hexoxidase is precipitated by saturated $(NH_4)_2SO_4$ solution; it oxidizes not more than about 25% of the substrate, whereas the enzyme of the squash oxidizes 100% very rapidly. Moreover, the kinetics of our preparation point to the presence of a single enzyme. Substances thus far tested, such as cysteine, tyrosine, glutathione, and phenols, are not affected. We suggest, therefore, that the enzyme responsible be designated "ascorbic acid oxidase".

It requires the presence of atmospheric oxygen, which plays the rôle of hydrogen acceptor. The oxidized ascorbic acid may be reduced to its original state by hydrogen sulphide. The enzyme is remarkably stable to dialysis, oxygen and carbon monoxid. Hydrogen sulphide, however, inactivates it.

For ascorbic acid estimation, Tillmans and associates'[2] 2,6-dichlorobenzenoneindophenol method was employed.

7770 P

Carcinomatous Changes in Virus-induced Papillomas of the Skin of the Rabbit.

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The Shope rabbit papilloma, a skin growth caused by a virus,[1] has been shown to possess the characters whereby tumors are recognized.[2] When given opportunity, as on implantation within the host, the growth frequently looks and behaves like a malignant neoplasm. The present report is concerned with instances in which skin papillomas caused by the virus have spontaneously become carcinomatous. The change has been noted in 5 of 10 domestic rabbits with growths existing 4 to 8 months.

During the early weeks of its development after virus inoculation the papilloma enlarges laterally, but later it is restricted by scar tissue and builds outwards only. At first it overlies the skin appendages, but these disappear after a time and it becomes bedded somewhat more deeply. The malignant change may first attract attention when a fissure exuding serosanguineous fluid opens in the midst

[2] Tillmans, J., Hirsch, P., and Hirsch, W., *Z. Untersuch. Lebensmittel*, 1932, **63**, 1.

[1] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

[2] Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701.

of the papilloma; but more often there occurs a generalized, fleshy, discoid thickening of the base of the growth, which gradually raises the jagged, dry, papillomatous tissue some millimeters above the skin surface, and also bulges downwards. Soon the animal gnaws at this portion of the growth, opening in the one case a depressed ulceration with firm, gristly walls, and in the other laying bare a high, fungoid mass. On biopsy a squamous cell carcinoma is found, or an invasive, papillomatous, epithelial tumor, or most frequently, the 2 intermixed, with every gradation between them. The growth may for some weeks remain circumscribed and fungating, or it may rapidly extend under the skin, involving it and the muscle and becoming fixed upon the deep tissues. Some cancers less than 2 months old are already 5 cm. across and 2-3 cm. deep. As a rule they become infected with pus-producing organisms and the health of the host suffers, though most of our rabbits are still alive. Metastasis to a regional lymph-node (confirmed by section) has occurred once. Transplantation to the leg muscle of the host has resulted in a secondary, highly invasive, squamous cell carcinoma.

In rabbits carrying numerous discrete papillomas, as result of tattooing a virus fluid into the skin at many spots, nearly all of the growths undergo malignant changes within a few weeks of one another. The course of events has been followed by frequent biopsies. For a long time macrophages, lymphocytes and plasma cells have been assembling beneath the growth, and concurrently its epithelial folds have become less evenly ranked, its cells progressively more disordered in arrangement, and here and there proliferating tongues of them have thrust down a little further than the generality. Melanomatous growths cease to be pigmented. Frank invasion of the underlying tissue often begins at several places at once, and it occurs as a rule at points where focal bacterial infection has given rise to reactive connective tissue proliferation. The papillomatous structure may be retained even when the tumor has pushed down into the muscle; but more often the advancing processes progressively break up into irregular strands and groups of cells, and the growth becomes a frank, squamous cell carcinoma.

The virus engendering the Shope papilloma must be held primarily responsible for the carcinomas as well, but their proximal cause remains to be determined. In our previous work[3] the observation was several times made that newly engendered papillomas implanted in the leg muscles of the host promptly assumed the form of squamous cell carcinomas and invaded and replaced the indi-

[3] Rous and Beard, *loc. cit.*

vidual muscle fibres. This happened when there was associated with the growth a reactive connective tissue proliferation due to contaminating bacteria. It was plain that from an early period the cells of the papilloma possessed potentialities for malignant behavior. But rabbits appropriately tarred may develop carcinoma of the ear within 2 weeks;[4] and yet tar cannot be considered as the proximal cause of the cancer. Even if the Shope virus gave rise forthwith to carcinoma the view would still be tenable that it had done no more than provide the conditions requisite to a cancerous change of unknown cause. At the moment this much only seems certain: The papilloma virus gives rise to skin growths which for some time are benign tumors, though their cells have malignant potentialities. Gradually, by alterations which involve no discontinuity of form or behavior, the growths take on the character of typical carcinomas. Bacterial infection frequently acts to precipitate the change.

7771 C

Sanitary Significance of the Succession of Colon-Aerogenes Organisms in Feces.

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A study has been made of 57 specimens of feces from normal humans, from which by direct plating, 1454 strains of bacteria have been isolated. Eight of the 57 were stored at 37°C. and plated 60 times during the period of their viability, 2 to 3 months, yielding 592 strains. Duplicates of these 8 and 4 others of the 57 were kept in the ice box and on 162 platings have yielded 1086 strains. The period of viability for cold stored suspensions varies but may exceed 15 months. The feces were suspended in sterile saline to a heavy turbidity and platings have been made from these suspensions. The procedure diagrammed has been adopted. It provides for adequate purification of strains isolated, permits a comparison of the reactions of purified and original strains, and utilizes the tests now considered most significant for the group. Recognition of lactose-deficient organisms in normal feces[1] has led to the inclusion in the series of all organisms typical of the group which ferment dextrose.

[4] Bittman, O., *Z. f. Krebsforsch.*, 1925, **22**, 278.

[1] Parr, L. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1019.