lies between the two previously obtained

values, showing that in dog muscle the ratio between cytoplasmic and nuclear nitrogen is constant, in spite of considerable variations in the individual values obtained for total and purine-nitrogen.

DISCUSSION.

The above results show that in dogs the nuclear-plasmic ratio of liver and skeletal muscle is in both cases increased by liberal meat feeding as compared to carbohydrate feeding. Hence it follows that, in these circumstances, not only is no specific protein laid apart in the cytoplasm of the cells of the organs in question, but proportionally more nuclear substance is formed. A further significant difference which may be observed is the considerable rise in the nitrogen content of the muscle and liver in meat feeding, amounting to from 30 to 50 % for both nucleus and cytoplasm. This indicates that nitrogen retention may be due not only to hyperplasia but also to an actual higher concentration of nitrogen in the cells; this difference is, further, too great to be explicable on the basis of different glycogen content. That this is due partly to a diminution in water content is shown by the greater percentage content of dry substance, particularly in muscle. This dehydrating effect of a protein diet has been shown by Bischoff and Voit [1860], and the above results afford, therefore, a confirmation of this early work.

In starvation the water content of skeletal muscle is of the same order as in carbohydrate feeding, whilst the nitrogen and purine contents are only slightly below the meat level. The values found for the nuclear-plasmic ratio are not higher than those found in the previous series, so that here there is no evidence for the existence of protein reserves in muscle cells.

In the case of the liver, dilution cannot be observed in hunger, the mean value found for percentage of dry substance being higher than in carbohydrate feeding. All other values are higher than in the previous series; in particular, high values are found for purine-nitrogen, which is 19 % above meat value, whereas total nitrogen is only 11 % above. In this case, therefore, it would seem that the nucleus has lost proportionally less than the cytoplasm, and consequently that the liver cells may contain a limited store of reserve protein, whose amount cannot be increased by liberal protein feeding, and which is called upon in extreme emergency. On the whole, however, it appears that protein is stored by the body not as a specific deposit protein, but rather by the proliferation of cells, accompanied by greater protoplasmic content of the latter, this difference being over 40 % in the case of the liver, and close on 40 % in the case of skeletal muscle.

Viewed in this light, various arguments advanced in favour of protein storage to a large extent lose their point. Thus the experiments cited in the introduction (Junkersdorf, Friske, Terroine), in which the nitrogen content of muscle and liver rises after protein feeding, are confirmed, but as the increase affects both nucleus and cytoplasm to the same extent, they cannot be considered to afford any evidence whatsoever that a specific protein is laid down.

Nor can the gradual diminution in nitrogen excretion observed during specific nitrogen hunger be put forward as proof of protein storage. It has been shown in this paper that in protein feeding the cells may contain 40 % more nitrogen than in carbohydrate feeding. This 40 % probably corresponds to Rubner's [1911] "improvement quota," and appears to be used up in nitrogen hunger; at the same time nuclear material is, however, catabolised. In nitrogen equilibrium the nitrogen of the entire cell is excreted as urea and uric acid. In nitrogen hunger, on the other hand, amino-acids derived from the autolysis of body tissues are not deaminated but are used for the production of new protein in surviving cells; this re-utilisation of protein material appears to become more and more complete as the body cells approach the carbohydrate diet level of protein content. At the same time, uric acid excretion, which represents nuclear catabolism, should remain constant, since (cf. Part II) purines are synthesised with comparative facility by the mammalian organism, but are not available for resynthesis of nucleic acids if introduced into the blood-stream. The gradual fall in nitrogen elimination during nitrogen hunger, and the constant excretion of uric acid may, therefore, be explained without the necessity of introducing the conception of specific protein storage.

SUMMARY.

1. The chemical nuclear-plasmic ratio of the liver and of skeletal muscle of dogs has been determined, under various conditions of alimentation.
2. The total nitrogen and purine contents of the liver are respectively 43 and 47 % higher in dogs fed with meat than in those fed on carbohydrates.
3. The nuclear-plasmic ratio of liver tissue in meat-fed dogs is almost the same as on a carbohydrate diet, indicating that a liberal protein diet does not lead to the formation of specific deposit protein.
4. The content of solid substance is 14 % higher in skeletal muscle of dogs fed on a meat than on a carbohydrate diet, pointing to a certain dilution of the tissues in the latter case.
5. The total nitrogen and purine contents of skeletal muscle are respectively 35 and 49 % higher in dogs fed with meat than in those fed on carbohydrates.
6. The nuclear-plasmic ratio of skeletal muscle in meat-fed dogs is slightly higher than that encountered in carbohydrate feeding; hence specific reserve protein is not laid down in the muscles as a result of exclusive protein feeding.
7. In starvation, the nuclear-plasmic ratio of the liver is 10 % above normal, pointing to the possible presence of specific reserve protein in this organ.
8. Under similar conditions, the nuclear-plasmic ratio of skeletal muscle is unchanged, indicating that deposit protein is absent from this tissue.

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