Hypophysectomized and thyroidectomized male rats of the inbred August strain, weighing 200 g were treated for 15 days with daily injections of thioacetamide (10 mg/100 g of body weight). The animals were then killed and their livers removed for histological examination. Intact control animals were also treated as above and examined within the same period.

Figure 1 shows the uniform distribution of glycogen in the liver parenchyma of a normal, well fed rat. Figure 2 shows the typical glycogen distribution pattern in a TA-treated liver of an intact animal. There is complete disappearance of glycogen from the parenchymal cells around the central veins.

Hypophysectomized (Fig. 3) or thyroidectomized (Fig. 4) rats, when treated with TA for 15 days, failed to show centrolobular glycogen depletion.

These histological observations clearly indicate that changes in the animal hormonal imbalance influence not only the terminal stages of drug-induced liver lesions, as has been demonstrated by others, but also prevent the early manifestations of the treatment, like centrolobular glycogen depletion.

The fact that thyroidectomy alone can suppress the TA-glycogen effect seems to indicate that thyroxin is the key hormone involved in the complex mechanism responsible for such effect. Further investigations will attempt to clarify this point.

Zusammenfassung. Die Verarmung von Glykogen in der Leber als Ausdruck der toxischen Wirkung von Thioacetamid kann durch Hypophysektomie oder Thyroidektomie verhindert werden.

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Leucine Aminopeptidase in the Foetal Kidney of the Rat

Leucine aminopeptidase is a widely distributed enzyme, taking part in protein degradation and perhaps also in synthesis. Its existence in large amounts in the kidney of numerous animal species has long been described. Histochemically it was localized by NACHLAS et al.[1] (1957) using *L*-leucyl-2-naphtylamide, and later (1960) using *L*-leucyl-4-methoxy-2-naphthylamide as substrate[2].

Leucine aminopeptidase of the adult rat kidney is noted to be most active in the juxtamedullary portion of the cortex, while the outer cortical zone is less active and the medulla is negative. The presence and distribution of leucine aminopeptidase in the foetal kidney of the rat had not been studied and was the object of the present investigation. The enzyme was demonstrated by the method of NACHLAS et al. as published in 1957. Altogether 30 kidneys of foetal and young rats were studied.

Figure 1 shows a section from the kidney of an 18-day embryo, the incubation time being 10 min. The cortex is still narrow and almost completely without enzymatic activity. Here and there, near the juxtamedullary portion, however, some groups of the tubuli have a faint enzymatic activity. Figure 2 shows a section from the kidney of a small rat, 4 days after delivery. Its enzymatic activity is clearly very much greater and localized over the greatest part of the narrow cortex. To make a comparison possible, Figure 3 shows a section from the kidney of a 2 months old rat after a similar staining procedure. It shows a high activity in the juxtamedullary part of the cortex and some staining also in the outer tubular portions of the cortex. The white spots represent the localization of the glomerula.

[1] M. M. NACHLAS, D. T. CRAWFORD, and A. M. SELIGMAN, J. Histochem. *5*, 264 (1957).

[2] M. M. NACHLAS, B. MONIS, D. ROSENBLATT, and A. M. SELIGMAN, J. biophysic. biochem. Cytol. *7*, 261 (1960).

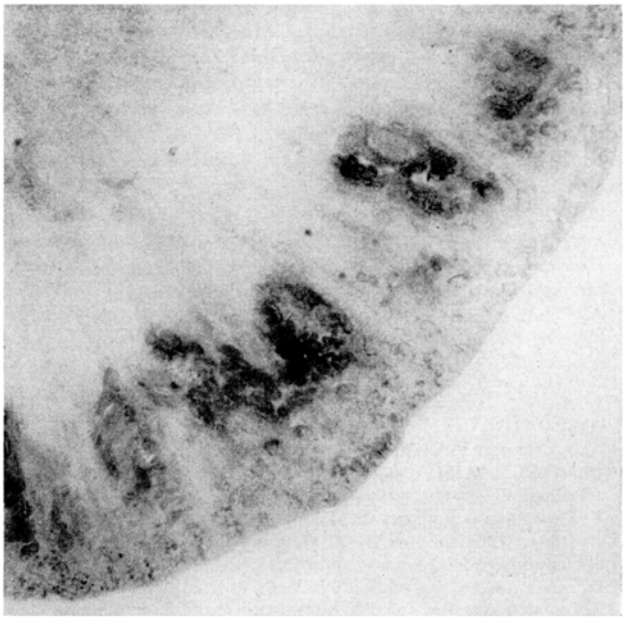

Fig. 1

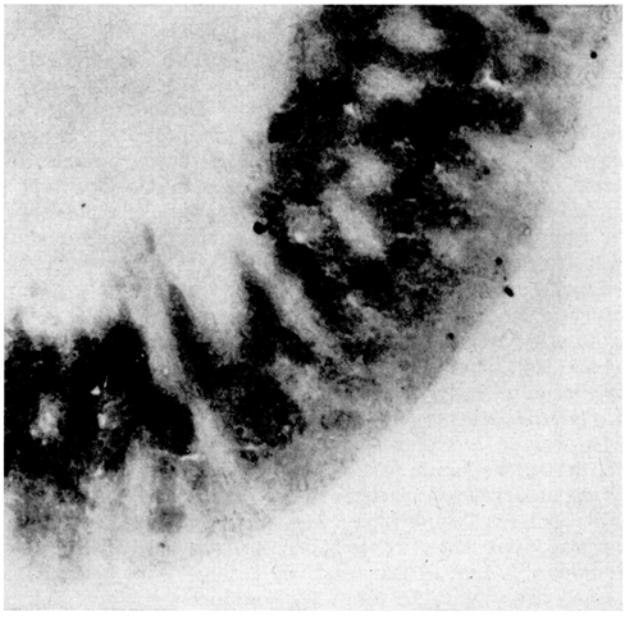

Fig. 2

These Figures show that the activity of leucine aminopeptidase in the kidney is very small during the foetal period of life. Further, they show that the occurence of leucine aminopeptidase happens regionally so that some few single, widely distributed tubular groups first show a moderate enzymatic activity, while the other parts of the cortex and the medulla still are fully negative. A high increase in the enzymatic activity occurs after delivery.

The observations made are interesting from the point of view of the foetal function of the kidney. Numerous investigations have shown that the function of the foetal kidney begins at a very early stage of pregnancy. One of the functions of the kidney during foetal life is supposed to be to participate in producing foetal fluid[3]. Our figures could be interpreted to show that in a rather late stage of pregnancy only very few single nefrons are capable of functioning where the presence of leucine aminopeptidase is needed and are thus competent for full function from this point of view. The tubuli of these nefrons are also shown to contain the enzyme in very low concentration, which may indicate that the function of these nefrons also cannot be intense.

The activity of leucine aminopeptidase increased soon after delivery, which is in good agreement with the earlier observations that the functional capacity of the kidney increases very intensely during the first days after delivery.

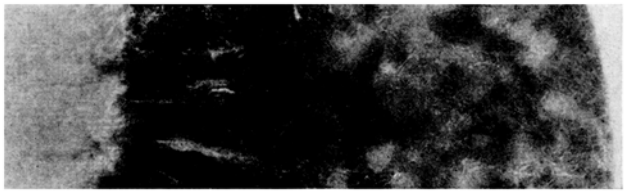

Fig. 3

Zusammenfassung. Histochemisch demonstrierbare Leucinaminopeptidase erschien in den letzten Tagen der Schwangerschaft in einzelnen Gruppen von Tubuli der Rinde der fötalen Rattenniere. Der grösste Teil der Rinde und das Mark erwiesen sich als negativ. Nach der Geburt verbreitete sich das Ferment über die ganze Rinde und vermehrte seine Aktivität stark.

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Department of Anatomy, University of Turku (Finland), January 24, 1961.

[3] R. E. SHAW and H. J. MARRIOT, J. Obstetr. Gynaec. *56*, 1004 (1949).

Increased Serum Diphenylamine Reaction in Patients with Leukemia

Normal human serum contains a variety of carbohydrate-reacting material some of which is tightly bound to peptide and protein to form the so-called mucosaccharide fraction. This mucosaccharide fraction can be estimated by a number of colorimetric reactions based on the sugar component; a comprehensive list of these reactions is given by BYWATERS and GLYNN[1]. In particular, the diphenylamine reaction[2] has often been used for obtaining an overall value of the level of mucosaccharides in serum[3-6].

Previous work on the diphenylamine reaction of human serum has shown that there is a considerable increase in the reacting component in the serum of patients with various types of malignant disease, acute rheumatoid arthritis and tuberculosis. Although NIAZI and STATE[4] included a considerable variety of malignant conditions in their study they did not differentiate between the conditions in listing the results; all their malignancy values were grouped together under one heading. Their investigation included one leukemic patient. We thought it worth while to study leukemia in more detail in this connection when serum from leukemic patients was made available to us by the courtesy of Dr. PRANKERD and of the Radiotherapy Department, U.C.H. Since this work was started a value for the *N*-acetyl neuraminic acid content of leukemic serum has been published[7] although as yet no details of the method used etc. are available to us.

Procedure. 5 ml of blood were mixed with 0.2 ml of 5% versene and then centrifuged to remove all cellular elements. 0.5 ml of the resulting serum[8] were heated for 15 min at 90° with 9.5 ml of 5% trichloroacetic acid. After cooling the mixture was filtered and the extract was used for estimating the mucosaccharide component as follows:

2 ml extract were boiled for 1 h with 4 ml of diphenylamine reagent[2] (1 g diphenylamine in 98 ml glacial pure acetic acid and 2 ml pure concentrated sulphuric acid). After cooling the absorption spectrum was determined using a Unicam-SP-500 spectrophotometer; the region covered was 360–700 mμ and the tubes were always read against a heated trichloroacetic acid blank boiled for the same period of time.

In agreement with earlier work[4], it was found that the cold TCA-soluble fraction of serum gave little absorption at 530 mμ on boiling with diphenylamine reagent. This indicates that the majority of the material producing the peak at 530 mμ is bound to protein and is extracted by hot TCA. Figure 1 shows the absorption spectra obtained by boiling hot TCA extracts of red blood cells, leucocytes and serum with diphenylamine reagent for 1 h. It can be seen that only the serum extract shows a marked 530 mμ peak.

[1] E. G. L. BYWATERS and L. E. GLYNN, in *Biochemical Disorders in Human Disease* (Ed. R. H. S. THOMPSON and E. J. KING, J. & A. Churchill Ltd., 1957), p. 634.

[2] Z. DISCHE, in *The Nucleic Acids* (Ed. E. CHARGAFF and J. N. DAVIDSON, Academic Press Inc., New York 1955), Vol. 1.

[3] N. W. PIRIE, Brit. J. exp. Path. *17*, 269 (1936).

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[5] W. AYALA, L. V. MOORE, and E. L. HESS, J. clin. Invest. *30*, 781 (1951).

[6] A. F. COBURN, L. V. MOORE, and J. HANINGER, Arch. int. Med. *92*, 185 (1953). – G. R. FEARNLEY, J. PIRKIS, N. DE COEK, R. LACKNER, and R. I. MEANOCK, Ann. Rheum. Dis. *14*, 226 (1955). – E. CECCHI and F. FERRARIS, Ann. Rheum. Dis. *14*, 267 (1955).

[7] P. BÖHM (1958) quoted by A. Gottschalk, in *The Chemistry and Biology of the Sialic Acids* (Cambridge University Press 1960), p. 93.

[8] In accordance with common practice this fraction is referred to as serum although the blood had been prevented from clotting. The sialic acid content of fibrin (and haemoglobin) is negligible compared to the serum level[7].