Studies on a Virus ("Polyoma") Inducing Multiple Tumors in Animals.* (25323)

LEON DMOCHOWSKI, CLIFFORD E. GREY AND LYMAN A. MAGEE†
Section of Virology and Electron Microscopy, University of Texas M. D. Anderson Hospital and Tumor Inst.; Dept. of Microbiology, Baylor University College of Medicine, Houston

Induction of leukemia, parotid gland and mammary tumors, and subcutaneous sarcomas in mice by cell-free preparations of mouse leukemic organs was first reported by Gross (1,2). Stewart(3,4,5) induced by similar means in mice neoplasms of similar types and tumors of adrenal, submaxillary, sublingual and exorbital lacrimal glands. These observations have been confirmed by a number of workers(6). Gross(7) induced leukemia, parotid gland tumors and/or sarcomas with cell-free extracts of parotid gland neoplasms. Although Stewart, Eddy *et al.*(8) failed to obtain similar results, they induced with extracts of the same tumors or leukemic organs of mice, following passage on monkey kidney or on mouse (NIH-Swiss) embryo cultures, with exception of leukemia, a great variety of tumors in mice and hamsters(8-10). The name "polyoma" virus has been proposed for this agent which induces cytopathogenic changes in monkey kidney and mouse embryo cultures (11) and agglutinates red blood cells of guinea pig, hamster, rabbit, mouse and man(12). The virus produces both proliferative and degenerative changes(13) and infected animals develop hemagglutination inhibition(12) and complement fixing antibodies(14). The observations of Gross and Stewart have been confirmed and extended to other strains of mice and rats(15-18). The question arose whether a number or only one virus(19) is responsible for the multiplicity of tumors. Separation of leukemia-inducing virus from parotid tumor-inducing virus, first reported by Gross(2), has been confirmed(8,15). Leukemia in mice comprises a number of histological types(20), and at least some of them are induced by different viruses, as there is no immunological relationship between leukemia-inducing agent of Gross or of Schwartz(21) and that of Friend(22-23). The relationship of parotid gland tumor virus of Gross and the agent described by Stewart is not clear and requires elucidation. The present studies were undertaken to characterize any submicroscopic changes and specific structures which may be found in tissues infected with polyoma virus and in tumors induced by this agent and to compare them with those already reported in parotid gland tumors induced by cell-free preparations of leukemic organs from mice with leukemia of Gross(24-25).

Materials and methods. Several strains of polyoma virus were obtained through the courtesy of Dr. Sarah E. Stewart, as lyophilized tissue culture fluids. For tissue culture embryos of mice of the following strains were used, NIH (Swiss) supplied by Dr. Stewart, Af/Dm, C3Hf/An, C3Hf/Gr, and C3HZb/Bi. Minced embryos were treated for 20 minutes at room temperature with minimal volume of trypsin solution (0.25% Difco Bacto Trypsin in Ca and Mg free Hanks' B.S.S.), centrifuged, washed in B.S.S., resuspended in Eagle's basal medium containing 20% inactivated horse serum and dispensed in 2 ounce prescription bottles. After good growth was obtained, medium containing 2% horse serum was used as maintenance medium to which polyoma suspension was added. Fluids from cultures of embryos showing cytopathogenic changes were tested for hemagglutination of guinea pig red blood cells and for tumor-inducing activity by subcutaneous inoculation in newborn hamsters (0.2 ml doses) and in newborn NIH (Swiss) mice (0.1 ml doses). Cells of cultures showing cytopathogenic changes and tumors induced by culture fluids were studied in ultrathin sec-

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† Rosalie B. Hite Fellow in Cancer Research. Present address: Dept. of Microbiology, University of Mississippi, Jackson.

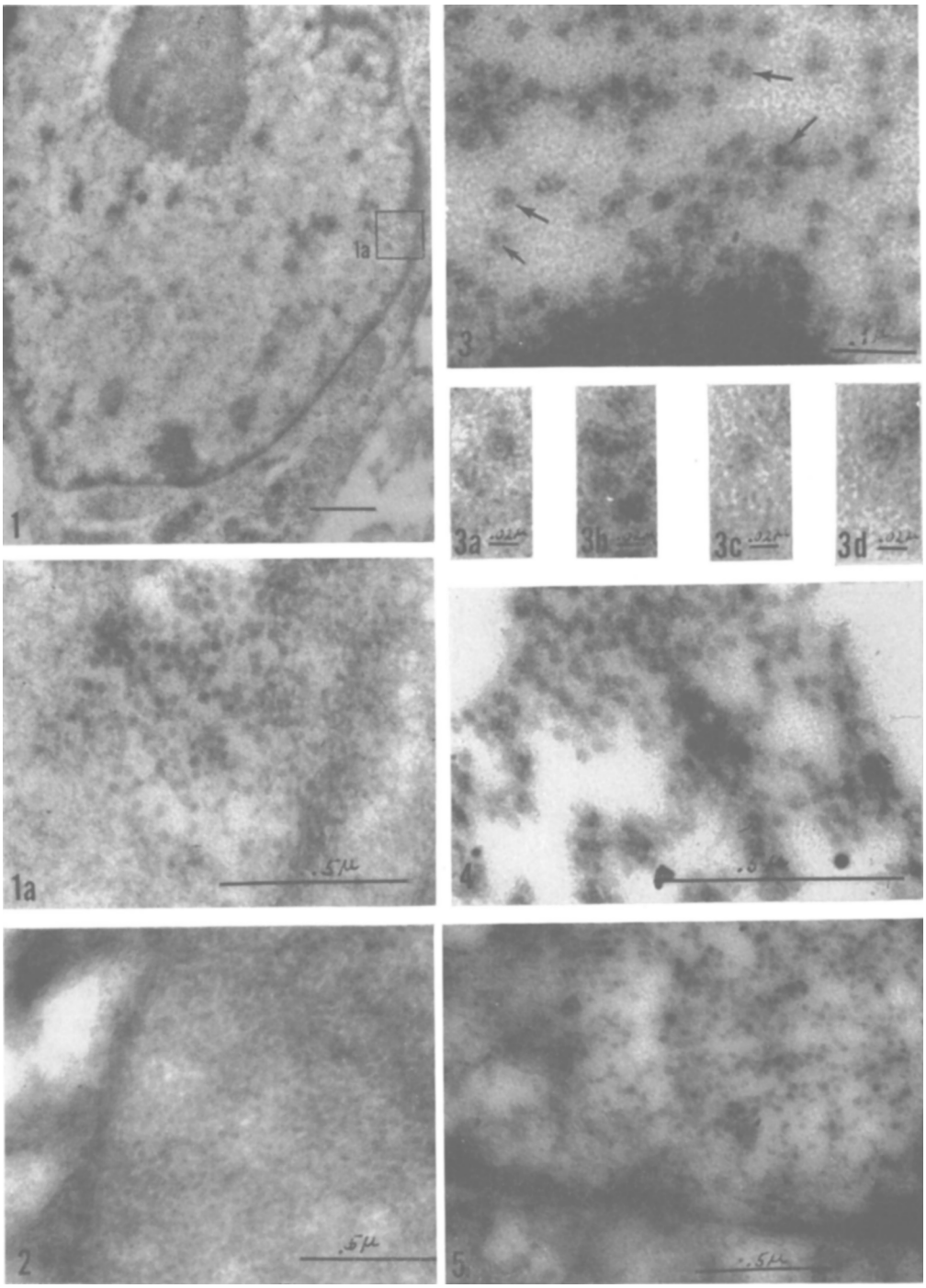

FIG. 1. Electron micrograph of section of a cell of Af strain mouse embryo grown *in vitro*, treated with polyoma virus. Particles scattered throughout nucleus and around nucleolus. × 10,000.

FIG. 1a. Part of Fig. 1 at higher magnification, showing characteristic particles near nuclear membrane. × 56,000.

FIG. 2. Part of nucleus of a cell from NIH (Swiss) mouse embryo culture, treated with polyoma virus, showing closely packed characteristic particles. × 40,000.

tions by means of RCA EMU-3E electron microscope. The method of tissue preparation for study in ultrathin sections was the same as previously described(24).

Results. Cytopathogenic changes, as described by Eddy *et al.*(11) have regularly been observed within 4 to 7 days in cultures of embryonic tissues of NIH (Swiss), Af/Dm, C3Hf/An, C3Hf/Gr, and C3HZb/Bi strain mice. Fluids from cultures of embryos from all strains of mice with cytopathogenic changes hemagglutinated guina pig red cells in variable titers of 1:80 to 1:2,560. Fluids from cultures of NIH (Swiss) and Af/Dm embryo mice, so far tested, induced within 2-3 months tumors in 5 out of 5 and 4 out of 4 surviving hamsters (sarcoma of the kidney and heart, lung adenocarcinoma, thymoma, hemorrhagic disease), and in aproximately 60% of surviving NIH (Swiss) mice, dwarfism, parotid gland and mammary tumors, and hemorrhagic disease.

An electron microscope study of sections of NIH (Swiss) and Af/Dm strain mouse embryo cells showing cytopathogenic changes revealed the presence of virus-like particles in many cells, chiefly in the nucleus, occasionally in the cytoplasm and outside the cells. The particles measure approximately 270 Å in diameter and are of low electron density. They may be scattered throughout nucleoplasm (Fig. 1 and 1a) or completely fill the nucleus (Fig. 2). At higher magnification, in some particles, an internal structure may be seen composed of a more dense center, measuring approximately 50 Å and surrounded by a less dense zone with outlines of a limiting membrane (Fig. 3, 3a, b, c, and d). Particles outside cells are of similar size and appearance (Fig. 4). Examination of cells grown *in vitro* from embryos of NIH and Af/Dm strain mice, untreated and maintained in tissue culture for similar intervals of time, failed to reveal any characteristic particles. Fluids from these cultures failed to hemagglutinate guinea pig red cells. In parotid gland(Fig. 5) and in mammary tumors induced by polyoma infected culture fluids, the nuclei and cytoplasm of cells showed characteristic particles observed in cells of infected tissue cultures. It is of interest that mammary tumors induced in NIH (Swiss) mice by polyoma virus have been found histologically different from mammary tumors induced by Bittner virus. The cells of these tumors progress from epithelial in character to fibroblast-like fusiform cells. The tumors contained only particles of 270 Å in size, but none of the larger size particles (900 Å) originally observed in mammary tumore of mice, known to harbor Bittner virus and developing, although at a much later age, a high incidence of mammary cancer(26).

Discussion. The parotid gland tumor agent of Gross and polyoma are known to share some physical properties, such as resistance to ether(2,7,12) and heating(2,12,27). Neutralization of the parotid gland tumor virus of Gross by sera of mice immunized against tissue culture preparations of polyoma(28) indicates antigenic similarity, but not necessarily identity of parotid gland tumor virus of Gross and of polyoma virus. The part played by normal mouse proteins in this neutralization effect still remains to be explored.

Electron microscope studies of leukemic organs of mice with spontaneous lymphocytic leukemia and of mice with similar type of leukemia induced by cell-free preparations of leukemic organs of mice revealed virus-like particles, similar in size and appearance (570 Å to 1650 Å), within and outside the cytoplasm of cells(29-30). In leukemia induced by cell-free filtrates of leukemic organs of mice with x-ray induced leukemia, the size of particles was smaller (400 Å to 670 Å)(31). In parotid gland tumors of mice induced by cell-free preparations of leukemic organs of mice, virus-like particles uniform in size and aver-

FIG. 3. Part of nucleus of cell from a mammary tumor induced in a NIH (Swiss) mouse with tissue culture fluid of NIH mouse embryo culture infected with polyoma. Some of the particles show internal structure. × 120,000.

FIG. 3a, b, c, d. Some of the particles at higher magnification. × 195,000.

FIG. 4. Extracellular particles in infected Af strain mouse embryo cells grown *in vitro*. × 72,000.

FIG. 5. Part of a cell from a parotid gland tumor induced in a NIH (Swiss) mouse with tissue culture fluid of NIH mouse embryo culture infected with polyoma. Group of particles adjacent to nuclear membrane. × 40,000.

aging 650 Å have been observed within and outside the cytoplasm of cells(24). Particles similar in size and appearance have also been found in high-speed centrifugal pellets of extracts of mouse leukemic organs which induced an incidence of 11% of parotid gland tumors in C3H/Gr strain mice(24-25). Similar particles have been observed, in confirmatory findings, in spontaneous(32) and induced leukemia in mice(33). The size of particles present in cells of a parotid gland tumor was not uniform and ranged from 750 Å to 1100 Å(33). Particles of similar size have also been demonstrated in other types of leukemia (34-35) and in plasmocytoma(36).

It appears of interest that electron microscope studies of parotid gland tumors induced in C3H strain mice by cell-free preparations of leukemic tissues from mice with spontaneous leukemia, failed to reveal the characteristic particles of 270 Å, chiefly observed in the nucleus of parotid gland tumors induced in NIH (Swiss) mice by fluids from polyoma infected NIH (Swiss) and Af/Dm embryo cultures. Thus, particles in parotid gland tumors, induced in mice of different strains by various means, differ not only in size but also in their relationship to various cell constituents, although these tumors are of similar histological appearance. While the characteristic particles have been observed in nuclei of numerous cells of mouse embryos grown *in vitro* and infected with polyoma, similar particles have only infrequently been found in nuclei of parotid gland and mammary tumor cells induced in mice by polyoma infected tissue culture fluids. It is not possible at this time to describe the frequency of occurrence of these particles in tumors induced by polyoma, as an extensive examination of tumor cells has not as yet been carried out. Recent electron microscope studies of tissue culture fluids from mouse embryo cells on which extracts of a parotid gland tumor and of a neoplastic mesenteric lymph node, both originally obtained from leukemic mice of Gross, have been passaged, showed in dried and shadowed with chromium preparations particles of 400 Å to 450 Å in diameter(37). Size of these particles in solution was 520 Å to 620 Å(37). Correlation of size of these particles with that of particles in sections of parotid gland tumors induced by extracts of Gross' leukemia(25) is difficult at this time. They do appear to be more closely related in size than with particles of 270 Å now reported in parotid gland and mammary tumors induced by polyoma. Further studies are required to correlate values of particles reported in this study with values of active particles reported in filtration of tissue culture fluids infected with polyoma through gradocol membranes(11). Should further studies demonstrate that the particles are indeed the agent responsible for multiple tumors, they would represent the smallest tumor-inducing virus so far observed.

Summary. Electron microscope examination of ultrathin sections of mouse (NIH-Swiss and Af/Dm strain) embryo cells grown *in vitro* and showing cytopathogenic changes following passage of polyoma virus preparations has been carried out. Spherical particles of 270 Å in diameter and of low electron density have been observed chiefly in the nucleus, also in the cytoplasm and outside the cells. In some particles an internal structure could be discerned, composed of a more dense center of 50 Å in diameter, surrounded by an electron translucent outer zone with outlines of a limiting membrane. Similar particles have also been found in parotid gland neoplasms and mammary tumors induced in NIH (Swiss) mice by polyoma infected tissue culture fluids. Particles of this type have not been observed in uninfected control cultures.‡

‡ During preparation of this paper, the results of Banfield, W. G., Dawe, D. J., and Brindley, D. C., submitted to J. Nat. Cancer Inst., came to our notice. They have observed similar particles (270 Å) in cells of a murine lymphoma strain grown *in vitro* and treated with polyoma preparations. In addition, they observed particles similar in size to some of those observed by us in spontaneous and induced mouse leukemia.

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Human Studies of Biologic Availability of Niacin in Coffee.* (25324)

GRACE A. GOLDSMITH, O. NEAL MILLER, WALTER G. UNGLAUB AND KAREN KERCHEVAL
Nutrition-Metabolism Unit, Depts. of Medicine and Biochemistry, Tulane University, New Orleans, La.

Teply *et al.*(1) found that roasted coffee contained about 10 mg niacin/100 g as measured biologically with *L. arabinosis* and later confirmed by rat assay(2). Niacin content increases with degree of roasting; values range from 7 to 46 mg/100 g(2). Coffee has not been considered as a source of niacin in the human diet. In view of its wide use as a beverage, it seemed desirable to determine the biologic availability of niacin in coffee for man. Niacin contributed by coffee might explain certain inconsistencies in epidemiologic studies relating dietary intake of niacin and

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