A Consideration of Virus-Host Relationships in Neoplasia at the Level of the Whole Animal

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"Come, my songs, let us speak of perfection—
We shall get ourselves rather disliked."


From the study of virus-induced malignant cells in tissue culture an idea may one day emerge, postulating a single key mechanism for triggering the process of carcinogenesis in vitro. The theory of such a single mechanism is untenable when consideration is given to the multiple cell structure which forms an organism. It would be out of place to stress the complexity of the machinery which the organism has at its disposal as compared with that of its isolated cells. Physiological, endocrine, and immunological factors, to mention only a few, are powerful forces which may change the process leading to malignant proliferation. This should deter anybody from consideration at present of a single mechanism governing the process of carcinogenesis in an animal organism. Readers who are interested in thinking about the concept of comparative study of cells and organisms vis à vis a virus infection are referred to recently published, excellent papers by Lwoff (38, 39).

The heterogeneous process of successive changes leading to neoplasia has rarely, if ever, been analyzed from a biological point of view. More recently, however, Foulds (16) successfully attempted to subject the neoplastic development in an organism to experimental analysis, and perhaps a summary of Foulds' lucid presentation may serve as a proper introduction to the subject of this paper.

The series of successive changes by which normal tissue becomes malignant has been termed "progressive." Foulds considers progression as the development of the tumor by way of a series of qualitative, stable, irreversible, and inheritable changes. He has laid down certain principles of progression, of which the first is by far the most important one. This concerns the independent progression of tumors. Through a qualitative change within the neoplastic tissue only a portion of multiple tumors present in the same host, and often in the same tissue, continue to grow at one time.

The second principle of Foulds postulates the independent progression of such characters of tumors as capacity to invade surrounding tissue, capacity to metastasize, sensitivity to drugs and to hormones, degree of differentiation, and others. In other words, while the tumor develops, these and other characters do not change together. This rule eliminates to a certain degree the necessity of classification of tumors as benign or malignant—a classification which tends more often to confuse than to help.

The third principle refers to the fact that progression is independent of growth. In simple language it says, as I understand it, that a tumor of small size which is found during the first phase of clinical inspection may be in a later stage of progression toward malignancy than a tumor of larger size observed after a longer period of clinical observation. An endocrine-dependent tumor in a latent stage may undergo rapid progression toward malignancy if the endocrine imbalance occurs after a period of correction.

Continuous or discontinuous progression, the fourth principle, refers to the fact that certain characters in tumors which are, for instance, being transplanted, undergo continuous change or progression, whereas in the case of the above-mentioned hormone-dependent tumors progression may be arrested, and, later on, change toward malignancy may occur abruptly.

The last two principles mentioned by Foulds state that progression follows one of alternative paths of development and that it does not always advance to an end-point within the lifetime of the host. It is understandable that a tumor trans-
planted after the death of its original host may undergo further progression in the course of subsequent passages. More often than not progression of tumors does not reach a definitive stage in the original host. The alternative paths of development have already been mentioned in connection with independent progression of character. The best example for this rule may be found in the follow-up of hyperplastic nodules of the breast in mice—a study to which I shall return later. We shall attempt in the course of this presentation to refer to Foulds' principles in relation to the progression of virus-induced tumors of the host.

**TABLE 1**

**EXAMPLES OF VIRUSES ACTING FROM WITHIN AND FROM WITHOUT**

<table>
<thead>
<tr>
<th>Mode of action</th>
<th>Tumor viruses</th>
<th>Non-tumor viruses</th>
<th>Mode of transmission in the vertebrate host organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within</td>
<td>Mammary tumor agent Mouse lymphatic leukemia</td>
<td></td>
<td>Congenital or neonatal, but LCM is also contagious</td>
</tr>
<tr>
<td>Without</td>
<td>Fowl tumor viruses (Rous sarcoma, myeloblastosis, erythroblastosis, and lymphomatosis)</td>
<td>Most viruses</td>
<td>Contagion, but age and genetic make-up of the host play a definite role. Lymphomatosis may be transmitted through the egg</td>
</tr>
<tr>
<td></td>
<td>Poloma virus Shope papilloma</td>
<td></td>
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</tr>
</tbody>
</table>

**VIRUSES FROM WITHOUT OR FROM WITHIN**

The task of finding a key to understanding the role of viruses in neoplasia would be much easier if we would and could make up our minds whether the viruses operate without or within. In other words, do tumor viruses act as infectious agents which can be transmitted from one individual to another following an epidemiological pattern not dissimilar to that observed in the case of other "noncacinogenic" viruses? Or are tumor viruses "endogenous" for the animal organism, inherited through generations as other characters are inherited? There is available evidence supporting both viewpoints (Table 1). If one wants to become more heretical one may postulate that viruses capable of acting as tumor viruses under certain conditions of "imbalance" (see below) form an integral part of every organism. It would be difficult, if not impossible, to obtain experimental evidence to support the latter hypothesis, and the decision as to its acceptability may depend largely on the agreement concerning the concept of the virus itself.

**VIRUSES FROM WITHOUT**

The peregrinations of polyoma virus in a mouse colony may be cited as an instance where tumor-viruses and non-tumor viruses behave in a similar manner. Evidence presented by Rowe and associates (49) seems to indicate that mother mice nursing newborns infected with mouse salivary gland tumor virus uniformly became infected through contact within 4 weeks of first exposure. The apparent contagiousness of the polyoma virus has also been confirmed through detection of antibodies against the virus in mice which are kept in the same room but not necessarily in the same cage with polyoma-infected newborns. These epidemiological observations are in agreement with other data indicating that newborn mice excrete the virus and can become a source of intense environmental contamination (49).

The viruses of fowl erythroblastosis, fowl myeloblastosis, and Rous sarcoma were also found to be highly contagious. In recent studies conducted by Burmester and associates (8), 28 per cent of birds kept in contact with erythroblastosis-inoculated chickens developed visceral lymphomatosis, and 10 per cent developed erythroblastosis. What is even more remarkable is the high contagiousness of Rous sarcoma virus observed by the same investigators.

Another interesting aspect of the same study (8) relates to the location and types of tumor produced by these fowl tumor viruses in contact birds. Visceral lymphomatosis, osteoporosis, and renal adenocarcinoma were found in contacts of birds infected with the myeloblastosis virus; and half of the birds kept in contact with Rous sarcoma-infected chickens, instead of developing "surface" tumors as expected, died of tumors developing internally.

In the case of polyoma virus, mice whose blood had specific antibody acquired by contact infection did not develop tumors. Apparently, in order to cause neoplasia this virus has to be implanted into very young animals and then in all probability in very large doses. Although the quantitative relationship between the host and the polyoma virus in the induction of neoplasia has not as yet been satisfactorily determined, there are data available related to other tumor viruses.

Table 2 is reproduced from Isaacs' (25) review.
on “Particle Count and Infectivity Titrations for Animal Viruses.” Figures obtained for the number of particles per ID₅₀ of noncarcinogenic viruses are strikingly low as compared with those shown for the two tumor viruses. These data have been further expanded by Beard and his associates (4), who calculated that 26 million particles are needed to cause myeloblastosis in 50 per cent of 3-day-old chickens given inoculations of the virus. Extrapolation of their data to the value corresponding to the 5 per cent level of disease incidence gives the figure of 6,000 particles per inoculum. To infect 95 per cent of the chickens the calculated number of particles rises to the astronomical figure of 145 billion particles.

Similar data obtained by Bryan (6) for Rous sarcoma virus yield the figures of 10 million particles for the induction of sarcoma in 50 per cent of chicks of a more resistant line and 600 particles in a more susceptible host.

Table 2

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Technic of particle count</th>
<th>Technic of infectivity titration</th>
<th>Approx. no. of particles per ID₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menigopneumonitis</td>
<td>Spray: E.M.</td>
<td>Chick embryo: yolk sac</td>
<td>45</td>
</tr>
<tr>
<td>Feline pneumonitis</td>
<td>Spray: Light microscopy</td>
<td>Chick embryo: yolk sac</td>
<td>100</td>
</tr>
<tr>
<td>Vaccinia</td>
<td>Calculation from particle weight</td>
<td>Rabbit intradermal</td>
<td>42</td>
</tr>
<tr>
<td>Cowpox</td>
<td>Calculation from nitrogen estimation</td>
<td>Rabbit intradermal and chick chorio-allantoic</td>
<td>366</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Calculation from particle weight</td>
<td>Mice: Intranasal; Chick allantoic</td>
<td>10-16</td>
</tr>
<tr>
<td>Influenza A and B</td>
<td>Spray: E.M. and red cell absorption</td>
<td>Chick allantoic</td>
<td>10</td>
</tr>
<tr>
<td>Influenza C</td>
<td>Spray: E.M.</td>
<td>Chick amniotic</td>
<td>50</td>
</tr>
<tr>
<td>Newcastle disease of fowls</td>
<td>Calculation from light scattering</td>
<td>Chick allantoic</td>
<td>5</td>
</tr>
<tr>
<td>Fowl plague</td>
<td>Spray: E.M. and red cell absorption</td>
<td>Chick allantoic</td>
<td>5</td>
</tr>
<tr>
<td>Mumps</td>
<td>Spray: E.M.</td>
<td>Chick allantoic</td>
<td>10</td>
</tr>
<tr>
<td>Sendai</td>
<td>Spray: E.M. and red cell absorption</td>
<td>Chick allantoic</td>
<td>100</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>Spray: E.M.</td>
<td>Chick allantoic</td>
<td>10</td>
</tr>
<tr>
<td>Rous sarcoma</td>
<td>Pocks on chorioallantois</td>
<td>Cotton rat intracerebral</td>
<td>20,000</td>
</tr>
<tr>
<td>Shope papilloma</td>
<td>Calculation from particle weight</td>
<td>Tumors in chicken 600-10,000,000</td>
<td>Rabbit intradermal</td>
</tr>
<tr>
<td>Erythromyeloblastic</td>
<td>Centrifugation, E.M.</td>
<td>Latent period after inoculation of fowls</td>
<td>&gt; 1,000,000</td>
</tr>
<tr>
<td>leukemia of fowls</td>
<td></td>
<td></td>
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</tbody>
</table>


The analysis of the relationship between “the virus quantity and level of host susceptibility” (6, 7) in the case of the fowl tumor viruses has been extremely illuminating from the point of view of a better understanding of the tumor-virus-host interaction. For instance, a direct relationship has been established not only between the concentration of virus in the inoculum and the incidence of Rous sarcoma, but also between the concentration of the virus and the rate of tumor growth, the latency period, and the types of tumors produced. The most striking quantitative relationship has also been established between the initiatory dose of Rous virus and the recovery of the virus from the induced tumors. The use of large amounts of virus in the inoculum permits uniform recovery of the virus from the resulting tumor, and, since such a virus material prepared by Dr. Bryan was made available to other laboratories, workers in this field have been able to obtain maximal benefits from their respective studies. The results of these quantitative investigations have revealed one fact often overlooked by scientists studying the virus-host relationship: variation exists in susceptibilities among hosts of different genetic make-up and among individuals of genetically similar though not identical background. It would be possible to write a volume on the pitfalls encountered in the past through disregard of the genetic constitution of the host. Although this was excusable during the period when knowledge of animal genetics was very scanty indeed, genetically defined inbred lines of animals became available to investigators some time ago, and one should now look askance at work on tumor viruses in hosts of ill-defined origin. Although the present paper is written for other purposes than discussion of the genetic factors of the host, it should be mentioned briefly that, if acceptability of skin grafts can be considered as evidence for genetic homogeneity, inbred lines of chickens, guinea pigs, hamsters, rats, and, of course, mice are available for scientific investigations. Use of a host of uniform genetic make-
up would remove at least one variable from experimental studies of virus-host relationships.

Returning to the studies of quantitative aspects of host-virus relationships, I would like to quote directly from one conclusion of Bryan's paper (6): "In the instance of one of the tumor viruses which has been most extensively investigated by means of quantitative biological methods, namely, the Rous sarcoma virus, and possibly also in the instance of certain other rapidly acting tumor viruses, certain facts and relationships are revealed which are contrary to long-held opinions and assumptions regarding the role of tumor viruses in the induction of neoplasia. Thus it is apparent that this particular virus is the direct activating cause of the cancerous reaction which it elicits, in all of its specific manifestations which have been critically investigated in this respect. This is contrary to the belief of many that all tumor viruses, like many other types of cancer provoking agents, act indirectly by 'triggering' some other activating mechanism which is inherently present and potentially activatable, within most living cells of higher organisms."

This statement has been fully supported by the data obtained with Rous sarcoma virus and the two other fowl tumor agents. It applies to fowl tumor viruses which act from without and which act rapidly, as stressed by Bryan.

Data now being obtained for tumor viruses affecting mammals, such as Shope papilloma, may undergo some revision; the number of virus particles required to cause a tumor may be much smaller than originally stated in the table. Similar quantitative relationships have not been established for other "mammalian" tumor-virus systems. One of the important projects in this field is to conduct investigations which may elucidate the relationship between the tumor-inducing dose of a virus and its host. However, it is possible that data obtained for one tumor virus-host system cannot serve as a model for another because of the enormous diversity of the relationship, particularly the complexity of the reacting host as related to its species and genetic make-up.

**HOST CONTROL**

If one takes into consideration all the reservations mentioned above, in comparison with non-neoplastic viruses, the number of particles of tumor-viruses needed to produce neoplasia seems large, probably reflecting the high degree of resistance of even the so-called susceptible host to tumor-virus infection. Compare the figure of $5 \, \text{ID}_{50}$ for Newcastle virus with that of 26,000,000 \text{ID}_{50} for the myeloblastosis virus. What is the mechanism of this resistance? Is the host reacting against the virus itself, or is the control exercised at the initial phase of cellular proliferation? The paucity of data does not permit a final and definitive answer to this question, but results obtained by Defendi and myself (11) seem perhaps to shed some light on this problem in relation to infection of hamsters with polyoma virus.

In newborn hamsters the polyoma virus will induce tumors within 2-4 weeks after inoculation. Tumors will become localized in liver, heart, kidney, subcutaneous tissue, and at the site of inoculation, liver lesions representing the acute phase of the neoplasia. The maximum yield of polyoma virus is obtained 5-6 days after inoculation.\(^1\) From then on progressively lesser amounts of virus are recovered. Antibodies are observed in blood as early as the 7th day of infection. Presence of intranuclear inclusions seems to indicate some cytotoxic action within 6-10 days after inoculation (40); this seemingly cytotoxic effect is followed, however, by proliferative changes.

When hamsters were given injections, on the day of birth, of large amounts (50-80 million) of cells obtained from adult hamster lymph nodes and spleen, followed 2 days later by inoculation of polyoma virus, the following events took place: There was a marked decrease in the incidence and delay in the appearance of tumors in the treated group as compared with the control group during an observation period of 45 days. However, when survivors were sacrificed on the 70th day after inoculation, the difference in the incidence of tumors between the two groups was nil (11). The use of adult lymphoid tissue was motivated by the fact that the appearance of polyoma-induced tumors in adult hamsters is much delayed as compared with the time of appearance in the newborn animal.

If the tolerance of a neonatal host such as the newborn mouse permits polyoma virus entering its cells to cause proliferation, then one may postulate that adult lymphoid tissue, through a fast-working immunological mechanism of neutralization of the injected virus, has provided a more efficient method of counteracting tolerance and combatting the virus-tumor effect. However, no evidence was obtained indicating that the virus causes viremia in hamsters later than 3 days after inoculation, and the mechanism of "adaptive immunity" is the more puzzling because of the late occurrence of the polyoma-induced tumors. If af-

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1. W. P. Rowe, personal communication.
after inoculation the virus probably multiplies in many organs, inducing cytopathic effects in some, and this initial phase of infection stimulates antibody response, which in the case of newborns bearing lymphoid grafts from adults neutralizes a large amount of virus, then what next follows remains in the realm of pure speculation; it will be discussed later in connection with the persistence of tumor viruses in tumor tissue.

**The Receptive Organ and Viruses from Within**

Lwoff's (38) statement that "An organism is sensitive to a virus only if some of its cells at least are able to adsorb the virus and allow its reproduction" is particularly pertinent to the study of the susceptible organs and tumor viruses, especially those acting from within.

As an example, the relationship between the thymus and mouse lymphatic leukemia will be briefly discussed. The physiological functions of the thymus are not very well understood; however, in 1944 McEndy et al. (41) had already shown that thymectomy causes a remarkable drop in the incidence of spontaneous leukemia in the AK, so-called high-leukemic, strain of mice. Their findings were extended by Furth and other workers to other types of spontaneous leukemia (17, 35), and radiation- (26) and carcinogen-induced leukemias (34). It was demonstrated that lowering of the incidence of leukemia could be reversed by implantation of thymus into thymectomized mice (27, 34).

The role of the thymus as an organ involved in the etiology of mouse lymphatic leukemia has been determined in experiments in which leukemia developed in irradiated, thymectomized mice carrying either thymus isografts (28) or thymus grafts of one of the parental strains (33). Recently (21, 37), it has been possible to isolate a leukemia virus from the thymus tissue of irradiated C57BL mice, a strain with a low incidence of spontaneous leukemia. Presence of the virus was demonstrated when the incidence of leukemia rose 20 per cent (37) after inoculation of embryos of the C57BL strain with filtrates of irradiated thymus tissue. Injection of a carcinogen, 9,10-dimethyl-1,2-benzanthracene, directly into the thymus of several mouse strains markedly increased the incidence of leukemic tumors or leukemias, which in a normal colony of the same strain of mice did not exceed 1 per cent (46, 47). This effect was not observed when thymic grafts were transplanted subcutaneously and then treated in the recipient mice with the carcinogen (48). The final proof that the thymus is the "receptive" organ for mouse leukemia came from experiments conducted by Levinthal, Buffett, and Furth (36) and Gross (20), who were unable to induce leukemia by injection of the virus into newborn mice which were thymectomized 1 month later.

However, it should be pointed out that if thymectomized mice of the AKR strain, injected with lymphatic leukemia virus, are observed for a sufficiently long period of time, they will ultimately develop other types of tumors. Myeloid leukemia has been observed by Gross in three thymectomized AKR mice, and Furth has noted occurrence of lymphomas and neoplasms never before seen in AKR mice which had been thymectomized. Gross has found that reimplantation of the thymus in thymectomized, virus-injected mice restores their capacity to develop lymphatic leukemia, indicating that the thymus "activates" the effect of the virus rather than serving as the locus of virus multiplication. But what does it all mean?

At this time a nonviral digression seems to be in order. In a series of extremely interesting papers Metcalf (42) described the presence of a factor which he called the lymphocytosis-stimulating factor (LSF). When newborn mice were given injections intracerebrally of a cell-free suspension of thymus, a 50-150 per cent increase in circulating lymphocytes was observed 6 days later. The LSF is present in plasma, and the production of thymic LSF is controlled by the levels of LSF in plasma. Thymus seems to be the only LSF-producing organ. LSF is not species-specific, since inoculation of human plasma or thymus obtained from cases of lymphosarcoma and chronic lymphatic leukemia into mice causes lymphocytosis (inoculation of normal plasma and normal thymus has no effect).

A very interesting phenomenon has been demonstrated by Metcalf (42) in connection with the so-called high-leukemic strains of mice; these mice do not respond to the inoculation of thymus by lymphocytosis, since the "normal" level of their LSF is much higher than that observed in low-leukemic strains. Although these mice are very young when these observations are conducted and show no signs of leukemia, their thymus contains an excessively high concentration of LSF.

Irradiation of mice of the low-leukemic strains creates the same condition as in the high-leukemic strain in relation to unresponsiveness to the stimulation of LSF. Although I do not know whether anybody has shown excess production of LSF in mice injected with mouse leukemia vi-
rus, the fact is that excess LSF is formed in the thymuses of mice which are apparently carrying the leukemia virus within, but are still in the asymptomatic though "pre-leukemic" stage.

Thus, within the limits of speculative hypothesis it may be conceived that infection with a leukemic virus may cause excess production of LSF by the thymus. Whether this excess is produced when virus infection causes the appearance of an "abnormal" cell, or whether the virus simply stimulates division of normal thymus cells, thereby increasing production of LSF, is, of course, a matter of careful histologic investigation.

The nature of LSF is elusive. Does it act as a hormone or as a "transforming" agent? Is the LSF inducive or permissive to virus infection? Is the prolonged stimulation of the lymphopoietic system by virus-infected, LSF-excreting thymus sufficient for the development of neoplasia, or is it essential to have a "secondary" infection with the virus of the lymphoid tissue of the mice in order to produce truly malignant cells? There are no answers as yet obtainable to such questions, and yet the importance of this problem is obvious. It should be mentioned in passing that filtrates of malignant mouse tumors (19) or of leukemic tissue extract (32) were known to produce leukemia when injected into newborns. An abdominal mouse lymphoblastosis can also be induced in mice by inoculation of extracts of human leukemic tissue (50), particularly by the intracerebral route, the same route used by Metcalf to demonstrate the presence of LSF. In the experiments of Schwartz (50) serial passage of mouse abdominal lymphoblastosis induced originally either by cell-free extracts from leukemic mouse brain or leukemic human brain could be carried out successfully only if brain tissue of the infected mouse, but not the tumor, was used as inoculum. Schwartz apparently did not make an attempt to determine the LSF of the thymus of these mice, and perhaps such a determination would have shown an increase in LSF. The fact that brain tissue contains the virus is not surprising, since the microglia may perhaps act as virus-receptive cells parallel to other cells of the reticuloendothelial system.

The Virus-Susceptible Tissue-Contact Relations-Hormones

According to the first postulate of Foulds (16), a tissue infected with tumor virus should undergo qualitative change leading to the independent progress of the neoplastic lesion. There are, however, other factors involved, and they will be correlated in a discussion of the mammary neoplasia of mice. About 55 years ago, Apolant (3) described the existence of hyperplastic areas in the mammary glands of mice; Haaland (23), 5 years later, considered these areas, which today are called hyperplastic nodules, as being intermediate stages of development between normal and neoplastic tissues.

Recently, DeOme and his associates (3, 12, 15) studied the progression of these hyperplastic nodules in mice infected with mammary tumor virus. Choosing an ingenious technic of separation of hyperplastic nodules within mammary glands, DeOme (3) transplanted selected portions of mammary gland from old C3H mice into the dorsal subcutis of young females of the same inbred strain. Results of a large series of experiments failed to reveal a higher incidence of tumors developing in transplants from nodules than in transplants from normal mammary tissue. Actually, only one tumor was observed in the entire series. DeOme (3) ascribed the failure to produce tumors to the placing of transplants in an unusual environment—the dorsal subcutis. When the hyperplastic nodules were transplanted into another mammary gland no outgrowth was observed, although the morphological integrity of the transplanted nodule was retained. However, when one of the mammary glands of the recipient animals was surgically removed and the cleared fat pad of the host was used as a transplantation site for the nodules or for normal mammary tissue, the situation was radically changed. Eleven mammary carcinomas developed from nineteen transplants of mammary glands containing hyperplastic nodules; in contrast, two tumors developed from nineteen transplants of normal mammary tissue. A careful morphological study of the tissue indicated that, whereas rapid growth of both types of transplants filled the cleared fat pads within 5–12 weeks after implantation, normal tissue produced normal outgrowth, and altered cells of the nodules apparently gave rise to hyperactive outgrowth. The tumors often did not arise from the nodule itself but appeared at points distant from the transplantation site. This again reminds us of the principle of the independent progression of tumors.

Since the hyperplastic alveolar nodules observed in old C3H virgin mice bear some resemblance to normal lobules encountered in pregnant mice, which regress after weaning, the hormone sensitivity of the mammary gland cells has been investigated (3). Results of this study indicated that alveolar hyperplastic nodules which
regress in hypophysectomized-ovariectomized-adrenalectomized mice without treatment can be maintained in mice which are treated with such substances as cortisol and somatotropin. These hormones are lactogenic. Indirect evidence from organ culture experiments suggests that the hormone-sensitive population probably arose through selection of altered cells in the terminal ducts. Results of transplantation experiments show that the neoplastic progression may also be related to the hormonal action of pituitary and/or adrenal and ovary. But where does the mammary tumor virus fit into this neoplastic process? Electron microscope studies conducted by Pitelka et al. (45) revealed the presence of virus-like particles in the cellular cytoplasm or lumina of all examined nodules, but in only nine out of 40 normal mammary transplants. In the hyperactive outgrowth derived from transplanted nodules the virus-like particles were in high concentration, often occurring extracellularly.

It should be mentioned in passing that the normal tissue-hyperplastic nodule-carcinoma sequence observed in the mammary gland is not the only example of gradual progression toward neoplasia. A parallel situation was also demonstrated by the ease of chemical production of cancers of the uterine cervix of mice, where the progression from cervical dysplasia through carcinoma in situ to cervical cancer was demonstrated by several investigators (31). It is probable that the cervical carcinoma in situ may correspond to the hyperplastic nodule in mammary tissue in the course of neoplastic development. Although direct evidence for the presence of virus-like particles in carcinoma in situ has not been obtained, investigations in this connection have been scanty and, with the development of better technics, may lead to the demonstration of virus-like particles similar to those observed in the hyperplastic nodule.

Virus fiat ubi cult.—Reaching the end of the factual data presented in such a convincing way by DeOme, one may now proceed to indulge in speculation concerning progression of mammary neoplasia. The hypothetical steps in the progression would be as follows:

1. The mammary tumor agent, which is a virus from within, acquired congenitally, causes formation of the hyperplastic nodule in a susceptible organ, such as the mammary gland.

2. The nodules may regress, persist as nodules, or develop into carcinomas, as determined by two factors:
   a) Retention or loss of contact relations with neighbor cells.
   b) Endocrine balance or imbalance.

Analysis of this hypothesis requires careful scrutiny of all steps involved in the progression of the neoplastic development, including scrutiny of the conflicting evidence as to the omnipresence of the endogenous virus. In one of their recent publications Mühlböck and Boot (44) state, “It has been made clear by recent investigations that the presence of the mammary tumor agent (MTA) is not necessary for the development of mammary carcinoma in mice; the MTA is assumed to act only as an accelerator or ‘intensifier.’” In the work described in this paper, the authors achieved through subcutaneous implantation of isologous pituitaries a dramatic increase in the incidence of mammary carcinoma in mice which were supposed to be virus-free. The tumor incidence rose from 3 to 75 per cent in some strains and from 40 to 100 per cent in others. The modus operandi was again based on the increased level of secretion of lactogenic and/or luteotrophic hormones by the hypophyseal graft, which apparently stimulated the mammary gland to neoplastic progression. Suspensions from the resulting mammary tumors failed to cause neoplasia in mice into which they were injected. The statement referring to the nonviral origin of the mammary carcinomas was based on this fact. It is highly debatable whether mammary tissue of the so-called milk tumor agent-free mice is really free of virus. It must be remembered that, after irradiation of the low leukemic strain of mice, the leukemic virus was demonstrated in the thymus by subinoculation of this organ into newborn mice of the isologous strain (37). Perhaps if organ culture methods, supported by electron microscopy, were used for detection of mammary tumor virus in the mammary tissues, the results might be more successful.

If we accept the principal hypothesis that congenital infection with mammary tumor agent occurs in many more instances than are actually suspected, it may then be postulated that the hyperplastic nodule eventually becomes a virus lesion, consisting of virus-infected “altered” cells. The nature of the alteration remains within the realm of pure hypothesis; however, we may be tempted to speculate upon the change in the metabolic pattern of the cell as the result of virus infection. Perhaps the function of the virus is to switch the metabolism of the cells from a differentiated type of activity to a growth and division activity (51). In other words, the virus-infected cells are set to synthesize much more effectively the mitotic protein, i.e., the less differentiated elements required for cell division only and not in-
volved in the mechanism of differentiation. This shift may be considered as one final step of the progression toward neoplasia.

Evidence that lack of cell contact is essential for the development of mammary carcinoma from the hyperplastic nodule has been presented by DeOme (3). The nature of this cell contact remains somewhat mystical. The hypertrophic growth of the nodule in the fat pads cleared of mammary glands fits well the template-antitemplate theory of Weiss (53) that removal of part of the normal organ reduces the rate of production of antitemplates, so that intracellular templates predominate and growth is stimulated, with a selective advantage for the virus-infected cells. One has to consider, however, other factors in contact relations, such as changes in the electrical charges of cells which are in the process of progression toward neoplasia (2), and many others which may change the milieu intérieur in which the few cells of the nodule with their neoplastic potentialities reside.

The action of hormones may be sequential, concomitant to, or independent of the effect of contact relations. The mitogenic action of some hormones on normal cells either in vivo or in vitro (52) will, of course, explain stimulating activity on the hyperplastic nodule, an effect which may occur easily under nonexperimental conditions during an endocrine imbalance.

FATE OF THE VIRUS

Thus, we have arrived at a point where we must determine the fate of the virus in a growth it has initiated. Here we have to consider results which have been mentioned before and which refer to the fact that large doses of Rous sarcoma virus introduced into a breed of chicks of uniform susceptibility will cause formation of tumors yielding a large quantity of the virus (6). If the inoculum contained small amounts of virus a certain proportion of the resulting tumors yielded no virus at all (7). A similar relationship between the amount of infective Rous virus obtained from the host and the infecting dose was found in the case of intracerebrally inoculated chicks whose brains yielded no virus when the inoculum was small (22). Tumors developing in hamsters infected with polyoma virus usually yield no virus (23), whereas virus can be demonstrated in polyoma tumors of mice after their excision and cultivation in tissue culture. Another interesting observation relates to the fact that, although myeloblasts obtained from infected chickens yield large amounts of virus in tissue culture, relatively few virus particles are encountered in apparently infected myeloblasts in the circulating blood of the bird, although plasma of the same bird has a large concentration of the virus (4).

Two hypotheses may be advanced to explain the fate of the virus in tumors. One would postulate that, once the proliferation of the cells is stimulated by virus infection, the role of the virus is finished, and the altered cells will continue to divide and metastasize in any organs of the body where there is adequate vascular supply without the need of carrying virus with them. The yield of Rous virus from tumors induced under experimental conditions is an argument against this hypothesis. Arguments supporting this hypothesis have been advanced for years by scientists who apparently thought they could induce neoplasia without virus involvement. The studies of Mühlböck et al. mentioned above are illustrative in this instance.

The second hypothesis relating to the fate of the virus would postulate that tumor viruses are always present in the proliferating cells at all stages of neoplasia, but their detection is made difficult because of an inhibitor or inhibitors produced by the animal concomitantly with the development of the tumor. Antiviral antibody may be cited as one such inhibitor. Studies discussed above and related to the development of antibody in polyoma-infected newborn hamsters should be mentioned in this connection. A more specific example has recently been furnished by Groupté and Rauscher (22), who have investigated the puzzling phenomenon of the "disappearance" of Rous sarcoma virus in tumors induced in turkeys with large amounts of virus. Absence of the extractable virus was particularly puzzling, because the dilution end-point was identical for tumor production in chicks and turkeys. Extracts of turkey sarcoma induced with large virus inoculum were found to contain high concentrations of antibody which could neutralize large quantities of virus and which appeared in the tumor and serum of the bird 2 or more weeks after infection. Although the antiviral serum itself may have no effect on the malignant cell, it may account somehow for the inactivation of the virus produced by the growing tumor. Other inhibitors should not be overlooked in this connection. For instance, factors related to the temperature of the tumor tissue under different conditions may influence the rate of inactivation of the virus. Finally, interferon may play a role as an inhibiting factor, particularly since data have been obtained indicating that one of the tumor viruses, the polyoma virus, causes inter-
ference and possibly production of interferon in tissue culture (13).

These considerations apply to viruses acting from without, and it is conceivable that the whole matter of virus persistence may be solved quite satisfactorily once quantitative studies of animal tumor virus similar to those conducted with some of the fowl tumor viruses are completed. The problem becomes much greater in magnitude in connection with virus acting from within. However, even there it may be essentially a problem of technics. It should be remembered that a mouse leukemia virus was extracted from Sarcoma 37 ascites cells by use of chemical fractionation procedures which were found to be so successful in the purification of Rous sarcoma virus (43).

WHAT IS A SUSCEPTIBLE CELL?

Quite obviously it is a cell in a susceptible organ which can become infected by a virus, but this unfortunately represents a target which can be missed by a wide margin in the animal organism. It would be tempting to speculate that, in a susceptible organ, those cells may become infected with a tumor virus which are moving through the division cycle, and that therefore the effect of the virus in activating the specific synthetic ability of elements such as mitotic protein which are required for division but not for differentiation or specialization would be facilitated. There are, unfortunately, no data which would support or destroy this hypothesis, since apparently no calculations of mitotic indices have been made on organs of animals infected by the endogenous or exogenous tumor virus. A parallel with the action of mitogenic hormones may be drawn. From the studies of mitogenic activity of hormones two patterns of response seem to be discernible. This can best be understood by citing the effect of a single injection of testosterone propionate upon two organs of a castrated male mouse, the malpighian layer of the epidermis and the seminal vesicle or coagulating gland (1). The mitotic index of resting cells of the malpighian layer is 0.83/1000 nuclei, and the mitotic rate rises within 6 hours after hormone administration, but only to a value about twice that observed in controls. Conversely, the mitotic index of the resting cells of the coagulating gland was only 0.018/1000 nuclei, i.e., approximately 1/50 of that of the malpighian layer. Following administration of hormone there is a lag period of approximately 36 hours, after which there is intense stimulation of mitosis as evidenced by a more than 100-fold rise in the mitotic index.

The second type of response has also been observed in the study of the effect of the pituitary growth hormone on the pancreatic islets (9). In analyzing the action of mitogenic hormones, Swann (51) postulates that the second pattern of response, i.e., an intense rise in mitotic activity after a considerable lag period, may indicate that hormones act through induction of a different pattern of synthesis of intracellular elements oriented toward cell division. Two questions may be asked: Are such cells particularly susceptible to infection with tumor-virus? Are the two stimuli, i.e., the hormonal and viral, sufficient to cause loss of growth control by the organ and formation of a tumor? The difficulty in answering such questions is explainable by our ignorance as to which of the two factors is inducive. Since the independent progression of tumors postulates gradual qualitative changes within the neoplastic tissue itself, the stimulating mitogenic substance may ultimately be produced by the tumor tissue itself. If such were the case, development of tumors in animals subjected to inoculation with products of human neoplasia (18, 50) would be explainable by the stimulating (mitogenic?) action of the donor tissue on animal cells probably harboring one of the animal tumor viruses. A more quantitative determination of mitogenic activity of the donor tissue extracts would perhaps enable us to solve this riddle, particularly in view of the results of Duplan (14) who demonstrated the mitogenic effect of a rat tumor on the normal liver of its host.

IMMUNOLOGIC CONSIDERATIONS

Several reviews published recently (10, 29, 50) deal with speculations that certain neoplastic conditions such as leukemias, Hodgkin's disease, and other malignant lymphomas are expressions of an immunologic fault of the host, resulting in auto-immunization. Although these neoplastic diseases and experimentally induced conditions in animals, such as "runt" and "secondary disease," are similar in their clinical syndromes, the arguments do not explain satisfactorily the two operational systems involved in the diseases: the nature of the reaction of the lymphoid apparatus of the host and the nature of the substance in the antigen that causes the host to react. It should be remembered that, in both "runt" and "secondary disease," homologous lymphoid tissue has been introduced into the recipient; his clinical condition is caused by a possible "attack" of the donor's proliferating lymphoid cells against the recipient. It is difficult to visualize the modus operandi of such a system in an animal who was
not exposed after birth to homologous tissue. Whether a virus could trigger an auto-immunological mechanism consisting of proliferation of lymphoid tissue of the host, and leading to its ultimate destruction, remains within the realm of hypothesis. The evidence so far accumulated points to the fact that a virus-infected, malignant cell may make the host immunologically tolerant. Although there is no convincing evidence available that virus particles per se induce tolerance, tolerance may be "elicited" toward the virus-infected cell rather than the virus itself. In the two cases in question, i.e., lymphocytic choriomeningitis and mouse lymphatic leukemia (5), cells of the lymphoid tissue have become virus-infected probably during fetal life or immediately after birth. In both cases, the adult host seems to be unable to react. In lymphocytic choriomeningitis the virus persists during the lifetime of the host, and the circulating antibody is either present at low concentration or absent. The case for tolerance toward the infected mouse leukemia cells is based on less firm ground; however, the loss of growth control regulations and the development of the neoplastic condition may be accounted for by the inability of the host to react, as in the case of neoplastic cells of other origin (30).

Actually, one of the most puzzling phenomena to investigators is the apparent tolerance elicited by mammalian cells turned malignant after cultivation in vitro. A normal tissue homograft is promptly rejected by its mouse recipient, but the same homograft turned malignant after prolonged cultivation in tissue culture causes tolerance and can induce tumor formation and death in a homologous strain of mice. Is the surface of the cells changed? Would studies of the possibly altered composition of cell surface lead to explanation of the puzzle? This is a fertile field for further investigations by competent scientists.

ENVIO

This is not a review article. The author is not even certain that all facts which form the background of many hypotheses advanced for the purpose of discussion are still correct. There is no other field of scientific endeavor to which Mencken's statement "The truth that survives is simply the lie that is pleasantest to believe" would be more applicable than that of the study of tumor viruses. However, it is the feeling of the author that solution of the tumor-virus problem will come, not from the indiscriminate collection of laboratory data, but from its analysis and from attempts at synthesis, even though such attempts may often seem to be concerned with the purely hypothetical.

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A Consideration of Virus-Host Relationships in Neoplasia at the Level of the Whole Animal

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