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.

J. NIENHAUS, M. STOLL
and F. VESTER[17]

*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

[1] G. S. DAWES, *Foetal and Neonatal Physiology; A Comparative Study of the Changes at Birth* (Year Book Medical Publishers Inc., Chicago 1968), p. 164.
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along the entire length of the ductus. However, chemical assay of catecholamines and uptake of ³H-norepinephrine by the tissue suggested a nonuniform distribution of adrenergic nerves[3].

Although the quantity of smooth muscle in relation to the supporting elements varies among vessels from different locations, the muscular vessels of medium size generally have specific fluorescent fibers in the media while elastic vessels have none in the same area[4]. The
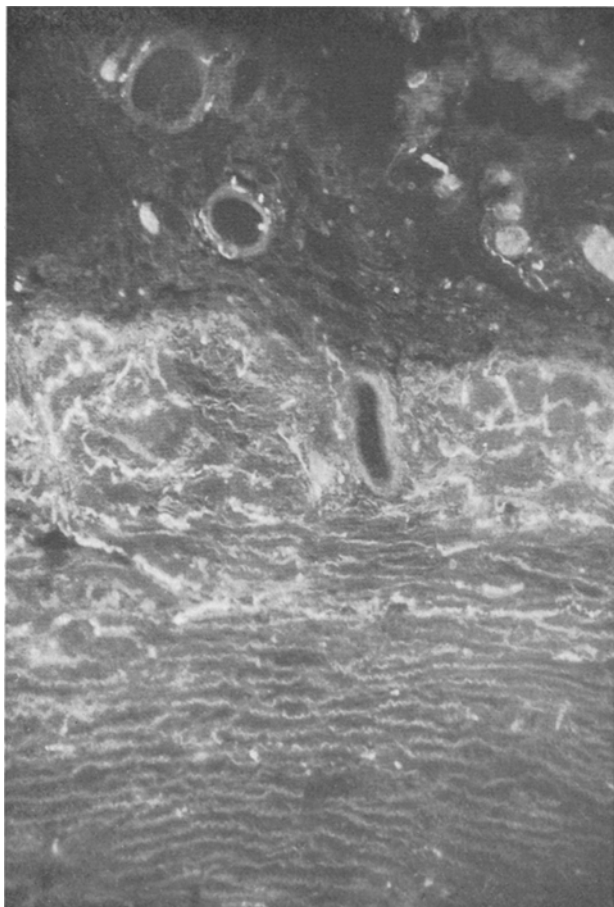
Cross-section of the ductus arteriosus of the fetal lamb. A large number of nerve fibers with intense specific fluorescence are seen around vasa vasorum in the adventitia (top) and among the auto-fluorescent elastic fibers and the nonfluorescent smooth muscle cells of the media (bottom). Fluorescence microphotograph. × 315.

ductus thus shares with other muscular vessels the feature of numerous fluorescent fibers associated with the media.

Using the Cajal technique, BOYD[5] described a sensory innervation in the rabbit ductus similar to that found in the carotid sinus and aortic arch. In addition, he found fine fibers terminating in the musculature of the ductus which he presumed to be efferent but whose nature and origin were unclear. KENNEDY[6] also observed fine myelinated fibers in the ductus and suggested that the muscle fibers are innervated; but, the small number of fibers observed does not seem to justify this conclusion. He postulated that since closure of the ductus followed inflation of the lungs, a reflex might be involved. Subsequently, KENNEDY[7] attempted denervation of the ductus and stressed the importance of oxygen tension as a major determinant of closure.

Thus, the neural contribution to ductus closure remains obscure. However, the present demonstration of adrenergic innervation associated with the well developed musculature suggests that neural control may be a factor in the initiation and/or maintenance of contraction of the ductus[8-10].

Zusammenfassung. Nachweis adrenergischer Nervenfasern in der Media des Ductus arteriosus von Schafföten mit Falck'scher histochemischer Fluoreszenzmethode. Zusammen mit der gut entwickelten Muskulatur macht dies wahrscheinlich, dass Nerven am Kontraktionsgeschehen des Ductus arteriosus teilnehmen.

M. IKEDA

Department of Physiology,
School of Medicine, University of California,
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[10] After this paper was submitted for publication, a report appeared (L. O. BORÉUS, T. MALMFORS, D. M. McMURPHY and L. OLSON, Acta physiol. scand. *77*, 316 (1969)) on the presence of specific adrenergic nerve fibers in the media of the human fetal ductus arteriosus.

Rosette Formation in White Blood Cell Cultures from Patients with Acute Myeloblastic Leukemia

Rosette-formation of cells in vitro is a well-known immunological phenomenon observed under various experimental conditions[1-3]. In cultures of human leukocytes, for example, typical rosettes develop after addition of red blood cells which have been coated with immunoglobulin G. It was assumed that the mononuclear cells (macrophages, monocytes, large lymphocytes) participating in the formation of these rosettes, carry specific surface receptors for immunoglobulin G which, in vivo, may

be involved in the apprehension and destruction of antibody-coated red blood cells or particles[4].

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