mental interference in the function of chondrocytes and osteocytes rather than by their mere deposition in bone.

Zusammenfassung. Nachweis, dass hohe Strontiumkonzentrationen die Kalzifikation der Knochen neugeborener Mäuse und diejenige in Knochengewebskulturen hemmen. Im Gegensatz dazu und zu anderen knochenaffinen Metallen wirkt Strontiumnitrat in Dosen von 25, 50, 100 and 200 mg/kg, verabreicht während des 9. bis 19. Trächtigkeitstages, bei Ratten nicht teratogen.

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Esterases in the Hearts, Lungs and Livers of Human Foetuses Before Mid-Term

BLANCO and ZINKHAM[1] have presented the non-specific esterase zymograms of various human tissues including some from foetal specimens of advanced gestational age (5 months and onwards). All tissues contained multiple bands of esterase activity in patterns which increased in complexity as development proceeded. Variations in pattern between different tissues were observed at all ages and their findings are comparable with those of MARKERT and HUNTER[2] who studied the mouse. However, PAUL and FOTTRELL[3] were unable to demonstrate any differences between the esterases in human tissues of either adult or foetal origin.

This paper presents evidence for the existence of esterases in much younger human foetuses and demonstrates the changes that take place in the period of gestation prior to that examined by BLANCO and ZINKHAM.

Ten normal human foetuses obtained at hysterotomy were used in this study. Their crown rump lengths varied between 40 and 160 mm. Both lyo and desmo tissue extracts were prepared as described earlier[4]. Starch gel electrophoresis of all specimens was carried out at pH 7.5 with a horizontal run at 4°C for 4 h employing a constant current with an average voltage drop of 12 volts/cm.

Simultaneous coupling azo dye reactions were performed at pH 7.5 using α-naphthyl acetate and proprionate as substrates (0.25 mg/ml) with Fast Blue 2B (1 mg/ml) as the diazonium salt in each case. The effects of the inhibitors eserine and E600 (Diethyl 4-nitro phenylphosphate) were both studied at molar concentrations of $1 \times 10^{-5} M$. Mipafox [*bis* (isopropylamine) phosphinocofluoridate] was employed at $2 \times 10^{-5} M$.

Esteraseswere demonstrated in all the specimens so examined. The desmo fraction exhibited fewer and less prominent bands than the lyo fraction. However, all these 'desmo' bands were represented in the 'lyo' zymogram and responded similarly to inhibition. The progression in complexity may be divided into 2 stages, firstly at 40–80 mm and secondly, from 100 to 160 mm crown rump length. At the 40 mm stage each of the 3 organs studied has a quite specific zymogram pattern (Figures 1, 2 and 3). With α-naphthyl acetate as substrate a total of 4 isozymes are present (A, B, C and D). Some of the isozymes are sensitive to treatment with E600 (Figure 1). The response of any one band to inhibition may, however, differ from one organ to the next, for example, the bottom band C of activity in the lung (Figure 3) as visualized with the α-naphthyl acetate substrate shows no inhibition by E600. In contrast, the equivalent band C in the liver (Figure 2) is completely inhibited by this compound using the same substrate.

Three isozymes A, B and C are found when the α-naphthyl proprionate substrate is employed. The two most slowly migrating bands A and B are both insensitive to E600 in all 3 tissues whereas the bottom band C is consistently inhibited. An individual band may however manifest differential sensitivity to E600 with different substrates. The bottom band C in the lung (Figure 3) is not inhibited by E600 using α-naphthyl acetate but is inhibited when the

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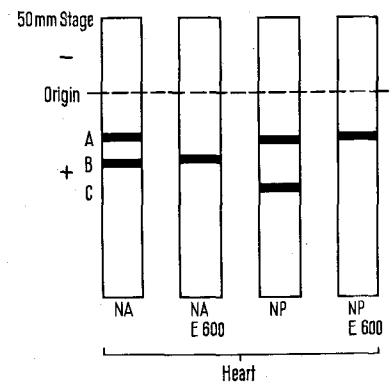

Fig. 1. 50 mm Human heart. Zymogram of 'lyo' extract showing 3 isozymes A, B and C. α-naphthyl acetate (NA) and propionate (NP) were employed as substrates.

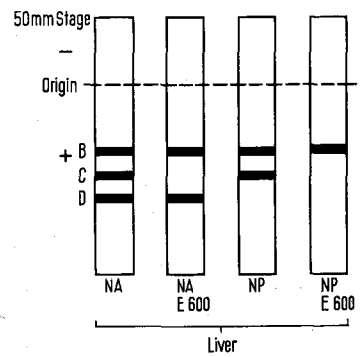

Fig. 2. 50 mm Human liver. Zymogram of 'lyo' extract containing 3 isozymes B, C and D.

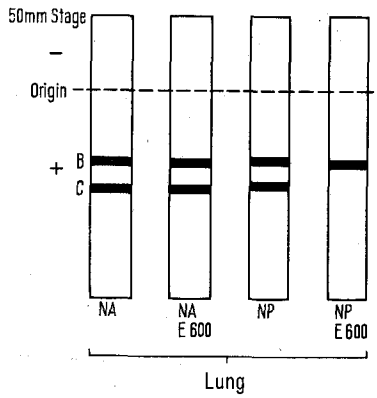

Fig. 3. 50 mm Human lung. Zymograms of 'lyo' extract reveals 2 isozymes B and C. Each is present in the heart, liver and lung at this stage although considerable variation is found in their substrate preference and response to E600 inhibition.

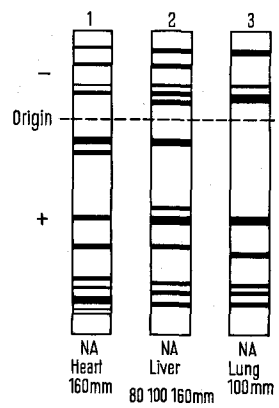

Fig. 4. Zymograms of heart, liver and lung at the stages indicated. Multiple bands of activity are seen with the α-naphthyl acetate substrate.

proprionate substrate is employed. Nevertheless in the liver (Figure 2) the same band C, electrophoretically identical, is totally inhibited using either substrate. The reasons for such differential inhibition have been the source of considerable speculation (CHOUDHURY and LUNDY[5]). Although this phenomenon has been described in both the adult rat and rabbit, BLANCO and ZINKHAM[1] noted that the bands they found in human tissues showed a remarkable similarity in enzymic activity and in sensitivity to inhibitors.

The period between the 100 and 160 mm crown rump length stages is one of intense activity so far as the development of a multiplicity of esterase enzymes is concerned (Figure 4). For the first time bands which migrate cathodally are observed. In all 3 tissues these cathodal bands but no others are totally inhibited by MIPAFOX at $2 \times 10^{-5} M$. At the same time, no band in any tissue appears to be affected by eserine at $1 \times 10^{-5} M$ or indeed at any concentration up to $1 \times 10^{-2} M$. This is not in accord with the findings of BLANCO and ZINKHAM who consistently showed deletion of bands using eserine at $1 \times 10^{-4} M$ at a pH of 7.3.

Cholinesterases are known to be present in several organs in the human foetus from the 28 mm stage onwards[4,6,7]. However, bands of cardiac cholinesterase activity of both true and pseudo types demonstrated by the method of KARNOVSKY and ROOTS do not coincide in position with any band of activity seen after incubation using α-naphthyl ester substrates[4].

If, therefore, both the observations described above and those of BLANCO and ZINKHAM are valid, the apparent contrast in reactivity and sensitivity to inhibition can only be explained on the basis of some change having taken place in the enzymes themselves. Presumably, cholinesterases initially exhibit highly selective activity with respect to possible substrates and lose this specificity as development continues.

The zymograms from the most advanced foetus are complex (Figure 4), more so than in the youngest such studied by BLANCO and his colleague. Further examination of the whole series reveals that certain bands of activity disappear in the later specimens. This is seen, for example, in the lung (Figure 3) where the only bands present at the 50 mm crown rump length stage (B and C) are both deleted by the 100 mm stage. Recently O'HARE, NEWMAN, VATTER, and REISS[8] have made similar observations in their study of the esterases in the developing rat lung.

From a developmental viewpoint, it might be expected that those enzymes essential for fundamental foetal metabolic pathways would be the ones demonstrable during the early stages of gestation. The isozymes described at the 40 and 50 mm stages would come within this basic category. As development proceeds, more complex isozymes appear and those which sufficed at earlier stages gradually disappear.

It has been suggested that the non-specific esterases may be implicated in the control of tissue growth factors[9] and O'HARE et al.[8] have commented on the intimate relationship between biochemical and morphological changes in the developing rat lung.

The esterases are a very complicated family having in many cases a multisubstrate reactivity and a wide range of sensitivity to various organophosphorous poisons. Some groups of enzymes such as the lactic dehydrogenases have been extensively studied during development but the esterases have been relatively neglected. Some of the important features noted during this preliminary study of the non-specific esterase patterns have been emphasized. Further work is proceeding to elucidate these problems and the study is being extended to other tissues and beyond mid-term to include the whole period of gestation.

Zusammenfassung. Verschiedene Fraktionen von Esterasen in embryonalem Gewebe und enzymatische Reifungsprozesse während der Ontogenese werden beschrieben.

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[10] I wish to thank Professor G. A. G. MITCHELL, Dr. S. R. CHOUDHURY and Mr. A. M. LUNDY for their help and advice.