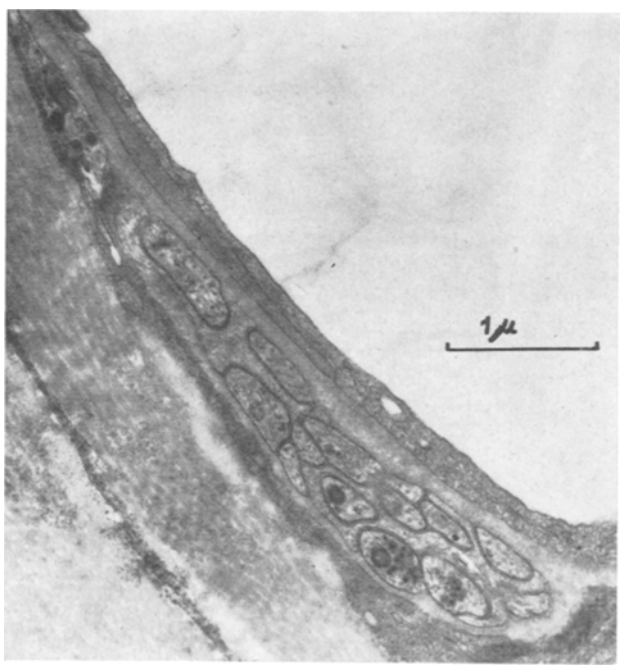
Innervation of Pulmonary Capillaries

In the dog, branches of the pulmonary artery down to 30 μ in diameter have smooth muscle cells in their wall[1] and receive a cholinergic and noradrenergic innervation[2]. At this diameter they lose their smooth muscle coat and their innervation. The arteries are encircled by bundles of non-myelinated axons which often appear to be more closely related to portions of the lung parenchyma than to the artery they are innervating. Nerve fibres intimately associated with pulmonary capillaries have been described in the kitten[3] and the snake[4].

The relation of nerve fibres to pulmonary alveoli and their capillaries was examined with the electronmicroscope. Lung lobes in anaesthetized dogs were perfused through the pulmonary artery with 2.5% glutaraldehyde in 0.1 *M* phosphate buffer; small pieces of tissue were postfixed with 1% OsO_4, embedded in araldite, and thin sections stained with lead and examined in an AEI 6B electronmicroscope. Between the smooth muscle layer of pulmonary arteries 100–200 μ in diameter and the pulmonary alveoli and their capillaries, there is a space of 3–4 μ occupied by collagen fibres, processes of fibrocytes and bundles of non-myelinated axons enclosed in Schwann cells. The axon bundles run at varying distances from the smooth muscle cells of the pulmonary arteries.



Bundle of axons, some containing vesicles, lying between capillary endothelium and process of a pericyte. Glutaraldehyde-OsO_4 fixed dog lung. Calibration 1 μ.

In the Figure a bundle of axons is seen in close apposition to a capillary and separated by the full width of the collagen layer from the smooth muscle cells of the pulmonary artery. The axon bundle lies between the capillary endothelium and the process of a pericyte. Several of the axon profiles are partially bare of Schwann cell cytoplasm and contain vesicles. One such axon is within 1000 Å of the endothelial cell and another within 450 Å of the pericyte. The axons are of 2 kinds depending on their vesicle content: one kind contains agranular vesicles 500 Å in diameter and occasional vesicles around 1000 Å in diameter with a moderately electron-dense core. This is the appearance characteristic of cholinergic terminal fibres[5]. The other kind of axon contains 3 types of vesicles: vesicles 500 Å in diameter with an intensely electron-dense core; vesicles of 1000 Å with a moderately electron-dense core, and vesicles 850–1000 Å in diameter with a more electron-dense core. This is the appearance characteristic of noradrenergic terminal fibres[5,6].

Such bundles of vesicle-filled axons closely related to capillaries are not infrequent. The capillaries, which are close to pulmonary arteries or arterioles, in addition to their endothelial lining are always surrounded by processes of pericytes, but have no smooth muscle cells related to them.

The great majority of alveolar capillaries are not innervated[2], but the present findings suggest that there are some vessels in the lung parenchyma, lacking a smooth muscle coat, and therefore called capillaries, which receive a double motor innervation. These capillaries all have pericytes and it may be these cells which respond to the released transmitter[7].

Zusammenfassung. Nachweis einer doppelt motorischen Innervation eines Teiles der Kapillaren in der Hundelunge: Bündel markloser Nervenfasern mit elektronenmikroskopischer Charakteristik cholinerger und noradrenerger Nervenendigungen bis 1000 Å Entfernung von der Kapillarwand.

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Cell Proliferations in *Lymantria dispar* L. Larvae Infected with the Nuclear Polyhedrosis Virus

In invertebrates both the proliferating processes and their causes have been less studied. The malignant degeneration of some tissues in this group is difficult to demonstrate, and because of lack of material some alterations which have been observed especially in insects cannot be compared with the true malignant processes in vertebrates[1].

There are tumours known to be produced in insects by some parasites or experimentally induced by inoculating nucleic acid extracts[2–5]. PAILLOT[6] described active neoplasm-like tissue proliferations induced by viruses in *Agrotis segetum* L., and BIRD[7] observed abnormal cell growth and proliferation of the midgut in the regenerative nidi area. In 1959, L'HELIAS[8] succeeded in purifying a

tumour-inducing viral factor in *Pieris brassicae* L. and later HUKUHARA[9] recorded tumoral formations in the hypodermis of *Bombyx mori* L. larvae infected by the *Tipula iridescent* Virus.

During our investigations on the nuclear polyhedrosis of *Lymantria dispar* L. we observed obvious cell proliferating processes in some kinds of tissues. In the following experiment, the *L. dispar* L. larvae infected experimentally with the nuclear polyhedrosis virus by a procedure previously described[10], were fixed in Carnoy's and Duboscq Brasil's fixatives and embedded in paraffin. 6 micron sections were stained with ferric hematoxylin-eosin and with hemalaun-eosin-light green or methylene blue. Feulgen's histochemical reaction and staining with methyl-green pyronine were also used.

Following viral infection, the nuclei show pronounced hypertrophy. In the nuclear centre there emerged from the nucleolus the virogenic stroma, a central mass initially rich in RNA and later strongly enriched in DNA, where viral inclusions were formed.

Proliferating processes were found in hypodermis and tracheae, appearing at first as a mass of accumulating cells hanging in the body cavity upon other tissues (Figure 1). At a later stage, these cell accumulations took up a larger volume and such hypodermic areas appeared to be pluristratified. The subsequent evolution of the pathological process gave the hypodermis the appearance of an invaded tissue with unlimited growth, pressing upon the adjacent tissues (Figure 2).

In a very large number of tracheae, pronounced proliferating processes occurred. These are obvious in cross and longitudinal sections of the tracheal epithelium (Figures 3 and 4).

From these results it is obvious that, in *L. dispar* L. larvae infected with the nuclear polyhedrosis virus, destructive cell proliferations (similar to neoplasm) occur in the hypodermis and tracheal tissues.

In both the hypodermis and tracheal epithelium, the new cells resulting from abnormal divisions show the pathological alterations usually induced by the nuclear polyhedrosis virus. These changes are characterized by nuclear and nucleolar hypertrophy and the formation of a virogenic stroma followed by the appearance of viral inclusion bodies. This observation suggests that the neo-

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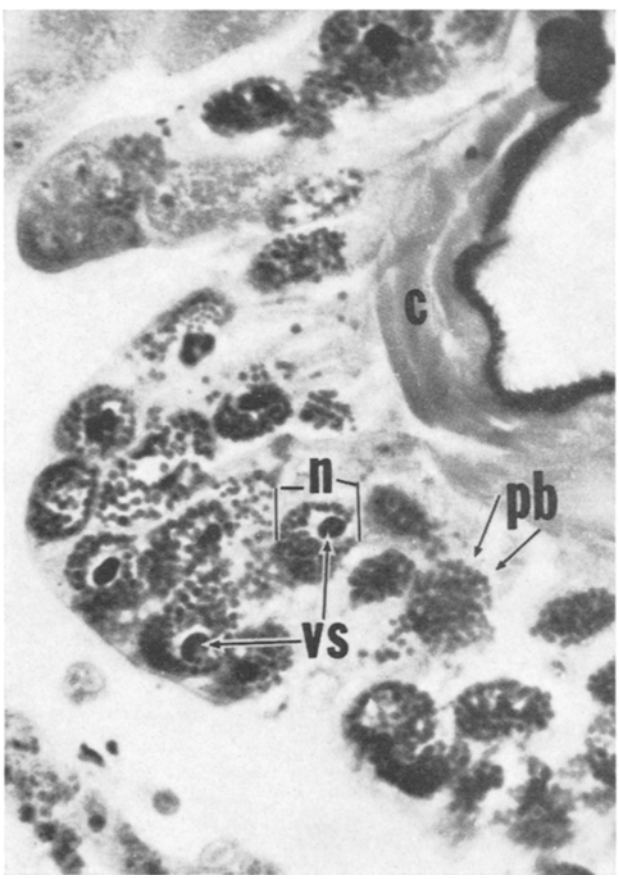

Fig. 1. Cross section of the infected larva showing cell proliferation of the hypodermis. The hypodermis usually formed by a single row of cells becomes pluristratified after infection. The cells show characteristic changes to the level of nucleus (n) with virogenic stroma (vs) and polyhedral bodies (pb). c, cuticle.

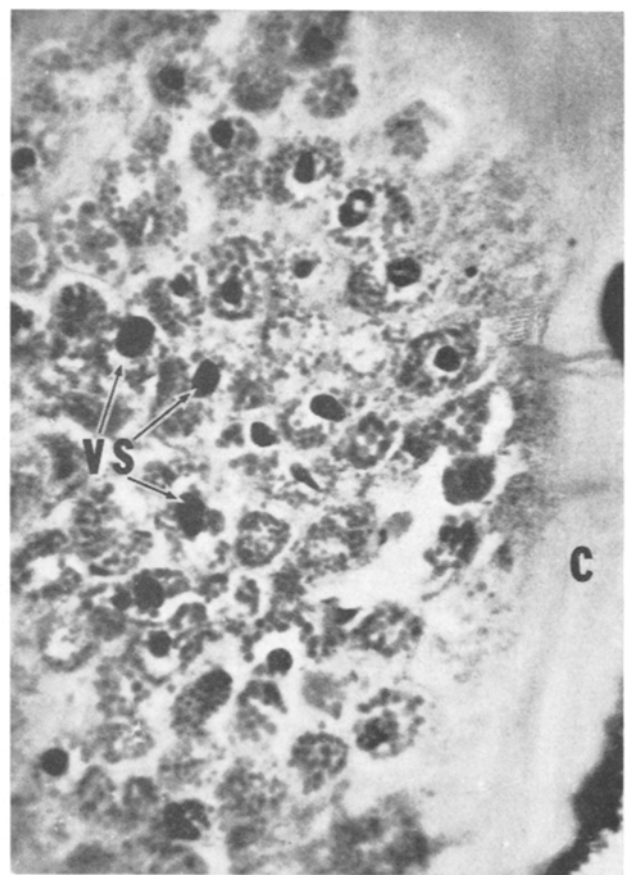

Fig. 2. Massive proliferations which give the hypodermis the appearance of a tissue with unlimited growth. vs, virogenic stroma; c, cuticle.

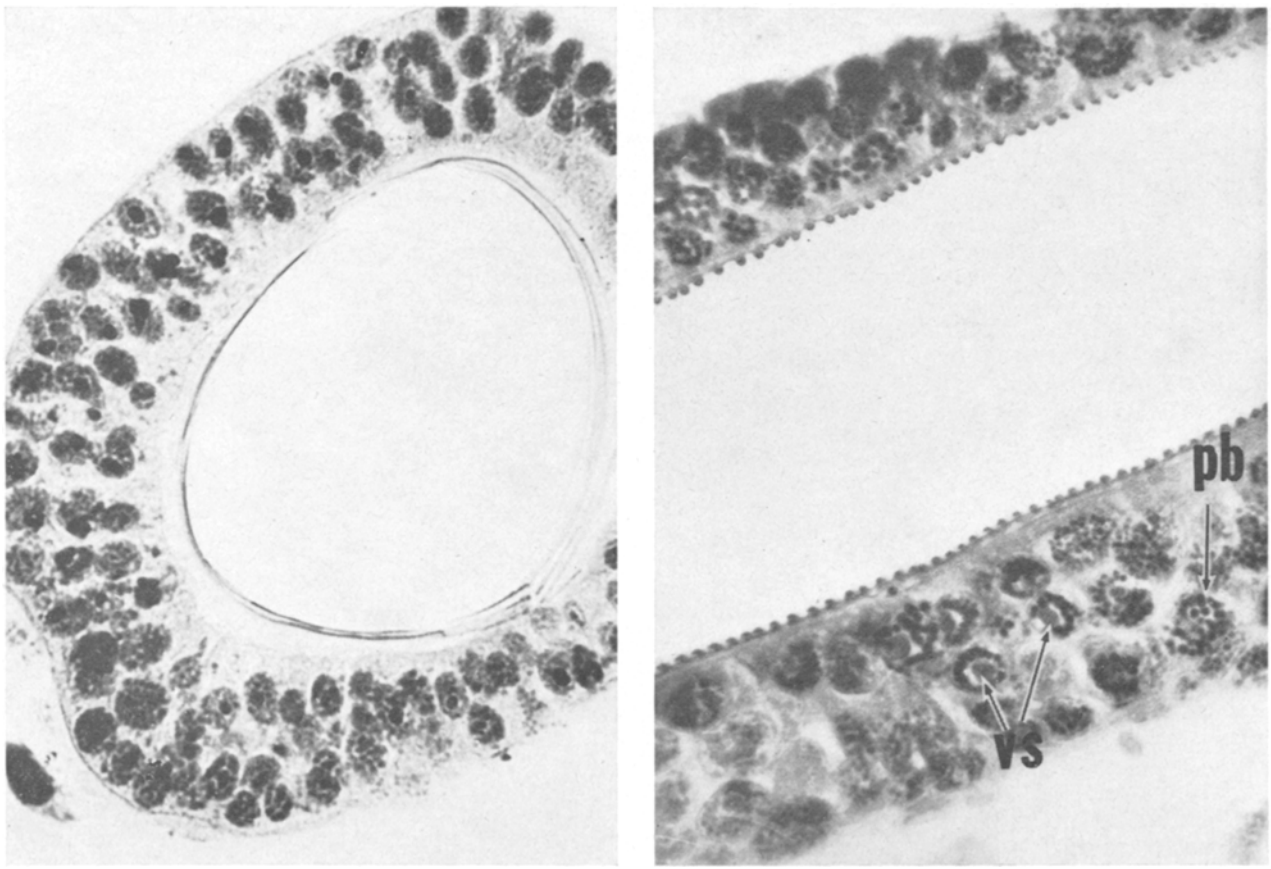

Fig. 3 and 4. Cross and longitudinal sections of the trachea. The tracheal epithelium is formed of many rows of cells. The nuclei show hypertrophy having virogenic stroma (vs) and polyhedral bodies (pb).

plasm-like proliferating processes are induced by nuclear polyhedrosis virus.

In an attempt to correlate the alterations observed by us with the alterations induced by other viruses which are known to generate malignant tumours, it is found that only the viruses containing DNA or double stranded RNA can induce tumours, for example the viruses from the variola-vaccina and Papova group. The viruses inducing the nuclear polyhedrosis in insects also have DNA[11-12]. On the other hand, it was reported that the pathological alterations which occur in general in both the cell nuclei infected with the polyhedral viruses, and the proliferating cells, are similar to the alterations recorded in mammalian tumours. Thus the nuclear hypertrophy, the chromatin margination process, the increase of DNA which are always found in the nuclear polyhedrosis of Lepidopterous larvae[13-17], are also specific alterations in malignant tumours[18-20]. Moreover, the occurrence of chromatic mass in the nuclei of malignant cells, similar to virogenic stroma from the polyhedral infection, was also reported in rat tumours induced by the polyoma virus[21], in the green monkey kidney cells infected in vitro with SV 40[22].

The great similarity of the alterations reported above to those of a malignant kind suggests that the nuclear polyhedrosis virus of *L. dispar* L. can induce tumours. This is reported for first time in the present investigation. A question which remains to be answered is whether our virus strain, which was always present in these neoplasm-like cell proliferations, is alone involved in the etiology of these tumours. Further studies are in progress in an attempt to answer this question.

Résumé. Dans l'hypoderme et l'épithelium trachéal de chenilles de *Lymantria dispar* L. infectées expérimentalement avec le virus de la polyhédrose nucléaire, nous avons découvert des proliférations cellulaires de type néoplasmique. Dans les cellules des tissus de prolifération on observe une hypertrophie du noyau et du nucléole, la formation d'une strome virogénique et l'apparition d'inclusions virales – modifications caractéristiques produites par le virus de la polyhédrose nucléaire.

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