activity in reversing SM inhibition. The reversing activity of the amino acid was proportional to the concentration used, within the limited range tested. A concentration of 100 μg per ml agar was toxic in the growth control plates. Also, a commercial yeast autoylsate (Basamine-Busch) was 8-fold more effective in reversing SM than DSM. In other experiments, varying concentrations of the reversing agents did not alter the qualitative specificity of the results.

Discussion. The evidence here, together with the absence of cross-reversal with SM- and DSM-reversing substances produced by homologous resistant strains described elsewhere(13), while not conclusively establishing different mechanisms of action of SM and DSM, does contradict the widespread assumption that both act in identical fashion. The reversing compounds may well be related to the biochemical mechanisms interrupted when the cells are inhibited by threshold concentrations of the antibiotics.

Summary. Several purine and pyrimidine derivatives reversed the growth inhibition of *Aerobacter aerogenes* caused by streptomycin under specified conditions. Inhibition by dihydrostreptomycin was not reversed under the same conditions. L-phenylalanine reversed the inhibition caused by dihydrostreptomycin and not that caused by streptomycin.

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Received October 26, 1953. P.S.E.B.M., 1953, v84.

Isolation of a Cytopathogenic Agent from Human Adenoids Undergoing Spontaneous Degeneration in Tissue Culture. (20714)

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During the course of a study of the growth of human adenoid tissue in roller tube culture, a characteristic degeneration has been encountered which has been found to be serially transmissible in other tissue cultures.

Methods. Adenoids were obtained during the winter and spring of 1952-53 from operations on young children.† The tissues were

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† We are glad to acknowledge the cooperation of Dr. E. Clarence Rice, pathologist, Children's Hospital, Washington, D. C., the Research Foundation, Children's Hospital, Washington, D. C., and the U. S. Naval Hospital, Bethesda, Md., in supplying the adenoids.

minced, washed 3 times in nutrient media, and cultured by the chicken plasma clot technic. The majority of adenoids and all other tissues were grown in a modification of the beef amniotic fluid medium described by Enders (1);‡ a few adenoids were cultured in media that were modified from those of Youngner *et al.*(2).§ Explant cultures of other tissues were prepared in the same manner; HeLa cell cultures(3) were obtained from Microbiological Associates, Bethesda, Md. Culture fluids were changed twice weekly, and the supernatant fluids were stored in screw-capped vials at −20°C. Passages were made by transfer of supernatant fluid in a dilution of 10^{-1}.

Results. During the first week of culture most adenoid cultures demonstrated sheets of epithelium, often ciliated, with a few areas of fibroblastic outgrowth. After 8 to 28 days in culture, 33 of the 53 (62%) adenoids observed for this period demonstrated a characteristic rounding of the peripheral epithelium, progressing to complete destruction of the epithelium within 7 to 10 days. The morphological appearance of the round cells was distinctive, and the appearance of a few of these cells almost invariably was followed by progressive replacement of the epithelium by the same type of cell. These cells were large, round or slightly ovoid, with a smooth, distinct margin, clear peripheral cytoplasm, and a densely granular center, the nucleus obscured by the granulation.

Culture fluids of 14 adenoids demonstrating this degeneration have been transferred to fresh cultures of adenoids, human embryonic tissue, or HeLa cells, and in 13 instances characteristic changes were produced in the recipient cultures. The changes observed in the recipient adenoid or embryonic tissues were identical with the picture of the original degeneration of the adenoids. The changes in HeLa cell cultures consisted of clumping and rounding of the cells, followed by dislodgement of the clumps from the wall of the tube. One isolation (strain No. 2) of the agent was obtained from an adenoid which degenerated on the eighth day of culture, by passage of the supernatant fluid to cultures of a second adenoid, the controls of which did not undergo spontaneous degeneration during 69 days of observation and from which no cytopathogenic agent could be isolated. This strain has been carried through a total of 17 passages, the first in adenoid, one in human embryo trachea, and the last 15 in HeLa cells, with production of the typical changes in every passage. Degeneration of the seventeenth passage tissue occurred one day after infection with fluid representing a theoretical dilution of the original adenoid fluid of 10^{-32}. Fluid of the eleventh passage when passed to cultures of human fetal trachea reproduced the typical round cell degeneration after 3 days. Similarly, other strains of the agent have been carried without difficulty through serial passages, and have consistently reproduced the degeneration. In both human embryo skin and HeLa cell cultures the agent has been found to reach a titer in the supernatant fluid of more than 10^5 infectious units per ml when titrated in HeLa cells.

The incubation period of the cytopathogenic effects in human epithelium is usually 4 to 8 days; with higher dilutions of the inoculum the incubation period may be as long as 23 days. In cultures consisting almost entirely of human embryo fibroblasts the incubation time is longer, ranging from 8 to 15 days for the lowest dilution, to as long as 40 days with higher dilutions. Incubation time in HeLa cells varies from 2 to 20 days, depending on the concentration of the agent. The following tissues have been found to show cytopathogenic effects after infection with the agent: human adenoid; human embryonic nose, pharynx, palate, tongue, trachea, skin, muscle, and pancreas; new-born human prepuce; HeLa cells; suckling rabbit kidney and trachea; suckling hamster trachea, lung, kidney, skin, and muscle; and chick embryo lung and skin. The effects seen in hamster and chick embryo

‡ The medium consisted of beef amniotic fluid 85%, beef embryo extract 10% and inactivated horse serum 5%.

§ During the first week of culture the following medium was used: Hanks' solution 40%, inactivated horse serum 40%, and chick embryo extract 20%. After this time the medium was changed to the following: Hanks'-Sims' solution (3:1) 85%, inactivated horse serum 5%, and chick embryo extract 10%. All media contained phenol red (0.001%), penicillin and streptomycin (50 to 250 units of each), and soybean trypsin inhibitor (0.005%).

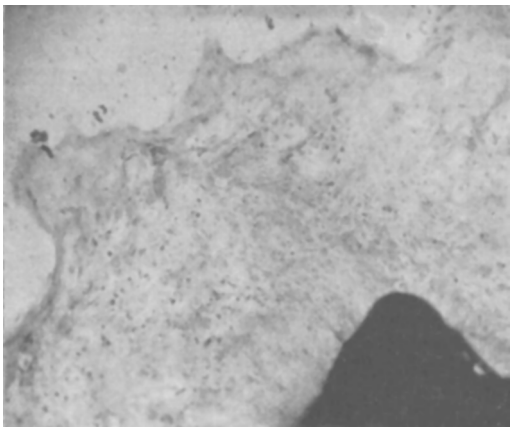

FIG. 1. Epithelial sheet of uninoculated human fetal trachea, 48th day of culture. H & E stain.

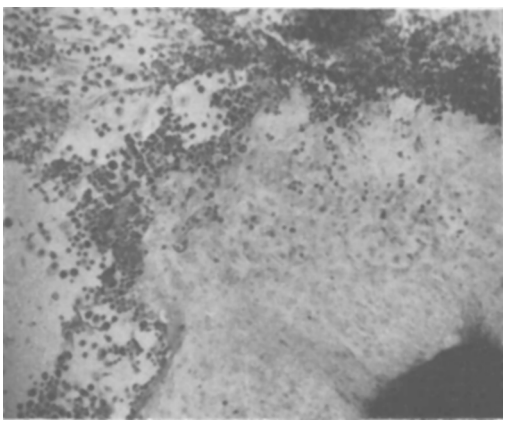

FIG. 2. Human fetal trachea, 48th day of culture, 19 days after infection with A.D. agent, strain #2. H & E stain. ×37.

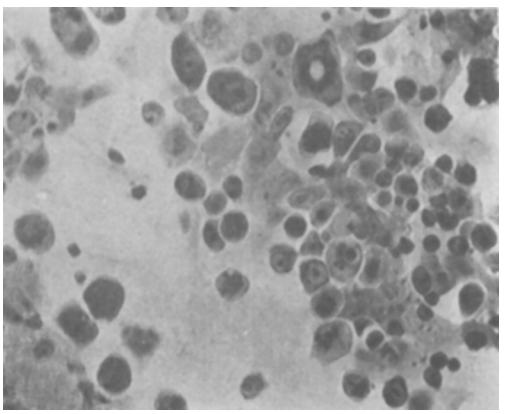

FIG. 3. Higher power photomicrograph of tissue shown in Fig. 2. H & E stain. ×146.

cultures were not distinctive, being characterized by gradual death and disappearance of tissue with lysis of the plasma clot. The following tissues have shown no evidence of cytopathogenic changes following inoculation with the agents: beef embryonic trachea, mouse embryo mixture, mouse glioblastoma, monkey testis, and guinea pig trachea and kidney.

Hematoxylin-eosin stained preparations of infected human embryonic tracheal epithelium cultures have been examined by Dr. Henry Pinkerton, St. Louis University School of Medicine, who made the following description: "The normal cells take the hematoxylin stain, both nucleus and cytoplasm. The affected cells show *eosinophilic* swollen nuclei, with what I take to be nuclear inclusions. These are usually basophilic, but occasionally eosinophilic. There is margination and fragmentation of the nuclear chromatin (which stains deeply blue-black), often wrinkling of the nuclear membrane, and swelling of the nuclei. The cytoplasm stains deep reddish purple, and occasionally contains 10 to 12 small pale blue inclusions'. . . . Often, also, the nucleus is blended with the cytoplasm to form a granular purplish mass in which no nucleus can be recognized". Photomicrographs of stained roller tube cultures are shown in Fig. 1-3.

The agent has not been found to grow on bacteriological media, including repeated cultures on thioglycollate broth, blood agar, and several types of pleuropneumonia media.‖ Activity of the agent was destroyed by heating at 62°C for 30 minutes; the agent was filterable through a Mandler No. 14 candle with some loss in titer. No loss of activity was found after storage at 3°C for a week or after three cycles of quick-freezing and thawing. No clinically recognizable disease has been produced by the agent inoculated by various routes into experimental animals, including embryonated eggs, suckling and adult mice, suckling hamsters, guinea pigs, rabbits, rhesus monkeys, and a chimpanzee.

Hyperimmunization of rabbits with agent-containing tissue culture fluids resulted in the production of antibodies capable of neutralizing the agent in tissue culture, whereas immunization with control culture fluid did not.

‖ We are indebted to Drs. Ruth Wickelhausen and Ella Verder for the attempts to isolate pleuropneumonia-like organisms.

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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Received October 26, 1953. P.S.E.B.M., 1953, v84.

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

* Published with approval of the Director of the Tennessee Agricultural Experiment Station. The Y^{91} was obtained from Oak Ridge National Laboratory on allocation by the U. S. Atomic Energy Commission.

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