and growth hormone possible by present bioassay technics.

Summary. FSH and ICSH and growth hormone-like activity were demonstrated in the cavernous sinus plasma of gonadectomized male sheep, and in apparent higher concentration than found in peripheral plasma.

1. Emery, F. E., *Am. J. Physiol.,* 1932, v101, 246.
2. Hellbaum, A. A., Greep, R. O., *Endocrinology,* 1943, v32, 33.
3. Contopoulos, A. N., Simpson, M. E., Koneff, A. A., *ibid.,* 1958, v63, 642.
4. Cozens, D. A., Nelson, M. E., PROC. SOC. EXP. BIOL. & MED., 1958, v98, 617.
5. Soliman, F. A., Ghanem, Y. S., *Nature,* 1956, v178, 745.
6. Martins, T., *Compt. rend. soc. biol.,* 1929, v102, 605.
7. Antoniades, H. N., *Hormones in Human Plasma,* Little, Brown & Co., Boston, Mass., 1960.
8. Gray, C. H., Bacharach, A. L., *Hormones in Blood,* Academic Press, New York, 1961.
9. Lozinski, E., Holden, G. W., Macallum, E. N., *Rev. Canad. Biol.,* 1942, v1, 677.
10. Cole, H. H., Goss, H., *Am. J. Physiol.,* 1939, v127, 702.
11. Rimington, C., Rowlands, I. W., *Biochem. J.,* 1944, v38, 54.
12. Santolucito, J. A., Ph.D. Dissertation, Univ. of California, Davis, 1957.
13. Gemzell, C. A., Heijkenskjold, F., Strom, L., *J. Clin. Endocrinol. & Metab.,* 1955, v15, 537.
14. Trenkle, A., Burroughs, W., Melampy, R. M., Quinn, L. Y., *J. Animal Sci.,* 1960, v19, 1338.
15. McFarland, L. Z., Clegg, M. T., Ganong, W. F., PROC. SOC. EXP. BIOL. & MED., 1960, v103, 538.
16. Evans, H. M., Simpson, M. E., Tolksdorf, S., Jensen, H., *Endocrinology,* 1939, v25, 259.
17. Simpson, M. E., Li, C. H., Evans, H. M., *ibid.,* 1942, v30, 977.
18. Contopoulos, A. N., Simpson, M. E., *ibid.,* 1957, v61, 765.

Received May 28, 1962. P.S.E.B.M., 1962, v110.

Attempts to Enhance Infectivity of Adenovirus Type 8 for Cell Cultures.* (27625)

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Adenovirus type 8 shares many characteristics (*e.g.*, size, morphology, ether-resistance, cytopathogenicity, complement-fixing group antigen) with other adenoviruses. However, it differs from other types in producing a specific and unique eye disease in man. Adenovirus type 8 is the principal and perhaps the sole infectious agent capable of regularly producing typical epidemic keratoconjunctivitis (EKC) in man after natural or experimental infection(1).

Isolates of type 8 from many parts of the world, studied in different laboratories, have shown uniformly low infective titers for many different cell lines and for some primary cells in culture. It has been suggested that this uniformly low infectivity is a function of the exceedingly small proportion of fully infective particles in the virus population grown in tissue culture(2). Much evidence indicates that virus populations growing in the adult human eye may also have only a minute proportion of fully infective particles. This small amount of infective virus released accounts for the low spontaneous communicability of the infection and the importance of iatrogenic spread(3).

The question arises whether propagation in cell culture at selective conditions might raise the infective titer of adenovirus type 8. To that end we have performed 2 series of experiments reported here. In one we evaluated the effect of different incubation temperatures, in the other the effect of inoculum size, comparing limiting dilution to undiluted tissue culture.

Materials and methods. Viruses. Two strains of adenovirus type 8 were employed. One was the prototype strain (Trim) isolated in this laboratory(4), the other a Japanese

* Supported by grants from U.S.P.H.S. and Burroughs Wellcome Fund.

virus (Ijima) sent to us by Dr. Mitsui(5). Initial inocula consisted of infected and degenerated 10th-12th passage cultures of Maben cells, stored at –40°C.

Cell cultures. Maben cells were grown in stationary tubes at 36°C in 10% human serum,† 45% mixture 199, and 45% Hanks' BSS for 3-4 days. The cultures were maintained in 2% horse serum, 0.5% dextrose, 0.5% peptone, 40% mixture 199 in Hanks' BSS for from 4 to 16 days. Cultures to be assayed were homogenized by 3 cycles of freezing and thawing before dilutions were prepared.

Infectivity titrations. Serial 3.2-fold (half log 10) dilutions of homogenized cultures were prepared in mixture 199 and 0.1 ml of each dilution was inoculated into 3 or 4 tubes of Maben cell monolayers. Maintenance medium 0.9 ml was then added and the tubes were incubated at 36°C in a stationary position and inspected daily for specific cytopathic effects. The infectivity titer end-point ($TCID_{50}$) was calculated as the highest dilution of virus which infected one-half of the tubes inoculated, resulting in typical cytopathic effects.

Serologic tests. Fluids from tissue cultures assayed for infectivity were also titrated for antigen content by means of a complement fixation procedure described elsewhere(2). A single pool of human serum with a complement fixing titer of 1 in 160 was used in this test in a dilution of 1 in 40.

Results. Experiment A. Virus was grown in 3 series of cultures kept, respectively, at 31, 36, and 39°C for 10 consecutive passages. At each passage level individual tubes were harvested when cell degeneration was far advanced, and were passed to new monolayer cultures. On portions of each harvest, infectivity titration and assay of antigen content were performed. The results obtained in 10 passages of strain Trim are summarized in Table I. It appears that CF antigen consistently reached higher titers at 36°C than at 31°C whereas the levels at 39°C were irregular. The infective titer of the seed virus was

† All tissue culture media were obtained from Microbiological Associates.

TABLE I. Serial Passage of Adenovirus Type 8 (TRIM) at 3 Temperature Levels.

Incub. temp., °C		Passage No. 1	3	6	8	10
		Inoculum dilution 1:6	1:8	1:15	1:26	1:10
31	Day*	5	3	5	4	16
	CP†	4+	4+	4+	3+	3+
	CF‡	80	40	80	20	20
	$TCID_{50}$/ml§	33		10		10
36	Day	5	2	5	4	16
	CP	4+	4+	4+	4+	3+
	CF	160	160	160	160	80
	$TCID_{50}$/ml	330		100		10
39	Day	5	2	10	4	16
	CP	4+	4+	2+	4+	4+
	CF	160	160	80	160	20
	$TCID_{50}$/ml	100		100		10

* Day of maximum CP and harvest.
† Intensity of cytopathic effect, 0 to 4+.
‡ Reciprocal of highest dilution of culture fixing complement in presence of specific antiserum(2).
§ No. of tissue culture infective doses/ml.

10-20 $TCID_{50}$/ml and that of the 1st, 6th, and 10th passages was within 1 log of it. In no case was there an increase in infective titer above $10^{2.5}$ $TCID_{50}$/ml in spite of occasional prolonged incubation. In order to slow cytopathic effects and permit prolonged incubation, inocula were arbitrarily diluted from 1 in 6 to 1 in 26 in inverse ratio to the rapidity of cell degeneration of the preceding passage. In spite of these efforts the infective titer after 10 passages had not increased, but rather declined slightly.

Experiment B. The failure of Experiment A might have been caused by passage of material containing a predominance of non-infective virus or other interfering agents. To explore this possibility we compared the results of serial passage at limiting dilutions with passage of concentrated material. Ijima seed virus was employed in an initial infective titer of $10^{3.5}$ $TCID_{50}$/ml, the highest we have ever encountered with any strain of adenovirus type 8. The results of 3 consecutive passages at 36°C are shown in Table II.

Cultures diluted to the limits of infectivity (lowest part of Table) sometimes yielded small amounts of infective virus in the absence of evident cytopathic effects. Neither this passage series nor that employing a 10-fold higher concentration of inoculum resulted

TABLE II. Effect of Inoculum Dilution on Serial Passage of Adenovirus Type 8 (IJIMA).

Passage No. 0			1			2			3		
Inoc. dilution	CP	CF	Inoc. dilution	CP	CF	Inoc. dilution	CP	CF	Inoc. dilution	CP	CF
10^{-1}	4+	160 →	10^{-1}	4+	160 →	10^{0}	3+	160 →	10^{-1}	4+	160
									10^{-2}	2+	80
									10^{-3}	1+	10
						10^{0}	4+	160 →	10^{-1}	4+	160
									10^{-2}	1+	160
									10^{-3}	?	10
			10^{-1}	4+	160				10^{-1}	4+	160
10^{-4}	1+	40 →	10^{-2}	2+	80	10^{-1}	1+	80 →	10^{-2}	1+	40
			10^{-3}	?	10 →				10^{-3}	—	10
									10^{-0}	4+	160
						10^{-2}	?	10 →	10^{-1}	?	40
									10^{-2}	—	10

in an increment of infective titer. On the contrary, passage of dilute seed tended to reduce infective titer. Serial passage of concentrated seed did not further reduce infectivity, as might be expected if the seed had contained an excessive concentration of interfering material.

Discussion. Experiment A was carried out at the suggestion of Dr. A. Lwoff who felt that passage at elevated temperature might favor selection of the more pathogenic particles in the viral population(7). The results of that experiment supported the belief that propagation of infective adenovirus type 8 occurs optimally at 36°C rather than at higher or lower temperatures. The results of Exp. B lent no support to the hypothesis that the presence of an interfering substance is responsible for the low yield of infective virus. The negative results of these studies favor the interpretation that the biochemical environment of the many types of cell cultures employed in growing adenovirus type 8 in various laboratories is specifically unfavorable for production of infective type 8, but not of most other adenovirus types. The only apparent exception is the claim of Clyde and Denny(8) to have grown adenovirus type 8 to infective titers of 10^3-10^5 $TCID_{50}$ in aged primary human amnion cultures incubated for prolonged periods. Details of their work have not yet appeared.

Studies on adult EKC have repeatedly illustrated the very low yield of infective virus released from the adult eye(6). The low temperature of the eye's surface structures (near 31°C) does not appear to account for this feature. It must be asumed that the cellular environment of the human conjunctiva is as unfavorable for synthesis and release of infective adenovirus type 8 as the cell cultures tested in many laboratories. Thus it appears that adenovirus type 8 possesses some unique biological feature which separates it from most other types—just as its pathogenetic potential for the regular production of EKC seems unique among adenoviruses.

Summary. The prototype strain of adenovirus type 8 grew in Maben cells best at 36°C but yielded maximal infective titers of only 100-300 $TCID_{50}$/ml. Serial passage at 31 or 39°C did not enhance the infective titer. Serial passage of undiluted degenerated cell culture gave no evidence of an interfering material. The consistently low titer of infective adenovirus type 8 in many different cell lines appears to be a specific characteristic of that agent.

1. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., Kimura, S. J., *Am. J. Ophth.*, 1957, v43, 79.

2. Jawetz, E., Hanna, L., Nicholas, A., Hoyt, R., *Am. J. Hyg.*, 1958, v67, 276.

3. Jawetz, E., *Brit. Med. J.*, 1959, 1 (#5126), 873.

4. Jawetz, E., Kimura, S., Nicholas, A., Thygeson, P., Hanna, L., *Science,* 1955, v122, 1190.

5. Mitsui, Y., Jawetz, E., *Am. J. Ophth.*, 1957, v43, 91.

6. Jawetz, E., Hanna, L., Sonne, M., Thygeson, P., *Am. J. Hyg.*, 1959, v69, 13.

7. Lwoff, A., *Bacteriol. Rev.*, 1959, v23, 109.

8. Clyde, W. A., Denny, F. W., *Fed. Proc.*, 1960, v19 404.

Received May 31, 1962. P.S.E.B.M., 1962, v110.