Die Zellkerne, die für Phosphoriboisomerasemessungen dienten, wurden zur Beseitigung des Rohrzuckers zweimal in Ringerlösung gewaschen und anschliessend in Ringerlösung suspendiert (0,8–1,1 mg N/ml, kjeldahlometrisch bestimmt). R1P wurde durch Phosphorolyse von Guanosin gewonnen[4] und als Kaliumsalz verwendet (5 μM/ml).

Das Reaktionsgemisch für die Mutaseteste (5 ml 0,2 *M* TRIS-HCl-Puffer pH 7,4, 2 ml R1P-Lösung und 2 ml 0,005 *M* $MgCl_2$-Lösung) wurde 10 min auf 37°C vorgewärmt. Nach Zugabe von 1 ml Proteinlösung wurde in bestimmten Zeitabständen je 1 ml Probe entnommen, mit gleicher Menge eiskalter, 5%iger Perchlorsäure enteiweisst und der Gehalt an anorganischem und leicht säurehydrolysierbarem Phosphat bestimmt[7] (Hydrolyse 10 min bei 100°C in 1 *n* schwefelsaurer Lösung).

Die Phosphoriboisomeraseteste wurden nach BRUNS[8] ausgeführt: Im Ansatz waren 1 Vol. 2%iger TRIS-HCl-Puffer pH 7,5, 1 Vol. 0,03 *M* Na-R5P-Lösung und 1 Vol. Zellkernsuspension. Während der Inkubation (mit 90 Schwingungen/min schüttelnd bei 37°C) wurden alle 5 min eine Probe (1,5 ml) entnommen, mit 8,5 ml eiskalter, 3%iger Trichloressigsäure enteiweisst und in 1 ml Überstand der Gehalt an Ketozucker mit Hilfe der Carbazolreaktion[9] bestimmt.

Ergebnisse. Phosphoribomutase: Während der Inkubation nahm der Gehalt an säurelabilem Phosphat linear zur Zeit und zur Enzymkonzentration ab. Die spezifischen Aktivitäten der aus Leber- und Nierenzellkernen dargestellten Proteinfraktionen waren einander ähnlich und lagen in derselben Grössenordnung wie eine ebenso hergestellte Proteinfraktion aus Gesamthomogenat (Tab. I). Im Gegensatz zu den Befunden von GUARINO und SABLE[4] wurde bei Zugabe von Mg-Ionen eine Steigerung der Enzymaktivität erreicht (Tab. I). Die Bildung von R5P konnte papierchromatographisch bestätigt werden.

5-Phosphoriboisomerase: Ketozuckerbildung war sowohl mit Leberzellkernen als auch mit Nierenzellkernen nachzuweisen. Die spezifischen Aktivitäten der beiden Enzymquellen zeigten keine charakteristischen Unterschiede (Tab. II). Das Reaktionsprodukt des Carbazoltestes ergab das für Ribulose charakteristische Absorptionsmaximum bei 540 mμ. Ribose als Substrat gab keine messbare Ribulosebildung; R1P wurde in geringem Masse zum Ketozucker isomerisiert infolge vorangehender Phosphoribomutasereaktion. Auf dem Papierchromatogramm wurde Ribulose-5-Phosphat gefunden.

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Physiologisch-chemisches Institut der Johannes-Gutenberg-Universität Mainz, 26. Januar 1958.

Summary

The existence of phosphoribomutase and 5-phosphoriboisomerase has been demonstrated in isolated cellular nuclei from rat liver and swine kidney. Phosphoribomutase has been shown to require Mg-ions for optimal activity.

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On the Location of Cholinesterase in Fishes

There seem to be few reports in the literature about the cholinesterase (ChE) activity in fish muscles[1]. However, AUGUSTINSSON[2] has reported an unusually high activity of acetylcholinesterase (AChE) in the guppy, *Lebistes reticulatus* and NACHMANSOHN[3] has found the same in the goldfish, *Carassius auratus*. The activities amount to 20 to 50 mg acetylcholine (ACh) hydrolyzed per 100 mg tissue/h (PROSSER[4]). Similar results have been obtained by the present author (LUNDIN[5]). It is remarkable that the activity per unit weight is as high as about $^1/_{10}$th that of the electric organ in the electric eel (*Gymnotus electricus*), which is the richest source of ChE as yet known (e.g. PROSSER[4]).

The present paper comprises a preliminary study on the basis for the high ChE activity in the muscles of guppy and goldfish and on the location of the activity.

The dependence of the ChE activity on the substrate-concentration and the inhibiting power of Mipafox [bis-(mono*iso*propylamido)-phosphoryl fluoride] and B & W 284C51 [bis-(p-N-dimethyl-allyl-ammoniumbenzyle)-aceton dibromide] have been recorded by means of an electrometric method (TAMMELIN[6]).

The tail muscles of a number of fishes, of which only guppy, goldfish and bream (*Abramis brama*) will be mentioned here, have been examined histochemically for ChE activity according to the method of KOELLE[7] as modified by HOLMSTEDT[8].

The biochemical investigations show that an esterase in the homogenized tail muscles of guppy and goldfish have the same properties as AChE (e.g. AUGUSTINSSON[9]). Thus the enzyme is completely inhibited by 10^{-5} M eserine and the substrate curves for ACh, propionylcholine and acetyl-β-methylcholine are bell shaped. Butyrylcholine is hydrolysed very slowly if at all. Thus it seems probable that no or only insignificantly small amounts of butyrylcholinesterase (BuChE) are present.

Furthermore, using the two specific inhibitors mentioned above, and after an incubation time of 30 min, it is observed that B & W 284C51, which inhibits AChE when acting on the enzyme in the fish muscle and on AChE from electric organ, gives inhibition curves that coincide fairly well between $pI_0 \simeq 7$ and $pI_{100} \simeq 5$. The same is true for Mipafox, the corresponding values being about 5 and 3. BuChE from serum is otherwise inhibited by Mipafox in far smaller concentrations, pI_{100} being about 6·5. (The values on electric organ and serum were kindly given by Dr. Bo HOLMSTEDT.)

The results of the histochemical investigation are shown in Figures 1 to 4. Figure 1 shows the location of the AChE

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[2] K.-B. AUGUSTINSSON, Arch. Biochem. *23*, 111 (1949).

[3] D. NACHMANSOHN, C. W. COATES, and R. T. COX, J. gen. Physiol. *25*, 75 (1941).

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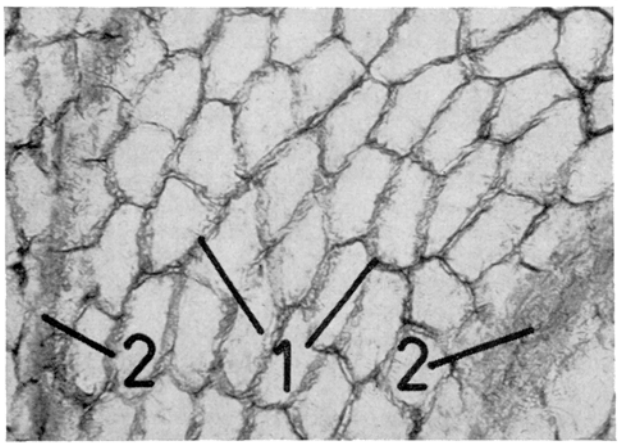

Fig. 1.—Transverse section from tail muscle of guppy. × 450. Enzyme activity demonstrated with acetylthiocholine according to KOELLE and HOLMSTEDT. 1. Endomysium. 2. Septae.

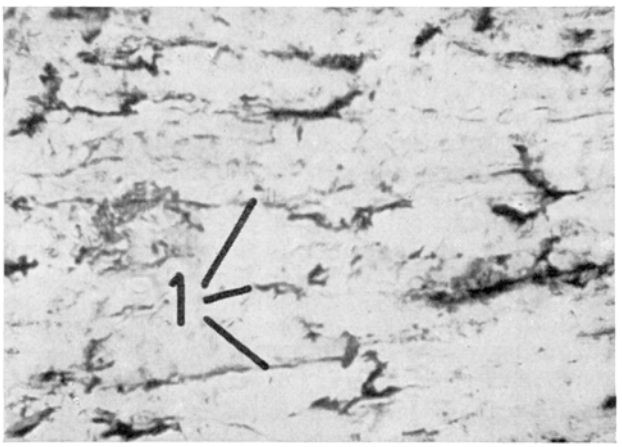

Fig. 3.—Longitudinal section from tail muscle of goldfish. × 450. See Figure 1.

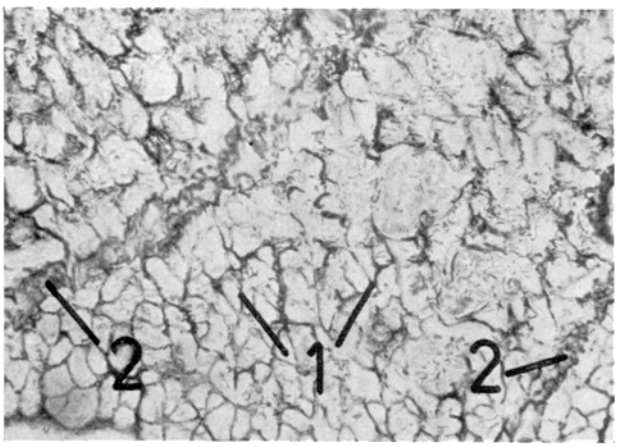

Fig. 2.—Transverse section from tail muscle of goldfish. × 450. See Figure 1.

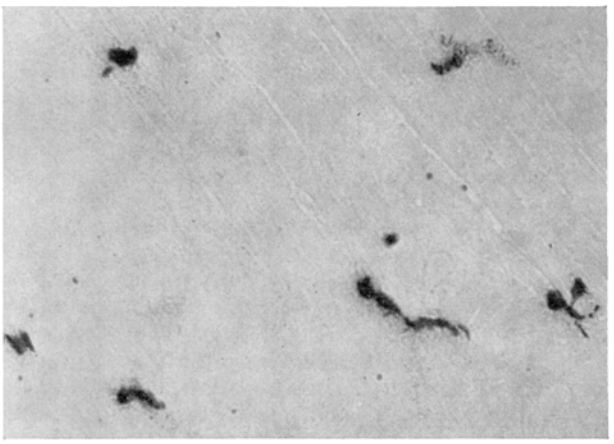

Fig. 4.—Longitudinal section from tail muscle of bream. × 450. See Figure 1.

activity in guppy as obtained on a transverse section from the tail muscle of guppy. The activity is localized to the endomysium around the muscle fibers. Some activity is obviously also found in the septae. A similar picture is obtained in a transverse section from goldfish muscle. (Fig. 2). A longitudinal section from goldfish is shown in Figure 3. The activity is clearly localized to the endomysium along the muscle fibers. These findings are interesting to compare with the results obtained by SIOU[10], who, with a biochemical method, has found a considerable ChE activity in the aneural zone of the muscle of the common eel.

In sharp contrast hereto is the picture of a longitudinal section from bream as shown in Figure 4. The activity is here located in a way very reminiscent of that obtained at endplates in mammalian muscles. Apart from these heavily stained centers of CHE activity, the picture is completely blank.

From the above findings the following conclusions can be drawn. The muscles of goldfish and guppy have a high enzyme activity, which can be characterized as AChE activity. No or little activity from BuChE seems to be present.

In guppy and goldfish, a high proportion of the AChE activity is localized to the endomysium of the muscle fibers. The septae contain diffusely distributed AChE activity.

Studies concerning the possible role of this AChE activity for nerve transmission, and some phylogenetical and ecological problems, are under way, the results of which are to be published elsewhere.

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The Medical Division, Research Institute of National Defence, Dept. 1, Sundbyberg (Sweden), January 28, 1958.

Zusammenfassung

Es wird eine sehr hohe Acetylcholinesteraseaktivität im Schwanzmuskel von Guppy und Goldfish gefunden. Die Aktivität ist in Endomysium und Septa lokalisiert.

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