too, the concentration of cAMP measured with both methods in the isolated storage organelles exceeded that in the whole platelets by more than 100 times.

The identity of the cAMP in the storage organelles was substantiated by the findings that a) the values determined with the saturation method were not significantly different ($p > 0.05$) from those obtained with the radioimmune-assay (Table) and b) no cAMP could be detected by either method after incubation of the nucleotide extracts with phosphodiesterase. Furthermore, the cAMP was probably not formed from ATP during the precipitation procedure (with $ZnSO_4$ + Ba $(OH)_2$) used for the saturation method[8,13]. Thus, the cAMP values showed no difference whether the ATP had been removed by precipitation or by absorption on Dowex AG50 WX4. A reduction by the ultrasonication of the extragranular cAMP is unlikely since platelets homogenized by freezing and thawing exhibited the same cAMP content as those subjected to ultrasonication.

These results indicate that cAMP accumulates together with 5'-phosphonucleotides like ATP and a biogenic amine (5HT) in subcellular storage organelles. Thereby, the concentration of the cyclic nucleotide is about 4000 times lower than that of ATP (8.5×10^6 *p*moles/mg protein)[5]. According to preliminary results with rabbit platelets, no measurable adenyl cyclase activity (using α-^{32}P-ATP as a substrate)[14] could be detected in the isolated storage organelles or in their membranes, even in the presence of NaF or prostaglandin E_1[15]. In the isolated cytoplasmatic membranes, however, adenyl cyclase activity was present and markedly activated by NaF as well as prostaglandin E_1. Therefore, the site of formation of the cAMP found in the storage organelles is not yet clear.

cAMP possibly has an influence on platelet function, e.g. in platelet aggregation, but its physiological and pathophysiological role is still unsettled[4,16-20]. Storage in subcellular organelles might result in biological inactivation of the cyclic nucleotide. Therefore, the organelles, due to their capacity of accumulating and possibly releasing cAMP, may be part of a system controlling the biological activity of the cyclic nucleotide in the platelets. It remains to be elucidated whether other types of organelles, e.g. the vesicles storing ATP and catecholamines in sympathetic nerve endings or brain, behave like platelet organelles with regard to cAMP, and if so, whether the presynaptically stored cAMP has a function in neurohumoral transmission.

Zusammenfassung. Die subzellulären Organellen, welche in Blutplättchen von Ratten und Meerschweinchen 5-Hydroxytryptamin und Adenosin-5'-triphosphat speichern, enthalten bis über 100 Mal mehr cyclisches Adenosin-5'-monophosphat (cAMP) als die ganzen Plättchen und die übrigen subzellulären Fraktionen. Durch die Speicherung in subzellulären Organellen kommt es möglicherweise zu einer biologischen Inaktivivierung von cAMP in den Plättchen.

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Content of cyclic AMP in isolated blood platelets and storage organelles of rabbits

Method	Blood platelets	Storage organelles
Saturation assay	24 ± 5 (10)	2501 ± 640 (8)
Immunological assay	9 ± 1 (8)	1975 ± 293 (7)

The values represent averages with S.E. and are expressed in *p*moles/mg protein. Number of experiments in parentheses.

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Cyclic AMP: its Effect on an Estrogen-Sensitive Antigen in Organ Cultures of the Cervicovaginal Epithelium from Neonatal Mice[1]

Cyclic AMP is known to regulate important activities in several different cells and to be essential in the action of many hormones. Its role for the expression of the sex steroid effects has recently been dealt with[2,3].

Immunofluorescence studies have shown that the cervicovaginal epithelium of neonatal and adult mice contains antigenic material(s) (CVA) specific for this epithelium. Antibodies against CVA do not cross-react with any antigen in several tissues tested (uterus, kidney, intestine, submaxillary gland etc.)[4]. In estradiol injected animals, the amount of CVA is considerably increased but still restricted to the cervicovaginal epithelium. When the uterine cervix from neonatal mice was cultured in vitro in a medium containing cyclic AMP, the amount of CVA was likewise increased compared with the controls[5].

In organ cultures of the neonatal uterine cervix, the cyclic AMP induced CVA was localized to the most apical part of the epithelial cells and to the lumen. This prompted an ultrastuctural study on the relation between CVA and the cell surface.

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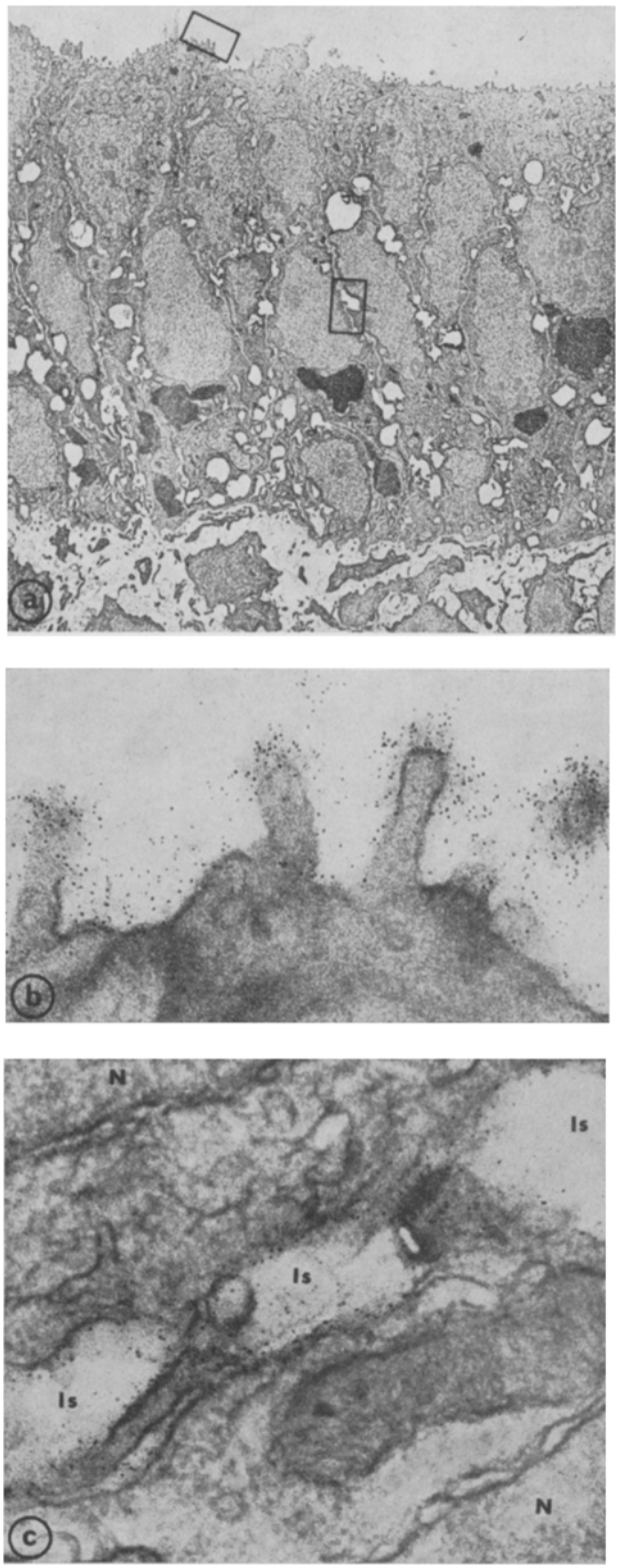

Fig. 1. Epithelium of the uterine cervix from neonatal mice after 40 h culture in vitro. a) Low power electron micrograph. Stained with lead citrate. ×2000. b) Detail of an area similar to the upper rectangle in a) showing the surface membrane with microvilli. Ferritin particles adherred to the surface membrane and to extracellular material in the lumen. Unstained. ×32000. c) Detail of an area corresponding to the lower rectangle in a) showing intercellular spaces between 2 adjacent epithelial cells. Ferritin particles adherred to the outside of the plasma membrane. N, nucleus; Is, intercellular space. Unstained. ×32000.

Material and methods. Explants of the cervicovaginal anlage and uterine horns from neonatal mice (NMRI strain) were cultured on Millipore filters placed on the top of Spongostan® for 30–45 h in a standard culture medium (SCM) made up of 70% Eagle L, 20% fetal bovine serum (Microbiological Associates) and 10% chicken embryo extract. In test cultures, the medium was supplemented with 0.1 m*M* sodium salt of N^6, O^2-dibutyryl adenosine 3′:5′-cyclic monophosphoric acid (dcAMP, Sigma).

Rabbits were immunized with vaginal epithelium from adult ovariectomized mice which had been given daily s.c. injections of 5 μg estradiol-17β in 0.02 ml olive oil for 2 days before the day of sacrifice. A crude IgG fraction (immune IgG) was prepared from the immune serum through salting out with saturated ammonium sulfate[4].

Conjugation of ferritin (Serva) to the crude immune IgG and to normal serum (rabbit) was performed through a one-step-coupling with *p,p′*difluoro-m,m′dinitrodiphenylsulfone[6]. For the controls, normal rabbit serum was preferred to crude non-immune IgG, because the IgG amounts in the normal serum used were proved to be similar to that in the immune IgG solution used; moreover, serum may contain unspecific antibodies that are lost during preparation of crude IgG. Antibody activity and homogeneity of coupled antibodies were checked by immunoelectrophoresis[7].

Formalin has proved to be the fixative that most adequately holds soluble antigens to their original sites during labelling and processing for electron microscopy[8]. Organ cultures were prefixed in 4% paraformaldehyde in buffered and isotonic phosphate solution (pH 7.3). Prefixed organs were sliced with razor blades under the dissecting microscope. Some of the slices were submitted to ultrasound for 1–2 min in order to open the plasma membrane and make the cells permeable to the conjugate. The tissue slices were incubated with ferritin conjugated antibody solution for 30 min at room temperature, washed, and further processed for electron microscopy in a conventional way.

Results. The ultrastructure of the cervicovaginal epithelium in cultures supplemented with dcAMP was similar to that seen after estradiol injections to neonatal mice (unpublished observations). Thus the free surface of the epithelial cell became convex and developed distinctive club-shaped microvilli. In the apical part of the cells, many vesicles, varying in size, appeared.

The following control experiments were set up: 1. slices from explants cultured in a medium containing dcAMP were incubated in ferritin-conjugated normal serum, 2. slices from explants cultured in a medium without dcAMP were incubated in ferritin-conjugated normal serum, 3. slices from explants cultured in a medium without dcAMP were incubated in ferritin-conjugated immune IgG. None of these experiments showed any signs of binding of ferritin-conjugated antibodies. Nor was any binding seen in explants of the uterine horns which had been cultured in a medium containg dcAMP and incubated with ferritin-conjugated IgG. The picture was quite different when slices from the cervicovaginal anlage, cultured in a medium containing dcAMP, were incubated in ferritin-conjugated immune IgG. A rich amount of ferritin

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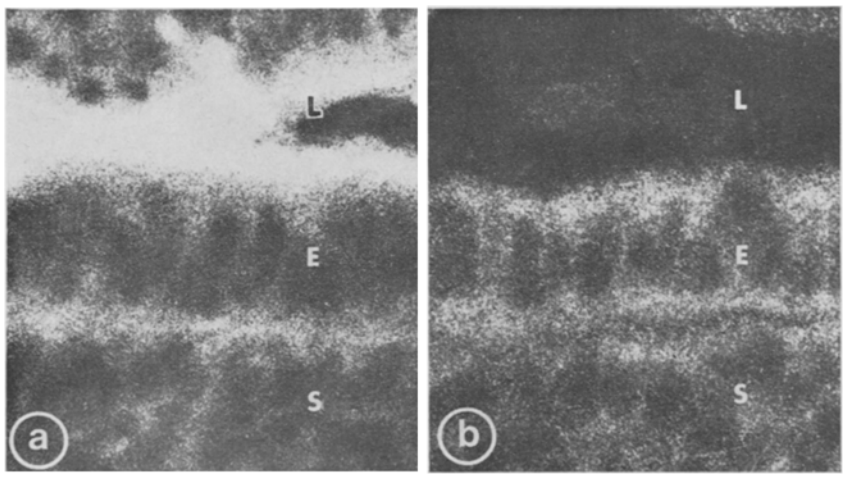

Fig. 2. Epithelium (*E*) of uterine cervix from neonatal mice after 40 h in vitro. L, lumen; S, stroma. (The micrographs are black and white copies from multi-colour Ektachrome film). ×680. a) Fluorescence at the apical part of the columnar cells and in the lumen. Incubation medium: SCM+dcAMP. Immune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