The absorption of benorylate from everted sacs of rat intestinal

The recently introduced anti-inflammatory compound 4-acetamidophenyl-*O*-acetylsalicylate (benorylate) has been shown to be similar to aspirin in its analgesic (Hart & Nicholson, 1971), antipyretic (Weill, 1968) and anti-inflammatory properties (Bain & Burt, 1970; Beales, Burry & Grahame, 1972). In the treatment of rheumatism and arthritis, benorylate has a major advantage over aspirin in that it does not cause gastric bleeding (Croft, Cuddigan & Sweetland, 1972; Danhof, Kailey & Guinn, 1972). The most satisfactory dose form appears to be a 40% w/v suspension (Benoral, Sterling–Winthrop). The compound is thought to be absorbed intact from the intestine into the blood stream, where it undergoes rapid hydrolysis catalysed by plasma esterases to its parent compounds aspirin and paracetamol (Robertson, Glynn & Watson, 1972). The present experiments were carried out as preliminary investigations into the mechanism of absorption of benorylate.

Studies were made using the everted sac technique of Wilson & Wiseman (1954). Mature Wistar strain rats of either sex, 200–300 g and previously fasted for 24 h, were used. Six everted sacs of 5 cm length were constructed from measured intervals along the length of the small intestine. Each sac was filled with 1 ml Krebs Ringer bicarbonate buffer solution and incubated at 37° for 45 min in agitated flasks containing 20 ml Krebs buffer solution gassed with 5% CO_2 in oxygen. Mucosal scrapings were produced by opening 5 cm lengths from measured intervals along the small intestine, rinsing with buffer solution and scraping the mucosal lining to remove the mucosal cells. After weighing, the mucosal scrapings were placed in 20 ml Krebs buffer solution at 37° for 45 min. In each experiment, drugs were added to the mucosal fluid of all flasks except that containing the sac or scrapings taken from the pyloric end of the intestine. This proximal intestinal tissue served as a control for the analyses of serosal fluids.

Thin layer chromatography (t.l.c.) on dichloromethane extracts from pooled aliquots of serosal or mucosal fluids was carried out on MN Polygram Silica Gel sheets, N–HR/UV254 (200 μg thick), developed in a solvent system of benzene–diethyl ether–glacial acetic acid–methanol (70:20:9:1) at 20° and examined under ultraviolet light. The salicylate concentration of diluted mucosal and serosal fluids was measured by means of an Aminco–Bowman spectrophotofluorimeter, the relative fluorescent intensity obtained at 295 nm (activation) and 400 nm (transmission) being converted to salicylate concentration with reference to standard solutions of sodium salicylate in water.

T.l.c. of serosal and mucosal fluids after incubation of everted sacs with 1 or 5 mg benorylate suspension ml^{-1} Krebs solution in the mucosal fluid revealed the presence of the major metabolites salicylate and paracetamol in both fluid compartments in all samples tested. However, benorylate itself could not be detected in the serosal fluid. The concentration of salicylate in the serosal fluid of everted sacs was correlated with distance of the sac from the ileo-caecal valve whether the mucosal solution contained 1 mg ($n = 30$) or 5 mg ($n = 25$) benorylate suspension ml^{-1}, and the salicylate transport was greater in sacs incubated in high benorylate concentration than in low concentration (Fig. 1). At the end of the incubation time the mucosal fluids contained salicylate at concentrations 2–4 times greater than that found in the serosal fluids, but the concentrations were not significantly correlated with distance along the gut (concentration from 1 mg, benorylate ml^{-1}: mean 141 $\mu g\ ml^{-1}$ s.e. $\pm$ 13, $n = 10$; from 5 mg benorylate ml^{-1}: mean 213 $\mu g\ ml^{-1}$, s.e. $\pm$ 22, $n = 10$).

Incubation of 1 mg benorylate ml^{-1} Krebs solution with mucosal scrapings produced salicylate, but again the amount formed was not significantly correlated with distance

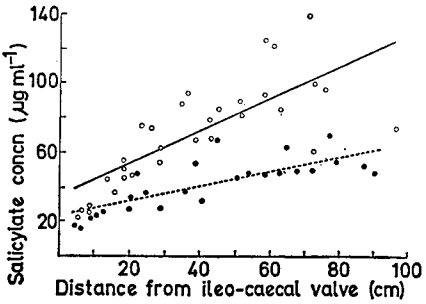

FIG. 1. Salicylate concentrations in serosal fluids of everted intestinal sacs of the rat incubated in mucosal fluids containing 1 mg (●) or 5 mg (○) benorylate ml^{-1} Krebs solution. The lines are calculated regression lines: 5 mg ml^{-1}, m = 0·9332, c = 34·8, r = 0·7316, $P < 0·001$; 1 mg ml^{-1}, m = 0·4333, c = 23·5, r = 0·7580, $P < 0·001$.

along the gut (mean 202 μg per 100 mg cells, s.e. $\pm$ 37, n = 20) and was not altered by addition of 0·5 ml rat blood to the mucosal scrapings (mean 173 μg per 100 mg cells, s.e. $\pm$ 5·5, n = 5, $P > 0·5$).

Aspirin, salicylic acid and sodium salicylate added to the mucosal fluid of everted sacs in a concentration of 1 mg ml^{-1} Krebs solution also showed no differential absorption along the length of gut (mean serosal salicylate concentration 358 μg ml^{-1}, s.e. $\pm$ 17, n = 21).

From these experiments, it would seem that there is no evidence for the transport of the intact benorylate molecule across the intestinal wall in rats. Since the major metabolites were found in the serosal fluid, it is possible that benorylate could have been hydrolysed to aspirin and paracetamol in the mucosal fluid before absorption, or that benorylate could have been absorbed into the mucosal cells and, following intracellular hydrolysis, appeared in the serosal fluid as free salicylate. The increase in salicylate concentration towards the pylorus (Fig. 1) could have been the result of a differential rate of benorylate hydrolysis along the intestine, either within the mucosal fluid (intralumenal) or within the cells. However, since mucosal scrapings produced the same degree of hydrolysis in different regions of the gut, it would seem unlikely that the promoted transport towards the pylorus was a result of increasing enzyme content of the mucosa. Furthermore, since there was no significant variation in the salicylate concentrations formed in the mucosal fluids of everted sacs, there would appear to be no difference along the gut in the enzyme secretion into the "lumen" or in the activity of this secreted enzyme. It is also unlikely that there is a differential absorption of the formed salicylates along the gut, since aspirin, salicylic acid and sodium salicylate showed no differential absorption. Thus, although the concentration of free salicylate on the mucosal side of 2–4 times greater than that found on the serosal side indicates the possibility of a passive diffusion gradient, there is little supportive evidence that this mucosal salicylate is responsible for the *increase* in serosal salicylate towards the pylorus.

It remains possible that the increase in salicylate transport towards the pylorus was a result of differential absorption of benorylate into the mucosal cells and, following intracellular hydrolysis, the salicylate appeared in the serosal fluid. The greater transport of salicylate at the higher benorylate concentration (Fig. 1) could have been a reflection of the greater rate of entry of benorylate at this higher concentration. From the present data, it is evident that in the rat there are present in mucosal cells esterases capable of effecting hydrolysis of benorylate. Since 0·5 ml blood added to the incubation medium containing mucosal scrapings did not enhance the hydrolysis, it is unlikely that the small volume of blood present in mucosal vessels in everted sacs

was sufficient to account for the observed hydrolysis. Whether the hydrolysis is intracellular or intravascular (Robertson & others, 1972) the entry of the intact benorylate molecule into mucosal cells appears to be an important step in the absorptive mechanism. The physicochemical form of presentation of intact benorylate at the mucosal cells would therefore be an important factor in the rate of its absorption into cells.

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The effect of ticarcillin on the haemostatic mechanism

Ticarcillin (disodium-α-carboxy-3-thienyl methyl penicillin) is a new semi-synthetic antibiotic with a structure and spectrum of antibacterial activity similar to that of carbenicillin (disodium-α-carboxy benzyl penicillin). It is as active as carbenicillin against *E. coli*, *Enterobacter* and indole-positive proteus species, but has at least a two fold greater activity against strains of *Pseudomonas aeruginosa* (Neu & Winshell, 1970; Sutherland, Burnett, & Robinson, 1971; Lynn, 1973). Adverse effects on the haemostatic mechanism have been reported during carbenicillin treatment when serum concentrations of the drug are of the order of 200 μg ml^{-1}. Such effects include the onset of a haemorrhagic diathesis (Gordon, 1970; Lurie & Goldberg, 1970; Waisbren, Evani & Ziebert, 1971; Yudis, Mahood & Maxwell, 1972; Brown, Natelson & others, 1974; Demos, 1971), prolongation of the thrombin, prothrombin, kaolin cephalin clotting time and bleeding time (Lurie, Gold & others, 1970) and depressed platelet aggregation to ADP (McClure, Casserly & others, 1970; Lederer, Davies & others, 1973) both *in vivo* and *in vitro*. In view of the reported effects of carbenicillin on the haemostatic mechanism, and the structural similarity and usage of ticarcillin, the *in vivo* and *in vitro* effects of ticarcillin on the haemostatic mechanism have been investigated.

Procedures for coagulation and fibrinolytic assays have been described previously (Lederer & others, 1973). Serum concentrations of ticarcillin were estimated by a microbiological assay using *Pseudomonas aeruginosa* as test organism.