On the other hand, when one considers the inhibition of the peripheric cholinergic effects of oxotremorine in mice, the antagonistic activity of diazepam is only 10 times lower[8] than that of atropine. However, according to our experimental results the IPSC 50 of atropine is 0.12 mg/kg, which means that diazepam has an anhydrotic activity 43.4 times lower than atropine. The most active and the least active benzodiazepines tested on PSC – nitrazepam and medazepam – are respectively 31.8 and 876 times less active than atropine. So the hypothesis of an anticholinergic mechanism to explain the antisweating effect of benzodiazepines appears to be ruled out. Could this phenomenon be attributable to the α-adrenolytic activity of benzodiazepines[8–10]? Indeed α-adrenolytics likewise present an IPS[11]. The IPSC 50 of moxisylyte and hydergine are respectively 32.32 and 13.95 mg/kg and are therefore closer to those of benzodiazepines. Finally, one must not discount the possibility that the IPS of benzodiazepines originates centrally on account of the central depressive activity of these drugs. Indeed, hypnotics likewise present an IPS. The IPSC 50 of phenobarbital, pentobarbital and methaqualone are respectively 53.71, 27.90 and 27.92 mg/kg[12].

Résumé. Les benzodiazépines inhibent la sécrétion palmaire cutanée chez la souris proportionnellement à la dose administrée. Les différents mécanismes (cholinergique, α-adrénergique, central) qui peuvent être en jeu sont discutés.

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The Effect of Hydrocortisone on Tension and Cyclic AMP Metabolism in Tracheal Smooth Muscle

The mode of action of corticosteroids in treatment of bronchial asthma is obscure. In high concentrations, corticosteroids increased the β-adrenoceptor response of isolated human bronchial muscle[1,2]. The relaxation following stimulation of β-adrenoceptors in bronchial[3] as well as in vascular[4,5] and intestinal[6] smooth muscles is probably mediated by cyclic AMP. The nucleotide probably induces relaxation by reducing the free myoplasmic Ca^{2+} concentration by stimulating sequestering of Ca^{2+} to microsomal fractions[7]. This work was performed to investigate if glucocorticoids had any relaxing action and whether they influenced the cyclic AMP metabolism of tracheal and bronchial smooth muscles.

The action of hydrocortisone (Hydrokortisonsuccinat®, Roussel) was tested on human bronchial, muscles tracheal rings from guinea-pig and bovine trachea. The preparations were suspended in Krebs bicarbonate buffer aerated with 95% O_2 and 5% CO_2 at 37 °C. The isometric muscle tension was measured by Grass FT 03 transducers and registered on a Grass polygraph Model 7. In all these preparations, the effect of hydrocortisone was tested in a concentration range of 5×10^{-7}–5×10^{-5} *M* both on spontaneous tension and on histamine contracted muscles. The human bronchial muscle was most sensitive to hydrocortisone; in a concentration of 1.0 μ*M* a complete relaxation was produced both in muscles with spontaneous tone (Figure 1) and in muscles contracted by histamine (1×10^{-6} g/ml). In tests on bovine and guinea-pig trachea, higher concentrations of hydrocortisone were needed (Figure 1). If the preparations were pretreated with the β-adrenoceptor blocking agent sotalol (1.2×10^{-5} g/ml), the relaxing effect of hydrocortisone was decreased by about 50%. The relaxing effect of isoprenaline on guinea-pig (Figure 2) and bovine tracheal muscle (Figure 3) was potentiated if the muscles were pretreated with hydrocortisone in a concentration of 6×10^{-5} *M*.

The effect of hydrocortisone, or a combination of hydrocortisone and isoprenaline, was studied on cyclic AMP content and tension of bovine tracheal muscles, which had been suspended for 60 min in Krebs buffer solution. The muscles were frozen in frigen 12 and solid CO_2 at fixed times after administration of the drugs. The frozen tissues were homogenized in 5% trichloroacetic

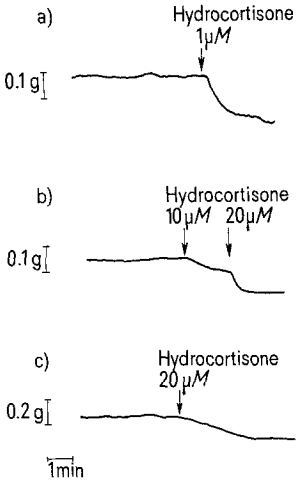

Fig. 1. The relaxing effect of hydrocortisone on different smooth muscle preparations with spontaneous tone. a) Segmental bronchi from humans. b) Tracheal rings from guinea-pig. c) Bovine tracheal muscle.

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acid and centrifuged at $6000 \times g$ for 10 min. The supernatants were extracted with ether to remove the trichloroacetic acid. Cyclic AMP was determined by the protein binding technique[8]. In some experiments the tissue extracts were chromatographed on Dowex 50WX8 before the cyclic AMP determinations, to control that no other nucleotides influenced the binding assay.

The cyclic AMP concentration regularly increased in the spontaneously contracting muscle after addition of 6×10^{-5} M hydrocortisone, even after a period as short as 1 min (Table). In order to correlate the relaxing action with the cyclic AMP changes, experiments were performed on histamine contracted muscles. In the histamine-contracted preparations, hydrocortisone also increased the cyclic AMP content. Both relaxation and the increase of the cyclic AMP content following isoprenaline were significantly increased in the preparations pretreated with hydrocortisone in comparison with the untreated controls (Figure 3).

The mechanism for the cylic AMP increasing action of hydrocortisone may involve an inhibition of the cyclic AMP hydrolyzing enzyme, phosphodiesterase (PDE), or an activation of synthetizing enzyme adenyl cyclase. The effect of hydrocortisone was tested on a crude PDE from bovine tracheal muscle according to the method of PÖCH[9]. No effect, however, was found on the PDE activity determined at a substrate concentration of 1×10^{-6} M. The activity in control tissue was 1.05 $\pm$ 0.1 nmol/mg protein/min and that after treatment with hydrocortisone in the concentration range 10^{-6}–10^{-4} M was 1.06 $\pm$ 0.1 nmol/mg protein/min. Adenyl cyclase activity was determined as described earlier[10]. The basal adenyl cyclase activity of a homogenate of tracheal muscle was found to be 0.54 $\pm$ 0.06 nmol/mg protein/10 min. After addition of hydrocortisone (6×10^{-5} M) to the homogenate, no significant changes of adenyl cyclase activity was observed (0.56 $\pm$ 0.05 nmol/mg protein/10 min).

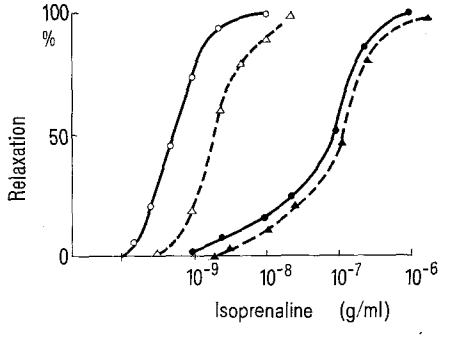

Fig. 2. Dose-response relationship for isoprenaline alone (△—△) or in combination with 1.2×10^{-5} g/ml sotalol (▲—▲); with 6×10^{-5} M hydrocortisone (○—○); with hydrocortisone + sotalol (●—●) on guinea-pig tracheal rings.

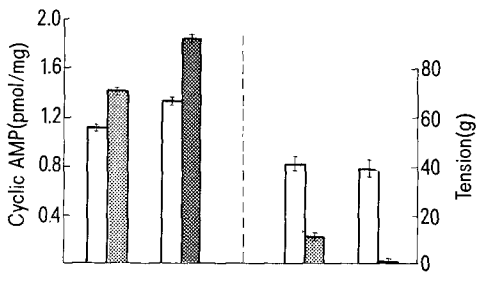

Fig. 3. The effects of isoprenaline alone (▩) or in combination with 6×10^{-5} M hydrocortisone (▩) on cyclic AMP content and tension in bovine tracheal muscle. Mean $\pm$ S.E. ($n = 6$).

The effects of hydrocortisone on the PDE and adenyl cyclase activities of the homogenate cannot explain the increasing action of the drug on the cyclic AMP level in the intact muscle. The reason may be that the hormone receptors are labile to homogenization, or that the hydrocortisone-induced increase of the cyclic AMP level in intact muscle is mediated by an influence on some endogenous substance.

Since blockade of β-adrenoceptors reduced the relaxing action of hydrocortisone, some of its effects might either depend on a release of catecholamines or reduced inactivation of adrenergic transmittor substance. It was recently shown that hydrocortisone impeded movements of noradrenaline to the sites of enzymatic inactivation in vascular smooth muscle[11].

In order to test if the relaxing effect of hydrocortisone was dependent on an increased amount of adrenergic transmittor at the receptor sites, the relaxing effect of hydrocortisone was tested on tracheal preparations from reserpinized guinea-pigs (5 mg/kg Serpasil®, 20 h before killing the animals). In these preparations hydrocortisone still produced a weak relaxation. This effect was not inhibited by adrenergic β-receptor blocking agents. To investigate the influence of hydrocortisone on the prostaglandin (PG) metabolism, the relaxing effect of hydrocortisone was tested on tracheal rings treated with PG-synthesis inhibitor indomethazin[12] (3×10^{-6} g/ml) for 60 min. In these preparations the relaxing effect of hydrocortisone was potentiated.

Corticosteroids have been found to have a permissive effect on the gluconeogenic response of exogenous cyclic AMP in rat liver[13]. It was therefore of interest to examine whether the steroid influenced the response of exogenous cyclic AMP. Cyclic AMP relaxed tracheal rings from guinea-pigs at a concentration of 10^{-4} M. Hydrocortisone did not potentiate this effect of the nucleotide.

The results of this study indicate that hydrocortisone can increase the level of cyclic AMP in tracheal smooth muscle. In human lymphocytes, corticosteroids have earlier been shown to produce a similar effect[14]. The mechanism by which hydrocortisone alters the cyclic AMP metabolism and improves catecholamine responsiveness is not clear, but the β-adrenoceptors are probably partly involved. A direct effect of hydrocortisone on cyclic nucleotide metabolism is also probable, since the effect of the steroid was still present after blockade of β-adrenoceptors, as well as in reserpinized animals.

It is possible that the effect of hydrocortisone on cyclic AMP metabolism is especially valuable in treatment fo bronchial asthma, since it seems to be a relationship between cyclic AMP metabolism and altered smooth

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muscle function in this disease. SZENTIVANYI[15] has thus suggested that in bronchial asthma there is a reduction of the sensitivity of β-adrenoceptors. It has also been demonstrated that leukocytes from patients with bronchial asthma have a decreased cyclic AMP response to β-adrenoceptor stimulators[16]. It is evident that most of the therapeutic agents used in bronchial asthma act via the cyclic AMP system. This applies to catecholamines[6], ACTH[17], as well as hydrocortisone which increase the cyclic AMP level in bronchial smooth muscle and decrease broncho-constriction.

Effect of hydrocortisone (6×10^{-5} *M*) on cyclic AMP content in bovine tracheal smooth muscle

Addition		Cyclic AMP (pmol/mg)
None (control)		0.85 ± 0.07
Hydrocortisone	1 min	1.02 ± 0.11 [a]
	5 min	0.97 ± 0.10 [a]
	10 min	1.01 ± 0.11 [a]

Each value represents the mean ± SEM of assays on 6 tissue samples.
[a] Differed from the control ($p < 0.05$).

Zusammenfassung. Untersuchung des Wirkungsmechanismus von Hydrocortison auf die Relaxation der Trachealmuskulatur von Kuh und Meerschweinchen, wobei stets eine Zunahme des zyklischen AMP-Gehalts gefunden wurde. Es wird angenommen, dass sich dadurch die intrazelluläre Sequestrierung von Calcium erhöht und der Hydrocortisoneffekt zum Teil von einer β-Rezeptorstimulation abhängig ist.

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Paracrystalline Inclusions in Mitochondria of Frog Oocytes

The main feature of amphibian oogenesis is certainly the formation of yolk platelets. In various amphibians, e.g. *Triturus viridescens, Rana temporaria, Rana esculenta* and *Triturus vulgaris*, the yolk platelet precursors are represented by multivesicular bodies which are as a rule formed through coalescense of pinocytotic vesicles[1–3]. Although it could be clearly demonstrated that the majority of vesicles incorporated into the yolk precursors is generally derived through pinocytotic activities, for most cases a participation of intraoocytic membrane systems in the formation of yolk could not be ruled out conclusively. Most ultrastructural investigations on amphibian oogenesis have been able clearly to demonstrate two or more different ways for the formation of yolk. In the case of *Xenopus laevis*, for instance, we too were able to demonstrate a dual mode of yolk formation[4]. One mode is represented by the transformation of mitochondria which comprises the disintegration of intramitochondrial membranes, the uptake of a large number of vesicles from various sources, and the formation of a paracrystalline lattice, resulting in yolk platelets whose mitochondrial origin is no longer depictable. In contrast to the formation of a paracrystalline lattice subsequent to the transformation of mitochondria, it is the direct intramitochondrial formation of paracrystalline lattices which is the subject of our present study carried out on a total of 10 different anuran species. (*Rana erythrea, R. graeca, R. adspersa, R. cyanophlictis, R. esculenta, R. temporaria, Rhacophorus maculatus, Hyla arborea, Bufo bufo, Bombina bombina*).

Intramitochondrial paracrystalline inclusion bodies (ICIB) were first described in 1958 by LANZAVECCHIA and LE COULTRE[5] in a study of embryogenesis in *Rana esculenta*. During the course of our investigation we were able to detect ICIB in 6 of the 10 species used. With respect to both the intramitochondrial localization and the centre to centre distance of the crystalline lattice, we were able to distinguish 2 different types of ICIBs. The main type of ICIB, occurring in all 6 species, is either formed in the intracristal or intermembrane space and has a centre to centre distance of the crystalline lattice which varies between 85 and 100 Å. In 5 of the 6 species mentioned, we were only able to observe the formation of ICIB's in the intracristal space. In *Rana erythrea* oocytes, the formation of ICIBs in the intermembrane space could, however, frequently be detected. On the basis of their morphology, that is the centre to centre spacing of the crystalline lattice and their size and shape, we were not able to detect any differences between the ICIBs that were formed in the intracristal space and those that were formed in the intermembrane space. Great differences were, however, found with respect to the largest crystals present in the different species. The size ranged between 0.2 μm × 0.5 μm in *Rana adspersa* and 1.5 μm × 5.0 μm in *Rana erythrea*. Although a large variety of different shapes of the ICIBs could be found even within the same oocyte, we were able to observe, in each of the 6 crystal-forming species, a crystal shape which appeared to be typical for the individual species, as can be seen in the Figure. Thus we found in *Rana adspersa* a large number of round to oval inclusions, in *Rana graeca* rectangular, in *Rana esculenta* and *temporaria* hexagonal, and in *Rana erythrea* most of the crystals were polymorphous. Quite outstanding were the crystals found in the intracristal space of *Rhacophorus maculatus*, and sometimes in *Rana esculenta* and *temporaria*, because of their rod-like shape. In *Rana temporaria* and *Rana esculenta*, as well as in *Rhacophorus maculatus*,

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