cortisone, the amount of radioactivity in aldosterone areas from "control" media was greater than that of right head kidney media. This radioactivity persisted despite 2 chromatographies as the free alcohol, and 2 as the diacetate. However, the magnitude of the differences between right head kidney and "control" results for aldosterone diminished after acetylation. These findings would indicate that either further purification is necessary to establish the identity of the aldosterone believed present or that the "control" tissue can synthesize this mineralocorticoid despite little or no ability to synthesize glucocorticoids. If radioactivity is indicative of aldosterone it raises the question as to whether aldosterone plays a role in electrolyte homeostasis in the fish.

Conclusions. With the above reservations in mind, the following tentative conclusions can be drawn. 1. It seems probable that cortisol, in plasma obtained from *Fundulus heteroclitus,* is synthesized by interrenal tissue of head kidney. 2. Production of cortisol and probably cortisone by both left and right head kidneys suggests that interrenal tissue is present in both, although in lesser amounts on the left. If this is so, no explanation can be given at present for slightly larger amounts of aldosterone produced by left head kidneys. 3. Interrenal tissue of Fundulus possesses enzyme systems, capable of converting progesterone-16-H^3 to tritiated cortisol, cortisone, and aldosterone through biosynthetic pathways not known.

The authors are grateful to Dr. P. K. Bondy and Dr. G. E. Pickford for help and encouragement, and to Albert Kuljian for technical assistance.

1. Chester Jones, I., Philipps, J. G., Holmes, W. N. *Symposium on Comparative Endocrinology,* John Wiley and Sons, 1959, in press.

2. Pickford, G. E., *Bull. Bingham Coll.,* 1953, vXIV, art. 2.

3. Pickford, G. E., Atz, J., *Physiology of Pituitary Gland of Fishes.* N. Y. Zool. Soc., 1957.

4. Chester Jones, I. *The Adrenal Cortex. C.U.P.* 1957.

5. Giroud, C. J. P., Stachenko, J., Piletta, P. *An International Symposium on Aldosterone.* Little Brown and Co., 1958.

6. Burton, R. B., Zaffaroni, A., Keutman, E. H., *J. Biol. Chem.,* 1951, v188, 763.

7. Bush, I. E., *Biochem. J.,* 1952, v50, 370.

8. Chen, P. S., Shedl, H. P., Rosenfeld, G., Bartter, F. C., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 683.

9. Bush, I. E., Ferguson, K. A., *J. Endocrin.,* 1953, v10, 1.

10. Hechter, O., Pincus, G., *Physiol. Rev.,* 1954, v34, 459.

11. Phillips, J. G., Chester Jones, I., *J. Endocrin.,* 1957, v16, iii.

Received April 3, 1959. P.S.E.B.M., 1959, v101.

Bovine Ocular Squamous Cell Carcinoma. III. Tissue Culture Studies of Carcinoma.* (24905)

J. A. SYKES, L. DMOCHOWSKI AND W. O. RUSSELL†‡

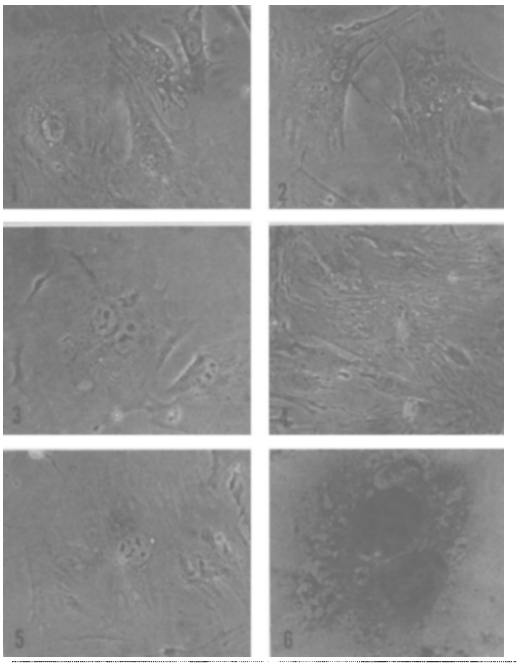
Section of Virology and Electron Microscopy, and Dept. of Pathology, University of Texas M. D. Anderson Hospital and Tumor Inst.; and Department of Microbiology, Baylor University College of Medicine, Houston, Texas

Genetic studies of bovine ocular squamous cell carcinoma have demonstrated relationship of incidence of this disease to age of animal and degree of ocular and circumocular pigmentation(1). Studies on pathological anatomy revealed presence of eosinophilic, cytoplasmic inclusion-like bodies in cells of both benign precursor and malignant lesions(2). Tissue culture studies of cells from benign precursor lesions, plaque and papilloma, have shown cytoplasmic changes and inclusions suggestive of presence of a virus(3,4,5,6). Dis-

* This work was supported by Research Grants from Nat. Cancer Inst., N.I.H., U.S.P.H.S.

† Invaluable cooperation of ranch owners in this study and of Dr. E. S. Wynne in histological diagnosis of specimens is gratefully acknowledged.

‡ Publication No. 9 of Cancer Eye Study Section.



Photographs were made with phase optics, Zeiss Microscope, Model No. GFL-65, using 35 mm camera attachment.

FIG. 1. Cells derived from bovine ocular squamous cell carcinoma. 3rd passage, 10 day S-M chamber culture. × 130.

FIG. 2. Cells showing cytoplasmic vacuoles with darkly refractile inclusion material. 3rd passage, 10 day S-M chamber culture. × 130.

FIG. 3. Cell showing "crystalline" cytoplasmic inclusion. 8th passage, 6 day S-M chamber culture. × 130.

FIG. 4. Large cell showing well developed tonofibrils. 4th passage, 7 day S-M chamber culture. × 130.

FIG. 5. Large cell showing fine granulation of cytoplasm in perinuclear region. 3rd passage, 10 day S-M chamber culture. × 130.

FIG. 6. May-Gruenwald-Giemsa stained very large cell from 7th passage, 3 day S-M chamber culture of bovine ocular squamous cell carcinoma, showing bizarre pattern of nucleoli. × 325.

appearance, reappearance and bi-laterality of benign precursor lesions may also be suggestive of a viral agent(7). These findings led to tissue culture study of the final, malignant, stage of the disease to determine whether cellular changes similar to those reported in benign precursor lesions are present in cells of bovine squamous cell carcinoma.

Materials and methods. Methods for removal of lesions in the field, their treatment for transportation to and subsequent treatment in the laboratory; also detailed study of cellular outgrowths from benign precursor lesions have already been described(3,4,5,6).

Results. Phase contrast microscopy of cells from carcinoma grown in specially designed chambers(8) has extended and confirmed earlier observations on stained tissue culture preparations in T-30 culture flasks made at low magnifications by bright field microscopy (3,4). Cells derived from carcinomata are epithelioid in character and of 2 sizes (Fig. 1). Large cells measure as much as 0.04 mm in diameter and small cells are of 0.01 mm average diameter. Multinucleated cells occur much less frequently than in cultures derived from benign precursor lesions. Vacuolization of cytoplasm, when encountered (Fig. 2), commonly precedes occurrence of inclusions in the cytoplasm and death of culture. Cytoplasmic inclusions, occasionally observed, are of two types, one preceded by vacuoles, the other irregular in outline and not associated with vacuolization (Fig. 3). The inclusions resemble those found in cultures of cells from papillomata. Cytoplasm of cells derived from squamous cell carcinomata commonly shows well developed tono-fibrils (Fig. 4) and much finer perinuclear granulation (Fig. 5) than that found in cells derived from plaque and papilloma. Occasionally an aggregation of granules occurs in the cytoplasm and is concentrated in the perinuclear region. Cytoplasmic bridging is well marked. Multiple nuclei even in large cells are uncommon. Nucleoli are usually multiple and may exhibit bizarre patterns (Fig. 6). Margination of chromatin, if it occurs, is not definite as in plaque lesion cultures. Cultures derived from carcinomata are stable, number of large and small cells showing little variation over long periods of time and many subcultures. A total of 52 carcinomata have been cultured. Thirty have survived 4 months or less with a maximum of 11 passages. Cytopathogenic changes have occurred in 3 of these cultures between passages one and nine. Twelve cultures have survived for 5 to 8 months with a maximum of 28 passages; 10 of these cultures have shown cytopathogenic changes between passage 4 and 14. Five cultures have been maintained for more than 12 months and 43 passages. Cytopathogenic

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.

1. Anderson, D. E., Chambers, D., Lush, J. L., *J. Animal Sci.,* 1957, v16, 1007.

2. Russell, W. O., Wynne, E. S., Loquvam, G., *Cancer,* 1956, v9, 1.

3. Sykes, J. A., Dmochowski, L., Wynne, E. S., Russell, W. O., *Proc. Tiss. Cult. Assn.,* 1958, 16, Abst.

4. Dmochowski, L., Sykes, J. A., Wynne, E. S., Russell, W. O., *Am. J. Path.,* 1958, v34, 602, Abst.

5. Sykes, J. A., Dmochowski, L., Russell, W. O., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 527.

6. ———, *ibid.,* 1959, v101, 192.

7. Wynne, E. S., personal communication.

8. Sykes, J. A., Mocre, E. B., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 125.

Received April 6, 1959. P.S.E.B.M., 1959, v101.

Carcinogenic Effects of Petroleum Asphalt.* (24906)

M. H. SIMMERS, EILEEN PODOLAK AND RIOJUN KINOSITA

Oncology Lab., College of Osteopathic Physicians and Surgeons, Los Angeles, Calif.

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,

* This investigation was supported by Research Grant, Dept. of H.E.W., P.H.S., Nat. Inst. of Health.