Short communications

Prostaglandin biosynthesis by human skin and its inhibition by corticosteroids

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Homogenates of human breast skin form prostaglandins E_2 and $F_{2\alpha}$ in the presence of arachidonic acid substrate. Significant inhibition of biosynthesis of prostaglandin E_2 and $F_{2\alpha}$ was observed in the presence of the anti-inflammatory corticosteroid fluocinolone acetonide. This effect was dose-related. By contrast no significant inhibition was observed in the presence of an approximately equimolar concentration of hydrocortisone.

Since prostaglandins have been recovered from several types of inflammatory reactions and are capable of mediating most of the components of inflammation the possibility arises that corticosteroids may owe their anti-inflammatory activity at least in part to inhibition of prostaglandin biosynthesis.

There is increasing evidence that some of the prostaglandin group of compounds are important mediators of inflammatory reactions (Ängåard, Arturson & Jonsson, 1970; Di Rosa, Giroud & Willoughby, 1971; Di Rosa & Willoughby, 1971; Ängåard & Jonsson, 1971; Anderson, Brocklehurst & Willis, 1971). Recently we have used a skin perfusion technique in man to demonstrate the presence of increased concentrations of prostaglandin-like activity in inflamed skin *in vivo* (Greaves, Søndergaard & McDonald-Gibson, 1971; Greaves & Søndergaard, 1970). Thin layer chromatography and bioassay studies indicated that this activity consisted of a mixture of prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$. Prostaglandin concentrations in normal skin are extremely low (Van Dorp, 1971; Greaves & McDonald-Gibson, unpublished observations) and it is probable that increased prostaglandin activity in skin as well as in other tissues is frequently the result of increased local prostaglandin biosynthesis.

Prostaglandin biosynthesis by skin *in vitro* was first demonstrated by Jessup, McDonald-Gibson, Ramwell & Shaw (1970) and by Van Dorp (1971). More recently we have demonstrated biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ by homogenates of rat skin from arachidonic acid (Greaves & McDonald-Gibson, 1972b). We report here evidence that normal human skin contains prostaglandin-synthesizing enzymes. Since the corticosteroids fluocinolone acetonide and hydrocortisone show anti-inflammatory activity following topical application to human skin we also describe studies of the inhibitory effects of these agents on *in vitro* prostaglandin biosynthesis by human skin.

Methods.—Samples of healthy human skin were obtained from mastectomy operations and used within 2 hours of excision. After removal of subcutaneous fat the skin was weighed and cut (with scissors) into small fragments which were placed in phosphate buffer (*p*H 7·4) containing hydroquinone (0·27 mM) and glutathione (0·325 mM) as described by Foss, Takeguchi, Tai & Sih (1971). The skin was homogenized in a Polytron tissue homogenizer and bovine serum albumin was added to a final concentration of 5 mg/ml. The homogenized skin was incubated aerobically with an excess of arachidonic acid containing tritium-labelled arachidonic acid tracer (New England Nuclear Inc.) for 40 min at 37° C in a shaking water bath. The final concentration of arachidonic acid was 12·5 μg/ml and the reaction volume was 2 ml. The reaction was terminated by adding 10 ml ice-cold ethanol and the mixture was kept at 4° C for 4 hours. The supernatant was then recovered and the residue washed with ice-cold ethanol. Acidic lipids were extracted from the combined washings and supernatants with petroleum ether and diethyl ether followed by thin-layer chromatography as described by Greaves & McDonald-Gibson (1972a). Prostaglandin E_2 and prostaglandin $F_{2\alpha}$ activity was estimated by elution of zones of the chromatoplate corresponding to simultaneously developed standard prostaglandins E_2 and $F_{2\alpha}$. Radioactivity was determined in these eluates with a Packard Tricarb Liquid Scintillation counter. In order to confirm that the prostaglandin E_2 and $F_{2\alpha}$ zones of the chromatoplate contained, respectively, prostaglandin E and F activity, results obtained by measurement of radioactivity were compared with bio-

assay results for the same eluates on a rat isolated uterus preparation. In a single experiment results obtained by the two methods for prostaglandins E and F were identical.

In preliminary experiments the mean recoveries of prostaglandins E_2 and $F_{2\alpha}$ added to skin prior to incubation both exceeded 90%. Variations in the rates of prostaglandins E_2 and $F_{2\alpha}$ synthesis between replicates of the same skin sample under identical conditions were below 10%. The effect of hydrocortisone or fluocinolone acetonide on biosynthesis of prostaglandins was studied by adding the drug to the reaction mixture immediately prior to incubation. All experiments included negative controls in which arachidonic acid and other reagents were incubated with buffer alone before addition of skin homogenates, extraction and thin-layer chromatography.

Results.—The results are summarized in Table 1. Experiments were carried out on skin from 6 donors. Formation of prostaglandin by skin from arachidonic acid took place in all experiments. The mean total prostaglandin activity synthesized in 40 min was 1·38 μg/g wet weight of skin, of which 50·5% was prostaglandin E_2 and 49·5% prostaglandin $F_{2\alpha}$. There was little or no synthesis of prostaglandins by skin homogenates in the absence of added arachidonic acid.

In the presence of hydrocortisone (0·28 mM) there was no significant inhibition of rate of synthesis of prostaglandin E_2 or prostaglandin $F_{2\alpha}$. By contrast addition of fluocinolone acetonide (0·22 mM) caused significant inhibition of prostaglandin biosynthesis by skin. The mean % inhibition of rate of total prostaglandin synthesis from arachidonic acid substrate was 34·9±10·4 S.E.M. ($P<0·05$). The corresponding figures for % inhibition of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ synthesis by fluocinolone were 44·2±10·7 S.E.M. ($P<0·025$) and 25·4±9·6 S.E.M. ($P<0·05$). In a further 3 experiments the inhibitory effect of fluocinolone was shown to be dose-related. A fourfold increase in the concentration of fluocinolone caused a 50% increase in the inhibition of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ synthesis.

Discussion.—The present results establish that human skin like the skin of other species (Jessup *et al.*, 1970; Van Dorp, 1971) contains an enzyme system capable of converting arachidonic acid substrate into prostaglandin E_2 and prostaglandin $F_{2\alpha}$. Increased local biosynthesis could therefore explain the increased concentrations of prostaglandin observed in the inflamed skin of man and other species. The precise mechanism of increased biosynthesis in inflammation remains speculative. It has been suggested (Anderson *et al.*, 1971; Vogt, Meyer, Kunze, Lufft & Babilli, 1969) that tissue injury might release lysosomal phospholipases which could in turn bring about increased local concentrations of polyunsaturated fatty acid prostaglandin precursors through action on cell membrane phospholipids. In support of this suggestion Vogt has demonstrated release of prostaglandin from lungs perfused with phospholipases.

The demonstration of significant inhibition of prostaglandin biosynthesis in human skin by the anti-inflammatory

TABLE 1. *Prostaglandin synthesis ((ng/g wet wt)/40 min) in human skin*

A	Control	Hydrocortisone (0·28 mM)	Fluocinolone (0·22 mM)
* Total	1,383·1±199·1	1,373±313·0	858·3±17·1 ($P<0·05$)
* Prostaglandin E_2	700·5±114·6	678·9±184	359·7±84·0 ($P<0·025$)
* Prostaglandin $F_{2\alpha}$	682·4±89·4	694·2±132·6	498·6±89·0 ($P<0·05$)

B	Control	Fluocinolone	
		0·11 mM	0·44 mM
† Prostaglandin E_2	558±88	364±125	267±73
† Prostaglandin $F_{2\alpha}$	674±248	343±174	174±97

* Each value represents mean±S.E.M. of 6 experiments. † Each value represents mean±S.E.M. of 3 experiments.

corticosteroid fluocinolone acetonide is a finding of great interest and is in agreement with other results of ours in similar experiments using rat skin (Greaves & McDonald-Gibson, 1972b). Vasoactive prostaglandins have now been recovered from inflamed tissues in a wide range of experimental situations. Prostaglandins are capable of mediating most of the components of the inflammatory reaction including vasodilation (Crunkhorn & Willis, 1971 ; Søndergaard & Greaves, 1971), increased vascular permeability (Horton, 1963 ; Kaley & Weiner, 1967), leucotaxis (Kaley & Weiner, 1971 ; Di Rosa & Willoughby, 1971), pain (Collier, 1971) and fever (Feldberg & Saxena, 1971). The possibility therefore arises that the anti-inflammatory efficacy of fluocinolone acetonide in skin may be due at least in part to its ability to inhibit local formation of prostaglandins. The observed ability of fluocinolone to reduce the rate of synthesis of prostaglandin E_2 to a greater extent than prostaglandin $F_{2\alpha}$ is also of great interest and possible significance since prostaglandin $F_{2\alpha}$ shows extremely low vasoactivity in skin compared with prostaglandin E_2 (Crunkhorn & Willis, 1971).

We were unable to demonstrate significant inhibition of prostaglandin synthesis by hydrocortisone. Similar results were obtained recently by Vane (1971) who used cell-free supernatants of homogenized guinea-pig lung tissue as a source of prostaglandin-synthesizing enzymes. Vane was unable to demonstrate inhibition either by hydrocortisone, or more recently by the more potent anti-inflammatory corticosteroid triamcinolone (Vane, personal communication). However, the source of prostaglandin-synthesizing enzymes used by Vane was cell-free and it is possible that cell membrane factors may be important in producing the observed inhibiting effects of fluocinolone. In other experiments Vane found that some non-steroid anti-inflammatory drugs including aspirin and indomethacin did inhibit synthesis of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ using guinea-pig lung as a source of prostaglandin-forming enzymes and we have obtained similar results using rat skin for prostaglandin biosynthesis (Greaves & McDonald-Gibson, in preparation). The possibility therefore arises that inhibition of prostaglandin biosynthesis may be a property shared by a wide range of anti-inflammatory drugs.

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