CXCIII. CHANGES IN THE NUCLEAR-PLASMIC RATIO OF MAMMALS DURING HUNGER.

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IN the preceding paper [1928] upon changes in the nuclear-plasmic ratio of vertebrate poikilotherms during hunger considerable differences were observed in the behaviour of frogs, axolotls and trout. Thus the nuclear-plasmic ratio of amphibia remained practically constant during inanition, whilst in the case of trout it at first rose, falling to the normal value in the later stages of hunger. This result was interpreted as being evidence for the possession by trout of protein reserves.

In view of the smaller part played by protein in the metabolism of mammals, it appeared probable that the corresponding changes in their nuclear-plasmic ratio would be inconsiderable. Truszkowski's researches [1926, 1927], in which the nuclear-plasmic ratios of whole rats subjected to total inanition and of skeletal muscle and liver of dogs, fed on carbohydrates or proteins, or starved, were determined, appear to confirm this supposition. This author, who found that in skeletal muscle the nuclear-plasmic ratio remained constant under the above conditions, drew the conclusion that protein reserves do not occur in these tissues; the possibility of a limited storage of protein in canine liver was not, however, excluded. These results, are, on the whole, in accordance with generally accepted views [cf. Caspari and Stilling, 1925].

Deuel *et al.* [1928] found, on the other hand, that injections of thyroxine have the effect of increasing urea-nitrogen excretion without raising uric acid-nitrogen excretion, and this effect may be obtained even on persons previously subjected to prolonged nitrogen hunger. These results are, in spite of certain objections, such as that purine-nitrogen was not determined in the urine and that the residual nitrogen content exhibits a considerable increase after injection of thyroxine, strong evidence for the presence of protein reserves in man, and calculation shows that these must be to a considerable extent localised in the muscles.

Certain of Truszkowski's results necessitated additional research, chiefly as to the study of the nuclear-plasmic ratio during the various stages of hunger, and not only at death from inanition, and this question is, amongst others, the subject of this paper.

It is known that the part played by protein in hunger metabolism depends to a large extent upon the stage of inanition, and that it increases rapidly as fat reserves approach exhaustion; this is, according to Voit [1901], the cause of the premortal increase in nitrogen excretion, although Schultz [1899, 1901] and Serio [1923] ascribe the latter to the toxic effect of nitrogen hunger, which leads to the wholesale breakdown of cells.

The nuclear-plasmic ratio might increase to a varying extent, according to the slower or faster exhaustion of protein reserves, and might finally, during the pre-mortal period of widespread cell destruction, possess its normal value, or even a lower value (Truszkowski).

In order to throw light upon this matter the nitrogen excretion of fasting animals was determined parallel to their nuclear-plasmic ratio.

The chemical nuclear-plasmic ratio, expressed as $N_p/(N_T - N_p)$, does not, as Schaeffer and Le Breton [1923] have pointed out, express the true ratio of nuclear to cytoplasmic nitrogen. Accepting quite arbitrarily for technical reasons that purine-nitrogen is a measure of nuclear mass (in reality it amounts to only two-thirds of the total nucleic acid-nitrogen, the remainder being pyrimidine base-nitrogen), and that total nitrogen is expressive of the cytoplasmic mass, we obtain figures divested of any intrinsic significance. Only changes in the ratio can be considered as having any significance in the investigation of such questions as that of the existence of reserve protein. In order, however, for the figure obtained for purine-nitrogen to be as representative as possible of the nucleus, it should be derived from the nuclein fraction only, and would consist of guanine and adenine only; oxypurines such as hypoxanthine, xanthine and uric acid should be excluded, as well as the adenine of adenylic acid [Embden and Zimmerman, 1927, 1, 2].

Schaeffer and Le Breton, who worked on embryos, could neglect this second fraction, but point out that for adult animals it may be considerable, and this would be the more so for determinations of the chemical nuclear-plasmic ratio of skeletal muscle.

Experiments were therefore carried out in order to verify whether the results obtained for skeletal muscle, after removal of soluble purines and non-protein nitrogenous substances, such as carnosine, carnitine, creatine, etc., are similar to those obtained for unextracted muscle.

EXPERIMENTAL.

Well nourished rabbits were taken as experimental material. They were kept in small cages at room temperature, under conditions of absolute inanition, *i.e.* without food or water, and were weighed daily. The urine was collected quantitatively and nitrogen determined every few days; towards the latter stages of hunger determinations were as far as possible made every day. Seven rabbits were killed after periods of hunger varying from 7 to 14 days, loss of weight of 17.8 to 28.4 %, four after 10–18 days, 40 % loss of weight. Of these, one was taken for analysis 1–2 hours after death from inanition, and

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three in the last stages of exhaustion—these exhibited a distinct pre-mortal rise in nitrogen excretion. The animals were killed by injection of chloroform into the heart, the skin being then immediately removed and skeletal muscle taken from the limbs, as far as possible free from tendons and fat. The material was then passed through a mincing machine, and 50–100 g. were weighed out and placed in boiling 4.5 % sulphuric acid.

In certain cases the carcasses were first chilled to 0°, and the above operations were repeated at a low temperature. This technique did not, however, affect the values obtained for purine-nitrogen.

The livers were taken as a whole, after the removal of the gall-bladder. Total and purine-nitrogen contents were determined as before, using for the latter determination a modification of Krüger and Schittenhelm's method.

Where water-extraction was applied the procedure adopted was as follows. The comminuted tissue was thrown into 500 cc. of boiling water, and the whole filtered after 10–15 minutes' boiling. This operation was twice repeated using 100–200 cc. of water, the united filtrates were freed from protein by boiling after feeble acidification with acetic acid, and the residue of coagulated protein was added to the original residue, which was then treated as before for total and purine-nitrogen estimation.

Table I. *Rabbit skeletal muscle.*

No.	% solid sub- stance	% N live weight	% N dry weight	Purine- N mg. per 100 g. live weight	Purine- N mg. per 100 g. dry weight	Nuclear- plasmic ratio	Initial weight g.	Final weight g.	% loss	Days hunger
I	24.7	3.826	15.43	110.5	447	29.73×10^{-3}				
II	24.4	3.755	15.39	114.57	470	31.19×10^{-3}				
III	24.4	3.830	15.70	119.1	488	32.10×10^{-3}				
IV	25.3	3.740	14.78	109.75	434	30.20×10^{-3}				
V	25.3	3.720	14.43	87.35	340	24.01×10^{-3}				
V _a	23.3	—	—	85.8	368	—				
Mean	24.63	3.774	15.13	104.5	424	28.05×10^{-3}				
VI	24.8	3.89	15.65	136.7	551	36.42×10^{-3}	1910	1570	17.8	10
♀ VII	27.7	3.555	13.8	110.9	400	29.31×10^{-3}	2890	2050	28.4	14
♀ VIII	23.75	3.605	15.18	123.3	520	35.4×10^{-3}	2140	1670	22.0	9
♂ IX	25.0	3.90	15.36	95.5	383	25.1×10^{-3}	3030	2300	24.0	9
X	22.5	3.65	16.22	82.42	366	23.08×10^{-3}	2450	1820	23.4	11
XI	27.8	4.04	14.53	91.97	331	23.30×10^{-3}	4250	3450	18.8	7
Mean	25.26	3.773	15.12	106.8	438	28.77×10^{-3}				
♀ XII	—	3.71	—	103.0	—	28.7×10^{-3}	2760	1630	40.9	10
♀ XIII	28.35	3.84	13.5	100.16	350	26.0×10^{-3}	2100	1230	41.4	18
♂ XIV	21.2	3.936	18.5	100.0	472	25.71×10^{-3}	2620	1550	40.8	18
XV	26.3	3.776	14.32	103.0	392	28.02×10^{-3}	2850	1700	40.3	18
Mean	25.28	3.815	15.44	101.5	401	27.34×10^{-3}				

The results, given in Table I, are in almost every case the mean of 2–3 concordant determinations. Considerable individual differences in purine-nitrogen content exist, this varying from 85.8 to 119.1 mg. per 100 g. The mean value of 104.5 mg. is higher than that found by Kikuchi [1923], viz. 66 mg. with variations of 58–83 mg.; this author, however, used Burian and

Hall's method, which, according to Schaeffer and Le Breton [1923], gives low results. Rabbit muscles, however, appear to have a higher purine-content than those of other mammals studied [Truszkowski, 1927, p. 1043].

As to inanition, the values found are constant for all stages of hunger, this applying to total and purine-nitrogen contents and to the nuclear-plasmic ratio. A general tendency towards increase in the latter in hunger appears, however, in liver tissue. Truszkowski obtained similar results for dogs. The marked lack of constancy in the results does not allow, however, of the drawing of any more definite conclusion (Table II).

Table II. *Rabbit liver.*

No.	% solid substance	% N live wt.	% N dry wt.	Purine-N mg. per 100 g. live wt.	Purine-N mg. per 100 g. dry wt.	Nuclear-plasmic ratio	Remarks
I	27.5	2.58	9.39	121.4	441	49.3×10^{-3}	Fed animals
II	24.6	3.12	12.68	161.4	655	54.5×10^{-3}	"
III	26.8	2.75	10.26	137.2	528	52.5×10^{-3}	"
IV	29.1	3.38	11.68	153.8	524	47.7×10^{-3}	"
Mean	27.0	2.96	10.96	143.45	531	50.92×10^{-3}	
VI	29.1	3.64	12.5	177.5	610	51.3×10^{-3}	Inanition
VII	29.9	3.33	11.13	109.7	370	34.1×10^{-3}	"
VIII	33.0	2.96	8.96	178.0	538	64.0×10^{-3}	"
IX	28.6	3.67	13.10	145.0	520	41.03×10^{-3}	"
XII	23.0	3.21	13.36	168.8	734	55.5×10^{-3}	"
XIII	26.0	3.46	13.32	169.0	650	51.3×10^{-3}	"
XIV	25.55	3.67	14.37	208.4	810	60.2×10^{-3}	"
XV	26.0	4.16	16.01	140.3	539	34.86×10^{-3}	"
Mean	22.64	3.51	12.70	162.1	586	48.42×10^{-3}	

Previous extraction with water did not, with the exception of determinations 1 and 8, where the difference observed may be due to some experimental error, to any great extent affect the results obtained for the nuclear-plasmic ratio of unextracted muscle. The purine-nitrogen content whether of the muscles of fed or of starved animals was uniformly about half that found before extraction, whilst the total nitrogen content fell in all cases by about 15 %. The nuclear-plasmic ratio, although now expressed by a different figure (Table III), exhibited the same constancy with respect to different conditions of nutrition as did that of unextracted muscle.

Table III. *Extracted rabbit skeletal muscle.*

No.	% solid substance	% N live wt.	% N dry wt.	Purine-N mg. per 100 g. live wt.	Purine-N mg. per 100 g. dry wt.	Nuclear-plasmic ratio	Remarks
I a ♀	25.0	3.212	12.84	70.40?	281?	22.41×10^{-3} ?	Fed animals
IV ♂	25.3	3.204	12.66	39.07	154	12.03×10^{-3}	"
V	25.8	3.286	12.74	43.78	171	13.68×10^{-3}	"
Mean	25.4	3.234	12.75	41.42	162	12.85×10^{-3}	
VIII	23.75	3.17	13.40	74.19?	312?	23.97×10^{-3} ?	Inanition
IX	25.0	3.38	13.50	46.47	185	14.37×10^{-3}	"
XI	27.8	3.36	12.08	39.28	141	11.8×10^{-3}	"
XV	26.3	3.34	12.7	47.22	180	14.3×10^{-3}	"
Mean	25.71	3.31	12.92	43.32	169	13.29×10^{-3}	

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Similar results were obtained for the skeletal muscle of dogs, some of which had been fed for 3 months exclusively on oatmeal, whilst others were fed upon meat for 2 months. The same uniformity in the nuclear-plasmic ratio was found for yearling trout, starved for 6 months, and starved for 1 month, after which they were fed with liver for 5 months (cf. Table IV).

Table IV.

No.	% solid substance	% N live wt.	% N after extraction	After extraction				Nuclear-plasmic ratio after extraction	Remarks	
				Purine-N mg. per 100 g. live wt.	Purine-N mg. per 100 g. dry wt.	Purine-N mg. per 100 g. live wt.	Purine-N mg. per 100 g. dry wt.			
1 ♂	—	3.50	3.0	79.13	—	30.61	—	23.12	10.14	Skeletal muscle of dog fed on oatmeal
2 ♂	26.2	3.71	3.10	79.37	303	32.32	123	21.80	10.50	Skeletal muscle of dog fed on meat
3	17.0	2.61	2.09	88.5	520	52.55	309	35.08	25.7	Whole trout, six months' inanition
4	20.17	2.92	2.20	88.7	440	50.35	250	31.3	23.36	Whole trout, one month's inanition, five months' restitution

DISCUSSION.

The above results on the whole confirm those of Truszkowski [1927], who found that the nuclear-plasmic ratio of mammalian skeletal muscle remains constant throughout starvation. This would militate against the protein reserve theory on the assumption that the purine-nitrogen content of the cells remains unchanged. Whilst this might be conclusive evidence for many tissues, in which all or the great majority of purines present are of nuclear origin, it can in no case apply to muscular tissue, in which, as will be shown in a subsequent paper, the quantity of purines not belonging to the nucleus is exceptionally high, amounting to 50 % or more of the total purine content. Should, however, the total purine content remain constant during hunger, the interpretation of the constancy of the nuclear-plasmic ratio as evidence against the existence of a protein reserve would be justifiable. Since muscles, after extraction of all non-protein nitrogen derivatives and extramuscular purines, exhibit the same general constancy of nuclear-plasmic ratio as without extraction, it would seem that the above conclusions as to the absence of reserve protein in these tissues remain valid.

The parallelism existing between changes in total purine-nitrogen content and in nuclein-phosphorus content has been frequently observed. Thus Burian and Schur [1897] observed that the nuclein-phosphorus content of puppies increased 3.5 times during a period of growth over which the purine-nitrogen content increased 2.5–3 times. Masing's [1911] figures for the fall in nuclein-phosphorus content of rabbit foetuses with age correspond to those found by Schaeffer and Le Breton for purine-nitrogen, and Javillier's [1923] researches upon the nuclein-phosphorus content of mice during lactation are similarly in agreement with the results of these authors.

This parallelism is apparently due to the content of extranuclear purine substances rising together with the growth of tissues, and remaining constant after growth has ceased. These extranuclear purines cannot therefore be considered as being entirely independent of other cell constituents.

This constancy of extranuclear purine-nitrogen content is an extremely favourable factor, inasmuch as it enables one to make use of changes in the ratio $N_p/(N_T - N_p)$ for the study of reserve protein. In those cases where considerable increase in this ratio is found, amounting in the case of trout to 40 % [Dmochowski, 1928], it is extremely improbable that the presence of extranuclear purine substances could in any way affect the conclusions drawn from this increase. Where, however, as for amphibia and mammalian muscle, this ratio remains constant or falls slightly, further examination of the extranuclear purine content would be necessary before conclusions as to the absence of reserve protein could be accepted without reserve.

SUMMARY.

1. The chemical nuclear-plasmic ratio of liver and skeletal muscles of rabbits, taken at various stages of total inanition up to the final stage, marked by the pre-mortal rise in nitrogen excretion, has been determined.

2. The purine-nitrogen content of rabbit skeletal muscle has an average value of 104.6 mg. per 100 g., varying from 85 to 119 mg.; this value is higher than that of other mammals studied.

3. The mean value of the nuclear-plasmic ratio is 28.55×10^{-3} , and this value remains constant throughout the duration of inanition. The same applies to the purine-nitrogen content.

4. The purine-nitrogen content of rabbit liver exhibits even more considerable individual variations than that of muscular tissue; it varies from 149.5 mg. per 100 g. in fed animals to 162.1 mg. in inanition. The mean nuclear-plasmic ratio is respectively 50.9 and 48.4×10^{-3} before and after starvation.

5. Over 50 % of the purines of muscle tissue are extracted by hot water, and are not therefore contained in the nucleus.

6. Parallel results were obtained on muscle from which extranuclear purine substances had been removed by extraction with hot water; in this case, too, the value found for the nuclear-plasmic ratio remains constant.

7. The agreement between change of nuclein-phosphorus content and purine-nitrogen content observed by a number of authors is probably due to the relatively constant content of extranuclear purine substances.

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