tained with 4 vaccinia strains as calf's lymph, whose final infectious titer was in between $10^{4.4}$ and $10^{6.2}$ (Figure 1).

Growth rate of viruses prepared in different cells: The Sc strain of vaccinia virus as lyophilized calf's lymph was propagated on membrane of 12-days-old embryonated eggs. The titration of calf's lymph virus and the egg passage shows that the final titer of the first one is one $\log_{10}$ times higher but the maximal plaque number was reached more gradually.

Growth rate of the same viruses with different agar overlay media: The effect of medium on the formation of plaques (WALLIS et al.[3]) was confirmed because ECHO virus type 4, which is not able to plaque on our standard overlay medium, produced plaques in cultures where bovine albumine was replaced by skim milk, and grew more rapidly and reached higher titers if Noble agar was replaced by Bacto agar. The other ECHO types 13, 17 and 19 were apparently not too much influenced by the different media (Figure 2).

Growth rate of virus mixture: A preparation of equal volumes of vaccinia (strain SI) and ECHO type 13 virus suspensions was inoculated by the usual technique. Differential counts of plaque made daily showed the normal growth of the single viruses (Figure 3). In cultures inoculated with the mixture, the growth of single viruses was apparently normal but on the fourth counting day it was impossible to report the number of plaques of vaccinia because of the plaque obscuring phenomenon (BERGH, HARRIS et al.[4]).

Evaluation of plaque cinetic index: By combining the plaque method with the calculation described for evaluating the cytopathic index (BALDUCCI, ANDREONI et al.[1]), it is possible to determine a 'plaque cinetic index' (Table). By this method the plaque cinetic index can be evaluated for each virus-cell combination or the influence of agar overlay media, temperature, light, interference by other viruses, etc., can be determined.

Example of calculation of plaque cinetic index obtained with Coxsackie B_3 in monkey kidney cultures

Days of observation	Daily no. of plaques	Deviation from daily mean		Square of deviation from daily mean	Production of deviation from daily mean
		x	y	x^2	xy
2	$10^{5.09}$	− 1.5	− 0.57	2.25	0.85
3	$10^{5.33}$	− 0.5	− 0.33	0.25	0.16
4	$10^{5.86}$	+ 0.5	+ 0.20	0.25	0.10
5	$10^{6.38}$	+ 1.5	+ 0.72	2.25	1.08
14	$^{32.66}$			5	2.19

3.5 avrg. 5.66 avrg.

The regression coefficient: $K = \frac{\sum xy}{\sum x^2}$ is $\frac{2{,}19}{5} = 0.45$.

End point of number of plaques × regression coefficient = plaque cinetic index, 6.38 × 0.45 = 2.87.

Riassunto. La velocità di sviluppo del numero di placche è una caratteristica biologica e può essere riferita con una espressione numerica qui dimostrata e indicata come indice placcocinetico.

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Istituto Superiore di Sanità, Laboratorio di Microbiologia, Roma (Italy), November 9, 1964.

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An Unusual Neoplasm in *Lumbricus terrestris*

The following neoplastic conditions have been reported for *Lumbricus terrestris*: (1) epithelial hyperplasia induced with benzpyrene[1], (2) adenocarcinoma and a chlorogen cell tumor with methylcholanthrene[2], (3) myoblastomas with X-irradiation[2,3], and (4) a pharyngeal tumor[4]. This report is concerned with the occurrence of a fifth neoplastic lesion of an undetermined kind. It occurred in an individual from a group of worms that had received 2 weeks of daily paintings on the 5–10th somite region with a saturated methylcholanthrene-acetone solution.

The tissue appeared as a 3 mm in diameter, glossy, red mass protruding ventrally from the region of the paintings. Microscopic examination of the abnormal tissue revealed its invasion into the entire ventral coelomic area (Figure 1). The esophagus (center of the section) and ventral nerve cord and ventral vessel (center of the new tissue) have been spared. Present also was the loss of the longitudinal and circular muscle layers on the ventral aspect. The mass is comprised of two major cell types. One of these consists of spermatogonial cells, other maturation phases, and even mature sperm, all of which resemble the contents of the seminal vesicle. Yet, there is a noticeable loss of pattern normally present, formed by groups of sperm cells (morulae) in maturation. Figure 2 is a section of the normal seminal vesicle contents from which a comparison can be made. It is quite curious that great numbers of mature sperm are apparently being formed in this mass. The other cell type appears in small clusters scattered throughout the major portion of the abnormal tissue, especially in certain peripheral regions of the tumor which is homogenous with respect to this cell type. These cells are arranged in cord-like arrays and contain large extremely dense nuclei. They are distinctly different from

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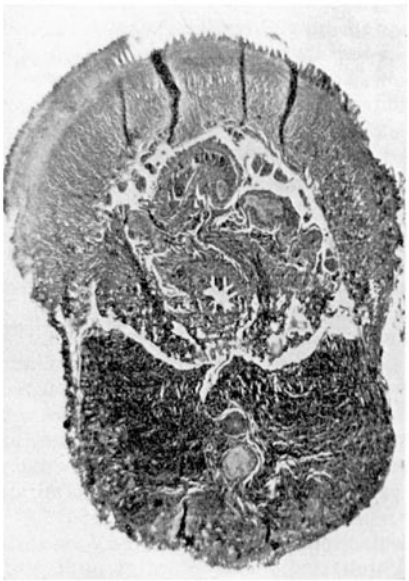

Fig. 1. Transverse section through worm and tumorous mass. Approx. ×12 magnification. Hematoxylin and eosin stained.

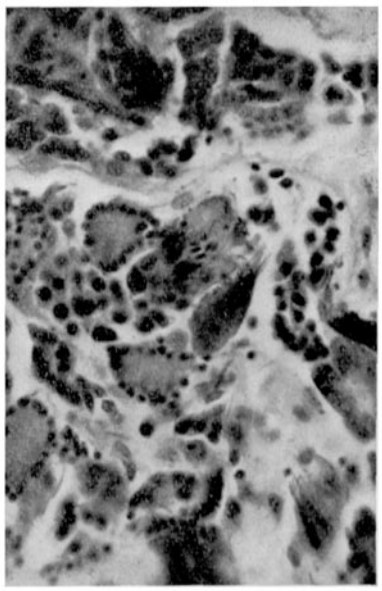

Fig. 2. Section of normal seminal vesicle contents containing clusters of spermatogenic cells at varying maturation stages. Approx. ×200 magnification. Hematoxylin and eosin stained.

aggregates of amoebocytes that sometimes occur in foreign body reactions or at sites of infection in earthworms.

Further studies will probably prove this to be a relatively rare neoplastic lesion for the earthworm. However, it will be of interest to determine if more common neoplasms such as benign fibromas and lipomas or malignant forms such as melanomas and sarcomas of vertebrates can occur in the phylum Annelida. Experiments with treatments of long duration are now in progress in an attempt to derive such tumors.

Zusammenfassung. Regenwürmer, die mit Methylcholanthren behandelt wurden, zeigten neoplastische Prozesse. Zwei verschiedene Zelltypen wurden gefunden: Einmal Spermatogonien mit ausgereiftem Sperma, daneben Zellen mit dichten Kernen, welche keinem normalen Zelltyp zugeordnet werden konnten.

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A New Species of Protomycopsis from India

A purple leaf spot disease was repeatedly noticed on the leaflets of *Sesbania grandiflora* Pers. in the University Campus from September to October over the past few years. Initial symptoms appeared as small pale greyish-green spots on the pinnules, later turning greyish purple and opaque (Figure 1). These resembled those incited by a *Cercospora* species, which brought about premature defoliation of the host, while the spots turned dull purple on the withering leaflets. Examination of the infection spots indicated a species of *Protomycopsis* as the disease incitant. The chlamydospores developed abundantly in the intercellular spaces of the mesophyll obscuring the host tissues. They were globose to oval and cinnamon brown having a thick exospore ornamented with hyaline to yellowish tinged, bluntly conical processes which were sometimes discontinuous (Figure 3). Intercellular hyphae were still discernible, often appearing as tail-like appendages on the chlamydospores (Figure 2).

Occurrence of *Protomycopsis ajmeriensis* Gupta, reported on this host genus from Rajasthan, incites development of rough warts on the leaflets. Its chlamydospores possess reticulate walls with small areoles[1]. Other species inciting opaque purple spots on leguminous hosts as in the present case but possessing reddish brown warty chlamydospores have been reported from elsewhere in the country[2–5]. Comparative observations indicate that this

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