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Isolation of an Unclassified Enterovirus from Healthy Children.* (31865)

ANDREW S. ABRAHAM† (Introduced by F. S. Cheever)
Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

During a long term epidemiological study of viral infection in a semiclosed community (Detention Home of the Juvenile Court of Allegheny County) in Pittsburgh, Pa., carried out during 1960-61, an antigenically distinct enterovirus apparently unrelated to any of the recognized human types was isolated from 2 healthy children by the inoculation of primary monkey kidney cell cultures. This paper describes the circumstances of isolation, and the attempts to characterize and classify this virus.

Materials and methods. The sources of prototype viruses and immune sera, assay of viruses, the technique of neutralization tests, preparation of viruses for electron microscopy, and the method of preparation of immune sera and cell cultures have been described(1). The Caldwell virus(2) and immune serum were received from Dr. P. S. Kamitsuka, and the Bryant virus from Dr. G. S. Hsiung(3).

Experimental results. Isolation of the virus: The first virus of the Hu 2080 group (Hu 1967) was isolated in primary rhesus monkey kidney cultures from a rectal swab taken from a child during a routine weekly sampling. He had been a resident for many months in this institution, and left a few days after the last swab (from which the virus Hu 1967 was isolated) was taken. Another child, also a resident for many months, yielded the second virus of this group (Hu 2080) in April, 1961, a week after the release of the first mentioned child. This child remained a resident for 2 more months in this institution, but none of the rectal swabs taken subsequently at weekly intervals yielded this virus again.

Identification of the virus: Both the Hu 1967 and Hu 2080 agents were propagated in primary rhesus monkey kidney cell cultures, and carried through fourteen terminal dilution passages. After both the fourth and the fourteenth passages neutralization tests were carried out employing immune sera prepared against the recognized human enterovirus types with the exception of ECHO 28 and 29 for which no antisera were available. The results were uniformly negative. Potent immune sera were prepared against both viruses in rhesus monkeys. The two agents proved to be serologically identical and Hu 2080 was arbitrarily designated as the prototype. No immunological relationship was detected when these two sera were tested against all human prototype enteroviruses. Several other enterovirus-like agents, isolated in this laboratory and elsewhere, were also tested in cross-neutralization tests. These

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† Present address: Peter Bent Brigham Hospital, Boston, Mass.

TABLE I. Cross-Neutralization Tests Conducted in Identification of Hu 2080 Virus.

1. Prototype immune sera	Hu 2080 virus
Coxsackie A, Types 1-21, and B Types 1-6*	Negative
Poliomyelitis, Types 1-3	"
ECHO Types 1-32†	"
H 136, PR 17, 20, 22, 28‡	"
HSO, Pett, C-18, Hu 39, Hu 504, Hu 659	"
2. Prototype viruses	**Hu 2080 immune serum**
Coxsackie A, Types 2, 3, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21 and Coxsackie B, Types 1-6§	Negative
Poliomyelitis, Types 1-3	"
ECHO Types 1-32	"
H 136, PR 17, 20, 22, 28	"
HSO, Pett, C-18, Hu 39, Hu 504, Hu 659, Bryant	"

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

† In the case of ECHO Type 4, 1:5 diluted immune serum was used, and a complement fixation test was also performed with type specific guinea pig immune serum. Types 28 and 29 were not tested.

‡ (6).

§ Coxsackie A, Types 2, 3, 8, 12, adapted to primary human amnion cell cultures, failed to grow in FL human amnion cell line used to propagate viruses not pathogenic for monkey kidney cell cultures. In the case of these 4 viruses, the cross-neutralization tests were done in human amnion primary cell cultures.

viruses were: H 136(4), HSO(5), Bryant(3), Pett(6), Thai C-18(7), Hu 39(1), Hu 504 and Hu 659.‡ No cross-neutralization was detected with the two viruses of the Hu 2080 group. The results of the cross-neuralization test are summarized in Table I.

Cytopathogenicity of the viruses: In primary rhesus monkey kidney cell cultures the typical enterovirus-like cytopathogenic effect (CPE) became evident after the second day following inoculation of approximately 100 $TCID_{50}$ of virus and the destruction of the cell sheet was complete by the third or fourth day. The virus titer in the supernatant fluid was found to be about 10^{-7} $TCID_{50}$ per ml.

‡ These last 2 viruses (Hu 504 and Hu 659) are possible "prime" strains of Coxsackie A Type 17 virus isolated in this laboratory. The Mill virus isolated in 1963 by Murphy in Australia has been shown to be identical with Hu 659(8).

In hematoxylin and eosin stained preparations of cell cultures infected with Hu 2080 virus an eosinophilic mass could be demonstrated in the cytoplasm of the infected cells with displacement of the shrunken nucleus and the cytoplasm to the periphery of the cell—a lesion characteristically produced by human enteroviruses propagated in human or simian cell cultures(9). Both viruses multiplied and produced CPE in primary cell cultures of rhesus monkey kidney and testis as well as in $LLCMK_2$ monkey kidney stable line cells(10), but they failed to produce CPE in primary human amnion and FL amnion stable cell line cells.

Characteristics of the virus: The average size of the virus particles was found to be 34.1 ± 8.0 mμ when individual particles were measured by the electron microscope in air dried preparations. No hemagglutination was observed using chick, rhesus monkey, mouse, guinea pig, sheep and bovine red blood cells at 4°C, room temperature or 37°C, by the methed of Goldfield(11). There was plaque formation by either of the 2 viruses of Hu 2080 group. Heat inactivation studies carried out as previously described(1) indicated a greater sensitivity than that associated with polioviruses and Coxsackie viruses. As regards other characteristics tested, however, the two strains resembled enteroviruses. These included: resistance to inactivation by ether and by high concentrations of hydrogen and hydroxyl ions, stabilization against heat inactivation (55°C for 120 minutes) by the presence of magnesium ions in 1 molar concentration(5), failure of 5 fluorodeoxyuridine (10 gamma per ml) to inhibit multiplication of the viruses, and in contrast, the ability of guanidine (10 μg per ml) and 20 (alpha-hydroxybenzyl)-benzinidazole (200 μg per ml) to do so(12).

Prevalence of Hu 2080 virus: Attempts were made to demonstrate the presence of antibodies in blood drawn from residents of Allegheny County in Western Pennsylvania. Of the 219 sera drawn in 1961 40 (18%) showed the presence of antibodies as compared to 23 (16%) of the 145 sera drawn in 1963. The antibody titers were low for the most part but occasional high titers were en-

countered. No sera were available for testing from the children from whom the viruses were isolated.

Summary. A viral agent was isolated in primary rhesus monkey kidney cell cultures from rectal swabs obtained from 2 healthy children resident in Western Pennsylvania. Although the agent had many of the characteristics of the human enterovirus group no serological relationship to any of the recognized protypes was demonstrated. Sera drawn from 2 normal adult population groups showed the presence of antibodies in a significant proportion (16-18%) against the virus which was successfully reisolated from the 2 original specimens. The evidence suggests that this as yet unidentified agent is a member of the human enterovirus group.

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Capillaries in Heart and Skeletal Muscle of Dog and Rabbit.* (31866)

K. RAKUSAN AND W. DU MESNIL DE ROCHEMONT
(Introduced by R. J. Bing)
Department of Medicine, Wayne State University School of Medicine, Detroit, Michigan

Since Krogh's pioneer work it is believed that the capillary density in small animals is higher than in the larger ones and that this is related to the higher metabolic rate of smaller animals. Krogh(1) found this difference in capillary counts per square mm of cross section in skeletal muscles of the horse, the dog and the guinea pig. Similar results were obtained by Paff in the rat, the guinea pig and the cat(2). Schmidt-Nielsen and Pennycuik(3) studied a large series of mammals and found that capillary density in different skeletal muscles was highest in the two smallest animals (bat and mouse). On the other hand, no definite trend was discernible in other animals examined. They called attention to many other factors influencing capillary density such as activity of the animal, acclimatization and size of the muscle fibers. Similar studies on the heart muscle are lacking.

In the experiments reported here we compared capillary density of heart and skeletal muscle in two animals of different size and physical activity: the dog and the domestic rabbit.

Methods. The capacity of the terminal vascular bed in per cent of tissue volume was used as an indicator of the capillary density (4). The procedure, identical for both species, was as follows: the animals were anesthetized with diabutal (sodium pentobarbital), artificially ventilated and the heart was exposed *via* left thoracotomy. Albumin I131

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