Virus-Cell Interaction with a Tumor-Producing Virus

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To establish the complete stability of a system one needs (i) to establish that all solutions are bounded for $t \geqq 0$ and (ii) to construct a function $V(x)$ satisfying the conditions of Theorem 3 and such that M is the origin. Here again one may be able to conclude from the Liapunov function $V(x)$ itself that all solutions are bounded for $t \geqq 0$. This is true, for instance, if $V(x) \to \infty$ as $||x|| \to \infty$, although it often is easier to consider (i) and (ii) as separate problems. Boundedness is a type of stability and can itself be investigated by Liapunov methods.[3]

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[1] Among the available references on Liapunov's method may be mentioned, (*a*) Hahn, W., *Theorie und Anwendung der Direckten Methode von Ljapunov* (Berlin: Springer-Verlag, 1959). (*b*) Antosiewicz, H. A., "A survey of Liapunoc's second method," in *Contributions to the Theory of Nonlinear Oscillations IV*, Annals of Math. Studies No. 41 (Princeton University Press, 1958). (*c*) Cesari, L., *Asymptotic Behavior and Stability Problems in Ordinary Differential Equations* (Berlin: Springer-Verlag, 1959). (*d*) Malkin, I. G., *Theory of Stability of Motion*, AEC Translation Series, AEC-t-3352 (translated from a publication of the State Publishing House of Technical-Theoretical Literature, Moscow-Leningrad, 1952).

[2] In a paper by the author to appear in the latter part of 1960 in a Special Nonlinear Issue of the *Proc. of the IRE.*

[3] This has been studied extensively by Taro Yoshizawa. See his paper on "Liapunov's Function and Boundedness of Solutions," *Funkcialaj Ekvacioj* **2**, 95–142 (1959).

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*VIRUS-CELL INTERACTION WITH A TUMOR-PRODUCING VIRUS**

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The polyoma(PY) virus or parotid tumor agent[1, 2]—a DNA-containing virus[3, 4]—is characterized by a duality of action: it produces neoplasias of various types in different species of rodents,[5] and causes cell degeneration in mouse embryo tissue cultures.[6] In the experiments to be reported here, it was possible to obtain in cellular cultures *in vitro* the oncogenic effect of the virus; this afforded the possibility of studying the relationship between the oncogenic and cytocidal effect of the virus. The results so far obtained reveal a situation novel in animal viruses and suggest the existence of a host-virus interaction with characteristics reminiscent of temperate bacteriophage.

Material and Methods.—The PY virus was obtained from Dr. Rowe of the National Institutes of Health. A stock was prepared from a single plaque and serial passages of this stock in mouse embryo tissue cultures were used for the experiments. In these passages, the virus maintained both the cytocidal activity in mouse embryo tissue cultures and the property of eliciting heart, liver, and kidney sarcomas within a few weeks after injection into newborn Golden hamsters. The virus was assayed by plaque formation on mouse embryo monolayer cultures by means of the technique previously described.[7]

Cultures from trypsinized whole embryos of either Swiss mice or Golden hamsters were prepared as previously described.[7] All experimental cultures were secondary cultures at the time of the first exposure to the virus. Plaque assays were done on monolayer cultures of mouse embryo cells which had been serially transferred two to five times. The growth medium for the cells consisted of reinforced Eagle's medium[8] supplemented with 20 per cent calf serum. Experimental cultures were grown in Eagle's medium supplemented with 10 per cent calf, horse, or fetal bovine serum, as indicated later.

Results.—The multiplication of the virus: Confluent monolayer cultures of either mouse or hamster cells were infected with virus at a multiplicity of about 10 mouse plaque-forming units per cell. After an adsorption period of forty minutes, the layers were washed to remove most of the non-adsorbed virus, and were covered

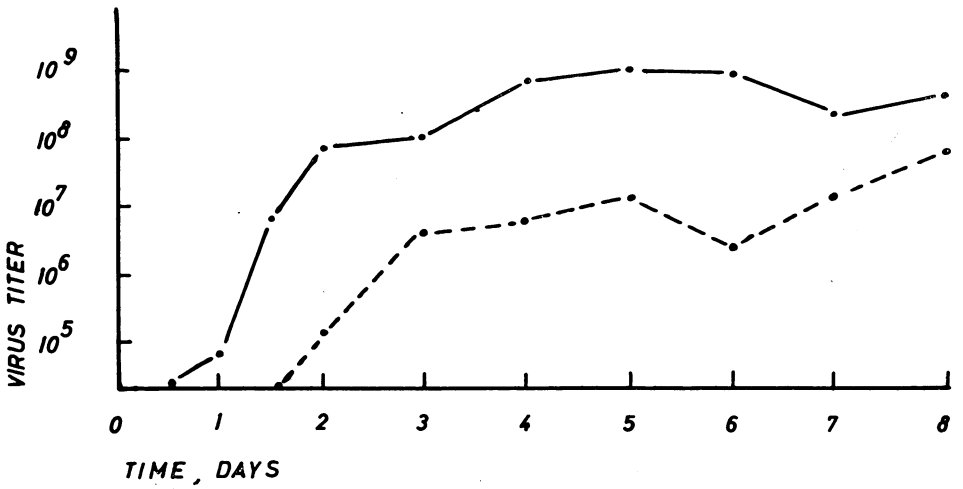

FIG. 1.—Extra-plus intracellular virus per culture at various times after infection.——Mouse embryo culture. - - - Hamster embryo culture. Fluid changes were done on the third and sixth day after infection. The growth medium was Eagle's medium supplemented with 10 per cent horse serum.

with Eagle's medium supplemented with 10 per cent of either calf, horse, or fetal bovine serum. The cultures were subsequently incubated at 37°C and the virus in the supernatants and in the cells (after disruption by three cycles of freeze-thawing) determined at various time intervals.

The growth curves obtained are reported in Figure 1, where the titer of intra- plus extracellular virus per culture is given. The curves show a long latent period, as found for another tumor-producing virus, the Rous virus.[9] The virus production was initially much greater in the mouse cultures; after about a week the difference decreased. At that time, however, the hamster cultures, which had continued to grow, contained many more cells than the mouse cultures, so that the average production of virus per cell was always much smaller in the hamster cultures.

Early behavior of the infected cultures (first week): The mouse cultures appeared unchanged for the first day after infection. Infected cultures containing a number of cells sufficiently low to allow the counting of the cells *in situ* showed after 24 hours a threefold increase in cell number, equal to that of uninfected cultures. The

cell number stopped increasing on the second day after infection. Degenerative changes became visible in the majority of the cells during the third and fourth day, simultaneously with the increase in virus titer. A proportion (about 20%) of the cells, however, remained unaltered as already observed by using fluorescent antibodies.[10] These unaltered cells multiplied in the subsequent days— in fact, frequent mitoses were observed—while more cells underwent degeneration: a balance of these two processes maintained the culture in a steady state.

The hamster cultures, in contrast, continued to increase in cell number during the whole week after infection. Cell counts on parallel cultures with lower cell concentrations showed no difference in the division rate of infected and control cultures for six days of observation. Neither did the infected hamster cultures show obvious signs of degeneration. Since, by plating the cells several hours after infection, most cells proved to be virus-yielders, it must be concluded that they released virus in small quantities without degeneration.

Late behavior of the infected cultures: The mouse cultures remained in the steady state for about four weeks. During this period the virus titer in the supernatants remained constant at a level of about 10^8 PFU per culture, in spite of the fluid changes. This prolonged existence of the steady state in the cultures is by itself of special interest and is being investigated in greater detail. The observations can, at present, be interpreted as follows: It is unlikely that the multiplying cells belonged to a virus-resistant type which was originally present in the culture, since such cells would have replaced the sensitive cells in a few days. It is also unlikely that the multiplying cells represented either partly resistant cells—like those occurring in several virus-carrying cultures of poliovirus[11] and Newcastle disease virus[12]—or a non-infected fraction of sensitive cells. In both cases, in fact, they would have been infected after a few days by the progeny virus accumulating in the medium in large amounts. It is therefore concluded that the surviving cells are infected cells which do not undergo degeneration. The conclusion that the surviving cells are altered cells is also indicated by the finding that all cells of a mouse embryo culture infected with PY virus become resistant to vesicular stomatitis virus.[10] It seems, furthermore, that the cells carry the virus in a state in which the fate of the virus-cell complex is undetermined (see below). Cells containing the virus in this "uncommitted" state can divide but have a considerable chance of being killed by a shift of the virus to the state of extensive, cytocidal multiplication.

A gradual change was observed in the mouse cultures after four weeks. The proportion of degenerating cells decreased significantly together with the titer of intra- and extracellular virus of the cultures. The cell number increased and weekly transfers became possible. The outgrowing cells were of a new type, more elongated than the original cells and with a tendency to form interwoven net-like structures when the cultures became more crowded. Ten weeks after infection, the cultures appeared to be made up entirely of the new cell type. They could be transferred twice weekly, contained no more degenerating cells than normal ones, and produced little virus.

The hamster cultures, at the end of the first week after infection, were made up of a single dense layer of cells indistinguishable from the controls. During the second week a striking difference between the infected and control cultures became apparent. Whereas the number of cells in the control cultures appeared to increase

only slightly during this time, the infected cultures continued to increase in cell number to a value approximately three times that of the control cultures. This led to strong acidification of the medium of the infected cultures. The new cells were more elongated than the control cells and had a tendency to grow in interwoven netlike structures similar to those described above for the infected mouse cells. Whorls of heavy cell strands became noticeable above the continuous cell layer in the infected cultures, but not in the control cultures.

After fourteen days, the cultures were dispersed with trypsin for injection into animals (see below), or for further transfers. No difference between the growth rate of control and infected cultures was observed during the first week after the transfer. In the second week after the transfer, however, whorls of heavy cell strands again appeared in the infected cultures but not in the control cultures; at the same time the medium became strongly acidified. The virus titer decreased drastically in the transformed cultures. Frequently no extra- or intracellular virus could be detected; in other cases titer of 10^3 PFU or less per 10^6 cells were obtained.

This proliferative response in hamster embryo cells to PY virus was first observed in experiments carried out in collaboration with Dr. G. Freeman. It has since been observed and followed in four independent experimental series, occurring in calf or fetal bovine serum medium but not in horse serum medium. The proliferative response failed to appear in two other experimental series. The observations tend to show that the occurrence of a proliferative response depends on the physiological state of the cells at the moment of infection, a point which is under further investigation.

Nature of the transformed cells: As to the nature of the new cells which became established both in infected mouse and hamster cultures, it is unlikely that they were originally present; in fact, no selective conditions favoring multiplication of such cells were present in the hamster cultures in which no cell destruction took place. It is more likely that these new cells were cells of the original type transformed or converted by the virus. Since the transformed cells of the mouse cultures became established in the presence of high virus concentrations, it can furthermore be assumed that these cells are resistant to superinfection with PY virus. This conclusion is also supported by the fact that superinfection of transformed hamster cells with PY virus failed to give evidence of virus production during the first week after infection.

The ability of the transformed cells to grow under conditions unfavorable to the growth of the control cells suggested that they were of neoplastic nature. To test this possibility, 10^6 to 4×10^6 cells of six independently transformed hamster embryo cultures were inoculated under the skin of 18–20 day old hamsters. All six cultures gave rise to tumors at the site of inoculation; five tumors were palpable six days, one tumor two weeks, after the inoculation. All six tumors grew progressively without any signs of regression. In three cases, the animals were killed when the neoplasias had reached a diameter of about 3 cm. The neoplasias were well localized; no metastases or tumors at other locations were found. Histologically, the neoplasias were constituted by fusiform cells. Fragments of the tumors were trypsinized, and tissue cultures were prepared from the cell suspensions. The outgrowing tumor cells formed whorls similar to those of the original cultures whenever the cell layers became crowded. Frequently no virus or only very small

amounts (100 PFU per 10^6 cells) of virus could be demonstrated in these cultures.

As controls, more than sixty hamsters of the same age were inoculated with 10^6 to 10^7 cells of uninfected hamster embryo cultures from the same culture batches in which the transformed cultures had been contained. In none of the hamsters were nodules formed. Four infected cultures in which no *in vitro* transformation had been observed likewise failed to induce tumor formation in the animal. Neither did ten hamsters inoculated with 5×10^7 to 10^8 PFU of PY virus form any tumors at the site of inoculation. The hamsters are still alive and it is therefore unknown whether they formed tumors of the organs normally affected by PY virus.

Conclusions.—In these experiments, the PY virus gave rise to two types of virus-cell interaction: a cytocidal interaction, leading to extensive virus synthesis and cell degeneration, and a moderate interaction leading to the transformation of the cells into neoplastic cells, usually unable to produce detectable virus and resistant to superinfection with the same virus. The cytocidal interaction is most frequent in the mouse cultures, the moderate interaction in the hamster cultures. The different reactions of the mouse and hamster cultures reproduce and clarify the events occurring in the animal: it is in fact known that in the newborn mouse the virus produces extensive cell degeneration and only later—after several months—tumors; whereas in the newborn hamster the virus produces few degenerative phenomena but within a few weeks leads to formation of tumors. The results obtained extend those of Dawe and Law[13] in showing that differences in virus-cell interaction explain the various effects of the virus in the animal, including the disappearance of the virus from hamster tumors.[14, 15, 16]

The following hypothesis is put forward to explain the results of our experiments: upon entering a cell, the virus assumes an "uncommitted" state in both hamster and mouse cells, during which it may undergo a limited multiplication without appreciably affecting the normal properties of the cell. From this "uncommitted" state, the virus has the choice of entering either the state of cytocidal multiplication or the integrated state. The late transformation of the mouse cultures would be due to the selection of a few transformed cells. In the hamster cultures, on the other hand, the choice would be almost exclusively toward the integrated state, both in the animal and in the tissue culture.

The state of the virus in the transformed cells is unknown. Two properties of cultures of these cells, i.e., the absence or low level of virus production and the resistance to superinfection, are similar to the properties of lysogenic bacterial cultures and suggest that the integrated virus exists as provirus. Other hypotheses are, however, not excluded: the virus could ultimately be lost from the transformed cells and resistance to superinfection could be a secondary consequence of the transformation.

The transformed, neoplastic cells are able to grow above the monolayer to which the normal cells are usually confined. This suggests that *in vitro* the normal cells are still subjected to growth-regulating mechanisms from which the transformed cells can escape. This property of PY-transformed cells, which is also characteristic of cells transformed by the Rous virus, may be general for neoplastic cells *in vitro;* if so, it may open a wider approach to the study of neoplasia-producing viruses.

These experiments will be reported in detail elsewhere.

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THE STABILITY OF NON-DISSIPATIVE COUETTE FLOW IN THE PRESENCE OF AN AXIAL MAGNETIC FIELD

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1. The effect of an axial magnetic field on the stability characteristics of Couette flow in the limiting case of zero viscosity and infinite conductivity has been examined recently by Chandrasekhar.[1] He showed that an adverse gradient of angular velocity can always be stabilized by a sufficiently strong magnetic field and that the required field strength can be derived from the solutions of the related nonmagnetic problem. Within the framework of the "small gap" approximation, the nonmagnetic problem has recently been solved exactly,[2] and in this paper, therefore, we present a determination of the magnetic field strength required to completely stabilize the flow.

2. In the small gap approximation it is assumed that the gap, $d = R_2 - R_1$, is small compared to the mean radius, $R_0 = {}^1/_2 (R_2 + R_1)$. The angular velocity distribution can then be approximated by the linear profile

$$\Omega = \Omega_1 [1 - (1 - \mu)\zeta], \tag{1}$$

where

$$\mu = \Omega_2/\Omega_1 \quad \text{and} \quad \zeta = (r - R_1)/d. \tag{2}$$

In the absence of a magnetic field, Rayleigh's criterion shows that the angular velocity distribution (1) is unstable for $\mu < 1$ and we wish to determine, therefore, the magnetic field strength required to stabilize the flow under these conditions.