also retard the attack by hydroxyl ions on the activated oxygen complex.

These results have demonstrated that the degradation kinetics of promethazine hydrochloride are modified by the physical state of the drug molecules in solution. This effect is likely to be observed with all drug systems that are prone to aggregation and it is thus necessary to carry out both physical and kinetic studies under identical conditions before interpretation of accelerated stability data obtained with such systems can be properly evaluated.

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The determination of salicylic acid and benzoic acid in pharmaceutical formulations by spectrofluorimetry

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Methods of extraction from pharmaceutical formulations and subsequent determination of benzoic acid and salicylic acid by spectrofluorimetry are described. The recovery of benzoic acid, in the presence of salicylic acid, was 99·3%, with a coefficient of variation of 1·04%, while the recovery of salicylic acid, in the presence of benzoic acid, was 97·7%, with a coefficient of variation of 0·68%.

The determination of benzoic acid when formulated with salicylic acid in pharmaceutical preparations can be troublesome. The assay of benzoic acid in compound benzoic acid ointment of the British Pharmaceutical Codex (1973) relies on an indirect method. The salicylic acid is determined by its bromine absorption, (Kolthoff, 1921) and subsequently a correction for the calculated salicylic acid content is made in the titration of the total acid. Such a procedure suffers from possible interference (in some formulations) from either acids or substances that react with bromine. The fluorescence of salicylic acid in organo-chlorine solvents is enhanced by the presence of aliphatic carboxylic acids while benzoic acid fluoresces at a lower wavelength but less strongly than salicylic acid (Schenk, Boyer & others, 1972).

MATERIALS AND METHODS

Reagents

The benzoic acid and salicylic acid employed were of B.P. quality. Glacial acetic acid, hydrochloric acid and methanol were 'Pronalys' grade while iso-octane and diethylamine were reagent grade (May and Baker Ltd, Dagenham). Dichloromethane was glass distilled, suitable for fluorimetry (Rathburn Chemicals, (Walkerburn) Ltd, Peebleshire).

The fluorimetric solvent consisted of 1% glacial acetic acid in dichloromethane.

Instrumentation settings

The instrument used was a Perkin Elmer MPF-3 spectrofluorimeter. The settings for the determination of benzoic acid were as follows: excitation wavelength, 282 nm; slit width 7, emission wavelength, 305 nm; slit width, 7; sensitivity 10. The settings for the determination of salicylic acid were as follows; excitation wavelength, 315 nm; slit width, 7; emission wavelength, 445 nm, slit width, 7, and sensitivity, 3.

Extraction of analytes

(a) *Creams and ointments.* A portion of the sample, equivalent to 100 mg benzoic acid and/or 100 mg salicylic acid, was accurately weighed into a 100 ml separator and dispersed in 40 ml iso-octane.

The solution was extracted with three 25 ml aliquots of methanol which were bulked and made up to 100 ml with methanol.

(b) *Aqueous solutions.* A suitable volume, equivalent to approximately 100 mg benzoic acid and/or salicylic acid, was pipetted into a 100 ml separator, made acid with dilute hydrochloric acid and extracted with three 25 ml aliquots of dichloromethane. The combined extracts were made up to 100 ml with dichloromethane.

Analysis

5 ml of the extract was pipetted into a 200 ml volumetric flask and diluted to volume with fluorimetric solvent (Solution A).

(a) Salicylic acid: 5 ml of solution A was pipetted into a 100 ml volumetric flask and diluted to volume with the fluorimetric solvent. The relative fluorescence of this solution was measured under the conditions described and compared with that of a standard solution of salicylic acid (1·25 μg ml^{-1}).

(b) Benzoic acid: Five 2 ml aliquots of Solution A were pipetted into each of five 50 ml volumetric flasks containing 0, 1, 2, 3 and 4 ml benzoic acid standard solution (25 μg ml^{-1}) and made up to volume with the fluorimetric solvent. The relative fluorescence intensities, of the solutions, 4 ml in a 10 mm silica cuvette, were measured, under the conditions described, before and after the addition of 3 drops of diethylamine. The second reading was

* Correspondence.

subtracted from the first reading and a graph constructed of the difference fluorescence reading versus the concentration of added standard. The concentration of benzoic acid in the sample solution, and hence in the original product, was determined from the intercept on the concentration axis when the plotted points were extrapolated.

Fluorescence quenching experiments

(i) A solution of benzoic acid (25 μg ml^{-1}) in the fluorimetric solvent was prepared. 2 ml aliquots were pipetted into each of four 50 ml volumetric flasks to which was added 0, 10, 15 and 20 ml of a solution of salicylic acid in the fluorimetric solvent, (25 μg ml^{-1}) and made up to volume with the solvent. The fluorescences of the solutions were read under the conditions previously described for the determination of benzoic acid. From the data, plots (Fo/F)-1 *vs* [Q] and (Fo/F-1)[Q] *vs* [Q] were constructed. Fo is the fluorescence of the solution without salicylic acid, F is the fluorescence of the solution with salicylic acid added, and [Q] is the concentration of added salicylic acid.

(ii) The following solutions were prepared.

(a) benzoic acid solution in the fluorimetric solvent (50 μg ml^{-1});

(b) as (a) with (200 μg ml^{-1}) salicylic acid added;

(c) salicylic acid solution in the fluorimetric solvent (200 μg ml^{-1}).

The fluorescence of solutions (a) and (b) were measured at the excitation and emission maxima for benzoic acid. The fluorescences of solutions (b) and (c) were measured at the excitation and emission maxima for salicylic acid. The fluorescences of solutions (b) and (c) were measured at the emission maximum for salicylic acid and excited at the excitation maximum for benzoic acid.

RESULTS AND DISCUSSION

Benzoic acid exhibits very weak fluorescence in dichloromethane but with the addition of glacial acetic acid there is a 14-fold increase in its relative fluorescence intensity, making its analysis in the presence of salicylic acid feasible since their emission maxima are well separated (Fig. 1). However, salicylic acid quenched the fluorescence of benzoic acid and vice versa but there was no increase in the fluorescence of salicylic acid in the presence of benzoic acid, when excited at the wavelength of maximum excitation for benzoic acid. This would tend to indicate that trivial processes of quenching were not involved e.g. reabsorption of the benzoic acid fluorescence by salicylic acid molecules with the

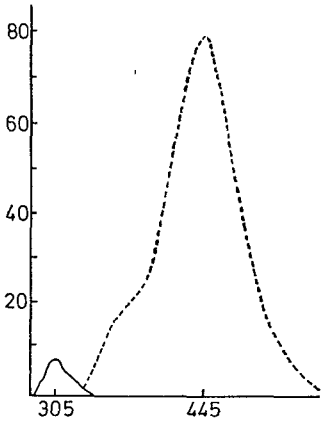

FIG. 1. The relative fluorescence intensity (ordinate) of benzoic acid (—) excited at 282 nm and the relative fluorescence intensity of salicylic acid (– – –) excited at 315 nm. Abscissa: Emission wavelength (nm).

subsequent emission of fluorescence characteristic of salicylic acid. Also, a radiationless transfer mechanism, by association of the molecules, was discounted since a plot of (Fo/F)-1 *vs* the added quencher concentration exhibited no deviation from a straight line (Fig. 2). This plot is based on the Stern-Volmer (1919) relation where

$$Fo/F = 1 + Kq[Q]$$

and Kq is the quenching constant. A plot (Fo/F)-1/[Q] gave a straight line with zero slope which according to Moon, Poland & Scheraga (1965) indicated that collisional processes are involved. According to these authors this is a more sensitive means of detecting association of the molecules, which is manifested by deviation from a zero slope.

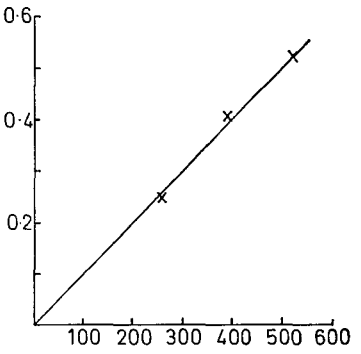

FIG. 2. The Stern-Volmer plot for benzoic acid at a concentration of 1 μg ml^{-1}, in the presence of increasing amounts of quencher (salicylic acid). Ordinate—(Fo/F) —1. Abscissa: Salicylic acid concentration (μg per 50 ml).

Since mutual quenching occurs it has to be corrected by the use of the standard addition technique for the analysis of benzoic acid. This technique overcomes errors of a proportional nature. The quenching of salicylic acid by benzoic acid was negligible at the concentrations used so that salicylic acid could be determined directly.

A sample of compound benzoic acid ointment, B.P.C., was analysed for its benzoic acid content by the method but without diethylamine. Thus, the unquenched benzoic acid fluorescence plotted against added standard concentration gave a straight line but high results were obtained, suggesting that some other component, co-extracted with the organic acids, caused an appreciable background fluorescence. By addition of diethylamine to the sample solution only the benzoic acid fluorescence was quenched. Thus, by measuring the difference between the original fluorescence and the fluorescence on the addition of diethylamine a result consistent with the stated label strength was obtained.

Using this technique, benzoic acid in a solution of benzoic and salicylic acids of known strength (1% of each) was assayed. The salicylic acid was also determined but, in this case, the interference from background fluorescence had a negligible effect and the addition of diethylamine was not necessary. Six replicates of benzoic acid and salicylic acid gave results of good accuracy and reproducibility. The recoveries were 99·3 and 97·7% and the coefficients of variation were 1·04 and 0·68% for benzoic acid and salicylic acid respectively.

Having ascertained the accuracy and precision of the method on a solution of known composition the method was used to determine the acids in a number of pharmaceutical formulations and, where applicable, compared with the results obtained by means of compendial methods (Tables 1 and 2). The recoveries of salicylic acid and benzoic acid in two 'official' ointments were in good agreement with the compendial titration method and the recoveries in the other preparations were good with one exception. In the case of the proprietary cream a low benzoic acid content was found but this was likely to be a true result since the absorbance of an extract was less than that of a standard solution of salicylic acid and benzoic acid made up to the same concentration. The official analytical technique could not be applied to this cream since another organic acid was present that interfered with the titration method.

The separation of the acids from ointment and cream base by partition between methanol and iso-octane gave an emulsion-free, clean separation and allowed for the rapid accurate and precise determination of benzoic acid and salicylic acid in the same preparation by spectrofluorimetry.

Table 1. *Comparison of the proposed method with the official method for the determination of salicylic acid in the presence of benzoic acid in a number of pharmaceutical formulations.*

Product	Salicylic acid content (%)	Proposed procedure Found	Proposed procedure Recov. (%)	B.P.C. method Found	B.P.C. method Recov. (%)
Co. benzoic acid ointment A	3·0	3·15	105·0	3·04	101·3
Co. benzoic acid ointment B	3·0	3·20	106·7	3·23	107·7
Proprietary cream	0·6	0·63	105·0	—	—
Co. glycerin of thymol A	0·49	0·47	95·9	—	—
Co. glycerin of thymol B	0·49	0·48	98·0	—	—

Table 2. *Comparison of the proposed method and the official method for the determination of benzoic acid in the presence of salicylic acid in some pharmaceutical products.*

Product	Benzoic acid content (%)	Proposed method Found	Proposed method Recov. (%)	B.P.C. method Found	B.P.C. method Recov. (%)
Co. benzoic acid ointment A	6·0	5·78	96·3	5·83	97·2
Co. benzoic acid ointment B	6·0	5·71	95·2	5·80	96·7
Proprietary cream	1·0	0·83	83·0	*	—
Co. glycerin of thymol A	0·68	0·75	110·3	—	—
Co. glycerin of thymol B	0·64	0·68	106·3	—	—

* See text.

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Gastric erosions induced by analgesic drug mixtures in the rat

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Gastric erosions after oral administration of analgesics separately and in admixture have been examined in adult rats. After administration of acetylsalicylic acid (aspirin), phenacetin, paracetamol and caffeine as single drugs, gastric erosions were only observed with aspirin. 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. The results for aspirin with paracetamol did not differ from those for the vehicle. Addition of caffeine to the combination of aspirin and phenacetin caused a significant increase in erosions, but when given with aspirin and paracetamol no erosions occurred. The mechanisms underlying the effects of these drugs on aspirin-induced erosions are discussed.

Gastric erosion as a side effect of acetylsalicylic acid (aspirin) treatment has been widely reported in clinical as well as in animal studies (Muir, 1963; Anderson, 1964; Brodie & Hooke, 1971; Cooke, 1976a). Furthermore, clinical reports (Douglas & Johnston, 1961; Duggan & Chapman, 1970) on the relations between gastric erosion and ingestion of mixtures containing aspirin, phenacetin and caffeine did not indicate simple addition of the erosive activities of the constituents. Therefore, we have examined the effects of phenacetin, paracetamol and caffeine on the aspirin-induced erosions in the glandular area of the rat stomach.

METHODS AND MATERIALS

Female Wistar rats of an inbred strain (Centraal Proefdierenbedrijf, TNO, Zeist), 180–200 g, starved for 36 h but allowed free access to water, were kept in cages (four to a cage; 0·32 × 0·20 × 0·20 m) with a metallic grid (8 × 8 mm) to avoid coprophagy. Room temperature was maintained at 21 ± 0·5°. The rats were killed 17 h after drug treatment, the stomachs removed and opened along the greater curvature. After they had been rinsed in a solution of 0·9% NaCl, they were stored in 4% formalin (buffered with 0·0067 M Sörensen buffer pH 7·0).

The gastric mucosa was examined with a magnifier (×10) the number of erosions per stomach counted and weighted for their severity according the scale of Bonta (1961).

All drugs used (Table 1) were from commercial sources and of a grade according the Ph. Ned. VII.

*Correspondence

Table 1. *Drugs and drug mixtures used.*

Drugs	Dose used: mg kg^{-1}	pH of suspensions
Tween-80	4% solution in water	6·8
Acetylsalicylic acid (ASA)	30; 60; 125; 250; 500 and 1000	2·3–2·4
Phenacetin (PHEN)	30; 60; 125; 250; 500 and 1000	6·6–6·7
Paracetamol (PAR)	75; 150; 300; 600; 1200 and 2400	6·4–6·5
Caffeine (CAF)	6·25; 12·5; 25; 50; 100 and 200	6·2–6·3

Drug mixtures	Dose-ratio	pH of suspensions
ASA + PHEN	1:1	2·3–2·4
ASA + PAR	1:1	2·3–2·5
ASA + CAF	5:1	2·3–2·4
ASA + PHEN + CAF	5:5:1	2·3–2·4
ASA + PAR + CAF	5:5:1	2·4–2·5

The drugs were ground (<90 μm) suspended in 4% Tween-80 and administered orally in a volume of 0·5 ml per 100 g weight. Control rats received 4% Tween-80 solution in a similar volume. The doses of drugs were within the range of pharmacological activity in the rat as measured in the carrageenan hindlimb oedema test and never exceeded their LD10-values (Table 2).

Mean erosion scores of the treated groups were obtained by dividing the cumulative score of a group by the number of animals. For ease of survey only mean values are shown in the figures. Rankit tests showed that the assumption that the individual erosion scores within treatment groups were normally distributed was justified. Regression lines were calculated using the least squares method and are presented only if tests on linearity and difference from zero regression coefficient were affirmative (P <95%, P <5% respectively). In the case of

Table 2. *LD10-values and anti-inflammatory activity of the drugs.*

Drugs	LD10* mg kg^{-1}	Dose used in carrageenan hindlimb oedema test mg kg^{-1}	Reduction of paw volume in carrageenan hindlimb oedema test** (n = 10) %
ASA	1000	62·5	19·2
		125	22·9
		250	36·1
PHEN	850	50	11·2
		100	16·6
		200	6·9
PAR	2400	50	0·1
		100	4·0
		200	12·5
CAF	210	not tested	—

* Acute oral LD50 (72 h) was determined according to Litchfield & Wilcoxon (1949) LD10-values are extrapolated graphically.
** Oedema was induced by injection of 0·1 ml of a 1% carrageenan solution (Marine Colloids Inc. lot no. 462106) 1 hour after oral drug treatment. Paw volume was measured 4 h after administration of the drugs. (Ham, unpublished).

non-linearity connecting lines are shown and a linear relation without a significant regression is indicated by the mean level line. Regression lines were compared according to Diem & Lentner (1969). Unless otherwise indicated, differences were assumed to be real if tests gave probability-levels of less than 5%.

RESULTS

The effects of single analgesics on gastric erosions

The erosive activity of single drugs after oral administration, was examined for the doses in Table 1 at ten rats per dose.

Fig. 1 shows that after aspirin-treatment the incidence of gastric erosions was significantly different from controls ($P < 0{\cdot}001$), was dose-dependent and linear over the dose-range. The parameters of the relations between erosion score and log dose are summarized in Table 3. Phenacetin, paracetamol and caffeine, even in lethal doses, did not cause gastric erosions significantly different from those of vehicle-treated rats.

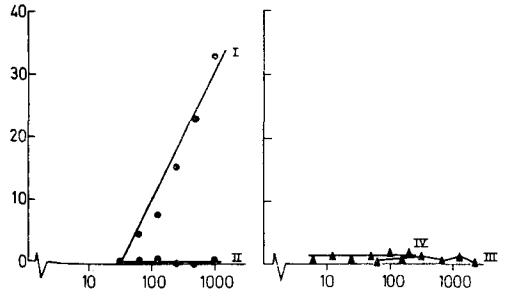

FIG. 1. Erosion scores as a function of log dose of the single drugs. aspirin ● (I), phenacetin ● (II), paracetamol ▲ (III) and caffeine ▲ (IV) n = 10, except for aspirin 1000 mg kg^{-1} n = 9. Ordinate: Erosion score. Abscissa: Dose (mg kg^{-1}).

Table 3. *Parameters of the relation between log dose and erosion score. Erosion score of rats treated with vehicle: 0·4 ± 0·1 (m ± s.e.m.).*

	Treatment	Regression coefficient with s.d.	Intercept with abcissa mg kg^{-1} ASA	Residual scatter s.d.
I	ASA	20·7 s.d. 2·0	35	8·2
II	PHEN	0·4 s.d. 0·2*	—	—
III	PAR	not linear	—	—
IV	CAF	0·8 s.d. 0·3*	—	—
V	ASA + PHEN	20·9 s.d. 7·4	26	14·1
VI	ASA + PAR	4·1 s.d. 2·5*	—	—
VII	ASA + CAF	47·3 s.d. 5·2	20	16·0
VIII	ASA + PHEN + CAF	65·7 s.d. 7·9	57·5	17·1
IX	ASA + PAR + CAF	0·9 s.d. 0·7*	—	—

* Regression coefficient not different from zero.

The effects of mixtures of analgesics on gastric erosions

The erosive activity of the combinations of analgesics, was studied using the mixtures in Table 1. For the dose-regimen of the mixtures the drugs were given with aspirin (30, 60, 125, 250 and 500 mg kg^{-1}) in the ratios shown in Table 1. Ten rats received each dose. The results are presented in Fig. 2 and Table 3.

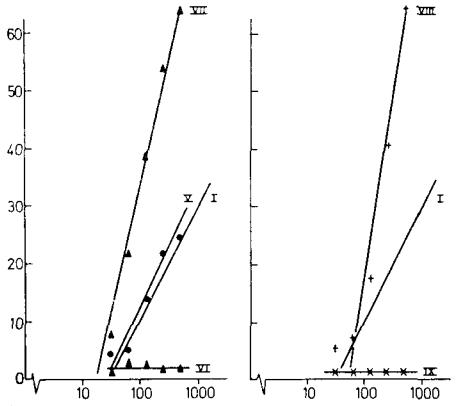

FIG. 2. Erosion scores as a function of log dose of the compound analgesics. aspirin + phenacetin ● (V), aspirin + paracetamol ▲ (VI), aspirin + caffeine ▲ (VII), aspirin + phenacetin + caffeine + (VIII) and aspirin + paracetamol + caffeine × (IX). For comparison, the log dose-response function of aspirin (I) (see Fig. 1) is also presented. n = 10 except for aspirin + paracetamol + caffeine 500 + 500 + 100 mg kg^{-1}, n = 9. Ordinate: Erosion score. Abscissa: Dose (mg kg^{-1} acetylsalicylic acid).

Of the dual combinations, only aspirin + paracetamol did not cause gastric erosions significantly different from controls. A log dose-dependent linear increase in erosion score was observed with the combinations of aspirin + phenacetin and aspirin + caffeine. The log dose response function for aspirin + phenacetin was identical to that for aspirin alone. The regression coefficient for aspirin + caffeine was significantly greater than that for aspirin alone ($P < 0.001$). The same results were obtained one month later (Table 4) when only mixtures based on doses of 60 and 125 mg kg^{-1} aspirin were used.

Table 4. *Erosion score after oral treatment with control, aspirin, aspirin + phenacetin and aspirin + paracetamol in two experiments with a time-interval of one month.*

Treatment	Dose mg kg^{-1}	First experiment mean ± s.e.m. ($n = 10$)	Second experiment mean ± s.e.m.
Control	4% Tween-80	0·4 ± 0·1	0·6 ± 0·1 ($n = 10$)
ASA	60	4·7 ± 0·9	4·1 ± 1·0 ($n = 10$)
	125	7·8 ± 1·3	8·3 ± 1·5 ($n = 10$)
ASA + PHEN	60 + 60	4·9 ± 1·1	5·9 ± 0·9 ($n = 9$)
	125 + 125	14·3 ± 2·6	16·3 ± 3·0 ($n = 9$)
ASA + PAR	60 + 60	1·8 ± 0·5	1·4 ± 0·8 ($n = 9$)
	125 + 125	0·9 ± 0·8	1·1 ± 0·7 ($n = 9$)

After treatment with triple combinations aspirin + phenacetin + caffeine and aspirin + paracetamol + caffeine, linear relations between the log dose and the erosion score were observed. The combination of aspirin + paracetamol + caffeine did not cause erosions significantly different from controls. On the other hand, the regression coefficient of the mixture containing aspirin + phenacetin + caffeine was significantly greater than that for aspirin alone ($P < 0.001$). By comparing the dose-response function of this combination with that of aspirin + caffeine, it was found that the functions were identical.

DISCUSSION

In the present study, the erosive activities of orally administered analgesic drug mixtures on the glandular area of the rat stomach were investigated. Paracetamol completely inhibited the erosive activity of aspirin, even in the presence of caffeine. On the other hand, caffeine had a pronounced potentiating effect on gastric erosions induced by aspirin. This dose-dependent potentiation was observed both in the presence, and in the absence of phenacetin. The enhancement of the erosive action of aspirin by caffeine was due to an increase in both the number and the severity of the erosions. In all cases the lesions were found only in the glandular area of the stomach. Repetition of some of the experiments, involving mixtures containing aspirin + phenacetin and aspirin + paracetamol, gave the same results.

Although the aetiology of aspirin-induced erosions has been widely studied, the pathophysiology is not fully understood. The most likely mechanism is that aspirin penetrates the mucosal cells of the stomach, breaks the gastric mucosal barrier and allows back diffusion of hydrogen ions (Davenport, 1967). If sufficient hydrogen ions penetrate the mucosa, bleeding and gastric damage will occur (Cooke, 1976b). Furthermore, Davenport (1967) found that hydrogen ions have two actions: (i) to facilitate the transfer of aspirin into the gastric mucosa thus breaking the mucosal barrier (ii) after penetrating through the broken barrier, to damage the submucosa.

As shown by the results, potentiation of aspirin's erosive action by caffeine could not be due to the latter's direct local irritating effects on the gastric mucosa, since treatment with it alone did not cause gastric erosions. This is in agreement with the observation that caffeine has no effect on the gastric mucosal barrier (Chvasta & Cook, 1972) and the absence of epidemiological data indicating ulcerogenic side-effects.

However, caffeine is a moderately strong stimulant of acid secretion in man and animals. It is thought to produce this effect by increasing the cAMP content in the gastric mucosa through inhibition of phosphodiesterase (Harris, Nigon & Alonso, 1969; Cano, Isenberg & Grossman, 1976). Thus, a possible explanation of the potentiating effect of caffeine is an enhancement of the hydrogen ion concentration in the gastric lumen.

Buffering aspirin to pH 6 and neutralization of the acid in the stomach prevents gastric damage (Hurley & Crandall, 1964; Thorsen, Western & others, 1968) and buffered aspirin did not affect gastric mucosal potential difference (Murray, Strottman & Cooke, 1974), nor did the mucosal barrier break down or the mucosa bleed (Davenport, 1969).

With paracetamol, the complete inhibition of the erosive activity of its combination with aspirin could not be the result of a neutralizing effect on the acidity of the drug mixture or gastric secretion, since all drug mixture suspensions have an acidity, near to pH 2·4. The reason for the inhibition is not known. In view of reports that in the rat paracetamol is the main metabolite of phenacetin (Smith & Timbrell, 1974) the lack of effect of phenacetin on

the aspirin-induced erosions is striking. This might indicate that the erosive action of aspirin can only be inhibited shortly after its oral administration.

Prostaglandins inhibit the formation of gastric and duodenal erosions induced by a variety of methods in the rat (Robert, Nezamis & Phillips, 1968; Robert, Stowe & Nezamis, 1971). This action may be related to the inhibition of acid secretion, since prostaglandins are possibly involved in the local control of gastric secretion by a negative feedback mechanism (Shaw & Ramwell, 1968). The exact role of the prostaglandins in normal gastric physiology or in the pathophysiology of gastric erosions is not known. However, the reduction of mucosal prostaglandin synthesis by aspirin-like drugs has been implicated in the formation of gastric erosions associated with the use of these anti-inflammatory drugs (Vane, 1971). The possibility that the effects of caffeine and paracetamol on aspirin-induced erosions are the result of altered prostaglandin synthetase inhibition may not be ruled out. Nevertheless, this hypothesis is not supported by enzyme studies, both *in vitro* and *in vivo*, reported by Vinegar, Truax & others (1976). These authors found a potentiation of the anti-inflammatory and analgesic activity of aspirin by caffeine in the rat, but this potentiation was shown not to be due to an enhanced inhibition of prostaglandin synthetase. In addition, attempts to ascribe the potentiating effects of caffeine to changes in the pharmacokinetics of aspirin were also negative. Caffeine did not significantly alter the rate of absorption required to achieve peak plasma concentrations of salicylate, nor the elimination of salicylate from plasma over 8 h (Vinegar & others, 1976). The effect of paracetamol on prostaglandin synthetase inhibition by aspirin has not been investigated as far as we know. As gastric acid appears to be an essential factor in causing aspirin-induced erosions (Cooke, 1973) the most plausible interpretation of the effect of caffeine on these lesions is stimulation of acid secretion.

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