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Failure of catecholamines to inhibit epidermal mitosis in vitro

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Skin samples were obtained from the forearm and leg of normal volunteers and patients with atopic eczema. The samples were incubated with ^3H -thymidine, and the radioactivity incorporated was measured at 48 hours. Isoproterenol, epinephrine, or norepinephrine was added in concentration of 10^{-5}M to 10^{-10}M . There was inhibition of deoxyribonuclear acid (DNA) synthesis in control samples with the catecholamines. In contrast, the catecholamines failed to inhibit DNA synthesis in the skin from patients with atopic eczema. It was concluded that inhibition of DNA synthesis is a beta adrenergic effect, and the cells of patients with atopic eczema are insensitive to beta stimulation.

In recent years we have been interested in the theory that the fundamental abnormality in the atopic diseases is a diminished target cell response to beta adrenergic stimulation, the "beta blockade theory of atopy."¹

Autonomic abnormalities occur not only in asthma but also in eczema. Specifically, in eczema there is exaggerated alpha adrenergic sensitivity manifested by vasoconstriction²⁻⁴ and pilomotor erection.⁵ There is also exaggerated cholinergic sensitivity of the sweat glands.⁶ Propranolol enhances acetylcholine stimulation of sweat glands of normal but not atopic subjects.⁷ This lack of response to propranolol could be explained if the beta receptors were already blocked. By extension of this line of reasoning, the physiologic abnormalities of eczema, both the increased alpha adrenergic and increased cholinergic sensitivity, could result from decreased response to counterbalancing beta adrenergic

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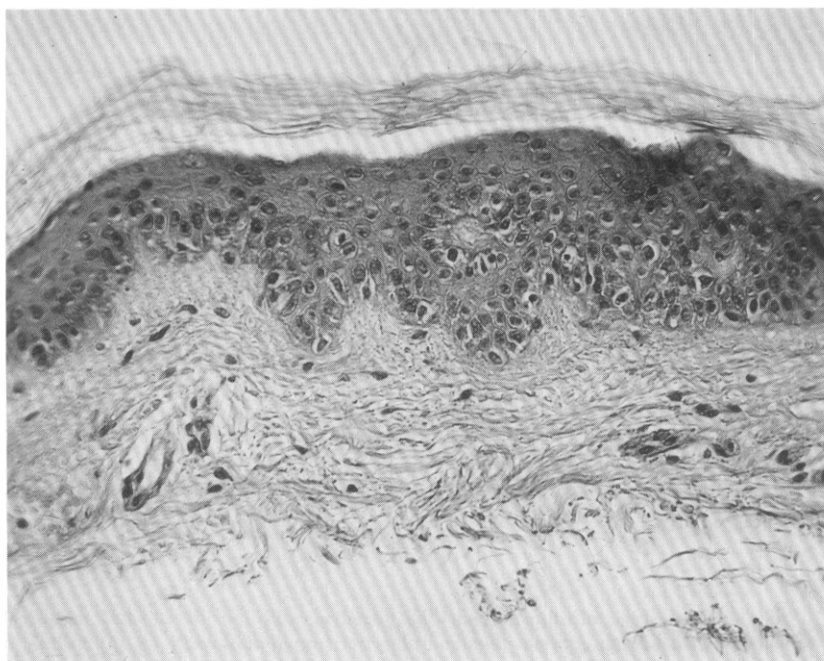


FIG. 1. Cross-section of skin biopsy. Skin from lesion of atopic eczema. The biopsy includes all of the epidermis and a thin, variable amount of dermis. (Hematoxylin and eosin stain.)

stimulation. Direct testing of this hypothesis can be done only *in vitro* where homeostatic responses can be eliminated.

In mice, epinephrine inhibits epidermal mitosis both *in vitro* and *in vivo*.^{8,9} The inhibition is a beta effect. In this regard epidermis, like HeLa cells,¹⁰ responds in the opposite way from the parotid gland in which mitosis is stimulated by beta receptor activation.^{11,12} Epidermal mitosis in man has a diurnal rhythm,¹³ suggesting that in man mitosis of the epidermis might also be moderated by beta receptors. If so, beta blockade could lead to an increased rate of mitosis, a possible explanation for the characteristic lichenification of eczema.

Epidermis has many advantages for a study of possible abnormal beta adrenergic responses in atopy because it is involved in the atopic state, it responds to adrenergic stimulation, and it can be readily obtained for *in vitro* study.

The experiments to be described were undertaken to determine whether mitosis of human epidermis is inhibited by beta adrenergic stimulation and whether this adrenergic response differs in atopic and normal skin.

METHODS

Five patients, 20 to 51 years of age, with long-standing atopic dermatitis, and 10 healthy control subjects, 21 to 35 years of age, volunteered for the study. The control subjects had no personal or immediate family history of asthma, hay fever, eczema, or other skin diseases.

The area of skin for biopsy was shaved, washed, with pHisoHex and 70 per cent alcohol,

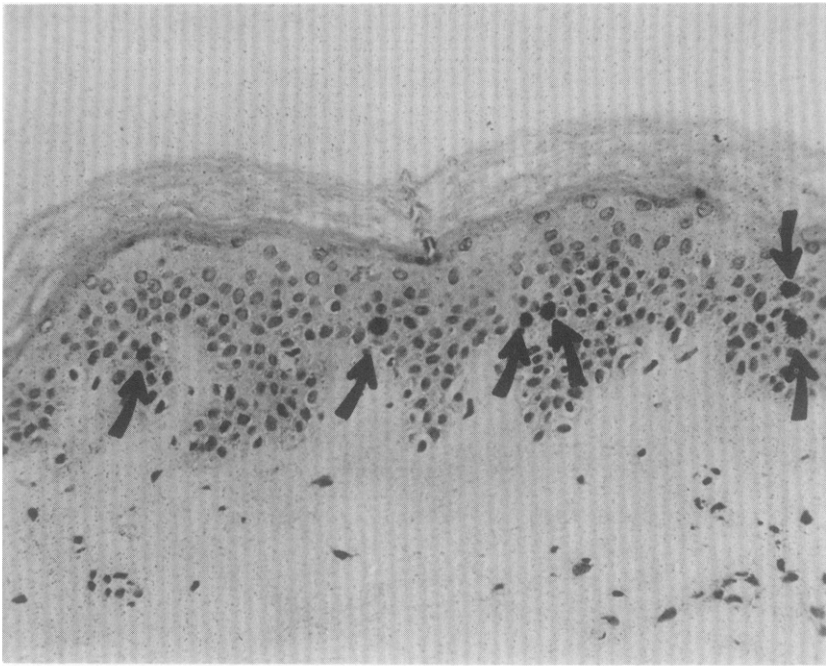


FIG. 2. Autoradiograph of skin biopsy from a normal subject incubated with ^3H -thymidine for 48 hours. Note heavy labeling of epidermal cells. More than 99 per cent of the labeled cells are in the epidermis.

and lubricated. For anesthesia the area of skin was covered with an ice bag for 20 minutes. Using a Castroviejo Electro-Keratome, a strip of skin approximately 20 by 70 by 0.3 mm. was removed from the volar surface of the left forearm and also the left midlateral thigh. In the atopic group the excision was made from the thigh where the skin appeared to be normal or nearly normal. The biopsies from the forearm and leg were taken at the same time, approximately 9 A.M. For one week prior to biopsy nothing, save water, had been applied topically to the chosen sites. The excised sheets of skin were immediately placed in ice cold medium 199 (GIBCO) containing antibiotics. As soon as possible, they were cut into squares 5 by 5 mm. These squares were placed in culture tubes (Falcon Plastics No. 2005) containing 1 ml. of culture medium. The culture medium consisted of medium 199 to which was added 5 μCi of ^3H -thymidine, specific activity 20 Ci. per milliliter (New England Nuclear), 80 units of penicillin, 80 μg of streptomycin, 0.2 μg of amphotericin B, and 20 per cent fresh heparinized autologous plasma. The catecholamines, 1-epinephrine, 1-isoproterenol, or 1-norepinephrine bitartrate (Winthrop-Stearns, Rensselaer, New York), were dissolved and diluted in isotonic saline containing 100 μg per milliliter of ascorbic acid and were sterilized by membrane filtration. To each culture tube was added 0.1 ml. of the appropriate dilution of the catecholamine solution to give final concentrations of 10^{-5} to 10^{-10}M . A similar volume of the ascorbic acid solution was added to triplicate control tubes. The tissue was incubated at 37° C. in an atmosphere of 5 per cent carbon dioxide in moist air.

Bioassay of catecholamine concentration by inhibition of spontaneous contractions of the excised rat uterus indicates that the concentration of dilute solutions of the catecholamines in this medium containing 20 per cent plasma remains relatively unchanged for 12 to 14 hours and then declines rapidly. Less than 10 per cent of the activity remains at 24 hours. Therefore, after 24 hours of incubation appropriate concentrations of catecholamines or their diluting fluid were added to restore the concentration to the initial level. At 48 hours the cultures were terminated.

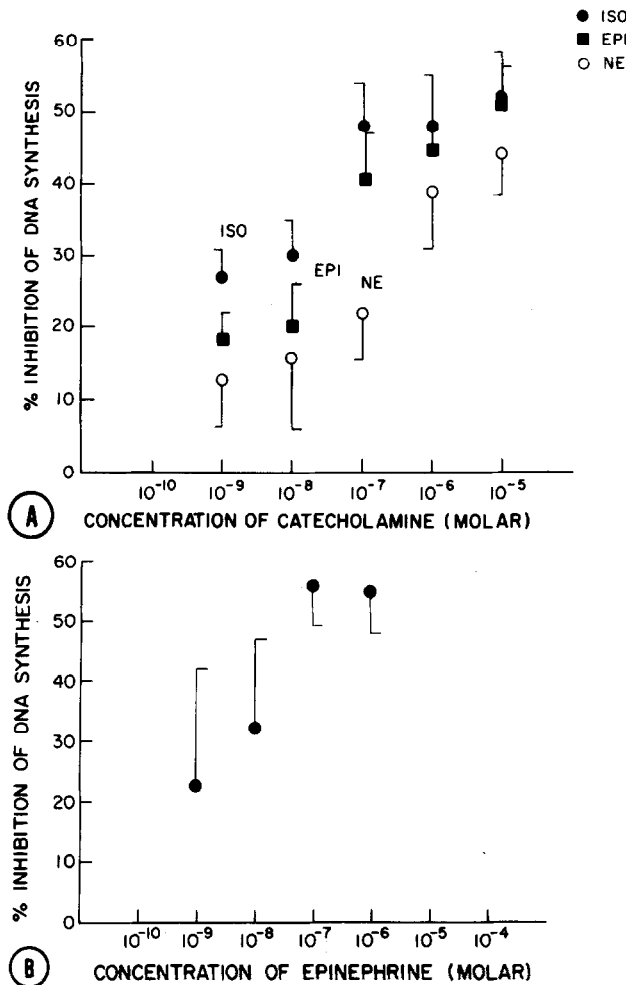


FIG. 3. Catecholamine inhibition of DNA synthesis by epidermis of normal subjects. Mean and S.E.M. of 10 subjects. At the maximal effective dose DNA synthesis was inhibited about 50 per cent. Isoproterenol is about 10 times more effective than epinephrine and about 100 times more effective than norepinephrine. **A**, Biopsies of the skin of the forearm. **B**, Biopsies of the skin of the leg.

The culture tubes, with their epidermal squares, were centrifuged at 4° C., and the culture medium was decanted. Isotonic saline was added, and, after centrifuging again, the tissue squares and sedimented cells were homogenized in ice cold 5 per cent trichloroacetic acid. The precipitate was collected by filtration on glass fiber discs (2.4 cm. in diameter, Reeve Angel No. 934 AH). The discs were washed 3 times with one milliliter of 5 per cent ice cold trichloroacetic acid and then 3 times with one milliliter of ice cold 80 per cent ethanol. They were then dried and placed in vials containing one milliliter of a toluene solution of Omnifluor (New England Nuclear), 4 Gm. per liter. The vials were counted in a scintillation counter.

After appropriate corrections, the data was expressed as counts per minute per square centimeter of tissue. The effect of the catecholamines was calculated by the formula:

$$\% \text{ inhibition} = 100 - \frac{\text{counts per minute in the experimental tube} \times 100}{\text{mean of counts per minute in 3 control tubes}}$$

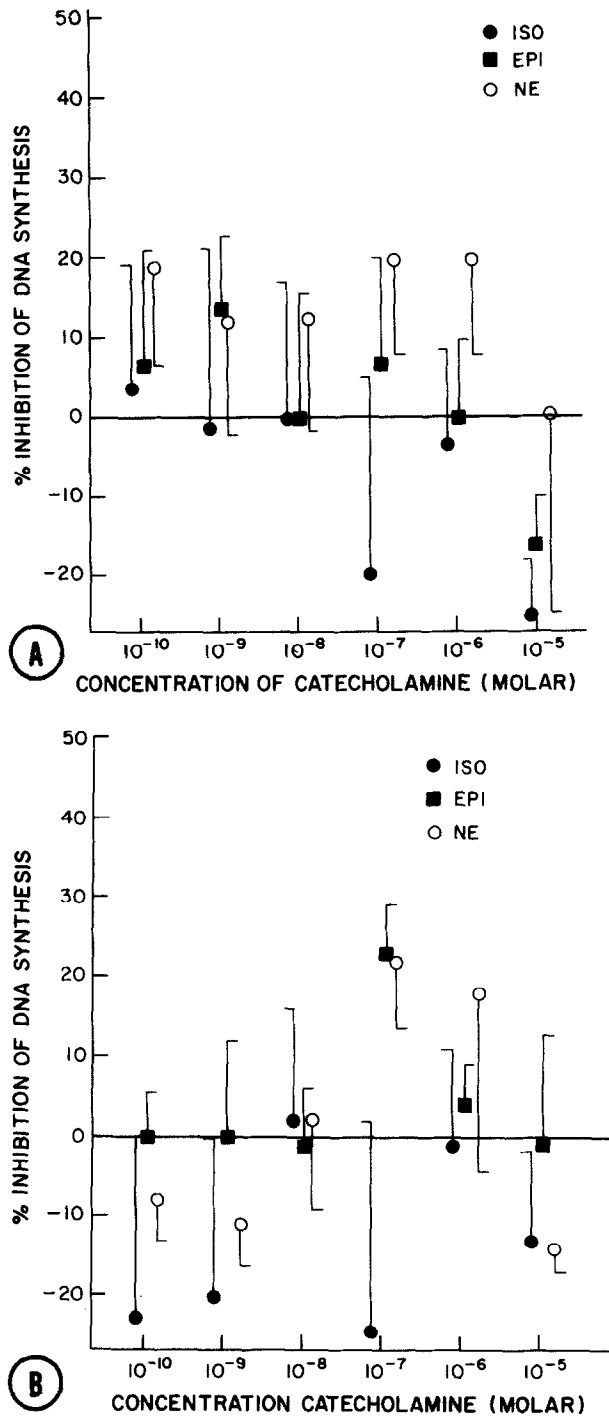


FIG. 4. Failure of catecholamines to inhibit DNA synthesis by epidermis of patients with atopic eczema. Mean and S.E.M. of 5 subjects. Conditions of incubation were identical to those of Fig. 3. **A**, Biopsies of lichenified skin of forearm. **B**, Biopsies of normal-appearing skin of thigh.

TABLE I. Incorporation of radioactivity into DNA by epidermis of normal subjects and patients with atopic eczema during incubation for 48 hours with ^3H -thymidine (5 subjects in each experiment)

Experiment 1	Counts per minute per cm. ² (mean $\pm$ S.D.)	Experiment 2	Counts per minute per cm. ² (mean $\pm$ S.D.)
Normal, arm	11,210 $\pm$ 2,016	Normal, arm	24,700 $\pm$ 1,900
Eczema, arm (lichenified skin)	10,728 $\pm$ 2,704	Normal, leg	18,700 $\pm$ 1,570
Eczema, leg (clear skin)	5,936 $\pm$ 2,680		

TABLE II. Per cent of 10 μM of epinephrine remaining in the culture fluid at 0, 4, 8, and 24 hours during incubation with skin from normal volunteers and patients with atopic eczema

Time	Per cent epinephrine remaining and S.D.	
	Normal (N = 5)	Eczema (N = 5)
0	100	100
4	81 $\pm$ 7	88 $\pm$ 5
8	80 $\pm$ 7	90 $\pm$ 7
24	66 $\pm$ 13	84 $\pm$ 7
0*	100	100
4	95 $\pm$ 2	86 $\pm$ 5
8	83 $\pm$ 8	87 $\pm$ 3
24	74 $\pm$ 8	64 $\pm$ 3

*Epinephrine 10 μM readded.

The metabolism of epinephrine by the control and eczema skin plugs was also determined. Skin squares from the forearm in both control and atopic patients were prepared as above and placed in incubation media with a final epinephrine concentration of 10^{-5}M . The incubation was terminated at 0, 4, 8, and 24 hours and again at the same times following the readdition of epinephrine. The concentration of epinephrine in the culture fluid was measured by the modified method of Von Euler and Lishajko.¹⁴ The amount of epinephrine remaining at the various times was expressed as the per cent of amount initially present.

RESULTS

The depth of cut of the biopsies includes all of the epidermis with a small amount of dermis containing a variety of cell types (Fig. 1). Biopsies of the eczematous skin contained more inflammatory cells. Autoradiography of biopsies incubated for 48 hours with ^3H -thymidine but without catecholamines showed heavy labeling of epidermal cells (Fig. 2). Only 3 cells, probably fibroblasts, of 575 labeled cells counted were in the dermis; the other labeled cells were epidermal. There was no qualitative difference in the labeling of cells of normal and atopic skin.

There was no difference in DNA synthesis in the control tubes without added catecholamines between the patients with eczema and the normal subjects. The skin of patients with eczema, however, showed greater DNA synthesis in the epidermis obtained from the arm, where the disease was active, than in epidermis from the thigh where the skin appeared normal (Table I, Experiment 1). The DNA synthesis in normal arm skin was also slightly more active than normal leg skin (Table I, Experiment 2).

Catecholamines inhibited DNA synthesis by normal epidermis from both

the arm and the leg. Comparison of the dose-response curves indicates that isoproterenol is about tenfold more potent than epinephrine and one hundred-fold more potent than norepinephrine (Fig. 3). In sharp contrast, the catecholamines had no effect on DNA synthesis of either lichenified or normal-appearing skin from patients with eczema (Fig. 4).

The decreased effect of the catecholamines was not due to increased destruction by catecholamine-O-methyl transferase or other enzymes by the eczematous skin since there was no difference in the rate of disappearance of epinephrine from the culture fluid (Table II).

DISCUSSION

The autoradiographs indicate that the uptake of ^3H -thymidine is, for all practical purposes, limited to the epidermal cells, although the skin biopsies do contain fibroblasts and other cells. Contrary to expectations, we did not find epidermal DNA synthesis increased in the skin taken from the lesions of atopic eczema compared to skin taken from the same site of normal subjects. DNA synthesis of skin from the arms was greater than the skin from the leg in both normal and atopic subjects, the difference being greater in the atopic subjects. The possibility remains that the rate of mitosis in the lesions might have been increased *in vivo* before the biopsy was taken and that the conditions of culture did not sustain the increase.

The sequence of potency of the three catecholamines, isoproterenol epinephrine, and norepinephrine, indicates that inhibition of mitosis in normal epidermis is a beta adrenergic function. Furthermore this sequence is similar to the sequence of potency of the beta agonists on tracheobronchial smooth muscle and glycogenolysis (beta 2) and different from the sequence of potency on the heart and lipolysis (beta 1).^{1, 15, 16} The adrenergic abnormalities of asthmatics also seem to involve the beta 2 type of response.¹⁷

The failure of the catecholamines to inhibit DNA synthesis by epidermis of atopic subjects *in vitro* indicates a deficient beta adrenergic response of cells isolated from all connections with the autonomic nervous system and that the autonomic abnormalities in eczema lie in the skin itself. Bamshad¹⁸ found the levels of catecholamine-O-methyl transferase to be increased in the lesions of eczema compared with normal skin. Increased catecholamine-O-methyl transferase activity has not been rigorously excluded as a possible explanation for these results. However, the metabolism of epinephrine was not different in normal and eczematous skin. An increased rate of destruction of the catecholamines would be unlikely to abolish their action so completely and could not explain the exaggerated alpha responses in the arterioles and piloerector muscles. In addition there is some evidence that the norepinephrine content of the lesions is increased.¹⁹ A defect in the beta adrenergic receptors of the target cells seems to us a more probable explanation for the findings.

These results appear to conflict with those of Mier and Urselmann,²⁰ who reported normal levels of adenylyl cyclase in the skin of atopic subjects. The adenylyl cyclase measured by their technique does not respond to epinephrine,²¹ so that the normal values obtained by their assay do not necessarily mean that beta receptor response is normal.

The sequence of biochemical reactions that occur between the stimulation of beta receptors at the cell surface and inhibition of DNA synthesis in the nucleus are as yet incompletely known. Thus, in a physiologic sense there is a blockade of the beta receptors of the epidermal cells in eczema. The biochemical basis for the defect remains to be identified.

Since the abnormal response was demonstrated not only in lesions but also in apparently normal skin, it is clear that the abnormal response to catecholamines alone does not account for the lesions. Additional features such as the trauma of scratching must be considered.

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