The absorption of warfarin from the rat small intestine *in situ*

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The *in situ* absorption from the rat small intestine of the weakly acidic drug, warfarin (pKa 5·05), at 200 μg ml⁻¹ in the instilled fluid with initial pH levels of 3, 5, 7 or 8 has been examined. These initial pH's in the buffer changed rapidly towards neutrality. The buffers at pH's 3 and 5 probably caused different amounts of warfarin precipitation, which resulted in different rates of warfarin disappearance from the instilled fluid which paralleled the initial rates of accumulation of warfarin in (or on) the intestinal wall. Where greater drug precipitation had probably occurred the initial rates of absorption into the plasma were slower. At the initial pH of 3 and by solubilization of warfarin with propylene glycol, the rate of absorption was similar to that from a fluid of pH 7. Propylene glycol in 15 % solution did not affect the system significantly. The relatively high transfer of warfarin into octanol from buffer solution at pH 7 might indicate that the small fraction of unionized drug (1 : 100) at pH 7 is enough for remarkable transfer of this highly lipid-soluble drug.

Since the results concerning the absorption of warfarin, a weakly acidic drug (pKa 5·05), in man (Pyörälä, Jussila & others, 1970) and *in vitro* (Julkunen, Pyörälä & others, 1971) could not satisfactorily be interpreted on the basis of the pH-partition theory (Schanker, Tocco & others, 1958; Hogben, Tocco & others, 1959) the effect of pH on the absorption of warfarin *in situ* has been investigated. The aim was to a certain degree also to simulate *in vivo* circumstances under which acidic drug solution containing hydrochloric acid passes from the stomach into the intestine.

MATERIALS AND METHODS

Animals
Male Spraque-Dawley rats, 180–260 g, maintained on a commercial pellet diet, were fasted for 16–20 h before the experiments, water being freely available.

Intestinal absorption procedure
A modification of the method of Doluisio, Billups & others (1969) was used. The small intestine was exposed by a midline abdominal incision under ethyl carbamate anaesthesia (1·2 g kg⁻¹, i.p., Riedel-DeHäen AG Scelze, Hannover) and an L-shaped polyethylene cannula was inserted through a small slit both at the duodenal and ileal end. A syringe fitted with a two-way stopcock was attached to each of the cannulae. The lumen of the intestine was rinsed with a continuous flow of Krebs bicarbonate saline (Krebs & Henseleit, 1932) pH 7·4, in which the KH_2PO_4 was replaced by equimolar amounts of CH_3COOH, and $MgSO_47H_2O$ by $MgCl_26H_2O$. This was expelled with air before the instillation of warfarin sodium (Orion OY, Helsinki) 200 μg ml⁻¹ in

Krebs at 37° to a total volume of 40 ml kg⁻¹. The pH of the fluid was adjusted to 3, 5, 7 or 8 with HCl or NaOH.

Samples of the luminal fluid, blood and whole intestine were taken 1, 2, 4, 8, 15, 30 and 60 min after the introduction of the fluid, a different rat being used at each sampling time. The blood sample was taken into a heparinized tube (2 IU ml⁻¹) from a quick heart puncture and the whole intestine was rapidly removed, drained and homogenized. In separate experiments blood and intestine were also examined 120, 240 and 480 min after the beginning of the experiment. All samples were stored at −20° for the assay of warfarin according to O'Reilly, Aggeler & others (1962).

Warfarin was also dissolved in propylene glycol (Fluga AG) and made up to a 15 % propylene glycol-buffer solution at either pH 3, to avoid precipitation, or at pH 7. At pH 3 precipitation was prevented for 10–15 min and samples were therefore taken at 2, 4 and 8 min.

Propylene glycol 15 % increased the apparent pKa of warfarin as determined in distilled water by microtitration (Beckman Zeromatic pH-meter), using 0·01 N HCl and 0·5 mequiv litre⁻¹(0·17 μg ml⁻¹) of warfarin sodium solution from 4·92 ± 0·00 to 5·14 ± 0·00 (s.e.m., n = 5), an effect too small to account for the results obtained.

Partitioning of warfarin between octanol and propylene glycol in water
Equal amounts (40 ml) of n-octanol (Merck) and buffer containing warfarin sodium (200 μg ml⁻¹), with or without 15 % of propylene glycol, were shaken for 5 min, the buffer solution and organic

solvent having previously been shaken and equili-
brated overnight. Shaking for 60 min and allowing
the solutions to stand for 24 h did not change the
results. The difference (%) between the contents of
warfarin in the buffer solution before and after
shaking was considered to represent the amount
diffused into octanol. The experiments were carried
out at 20–22°.

Refractive indices
No significant changes in refractive indices were
found between the warfarin buffer solution and war-
farin buffer-propylene glycol solution at pH 3, 6 or 7.
This suggests that no interaction occurs between the
compounds (Kakemi, Arita & Muranishi, 1965).

pH measurements
The initial pH's of the intestinal buffer solutions were
measured with a pH-meter (Titrator TTT 2, Radio-
meter, Copenhagen). The pH of the fluid at the end
of the experiment was measured with special pH-
indicator papers: Acilit (pH 0·5–5·0); (pH 5·4–7·0)
E. Merck AG; MN (pH 6·8–8·0) Mecherey, Nagel &
Co. The accuracy of the pH-papers was checked
from time to time with the pH-meter. Other pH
measurements were carried out by using the pH-
meter.

RESULTS

Warfarin concentration and pH in the intestinal fluid
The warfarin concentration in the instilled solution
declined rapidly at all pH values being faster at the
initial pH of 3 and 5 than at pH 7 or 8 (Fig. 1). Also
the initial pH values were rapidly neutralized (Fig. 2).
The decrease of warfarin concentration in the fluids
and pH changes became slower from 4-8 min on-
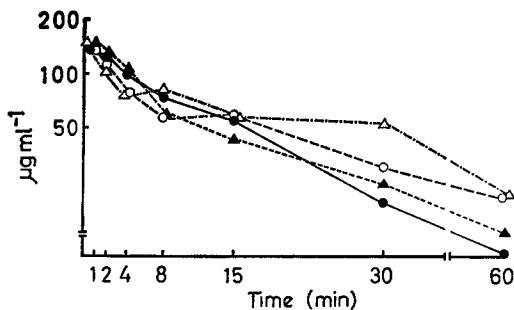wards. From 15 min onwards the drug concentra-

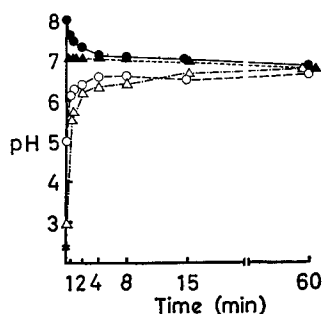FIG. 2.　Changes in the pH of the luminal solutions.
Means of 4–6 experiments; standard errors were 0·0–
2·5% of means. Initial pH values 3 ····△····, 5 -- ○ --,
7 --- ▲ ---, 8 —●—.

tions in fluids with initial pH values of 7 and 8 were
lower than in those with the initial pH values of
3 or 5.

Warfarin concentration in the plasma
The rise in the plasma warfarin concentration was
rapid and similar when the initial pH of the instilled
fluid was 7 or 8 (Fig. 3) with peaks being attained in
15–30 min. When the initial pH was 3 or 5 the rise
was markedly slower. The mean plasma concentra-
tions obtained with the initial pH of 3 in the fluid
were significantly ($P < 0.05$) lower than those
obtained with pH 7 or 8 at 1, 2, 4, 8, 15, 30 and 60
min. The values in experiments with pH 3 were also
significantly ($P < 0.05$) lower than those obtained
with pH 5 at 1, 8, 15 and 60 min.

Warfarin concentration in the intestinal wall
The warfarin concentration in the intestinal wall in-
creased with the increasing initial acidity of the
luminal fluid for 2 min and for 30 min it remained
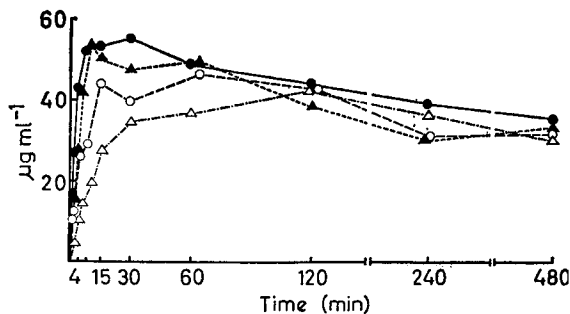higher with solutions of initial pH of 3 and 5 than

FIG. 1.　The decline of warfarin concentration (μg ml^{-1})
in intestinal fluid for initial pH values of 3 ····△····,
5 -- ○ --, 7 --- ▲ ---, 8 —●—. Means of 4-6
experiments; standard errors were 0·3–26% of means.

FIG. 3.　Plasma warfarin concentrations (μg ml^{-1}) in
experiments with initial pH of 3 ····△····, 5 -- ○ --,
7 --- ▲ ---, 8 —●—. in luminal fluids. Means of 4-6
experiments; standard errors were 5–28% of means.

with those of pH 7 or 8 (Fig. 4). After that time the wall concentrations became similar.
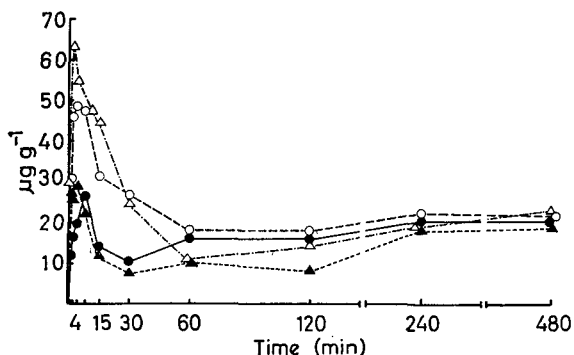

FIG. 4. Warfarin concentration (μg g^{-1}) in homogenate of intestine after instillation of warfarin sodium solution with initial pH of 3 ···△···, 5 ‑‑ ○ ‑‑, 7 ‑‑‑▲‑‑‑, 8 —●—. Means of 4–6 experiments; standard errors were 3–42 % of means.

Effect of propylene glycol on the absorption of warfarin

In the experiments with 15% propylene glycol the warfarin concentrations of the luminal fluid and plasma were not found to differ significantly when the initial pH was 3 or 7 (Table 1). With the initial pH of 7 the plasma concentrations indicated that propylene glycol caused a slight decrease in the rate of absorption when compared with that from Krebs solution. With the initial pH of 3 propylene glycol enhanced the rate.

Effect of propylene glycol on the partitioning of warfarin into octanol

The diffusion of warfarin into octanol was greater (99·6 ± 0·0 % vs 74·7 ± 0·4 %, means of 5 experiments ± s.e.m.) when the drug was mainly in its unionized form at pH 3 than when it was mainly in its ionized form at pH 7. Propylene glycol had no pronounced effect on the partitioning of warfarin

into octanol at either pH 3 (98·5 ± 0·5 %) or 7 (69·6 ± 0·3 %). No significant change took place in the pH of the buffer during the experiment.

DISCUSSION

The disappearance of drug from the intraluminal fluid of the rat small intestine *in situ* has been widely used in estimating the rate and amount of drug absorption (Schanker & others, 1958; Hogben & others, 1959; Doluisio & others, 1969). The present results indicate that measurement of luminal drug concentration alone may sometimes be misleading without concurrent measurement of the plasma concentrations. The initial rate of the decline of the warfarin concentration from the lumen was more rapid with acidic than basic or neutral solutions, that at pH 3 being greater than that at pH 5 > pH 7 or 8. This might be considered as indicating more rapid absorption from acidic than from neutral or basic intestinal contents. However, the curves obtained for the concentration of warfarin in the plasma clearly demonstrated the opposite: the initial rise in the plasma warfarin concentrations was slower with the acidic solutions.

Measurement of the warfarin content of the intestinal wall proved that with initially acidic solutions there was a fast and marked accumulation of warfarin in the intestinal wall, and at least part of the drug was visible as precipitates on the mucosa at pH 3 and pH 5. The precipitates probably dissolved later since solutions with widely different pH values were rapidly neutralized in the intestine, a fact also reported by Morishito, Yata & others (1971). The initial rates of absorption into the plasma were slower at the lower pH values (pH 3<5<7 = 8) probably due to the precipitation.

The apparent decrease in the rate of absorption after the initial phase in the luminal fluid concentrations was probably caused by proportionally greater absorption of water marked absorption of which was

Table 1. *Warfarin concentration in the intestinal solution and in the plasma with and without propylene glycol in the luminal fluid with the initial pH of 3 and 7.*

| Time (min) | With propylene glycol | | | | Without propylene glycol | | | |
| | Luminal fluid μg ml^{-1} ± s.e.m.* | | Plasma μg ml^{-1} ± s.e.m.* | | Luminal fluid μg ml^{-1} ± s.e.m.* | | Plasma μg ml^{-1} ± s.e.m.* | |
	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7
2	108·6 ± 5·6	111·0 ± 8·0	27·1 ± 4·9	23·1 ± 2·3	110·2 ± 9·7	132·6 ± 3·6	11·2 ± 2·7	28·7 ± 3·1
4	89·0 ± 1·0	96·6 ± 2·2	34·4 ± 1·2	29·7 ± 3·4	78·4 ± 13·4	106·8 ± 0·3	15·2 ± 4·2	42·5 ± 2·3
8	69·1 ± 4·3	76·0 ± 4·0	41·4 ± 2·3	44·5 ± 8·7	89·6 ± 7·6	60·2 ± 3·5	20·5 ± 1·2	54·8 ± 2·8

* Mean values of 4–6 animals at each time period.

suggested by the finding that no luminal fluid samples could be collected after 60 min. Although Doluiso & others (1969) reported neglible absorption of water with this kind of technique, other investigators have found it to be very marked (Levy & Perälä, 1970; Pelzmann & Havemeyer, 1972).

Prevention of the precipitation of warfarin in an acidic environment by first dissolving it in propylene glycol initially increased its rate of absorption from the pH 3 solution to equal that from the neutral pH 7 solution. The fact that the rate did not increase beyond that from neutral solution does not necessarily favour the interpretation that warfarin may have been absorbed in its ionized form, since the pH of the acidic luminal solution was almost neutralized within 4 min. The effect of propylene glycol was apparently not exerted through changes in the pKa or in the lipid-solubility of warfarin, but mainly through its solubilizing effect. Specific interaction between warfarin and propylene glycol as assessed by measurement of refractive indices also seemed to be excluded.

The pH-dependence of the diffusion of warfarin into octanol agreed fairly well with the luminal fluid concentrations, which suggested preferential 'absorption' from acidic solution compared with neutral fluid. However, plasma concentrations of warfarin did not support this, since higher initial values were found when the initial luminal pH values were more basic. The fact that there was not a big difference in the percentage of the drug partitioned into octanol from buffer solutions with pH 3 and 7 would suggest that the lipid solubility of warfarin is of such magnitude that even the small amount of unionized molecules (1:100) in the buffer of pH 7 might be sufficient to cause substantial transfer of the drug into the organic phase as with mecamylamine (Goldstein, Aronow & Kalman, 1974). The observations *in situ* suggest that knowledge of such physicochemical properties of drugs as their lipid solubility and degree of ionization, does not always allow the actual nature of the absorption of a drug to be forecast without studies on blood concentrations.

Keberle (1971) has presented several other examples in which the absorption of drugs could not be foreseen from their physicochemical properties. A 'virtual' pH of 5·3 at the mucosal surface of the intestine has been postulated to explain certain cases which would otherwise appear to indicate preferential absorption of the ionized form of the drug (Hogben & others, 1959). In fact, thermodynamic calculations do not support this explanation and direct measurements of the pH at the mucosal surface of the intestine at pH 7·0 of the intraluminal solution suggest that the pH at the surface is closer to 7·4 than to 5·3 (Smolen, 1973). The apparent differences in the absorption of warfarin from the pH-partition hypothesis as occurring in the plasma concentrations are probably explainable on the basis of drug precipitation at low pH values, which slows the absorption, and by high lipid-solubility of warfarin, which allows fast absorption even when the unionized fraction of the drug concentration is minimal at neutral or basic pH values.

As a result of the low solubility of the drug in an acidic environment the gastric absorption of this weakly acidic drug is slow (the peak plasma concentration obtained with the same dose of warfarin sodium as in this study and with the initial pH of 3 in the gastric fluid was only 5·5 μg ml^{-1}; Julkunen, unpublished results). Under *in vivo* conditions it would thus seem probable that the gastric contents with precipitated warfarin are rapidly neutralized in the intestine and the drug precipitates dissolve allowing fairly rapid absorption.

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