surface and not those in close proximity to the micelle surface, so giving a low hydrogen ion determination. These discrepancies between ORD and CD determinations are being further investigated.

(1) S. Bonkoski and J. H. Perrin, *J. Pharm. Pharmacol.*, **20**, 934(1968).

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The Biogenesis of Gramine

Keyphrases ☐ Gramine biogenesis—literature correction ☐ Tryptophan relation—gramine biogenesis

Sir:

Many years ago I demonstrated (1, 2) that *dl*-tryptophan-β-^{14}C fed to sprouting barley, is transformed into the alkaloid gramine in which the ^{14}C is located in one position, corresponding to that in the administered tryptophan, strongly suggesting that it was a precursor of gramine. This was one of the first demonstrations, using a radioactive compound, that an alkaloid could be formed from an amino acid.

In a recent paper (3), Digenis states "Based on other tracer experiments and the fact that tryptophan was formed in *Neurospora* by a condensation reaction between indole and L-serine, Bowden and Marion suggested a reversal of the above-mentioned tryptophan biosynthesis could possibly lead to indole and L-serine in barley. This suggested that the indole could subsequently react in a Mannich-type reaction with formaldehyde and dimethylamine to produce gramine." Later in the paper the author continues, "However, Leete and Marion were able to show that the bond between the 3-position of the indole nucleus and the side chain of tryptophan remained intact during the biosynthesis of gramine in barley, thus disposing of the hypothesis of Bowden and Marion described above."

An examination of the only two papers I have published (1, 2), to which Digenis refers, will show that in them no theory on the biogenesis of gramine from tryptophan is proposed or implied. The only conclusion reached was, I quote, "that tryptophan is a precursor of gramine in barley," a conclusion that has been amply justified by subsequent investigators.

(1) K. Bowden and L. Marion, *Can. J. Chem.*, **29**, 1037(1951).
(2) *Ibid.*, **29**, 1043(1951).
(3) G. A. Digenis, *J. Pharm. Sci.*, **58**, 39(1969).

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Drug Transport I: Effect of Potassium Ion on the *In Vitro* Transfer of Several Drugs Across the Rat Intestine: Preliminary Observations

Keyphrases ☐ Intestinal transport, drug—K^+ effect ☐ K^+ substitution of Na^+—*in vitro* intestinal transport

Sir:

Recent work in our laboratory concerning factors that affect drug transport has resulted in several interesting, preliminary findings as to the effect of replacing Na^+ with K^+ on the transfer of several drugs across the everted rat intestine.

Sprague-Dawley rats,[1] weighing approximately 250 g., were fasted 20–24 hr. prior to the experiment. Water was allowed *ad libitum*. The experimental method for preparing the everted rat intestine preparation has been described previously (1). After severing the intestine at the pyloric junction, the first 15 cm. of intestine are discarded and the following 20 cm. are divided into two 10-cm. segments. The most proximal segment is designated Segment 1, and the distal portion is designated Segment 2. A modified physiologic Kreb's bicarbonate buffer,[2] pH 7.4, was prepared to contain a total cation

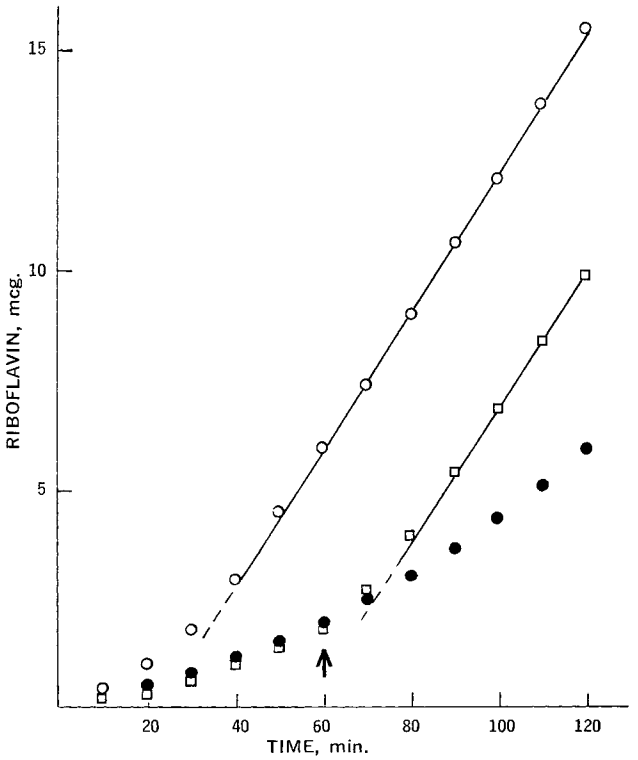

Figure 1—*Cumulative transfer of riboflavin across the everted rat intestine. Mucosal concentration maintained essentially constant at 20 mcg./ml. Key: (○), Na^+-buffer; (●), K^+-buffer; (□), K^+-buffer for 60 min., then placed into Na^+-buffer (arrow). See text for details.*

[1] Blue Spruce Farms, Altamont, N. Y.
[2] KCl, 5 m*M*; KH_2PO_4, 6 m*M*; $NaHCO_3$, 26 m*M*; NaCl *q.s.* 154 m*M* cation ($Na^+ + K^+$).

Table I—Cumulative Mucosal-to-Serosal Transfer of Various Drugs Across the Everted Rat Intestine at pH 7.4 from Na^+ and K^+ Buffers[a]

Drug	Amount Transferred ± SD[b]				Level of Significance[c]	
	Segment 1		Segment 2		Segment 1	Segment 2
	Na^+	K^+	Na^+	K^+	Na^+ *vs.* K^+	Na^+ *vs.* K^+
Riboflavin, mcg.	14.2 ± 2.5(5)	6.3 ± 2.0(5)	11.8 ± 0.8(5)	4.6 ± 2.0(5)	$p < 0.005$	$p < 0.001$
Salicylate, mg.	3.6 ± 0.7(5)	2.6 ± 0.4(5)	3.7 ± 0.5(5)	1.7 ± 0.3(5)	$p < 0.05$	$p < 0.001$
Sulfanilamide, mcg.	130.7 ± 5.4(5)	86.2 ± 23.5(4)	126.4 ± 23.7(4)	41.5 ± 2.0(4)	$p < 0.01$	$p < 0.001$

[a] Na^+-buffer is the modified physiologic Kreb's bicarbonate buffer (see *Footnote* 2). K^+-buffer is identical except that the Na^+ is quantitatively replaced by K^+. [b] Amount transferred in 2 hr. ± standard deviation of the mean. Parenthetic values denote number of experiments. [c] Unpaired comparison using Student's *t* test.

concentration of 154 m*M* (148 m*M* Na^+ and 6 m*M* K^+). A similar buffer solution was prepared in which the Na^+ was quantitatively replaced with K^+. The intestinal transfer rates of three drugs in these two buffer solutions were determined. The drugs and their respective concentrations in the mucosal solution were: riboflavin, 20 mcg./ml.; salicylate, 2.0 mg./ml., and sulfanilamide, 0.1 mg./ml. At pH 7.4, salicylate is completely ionized and sulfanilamide and riboflavin are essentially unionized. The mucosal solution pH before and after each experiment never varied by more than ±0.2 pH units and the concentration of the respective drugs also remained essentially constant throughout the experiment due to the large (100 ml.) volume of mucosal solution.

The results of these experiments are summarized in Table I. When Na^+ is replaced by K^+ in the buffer solution there is a significant decrease in the amount of drug transferred in each case. This apparent inhibitory effect is reversible as illustrated in Fig. 1, which is a plot of the cumulative amount of riboflavin transferred as a function of time from mucosal solutions of varying ionic composition. The marked influence of Na^+-replacement by K^+ is evident. When, after 60 min. in K^+-buffer, the intestinal segment is placed into a riboflavin solution in the conventional Na^+-buffer, the transfer rate markedly and rapidly increases and becomes virtually identical to that observed when the intestinal segment is present in the Na^+-buffer during the entire course of the experiment.

These observations may be due to a Na^+-dependent transport mechanism for the several unrelated drugs studied. Alternatively, there may be a K^+-mediated inhibition of the drug transfer process. The intestinal transport of several nutrients, particularly amino acids and sugars, is known to be Na^+-dependent. Replacement of Na^+ with K^+ has been shown to markedly depress amino acid (2) and sugar (3) transport. In the presence of ouabain, a glycoside known to specifically inhibit Na^+ transport (4), the transport of amino acids (2) and sugars (5) can be effectively reduced to the level observed when K^+ completely replaces Na^+. However, the affected processes involve specialized transport rather than transfer by passive diffusion as is presumed to be the case with salicylate and sulfanilamide. Preliminary studies during the course of this investigation indicate that the presence of 10^{-3} *M* ouabain in the Na^+ buffer (in both mucosal and serosal solutions) has no effect on the transfer of the three drugs studied. It would seem then, that there is no specific Na^+ requirement for the intestinal transfer of these drugs in the *in vitro* intestinal preparation used here; rather, there seems to be a K^+ inhibition of drug transfer.

We are presently engaged in extensive studies to elucidate the mechanism of the observed effects.

(1) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **58**, 425(1969).
(2) I. H. Rosenberg, A. L. Coleman, and L. E. Rosenberg, *Biochim. Biophys. Acta*, **102**, 161(1965).
(3) I. Bihler and R. K. Crane, *ibid.*, **59**, 78(1962).
(4) H. J. Schatzmann, *Helv. Physiol. Acta*, **11**, 346(1953).
(5) T. Z. Csaky, *Am. J. Physiol.*, **201**, 999(1961).

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