

D'une façon générale, l'activité MAO du nouveau-né de Lapin est plus proche de celle de l'adulte que celle du Rat nouveau-né⁵. Cela rend vraisemblablement compte des différences de maturité physiologique des nouveau-nés de ces deux espèces. Une diminution de l'activité MAO a déjà été mentionnée, au moment de l'éclosion chez le Poulet⁶ et au moment de la naissance dans plusieurs organes du Rat^{5,7,8}. Chez le Lapin, ce phénomène est particulièrement net et se reproduit au moins dans 3 organes de façon synchrone. Comme chez le Rat⁵ deux chutes successives ont lieu au voisinage de la naissance.

Il est difficile de déterminer les causes précises de ces diminutions d'activité puisque, d'une part de nombreux paramètres physiologiques varient au moment de la naissance et que, d'autre part, l'activité MAO est soumise à de multiples influences et en particulier à un contrôle hormonal. Ces diminutions ne semblent pas pouvoir être des artefacts dus au mode d'expression des résultats. Il semble en tous cas que les chutes observées sont trop rapides pour résulter de variations de la teneur en eau des tissus ou d'une diminution de la synthèse de l'enzyme. Des variations des conditions physico-chimiques intracellulaires ne semblent pas pouvoir non plus être en cause puisque le dosage est fait *in vitro* dans des conditions standardisées. Ces diminutions peuvent par contre s'expliquer par l'intervention d'inhibiteurs ou la disparition d'activateurs de l'activité enzymatique chez le nouveau-né. C'est ainsi que l'on connaît l'effet inhibiteur des hormones thyroïdiennes⁹ et des glucocorticoïdes^{10,11} sur l'activité MAO et l'on sait qu'au moment de la naissance, la thyroïde du Rat est activée¹² et le taux plasmatique de glucocorticoïdes est élevé chez le Rat, le Lapin et le Cobaye¹³. Enfin ces diminutions mesurées *in vitro* sont probablement des sous-estimations des chutes qui se produisent *in vivo*. En effet, l'activité MAO subit en plus l'effet de l'hypoxie néonatale et l'on sait que la teneur en oxygène influence cette activité^{14,15}.

Summary. The activity of enzyme monoamine oxidase was studied from 3 days before birth up to 2 days after birth in the heart, liver and kidney of albino rabbits. At the end of foetal life, the MAO activity of heart and liver expressed per unit of organ weight was nearly the same as the adult one. At birth and 1 day, the activity showed decrease in the 3 organs. The possible causes of these decreases are discussed.

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Chromatography of some Human Glycoproteins on DEAE-Sephadex A-25

The DEAE-Sephadex anion exchangers have a high capacity and low nonspecific adsorption. They are therefore extremely useful for column chromatography in the biochemical field.

The Sephadex anion exchanger A-25 is the more highly crosslinked gel and has a high capacity for low molecular

weight fraction – to 10,000¹, although other applications are also known². The DEAE-Sephadex A-50 is suitable for larger molecules and finds wide application in fractionating and preparing a great number of plasma proteins^{3–5}.

In this investigation, human serum was fractionated by column chromatography on DEAE-Sephadex A-25. The fractions were determined by measuring optical density and characterized by specific methods.

Material and methods. Normal human serum was obtained from 10 human donors and then pooled. Dry DEAE-Sephadex A-25 medium (AB Pharmacia, Uppsala) was suspended in distilled water and washed with 0.5 M HCl, followed by water, then treated with 0.5 M NaOH and washed with water several times. Neutralization was made with 0.5 M CH₃COOH. Finally the material was washed with 0.02 M phosphate buffer (pH = 6.6).

A glass column (1.5 × 20.0 cm) before packing was filled with phosphate buffer in which the gel was poured.

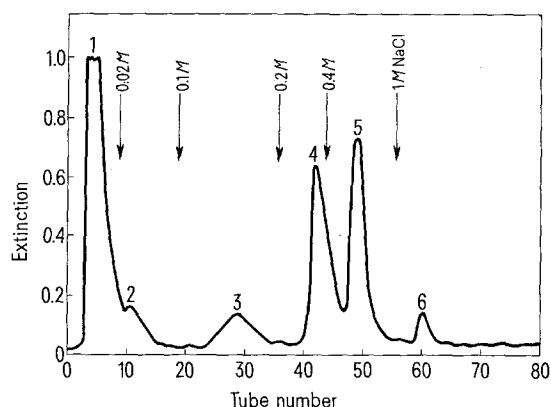


Fig. 1. Effluent diagram of normal human serum proteins chromatographed on DEAE-Sephadex A-25. Elution was started with 0.02 M phosphate buffer (pH 6.6) followed by a system of increasing molarity of NaCl.

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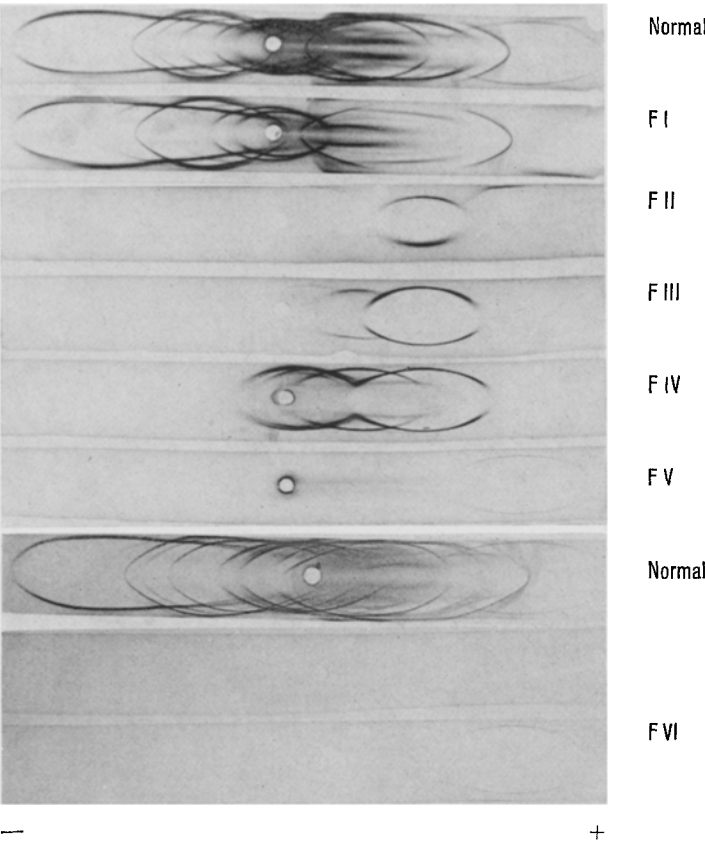


Fig. 2. Immunoelectrophoretic control of serum fractions after chromatography on DEAE-Sephadex A-25 (F, I-V) using a horse anti-human serum (Human Inst., Budapest). For the fraction VI antiserum was diluted 1:4.

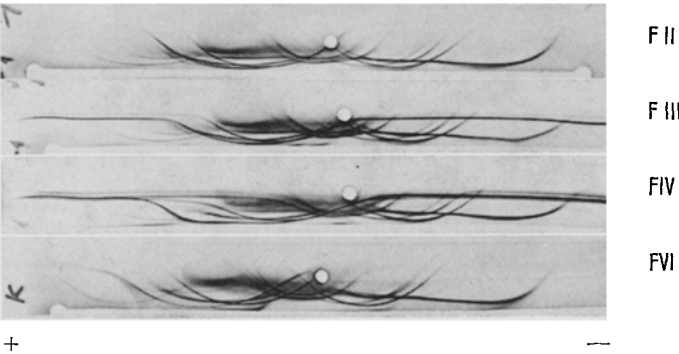


Fig. 3. The identification of individual fractions by the OSSERMAN¹⁰ technique.

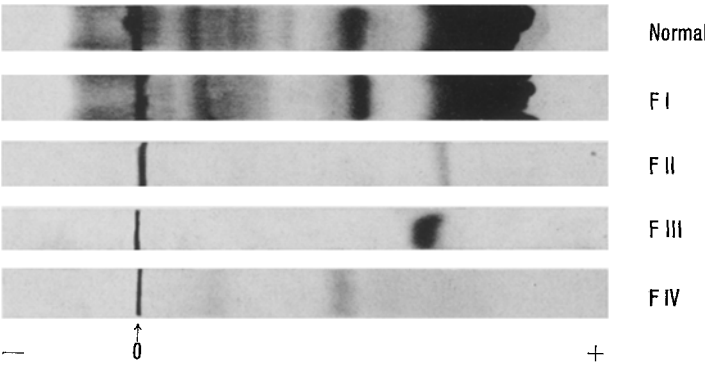


Fig. 4. Starch-gel electrophoresis of the individual fractions after column chromatography (20 mA, 200 V, 4 h at room temperature).

3 ml pooled normal human serum was applied to the column. 81 fractions of 5.5 ml were collected at flow rate of 30–35 ml/h. Chromatography was started with 0.02 M phosphate buffer and then a system of stepwise increasing NaCl concentration (0.02, 0.1, 0.2, 0.4, 1 M) was applied. Protein content of the effluent was determined by measuring optical density (OD_{280}^1) and by LOWRY method⁶.

After chromatography the peaks of each group were pooled and dialyzed. Concentration of the effluent fractions was made by lyophilization after dialysis. Each peak was analyzed by immunoelectrophoresis^{7–9}, using a horse antiserum, and the individual precipitate arcs were also identified by the method of OSSERMAN¹⁰. Starch-gel electrophoresis¹¹ was performed with a discontinuous buffer system.

Results and discussion. The absorption of the effluent (its protein content) separating the serum into 6 fractions (I–VI), is reproduced in Figure 1. The individual fractions analyzed by immunoelectrophoresis are demonstrated in Figure 2.

The bulk of the proteins passes through the liquid phase outside the molecular sieve and is eluted first. The immunoelectrophoretic analysis of the first fraction (I) develops almost a complete protein spectrum. The γ -region – IgA, IgM and IgG appears only in that fraction. The β_1 -region seems to be also unvaried, except for hemopexin, which is completely missing. The α_2 -region is lacking in many of its components. The α_0 , α_1 and prealbumin regions are also very poor. Prealbumin (α_1) is completely retained (Figure 2, I). When the first fraction is adsorbed with antiserum, or when the fractions II–VI are identified by means of direct immunoelectrophoresis^{7–9}, or using specific method of identification¹⁰, one can see the following: After perfect technical separation the 2nd fraction contains only traces of albumin (Figures 2 and 3). In the 3rd fraction albumin appears again, further α_1 -antitrypsin and a precipitation arc in the α_2 region. In the 4th fraction albumin, α_1 -acid glycoprotein, α_1 -antitrypsin, haptoglobins, ceruloplasmin and hemopexin can be demonstrated. The fraction V contains traces of albumin, α_1 -antitrypsin, haptoglobins again and prealbumin. Almost pure prealbumin appears in the fraction VI. By the stepwise increasing NaCl concentration used, it is the protein which is eluted last (Figure 3, VI). The identification of prealbumin by means of direct immunoelectrophoresis is possible only after dilution of antiserum (Figure 2, VI).

The results obtained by starch-gel electrophoresis, are nearly the same as those obtained by immunoelectrophoresis (Figure 4).

Human serum proteins are partly retained on DEAE-Sephadex A-25. The character of the retained proteins shows relationship to the glycoproteins. α_1 -acid glycoprotein, α_1 -antitrypsin, haptoglobins, ceruloplasmin and hemopexin are retained most of all. The immunoelectrophoretic analysis also revealed the retention of some proteins, which were usually not grouped together with glycoproteins, though albumin or its traces were obtained

in the fractions I to V. It is likely that a high degree of heterogeneity, due to complexes of albumin with other small molecules, is responsible for this behaviour, or it is the phenomenon observed at the partial dissolution of these proteins in perchloric acid due to the protective property of the glycoproteins^{12–17}. It is of special interest that it was possible to separate almost pure prealbumin in the last fraction, although prealbumin has a carbohydrate composition which is very different from the average of all plasma proteins, with a very low hexosamine to hexose ratio and no sialic acids¹⁸.

Much work has been carried out in recent years in connection the pathological variables with glycoproteins in patients^{19–21}, nevertheless the pathological significance is not yet exactly understood. The method described may possibly give more significant information about the levels, presence, or absence of individual glycoproteins.

Zusammenfassung. Zur Fraktionierung der Humanplasmaproteine wurde DEAE-Sephadex A-25 verwendet und die Fraktionen I–VI durch Immuno- und Stärkegelelektrophorese charakterisiert. Nach Eluierung der proteinreichen 1. Fraktion wurden zahlreiche Glykoproteine gefunden und die Abtrennung von α_1 -Präalbumin erreicht.

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Histone Synthesis in Amphibian Oocytes and Early Embryos¹

Amphibian embryos have been demonstrated to synthesize protein at substantial rates during both the pre- and post-fertilization stages of early development². Modulations in the electrophoretic patterns of newly synthesized protein have also been observed during the early cleavage stages of those embryos. Some of those

modulations require the presence of functional chromosomes³, while others are influenced by the composition of the egg cytoplasm⁴.

A more complete understanding of the regulation of early protein synthesis in amphibian embryos might be achieved if the identity of some of the newly synthesized