of the spleen in animals to which PMAA was administered. These findings resemble those recorded after treatment with dextran sulfate (*6*).

Like the polysaccharide polysulfates, PMAA mobilizes lymphocytes from the reservoirs into blood. The majority of the mobilized small lymphocytes reach the blood through the lymphatic vessels.

The finding of a simultaneous increase of the lymphocyte count in the peripheral blood of the rats with thoracic duct cannulas indicates another route from the lymphatic tissues to the blood, as described earlier (*7*).

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8. We thank M. Claesen, Rega Institute for Medical Research, Leuven, Belgium, for supplying PMAA. S.O. is a fellow of the Hungarian Academy of Sciences. E.D. is an "Aspirant" of the Belgian N.F.W.O.

22 October 1968

Secretory Activity and Oncogenicity of a Cell Line (MDCK) Derived from Canine Kidney

Abstract. *A cell line (MDCK) of dog kidney origin grows on a glass surface as a mosaic of epithelium with many multicellular hemispherical vesicles. The cells lining the blisters actively secrete into the cyst cavities. Suspensions of these cells injected intravenously in the chick embryo produce brain metastases resembling adenocarcinoma.*

The cell line MDCK (Madin-Darby canine kidney) was derived in 1958 from the kidney of a normal male cocker spaniel and has been used subsequently for virologic study (*1*). Confluent sheets of cells on glass developed multiple lesions described as "ulcers." We recognized that these focal lesions were not ulcers but hemispherical vesicles or blisters composed of numbers of cells. The blister-like character of the structures was appreciated best by examining the growth of living cultures on a microscope slide in a large flat tube (*2*; Fig. 1a).

We anticipated that any polarization suggesting renal tubular organization of the cells making up the wall of the blister would find the surfaces of the cells bordering the vesicle contents covered with microvillus processes. Polarization was readily demonstrated in the cells of the cyst wall, as well as in cells adherent to glass, but the orientation was the opposite of our expectation. Numerous microvillus processes and tight junctions were consistently found on the convex surfaces of the blisters and on the surface of the epithelial sheets bathed by medium. No basement membrane was evident on either surface of the epithelial sheet (Fig. 1b).

To discover whether the vesicles once formed were relatively stable structures, we examined them for periods of 1 to 2 days with low magnification time-lapse microscopy. Innumerable vesicles were observed to form, expand, distend, and collapse. The picture resembled the bubbling surface of gently boiling oatmeal. The polarized functional cells seem to resemble renal tubular epithelium while the medium is anatomically and functionally analogous to a glomerular filtrate. The vesicles are interpreted as interstitial collections of "reabsorbed" components of the medium, "glomerular filtrate," which accumulate in a plane of cleavage between cells and glass until the distended secreting epithelial membrane covering the confined fluid develops a significant break. Then the vesicle collapses as fluid leaks into the medium. The cells return to the glass surface. The defect between cells is repaired, and the accumulation of fluid between the sheet of cells and the glass surface is resumed.

Oncogenicity of MDCK in vivo has been tested by injecting suspensions of single cells (removed from glass surfaces with EDTA-trypsin) intravenously in chick embryos. In one experiment, 3×10^6 cells were injected into each of a series of 12-day-old embryos. Histologic examination of the brain and liver of 12 animals was made 1 week later. Multiple foci of metastases were seen in 11 of 12 brains (Fig. 2); none was found in any of the livers. The lesions

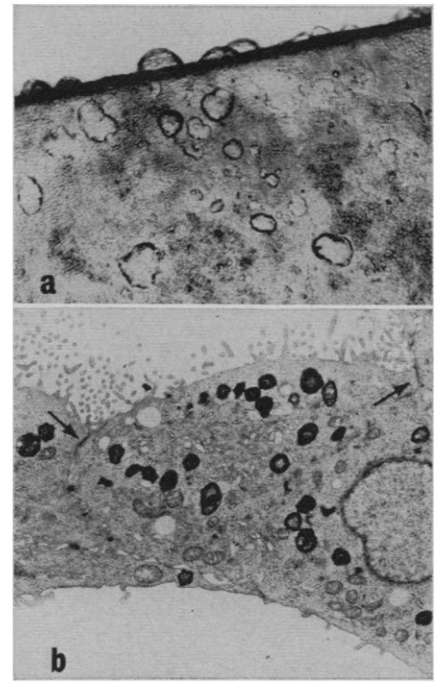

Fig. 1. (a) Living culture of MDCK growing on the surface of a microscope slide contained in a large flat tube photographed with an inverted microscope. In most of the field, the flat surface of the slide is seen completely covered with epithelium. The irregular, oval, scalloped structures (vesicles) seen on the flat surface appear as a series of hemispherical blisters on the edge of the slide (approximately × 25). (b) Vesicle wall grown on a glass slide. The convex surface on the top, the outer (medium-bathed) surface of the vesicle, is covered with numerous microvilli. Two tight junctions (arrows) join cell borders near the surfaces with many microvilli. The pattern of many microvilli and adjacent tight junctions indicate that the tissue culture medium is anatomically analogous to a glomerular filtrate. At the bottom of the figure, occasional microvilli are seen in the concave surface lining the fluid cavity of the blister. The fluid-filled cavity is perhaps analogous to an interstitial cyst. The dark structures in the cytoplasm are cytosomes (lysosomes) (× 3900).

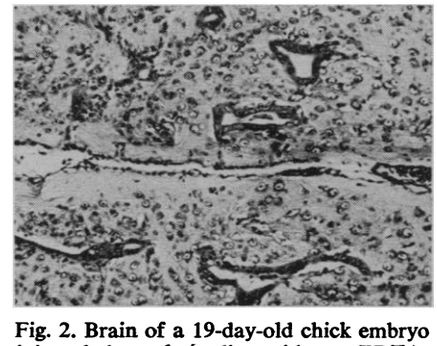

Fig. 2. Brain of a 19-day-old chick embryo injected 1 week earlier with an EDTA-trypsin produced, single-cell suspension of MDCK. Epithelial glandular structures are consistent with a histopathologic diagnosis of metastatic adenocarcinoma (hematoxylin and eosin; × 85).

in the brain were diagnosed as foci of metastatic adenocarcinoma.

The MDCK cells may be a useful tool in both renal pharmacology and oncology.

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9 October 1968

Thermoregulation: Effects of Environmental Temperature on Turnover of Hypothalamic Norepinephrine

Abstract. *The hypothesis that norepinephrine is a transmitter in the temperature-regulating center of the hypothalamus is based on observations of changes in the rectal temperatures of animals after injections of norepinephrine into the hypothalamus. By introducing tritiated norepinephrine as a label into the endogenous norepinephrine stores in the brain and then measuring the disappearance of tritiated norepinephrine from discrete areas, one can monitor the activity of norepinephrine-containing neurons in those areas. In the rat exposed to heat, the turnover of endogenous norepinephrine appears to be increased selectively in the hypothalamus, whereas exposure to cold has no effect.*

The hypothalamus plays a role in temperature regulation, although the details of the regulating mechanisms are not fully understood (*1*). Feldberg and Myers (*2*) have postulated that the normal body temperature of a cat is maintained by a balance between the secretion of norepinephrine (NE) and serotonin in the hypothalamus. This hypothesis was initially based on evidence that serotonin, when injected into the anterior hypothalamus, caused shivering and that the shivering could be abolished by injection of NE into the same area. Injection of NE into the cerebral ventricles of rats elicits a biphasic response in rectal temperature (*3*). Low doses cause a transient fall in renal temperature followed by a rise, but with increasing dosage, the fall becomes more pronounced and more prolonged. Attempts to demonstrate temperature-dependent changes in the concentration of endogenous NE in the whole brain or hypothalamus of the rat have been unsuccessful (*4*), but increased activity of NE-containing neurons in several regions of brain has been reported in rats exposed to a temperature of 40°C (*5*). In the latter experiments, however, the inhibition of both central and peripheral synthesis of NE by α-methyltyrosine impaired normal thermoregulation.

As an alternative approach, we have attempted to obtain an index of the turnover of endogenous NE in the hypothalamus of rats exposed to different environmental temperatures. A small dose of ^{3}H-NE (*6*) (2.5 μc, 0.044 μg) was injected intracisternally (*7*), under ether anesthesia, into male Wistar rats (200 to 250 g). This dose was much less than that required to induce a change in the rat's body temperature (*3*) but was sufficient to label the endogenous NE stores throughout the brain (*8*). After ½ hour, when the rats had fully recovered from the anesthetic, they were placed in individual cages in an environment of 9°, 24°, or 32°C, controlled to within ±2°C. At 2, 4, and 6 hours after injection, rats were stunned and decapitated, their rectal temperatures having first been measured with a Grant thermistor thermometer. The brains were rapidly removed, rinsed, and chilled. Cerebellum and medulla were discarded since they contained a disproportionately high concentration of ^{3}H-NE due to their close proximity to the site of injection. The hypothalamus (mean weight, 66 mg) was dissected from the rest of the brain as described by Glowinski and Iversen (*8*), except that the rostral limit was placed at the caudal end of the optic chiasma to exclude most of the preoptic area. The rest of the brain (minus hypothalamus, preoptic area, cerebellum, and medulla) was taken as a whole. The tissues were homogenized in 15 volumes of 0.4*N* perchloric acid containing 0.1 percent ethylenediaminetetraacetate, and the ^{3}H-NE in the extracts was separated from its metabolites by cation-exchange chromatography (*9*). The amount of ^{3}H-NE present was estimated by liquid scintillation counting, the tissue content of ^{3}H-NE being expressed as disintegrations per minute (dpm) per milligram of tissue.

The disappearance of ^{3}H-NE from the hypothalamus and the rest of the brain is shown in Fig. 1. The tissue content of ^{3}H-NE at 4 and 6 hours after injection is expressed as a percentage of that at 2 hours. The values of rate constant (*k*) (*10*) derived from the slopes of these lines are given in Table 1. The disappearance of ^{3}H-NE from the hypothalamus at 32°C is significantly faster than at 24°C. This is associated with an elevation of 2.9°C ($P < .001$) in rectal temperature at 32°C. There was no significant difference in the rates of disappearance of ^{3}H-NE from the hypothalamus at 24° and 9°C, although rectal temperatures fell by 1.3°C ($P <$

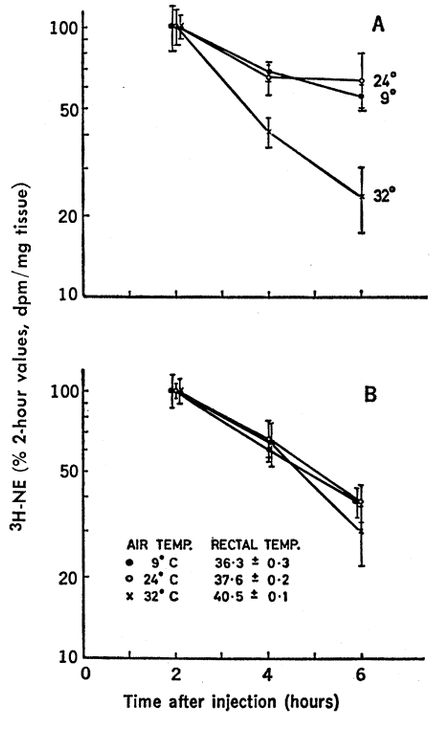

Fig. 1. Disappearance of ^{3}H-norepinephrine (NE) from hypothalamus (A) and rest of brain (excluding cerebellum and medulla) (B) of rats at various environmental temperatures. For the purposes of illustration, the ^{3}H-NE concentrations ± the standard error of the mean are plotted as percentages of the concentration at 2 hours. Each value is the mean for five to eight animals. Rectal temperatures are the overall means from rats killed at 2, 4, and 6 hours after injection.