Electronmicroscopic observations on the effects of orally administered aspirin and aspirin-bicarbonate mixtures on the development of gastric mucosal damage in the rat

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SUMMARY The effects of administering a single dose of (200 mg to 50 mg/kg body weight) aspirin or an equimolar mixture of aspirin (200 mg/kg body wt) with sodium bicarbonate on the fine structure of the rat gastric mucosa were investigated in order to establish the role of particles of the drug in the development of gastric damage. The sequence of cellular events involved in the development of a lesion and the influence of short-term starvation were also investigated. Aspirin-bicarbonate solutions produced much less damage in starved rats than aspirin suspensions given at low (50 mg/kg body weight) or high therapeutic doses (200 mg/kg body weight). Also, when non-starved rats were given 200 mg/kg aspirin, only slight damage was observed. The presence of particles of the drug in intimate contact with the mucosa is thus important in the development of gastric damage. A sequence of events with time involving direct physical exfoliation of mucosal cells and selective structural damage to parietal cells followed by structural damage indicative of a disturbance to oxidative and biosynthetic functions in cells near the developing erosion was observed. The implications of these results on the development of aspirin-induced lesions are discussed.

It is well known that aspirin causes damage to the gastric mucosa which can lead to bleeding from the gastrointestinal tract (Smith, 1966; Salter, 1968). Systemic as well as local effects have been suggested as a possible mode of action of the drug (Smith, 1966). A local effect of the drug has been demonstrated from studies of the application of aspirin particles to explanted gastric mucosa (Stephens, Milton, and Loewenthal, 1966) or directly through fistulae onto the gastric mucosa of experimental animals (Roth, Valdes-Dapena, Pieses, and Buchman, 1963; Roth and Valdes-Dapena, 1963). Systemic action could also be invoked but this is debatable (see Smith, 1966). Several mechanisms have been proposed to account for the development of gastric damage, among them direct physical damage by particles and loss of the protective mucus layer (Roth and Valdes-Dapena, 1963), cellular exfoliation (Croft, 1963; Croft and Wood, 1967), permeability changes and the influence of acid (Davenport, 1967), defective mucus production (Menguy and Masters, 1965; Håkkinen, Johansson, and Pantio, 1968), and an increased haemorrhagic tendency (Quick and Clesceri, 1960; Weiss and Aledort, 1967). Some of these factors appear important at one stage or another.

Few studies have been performed to establish if the development of gastric damage is related to some of the well known biochemical effects of salicylates (Smith, 1966; Smith and Dawkins, 1971) which could affect cell growth and mucus production (Kent and Allen, 1968; Rainsford and Smith, 1969). The present work is part of a study in which the biochemical effects of aspirin administration are being related to structural damage observed under the light and electron microscopes. Hingson and Ito (1971) made some electronmicroscopic observations on the early stages of development of gastric damage after the oral administration of aspirin to mice. They concluded that permeability changes underlie the development of gastric damage and

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discounted the possibility of effects on biosynthetic machinery in the mucosa. The aim of the present study is to extend these observations and establish the effects of the following factors on the development of gastric damage: (1) the role of aspirin in solution with sodium bicarbonate compared with particles of the drug; (2) the effects of prior starvation; and (3) the time sequence of structural events in the mucosal cells leading to the development of an erosion. In the last case the cells bordering an erosion were examined for structural changes which could be linked with the inhibition of biochemical functions known to occur in vitro and in some other systems in vivo (Smith, 1966; Kent and Allen, 1968; Rainsford and Smith, 1969; Rainsford, 1970).

Methods

Male hooded Wistar rats of 200 ± 5 g body weight were used for the experiments. In all the experiments the animals were starved from 1600 hours on the day before the experiment, with the exception of a non-starved group. They were given water ad libitum until the time of the experiment when the water was withdrawn. At least six to 10 animals were used for each experimental treatment. The experiments were arranged as follows:

1. Aspirin (acetylsalicylic acid) was given orally in single doses of 50, 150, and 200 mg/kg body weight to starved animals. The animals were then killed and the stomachs removed at time intervals of 10 min (excepting 50 mg/kg body weight dose) and one, two and a half and five hours after dosing. The control animals were given an equivalent quantity (1·0 ml) of distilled water.

2. Aspirin was given orally at a dose of 200 mg/kg body weight to non-starved rats and the animals were killed at time intervals as above.

3. An equimolar solution of aspirin and sodium bicarbonate was given orally at a dose of 200 mg/kg body weight of aspirin and the animals were killed at time intervals of one, three, and five hours after administration.

The aspirin suspension was prepared by adding a small quantity of distilled H2O aspirin BP (May and Baker Ltd, Dagenham, England) to make a thick paste and adding water to make a suspension. In this way an even suspension of aspirin could be obtained with no settling of large particles or foaming. For administering the dose, the suspension was stirred immediately before withdrawing an aliquot into a 1 ml tuberculin syringe on which was attached an 18-gauge needle with a 3-cm length of plastic catheter tubing over the needle. In other experiments (Rainsford, 1975) the distribution in blood and gastric contents of salicylates was followed after the oral administration of aspirin in dosages employed in the present study. It was found that the dosing procedure used here gave a low error in dosing and resulted in consistent blood levels of salicylates.

The aspirin-bicarbonate mixture was prepared and administered in the same volumes of H2O as above. The pH of the solution was pH 5·6. In all experiments, the aspirin and aspirin-bicarbonate mixtures were prepared and given immediately afterwards in order to minimize hydrolysis to salicylic and acetic acids.

The animals were killed by stunning and cervical fracture and the stomachs rapidly excised, cut along the lesser curvature and contents washed out with a 0·9% w/v aqueous NaCl solution. Sections of the glandular mucosa were quickly selected, excised, and the tissue placed immediately into ice-cold fixative. Smaller sections were then cut using a sharp scalpel over dental wax and placed into fresh ice-cold fixative. The time between stunning and placing the tissue into fixative averaged 17 seconds.

Some experiments were performed in which the stomach of Nembutal-anaesthetized rats was perfused with fixative in situ before sectioning the tissue. The technique involved slitting the duodenum and inserting an 18-gauge needle covered with 6 cm of plastic catheter tubing (coupled to a 20-ml syringe) into the stomach through the pylorus and tying cotton thread over the duodenum and needle. A small slit was made in the non-glandular region of the stomach and the stomach contents were washed out with 40 ml 0·9% w/v aqueous NaCl. The stomach was emptied and forceps were applied over the slit in the non-glandular area. The stomach was then slowly perfused with 30 ml 2% w/v formaldehyde + 2·5% v/v glutaraldehyde fixative (at room temperature), the forceps were released to drain out the fixative, and the stomach was perfused again. Shortly after, the stomach (filled with fixative) was tied off with thread and removed. The stomach was opened along the lesser curvature and sections of tissue were removed and later sectioned (as above) before placing in a fresh batch of ice-cold fixative. In all experiments, the tissues obtained were subsequently cut into small rectangular solids about 1 × 2 mm so that the side parallel to surface mucosa was longer than any of the other sides. This enabled sections for electronmicroscopy to be taken in a plane perpendicular to the surface of the mucosa.

In preliminary experiments, tissues from both control and aspirin-treated rats were treated with several formaldehyde and/or glutaraldehyde fixatives including those employed by Karnovsky (1965) and Ito and Karnovsky (1971). Fixation was performed at room temperature or at 4°C for one to five hours.

The fixatives were prepared in 0·08 M cacodylate...
buffer pH 7.4 with 3 mM CaCl₂ or 0.08 M-phosphate buffer pH 7.4. Poorer fixation was achieved with glutaraldehyde alone than with formaldehyde-glutaraldehyde fixatives. Some shrinkage of mucosal and parietal cells was evident in tissues fixed in glutaraldehyde-formaldehyde fixatives of high osmolarity, especially in the aspirin-damaged regions. This effect has been noted in other tissues by Karnovsky (1965). Satisfactory results were obtained using a half-concentration mixture of Karnovsky’s (1965) fixative at 4°C and this was generally employed.

After fixing for four to six hours the tissues were washed three times in 0.1 M phosphate buffer pH 7.4 with 8.2% sucrose at 4°C over 16 hours and post-fixed in 1.0% OsO₄ in 0.1 M phosphate buffer, pH 7.4, at 4°C for two hours. Finally, the tissues were washed and dehydrated in a graded series of alcohols and embedded in araldite (Glaueer, 1965). Thick and ultrathin sections were cut on an LKB Ultratome (LKB Produkter, Sweden) for light and electron microscopy respectively. The thick sections were stained with 1% w/v toluidine blue + 1% w/v Pyronin B in 1% w/v aqueous sodium borate. The ultrathin sections were stained with lead citrate (Reynolds, 1963) and uranyl acetate (Watson, 1958).

The sections were viewed under an Hitachi HU-12 electron microscope on a liquid N₂-cooled stage at an accelerating voltage of 75 kV.

**Results**

**MORPHOLOGICAL APPEARANCE OF GASTRIC DAMAGE PRODUCED BY ASPIRIN**

The oral administration of 50-200 mg/kg body eight aspirin to starved rats produced focal erosions in the corpus and body with evidence of bleeding in and around the eroded areas one hour after administration of aspirin. No erosions were evident at 10 min after dosing but there was extensive sloughing of the mucus layer. The erosions were clearly visible to the naked eye and were generally focal (approximately 1.0 mm in diameter) or extended lengthwise (about 2 mm long and 1.0-0.5 mm wide) down the mucosa. No damage was observed in the forestomach. Most of the damage occurred in the middle of the greater curvature in the corpus with occasional damage in the antrum and pylorus. For this reason, the sections that were taken for light and electron-microscopy were from the corpus. The damage was often present on the superficial ('upper') areas of the

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**Table**  Lesions produced in rats used for electronmicroscopy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>Average Number of Lesions per Rat</th>
<th>Average Intensity of Lesions</th>
<th>Frequency of Lesion Development</th>
<th>n</th>
<th>Lesion Indexa</th>
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<tbody>
<tr>
<td>Control (starved)</td>
<td>10 min</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<td>2 hr</td>
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<td>0</td>
<td>10</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Control (not starved)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
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<td>3 hr</td>
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<td>5 hr</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>200 mg/kg ASA (starved)</td>
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<td>0-20</td>
<td>1-20</td>
<td>10%</td>
<td>10</td>
<td>2-40</td>
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<tr>
<td></td>
<td>1 hr</td>
<td>0-15</td>
<td>2-46</td>
<td>92.3%a,b,c</td>
<td>13</td>
<td>17-94</td>
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<tr>
<td></td>
<td>2 hr</td>
<td>0-90</td>
<td>1-80</td>
<td>100%a,b</td>
<td>5</td>
<td>20-80</td>
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<td></td>
<td>5 hr</td>
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</tr>
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<td>NDa</td>
<td>NDa</td>
<td>35</td>
<td>NDa</td>
</tr>
<tr>
<td>200 mg/kg ASA (not starved)</td>
<td>2 hr</td>
<td>1-33</td>
<td>1-00</td>
<td>33-3%b</td>
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<td>5-33</td>
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</tr>
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<td>0-66</td>
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<tr>
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<td>0</td>
<td>0</td>
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<td>6</td>
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<td>50 mg/kg ASA (starved)</td>
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<td>0</td>
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<td>1 hr</td>
<td>1-36</td>
<td>0-64</td>
<td>45.5%a</td>
<td>11</td>
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<td>0-60</td>
<td>40.0%a,d</td>
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<td>5-80</td>
</tr>
<tr>
<td></td>
<td>5 hr</td>
<td>1-50</td>
<td>0-25</td>
<td>25-0%a,e</td>
<td>4</td>
<td>4-25</td>
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<tr>
<td>50 mg/kg ASA (not starved)</td>
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</tr>
</tbody>
</table>

1. n = number of animals used for each treatment
2. Lesion index calculated by the method of Robert, Nezamis, and Phillips (1968), which is the sum of (1) the percentage incidence/10 of animals with ulcers, (2) average severity of the lesions on an arbitrary scale 0 to 4, and (1) average number of lesions per stomach.
3. Statistically significant difference assessed by the Chi-square 2×2 contingency test in numbers of animals with lesions in: 1 hr 200 mg/kg aspirin compared with 1 hr aspirin-bicarbonate group, P < 0.003 (designated a); 1 hr 200 mg/kg aspirin starved versus non-starved, P < 0.025 (designated b); and 1, 2, and 5 hr 200 mg/kg aspirin versus corresponding times of 50 mg/kg aspirin treatments, all P < 0.05 (designated c, d, and e respectively).
4. ND = not determined.
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Fig la Part of an erosion in the rat gastric mucosa five hours after a single oral dose of 200 mg/kg body weight aspirin. The eroded area extends from one-third to half way into the mucosa. The area on the left is almost normal apart from the loss of some superficial mucosal cells. Extensive damage occurred and the damaged cells in the erosions stained poorly. The cells deeper in the mucosa below and adjacent to erosions appeared intact and stained normally. × 190

Fig lb Enlargement of the area between the damaged and intact cells at the base of an erosion in the right-hand side of figure la. There is a sharp line of distinction between damaged and undamaged cells. A few parietal cells have been sloughed away but the remainder have clearly been either damaged severely in the eroded area or remain intact. × 660

Fig lc The superficial region of the gastric mucosa from a rat after oral administration of 50 mg/kg body weight aspirin for two hours. The damage is confined to the superficial mucosal cells and a few associated cells. Some sloughing of mucosal cells occurred and the internal cytoplasm of the superficial mucous cells disrupted as a consequence of discharging large numbers of mucous granules. Damaged areas of the kind shown here are as with areas of the kind shown in fig la, only present in isolated areas and not over the whole mucosa. There is no other damage to the underlying mucosal tissue. × 625
ral folds. In severely damaged stomachs the erosions extended lengthwise on top of the rugal folds. A quantitative assessment of damage, as measured by the number of erosions and their size (determined on an arbitrary scale), and the number of animals with lesions present is shown in the table.

Extensive focal damage occurred at intervals ranging from one to five hours after the administration of 150 and 200 mg/kg aspirin suspension to starved rats. Less damage was evident in starved rats given 50 mg/kg body weight aspirin. The peak in the development of damage occurred at one to two hours in starved animals given 200 and 50 mg/kg aspirin (table). Very little, if any, damage was observed in non-starved animals given 200 mg/kg body weight aspirin suspension, starved animals given aspirin (200 mg/kg body weight) with bicarbonate, or in control animals (table).

When observed under the light microscope the erosions in starved rats given 200 mg/kg aspirin appeared to extend to about a third to half way into the mucosa (fig 1a). The cells present in the eroded area showed extensive internal disruption and did not stain as well as normal cells (figs 1a, 1b). Some of the superficial mucous and neighbouring cells were sloughed away and there was a reduction in the quantity of mucus-containing granules in the surface mucosal cells near an erosion. Generally, the remainder of the gastric mucosal cells away from an erosion appeared morphologically similar to those in tissues from control animals (fig 1a, 1b). In starved rats that were given 50 mg/kg aspirin for one to five hours, damage was confined to the superficial mucous layer (fig 1c). Occasionally, small focal erosions were present which extended to about one-fifth the way into the mucosa.

The surface mucous layer assumed a whitish appearance 10 minutes after the administration of aspirin suspensions. This denatured mucus could be readily removed by scraping the surface of the mucosa with a smooth glass rod. The mucous layer from control animals could not be removed in this way. When viewed under the binocular microscope it could be seen that aspirin particles had become embedded in the mucous layer and in the superficial region of the gastric pits.

Extensive oedema was evident in the mucosa of rats given 150 and 200 mg/kg body weight aspirin. In these animals the swelling of the stomach, as assessed by an increase in the volume of the contents (resulting from the presence of both fluid and gas) and swelling of the mucosal tissues, could be clearly seen by the unaided eye. The degree of swelling was much reduced in non-starved animals given 200 mg/kg aspirin or in starved animals given 200 mg/kg aspirin with bicarbonate or 50 mg/kg aspirin alone.

When viewed from the serosal side, the blood vessels in the stomach were extensively swollen in all rats given the aspirin suspension. Swelling of the blood vessels was also observed in sections viewed under the light microscope.

The gastric mucosa from animals given 200 mg/kg body weight aspirin with equimolar sodium bicarbonate appeared essentially undamaged when examined under the light microscope. Occasionally, a few surface mucous cells were sloughed away and slight swelling of the mucosal tissue occurred. However, the damage was minor, especially compared with that produced by aspirin alone.

**Fine structural changes induced by aspirin**

In rats given 200 mg/kg body weight aspirin the first signs of damage to the superficial mucosal cells were evident 10 min after oral administration of the drug although, as noted earlier, no erosions were visible to the unaided eye or in light microscope sections. Extensive cellular damage was evident in the region of an erosion in the mucosa of rats one hour after administering aspirin, 200 mg/kg body weight, as a suspension (fig 2a). In eroded areas, a large number of cells were sloughed away (fig 2a) and these usually stained poorly with lead citrate and uranyl acetate. Aggregation of the nuclear chromatin material occurred and the organelles of most of these cells were extensively damaged to the extent that positive identification of the cell types was difficult to ascertain (fig 2a).

However, the point of particular interest was to examine the cells in the region adjacent to cells which had actually been disrupted, ie, in a radial direction away from an erosion. Observations in this region would most likely yield information on the sequence of biochemical changes occurring in the development of gastric damage. In most cases, changes were observed in the layer of several cells around an erosion which were still intact (fig 5). This is in the area that corresponds to undamaged cells situated immediately around erosions seen in light microscopic sections (fig 1a, 1b). The observed damage was confined to animals that were given 200 mg/kg aspirin for one hour or longer. The most striking changes were observed in parietal cells (fig 3a). The mitochondria and canaliculi were swollen and there was a marked reduction in the content of matrix material in mitochondria. The matrix in the canaliculi was aggregated into small clumps and the internal tubular structures were often extensively disrupted. Damage to the canaliculi often preceded damage to the mitochondria in less-affected parietal cells. In some cells, there was a reduction in the number of canaliculi con-
Fig 2a. Section of an erosion showing damage to the mucosa one hour following the oral administration of 200 mg/kg aspirin. Extensive damage is evident in mucosal cells to the extent where positive identification of cell types is difficult to ascertain. Marked aggregation of nuclear contents and 'vacuolation' was evident. Considerable displacement or sloughing of cells also occurred. × 9200
Fig 2b  Surface mucous cells after oral administration of aspirin 200 mg/kg and sodium bicarbonate showing swelling of the endoplasmic reticulum and areas of 'vacuolation'. Note the absence of any damage to the surface membranes or disruption of intercellular contact as seen in the mucosa of rats given aspirin only. × 15 000
Fig 3a  Parietal cells in the otherwise undamaged areas of the gastric mucosa of a rat given an oral dose of 200 mg/kg body weight aspirin for one hour showing marked damage to the mitochondria and clumping of nuclear material in these cells. × 16 500

Fig 3b  Parietal cell from a control rat given 1 ml H₂O for one hour for comparison with figs 3a and 3b. × 23 000
Fig 4 Mitochondria in a parietal cell in otherwise undamaged region in the gastric mucosa of a rat given 200 mg/kg aspirin for two and a half hours showing extensive disruption to the matrix contents and associated swelling. × 66 000
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Fig 5  Edge of erosion deep in the mucosa bordering undamaged mucosa showing damaged parietal cells and extensive 'vacuolation' in blood vessels and associated cells. × 5000
comitant with an increase in the numbers of vacuoles. ‘Vacuolation’, ie, the appearance of membrane-bound vacuoles often containing what is presumably debris, was evident in parietal cells. This was often present in parietal cells which had not been severely affected, ie, in which the canaliculi and mitochondria were largely unaffected. The ribosomes on the endoplasmic reticulum were disrupted and distributed about adjacent areas of the parietal (fig 4) and mucous cells. In the mucous cells, the mucus-containing globules were disrupted and, in some cells, away from an erosion, the contents of the globules were released. Occasionally, swelling of the rough endoplasmic reticulum of chief (zymogen) cells occurred in deeply eroded areas. These changes bordering an erosion varied considerably in the number of cells affected.

The capillaries in and adjacent to eroded regions in the mucosa showed extensive vacuolation (fig 5). Some capillaries were completely disrupted and there was associated loss of red blood cells into the adjacent interstitial areas. Surprisingly, some of the capillaries were disrupted in areas near an erosion which contained apparently normal mucous and connective tissue cells.

More cells seem to be affected at later time intervals (one to two hours) after the administration of aspirin suspensions. Apart from the effects seen in eroded areas and cells bordering erosion already mentioned, the remainder of the gastric mucosa appeared unaffected in aspirin-treated rats as compared with that from control animals. Examination of non-eroded areas revealed very little, if any, damage to the mucosa of aspirin-treated rats. Occasionally, there was disruption of superficial mucous cells and mucous granules and some changes in parietal cells of the kind previously mentioned.

The administration of aspirin (200 mg/kg body weight) with bicarbonate did not cause the marked changes observed in rats given aspirin suspensions. A few surface mucous cells were sloughed away from the mucosa. Swelling of the endoplasmic reticulum was evident in some of the remaining mucous cells (fig 2b). Occasionally, some parietal cells in the region immediately below the damaged areas were affected in the same way as that observed in rats given the aspirin suspension. However, apart from these minor changes, the rest of the gastric mucosa was totally unaffected by the drug. Similarly, very little damage was observed in the gastric mucosa of non-starved rats given aspirin alone (200 mg/kg body weight) over the time period studied and this involved only some minor disruption of a few superficial mucous cells bordering the gastric lumen.

Discussion

The most striking effects seen in the gastric mucosa of animals given aspirin alone were the sloughing of intact cells and destruction of some of these cells (fig 1a, 2a). These changes are similar to those observed by Hingson and Ito (1971) in the mouse at eight to 10 minutes, and in the ferret by Pfeiffer and Weibel (1973) up to 45 minutes after oral administration of the drug. However, the changes in cells bordering an erosion (seen in fig 3a and 4), especially the selective damage to the parietal cells seen near an erosion, were not reported by these authors. This could be due to the shorter time intervals of the experiments. Damage to some other cells in the gastric mucosa was observed by Pfeiffer and Weibel (1973), but not of the kind observed here. However, Pfeiffer and Weibel (1973) dosed the animals they used with aspirin suspended in 5 ml 0.1 M HCl. It is difficult to establish what role the addition of this substantial quantity of acid has in the development of gastric damage, especially as the drug is usually taken with water.

The changes observed in this present work are particularly interesting when considering possible biochemical effects of the salicylates. The changes in the parietal cell mitochondria of aspirin-treated rats (fig 3a and 4) may be a reflection of disturbances of oxidative metabolism and would be expected to result in disruption and swelling of mitochondria, a phenomenon which is known to occur with salicylates in vitro (Lutwak-Mann, 1942; Brody, 1956; Bryant, Smith, and Hines, 1963). Structural changes of the type seen in the present study occur with agents which disturb oxidative metabolism (Kaltenbach and Harman, 1955; Gómez-Puyou, Tuena, Guzmán-García, and Peña, 1963; Kimber, Loud, and Weiner, 1968). Another possibility is that release of lysosomal enzymes by salicylates which occurs in vitro (Brown and Schwartz, 1969; Lee and Spencer, 1969; Harford and Smith, 1970) could result in uncoupling and changes in mitochondrial morphology. Mellors, Tappel, Sawant, and Desai (1967) observed that lysosomes caused uncoupling of oxidative phosphorylation and concomitant irreversible high amplitude swelling of isolated mitochondria in vitro.

Structural changes in mitochondria of the kind seen in figs 3a and 4a have been observed in the liver and kidneys of rats given high doses of aspirin on the long term (Gutowska-Grzegorczyk and Kalczak, 1968) and in fibroblasts of regenerating tissue of rats given repeated doses of salicylate (Jorgensen, 1964). Stockinger (1964) found that solutions of 0.5% w/v concentration of various salicylates instilled into the colonic sacs of anaes-
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The presence of epithelium resembling those observed in aspirin-treated rats in the present study. The changes seen in the morphology of the parietal cell mitochondria and canaliculi could reflect a change in the secretory state of these cells. An increase in the number and length of canaliculi occurs during acid secretion (Sedar and Freidman, 1961). Vial and Orrego (1963) observed identical changes in parietal cells to those seen in the present study in rats four hours after a single dose of 30 mg/kg body weight 2,4-dinitrophenol with a concomitant decrease in the acid output. A reduction in the titratable acid in the stomach has been observed after the administration of aspirin to dogs (Davenport, 1964), guinea pigs (Anderson, 1964), and rats (Brodie and Chase, 1967). Davenport (1964, 1967) has suggested that this could be a consequence of loss of hydrogen ions by back diffusion into the mucosa. However, it is also possible that there is some impairment in the oxidative functions of the parietal cell mitochondria leading to a block in the production of acid.

An intriguing aspect observed here is that the parietal cell seems much more sensitive to the changes mentioned above than other cells in the gastric mucosa. This effect could be due to differences in the rate of penetration of aspirin into different cells. It is known that an acid pH in the stomach favours absorption of the drug in its non-ionized form (Hogben, Schanker, Tocco, and Brodie, 1957; Schanker, Shore, Brodie, and Hogben, 1957). The parietal organelle(s) involved in the secretion of hydrogen ions, especially the parietal cell canaliculi, would be expected to have a low pH (Ito, 1967; Nakamura, Wakabayashi, Tsutumi, Fujihira, and Kawabe, 1970). The low pH in these structures and in the outside milieu of the gastric pit adjacent to the parietal cell would favour increased uptake of the drug in this cell compared with other cells of the gastric pit. That damage to the parietal cell canaliculi appears to precede the damage to mitochondria in less affected cells is indicative of the greater sensitivity of these structures to effects of the drug caused by the presence of acid.

Another surprising aspect is that evidence in vitro strongly indicates that the biochemical effects mentioned above would be most likely to occur in the entire gastric mucosa. It is also surprising that the mucosa is not eroded away by more particles. The number of erosions that can be produced above a dose of 150 mg/kg appears to be the same up to the toxic limits (Anderson, 1964). It appears that the gastric mucosa develops a protective response to the presence of particles of the drug which could be due to the release of mucus from the mucosal granules and an increase in the total fluid content of the stomach (see Results) leading to a reduction in the contact of particles with the mucosa. The appearance of fluid and mucus around tablets has been observed in gastroscopic studies on human subjects (Thorsen, Western, Tanaka, and Morrissey, 1968), in anaesthetized rats and through gastric fistulae of tranquillized pigs (Rainsford, 1970 and unpublished observations).

The protective response in the form of mucus discharge on the one hand is presumably countered in areas where an erosion is developing by sloughing of the mucous layer (fig 1a). The sloughing is probably a consequence of changes in the physico-chemical properties of salicylates in solution which cause an aggregation of components of mucus (Rainsford, Watkins, and Smith, 1968). Also, release of mucus globules may, where the mucous layer is sloughed away, actually expose the underlying cells to the corrosive aspirin particles.

The selective damage to parietal cells near an erosion could follow breakdown in the mucosal barrier thereby exposing the parietal cells to local high concentrations of the drug. Superimposed upon this the parietal cells could, because of the presence of acid, concentrate the drug. Through this mechanism the parietal cell could become the focus for the further development of damage by 'explosive' damage to neighbouring cells. Impairment of protein and glycoprotein biosynthesis which occurs with salicylates (Rainsford and Smith, 1969; Dawkins, McArthur, and Smith, 1971) could occur in the mucosal cells exposed to high concentrations of the drug and account for the observed swelling of the endoplasmic reticulum (fig 2b).

Many authors have observed a marked reduction in gastric damage by aspirin when an excess of bicarbonate is given as a solution of the drug to human subjects (Matsumoto and Grossman, 1959; Wood, 1963; Thorsen et al, 1968; Leonards and Levy, 1969) or guinea pigs (Anderson, 1963). However, in the present study, equimolar mixtures of aspirin and sodium bicarbonate were found to cause a marked reduction in the damage compared to that produced by aspirin suspensions.

A combination of factors may be responsible for this effect. Enhanced gastric emptying at the higher pH has been suggested as a factor (Anderson, 1964). However, the pH of the bicarbonate-aspirin mixture used was 5-6 and this would not appear to be high enough appreciably to stimulate gastric emptying (Hunt and Knox, 1962 and 1969). In studies reported elsewhere (Rainsford, 1975), the distribution of $^{14}$C-carboxy-aspirin in the blood was comparable whether sodium bicarbonate was given with or without aspirin at the same doses and times used in
the present work. It was found that three to four times the quantity of 14C-salicylates was present in the gastric contents up to three hours after the administration of 14C-aspirin with bicarbonate compared to that produced by aspirin alone. Although the quantity of 14C-salicylates in the intestinal contents was increased up to one hour in the aspirin-bicarbonate group, this was only a small percentage of the total dose. Uptake of the drug into the gastric mucosal cells must be, in part, a factor in the development of gastric damage, since a considerable quantity of the drug is still present in the stomachs of aspirin-bicarbonate-treated animals. Enhanced gastric emptying of aspirin-bicarbonate mixtures is apparently not a major factor in reducing gastric damage under the conditions used here. The absence of particles of the drug in the aspirin-bicarbonate mixtures is clearly a major factor. A consequence of this is that there would be an increase in the spread of the drug over the mucosal surface and gastric pit, thereby reducing the amount of the drug in concentrated particles, which seems important in the development of focal damage.

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References


cylocwego. Rheumatologia (Warsz), 6, 181-184.


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Notes and activities

Twenty-fifth anniversary

On 30 May 1975 the Gastrointestinal Unit at the Western General Hospital, Edinburgh, celebrated the 25th anniversary of its foundation with a Festschrift in honour of the founders Sir John Bruce and Professor W. I. Card. A scientific meeting was attended by former members of the clinical staff and research fellows coming from as far afield as South Africa and Canada. At the evening banquet the founders were presented with specially designed silver medals bearing the unit coat of arms and the other participants received bronze replicas. For the occasion a bibliography was printed of the unit’s research publications since its foundation.

The unit was the first truly combined medical and surgical gastroenterology unit in the world. In 1970 the University of Edinburgh accorded it the status of a University Unit with an independent budget. Several other units around the world have been modelled on the same pattern.

Six years ago through the generosity of the Wolfson Foundation a suite of laboratories with observation rooms and symposium/library was built immediately adjacent to the wards. In 1972 a diagnostic suite was commissioned in the new outpatient department which includes day beds, twin endoscopy theatres, a room for radiologically-controlled endoscopy procedures, a motility room with analogue to digital computerized motility facilities, and an additional academic laboratory. A team of staff nurses specially trained in a wide range of investigative procedures and directed by a sister in charge has allowed major developments in the investigation of gastrointestinal problems without having to admit the patient. The spectrum of both service and research interests in the unit remains broad, representing the surgical and medical aspects of digestive diseases.

Professor C. V. Perrier

Professor Claude Perrier died at the age of 45 in an accident on 26 July 1975. He was educated first in law and then in medicine at Lausanne, and did postgraduate work there, in Zurich, and in Geneva where he wrote his thesis on laxative-induced hypokalaemia. He spent 1961 and 1962 in the Gastroenterology Department and Pancreatic Laboratory in Mount Sinai Hospital, New York, with Doctors Drelling and Janowitz with whom he studied pancreatic and gastric secretion, and wrote the standard monograph, Pancreatic Inflammatory Disease (Hoeber, 1964). He then went back to Geneva, but returned to the USA in 1967 and worked with Dr. Laster at the NIH, studying gastric mucosal adenyl cyclase activity.

On his return to Geneva in 1970 he was appointed to a personal chair in charge of the new Department of Clinical Pharmacology. In the last five years he built up a powerful clinical research and teaching department in the University Cantonal Hospital, and was heavily involved in local and federal medical commissions. In 1972 he created Pharma-flash, a critical therapeutic newsletter in French that now has a circulation of 1500, a lasting monument to his intellect energy and initiative.

He was a frequent visitor to Britain, particularly in relation to courses at the Royal Postgraduate Medical School, and a regular attender of meetings of the British Society of Gastroenterology to which he was elected as Corresponding Member in 1973.

Above all, Claude Perrier is mourned as an intensely live and warm personality, a devoted husband and father, and a good friend. J.H.B.

Academic appointments

Gastroenterology is well represented in recent professorial appointments. We congratulate Mr M. Hobsley on his appointment to a personal chair in Surgical Sciences at the Middlesex Hospital, London; Surgeon Commander G. J. Milton-Thompson, RN who has been appointed Professor of Naval Medicine at RN Hospital, Haslar; and Dr Graham Neale who goes to Trinity College Dublin as Professor of Medicine. Professor A. J. Zuckermand has been appointed to the Chair of Microbiology at the London School of Hygiene and Tropical Medicine, where he has been Professor of Virology since 1972. He is also Director of the WHO Collaborating Centre for Reference and Research on Viral Hepatitis and Consultant Clinical Virologist to the West London Hospital and North-East Metropolitan Blood Transfusion Centre. They are all members of the British Society of Gastroenterology.

Notes on Books

Refined carbohydrate and disease. Some implications of dietary fibre. Edited by D. P. Burkitt and H. C. Trowell. (Pp. xiii + 356; illustrated: $7-80). Academic Press: London. 1975. This book is a most important publication, setting out all the background scientific and clinical data in relation to the role of refined carbohydrate foods and disease. It will facilitate the research work now in progress and will have much appeal to clinical gastroenterologists around the world.

Recent progress in anaesthesiology and resuscitation. Editors: A. Arias, R. Llaurado, M. A. Nalda, and J. N. Lunn. (Pp. XXIII + 875; illustrated: $110/75 Dfl.255.00) Excerpta Medica: Amsterdam. 1975. This book publishes the proceedings of the IV European Congress in Anaesthesiology held in Madrid in September 1974. Although primarily of interest to anaesthetists, nevertheless, the studies on organ transplantation and resuscitation have relevance in gastroenterology.

Moments of decision in cancer of the esophagus Principal author: Seymour H. Levitt (Pp. vii + 108; illustrated: price not stated) American College of Radiography: Chicago. 1975. This is a self-testing and self-instructional unit with the answers overleaf. A most interesting and well-produced publication which should attract attention.

Correction

We regret the following errors in the July issue in the article 'Electromicroscopic observations on the effects of orally administered aspirin and aspirin-bicarbonate mixtures on the development of gastric mucosal damage in the rat', by K. D. Rainsford (Gut, 1975, 16, 514-527): the legends to Fig. 2b and Fig. 4 were transposed, as were those of Fig. 2a and Fig. 5.