CXCII. CHANGES IN THE NUCLEAR-PLASMIC RATIO OF VERTEBRATE POIKILOTHERMS DURING HUNGER.

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INTRODUCTION.

THE metabolic processes of poikilotherms differ considerably from those of homeotherms. Whilst during hunger proteins, carbohydrates and fats are metabolised by warm-blooded animals, yet the relative consumption of these constituents changes greatly during the course of inanition. Thus glycogen and fats are metabolised during the first or middle stage of inanition, and, as these reserves are consumed, protein metabolism begins to predominate. In the case of poikilotherms, conditions are different. The work of Reuss and Weinland [1912], Knauth [1898], Białaszewicz [1919] and others, has shown that the part played by protein in hunger metabolism is several times greater than for homeotherms, and recently Przylecki and Karczewski [1923] and Przylecki and Opienska [1926] have shown that, only under certain abnormal conditions, vertebrate poikilotherms are able, using fat and carbohydrate reserves, to spare the utilisation of protein in hunger as a source of energy.

A higher percentage of the organic constituents of poikilotherms is represented by protein than is the case for homeotherms. This is illustrated by Table I, where percentage protein and percentage protein metabolism in hunger are compared for various animals [Białaszewicz, 1919].

Table I. *Protein content and percentage protein metabolism.*

Animal	% of protein in organic constituents	% of total hunger metabolism due to protein	Author
<i>Lepus caniculus</i>	71.8	20.5*	Rubner [1881]
<i>Gallus domesticus</i>	66.8	18.8*	Kückein [1882]
<i>Anguilla vulgaris</i>	84.6	83.1	Reuss and Weinland [1912]
<i>Salmo salar</i>	63.3	73.1	Miescher [1897]
<i>Hirudo medicinalis</i>	90.5	84.5	Białaszewicz [1919]
<i>Amblystoma</i>	88.6	89.1	Librach [1922]
<i>Rana esculenta</i>	86.2	85.5	Librach [1922]

* For first few days of hunger.

We see that protein represents 80–90 % of the organic constituents of poikilotherms, and that about the same percentage of the total hunger

metabolism is due to protein. The loss of weight on death from inanition is much greater than for homeotherms, being 55–75 % of the original weight for frogs, axolotls and newts [Librach, 1922], whilst for birds and mammals it does not exceed 40–50 %.

The question arises whether the difference in the loss of proteins accompanying inanition between homeotherms (about 40 %) and poikilotherms (60–70 %) is due to a larger protein reserve possessed by the latter class of animals. Should this be so, the exhaustion of this reserve should lead to a marked increase in the nuclear-plasmic ratio. Research on this question has been conducted in this laboratory for some time, along lines initiated by Schaeffer and Le Breton [1923]. Truszkowski [1928] found that the nuclear-plasmic ratio of frogs not only did not rise, but even fell at the end of winter, from the average value of 37.8×10^{-3} to 35×10^{-3} in March and 30.5×10^{-3} in April. This paper describes further similar researches on protein reserve, the number of different species examined being greater, and the period of inanition longer. A wider range of temperatures of the surroundings was also taken.

EXPERIMENTAL.

Frogs (*Rana esculenta*), axolotls (*Amblystoma*) and trout (*Salmo officinalis*) were taken as experimental material. The technique adopted was that described by Truszkowski [1926], being a slight modification of Schaeffer and Le Breton's method.

1. *Frogs*. Four series of experiments were carried out on frogs. The first group represented fed animals, taken in October and November, the second group was of frogs not fed for 26 days, kept at a temperature of 25° (December), the third represented 53 days of hunger at 15° (January), and the fourth 73 days of hunger at 0° (February). A few animals were taken for each determination, being minced immediately after removal of the testes or ovaries, and of the contents of the alimentary canal. The results, given in Table II, show that, as Truszkowski found, no great variations occur in the nuclear-plasmic ratio, the values found for the above four groups being respectively 35.89, 36.07, 33.37 and 36.77×10^{-3} .

Table II. *Nuclear-plasmic ratio of frogs at various stages of inanition.*

No.	Days hunger	% solid substance	% 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	Remarks
1	0	21.80	2.633	12.08	98.76	453	39.0×10^{-3}	
2	0	21.15	2.954	13.97	90.30	427	31.52×10^{-3}	
3	0	21.30	2.758	12.95	98.85	464	37.17×10^{-3}	
Mean	0	21.42	2.782	13.0	95.97	448	35.89×10^{-3}	
4	26	20.20	2.573	12.49	88.21	437	36.07×10^{-3}	At 25°
5	53	20.23	2.651	13.14	85.24	425	33.37×10^{-3}	At 15°
6	73	22.10	2.627	11.83	92.98	420	36.77×10^{-3}	At 0°
Mean	—	20.84	2.617	12.49	88.81	427	35.4×10^{-3}	

The figures given by Scaffidi [1910] for the purine-nitrogen content of frog muscle (35 mg. %) are too low, this difference being due to the fact that he calculated N_p as the sum of that of the extracted muscles plus that of the unhydrolysed extract.

2. *Axolotls*. A considerably longer period of inanition was taken for axolotls. As for frogs, four series of determinations were made. The first group of animals was analysed before hunger; the remainder was divided into three groups, each member of which was placed in a separate vessel with water at room temperature, which was changed every few days. These groups were taken for analysis after 145, 280 and 340 days of hunger. Our observations upon axolotls are to a certain extent divergent from those of Librach, who found that no individual survived more than 173 days at 25°, when 75 % of the original weight had been lost; in our case, after 340 days of hunger only 56.6 % had been lost. Further, Librach did not find a diminution in total dry content, which in our case fell from 13.5 to 9.1 %.

The results, given in Table III, show that the nuclear-plasmic ratio rises from 33.0 to 38.0×10^{-3} after the first 5 months of hunger, and then falls to 28.5×10^{-3} after 11 months. Owing to an accident, values were not obtained for 9 months of hunger, and also unfortunately material was not available for the study of the final stages of inanition, which would have been particularly interesting in view of the pre-mortal rise in nitrogen elimination.

Table III. *Nuclear-plasmic ratio of axolotls at various stages of inanition.*

No.	Days hunger	% loss in weight	% solid substance	% 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
1	0	—	13.80	1.702	12.33	52.38	380	31.75×10^{-3}
2	0	—	13.83	1.679	12.07	56.66	410	34.93×10^{-3}
3	0	—	13.60	1.774	13.06	55.57	408	32.32×10^{-3}
Mean	0	—	13.74	1.718	12.49	54.87	400	33.0×10^{-3}
4	145	35.5	13.50	1.732	12.83	63.48	471	38.0×10^{-3}
5	280	55.2	10.20	1.282	12.57	—	—	—
6	340	56.6	9.09	1.197	13.17	33.27	366	28.5×10^{-3}

3. *Trout*. Trout were obtained from the Zloty Potok breeding ponds. The fish, which were in a good state of nutrition, consisted of small fry about 5 g. weight each, one-year-olds, weighing 100–150 g., and two-year-olds weighing about 300 g. These were kept in running water aquaria which the Ichthyological Laboratory of the Chief Agricultural School kindly placed at our disposal. The first group of trout were analysed at once (Nov. 25th to Dec. 13th); the remaining fish were subjected to inanition.

50–100 g. of fresh mince were taken for each determination. The roe was removed before mincing, as well as the contents of the alimentary canal. The results, given in Table IV, indicate a 36.3 % increase in the nuclear-plasmic ratio of the young fish, which rises from 38.6×10^{-3} for fed fish to 52.5×10^{-3} after 80 days of inanition, involving a loss of body weight of 30 %. In one case, where the fish were analysed several hours after death, the nuclear-

Table IV. *The nuclear-plasmic ratio of trout fry at various stages of inanition.*

No.	Days hunger	% loss in weight	% 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	Remarks
1	—	—	21.0	2.78	13.24	105.18	501	39.32×10^{-3}	
2	—	—	—	2.58	—	97.65	—	39.34×10^{-3}	
3	—	—	21.67	2.64	12.19	113.5	524	44.91×10^{-3}	
4	—	—	21.53	2.57	11.95	86.19	401	34.70×10^{-3}	
5	—	—	21.8	2.72	12.48	93.65	430	35.66×10^{-3}	
Mean	—	—	21.49	2.66	12.46	99.23	464	38.78×10^{-3}	
6	80	30	18.3	2.46	13.31	123.12	673	52.49×10^{-3}	
7	80	30	17.65	2.30	13.03	97.4	551	44.21×10^{-3}	Dead fish taken

Table V. *The nuclear-plasmic ratio of two-year-old trout at various stages of inanition.*

No.	Days hunger	% 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	Remarks
1 ♀	—	22.15	2.79	12.6	88.0	397	32.2×10^{-3}	
2 ♀	—	19.4	2.42	12.38	53.11	274	22.44×10^{-3}	Dead fish taken
3 ♀	—	21.7	2.83	13.04	74.0	341	26.82×10^{-3}	Dead fish taken
4 ♀	80	22.0	2.82	12.83	121.5	552	45.0×10^{-3}	

Table VI. *The nuclear-plasmic ratio of yearling trout at various stages of inanition.*

No.	Days hunger	Days subse- quent feeding	% solid substance	% 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
1	90	—	17.7	2.525	14.27	113.5	641	47.06×10^{-3}
2	180	—	17.0	2.61	15.36	88.5	520	35.09×10^{-3}
3	30	60	19.7	2.58	13.1	116.5	592	47.15×10^{-3}
4	30	150	20.17	2.92	14.49	88.72	440	31.3×10^{-3}

plasmic ratio was only 44.2×10^{-3} , indicating rapid *post mortem* purinolysis or deamination. Similar results were obtained for two-year-old fish (Table V). Thus, before inanition, a nuclear-plasmic ratio of 32.2×10^{-3} was found for a female trout, whereas after 80 days of hunger this value had increased by 40.1 % to 45.0×10^{-3} . Here also fish taken for analysis some hours after death had very low purine contents. After 90 days of inanition the nuclear-plasmic ratio of one-year-old fish (Table VI) was 47.06×10^{-3} ; after 180 days when the fish were in the last stages of exhaustion, this value falls again to 35.09×10^{-3} . After 30 days' hunger followed by 60 days' feeding with liver, the nuclear-plasmic ratio was 47.15×10^{-3} , whilst after 150 days of restitution it was 31.3×10^{-3} . Owing to lack of material, separate determinations of the nuclear-plasmic ratio of one-year-old fish were not made; these presumably differ little from the values obtained for fry and for two-year-olds, or from those yearlings subjected to prolonged restitution. There is thus an undoubted increase in the value of the nuclear-plasmic ratio during the first stages of hunger, followed by a diminution to the normal value or slightly below. Restitution takes place slowly, pointing to an early depletion of reserves.

DISCUSSION.

A consideration of the above results, both from the points of view of protein reserve and of the part played by nuclear and plasmic constituents in hunger metabolism, shows that the animals studied may be divided into two groups—amphibia, which exhibit a practically constant nuclear-plasmic ratio throughout the duration of inanition, and fish, whose nuclear-plasmic ratio undergoes marked variations.

Certain differences could, however, be distinguished within the former group. Thus the nuclear-plasmic ratio of frogs was practically constant over the period of hunger, whilst in the case of axolotls, it rose by 15 % after 5 months, to fall again to 15 % below normal after 11 months of hunger. This decline may be observed for all animals so far studied, trout also exhibiting it, and Truszkowski, who found the same for rats and frogs, explains it as being due to the disintegration of a certain number of cells, with elimination of nuclear constituents and resorption of plasmic constituents by other cells; this process would prevent the excessive increase of the nuclear-plasmic ratio. It is also, however, possible that this fall is due to elimination of extra-nuclear purines. The constancy of this ratio for the frog would indicate that this animal has a more economical hunger metabolism than the axolotl, and is able, by a more uniform oxidation of its constituents to achieve a better regulation of its hunger metabolism. This view is further supported by Librach's observation that a tendency exists in these animals towards the reduction of their nitrogen metabolism during the last stages of hunger; it may be, however, that the protein reserve of frogs is very small, if it exists at all.

On the whole, our results for amphibia diverge considerably from those obtained by other authors, who, on the basis of measurements of the surface or volume of nucleus and cytoplasm, found that hunger in all cases led to an increase in the nuclear-plasmic ratio [Vieweger, 1921 (infusoria); Morgulis, 1911 and Leonard, 1887 (amphibia)]. Our gravimetric determinations would rather support the work of Białaszewicz and of Librach, who found that the chemical composition of fed and starved organisms is practically identical, and who connected this fact with the ability to withstand protracted inanition.

The results for trout are quite different. All individuals, irrespective of age, exhibited a considerable though transient increase of nuclear-plasmic ratio, amounting to about 40 %, followed by a return to normal values. So considerable a change in a fish closely related to the salmon, which has served as the classical example of the existence of reserve protein, must, in our opinion, be considered as a further, definitive proof of the existence of this reserve protein.

Both the chemical and histological evidence adduced in support of the reserve protein theory by Miescher [1897], Paton [1898], C. W. Greene [1919] and C. H. Greene [1919], have been considered to constitute a sufficient basis for the acceptance of this theory. In our opinion, however, certain objections might be raised against the validity of these proofs.

Firstly, the fact that the solid content of tissues falls continuously during hunger to a large extent vitiates conclusions based upon morphological evidence, as Schaeffer and Le Breton have pointed out. For the same reason, the observation that 30 % of the relative protein content is lost, taking as the basis for comparison the whole of the remaining fat-free protein (C. W. Greene's "protoplasm basis") cannot be accepted as evidence for the existence of reserve protein. C. W. Greene considers the constant mineral content of starved animals to be a sufficient proof that no change has occurred in the degree of hydration of the cytoplasmic protein colloids, but it may be considered that even minimal changes in the relative contents of the particular mineral constituents may lead to an increase in the water-content of the tissues.

The results of our research, based upon the determination of changes in the ratio of the weights of substances independent of the degree of hydration of the tissues, support the hypothesis of the existence of reserve protein, at least in fish, and their agreement with the results of other authors shows that the method applied by us to the study of this question is, in spite of all its short-comings, suitable for the purpose in question. These defects will be discussed elsewhere, and deal chiefly with extra-nuclear purines.

SUMMARY.

1. In view of their higher protein content, and of the greater part played by protein in the hunger metabolism of poikilotherms than in that of homeotherms, as well as of the greater loss of weight attainable, it would appear that the former afford a convenient material for the study of protein reserve.

2. The effect of inanition upon the nuclear-plasmic ratio of amphibia (*Rana esculenta* and *Amblystoma*) and of fish (*Salmo officinalis*) was investigated.

3. The nuclear-plasmic ratio was practically constant for frogs; in the case of axolotls it rose by 15 % after 5 months of hunger, and fell to 15 % below normal after 11 months of hunger.

4. The nuclear-plasmic ratio of trout after 80 days of hunger rose by 30 % for fry and 49 % for two-year-olds. Yearling trout exhibited an increase of about 50 % after 90 days of hunger. This rise in the nuclear-plasmic ratio is transient, values obtained for the final stages of hunger being subnormal.

5. A short period of restitution does not reduce the nuclear-plasmic ratio of trout to its normal value; this, however, takes place after a prolonged period of restitution.

6. The results obtained for trout are, in view of the elimination of the effect of tissue dilution accompanying hunger, satisfactory proof of the existence of reserve protein.

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