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# Effects of phenacetin, paracetamol and caffeine on the erosive activity of acetylsalicylic acid in the rat stomach: dose-response relationships, time course of erosion development and effects on acid secretion

A. J. M. SEEGERS*, L. P. JAGER† AND J. VAN NOORDWIJK

*National Institute of Public Health, P.O. Box 1, 3720 BA Bilthoven, The Netherlands and †Department of Pharmacology, Subfaculty of Pharmacy, State University of Utrecht, The Netherlands

Adult male and female Wistar rats were equally susceptible to gastric injury induced with acetylsalicylic acid (aspirin). Both in male and in female rats simultaneous administration of caffeine and aspirin caused significantly more gastric erosions than the same dose of aspirin alone; likewise addition of paracetamol to aspirin decreased the incidence of gastric lesions in either sex, and addition of phenacetin to aspirin had no effect. The potentiation by caffeine and the inhibition by paracetamol were both dose-dependent and only markedly influenced the development of erosions after 3–4 h. Pretreatment with phenacetin or paracetamol 1 h before administration of aspirin did not affect its erosive activity. Administration of benorylate caused no more gastric erosions than the vehicle or than equivalent mixtures of aspirin and paracetamol. The histamine-stimulated acid output of the stomach during gastric perfusion with aspirin was rapidly diminished. Neither paracetamol nor caffeine initially affected this decrease in acid output. However, 30 min after perfusion with aspirin and caffeine, acid secretion increased approximately as strongly as after caffeine alone. Caffeine potentiates aspirin-induced erosions by its stimulatory effect on acid secretion whereas paracetamol inhibits these erosions by preventing their growth.

Numerous clinical and laboratory studies have demonstrated that acetylsalicylic acid (aspirin) damages the gastric mucosa. A single oral dose can cause visible erosions of the epithelial lining of the stomach (Anderson 1964; Thorsen et al 1968), an increase in the rate of exfoliation of gastric mucosal cells (Croft 1966) and gastric bleeding (Grossman et al 1961; Davenport 1969). On the other hand little information is available about the erosive activities of drug mixtures containing aspirin. Recently we have shown that, in adult female rats, the combination of aspirin with phenacetin did not change, that of aspirin with caffeine significantly increased, and aspirin with paracetamol significantly decreased the incidence of gastric lesions compared with aspirin alone (Seegers et al 1978).

One of the purposes of the present study was to find whether the same results could be obtained using adult male rats, since it is known that sex as well as age and environmental conditions may influence the lesion-producing action of drugs on the glandular area of the rat stomach (Reilly et al 1969; Wilhelmi & Menassé-Gdynia 1972; Rainsford 1977). In addition, we studied the dose effect relationship of the potentiating effect of caffeine and of the inhibitory effect of paracetamol on aspirin-induced erosions as well as the interactions between aspirin and these compounds during the development of erosions. Furthermore, the erosive activity of benorylate (2-acetamido-2-acetoxybenzoate), a recently introduced anti-inflammatory compound (Malcolm 1974) with antipyretic (Alexander et al 1970) and analgesic properties (Addis-Jones et al 1973) was compared with that of equivalent mixtures of aspirin and paracetamol. After oral administration benorylate is slowly absorbed from the gastrointestinal tract and hydrolysed rapidly and completely in the blood, both in man (Robertson et al 1972) and in rats (Liss & Robertson 1975). It probably owes much of its pharmacological activity to this rapid hydrolysis to salicylic acid and paracetamol, but it may also have a pharmacological activity of its own (Khalili-Varasteh et al 1976).

Because the amount of acid present in the gastric lumen plays an important role in the pathogenesis of aspirin-induced erosions (Cooke 1973), the effects of gastric perfusion with analgesics alone and in combinations on the histamine-stimulated

* Correspondence.

acid output of the stomach were examined. The effects of paracetamol on aspirin-lethality and -anti-inflammatory activity were studied for the purpose of comparing these effects with its protective activity against aspirin-induced erosions.

## MATERIALS AND METHODS

### *Animals and drugs*

Male and female Wistar rats of an inbred strain (Centraal Proefdierenbedrijf TNO, Zeist), 170–200 g, were acclimatized for at least 1 week before each experiment during which time they had free access to food and water. Room temperature was 21 ± 0·5 °C.

Aspirin, phenacetin, paracetamol and caffeine were obtained commercially and their quality complied with the requirements of Ph.Ned.VIII. Benorylate was kindly provided by Winthrop Laboratories (lot no. 6670). Before use the drugs were ground (<90 $\mu$m) and in the gastric erosion-, lethality- and anti-inflammatory activity studies they were suspended in 4% Tween 80 and administered orally in a volume of 0·5 or 1·0 ml (lethality studies) per 100 g weight. Control rats received a similar volume of 4% Tween 80 solution. Drugs administered in the gastric perfusion studies were dissolved in 0·9% NaCl (saline).

### *Gastric erosions*

Food was withheld for 36 h before experiments but the animals were allowed free access to water, they were kept in cages (three to a cage; 0·32 × 0·20 × 0·20 m) with a metal grid (8 × 8 mm) to avoid coprophagy. The rats were killed 17 h after drug treatment (in the erosion-development studies: at 0·5, 1·5, 4, 8 and 17 h after treatment), the stomach was removed and opened along the greater curvature. After it had been rinsed in saline, it was stored in 4% formalin (buffered with 0·0067 M Sörensen buffer pH 7·0). The gastric mucosa was examined with a magnifier (10×) for the presence of lesions and erosion scores were calculated (Bonta 1961).

### *Gastric acid secretion*

Male rats were starved for 24 h but allowed free access to water. Atropine sulphate (30 mg kg$^{-1}$ i.p. as a 3% solution; Merck) was injected to block vagal influence on acid production. Anaesthesia was induced with pentobarbitone sodium (60 mg kg$^{-1}$ i.v. as a 1·2% solution into a tail vein; Abbott) and the trachea was cannulated.

The gastric lumen was perfused with saline pH 7·0, at a rate of 1·1 ml min$^{-1}$, using a technique (Aarsen 1959) similar to that described by Ghosh & Schild (1958). The fluid emerging from the pylorus passed over a glass electrode which recorded pH continuously. Perfusates were collected at 10 min intervals and the acid content was determined by potentiometric titration with 0·1 M NaOH to pH 7·0. The volume of the cannula from the tip in the pylorus to the point of sample collection was 0·8–0·9 ml. The time lag between administration of 0·1 ml of 0·1 M HCl via the tip of the oesophageal cannula and detection of a decrease in pH was 2 min.

Gastric acid secretion was stimulated with an infusion of histamine dihydrochloride (1·25 or 2·50 × 10$^{-7}$ M kg$^{-1}$ min$^{-1}$, expressed as base; Merck) via a cannula into an external jugular vein. The cannula was also used for repeat pentobarbitone sodium administration during the experiment. With saline-perfusion, histamine-stimulated acid secretion was determined over a period of 50 min after plateau values had been reached. The gastric lumen was then perfused for 50 min with saline to which the drugs had been added (pH 7·0). Afterwards, perfusion with saline was continued until control values were reached.

### *Lethality*

Male rats were kept separately in cages and allowed food and free access to water during the experiments. The oral LD 50 of aspirin alone and of aspirin + paracetamol was determined. The LD 50, the slope of the dose-response curve and 95% confidence limits were determined from the cumulative 10-day mortality according to Litchfield & Wilcoxon (1949).

### *Anti-inflammatory activity*

Using the method of Winter et al (1962), we measured the reduction of carrageenan-induced hindpaw oedema in male rats treated with aspirin, paracetamol and aspirin + paracetamol (1:1), respectively. The drugs were administered at zero time; 1 h later oedema was induced by subplantar injection of 0·1 ml of a 1% carrageenan solution (Marine Colloids Inc. lot no. 462106) into the right hindpaw. After 5 h paw-diameter was measured. Drug effects were calculated as percentage inhibition taking the swelling of the control group as 100%.

### *Plasma- and gastric tissue-concentrations*

Phenacetin and paracetamol plasma- and gastric tissue-concentrations were determined by gas-

liquid chromatography after silylation with bis(trimethylsilyl)trifluoro-acetamide (Supelco Inc.) on a 3% OV-17 column at 200 °C (Grove 1971; Prescott 1971; Thomas & Coldwell 1972). *p*-Bromo-acetanilide (Koch-Light Ltd.) was used as an internal standard.

Blood samples were taken by orbital puncture from groups of five starved rats (5–360 min after dosing); a 1·0 ml blood sample from each rat was collected in a beaker containing 0·02 ml of heparin solution (5,000 U.S.P.-units $ml^{-1}$), stored in an ice bath and centrifuged at 1000 *g*. After blood samples had been taken the animals were decapitated and the stomach dissected. Plasma and gastric tissue were stored at −20 °C until analysis.

Tissue samples were homogenized in 4 ml of phosphate buffer (1·0 M, pH 7·4) in a Potter-Elvehjem-type homogenizer. Homogenate (2 ml) or plasma (0·5 ml) with phosphate buffer (2 ml) was extracted with freshly distilled diethylether containing *p*-bromo-acetanilide ($0{\cdot}5 \times 10^{-5}$ M). After standing overnight an aliquot of 1 $\mu$l was chromatographed.

Recovery was determined by the addition of known amounts of phenacetin or paracetamol to plasma or homogenate and assayed as above. Recovery from plasma or tissue homogenate was essentially complete for phenacetin (99 ± 5%) and 70 ± 4% for paracetamol; these recoveries agreed well with those from aqueous solution.

Drug concentrations were determined by the Laboratory of Pharmacology of the National Institute of Public Health.

*Statistical analysis*

Regression lines of erosion score on log dose are presented only when there was a significant slope ($P<0{\cdot}05$) and no significant deviations from linearity ($P>0{\cdot}05$). In the case of non-linearity, connecting lines are shown; a horizontal line through the mean for all doses used indicates absence of a significant slope. Regression lines were compared according to Diem & Lentner (1969). The significance of the differences observed in the studies of erosion development, gastric acid secretion and carrageenan-induced oedema was evaluated using Student's *t*-test. For caffeine regression of acid secretion-stimulation on log concentration was also calculated. The lethality data of aspirin and of the combination aspirin + paracetamol were fitted to curves by logit analysis. The goodness of fit of the curves to the experimental data was tested with a Chi-square test. Unless otherwise indicated, differences were assumed to be real when tests indicated probability levels of less than 5%.

## RESULTS

*Gastric erosions*

*Gastric erosions in male and in female rats.* The erosive activities of aspirin, and of the combinations: aspirin + phenacetin, aspirin + paracetamol and aspirin + caffeine, were examined after oral administration to male and female rats. The animals were 9–11 weeks old and weighed 187 (8)g and 183 (7)g respectively (mean with s.d.). For the dose-regimen of the combinations phenacetin, paracetamol or caffeine were mixed with aspirin (60, 125, 250 and 500 mg $kg^{-1}$) in the ratios 1:1, 1:1, 1:5, respectively. Ten rats of either sex received each dose.

The log dose-response functions of female rats did not significantly differ from those of male rats (Fig. 1). Furthermore, these data confirm some of our earlier observations (Seegers et al 1978).

*Interaction of caffeine with aspirin.* As indicated by the increased slope of the log dose-response curve (Fig. 1), caffeine potentiates the erosive activity of aspirin. This potentiation could not be due directly to locally irritating effects of caffeine on the gastric mucosa, since treatment with it alone did not cause gastric erosions (Seegers et al 1978). To

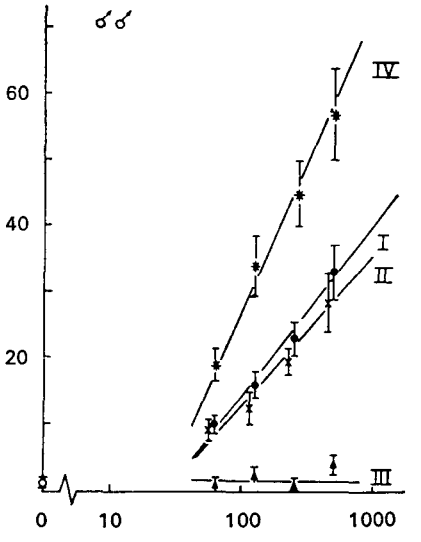


FIG. 1. Mean erosion scores (± s.e.m.) as a function of log dose in male rats. Aspirin ● (I), aspirin + phenacetin × (II), aspirin + paracetamol ▲ (III) and aspirin + caffeine * (IV), n = 10. Ordinate: erosion score, 4% Tween 80 ○. Abscissa: log dose aspirin (mg $kg^{-1}$). Results for female rats were not significantly different.

elucidate the nature of this potentiation we studied the effects of different doses of caffeine (6·25, 12·5, 25, 50 and 100 mg $kg^{-1}$) on the erosive activity of the same dose of aspirin (250 mg $kg^{-1}$). In addition the effects of fixed doses of caffeine (6·25 and 25 mg $kg^{-1}$) on the log dose-response curve of aspirin were investigated. Nine male rats received each dose.

The erosion scores of all doses of aspirin increased after combination with a fixed dose of caffeine by a constant amount (Fig. 2). The extent of this increase depends on the dose of caffeine.

*Interaction of paracetamol with aspirin.* Paracetamol inhibits the erosive activity of aspirin (Fig. 1). To elucidate the nature of this inhibition we studied the effects of different doses of paracetamol (15, 30, 60, 125 and 250 mg $kg^{-1}$) on the erosive activity of the same dose of aspirin (250 mg $kg^{-1}$). In addition the effects of fixed doses of paracetamol (30 and 125 mg $kg^{-1}$) on the log dose-response curve of aspirin were examined. Ten male rats received each dose.

Paracetamol, administered simultaneously with aspirin, produced a log dose-dependent linear decrease in erosion score (Fig. 3). However, in

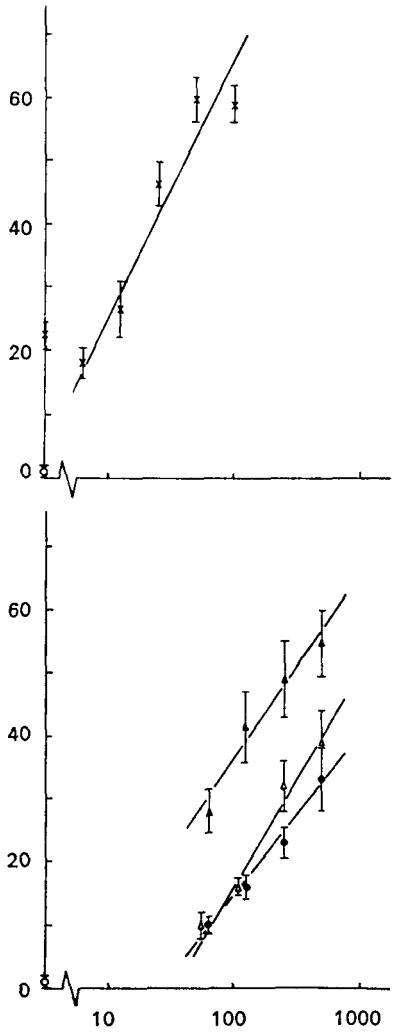


FIG. 2. Mean erosion scores (± s.e.m.) as a function of log dose of caffeine and aspirin. Aspirin 250 mg $kg^{-1}$ + caffeine ×, aspirin ●, caffeine 6·25 mg $kg^{-1}$ + aspirin △, and caffeine 25 mg $kg^{-1}$ + aspirin ▲, n = 9. Ordinate: erosion score, 4% Tween 80 ○. Abscissa: log dose caffeine (mg $kg^{-1}$) (upper figure) and aspirin (lower figure).

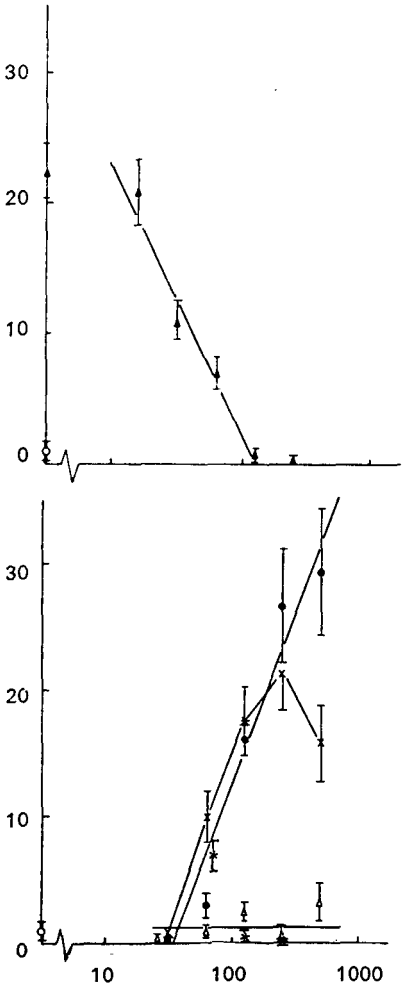


FIG. 3. Mean erosion scores (± s.e.m.) as a function of log dose of paracetamol and aspirin. Aspirin 250 mg $kg^{-1}$ + paracetamol ▲, aspirin ●, paracetamol 30 mg $kg^{-1}$ + aspirin ×, and paracetamol 125 mg $kg^{-1}$ + aspirin △, n = 10. Ordinate: erosion score, 4% Tween-80 ○. Abscissa: Log dose paracetamol (mg $kg^{-1}$) (upper figure) and aspirin (lower figure).

contrast with the relation between caffeine and aspirin, the effect of a constant dose of paracetamol depends on the dose of aspirin: paracetamol, 125 mg $kg^{-1}$ caused a complete inhibition of the erosive activity of aspirin over the dose range used, whereas a dose of 30 mg $kg^{-1}$ only inhibited the erosive action of 500 mg $kg^{-1}$ of aspirin partially (but nevertheless significantly).

*Benorylate and gastric erosions.* The erosive activity of benorylate was examined with doses, corresponding to 60, 125, 250 or 500 mg $kg^{-1}$ of aspirin. Ten male rats received each dose.

Benorylate in these doses did not cause gastric erosions significantly different from those of the vehicle-treated rats, nor from those treated with equivalent mixtures of aspirin with paracetamol.

*Effects of pretreatment with paracetamol or phenacetin on aspirin-induced erosions.* The inhibition of aspirin-induced erosions, observed when rats were treated simultaneously with paracetamol, might be due to: (i) an extracellular action of paracetamol, such as an interaction with aspirin within the stomach or masking the mucosal cells; (ii) an intracellular action of paracetamol which renders the mucosal cells insensitive to the erosive action of aspirin.

According to the second possibility, the inhibitory action of paracetamol does not depend on its simultaneous administration with aspirin, but on its presence in the gastric mucosal cells. So protection against aspirin-irritancy might be expected after pretreatment with paracetamol or with phenacetin, which is known to be converted mainly into paracetamol (Raaflaub & Dubach 1975; Smith & Griffiths 1976).

Fig. 4 shows that administration of 250 mg $kg^{-1}$ of either paracetamol or phenacetin caused similar paracetamol-plasma concentrations after 1–1·5 h. Furthermore, the concentrations reached after 1 h were hardly different from peak-values. Pretreatment (1 h) with paracetamol (125 mg $kg^{-1}$) or with an equimolar amount of phenacetin (147 mg $kg^{-1}$) did not inhibit the erosive activity of aspirin in male rats.

*Interactions of paracetamol and caffeine with aspirin during development of erosions.* The erosive activities of aspirin (250 mg $kg^{-1}$), paracetamol (125 mg $kg^{-1}$), caffeine (50 mg $kg^{-1}$) and of the combinations aspirin + paracetamol (250 + 125 mg $kg^{-1}$) and aspirin + caffeine (250 + 50 mg $kg^{-1}$) in male rats

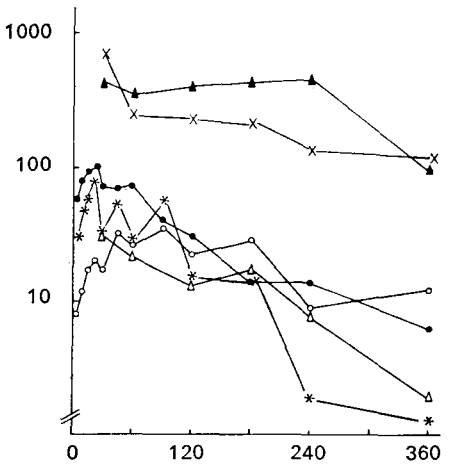


FIG. 4. Mean concentrations of paracetamol (lefthand figure) and phenacetin (righthand figure) in plasma and gastric wall after oral treatment (250 mg $kg^{-1}$). Paracetamol in plasma after paracetamol orally. ●, paracetamol in plasma after phenacetin orally ○, phenacetin in plasma after phenacetin orally *, paracetamol in gastric wall after paracetamol orally. ▲, paracetamol in gastric wall after phenacetin orally △, and phenacetin in gastric wall after phenacetin orally ×, n = 5. Ordinate: concentration ($\mu$g $g^{-1}$). Abscissa: time (min).

were determined at 0·5, 1·5, 4, 8 and 17 h after administration. For each time 10 animals received each dose. The results are presented in Fig. 5.

Within 1·5 h after aspirin treatment small erosion-foculi (≤2 mm) appeared in the glandular mucosa. The maximal erosion-response was reached after 8 h. Paracetamol hardly influenced the number of

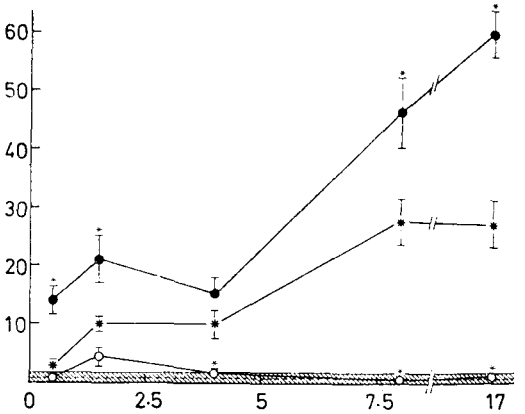


FIG. 5. Mean erosion scores (± s.e.m.) at 0·5, 1·5, 4, 8 and 17 h after treatment with aspirin 250 mg $kg^{-1}$*, aspirin + paracetamol 250 + 125 mg $kg^{-1}$ ○ and aspirin + caffeine 250 + 50 mg $kg^{-1}$ ●. Shaded area: Mean (– –) and 95% confidence limits of 4% Tween 80. n = 10. Ordinate: erosion score. Abscissa: time (h). *$P < 0.01$ compared with aspirin value. The erosion scores of paracetamol 125 mg $kg^{-1}$ and of caffeine 50 mg $kg^{-1}$ did not differ significantly from 4% Tween 80 value.

Table 1. Effects of gastric perfusion (1·1 ml $min^{-1}$, saline pH 7·0) with aspirin, paracetamol and caffeine as single drugs and in admixture on histamine-stimulated acid secretion in the rat.*

| Treatment | Concn (mM) | n | Mean histamine-stimulated acid secretion ($\mu$equiv $H^+$ 10 $min^{-1}$) | Δ Acid secretion ($\mu$equiv $H^+$ 10 $min^{-1}$) | Time between start of drug perfusion and max effect (min) |
|---|---|---|---|---|---|
| Aspirin | 9·2 | 7 | 9·8 ± 1·6† | −8·5 ± 0·8†‡ | 31 ± 2† |
| Paracetamol | 11·0 | 4 | 5·3 ± 1·6 | +0·4 ± 0·2 | — |
| Caffeine | 27·2 | 4 | 8·0 ± 1·2 | +2·7 ± 0·2‡ | 87 ± 8 |
| Aspirin + paracetamol | 9·2 + 11·0 | 4 | 9·7 ± 0·7 | −7·5 ± 0·4‡ | 35 ± 3 |
| Aspirin + caffeine | 9·2 + 27·2 | 4 | 8·8 ± 0·6 | −6·7 ± 0·5‡ | 35 ± 6 |
| | | | | +3·1 ± 0·2‡ | 92 ± 5 |

* Acid secretion was stimulated with histamine 2·5 × $10^{-7}$ M $kg^{-1}$ $min^{-1}$ i.v.
n = number of animals.
† Mean ± s.e.m.
‡ Δ Acid secretion significantly different from zero ($P < 0·05$).

erosion-foculi at 1·5 h, but after 4 h mucosal damage could not be observed anymore. Within 0·5 h caffeine potentiated the erosive action of aspirin by increasing both the number and the severity of the erosions.

*Effects of analgesics on gastric acid secretion during histamine stimulation*

Table 1 shows the effects of gastric perfusion with aspirin (9·2 mM), paracetamol (11·0 mM), caffeine (27·2 mM) and with the combinations aspirin + paracetamol (9·2 + 11·0 mM) and aspirin + caffeine (9·2 + 27·2 mM) on sub-maximal histamine-stimulated (2·5 × $10^{-7}$ M $kg^{-1}$ $min^{-1}$) acid secretion in male rats.

During gastric perfusion with aspirin a progressive decrease in histamine-stimulated acid output which reached plateau levels within 30 min was observed. On the other hand with caffeine a slight, but nevertheless significant increase in acid output over 80–130 min after starting its perfusion was found. In addition the effects of increasing concentrations of caffeine (6·8, 13·6, 27·2 and 54·4 mM) on half-maximal histamine stimulation (1·25 × $10^{-7}$ M $kg^{-1}$ $min^{-1}$) were studied. From the linear regression of the increment of acid secretion on log concentration ($\Delta$ $\mu$equiv $H^+$ 10 $min^{-1}$ = 2·26 (log [caffeine]) + 1·16; n = 10, r = 0·91) it appeared that the stimulatory effect of caffeine was dose-dependent ($P < 0·05$). Perfusion with paracetamol did not affect histamine-stimulated acid output.

The combination of aspirin with either paracetamol or caffeine showed a reduction of acid output comparable to that observed with aspirin alone. However, within 80 min after starting perfusion with the combination of aspirin + caffeine an increase in acid secretion was found, not significantly different in magnitude and duration from the effect of caffeine alone.

*Effect of paracetamol on aspirin lethality*

The oral LD50 of aspirin alone and after simultaneous treatment with a subtoxic dose of paracetamol (200 mg $kg^{-1}$) was determined. The dosages of aspirin ranged from 1000 to 3310 mg $kg^{-1}$. Five groups of 15 male rats per group were dosed for each LD50 determination.

A protective effect of paracetamol against aspirin-induced mortality could not be demonstrated statistically (Table 2).

*Effect of paracetamol on aspirin-anti-inflammatory activity*

The reduction of carrageenan-induced hindpaw oedema was measured after treatment with aspirin (125 and 250 mg $kg^{-1}$), paracetamol (125 and 250 mg $kg^{-1}$) and aspirin + paracetamol (125 + 125

Table 2. Effect of a subtoxic dose of paracetamol on the lethality of aspirin in male Wistar rats*.

| | Treatment | |
|---|---|---|
| | Aspirin | Aspirin + paracetamol |
| Number of animals | 75 | 75 |
| Slope | 1·30 | 1·53 |
| Confidence limits (95%) | 0·90–1·87 | 0·99–2·37 |
| LD 50 (mg $kg^{-1}$ aspirin) | 1980 | 2050 |
| Confidence limits (95%) | 1737–2257 | 1730–2429 |

* Paracetamol (200 mg $kg^{-1}$) was orally administered simultaneously with aspirin, lethality was determined from the cumulative 10-day mortality.

and 250 + 250 mg kg$^{-1}$). Eight male rats received each dose.

The results (Table 3) show that these doses of aspirin and of paracetamol produced significant reductions in the development of the paw-swelling. After combination of the drugs the response was equal to at least the sum of the responses of the drugs given separately.

Table 3. Anti-inflammatory activity of aspirin, paracetamol and aspirin + paracetamol (1 : 1) in the rat.

| Treatment | Dose (mg kg$^{-1}$) | n | Reduction of paw-diameter in carrageenan hindpaw oedema test* (%) |
|---|---|---|---|
| 4% Tween-80 | — | 24 | 0 ± 3·2† |
| Aspirin | 125 | 8 | 24·4 ± 7·5‡ |
| | 250 | 8 | 41·9 ± 5·3¶ |
| Paracetamol | 125 | 8 | 19·9 ± 7·6‡ |
| | 250 | 8 | 20·9 ± 7·9‡ |
| Aspirin + paracetamol | 125 + 125 | 8 | 53·5 ± 8·7¶ |
| | 250 + 250 | 8 | 69·6 ± 6·5¶ |

* Oedema was induced by injection of 0·1 ml of a 1% carrageenan solution 1 h after oral drug treatment. Paw-diameter was measured 4 h after administration of the drugs.
n = number of animals.
† Mean ± s.e.m.
‡ $P < 0{\cdot}01$ compared to control value.
¶ $P < 0{\cdot}001$ compared to control value.

## DISCUSSION

The results indicate that adult male and female Wistar rats are equally susceptible to gastric injury by aspirin. A sex difference in erosion score, similar to the observations of Reilly et al (1969) after the administration of reserpine to 7- and 20-week old rats of the Osborne-Mendel strain was not observed in our Wistar rat of 10 weeks. Wilson (1966) reported the production of erosions by immobilization stress in Wistar rats at six or eight weeks of age to be independent of sex. Whether these differences are due to differences in strain or in erosive agent is not clear. In male and in female animals simultaneous administration of phenacetin with aspirin did not change the incidence of gastric lesions compared with aspirin alone. Also in either sex, simultaneous administration with paracetamol inhibited the erosive activity of aspirin, whereas caffeine had a pronounced potentiating effect on gastric erosions induced with aspirin.

The increased slope of the log dose-response curve of the aspirin-caffeine mixture (5 : 1), compared with that of aspirin alone, indicates a dose-dependent potentiation of aspirin-induced erosions by caffeine. In agreement with this observation we found a caffeine dose-dependent increase in erosions induced with a constant dose of aspirin. Conversely, addition of a constant dose of caffeine to increasing doses of aspirin caused a parallel upward shift of the regression line of erosion score on log dose of aspirin. From this shift it is likely that the relation observed with the aspirin-caffeine mixture (5 : 1) actually constitutes a cross-section through several parallel shifted relations. The present data also show that a relatively low dose of 25 mg kg$^{-1}$ of caffeine induced a threefold increase in erosion score. Thus, the erosive activity of 60 mg kg$^{-1}$ aspirin in admixture with 25 mg kg$^{-1}$ caffeine is similar to that of 500 mg kg$^{-1}$ aspirin alone.

Gastric acid is an important factor in the development of aspirin-induced erosions (Cooke 1973). After aspirin has penetrated the mucosal cells of the stomach hydrogen ions diffuse back (Davenport 1967). If sufficient hydrogen ions penetrate the mucosa, bleeding and gastric damage will occur (Cooke 1976). Gastric acid output is the resultant of two fluxes: hydrochloric acid secretion by parietal cells minus that which is absorbed and neutralized. The observed rapid decrease in acid output during gastric perfusion with aspirin is probably the result of both a decrease in the secretion of hydrochloric acid (Flemström & Marsden 1973) and an increase in back diffusion of hydrogen ions into the mucosa (Davenport 1967). Caffeine does not affect this rapid reduction in net acid output. As caffeine alone does not increase acid output until 80 min after the beginning of perfusion, and has no effect on the gastric mucosal barrier (Chvasta & Cooke 1972), it seems unlikely that this delayed stimulation of acid output is due to changes in hydrochloric acid secretion and not to changes in back diffusion of hydrogen ions. These data strongly indicate that the observed potentiating effect of caffeine on the development of aspirin-induced erosions with time is caused by a delayed stimulation of acid secretion by caffeine administered orally.

In contrast with caffeine, the log dose-response curve of the aspirin-paracetamol mixture (1 : 1) only indicates an inhibition of the aspirin-induced erosions by paracetamol within the dose-range used. With identical doses of aspirin, a dose-dependent relation was found for the decrease in erosion score by paracetamol. This inhibition could not be due to a change in acid secretion since paracetamol did not affect the acid output nor the aspirin-induced reduc-

tion of net acid output. Whatever the mechanism of the inhibitory action of paracetamol on aspirin-induced erosions may be our results suggest that it is non-competitive.

Regarding the localization of the protective action of paracetamol two possibilities were taken into consideration:

(i) an extracellular action and
(ii) an intracellular action of paracetamol.

The latter (ii) could not be confirmed by the results of the pretreatment experiments. Relevant for possibility (i) is the recent report of Brune et al (1977) that during salicylate absorption salicylate anions were rapidly trapped in the glandular part of the gastric mucosa. Very high concentrations of salicylate were found in the parietal cells for a few minutes after oral administration. These authors suggested that this transient accumulation initiates decay of parietal cells and that reduction of this accumulation may diminish gastric toxicity of aspirin. However, it is unlikely that paracetamol inhibits the incidence of aspirin-induced erosions by reducing salicylate-trapping in parietal cells, since administration of paracetamol with aspirin did not affect the absorption of salicylate into the glandular mucosa (Seegers et al in preparation).

Summarizing the results obtained with paracetamol, we conclude that paracetamol protects the gastric mucosa against irritation by aspirin only when it is administered orally with the aspirin. As the genesis of aspirin-induced erosion-foculi is hardly influenced by paracetamol, this protection is possibly caused by preventing the development of erosion-foculi into larger erosions and/or by stimulating the process of erosion-healing. This observation also corroborates the suggestion that the mechanism of the inhibitory action of paracetamol is non-competitive. In addition, the protective action of paracetamol in the dose-range on aspirin-induced erosions in rats is accompanied by favourable effects on aspirin-lethality and -anti-inflammatory activity.

The erosive activity of benorylate was not significantly different from that of the vehicle, nor from that of an equivalent mixture of aspirin and paracetamol. Since benorylate is hardly hydrolysed into salicylic acid and paracetamol within the gastric lumen (Davison et al 1977), we concluded that, under the conditions used, it has no erosive activity of its own. Its relatively slow absorption, in comparison with that of a mixture of aspirin and paracetamol (Liss & Robertson 1975) might contribute to this favourable effect. However, during absorption through the gastric wall benorylate is hydrolysed within the mucosa (Humphreys & Smy 1975). As no erosive activity was observed, the following conclusions may be drawn:

(i) the effective concentration of salicylate within the gastric mucosa is too low to produce erosions;
(ii) the erosive activity of salicylate is inhibited by the second product of hydrolysis: paracetamol.

However, the observation that administration of paracetamol 1 h before aspirin does not prevent gastric erosions in spite of its presence in the gastric wall (see Fig. 4) makes the second conclusion less likely. On the other hand the first conclusion is not invalidated by the observation that an equivalent mixture of aspirin and paracetamol does not cause gastric erosions.

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