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Transplantable Animal Cancer, the Primary Standard

*Guest Editorial**

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Two previous editorials have appeared under the titles "Human Cancer, the Primary Target" (3) and "Animal Cancer, the Primary Tool" (1). These essays represent commendable contributions in the area of critical writing in the field of cancer research. We need much more discussion on the relative merits of the different approaches to the cancer problem simply because the number of possible combinations of measurements and materials is for all practical purposes infinite.

I believe that emphasis on human cancer (3) or on primary animal cancer (1) without vigorous parallel developments in the field of transplantable animal cancer would be a mistake. This view is in contrast to that of the previous authors, since Day (1) believes that "we should begin limiting the use of the trocar, not expanding it. The foreignness of the tumor explant superimposes such a multitude of extraneous features upon the 'tumorness' of the tissue and wrests such a multitude of pertinent features from it that it can serve only poorly as a physiological model for cancer in man. . . . Those subtleties inherent in cancer which distinguish it from normal tissue may emerge clearly and simply once we revert to systematic studies with autochthonous tumors in our laboratory animals." Holland and Heidelberger (3) are not explicitly opposed to the use of transplanted tumors, but they "doubt the wisdom of accepting biochemical information derived from transplantable tumors when the data apparently are not consistently applicable to spontaneous neoplasms in the same species" and suggest that "the convenience of these tumors as research tools tends to obscure the need for cancer research in man."

Hepatoma studies.—On the basis of recent studies on rat hepatomas in cooperation with Dr. Harold Morris and Dr. Henry Pitot (5, 7–9) it is not surprising that many transplantable tumors are not identical with spontaneous or primary induced tumors in the same species (3), and it is no longer possible to affirm categorically that all transplantable tumors have lost "a multitude of pertinent features" because of the "foreignness of the tumor explant" (1). There are now available transplantable rat hepatomas showing fewer biochemical and morphological deviations[1] from parenchymal liver cells than any primary hepatomas previously examined (6–10). The primary tumors from which they were derived were produced

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[1] The word *deviation* is used because it is a more general term than the word *deletion*, which was introduced by Miller and Miller (4) and which implies loss of specific properties (8). Deviation is intended to be synonymous with the word change and is not committed to any particular mechanism of change. The terms are compatible with the concept of successive somatic mutations, which could conceivably result in either loss, gain, or mere alteration of specific cell properties. Operationally, one can assay the tumors for enzyme activity under standard conditions or one can assay for the ability to produce increased enzyme activity in response to some kind of a stimulus. The "Deletion Hypothesis" is extremely useful as a guide for enzyme studies on minimal deviation tumors, since the loss of enzyme activity is much more readily detected than a slight change in physico-chemical properties or amino acid sequences in specific enzymes. The word *deviation* should be used in theoretical discussions, and the word *deletion* seems appropriate to describe actual situations where a given enzyme function appears to be lost. The words *addition* or *alteration* might also be reserved for actual examples.

either with a slowly acting carcinogen (6) or with a short intermittent exposure to a very active carcinogen (10). The Morris hepatoma 5123 (6) has been carried for 23 transplant generations in the highly inbred Buffalo strain of rats and has apparently maintained its biological, morphological, and biochemical characteristics. However, the primary tumor and the first transplants were not analyzed, and there may have been significant changes at any time after the primary event; of course the possibility of change in future generations cannot be denied. All of the transplants have grown slowly, but they are capable of invasion and metastasis and are able to kill the host in about 3 months (6). The tumor is now being carried in four parallel lines, and specimens have been placed in deep frozen storage to preserve the original strain if mutants should appear (H. P. Morris, personal communications). The Morris hepatoma 5123 may correspond to what Firminger (2) has referred to, on morphological grounds, as the "early" type of induced hepatoma, and it is possible that such tumors differ from the "later" hepatomas (2, p. 1430) in having fewer deviations from their cell of origin. The Morris hepatoma is one of a series of hepatomas that was surveyed in an attempt to find one with *minimal deviations*, since it was postulated (8) that many enzymatic changes seen in transplanted tumors are indeed irrelevant to the problem of carcinogenesis and that in these tumors it is impossible to separate the relevant from the irrelevant, a view not incompatible with the view of Day (1) quoted earlier. It was postulated that, if a hepatoma with minimal deviations possessed enzymes lacking in other hepatomas, the changes seen in the other hepatomas could not be claimed as essential to the carcinogenic process. By the same line of reasoning, a change seen in an "early" tumor cannot be claimed as essential to carcinogenesis until meaningful metabolic patterns have been demonstrated and until similar changes have been found in other "early" hepatomas.

In the survey mentioned (7–9) about ten different types of rat hepatoma were assayed for a variety of enzymes and exhibited a complete spectrum of enzyme patterns ranging from only a few deviations from normal liver to deviations so extensive that it has not been possible to state that the hepatoma cells were derived from liver cells. In particular the Novikoff hepatoma fell in this category and in addition contained deoxycytidylic deaminase, an enzyme that is considered to be associated with proliferating bile duct epithelium (7–9). It will be convenient to refer to the hepatomas that are most similar to liver as "minimal deviation" tumors and hepatomas that are very dissimilar from liver as "multiple deviation" tumors, recognizing that a tumor that is now minimal may be replaced by a tumor that represents a new minimum, and what is now a multiple deviation hepatoma may turn out to be derived from a different cell of origin.

Objective: carcinogenesis or chemotherapy?—The above studies suggest that, for understanding the specific mechanisms of carcinogenesis, we need to identify with certainty the cell of origin and to have cancers that differ as little as possible from their normal progenitors in order to narrow the number of possible irrelevant changes. The Morris 5123 hepatoma (5) and the Reuber hepatoma (10) appear to be examples of such minimal-deviation tumors and should be useful progenitors for studying the possibility of successive deviations all the way to the multiple-deviation type. At present, the "multiple-deviation" hepatomas such as the Novikoff present uncertainty, because their cells of origin are not established. This criticism applies to the vast majority of transplantable tumors, as well as to a large number of primary tumors in animals and in humans. Such cancers may present great difficulties if used to try to unravel specific mechanisms of carcinogenesis. On the other hand they represent *de facto* enzyme patterns that must be coped with in the realm of chemotherapy; and their enzyme patterns are legitimate objects of inquiry, especially in relation to correlations between the sensitivity and resistance to specific drugs and the enzymes that interact with the drugs (3).

Minimal-deviation tumors as standards.—Transplantable cancers such as the Morris 5123 constitute standards that can be produced in quantity, distributed widely, monitored for constancy, and compared with other minimal-deviation tumors derived from the same type of cell. They constitute a standard against which primary hepatomas, both human and rodent, can be compared. If a sufficient number of minimal-deviation hepatomas are characterized enzymatically, biochemists may find the least common denominators of enzymatic change that are associated with the carcinogenic process. Such studies are at present under way in several biochemical laboratories in collaboration with Dr. H. P. Morris. If minimal deviation tumors can be induced under reproducible conditions it will be possible to test not only the primary tumors but also the subsequent transplant generations from the identical tumor in order to learn whether the transplant line is stable or unstable.

It is suggested that the recommendations by Day (2) should be modulated slightly in that ex-

perimentalists should not merely rely upon "the large variety of carcinogenic agents for providing themselves with animal tumors while they are waiting for a wide spectrum of spontaneous tumors to become commercially available." They should determine the lowest dose of known carcinogens, as well as the mode of administration that will induce minimal deviation tumors in specific tissues when given with the proper diet to highly inbred animals of specified strain and sex. The resulting tumors should then be transplanted and tested for evidences of progression. The emphasis should be on reproducibility and understanding, not variety, at this stage. If the specific enzymatic defect in only *one* type of cancer could be established it would be worth more than the cataloging of hundreds of examples. It will serve no purpose to study cancers that may never be seen again, to produce data that are not subject to confirmation, to leave the escape hatch of uniqueness open to the careless investigator. This is the grave danger with specimens of human tumor material, as indeed it is with primary animal tumors.

Minimal-deviation primary tumors from various cells of origin should be looked for and should be transplanted in highly inbred animals of the same strain. It is postulated that such transplantable tumors can serve as the parent strains for multiple-deviation tumors and can become standardized as to growth rate, morphology, enzymatic pattern, and chemotherapeutic response. They can become the standards and the tools by which we can guide studies on the control and cure of human cancer.

The keystone of science is reproducibility of experiments. If cancers that were identical at the molecular level could be identified in human patients or produced at will in animals there might be no need for transplantable tumors. Until that day, at least some experimentalists should devote their energy to perfecting and using the only standard that we have—the transplantable animal tumor.

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