marquaient moins nettement sur des feuilles attachées à leur pétiole que sur des disques foliaires. Une telle différence de réactivité peut être attribuée[2] au fait que les feuilles non sectionnées sont bien plus riches en cytokinines – dont on sait qu'elles freinent la sénescence cellulaire[16] – que les feuilles découpées. C'est pourquoi, dans une troisième série d'essais, une cytokinine synthétique, la kinétine (6-furfurylaminopurine) a été ajoutée (10 µg/10 ml) au milieu d'incubation. Les résultats (Tableau III) montrent que: 1. la diminution de l'ATr, pour des racines isolées, est nettement freinée par la kinétine; 2. les effets de l'ABA – particulièrement marqués pour des racines isolées – ne sont plus significativement observables lorsque ces racines ont subi l'action de la kinétine.

Ainsi l'ABA n'agit sur les transaminases – en accélérant la chute de leur activité – que lorsque les cellules, au niveau desquelles elles opèrent, présentent des caractères de sénescence. Ces observations permettent donc de rendre compte, dans une certaine mesure, des divergences relevées quant à l'intervention de l'ABA sur certains systèmes enzymatiques[6]. En effet, l'âge – rarement précisé et souvent fort différent d'un type d'essai à l'autre – du matériel, employé dans l'extraction des enzymes testées et traité par l'ABA, est un facteur de variation non négligeable.

Summary. Abscissic acid (ABA) was found to increase the fall of the 'GOT' transaminase activity in the root tissues. In combination with ABA, kinetin counteracted the ABA inhibition of transaminase reaction, which was discussed in relation to cell aging.

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Further Studies on Polyacrylamide Gel Electrophoresis of Water-soluble Proteolipid-Protein from Bovine Brain White Matter

Our previous studies[1] have shown the polyacrylamide gel electrophoretic pattern of lyophylized sample of water-soluble proteolipid-protein. The electrophoresis was carried out by the procedures of Takayama et al. with a little modification. This electrophoretic pattern of proteolipid-protein indicated the evidence of one broad band on either 5% or 7.5% acrylamide gel containing 8*M* urea (pH 3.8). However, we did not directly use water-soluble proteolipid-protein without lyophilization. In this report we shall describe the electrophoretic pattern of water-soluble proteolipid-protein without lyophilization.

Water-soluble proteolipid-protein was isolated from bovine brain white matter by the slightly modified procedure of Tenenbaum and Folch[2]. Fresh brain white matter was extracted with 19 volumes of chloroform-methanol (2:1, v/v) and the extracted solution was partitioned with 0.2 volume of distilled water. The lower phase was washed 3 times with theoretical upper phase. The extract containing proteolipid-protein was concentrated to $^1/_3$ volume using a rotary evaporator. The concentrated extract was dialyzed against chloroform-methanol mixtures for 7 days and then against acidic chloroform-methanol (chloroform-methanol-HCl, 200:100:1, v/v/v) for 6 days. The transfer of the lipid-free protein to aqueous solution was carried out by successive dialysis in solution of increasing methanol and water and finally in water alone. The pH of the final clear solution was just neutral. This final proteolipid-protein solution was concentrated to 300–500 µg/ml by vacuum evaporation or dialysis against 20% Arabia gum solution. The $E^{1\%}_{1\,cm}$ of proteolipid-protein gave 10.5 at 278 nm wave length which showed the maximum absorption value. The concentrated proteolipid-protein was not lyophilized and stored at 4 °C with a few drops of chloroform. Polyacrylamide gel electrophoresis of water-soluble proteolipid-protein obtained with above procedures was carried out at pH 8.3 according to the method of Davis[3]. Proteolipid-protein in 5% sucrose solution was used as a sample for the electrophoresis. Current was applied for 2 h at 4 mA per gel. Gels were stained for 60 min with 1% Amido Black in 7% acetic acid and destained by diffusion in 7% acetic acid.

Figure 1 illustrates the electrophoretic pattern on 7.5% polyacrylamide gel at pH 8.3 and its densitometric pattern. 2 sharp bands were observed. The electrophoretic

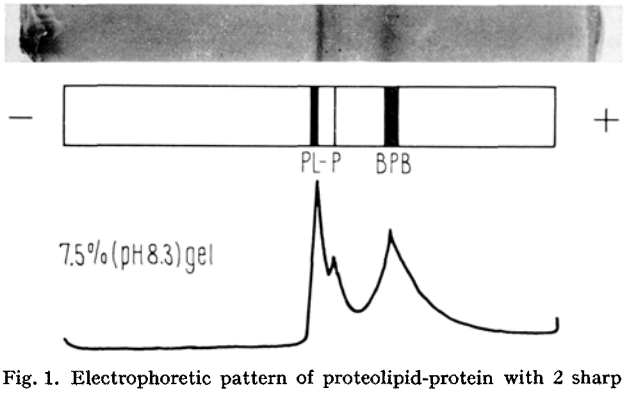

Fig. 1. Electrophoretic pattern of proteolipid-protein with 2 sharp bands.

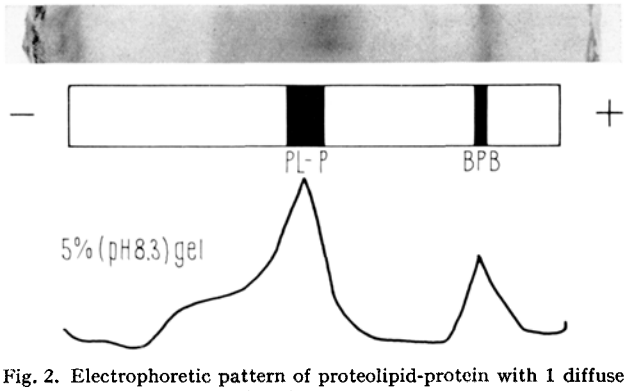

Fig. 2. Electrophoretic pattern of proteolipid-protein with 1 diffuse band on 5% polyacrylamide gel.

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

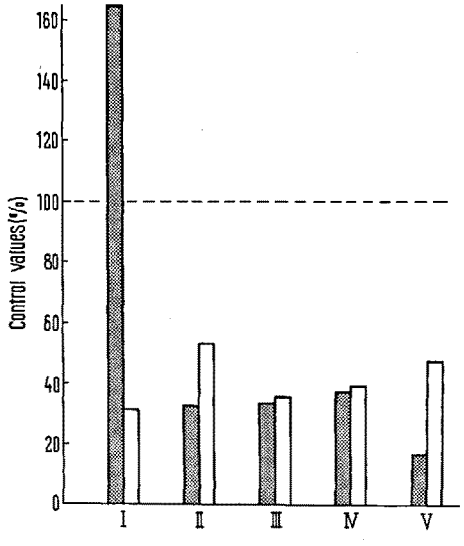

Fig. 1. Effect of monoamines on activity of γ-amylase in rat liver (shaded bars) and heart muscle (white bars). I, adrenaline; II, noradrenaline; III, dopamine; IV, tyramine; V, tryptamine. Mean values from the results of 3 parallel experiments are presented as % of control values (about 10 μmoles of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

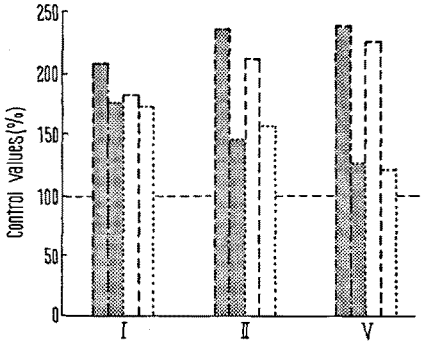

Fig. 2. Effect of monoamines on activity of γ-amylase in rats pretreated with iproniazid (30 mg/kg; dashed bars) or pargyline (2.5 mg/kg; dotted bars). Other designations as in Figure 1.