weight) or tissue nitrogen values (33.5-37.4 mg nitrogen per g tissue dry weight) was observed.

A comparison of tissue MDH activities with previously determined oxygen consumption changes is given in Fig. 1. On day 8 post-thyroidectomy the liver MDH capacity rose to a significant value of 121% of control whereas the corresponding QO_2 continued to decrease to a value of 80% of the control. These values for MDH capacity and tissue QO_2 remained approximately at these levels throughout the remainder of the experimental period. There is apparently a complete lack of relationship between liver QO_2 and MDH activity since there was a progressive decrease in oxygen uptake from days 2 to 12 before plateauing, while the enzyme activity showed no change until an abrupt rise on day 8.

An *in vitro* study reported by Wolff and Ball(2) using heart homogenates showed a decrease in MDH capacity with addition of thyroxine to the system. The present *in vivo* study of liver revealed that a physiological reduction of circulating thyroxine after thyroidectomy resulted in an increase in MDH activity. Because of the difference in assay systems and tissues used, it is not certain whether the mechanisms involved in the two studies are the same.

Summary. Changes in MDH capacity of rat liver homogenates were followed for 28 days post-thyroidectomy. After an initial period of inconclusive results, liver showed an elevation in MDH capacity which occurred while the tissue QO_2 was decreasing. In this study of developing hypometabolism it is not possible to attribute the increased MDH capacities to a decreased oxygen consumption. Our findings do, however, support the concept that a thyroxine deficiency may have a stimulating influence on malic dehydrogenase capacity.

1. Barker, S. B., Physiol. Rev., 1951, v31, 205.
2. Wolff, E. C., Ball, E. G., J. Biol. Chem., 1957, v224, 1083.
3. Wolff, J., Wolff, E. C., Biochim. Biophys. Acta, 1957, v26, 387.
4. Nielson, R. R., Loizzi, R. F., Klitgaard, H. M., Am. J. Physiol., 1961, v200, 55.
5. Umbreit, W. W., Burris, R. H., Stauffer, J. F., in Manometric Techniques, Burgess Publishing Co., Minneapolis, 1957, p176.
6. Folin, O., Wu, N., J. Biol. Chem., 1919, v38, 81.

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A Stable Human Cell Line as a Host System for Sendai Virus.* (29943)

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Although Sendai virus (parainfluenza 1) has been thought by some investigators to be an agent of human disease (cf. 1,2), it has shown little capacity for multiplication in stable human cell lines. Cultivation of the virus and study of its multiplication usually have been performed in chicken embryos or in primary cultures of animal cells(3-9). In primary cultures of certain human cells it has been shown that a complete cycle of multiplication can be obtained(5,6,10,11), but even in primary cultures an incomplete cycle frequently occurs(10,11,12), and an incomplete cycle has been the regular result of inoculation of stable human cell lines(2,6,13, 14,15,16).

Data presented here demonstrate (a) that serial cultivation of Sendai virus can be accomplished in Chang's stable human conjunctiva cell line, (b) that the multiplication cycle in these cells is a complete one, (c) that once adapted to growth in conjunctiva cells the virus can then be cultivated readily either in conjunctiva cells or in chicken em-

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bryos, and (d) that the conjunctiva cells provide a more sensitive assay system for the adapted virus than do chicken embryos even though the virus grows to a higher titer in the latter.

Materials and methods. Cell culture. Human conjunctiva cells(17) were grown in prescription bottles in Eagle's basal medium (18) supplemented with 20% horse serum. Cells were subcultured weekly using standard methods and were maintained free of pleuropneumonia-like organisms by incorporating 50 μg kanamycin per ml of growth medium for 7 of every 28 days of cultivation.

Viruses. An egg-adapted Sendai strain of parainfluenza 1 virus was obtained from Dr. M. Kuroya of Sendai, Japan. Subsequently, it had been passed an unrecorded number of times in the allantoic sac of embryonated eggs. The tissue culture adapted strain, used exclusively in these experiments, was grown in human conjunctiva cell cultures maintained in Eagle's medium containing 5% horse serum at 37°C; fluids were harvested 5 to 7 days post-inoculation when peak cytopathic changes were evident. Stocks of both egg-adapted and variant virus were diluted with an equal volume of inactivated (56° C, 30 minutes) horse serum and were stored at —70°C.

Infectivity titrations in eggs. Egg infectious units were determined by inoculating 0.1 ml of each serial 10-fold dilution into 6 ten-day-old embryonated eggs. After incubation of the eggs for 48 to 72 hours at 35°C, samples of allantoic fluid were tested for hemagglutinin. The 50% egg-infective dose (EID_{50}) was calculated according to Reed and Muench(19).

Plaque assay. Confluent monolayers of human conjunctiva cells in 2 or 3 ounce prescription bottles were washed free of growth medium with Earle's saline. An appropriate dilution of virus suspension was added to each culture in a 1.0 ml volume and held for 40 minutes at room temperature. The bottles were rocked intermittently during this adsorption period. After decanting the inoculum, a soft agar overlay was added consisting of medium 199, 15% tryptose phosphate broth, 0.3% purified agar (Difco), antibiotics (penicillin, 100 units; streptomycin, 100 μg; amphotericin B, 50 μg per ml), and sodium bicarbonate to adjust the pH to 7.8. The bottles were left undisturbed at 35°C. On the fifth day the soft agar overlay was poured from the bottles and the cell sheet was washed with Earle's saline. One ml of 0.5% chick erythrocytes suspended in Earle's saline was added to each monolayer. After 30 minutes at 4°C, unadsorbed erythrocytes were washed from the monolayers with cold saline, and plaques were counted immediately. In later experiments the cells and adsorbed erythrocytes were fixed to glass by addition of methanol for 10 minutes. To increase the contrast between cells and erythrocytes, Kingsley stain (Harleco, Philadelphia) was added to cover the fixed cell sheet. After 30 to 50 seconds, the excess stain was removed with water. The erythrocytes adsorbed to the foci of infection stained magenta against the blue conjunctiva cells. Plaques of 2 sizes resulted and are shown in Fig. 1; small plaques ranged in sizes from 0.5 to 1.6 mm and large plaques from 2 to 4 mm. The small plaques, normally difficult to visualize, were detected readily by the staining con-

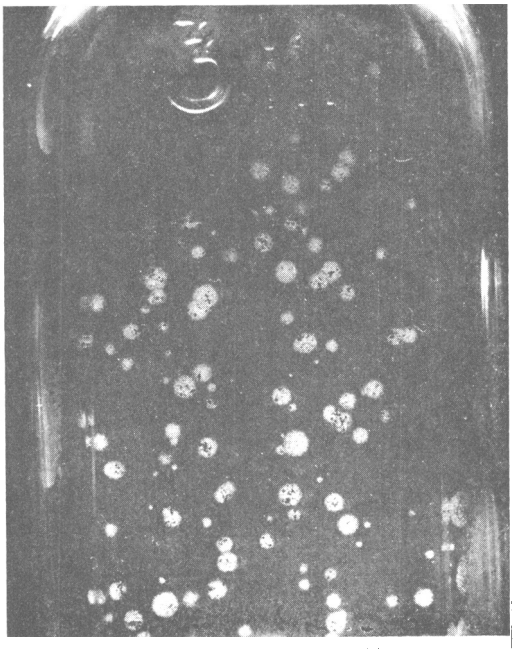


FIG. 1. Foci of infection of Sendai virus in human conjunctiva cells developed under 0.3% agar overlay in 5 days at 35°C and demonstrated by adsorption of chick erythrocytes. Kingsley stain.

TABLE I. Precision of Plaque Assay.

Virus dilution	Plaques per bottle					Mean	Variance	Standard deviation	Confidence limits* No. of bottles	Plaques per bottle	% of mean
1×10^{-7}	169	159	158	157	152	149.0	95.5	9.8	N=2	14.9	10.0
	152	151	148	147	144				N=3	12.1	8.1
	142	141	139	132					N=4	10.9	7.3
									N=5	10.0	6.7
1.48×10^{-7}	117	116	116	116	114	106.6	72.0	8.5	N=2	12.8	12.0
	112	111	110	106	102				N=3	10.4	9.8
	102	102	98	96	94				N=4	9.1	8.5
	93								N=5	8.0	7.5
2×10^{-7}	96	94	90	89	87	83.2	67.2	8.2	N=2	12.7	15.3
	85	85	84	77	76				N=3	10.4	12.5
	75	73	70						N=4	8.9	10.8
									N=5	8.1	9.7
4×10^{-7}	49	48	47	43	41	38.4	36.0	6.0	N=2	9.2	23.9
	39	38	38	37	37				N=3	7.5	19.6
	37	36	32	31	31				N=4	6.4	16.7
	30								N=5	5.8	15.1

* Confidence limits at 95% confidence = mean $\pm$ $t_{0.05}$ times standard error of mean for N number of replicates.

trast. Staining revealed significantly greater numbers of plaques than were visible on unstained monolayers. Assay bottles prepared in this way could be stored for later counting and observation since plaques retained the stain and remained clearly countable for over one year.

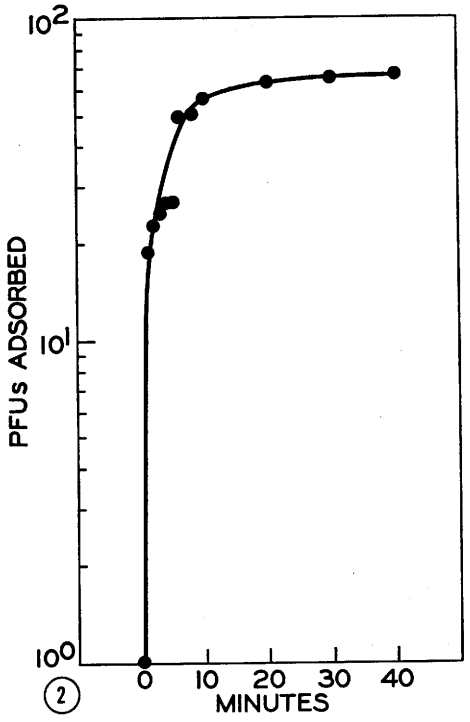
Antiserum. Guinea pigs were infected with egg-adapted Sendai virus by intranasal inoculation. Fourteen days later they were bled by cardiac puncture and their sera were pooled. Pre- and post-inoculation sera were inactivated at 56°C for 30 minutes and stored at —20°C.

Hemagglutination test. Hemagglutinin (HA) titers were determined by serial 2-fold dilutions of test materials in 0.4 ml phosphate buffered saline. An equal volume of 0.5% chick erythrocytes was added and the pattern was examined after 1 hour at 4°C. Titrations of cell-associated HA included pretreatment of cellular material with sodium periodate to destroy inhibitors as described by Granoff(20).

Complement-fixation test. Titrations of complement-fixing virus antigen in cells and fluids were performed as described by Chanock *et al*(21), using 4 units of antibody, 2 units of complement and overnight fixation at 4°C.

Results. After inoculation of cultures of conjunctiva cells with egg-adapted virus, the cytopathic effect (CPE) was mild and irregular for the first one or two subcultures, but thereafter the CPE regularly appeared on the 5th to 7th day after inoculation and was similar to that described for Sendai virus in other cell types(6). In the development of a plaque assay for the virus it was found, however, that under an agar overlay the CPE was insufficient to provide reliable recognition of foci of infection. Use of hemadsorption to reveal foci resulted in reliable and reproducible assays. The precision of the assay using hemadsorption plaques is shown in Table I. In replicate assays made from a series of virus dilutions, the variance was found to approximate the mean number of plaques through the range of 40 to 150 plaques per bottle. This suggests that the distribution of plaques was Poissonian and that each plaque was initiated by a single infectious particle. The calculated confidence limits revealed that at the 5% significance level the mean of 4 assay bottles did not vary by more than 17% from the mean count of 16 bottles, even when the mean number of plaques was only 35.

The temperature of incubation was important when conjunctiva cells were used for the assay of Sendai virus. Incubation at 35°C resulted in about 50% more plaques (Table II) than were found in assay cultures held at 37°C. Plaques formed at 35° were generally



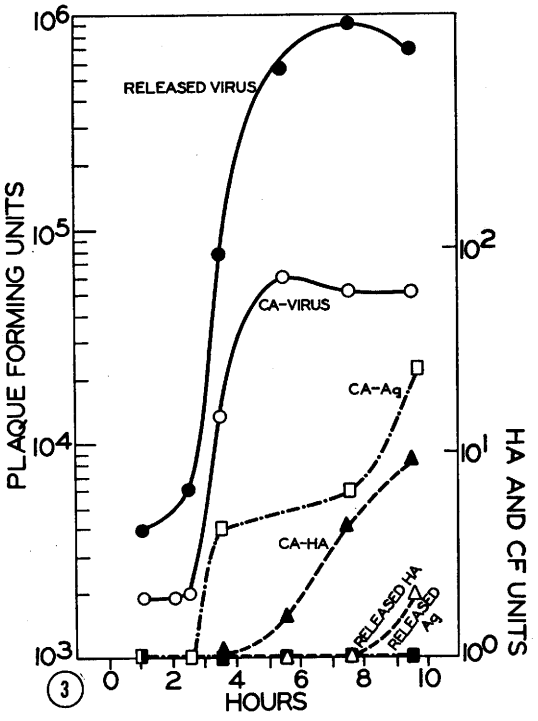


FIG. 2. Adsorption rate of Sendai virus to human conjunctiva cells. The inoculum, diluted to contain 70 PFU/ml, was added to monolayers of conjunctiva cells and held at room temperature for indicated times. Each point represents average of 4 assays.

FIG. 3. Growth curve of Sendai virus in human conjunctiva cells. CA = cell associated virus; HA = hemagglutinin; Ag = complement-fixing antigen.

smaller than at 37°C, but were large enough to count easily.

The adapted virus adsorbed rapidly to monolayers of conjunctiva cells; 50% of an inoculum was adsorbed in 5½ minutes and 90% in 15 minutes (Fig. 2). Adapted virus did not differ from the egg line of Sendai virus in its stability at 37°C, and the half-life at 37°C at pH 7.2 was 14 hours.

One-step growth cycle. Since Sendai virus had not been shown previously to multiply in cells maintained in serial cultivation, it was important to determine the characteristics of its multiplication in human conjunctiva cells. Actively growing cells were released from glass with trypsin-versene and were sedimented at 600 × *g* for 6 minutes. The cells were suspended in an inoculum containing $10^{6.8}$ PFU (10 PFU/cell). Immunofluorescent studies indicated that this inoculum infected 100% of cells. After 90 minutes at room temperature, the cells were centrifuged from the inoculum, washed 3 times with Earle's saline, and suspended in 4 ml of a 1:10 dilution of antiserum for 15 minutes at 37°C. The cells were then washed twice with Earle's saline and suspended in 10 ml of Eagle's medium supplemented with 5% horse serum. Thereafter, the cells ($10^{5.63}$ cells) were maintained in suspension in a paraffin-coated flask under 5% CO_2 in air at 37°C. At intervals samples were removed and centrifuged immediately to separate cellular and fluid phases. The cells were washed and resuspended in ½ of the original sample volume of Earle's saline and were disrupted by

TABLE II. Effect of Temperature of Incubation During Plaque Development on Number of Plaques Obtained.

Virus dilution	Avg No. of plaques per 5 replicate bottles incubated at: 35°C	37°C
1:4	143	107
1:8	94	47
1:16	35	30
1:32	20	5

TABLE III. Effect of Adaptation of Sendai Virus to Human Conjunctiva Cells on Capacity of the Virus to Multiply in Human Conjunctiva Cells and in Chick Embryo Allantois.

Host and passage No.	Assay system: CE allantoic sac (EIU/ml)*	Assay system: Human conjunctiva cells (PFU/ml)	Ratio PFU/EIU
E>35	2.0×10^7	5.0×10^1	2.5×10^{-6}
E>35, C12	2.8×10^6	3.0×10^6	1.1
E>35, C17	1.2×10^6	3.3×10^7	26.8
E>35, C11, E1†	1.3×10^7	1.4×10^8	11.0
E>35, C11, E5	9.7×10^7	8.0×10^8	8.3

* EIU = egg infectious unit: $EID_{50} \times 0.69$ according to Isaacs and Edney(24).
† E>35, C11, E1 = virus after >35 egg passage, then 11 passages in conjunctiva cells, and 1 passage in eggs.

3 cycles of freezing and thawing. All fractions were stored at —70°C until assayed.

Production of new infectious virus was clearly evident (Fig. 3) at 2½ hours after infection. For the next 3 hours the increase was very rapid. From the rate of virus release during that time, it seemed likely that the majority of the infectious units detected at one hour were unneutralized residue of the inoculum. Beginning with the 5th hour production was less rapid, but was continuous for another 20 hours (not shown in Fig. 3), at which time marked cytopathic effect was evident. Cell-associated infectious virus increased rapidly from 2½ to 5½ hours after inoculation and then reached a plateau. Cell-associated virus was always at concentrations much below those found in the fluid medium, thus indicating rapid release of newly-formed infectious units.

Cell-associated hemagglutinin reached measurable levels 5½ hours after infection and increased steadily over the next 4 hours, but hemagglutinin was not detectable in the medium until after 9½ hours. Complement-fixing antigen, on the other hand, accumulated in the cells quite early and was at a titer of 1:4 only 3½ hours after infection.

Effect of passage in conjunctiva cells. Adaptation of myxoviruses to a particular animal or cell host has frequently resulted in loss of capacity of the virus to multiply well in previously employed hosts(22,23). A small degree of this effect was observed during adaptation of Sendai virus to conjunctiva cells, but in the main, the virus gained the capacity for multiplication in conjunctiva cells without marked loss of capacity for multiplication *in ovo* (Table III). Virus obtained after 12 passages in conjunctiva cells had the same titer when assayed in eggs as when assayed on conjunctiva-cell monolayers. The yield of virus from the 17th passage in conjunctiva cells contained 26.8 PFU for each EIU and additional passages in conjunctiva cells beyond the 17th did not alter that ratio. Once the virus was adapted to conjunctiva cells, it could then be cultivated easily in either conjunctiva cells or *in ovo,* but the egg yielded higher titers of virus. Whether produced *in ovo* or in cultures of conjunctiva, however, the conjunctiva cell-adapted virus was 10 times more effective in producing plaques in conjunctiva cells than it was in initiating infection in the egg.

Discussion. The data presented indicate that Sendai virus can be cultivated serially in a stable line of human cells, Chang's human conjunctiva cells. The fact that multiple serial passages were possible suggests that the multiplication cycle of the virus in these cells is a relatively complete one. This is further supported by the ability of the virus to initiate the formation of macroscopic foci of infection in cell monolayers and, particularly, by the characteristics of the one-step growth cycle of the virus. Although the yield of infectious units per infected cell was low, the ratio of infectious units to hemagglutinating units was $10^{5.95}$ at the peak of infectious virus titers. Low yields of infectious virus from cells cultivated *in vitro* are not an uncommon finding for myxoviruses previously adapted to embryonated eggs.

An important point in the data presented here was the finding that once adapted to growth in conjunctiva c , Sendai virus could then be cultivated re ly in either con-

junctiva cells or in the allantoic sac of the chicken embryo. This is uncommon among myxoviruses. It represents a distinct advantage since in the egg the virus can be cultivated in large quantity with ease, and then the virus can be used for experiments in human cells. A somewhat surprising aspect of this flexibility of the adapted virus is the finding that, whether the virus is grown in conjunctiva cells or in the egg, the conjunctiva cell monolayer is considerably more sensitive in detecting infectious virus particles than is the egg.

Summary. Sendai strain of parainfluenza 1 virus previously passed in embryonated eggs was found to be adaptable to the stable line of human conjunctiva cells. Evidence for its multiplication in these cells was based on its capacity to produce typical CPE in 5-7 days throughout 17 serial passages, on its ability to initiate macroscopic plaque formation and on the nature of its single step growth cycle. The adapted virus could be cultivated in either conjunctiva cells or chick embryos. The former was more sensitive to viral infection (ratios of PFU:EIU up to 27) whereas higher virus titers were obtained from the latter.

1. Matsumoto, M., Am. Rev. Resp. Dis., 1963, v88, 46.
2. Chanock, R. M., Parrott, R. H., Johnson, K. M., Kapikan, A. Z., Bell, J. A., ibid., 1963, v88, 152.
3. Jensen, K. E., Minuse, E., Ackermann, W. W., J. Immunol., 1955, v75, 71.
4. Shimizu, T., Ishizaki, R., Kono, Y., Kumagai, T., Arai, S., Sasahara, J., Ishii, S., Matumoto, M., Japan J. Exp. Med., 1955, v25, 211.
5. Bukrinskaya, A. G., Acta Virol., 1958, v2, 208.
6. Heath, R. B., Tyrrell, D. A. J., Arch. Virusforsch., 1959, v8, 577.
7. Mannweiler, E., ibid., 1961, v10, 647.
8. Matsumoto, T., Maeno, K., Japan. J. Microbiol., 1961, v5, 89.
9. Zhdanov, V. M., Bukrinskaya, A. G., Prob. in Virol., 1960, v5, 245.
10. Zhdanov, V. M., Bukrinskaya, A. G., Azaslova, N. B., J. Immunol., 1961, v81, 647.
11. Gresser, I., Proc. Soc. Exp. Biol. and Med., 1961, v108, 303.
12. Bukrinskaya, A. G., Zhdanov, V. M., Nature, 1963, v200, 920.
13. Henle, W., Henle, G., Deinhardt, F., Bergs, V. V., J. Exp. Med., 1959, v110, 525.
14. Ho, Y. D., Gorbunova, A. S., Acta Virol., 1962, v6, 193.
15. Ishida, N., Homma, M., Virology, 1961, v14, 486.
16. Demont, G., Berkaloff, A., Colobert, L., Compt. Rend., 1962, v255, 421.
17. Chang, R. S. M., Proc. Soc. Exp. Biol. and Med., 1954, v87, 440.
18. Eagle, H., Science, 1955, v122, 501.
19. Reed, L. J., Muench, H., Am. J. Hyg., 1938, v27, 493.
20. Granoff, A., Virology, 1955, v1, 516.
21. Chanock, R. M., Parrott, R. H., Cook, K., Andrews, B. E., Bell, J. A., Reichelderfer, T., Kapikan, A. Z., Mastrota, T. M., Huebner, R. J., New Eng. J. Med., 1958, v258, 207.
22. Henle, W., Deinhardt, F., Henle, G., Internat. Cong. for Microbiol., Stockholm, 1958, 7th, 268.
23. Matsumoto, T., J. Immunol., 1961, v87, 590.
24. Isaacs, A., Edney, M., Aust. J. Exp. Biol. Med. Sci., 1950, v28, 635.

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In vitro Production of Coagulation Factors VII-X by Liver Slices from Rats Fed Atherogenic Diets.* (29944)

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Previous reports from this and other laboratories indicate that rats fed atherogenic diets develop high levels of many blood coagulation factors (1-5) including Factor VII-X. Theoretically, the elevated levels could result from increased synthesis, diminished degradation, or both. We have therefore meas-

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