propylthiouracil and radiothyroidectomy reduced salivary flow and increased salivary viscosity. This can be reversed and function restored by administration of thyroxine. Testosterone partially restores salivary flow. 2) These data provide one explanation for the mechanism of alteration in dental caries incidence in the rat following disturbance in thyroid function, although the relationship is probably far more complex than this.

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Isolation of Tumor Cell Colonies in Tissue Culture.* (24005)

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Marcus, Puck, and Cieciura(1,2) described methods for isolation of "clones" of HeLa cells in petri dish tissue culture. They emphasized the necessity for the presence of more than a single cell to obtain growth. Goldstein(3) pointed out that it is not possible with this procedure to be certain that colonies originated from a single cell. He described a method for isolation of clones by which all but a single cell in a Carrel flask are destroyed by local heat with electric soldering iron. However, this cannot be done if single isolated cells fail to grow. The present study was made in an attempt to obtain colonies of cells which failed to grow singly and to avoid the difficulty in microscopic observation of cells grown in a petri dish. The ascitic form of a mouse ependymoma grown in A mice(4) previously could not be propagated from single cells isolated from droplets in a micropipet and injected subcutaneously in suckling mice (unpublished data). This procedure was successfully used elsewhere for growth of single tumor cells in mice(5,6). Likewise our attempts to grow single cells in a variety of media in capillary pipets(7) or on cover slips failed. These ependymoma cells are readily cultivated on glass in tissue culture. Isolated colonies originating from single cells were obtained as follows.

Materials and methods. After good growth of cells was obtained at 37°C in Kolle flasks containing a casein hydrolysate medium(8) with 10% horse serum, the cells were removed from the glass by contact at 37°C with 0.25% trypsin, pH 8, for 2-3 minutes. Serial dilutions of cells were made in chicken plasma, one drop placed near one end of each of 7-8 rectangular cover slips (11 x 32 mm) contained on moist filter paper in a small petri dish. An attempt was made to have 1-20 cells/drop after dilution. A second drop of plasma containing several hundred cells was placed near the other end of each cover slip. A drop of filtered chick embryo extract (CEE) was added first to the plasma drop containing diluted cells. The drop was mixed (with a fine glass rod bent to form an L) and spread

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over approximately one-half the area of cover slip. The other drop with many cells was then mixed with CEE and spread over the remainder of the slip. All slips were examined under a microscope to determine number and distribution of cells. Those which contained too many cells or cells not well separated from each other were discarded. After fibrin clots were formed, each slip was placed in a flat-sided (Leighton) test tube so that the cells were not in contact with the tube. Each tube then received 1.5-2 ml of above culture medium and closed with a rubber stopper and incubated. Cultures were observed under the microscope daily and culture fluid was changed twice weekly. Vigorous growth usually was observed after 2-3 days on portions of the slips heavily seeded with cells. Growth of individual cells on lightly seeded areas of the slip occurred in 4-7 days. After 14-20 days grossly visible colonies of cells could be seen. To transfer a single isolated colony, the cover slip was washed twice with fresh medium and removed from its tube to an open petri dish. The colony to be selected was located by use of dissecting microscope. A small piece of sterile tissue paper held with fine-pointed forceps was pressed against the colony and then placed in a drop of fresh plasma on another cover slip. After coagulation of the drop with CEE, the cover slip was placed in a fresh tube with culture medium and was incubated. Further subcultures were made directly onto glass without plasma. After several months the cells were routinely maintained in Eagle's medium with 10% horse serum. For mass production, cells were grown in 50 ml of medium contained in 32 oz. (1 liter) prescription bottles placed on a flat side. The virus used was a mouse brain tissue culture passaged strain of Theiler's GDVII mouse encephalomyelitis virus. Virus titer were assayed by hemagglutination with human rbc. Chromosomes of cells grown on cover slips were stained with 1% orcein in 50% acetic acid.

Results. Twenty-one colonies of cells were isolated of which 5 labeled A, O, R, U, and W were maintained and compared. The first 4 were indistinguishable morphologically. The W strain differed from the others in that cells were more definitely polygonal in shape and were more widely separated from each other when grown on glass. The cells also tended to spread out more and did not initially form closely packed colonies. Approximately 4% of cells in the original line were polyploid; none of the isolated colonies was. The A, O, and W strains grew at approximately the same rate, while the U cells gave the best growth and the R cells the poorest. During continuous growth the maximal cell counts attained were 3.7, 1.5 and 0.7 million per ml respectively for U, A and R strains. In a 32 oz bottle containing 50 ml of fluid approximately 100 mg wet wt. of cells were produced by the A strain.

The 5 strains of cells differed in their capacity to support growth of GDVII virus. Virus was grown in approximately equal numbers of cells for 3 days in Leighton tubes and the pooled supernatant fluid from 3 tubes was titered. Hemagglutination titers were as follows: A - 64, O - 256, R - 128, U - 32, W - 0, one day mouse brain - 1024. The W strain did not produce any virus.

The parent culture of cells from which the colony strains were isolated was obtained from ascitic fluid. It retained the capacity after 3 months, *in vitro,* to grow intraperitoneally in mice and to produce solid tumor on the serous membranes. After isolated colonies were maintained, *in vitro,* for 9-10 months, attempts were made to pass them intraperitoneally in mice by injection of each animal with 2-3 million cells in 1 ml. Mice examined 3 weeks later had growth of solid tumors on the serous membranes and 1-4 ml of ascitic fluid containing tumor cells with A, O, and R strains of tumor. W cells formed solid tumors but only a few drops of ascitic fluid. Mice examined 3 and 9 weeks after injection with the U strain of cells had no remaining tumor cells found.

Discussion. Practically, cell variations may occur so rapidly that strictly homologous cell collections will not be obtained. However, if the limitations of the procedure are recognized, useful information may be obtained from studies of variant cell populations. The

derivation of cell variants can be a laborious task, particularly if individual isolated cells fail to grow. Growth of isolated cells in the environment of a nutritive colony as described here permits the development of colonies under direct observation. Detachment and wandering of cells is limited by the presence of plasma clots. Presumably each colony originated from a single cell. Because other cells are present in the culture it is not possible to be certain the colonies obtained are clones. At present there is no technic available which will obtain clones of cells which fail to grow as single isolated cells. In the meantime procedures of the type described here permit the derivation of cell populations which may differ considerably in regard to various characteristics.

Summary. A procedure is described for isolation of colonies of mammalian cells in tissue culture. Cell lines of a mouse ependymoma obtained in this way differed in growth characteristics, in capacity to grow, *in vivo*, and in susceptibility to Theiler's GDVII virus.

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Effect of Epinephrine on Adrenal Ascorbic Acid Following Premedication With Lysergic Acid Diethylamides or 5-Hydroxyindolalkylamines. (24006)

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Slight changes in molecular configuration of psychotomimetic drugs may alter their specific activity(1). Thus the interrelations between chemical structure and pharmacological properties of psychotomimetic analogs can be used as pharmacological tools to reveal biochemical mechanisms underlying behavioral changes induced by drugs. New findings have challenged the theories which propose serotonin antagonism as an explanatory clue for the mechanism of d-lysergic acid diethylamide (LSD) induced-psychosis(2). By comparing the pharmacology of d-2-brom LSD (BOL) and LSD it appears evident that increase of central sympathetic tonus following LSD injections fails to occur after BOL(1). It is, therefore, interesting to investigate the influence of various psychotomimetics on the actions of catecholamines on different neurohumoral mechanisms. Indolalkylamines are a class of chemicals including such psychotomimetic drugs as bufotenin(3). Lecomte demonstrated that serotonin sensitizes the nictitating membrane of the cat to epinephrine(4). Both bufotenin(5) and serotonin (6) can release epinephrine *in vivo* and the former is the more active compound. In contrast to bufotenin, serotonin does not cause hallucinatory experiences or behavioral changes. Thus, experiments were designed to follow changes in adrenal ascorbic acid content caused by epinephrine in rats premedicated with LSD, BOL, bufotenin and serotonin. In cats premedicated with indolalkylamines the variation of the contractive response of nictitating membrane to injected epinephrine was also investigated.

Method. Ascorbic acid was determined according to Roe and Kuether(7) on 350 Wistar rats decapitated 2 hours after intraperitoneal

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