LIX. STUDIES IN PURINE METABOLISM.

I. VARIATIONS IN THE NUCLEAR-PLASMIC RATIO IN THE ADULT ALBINO RAT.

II. SYNTHESIS OF PURINES BY THE ADULT MAMMALIAN ORGANISM.

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I. THE NUCLEAR-PLASMIC RATIO.

ONE of the most fundamental problems of biology is that of the interdependence between nucleus and cytoplasm. The most striking equilibrium of the cell is that between nucleus and cytoplasm, and it is at once obvious that any factor affecting the vitality of a cell must of necessity tend also to affect the relative proportions of the above two phases. Thus the nuclear-plasmic ratio might to a certain extent be considered as an index of vitality, and as such it was put forward by Hertwig [1903], who assumed that in the case of monocytes a tendency exists towards the conservation of a constant nuclear-plasmic ratio, *i.e.* that changes in the nucleus must bring about corresponding changes in the cytoplasm, and *vice versa*. Hertwig, and the majority of subsequent workers, measured nuclear and cytoplasmic mass in terms of the areas occupied by each phase in microscopic preparations, and many interesting and far-reaching conclusions have been drawn from results so obtained. In particular, the question of the existence of protein storage has greatly occupied the attention of biologists, and it will not be out of place shortly to describe the development of research on this subject.

Rubner [1908] stated that well-nourished cells possess a reserve of proteins which may amount to about 50 % of the total cytoplasmic proteins, and which may be lost without damage to the vital functions of the cell. In the same paper he states that the dimensions of a cell are determined by its nuclear-plasmic ratio, but since the latter must necessarily be approximately doubled by the loss of half the cytoplasmic proteins, it appears difficult to reconcile this statement with that preceding. Such a condition, in which the nucleus is greatly increased relatively to the cytoplasm, has been shown by Hertwig [1904] to be fatal to *Actinosphaerium eichhornii* and other protozoa, and, as we shall later endeavour to show, should lead in these conditions (hunger) to the destruction of cells in general.

Rubner's contention is supported by the following observations. Boyd [1895], Miescher [1906] and Greene [1919] have shown that in the salmon, during the period of spawning, muscular tissue undergoes profound atrophy, in which the volume but not the number of muscle fibres diminishes. Moulton [1923] showed that cattle behave similarly in this respect, their muscle-cell volume being respectively as 14:8 and 6:1 in fat and thin and starved individuals respectively. Since in these cases in restitution the cells regain their original volumes it was assumed that the nuclei remain intact, the loss being chiefly sustained by the cytoplasm. Further observations supporting the view that in hunger the nuclear-plasmic ratio must increase are those of Morgulis [1911, 1913] on salamanders and rats, of Leonard [1887] on frogs, of Morpurgo [1888] on pigeons, and of Carlier [1892] on hedgehogs. In all these cases, with the exception of rats, the cells of the organs examined exhibited greater loss of cytoplasm than of nucleus, although these differences were not of the order indicated by Rubner.

Other workers who have on rather less definite grounds decided in favour of the existence of a protein reserve are Pflüger [1903], who held that the liver may serve as a storehouse not only of glycogen but also of proteins, Schreuer [1905], who from a study of the respiratory quotient concluded that a certain temporary and limited storage of proteins may take place in the tissues after liberal protein feeding, and Seitz [1906], who showed that the nitrogen content of the liver of starved hens and ducks is smaller in hunger than after liberal protein feeding. The evidence, however, on which the two former workers base their conclusions is both insufficient and inconclusive, whilst Seitz's results prove nothing but that the weight of the liver, and with it its nitrogen content, diminishes in hunger, and indeed, in the case of cats exactly the contrary has been stated by Schryver [1906]. For these reasons the statements of the above three workers will not here be further considered. As to the other observations cited, they cannot be considered as being so finally and decisively in favour of Rubner's theory as Robertson [1923] and Schaeffer and Le Breton [1923] contend.

In the first place, E. Godlewski jun. [1908] found that for polycellular organisms (sea-urchins) it appeared that the nuclear-plasmic ratio of the entire embryo as a whole determined its further development, and that for the study of such organisms conclusions drawn from the study of individual cells may be misleading. Further, as Robertson pointed out, the choice of organ in most of the above cases was singularly unfortunate, the liver being usually taken. As is known, liver cells serve to a certain extent as places of storage of glycogen, which may represent a considerable portion of the cytoplasm, and which would disappear at a comparatively early stage of hunger. While, therefore, it may be true that in hunger liver cells lose cytoplasm more rapidly than nucleus, it by no means follows that the same holds for other cells of the body, not possessed of a glycogen reserve.

It will also be observed that these observations are made on somewhat

heterogeneous material, the least well represented being mammals, and we may add that in the case of the rat (*Morgulis*) the results obtained were somewhat inconclusive, as both nucleus and cytoplasm were diminished almost equally in hunger.

Finally, both nuclear and cytoplasmic material is to a certain extent diluted in hunger, and we have no reason *a priori* to assume that this dilution is equal for both constituents. This objection renders extremely questionable the value of observations based on morphological methods, and indicates the necessity of some gravimetric procedure.

The first research in this direction is that of Masing [1910], who took as representative of the cytoplasm, protein, *i.e.* total nitrogen, and nucleic acid phosphorus as characteristic of the nucleus. That nucleic acid is a true measure of nuclear mass has been shown by the experiments of Kossel [1886], Tichomiroff [1885], Burian and Schur [1897], and Fridericia [1912]. Schaeffer determined nucleic acid as purine nitrogen, and since this method presents certain advantages to that of Masing it has been used in this research.

Purine storage. A second problem connected with the nuclear-plasmic ratio is that of the possibility of storage of nuclear material. Should this exist, feeding with foods of high purine content should lead to an increase in the nuclear-plasmic ratio. Numerous researches on purine metabolism have yielded a mass of confused and often conflicting evidence as to the fate of ingested purine bodies. Thus it is generally known that in man uric acid excretion falls to a minimum on a purine-free diet, that only a portion of the ingested purines is excreted as uric acid, the remainder being destroyed in the intestine by bacterial action, and that protein catabolism is accelerated by purines, as also the reverse [Underhill and Farrell, 1922]. Finally, it is known that injection or ingestion of purines leads to a considerable increase in the number of leucocytes, which on destruction yield a further quota of uric acid.

From this evidence it is difficult to arrive at any conclusion as to the possibility of a purine reserve, and, in order to decide this question, a series of rats, previously kept for some months on a purine-free diet, have now been given purine-containing foodstuffs, and their nuclear-plasmic ratio compared with that of control animals.

Experimental.

Albino rats were used, as they had the advantage of being able rapidly to degrade extracellular purines to allantoin, so that the values obtained for purine nitrogen represent almost exclusively those purines which are actually in the nucleus. These rats were fed for several weeks with purine-free foods, *i.e.* bread and milk. They were killed by strangulation in order to avoid loss of blood or introduction of foreign substances, and were immediately weighed, the contents of the alimentary tract removed, and again weighed. They were

then minced, the mince well mixed, and two portions of 50 g. and 20 g. weighed out, serving for hydrolysis and determination of percentage solid substance respectively. The first portion was hydrolysed by boiling for 4 hours with 500 cc. 4 % sulphuric acid, and from the hydrolysis mixture two samples were taken for the determination of total nitrogen by Kjeldahl's method. This procedure is necessarily somewhat crude, but it is difficult to conceive of any better method of obtaining a representative sample in small enough quantity for this purpose. In certain cases, however, the following procedure was adopted as a check, in addition to that preceding. A weighed portion of mince was moistened with dilute hydrochloric acid, in order to prevent loss of ammonia, and dried to constant weight at 100°. The dried mass, together with the vessel, was then again weighed, the residue powdered in a mortar, and aliquot portions taken for analysis. The values so obtained differed very slightly from those obtained from the hydrolysate.

The purine-nitrogen was in all cases determined by the method of Krüger and Schittenhelm [1905], in which the purines are doubly precipitated as the copper salt, the nitrogen being then determined by the Kjeldahl method.

The proportion of solid substance was determined by drying to constant weight at 100°. Under these conditions not all the water was removed, since at 110° a further small decrease in weight could be obtained. However, the difference was not great, and for comparative purposes drying at 100° was quite adequate, and for technical reasons preferable.

The calculations are as follows. The total volume of the hydrolysate at room temperature was 550 cc., and from this two portions of 20 cc. were taken. Each portion represented 2/55 of 50 g. of mince, and the percentage of nitrogen was therefore obtained by multiplying the nitrogen content of each sample by 55. The value obtained for purine-nitrogen was corrected by multiplying by 55/51, and doubled, to give percentage. As to the nuclear-plasmic ratio, this is not given exactly by the division of purine by total nitrogen, since the latter includes also the former. Schaeffer expresses this ratio as $N_P/N_T - N_P$, but, as he points out, this also is not exact, since nucleic acid contains also pyrimidine-nitrogen, and, further, certain nitrogenous substances other than nucleic acid may be present in the nucleus, so that the absolute values would undoubtedly be higher than those calculated on the above basis.

For comparative purposes, however, the form in which the nuclear-plasmic ratio is expressed is not of essential importance, and, in this case, that used by Schaeffer has been adopted.

Control animals.

The values obtained for control animals are given in Table I. As can be seen, the sex of the animals does not influence the results, and, whilst wide variations exist in the percentage of solid substance, from 30.4 to 37.5 %, in the nitrogen content from 3.0838 to 3.5944 % live weight and from 8.253 to 11.600 % dry weight, and in the purine nitrogen content from 59.0 to

72.8 mg. % live weight, and from 168.5 to 224.0 mg. % dry weight, yet the nuclear-plasmic ratio is fairly constant, varying from 18.30 to 22.34×10^{-3} , with a mean value of 20.62×10^{-3} , and this is taken as the normal figure.

Table I. *Control Animals.*

No.	Weight without contents of alimentary canal, g.	% solid substance	% N live weight	% N dry weight	Purine-N, mg. % live weight	Purine-N, mg. % dry weight	Nuclear plasmic ratio
1 ♂	166.4	30.4	—	—	62.1	204.3	—
2 ♂	161.2	31.9	—	—	61.3	192.0	—
3 ♂	172.0	34.5	—	—	65.0	189.45	—
4 ♂	172.4	31.0	3.3370	10.7655	67.8	218.7	20.74×10^{-3}
5 ♀	154.4	37.5	3.0958	8.253	62.3	166.1	$20.52 \times$ „
6 ♂	160.5	35.05	3.3310	9.501	72.8	207.6	$22.34 \times$ „
7 ♂	167.7	33.5	3.3018	9.856	—	—	—
8 ♀	135.7	35.05	3.3048	9.428	59.0	168.5	$18.20 \times$ „
9 ♂	124.0	31.0	3.5944	11.600	69.4	224.0	$19.70 \times$ „
10 ♀	147.3	33.0	3.0838	9.346	65.8	199.5	$21.81 \times$ „
11 ♀	135.4	35.05	3.2278	9.208	66.4	189.5	$21.01 \times$ „
Mean	154.5	33.45	3.2846	9.745	65.2	196.0	$20.62 \times$ „

Starvation.

Table II gives the values obtained for rats subjected to inanition. These rats lived for from 74 to 216 hours, this depending upon the room temperature and the initial state of nourishment, and lost from 20 to 40 % of their original weight. Here the percentage of solid substance varies from 28.9 to 31.6, on the average 30.45, pointing to a certain dilution of the tissues. The total nitrogen, which varies from 3.2796 to 4.1195 and 10.840 to 13.764 % live and dry weights respectively, has the average values of 3.7185 and 12.215—the latter being almost 25 % above the normal value. Purine-nitrogen is, on the whole, lower, being from 54.55 to 72.16 mg. % live weight, but calculated on dry substance is also higher than normal, namely from 176.0 to 232.8, on the average, 207.7 mg. %. The effect of this increase in total nitrogen, together with a slight decrease in purine-nitrogen, is to lower the nuclear-plasmic ratio to a mean value of 17.77×10^{-3} , varying from 16.35 to 21.41×10^{-3} . This mean value is 15 % below normal, and as, according to Schaeffer, the margin of experimental error is ± 5 %, this difference must be considered as being substantiated.

Table II. *Inanition.*

No.	Hours hunger	Initial weight g.	Final weight g.	% loss	% solid substance	% N live weight	% N dry weight	Purine-N mg. % live weight	Purine-N mg. % dry weight	Nuclear-plasmic ratio
1 ♂	89.0	155.5	116.4	29.97	31.0	3.3774	10.895	54.55	176.0	16.42×10^{-3}
2 ♂	74.5	138.0	109.0	20.29	29.8	3.3726	11.318	57.46	192.9	$17.33 \times$ „
3 ♂	78.0	202.5	159.4	21.28	28.88	3.2796	11.360	—	—	—
4 ♂	134.0	159.2	106.0	33.41	30.5	3.8494	12.621	63.88	209.4	$16.88 \times$ „
5 ♂	139.0	119.3	79.0	33.72	31.11	3.9501	12.697	—	—	—
6 ♂	96.0	177.5	118.8	33.06	31.0	3.6844	11.898	72.16	232.8	$21.41 \times$ „
7 ♀	223.0	182.9	114.7	37.27	30.25	4.0664	13.443	—	—	—
8 ♀	216.0	173.7	103.1	40.67	29.9	4.1195	13.764	66.28	221.7	$16.35 \times$ „
9 ♀	186.5	145.9	101.1	30.68	31.6	3.4265	10.840	65.26	206.5	$19.42 \times$ „
10 ♀	186.0	179.7	114.5	35.45	30.5	4.0594	13.310	65.52	214.8	$16.40 \times$ „
Mean	—	—	—	—	30.45	3.7185	12.215	63.6	207.7	$17.77 \times$ „

Purine feeding.

The results of purine feeding are found in Table III. These rats were fed for from 6 to 14 days with bread and milk to which nucleic acid had been added, then for three days without purines to clear the body of unassimilated purines which might transitorily remain, and then killed.

The values obtained do not differ greatly from the normal, the nitrogen content being on the average 3.410 and 10.281 % live and dry weights respectively, and varying from 3.057 to 3.931 and 9.306 to 11.911 %. This slight increase in total nitrogen is accompanied by a similar slight decrease in purine-nitrogen, which is 63.56 and 191.9, varying from 59.64 to 70.74 and 170.1 to 214.3 mg. % live and dry weight. The nuclear-plasmic ratio is thus lowered to 18.85×10^{-3} , varying from 18.32 to 20.97×10^{-3} .

Table III. *Purine feeding.*

No.	Period of purine feeding, days	Weight g.	% solid substance	% N live weight	% N dry weight	Purine-N mg. % live wt.	Purine-N mg. % dry wt.	Nuclear- plasmic ratio
1 ♂	19	214.0	35.05	3.2617	9.306	59.64	170.1	18.62×10^{-3}
2 ♂	21	195.9	34.5	3.3349	9.665	61.51	178.3	$18.79 \times$ „
3 ♂	24	185.8	31.0	3.0570	9.860	62.78	202.5	$20.97 \times$ „
4 ♀	6	147.0	32.5	3.4650	10.663	63.12	194.2	$18.55 \times$ „
5 ♂	8	191.9	33.0	3.9308	11.911	70.74	214.3	$18.32 \times$ „
Mean	—	—	33.1	3.410	10.281	63.56	191.9	$18.85 \times$ „

Table IV. *Synthesis.*

No.	Period of purine feeding, days	Weight g.	% solid substance	% N live weight	% N dry weight	Purine-N mg. % live wt.	Purine-N mg. % dry wt.	Nuclear- plasmic ratio
1 ♂	—	164.9	32.0	3.2017	10.006	70.2	218.9	22.42×10^{-3}
2 ♂	—	138.9	36.8	3.2047	8.708	64.9	176.4	$20.63 \times$ „
3 ♂	—	131.1	30.5	3.2917	10.790	73.52	241.1	$22.84 \times$ „

II. SYNTHESIS OF PURINES.

The question of whether the adult mammalian organism is capable of synthesising purines, or whether these have to be supplied with the food, has for many years been debated, and in spite of numerous researches, carried out mainly on man, no definite conclusion can so far be drawn. These researches consisted mainly in a comparison of the amounts of administered and excreted purines over a given period of time, and on such grounds Schreiber and Waldvogel [1899], Burian and Schur [1900], Fauvel [1906], Ueber [1909], Benedict [1916], and Kollman [1921] have decided in favour of purine synthesis. Owing, however, to the difficulty of supplying food of constant and known purine content over a long period of time, and to the impossibility of the quantitative determination of excreted purines, these results cannot be considered as being entirely conclusive. In this group, the

best substantiated work is perhaps that of Benedict, who kept a Dalmatian dog for over a year on a so-called purine-free diet, and found that over this period 100 g. of uric acid had been excreted.

On the other hand, Abderhalden [1914], Fürth [1915] and Hammarsten [1914] consider that the synthesis of purines by adult mammals cannot be considered as proved. Since, therefore, this might still be regarded as an open question, restitution experiments have been carried out in the present research, consisting in the partial inanition of a number of rats, which were then fed until sufficient restitution of weight had taken place, when they were killed and their purine content determined. One of the chief advantages of this procedure is that the period over which experiments are conducted is extremely short in comparison to that necessary for metabolism experiments in which a purine balance is drawn up, and which necessarily involve the introduction of larger quantities of foodstuffs whose purine content it is difficult to determine with any degree of accuracy.

Experimental.

A number of rats were kept without food until their weight had fallen to about 80 % of the original value. They were then fed with a diet adequate in all respects, consisting of sugar, starch, caseinogen, Witte's peptone, purified lard, cellulose, and mineral salts (Osborne and Mendel's formula) together with the necessary vitamins, which were supplied either by a known small quantity of milk, or by lemon juice.

On this diet restitution took place fairly rapidly, and, when the weight had sufficiently exceeded that at the end of the period of hunger, the animals were killed and their purine content determined.

Table IV shows that the total and purine-nitrogen contents are similar to those of Table I, as is the nuclear-plasmic ratio. Table V gives the purine-nitrogen contents of the same animals at the beginning of the experiment and at the end of the hunger period, calculated from the average values found in Tables I and II. In calculating these values, the average weight of the contents of the alimentary canal, namely 10 g. for control and 4 g. for starved animals, was subtracted from the weight, and the result multiplied by 0.65 and 0.636 respectively. The final purine-nitrogen content was obtained by direct determination, and by subtracting from this the hunger value we have the gain in purines over the period of feeding.

Table V. *Synthesis of purines.*

No.	Original weight	Weight after hunger	% loss	Weight after restitution	Original N _P (65 mg. %)	Hunger N _P (63.6 mg. %)	N _P					Hours feeding
							Resti- tution N _P	N _P /gain during feeding	given in food	N _P synthesised	% gain	
1 ♂	187.0	138.6	26.2	164.9	121.7	88.16	115.7	27.5	3	24.5	27.8	341
2 ♂	162.0	106.0	34.57	138.9	105.3	67.4	90.2	22.8	3	19.8	29.38	486
3 ♂	141.0	110.0	22.0	131.1	91.6	70.0	96.4	26.4	0	26.4	37.7	360

The first rat weighed at the end of the period of starvation 138.6 g., and was killed after feeding for 340 hours, in which period 400 cc. of milk were consumed, the gain in weight being 26 g. During this time 27.5 mg. of purine-nitrogen had been gained, and if from this we subtract 3 mg. of purine-nitrogen contained in the milk this leaves 24.5 mg. purine-nitrogen synthesised, or an increase of 27.8 % of the purine content at the end of the period of hunger. Similarly, the second rat after starvation weighed 106 g., and after feeding for 486 hours, 400 cc. of milk being also consumed in this time, 33 g. more. Here the gain is 22.8 mg., and the amount synthesised, after subtracting 3 mg. for the milk, is 19.8 mg., an increase of 29.4 %. The third rat received the same diet, with the difference that in place of milk, lard containing a certain amount of vitamin A was given, and in this case the purine content of the food was entirely negligible. The period of feeding was in this case 360 hours, during which time the weight rose from 110 to 131 g. Here the amount of purine-nitrogen synthesised was 26.4 mg., representing an increase of 37.7 %.

DISCUSSION.

Inanition. In view of the results shown in Table II it appears that Rubner's theory of protein storage cannot be considered to be substantiated, at least so far as mammals are concerned. On the contrary, it appears that in hunger rats exhibit a greater loss of nucleus than cytoplasm. It becomes therefore necessary critically to consider the theories and experimental findings of Rubner and of other workers in this field, and to attempt to reconcile these data with our own findings.

If one accepts that in hunger the cytoplasm is used up more rapidly than the nucleus, a time must arrive when the cell can no longer remain in equilibrium with the latter.

It is well known that the nucleus is the seat of perhaps the most important reactions of life, and that the cytoplasm must supply the energy demands of its nucleus. In this connection it is not without interest to cite certain experiments of Gerassimow [1902] on infusoria, in which the nuclear-plasmic ratio of those cultivated at 8° was 1/7, whilst at 25° it fell to 1/13. This points to the retardation of chemical processes at lower temperatures, necessitating a nucleus almost twice as great as that which suffices at 25°, and might to a certain extent explain the low energy loss of poikilothermic animals at lower temperatures, since their nuclei become then insufficient to carry out all the reactions necessary to normal life.

Robertson considers that a cell, which, as a result of starvation, has suffered disproportionate loss of cytoplasm, is rejuvenated, and should tend to undergo mitosis. It is difficult to conceive in what way this could lead to the restoration of the equilibrium of the cell, since the nuclear-plasmic ratio remains constant with each division, and, on the contrary, since the nuclear surface increases relatively to its mass, each mitosis should intensify the pathological condition of the cell.

Since, in our case, cytoplasmic material is not available, the cell can regain equilibrium only by the destruction or extrusion of nuclear material. Should it be unable to do this, the cell must break up as a whole and, in that case, its cytoplasmic constituents may be utilised by other cells, whilst the nuclear material is in all probability rejected. Such a condition would necessarily lead to a diminution of the nuclear-plasmic ratio for the entire organism.

In support of the above reasoning may be cited certain observations of Hertwig [1904] and of Popoff [1908, 1909] in which it is shown that in infusoria nuclear hypertrophy leads either to the degeneration of part of the nucleus to chromidium (unorganised nuclear matter) which is distributed throughout the cytoplasm, or to the death of the organism as a result of the extrusion of the entire nucleus. Further, in so-called depressed infusorial cultures, in which multiplication has ceased, the organisms exhibit nuclear hypertrophy, so that, at least in this case, increase in the nuclear-plasmic ratio does not lead to mitosis, but, on the contrary, inhibits it. Popoff was also able to show that in infusoria the removal of a portion of cytoplasm immediately before mitosis considerably retarded the latter.

Finally, it is in this connection interesting that in man the uric acid content of the blood rises during starvation, particularly during the pre-mortal period [Lennox, 1924]. This is obviously due to augmented nuclear destruction, and it appears that in rats a similar process takes place, with the difference that uric acid is in this case degraded to allantoin.

Purine feeding.

The nuclear-plasmic ratio falls as a result of purine feeding to 18.85×10^{-3} , or 9 % below normal. This result excludes the possibility of any storage of purines taking place in the rat, and further suggests that introduced purines are not anabolised by the mammalian organism.

As to the reason for an actual relative reduction of purines, it would appear either that these have an accelerating effect upon cellular, particularly nuclear, metabolism or that the leucocytosis observed by Schittenhelm after the ingestion of purines leads to enhanced excretion of endogenous uric acid or allantoin, and so diminishes the purine content of the entire organism.

SUMMARY.

1. The nuclear-plasmic ratio has been determined for albino rats under various conditions.
2. In hunger the nuclear-plasmic ratio falls slightly below normal, indicating a greater relative loss of nuclear than of cytoplasmic material.
3. The protein reserve theory postulated by Rubner must in view of this be reconsidered.
4. Purine feeding does not lead to any increase in the nuclear-plasmic ratio, whence it follows that purine bodies are not assimilated and stored by the mammalian organism.

5. Purine bodies are synthesised with comparative facility by the adult albino rat.

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