sure. On the other hand, a pure blocking effect on peristalsis is indicated by "C_6" at all concentrations.

Results of a similar nature are shown in Fig. 2, obtained in experiments by adding DMPP or "C_6" during peristalsis. These graphs are given to show particularly the return of peristalsis, which had been suppressed with "C_6", by a further increase of pressure in the gut or by DMPP. When once suppressed by DMPP, on the other hand, no resumption of peristalsis occurred by the further increase of pressure.

In view of the antagonistic action of physostigmine toward d-tubocurarine at the neuromuscular junction, the influence of physostigmine upon the suppressive effect of DMPP, nicotine, "C_6" and d-tubocurarine on peristaltic reflexes was investigated. As shown by the recordings in Fig. 3, physostigmine antagonized the blocking effect of d-tubocurarine on peristaltic reflexes but did not affect that of DMPP, nicotine, or "C_6". It suggests the possibility that the latter agents and d-tubocurarine inhibit the ganglionic response of the ileum to increasing pressure in the lumen by different mechanisms. This awaits further investigation.

Summary. DMPP has been shown, like nicotine, to augment the peristaltic reflex of an isolated ileum to increasing pressure at low concentrations but inhibits it at high concentrations. This is apparently due to its stimulating and paralyzing action on the parasympathetic ganglion at low and high concentrations, respectively. The fact that the paralyzing effect of DMPP on the ganglia was not evident on repeated administrations in animals of submaximal doses may be explained by the rapid elimination of it from the site of action. Whereas physostigmine antagonized the blocking effect of d-tubocurarine, it does not influence that of DMPP on peristaltic reflexes of the ileum.

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Influence of Incubation Time and pH Changes on Uptake of P-aminohippurate by Renal Slices. (20845)

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Cross and Taggart(1) have determined the various factors which influence the uptake of p-aminohippurate (PAH) in rabbit kidney slices. These authors have stated that the maximal uptake of PAH by renal slices occurs following a 120-minute experimental period. Furthermore, they state that pH variations between 7.0 and 7.8 have no effect on PAH slice:medium ratio (S:M Ratio) of renal slices of the rabbit. Under certain conditions the time factor as well as the pH of the buffer solution have marked effects on the S:M ratio and this report represents the data on the influence of these factors on the PAH S:M ratio determined with dog renal slices.

Methods. In some of the experiments the phosphate buffer devised by Cross and Taggart(1) was employed. In another series of experiments a bicarbonate buffer (NaCl = 0.92%, KCl = 0.042%, $CaCl_2$ = 0.024%, $NaHCO_3$ = 0.05%(2) and a 95% oxygen, 5% CO_2 gas phase were used. About 100-150 mg of kidney slices obtained from 9-18 kg dogs were placed in 20 cc beakers containing 2.8 cc of buffer and 0.0013 M PAH. Various substrates were neutralized and added in a

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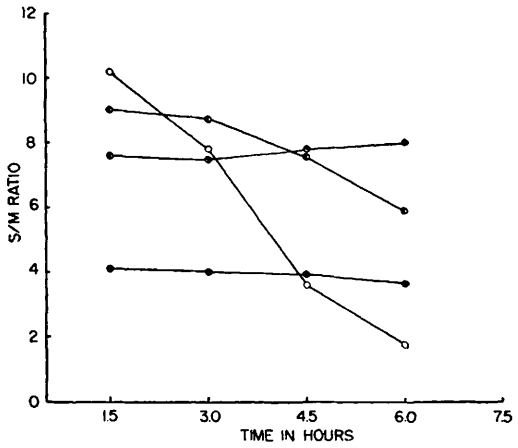

FIG. 1. Effect of incubation time on Slice:Medium Ratio of PAH. Dog renal slices, phosphate buffer according to Cross and Taggart(1). ● = no added substrate; ○ = substrate—sodium acetate .01M; Circle (shaded, left side) = substrate—sodium pyruvate .01M; ⊙ = substrate—sodium lactate (dl) .01M.

0.01 M concentration to the buffer. The beakers were shaken for varying periods in a Dubnoff metabolic shaking machine. In all these experiments the bath temperature was 25°C. Otherwise, the procedures were similar to those used by Cross and Taggart(1). Slices were prepared by means of a Stadie-Riggs tissue slicer. The S:M ratio was based on the final wet weight of the tissue.

Results. The observation was made that in the presence of sodium acetate as substrate the S:M ratio declined rather rapidly with the time of incubation. This decrease was much less marked or absent in those experiments where no substrate had been added or in the presence of sodium lactate or sodium pyruvate. Typical examples of such experiments are given in Fig. 1. The marked decline in the S:M ratio in the presence of acetate was

TABLE I. Effect of Sodium Acetate Concentration on Rate of Decline of S:M Ratio of PAH with Time. Dog renal slices, phosphate buffer according to Cross and Taggart(1). Bath temp. 25°C.

Incubation time, hr	NaAc 0.005M, S:M ratio	NaAc 0.015M, S:M ratio
1.5	9.2	8.7
3.0	6.6	6.7
4.5	4.4	4.0
6.0	2.1	1.8

TABLE II. Influence of Time on S:M Ratio and pH of the Buffer. Dog kidney slices in phosphate buffer according to Cross and Taggart(1). Bath temp. 25°C.

Incubation time, hr	No substrate (6)* S:M ratio	No substrate (6)* 7.34† Δ pH	Sodium acetate .01M (5)* S:M ratio	Sodium acetate .01M (5)* 7.34† Δ pH	Sodium pyruvate .01M (4)* S:M ratio	Sodium pyruvate .01M (4)* 7.33† Δ pH	Sodium lactate .01M (4)* S:M ratio	Sodium lactate .01M (4)* 7.43† Δ pH
1.5	3.99 (2.63-4.97)	.03 (-.12)	9.63 (7.03-11.95)	.40 (.35-.47)	8.53 (7.21-8.85)	.36 (.32-.42)	7.83 (5.73- 9.53)	.08 (.04-.12)
3.0	3.78 (2.93-4.44)	.11 (.07-.17)	4.90 (4.30- 5.86)	.64 (.63-.65)	7.40 (6.05-9.45)	.58 (.48-.65)	8.0 (6.0 - 9.86)	.20 (.20-.20)
4.5	3.88 (2.87-4.79)	.21 (.08-.27)	2.30 (1.41- 3.88)	.79 (.70-.85)	7.15 (5.89-8.20)	.70 (.63-.78)	8.55 (7.88-10.35)	.34 (.32-.36)
6.0	3.56 (2.47-3.76)	.19 (.09-.27)	1.47 (.75- 2.64)	.85 (.75-.95)	5.85 (3.88-7.85)	.79 (.70-.88)	8.15 (7.50- 9.20)	.45 (.32-.50)

* No. of experiments.
† Initial pH of buffer.

TABLE III. Effect of pH of Buffer Solution on PAH Uptake. Dog kidney slices in phosphate buffer(1). Incubation time 2 hr. Bath temp. 25°C.

Initial pH avg	No substrate added (4)*	Sodium acetate .01M (4)*	Sodium pyruvate .01M (3)*	Sodium lactate .01M (3)*
6.49	3.40 (2.10-4.2)	10.95 (8.65-14.5)	—	9.6 (6.3-11.5)
7.02	3.38 (2.10-4.10)	10.8 (6.56-10.80)	10.9 (7.2-13.2)	8.3 (5.6- 9.9)
7.49	3.25 (2.20-4.40)	8.75 (6.58-10.80)	9.8 (7.4-11.4)	8.0 (5.8-10.0)
8.01	2.68 (2.42-3.10)	5.76 (3.54- 8.29)	8.9 (7.2-11.2)	7.0 (4.7- 9.4)
8.52	2.1 (2.0 -2.3)	3.92 (1.4 - 6.4)	8.2 (5.4-11.7)	6.4 (4.2- 8.9)
9.02	—	1.61 (0.7 - 4.3)	6.4 (5.7- 8.4)	—

* No. of experiments.

studied further and it could be shown that neither calcium, pantothenate (0.0005 M) or α ketoglutaric acid (0.0015 M) prevented this decline. The possibility that substrate exhaustion could account for this phenomenon was considered. Since the rate of decline of the S:M ratio with time in the presence of 0.005 or 0.015 sodium acetate was the same, it is unlikely that substrate depletion was the major cause of this phenomenon (Table I).

It is apparent that the decline in the S:M ratio with time is much greater with sodium acetate than with any of the other substrates studied. The oxidation of every mole of sodium acetate would liberate one equivalent of hydroxyl ion. The determination of the pH of the buffer at the beginning and end of the observation period has shown that in the presence of substrate the pH of the phosphate buffer increased with time. In the presence of sodium acetate the increment in pH amounted to about 0.85 unit over a period of 6 hours (Table II). This change in pH could be the cause of the depressions of the S:M ratio with time. We have thus determined the effect of pH variations in the buffer solution on the S:M ratio in the absence of added substrate and in the presence of 0.01 M sodium acetate, pyruvate or lactate. Table III summarizes the data obtained and it is clear that, in the presence of acetate, an increase in pH produced a more profound depression than in the presence of the other substrates studied.

One possible reason for the decline of the S:M ratio, with acetate as buffer, could be acetylation of PAH which then would simulate a decline in the PAH S:M ratio. This was not likely since recovery of PAH in all these experiments was about 95-100%. To further exclude this possibility we have repeated these experiments with acetyl p-aminohippurate (AcPAH). Table IV shows that the S:M ratio of AcPAH declines rapidly in the presence of sodium acetate but does not do so in the presence of sodium lactate. The fact that the recovery of PAH is complete and that AcPAH behaves in a manner similar to PAH definitely eliminates acetylation of PAH as being the cause of the decline in S:M ratio when sodium acetate is the substrate.

TABLE IV. Effect of Incubation Time on the S:M Ratio of Acetyl p-Aminohippurate Determined with Dog Renal Slices.

Incubation time, hr	Sodium lactate .01M S:M ratio	Δ pH	Sodium acetate .01M S:M ratio	Δ pH
1.5	4.22	.14	5.87	.33
3.0	4.82	.34	4.88	.42
4.5	4.85	.29	2.4	.58
6.0	3.64	.42	1.67	.64

Since the increase in pH was the likely cause of this decline in S:M ratio when sodium acetate was the substrate, we have attempted to prevent pH changes by means of a bicarbonate-CO_2 buffer system. Fig. 2 gives the average results of two such experiments and it is clear that in the presence of bicarbonate buffer the pH increase seen with phosphate buffer does not occur to the same extent and furthermore the decline in the S:M ratio is also prevented. If 100% oxygen was used in conjunction with bicarbonate buffer the pH of the buffer rapidly increased from about 7.2 to 8.25 and the S:M ratio was depressed below 1.0.

It could also be shown that in bicarbonate buffer and in the presence of sodium acetate

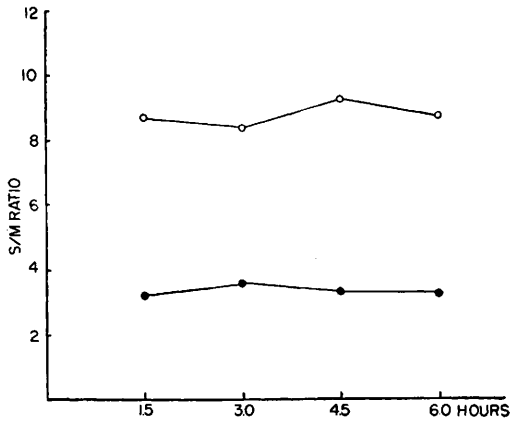

FIG. 2. Effect of incubation time on Slice:Medium Ratio of PAH in presence of bicarbonate buffer (pH 7.2). Average change in pH over a 6 hr period was 0.14 pH unit. Dog renal slices. ● = no added substrate; ○ = substrate—sodium acetate .01M.

(.01 M) the decline of the S:M ratio of AcPAH did not occur and the pH of the buffer remained relatively constant.

Discussion. The data presented have shown that the decline in the S:M ratio of PAH with time is related to the pH changes in the buffer solution occurring in the presence of the various substrates. However, this pH change is not the only factor since acetate and pyruvate behaved differently although the increment in pH change was about the same for both substrates. A possible mechanism is the higher resistance of the pyruvate system towards pH changes demonstrated in Table III. The possibility that acetylation of PAH may account for this decrease of the S:M ratio in presence of acetate as substrate was considered. Calculations of the recovery of PAH in the system did not support this hypothesis since the same amount of PAH was recovered (95-100%) in 1.5-hour experimental series as in the 6-hour series. Further evidence that acetylation of PAH is not the cause was presented in Table IV where acetyl PAH showed a decline similar to that of PAH in the presence of sodium acetate. The demonstration that in the presence of bicarbonate buffer the pH changes as well as the decline in the S:M ratio were absent strongly indicated that the main factor explaining this decline observed in phosphate buffer and sodium acetate as substrate were the pH changes. The differences in sensitivity to pH changes between lactate and pyruvate on the one hand and acetate on the other are shown in Table III. This difference in sensitivity to pH changes explains why in the presence of pyruvate and lactate the decline in the S:M ratio with time was much less than in the presence of sodium acetate as substrate. Why the pyruvate and lactate systems are more resistant than the acetate system to pH changes is not clear. It is possible that a fraction of the pyruvate and lactate may be oxidized by metabolic pathways different from those of acetate.

Summary. In the presence of sodium acetate as substrate the S:M ratio of PAH declines rapidly with time. With no added substrate and with sodium lactate or sodium pyruvate as added substrate this decline in the S:M ratio is much less, or even absent. It is shown that the increase in pH occurring in phosphate buffer is the cause of the decline of the S:M ratio.

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