THE CARCINOGENIC EFFECT OF METHYLCHOLANTHRENE AND OF TAR ON RABBIT PAPILLOMAS DUE TO A VIRUS[1]

WESTERN cottontail rabbits frequently carry on the skin large epidermal papillomas caused by a virus,[2] which have the immediate character of neoplasms.[3] The growths closely resemble in structure and behavior the papillomas of unknown cause which are elicited on rabbit skin by tar and methylcholanthrene,[4] and after growing for a while they sometimes become carcinomatous,[5] as do these latter, undergoing histological changes of the same sort then, with result in malignant tumors of like kind. As bearing on the theoretical possibilities, it has seemed desirable to learn whether chemical carcinogens will cause virus papillomas to become cancerous.[6]

Two groups of domestic rabbits were inoculated with papilloma virus from different sources by rubbing it into four to six scarified areas of skin; and as soon as healing had taken place tar and 0.3 per cent. methylcholanthrene[7] were applied to some of the inoculated areas, while others on the same animals were painted with the solvent as such, or with a mixture in equal parts of turpentine and acetone which had been previously tested and found mildly irritant and non-carcinogenic for normal rabbit skin.[8] Untreated papillomatous areas served as further controls. The applications were repeated thrice weekly and were kept up for 2 to 4½ months, with stripping away from time to time of the keratinized layer overlying all the growths. Fifteen rabbits survived to the end of the tests. In no instance did cancer develop from the untreated papillomas or from those receiving ether and paraffin oil or turpentine and acetone, though this last mixture induced notably vigorous proliferation of the growths. In contrast with these results, malignant changes not infrequently occurred in the papillomas to which tar was applied, and they took place regularly and with unprecedented rapidity in those receiving methylcholanthrene. Under the influence of this last numerous cancers often arose in a single papillomatous mass, and some had exceeded a centimeter in diameter by the 63rd day after virus inoculation, that is to say, by the 55th day after papillomas had first become visible in the gross. In one instance a metastasis 1.2 cm in diameter was found in a regional lymph node on the 71st day. Sections of it disclosed squamous cell carcinomatosis like that already present at several spots amidst the papillomatous masses treated with tar and methylcholanthrene.

The cancers derived directly from the virus-infected cells, as do those which arise eventually in the ordinary course of events. Any interference which stimulates these cells may hasten malignancy[5]—a fact which holds true of tar papillomas also.[4] The question comes up of whether the chemical carcinogens did not act in this non-specific way in the present experiments; for they induced a more vigorous proliferation than occurred in the control growths. But highly effective non-specific stimulation falls far short of methylcholanthrene in bringing on malignant changes. During several years we have subjected virus papillomas to various stimulative procedures with the aim of procuring cancers as soon as possible, but have never succeeded in reducing the interval between inoculation and cancer to less than 4 months, and usually it has amounted to several months more. The repeated injection under papillomas of Scharlach R in olive oil, a non-carcinogenic mixture as the experience of many workers has shown, results in extraordinarily exuberant proliferation, and the appearance of cancer is often hastened, though not nearly so much as in the present experiments in which stimulation was less pronounced. The tar brought on fewer cancers than did methylcholanthrene, a finding which accords with the known carcinogenic effectiveness of the two agents.[9] These had been applied, not only to the virus papillomas but to the skin of the animals, and here by the end of the tests they had frequently evoked benign tumors of the sorts they usually call forth.

Of the known factors concerned in the production of the cancers the virus was obviously the most responsible and effective. Methylcholanthrene as such, when applied to the skin of domestic rabbits, does not give rise to visible papillomas for nearly two months at least—a fact exemplified in the present animals—and usually they do not appear until much later, while

[1] Work done with the aid of a fund from the Staff of Public School No. 158, Borough of Brooklyn, New York City.

[2] R. E. Shope, *Jour. Exp. Med.*, 58: 607, 1933.

[3] P. Rous and J. W. Beard, *Jour. Exp. Med.*, 60: 701, 723, 741, 1934.

[4] P. Rous and J. G. Kidd, *Jour. Exp. Med.*, 69: 399, 1939.

[5] P. Rous, J. W. Beard and J. G. Kidd, *Jour. Exp. Med.*, 64: 401, 1936.

[6] Green has injected methylcholanthrene under virus papillomas with negative results (H. N. Green, Report of the Yorkshire Council of the British Empire Cancer Campaign, Hunters Armley, Ltd., Leeds, 1940–41, 13).

[7] The methylcholanthrene (Eastman Kodak Company) was dissolved in ether containing 2 per cent. of mineral oil. For some of the tests the tar was diluted with this solvent in a 1 in 10 proportion. It came from the Ostergasfabrik of Amsterdam—a gift from Dr. Karl Landsteiner.

[8] P. Rous and J. G. Kidd, *Jour. Exp. Med.*, 73: 365, 1941.

[9] J. W. Cook, G. A. D. Haslewood, C. L. Hewett, I. Hieger, E. L. Kennaway and W. V. Mayneord, *Am. Jour. Cancer*, 29: 219, 1937.

cancer supervenes only after 9 months to 2 years, and infrequently then.[10] The tar we employed sometimes elicits papillomas after a few weeks, being stimulative as well as oncogenic,[4] but cancer is a very late and rare occurrence. The virus, in contrast, causes papillomas at once, and if nothing further be done and the growths prosper (for some retrogress), they nearly always become cancerous after 6 months to a year. The chemical carcinogens hurried along a process which would have occurred anyhow.

The skin papillomas and cancers of unknown cause which are evoked by tar and benzpyrene undergo singular alterations when they are experimentally infected with the papilloma virus.[11] Most of the papillomas suddenly begin to grow with great rapidity, and many of them alter histologically in ways more or less clearly indicative of the presence of the virus, with result in widely diversified, papillomatous neoplasms. Not a few of the tar papillomas which were previously benign, and which would have disappeared if let alone, change at once to squamous cell carcinomas; and tumors which were of the latter sort at time of infection may suddenly start to grow very fast and undergo cytological changes referable to the virus. Evidently the actuating cause or causes for the tumors evoked by the chemical carcinogens works in concert with the virus to cause these phenomena. The question arises of whether a similar joint action, with the association occurring in reverse order, may not have been responsible for the present findings. The facts give no support to this hypothesis. The application of tar and methylcholanthrene to the virus-infected cells did not result in the highly diversified and rapidly growing papillomatous neoplasms mentioned above, and such cancers as arose wholly resembled those ordinarily developing, in histology and behavior.

It seemed possible that the virus might have exerted so dominating an influence upon the cells with which it was already associated as to obscure the effects of any superimposed neoplastic changes referable to the carcinogens. The changes produced by the latter in the uninfected epidermis of the test rabbits assume importance in this relation; for the response of rabbit skin to the chemical carcinogens, as expressed in growths elicited, varies widely from animal to animal, and the local findings provide an index to the general potentialities of the tissue.

To learn the responsiveness of the skin of our rabbits the carcinogens were applied to areas situated immediately next virus papillomas that were similarly treated, or opposite them on the other side of the belly. The range of response proved great, many tar and methylcholanthrene papillomas developing on the skin of some of the animals after relatively brief exposure to the carcinogens, while in other cases none appeared throughout the period of the applications. The happenings in the virus papillomas varied independently of the cutaneous phenomena. Some of the rabbits which had most cancers developing from the virus-infected epidermal cells were nearly or quite free from skin tumors due to tar or methylcholanthrene, whereas the animal in which cancer appeared last of all, and then at one spot only, had skin notably responsive to the chemical carcinogens, numerous papillomas arising early where they were painted on. These results, like the character of the growths arising from the virus papillomas, speak against the possibility that the chemical carcinogens acted by bringing about neoplastic changes additional to those which the virus-infected cells would have undergone in the ordinary course of events.

The influence of many carcinogens, even those of widely differing character (as, *e.g.*, ultraviolet light and methylcholanthrene, beta radiation and benzpyrene), can be combined or summated; and within limits one such agent can often be substituted for another with result in tumors of the usual sorts. Conceivably the malignant alterations in the virus papillomas were due to some such process, the chemical carcinogens and the virus acting in a similar way, and together, to elicit cancers due intrinsically to neither of them. One would have to suppose, though, that the epidermal cells infected with the virus were so altered thereby that those least responsive to the influence of tar and methylcholanthrene under ordinary circumstances were now often markedly susceptible and *vice versa*.

The role of the virus in the carcinogenesis remains to be considered. It impelled the cells to a lively neoplastic proliferation throughout the period while tar and methylcholanthrene were applied to them, and many of the derivative cancers had a histology[4] which indicated its continued morphological influence. The possibility has to be thought upon that the chemical carcinogens acted by influencing the virus, either directly or through induced pathological alterations in the cells which are its milieu and medium of expression. Many facts point to virus variation as the cause for the changes in cell behavior and morphology occurring when cancers arise spontaneously from virus papillomas.[12]

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[10] Unpublished personal experience.

[11] P. Rous and J. G. Kidd, *Jour. Exp. Med.*, 67: 399, 1938; *ibid.*, 68: 529, 1938; *ibid.*, 71: 787, 1940; A. Lacassagne and W. Nyka, *Bull. Assn. franc. étude cancer*, 26: 154, 1937; J. McIntosh, *17th Ann. Rep. Brit. Empire Cancer Campaign*, London, 1940, 44.

[12] J. G. Kidd and P. Rous, *Jour. Exp. Med.*, 71: 469, 1940.