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# THE pH DEPENDENCE OF RECTAL ABSORPTION OF THEOPHYLLINE FROM SOLUTIONS OF AMINOPHYLLINE IN SITU IN RATS

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SUMMARY

The rectal absorption of theophylline was investigated in situ in rats as a function of luminal pH. As the pH near the membrane may deviate from the luminal pH by secretion, the neutralizing capacity of the rectum was determined by perfusing phosphate buffers with initial pH 5 or pH 9 for 2 h. The secretion of basic or acidic agents appeared to be strongly dependent on the deviation of the luminal pH from the physiological pH. In the first hour, respectively, 2.3 μmol of basic and 1.5 μmol of acidic agents per mg dry weight were produced, perfusing with pH 5 or pH 9 solutions. The secretion decreased when the pH of the perfusate approached physiological values, i.e. about pH 7.4.

The influence of the composition of the perfusate on the absorption rate of theophylline (TP) from aminophylline (AP) solutions containing 10 mg TP/30 ml perfusate was studied. No difference in the absorption rate could be shown when phosphate buffers differing in strength and pH (between 5 and 10) were used. However, with solutions containing 50 mg TP and 130 mg TP per 30 ml, the absorption rates of TP from isotonic phosphate buffers with pH 7, were higher than with pH 10, but far less than predicted by the pH–partition hypothesis (pKa theophylline = 8.4).

The percentage of TP absorbed varied between 12.0 and 14.7% per 2 h for the pH 7 solutions and 8.2 and 12.4% per 2 h for the pH 10 solutions. The fractions absorbed at equal pH did not show a concentration dependency. Two explanations are proposed for the observed small pH dependency. Firstly, assuming that only TP molecules pass through the membrane, the absorption rate of TP is not dependent on the pH when a 'microclimate' exists near the membrane with a pH not, or only slightly, dependent on the bulk pH. Because of the relatively large secretion of acidic agents by the mucosa during perfusion with basic solutions such a 'microclimate' is expected to exist. Secondly, TP ions might also be able to pass through the membrane as fast as TP molecules.

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## INTRODUCTION

The absorption rate of drugs after oral and rectal administration can be limited by their transport through the gastrointestinal membrane. In spite of many investigations on the mechanism of this transport, only a few general rules have emerged. The pH–partition hypothesis, reviewed by Brodie (1964), states that for most drugs the membrane acts as a lipophilic barrier, which favours the transport of unionized species of high lipid solubility.

Several investigators measured the absorption rate of drugs after rectal administration to clarify the details of the transport process in the rectal area, in particular the pH dependence. Rectal absorption rates of some acidic and basic drugs were investigated in situ in rats (Kakemi et al., 1965, 1969) and a strong pH dependence of the absorption rates was found. As an explanation it was proposed that the absorption rates of the ionized species were low relative to the unionized species. The same authors suggested that with some xanthines the absorption rates agreed with the pH–partition theory. However, nothing was mentioned about a possible pH dependence.

In man, the rectal area was perfused with salicylic acid in solutions of different pH (Bechgaard, 1973a, b). A distinct pH dependence was found, but not as large as was expected when only the unionized molecules had been involved in the transport process. Still, salicylic acid was absorbed from solutions hardly containing any unionized molecules. Thus, the pH appears to be an important parameter in the rectal absorption process, but the exact manner in which it influences the absorption is still unknown. Important factors appear to be the existence of a 'microclimate' – a region near the membrane with a pH deviating from the bulk pH – the dimensions of an unstirred layer at the membrane, and the distribution ratio between barrier and bulk phase (Winne, 1977).

In particular, the existence of a 'microclimate' adjacent to the surface of, for example, the small intestine, is a point of controversy (Smolen, 1973; Lucas et al., 1975a, b). It is not known whether the rectum has a substantial capacity for neutralizing solutions with a pH deviating from the physiological value. If it has, a 'microclimate' near the membrane may be possible. Recently Schürmann and Turner (1978) proposed a new membrane model for buccal absorption, arguing that an aqueous pH-buffering surface system exists.

From other sources it appeared that not only the pH itself is important, but that the absorption rate can also be influenced by the composition of the perfusate (Kakemi et al., 1965; Perrin, 1972; Beckgaard, 1973b; Yotsuyanagi et al., 1975; Kojima and Miyake, 1976).

The purpose of the present study was to investigate the rectal absorption mechanism in rats of theophylline (TP) from solutions of aminophylline (AP), a drug substance often administered rectally. Firstly the magnitude of the neutralizing capacity of the rectal area, and secondly the influence of the pH, the concentration of aminophylline (AP), and the buffer composition on the absorption rate in situ, were investigated.

## MATERIALS AND METHODS

### *Procedure to measure the neutralizing capacity*

The experimental technique used was adapted from the one described in more detail

by Van Rees et al. (1974) for the perfusion of the rat small intestine. Male Wistar rats weighing about 200 g were used. They were fasted for 24 h, but water was given ad libitum. The rats were anaesthetized with urethane (3.2 g/kg). The rectum was exposed by an abdominal incision, a plastic cannula was inserted in distal direction and tied firmly to keep it in position. Through the anus a second cannula was entered 1 cm inside the rectum and secured by ligation. Thus, about 4 cm of the rectum was exposed to the perfusate. Care was taken to handle the rectum gently in order to maintain an intact blood supply. To monitor the pH, a glass electrode was placed in the circulating fluid. The amounts of acid, or base secreted by the rectum were calculated from the titration curves of the two buffer solutions used as perfusate, consisting of 1 part isotonic phosphate buffer, mixed with 9 parts of isotonic NaCl solution, one adjusted to pH 5 and the other to pH 9. The perfusate (30 ml) was circulated at a rate of 10 ml/min at 37°C.

After 2 h the perfusion was stopped, the perfusate collected and the volume measured. After killing the rat with ether, the perfused part of the rectum was isolated. By gently pressing on filter paper, the adhering fluid and mucus was removed and the wet weight was established.

*Procedure for the rectal absorption studies*

The same procedure for perfusion was used. The loss of theophylline (pKa = 8.4) was recorded by taking 0.1 ml samples every 15 min over a period of 2 h. The solutions used to study the influence of the composition of the perfusate on the absorption rate were (the concentration of AP given as the equivalent amount of TP is 10 mg TP/30 ml):

(1) isotonic NaCl solution

(2) a, 1 part isotonic phosphate buffer + 9 parts isotonic NaCl solution: pH 9 (diluted phosphate buffer)
b, 1 part isotonic phosphate buffer + 9 parts isotonic NaCl solution: pH 5 (diluted phosphate buffer)

(3) a, isotonic phosphate buffer pH 10
b, isotonic phosphate buffer pH 7

In the experiments investigating the influence of the pH and the concentration of AP on the absorption rate, isotonic phosphate buffers of pH 10 and 7 containing 10, 50 and 130 mg TP in 30 ml were used. $Na_2HPO_4 \cdot H_2O$ was reagent grade; NaCl was aminophylline Dutch Pharmacopoeia grade. The contribution of aminophylline to the tonicity of the perfusate was small and could be neglected. The disappearance of TP from the lumen of the rectum does not necessarily imply that this amount actually reaches the blood circulation. However, it was reported by Kakemi et al. (1969) using the same perfusion technique with some closely related xanthine derivatives, that storage of these substances in the rectum wall was negligible.

*Analytical methods*

After dilution with 0.1 M NaOH, the concentrations of AP in the 0.1 ml samples from the perfusate were directly determined spectrophotometrically at 273 nm as TP. Our direct method agreed well with an analysis of TP after extraction from the perfusate (Kakemi et al., 1969).

To establish to what extent the volume of the perfusate might change, the final

volume of the solution was measured and, when phosphate buffers were used, the concentration of phosphate was determined by the method of Hurst (1964), modified by Wolff (1973), at the beginning and at the end of the experiments. Generally, the change in volume of the perfusate after 2 h was negligible, according to both the measurement of the final volume and the analysis of the phosphate concentration.

## RESULTS AND DISCUSSION

### *Measurement of the neutralizing capacity*

The change of the pH in the perfusate for the two buffer solutions with, respectively, initial pH values of 5 and 9, is shown in Fig. 1.

The rectal area appears to secrete acid or base to neutralize solutions with a pH deviating from the physiological pH. The amounts of acid or base secreted every 15 min are given in Fig. 2.

The standard deviations shown for each 15 min period are relatively large. Relating the secretion to the wet weight of the perfused part of the rectum, or the weight after drying at 100°C, did not bring about any improvement. For the pH 5 measurements, the amounts of base secreted every 15 min varied between about 80 and 60 μmol per kg rat per 4 cm rectum at, respectively, t = 0 and t = 2 h.

For the pH 9 experiments, the amounts of acid secreted were between 55 and 25 μmol per 15 min per kg rat per 4 cm rectum. Changes in the pH by external causes, as could occur, for example, by $CO_2$ attraction, were small compared to those produced by the rectum. Calculated on dry weight the amounts of base (pH 5) and acid (pH 9) produced were, respectively, 2.3 and 1.5 μmol per mg dry weight of rectum during the first hour. There are no directly comparable literature data available.

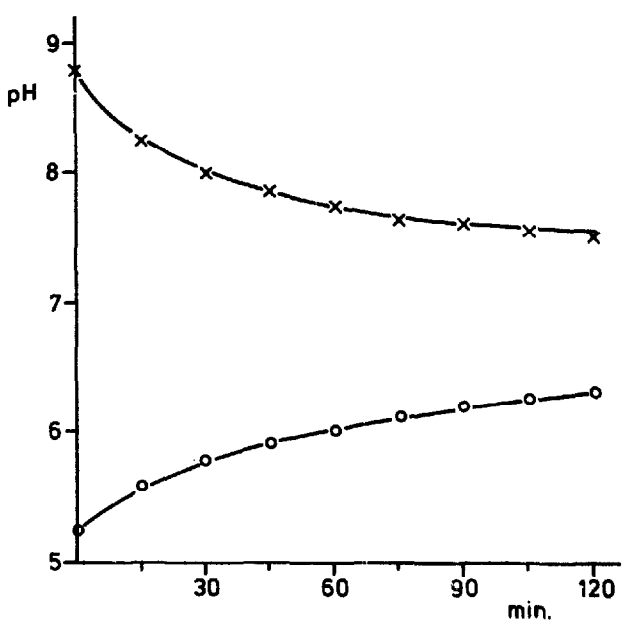


Fig. 1. pH in the perfusate versus time (diluted phosphate buffers). x, initial pH = 9; o, initial pH = 5. Each curve is the mean of 9 (pH 5) and 10 (pH 9) experiments.

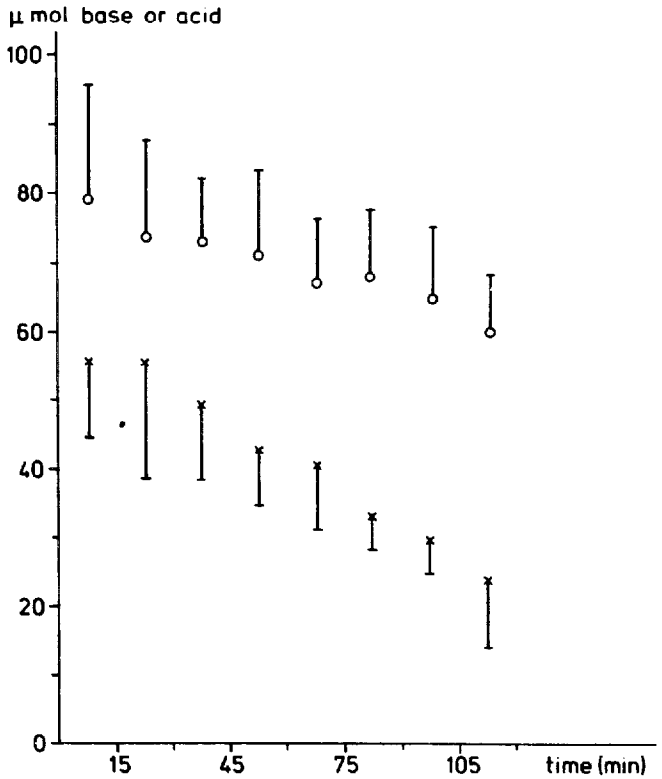


Fig. 2. µmol acid or base produced per kg rat and per 15 min for 4 cm of perfused rectum, versus time in min. Diluted phosphate buffers. ×, initial pH = 9; ○, initial pH = 5. Mean values are given in ± standard deviations calculated from 9 (pH 5) and 10 (pH 9) experiments.

With both the pH 5 and the pH 9 experiments, a decrease in the secretion with time occurred. When the pH of the bulk approaches the physiological pH (7.0–7.4), the secretion of acidic or basic agents decreased due to a decreasing stimulus, as is illustrated in Fig. 3. This decrease could not be ascribed to exhaustion of the rats, since replenishing the medium restored the neutralizing action.

In the experiments with the isotonic unbuffered NaCl solutions (see below) with 10 mg TP/30 ml perfusate, the pH fell from 8.09 to 7.38 in the first hour. In the second hour hardly any change occurred, indicating no further production of acid.

It can be concluded that the pH of the perfusate strongly influences the secretion of acid or basic agents, and consequently may determine whether a 'microclimate' exists near the membrane.

*The influence of the composition of the perfusate*

For studies on the possible effect of the composition of the perfusate on the absorption rate of TP, the perfusates as described in Materials and Methods were used, i.e. an isotonic NaCl solution, diluted phosphate buffers (pH 5 and 9) and isotonic phosphate buffers (pH 7 and 10). Some of the results are shown in Fig. 4, which gives a typical

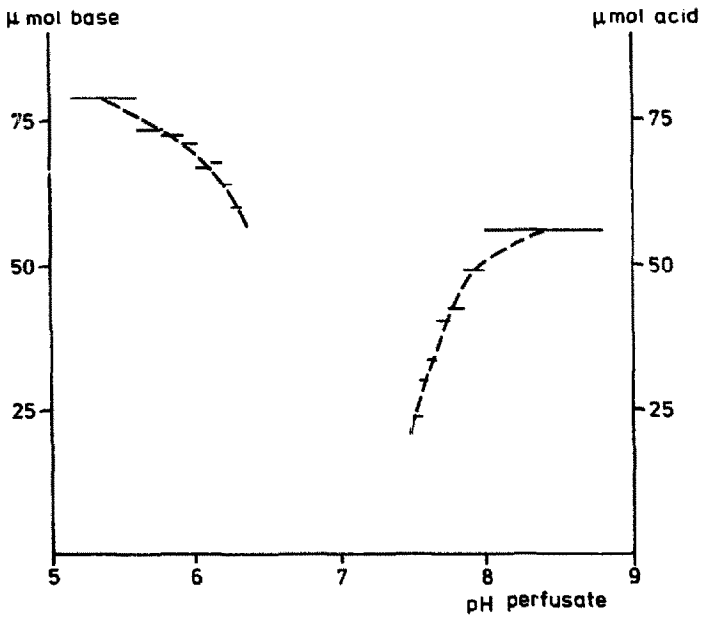


Fig. 3. µmol acid or base secreted per kg rat and per 15 min for 4 cm of perfused rectum versus the pH of the perfusate during a 15 min interval. The straight horizontal lines represent the mean pH values of 9 (pH 5) and 10 (pH 9) experiments during the 15 min intervals.

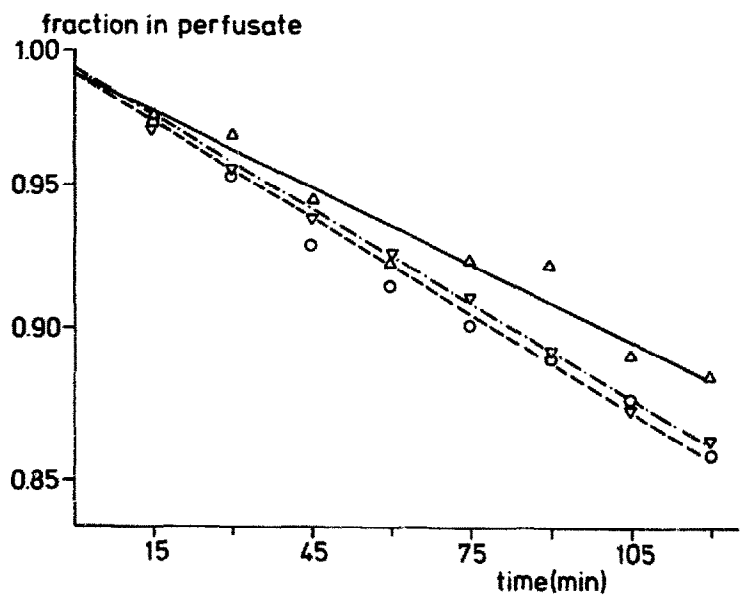


Fig. 4. Semi-logarithmic plots of the disappearance of theophylline from the perfusate. The data points represent the mean of 4 experiments. Least squares regression lines have been drawn. Concentration of aminophylline at t = 0: 10 mg TP/30 ml perfusate. △: pH 10, isotonic phosphate buffer; ▽: pH 9, diluted phosphate buffer; ○: isotonic NaCl solution.

example of all other curves obtained in this study. The least squares regression lines have been drawn, and from these the first-order absorption rate constants ($K_{abs}$) per 4 cm rectum have been calculated.

As can be seen from Table 1, the first-order rate constants are small and the standard deviations of the percentages absorbed after 2 h relatively large.

Relating the amount absorbed to the wet weight of the perfused part of the rectum, or the weight of the rat, did not lessen the differences between the 4 rats. Statistical analysis of the fractions of TP absorbed after 2 h (Student's *t*-test), did not show significant differences in the solutions under investigation.

Thus in this study no influence of the composition of the solutions perfused: isotonic NaCl solutions and phosphate buffers of different strength and pH, could be demonstrated.

*Influence of the pH and concentration of aminophylline on the absorption rate*

Isotonic phosphate buffers with three concentrations of AP were used: 10, 50 and 130 mg TP in 30 ml perfusate. The results of the experiments are given in Table 2.

The percentages absorbed in 2 h varied from 8.2 to 14.7%; the standard deviations of the absorbed fraction of TP after 2 h were large. Relating the amount of TP absorbed to the wet weight of the perfused part of the rectum or the weight of the rat, did not affect the differences between the rats. The pH remained constant when the buffers of pH 7 were perfused, but decreased with the buffers of pH 10. The fractions absorbed after 2 h of perfusing at pH 7 or pH 10 did not differ significantly (Student's *t*-test) at 10 mg TP/30 ml. But when the concentrations were 50 mg TP/30 ml or 130 mg TP/30 ml a significant difference, $0.02 < P_D < 0.05$ and $0.05 < P_D < 0.1$, respectively, occurred. Thus at 50 and 130 mg TP/30 ml the absorption rates were higher at pH 7 than at pH 10.

If the transport of TP through the membrane is governed by diffusion, the fractions absorbed during 2 h of perfusion have to be constant under sink conditions. In the present experiments, sink conditions were met because the TP concentrations in the serum measured, as described by de Blaey and de Boer (1976) after circulating a solution containing 130 mg TP/30 ml of pH 10 for 2 h, were about 8 $\mu$g TP/ml serum, i.e. very low compared with the concentration of TP (unionized) in the perfusate. In Table 2 the results of perfusion at pH 7 do not show a concentration dependence. For the experiments at pH 10 the low value of the absorption rate for the 50 mg TP/30 ml solutions was striking. However, from analysis of variance it appeared that the fraction absorbed was not dependent on the concentration, confirming one of the assumptions of the pH–partition theory. According to the same theory, a ratio between the absorption rates at pH 7 and pH 10 of about 1000 would be expected. However, only a small difference between the absorption rates was observed (Table 2). This means that our experimental results disagree with the statement of Kakemi et al. (1969) on the rectal absorption mechanism of xanthine derivatives. Two explanations for our results may be proposed.

First, it is generally assumed that mainly unionized species pass through the membrane. The results of our measurements of the neutralizing capacity indicate that a 'microclimate' can exist near the surface of the mucosa, when the luminal pH deviates from the physiological pH. Winne (1977) states that it is difficult to predict the pH

TABLE 1

THE INFLUENCE OF THE COMPOSITION OF THE PERFUSATE ON THE ABSORPTION RATE

Concentration of aminophylline at t = 0 is 10 mg TP/30 ml perfusate.

| Perfusate | pH (t = 0) ± S.D. | pH (t = 2 h) ± S.D. | % abs. (2 h) ± S.D. | $K_{abs}$ [a] ($min^{-1}$) | TP abs. [b] (mg/2 h) ± S.D. | TP abs. [c] (mg/2 h) ± S.D. |
|---|---|---|---|---|---|---|
| Isot. NaCl | 8.09 ± 0.02 | 7.37 ± 0.11 | 14.2 ± 2.4 | $12.2 \times 10^{-4}$ | 1.46 ± 0.20 | 3.84 ± 1.07 |
| dil. phosphate buffer pH 9 | 8.73 ± 0.06 | 7.69 ± 0.08 | 13.6 ± 3.5 | $12.0 \times 10^{-4}$ | 1.42 ± 0.38 | 3.54 ± 0.41 |
| dil. phosphate buffer pH 5 | 5.15 ± 0.04 | 6.10 ± 0.06 | 14.8 ± 2.9 | $13.7 \times 10^{-4}$ | 1.48 ± 0.25 | 3.71 ± 0.96 |
| isot. phosphate buffer pH 10 | 9.99 ± 0.02 | 8.46 ± 0.15 | 12.4 ± 0.8 | $9.9 \times 10^{-4}$ | 1.22 ± 0.11 | 3.23 ± 0.46 |
| isot. phosphate buffer pH 7 | 6.95 ± 0.00 | 6.96 ± 0.00 | 12.0 ± 3.0 | $10.2 \times 10^{-4}$ | 1.27 ± 0.35 | 3.32 ± 0.71 |

[a] per 4 cm rectum.
[b] per 200 g rat.
[c] per g wet rectum.

TABLE 2

THE INFLUENCE OF THE pH AND CONCENTRATION OF AMINOPHYLLINE ON THE ABSORPTION RATE

| Perfusate conc. (mg TP/30 ml) | pH (t = 0) ± S.D. | pH (t = 2 h) ± S.D. | % abs. (2 h) ± S.D. | $K_{abs.}$ [a] ($min^{-1}$) | TP abs. [b] (mg/2 h) ± S.D. | TP abs. [c] (mg/2 h) ± S.D. |
|---|---|---|---|---|---|---|
| 10 | 9.99 ± 0.02 | 8.46 ± 0.15 | 12.4 ± 0.8 | $9.9 \times 10^{-4}$ | 1.22 ± 0.11 | 3.23 ± 0.46 |
| 10 | 6.95 ± 0.00 | 6.96 ± 0.00 | 12.0 ± 3.0 | $10.2 \times 10^{-4}$ | 1.27 ± 0.35 | 3.32 ± 0.71 |
| 50 | 9.94 ± 0.02 | 9.11 ± 0.13 | 8.2 ± 2.2 | $7.4 \times 10^{-4}$ | 4.75 ± 1.13 | 12.9 ± 1.8 |
| 50 | 6.95 ± 0.00 | 6.98 ± 0.00 | 14.1 ± 3.9 | $12.1 \times 10^{-4}$ | 7.28 ± 1.80 | 21.6 ± 2.9 |
| 130 | 10.0 [d] | 9.50 [d] | 11.4 ± 3.i | $10.8 \times 10^{-4}$ | 16.0 ± 3.6 | 48.0 ± 12.6 |
| 130 | 6.98 ± 0.00 | 7.01 ± 0.00 | 14.7 ± 0.7 | $12.0 \times 10^{-4}$ | 18.3 ± 2.0 | 68.3 ± 7.2 |

[a] per 4 cm rectum.
[b] per 200 g rat.
[c] per g wet rectum.
[d] not available.

dependence of the absorption rate when secretion to neutralize the luminal contents occurs. Secretion of acids or bases can imply that the pH at the rectal mucosa is constant. Then the concentration of unionized TP at the surface will not depend on the luminal pH, and there will be no dependence on the pH of the absorption rate. In that case the neutralizing capacity of the rectum has to be sufficient to maintain this physiological pH of the mucosa. From the pH and the pKa values of the phosphate, TP and ethylenediamine, it can be calculated that for the 10 mg TP/30 ml solutions (pH 10) the capacity of the rectum to secrete acid was at least around 200 μmol/2 h. This amount far surpasses the acid required for the neutralization of the 7 μmol $TP^-$ absorbed in 2 h. The neutralizing capacity appeared to be sufficient with this low concentration of aminophylline. Table 2 shows a decreasing pH drop during the experiments, when the concentration of AP in the perfusate increased at constant phosphate concentration. The reason is that in the 50 and 130 mg TP/30 ml solutions, more acid was necessary for the neutralization of the $TP^-$ and the ethylenediamine. The role of the phosphate ions in buffering the perfusate decreased, and the $TP^-$ and ethylenediamine consumed more acid; in these cases information is lacking to decide whether the neutralization capacity has been high enough for the neutralization of the amount of theophylline absorbed.

Secondly, our results would be explained by assuming that the absorption rate of the ionized TP is not negligible compared to the rate of the unionized molecule TP. In a manner analogous to Crouthamel (1971) we calculated the absorption rate constants for the ionized and the unionized form. As the pH changed during the experiments, the $TP/TP^-$ ratio also varied, and consequently it is possible to calculate the absorption rate constant of the ionized and the unionized form of TP from one experiment. The mean values of the absorption rates of the TP molecule ($K_u$) and TP ion ($K_i$) calculated from the experiments with solutions of AP (10 mg TP/30 ml) with pH 10, pH 9 and isotonic NaCl, were: for $K_u$ $3.3 \times 10^{-5}$ and for $K_i$ $4.1 \times 10^{-5}$ litre per minute per 4 cm rectum. An estimation of $K_u$ and $K_i$ can also be made by comparing the absorption rates from experiments with nearly constant pH, and almost all theophylline present, either unionized or ionized, during perfusion. These conditions were approximated in the experiments with AP concentrations of 130 mg TP/30 ml at pH 7 and pH 10 (Table 2). The constants were, respectively, $3.6 \times 10^{-5}$ ($K_u$) and $3.2 \times 10^{-5}$ ($K_i$) liter per minute per 4 cm rectum. These results would indicate that the rates of absorption of the unionized and ionized form do not differ much. In the literature the absorption rate constants of the unionized species are nearly always reported as surpassing the ionized species in situ in rats (Crouthamel, 1971; Kakemi et al., 1969), however.

In conclusion, it may be stated that under the conditions met in this study:

(a) the rectum is able to secrete neutralizing agents, when the luminal pH deviates from the physiological pH. The degree of the secretion depends on the magnitude of the deviation;

(b) changing the composition of the perfusate from an isotonic NaCl solution into an isotonic phosphate solution did not influence the absorption rate of TP;

(c) the fraction of TP absorbed remained constant with increasing concentration; and

(d) the absorption of TP showed only a small pH dependence.

This last result (d) disagrees with the pH–partition theory. Two explanations were proposed. The results of the measurements of the neutralizing capacity of the rectum of a

rat give strong support to the 'microclimate' hypothesis. Recently also Schürmann and Turner (1978) have proposed an analogous mechanism for buccal absorption.

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