On adding to the sera 10 m*M* α-ketoglutarate, a similar difference was found between the groups studied as in the case of lactate.

Discussion. It is evident from the results of these experiments that either the transmembrane transfer of glucose, or glucose phosphorylation by hexokinase are the sites of inhibition of glucose utilization in the diaphragm incubated in the uremic serum. Though it was not examined, it seems more probable that the site of metabolic block is on the level of transmembrane transfer, as there are evidences that patients with renal insufficiency react poorly to the applied insulin[2–4]. Whatever the exact mechanism, there is no doubt that the carbohydrate metabolism of muscle incubated in uremic serum is abnormal.

Diaphragm compensates decrease in the utilization of glucose by an increased utilization of other substrates and so ensures an unaltered oxygen consumption. This finding corresponds also to the in vivo conditions, as it was shown that the basal metabolic rate of patients with renal insufficiency is not significantly altered[8].

THÖLEN et al.[9,10] found in patients with renal insufficiency also inhibition of pyruvate oxidation with the secondary increased formation of acetoin. The results with kidney cortex slices incubated in uremic sera[11] are in accordance with this view. An increased concentration of some acids of citric acid cycle in the blood of uremic patients testifies to the inhibition of oxidative processes[12]. On the basis of the experiments presented, the block on this level in the muscle is improbable. However, it is a question whether this block is not valid only in some organs, i.e. liver and kidney, which have a suitable cell structure as well as active transport processes for the uptake of these substrates.

Zusammenfassung. Das Serum von Uremikern inhibiert die Glukoseutilisation im Rattendiaphragma. Der Sauerstoffverbrauch ändert sich nicht trotz niedrigerer Glukoseutilisation, da dieselbe durch eine erhöhte Auswertung anderer Substrate im Diaphragma kompensiert wird. Ein erhöhter Glykogen-, Laktat- und α-ketoglutarat-Verbrauch konnte bewiesen werden.

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28th April 1967.

[8] S. FANKHAUSER, Schweiz. med. Wschr. *96*, 243 (1966).
[9] H. THÖLEN, F. BIGLER and H. STAUB, Experientia *17*, 359 (1961).
[10] H. THÖLEN and F. BIGLER, Schweiz. med. Wschr. *94*, 65 (1964).
[11] D. RENNER and R. HEINZ, Klin. Wschr. *44*, 1204 (1966).
[12] R. NORDMANN, M. ARNAUD and J. NORDMANN, Clinica chim. Acta *12*, 304 (1965).

The Role of Renal Hyperaemia and Plasma Oncotic Pressure in Proximal Tubular Reabsorption in the Rat

In 1966 EARLEY and FRIEDLER[1] postulated an important role of renal vascular resistance in the regulation of sodium reabsorption. They expressed the idea, which was later worked out in more detail by RECTOR et al.[2], that there is an inverse relationship between the capillary blood volume and tubular volume: tubular volume decreases during vasodilatation and vice versa. Since RECTOR et al.[3] showed that proximal tubular reabsorption is a function of tubular volume – as had been presumed by GERTZ[4] – and since further experiments existed indicating that renal hyperaemia was followed by natriuresis[5,6,1], we measured the tubular flow rate and the intrinsic reabsorptive capacity in the proximal tubule of the rat after the administration of acetylcholine, bradykinin and Pyrogen and after total body heating; these maneuvres are known to produce renal hyperaemia. The transit time of the fluid in the proximal tubule was measured with Lissamine green according to STEINHAUSEN's method[7], modified by GERTZ et al.[8]. The intrinsic reabsorptive capacity was estimated by means of the shrinking-drop technique as described by GERTZ[9]; in this method the half-time of the intratubular shrinkage of an isotonic saline drop injected between two oil drops is measured. The original method of GERTZ based on photographic recording of drop-shrinkage was replaced by direct measurements with an ocular micrometer. Fractional reabsorption in the proximal tubule was calculated according to the equation of BRUNNER, RECTOR and SELDIN[10] originally introduced by GERTZ et al.[8]:

$$\%\ \text{reabsorption} = \left(1 - \frac{1}{\text{antilog}\,(0.301\ T/t_{1/2})}\right) \cdot 100$$

where T is transit time of Lissamine green in the proximal tubule, and $t_1/_2$ is half-time of the drop shrinkage. As is obvious from the Table, no change was found in the in-

	IRC	TT	FR
Controls	9.10 ± 1.37	9.00 ± 1.10	49.6
Pyrogen	9.31 ± 0.95	6.89 ± 0.48	40.0
Acetylcholine	9.88 ± 1.45	6.50 ± 0.31	36.4
Bradykinin	9.81 ± 1.17	7.48 ± 0.25	40.9
Overheating	9.40 ± 1.11	6.24 ± 0.52	36.6
Saline infusion	14.60 ± 2.18	6.60 ± 0.55	26.7
Saline and albumin infusion	11.92 ± 1.84	8.11 ± 0.76	37.5

IRC, intrinsic reabsorptive capacity as measured by shrinking-drop technique ($t_1/_2$ in sec); TT, transit time of Lissamine green (sec); FR, calculated % reabsorption in proximal tubule. Values presented as mean ± residual standard error.

[1] L. E. EARLY and R. M. FRIEDLER, J. clin. Invest. *45*, 542 (1966).
[2] F. C. RECTOR JR., J. C. SELLMAN, M. MARTINEZ-MALDANDO and D. W. SELDIN, J. clin. Invest. *46*, 47 (1967).
[3] F. C. RECTOR JR., F. B. BRUNNER and D. W. SELDIN, J. clin. Invest. *45*, 590 (1966).
[4] K. H. GERTZ, in *Normale und pathologische Funktion des Nierentubulus* (Ed. K. J. ULLRICH and K. HIERHOLZER; Hans Huber Verlag, Bern 1964), p. 141.
[5] J. R. GILL JR., D. T. MASON and F. C. BARTTER, J. clin. Invest. *43*, 177 (1964).
[6] A. J. VANDER, Am. J. Physiol. *206*, 492 (1964).
[7] M. STEINHAUSEN, Pflügers Arch. ges. Physiol. *277*, 23 (1963).
[8] K. H. GERTZ, J. A. MANGOS, G. BRAUN and H. D. PAGEL, Pflügers Arch. ges. Physiol. *285*, 360 (1965).
[9] K. H. GERTZ, Pflügers Arch. ges. Physiol. *276*, 336 (1963).
[10] F. P. BRUNNER, F. C. RECTOR JR. and D. W. SELDIN, J. clin. Invest. *45*, 603 (1966).

trinsic capacity as judged from the shrinking-drop technique after the maneuvres 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 m*M* $NaHCO_3$ and 110 m*M* 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]. STARLING[11] 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 method[19] 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 Ölsäule 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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Institute for Cardiovascular Research, Praha 4-Krc (CSSR), 28th June 1967.

[11] E. H. STARLING, *The Fluids of the Body* (Herter Lectures, W. T. Keener & Co., New York-Chicago 1909).
[12] A. V. N. GOODYER, E. R. PETERSON and A. S. RELMAN, J. appl. Physiol. *1*, 671 (1949).
[13] R. G. PETERSDORF and L. G. WELT, J. clin. Invest. *32*, 283 (1953).
[14] E. BOJESEN, Acta physiol. scand. *32*, 129 (1954).
[15] J. R. ELKINTON, A. P. CROSLEY JR., H. G. BARKER and J. K. CLARK, Fedn. Proc. Fedn Am. Socs exp. Biol. *9*, 37 (1950).
[16] J. ORLOFF and W. D. BLAKE, Am. J. Physiol. *164*, 167 (1951).
[17] I. H. MILLS, H. E. DE WARDENER, C. J. HAYTER and W. F. CLAPHAM, Clin. Sci. *21*, 259 (1961).
[18] N. G. LEVINSKY and R. C. LALONE, J. clin. Invest. *42*, 1261 (1963).
[19] A. G. CORNALL, C. J. BRADAWILL and M. M. DAVID, J. biol. Chem. *177*, 751 (1949).
[20] H. E. DE WARDENER, I. H. MILLS, W. F. CLAPHAM and J. C. HAYTER, Clin. Sci. *21*, 249 (1961).

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 lines[5] and is consequently obtained when analyzing the cell types of the blood[6] and approximately so when analysing isolated regions of an organ[7].

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

[1] E. APELLA and C. L. MARKERT, Biochem. biophys. Res. Commun. *6*, 171 (1961).
[2] C. L. MARKERT and E. APELLA, Ann. N.Y. Acad. Sci. *94/3*, 678 (1961).
[3] C. L. MARKERT, Science *140*, 1329 (1963).
[4] R. CAHN, N. O. KAPLAN, L. LEVINE and E. ZWILLING, Science *136*, 962 (1962).
[5] R. YASIN and G. GOLDBERG, Nature *211*, 5055, 1296 (1966).
[6] E. S. VESSEL, Proc. Soc. exp. Biol. Med. *11*, 100 (1962).
[7] I. H. FINE, N. O. KAPLAN and D. KUFTINEC, Biochemistry *2*, *1*, 116 (1963).