thermal stability, metal ion effect and affinity towards some substrates (unpublished observations).

These results point to the fact that at least in some tissues the heterogeneity observed is not solely due to the presence of terminal sialic acid residue. It may be mentioned here that the intestinal alkaline phosphatase isoenzymes are devoid of sialic acid[16,17]. The possibility of minor differences in the protein architecture itself seems to be gaining ground.

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[18] Acknowledgements. We wish to record our sincere thanks to Sigma Chemical Company, U.S.A., for their generous gift of neuraminidase. We are also thankful to the Customs authorities, Madras, India, for releasing the gift sample without any procedural delay. The work was financed by U.S.P.L. No. 480.

Zusammenfassung. Es wurde gefunden, dass von den 3 Alkali-Phosphatase-isoenzymen nur das bei der Gelelektrophorese auf Polyacrylamid sich langsam bewegende Isoenzym ein Sialoprotein war. Die kinetischen Eigenschaften der normalen und der mit Neuraminidase bearbeiteten Enzyme waren ähnlich.

C. S. RAMADOSS, R. SELVAM,
K. RADHA SHANMUGASUNDARAM and
E. R. B. SHANMUGASUNDARAM[18]

University Biochemical Laboratories, A. C. College Campus, Madras – 600025; and
P.G. Institute of Basic Medical Sciences, Madras – 600005 (India),
7 September 1973.

Autolysis of *Coprinus comatus* Sporophores

The release of basiodiospores from species of the genus *Coprinus* is accompanied by a rapid autolysis of the cap of the fruiting body leaving only the stipe intact. This phenomenon has been examined in detail with respect to *Coprinus lagopus* (*C. cinereus*)[1,2]. From this work it was concluded that the main agents responsible for the autolysis of the sporophores of *C. lagopus* were chitinolytic enzymes located in the vacuoles of the fungus. These enzymes appeared shortly before the autolysis of the gills.

In view of the speed at which the autolysis of the gills of *Coprinus lagopus* occurs (4–5 h)[2], it is surprising that chitinases play such an important part in this process. Chitin is a substance notable for its resistance to degradation[3]. In Ascomycetes and Basidiomycetes it forms part of the wall, together with glucan, though it is usually present in smaller quantities than the latter[4]. ITEN and MATILE[2] assayed chitinase by measuring the release of reducing groups from purified *C. lagopus* walls, and chitobiase by the release of N-acetyl glucosamine from the same substrate. They found 30 times more reducing groups than N-acetyl glucosamine and interpreted this as meaning that chitinase was present in much greater quantities than chitobiase. The possiblity that the release of reducing groups was due to other carbohydrases (e.g. glucanase) does not seem to have been considered. As β-glucan is normally present as a prominent wall constituent of filamentous fungi[4], it is proposed that β-glucanase may have a significant part to play in the autolysis of *Coprinus* sporophores.

Enzyme activities detected in the autolysate of *Coprinus comatus* sporophores

Substrate	Enzyme	Activity/ml autolysate
Laminarin	β-(1 → 3) glucanase	1.30
Pustulan	β-(1 → 6) glucanase	0
Carboxymethyl Cellulose	β-(1 → 4) glucanase (cellulase)	0
Lichenin	β-(1 → 4), β-(1 → 3) glucanase	0.15
Colloidal Chitin	Chitinase	0.05
Azocoll	Protease	+

In order to test the validity of this idea, several sporophores of *Coprinus comatus* were collected from a wood near Montreux, Switzerland. Two of the best specimens, weighing 140 g each, were allowed to autolyse overnight at room temperature. After this time the sporophores were completely liquified. Microscopic examination showed the presence of spores and some fragments of hyphae, which were removed by centrifugation at 10,000 rpm for 30 min. The pellet was washed with water and the supernatant and washings were made up to 200 ml. This was tested for activity against the following substrates: laminarin and lichenin (Koch Light), pustulan and Azocoll (Calbiochem), carboxymethyl cellulose (Fluka) and colloidall chitin prepared from chitin (Koch Light) by the method of HOWARD and GLAZER[5]. All substrates except the Azocoll were dissolved or suspended in 0.05 *M* acetate buffer pH 5.0, and 10 ml were mixed with 1 ml of autolysate. The release of reducing groups from these substrates was followed at 30 °C by the NELSON SOMOGYI method[6], using the appropriate standard curve. One unit of activity was defined as that amount of enzyme which caused the release of 1 µMole reducing group/min. The Azocoll was used according to the manufacturer's instructions, though quantitative measurements could not be made due to interference from the colour of the autolysate. The results of these assays are shown in the Table.

No release of reducing groups was detected from pustulan or carboxymethyl cellulose, even after 24 h. The reducing groups liberated from lichenin must therefore have been due to the β-(1–3) linkages cleaved in this polymer. There appeared to be about 26 times as much β-(1–3) glucanase as chitinase in the 24 h autolysate, a figure similar to that of ITEN and MATILE[2] for the release of reducing groups and N-acetyl glucosamine from *Coprinus lagopus* walls. However, if the frozen residue was thawed, suspended in water and allowed to stand for a further 48 h, the ratio of β-(1–3) glucanase to chitinase dropped to 5 to 1. It thus appears that the β-(1–3)-glucanase plays a major role in the part of autolysis

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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*.

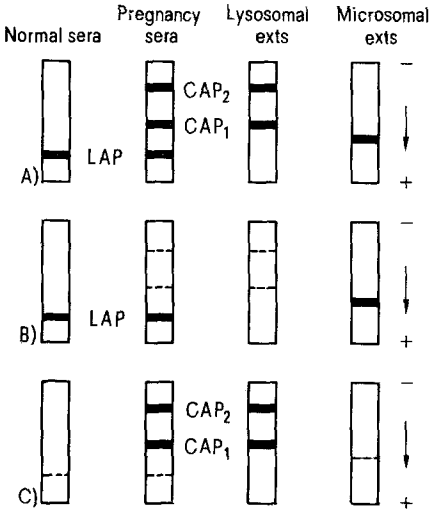
Résumé. Il parait évident que la β-(1–3)-glucanase est impliquée dans l'autolyse des sporophores du champignon *Coprinus comatus*.

D. A. Bush

Research and Development Department, Nestlé Products Technical Assistance Ltd., CH-1000 Lausanne (Switzerland), 21 March 1974.

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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