Zusammenfassung

Gegenwärtig vorliegenden Daten gemäss kann der Mechanismus der äusseren Sekretion der Bauchspeicheldrüsen am besten durch die Hypothese erklärt werden, dass zuerst eine bikarbonatreiche Lösung gebildet wird, die dann auf dem Weg durch die Gänge der Bauchspeicheldrüse mit dem extrazellulären Chlorid ausgetauscht wird. Die Wände der Gänge wirken als eine anionendurchlässige Membrane.

Absorption of L-Lysine in the Small Intestine of Rats

WISEMANN[1] reported that isolated loops of rat's intestine transfer monoamino acids but not diamino acids, lysine, and ornithine, against a concentration gradient. Since L-lysine is essential to animals, it seemed worth while to carry out further investigations upon *in vitro* and *in vivo* L-lysine intestinal absorption.

Methods and results. Male rats, weighing 150–200 g were used, they were fed on a standard diet with at least 12 h fasting before the experiments.

1. Perfusion of isolated surviving rat small intestine. Each animal was killed by decapitation and bled.

The apparatus used for the intestinal perfusion was based on the original DARLINGTON and QUASTEL one[2], but substantially modified to make it more practical (see Figure).

Loops of small intestine of about 25 cm, adjacent to duodenum, were used. As soon as removed from an intact animal, a loop was placed in a small beaker and the inside was washed out with the solutions to be used in the experiments. The upper and lower ends of a loop were then tied on (S) and (S') of the apparatus with a silk thread. Description in fuller details may be found elsewhere[3]).

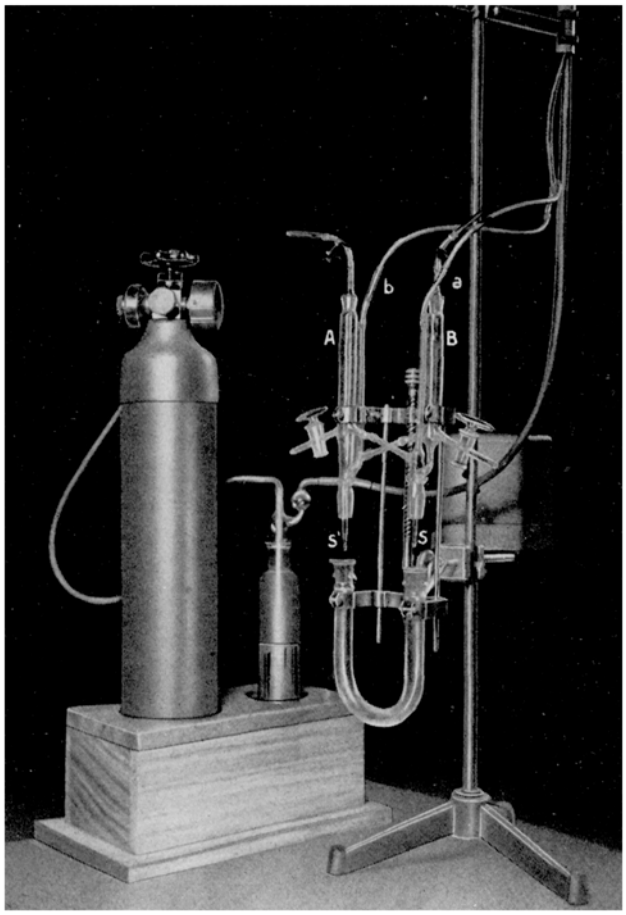
On assembling the intestinal segment in the apparatus, two independent systems are involved: the former inside, the latter outside the intestinal lumen. In both systems, the perfusion fluid runs in opposite directions, if a gas flow is led to the apparatus under a moderate pressure. Gas flows through (a) and (b), which are connected to the reservoirs (A) and (B) by two side connections. Therefore, in passing through (A) and (B) filled with a perfusion fluid (Ringer-bicarbonate), a gas carries some fluid with it in such a way that the level in the reservoirs (A) and (B) are made higher than in (a) and (b). By the difference between the fluid levels, two circulations result: the inner solution running through the intestinal lumen and the outer solution outside.

The apparatus, when in use, is kept in a bath maintained at 38°C.

Samples of inner and outer solutions for lysine estimation are taken through two glass coks on the apparatus; L-lysine is analyzed by the method reported by ALLPORT and KEYSER[4].

Perfusion results are given in Table I, where data of experiments, each corresponding to a different L-lysine concentration are reported.

2. Uptake of L-lysine by intestinal tissue. The small intestine was removed with the same care taken in the previous experiments. After dipping in a Ringer-bicarbonate solution at 38°C, and drying on a filter paper, an isolated loop was then cut into pieces of ÷ 0·25–0·5 cm ÷ 0·25 to 0·50 cm with scissors.



The intestinal tissue was transferred to Warburg flasks of 50 ml capacity, which contained Ringer-bicarbonate buffer, with or without L-lysine.

The flasks were kept in a bath at a constant temperature (38°C). At intervals, samples of the fluid were taken for lysine estimations. At the end of an experiment, the tissue was precipitated with 10% TCA (0·5 ml), then separated from the incubation fluid by centrifugation, and finally minced with Quartz sand in a mortar containing distilled water. Extracts were made, and analyzed for lysine. The volumes of the extracts correspond to the

Table I. Absorption of L-lysine from isolated surviving rat small intestine.

Inner solution: L-lysine as shown in the 1st line, in Ringer-bicarbonate, pH 7; 20 ml. Outer solution: Ringer-bicarbonate, pH 7; 60 ml. Gas: 95% O_2 + 5% CO_2. Duration of an experiment: 60 min; temperature 38°C.

Inner solution	μM L-lysine before the experiment	130	300	653	910	1317	1750
	μM L-lysine/h found after the experiment	90	144	215	316	622	675
Outer solution	μM L-lysine/h found after the experiment	19	58	84	96	74	21

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[2] W. A. DARLINGTON and J. H. QUASTEL, Arch. Biochem. *43*, 194 (1953).

[3] S. DI BELLA, Arch. Sci. biol., in press.

[4] ALLPORT and J. W. KEYSER, *Colorimetric Analysis*, vol. 1, (Chapman and Hall Ltd., London), p. 43.

volumes of the incubation mixtures. The results of an experiment, referring to different L-lysine concentration used, are shown in Table II.

Table II. Uptake of L-lysine from the rat small intestine tissue. Fresh intestinal tissue: g 1; Substrate: L-lysine as shown in the 1st line, in Ringer-bicarbonate buffer, pH 7; 10 ml; Gas: 95% O_2 + 5% CO_2; duration of an experiment: 60 min; temperature: 38°C.

	μM L-lysine				
Before uptake	90	135	450	810	900
After uptake (60th min)	86	125	337	791	873
Uptake, i.e. theoretical amounts of L-lysine assumed during the experiment	4	10	13	19	27
L-lysine found in intestinal tissue slices after 10% TCA precipitation	2	9·5	11·5	17·5	25

3. L-lysine absorption tests in intact animals. The rats were anesthetised with ether, and the abdomen opened in its midline. The small intestine was then tied off at about 5 cm from the stomach, and in a similar manner at a lower level close to the ileo-cecal valve, with a silk thread. Just near both ligatures a little stoma on the intestinal wall was made, in order to place two small glass funnels at each open end of the intestine. A solution containing RINGER-bicarbonate buffer plus L-lysine was then delivered at the upper end of the intestine. Samples of lysine solution for analysis were taken from the lower glass funnel at fixed times. The results of three experiments are given in Table III.

Table III. Absorption of L-lysine from the rat small intestine *in vivo* L-lysine: 520 μM, in Ringer-bicarbonate buffer pH 7, 5 ml; duration of an experiment: 60 min.

Experiments	1	2	3
	μM L-lysine/h		
L-lysine found in the intestinal lumen after the experiment	73	82	86
Theoretical amounts of absorbed L-lysine	447	438	434

Conclusions. Experiments carried out on the rat small intestine, using different methods, show that L-lysine is taken up actively by the epithelial cells of the mucosa.

The uptake and absorption rates depend on the L-lysine present at various concentrations in the experimental systems.

A relationship has also been found between L-lysine concentration in the intestinal lumen and the rate of its appearance in the outer solution, i. e. the diamino acid transport through the intestinal wall. It is apparent, therefore, that an important limiting factor of the transport reactions in intestinal villi is the lysine concentration.

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Zusammenfassung

L-Lysin wird durch die Rattendarmschleimhaut mit einer je nach den Anfangskonzentrationen verschiedenen Geschwindigkeit absorbiert. Die anfänglichen Lysinkonzentrationen stellen beim Transport der Diaminosäure durch die Darmvilli ein die Reaktionen abschwächendes Moment dar.

The Antagonism of Adrenergic Blockade by Dichloroisoproterenol (DCI)[1]

The compound dichloroisoproterenol (DCI)[2] has been described as a blocking agent of inhibitory adrenergic receptors[3] as well as of the inotropic and chronotropic receptors of the heart[4]. These results suggest that this drug is a specific antagonist of the hypothetical beta receptors (as described by AHLQUIST[5]) without exerting appreciable effects on the excitatory (alpha) adrenergic receptors. Thus, the vasodepressor and cardiac stimulant actions of isoproterenol or epinephrine are effectively antagonized by DCI, but no effect is seen against the pressor activity of epinephrine or norepinephrine.

In this report we wish to describe the effect of DCI in antagonizing the blockade of the epinephrine and norepinephrine pressor responses by several of the adrenergic blocking agents. All of these experiments were carried out in pentobarbital anesthetized dogs, and blood pressures were measured from the carotid artery with a mercury manometer. As can be seen in the Figure, the pressor responses to epinephrine and norepinephrine are reversed by a 10 mg/kg dose of dibenzyline. The vasodepressor effect of isoproterenol is not altered by the adrenergic blocking agent. When administered after dibenzyline DCI in doses of 5 to 15 mg/kg was found to produce a depressor response. Subsequent injections of epinephrine and norepinephrine no longer exhibited depressor responses, but were reconverted to pressor effects, indicating that the blockade of excitatory receptors by dibenzyline had been removed. This effect was seen whether the DCI was given 1, 3, or 5 h after the dibenzyline. In all instances the depressor response of isoproterenol was reduced or abolished after the DCI treatment. The magnitude of the epinephrine and norepinephrine pressor responses after the dibenzyline-DCI pretreatment varied with the different experiments, but in general they were at least 50 to 75% of control, and occasionally equal to control responses. If further doses of the adrenergic blocking agent were administered the reconverted pressor effects again decreased, but depressor responses to the catecholamines did not reappear. Similar results were obtained when dihydroergotamine or benzodioxane were employed as the adrenergic blocking agent.

These findings indicate that DCI alters not only the inhibitory (beta) adrenergic responses but also the excitatory effects of epinephrine and norepinephrine after adrenergic blockade. This is especially interesting with dibenzyline since blockade produced by drugs of this series is generally considered to be of the nonequilibrium type[6] with extremely prolonged durations of action[7,8]; yet the administration of DCI immediately restores the vasopressor actions of epinephrine and norepinephrine. The true nature of the observed antagonism of adrenergic

[1] This study was supported in part by a Research Training Grant from the National Institutes of Health.

[2] Dichloroisoproterenol is 1-(3,4-dichlorophenyl)-2-isopropylaminoethanol hydrochloride (Lilly 20522) and was generously supplied by Dr. I. H. SLATER of the Lilly Research Laboratories.

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