LETTERS TO THE EDITORS

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Influence of the Nucleus on Anaerobic Breakdown of Adenosine Triphosphate

WE have shown earlier¹ that non-nucleated fragments of *Amoeba proteus* maintain a normal oxygen consumption for as long as ten days; since the removal of the nucleus quickly reduces the uptake of phosphorus-32 by the cytoplasm², we concluded that the nucleus plays a part in the coupling between oxidations and phosphorylations, possibly by the production of some co-enzyme. Later experiments³ showed, however, that such a simple hypothesis can scarcely be correct, since the adenosine triphosphate content of nucleated and non-nucleated halves remains almost identical for a considerable length of time (twelve days).

The recent publication by Strehler and Totter⁴ of a sensitive and quick method for the estimation of adenosine triphosphate (based on the luminescence of fireflies extracts in the presence of that substance), and the description by Benesch and Benesch⁵ of a simple technique for maintaining anaerobic conditions, led us to study the adenosine triphosphate content of nucleated and non-nucleated halves of *A. proteus*, both in aerobiosis and in anaerobiosis. The method of Benesch and Benesch⁵ is based on the removal of oxygen from solutions by the glucose-glucose oxidase-catalase system; control experiments have shown that neither these enzymes nor glucose—when taken separately—exert any measurable effect on the adenosine triphosphate level in the amoebae. When amoebae are placed in the medium of Benesch and Benesch⁵, they behave exactly as when they are subjected to usual anaerobic conditions⁶.

The results obtained after a period of 16 hr. anaerobiosis are given in Table 1; in each experiment, two hundred fragments were finally suspended in 75 μ l. of Chalkley's medium and placed in a boiling water-bath for 2-3 min. in order to extract the adenosine triphosphate; the estimations of the adenosine triphosphate content, which were kindly performed by Dr. M. Errera, were made in duplicate on 30- μ l. samples. The results in Table 1 are expressed in γ ATP/200 fragments/ml.

Table 1

Days after sectioning	Type of fragments	No. of expts.	ATP in aerobiosis	ATP in anaerobiosis	ATP lost in anaerobiosis (per cent)
0-1	Nucleated	6	0.057	0.047	17.5
0-1	Non-nucleated	6	0.058	0.018	69
4-9	Nucleated	5	0.065	0.033	50
4-9	Non-nucleated	5	0.062	0.019	70

These results confirm that the adenosine triphosphate content of nucleated and non-nucleated halves of amoebae remains the same in the presence of air; but a very striking difference occurs in anaerobiosis: the nucleated fragments then retain a much larger proportion of their adenosine triphosphate in the phosphorylated condition than the non-nucleated halves; this is especially true for a short time after the amoebae have been cut, that is, when they still have a large store of carbohydrate reserve.

It is likely that the maintenance of a high level of adenosine triphosphate in anaerobiosis is linked to glycogenolysis; previous work⁷ has shown that glycogen utilization by nucleated halves is faster during the first three days than later; the non-nucleated fragments are only capable of a very limited utilization of their carbohydrate reserve. Preliminary cytochemical observations on the glycogen content of both types of fragments in anaerobic conditions have clearly shown that nucleated halves from well-fed amoebae (0-1 day after sectioning) lose a very large proportion of their glycogen during a 16-hr. anaerobiosis period; such striking staining changes never occur in the case of non-nucleated fragments or of four-day old nucleated halves. Apparently, removal of the nucleus quickly and simultaneously impairs both glycogen breakdown and ability to keep adenosine triphosphate in the phosphorylated state in anaerobic conditions.

The conclusion that the nucleus controls certain steps of the glycolytic mechanism would be in agreement with Stern and Mirsky's⁸ finding that wheat-germ nuclei are richer than the cytoplasm in various glycolytic enzymes; however, it was found (Table 2) that at any rate enolase, which according to Stern and Mirsky⁸ is definitely accumulated in these nuclei, is almost equally distributed between nucleated and non-nucleated halves of amoebae.

Table 2. ENOLASE ACTIVITY IN 10^{-3} μ M PHOSPHOGLYCERIC ACID/100 FRAGMENTS/5 HR. AT 36°

Days after sectioning	Nucleated halves	Non-nucleated halves
2	5.3	4.4
3	3.9*	3.1*
6	6.7	6.3
7	7.3	6.3
11	4.2	3.6

* Experiment performed at 30°.

This negative finding leads us to believe that the nucleus probably plays a part in the activation of glycolytic enzymes (by production of co-factors) rather than in their actual synthesis. Further work will be required to elucidate at which step the nucleus is acting; both the phosphorylating systems and the steps involving diphosphopyridine nucleotide (DPN) are possibilities, since there have been reports that diphosphopyridine nucleotide is synthesized and accumulated in the nucleus⁹⁻¹⁰.

Finally, it is worth pointing out that decreased capacity for keeping adenosine triphosphate in the phosphorylated form during anaerobiosis and reduced glycogen utilization probably are characteristic biochemical abnormalities whenever the nucleus is severely damaged, since similar metabolic difficulties have been found in strongly lethal interspecific frog hybrids¹⁰.

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¹ Brachet, J., *Nature*, **168**, 205 (1951).

² Mazia, D., and Hirshfield, H. L., *Science*, **112**, 297 (1950).

³ Brachet, J., *Biochim. Biophys. Acta*, **9**, 221 (1952).

⁴ Strehler, B. L., and Totter, J. R., *Arch. Biochem.*, **40**, 28 (1952).

⁵ Benesch, R. E., and Benesch, R., *Science*, **118**, 447 (1953).

⁶ Pantin, C. F. A., *Proc. Roy. Soc. B*, **105**, 538 (1930). Hulpieu, H. R., *J. Exp. Zool.*, **56**, 321 (1930). Kitching, J. A., *J. Cell. Comp. Physiol.*, **14**, 227 (1939).

⁷ Linet, N., and Brachet, J., *Biochim. Biophys. Acta*, **7**, 607 (1951).

⁸ Stern, H., and Mirsky, A. E., *J. Gen. Physiol.*, **36**, 181 (1952).

⁹ Hogeboom, G. H., and Schneider, W. C., *J. Biol. Chem.*, **197**, 611 (1952). Stern, H., Allfrey, V., Mirsky, A. E., and Saetren, H., *J. Gen. Physiol.*, **35**, 559 (1952).

¹⁰ Barth, L. G., and Jaeger, L., *Physiol. Zool.*, **20**, 133 (1947). Gregg, J. R., *J. Exp. Zool.*, **109**, 119 (1948).