Enzyme Localization in Proteins Separated by Paper Electrophoresis in Human Seminal Plasma

Several enzymes have been detected in human seminal plasma. This fluid is very rich in glutamic oxalacetic (GOT), glutamic pyruvic (GPT) transaminases[1,2] and α-amylase[2,3] and we therefore found it of interest to report the localization of these enzymes in normal human seminal plasma after separation of proteins by paper electrophoresis.

Materials and Methods. Paper electrophoresis was performed in a Spinco paper electrophoresis apparatus with a veronal buffer (pH 8.6 and ionic strength 0.05), at 4 mA. Complete separation was attained after 16 h.

Amounts of 0.01 ml of seminal plasma were applied to seven strips as opposed to 0.006 ml of human serum applied to one of them. This strip and one of the others were dried and fixed at 100°C for 15 min and stained with 1% bromphenol blue[4]. The other six strips were not fixed but cut in 1 cm segments and eluted in buffers for GOT[5], GPT[5] and α-amylase[6] determinations. After this, lectures in optical density were plotted against distance in cm from origin.

Results. Results are presented in the Table and are the average values from 5 determinations in seminal plasma obtained from men with normal samples (after being submitted to several criteria of normality: spermatozoa number, volume of ejaculate, percentual quantity of abnormal and immobile forms, vitality after 12 and 24 h, at 4°C and 37°C).

Amylase was localized in every fraction but GOT and GPT were situated in α-2 globulin and albumin, respectively.

Localization of enzymes in seminal plasma

	Amylase %	GOT %	GPT %
Albumin	16.3		100
α-1 Globulin	20.1		
α-2 Globulin	11.8	100	
β-Globulin	8.7		
γ-Globulin	26.8		
O-Fraction (origin)	16.3		

Résumé. Les transaminases α-glutamo-oxaloacétique (GOT) et glutamo-pyruvique (GPT) ont été déterminées dans les protéines du liquide spermatique de l'homme après séparation par électrophorèse sur papier. L'amylase se trouve dans toutes les fractions, mais la GOT et la GPT sont localisées respectivement dans la globuline α-2 et l'albumine.

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Effect of Cold Acclimatization on the Succinic Dehydrogenase Activity in the Liver and Brain Tissues of Mice

Little is known about the cold acclimatization in mammals other than rats. In addition, studies on the effects of the cold acclimatization on tissue metabolism have been confined to skeletal muscles and visceral tissues, as appears from the recent reviews[1] on this subject. It is a well-established fact that the succinic dehydrogenase activity in the liver tissue of rats increases during the cold acclimatization[2]. The aim of the present experiments was to find out whether this change also occurs in the liver and brain tissues of mice.

Groups of male albino mice were kept for 7 to 12 days at + 5°C in a refrigerator. The control males, similar in age and in weight, were kept in the animal room at + 24°C. Tissue homogenates (5%) were prepared from the liver and from the whole brain of decapitated animals in ice-cold buffer. The succinic dehydrogenase activity was measured with the method of KUN and ABOOD[3]. The reaction tubes contained 0.5 ml 0.2*M* Na-succinate, 1 ml tissue homogenate in 0.13*M* phosphate buffer (pH 7.4) and 1 ml 0.1% triphenyltetrazolium chloride (TTC). The reaction was stopped and the formazan brought into solution by adding 8 ml ethanol. The linearity of the concentration of red formazan in relation to the incubation time (at 38°C) was checked. The incubation times chosen were 10 min for liver and 20 min for brain homogenates. The extinction was read at 490 mμ with a Beckman DU spectrophotometer. All determinations were run in duplicate or triplicate together with the necessary standards and blanks. The determinations were made at the same time with one cold acclimatized and one control animal. The mean results with their standard errors are presented below. The number of animals is shown in brackets.

The enzyme activities are higher in the liver and lower in the brain of mice than those found in the corresponding tissues of rats with the same technique[3]. The succinic dehydrogenase activity is 72% higher in the animals

Acclimatization temperature	Succinic dehydrogenase activity (μg reduced TTC per mg fresh tissue in 10 min) Liver	Brain
5°C	3.30 ± 0.21 (10)	0.30 ± 0.02 (14)
24°C	1.92 ± 0.18 (10)	0.32 ± 0.02 (14)

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acclimatized to cold ($t = 4.98$, $p < 0.001$). On the other hand, the difference between the enzyme activities in the brain homogenates from the cold acclimatized animals and from the controls is not significant ($t = 0.70$).

The present results are in agreement with the results obtained with rat liver[2], although the increase in the succinic dehydrogenase activity during the cold acclimatization in the mouse liver is greater than has been observed in the rat liver after a stay of 2 to 70 days at 1.5° to 5°C[2]. This may reflect the fact that the cold acclimatization obviously involves a greater compensatory increase in the basal heat production in mice than in rats which have a relatively smaller rate of heat loss in the cold. The acclimatization to cold did not cause any difference in the activity of the succinic dehydrogenase in the brain, a fact which indicates the relative stability of the metabolism of the central nervous system in spite of general compensatory adjustments in the other parts of the body.

Résumé. L'activité du déshydrogénase succinique dans le foie des souris mâles adultes acclimatisés pendant 7–12 jours à + 5°C est augmentée de 72%. L'activité du même enzyme dans le cerveau n'est pas altérée par cette acclimatisation au froid.

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A New Type of Antigen Induced by Chemical Linkage of *Mycobacterium tuberculosis* and γ-Globulin

The search for more effective methods to produce vaccines is very important. Hence, the authors attempted to examine whether it would not be possible to produce a new kind of chemospecific antigen which would change the antigen structure of the micro-organism and influence its ability to immunize favourably, and which could be bound by chemical linkage to a large molecule having the property to produce antibodies in considerable amounts.

Of the methods of chemical linkage which may be considered, the authors chose Pauly's[1] diazoreaction by the use of which the chemical linkage of a protein molecule to the body of the *Mycobacterium tuberculosis* (*M*) is possible[2–4].

The linkage between the *M* and γ-globulin was performed as follows: The human pathogen, virulent *M* suspension (Type: H III) was washed 5 times with distilled water and dried. Subsequently, in order to remove the lipoid layer on the surface, an amount of 5 g dry weight of the bacterium was washed with petroleum ether and then the traces of the petroleum ether were evaporated. This substance was suspended in 100 ml of 10% hydrochloric acid, and, after stirring it for 1 h in ice, 30 ml of a saturated solution of sodium nitrite was added during 4 h. 3 ml of a 10% human γ-globulin solution ('Human' Oltóanyagtermelö és Kutató Intézet, Budapest, Hungary) was dissolved in 60 ml 5*n* sodium hydroxide and added to the above mixture. After mixing for 30 min, it was centrifuged and the protein which had not been chemically bound to the bacteria was removed by washing in saline.

The chemical binding of γ-globulin to *M* was proved as follows: (1) By morphological studies of the bacterium. The *M* linked to γ-globulin loses its acid-fast character and stains well with aniline dyes. Electronmicroscopic studies show that the bacterium which, owing to the treatment with petroleum ether, absorbed electrons only poorly (Figure 1), changes again to a good absorber of electron rays through the γ-globulin coating of its surface (Figure 2).

(2) By immune electrophoresis. The *M* suspension bound to γ-globulin was incubated for the sake of comparison in a 2% γ-globulin solution for 24 h and then washed and submitted to immune electrophoresis. On the action of the current, the γ-globulin separated from the surface of the *M* incubated in it; however, the current could not separate the γ-globulin from the bacterium linked to it, but it migrated simultaneously with the *M*.

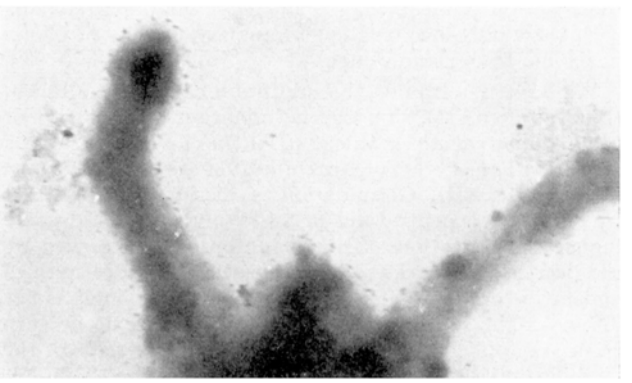

Fig. 1. Diazotized *Mycobacterium tuberculosis*. Examined without shading with a Zeiss electronmicroscope type D_2; basic magnification 12000, magnified to 20000.

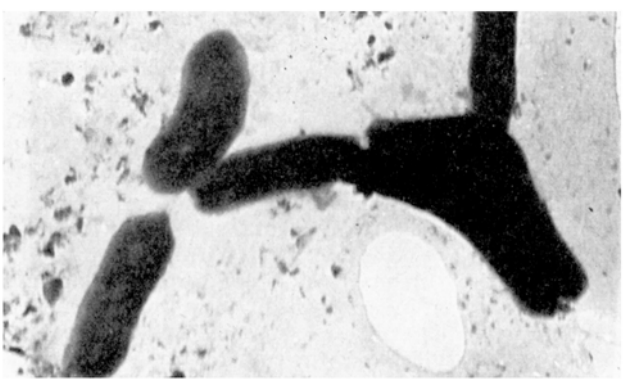

Fig. 2. *Mycobacterium tuberculosis*, linked chemically to γ-globulin. Similar data as in Figure 1.

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