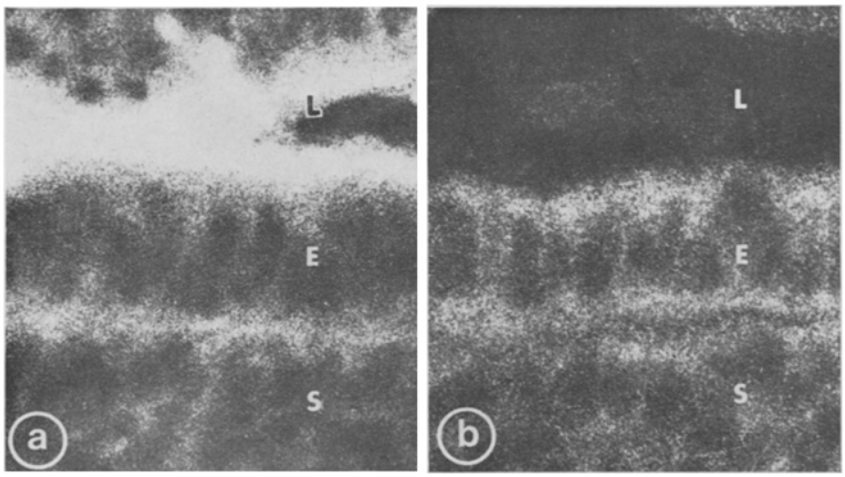

Fig. 2. Epithelium (*E*) of uterine cervix from neonatal mice after 40 h in vitro. L, lumen; S, stroma. (The micrographs are black and white copies from multi-colour Ektachrome film). ×680. a) Fluorescence at the apical part of the columnar cells and in the lumen. Incubation medium: SCM+dcAMP. Immune IgG. b) Control with only faint fluorescence. Incubation medium: SCM. Immune IgG.

particles, indicating the presence of antigen-antibody complexes, were seen in the fuzz filamentous material attached to the microvilli, and along the surface membrane of the epithelial cells. Moreover, ferritin granules were found along the cell membrane bordering the intercellular spaces (Figure 1a–c). In contrast to this, no ferritin particles were seen along the basal part of the cell membrane or in the basal lamina. Nor were ferritin particles found to adhere to organelles, vesicles, or membranes inside the cells. The location of ferritin in the organ cultures supplemented with dcAMP was similar to that obtained after estradiol injections to neonatal mice.[7]

Discussion. Although cAMP is involved in the action of several hormones, sex steroids seemed for some time to be an exception. Recent evidence suggests the involvement of cAMP in sex steroid action. Studies on rat uterus have shown that cAMP imitates many of the estradiol induced effects[9,10].

The present ultrastructural study with a ferritin-conjugated antibody demonstrates that the specific cervicovaginal antigen (CVA) is localized to the cell membrane, the part facing the lumen as well as the part facing the intercellular spaces, and is also associated with the fine filamentous material on the epithelial surface. The immunoferritin studies confirm results obtained with immunofluorescence technique (Figures 2a and b). When dcAMP was added to a synthetic culture medium lacking estradiol, a considerable increase in the amounts of CVA was observed in the cervicovaginal anlage from neonatal mice[5]. Estradiol injected to neonatal mice produced a similar increase of CVA in the cervicovaginal epithelium[4]. The estradiol effect in vivo is thus simulated by dcAMP in vitro. Neither the in vivo nor the in vitro experiments influenced the uterine epithelium to produce CVA. Our studies support the interpretation that cAMP is involved in the mechanism of estradiol action. Further studies concerning the involvement of dcAMP in this system are in progress in our laboratory.

Zusammenfassung. Untersuchungen mit Immunelektronenmikroskopie haben erwiesen, dass cyclische AMP in vitro die Wirkung von Östradiol in vivo simuliert. Das zeigt sich in einer Vergrösserung der Menge antigenen Materials, dessen Bildung für das cervicovaginale Epithel spezifisch ist. Der mit Ferritin konjugierte Antigen-Antikörper-Komplex war an der Oberfläche der Zellen lokalisiert.

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Possible Role of Glucose-6-Phosphate in the Anti-Anaphylactic Mechanism Mediated by Cyclic AMP

Cyclic AMP enhances the synthesis of glucose-6-phosphate via glucose-1-phosphate[1,2]. There is now a great deal of evidence[3–10] that cyclic AMP inhibits anaphylactic phenomena. We report here the results of investigation of anaphylactic mechanisms by glucose-6-phosphate.

Materials and methods. To test the action of glucose-6-phosphate on anaphylaxis in vitro, we used Schultz-Dale technique as described in a previous paper[11], whereby D-glucose-6-phosphate (Sigma) in the final concentration of 4.65 μM would influence anaphylactic reaction of passively sensitized smooth muscle from guinea-pi uterus. Size of the isotonic anaphylactic muscular contraction was expressed in histamine equivalent determined by interpolation to a dose-response curve obtained with histamine dihydrochloride on the same muscle piece.

Using normal non-sensitized smooth muscle pieces of guinea-pig uterine horns, influence of glucose-6-phosphate on isotonic histamine contraction was observed in the final concentration of 4.65 μM. The results were expressed by the change of 50% contractive dose (CD_{50}) of histamine dihydrochloride. Finally, release of ana-

Inhibition of anaphylactic mechanisms by glucose-6-phosphate

	Control (C)	Glucose-6-phosphate added (*T*)	Difference	*t*-test against control (*P*)
Histamine equivalent[a] of Schultz-Dale reaction	2.85 ± 1.21[b]	2.05 ± 0.99[b]	0.80 ± 0.33[c]	< 0.001
Histamine equivalent[d] of anaphylactic mediator released from sensitized guinea-pig lung[e]	0.331 ± 0.176[b]	0.266 ± 0.170[b]	0.066 ± 0.048[c]	< 0.02
CD_{50}[a] of histamine contraction	6.7 ± 3.1[b]	11.4 ± 8.3[b]	4.6 ± 5.5[f]	< 0.1

[a] μg of histamine dihydrochloride in 20 ml organ bath. [b] Mean value of 7 experiments on 7 animals ± standard deviation. [c] Mean value of 7 (*C*–*T*) obtained on 7 animals ± standard deviation. [d] μg of histamine dihydrochloride. [e] Per 80 mg of chopped tissue. [f] Mean value of 7 (*T*–*C*) obtained on 7 animals ± standard deviation.

phylactic mediator from sensitized guinea-pig lung in vitro in the presence of 0.19 m*M* of glucose-6-phosphate was studied. Guinea-pigs were stunned and exsanguinated. The lungs were removed and chopped into rods approximately 0.3 to 0.5 mm^3. The chopped tissue was sensitized with the equal amount in weight of anti-BSA rabbit antiserum which had been prepared as shown in the previous paper[11]. This sensitization in vitro was made for 2 h at 37°C. The tissue was washed with Tyrode's solution, and aliquots of 400 mg were mixed with 2.5 ml of Tyrode's solution with either glucose-6-phosphate for test or isoosmotical amount of sodium chloride for control. After 5 min of equilibration in a 37°C water-bath, 0.25 ml of 1% BSA solution was added to each tube and the tubes were kept at 37°C for 15 min. The tissue was then removed with filter paper, and the filtrates were plunged into and kept in 100°C water-bath for 3 min.

To control possible interference of glucose-6-phosphate with bioassay of the anaphylactic mediator, the same procedure was simultaneously performed on other aliquots of the sensitized tissue without the addition of BSA. One of the supernatants of these blank tests, which contained glucose-6-phosphate, was mixed with the equal amount of control specimen. The other supernatant, without glucose-6-phosphate, of the blank tests was mixed with the test preparation which contained the initially added glucose-6-phosphate. Bioassay of the anaphylactic mediator was performed with guinea-pig uterine horns, and the amount of the active substance was expressed in the comparative amount of histamine dihydrochloride which was capable of eliciting the same size of isotonic muscular contraction. All control and test experiments with the lung from one guinea-pig were performed in duplicate, respectively, and the mean values were used as representing the animal.

Throughout these experiments, unless otherwise noted, Tyrode's solution containing 0.1% of glucose was used for all the solutions and organ baths.

Results and discussions. The results are summarized in the Table. Glucose-6-phosphate showed statistically highly significant inhibitory action on the Schultz-Dale reaction, and release of anaphylactic mediator from sensitized lung tissue was also inhibited. Although the difference of the mean of CD_{50} of histamine contraction of smooth muscle in the presence of glucose-6-phosphate was not significant enough under our experimental conditions, all 7 trials with histamine dihydrochloride had been uniformly inhibited by glucose-6-phosphate, indicating a tendency of the compound to inhibit smooth muscle reaction to histamine.

Inhibitory action of cyclic AMP on smooth muscle motility is well established. Studies[3–10] have shown that cyclic AMP suppresses anaphylactic release of histamine and SRS-A. Furthermore, as mentioned above, synthesis of glucose-6-phosphate is activated by cyclic AMP. Thus, in conclusion, the resemblance of mode of inhibition of anaphylaxis in vitro and close relationship in metabolism might indicate that major effect of cyclic AMP on anaphylaxis is brought about by increase in intracellular glucose-6-phosphate level[12].

Zusammenfassung. Nachweis der Hemmung von D-Glucose-6-Phosphat auf die Schultz-Dalesche Reaktion, die Freilegung des anaphylaktischen Mediators und die Histaminkontraktion des glatten Muskels.

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