and densitometric pattern shown in Figure 2 illustrates 1 diffuse band on 5% polyacrylamide gel. However, some portions of proteolipid-protein did not penetrate the gel even after long run. When 8 *M* urea gel system (pH 3.8) was used, almost all of proteolipid-protein penetrated the gel. However 2 harp bands were not observed as in Figure 1.

Since our previous study indicated that the proteolipid-protein obtained with the method of TENENBAUM and FOLCH[2] did not contain the myelin basic protein, the electrophoretic pattern of proteolipid-protein in the present study did not mean the contamination of the basic protein. Recently BRAUN and RADIN[4] reported that proteolipid-protein obtained by the method of TENENBAUM and FOLCH did not exhibit faster migrating band in the 5% and 7.5% acrylamide gel containing 5 *M* urea and 0.5% Triton X-100. EICHBERG[5] reported that almost all of the proteolipid-protein of beef heart penetrated the gel and exhibited the multiple bands pattern on the polyacrylamide gel electrophoresis in a phenol-acetic acid-water-urea (56.6:25:19.4:30, v/v/v/w) system. He used chloroform-methanol-90% formic acid (49:49:2, v/v/v) to re-dissolve the proteolipid-protein precipitated with excess ether.

In our present study, the water-soluble proteolipid-protein from bovine white matter revealed 2 migrating bands pattern and some non-migrating portions on disc polyacrylamide gel electrophoresis at pH 8.3. Therefore, it may be concluded that our water-soluble proteolipid-protein from bovine brain white matter is heterogenous protein.

Zusammenfassung. Wasserlösliche, proteolipide Proteine wurden von der weissen Hirnsubstanz durch Isolierungsmethoden von TENENBAUM und FOLCH[2] gewonnen. Ohne Lyophilisation ergab die Disk-Elektrophorese bei pH 8.3 zwei Banden.

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Effect of Biogenic Amines on γ-Amylase (Acid α-Glucosidase)

It has been established that adrenaline participates in regulation of glycogen hydrolysis by γ-amylase (acid α-glucosidase)[1]. Parenteral administration of the hormone stimulates the enzymatic activity in liver or skeletal muscles but inhibits this activity in heart[2]. The well recognized difference between metabolic pathways of catecholamines in liver and heart muscle[3] has been considered as a possible cause of this phenomenon. It seemed also probable that the regulatory effect belongs not to the molecule of adrenaline but to the products of its catabolism[4]. Purpose of this work was to compare the effects on γ-amylase of adrenaline and other biogenic amines (including precursors of adrenaline – noradrenaline, dopamine – and tyramine or tryptamine which are metabolized only via oxidative deamination[5] both in normal animals and in conditions of inhibition by specific monoamine oxidase inhibitors of enzymatic deamination in rat liver and heart.

Adrenaline. HCl (0.1 ml of 0.1% solution/200 g body wt.), equimolar amounts of other amines (or 0.9% NaCl) were injected s.c. either into control 200–220 g male white rats or into animals pretreated with one of monoamine oxidase inhibitors (iproniazid or pargyline s.c., 18 h before the experiment). The animals were sacrificed 30 min

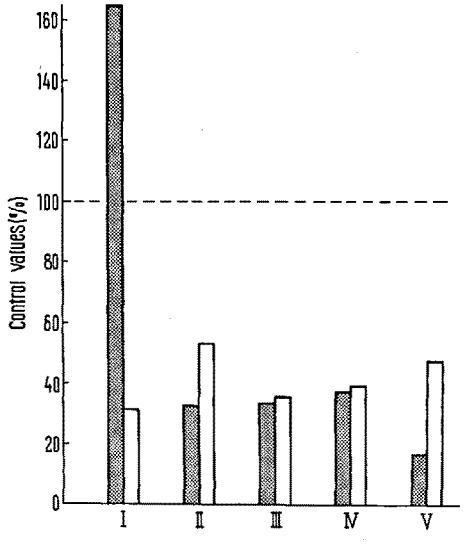

Fig. 1. Effect of monoamines on activity of γ-amylase in rat liver (shaded bars) and heart muscle (white bars). I, adrenaline; II, noradrenaline; III, dopamine; IV, tyramine; V, tryptamine. Mean values from the results of 3 parallel experiments are presented as % of control values (about 10 μmoles of glucose were liberated during 3 h incubation with 20 mg glycogen) indicated by a horizontal dashed line.

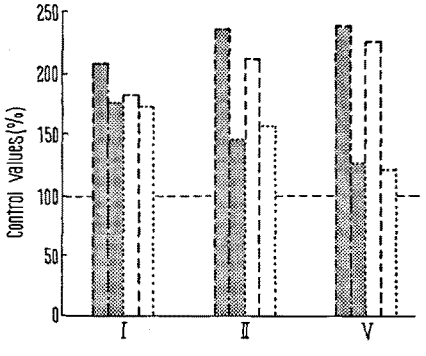

Fig. 2. Effect of monoamines on activity of γ-amylase in rats pretreated with iproniazid (30 mg/kg; dashed bars) or pargyline (2.5 mg/kg; dotted bars). Other designations as in Figure 1.

after the injections of amines. Partial purification and estimation of activity of γ-amylase in liver and heart were carried out as described previously[2].

Stimulation by adrenaline of γ-amylase activity in rat liver is not reproduced by equimolar doses of other amines which, in contrast to adrenaline, markedly inhibit hydrolysis of glycogen by the acid α-glucosidase in liver (Figure 1). Inhibitory effect on γ-amylase in heart muscle is observed in experiments with all the monoamines tested (Figure 1). This effect is prevented by monoamine oxidase inhibitors in doses causing strong inhibition of enzymatic deamination of monoamines[6]. Monoamine oxidase inhibitors also prevent the effect of noradrenaline and tryptamine on γ-amylase in liver (Figure 2).

The data obtained suggest that the inhibitory effects of adrenaline on γ-amylase, exhibited also by other monoamines, are possibly caused by the deaminated metabolic products. The stimulatory effect of adrenaline on liver γ-amylase is specific for the hormone, and may not be prevented by inhibition of monoamine oxidase activity in liver.

Выводы. Норадреналин, дофамин, тирамин и триптамин тормозят γ-амилазу печени и сердца крыс. Ингибиторы моноаминоксидазы предупреждают этот эффект.

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Alteration of Cyclic 3′,5′-Adenosine Monophosphate Action on Adenosine 5′-Triphosphate Induced Muscular Contraction by a Serum Enzyme

The interaction between myosin and actin which results in the formation of actomyosin is catalyzed by the presence of adenosine 5′-triphosphate (ATP). Variations in ATP concentrations influence adenosine 5′-triphosphatase (ATPase) and superprecipitation of actomyosin. Both of these parameters are limited by the availability of the substrate[1].

Several accessory proteins were reported to play various roles in the functions of the myofibrils. For instance, α-actinin was shown to play a role in the structure of the Z-band and its attachment to the I-filament[2–4]. A troponin-tropomyosin factor was shown to be active and to delay the onset of superprecipitation in certain tropomyosin preparations[5]. Troponin is another protein which plays a role in the On-Off control by calcium ions of the contraction cycle[6,7].

A serum enzyme was shown to enhance the creatine-phosphotransferase-ATP regeneration and the phosphoenol pyruvate-pyruvate kinase system and muscular contraction[8]. The enzyme is shown in the present studies to reverse excess substrate inhibition and cyclic-3′, 5′-adenosine monophosphate (cyclic 3′ 5′-AMP) inhibition of the ATP-induced development of tension by glycerinated muscle fibers.

The sensitivity of glycerinated fibers to ATP was shown to be influenced by a toxic glycoprotein from scalded human skin[9]. The degree of inhibition of the ATP-induced muscle contraction was dependent on the concentration of the toxic glycoprotein[10]. Immuno sera and purified immunoglobulin against the toxic glycoprotein prepared by i.m. injections of the glycoprotein neutralized the inhibitory effects of the antigen. When used in combination with the immune serum or with the immunoglobulin, the toxic glycoprotein failed to inhibit the ATP-induced development of tension by glycerinated fibers[10].

Materials and methods. Glycerinated rabbit psoas muscle fibers were prepared according to the method of Szent-Gyorgyi[11]. Actomyosin was prepared from rabbit striated skeletal muscle following the method of Ebashi[12]. The serum enzyme was obtained from either human or calf serum following the method described by Hakim[8]. The serum was adjusted to pH 5.0, then made up to 25% saturation with ammonium sulfate. A precipitate was separated by centrifugation at 10,000 g for 30 min at 4 °C. The precipitate was dissolved in cold 0.9% sodium chloride and dialyzed exhaustively against 0.9% NaCl. The dialyzed sulfate free solution was then adjusted to pH 4.0 and centrifuged at 15,000 *g* for 60 min. The precipitate obtained was dissolved in 0.9% NaCl to produce a solution

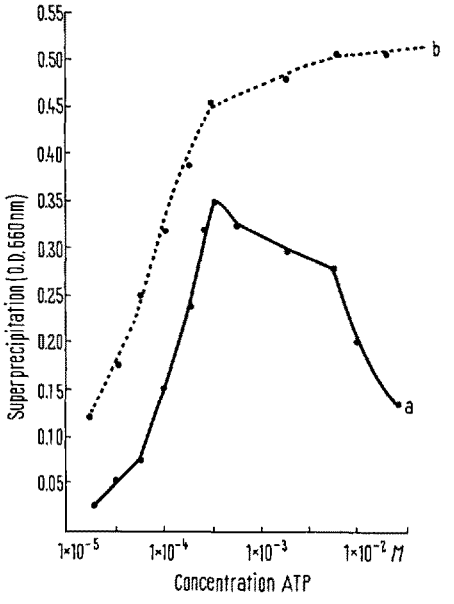

Fig. 1. Action of serum enzyme on the superprecipitation of actomyosin. a) Superprecipitation of actomyosin in a system containing 0.05 *M* KCl, 1.0 m*M* $MgCl_2$, 0.016 *M* *Tris*-phosphate buffer of pH 7.0 and 0.15 mg/ml of actomyosin in a total volume of 4.0 ml and ionic strength of 0.080 µm. The final ATP concentrations employed are indicated on the abscissa. Superprecipitation of actomyosin assayed in presence of 0.25 mg protein of serum enzyme produced the data of curve b).