CLV. ARGINASE AND ARCAIN.

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INTEREST in the enzyme arginase has recently been considerably stimulated, firstly by the discovery of argininephosphoric acid in a number of invertebrates [Meyerhof and Lohmann, 1928; Needham *et al.*, 1932], and secondly by the realisation of its importance in the elaboration of urea [Krebs and Henseleit, 1932]. A survey of the invertebrate phyla is now in progress in this laboratory with a view to ascertaining the distribution of arginase.

Arnold and Luck [1933] have already examined many invertebrates for the presence of arginine, since such a study is of the greatest importance for the comparative biochemistry of muscle. Their method [Kiech *et al.*, 1931] assumes the perfect specificity of arginase. Tissue extracts are submitted to the action of that enzyme, urea being then isolated as dioxanthidylurea, followed by a volumetric estimation of that compound. In several respects their results appear to conflict with those of other authors; thus the polychaete worms, represented by *Nereis*, were found to contain no arginine, whereas Needham *et al.* [1932] demonstrated the presence of a phosphagen which showed the behaviour typical of argininephosphoric acid. Again, the cephalopoda proved to contain arginine in considerable quantity, whereas Baldwin [1933] has shown that in all probability the cephalopod phosphagen is not argininephosphoric acid. The possibility that other phosphagens may be present in the invertebrate phyla has, in the author's opinion, not been investigated with sufficient thoroughness. The harmonisation of certain of Arnold and Luck's results with those of Needham *et al.* [1932] must depend largely upon such studies.

Of special interest in this connection is the lamellibranch class of the Mollusca, for here very variable results have been obtained by various workers. Meyerhof [1928] found argininephosphoric acid in *Pecten* but could detect no labile phosphate whatever in *Pinna*, *Cytherea* or *Anodonta*, while Borsuk *et al.* [1933] have confirmed Meyerhof's findings with regard to *Pecten*. Arnold and Luck [1933] report that among nine such molluscs examined by them arginine was found in every case. The lack of concordance between the distribution of arginine itself and that of its phosphoric acid compound suggested that some other compound might be involved, and arcain, the substance recently isolated from *Arca noae* by Kutscher, Ackermann and Flössner [1931], at once suggested itself, particularly since it appears to be of random distribution. Thus it is present in *Arca noae* but absent from *Mytilus* [Kutscher and Ackerman, 1931]. Arcain has been shown by Kutscher, Ackermann and Hoppe-Seyler [1931] to be identical with tetramethylenediguanidine, which was first synthesised by Kiesel [1921, 1] and found by him to be attacked by a preparation of *Aspergillus* arginase, giving urea and agmatine [1921, 2].

It therefore seemed possible that arcain might also be attacked by animal arginase, and would in this case interfere with the estimation of arginine, since

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it too would give urea on hydrolysis. Arnold and Luck [1933], Meyerhof [1928], Baldwin and Needham [1933] and many more have used arginase as a specific test for the presence of arginine, for its specificity appeared to be absolute. Even γ -guanidinobutyric acid, which was formerly believed to be attacked [Thomas, 1913] has since been shown not to be affected [Thomas *et al.*, 1922], and repeated observations with many possible substrates have only confirmed the apparently complete specificity of arginase [Haldane, 1930]. Nevertheless it cannot be too greatly stressed that material of practically unknown composition, such as is presented by invertebrate tissues in general, may well prove to contain potential substrates of arginase, and it is therefore of the greatest importance that any newly discovered bases allied to guanidine should be submitted to its action, if the significance of such results as those of Arnold and Luck is to remain unmodified.

In view of the structural relationship between arcain and arginine, and especially since arcain is attacked by *Aspergillus* arginase, I have submitted arcain to the action of an arginase preparation obtained from fresh guinea-pig liver according to the directions of Edlbacher and Röthler [1925]. The arcain solutions, with added arginase, were incubated overnight at 37° with glycine buffer at p_H 9 under a layer of toluene. Next day, after removal of the proteins, urea was estimated in the filtrates by the manometric technique of Krebs and Henseleit [1932]. The amount of urea found never exceeded that in controls carried out on distilled water, and corresponded to the quantities to be expected from the amounts of arginase taken. Controls performed upon equivalent quantities of arginine showed that this substance was completely hydrolysed by the enzyme preparation in half an hour.

It is therefore concluded that arcain is not attacked by mammalian arginase, and that the results of Arnold and Luck and of the other workers to whom we have alluded must stand, unless subsequent work may show that other substrates of arginase are present in invertebrate materials.

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