Table 2. *Prevention of indomethacin-induced small intestine ulceration by sodium salicylate.*

Treatment	n	Dose mg kg^{-1}, oral	Tensile strength (mm Hg)
Normal animals	40	—	181 ± 4·5
Indomethacin	20	3 × 10	38 ± 7·6†
Ind + SS	20	3 × (10 + 25)	156 ± 7·4*
Ind + SS	10	3 × (10 + 50)	176 ± 4·7
Ind + SS	10	3 × (10 + 100)	181 ± 5·4

* $P<0·01$ when compared to indomethacin group.
† 25% mortality.

sodium salicylate (3 × 200 mg kg^{-1}) completely eliminated the toxic effect of indomethacin and there were no deaths.

An essential role in the main and side-effects of the aspirin-like anti-inflammatory drugs is attributed to prostaglandins (Vane, 1971). That the intestinal lesions produced by these agents could be due to a local prostaglandin deficiency is a possibility (Robert, 1974). Sodium salicylate and aspirin have almost equal anti-inflammatory actions on animals (Vane 1971; Smith, Ford-Hutchinson & Elliott 1975), but salicylic acid, in contrast to aspirin, is practically ineffective as a prostaglandin synthetase inhibitor.

To explain the antagonism described we suppose that certain types of prostaglandins play a role in maintaining the integrity of the gut and that aspirin-like drugs separately induce ulcers by unbalancing prostaglandin equilibrium. When salicylic acid and aspirin-like drugs are combined disturbances in the equilibrium are not strong enough to cause damage to the gut wall.

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Stimulation by hydrocortisone of the rate of collagen synthesis in cultured fibroblasts

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Nakagawa, Ikeda & Tsurufuji (1975) recently reported that dexamethasone sodium phosphate produced an apparent stimulation of collagen synthesis in granulation tissue *in vitro*. We wish to present the results of some experiments we have made using another water soluble anti-inflammatory steroid, hydrocortisone sodium succinate, in which stimulation of collagen synthesis by cultured human fibroblasts was observed.

Early passage human foetal skin fibroblasts were obtained by trypsinization of the skin from 3 to 5 month old human embryos, all experiments were performed on cells which had been passaged between 5 and 10 times. The cells were maintained in monolayer culture in Dulbecco's modification of Eagle's minimal essential medium (Gibco-Biocult) containing 10% foetal calf serum (Flow Laboratories), 100 μg ml^{-1} penicillin and 50 μg ml^{-1} streptomycin sulphate in an atmosphere of 5% CO_2 in air at 37°. For experiments cells were trypsinized and suspended in medium at a concentration of 2·5 × 10^5 cells ml^{-1} and 2 ml of this suspension was added to plastic culture tubes with a growth area of 5 cm^2. Three to 5 days later when the cells had formed a dense confluent monolayer, the medium was removed and replaced by 2 ml of fresh medium containing 50 μg ml^{-1} ascorbic acid plus the drug. The sodium succinate salt of hydrocortisone was used since it is freely soluble in water and so does not require the addition of organic solvents to the medium. One h after the addition of the drug 5 μCi of tritiated proline ([L-G-^{3}H]proline, 653 mCi mmol^{-1}, Radiochemical Centre, Amersham) was added in 100 μl of phosphate buffered saline. To terminate the incubation the tubes were transfered to crushed ice and 2 ml of ice cold 0·9% NaCl containing 0·1% proline and 0·2%

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ethylenediaminetetra-acetic acid was added. The cells were suspended by freezing and thawing followed by sonication and then the contents of the tubes were dialysed to remove un-incorporated proline, hydrolysed in 6M HCl at 130° for 3 h and analysed for total incorporation of radioactivity and incorporation into hydroxyproline by the method of Juva & Prockop (1966). Results were analysed using Student's *t*-test.

In the absence of drug the rates of collagen synthesis ([^{3}H]proline incorporation into hydroxyproline) and non-collagen protein synthesis (total incorporation minus incorporation into collagen) were linear with time throughout the experimental period (11 h). 65% of the collagen synthesized was found in the medium (soluble collagen) while the remaining 35% was found in the cell layer (fibrous collagen). All of the non-collagen protein synthesized was retained in the cell layer.

Hydrocortisone sodium succinate at concentrations between 10^{-7}M and 10^{-4}M did not inhibit either collagen or non-collagen protein synthesis, when the total incubation period was 5 h. But, at 10^{-5}M a statistically significant stimulation of collagen synthesis was found (133% of control, $P < 0.02$, N = degrees of freedom = 10) was found. There was also a slight (107% of control) stimulation of non-collagen protein synthesis, but this was not statistically significant. To examine this effect in greater detail an experiment was performed in which the total incubation period was 11 h and the medium and cells were analysed separately. It was found that fibrous collagen (134% of control, $P < 0.01$, N = 6), soluble collagen (151% of control, $P < 0.01$, N = 6) and non-collagen protein (142% of control, $P < 0.01$, N = 6) synthesis was stimulated by 10^{-5}M hydrocortisone sodium succinate. When examined by phase contrast microscopy no morphological alterations in the steroid-treated cells could be detected.

Although other studies have shown stimulation of collagen synthesis with anti-inflammatory steroids (Harvey, Grahame & Panayi, 1974; Nakagawa & others, 1975), most *in vitro* studies have demonstrated inhibition of either collagen synthesis or protein synthesis by fibroblasts (reviewed by Nacht & Garzon, 1974; Trnavsky, 1974). It is not clear why there should be such variation in the findings, but it has been pointed out (Cunningham, Thrash & others, 1974) that the transformed cells used in many of the studies respond in different ways compared with normal cells as used in this study. In addition, Pratt & Aronow (1966) using L cells (transformed) found that although anti-inflammatory steroids reduced the rate of protein synthesis per flask, this was due to inhibition of cell division which masked a stimulatory effect on protein synthesis when expressed on a per cell basis. In the present experiment the cells were non-dividing at the time of drug addition and stimulation was found as early as 5 h after addition of drug, by which time cells could not have divided. Furthermore, since proline does not bind to serum proteins (McArthur, Dawkins & Smith, 1971), the effects described here cannot be explained in terms of competition between the steroid and the radioactive substrate for binding sites on the proteins in the medium.

Nakagawa & others (1975) recently reported that dexamethasone disodium phosphate caused an apparent increase in the rate of collagen synthesis by granulation tissue incubated *in vitro*. These authors suggested that this effect was due to protection by the drug of the collagen synthesizing mechanism of the cells from the adverse effects of the incubation conditions. The stimulation found in the present study cannot be explained by the same mechanism since the incubation conditions did not cause damage to the synthetic apparatus of the cells, as indicated by the constant rate of synthesis.

The relevance of this *in vitro* effect to the *in vivo* actions of hydrocortisone is not known but the question deserves further study since it has now been obtained by three independent laboratories.

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