responsible for shedding the spores from *C. comatus*, but that the role of chitinase may increase in importance in later stages.

From these results it would seem to be worthwhile undertaking a re-examination of the enzymes present in *Coprinus lagopus*, autolyzing fruiting bodies, and a more detailed study of the wall composition of *C. lagopus* and *C. comatus*.

*Résumé.* Il parait évident que la β-(1–3)-glucanase est impliquée dans l'autolyse des sporophores du champignon *Coprinus comatus*.

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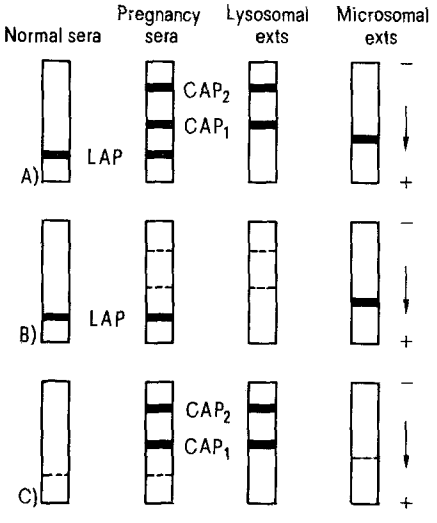
## Human Placental Aminopeptidase Isozymes

Serum aminopeptidase (AP), which hydrolyzes L-leucyl-β-naphthylamide (leucine aminopeptidase, LAP) or L-cystine-di-β-napthylamide (cystine aminopeptidase, CAP or oxytocinase) increases progressively as pregnancy advances[1–4]. Electrophoretic studies of human pregnancy sera exhibit 3 distinct bands[5,6]: the first moving LAP band, which shows negligible CAP activity, is found in all human sera and the other 2 CAP bands ($CAP_1$ and $CAP_2$), which have also LAP activity, appear only during pregnancy.




Polyacrylamide gel electrophoresis[10] of AP in normal sera, pregnancy sera, lysosomal extracts and microsomal extracts. The concentration of acrylamide monomer was adjusted to 6.5% solids. Electrophoresis was carried out at a constant current of 2 mA per tube for about 2 h. LAP activity was stained as follows; the gels were incubated at 37 °C for 2 h in 100 ml of 0.2 *M* sodium phosphate buffer (pH 6.8), containing 20 mg of L-leucyl-β-naphthylamide-HCl and 50 mg of Fast Blue BB. CAP activity was stained by the method of Kleiner and Brouet-Yager[6]. A) Neither heating nor inhibitor. LAP band of normal sera and pregnancy sera showed only LAP activity; the other bands both LAP and CAP activities. B) Each sample was heated at 60 °C for 30 min before electrophoresis. 2 CAP bands ($CAP_1$ and $CAP_2$) of both pregnancy sera and lysosomal extracts disappeared. C) The gels were stained in the presence of 0.02 *M* L-methionine. Serum LAP and microsomal bands disappeared.

Table I. Intracellular distribution of LAP and CAP activities in human placenta

| Fractions | Total activity (%) | |
|---|---|---|
| | LAP | CAP |
| Nuclear | 7.8 | 5.1 |
| Mitochondrial | 3.0 | 1.4 |
| Lysosomal | 21.3 | 14.9 |
| Microsomal | 16.6 | 11.5 |
| Supernatant | 51.3 | 67.1 |

*Materials and methods.* Homogenates of human placentae were separated into 5 fractions by the differential centrifugation according to the method of de Duve et al.[7]. LAP and CAP activities were determined essentially according to the method of Takenaka[8]. Lysosomal enzyme was obtained by freezing and thawing 10 times from the lysosomes prepared by the method of Ragab et al.[9]. Microsomal enzyme was solubilized by treating the microsomes with 5% sodium deoxycholate.

*Results and discussion.* The results for the intracellular distribution of LAP and CAP are given in Table I, which indicates the existence of 3 main sources of AP in human placentae; the lysosomal, microsomal, and supernatant fractions.

Figure (A) represents disc electrophoretic pattern of AP isozymes. Pregnancy sera (at term) displayed 3 distinct LAP bands. The fastest moving band (LAP band of Page et al.[5]), which showed no detectable CAP activity, was also present in normal non-pregnancy sera. The other 2 bands (the faster, $CAP_1$ and the slower, $CAP_2$), which had also CAP activity, were demonstrated in lysosomal extracts, too. Microsomal AP was stained as a single band with both LAP and CAP activities, which migrated at a location between the fast-moving LAP and $CAP_1$ bands.

Several enzymatic properties of serum AP including heat stability[11] and L-methionine inhibition[12] have been

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Table II. Effect of heat inactivation and L-methionine inhibition on the activity of LAP and CAP

| Samples | Remaining activity (%) | | | |
|---|---|---|---|---|
| | LAP | | CAP | |
| | Heating[a] | Methionine[b] | Heating[a] | Methionine[b] |
| Normal serum | 96.2 | 21.5 | — | — |
| Pregnancy serum | 32.0 | 65.8 | 8.6 | 77.6 |
| Lysosomal extract | 18.3 | 64.5 | 8.2 | 72.5 |
| Microsomal extract | 91.4 | 21.2 | 88.7 | 12.8 |

[a] 60 °C for 30 min. [b] 0.02 *M*.

reported. Our preliminary experiments revealed different rates of heat inactivation and L-methionine inhibition among serum and placental enzymes (Table II). Successively, we examined and compared effects of heating at 60 °C for 30 min as well as 0.02 *M* L-methionine on serum and placental AP isozymes with the use of disc electrophoresis. As can be seen in Figure (B) and (C), it is evident that normal serum LAP band and microsomal band were heat-stable and sensitive to L-methionine inhibition, while 2 CAP bands of pregnancy sera and lysosomal extracts were heat-labile and insensitive to inhibition by this amino acid.

RYDÉN[13] described the subcellular localization of CAP in human placenta. However, no experiments were performed on the characterization of properties of each enzyme. From the results obtained in our study, the 2 bands ($CAP_1$ and $CAP_2$) of pregnancy sera and the 2 lysosomal bands shared the same enzymatic characteristics, such as heat stability, L-methionine inhibition and electrophoretic pattern. This finding suggests that the increased AP in pregnancy sera may be derived from the lysosmes of placenta. These 2 lysosomal bands might represent 2 different conformational forms of a single enzyme, whose rate of migration is in a pH dependent equilibrium with each other, as discussed on retroplacental CAP by SJÖHOLM and YMAN[14]. The difference in the enzymatic properties between the lysosomal and the microsomal enzymes indicates the presence of multiple molecular forms of AP within a single tissue, i.e. the placenta. From the fact that LAP band in all human sera has practically no CAP activity, the normal serum LAP seems to be distinct from the placental enzymes.

The supernatant showed exactly the same electrophoretic behavior as the pregnancy serum. This observation is supposed to be due to the contamination of retroplacental blood, which contains a large amount of AP and in addition the soluble enzyme derived from ruptured lysosomes during fractionation procedures.

*Conclusions.* Evidences for the existence of 2 AP isozymes in human placenta were presented: the lysosomal and the microsomal isozymes, which are distinct from the normal serum LAP. The increased AP in pregnancy sera possessed the same enzymatic properties as the lysosomal enzyme in human placenta, with regard to heat resistence, sensitivity to L-methionine inhibition and electrophoretic pattern. These similarities suggest that the pregnancy serum AP may originate from the placental lysosomes.

*Zusammenfassung.* Charakterisierung zweier Arten von Aminopeptidase (AP)-Isozymen in den Lysosomen und Mikrosomen der menschlichen Plazenta. Die im Serum schwangerer Frauen auftretende AP hatte ähnliche Eigenschaften wie die Lysosomen-AP. Die Erhöhung der Aminopeptidase beruhte folglich auf dem Austritt lysosomaler Enzyme aus der Plazenta.

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## Stimulation of Phosphoenolpyruvate Carboxylase Activity in Rust-Infected Wheat Leaves

Rust infected wheat leaves retain chlorophyll at the periphery of uredosori in regions termed 'Green Islands'[1]. This area of the infected leaf is active metabolically and can be considered a 'sink' for all metabolites[2–4]. *Erysiphe graminis* infected wheat leaves showed no decline in photosynthetic activity per unit of chlorophyll[5]. Since the chlorophyll content of leaves is reduced by infection, it is only the overall photosynthetic activity that decreases[3, 5, 6]. Therefore it is entirely possible that the chlorophyll retained[7] or reformed[5], in the green islands may be more efficient in photosynthesis. Stimulation of the photosynthetic $CO_2$ uptake was reported in infected organs of bean and safflower prior to the sporulation of the fungus[8]. LIVINE speculated that the flow of carbon during the photosynthesis of the green island, follows a

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