[7] Tilney, L. G., and Mooseker, M., *Eleventh Ann. Meeting Amer. Soc. Cell Biol.* (1971).
[8] Allison, A. C., Davies, P., and de Petris, S., *Nature New Biology*, **232**, 153 (1971).
[9] Behnke, O., Forer, A., and Emmersen, J., *Nature*, **234**, 408 (1971).
[10] Gawadi, N., *Nature*, **234**, 410 (1971).
[11] Lowey, S., and Cohen, C., *J. Mol. Biol.*, **4**, 293 (1962).
[12] Fine, R. E., and Bray, D., *Nature New Biology*, **234**, 115 (1971).
[13] Lasek, R. J., *Intern. Rev. Neurobiol.*, **13**, 289 (1970).
[14] Samson, F. E., *J. Neurobiol.*, **2**, 347 (1971).
[15] Fernandez, H. L., Burton, P. R., and Samson, F. E., *J. Cell Biol.*, **51**, 176 (1971).

Effect of Prostaglandins E_1 and $F_{1\alpha}$ on Biosynthesis of Collagen

THE prostaglandins (PG) are possible mediators of inflammation. Prostaglandins E and F are present in inflammatory exudates[1-3] and could be related to the increase of collagen biosynthesis associated with inflammation. Vane and his colleagues[4-6] recently observed that indomethacin, aspirin and sodium salicylate potently block the biosynthesis of prostaglandins. These anti-inflammatory drugs are also inhibitors of collagen biosynthesis[7,8]. Morphological studies[9] have revealed increased deposition of collagen or collagen-related elements in organ cultures of chick embryo skin containing prostaglandins E_1 and B_1. We report here results which indicate stimulation of collagen biosynthesis by prostaglandins E_1 and $F_{1\alpha}$ evaluated by hydroxylation of proline and lysine and glycosylation of hydroxylysine in 10 day chick embryo tibiae.

10-day-old chick embryo tibiae (Statens Seruminstitut, Copenhagen) were preincubated in Tyrode solution at 37° C for 1 h. Prostaglandins dissolved in Tyrode solution were added and Tyrode solution alone was added to controls. Preincubation was continued for an additional 30 min. 5 μCi L-^{14}C-proline (180 mCi/mM) or L-^{14}C-lysine (220 mCi/mM) (New Zealand Nuclear Corporation) was added and the incubation continued for 2 h. Tissues were then homogenized and dialysed against running tap water overnight, and undialysable samples containing ^{14}C were hydrolysed. Total radioactivity (^{14}C-proline or ^{14}C-lysine) was determined on an aliquot of the undialysable material. Samples for determination of ^{14}C-hydroxyproline (Hypro)[10] and total ^{14}C-hydroxylysine (total Hylys)[11] were hydrolysed in 6 M HCl at 110° C for 16–18 h in sealed ampoules. Unglycosylated ^{14}C-Hylys was determined after alkaline hydrolysis and neutralization[11].

Prostaglandins E_1 and $F_{1\alpha}$ increased total uptake of ^{14}C-Pr and ^{14}C-Lys, both indicators of total protein biosynthesis. The increase was parallel to ^{14}C-Hypro and ^{14}C-Hylys formation, specific indicators of collagen biosynthesis (Table 1). Stimulation of collagen is further reflected by increased glycosylated hydroxylysine, glycosylation being a terminal step of collagen biosynthesis related to the normal process of collagen extrusion from the fibroblasts.

Our results indicate that PGE_1 and $PGF_{1\alpha}$ increase collagen biosynthesis as evaluated by hydroxylation of proline and lysine residues to produce the two characteristic amino-acids, hydroxyproline and hydroxylysine, of collagen and glycosylation of hydroxylysine residues. The increase in collagen formation induced by PGE_1 and $PGF_{1\alpha}$ may explain the increased collagen biosynthesis induced by inflammation. Several other substances including histamine, 5-hydroxytryptamine and the kinins may act as mediators of inflammation and may also affect collagen metabolism. Histamine and 5-hydroxytryptamine, however, which are mainly involved in short lasting inflammatory processes (for review see ref. 12), had no effect on collagen formation in our system, whereas bradykinin caused an increase of collagen biosynthesis almost of the same order as PGE_1 and $PGF_{1\alpha}$ (Blumenkrantz and Søndergaard, unpublished).

Table 1 Effect of Prostaglandins E_1 and $F_{1\alpha}$ on *in vitro* Biosynthesis of Collagen

a, Biosynthesis of (^{14}C) hydroxyproline (Hypro)

Addition	No. of determinations	Total (^{14}C)Pr uptake % of control	(^{14}C) Hypro % of control
PGE_1	6	128 ± 12*	131 ± 12*
$PGF_{1\alpha}$	7	135 ± 10*	145 ± 15*

b, Biosynthesis of (^{14}C) hydroxylysine (Hylys)

Addition	No. of determinations	Total (^{14}C) Lys uptake % of control	(^{14}C)Hylys % of control	
			Total	Glycosylated
PGE_1	6	165 ± 22*	132 ± 6*	117 ± 6*
$PGF_{1\alpha}$	7	169 ± 14*	140 ± 6*	116 ± 6*

10-day-old chick embryo tibiae were preincubated in Tyrode solution containing 50 μg PGE_1 or $PGF_{1\alpha}$/3 ml. incubation medium. Tyrode solution without prostaglandins was used for control experiments. After preincubation, 5 μCi of L-^{14}C-lysine (Lys) or 5 μCi of L-^{14}C-proline (Pr) was added for an incubation period of 2 h. Tissues were homogenized, dialysed and assayed as described in the text. The figures represent mean values of the number of experiments performed ± standard error.

* Significantly different from controls at the 5% level of probability.

Our results support the morphological findings of Kischer[9], who observed increased collagen deposition under the effect of PGE_1 and PGF_1. The stimulating effects of PGE_1 and $PGF_{1\alpha}$ observed by us coincide with a high affinity of the connective tissue to radioactive material after administration of ^{3}H-labelled PGE_1 to mice[13].

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N. BLUMENKRANTZ
J. SØNDERGAARD

Department of Dermatology,
University of Copenhagen,
Rigshospital,
2100 *Copenhagen*

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[1] Willis, A. L., *J. Pharm. Pharmacol.*, **21**, 128 (1969).
[2] Søndergaard, J., and Greaves, M. W., *Int. Arch. Allergy*, **39**, 56 (1970).
[3] Greaves, M. W., Søndergaard, J., and McDonald-Gibson, W., *Brit. Med. J.*, **2**, 258 (1971).
[4] Vane, J. R., *Nature New Biology*, **231**, 232 (1971).
[5] Smith, J. B., and Willis, A. L., *Nature New Biology*, **231**, 235 (1971).
[6] Ferreira, S. H., Moncada, S., and Vane, J. R., *Nature New Biology*, **231**, 237 (1971).
[7] Nakagawa, H., and Bentley, J. P., *J. Pharm. Pharmacol.*, **23**, 399 (1970).
[8] Whitehouse, M. W., and Boström, H., *Biochem. Pharmacol.*, **14**, 1173 (1965).
[9] Kischer, C. W., *Devel. Biol.*, **16**, 203 (1967).
[10] Juva, K., and Prockop, D. J., *Anal. Biochem.*, **15**, 77 (1966).
[11] Blumenkrantz, N., and Prockop, D. J., *Anal. Biochem.*, **30**, 377 (1969).
[12] Wilhelm, D. L., in *The Inflammatory Process* (edit. by Zweifach, B. W., Grant, L., and McCluskey, R. T.), 389 (Academic Press, London, 1965).
[13] Hansson, E., and Samuelsson, B., *Biochim. Biophys. Acta*, **106**, 379 (1965).

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