of UV-doses reaching maximum increases over spontaneous frequencies of 500 times for rifampicin and about 1000 times for nalidixic acid. The induced mutation frequencies in the *Proteus* strains remained low over the range of UV-doses; none of the frequencies was increased over the spontaneous frequencies by more than 15 times, and the average maximum increases for the 5 strains were 9.0 times for rifampicin and 7.7 times for nalidixic acid. It should be noted that lysogeny is reportedly common among *Proteus* strains[10]; however, no evidence for UV-induction of prophage was observed in any of these experiments. In contrast to UV-irradiation, nitrosoguanidine proved to be an effective mutagenic agent for *Proteus*. Exposure of these strains to nitrosoguanidine (25 or 50 µg/ml for 30 min at 37 °C) caused increases in mutation frequencies to rifampicin resistance that ranged from 300-to 3000-fold.

Recent investigations by Hofemeister et al.[11] indicate that resistance to UV-mutagenesis in *P. mirabilis* is caused by the absence of specific genetic components that, in other bacteria, are associated with error-prone repair of UV-induced lesions in DNA, and the results from our study suggest that this trait is a distinctive feature of all *Proteus* species. Because of significant physiological differences between the organisms in the *Proteus* group, their assignment to a single genus, as proposed in the latest edition of Bergey's Manual, is not uniformly accepted. The observation that these organisms all share the unusual characteristic of resistance to UV-mutability is, therefore, significant and could be a consideration in their classification.

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## Isozymes of cathepsin B1 in developing human placenta

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*Summary.* Cathepsin B1 was purified from human placentas of different gestational ages and analyzed by isoelectric focusing on polyacrylamide gel. The enzyme was shown to consist of 3 or 4 isozymes with pI values between 5.1 and 5.7.

Cathepsin B1 (EC 3.4.22.1), a lysosomal thiol-dependent proteinase, has been found in various mammalian tissues including human placenta[1,2] and fetal membranes[3]. In view of changes in the cathepsin B1 activity during the development of the placenta[4] and the presence of isozymes in chorion[3] it seemed worthwhile to investigate changes in the isozyme pattern in placentas from different gestational ages. The results are reported in this paper.

*Materials and methods.* Human placentas were obtained from the Institute of Obstetrics and Gynecology immediately after delivery. The placenta was washed with cold water to remove adherent blood, separated from fetal membranes and stored at −20 °C until preparation. Cathepsin B1 activity was assayed according to Barrett[5] with α-N-benzoyl-DL-arginine-β-naphthylamide hydrochloride as a substrate. Protein concentration was determined by the procedure of Lowry et al.[6]. Isoelectric focusing in polyacrylamide gel was performed at 4 °C according to Drysdale et al.[7]. Ampholine of pH 4–6 was used at the concentration of 2%. The cathepsin B1 activity was located as described by Barrett[8]. For the pH determination the gels were cut into 30 or more sections and immersed in 1 ml of distilled water. Purification of cathepsin B1 was carried out at 4 °C in the presence of 1 mM EDTA. Frozen human placenta was thawed and homogenized in 0.2 M acetate buffer, pH 4.3 (2 ml/g). The homogenate was incubated for 16 h at 37 °C. Early placentas (5–13 weeks) were centrifuged immediately after homogenization. In this case autolysis was not necessary because in the preliminary experiments no increase of the activity had been observed. After centrifugation the fraction precipitated between 0.4 and 0.7 ammonium sulphate saturation was dialyzed

Isoelectric points of cathepsin B1 isozymes during the development of the human placenta

| Weeks of gestation | Activity* of homogenate units/g wet tissue | Specific activity** after purification | Isoelectric points of isozymes | | | |
|---|---|---|---|---|---|---|
| | | | I | II | III | IV |
| 5–13, formation of placenta, n = 1 | 0.083 | 0.86 | 5.1 | 5.2 | *5.4* | |
| 21, growth and differentation, n = 1 | 0.045 | 0.64 | 5.1 | *5.2* | 5.4 | |
| 40, fully developed placenta, n = 3 | 0.034 | 0.40 | 5.1 | *5.2* | *5.4* | |
| 42, involution of placenta, n = 3 | 0.027 | 0.32 | 5.1 | 5.2 | *5.4* | 5.7 |

Main fractions are in italics. In the case where n > 1, the data are mean values. *Activity unit is expressed in µmoles of substrate hydrolyzed per min. **Specific activity was calculated in units per mg of protein.

against 0.05 M acetate buffer, pH 5.5 with 0.2 M NaCl and was then chromatographed on a Sephadex G-75 column equilibrated with the same buffer. The active fractions were pooled and run on a column of organomercurial-Sepharose under conditions employed by Barrett[8], then the cathepsin B1 preparation was rechromatographed on Sephadex G-75 column.

*Results and discussion.* As can be seen in the table the activity of homogenates from early placentas was higher than the activities of term and post-term homogenates, respectively. Samples of purified enzyme (about 0.03 units) were run in isoelectric focusing gels. The distribution of activity was demonstrated and the pI determined. Present data (table) indicate the presence of 3 forms of cathepsin B1 in the developing or mature placenta and 4 in the involuting one. The forms differ in isoelectric points, the pI-values ranging from 5.1 to 5.7. Cathepsin B1 isolated by Evans and Etherington[2] from human placenta had a pI of 5.4. The authors have not detected isozymes on isoelectric focusing of the crude samples of the placental enzyme.

Previously we demonstrated 3 isozymes of cathepsin B1 in human fetal placenta membranes and chorion, and only 1 isozyme in amnion[3]. The nature of the microheterogeneity of cathepsin B1 is not clear at this time. Takahashi et al.[9] suggested that the charge difference between isozymes may be the result of deamidation and the forms of cathepsin B may represent a part of the activation and degradation of the enzyme. On the other hand, Tawatari et al.[10] working with the crystalline cathepsin B1 from rat liver ascribed this microheterogeneity to the changes in the carbohydrate content during the solubilization step. Changes in the cathepsin B1 activity as well as the variety in isozyme pattern may reflect the alteration in the metabolism of the developing placenta. Early in gestation the placenta carries on many metabolic functions that are later taken up by the fetal organs. The endopeptidase activity of cathepsin B1 is well established[11], and peptidylodipeptidase activity against glucagon[12] and fructose-1,6-bisphosphate aldolase[13] has recently been observed.


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## Microdensitometric analysis of denervation effects on newt limb blastema cells


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*Summary.* This report examines the fate of cycling cells in normal and denervated blastemas of adult newts. Cells are found to accumulate in $G_1$ in blastemas which are nerve independent. No stage specific accumulation different from controls is found in limbs with nerve-dependent blastemas.


Regeneration of an amphibian limb is dependent upon nerves for its completion[1]. Early phases of dedifferentiation and initiation of DNA synthesis occur in the absence of nerves, but normal mitotic activity, blastema formation, and growth fail to occur in denervated limbs[2,3]. Limbs are considered to be nerve-dependent until 15 days after amputation (8 days for larvae)[4,5], yet are generally capable of producing morphologically normal but smaller regenerates following denervation at 17 days post-amputation; i.e., become nerve-independent. Therefore, the period from 15-17 days has been termed the transition period[4] when the limb switches from an absolute to a conditional dependence on innervation. The fate of cells in nerve-dependent blastemas after denervation is unclear. Tassava and Mescher[6] proposed a model suggesting that the nerve facilitates passage of dedifferentiated cells through the $G_2$ phase of the cell cycle and allows them to divide in M. This hypothesis suggests that following denervation cells might accumulate in $G_2$. Mescher and Tassava[2] were unable to demonstrate the presence of such cells in sections of blastemas using microspectrophotometric techniques. Recently, Maden[7] has reported that cells in denervated axolotl limbs may block in $G_1$. These alternative results are clouded by differences in experimental methodology which seriously affect their interpretation. In order to reconcile the differences between these authors, we undertook a detailed search, using microdensitometry, for discrete populations of cells in amputated, denervated limbs of adult newts which might differ from those found in amputated, normally regenerating limbs.

One of the differences in previous experimental methodologies is that Maden measured whole nuclei in dispersed preparations while Mescher and Tassava measured nuclei in sectioned material. A more important factor is that Maden denervated well developed blastemas which may have been nerve-independent. Mescher and Tassava, on the other hand, did denervations at the time of amputation, before blastema formation, when the limbs were clearly nerve-dependent.

Right and left limbs of adult newts were amputated midway between the wrist and the elbow. In 1 series of experiments left limbs were denervated at 15 days post-amputation, when blastemas had reached the early bud stage. In a 2nd, left limbs were denervated at 19-21 days post-amputation, when regenerates had reached the late bud stage[1] (comparable to the cone stage referred to in the