cult to explain because the $\alpha^5$-pyridoxalacetic acid has a chemical structure very similar to that of pyridoxal-P and also shows a high affinity for the apotransaminases tested. These data suggest that the analogue binds at the pyridoxal-P binding site.

FURBISH et al.[13] have observed that the $\alpha^5$-pyridoxalacetic acid-apoAAT from pig heart complex undergoes reversible transamination by L-glutamate: this observation is consistent with the above suggestion.

The $\alpha^5$-pyridoxalacetic acid-apoAAT from pig heart complex shows an efficient but reduced catalytic activity, evidenced as using, not the glutamate which has an extremely high rate of transamination[14], but a more suitable substrate, the DL-alanine, as well as the possibility to form the intermediate complex enzyme-substrate, proposed by JENKINS[15] in the transamination reaction.

Furthermore, if we compare the ratio between $[I]_{50}$ of the $\alpha^5$-pyridoxalacetic acid and its pyridoxyl derivative and the ratio between the dissociation constants for pyridoxal-P and P-pyridoxyl-L-phenylalanine[12] we found a similar value. This observation suggests that the substitution of the phosphate with a carboxyl group does not hinder the formation of the enzyme-substrate complex, but affects the catalytic activity.

It is possible that the binding of the $\alpha^5$-pyridoxalacetic acid to the active site has some peculiar characteristics which could result in an imperfect fit at the active center so as not to allow an efficent interaction and catalysis to take place. The extent of the binding is much more lower in apoTDC, as compared to apotransaminases, according to the evidence reported by GROMAN et al.[2].

*Riassunto.* I risultati ottenuti dall'interazione dell'$\alpha^5$-piridossal acido acetico e dall'$\alpha^5$-piridossil-L-fenilalanina acido acetico su alcuni enzimi $B_6$ dipendenti suggeriscono che la sostituzione del fosfato con un gruppo carbossilico nel piridossal-5'-fosfato non impedisce la formazione del complesso enzima-substrato, ma influenza l'attività catalitica.

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## Molecular Heterogeneity of Human Placental Aminopeptidase Isozymes

An aminopeptidase (AP) that hydrolyzes L-leucyl-$\beta$-naphthylamide (leucine aminopeptidase, LAP) or L-cystine-di-$\beta$-naphthylamide (cystine aminopeptidase, CAP or oxytocinase) appears in maternal sera during pregnancy[1–3]. GOEBELSMANN and BELLER[4] successfully used chromatography on Sephadex for separating this pregnancy serum AP from an LAP which is present in all human sera. In an earlier report we presented evidence for the existence of 2 AP isozymes in human placenta: the lysosomal and microsomal isozymes, which were distinct from normal serum LAP[5]. In this paper we present new data on the molecular heterogeneity of placental AP isozymes obtained from a gel filtration study.

Lysosomal and microsomal extracts were prepared from homogenates of human placentae as previously prepared[5]. Sepharose 6B column (*2.5 × 100 cm*) was prepared and equilibrated with 0.1 *M* sodium phosphate buffer (pH 7.0). Elution was carried out with the same buffer and the effluent was collected in 4-ml-fractions. LAP and CAP activities were determined by the method of TAKENAKA[6] with slight modifications. Techniques for disc electrophoresis and the enzyme staining were also described previously[5].

Figure 1 represents a typical elution pattern of AP isozymes by chromatography on Sepharose 6B column. Non-pregnancy sera and fetal sera exhibited only 1 peak of LAP activity. Pregnancy sera at term showed in addition another tall LAP peak (Peak I) which was separated from the LAP peak in non-pregnancy sera (Peak II). Corresponding to Peak I, a peak of CAP activity was found in pregnancy sera, whereas non-pregnancy sera and fetal sera had no CAP activity in any fraction. Lysosomal LAP and CAP were eluted in the same position as Peak I; microsomal LAP and CAP as Peak II.

Since only pregnancy sera exhibited biphasic LAP peaks, these 2 separated peaks were subjected to disc electrophoresis. As is presented in Figure 1, Peak I showed 2 CAP bands ($CAP_1$ and $CAP_2$)[7] as the lysosomal enzyme did; Peak II one LAP band.

These results support our view that the origin of pregnancy serum AP is the lysosomes of placenta, from which the enzyme is released into maternal circulation during pregnancy[5,8]. Furthermore, it was shown that the pregnancy serum AP of lysosomal origin displaying 2 CAP bands can be separated from the LAP in all human sera by gel filtration. This is in agreement with the results of GOEBELSMANN and BELLER[4].

As is shown in Figure 2, according to the method of ANDREWS[9], the molecular weight of the lysosomal enzyme was estimated to be approximately 320,000; that of the microsomal enzyme and normal serum LAP to be approximately 145,000. The former is nearly equal to the molecular weight of retroplacental CAP as estimated to be 325,000 by YMAN and SJÖHOLM[10]. From the present gel filtration experiment, the lysosomal and microsomal isozymes are apparently multiple molecular forms differing in their molecular sizes.

Absence of Peak I (2 CAP bands) in fetal serum suggests that this enzyme does not leak into fetal circulation. It is likely that the high molecular weight of pregnancy serum AP is also responsible for its failure to pass from the blood of pregnant women through the placental barrier into fetal circulation.

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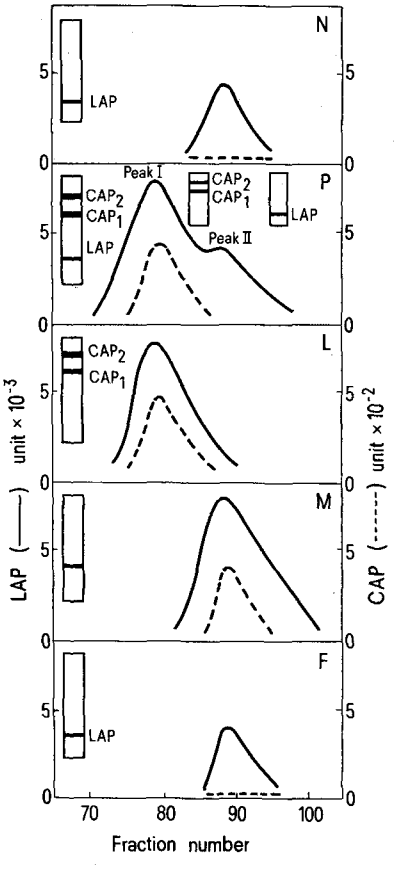


Fig. 1. Sepharose 6B gel filtration of LAP and CAP in non-pregnancy serum (N), pregnancy serum (P), lysosomal extract (L), microsomal extract (M) and fetal serum (F). Column: 2.5 × 100 cm; eluant: 0.1 *M* sodium phosphate buffer (pH 7.0); fraction volume: 4 ml. For the sake of reference, the electrophoretic pattern of LAP in each sample[5] is presented to the left side of the elution diagram. Disc electrophoresis of pregnancy serum (P) exhibits three bands with LAP activity. In the present electrophoretic experiment of 2 separated LAP peaks of pregnancy serum, Peak I showed $CAP_1$ and $CAP_2$ bands; Peak II LAP band.

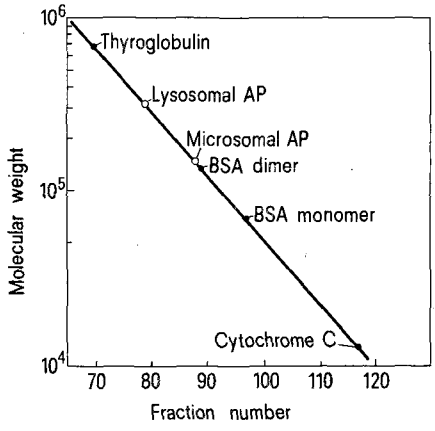


Fig. 2. Molecular weight estimation of AP by gel filtration. The molecular weight was calculated by plotting the fraction numbers of marker proteins against the logarithmic values of their molecular weights. The markers used were cytochrome C (mol. wt. 12,400), bovine serum albumin (BSA, mol. wt. 67,000) and thyroglobulin (mol. wt. 680,000). Cytochrome C was measured by reading the the absorbance at 410 nm; BSA and thyroglobulin at 280 nm.

*Zusammenfassung.* Mit Hilfe von Gelfiltration wurden 2 verschiedene Molekularformen von Aminopeptidase (AP)-Isozymen in der menschlichen Placenta charakterisiert: die Lysosomen-AP (Molekulargewicht ca. 320,000) und die Mikrosomen-AP (Molekulargewicht ca. 145,000).

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## Chromatin-Free Synaptonemal Complex in Whole Mount Preparations

The synaptonemal complex in meiosis has been largely studied in thin section and recently in whole mount preparations. In general the aspects and dimensions of the tripartite structure are very regular in the eukaryote species investigated (see review of WESTERGAARD and VON WETTSTEIN[1]).

In mammals, the reconstitution of the synaptonemal complex (SC) from serial sections of the mouse has been elegantly shown by SOLARI[2]. COMINGS and OKADA[3] described the SC in whole mount preparations of mice. By enzymatic treatment they demonstrated the proteic nature of this structure.

Recently COUNCE and MEYER[4] in testing several saline hypophases as spreading media obtained chromatin-free SC, in *Locusta migratoria*. The authors described the lateral elements, their terminal attachment points to the nuclear membrane and the morphological evolution of the kinetochore during meiotic prophases.

MOSES et al.[5], applying the same technique, described in human chromosome preparations the SC, the kinetochore, the attachment points to the nuclear membrane and the XY sex bivalent, paired by the short arm of the *X*.

In this paper we describe aspects of the chromatin-free SC in the male Swiss albino mouse, obtained by the water spreading technique. These aspects are similar to those obtained by COUNCE and MEYER[4] with the use of diluted saline solutions as spreading medium.

Dissected seminiferous tubules are immediatly immersed in Ringer solution (0.9%). Fragments of about 2 mm are rapidly dipped in 10 successive baths of Ringer for 1 min each. The fragments are then mechanically pulverized on slides which are then dipped in distilled water pH 6.4, in a plastic tray with Teflon bars.

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