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PARIETAL CELLS OF THE STOMACH TRAP SALICYLATES DURING ABSORPTION

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Gastric irritation, ulceration and bleeding are frequent side effects of aspirin and other acidic, non-steroid anti-inflammatory drugs (NSAID)[1]. These compounds are contained in most analgesic, antirheumatic drugs in medical use. An understanding of the cellular and molecular mechanisms involved in the development of gastric damage is essential if the therapeutic effectiveness of known NSAID is to be improved and less toxic compounds developed. Many theories have been proposed to explain why NSAID damage the gastric mucosa. Some of them leave crucial events unexplained, while others lack experimental proof. For example, it has been suggested that aspirin blocks the synthesis of prostaglandins, which normally restrain secretion of acid and thus inhibit gastric irritation[2]. This cannot be accepted as an adequate explanation because (a) non-acidic inhibitors of prostaglandin synthesis do not cause gastric irritation, and (b) acidic NSAID apparently do not - as would be expected - increase, but decrease acid secretion[3]. The attractive hypothesis put forward by Martin[4], on the other hand, lacks experimental proof. On the basis of biophysical considerations he concluded that trapping of large amounts of salicylate anions together with protons would occur in cells of the stomach mucosa during absorption. These molecules would then disturb the buffer system of the cell, interfere with mitochondrial functions and eventually lead to cell death. However, not all cell types of the stomach mucosa appear to be equally sensitive to salicylates and to show signs of decay after aspirin administration. One explanation is that not all types of cells accumulate salicylates to the same extent during absorption. Theoretical[5], clinical[6] and morphological[7] observations indicate that parietal cells of the stomach which are actively secreting protons may take up particularly high concentrations of salicylate during absorption and that damage to the gastric mucosa starts with the decay of these cells. In this communication, evidence is presented that such selective accumulation of salicylates does take place and that it can be visualized by autoradiographic methods. Commercially available radioactive salicylic acid was used in these experiments. Whole-body auto-

radiographs of young rats (40 g body weight) taken 45 min. after the administration of ^{14}C-salicylic acid* show accumulation of activity in the kidney and especially in the stomach wall. An example is shown in fig. 1. Interestingly, little activity is found at this time in the stomach lumen or the gut.

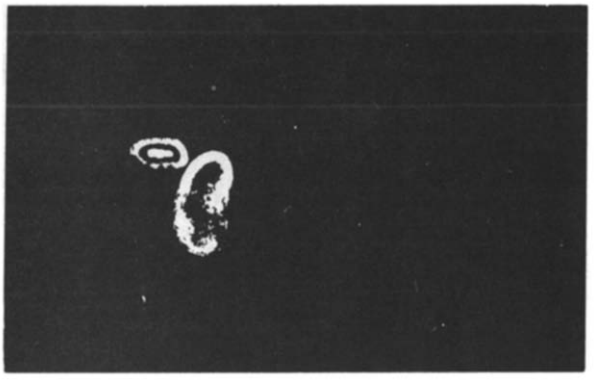

Fig. 1. Autoradiograph of a rat treated with ^{14}C-salicylic acid. At time 0 the drug (10 µCi in 1mg/100g) was administered by stomach tube. Forty-five minutes later the rat was exsanguinated, deep frozen and prepared for autoradiography. Slices (100µ) were mounted on X-ray film and exposed for three weeks. The autoradiograph obtained shows high activity in the stomach and the kidney.

The high concentration of salicylic acid in the stomach wall prompted further investigation of drug distribution between the different compartments of the stomach wall, i.e. the non-glandular part (rumen), which has a cornified epithelium, and the glandular part, the mucosa of which is comparable to that found in humans. To establish the time course of quantitative changes in drug concentration during absorption, the concentrations in the glandular and non-glandular tissues of rat stomach were measured at different times following administration. The results are given in fig. 2. The two different parts of the stomach show a strikingly different pattern of drug concentration. There was a slow increase of activity in the non-glandular part of the stomach, reaching a maximum at about 45 min. after drug administration. By contrast, in the glandular part of the stomach, the highest concentrations were found only one minute after administration. Thereafter, the concentration declined rapidly and had fallen to almost blood levels after about 45 min.

These findings suggested that in certain cells of the glandular stomach particularly high drug concentrations might be expected shortly after drug administration. Hence, we tried to obtain autoradiographic recordings of thin (5µ) slices of the stomach wall removed 1,5 and 15 min. after administration of ^{3}H-salicylic acid*. The biochemical data indicated very rapid diffusion of salicylic acid across the stomach wall and a suitable method was therefore sought which would provide immediate fixation of tissue and drug for autoradiography purposes. Preliminary experiments using different fixation and embedding techniques showed that immediate deep freezing of the stomach

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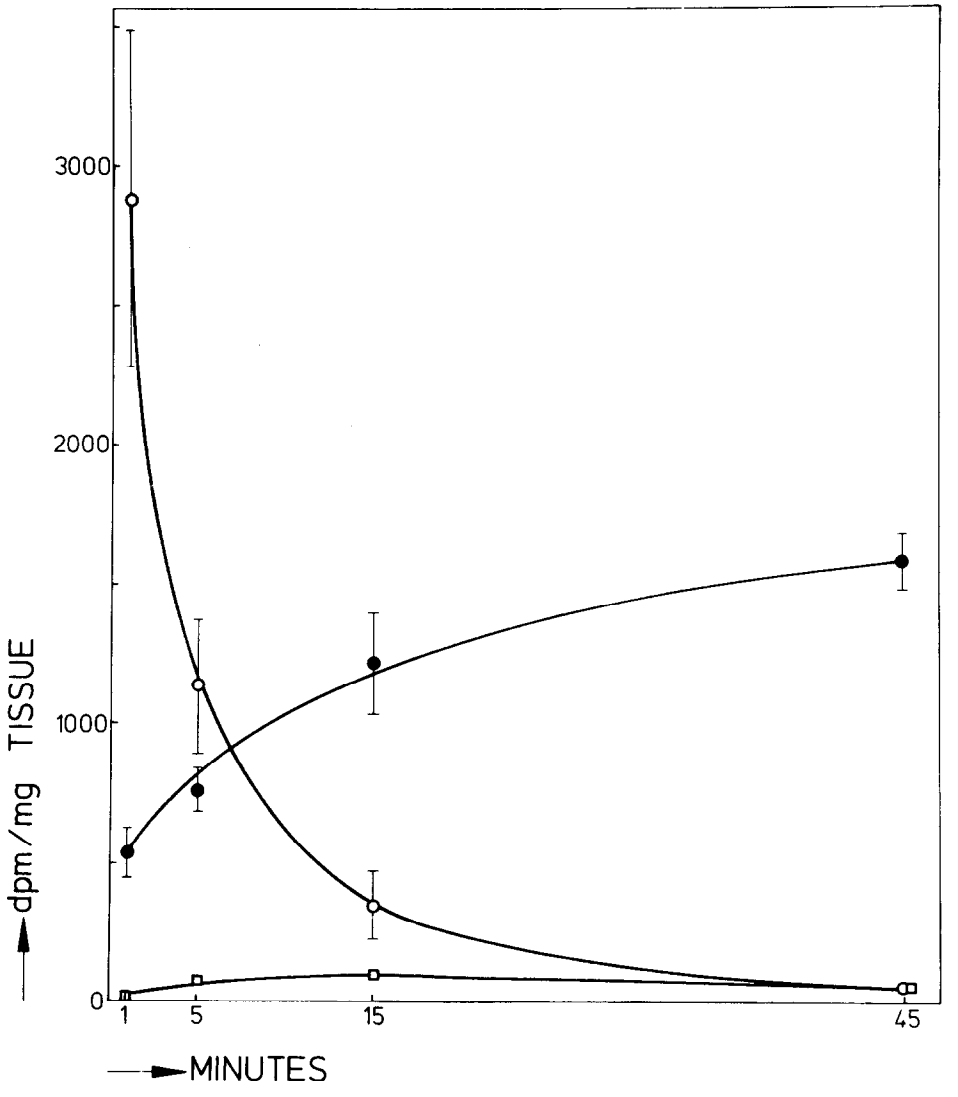
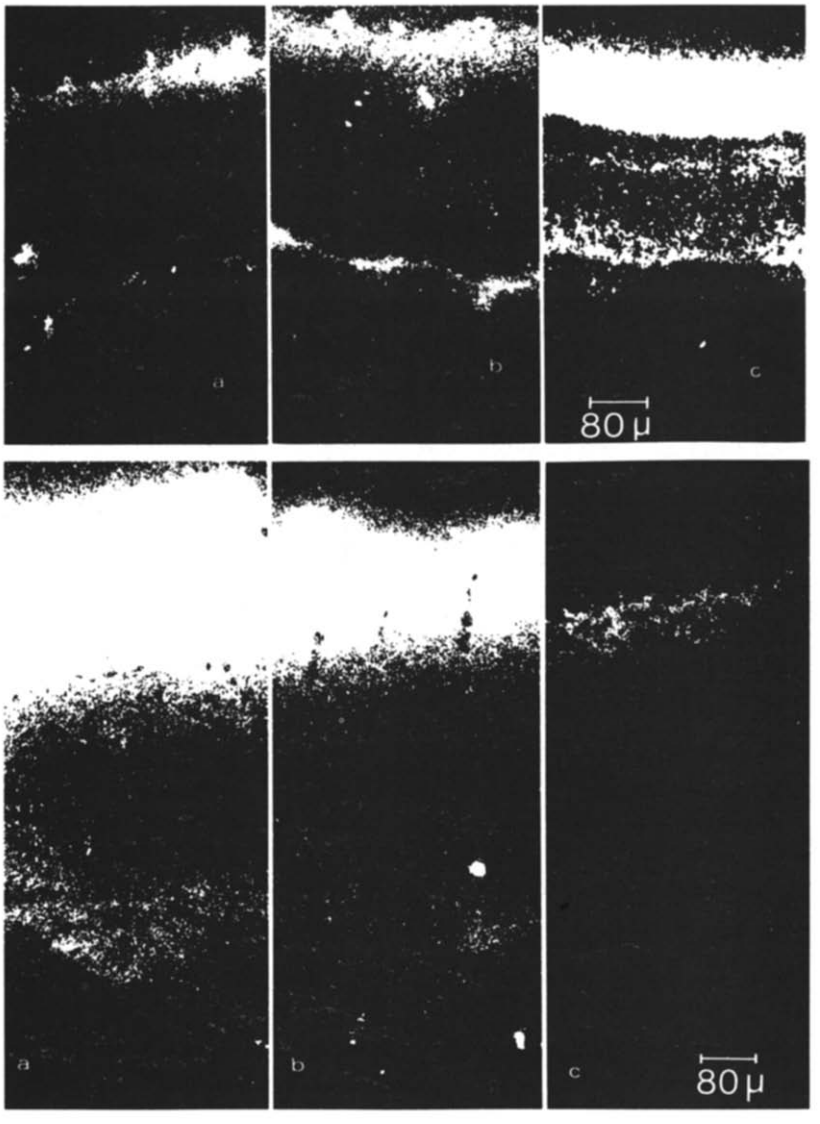
Fig. 2. Time course of ^{14}C-salicylic acid concentration in the glandular (O) and non-glandular (●) stomach of rats and in blood (□). Rats (100g body weight) were fasted for 16 hours, dosed with 2 µCi in 1mg salicylic acid in 0,5 ml saline by stomach tube and sacrified after different time intervals. The stomach was removed, rinsed with saline and cleaned with tissue paper. Samples from both parts of the stomach (whole wall) and blood were weighed, processed in a sample oxidizer and counted in a liquid scintillation spectrometer. The results were corrected for quenching and background, and means ± S.E. were calculated (n = 5). S.E. of the means of the blood values were too small to be shown.



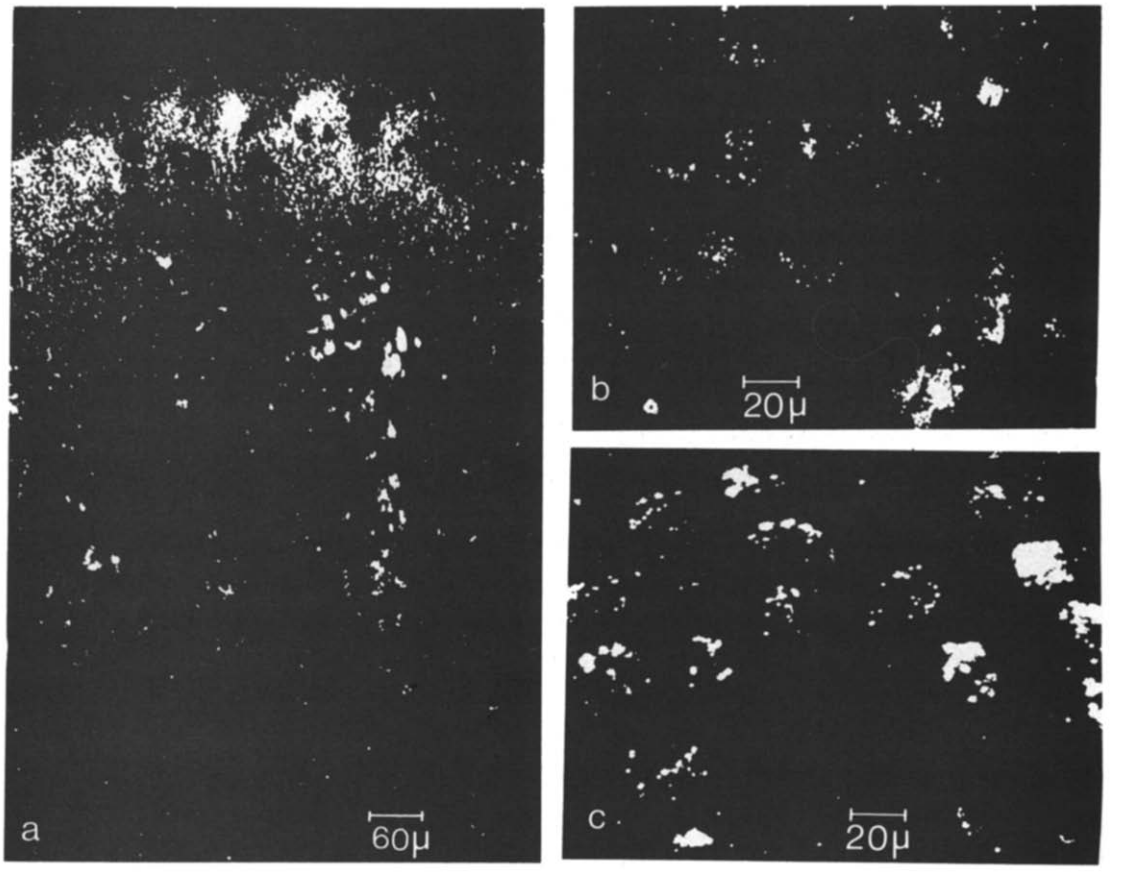
wall of treated animals in liquid propane, cutting in a cryostat at -25°C, mounting on emulsion and exposure at the same temperature (details see ref.8) prevented diffusion of the radioactivity within the tissue. (By contrast, freeze drying and embedding in epon - the method used by others 9 - allowed ^{3}H to diffuse even into the embedding medium). Using our technique we found, as expected on the basis of the biochemical studies, a steady increase in drug concentration in the (cornified) surface layer of the non-glandular stomach (fig.3).However, drug penetration into deeper layers containing vital cells and blood vessels was apparently small.(This finding is in keeping with the fact that ulcers due to salicylates do not occur in the rumen of rats.)

Fig. 3. Autoradiographs of sections of the non-glandular (upper series) and glandular part of rat stomach (lower series). ^{3}H-salicylic acid (40 µCi in 1 mg/100g) in 0,5 ml saline was administered at time 0 to fasted (16 hours) rats. The animals were killed one (a), five (b) or fifteen (c) minutes later. The stomach was removed within 50 sec., carefully cleaned by wiping with tissues and frozen in liquid propane. The tissue was transferred into a cryostat, cut at -25°C, and 5µ sections were mounted on emulsion covering micro slides (for details see ref. 8). After exposure at -30°C for 14 days the sections were quenched in methanol-collodium and the emulsion developed at room temperature. Darkfield pictures of unstained autoradiographs are shown. Silvergrains resulting from ^{3}H disintegrations appear as light dots.



In contrast, autoradiographs of the glandular part of the stomach revealed high drug concentrations throughout the glandular mucosa only 1 min. after drug administration (fig.3). At 5 min. the overall drug concentration was considerably lower than at 1 min.. As expected, high drug concentrations persisted in particular compartments. The surface mucus, together with the mucus-producing superficial cells was loaded with activity. More striking, however, was the accumulation of activity in many parietal cells in some areas of the mucosa, as shown in fig. 4, especially since the extracellular

Fig. 4. Autoradiograph of a particular area in the glandular stomach of a rat treated as explained in fig. 3. The specimens were obtained 1 min. (a,b) and 5 min. (c) after drug administration. In 4a the drug has accumulated in a line of parietal cells below the mucus layer. Higher magnification of these cells (4b) shows the location of activity in individual parietal cells. Such areas were found in serial sections of the glandular stomach of all rats killed 1 min. after drug administration and in 2 out of 3 rats killed 5 min. after drug administration (4c). The stomachs of all 3 rats investigated 15 min. after drug administration showed no such localized drug accumulation.



space, connective tissue cells and pepsinogen-producing cells in these areas were practically devoid of activity (seen in stained sections). The conclusion must be that selective trapping of salicylate molecules in parietal cells in some areas does indeed occur. It is, however, a very short-lived event. Already 15 min. after drug administration comparatively low levels of activity, which appeared to be randomly distributed, were observed. Sections of the glandular stomach of 3 non-drugged animals and of 3 animals given "cold" drug only did not contain areas exhibiting similar "grain accumulation" in dark field pictures or stained specimens. The areas where accumulation occurs may contain actively secreting parietal cells and they may well be the foci from which ulcers of the stomach mucosa develop.

These findings represent the first experimental evidence that the trapping of salicylate anions occurs in stomach cells after ingestion, as proposed by Martin years ago [4]. (Previous research was probably unable to provide this evidence because the techniques used [9] did not prevent diffusion of salicylic acid). Moreover, our findings show that absorption of salicylic acid may take place within a few minutes and that trapping of salicylate anions occurs in parietal cells in some areas of the stomach during that time. This may well cause the immediate decay of these cells. Hence our observation supports the assumption that functioning parietal cells are essential for the development of ulcers due to salicylate medication [5], which explains why achlorhydric patients hardly experience gastric side effects from aspirin [6,10] and why pharmacological [13] or surgical vagotomy [14] reduces both, parietal cell activity and mucosal damage due to salicylates. It also explains why reduced acidity together with increased concentrations of Na^+ and K^+ in gastric fluid characterize the early stages of mucosal damage due to salicylates. These events are possibly not causes [13] but consequences of parietal cell decay. In addition, our observations might explain why recent approaches to the problem of reducing salicylate toxicity have been successful. Slow-release forms of aspirin 6 and its esterification with other chemicals 15 retard absorption and may thus reduce trapping of drug anions in parietal cells. Finally, it is hoped that the data presented will add to the understanding of the cellular events leading to gastric damage by salicylates and by acidic NSAID in general, and thus provide a rationale for the development of less toxic NSAID. However, further research is needed to elucidate the reasons why drug accumulates in the parietal cells of selected areas only. The causal relationship between intracellular drug accumulation and cell decay also remains to be established.

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