To see whether the immunological effect observed in our experiment was the result of a humoral response, we adsorbed the same polynucleotide doses on sheep RBC, and then inoculated mice with  $1\times10^3$  of these treated cells. As the table shows, after 8 days no significant difference in hemagglutinin titer between the treated groups and the controls was noted. Moreover, the sera from the groups of animals pretreated with the ADK-1t cells adsorbed with the polynucleotides and from the controls were tested for cytotoxic antibodies, in terms of the trypan blue dye exclusion test. In no instance were tumour-specific antibodies directly demonstrable in response to immunization with tumour cells, polynucleotides-treated or not.

Discussion. Our results suggest that the protective effect observed by mice pretreatment with tumour cells adsorbed with poly I:C is essentially due to the poly C component, because this is the only strand that enhances immunogenicity when used alone. By contrast, it has been demonstrated that poly I is more important than poly C in the poly I:C induction of Interferon<sup>7.8</sup>. This observation shows that 2 phenomena are distinct. The different effect of this polynucleotides on tumour cells immunogenicity could, of course, be ascribed to the different membrane affinity. It is therefore possible that poly I, which has a higher membrane affinity compared with poly I:C and especially poly C<sup>9</sup>, ends up by masking rather than modifying important structures on the surface of tumour cell membrane. Moreover, the different antigenic characteristics of the 3 polynucleotides should not be overlooked<sup>5,6</sup>.

Since BALB/c mice pretreated with sheep RBC adsorbed with polynucleotides do not show a serum hemagglutinin titer variation when compared to controls, and cytotoxic

antibodies to ADK-1t cells in animals pretreated with tumour cells adsorbed with the same polynucleotides are not detectable, it is possible that the immunogenic effect of poly C adsorbed to tumour cell membrane is primarily related to the cell-mediated response. In previous work<sup>2</sup>, in fact, we have demonstrated that lymph node cells from mice immunized with poly I:C-treated tumour cells were significantly more active (about twice) than those from mice immunized with untreated cells.

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## Elaunin fibres in the basement membrane of sweat gland secretory coil are rich in disulfide-groups

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Summary. Disulfide-groups of elaunin fibres of sweat gland basement membrane are demonstrated a) by thiosulfation/aldehyde-fuchsin staining or thiosulfation/Alcian Blue+0.8 M MgCl<sub>2</sub> staining, and b) by identifying SH-groups after reduction with sodiumthioglycollate. Elaunin fibres share this staining behaviour with 'elastic fibre microfibrils' and with oxytalan fibres.

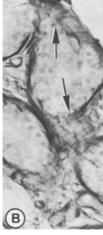
The elastic system of the dermis is composed of 3 different types of fibres: oxytalan, elaunin and elastic fibres<sup>1</sup>. Elaunin fibres stain with aldehyde-fuchsin, resorcin-fuchsin and orcein, but not with Verhoeff's hematoxylin which, on the other hand, stains true elastin<sup>2</sup>. The basement membrane of the sweat gland secretory coil has attracted attention because elaunin fibres are present in this region, but no elastin<sup>3</sup>.

At the fine structural level, oxytalan fibres are represented by bundles of microfibrils, 10–12 nm in diameter<sup>4</sup>. Elaunin fibres consist of similar bundles of microfibrils, but centrally located fibrils are embedded in few amorphous material which morphologically resembles elastin<sup>4</sup>. Elastic fibres are seen in the EM as amorphous material with 'elastic fibre microfibrils' attached to their periphery<sup>5</sup>. Chemical analysis of 'elastic fibre microfibrils' revealed 48.2 half-cystine residues/1000, whereas elastin contains only 4.1 half-cystine residues/1000<sup>5</sup>. This remarkable cystine content has been used histochemically to identify 'elastic fibre microfibrils' with acidic dye solutions (Alcian Blue or aldehyde-fuchsin) after thiosulfation<sup>6</sup> of disulfide-groups<sup>7,8</sup>. Methods to demonstrate disulfide-groups have been applied to base-

ment membranes of sweat glands to get information whether microfibrils of elaunin fibres are also characterized by high cystine content. This would be a further indication that microfibrils of elaunin fibres are identical with 'elastic fibre microfibrils'.

Freshly obtained material from 3 baboons and human autopsy material was used. Biopsies were taken from toes and finger tips within 3 h p.m., fixed in unbuffered formalin and embedded in Paraplast. 10 µm sections were submitted to the following procedures: a) Resorcin-fuchsin staining<sup>8</sup>, b) Aldehyde-fuchsin staining either without pretreatment or after thiosulfation<sup>7</sup> or permanganate oxidation<sup>8</sup>, c) Verhoeff's hematoxylin staining<sup>8</sup>, d) Alcian Blue 8GX staining, using a 1% solution in 3% acetic acid with 0.8 M MgCl<sub>2</sub> added to prevent staining of negatively charged groups other than  $-SO_3$  or  $-S.SO_3$ , sections were stained without pretreatment, after thiosulfation<sup>6</sup>, or after thiosulfation followed by methylation<sup>8</sup>, e) SH-groups were identified by means of the ferric-ferricyanide method in untreated sections or after reduction with sodium thioglycollate; blockade of SH-groups was performed with N-ethylmaleimide<sup>8</sup>.





Aldehyde-fuchsin staining after thiosulfation. Basement membranes of sweat gland secretory coils are strongly reactive. In tangentially sectioned regions, positively stained material is seen to form circularly running fibres (arrows). A:  $\times$  120; B:  $\times$  480.

The basement membrane of secretory coils of sweat glands stains with resorcin-fuchsin or with aldehyde-fuchsin after permanganate oxidation. The thickness of elastica-positive layers, as well as its staining intensity, decreases where the secretory coil transforms to excretory duct. Elastica-positive fibres are seen to form parallel, tightly packed rings which are filed along the tubular gland. The basement membrane does not stain with Verhoeff's hematoxylin. Aldehydefuchsin does not tinge this region in untreated sections, whereas resorcin-fuchsin does.

Alcian Blue evinces only pale colouration of basement membranes of sweat gland secretory coils when used in the presence of 0.8 M MgCl<sub>2</sub>. Mast cell granules in the same section are stained under these conditions. After thiosulfation, basement membranes are intensively stained both with Alcian Blue + 0.8 M MgCl<sub>2</sub> and with aldehyde-fuchsin (figure). Positively reacting fibres form parallel rings filed along the secretory coils. Methylation after thiosulfation

abolished staining with Alcian Blue or with aldehyde-fuchsin.

SH-groups in sweat gland basement membranes can be visualized with ferric-ferricyanide reagent. In untreated sections, myoepithelial cells are faintly reactive. These cells are seen to be wedged into secretory cells and to rest on an almost unreactive basement membrane. Pretreatment with N-ethylmaleimide abolishes the reactivity of the basement membrane and decreases staining of myoepithelial cells. Basement membranes become strongly reactive after reduction with sodium thioglycollate.

Fine structural studies<sup>4</sup> have shown that elaunin fibres can be aligned between oxytalan fibres and elastic fibres. As a result of studies on elastogenesis, Gawlik<sup>2</sup> has suggested that primordial oxytalan fibres are replaced by elaunin fibres and finally by elastic fibres. In the EM this series is initially characterized by bundles of fibrotubules, 10-12 nm in diameter (oxytalan fibres). After this, increasing amounts of homogeneous amorphous material (elastin) are observed within these bundles and centrally located fibrotubules are encemented: gradual transformation from elaunin fibres (small amorphous cores within fibrotubular bundles) to elastic fibres (amorphous material predominates) takes place. During the course of aging, the amount of fibrotubules at the surfaces of elastic fibres decreases and fibrotubules finally completely disappear<sup>5</sup>. Oxytalan fibres, fibrotubules of elaunin fibres and 'elastic fibre microfibrils' of elastic fibres morphologically correspond. These microfibrils are also closely related concerning their high histochemically demonstrable content of disulfide-groups.

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## Uptake of exogenous protein by supraependymal cells of the feline area postrema

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Summary. Supraependymal cells occurring on the surface of the feline area postrema were examined for phagocytic ability. It was shown that they could ingest exogenous horseradish peroxidase that was experimentally introduced into the brain ventricular system. The cells thus bear functional as well as ultrastructural attributes of macrophages, similar to those found in the third ventricle and subarachnoid space.

Macrophage-like cells have been found in various spaces which contain cerebrospinal fluid (CSF) both within and around the vertebrate brain. These include cells lying in the subarachnoid space<sup>3,4</sup>, on the ventricular surface of the choroid plexus<sup>5,6</sup>, and on the ependymal surface of the 3rd ventricle<sup>7-11,23</sup>. Recent electron microscopic studies<sup>8,9,12-15</sup> have shown that certain subarachnoid, supraplexus and supraependymal (SE) cells have similar ultrastructural characteristics typical of macrophages. Tracer studies using latex beads<sup>9</sup> and India ink, Thorotrast and ferritin<sup>16</sup>, and

studies challenging macrophage-like cells with live Bacillus Calmette-Guerin<sup>17,18</sup> have confirmed that the cells are phagocytic in nature.

In studies of the ependymal surface of the feline area postrema<sup>19</sup> (AP) a population of SE cells was described on the caudal part of the organ. The cells resembled SE cells of the mammalian third ventricle<sup>7-11</sup>, cells lying on the surface of the choroid plexus<sup>12,16</sup>, and macrophages of the subarachnoid space<sup>13,17</sup>. To examine the phagocytic ability of the SE cells of the cat AP, we presented an exogenous