LETTERS TO THE EDITORS

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Biochemical Evolution : Position of the Tunicates

THE biochemical differentiation[1] between the vertebrates and the invertebrates rested upon the finding that phosphocreatine and phosphoarginine were characteristic of these two classes respectively. Recently, the discovery of phosphagens other than phosphoarginine in invertebrates has suggested some modification of this generalization ; but there are no reports in the literature suggesting the presence of a phosphagen other than phosphocreatine in the vertebrates.

The work[2,3] on members of the intermediate group of protochordates, which indicated the presence of phosphoarginine in the atrial muscle of tunicates, created an anomalous situation, inasmuch as the tunicates appeared to reverse the general chordate pattern[4] biochemically characterized by the presence of phosphocreatine.

In connexion with investigations on creatine phosphokinase[5] and arginine phosphokinase (unpublished results), the opportunity occurred to examine two tunicates, *Pyura* sp. and *Pyura stolonifera* (Heller), for the presence of arginine phosphokinase and phosphoarginine. The atrial muscles were removed immediately after collection (from the south coast of New South Wales) and frozen in liquid nitrogen to prevent decomposition of the phosphagen. A portion of this frozen material was extracted in the cold with trichloroacetic acid (10 per cent) and the extracts neutralized after filtration. A water-soluble ethanol-insoluble fraction of barium salts was obtained which contained a phosphorus compound unstable in 0·1 *N* hydrochloric acid at 60°. The compound was identified as phosphocreatine by ascending paper chromatography in a number of solvent systems. The compound, moreover, did not react with adenosine diphosphate in the presence of arginine phosphokinase isolated from crayfish muscle, but did react with it in the presence of creatine phosphokinase from rabbit muscle.

Another portion of the muscle was extracted with boiling water, and from the filtered extracts, after treatment with ion-exchange resins, a picrate was obtained which possessed a melting point identical with creatine picrate. Chromatography of the initial extract in six solvent systems revealed only one spot reacting with α-naphthol and diacetyl and corresponding in position with authentic creatine. This spot did not give a Sakaguchi reaction, nor did it react with ninhydrin. The guanidine was phosphorylated by creatine phosphokinase but not by arginine phosphokinase.

A third portion of the frozen muscle was finely ground and extracted with 1·5 vol. of water in the cold room for 18 hr. After centrifugation, the extract was treated with ammonium sulphate and a protein fraction obtained which showed creatine phosphokinase activity. No trace of arginine phosphokinase activity was detected in either the crude or partially purified extracts. The enzyme was active not only with authentic phosphocreatine and creatine as substrates, but also with the free base and its phosphorylated derivative isolated from the muscle.

The generalization of Needham *et al.*[3] (that the tunicates contain phosphoarginine) is based on equivocal chemical evidence gained from an investigation of one species but has been generally accepted[4,6]. The present results indicate the absence of arginine, phosphoarginine and arginine phosphokinase in the tunicates as represented by two species. On the other hand, they prove conclusively the presence of creatine, phosphocreatine and an enzyme possessing creatine phosphokinase activity.

Though it is perhaps not permissible to conclude that the tunicates as a class possess creatine, etc., the present work is consistent with, and provides biochemical support for, the accepted phylogenetic classification of the tunicates and therefore removes the anomaly referred to by Wald[4].

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Effect of X-Rays on the Metabolism of Cell Nuclei of Non-dividing Tissues

ADULT mice were given single intraperitoneal injections of either a solution containing ^{35}S-DL-methionine or [8-^{14}C]-adenine. Some of the animals were given whole-body irradiation immediately before injection. The animals were killed at various times after injection, and organs fixed for 1 hr. in acetic acid–alcohol (1 : 3) followed by formol saline ; autoradiographs were prepared by the stripping film technique. The methods employed have been described previously[1].

After injection of labelled methionine some tissues with extremely low mitotic indexes, such as thyroid, seminal vesicle, tracheal epithelium, nerve cells and epididymis, give nuclear autoradiographs suggesting protein metabolism. Although nucleus and cytoplasm are labelled, the resolving power of the method is undoubtedly sufficient to localize sulphur-35 in parts of the cells. Only some of the nuclei are positive in those tissues, the proportion varying within wide limits.

The effect of X-rays on nuclear protein metabolism in the columnar epithelium in the trachea 4 hr. after irradiation and injection is shown in Figs. 1 and 2 ; numerical values are given in Table 1. Weakly positive nuclei were scored as negative, since the photographic grains above them might have been due to sulphur-35 in adjacent cytoplasm. The strength of the autoradiograph above the cytoplasm of the epithelial cells in the trachea is not markedly affected by the irradiation (cf. Figs. 1 and 2). It is about equal to that in positive nuclei in animals killed four hours after injection. Two hours after injection the concentration of sulphur-35 is higher in the cytoplasm of all cells than in positive nuclei. Fewer

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