extractable in fraction F3. The pellets obtained from fractions N1, F1, F2 and F3 were studied with the electron microscope. For this purpose they were fixed in osmium tetroxide, dehydrated, and embedded in Epon 812[6]. These steps were performed in a small (0.3 ml) plastic tube which was centrifuged every time in a Beckman Microfuge before each change. A barely visible dark pellet was finally obtained in the hardened Epon at the bottom of the tube.

While fraction N contained mostly nuclei and cellular debris, which included some granules, F1 was the richest in granules, F2 contained far less and in fraction F3 only occasional ones were seen. Figure 2 shows a micrograph of the pellets obtained in fraction F1. The contaminant mitochondria and the still high percentage of hypertensive activity in the final supernatant (50%) suggest that the techniques of separation and preservation of the granules must be improved. There is evidence, however, that (1) a correlation exists between the levels of hypertensive activity in the fractions and the presence of granules in the respective pellets, and (2) the granule-containing pellets release the hypertensive activity after osmotic shock. The findings indicate that these bodies contain factors active on rat blood pressure. The question remains whether the endothelial cells of the arteries of the toad and their granules play some role in the blood pressure regulation[7,8].

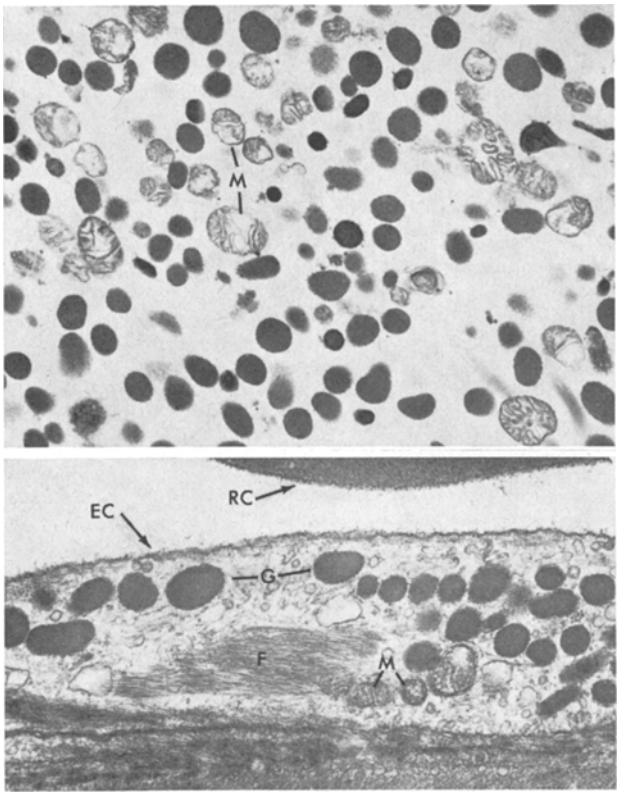

Fig. 2. Above: cytoplasmic granules obtained from aorta homogenates of the toad and recovered in fraction F1. Below: Portion of an andothelial cell (EC) of the toad aorta. The nucleus is not visible. RC, red blood cell; G, granules; M, mitochondria; F, filaments.

Resumen. Los gránulos citoplasmáticos de células endoteliales de aorta de sapo fueron separados en fracciones subcelulares, obtenidas por centrifugación diferencial, de homogenados de esa arteria. Los sedimentos que contienen los gránulos, liberaron factores hipertensores bajo el efecto de un «shock» osmótico.

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8 This work has been supported in part by the Consejo de Investigaciones Cientificas y Técnicas, Buenos Aires, Argentina.
9 Members of the Research Career Program of the Consejo Nacional de Investigaciones Cientificas y Técnicas, Buenos Aires (Argentina).

Thymus Stimulation and Cancer Prophylaxis by *Viscum* Proteins

The tumour-inhibiting effects of native basic protein fractions from the semi-parasite plant *Viscum album* have been repeatedly described[1-4]. Both in vitro and in vivo studies show cancerostatic, cytostatic and toxic activities independent of each other and with extremely low doses (down to 10^{-15} mol/kg), as well as high immunogenic activity. The cancerostatic effect, however, is strongly dependent on the native state of the proteins.

These findings suggested an interference of the active components with the cellular information mechanism at the nuclear level[4]. We described recently a striking influence of some of these protein components on RNA and DNA synthesis, showing a transcription inhibition in ascites-[5] and HeLa-cells, as well as in fibroblasts[6]. In the latter, however, at very low doses, the picture shifted to a 200% increase of RNA synthesis at the expense of DNA synthesis. We therefore abandoned testing the maximum tolerated dosis in vivo and turned to investigations in the lower dosis range (5–10% of the LD 50). In the present paper the tumour prophylactic effects of purified *Viscum album* proteins and their stimulating effect on thymus growth are described.

Material and methods. Selected protein preparations isolated from cancerostatic fractions of *Viscum album* have been used. They correspond with purified steps already described elsewhere[7] and to fractions of them,

separated by free-flow electrophoresis (VaP II-device according to HANNIG[8,9]). For reason of comparison, press juice preparations of the commercially available *Viscum* drug Iscador® were chosen. This drug is known from its clinical use in cancer treatment[10].

The compounds were dissolved in physiological NaCl-solution under sterile conditions and stored at 2–4 °C until used. Test animals: white male inbred 'Varese' mice, 4 months old, 20 ± 1 g were used. Standard diet with addition of antibiotics was fed. Application: s.c., i.m., i.p. and i.v. injections in the tail vein and intracardial (i.c.), in each case under sterile conditions. Tumour implantation: 2×10^7 ascites cells (sarcoma 180) per animal s.c. in the dorsal area, where a well-controllable solid tumour develops. 16 days after implantation the animals were killed, the tumours excised and weighed.

Determination of the LD 50. The Viscum proteins show 2 kinds of toxicity as already described elsewhere[3]: the 'typical' toxicity (death after 3–4 days under marasmu-like symptoms) and the 'atypical' toxicity (immediate death by respiratory paralysis under tonic spasms, with far higher doses). The latter is due to a non-cancerostatic side-component of the protein complex[9].

The LD 50 was determined in long-term toxicity tests. The compatibility of the protein fractions is strongly dependent on the mode of application (Table I).

After s.c. and i.m. application at least 20 times higher doses were tolerated than after applications avoiding the lymphatic system (i.v., i.p., i.c.). For Iscador® this striking difference was not found. In all cases of s.c. application of toxic doses an encapsulation at the injection site as well as connective tissue-like neoplasms and local inflammatory reactions were observed. Following i.m. application the lymph-nodes adjoining the injection site began to swell.

Determination of the 'therapeutic' range by autopsy data, after single doses of *Viscum* protein [4514-16-6].

(a) *High toxic doses* (LD 50 and higher): Necropsy was performed 10 days after treatment and the results compared with those in control animals. Liver: increasing anaemia until greyish appearance and scattered haemorrhage. Stomach: white spotted, wrinkled. Intestine: increasingly haemorrhagic, eventually blood-filled. Thymus: increasing fatty degeneration until decomposition and complete disappearance. Other findings independent of observation time: pancreas dried out and reduced,

Table I. Toxicity assay (LD 50) of *Viscum album* fractions in mice

Code No. of our preparations[7]	LD 50 in µg/mouse (20 g) s.c.	i.m.	i.p.	i.v.	i.c.	Type of toxicity
3011–16	100	75	1.5	1.5	1.5	typical
3410–16	100	85	5.0	5.0	5.0	typical
4510–16	25	20	1.5	1.5	1.5	typical
4513–16–5	50	50	1.0	1.0	1.0	typical
4514–16–6	100	50	5.0	5.0	5.0	typical
For comparison: Iscador® No. Q-2[a]	1.5 ml	0.6 ml	0.6 ml	0.22 ml	0.25 ml	atypical

[a] We thank Dr. R. LEROI, Verein für Krebsforschung, Arlesheim, for kindly giving of samples of Iscador®.

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Table II. Inhibition of transplanted tumours and growth-stimulation of thymus and spleen by i.p. treatment with *Viscum album* protein (Code[7]-No. 4514-16-6)

Type of treatment	No. of mice	Average tumour weight (mg)	Necropsy data: Tumour	Thymus (normal 22 mg)	Spleen (normal 85 mg)
Pre-treatment:					
For 6 days daily 0.5 µg protein.	12	110 ± 10	Encapsulated in firm connective tissue	40 ± 8	110–120
Tumour cells i.p. on day 6.	14	45 ± 5			
Animals killed on day 22	4	None			
Pre- and post-treatment:	12	110 ± 10	Encapsulated in firm connective tissue	50 ± 10	120–140
For 22 days daily 0.5 µg protein.	6	40 ± 5			
Tumour cells i.p. on day 6.	6	20 ± 5			
Animals killed on day 22	6	10 ± 2			
Post-treatment only:	18	500	Encapsulated in firm connective tissue	22 ± 3 (normal)	130–160
Tumour cells i.p. on day 6.	3	250			
Then for 16 days daily 0.5 µg protein.	3	200			
Animals killed on day 22	3	60			
	3	50			
Controls:	15	1100 ± 100	Surrounded by soft connective tissue	22 ± 3 (normal)	120–140
Tumour cells injected on day 6.	15	650 ± 150			
Animals killed on day 22					

hypotrophy of the kidneys to $^1/_3$, spleen reduced to $^1/_2$; heart, lungs, brain, pituitary gland normal.

(b) *Low toxic doses* (LD 50 and lower) with decreasing doses these effects disappear gradually. Below 20% of the LD 50 gross alterations are no longer detectable.

(c) *Subtoxic doses*. With still lower doses (10 to 5% of the LD 50, i.v. or i.p.) new effects appear about 6 days after application in form of startling reactions of the immune apparatus: 200 necropsies revealed without exception: (1) increase in spleen size from normally 85 ± 5 mg up to values between 120 and 190 mg; (2) increase in thymus size from normally 22 ± 3 mg up to values between 30 and 60 mg. Histological and cytological observations show a significant hyperplasia of the thymocytes. As far as we know, a drug effect of this type has never been observed.

When compared with the controls, the treated animals appear more vital. The testes are strikingly tight and enlarged and the general condition is excellent.

(d) *'Therapeutic' doses*. Doses of 10% of the LD 50 were used therefore as 'therapeutic' doses against implanted tumours, and applied i.p. once daily on 6 subsequent days.

Tumour inhibition. 120 male mice of 20 g were divided into 4 groups of 10, respectively 20 animals each, for 2 subsequent test-series. The type of treatment and the corresponding results including data from necropsy are seen in Table II.

Our findings show that *Viscum album* proteins exert an inhibitory effect on transplanted tumour cells. Prophylactic injections prior to transplantation of the tumours are the most efficient. In this case the tumour cells either grow only up to 1–10% of the weight of the control tumours or they are even entirely rejected. Without this pretreatment an average of about 50% growth inhibition can be obtained (as also described earlier[1,4]).

Similar effects were noticed with the viscum press juice preparation Iscador®. With our proteins we obviously have the effective components of those preparations in our hand.

The data presented raise several questions as to the mechanism of the tumour inhibition and concomitant thymus growth observed. Is the prophylactic effect against tumour growth of these basic *Viscum* proteins in 'therapeutic' doses connected with the observed stimulation of the thymus and the strong immunogenic effect? Furthermore, if and how are the reported phenomena associated with the specific effects of the *Viscum album* proteins on RNA and DNA synthesis, as reported elsewhere[5,6]?

In view of the significance of the thymus for cell-mediated immunity, the thymus stimulation may be more than a mere coincidence. Interdependences of this kind, like regression of thymus with tumour growth[11], enlarged thymus in tumour resistant animals[12] as well as increase in tumour-susceptibility of hamsters[13] or rats[14] after neonatal thymectomy have been described. We should also like to draw attention to the possibility of tumour prophylaxis and therapy by immunological methods as has been suggested, e.g. by BAUER[15], and discussed by SORKIN[16].

Zusammenfassung. Die cancerostatischen Viscum-Proteine induzieren im Tierversuch mit geringsten Dosen eine Proliferation des Thymus und verhindern prophylaktisch das Angehen von Tumoren. Höhere Dosen zerstören dagegen den Immunapparat durch Überbeanspruchung und die Tumorhemmung bleibt aus.

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Arbeitsgruppe für Krebsforschung als Gastgruppe am Max-Planck-Institut für Eiweiss- und Lederforschung, D-8 München 15 (Germany), 4 December 1969.

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Adrenergic Innervation of the Ductus Arteriosus of the Fetal Lamb

Several factors have been implicated in closure of the ductus arteriosus at birth: sensitivity of the ductus to oxygen and vasoactive agents, hemodynamic changes in systemic and pulmonary circulations, and direct innervation[1]. Since the role of neural control is especially uncertain, it appeared worthwhile to investigate, as a new approach, the existence and extent of adrenergic innervation in the wall of the ductus.

Near-term fetal lambs were delivered by caesarian section with the placental circulation intact. Breathing of the fetus was prevented by placing a rubber membrane over its head. The chest was opened and the ductus was dissected carefully. Immediately after the umbilical cord was tied, the ductus and adjacent portions of the pulmonary trunk and aortic arch were removed. Specimens were prepared by FALCK's fluorescence histochemical method[2].

After treatment with formaldehyde gas, adrenergic nerve fibers in the wall of the freeze-dried ductus exhibited strong specific fluorescence and characteristic varicosities at their terminals. Nonspecific autofluorescence was weak and sparse compared with that of elastic vessels such as the adult aorta.

In addition to specific fluorescence in the perivascular plexus around the vasa vasorum of the ductus, nerve fibers with specific fluorescence were seen in the adventitia and, in large number, in the outer third to outer half of the media (Figure). Specific fluorescence was not seen in the inner portion of the media. No appreciable difference in the density of specific fluorescent fibers was noted

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