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Purification and Biophysical Properties of Rhinoviruses.* (31068)

SANDY MCGREGOR, C. ALAN PHILLIPS AND HEATHER D. MAYOR
(Introduced by Joseph L. Melnick)
WHO International Reference Centre Enteroviruses, Baylor University College of Medicine, Houston, Texas

Rhinoviruses have been established recently as a subgroup of the picornaviruses(1,2). New serotypes continue to be recognized(3). Rhinoviruses contain RNA and protein and display cubic symmetry of the icosahedral pattern(4). They are approximately 18-23 mμ in diameter, ether stable and acid labile. Their low titer, lability, and specialized growth requirements(2,5) have not encouraged extensive study by routine biophysical procedures. This report describes methods that we have developed for obtaining partially purified preparations of human rhinoviruses without significant losses in titer and presents preliminary biophysical data obtained with these agents.

Materials and methods. Virus. Two rhinovirus strains, B 632 and Baylor 1, were used in this study. Strain B 632, one of the original Salisbury strains, was isolated from a person with a common cold(6). The virus was passed twice in rhesus monkey kidney cells and 6 times in KB cells before use. The Baylor 1 type, strain Tippett, was isolated in human aorta cells from a patient with a common cold in Houston, Texas(7). The virus was passed 4 times in aorta cells and 10 times in human lung fibroblast cells (WI-38) before use.

Cell lines, virus growth and assay. The WI-38 cells were cultivated in roller tubes, the KB cells both in roller tubes and spinner culture. Both cell lines were grown in Eagle's medium containing 10% calf serum and 0.075 g% sodium bicarbonate. The cultures were maintained in Eagle's medium containing 2% fetal bovine serum and 0.113 g% sodium bicarbonate.

Virus was grown primarily in WI-38 roller tubes at 33°C in maintenance medium. The titer of the B 632 strain was boosted from 10^5 $TCID_{50}$/ml to $10^{7.5}$/ml by 3 passages in KB cells. An increase in titer was not achieved by similar treatment of the Baylor 1 strain which maintained an average titer of $10^{4.5}$/ml. Viruses were harvested by a single cycle of freezing and thawing when cytopathic effects (CPE) were judged to be at a maximum, usually within 48 hours after inoculation.

Titrations were carried out in roller tubes at 33°C. KB cells were used for the B 632 strain, WI-38 for the Baylor 1 strain. Fifty per cent endpoints were determined.

Purification and concentration. Crude virus harvests were clarified by low speed centrifugation in an International clinical centrifuge for 5 to 10 minutes. After extraction with an equal volume of fluorocarbon (Genetron 113) the preparations were concentrated by dialysis against carbowax (polyethyleneglycol compound, 20 M) or by sedimentation in the Spinco model L ultracentrifuge for 3 hours at 105,000 × *g*. The pellets produced by ultracentrifugation were resuspended either by adding 1 ml of physiological saline and freezing and thawing (Method I, Table I), or by soaking overnight at 4°C in a 1 ml volume of physiological saline (Method III, Table I). Both methods were followed subsequently by mechanical agitation. The suspensions were layered over preformed gradients, prepared by layering 6 CsCl solutions varying in density from 1.46 to 1.25 g/cm^3, and

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TABLE I. Purification Procedures for Human Rhinoviruses.

	Titer ($TCID_{50}$/1.0 ml)	Volumes (ml)
Method I		
Clarified tissue culture fluid	5.00	100
Fluorocarbon extraction	5.75	100
Resuspended pellet from ultracentrifugation	2.50	1
Method II		
Fluorocarbon extracted culture fluid	5.00	100
Sediment from CsCl cushion	6.75	3
Supernatant fluid	2.70	95
Method III		
Clarified tissue culture fluid	4.75	100
Fluorocarbon extraction	5.00	100
Mildly resuspended* pellet from ultracentrifugation	7.00	1

* Extraction overnight at 4°.

spun for 6-12 hours at 39,000 RPM. Fractions of approximately 0.1 ml were collected by bottom puncture of the tube and the density of these fractions computed from measurements of refractive index.

Electron microscopy. Negatively-stained specimens were prepared by the loop-drop method of Brenner and Horne(8) or the pseudoreplication procedure of Smith and Melnick(9).

Results. Preliminary density gradient ultracentrifugation of clarified fluorocarbon-treated tissue culture fluids in the Spinco SW-39 rotor failed in general to produce visible bands at equilibrium. On the rare occasions when faint visible bands were detected subsequent examination in the electron microscope failed to detect virus-like particles. The effects of various gradient salts on rhinovirus infectivity were therefore studied.

Tissue culture harvests of B 632, Baylor 1 and several other rhinovirus strains were diluted into cesium chloride, potassium tartrate and potassium citrate to give final densities comparable with those achieved during gradient centrifugation, and maintained overnight at 4°C. Subsequent titration indicated no loss in infectivity. Titers obtained at various stages of the purification procedure indicated that most of the infectivity (greater than 99%) was being lost after sedimentation and resuspension of the virus (Table I, Method I). In order to determine the effect of ultracentrifugation, similar material was sedimented onto a preformed cushion of cesium chloride(10) which led to quantitative recovery of infectivity (Table I, Method II). This suggested that the resuspension technique was destroying the infectivity, and when a milder technique (soaking overnight at 4°C and mechanical agitation) was employed it resulted in quantitative retention of titer. This method was then instituted routinely. The scheme we finally adopted is illustrated together with typical infectivity data in Table I, Method III.

Strain B 632 whose titer had been boosted to $10^{7.5}$ $TCID_{50}$/ml by passage in KB cells was inoculated onto WI-38 yielding a harvest with a titer of 10^6 $TCID_{50}$/ml.

CsCl density gradient centrifugation of this material after treatment according to Method III, Table I yielded two visible bands in the tube. The lower band with density 1.38-1.39 g/cm^3 was found by electron microscopic examination to contain morphologically complete virus particles (Fig. 1 and 2). This band coincided with the peak of virus infectivity (Fig. 2). An upper band at a density of 1.28-1.30 was found to contain empty vi-

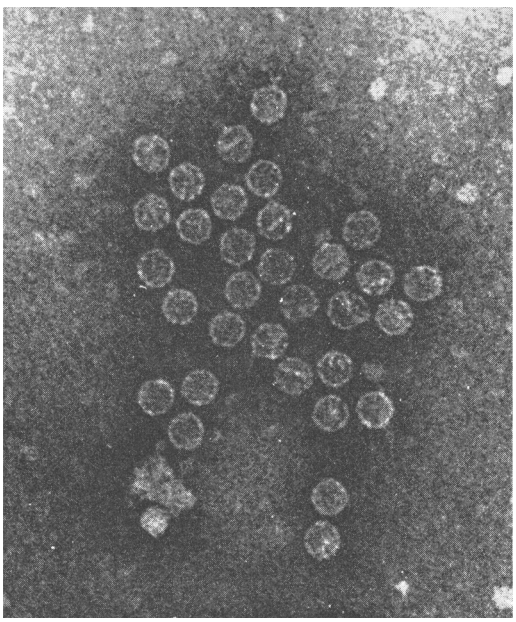

FIG. 1. Negatively-stained preparation of B 632 strain rhinovirus. Virus particles are 22-23 mμ in diameter. × 210,000.

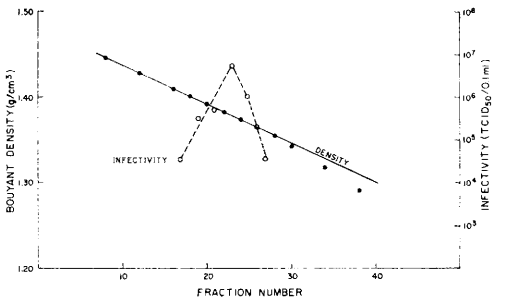

FIG. 2. Plots of buoyant density and infectivity against fraction number for a typical experiment with strain B 632.

rus particles and was non-infective. This experiment has been repeated 3 times with essentially the same results.

Similar preliminary findings have been obtained with the Baylor 1 strain except that the peak of infectivity and location of mature particles appear to coincide in the density range, 1.40-1.41 g/cm^3. The virions of both Baylor 1 and B 632 appear to be 20-23 mμ in diameter and preliminary examination indicates that their capsid displays icosahedral symmetry identical to that previously reported for some acid-insensitive (entero-) as well as acid-sensitive (rhino-) picornaviruses (4).

Discussion. The buoyant density (range 1.38-1.41 g/cm^3) found for rhinoviruses in CsCl is significantly greater than that reported for a number of acid-insensitive picornaviruses (1.33 - 1.34 g/cm^3) (11,12). Plummer(13) has tentatively classified foot-and-mouth disease virus as a rhinovirus. Trautman and Breese(14) have demonstrated that this bovine picornavirus has a density of 1.43 g/cm^3 in cesium chloride. In view of the present data this property coupled with its acid-sensitivity lend support to the suggestion that foot-and-mouth disease virus should be classified as a rhinovirus.

The finding of the higher buoyant density for rhinoviruses is of interest and 3 possible explanations are being investigated.

1. Rhinoviruses have a lower protein/RNA ratio than acid-insensitive picornaviruses. This could possibly result in less structural protein for protection of viral RNA and account for acid-sensitivity due to the increased accessibility of the RNA to denaturing agents. However, to date there appear to be no significant differences in the morphology of rhinoviruses and other picornaviruses(4).

2. Rhinoviruses contain more RNA and are hence denser than acid-insensitive picornaviruses. Poliovirus has been shown to contain 2×10^6 daltons of RNA(15). As yet there are no figures available for rhinoviruses, but it is to be hoped that now that methods are available for obtaining large quantities of high titer rhinoviruses, relevant data will become available. Increased density could also be a reflection of increased binding by rhinoviruses of heavy ions of density gradient salts.

3. Rhinoviruses contain an RNA whose secondary structure is different from that of acid-insensitive picornaviruses.

The finding of the high density of human rhinoviruses has been made independently in 4 laboratories that have been studying a number of different serotypes. The work from Dr. Robert Chanock's and Dr. David Tyrrell's laboratories was mentioned at the annual meeting of the Directors of the WHO International and Regional Virus Laboratories, Copenhagen, September 27-30, 1965, attended by Dr. Joseph L. Melnick, and subsequent work on this subject was discussed by Dr. Paul Chapple (Nature, 1966, v209, 790) and Dr. John Gerin at the Third Annual Conference, Vaccine Development Program, NIAID, October 8-9, 1965.

Summary. Two strains of human rhinoviruses, B 632 and Baylor 1, have been purified and concentrated by fluorocarbon extraction, ultracentrifugation, and cesium chloride density gradients without significant losses in titer. Biophysical studies indicate that they have a buoyant density in cesium chloride of approximately 1.4 g/cm^3 while acid-insensitive picornaviruses (enteroviruses) have a buoyant density of approximately 1.34 g/cm^3. Measurements of electron micrographs show that the virions of Baylor 1 and B 632 are 20-23 mμ in diameter.

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Metabolism of I-131 by Human Thyroid Tissue in Organ Culture.* (31069)

WELLINGTON HUNG, THEODORE WINSHIP, KEITH BOWEN AND JOHN C. HOUCK
Section of Endocrinology and Biochemical Research Laboratory, Research Foundation of Children's Hospital of D.C., Department of Surgical Pathology, Washington Hospital Center, Departments of Pediatrics and Pathology, George Washington University and Department of Biochemistry, Georgetown University, Washington, D.C.

Organ culture techniques have been used to study the metabolism of certain human endocrine glands(1-3). To our knowledge, only one study of human thyroid tissue in organ culture has been reported(2). Iodine metabolism by human thyroid tissue maintained in organ culture has not been studied extensively.

This report describes the metabolism of radioactive iodine by normal and pathological human thyroid explants maintained in organ culture for varying periods.

Material and methods. Normal thyroid tissue was obtained from radical neck dissection for cancer of the head and neck. Thyrotoxic tissue was obtained from a patient who had received an antithyroid drug and iodine solution prior to surgery. All of the thyroid tissues were obtained from adults. None of the tissues contained I-131 prior to these studies as determined by counting in a well-type scintillation counter.

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Thyroid tissue was collected in cold sterile Dulbecco's(4) phosphate buffered saline containing potassium penicillin G (100 units/ml) and streptomycin sulfate (100 μg/ml). The thyroid tissue was immediately dissected free of surrounding connective tissue and thinly cut, using micro dissecting scissors. Each slice measured approximately 10 $\times$ 5 $\times$ 1 mm. The watch-glass technique of Fell and Robison(5) and the stationary steel grid method of Trowell(6) were used for organ culture with some minor modifications. Stainless steel gauze grids, 4 mm in height with a platform measuring approximately 20 $\times$ 10 mm, were placed inside a 30 mm tissue culture dish. Two tissue culture dishes were placed in each 100 $\times$ 20 mm Petri dish which was lined on the bottom by absorbent paper moistened with sterile saline. One thyroid slice was placed on each stainless steel grid (Fig. 1). The Petri culture dishes were then placed in a jar and a cover was sealed in place. A mixture of 95% O_2 and 5% CO_2 was gassed into