when inoculated either intracerebrally or intraperitoneally; hemadsorbs guinea pig erythrocytes and is inactivated by ether. Although these properties suggest inclusion of the peromyscus agent in the myxovirus group, no antigenic relationship was demonstrated in cross hemadsorption inhibition and complement fixation tests with any of 10 myxoviruses or with any of a number of other viruses with which the new agent was compared.

The occurrence of antibody against peromyscus virus in the sera of white-footed mice and its absence in the sera of any of a number of other wild animals or in the sera of any of 10 strains of laboratory mice indicate that the natural host range of this virus is limited. It is of special interest that antibody directed against peromyscus virus was present in the sera of 20 of 122 human beings examined in this study. This finding suggests that peromyscus virus or a serologically related agent infects man. However, the possible relationship of peromyscus virus to disease in either white-footed mice or man remains to be determined.

*Summary.* A transmissible agent with biological and physical properties similar to those of viruses in the myxovirus group was recovered from the pooled tissues of 2 white-footed mice (*Peromyscus leucopus*) trapped in Virginia in March, 1962. The recovered agent produced degenerative changes in a variety of primary and continuous cell cultures, induced fatal infections in suckling mice when inoculated intracerebrally and hamsters when inoculated intracerebrally or intraperitoneally. Five of 8 white-footed mouse serum pools contained hemadsorption inhibiting antibody directed against the new agent in titers ranging from 1:20 to 1:160 but sera obtained from laboratory mice and from wild animals other than *Peromyscus* did not. A number of human beings were found to have hemadsorption inhibiting antibody against the new virus in their sera. This observation is taken as evidence that these individuals had experienced infection with the new agent or one closely related to it.

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Received March 8, 1963. P.S.E.B.M., 1963, v113.

## Isolation of Three Unclassified Enteroviruses from Patients Suffering from Dysentery.* (28347)

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During a study of the gastro-intestinal viral flora of cases of proven or presumed bacillary dysentery, 122 stool specimens from Mexico City, D. F., Mexico, was received through the kindness of Dr. Jorge Olarte, of the Hospital Infantil de Mexico. These specimens were derived from children hospitalized because of diarrheal disease proven or presumed to be bacillary dysentery, and were collected during the acute phase of the disease. Among the numerous viral agents isolated were 3 which, although they appeared to have the characteristics of members of the enterovirus group, bore no demonstrable serological relationship to any of the presently recognized prototypes. This paper describes the more important properties of these 3 agents and discusses the possibility that one or more of them may represent new human enterovirus prototypes.

*Materials and methods.* The sources of

* This investigation was carried out under the sponsorship of the Commission on the Enteric Infections of Armed Forces Epidemiological Board, and was supported by Office of Surgeon General, Department of the Army.

viruses and immune sera, the assay of viruses, the technique of neutralization tests, and preparation of viruses for electron microscopy have been described(1). Trypsin dispersed monolayer monkey kidney cell cultures were prepared by a slight modification of Youngner's method(2). For plaque assay, monolayer cell cultures in 3 oz. bottle were employed, using an agar overlay medium consisting of 1% Noble agar, 2% calf serum, 0.0017% neutral red, 0.23% sodium bicarbonate and antibiotics in Earle's saline solution. The method for preparation of human amnion cell cultures (FL line) has also been described(1). Immune sera were prepared by inoculation of rhesus monkeys with concentrated and partially purified virus suspensions using the incomplete adjuvant technique. Each animal received 3 intramuscular 2.5 ml injections, with a 3 week interval between each. The animals were bled 2-3 weeks after the last injection. The antibody titer found was quite satisfactory with H 130 and H 136 viruses (1:1, 260), but in case of H 170 virus the antibody titer was found to be low (1:120) against approximately 100 $TCID_{50}$ of virus.

*Experimental results. Identification of viruses.* The viruses H 130, H 136, and H 170 were readily propagated in monkey kidney cell cultures in serial passage. Attempts to type out these viruses in the fourth passage in neutralization test using immune-sera prepared against the recognized human enteroviruses failed. Two of the viruses, H 130 and H 170, were then plaque purified 3 times and then terminal dilution passages were carried out (using one-half $\log_{10}$ dilutions) 10 times with all 3 viruses. Subsequent efforts to type out the 3 viruses failed to reveal immunological relationships with the recognized human enterovirus prototypes. Numerous other enterovirus-like agents isolated in this laboratory and elsewhere were also tested in cross neutralization tests, but no immunological cross reactions were found. These viruses were: Frater (3), PR 10, 17, 20, 22, 28(4,5), Hu 780, 2080(6), and several other viruses, non-pathogenic for monkey kidney cell cultures, such as: HSO(7), Thailand C-18(8), Giles(9), and several viruses recently isolated in this laboratory, Hu 39 (1), Hu 504, 659(10). The results of the cross neutralization tests are summarized in Table I. No immunological cross reactions were found between the 3 viruses (Table II).

*Properties of the viruses.* All 3 viruses showed the typical characteristics of human enteroviruses. They were found to be resistant to ether treatment (*i.e.*, 20% ether for 24 hours at 4 C° did not inactivate the viruses), indicating the absence of essential lipids in the viruses. Magnesium ions in 1 mole per liter concentration fully stabilized the viruses against heat inactivation at 55 C° for 120 minutes as has been shown to be a general property of human enteroviruses(11).

*Cytopathogenicity of the viruses.* All 3 viruses caused typical enterovirus-like CPE in monkey kidney cell culture (Fig. 1). Using 100 $TCID_{50}$ of virus, in the case of the H 136 virus the CPE was evident on the second day, when foci of rounded, shrunken, highly refractile cells appeared. The destruction of the cell sheet was complete by the third or fourth day as the cells sloughed off from the glass wall of the tube into the medium. The virus titer in the supernatant fluid was found to be high: $10^7$-$10^8$ $TCID_{50}$ per ml culture fluid. In contrast, significant cytopathogenic effects were first noted with cultures of H 130 and H 170 viruses on the third or fourth day. Although the process went on to complete destruction of the cell sheet, the final viral titers were lower, $10^5$-$10^6$ $TCID_{50}$ per ml of culture fluid. In hematoxylineosin stained preparations (Fig. 2, 3, 4), the appearance of an eosinophilic mass was noted in the center of the infected cells, which increased in size and by approximately 12 hours after infection displaced the shrunken nucleus, to one side of the cell and the cytoplasm to the periphery of the latter. This type of lesion, noted with all 3 viruses, was found to be characteristic of human enteroviruses propagated in cell cultures by Barsky(12). All 3 viruses multiplied and produced CPE on rhesus monkey kidney, testis primary cell cultures and also on monkey kidney cell strain $LLMCK_2$(13). No CPE was noted when embryonic human kid-

TABLE I. Cross Neutralization Tests Conducted in Identification of H 130, H 136, and H 170 Viruses.

| | Viruses | | |
|---|---|---|---|
| | H 130 | H 136 | H 170 |
| 1. Prototype Immune Sera | | | |
| Coxsackie A Type 1-21, B Type 1-6* | Negative | Negative | Negative |
| " " Type 22, 24, Gilest | Not done | Not done | Not done |
| Poliomyelitis Type 1-3 | Negative | Negative | Negative |
| ECHO Type 1-28, E 59 (JV 10)‡ | " | " | " |
| C-18, Hu 39, 504, 659, HSO, Pett | " | " | " |
| Frater, PR 10, 17, 20, 22, 28, Hu 780, 2080 | " | " | " |
| 2. Prototype Viruses | | | |
| Coxsackie A Type 2, 3, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 24,§ B Type 1-6 | Negative | Negative | Negative |
| Coxsackie A Type 1, 4, 5, 6, 19, 22 | Not done | Not done | Not done |
| Poliomyelitis Type 1-3 | Negative | Negative | Negative |
| ECHO Type 1-28, E 59 (JV 10) | " | " | " |
| Frater, PR 10, 17, 20, 22, 28, Hu 780, 2080 | " | " | " |
| C-18, Hu 39, 504, 659, HSO, Pett | " | " | " |

* In case of rabbit immune serum 1:15, monkey immune serum 1:25 dilution was used.

† Immune serum was not available for testing.

‡ In case of ECHO 4 : 1:5 diluted immune serum was used, and CF test was also performed with type specific guinea pig immune serum(16).

§ Coxsackie A Type 2, 3, 8, and 12 viruses, adapted to primary human amnion culture cells (17), failed to grow in FL human amnion line cells used in this laboratory to propagate viruses not pathogenic for monkey kidney cells. In the case of these 4 viruses the cross neutralization tests were done in human amnion primary cell cultures.

ney, human amnion FL line, HeLa, chicken embryo, guinea pig kidney and rabbit kidney primary cell cultures were inoculated with these viruses. None of the 3 viruses produced symptoms of illness when suckling mice (i.c., i. p.), adult mice (i.c., i.p.), rhesus monkey (i.m.), guinea pig (i.m., i.p.), rabbits (i.m., i.v.) were inoculated. Chick embryos inoculated intra-allantoically on the seventh day of incubation hatched at the expected time and the birds showed no visible abnormalities.

*Plaque formation.* The viruses H 130 and H 170 formed plaques on monkey kidney monolayer cell cultures. Clear, round plaques appeared on the third day and increased in size to 5-10 mm in diameter by the 5th-7th day. No plaque formation was observed with the H 136 virus, in spite of the use of different overlay media.

TABLE II. Cross Neutralization Test with H 130, H 136 and H 170 Viruses.

| Virus tested | H 130 | H 136 | H 170 |
|---|---|---|---|
| H 130 | 1:1, 280* | <1:10 | <1:10 |
| H 136 | <1:10 | 1:1, 280 | <1:10 |
| H 170 | <1:10 | <1:10 | 1:120 |

* Neutralization endpoint.

*Morphology of virus particle.* Pictures of air dried preparations of purified virus taken by the electron microscope revealed the presence of uniform round particles. The average size of the virus particles was found to be in case of H 130 : 36.5 $\pm$ 5.5 m$\mu$, H 136 : 32.4 $\pm$ 5.1 m$\mu$, and H 170 : 35.5 $\pm$ 4.3 m$\mu$, respectively (single particles measured). These values are very close to the size of poliovirus and other human enteroviruses.

*Hemagglutination.* The hemagglutinating activity of the virus was tested by the method of Goldfield(14) at 37 C°, room and 4 C° temperatures. No hemagglutination was observed in case of H 130 and H 170 viruses using chick, rhesus monkey, rabbit, guinea pig, rat, sheep, mouse, bovine and human Type O erythrocytes. In case of H 136 virus, a low hemagglutination titer of 1:16 was observed with mouse red blood cells at 4 C° but not at 37 C° or room temperature. Tests employing erythrocytes of other species were negative.

*Stability of viruses.* Heat inactivation studies were carried out using high titered virus in tightly stoppered Wassermann tubes

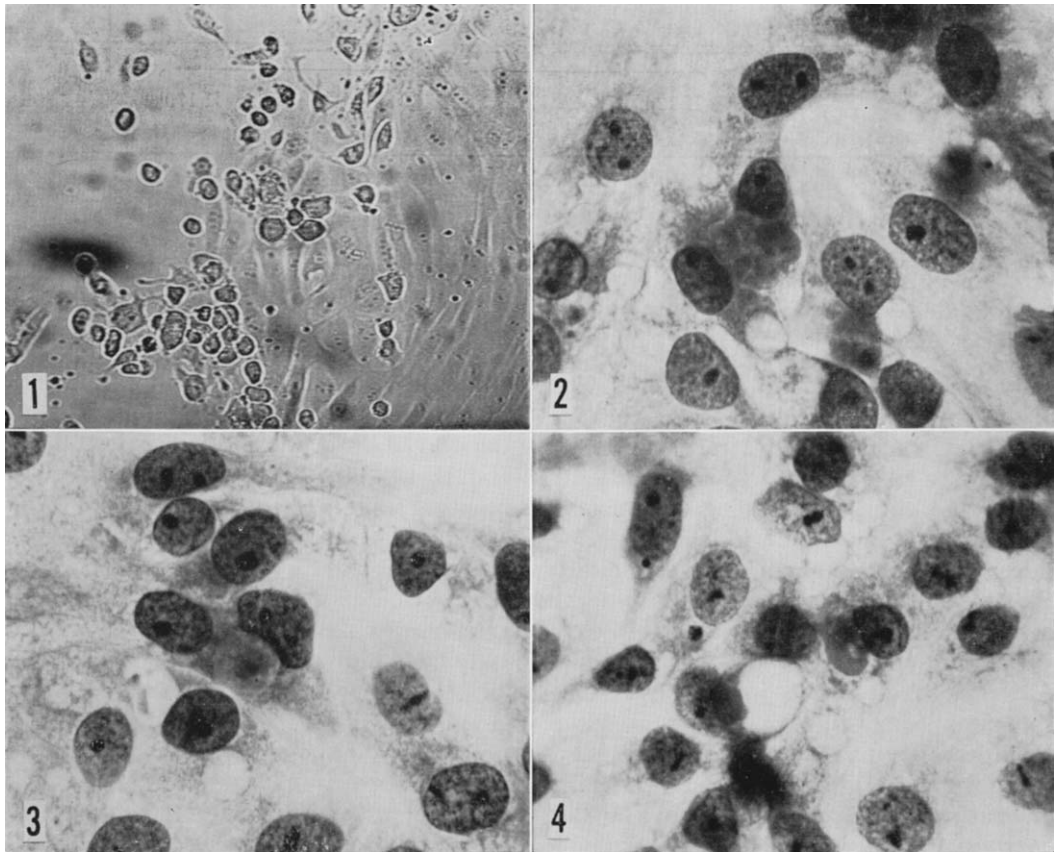


FIG. 1. Monkey kidney cell culture infected with H 130 virus, 12 hr after infection. Magnification × 185.

FIG. 2. H 130 virus infected monkey kidney culture cells, 12 hr after infection. Hematoxylin-Eosin stained preparation. Magnification × 1.300.

FIG. 3. H 136 virus infected monkey kidney culture cells, 12 hr after infection. Hematoxylin-Eosin stained preparation. Magnification × 1.300.

FIG. 4. H 170 virus infected monkey kidney culture cells, 12 hr after infection. Hematoxylin-Eosin stained preparation. Magnification × 1.300.

which were kept submerged in a water bath at various temperatures. Samples were withdrawn at regular intervals, chilled in crushed ice and amount of surviving virus determined. The results are presented in Fig. 5. The virus H 130 was more stable than H 136 and H 170. All 3 viruses were markedly more heat sensitive than most strains of poliomyelitis viruses(15).

*The nature of the viruses.* Indirect evidence indicated that the viruses were all RNA type viruses. Five-fluorodeoxyuridine (10 γ per ml) failed to inhibit the multiplication of these viruses in monkey cell cultures treated with the inhibitor 8 hours before virus infection. This concentration of 5-fluorodeoxyuridine inhibited multiplication of adenovirus Type 7, which inhibition could be reversed by addition of thymidine (50 γ per ml).

*Antibody studies.* A limited number of sera collected in 1957 in Mexico City from patients suffering from dysentery were tested for the presence of antibodies against these viruses. The results are summarized in Table III. An attempt was made to demonstrate the presence of antibodies in blood drawn in 1960-61 from residents of Allegheny County. Of the 211 normal adult sera tested, antibody was found in 11 cases against the H 130 virus, and in 20 against the H 136 virus. In no instance was anti-

TABLE III. Presence of Antibodies Against H 130, H 136, H 170 Viruses in Sera of Patients from Mexico City Suffering from Dysentery.*

| | Viruses | | | | | |
|---|---|---|---|---|---|---|
| | H 130 | | H 136 | | H 170 | |
| Patient No. | Acute | Conv | Acute | Conv | Acute | Conv |
| 77 | 1:12† | 1:128 | <1:8 | <1:8 | <1:8 | <1:8 |
| 83 | — ‡ | 1:96 | — | <1:8 | — | <1:8 |
| 117 | — | 1:64 | — | <1:8 | — | <1:8 |
| 76 | <1:8 | <1:8 | <1:8 | 1:64 | <1:8 | <1:8 |

* Total No. of Sera tested were 16.
† Neutralization endpoint.
‡ Acute serum specimen was not available.

body demonstrable against the H 170 virus at the 1:8 screening dilution level. The antibody titer found showed a range of 1:8 to 1:128 when tested against approximately 100 $TCID_{50}$ of virus. The finding of antibodies against H 130 and H 136 in human sera obtained in Western Pennsylvania indicated the possibility of infection with these agents or related ones in this area.

*Evidence of human origin of these viruses.* All these viruses were isolated from stool specimens obtained from patients suffering from dysentery in Mexico City, D. F., Mexico, in monkey kidney cell cultures. As the original specimens were no longer available, no attempt could be made to reisolate the viruses. No paired (acute and convalescent) sera were available from the patients from whom these viruses were isolated. Sera obtained from other patients, hospitalized with dysentery at the same time, showed the presence of antibodies against H 130 virus (Table III), and a significant rise in antibody titer against H 130 virus could be demonstrated in one case, and against H 136 virus in another. No antibody was found against the H 170 virus. Antibodies against H 130 and H 136 viruses were also found in sera of normal adult residents of Western Pennsylvania, but no antibody was demonstrable against the H 170 virus. Thus we have no direct evidence that these agents are human enteroviruses, but indirect evidence of antibody studies give indication that the H 130 and H 136 viruses may be members of this group.

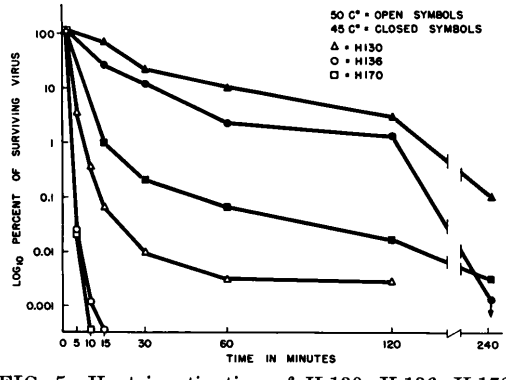


FIG. 5. Heat inactivation of H 130, H 136, H 170 viruses.

*Summary.* Three immunologically unrelated enterovirus-like agents, antigenically unrelated to any of the recognized human prototypes were isolated from stool specimens of patients suffering from bacillary dysentery in Mexico City in 1957. The viruses were not pathogenic for suckling mice nor for cultured cells of human origin. Indirect evidence based on antibody studies would indicate that 2 of these viruses may belong to the human enterovirus group. The relationship of these agents to the clinical disease observed remains undetermined.

The expert technical assistance of Mrs. Christine Ziegler is gratefully acknowledged.

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Received March 11, 1963. P.S.E.B.M., 1963, v113.

## Influence of Dietary Potassium and Sodium on Cesium-137 Retention in Rats.* (28348)

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Recently, Richmond and Furchner(1) reported that high dietary levels of potassium significantly increased the excretion of a single dose of $Cs^{137}$ in rats. In the above study, the normal diet (1% K) was supplemented with sufficient KCl to raise the potassium concentration in the diet to 5, 8 and 11% and thereby increase the corresponding salt content by 7.6%, 15.2% and 20.9%. It would seem, then, that such a high salt intake could have deleterious effects which, in turn, may account for the reported observations. Richmond and Furchner(1) gave no data on body weight changes or on feed and water intake to indicate if other physiological changes occurred. Previously, Wasserman and Comar(2) found that raising dietary $K^+$ from 0.2% to 1.9% decreased the retention of chronically ingested $Cs^{137}$ by a factor of 2; others had observed little or no effect of potassium on $Cs^{137}$ retention except when a potassium deficient diet was made adequate(3-5).

The present experiments were undertaken to determine the effect of either 5% dietary potassium or sodium on $Cs^{137}$ retention; like Richmond and Furchner(1), potassium did enhance $Cs^{137}$ turnover but sodium was equally effective, and both salts significantly increased water consumption.

*Materials and methods.* In the first experiment, female albino rats of the Carworth strain (about 6 weeks of age) were placed on either a diet consisting of ground Rockland diet (0.7% $K^+$ and 0.4% $Na^+$) or the Rockland diet containing 9.5% KCl. At one week, the rats were injected intraperitoneally with about 50 $\mu$c $Cs^{137}$ (carrier-free) and the body retention of the radionuclide followed for 34 days. Cesium-137 retention was determined by counting the rats in a quart screw-cap jar (with air holes) at 12 inches from a 2-inch NaI scintillation probe attached to a count rate meter and a single channel analyzer. The animals were counted against a known standard placed in the same geometric situation. The retention on any given day was calculated from the equation below:

$$Cs^{137}\ \text{retention}\ (\%) = \frac{\text{Rat count rate at time, t}}{\text{Rat count rate at t} = 0} \times \frac{\text{Std. count rate at t} = 0}{\text{Std. count rate at time, t}} \times 100$$

where time (t) is in days. The initial count was made immediately after radionuclide administration and taken as 100%.

The second experiment was identical to the first except that the basal diet was supplemented with 12.7% NaCl (5% $Na^+$).

* This study supported by AEC grant. The technical assistance of Philip Shapiro is gratefully acknowledged.