Plaque Cinetic Index in Cultures under Agar

We have described a 'cytopathic index' as a method to evaluate the differential virulence of virus for tissue cultures of different species (BALDUCCI, ANDREONI et al.[1]). The plaque under agar technique, introduced by DULBECCO[2], made possible quantitative determinations in viral studies with a precision and accuracy previously unobtainable under practical conditions.

Materials and methods. Cultures: Primary cultures under agar of kidney epithelial cells of *Macaca mulatta* (rhesus monkey) in 100 ml Erlenmeyer's bottles have been used. Each bottle received 2 million cells in 6 ml medium.

Viruses: Stock strains of poliovirus type 3, Saukett, attenuated type 3 Leon 12 a_1b, Coxsackie B_3, ECHO 13 and vaccinia strains IS, SM, SI, Sc, were repeatedly titrated.

The evaluation of the proposed plaque cinetic index is performed by multiplying the titer in PFU, as number of plaques of the virus suspension per 0.20 ml, by the regression coefficient calculated as previously described (BALDUCCI, ANDREONI et al.[1]).

Results. Growth rate of different families of viruses: Our experiments show that the growth rate of poliovirus type 3 Saukett, Coxsackie B_3, ECHO 13 and vaccinia is in some way more exactly analysed if the reading of plaque number is reported each day.

Growth rate of different strains of the same virus: Titration in parallel of virulent type 3 poliovirus and the corresponding attenuated strain Leon 12 a_1b of Sabin cultivated in monkey kidney cells were performed at 37°C. The differences between the progression of plaque numbers were not significant. A similar result was ob-

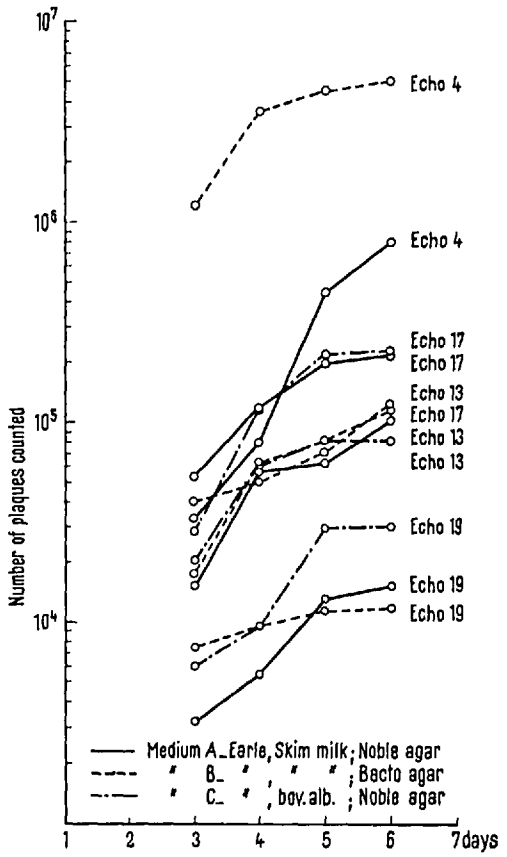

Fig. 2. Growth rate of the same viruses with different agar overlay media.

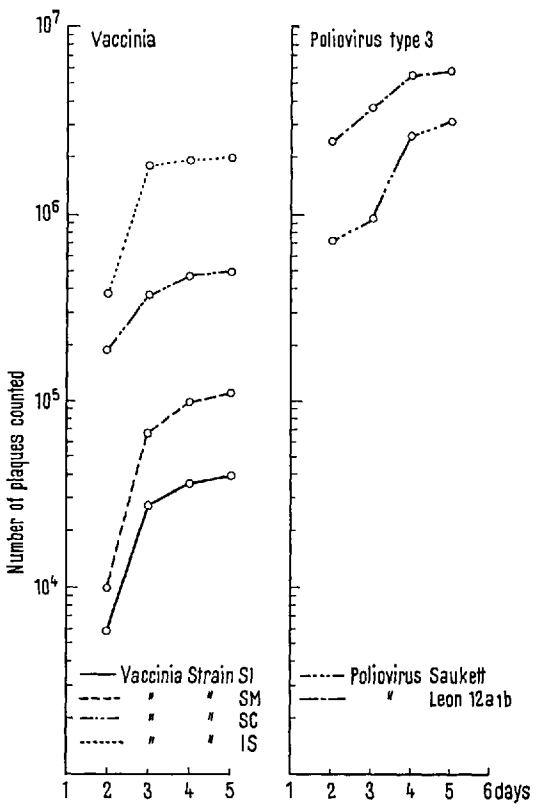

Fig. 1. Growth rate of different strains of the same virus.

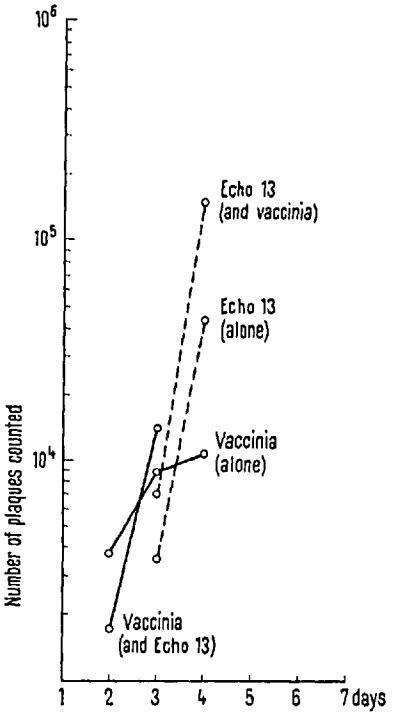

Fig. 3. Growth rate of virus mixture.

[1] D. BALDUCCI, G. ANDREONI, G. B. GORI, L. CASTELLI, and G. MANCINI, J. Bact. *79*, 379 (1960).
[2] R. DULBECCO, Proc. Nat. Acad. Sci. U.S. *38*, 747 (1952).

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.

[3] C. WALLIS, J. L. MELNICK, and M. BIANCHI, Texas Rep. Biol. Med. *20*, 693 (1962).
[4] G. BERG, E. K. HARRIS, S. L. CHANG, and K. H. BUSCH, J. Bact. *85*, 691 (1963).

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

[1] M. GERSCH, Naturwissenschaften *41*, 337 (1954).
[2] R. L. HANCOCK, Exper. *17*, 547 (1961).
[3] R. L. HANCOCK, Exper., *21*, 152 (1965).
[4] A. STOLK, Exper. *17*, 306 (1961).