The resistance of guinea-pigs to cortisone ulcerogenesis

It is generally, though not universally, accepted that the prolonged treatment of man and experimental animals with high doses of glucocorticoids can induce fresh peptic ulcers, reactivate previously quiescent ulcers or exacerbate latent ulcers (Sandweiss, 1954; Gray, 1961; Crean, 1963). We had observed that ulcers did not develop in the stomach of guinea-pigs treated with high doses of cortisone for 10 days, even when animals so treated were also subjected to pylorus ligation at the time of the last cortisone injection (Heisler & Kovacs, 1967a).

We have now subjected guinea-pigs to prolonged cortisone treatment alone or with semi-starvation, restraint and acetylsalicyclic acid administration, to observe whether cortisone produced gastric ulceration under any of these conditions.

Male guinea-pigs, initially 300–340 g, were used. Drugs were cortisone acetate (Cortone; Merck Sharp & Dohme) and acetylsalicyclic acid (Lymans Ltd.). These were always given at 9 a.m.; control guinea-pigs were treated with the same volume of physiological saline. For restraint, the technique described by Brodie & Hanson (1960) was used. Animals were fasted 24 h before the experiment: water was allowed at all times. Cortisone was injected immediately before restraint. Gastric ulceration was evaluated according to the "all or nothing" method of Bonfils & Lambling (1963). In some experiments fasted animals were subjected to pylorus ligation (Shay, Sun & Gruenstein, 1954) and then restrained. The duration of restraint in unligated guinea-pigs was 24 h and in pylorus-ligated animals 16 h. Animals in both groups were injected with physiological saline (3 ml, s.c.) to prevent dehydration. Gastric acidity was measured by titrating the samples, after centrifugation, with 0·1 N NaOH using Töpfer's reagent and phenolphthalein as indicators. The significance of differences between means was calculated by Student's *t*-test and in the "restraint" method the significance was estimated by the χ^2 test.

Prolonged treatment of 10 guinea-pigs with 100 mg/kg of cortisone injected subcutaneously once daily for 14–28 days did not bring about gastric ulceration.

The effect on gastric ulceration of cortisone treatment combined with semistarvation was determined in 12 guinea-pigs. Six guinea-pigs were injected once daily for 10 days with cortisone (100 mg/kg, s.c.), 6 animals received the same dose of cortisone by mouth, since according to Gray (1961) cortisone so given possesses a greater ulcerogenic liability than via the subcutaneous route. The guinea-pigs in both groups were fed for only 5 of every 24 h (12–5 p.m.) except on the last day of treatment when they were fasted all day. Animals were killed 24 h after the last injection of cortisone, the stomachs removed and examined macroscopically. No ulcers were found.

The effect of cortisone combined with restraint on gastric ulceration showed that in the control group (23 animals where restraint was for 24 h) 87% of the animals developed gastric ulceration—no particular anatomical distribution was noted. In the cortisone-treated group (14 animals, cortisone 100 mg/kg, s.c. for 3 days, the last dose before 24 h restraint) the incidence of ulceration was 42·9% which is a significant reduction ($P < 0.005$).

Since cortisone stimulates gastric acid secretion in pylorus-ligated guinea-pigs (Heisler & Kovacs, 1967a) we felt that a combination of restraint and pylorus ligation might increase the incidence of ulceration after cortisone administration. This experiment is illustrated in Table 1. Cortisone significantly increased the free and total acid output in comparison with the respective outputs in animals not receiving cortisone. The incidence of ulceration in the latter group was 85% and in the cortisone-treated group 50%. The hyperacidity observed in cortisone-treated guinea-pigs did not increase the incidence of gastric ulceration, rather, there was a definite

tendency for protection against ulceration in the cortisone treated group, though this protection was not statistically significant.

Table 1. *The effect of cortisone on acid secretion and ulcer incidence in pylorus-ligated and restrained guinea-pigs*

Treatment	No. of animals	Volume of gastric juice (ml)	Free HCl m-equiv	Total acid m-equiv	% showing ulceration
Control (pylorus ligated and restrained for 16 h)	7	13·30 ± 2·16	0·37 ± 0·10	0·64 ± 0·12	85·0
Cortisone, 100 mg/kg s.c. for 3 days. Last dose: at ligation	8	17·03 ± 2·10	0·92 ± 0·17	1·18 ± 0·16	50·0
Probability		N.S.*	< 0·02	< 0·05	N.S.

* N.S. = not significant.

Bleeding from the stomach may occur in about half the general population who take aspirin (Smith & Smith, 1966) and gastric erosions after acetylsalicylic acid commonly occur in man (Muir & Cossar, 1955) and experimental animals (Anderson, 1964). To determine whether cortisone becomes ulcerogenic when given with an agent which damages the gastric mucosa, cortisone was administered with acetylsalicylic acid. The results are illustrated in Fig. 1. Acetylsalicylic acid (100 mg/kg orally once daily for 4 days) administered to 4 guinea-pigs caused widespread gastric erosions, but no ulceration. Of the group which received cortisone (100 mg/kg s.c. once daily for 4 days) with acetylsalicylic acid, distinct gastric ulceration was found in 3 of 4 animals in addition to gastric erosions.

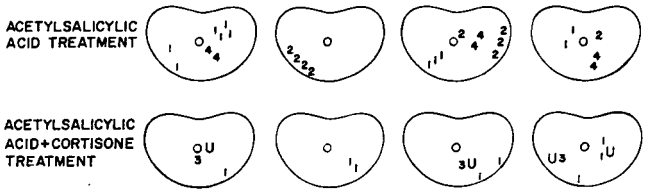

FIG. 1. The effect of acetylsalicyclic acid (100 mg/kg orally, once daily for 4 days) by itself and in combination with cortisone treatment (100 mg/kg s.c., once daily for 4 days) on the guinea-pig stomach. The guinea-pigs were fasted 5 h before and 3 h after treatments and were killed 2½ h after the last treatment.

It is concluded from these results, that in contrast to rats (Ingle, Prestrud & Nezamis, 1951; Robert & Nezamis, 1958) the intact guinea-pig stomach is insensitive to the ulcerogenic action of cortisone and also to some other ulcerogenic stimuli. According to Long (1956) there is a clear-cut species difference to the toxic effects of cortisone; man, monkey and guinea-pig are "cortisone-resistant" while the rat, mouse and rabbit are "cortisone-sensitive" species. Gastric mucosal damage, like that produced by aspirin may be a prerequisite in the guinea-pig, and perhaps also in man, for cortisone-induced gastric ulceration. A protective factor which was found in guinea-pig stomach but was absent in rat stomach (Heisler & Kovacs, 1967b) may also contribute to the relative insensitivity of guinea-pigs to certain ulcerogenic stimuli.

This work was supported by a grant from the Medical Research Council of Canada. We thank Dr. W. Dorian of Merck Sharp and Dohme, for the gift of cortone injections.

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January 30, 1969

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Prolonged oestrogenic activity in rats after single oral administration of ethinyloestradiol-3-cyclopentyl ether

The observation that relief of symptoms in menopausal patients persisted long after oral treatment with the 3-cyclopentyl ether of ethinyloestradiol had been discontinued was interpreted by Bompiani & Bubani (1961) to indicate its storage in and slow release from body depots.

Since then, Meli, Wolff & Honrath (1963) and Meli, Steinetz & others (1965) have shown that the drug is stored in and slowly released from body fat after oral administration to rats. This they considered the mechanism responsible for its increased and prolonged biological activity. Epstein (1967) also found prolonged oestrogenicity in women treated with the drug. Cohen, Bronstein & Leb (1966) showed a uterine-growth stimulating substance to be present in the fat of women taking the drug by mouth. The metabolic fate of the labelled drug in women indicated that it was stored unaltered in body fat depots (Williams, Layne & others, 1967).

While fat storage and prolonged oestrogenic activity occurs in women after the drug had been taken by mouth, no evidence, other than of fat storage, exists for the rat (Meli & others, 1963, 1965).

We now report prolonged oestrogenic activity in the rat given a single oral dose of the drug.

Female rats, 150–180 g, were ovariectomized two weeks before treatment. Groups of 5 animals each received a single oral treatment of ethinyloestradiol or its 3-cyclopentyl ether dissolved in sesame oil at doses of 1, 10, 100 and 1000 μg/animal.

Vaginal smears were taken daily and the animals were killed when vaginal cornification was no longer present. The uteri were then removed and weighed (after pressing out the intra-uterine fluid) to the nearest 0·1 mg on a torsion balance.

In other experiments, 2 groups of ovariectomized rats similarly treated with the 3-cyclopentyl ether at single oral doses of 100 or 1000 μg/animal were killed at 1, 5 and 13 days after the last day of cornified vaginal smear.

On the basis of vaginal cornification, ethinyloestradiol was ineffective at 1 μg dose (Table 1). The effect of the 10 and 100 μg doses lasted only for 24 h whereas that of the 1000 μg dose was effective for 72 h. Vaginal cornification occurred at all doses of the 3-cyclopentyl ether. Duration of action was proportional to the dose given and,