modification of the photoreceptor-cell outer segment. Whether or not they represent a developmental stage of the outer segment saccules could not be determined since definite intermediate stages between the 2 were not observed. Although the outer segments of photoreceptor cells are known to be susceptible to fixation artifact[5,13], the presence of ribosomes in the processes of these modified photoreceptor cells makes it unlikely that they are distorted outer segment saccules. The disorganized appearance of the cytoplasmic processes raises the question of whether these cells function in photoreception or in some other respect. Their functional significance cannot be determined by the present study.

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Immunofluorescent localization of the acid-stable proteinase inhibitor (antileukoprotease) of human cervical mucus[1]

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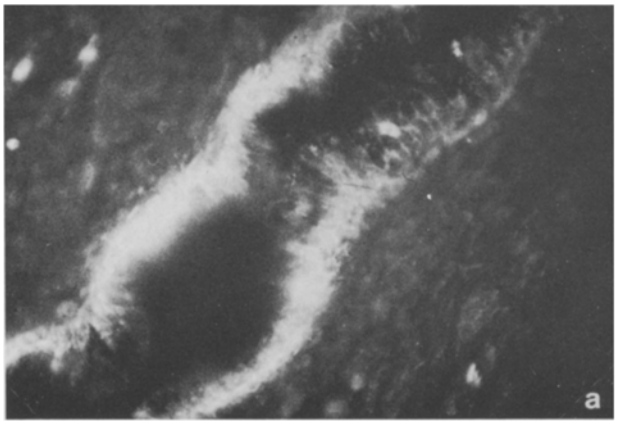
Summary. Localization of the acid-stable proteinase inhibitor of human cervical mucus within the epithelium of the upper cervix was possible by indirect immunofluorescence.

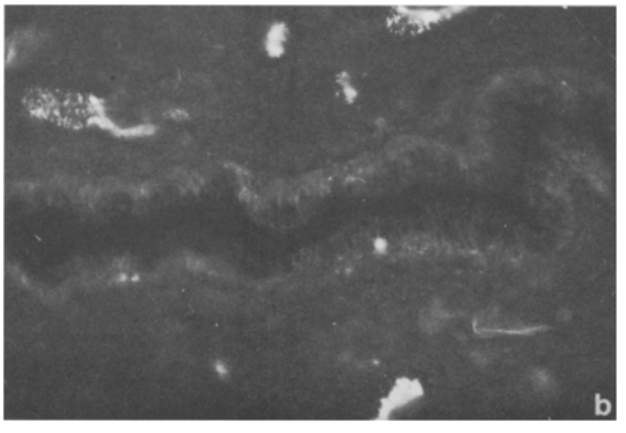
Human cervical mucus contains an acid-stable low molecular weight trypsin-chymotrypsin inhibitor[3] which has been recently characterized in more detail[4]. Inhibition characteristics, mol. wt, amino acid analysis and immunological properties indicate identity of this proteinase inhibitor with human seminal plasma inhibitor I (HUSI-I) produced by the seminal vesicles[5-8]. Remarkably, an acid-stable inhibitor with very similar characteristics is present in human nasal and bronchial secretions[9]. This class of inhibitors has a high affinity to neutral granulocytic proteinases (elastase, cathepsin G) present in most mucus secretions. The rapid and permanent inhibition of granulocytic proteinases by this antileukoprotease suggests a protective function of the cervical epithelium against proteinases liberated from disintegrating leukocytes[7].

In this communication, the side of secretion of the antileukoprotease in the uterine cervix is demonstrated by indirect immunofluorescent technique employing an anti-HUSI-I immunoglobulin obtained from rabbits immunized with highly purified HUSI-I.

Material and methods. The cervix of 5 women (32–45 years) at cycle day 8–10 undergoing hysterectomy for various reasons was collected immediately after surgery. Small tissue pieces of different parts of the cervix and the endometrium were preserved in isopentan/liquid nitrogen. The frozen material was transferred to a cryostate (SLEE, London). Slices of 8–10 µm thickness were cut, mounted on microscopic slides, air-dried and fixed with acetone for 10 min. The indirect immunofluorescent staining method according to Nairn[10] was performed, using either treatment



a Immunofluorescent localization of the acid-stable proteinase inhibitor (antileukoprotease) of human cervical mucus in the columnar epithelium of the upper cervix, using HUSI-I-directed immunoglobulins. Magnification 250:1.



b Control treated with HUSI-I-directed immunoglobulins incubated prior to the experiment with highly purified HUSI-I.

with inhibitor-specific antibodies, or for control unspecific rabbit 7S-γ-globulins (Behring Werke AG, Marburg) followed by appropriate washings with phosphate buffered saline. Further controls were done by using inhibitor-specific antibodies incubated prior to the experiments with highly purified HUSI-I. In a 2nd step, goat antiserum directed against rabbit 7S-γ-globulins conjugated with fluoresceinisothiocyanate (Hyland Lab. Inc., USA, Lot No.2232T004A) was employed. For the microscopical examination, a Leitz fluorescence microscope equipped with an automatic camera set (Orthomat, Leitz, Wetzler) was available. Immunodiffusion and immunoelectrophoresis showed monospecificity of the HUSI-I-directed antibodies with formation of a single precipitation line against cervical mucus and cervical homogenate[5,6].

Results and discussion. The results of the immunofluorescent studies are summarized in the figure showing corresponding findings in the 5 investigated female subjects. The antileukoprotease of the human cervical mucus could be localized in the epithelial cell-layer of the upper cervix. In the lower cervix and in the endometrium, no acid-stable proteinase inhibitor was found. This indicates that the antileukoprotease is a specific product of the cervical epithelium. However, immunofluorescence does not allow us to determine whether the identified molecules are biological active or inactive. The probability of active inhibitor molecules is supported from studies showing that the antileukoprotease is a rather stable molecule which is found only in an active form, with no evidence for precursor molecules[4,5,11].

Oestrogen-dependency of the antileukoprotease and changes during the normal menstrual cycle were recently investigated[11]. In contrast to the pattern of different serum proteins in cervical mucus during ovulatory cycles showing lowest concentrations during the periovulatory phase[12], the concentrations of the antileukoprotease remains relatively high around ovulation[11,13]. If the concentration of the acid-stable proteinase inhibitor is referred to albumin, an increased synthesis of the antileukoprotease by the epithelial cells at the time of ovulation can be demonstrated. Present evidence suggests that the cervical mucus proteinase inhibitor is not directly involved in the fertilization process, but is a part of a local defense mechanism of the cervical epithelium with the capacity to neutralize free proteinases.

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A synergistic interaction between the teratogenic effect of trypan blue and dietary deficiency in the rat

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Summary. There was an increased incidence, compared to controls, of exencephaly and micropthalmia in the offspring of rats fed a vitamin D deficient diet and injected with trypan blue on day 9 of gestation. Oral vitamin D did not reverse the effect.

Experimental studies have indicated that animals fed vitamin deficient diets or vitamin antagonists during pregnancy can show high incidences of congenital malformations[2]. However few studies have been made on the effect of vitamin D deficiency on pregnancy mainly due to the fact that a suitable diet was difficult to produce. With the recent development of better diets[3] and more accurate serum assays for vitamin D metabolites[4] it seemed reasonable to re-assess the effect of vitamin D deficiency on the outcome of pregnancy, particularly as Davies[5] had suggested that vitamin D precursors might be important for neurulation in the chick. As malformations like neural tube anomalies are probably multifactorial in origin[6] it was also decided to try the effect of injecting a known neural tube teratogen (trypan blue) into animals on the deficient diet.

Materials and methods. The diet used for the experiments was similar to diet II used by Lumb et al.[3] but was not ricketogenic. It consisted of, white flour 4.25 kg; egg albumen 0.5 kg; sodium chloride 0.1 kg; calcium carbonate 0.0475 kg; ferric citrate 0.01 kg; anhydrous disodium hydrogen orthophosphate 0.112 kg. To this was added 0.02 kg of a mixture of B vitamins[7], plus vitamins A (14 mg), E (0.2 g), and K (21 mg). There is no dietary requirement for vitamin C in the rat[8]. The whole mix was made into a dough with water and arachis oil.

5 groups of inbred Wistar-derived rats were used. The 1st group was maintained on normal pellet diet (Oakes PMD) before being timed mated (vaginal plug = day 1). The 2nd group was fed the deficient diet for 3 months from 70 days of age, and were then mated. Group 3 received normal