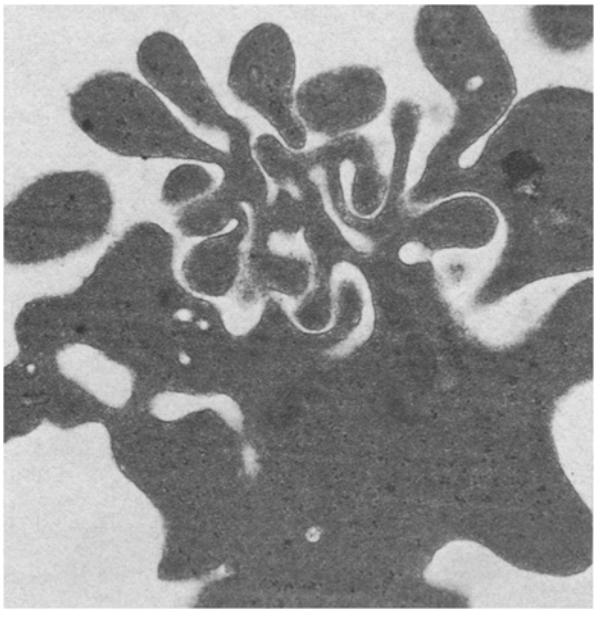

Fig. 3. Marked degree of ropalocytosis in reticulocyte in the peripheral blood. ×20,000.

ropalocytosis in erythrocytes and in cells exposed to the cytochalasins. The fact that this phenomenon has been found in leukemic blasts is more difficult to explain. There was no known exposure to drugs or chemicals prior to obtaining the specimen but some agent in the blood cannot be absolutely excluded. The occurrence of the clear vacuoles in the extremities of the processes is also intriguing. Formation of 'blisters' on cell surfaces is known to occur in malignant cells spontaneously or on exposure to certain agents[10] and formation and release of membrane bound vacuoles has been noted in lymphoblasts exposed to the vinca alkaloids[11], but one cannot say from the static photographs in our case whether the vacuoles have formed in the processes or whether active discharge is taking place.

Whatever the cause may be, it is evidently not the result of degeneration of the cell as both the nuclei and organelles show no evidence of damage. Nor can ropalocytosis be an artefact of fixation in gluteraldehyde as it has been seen in red cells with osmium fixation[2]. The cause may lie in the fact that cell surface alterations occur in malignant transformation and the formation of these club-shaped processes may be an expression of changes occurring in neoplastic cells making them more susceptible to unknown agents in the environment.

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Allergic reactions, cyclic AMP and histamine release[1]

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Summary. Both isobutylmethylxanthine and theophylline increased the level of cyclic AMP in the mast cell. Theophylline reduced the allergic histamine release, whereas isobutylmethylxynthine caused a pronounced potentiation of the histamine release. This indicates that the hypothesis of an inverse relationship between the level of cyclic AMP in mast cells and histamine release is too simple.

Cyclic AMP is assumed to modulate the release of allergic mediators, i.e. histamine and SRS-A (slow reacting substance of anaphylaxis) from mast cells and basophils in the allergic response. It is claimed that increase and decrease in the level of cAMP in these target cells would suppress and enhance, respectively, the release of histamine and SRS-A. This hypothesis is based on comparisons between the influence of drugs on cAMP level and release of allergic mediators in mast cells, peripheral leukocytes and lung tissue[2-6]. Thus, anti-allergic drugs increase the level of cAMP and concomitantly reduce the release of histamine and SRS-A. Different mechanisms are involved in the augmentation of the level of cAMP: beta-adrenergic agents (e.g. isoproterenol) increase cAMP formation by stimulating beta-adrenergic receptors and methylxanthines (theophylline, aminophylline) inhibit the breakdown of cAMP. Propranolol may promote attacks in asthmatic patients. In fact, this drug shows the reverse effects: a reduced cAMP level by blocking beta-adrenergic receptors and an increased release of allergic mediators[6]. However, this hypothesis of drug-induced alterations of histamine and SRS-A release by changes in cAMP level is as yet unproved.

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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.

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

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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.