XXVIII. STUDIES IN PURINE METABOLISM.

V. THE NUCLEAR-PLASMIC RATIO OF FROGS.

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INTRODUCTION.

IN Parts I and IV of this series [1926, 1927] it was shown that inanition does not in the case of rats and dogs lead to an increase in the chemical nuclear-plasmic ratio, as would have been the case had protein reserves been present in the cytoplasm of these mammals. In view of this result, it was interesting to verify whether the same was applicable to poikilothermic animals such as frogs. This material is, moreover, of particular interest, for several reasons. Firstly, as shown by Przylecki and Opieńska [1926], these animals have normally an almost exclusively protein metabolism, and do not possess any considerable fat reserves. Thus, if protein reserves exist, they should above all be present in the poikilotherms. Secondly, the period over which they can endure inanition is much greater for frogs than for mammals. Thirdly, it was mainly upon the basis of the histological examination of such material that the theory of protein storage was originally put forward [Leonard, 1887; Morgulis, 1911]. Finally, since the frog undergoes certain seasonal changes, such as hibernation, sexual activity, etc., the possibility might exist that the nuclear-plasmic ratio might undergo seasonal variation.

EXPERIMENTAL.

Rana esculenta was employed. Six or seven frogs were taken for each determination, being minced after removal of the ovaries or testes and of the contents of the alimentary tract. The experimental methods used were the same as in our previous papers, *i.e.* total nitrogen, N_T , was determined by the Kjeldahl method, purine nitrogen, N_P , by the method of Krüger and Schittenhelm, the ratio $N_P/N_T - N_P$ being taken as the chemical nuclear-plasmic ratio.

Determinations were made once or more every month from May, 1926, to June, 1927 only the average value for the month being given in the table.

Table I. *Nuclear-plasmic ratio of frogs from May, 1926, to June, 1927.*

Month	% solid substance	% N live weight	% N dry weight	Purine-N mg. % live weight	Purine-N mg. % dry weight	Nuclear-plasmic ratio
May 1926	—	1.935	—	77.2	—	41.55×10^{-3}
July „	20.4	2.551	12.50	98.2	481.2	40.02 „
Aug. „	20.5	2.700	13.17	90.6	442.0	34.83 „
Sept. „	20.7	2.423	11.71	95.8	462.7	41.15 „
Oct. „	22.3	2.521	11.30	92.1	413.0	37.92 „
Nov. „	21.3	2.638	12.38	98.1	459.9	38.56 „
Dec. „	20.0	2.585	12.98	96.8	483.7	38.82 „
Jan. 1927	20.1	2.561	12.85	100.9	502.0	41.20 „
Feb. „	18.3	2.657	14.52	97.0	530.3	37.91 „
Mar. „	18.3	2.296	12.54	78.5	429.1	35.42 „
Apr. „	20.7	3.034	14.62	89.7	433.4	30.48 „
June „	18.5	2.474	13.38	85.3	461.2	35.71 „
Mean	20.1	2.531	12.81	91.7	458.2	37.80 „

The percentage dry content, at first 20.4, attains a maximum of 22.3 in October, falling to 18.3 in February, March and 18.5 in June. The total nitrogen content is lowest in May, 1926, presumably owing to high water content, sufficient material having unfortunately not been available for the determination of this value. During the rest of the year the values of N_T obtained vary very little from the average of 2.531 %, the highest value being found in April (3.034 %). The total nitrogen content calculated on dry weight is lowest in September and October and highest in the spring, corresponding with the greatest and least non-protein contents respectively. The purine nitrogen content varies 10 % about the average value of 91.7 mg. %, the highest values being found during the winter months (Nov.–Feb.).

As to the nuclear-plasmic ratio, this, with one exception, is extremely close to the mean value of 37.8×10^{-3} . Between March and June, 1927, the values obtained are lower than this average, whilst from May, 1926, to February, 1927, they are higher.

DISCUSSION.

In Poland, frogs do not feed between November and April, and the alimentary tract of frogs taken during this period is empty. In March or April begins the period of sexual activity, *i.e.* before feeding, and in this period considerable quantities of eggs and sperm are produced. If, therefore, specific protein reserves exist within the cytoplasm of the frog, these should be exhausted in March or April, with a corresponding rise in the nuclear-plasmic ratio. The above results show, however, that the value actually found is lower from February to April than during the preceding months. In Part I of this series [1926] it was shown that rats behave similarly in this respect, inanition also leading to diminution in the nuclear-plasmic ratio. It would appear, therefore, that amphibia as well as mammals do not lay aside any specific deposit protein.

It is of interest, further, that the purine content of frogs is about 50 % higher than that of mammals; this is possibly due to their lower body temperature (Gerassimow [1902] found that the nuclear-plasmic ratios of infusoria cultivated at 8° and at 25° were as 13 : 7).

SUMMARY.

1. The chemical nuclear-plasmic ratio of *Rana esculenta* has been determined monthly over a year.
2. The nuclear-plasmic ratio has a value of approximately double that of mammals.
3. It is highest from May to January, the value falling slightly during the periods of winter-sleep, hunger, and sexual activity which follow.
4. The existence of specific reserve protein in the cytoplasm of the frog is not confirmed.

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