Effect of methylxanthines on cAMP level and on allergic histamine release

	Drug concentration mM	cAMP level (% of control)	Histamine release (% of control)
Isobutyl-methyl-xanthine	0.0004	100 ± 5	100 ± 10
	0.004	100 ± 4	97 ± 6
	0.008	115 ± 10	103 ± 8
	0.016	125 ± 6	100 ± 5
	0.03	235 ± 15	95 ± 8
	0.06	265 ± 32	114 ± 12
	0.13	312 ± 30	140 ± 6
	0.25	353 ± 37	162 ± 10
Theophylline	2.5	105 ± 10	97 ± 10
	10	208 ± 10	44 ± 8
	20	310 ± 15	5 ± 2

In all experiments, the mast cell suspension was incubated at 37 °C for 60 min with or without drugs before estimation of cAMP and histamine release. cAMP level and histamine release were compared with corresponding controls. In the controls the level of cAMP varied from 0.20 to 0.37 pmoles per 10^6 cells (0.30 ± 0.04) and histamine release varied from 29 to 46% (39 ± 3) of the total histamine content in the cells. Note: Isobutylmethylxanthine and theophylline did not liberate histamine in the absence of anaphylactic challenge. Mean of 4–8 experiments ± SEM.

Thus, only a qualitative but not quantitative evaluation of the relation between drug-induced changes in cAMP level and the release of allergic mediators has been considered. We have examined the effect of a new methylxanthine, isobutylmethylxanthine (3-isobutyl-1-methylxanthine), on cAMP level and histamine release from isolated mast cells, and the results of these experiments throw doubt on the hypothesis.

Material and methods. Rats were sensitized to horse serum and mast cells from the peritoneal, and pleural cavities were isolated by the Ficoll density gradient method[7]. The mast cell suspension consisted of 1–3 million cells/ml salt solution buffered to pH 7.0 with 10% Sørensen phosphate buffer and containing 0.131 M NaCl, 2.4 mM KCl, 1.0 mM $CaCl_2$, 4.7 mM Na_2HPO_4, 2.0 mM KH_2PO_4, 5 mM glucose, and 1 mg/ml gelatine. The cells were equilibrated at 37 °C for 60 min in the presence or absence of isobutylmethylxanthine or theophylline. Thereafter cAMP and histamine release were estimated. An aliquot of the suspension was heated to 94 °C and used for cAMP determination[8], and another part was simultaneously challenged with specific antigen and the release of histamine was estimated[7].

Results and discussion. Both isobutylmethylxanthine and theophylline increased the level of cAMP in the mast cells. Although a similar increase in cAMP level was obtained, theophylline reduced the allergic histamine release according to the hypothesis, whereas isobutylmethylxanthine caused a pronounced potentiation of the histamine release (table).

Our experiments were based on direct anaphylactic histamine release in the pure target cell, which is in contrast to other studies with reversed anaphylactic histamine release[3] or with mixed cell populations[2,4–6].

The above-mentioned hypothesis of a modulating effect of cAMP on histamine release was found to be inadequate and a more complex mechanism has to be considered. Thus, if cAMP is of significance for the secretion of histamine, its controlling function might be due to changes in the cAMP level of a subcellular fraction of the mast cell, i.e. in a specific cAMP pool.

7 S. Norn and P. Stahl Skov, Clin. exp. Immun. *18*, 431 (1974).
8 A. Geisler, R. Klysner, P. Thams and S. Christensen, Acta pharmac. tox. *40*, 356 (1977).

Inhibitory action of dehydroepiandrosterone (DHEA) on fibroblast growth[1]

P. Saenger and Maria New[2]

Division of Pediatric Endocrinology, The New York Hospital-Cornell Medical College, 525 East 68th Street, New York, N. Y. 10021 (USA), 24 December 1976

Summary. Dehydroepiandrosterone inhibits thymidine incorporation into human fibroblasts and may thus interfere with cellular growth.

The growth inhibitory properties of glucocorticoids on fibroblasts from different sources have been well documented[3–6]. Little is known about the action of C^{19} steroids on fibroblast growth in tissue culture. We, therefore, studied the effects of DHEA, a C^{19} steroid with androgenic properties, which occurs in amniotic fluid[7], on the growth of fetal fibroblasts.

Materials and methods. Full thickness skin biopsies of the upper extremity of 4 (2 males, 2 females), 10–16-week-old human fetuses, delivered after prostaglandin induced abortion, were obtained. Fibroblasts were cultured as previously described[8]. The 4 cell lines had undergone a mean of 4 subcultures prior to study. To a standardized cell concentration of 1×10^6 cells per flask, DHEA in 10 µl of ethanol was added 12 h after the last trypsinization. The final DHEA concentration ranged from 36 to 290 µg/5 ml. The rate of DNA synthesis was determined utilizing 48 h pulses of (3H) thymidine; 2 µCi (3H) thymidine (SA 6.0 Ci/mM) per flask was added to the medium. In previous experiments we determined that this concentration of ethanol in the medium did not affect the growth or morphology of cultured fibroblasts[8].

The incubation was terminated after 24 h by cooling to 4 °C. The medium was then removed for extraction of steroid metabolites. The remaining cells were harvested by treatment with 0.25% trypsin and disodium versenate[8,9]. Cell viability of fibroblasts incubated with DHEA and of controls was tested by adding a freshly prepared solution of trypan-blue to harvested cells in suspension. 100–300 cells were counted in a hemocytometer. Cells that stained with dye were scored as dead cells.

The cells were centrifuged at 800 rpm for 20 min. The cell pellet was washed once with 10% trichloroacetic acid, twice with 5% trichloroacetic acid and once with ethanol. The dried pellet was then dissolved in Soluene® (Packard) and counted in a toluene phosphate solution.

Results. Figure 1 shows a time study of the inhibition of (3H) thymidine uptake by DHEA. Maximum inhibition occurs at 24 h and thereafter the degree of inhibition remained constant. Effects of DHEA on cell viability are shown in the table. No increase in cell death occurred when fibroblasts were incubated with DHEA 2×10^{-4} M for up to 48 h.

Inhibitory action on cell growth at 24 h of increasing DHEA concentrations is shown in figure 2. At a minimal concentration of 2.5 10^{-5} M DHEA in the medium, the (3H) thymidine uptake was inhibited by 18% (SD ± 8). No sex difference is apparent in these preliminary studies.

Percent dead cells (±SD) at various periods, DHEA 2×10^{-4} M added.

Time (h)	Number of flasks	Percent
0	4	26.3 ± 2.7
18	4	23.7 ± 3.4*
24	4	28.3 ± 1.5*
48	4	24.5 ± 1.0*

*p>0.1 when compared to 0 time value.

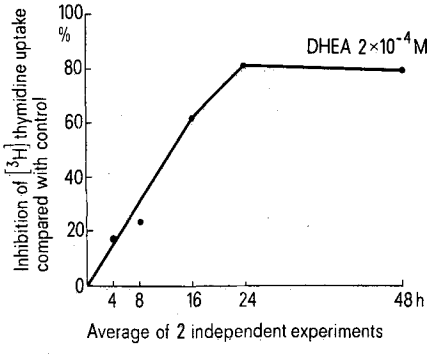

Fig. 1. Percent inhibition of (3H) thymidine uptake compared with controls at DHEA, 2×10^{-4} M, at 4, 8, 16, 24 and 48 h.

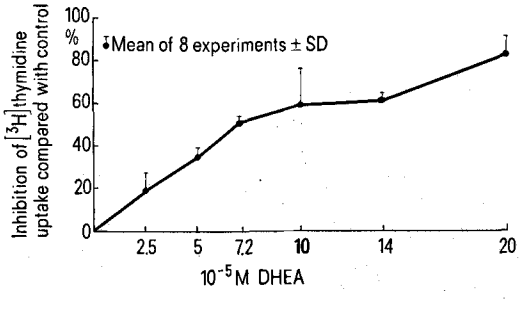

Fig. 2. Percent inhibition of (3H) thymidine uptake compared with control at 24 h with increasing DHEA concentration.

Discussion. The data demonstrate that cellular proliferation of normal skin fibroblasts can be inhibited by DHEA at concentrations ranging from 50 to 100 times that of normal adult plasma. The inhibitory action of DHEA on mitosis of cultured lymphocytes has been demonstrated previously[10].

DHEA interferes also with the growth of experimental tumors such as the Ehrlich Ascites tumor, possibly by inhibition of G6PD activity[11]. DHEA has been shown to inhibit G6PD activity in concentrations of 10^{-5} M, in human testes, ovary, placenta, and liver[12,13]. It is not known whether growth inhibiting properties in fibroblast cultures are mediated by the same mechanism. Glucocorticoids in concentrations higher than 10^{-4} M depress the incorporation of 3H thymidine into fibroblasts[6,14]. These investigators concluded also that cortisol does not interfere with the DNA biosynthetic pathway, but that it simply reduces the number of cells capable of incorporating thymidine. The data presented here do not allow accurate differentiation during which phase of the cell cycle DHEA displays its inhibiting action on fibroblasts growth. Further studies can be designed to determine whether the hormone interferes with cell growth in the S, G_1 or G_2 phase.

1 This investigation was supported in part by USPHS NIH training award AM 00329 and The New York State Health Research Council under contract # 272.

2 Acknowledgment. The authors wish to thank A. B. Rifkind, from the Department of Pharmacology at Cornell University Medical College for provision of skin biopsies.

3 M. Holden and L. B. Adams, Proc. Soc. exp. Biol. Med. *95*, 364 (1975).

4 B. I. Grosser, M. D. Sweat, D. L. Berliner and T. F. Daughaday, Archs Biochem. Biophys. *96*, 259 (1962).

5 O. Wieser and A. H. Taifour, Experientia *25*, 841 (1969).

6 S. Nacht and P. Garzon, in: Advances in Steroid Biochem., vol. 4. Ed. M. H. Briggs and G. A. Christie. Academic Press, New York 1974.

7 H. M. Gandy, Endocrinology of Pregnancy. Ed. F. Fuchs and A. Klopper. Harper Row, New York 1971.

8 D. D. Shanies, K. Hirschhorn and M. I. New, J. clin. Invest. *51*, 1459 (1972).

9 P. Saenger, D. D. Shanies and M. I. New, J. clin. Endocr. Metab. *37*, 760 (1973).

10 H. Holzmann, J. Fraenz, B. Morsches, G. W. Oertel and K. H. Degenhardt, Germ. med. Mon. *2*, 140 (1972).

11 I. Avenarius and E. Schwartz, Naturwissenschaften *62*, 44 (1975).

12 G. W. Oertel, P. Menzel and D. Bauke, Clinica chim. Acta *27*, 107 (1970).

13 P. Menzel, M. Gobbert and G. W. Oertel, Horm. Metab. Res. *2*, 225 (1970).

14 P. Garzon and D. L. Berliner, 51st Meeting of the Endocrine Society, 1969, p. 78.