Rabbit antisera against herpes simplex and vaccinia viruses did not neutralize the agent in tissue culture, and rabbit hyperimmune serum against strain No. 2 of the adenoid agent did not neutralize herpes simplex virus in suckling mice or vaccinia virus in tissue culture. The cytopathogenic effects of the agent were prevented or significantly delayed by addition to the cultures of human gamma globulin in a final concentration of 1:500 or some human sera in dilutions of 1:50 or higher, whereas other human sera showed no neutralizing capacity at dilutions of 1:25.

In this laboratory a variety of human viruses have been inoculated into human adenoid and embryo cultures and HeLa cell cultures, including herpes simplex, vaccinia, influenza A′ and B, Type 1 poliomyelitis, and members of the Coxsackie A and B groups, as well as human pleuropneumonia-like organisms and presumably virus-containing materials from cases of measles and varicella; in no instance has the picture produced by the adenoid agents been produced. The pattern of destruction of adenoid epithelium by herpes simplex and poliomyelitis viruses was somewhat similar to that of the adenoid agents, but their effects on HeLa cells did not resemble that produced by the adenoid agents.

Summary. 1. From the present evidence it appears that an unidentified, possibly new, tissue culture cytopathogenic agent has been isolated repeatedly from human adenoids undergoing spontaneous degeneration in tissue culture. The filterability and the inability to cultivate the agent on bacteriological media and to demonstrate organisms in stained tissue culture preparations would indicate that the agent belongs to the group of viruses or rickettsiae. It is tentatively proposed to designate the agent as the "adenoid degeneration agent", abbreviated as "A.D. agent". 2. That the agent is derived from the adenoid tissue rather than from the nutrient media is indicated by the fact that some adenoids and all human embryonic tissues cultivated in the identical media and at the same time have not undergone degeneration, although they are susceptible to infection with the agent; also, repeated attempts to isolate the agents from adenoid cultures not demonstrating degeneration have been uniformly unsuccessful. 3. Further investigation is in progress to determine the relation of the agent to the adenoids and to study their possible role in human disease; par ticularly upper respiratory infections.

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Effect of Buffer on Paper Electrophoretic Studies on State of Yttrium in Blood.* (20715)

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When an inorganic compound is injected into an animal its physico-chemical state may be altered by the blood, which is a highly complex fluid containing various inorganic ions and organic compounds, especially proteins. Substances introduced into blood may be complexed and thereby kept in solution or may aggregate to form colloids which are largely removed by the reticulo-endothelial system. To investigate the physico-chemical state of certain radioactive tracers in the blood stream, we have used filter-paper electrophoresis with

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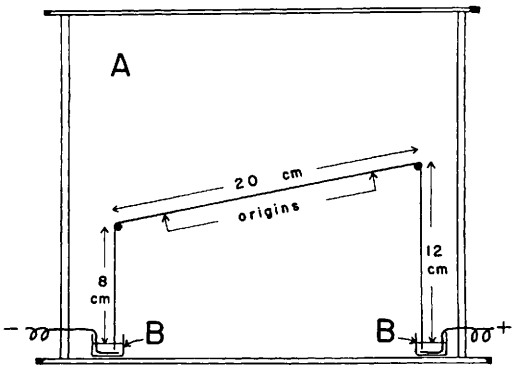

FIG. 1. Paper electrophoresis apparatus. A is a covered glass cylinder. B are cups or troughs of buffer solution containing carbon or nichrome electrodes. Paper strips supported on 2 glass rods.

a modified buffer system. In the electrical separation of plasma proteins, most investigators have used barbiturate (veronal) buffer of pH 8.5, but the possible effects of the buffer upon labeled plasma were not investigated. To determine whether results obtained by this technic are truly representative of the conditions in blood, we used radioactive yttrium as a test material to study the effects of different buffers upon the electrophoretic patterns.

Methods. The yttrium-91 used was carrier-free and therefore present in a low enough concentration so as not to affect the system under study. The chemical behavior of Y^{91} in pure solutions has been studied by Kurbatov and Kurbatov(1) and Schweitzer and Jackson (2) who found that at a pH above 3, a portion of the yttrium will no longer pass through filter paper and becomes partially removable by centrifugation. Such behavior is characteristic of radiocolloids. Thus, it is probable that yttrium at the pH of blood plasma exists, at least in part, in colloidal form unless one or more of the normal blood constituents prevent colloid formation by their complexing action.

The apparatus, Fig. 1, was essentially that of Gordon *et al.*(3). Evaporation of water from the strips was reduced, though not entirely prevented, by covering the walls of the outer container with moist filter paper. Filter paper strips were moistened with various buffers, then 5-10 μl of sample containing 0.1 μc of Y^{91} was applied in a narrow band at a marked position on the strip. A voltage gradient of 5 volts per cm was maintained during the experiment.

First we followed the movement of a pure solution of radioactive yttrium chloride dissolved in 0.05 N HCl and applied to individual paper strips A, B, and C in Fig. 2, buffered respectively with 0.05 N HCl, 0.2 M KH phthalate (pH 4), and 0.05 molar sodium barbital-barbituric acid (pH 8.5). The autoradiograms show that after 1 hour the yttrium had migrated only in the acid solutions, and the phthalate band was not sharp. There was no movement in the barbital buffer.

Next, sheep plasma was incubated *in vitro* 30 minutes with Y^{91} chloride, and an aliquot placed on strip D (Fig. 2), which was dipped into barbiturate buffer. As with strip C, no migration occurred. It is evident that yttrium in plasma and yttrium chloride behave similarly when the separations are carried out in the standard barbiturate buffer.

In an attempt to avoid possible alterations of the physico-chemical state of plasma yttrium, we then substituted heparinized sheep plasma for the barbiturate buffer, strip E. Labeled plasma from the same animal that supplied the buffer plasma was placed at the origin of this strip just as for strip D. Now a fraction of the plasma did migrate, forming a distinct band containing approximately 30% of the Y^{91} (as determined by mica-window GM tube counting). Evidently yttrium in plasma existed in at least 2 fractions, and the mobile fraction did not appear when electrophoresis was carried out in barbiturate buffer.

Discussion. Schulz and Wegner(4) pointed out that the migration of materials in filter paper electrophoresis is determined by 3 factors: by electromigration, by the movement of solvent on the strip caused by evaporation and electroendosmosis, and by interaction of some materials with paper. To determine whether the observed movements of yttrium were due primarily to solvent flow or to electrophoresis, applications of Y^{91} were made to 3 different locations on each of strips F and G, which were buffered in 0.05 N HCl and sheep plasma, respectively. After an hour, the mobile fractions of the endspots migrated toward the center

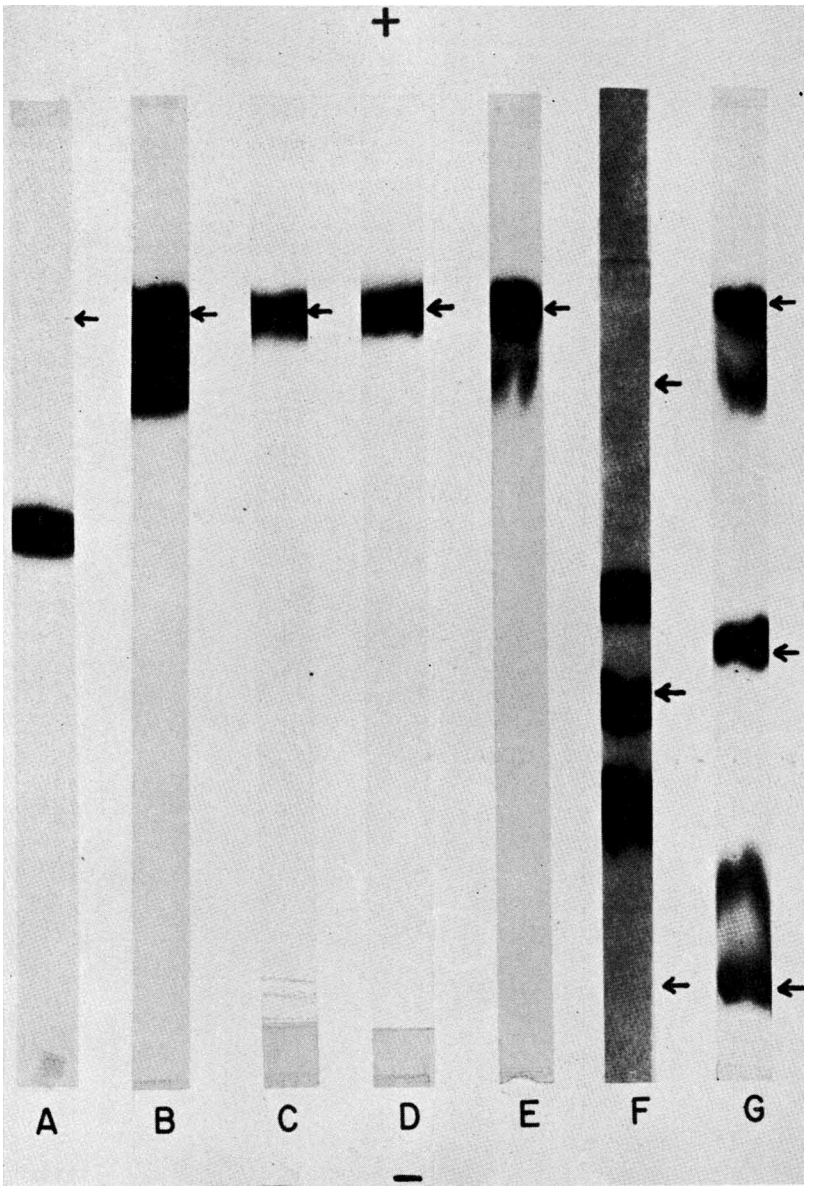

FIG. 2. Autoradiograms of paper strips. Arrows indicate points of application of samples.

while the center spot did not move. Such behavior would be characteristic of an uncharged or very slightly charged molecule which was simply washed along by the solvent, which flows toward the middle of the paper strip(4). Using the same technic with $Ca^{45}Cl_2$ we observed that the radioactive calcium always migrated toward the cathode, in plasma as well as in HCl buffers, though the rate of migration depended on the site of sample application. Thus, even though the movement of materials on paper strips is not necessarily the simple result of electromigration, the additional flow of solvent may be useful in the differentiation of ionic and neutral molecules.

Summary. The use of blood plasma as a buffer for filter paper electrophoresis makes it possible to study the physico-chemical state of injected materials or of native blood constituents without subjecting the blood fractions

to possible alterations by an artificial buffer. In veronal buffer yttrium appeared to exist in wholly immobile forms. In plasma buffer, yttrium existed in an immobile fraction and in an unionized fraction which moved with the solvent. Migration of materials as a result of solvent flow must be considered as a factor in the interpretation of electrophoretic studies of this type.

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Effect of 2,3-Dimercaptopropanol (BAL) upon Distribution and Excretion of Plutonium.* (20716)

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Introduction. The transuranic element, plutonium, presents a major health hazard due to its very toxic alpha radiation, long half life, high degree of retention by the skeleton, and extremely slow rate of elimination from the body(1). Although the sulfhydryl compound, 2,3-dimercaptopropanol (BAL) has been shown to have no significant influence on acute poisoning of animals with uranium salts(2) and strontium^{89}(3), it has proven effective in the treatment of systemic metal poisoning with arsenic(4-6), mercury(7), antimony(8), and polonium(9).

Because of the potential importance of plutonium poisoning, the following study was carried out to determine if therapy with BAL might alter the distribution and increase the excretion of this element.

Experimental. Seventeen young adult female Long-Evans rats ranging in weight from 200 to 240 g were each injected intramuscularly with 15 μg of hexavalent Pu^{239} (half-life 2.43 x 10^4 years) as the chloride into the right front leg. The volume injected in each case was 0.25 ml of physiological saline at pH 3. An amount equivalent to the injected dose was made up to volume in a volumetric flask and aliquots were taken to provide standards.

The animals were divided into 3 groups: 1) Controls. These received no treatment. 2) BAL treated. These animals each received 9 injections of 10 mg BAL in 0.1 ml peanut oil in the muscles of the hind leg, at 4-hour intervals following the injection of plutonium. This dose was well below the LD_{50} of 105 mg/kg reported for intramuscular injection in rats(5), and in the range of effective doses used in treatment of mercury and arsenic poisoning(2,5). 3) BAL-pretreated. These rats received a single injection of 10 mg BAL 5 hours prior to administration of plutonium, in order to ensure a high BAL level in the tissues at the time of injection of the metal. They then received the same treatment as the animals in group 2. Each rat was placed in an individual metabolism cage and fed *ad libitum* on the stock ration. Average weight loss was 5-6 g during the experiment. Urine and feces were collected separately at 2 days and 8 days following injection of plutonium. At the end of 8 days, the rats were sacrificed, and liver, kidneys, right femur, right foreleg and left foreleg were removed, dried and ashed at 650°C for 10 hours. The remainder of the animal or "carcass" and the urine and feces were also dried and ashed. The ashed samples

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