LETTERS TO THE EDITORS

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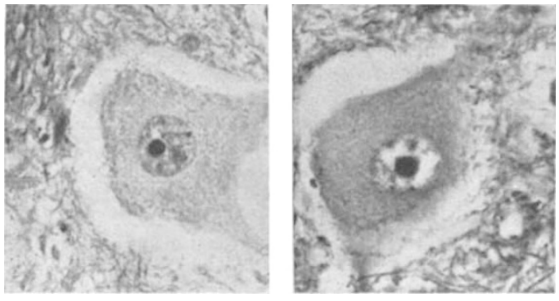
Histochemical Evidence of Increased Activity of Hydrolytic Enzymes in the Cells of Old Animals

Histochemical studies, mainly of dephosphorylating enzymes, have been made on sixteen different organs in young (three-months old) and old (three-years old) rats. Substrates used were glycerophosphate, œstrone phosphate, cortisone phosphate, pyridoxal phosphate, carbamyl phosphate and ethanolamine phosphate, and the *p*H used was 9·0. Evidence has been published[1,2] (or has been obtained but not yet published) from this laboratory that these compounds are dephosphorylated probably by specific enzymes.

The steroid phosphates and ethanolamine and carbamyl phosphates are dephosphorylated mainly in the nuclei. This activity is greater in the cells of old than in those of young animals. In addition there is increased activity in the cytoplasm in the former. Pyridoxal phosphate, which is dephosphorylated mainly in the cytoplasm, also gives an increased reaction in old animals when it is used as a substrate and this is so also for glycerophosphate. Similar results have been obtained for simple esterase.



(*a*) (*b*)

Fig. 1. Ethanolamine phosphatase preparations of cells from region of origin of motor root of 5th nerve in young (*a*) and old (*b*) rats. Greater intensity of reaction in both nucleus and cytoplasm is apparent in the latter

Byrbye and Kirk[3] have shown increased β-glucuronidase activity and Zorzoli[4] increased acid phosphatase activity in the cells of old animals.

It is of interest in this connexion that Weinbach and Garbus[5] have found a decrease in oxidative phosphorylation in liver but not in brain in old animals, but no change in either organ in the level of glycolytic phosphorylation. They also found no decrease in respiratory level in liver and brain in old animals. This latter finding can be supported from my observations[6,7] that there is no histochemical evidence of decrease in succinic dehydrogenase or cytochrome oxidase activity in the tissues of old animals and that in fact there is some evidence of increase.

Irrespective of whether oxidative phosphorylation decreases or remains constant, a progressively increasing rate of dephosphorylation of metabolically important substances would lead to a similarly progressive decrease in concentration of these substances in old cells resulting in inadequate functioning and eventually in death. It is possible, therefore, that this may be one of the fundamental processes involved in cellular senescence.

The present results will be presented in greater detail elsewhere.

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[2] Bourne, G. H., *Quart. J. Micro. Sci.*, **95**, 359 (1954).
[3] Byrbye, M., and Kirk, J. E., *J. Geront.*, **11**, 33 (1956).
[4] Zorzoli, A., *J. Geront.*, **10**, 156 (1955).
[5] Weinbach, E. C., and Garbus, J., *Nature*, **178**, 1225 (1956).
[6] Bourne, G. H., Proc. European Conf. Gerontology, *Experientia*, Supp. IV, 61 (Birkhäuser, Basel, 1957).
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In vitro Effect of Growth Hormone on the Glucose Uptake of Isolated Rat Diaphragm

There is a disagreement in the literature concerning the *in vitro* action of growth hormone on the glucose uptake of the isolated diaphragm of the normal rat. Ottaway[1,2] has reported that growth hormone added to the Stadie–Zapp buffer[3] will stimulate the glucose uptake of the isolated rat diaphragm. Park *et al.*[4] using Krebs–Henseleit bicarbonate buffer[5] and Randle and Young[6] using Gey and Gey buffer[7], which is qualitatively similar to the Krebs–Henseleit buffer, have reported that growth hormone *in vitro* is without effect on the glucose uptake of the isolated diaphragm from normal rats. It was of interest, therefore, to compare the effect of growth hormone added *in vitro* on the glucose uptake of the isolated rat diaphragm incubated either in Stadie–Zapp buffer[3] or in Gey and Gey buffer[7].

Normal female albino rats weighing 90–120 gm. were used. After a fasting period of 18–20 hr. each rat was killed by decapitation, the diaphragm removed, divided into two approximately equal portions and allowed to remain in freshly gassed buffer containing 250 mgm. of glucose/100 ml. for about 10 min. The Gey and Gey buffer was gassed with 93 per cent oxygen — 7 per cent carbon dioxide and the Stadie–Zapp buffer with 100 per cent oxygen. Each hemi-diaphragm was blotted gently and transferred to a beaker containing 1 ml. of the type of buffer in which it had been soaked. The hemi-diaphragms were incubated in a Dubnoff metabolic shaker at 37° C. for the next 60 min. The gas phase for each buffer was as shown above. Growth hormone, where indicated, was dissolved in the appropriate buffer just before it was used. The hormone was never in solution for more than 60 min. before the beginning of the incubation. At the end of the incubation period the glucose content of each beaker was measured by the method of Somogyi[8]. Each hemi-diaphragm was blotted and weighed on a torsion balance. The glucose uptake was calculated as mgm. glucose disappearing from the medium/gm. of wet diaphragm/hr. of incubation.

The results of the experiments are shown in Table 1. When the incubation was carried out in Stadie–Zapp buffer, a significant increase in the glucose uptake of the isolated rat diaphragm occurred in the presence of growth hormone at concentrations of 25 μgm. and 50 μgm. In no case did the addition of growth