labelled when ^{14}C-formate was injected, confirming that the site of injection was correctly chosen for substances to reach the liver.

It must therefore be concluded that the carcinogenic and alkylating actions of dimethylnitrosamine are not correlated in trout, as has already been suggested for other nitroso compounds in rats[13–16].

Zusammenfassung. Zur Klärung des Zusammenhanges zwischen carcinogener und alkylierender Wirkung von Dimethylnitrosamin wurde die Bildung von ^{14}C-7-Methylguanin in der Leber-RNA von Tauben, Fröschen und Forellen nach Gabe von ^{14}C-Dimethylnitrosamin untersucht. Dabei konnte ^{14}C-7-Methylguanin in der Leber-RNS von Tauben und Fröschen nachgewiesen werden, während RNA, DNS und Protein der Leber bei Forellen nicht markiert wurden.

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A Novel Type of Granules Observed in Toad Endothelial Cells and Their Relationship with Blood Pressure Active Factors

We previously found[1,2] that the endothelium of the aorta, iliac and renal arteries of the toad is made of typical cells, the cytoplasm of which contains abundant granules visible with conventional electron microscopy techniques. Since we are searching for similar structures in different species of this and other classes, we are also interested in knowing whether these granules are linked to any special function or contain biologically active substances. Though the chemical agents contained in these granules are not known, we found that these bodies can be recovered apparently intact, together with contaminant mitochondria, in the pellets of subcellular fractions obtained by differential centrifugation. These fractions are rich in hypertensive activity which becomes rapidly unsedimentable when the pellets are suspended in a hypotonic medium.

The toad aorta aqueous extracts exhibit a strong hypertensive activity as compared with other tissues and organs of the same amphibian. The activity in kidney (Table) is also high and it is well known that this organ contains factors which act on blood pressure[3].

The hypertensive activity of the toad aorta becomes sedimentable to a great extent when the homogenates are prepared with isosmotic sucrose. The homogenates were prepared and fractionated as follow. Six local common toads *Bufo arenarum* H. were demedullated; the segments of the aorta between the junction of the aortic arches and iliac bifurcation were removed and placed in a small mortar with 1 ml of ice-cool 0.25 *M* sucrose in 0.01 *M tris*-HCl buffer (pH 7.4). They were minced with scissors and gently homogenized with the glass pestle; the suspension was filtered through a folded cheese-cloth. The volume of the filtrate was made up to 4 ml with the buffered sucrose and centrifuged in a refrigerated Beckman Spinco centrifuge with the No. 40.2 rotor. 4 sedimentable fractions and a final supernatant were recovered. Proteins were measured by LOWRY's method[4] and the hypertensive activity was tested in rats by the method of DE VITO et al.[5]

As is shown in Figure 1, fraction F1 exhibited high specific activity. The pellets activity was 93% extractable by osmotic shock in fractions N, F1 and F2, and 75%

Hypertensive activity in different toad tissues

	Aorta	Kidney	Brain	Muscle	Spleen	Liver
mm Hg/mg of tissue	13.7	13.3	2.9	2.1	0	0
mm Hg/mg of protein	210	185	52	45	0	0

From 40–60 mg of tissue were sonicated in 2 ml of 0.01 *M tris*-HCl buffer (pH 7.4) and centrifuged at 25,000 rpm for 10 min. Proteins and hypertensive activity were measured in the supernatants.

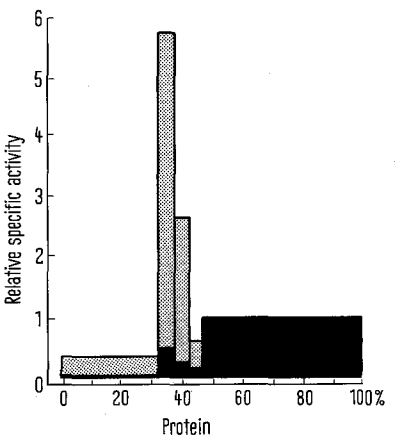

Fig. 1. Distribution of hypertensive activity in homogenates of *Bufo arenarum* H. aorta. The activity was measured in the fractions in mm of Hg/volume injected in the rat × volume of the fraction. Relative specific activity: percentage of total homogenate activity in the fraction/percentage of total homogenate protein in the same fraction. From left to right: the bars represent fraction N (600 *g*/5 min), F1 (9,959 *g*/3 min), F2 (39,825 *g*/7 min), F3 and the final supernatant (101,952 *g*/30 min). The first 4 fractions were suspended in 0.01 *M tris*-HCl buffer (pH 7.4) and spun at 101,952 *g*/30 min. The activity was tested in both supernatants and pellets (resuspended in the buffer) in order to calculate the percentage of released activity by osmotic shock (dotted area).

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].

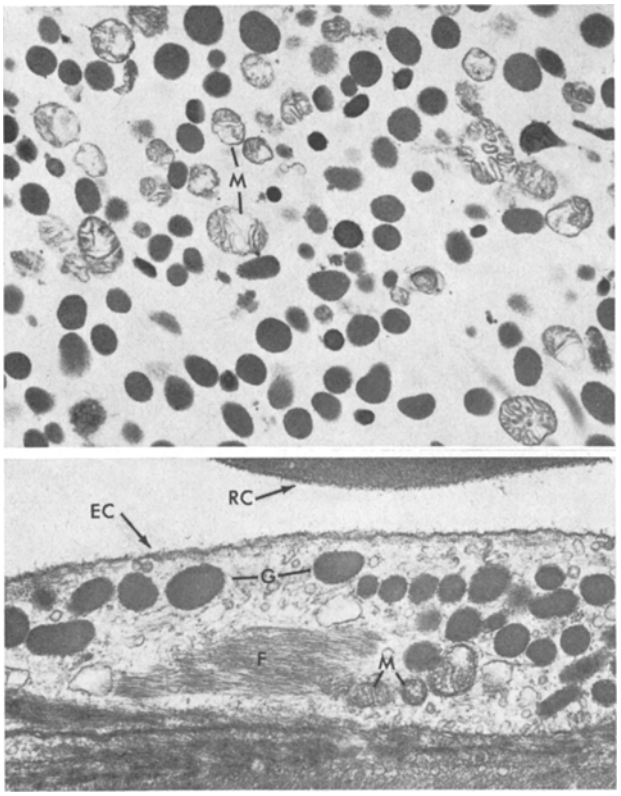

Fig. 2. Above: cytoplasmic granules obtained from aorta homogenates of the toad and recovered in fraction F1. Below: Portion of an andothelial cell (EC) of the toad aorta. The nucleus is not visible. RC, red blood cell; G, granules; M, mitochondria; F, filaments.

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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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,