changes have been noted in 4 of these cultures after the 15th but not after the 27th passage. Cytopathogenic changes observed in cultures of carcinomata do not lead to complete loss of culture. It is possible to preserve some cells and gradually regenerate the cell line. When changes occur early, between passages 1 and 7, it is not possible to save the culture. Seventeen cultures derived from bovine ocular squamous cell carcinoma are presently maintained. Average number of passages during more than 6 months is 25. In two cases 30 passages and in one case 43 passages have been possible in cell lines grown continuously for 10 months.

Summary. Behavior and properties of cells derived from bovine ocular squamous cell carcinoma grown *in vitro* are described. In these cells, unlike those from benign precursor lesions, plaque and pappilloma, cytoplasmic inclusions have seldom been observed. Multiple nuclei and margination of chromatin have been found only in rare instances. Changes, normally associated with cells of cultures of plaque and papilloma, have been found in cells from lesions diagnosed as transitional from plaque or papilloma to carcinoma.

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Carcinogenic Effects of Petroleum Asphalt.* (24906)

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Evidence indicates that some petroleum products are carcinogenic and that the carcinogenic effects of these products may vary with geographical source and refining procedures to which the crude oils have been subjected. Because of the enormous increase in use of petroleum asphalts, the question occurs as to whether these materials may be of importance in cancer causation. It is reported (1) that asphalt tonnage in the United States increased from 20,000 tons in 1902 to 9,000,000 tons in 1946. Interpretation of previous work in this field is often confused by using the terms pitch, tar and asphalt without defining precisely what each term means. An examination of the literature shows no reference to studies of carcinogenic effects of commercially manufactured petroleum asphalt, as defined here, used for paving, roofing, waterproofing, etc. This investigation seeks to answer the question: Is petroleum asphalt, as defined in the following paragraph and made from western United States crude oils, carcinogenic under the conditions of these experiments?

Methods. Asphalts, as used here, are (2): "Black to dark brown solid or semi-solid cementitious materials which gradually liquefy when heated, in which the predominating constituents are bitumens all of which occur in the solid or semi-solid form in nature or are obtained by refining petroleum, or are combinations of the bitumens mentioned with each other or with petroleum or derivatives thereof." "Bitumen is that portion of petroleum asphalt and tar products which will dissolve completely in carbon disulphide(3)." Manufactured, as contrasted with natural, asphalt is produced by distilling off the lower boiling point fractions of crude petroleum—gasoline,

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kerosene, and a variety of other oils—the residue is asphalt. In general, 2 methods of distillation are used. In steam-blown (straight-run) asphalt, distillation is accomplished in tubular stills. Distillation is aided by admitting steam and the lighter fractions are removed from the top of the column and the asphaltic fractions are continuously drawn off at the bottom. In this method of distillation, exposure to high temperatures which might result in cracking and alteration of the chemistry of the crude oil is avoided. The second method of distillation results in cracking and chemical alterations in the crude oil because of the high temperatures employed. Shell stills are used and hot air is blown into the material through perforated pipes. Such asphalts are known as "air-blown" or "oxidized." C-57 black mice, purchased from Rockland Farms, New City, N. Y., were the test animals used. The asphalt used is a pooled lot from 6 different samples supplied by Southern California refiners. Both steam and air-blown asphalts are represented in the pooled material. The mice have been exposed to asphalt in 2 ways: Group I, consisting originally of 68 mice (32 males and 36 females) have had asphalt, rendered liquid enough for handling by addition of benzene, applied with a glass rod to the skin of the interscapular region 2 times weekly. Group II, consisting of 63 mice (31 males and 32 females) were similarly treated with benzene alone. Group III, made up of 33 males and 29 females were injected subcutaneously in the interscapular region 2 times weekly with .2 cc of a 1% suspension of asphalt in olive oil. Frequency of injection was reduced to once weekly after 41 weeks because the volume of material under the skin became excessive. Group IV composed of 32 males and 28 females were treated as Group III except that olive oil alone was injected.

Results. Group I: There have been 12 epidermoid carcinomas form at site of painting. Fig. 1 A and B show the characteristics of the cancers in this group. Four(4) mice of this group are alive with well-developed papillomas and one mouse died with a prominent papilloma. Formation of the cancer was preceded by epilation, dryness, scaling and papilloma formation. Fifty-four weeks elapsed before appearance of the first epidermoid carcinoma. Slight epilation, dryness and scaling have been the only changes observed in Group II.

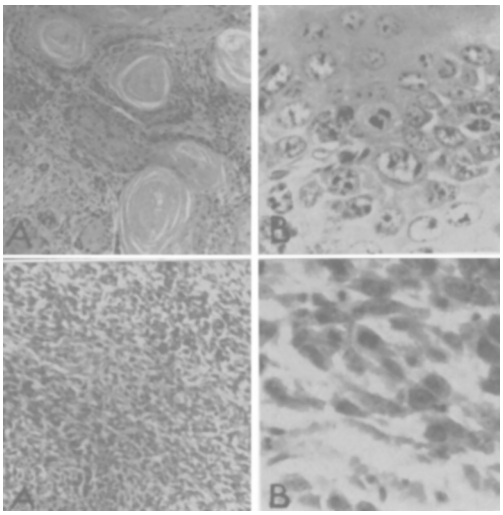

FIG. 1 (top). Epidermoid carcinoma, H and E stain. A, × 80; B, × 360.
FIG. 2 (bottom). Fibrosarcoma, H and E stain. A, × 160; B, × 720.

Group III: Eight sarcomas have occurred at site of injection. One of these is a rhabdomyosarcoma and the remainder are fibrosarcomas. Fig. 2, A and B show a representative example of these tumors. Thus far, distant metastasis has not been seen. One sarcoma has been successfully transplanted to a female C-57 black mouse. Thirty-six weeks of injection were required before occurrence of the first sarcoma. Thus far, Group IV has shown no evidence of tumor formation.

Discussion. These carcinomas and sarcomas conform to all of the conventional histological criteria for malignancy. One of the sarcomas has been successfully transplanted into a female mouse of the same strain. Transplantation has been attempted with but 2 of the sarcomas and one of the carcinomas. All tumors have occurred at site of treatment and no similar tumors have occurred in any of the control groups. One benzene-painted control died and microscopic examination of the organs disclosed leukemic infiltration of the salivary glands and the lungs.

Conclusions. A pooled sample of petroleum asphalt manufactured from western U. S.

crude oils contains a substance or substances which will cause epidermoid carcinomas when applied externally to the skin of C-57 black mice and sarcomas when injected subcutaneously into the same strain.

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Studies in Wound Healing. III. Contraction in Vit. C Deficiency. (24907)

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The closure of full thickness skin defects in mammals by spontaneous approximation of the wound edges—wound contraction—has been demonstrated to result from forces localized in the wound margins(4,8). Although the site of origin of the contraction process has been clearly identified, the mechanism remains obscure. Histologic study of the wound margin in the guinea pig has revealed an inactive dermis but a highly cellular area of proliferation of loose subdermal connective tissue (unpublished data), suggesting that the latter is the active layer within the margin. While our studies indicated that the collagen fibers composing the central mass of wound granulations were unrelated to the contraction process(4,8), they did not clarify the roles of individual components of the new forming connective tissue in the marginal area, nor did they exclude a possible function for new formed collagen fibers in this region. Experimental scurvy offers a method for partial dissection of components of connective tissue by inhibition of collagen formation(5,7,10). Abercrombie, Flint and James(1) stated that the contraction occurring in skin defects in scorbutic guinea pigs in the first 10 days after wounding did not differ significantly from that in normal animals. Woessner(9), on the other hand, concluded that "contraction took place normally in wounds of animals on ascorbic acid supplemented diets, but in scorbutic animals very little contraction took place." Edwards and Dunphy commented on conflicting reports of contraction in scurvy(3). This point is of obvious importance in determining relative roles of cells and fibers in the "picture frame" mechanism; we report here the results of systematic study of contraction in skin wounds in scorbutic guinea pigs.

Methods. Male guinea pigs weighing 300-400 g were used. The control animals were placed either on a standard laboratory diet of Purina pellets or on a MacDonald #5 Scorbutigenic Diet(2),‡ receiving supplementary Vit. A, B complex, D, and, in addition, ascorbic acid. Experimental animals were placed on the same diet omitting ascorbic acid for 7 and 12 days prior to wounding. Wounds 2 cm square were made on each side of the back through skin and panniculus carnosus. Wound outlines were traced initially and at intervals of one to 3 days. Areas were determined by planimetry. *Wounds without dressings.* For the control series of guinea pigs with undressed wounds and intact scabs, our previously published data(4) were used. In a second control series scabs were removed at

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