

trinsic capacity as judged from the shrinking-drop technique after the manœuvres leading to renal hyperaemia. On the contrary, the transit time and consequently the percent reabsorption decreased. From this point of view, the decrease of the fractional reabsorption due to simple body overheating is of the greatest importance, since the possible direct effects of the administered drugs on tubular reabsorption were thus excluded. The results of these preliminary experiments are in agreement with the above mentioned hypothesis that renal hyperaemia leads to a decrease of proximal tubular reabsorption.

In the second series of experiments rats were given an i.v. infusion of an isotonic solution containing 25 mM NaHCO₃ and 110 mM NaCl/l at a constant rate of 0.31 ml/min. After 60–90 min, the intrinsic reabsorption capacity, as measured by the shrinking-drop technique, was clearly reduced and the transit time in the proximal tubule was clearly shortened; this caused a decrease in the fractional reabsorption rate of about 46%. These findings entirely confirm analogous results published by RECTOR et al.². STARLING¹¹ already presented the conception that the mechanism of polyuria following an isotonic saline infusion is based on the fall of the tubular reabsorption in consequence of the decrease of the oncotic pressure of the plasma proteins; he called this polyuria 'dilution diuresis'. Since that time much contradictory evidence has been accumulated; in some reports an increase of tubular reabsorption after albumin administration was found^{12–14}, in others polyuria and natriuresis or no change at all after albumin infusion was described^{15–18}. In order to clarify this question, human serum albumin was added to the isotonic bicarbonate-saline infusion in such amounts that the total concentration of plasma proteins measured by the biuret method¹⁹ did not differ from the level of plasma proteins in non-infused control rats. In these rats the intrinsic reabsorptive capacity was constantly significantly decreased, as in rats after isotonic bicarbonate-saline infusion, but the transit time in the proximal tubule did not differ from the value found in the control non-infused rats. Calculated fractional proximal

reabsorption was then decreased but to a lesser extent than in rats after bicarbonate-saline infusion only. The cause of the decrease of the intrinsic reabsorptive capacity following these infusions is not clear; the easiest explanation appears to be the effect of the hypothetical 'natriuretic' factor postulated by DE WARDENER et al.²⁰.

Zusammenfassung. Die Resorptionsfähigkeit (RF) des proximalen Konvolutes der Rattenniere wurde mit der Methode der getrennten Ölsäure und die Passagezeit (PZ) der Tubuliflüssigkeit mit Lissamingrün gemessen. Aus beiden Werten konnte die prozentuale Resorption (PR) des proximalen Konvolutes berechnet werden. Mittels künstlich gesetzter Nierenhyperämie und nach Infusionen isotoner NaCl-Bikarbonat-Lösung (mit und ohne Albumin) wurden die Veränderungen von RF, PZ und PR geprüft.

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Lactic Dehydrogenase Isoenzymes in Various Cell Types of Mouse Liver

Lactic dehydrogenase (LDH; EC 1.1.1.27) has been shown to exist in at least 5 molecular forms or isoenzymes demonstrable by electrophoresis. The explanation for this has been forwarded by work from several laboratories^{1–4}. The enzyme is a tetramer molecule composed of a random combination of the 2 different polypeptides, A and B, which accounts for the characteristics of the 5 lactic dehydrogenase isoenzymes (AAAA, AAAB, AABB, ABBB and BBBB). The synthesis of the A and B subunits is controlled by 2 different genes and the resulting combination into the complete enzyme molecule occurs according to statistical laws giving a binomial distribution of the 5 isoenzymes in any one cell. Thus provided that a and b represent the relative activities of the 2 genes and that $a + b = 1$ the proportions of the 5 isoenzymes should be $a^4:4a^3b:6a^2b^2:4ab^3:b^4$. However such random distribution is only found in pure cell lines⁵ and is consequently obtained when analyzing the cell types of the blood⁶ and approximately so when analysing isolated regions of an organ⁷.

Thus the isoenzyme patterns of organ extracts from, for example, liver or kidney represent a mixed pattern from various cell types constituting the organ. Little work has been done on the combined LDH-activities of different cell types of solid organs by separating the different cells. While studying the possible isoenzyme content of the nuclei of liver cells, we managed to effect a separation into connective tissue cells and 'pure liver cells'.

In each experiment 4 or 5 mice were killed by decapitation, the livers were quickly removed and cut into small

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Sulphate content and activities of the different LDH-isoenzymes in various fractions of mouse liver

Fraction	Sulphate % of dry weight	LDH-isoenzyme number as % of total LDH-activity					Cathodally migrating band (see ¹⁰)
		1	2	3	4	5	
Whole liver	0.12 ± 0.005	0.36 ± 0.1	1.4 ± 0.3	3.0 ± 0.5	4.4 ± 0.5	90.7 ± 7.0	0.12 ± 0.06
Liver with connective tissues removed	0.096 ± 0.002	0.2 ± 0.08	0.31 ± 0.1	0.34 ± 0.1	1.8 ± 0.5	95.6 ± 8.0	1.7 ± 0.05
'Connective tissue'	0.83 ± 0.01	7.2 ± 1.0	7.5 ± 1.0	9.0 ± 4.0	20.0 ± 4.0	58.0 ± 5.0	

pieces. The connective tissues were removed by pressing the whole livers through a nylon gauze. The fractions were then separately freeze-dried for 60 h under a pressure of approximately 10 mm Hg, and were then analysed for their sulphate content according to the method of WAINER and KOCH⁸ after hydrolysis with formic acid. The amount of sulphate in each tissue was taken as an approximate measure of the content of connective tissue. The LDH-isoenzymes of the different fractions were separated by starch electrophoresis and were rendered visible and evaluated as described by KARLSSON and KJELLBERG⁹.

The sulphate content (the content of connective tissues) and the relative activities of the various liver fractions are shown in the Table. A marked diminishing in the activity of LDH-5 was noted, accompanied by an increase in LDH1, 2, 3 and 4 as the fraction becomes more dominated by the connective tissues. The sixth, cathodally migrating band, as described by AGRELL and KJELLBERG¹⁰ shows no correlation with the sulphate content of the material and will not be considered further here. In an attempt to evaluate the total activity of LDH in the various fractions, no significant difference was found.

From the results related it seems likely that the liver cells show only LDH-5 activity while the other tissues contribute to all 5 LDH-isoenzymes. The material also suggests that the 'connective tissues' in this report is not

a separate entity but contains material of several cell types, as the values do not approximate to a binomial distribution. This heterogeneity is, however, to be expected as the fraction contains not only the connective tissue cells but also other cells e.g. cells from blood vessels.

Zusammenfassung. Mit einer einfachen Methode wird demonstriert, dass Lebergewebe von Mäusen in 2 Fraktionen verschiedener Menge an Bindegewebe zu trennen ist. Die an Bindegewebe reiche Fraktion zeigt Enzymaktivität in LDH 1, 2, 3, 4 und 5. Nach Entfernung des Bindegewebes wird die Aktivität der LDH 1, 2, 3 und 4 Isoenzyme des restierenden Lebergewebes reduziert.

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Some Aspects of the Hydrolysis of Phosphomonoesters by Homogenates of Growing and Regressing Mesonephroi in Chick Embryos¹

Several investigators have obtained results which indicate that acid phenylphosphatase and acid β -glycerophosphatase activities are associated with different enzyme molecules. In a study of the hydrolysis of different phosphomonoesters by the microsomal fraction of rat liver preincubated at 37°C at pH 5, BEAUFAY and DE DUVE² concluded that the enzyme glucose-6-phosphatase acts upon phenylphosphate, but is unreactive towards β -glycerophosphate. A study with homogenates of rat liver and spleen by MACDONALD³ led that author to believe that an enzyme other than glucose-6-phosphatase is responsible for the hydrolysis of phenylphosphate. He compared the activities of glucose-6-phosphatase, acid phenylphosphatase, and acid β -glycerophosphatase, and examined their stability after preincubation at 37°C at pH 5 for 1 h. As a result of his investigation, he suggested

that each of the above enzymic activities is attributed to a different enzyme molecule. The study reported here on homogenates of growing and regressing chick mesonephroi indicates that acid phenylphosphatase and acid β -glycerophosphatase activities are associated with the same enzyme molecule.

The embryonic kidneys were homogenized in ice-cold distilled water, and then diluted with sodium acetate buffer (pH 5) to give 0.4% homogenates. The enzymic activities of the untreated homogenates and homogenates preincubated for 1 h at 37°C were then determined. The assay employed for β -glycerophosphatase activity was based on that described by FISKE and SUBBAROW⁴; that

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