Are Pepsinogens Activated in Gastric Mucosa After Aspirin-Induced Injury?

It has been known for some years that a number of substances, among them aspirin, may break the gastric mucosal barrier to hydrogen ion[1-5] and that administration of aspirin may be associated with the production of gastric mucosal erosions[1,5]. Consumption of aspirin has also been associated in man with upper gastrointestinal haemorrhage[6-8] and has been postulated as a factor in the development of chronic peptic ulcer[9,10]. It has recently been suggested that pyloric reflux may be important in the genesis of gastric ulcer and that[11,12] bile salts which enter the stomach produce back diffusion of hydrogen ion into the mucosa which is thus damaged. Although it is generally believed that entry of hydrogen ion into the mucosa produces damage[13-15], the intimate mechanism of this is unknown. Some experiments in rats by PALLARES[16] demonstrated that after pyloric ligation, intragastric instillation of HCl failed to produce mucosal lesions which, however, could be induced in such animals by gastric juice or a mixture of hydrochloric acid and pepsin. The enzymically active pepsins are produced from their precursor pepsinogens by activation with acid. The active pepsins can be distinguished from pepsinogens, by their intense milk-clotting activity, and their destruction at neutral or alkaline pH. The recent development of techniques for simultaneous electrophoresis of pepsinogens and pepsins has also made possible their distinction[17], the enzymes showing enhanced electrophoretic mobility when compared with the zymogens.

The present study was undertaken to determine if the mechanism of gastric mucosal injury in rats after aspirin could be explained by the conversion of pepsinogens to pepsins in the mucosa.

Materials and methods. Aspirin. The aspirin used was purified acetyl saticyclic acid purchased from Mallinckrodt Chemical Works, New York, N.Y. All chemicals and reagents were of analytical grade or better.

'pH 5.7 buffer' was developed with allowed simultaneous electrophoretic separation of pepsinogens and pepsins[17].

Agar gel electrophoresis for the simultaneous demonstration of pepsinogens and pepsins was carried out by a modification of URIEL's original method[18] as previously described[17] using pH 5.7 buffer. Agar gel electrophoresis of pepsinogens was done at pH 8.3 as before[19].

Milk coagulation. The milk coagulating capacity of the mucosal homogenates and a standard pepsin solution was measured by a modification of West's original technique[20].

Experimental designs. 40 male Holtzman rats (150–175 g) were used for the experiments, 20 in an experimental group and 20 serving as controls. All were fasted for 24 h before the experiment. Each of the 20 animals in the experimental group was given an i.m. injection of aspirin 600 mg/kg body wt.) suspended in 1% methyl cellulose. At the end of 4 h, anaesthesia was rapidly induced with diethyl ether, the abdomen was opened and the stomach removed. The stomach was opened along the lesser curvature and the mucosa immediately examined for the presence of lesions with a hand lens. The stomach was then quickly washed with ice cold pH 5.7 buffer, the mucosa rapidly removed from the glandular portion of the stomach by blunt dissection and homogenized in 5 ml of the same buffer. 20 animals served as controls; 10 were placed in cages and allowed free access to water but

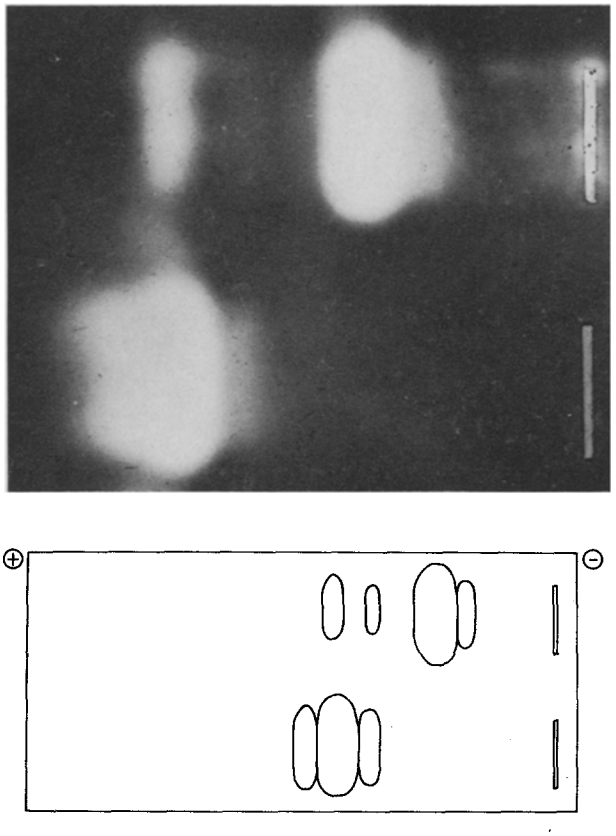

Fig. 1. Agar gel electrophoresis of rat gastric mucosa at pH 5.7 mixed buffer. First slot from top contains normal mucosa showing 4 bands of proteolytic activity. A similar pattern was seen in experimental group rats given 600 mg/kg of body weight of aspirin i.m. The second slot contains mucosa after activation to pH 2.0 in vitro by the addition of 0.2 *N* HCl. Note 3 bands of proteolytic activity and markedly enhanced mobility of these bands. Similar patterns were seen when mucosa was incubated in unbuffered aspirin. Tracing of Figure 1 is shown to show the relation of indistinct bands, not reproduced well in the picture.

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were not otherwise manipulated until they were anaesthetized 4 h later. The other 10 animals were given an i.m. injection of 1% methyl cellulose without aspirin in the same volume as that used for the aspirin injections. These were allowed free access to water. At the end of 4 h, they were anaesthetized and the stomach removed and treated as described above[21,22].

Time study. After giving 600 mg/kg body weight of aspirin i.m. 3 groups of 5 rats each were killed 1, 2 and 3 h later as described above. 2 rats, given 1% methyl cellulose, served as controls in each group. The mucosa from these animals were treated in the same manner as described in experimental design.

Effect of aspirin on mucosal homogenate in vitro. Aspirin was suspended in water and the pH adjusted to 7.0 by the addition of 0.1 *M* Na_2CO_3. Different quantities of this neutralized solution containing from 0.05 to 0.5 mg of aspirin were then added to 1 ml quantities of normal rat gastric mucosal homogenate. The mixture was homogenized further for 5 min in a tissue homogenizer and then stood at 37 °C for 4 h. A similar experiment was done using unbuffered aspirin. The pH of aspirin suspended in methyl cellulose was 3.4.

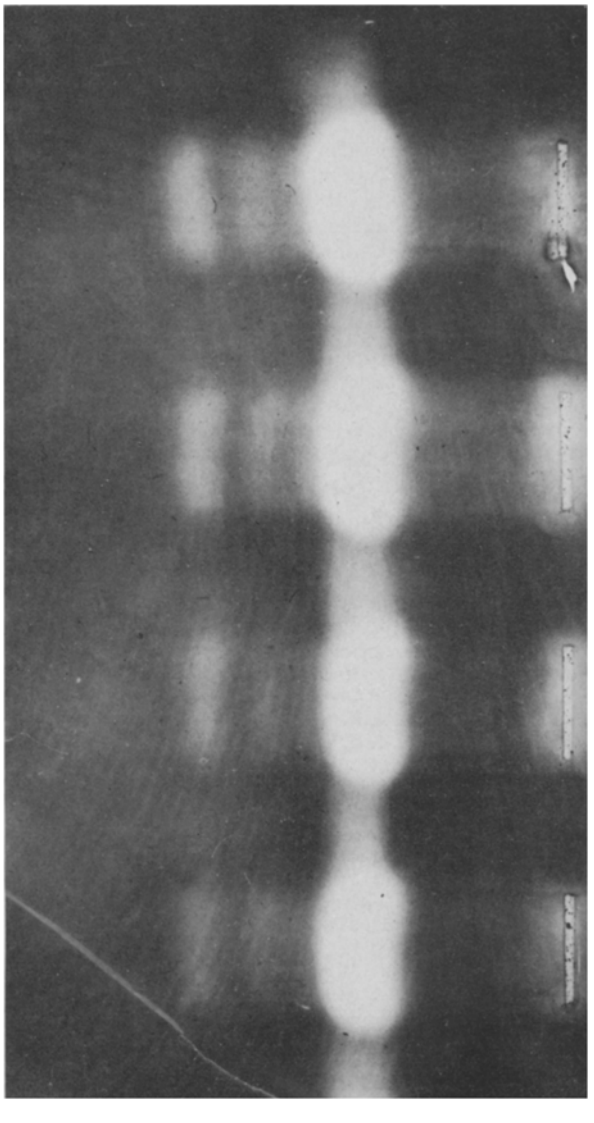

Fig. 2. Normal mucosa in the top slot. Note that no change in pattern was observed when 0.05, 0.2, and 0.5 mg of neutralized aspirin was homogenized with 1 cm³ of normal rat mucosal extract.

Results. On agar gel electrophoresis at pH 5.7 gastric mucosal homogenates from normal rats showed 4 major bands of proteolytic activity (Figure 1). The addition of acid to the normal gastric mucosal homogenates in vitro produced a change in this pattern of proteinases. The 4 bands of proteolytic activity seen in the normal gastric mucosal homogenates were no longer seen and were replaced by 3 bands of proteolytic activity with much enhanced electrophoretic mobility (Figure 1).

When aspirin (0.2–0.5 mgs/1 cm³ of gastric mucosal homogenate) was homogenized with mucosa, results identical to those observed after treatment of the mucosa with HCl were seen (Figure 1, second slot). However, the addition of neutralized solutions of aspirin in various concentrations had no effect on the pattern of proteinases observed on agar gel electrophoresis as compared to normal mucosa (Figure 2).

Normal rat gastric mucosal homogenates coagulated milk in the standard assay after some 2 min of incubation (normal human gastric mucosal homogenates do not coagulate milk), but the mucosal homogenates which had been acidified in vitro, all produced coagulation in less than 15 sec. Acid activation and neutralization of the normal rat gastric mucosal homogenate preparations in vitro resulted in a loss of some 60% of the proteolytic activity (in humans, such a treatment results in almost total loss of proteolytic activity). When these activated homogenates were subjected to electrophoresis at pH 8.3, only 1 band of residual proteolytic activity was visible (Figure 3), the other 2 bands having been destroyed by alkaline pH of 8.3. These experiments indicate that rat pepsinogens are different from human gastric mucosal pepsinogens. The disappearance of 2 components, however, confirms that acidification of the pepsinogens resulted in their activation to pepsins.

The stomach mucosae of the 18 rats in the control group appeared normal on examination with a hand lens, while 2 revealed 3 erosions in one, and 2 in another control animal. The gastric mucosal homogenates prepared from these animals coagulated milk slowly after some 2 or 3 min in a similar manner to other normal rat mucosal homogenates. On electrophoresis in both pH 5.7 and pH 8.3 buffer, these homogenates demonstrated the presence of the 4 bands of proteolytic activity seen in other normal rat mucosae. On electrophoresis at pH 8.3, the bands were still seen, indicating that they were due to the presence of zymogens. The 20 experimental animals given aspirin all showed between 15 and 45 gastric mucosal erosions when examined with a hand lens. The homogenates prepared from these mucosae all coagulated milk rapidly within 15 sec in a similar manner to the mucosa which had been acidified with HCl in vitro. On electrophoresis, none of the 20 mucosal homogenates prepared from these experimental animals showed 3 bands of proteolytic activity which corresponded in mobility to the bands seen in vitro acidified mucosa. Figure 3 is a diagrammatic representation of all our results on agar gel electrophoresis at pH 5.7.

Results of timed study. After 1 h 5–15 erosions were seen and after 2 h 10–25. After 3 h there were from 10–30 erosions. No difference in the pattern on agar gel electro-

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phoresis pattern was observed in the experimental and control animals. There was, therefore, no evidence of pepsinogen activation from 1 to 3 h after administration of aspirin.

Discussion. Human gastric mucosal pepsinogens are activated to pepsins by acid, the enzymes lose their activity on neutralization and cannot be detected on electrophoresis at pH 8.3[23], a pH 5.7 buffer being necessary to demonstrate them by agar gel electrophoresis[17]. In this latter buffer, both pepsinogens and pepsins may be separated and detected simultaneously, the pepsins showing a markedly greater electrophoretic mobility than the pepsinogens[24].

Although it seemed possible that intramucosal activation of pepsinogens to pepsins might occur on aspirin administration, due to a break in the mucosal barrier and the back diffusion of acid, no evidence was found in the present study to support this hypothesis. However, a lot of interesting data was collected during these experiments.

The experiments with normal rat gastric mucosa demonstrated that 4 bands of proteinase activity could be detected on electrophoresis at pH 5.7 and 8.3. This persistence of the bands at pH 8.3 indicates that under normal conditions these enzymes exist in the form of zymogens in the mucosa. Upon acidification and subsequent electrophoresis at pH 5.7, three more rapidly moving bands were identified. Two of these were destroyed by neutralization. This was confirmed by the electrophoresis at pH 8.3 which demonstrated the persistence of only 1 band in the acidified mucosa (Figure 3). The quantitative measurement of the persistence of proteinase activity in the acidified mucosal homogenate after neutralization also indicated that partial destruction of the proteinase activity occurred on neutralization. Further evidence of the activation of the mucosal zymogens by acid was given by the marked increase in milk-clotting activity observed in the preparations after acidification.

These results indicate that there are 4 major zymogens in rat mucosa and 1 zymogen may resemble more closely the zymogen of the human gastric mucosal cathepsin[23] which is resistant to destruction on acid activation and subsequent neutralization and shows enhanced mobility on agar gel electrophoresis after acid activation[24–27]. The presence of only 3 bands after acidification (Figure 1) only means that 2 pepsins merge with one another and cannot be separated by this technique.

While human pepsinogens do not clot milk after 2 min[20], rat mucosal pepsinogens were shown to clot milk after 2 min of incubation. Human pepsinogens are almost completely destroyed after acidification and subsequent neutralization[19], while the results of this study show that in a rat only 60% destruction occurs after such a treatment. Why we could not achieve the activation of pepsinogens to pepsins after aspirin injury to rat gastric mucosa needs further work to be understood.

Conclusions. Aspirin was postulated to produce gastric mucosal injury by back diffusion of H^+ ions[1–5]. HCl causes activation of pepsinogens to pepsins. Intramucosal activation of pepsinogens to pepsins was postulated as a mechanism of aspirin-induced rat gastric mucosal injuries.

No evidence of intramucosal activation of pepsinogens to pepsins in vivo was found by the present techniques; however, it was shown that rat gastric mucosal pepsinogens are activated to pepsins by unbuffered aspirin in the test tube, while neutralized aspirin did not alter the pepsinogens in the experiments done in this study. Rat pepsinogens were a little different compared to human gastric mucosal pepsinogens. While human pepsinogens are destroyed on acid activation and subsequent neutralization, not all rat pepsinogen behave in this way. One of the bands resists this treatment. Human pepsinogens do not coagulate milk even after 2 min, while rat mucosal pepsinogens were shown to clot milk after 2 min of incubation.

Résumé. La conversion intramuqueuse du pepsinogène en pepsine a été considérée comme l'un des facteurs responsables des lésions gastriques produites par l'aspirine chez le rat. Les expériences réalisées in vitro seraient en en faveur de cette hypothèse, mais elle n'a pas pu être confirmée in vivo.

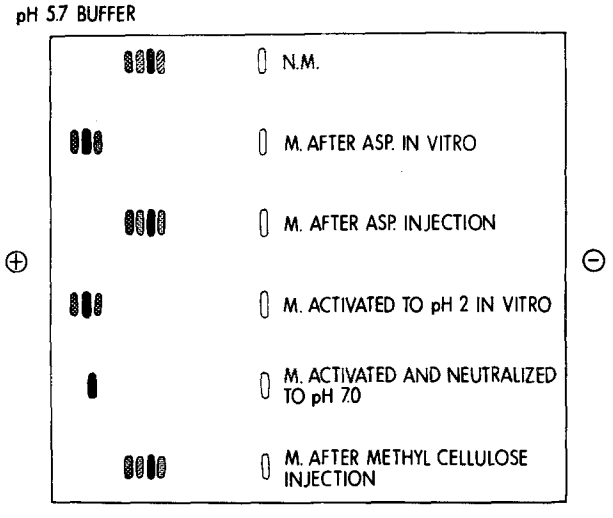

Fig. 3. Composite diagram of agar gel electrophoresis of rat gastric mucosa. Slot 1, normal mucosa. Slot 2, mucosa homogenized with unbuffered aspirin in vitro. Slot 3, mucosa after i.m. aspirin injection. Slot 4, mucosa after acid activation to pH 2 in a test tube. Slot 5, mucosa activated to pH 2.0, then neutralized to pH 7.0. Slot 6, mucosa after i.m. methyl cellulose injection N.M., normal mucosa; Asp., aspirin.

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