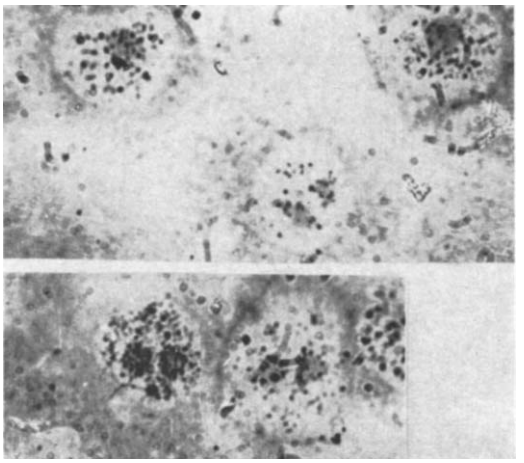

FIG. 3. Radioautographs of actinomycin-treated NDV-infected HEp-2 cells exposed to 10 μC/ml H^3-uridine from 4 to 10 hr after infection. × 600.

cytoplasm when it functions as a template for S- and V-antigen synthesis. This migration occurs more quickly than in the case of Sendai(4) and influenza(5) viruses.

Since the reproduction of Sendai virus occurs also in nucleoli(4), it may be assumed that nucleolar structures play an important role in RNA synthesis of paramyxoviruses.

The label found in nuclei during the first hours after infection seems to migrate also into the cytoplasm. The origin of this label is not clear; it may represent the synthesis of some early RNA. Such RNA was shown to be induced by Sendai and FPV(6).

Summary. The newly synthesized actinomycin-resistant RNA as determined by brief pulses of H^3-uridine at the period of NDV-induced RNA synthesis is detected in the nucleolus. When the contact with H^3-uridine is prolonged the label is found over the cytoplasm. It is suggested that the RNA of Newcastle disease virus replicates in the nucleolus and migrates into the cytoplasm.

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Growth and Cytopathic Effect of H Type Rhinoviruses in Monkey Kidney Tissue Culture. (31454)

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Rhinoviruses have been divided into two groups based on their capacity to multiply and produce cytopathic effect (CPE) in primary monkey kidney tissue cultures (MKTC)(1). Those virus strains which multiply in MKTC have been designated M strains and those which do not are called H strains. Both H and M type rhinoviruses have the capacity to propagate and produce CPE in tissue cultures of human origin. It is not clear whether this division represents true differences in biologic properties of viruses or is dependent on other factors. In addition, since differences in antigenicity and in the seasonal pattern of infection and illness have been reported between M and H type rhinoviruses (2,3), it was of interest to investigate the stability of the difference in capacity of rhinoviruses to grow in MKTC. In the present study it was possible to demonstrate growth and CPE in MKTC of 5 rhinovirus serotypes previously considered to be H strains.

Materials and methods. Source of virus. The H type rhinoviruses used in this study were prototype strains NIH 1734, 353, 11757, 1059, and 33342(4). Each strain had been

isolated in primary human embryonic kidney (HEK) tissue cultures, "purified" by triple terminal dilutions, and passaged a total of 14 to 22 times in HEK, human nasopharyngeal carcinoma (KB), or human embryonic fibroblast tissue cultures. The basis for classification of these isolates as H strains was as follows: attempts to isolate strains 33342 and 11757 in MKTC had been unsuccessful, and low passage HEK tissue culture material infected with each strain failed to produce CPE or viral interference in MKTC(4).

Nasopharyngeal wash specimens were obtained from each of 3 volunteers who had been inoculated with second human embryonic lung fibroblast (WI-26) passage harvest of rhinovirus NIH 1734(5). Selected specimens contained large amounts of rhinovirus as determined by virus titrations in WI-38 tissue cultures.

Tissue cultures. The tissue cultures employed in this study were human embryonic lung fibroblast cultures (WI-38, NIH media unit), Rhesus MKTC, and African green MKTC (Microbiological Associates, Inc., Bethesda, Md.). Unless otherwise indicated, maintenance medium consisted of 49% Medium No. 199, 49% BME, 2% inactivated (56°C, 30 minutes) calf serum, 0.2% SV-5 hyperimmune rabbit antiserum, and supplemental antibiotics (pH 7.2-7.5). Cultures were incubated at 33°C on a rotating drum at 12 rph and re-fed periodically. WI-38 cultures were observed for CPE for 14 days, and MKTC for a minimum of 3 weeks. Harvest fluids from CPE-positive MKTC were used for serological identification, acid lability tests, and further MKTC passage. Harvest fluids from negative MKTC were passaged an additional time in WI-38 cultures.

Other procedures. Virus titrations were performed by inoculation of two-tenths ml of serial 10-fold viral dilutions prepared in Hanks' balanced salt solution into each of 4 tissue cultures. The Spearman-Kärber method was used to determine 50 per cent tissue culture infectious doses ($TCID_{50}$).

Serological identification of the CPE-positive MKTC harvests was determined in WI-38 tissue culture by neutralization of 32 or more $TCID_{50}$ by 20 or less antibody units of specific hyperimmune guinea pig antiserum.

Acid lability was tested by the method described by Tyrrell and Chanock (first method)(6).

Results. Occurrence of CPE in MKTC. CPE, indistinguishable from that produced by M type rhinovirus, occurred in MKTC following an input inoculum of 10^4 or more $TCID_{50}$ of the rhinovirus prototype strains NIH 1734, 353, 33342, and 1059. In the case of strain 11757, an input inoculum of $10^{5.2}$ $TCID_{50}$ was required. Harvest fluids obtained after 21 days' incubation contained 10-100-fold more virus than had been inoculated indicating that viral replication occurred.

The type of CPE produced by each of these strains was indistinguishable. One or more foci of rounded refractile cells first appeared in 7 to 15 days at the periphery of the tissue monolayer. These foci then extended around the edge of the cell sheet which gradually became smaller as the infected cells fell off the glass. However, complete degeneration did not occur for any of the strains in the initial 21-day observation period. In one instance, a single focus of CPE was observed to disappear, but rhinovirus was recovered from the fluid harvest obtained at the time CPE was observed. Virus growth was never detected in the absence of CPE.

Effect of growth conditions. Testing with strain NIH 1734 revealed that CPE occurred in MKTC maintained with a variety of serum types and concentrations (0-5% chicken, horse or calf), and tissue culture media adjusted to final pH of 6.8 to 7.7. Rhesus and African green MKTC were similar in their ability to support viral growth and exhibit CPE; however, occasional lots of each type of MKTC differed in their sensitivity and necessitated testing of each lot of MKTC with a standard pool of rhinovirus NIH 1734.

Effect of tissue culture passage. To ascertain the importance of tissue culture passage on these results, studies were made with nasopharyngeal wash specimens obtained from volunteers infected with strain NIH 1734. As shown in Table I, it was not possible to isolate rhinovirus directly in MKTC from these specimens. However, after 2 passages in WI-38 cultures, CPE was observed in MKTC.

Some characteristics of MKTC-adapted

TABLE I. Effect of Tissue Culture Passage on Production of CPE in MKTC by Rhinovirus NIH 1734.

Source of virus	Volunteer A	B	C
Nasopharyngeal wash	0*(2.4)†	0 (3.7)	0 (3.2)
WI_1 harvest	0 (3.2)	0 (4.4)	0 (4.2)
WI_2 "	+ (4.4)	+ (4.2)	+ (4.9)

* 0 = no CPE in MKTC, + = CPE in MKTC.
† Quantity of virus inoculated (log_{10} $TCID_{50}$) based on titration in WI-38 cultures.

rhinovirus. Studies with NIH 1734 rhinovirus revealed that the first passage virus yield from MKTC was $10^{4.7-5.2}$ $TCID_{50}$ (titration in WI-38). In second passage, an inoculum of 10^0 to 10^1 $TCID_{50}$ was sufficient to infect the MKTC cultures. CPE appeared earlier than on initial passage, and often progressed to complete destruction of the cell sheet. The virus yield was $10^{5.7-6.2}$ $TCID_{50}$. However, in subsequent passage, there was no further enhancement in the rate or extent of occurrence of CPE or the quantity of virus produced. As shown in Table II, MKTC-adapted 1734 resembled more closely 2 M strains (ECHO 28 or HGP) than unadapted 1734, when each strain was tested by simultaneous titration in WI-38 cultures and MKTC.

In neutralization tests employing MKTC or WI-38 cultures and specific guinea pig hyperimmune antiserum the MKTC-adapted virus of each prototype was serologically identical to the parent strain. Each serotype remained acid labile(6) after MKTC passage. In WI-38 tissue culture, neutralization tests (7) with 6 paired human sera using NIH 1734 rhinovirus before and after passage in MKTC, no significant differences were observed between the titers of antibody detected with each of these viruses.

TABLE II. Comparative Titrations of Types of Rhinovirus in Human and Simian Tissue Cultures.

Virus	Type	Quantity of virus determined by simultaneous titrations in: WI-38	MKTC
1734	H	4.6*	1.2
ECHO-28	M	4.5	3.3
HGP	M	5.4	4.3
1734	MKTC-adapted (4th passage)	6.0	4.0

* Mean (log_{10} $TCID_{50}$ per ml) of 3 titrations.

Discussion. This study demonstrated that 5 rhinovirus serotypes, previously classified as H strains, will replicate and produce CPE in MKTC. It has been shown that this result is not dependent on narrowly defined tissue culture conditions, but is related in part to the quantity of input inoculum. Experience with the nasopharyngeal washings suggests that passage in HEK and KB cultures is not essential. Passage in WI-38 cultures, however, was necessary for the production of CPE in MKTC. It may be that this effect was due to the higher input inoculum available after passage in WI-38 cultures. However, since the first WI-38 harvest fluids for 2 of the 3 volunteer specimens which did not produce CPE contained quantities of virus similar to those from second WI-38 harvests which did produce this result, it may be that WI-38 culture passage enhances the ability of the virus to replicate in MKTC.

These results suggest that classification of rhinoviruses as M or H types may be misleading. An example of the confusion that can arise may be found in the reported isolation of both M and H strains of NIH 1734 and NIH 1200(8). However, since differences in antigenicity and in the seasonal pattern of infection and illness have been reported between M and H type rhinoviruses(2,3), an important distinction may yet exist between those strains that can be isolated in MKTC and those that cannot.

Summary. Growth and production of CPE in MKTC has been demonstrated for 5 rhinovirus strains, NIH 1734, 353, 11757, 1059, and 33342, previously considered to be H strains.

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Gastric Acid Response to 2-Deoxy-D-Glucose in Chronic Fistula Rats.* (31455)

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Hirschowitz and co-workers(1,2) recently established the usefulness of 2-deoxy-D-glucose (2-D.G.) as a gastric secretory stimulant in dog and man. Acid production after 2-D.G. equalled that after augmented histamine stimulation and exceeded that after insulin, while pepsin secretion was greater with 2-D.G. than after either histamine or insulin. The secretory response was abolished by bilateral vagotomy or atropine. The mechanism of the stimulatory action was not clear but it depended upon the competition of 2-D.G. with glucose at an intracellular site(3) in the brain. On the basis of these results 2-D.G. was proposed as a single stimulus which could be used to evaluate central neural mechanisms involved in acid and pepsin secretion, and to estimate the maximum secretory capacity of the stomach. We were attracted by the possibility that 2-D.G. might have similar gastric secretory effects in the rat and thus would be useful in studies of the central nervous control of gastric secretion. This paper is a report of the acid response of the chronic gastric fistula rat to varying doses of 2-D.G. (25-400 mg/kg). No dose of 2-D.G. in this range produced a significantly greater acid response than that produced by insulin (0.4 U/kg).

Materials and methods. Seven Sprague-Dawley rats, weighing 300-500 g, were equipped with chronic gastric fistulas. Gastric contents were collected according to the method of Brodie *et al*(4). Hourly volumes were measured directly. Acid concentration was determined by electrometric titration to pH 7.0. Animals were deprived of food for at least 18 hours before each experiment. All experiments had the following design: 1 hour control gastric collection followed by a subcutaneous injection of 0.9% saline, or U/40 regular insulin§ (0.4 U/kg) or 2-D.G.‖ (25-400 mg/kg), followed by 4 hourly gastric collections. Acid outputs were computed for each hour. The statistical significance of the differences in acid responses to the various stimuli was determined using the two-sample rank test.

Results. The gastric acid response to 2-D. G. was "all or none" over the range of 50-400 mg/kg (Table I). The peak response occurred in the second or third hour following the subcutaneous injection in most of the experiments. The response usually lasted longer than 4 hours; no attempt was made to measure the duration of the response more precisely.

Four rats were tested with both 2-D.G.

TABLE I. Acid Response to 2-D.G.

Treatment	No. of rats	"N"	Acid output (μEq/4 hr)
.9% Saline	3	9	40.1 ± 11.8
2-D.G. (25 mg/kg)	2	6	49.2 ± 15.9
2-D.G. (50 ")	7	8	184.9 ± 48.1
2-D.G. (100 ")	7	11	296.8 ± 80.4
2-D.G. (200 ")	7	10	136.5 ± 36.0
2-D.G. (400 ")	5	10	216.1 ± 77.7

Using the two-sample rank test, there is no significant difference between the acid responses to doses of 2-D.G. $\geq$ 50 mg/kg.

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§ Iletin, Eli Lilly & Co.

‖ Mann Research Laboratories, Inc., New York, and Sigma Chemical Co., St. Louis, Mo.