CXXXVII. STUDIES IN PURINE METABOLISM.

III. BASAL METABOLISM AND PURINE CONTENT.

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(Received June 23rd, 1927.)

BASAL metabolism has been found experimentally to be more nearly a function of body surface than of weight; this relation, the law of surfaces, is, as has been shown by Rubner [1883], Richet [1891], Frank and Voit [1901], Slowtsoff [1903], Lautanié [1905] and Benedict and Talbot [1915], only approximately exact. Further, since at thermal neutrality basal metabolism still remains a function of body surface, it would appear that thermolysis is not the cause of this constancy, but that this must be ascribed to some other factor. Very numerous explanations of the law of surfaces have been advanced. These may be divided into four groups—anatomical, physico-chemical, nervous and chemical. The first of these groups, represented by von Hösslin [1888], Terroine [1924, 1], and others, connects basal metabolism with the volume of blood passing a given area in unit time. The second group [Pfaundler, 1916, 1921] considers the cellular surface to be the deciding factor in thermogenesis, although Rubner's [1913] findings tend to discredit this view. The third group [Richet, 1889; Lefèvre, 1911; de Almeyda and de Fialtro, 1925] consider basal metabolism to be under nervous control.

The fourth group deals with chemical differences, and is mainly occupied by attempts at determining the "active mass" of an animal. According to these views, some portion of the complex chemical mechanism of the animal system is responsible for the energy output of the latter, the remainder serving various ends, such as structural, connective, and reserve tissues. If, therefore, the weight of this active portion of the animal could be determined, this should stand in some simple proportion to its basal metabolism.

Attempts at determining this active mass have been so numerous that I shall here cite only the more important papers on the subject. According to Terroine [1924, 2] neither the lipid content, nor that of lipid phosphorus can in any way be connected with basal metabolism. As to the mineral constituents, he finds that neither their total amount, nor the percentage of iron, which might be supposed to catalyse oxidations, can be considered to be the active mass, and in the same paper he states that there is no real reason for assuming purine content to be the active constituent in question, and concludes

that, since thermal neutrality is a condition almost never met with under natural conditions, the law of surfaces is nevertheless valid and rational and that it is thermolysis which determines thermogenesis. In certain other papers, however [Terroine and co-workers, 1923, 1924, 1926], the opinion is put forward that the active mass of the body may be expressed by its protein content. Since this is proportional to the body weight only in animals divested of reserves of all types, this type of metabolism, where the energy output is constant per unit weight of animal, is met with only in starvation. Hence, Terroine, Feuerbach and Brenckmann [1924] concluded that the average animal contains two types of material; one a vital, active portion, of constant composition, unalterable by the exigencies of life, and the other, consisting of reserves, whose relative proportion is variable and which normally mask the active mass of the animal. In 1923, however, the same authors found that, since the proportion of protein present in animals so different as mice and bulls differs very little, whilst the basal metabolism per unit mass differs very widely, the protein content cannot be considered to be representative of the active mass.

Le Breton [1926] is, however, of the opinion that body-proteins are divisible into two classes—active protoplasm and inactive protoplasm, or paraplasm. According to this hypothesis, the size of an animal is determined by the inherent metabolic intensity of its active protoplasm, whose heat production must be equal to loss of heat from body-surfaces. If, by the inclusion of paraplasm in the cytoplasm the animal can dilute its active mass, it can then grow larger until thermal equilibrium is attained.

Terroine and Roche [1925], however, conclude from measurements of the intensity of respiration of minced tissues of homeotherms of different sizes that the metabolic intensity of protoplasm is identical in the animals studied. These authors therefore concluded that the intensity of basal metabolism depends, not upon differences in protoplasm, but upon blood supply.

Terroine, in one of the above cited papers [1923] expressed the opinion that nuclear matter is the active mass in question. Indeed, a number of biologists have drawn attention to the importance of the nucleus in cellular metabolism. Thus, Palladine [1896] considered that the respiration of plant tissues is proportional to their nuclear content, Loeb [1913] and Osterhout [1917] found that the nucleus is the seat of the most intensive oxidative processes taking place in living tissues, and Robertson [1923] thought that oxidative activity should be proportional to the nuclear-plasmic ratio of a given tissue or cell. Warburg [1909] showed that nucleated blood corpuscles consume more oxygen than non-nucleated, and that, further [1912], if a cytolysed suspension of avian erythrocytes be centrifuged, respiration is confined to the lower layer, in which may be found the nuclei. On these grounds, therefore, one might expect the basal metabolism of an animal to be proportional to the nuclear content of the latter.

In our opinion, the magnitude of the nuclear-plasmic ratio would be an

even better index of metabolic intensity than merely the nuclein content, since it deals with only the actively reacting portions of the cell, excluding differences due to variable water and reserve contents among the various individuals examined.

Since we have been able to find no reliable values for the purine content or nuclear-plasmic ratio of various animals, this research was undertaken with a view to the confirmation or otherwise of the above hypothesis, connecting basal metabolism with purine content or nuclear-plasmic ratio.

EXPERIMENTAL.

Purine-nitrogen, which is taken as representative of nuclear content, the total nitrogen, and the nuclear-plasmic ratio were determined by the methods described in a previous paper [Truszkowski, 1926]. The animals examined were rats, guinea-pigs, rabbits, dogs, horses and cattle. The first two species were analysed whole, after removal of food remains from the alimentary tract and mincing. In the case of rabbits, dogs, horses and cattle, skeletal muscle and liver alone were analysed, since for technical reasons the analysis of whole animals larger than guinea-pigs presented too great difficulties: for comparative purposes, analyses were also made of the skeletal muscle of rats. It is realised that results obtained for one portion of an animal need not necessarily apply to the animal as a whole, but in view of the preponderance of muscular and glandular tissue among the nitrogenous constituents of the homeotherms, there is some justification for considering that the values obtained for muscle and liver are to a large extent representative of those which would be obtained for the entire animal.

The values for whole rats are taken from our previous paper (Part I, p. 441), and the mean values are given in Table II. Those obtained for skeletal muscle taken from six rats are shown in Table I. The water content, and total and purine-nitrogen are higher than for the whole animal, but the nuclear-plasmic ratio differs by less than 3 %. In the same table may be found the results obtained for guinea-pigs. Here the solid substance varies from 28 to 29.8 %, total nitrogen from 3.421 to 3.529 %, and purine-nitrogen from 72.18 to 77.47 mg. %; the nuclear-plasmic ratio varies from 21.56 to 23.63, on the average 22.60×10^{-3} .

The values for skeletal muscle and liver of rabbits are taken from some unpublished results, which Dr Dmochowski has communicated to me. As these will be published separately, and deal with a different problem, only the mean values will be given here (Table II), and the same applies to those obtained by Miss Rowinska and Mr Zdunkiewicz for cattle and by myself for dogs. In the latter case, the animals weighed from 5 to 10 kg. and were fed on a diet poor in protein (oatmeal, potatoes, etc.).

In the case of horses (Table I) fairly wide variations were found; for muscle, total nitrogen varies from 3.119 to 3.550%, calculated for dry tissue from 13.32 to 15.40 %; purine-nitrogen is from 77.0 to 83.7 mg. %, and the nuclear-

plasmic ratio varies from 22.17 to 26.48×10^{-3} . The liver has a lower water content (71.1 %) due obviously to the presence of some non-nitrogenous substances, since the nitrogen content is lower than in muscle, being 3.204 and 11.15 % for fresh and dry weight respectively. The purine content is considerably higher than for muscle, and is, on the mean of two determinations, 147 mg. %, and the nuclear-plasmic ratio, which shows wide variations for liver tissue in general, is in one case 42.12 and in the other 54.89, on the average 48.50×10^{-3} .

Table I.

Species	No.	Material taken	% solid substance	% N live weight	% N dry weight	Purine-N mg. % live weight	Purine-N mg. % dry weight	Nuclear-plasmic ratio
Rat	1	Skeletal muscle	28.0	3.850	13.90	81.7	294.9	21.68×10^{-3}
	2	"	28.0	3.842	13.80	78.2	282.2	20.76×10^{-3}
Guinea-pig	1♂	Whole	29.8	3.447	11.67	80.3	269.5	23.63×10^{-3}
"	2♂	"	28.0	3.529	12.57	77.5	276.7	22.51×10^{-3}
"	3♀	"	28.0	3.421	12.22	72.2	257.7	21.56×10^{-3}
Horse	1♀	Skeletal muscle	20.8	3.188	15.32	—	—	—
"	2♂	"	24.9	3.550	14.26	77.0	309.2	22.17×10^{-3}
"	3♂	"	23.4	3.119	13.32	—	—	—
"	4♂	"	23.2	3.142	13.54	81.0	349.3	26.48×10^{-3}
"	5♀	"	21.8	3.357	15.40	83.7	383.8	25.57×10^{-3}
"	6♀	"	21.8	3.342	15.30	81.9	375.8	25.14×10^{-3}
"	3♀	Liver	30.3	2.987	9.86	155.4	513.1	54.89×10^{-3}
"	4♂	"	27.5	3.421	12.44	138.3	502.9	42.12×10^{-3}

The mean values obtained by other authors and by myself are given in Table II. Rats and guinea-pigs give fairly concordant values, all figures obtained for the latter being slightly higher than for the former, with the exception of the percentage of solid substance. Thus, for whole animals, it would not appear that either purine content or nuclear-plasmic ratio is in any way connected with basal metabolism.

For muscular tissue much more divergent results are found.

Table II. *Mean values.*

Species	Material taken	% solid substance	% N live weight	% N dry weight	Purine-N mg. % live weight	Purine-N mg. % dry weight	Nuclear-plasmic ratio	Author
Rat	Whole	33.45	3.285	9.745	65.2	196.0	20.62×10^{-3}	Truszkowski
Guinea-pig	"	28.6	3.466	12.152	76.7	268.0	22.60×10^{-3}	Truszkowski
Rat	Skeletal muscle	27.7	3.846	13.85	80.0	288.6	21.22×10^{-3}	Truszkowski
Rabbit	"	24.5	3.777	15.41	115.5	471.3	31.54×10^{-3}	Dmochowski
Dog	"	21.2	2.514	12.06	54.2	264.0	22.01×10^{-3}	Truszkowski
Horse	"	22.6	3.283	14.53	80.9	358.8	25.33×10^{-3}	Truszkowski
Cattle	"	25.3	3.933	15.58	76.5	284.1	19.86×10^{-3}	Rowinska and Zdzunkiewicz
Rabbit	Liver	26.3	2.819	9.85	140.0	536.3	52.25×10^{-3}	Dmochowski
Dog	"	24.4	2.335	9.65	86.7	355.5	40.53×10^{-3}	Truszkowski
Horse	"	28.9	3.204	11.15	146.9	508.0	48.50×10^{-3}	Truszkowski
Cattle	"	24.8	3.115	12.56	118.8	478.9	39.71×10^{-3}	Zdzunkiewicz

No proportionality between size of animal and water, nitrogen, or purine content of muscles, or of their nuclear-plasmic ratio, appears to exist. Thus, whilst rat muscle has the highest percentage of solid substance, that of cattle

is the next largest, then that of rabbits, horses and dogs. The nitrogen content decreases in the order—cattle, rats, rabbits, horses, and dogs; and the purine content in the order—rabbits, horses, rats, cattle and dogs. The nuclear-plasmic ratio is highest for rabbits, *i.e.* 31.54×10^{-3} , then horses, dogs, rats and cattle, 19.86×10^{-3} . Thus we see that rats and cattle, whose average weights are as 1 : 2500 are the most similar to each other in purine and nitrogen content of muscle, and that generally, no regularity exists in the values obtained for the above series of animals.

The same may be observed in the values found for the liver. Here horses have the highest percentage of solid substance, next rabbits, cattle and dogs. The highest nitrogen content may be found in horses, then cattle, rabbits and dogs, whilst the purine nitrogen content decreases in the order—horses, rabbits, cattle and dogs. The nuclear-plasmic ratio is, as for muscle, highest in the rabbit (52.25×10^{-3}), then follow horses, dogs and cattle (39.71×10^{-3}). Here, also, there is no connection between the values obtained and the weight, *i.e.* the basal metabolism of the animal.

It is, however, interesting that the liver has a nuclear-plasmic ratio approximately double that of skeletal muscle, and this is in accordance with histological findings for the same tissues. No particular stress is laid upon the values other than this ratio, since it is realised that these depend upon the state of nutrition of the given animal at the moment when killed, and to a certain extent upon the previous history of the latter, which is, in the case of horses and cattle, unknown.

DISCUSSION.

The results given above show that considerable variations may exist in the nitrogen and purine contents of the liver and skeletal muscle of various animals and in their nuclear-plasmic ratios, but that these variations are in no way connected with the basal metabolism of the given animal. The values obtained are, however, to a certain extent constant for a given species, and appear, further, to be characteristic of the particular organ examined.

There exists, however, the possibility that, whilst basal metabolism does not depend upon nuclear mass, it may depend upon the active surface of the nucleus. Thus, admitting that reactions of oxidation take place within the nucleus, their intensity will depend not so much upon its mass as upon the nuclear-plasmic intersurface. It is therefore conceivable that equal nuclear masses need not be equivalent, their activity depending upon the development of their surfaces. This, again, may be influenced by two factors, namely, cell dimensions and concentration of active substances within the nucleus.

In the first case, it is obvious that the smaller the cell, the greater the relative nuclear surface, assuming the volumes of nucleus and plasma to diminish proportionally. Unfortunately very little work has been done in this field, and those values which I have been able to find are contradictory. Amelung [1893] and Strasburger [1893] found that cells of the same organ in

plants of different size but of the same species are of equal size. Rabl [1899] found that the dimensions of epithelial cells and lens fibres were the same for dogs of various sizes. On the other hand, Levi [1905], found that sections of cerebral cells of different mammals were greater, the greater the weight of the animal, that of the ox being 104.3μ , whilst that of the mouse is 37.25μ , and differences of the same order may be found for lens fibres. These differences are, however, insufficient to explain differences in basal metabolism, since, if we assume that the nuclei diminish in radius to the same degree as the cells, this would mean that the surface of unit weight is approximately three times greater for mouse than for ox nuclei, whereas the basal metabolism of the former animal is about 20 times as great as for the latter. However, in view of the lack of reliable data on this point, it is unprofitable further to discuss it, and the same applies to the possibility of enlargement of nuclear surface by dilution.

SUMMARY.

1. The chemical nuclear-plasmic ratios of whole rats and guinea-pigs differ little from one another.
2. The values obtained for purine- and total nitrogen, and for the nuclear-plasmic ratios of liver and of skeletal muscle of rats, rabbits, dogs, horses and cattle vary within wide limits, but are in no way connected with the basal metabolism of the animals in question.
3. The purine content of animals cannot therefore be identified with their "active mass."

I wish to express my sincere gratitude to Prof. St. J. Przylecki, who proposed the above research, and to whom I am indebted for constant help and advice.

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