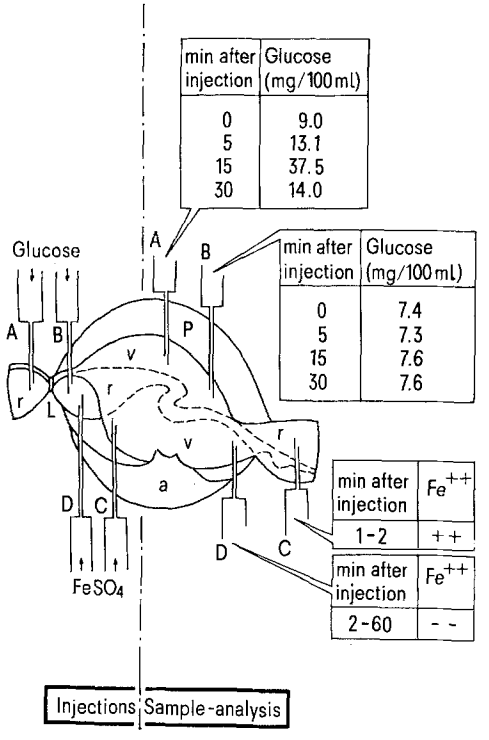

Deliberations for establishing the physiological significance of the piercing of ventricle by gut in *L. corrianus*. Injections: 0.5–1.0 ml in both types of experiments. a, auricle; L, ligature; p, pericardium; r, hind-gut; v, ventricle.

In the other set, ferrous sulphate was introduced in the portion of gut just posterior to the ligature, and blood samples, frequently tapped from the ventricle, were tested for ferrous ions (D in the Figure). The blood samples did not show the presence of ferrous ions even after 1 h of administration of ferrous sulphate solution, thus demonstrating that ferrous ions are not transported from rectal lumen to ventricular blood.

On comparison of the 2 sets of results, it can be said that since ferrous ions could be transported only from blood to rectum and not vice versa, their transport across the rectal wall is not likely to be passive in nature. So, while no direct absorption of nutrients, like glucose, occurs from hind-gut into blood within the ventricle, there is a rapid and selectively oriented transport of harmful material, like ferrous ions, directly from blood to rectum. It is, therefore, felt that passage of hind-gut through the ventricle in *L. corrianus* has some specific role in elimination of undesirable and harmful elements, thus enhancing the excretory efficiency of the mussel. This appears to have been possible due to some oriented permeability properties of the rectal wall.

Résumé. Les résultats suggèrent que le passage de l'intestin dans le cœur de *Lamellidens corrianus* facilite le transfert des éléments peu désirables du sang directement dans l'intestin.

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18 March 1974.

[1] Suggestions from Dr. A. B. DAS, Professor of Biological Sciences, University of Sambalpur, are acknowledged with thanks.

Effect of Insulin upon Renal Amino Acid Transport in Lambs

Active transport of amino acids by several mammalian tissues appears to be regulated by hormones[1]. For instance, in the rat insulin stimulates uptake of certain neutral amino acids into the muscle cell[2]. In the rat liver, cellular uptake of amino acids seems to be enhanced by both insulin[3] and glucagon[3,4]. Since it has not yet been established whether these hormones have an effect upon renal amino acid transport, we have studied uptake of the non-metabolizable α-aminoisobutyric acid by kidney cortex slices of lambs and sheep as affected by insulin and glucagon respectively.

Methods and material. Kidneys of 11 young lambs, 1–6 days old, 5 lambs aged 9 weeks and 5 sheep, aged 2.5–4 months were used for the experiments. 3 of the lambs aged 9 weeks had been fed milk[5] all the time, whereas 2 of these animals in addition to milk[5] had been fed hay and concentrate (33.3% soybean meal, 30.8% beat bulb dried, 16.7% barley, 16.7% oats, 2.5% salt-vitamine-mixture) up to the 6th week of age. From there on milk had been withdrawn. The young lambs had been fed milk[5]. The sheep had received hay, concentrate and maize silage.

The kidneys were removed under anesthesia (i.m. 1 mg Rompun[R,6] and 2.5 mg Ketanest[R,7]/kg body weight) and put between ice cubes. Immediately thereafter kidney cortex slices, 0.3–0.5 mm thick, were made with a Stadie-Riggs microtome. Thereupon the slices were preincubated for 10 min at room temperature in a 250 ml Erlenmeyer flask containing 100 ml KREBS-HENSELEIT[8] bicarbonate puffer (pH 7.4). Then 4 slices per flask were transferred to 50 ml Erlenmeyer flasks containing 5 ml Krebs-Henseleit bicarbonate puffer with 0.065 m*M* ^{14}C-labelled α-aminoisobutyric acid (specific activity: 0.769 mCi/m*M*ol). The flasks were filled with the appropriate gaseous phase (O_2:CO_2 = 95:5) and then closed with rubber stoppers and agitated (120 oscillations/min) for 80 min at 37 °C in a Köttermann metabolic incubator. At the end of the incubation, the slices were rinsed with saline, blotted on filter paper, weighed, transferred into counting vials and solubilized with 1.5 ml sample solubilizer (Soluene[R] 350,

[1] *Biochemical Actions of Hormones* (Ed. G. LITWACK; Academic Press, New York and London 1970), vol. 1, p. 197.
[2] *Handbook of Physiology*, Section 7: *Endocrinology* (American Physiological Society, Washington, D.C. 1972), vol. 1, p. 327.
[3] J. W. CHAMBERS, R. H. GEORG and A. D. BASS, Molec. Pharmac. *1*, 66 (1965).
[4] J. K. TEWS, N. A. WOODCOCK and A. E. HARPER, J. biol. Chem. *245*, 3026 (1970).
[5] Milk-replacer for lambs, Bayerische Milchindustrie GmbH. Landshut.
[6] Bayer, Leverkusen.
[7] Parke-Davis.
[8] H. A. KREBS and K. HENSELEIT, Hoppe Seyler's Z. physiol. Chem. *210*, 33 (1932).

Packard). Then 20 ml toluene scintillation fluid (5.5 g Permablend III (Packard) in 1 l toluene) was added and the ^{14}C-activity was measured in a liquid scintillation spectrometer (modell 3375, Packard).

The incubation medium was assayed for ^{14}C-activity using BRAY[9] scintillation fluid (1 ml incubation medium + 20 ml scintillation fluid). The ^{14}C-activity was corrected for quenching by external standardization (channels ratio method). Extracellular fluid space of the slices was determined by incubating slices with (carboxy-^{14}C)-inulin. The total tissue water was assessed by measuring the change in weight of slices after drying at 105 °C. By knowing extracellular space, one can correct total tissue ^{14}C-activity for radioactive α-aminoisobutyric acid trapped in the extracellular space and thus determine radioactivity in intracellular water. From the wet weight of the slices and the extracellular fluid space, calculation of the amount of intracellular fluid and the ^{14}C-activity per ml of intracellular fluid was made. From the ^{14}C-activity, the concentration of α-aminoisobutyric acid in the intracellular fluid and the incubation medium was calculated.

The concentration gradient of α-aminoisobutyric acid between the intracellular fluid and the incubation medium (= $[AIB_i]/[AIB]_o$) was used as parameter for α-aminoisobutyric acid uptake by kidney cortex cells. A gradient > 1 is indicative of an active transport process.

Hormones, which were obtained from Serva, Heidelberg, were added to the incubation medium at the following concentrations: Insulin 0.1 U/ml; glucagon 3×10^{-6} *M*.

Results. It is evident from Figures 1 and 2 that uptake of α-aminoisobutyric acid by kidney cortex cells occurred against a high concentration gradient in both lambs and sheep. Extracellular space of kidney cortex slices was significantly ($p < 0.05$) higher in sheep (33.9 ± 4.7%) than in young lambs, 1–6 days old (27.7 ± 3.9%). In 9-week-old lambs, which had been fed either milk or hay and concentrate, extracellular space of kidney cortex slices was 30.7 ± 3.1% and 31.5 ± 4.0%, respectively.

Insulin, when present in the incubation medium, enhanced renal uptake of α-aminoisobutyric acid in young lambs, but not in sheep and older lambs fed either milk or hay and concentrate (Figures 1 and 2). Addition of glucagon to the incubation medium remained without influence upon accumulation of α-aminoisobutyric acid by kidney cortex cells of lambs and sheep (Figures 1 and 2).

Discussion. Our results show that insulin, when present in the incubation medium, enhances accumulation of α-aminoisobutyric acid by kidney cortex cells in young lambs. Since, under our experimental conditions, accumulation of α-aminoisobutyric acid in the intracellular fluid depends on both the rate of influx and efflux of α-aminoisobutyric acid, the insulin effect described might be due either to an increased influx or to a decreased efflux of α-aminoisobutyric acid. It is also possible that both processes are affected by insulin. Further experiments are necessary to elucidate this problem.

The regression of the response of renal α-aminoisobutyric acid transport to insulin in the maturing sheep appears to be independent of rumen development, since neither in 9-week-old lambs with rudimentary rumen (= milk-fed lambs) nor in 9-week-old lambs with well developed rumen (= lambs fed hay and concentrate) did insulin influence α-aminoisobutyric acid accumulation by kidney cortex cells. Hence the insulin-sensitivity of renal amino acid transport appears to regress with advancing age in the ovine.

Several authors[10–12] have shown that plasma insulin levels are similar in lambs of different age and adult sheep. Thus the different response of renal α-aminoisobutyric acid transport to insulin in young lambs as compared to sheep and older lambs cannot be attributed to differences in plasma insulin levels.

Because α-aminoisobutyric acid has an affinity to the same renal transport systems which mediate transport of natural neutral amino acids[13], our results might also apply

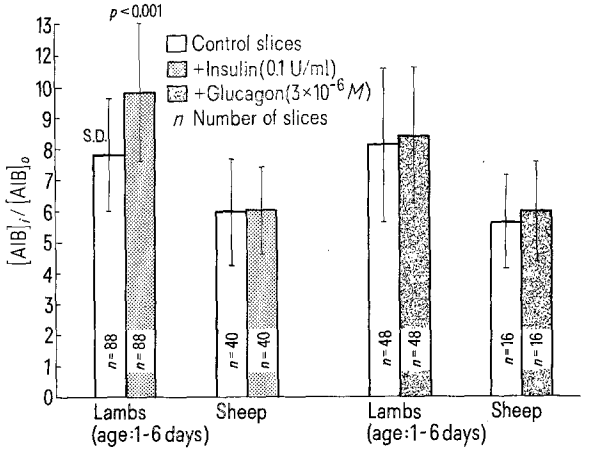

Fig. 1. Accumulation of ^{14}C- labelled α-aminoisobutyric acid by kidney cortex slices of young lambs (age: 1–6 days) and sheep as affected by the presence of insulin or glucagon in the incubation medium. Slices were incubated aerobically (O_2:CO_2 = 95:5) for 80 min at 37 °C in KREBS-HENSELEIT bicarbonate puffer. The columns represent concentration gradients of α-aminoisobutyric acid between the intracellular fluid of the slices and the incubation medium (= $[AIB]_i/[AIB]_o$).

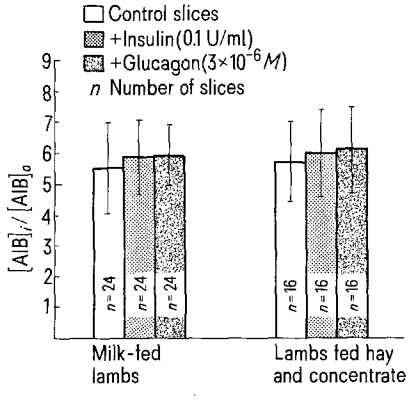

Fig. 2. Accumulation of ^{14}C- labelled α-aminoisobutyric acid by kidney cortex slices of 9-week-old lambs fed either milk or hay and concentrate as affected by the presence of insulin or glucagon in the incubation medium. Slices were incubated aerobically (O_2:CO_2 = 95:5) for 80 min at 37 °C in KREBS-HENSELEIT bicarbonate puffer. The columns represent concentration gradients of α-aminoisobutyric acid between the intracellular fluid of the slices and the incubation medium (= $[AIB]_i/[AIB]_o$).

[9] G. A. BRAY, Analyt. Biochem. *1*, 279 (1960).
[10] J. M. BASSETT and G. ALEXANDER, Biol. Neonate *17*, 112 (1971).
[11] F. HERTELENDY, L. MACHLIN and D. M. KIPNIS, Endocrinology *84*, 192 (1969).
[12] J. S. STERN, C. A. BAILE and J. MAYER, J. Dairy Sci. *54*, 1052 (1971).

to renal transport of these amino acids. To our knowledge, up to now nobody has reported on an in vitro effect of insulin upon renal amino acid transport.

Zusammenfassung. An Nierenrindenschnitten von Lämmern und Schafen wurde untersucht, ob die Anwesenheit von Insulin bzw. Glukagon im Inkubationsmedium die gegen ein Konzentrationsgefälle erfolgende Aufnahme von α-Aminoisobuttersäure in Nierenrindenzellen beeinflusst. Insulin stimulierte in diesen Versuchen (Inkubationszeit: 80 min) die Akkumulation von α-Aminoisobuttersäure in Nierenrindenzellen bei jungen Lämmern, nicht jedoch bei älteren Lämmern und Schafen. Für Glukagon konnte kein Effekt nachgewiesen werden.

E. SCHARRER, C. LANDES and H. SCHOLL[14]

[13] *Handbook of Physiology*, Section 8: *Renal Physiology* (American Physiological Society, Washington, D.C. 1973), p. 653.

[14] Acknowledgment. The investigation was supported by the Deutsche Forschungsgemeinschaft (SFB 147 Jungtierkunde).

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Release of Renomedullary Prostaglandins in Normal and Hypertensive Rats

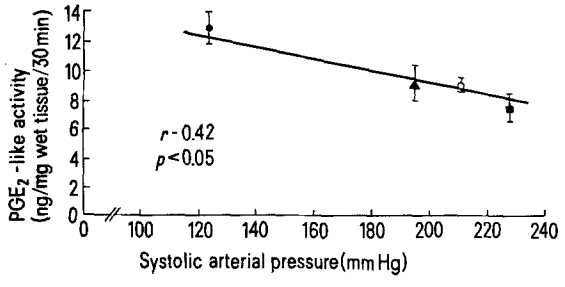
Previous studies have shown that prostaglandins might be involved in the control of arterial pressure. NEKRASOVA et al.[1–3] have demonstrated the presence of prostaglandin E-like lipids in the kidneys of renal hypertensive rabbits and have associated the onset of hypertension and elevated blood pressure with low renal prostaglandins. Similarly, ZUSMAN et al.[4] and LEE et al.[5] have postulated a deficiency of circulating prostaglandins in essential hypertension in man. On the other hand, incubated renal papillae of 'post salt' hypertensive rats (hypertension obtained after salted diet) released more prostaglandin E_2 than papillae from normotensive rats[6]. The present work was undertaken to study the effect of the first type Goldblatt hypertension (one kidney clamped and the other intact), the second type (one kidney clamped with contralateral nephrectomy) and spontaneous hypertension (genetically hypertensive rats, of the strain of Akamoto-Aoki and bred in our laboratory[7]) upon the release of prostaglandin E_2-like material from the rat renal papilla.

Methods. Albino rats of either sex weighing 100–125 g were divided into 4 groups: A) controls, B) second type Goldblatt hypertension, C) first type Goldblatt hypertension and D) genetically hypertensive rats. All operations were performed under ether anesthesia. The animals were given Purina chow and water ad libidum. Systolic arterial pressure was measured before each experiment with a tail cuff connected to a W. & W. blood pressure recorder. 4 to 5 weeks after clamping the renal artery, the surviving animals showed hypertensive values (higher than 160 mm Hg). The animals were then decapitated, and the kidneys were removed, weighted and put in cold Krebs solution. The papillae were then dissected out, cut in small pieces (1–2 mm) and incubated in Krebs solution at 37 °C for 30 min. Following incubation, prostaglandins in the medium were extracted and bioassayed according to SIROIS and GAGNON[8]. Results are expressed as prostaglandin E_2-like activity and are given as means ± S.E.M. Significance of differences and correlation coefficients were calculated according to STEEL and TORRIE[9].

Results and discussion. As demonstrated in the Table, the release of prostaglandins from renal papillae (pooled kidneys) of normotensive (blood pressure: 124 ± 5 mm Hg) rats (group A) averaged 12.8 ± 1.1 ng/mg wet tissue/30 min, whereas the renal papillae of animals with one kidney clamped and contralateral nephrectomy (group B) released only 7.4 ± 1.1 ng/mg. The rats of this last group had developed hypertension (blood pressure: 228 ± 8 mm Hg) and their total output of prostaglandins was decreased by 43% as compared to control animals ($p < 0.05$).

Rats which were submitted only to clamping of the left renal artery (group C) developed less severe hypertension (195 ± 11 mm Hg). Again, the release of prostaglandins from the left papillae of these animals was lower than that observed in control animals showing mean values of 9.9 ± 1.4 ng/mg. These values represent a 23% decrease in prostaglandins release as compared to controls.

When used in these experiments, the genetically hypertensive rats (group D) had a mean arterial pressure of 211 ± 7 mm Hg, a value that lies between those observed in groups B and C. Similarly, the release of prostaglandins (8.9 ± 0.5 ng/mg) was respectively higher and lower than that observed in rats of groups B and C. This



Release of renomedullary prostaglandins as a fonction of systolic arterial pressure in normal (●), clamped (▲), nephrectomized and clamped (■), and genetically hypertensive rats (○).

[1] A. A. NEKRASOVA and L. A. LANTSBERG, Kardiologiya *9*, 86 (1969).
[2] A. A. NEKRASOVA, E. N. NIKOLAEVA and V. V. KHUKHAREV, Kardiologiya *8*, 16 (1968).
[3] A. A. NEKRASOVA, Y. A. SEREBROVSKAYA, L. A. LANTSBERG and I. A. UCHITEL, Kardiologiya *10*, 31 (1970).
[4] R. ZUSMAN, D. SPECTOR, B. CALDWELL, L. SPEROFF, B. FORMAN, G. SCHNEIDER and P. MULROW, J. clin. Invest. *52*, 93a (1973).
[5] J. B. LEE, A. ATTALLOW, V. K. VANCE, C. ELLWOOD and A. PREZYNA, J. clin. Invest. *52*, 50a (1973).
[6] L. TOBIAN and S. AZAR, *Hypertension 72* (Eds. J. GENEST and E. KOIW; Springer-Verlag, New York 1972), p. 393.
[7] The initial breeding stock was kindly provided by Dr. F. HERR, Ayerst Laboratories, Montréal.
[8] P. SIROIS and D. J. GAGNON, Eur. J. Pharmac., in press (1974).
[9] R. G. D. STEEL and J. H. TORRIE, *Principles and Procedures of Statistics* (McGraw-Hill, New York 1960).