A Generalized Infection of Newborn Hamsters with Type 2 Adenovirus (34538)

JAMES D. CONNOR
(Introduced by M. M. Sigel)
University of Miami School of Medicine, Miami, Florida 33152

Characteristically, human adenoviruses do not cause overt disease when introduced by a variety of routes into animals of other species (1–3). The exceptions which have been reported are few (4–7).

At present, our efforts are directed towards an association between human adenovirus infection and a syndrome resembling clinical pertussis. During this study adenoviruses were isolated which at first typing were neutralized equally by both type 2 and type 12 reference antisera. For this reason, these strains were subjected to additional tests for identification, including tumorigenicity. Inoculation of early passage material into newborn Syrian hamsters resulted in sickness and death in a majority of animals.

Autopsy findings of these animals, at gross examination, were limited to pale, swollen, occasionally hemorrhagic livers and swollen kidneys. Focal necrosis of the livers was present in histologic preparations and intranuclear inclusion bearing parenchymal cells were present in these areas. Particles resembling adenoviruses were present in electron microscopic (EM) sections of liver and kidney. Viruses recovered from homogenates of liver and kidney tissue as well as the viruses originally isolated from the human were reidentified as type 2 adenoviruses. Further studies have confirmed the pathogenicity of these type 2 adenoviruses for the newborn hamster and are reported here.

Materials and Methods. Tissue cultures. PPLO-free KB cells and primary human amnion cell cultures (PHua) were provided by the laboratory, passaged and maintained in Eagle's minimal essential medium (MEM) with fetal bovine serum (FBS) 5–10%, penicillin G, 200 units/ml; streptomycin, 200 μg/ml; and Mycostatin, 100 μg/ml. During maintenance, media were replaced every 3–4 days. Primary human embryo kidney cells (HEK) were purchased from a commercial source[1] and maintained in basal medium (BME) or MEM with 5–10% FBS, with medium replacement once or twice weekly.

Animal studies. Pregnant female Syrian hamsters from the Lakeview Hamster Colony, New Field, New Jersey and from the Division of Oral Biology, University of Miami were obtained and separated prior to delivery. Within 24 hr after birth, newborn animals were inoculated with 0.1 ml of virus suspension (strain of type 2, P-3-66, or P-6-66) titering $10^{8.5}$ TCD_{50}/ml, posterior between the scapulae. The injected fluid could be observed to occupy the subcutaneous space. Animals were counted and observed every day for color, appearance, activity, and growth. All animals dead or missing within 24 hr after inoculation were tallied as traumatic deaths due to handling and inoculation. Portions of organs were used for viral isolation, other portions were placed in 10% buffered formalin for histologic examination and other portions were fixed in osmium tetroxide for electron microscopy. Histologic slides were prepared by the Research Histology Laboratory of the Department of Pathology, examined and interpreted by a pathologist. Electron microscopic preparations, studies, and microphotographs were performed by the staff of EM laboratory of the Department of Dermatology.

In several experiments newborn animals were inoculated and observed only for morbidity and mortality, comparing the P-3-66

[1] Microbiological Associates, Inc., Bethesda, Maryland.

virus (type 2) and prototype 2 adenovirus. In a series of experiments, animals were observed and harvested in groups of 6 at 12, 24, 48, 72, 96, 120, and 144 hr after inoculation and after gross examination, portions of organs were prepared for histology, EM, and infectivity studies.

Viral studies. Animals were killed by decapitation; blood from the cervical stump was collected dropwise directly into 1.0 ml of MEM and allowed to clot. The supernatant was used as serum inoculum without further treatment; the clot was removed, lysed in 0.5 ml of sterile distilled water, and clarified for inoculation into primary HEK stationary tube cultures. Organs were removed separately and aseptically, minced, ground, and 20% suspensions were made in MEM with 10% FBS containing penicillin G, 200 unit/ml; streptomycin, 200 μg/ml; and mycostatin 100 μg/ml. After clarification at 1000 rpm for 15–20 min, supernatants were inoculated into fresh HEK cultures immediately or frozen at —20° for further use. Inocula of 0.1-ml volume were used for all 1.0-ml stationary tube cultures and all were observed for 30 days or until cytopathogenic changes (CPC) appeared. Virus stocks were made by passage at 10^{-2} final dilution into bottle or suspension cultures of KB cells. Stocks were harvested at 75–100% CPC, sonicated, clarified, divided into 1–5-ml aliquots in sealed glass ampoules and stored at —20° for further use. Stocks and specimens were titered by the tube dilution method in primary HEK tube cultures using maintenance medium as diluent for virus inocula. After 30 days, all cultures with CPC of less then 50% were harvested, sonicated, and blind passaged at 1:4 dilution for further confirmation of virus presence.

Neutralization tests were performed in PPLO-free KB cells using virus inocula at a dilution (usually 1:20-1:100) proven to cause complete CPC to form in a majority of replicate control cultures in less than 6 days. Reference antisera used were obtained from the Reference Reagent Branch, NIH, Bethesda, and from the National Communicable Disease Center, Atlanta.

Results. Generally, animals inoculated with early passages of the P-3 or P-6 strains of type 2 virus became sick in 4–6 days, failed to suck and to thrive, were lethargic, had poor color, sometimes appeared to be jaundiced, and were runted. A majority of these animals died in 12 days. The gross pathology was limited to swollen, pale kidneys and livers with scattered hemorrhage into the hepatic capsule.

Microscopic examination of organs, including brain, lungs, heart, liver, and kidneys revealed lesions predominantly confined to the liver. Here there were multiple scattered foci of necrosis with or without hemorrhage. In these areas, disorganization of liver cords and inflammatory round cells were evident and the cells present in areas peripheral to necrotic centers contained large, amphorphilic intranuclear inclusions resembling those of adenovirus infection. No similar changes were observed in tissues of uninoculated animals prepared and processed in the same manner. Occasionally, cardiac muscle was also inflamed with cords of round cells infiltrating the muscle bundles.

These pathologic changes occurred as early as 72 hr after inoculation and had an inconstant correlation with the presence of physical signs of infection (Table I). In some animals studied in the later periods (120–144 hr), 20% of liver tissue was necrotic.

Virus particles and crystals of virus particles, morphologically resembling adenoviruses could be found in liver and kidney only (Fig. 1). This was the case despite careful selection of areas for low power screening (EM) and carefully examining 100 different fields of 20 cells each of each organ at the various time intervals. By this method, virus particles could be found only at 144 hr. The ratio of virus-bearing cells to cells containing no virus was approximately 1:20, in areas in which there was no necrosis and the architecture was maintained, thus indicating generalized infection of these organs. No EM studies were made of blood. Tissues of uninoculated animals did not contain virus bodies of any size or morphology.

In contrast, viral infectivity was quickly

TABLE I. Summary of Viral Infection.

Time after inoculation (hr)	Physical signs of infection[b]	Pathology			Virus infection	
		Site examined[a]	Gross	Microscopic	Titer[c]	Electron microscopy
12	None	S			6.5	ND[d]
		C			4.5	ND
		Li	Neg.	Neg.	1.2	Neg.
		K	Neg.	Neg.	1.6	Neg.
		B	Neg.	Neg.	Neg.	Neg.
		Lu	Neg.	Neg.	Neg.	Neg.
		H	Neg.	Neg.	Neg.	Neg.
24	None	S			<1.0	ND
		C			<1.0	ND
		Li	Neg.	Neg.	4.1	Neg.
		K	Neg.	Neg.	3.6	Neg.
		B	Neg.	Neg.	Neg.	Neg.
		Lu	Neg.	Neg.	Neg.	Neg.
		H	Neg.	Neg.	Neg.	Neg.
48		S			6.5	ND
		C			5.0	ND
		Li	Neg.	Neg.	7.8	Neg.
		K	Neg.	Neg.	8.12	Neg.
		B	Neg.	Neg.	3.13	Neg.
		Lu	Neg.	Neg.	3.12	Neg.
		H	Neg.	Neg.	3.1	Neg.
72	None	S			7.5	ND
		C			4.5	ND
		Li	Pos.	Pos.	5.3	Neg.
		K	Pos.	Pos.	5.95	Neg.
		B	Neg.	Neg.	5.74	Neg.
		Lu	Neg.	Neg.	5.95	Neg.
		H	Neg.	Neg.	6.49	Neg.
96	Yes	S			7.5	ND
		C			3.0	ND
		Li	Pos.	Pos.	9.63	Neg.
		K	Pos.	Pos.	6.45	Neg.
		B	Neg.	Neg.	4.21	Neg.
		Lu	Neg.	Neg.	5.45	Neg.
		H	Neg.	Pos.	3.94	Neg.
		Sp	Neg.	Neg.	6.96	ND
120	Yes	S			5.5	ND
		C			3.5	ND
		Li	Pos.	Pos.	9.1	Neg.
		K	Pos.	Pos.	7.43	Neg.
		B	Neg.	Neg.	5.1	Neg.
		Lu	Neg.	Neg.	6.34	Neg.
		H	Neg.	Pos.	5.8	Neg.
		Sp	Neg.	Neg.	6.96	ND

TABLE I (continued)

Time after inoculation (hr)	Physical signs of infection[b]	Pathology			Virus infection	
		Site examined[a]	Gross	Microscopic	Titer[c]	Electron microscopy
144	Yes	S			9.5	ND
		C			8.5	ND
		Li	Pos.	Pos.	9.54	Pos.
		K	Pos.	Pos.	9.84	Pos.
		B	Neg.	Neg.	4.1	Neg.
		Lu	Neg.	Neg.	6.34	Neg.
		H	Neg.	Pos.	6.9	Neg.
		Sp	Neg.	Neg.	6.8	Neg.

[a] S = serum, C = clot, Li = liver, K = kidney, B = brain, Lu = lung, H = heart, Sp = spleen.

[b] See text for description of physical signs of infection.

[c] $TCID_{50}$/ml (serum or clot)/g for organs.

[d] ND = not done.

detected in the animals, appearing in high titer in blood and in low titer in liver and kidney at 12 hr (Fig. 2). At 24 hr viremia had cleared and increasing amounts of virus were present in liver and kidney, with no virus present in other organs throughout this period. By 48 hr, replication was evident and virus was detectable in other organs. At 144 hr, high concentrations of virus were present in serum, clot, liver, and kidney. This correlated generally with sickness, pathology, and the findings of intracellular virus particles by EM.

Discussion and General Conclusions. These results indicate that adenovirus type 2 infection may be established in the newborn Syrian hamster as a result of parenteral injection of a relatively high virus dose. Virus was rapidly absorbed from the injection site and disseminated via the circulation and detected in blood, liver, and kidney before a single virus replication cycle had occurred. Preferential distribution to these organs is consistent with the magnitude of their blood supply (8).

Replication had occurred in liver and kidney by 48 hr and the infectivity of these organs continued to increase through 144 hr, generally parallel with infectivity of serum. Since gross pathological changes *and* virus particles were found in these organs only and viral inclusions were found in liver cells only it is concluded that the major sites of viral replication were in cells of these organs. The viremia which occurred after replication had been established in these organs probably was a reflection of this; however, viral replication in vascular endothelium or cellular elements of the blood may have occurred.

Infectivity of tissues of lung, liver, and heart appeared later and was of lower titer; this could have reflected viremia or intravascular virus production. Low infectivity in these organs corresponded well with absence both of pathologic changes and intracellular viral particles by EM. Whether or not infection of these organs was established could thus not be ascertained.

Pereira and Kelly (4) reported that rabbits inoculated with adenovirus type 5 developed complement fixing CF and neutralization antibody but failed to develop signs of infection. Virologic studies of homogenates of spleen were generally negative for infectious virus, whereas splenic cells when explanted into monolayer culture were observed to produce infectious virus in the absence of cytopathogenic changes after a 2-week latent period. Jennings and Betts (5) reported that adenoviruses type 1, 2, 4, and 6 caused silent pneumonitis in germ-free piglets when given intratracheally. Likewise, adenovirus types 2, 3, 4, and 7 apparently infected dogs, asymptomatically (6). Later, Pereira (7)

and others presented evidence for type 5 adenoviral infection resulting in necrotizing hepatitis, as a part of a generalized infection of newborn Syrian hamsters.

Whereas adenoviruses of other types particularly 12 and 18, are oncogenic for newborn hamsters (9–11) and to a limited extent, other rodents (12) these viruses do not

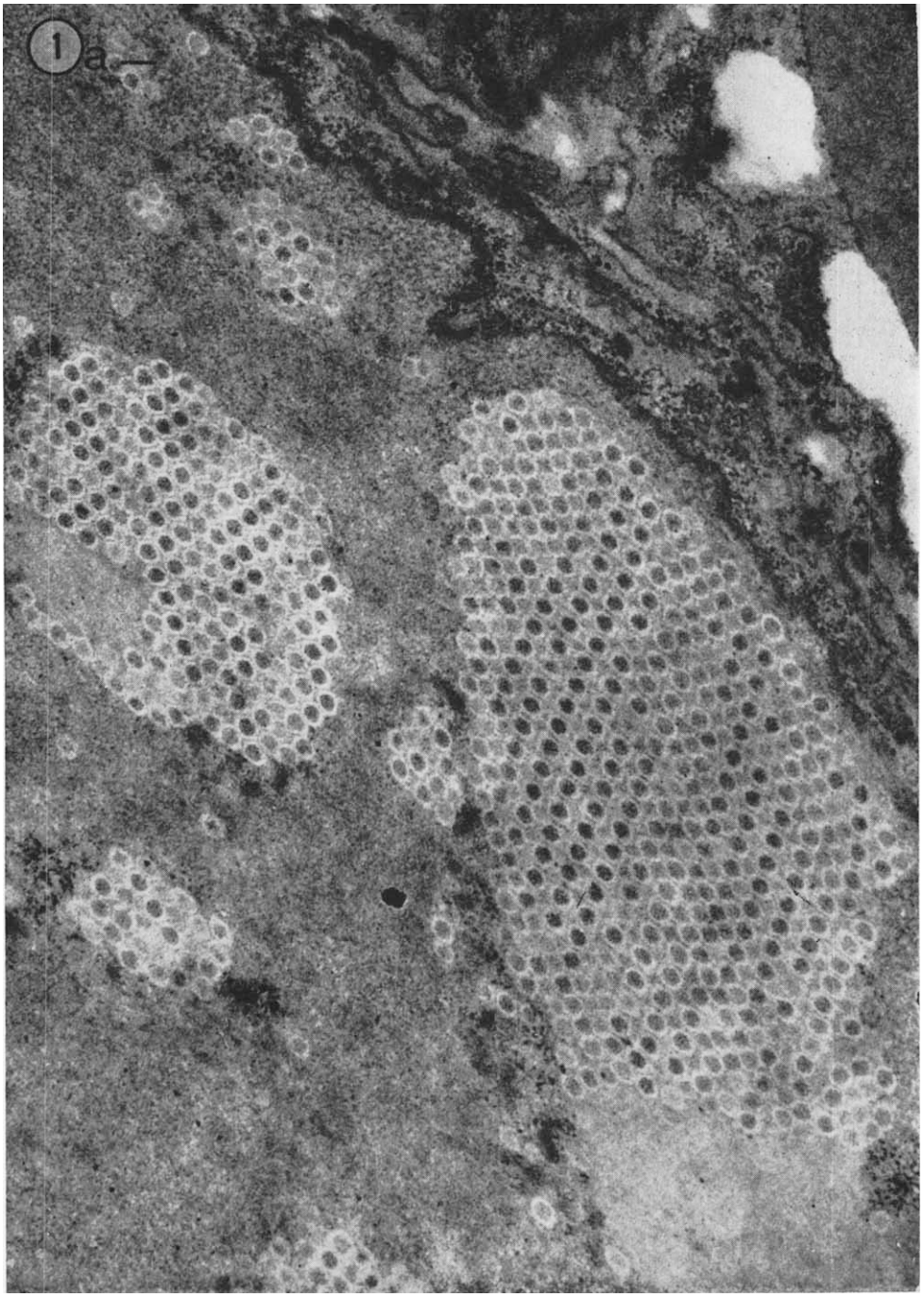

FIG. 1a. Virus-bearing parenchymal cell of liver 144 hr after inoculation; ×51,000; bar = 1000 Å.

cause productive infection in these animals.

The infection with a type 2 virus described here thus resembles the infection of newborn hamsters with type 5 as described by Pereira. Limited comparison of the P-3-66 virus with prototype 2 virus gave evidence that the prototype also is infective for the newborn hamster without evidence of progressive infection and without mortality. Likewise, the P-3-66 virus also produced silent infection in

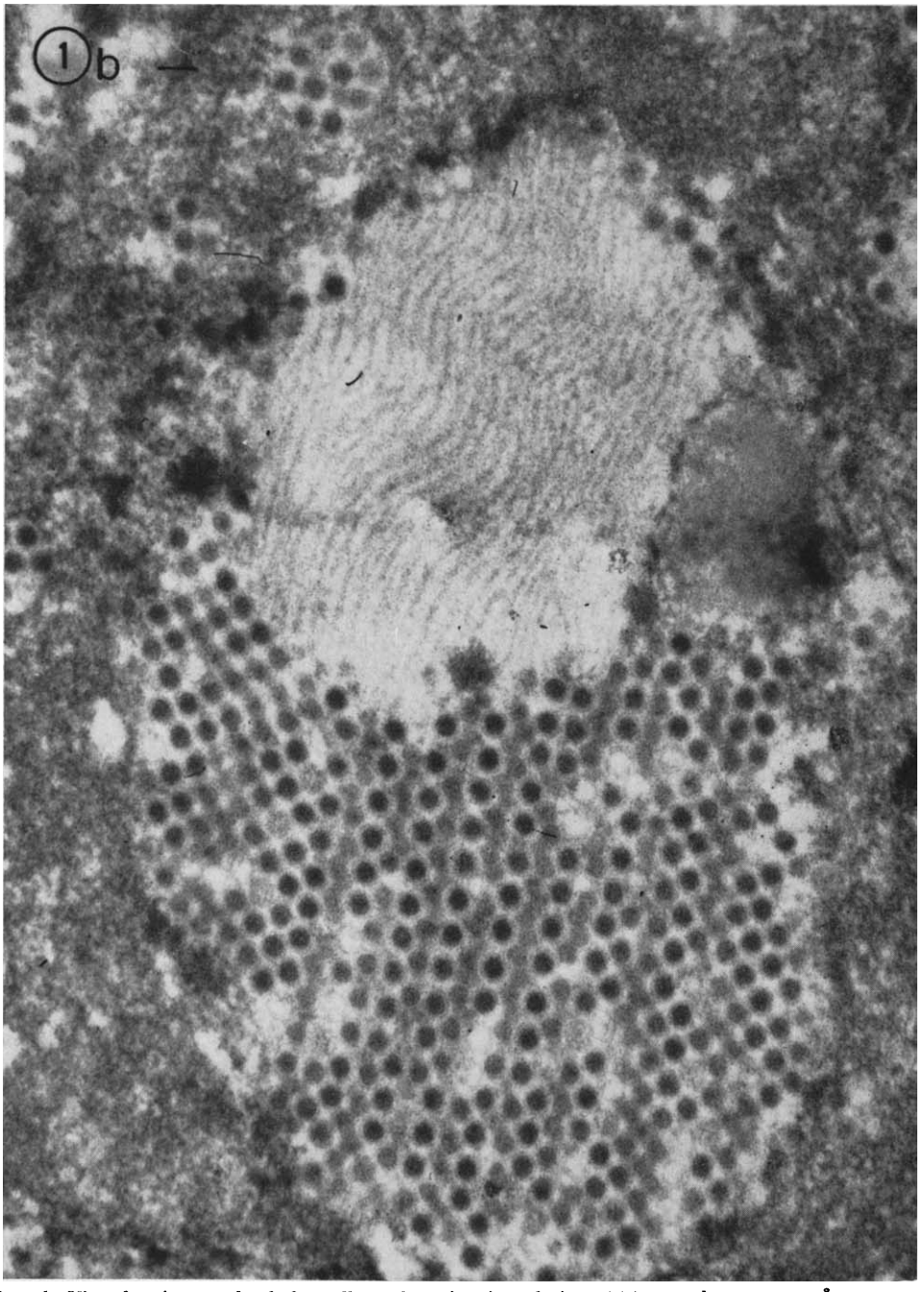

FIG. 1b. Virus-bearing renal tubular cell 144 hrs after inoculation; ×58,200; bar = 1000 Å.

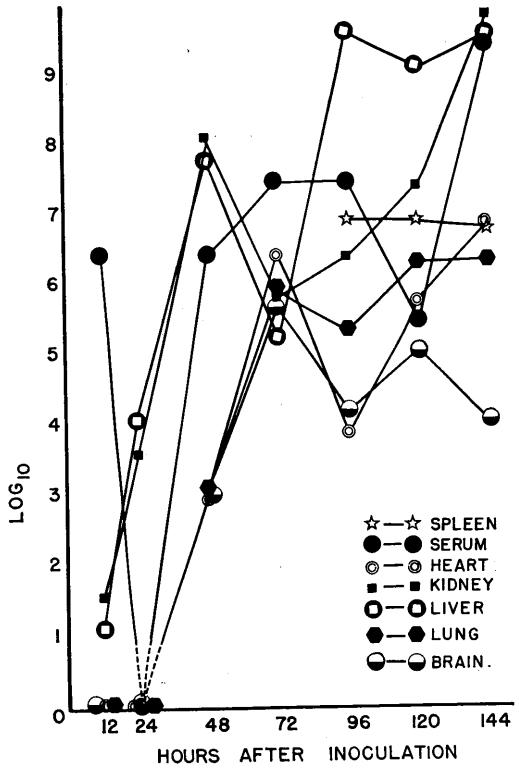

FIG. 2. Viral infectivity of organs at various intervals after inoculation of virus subcutaneously. Log_{10} refers to $TCID_{50}$/g of tissue from heart, kidney, liver, lung, brain, and spleen, and per milliliter for serum. End points calculated by the method of Reed and Muench (1938).

newborn Swiss mice. Neither virus infected adult hamsters.

This work was supported by HEW Grant No. 1 RO1-AI-HD-07719. The electron microscopy studies were supported in part by HEW Grant CA 04036 in the Department of Dermatology, Dr. H. Blank, Chairman, and performed by Mrs. Carol Collins. I should like to acknowledge the excellent technical asistance of Mrs. C. De la Cruz and Mrs. J. Glasser.

1. Rowe, W. P., Huebner, R. J., Hartley, J. W., Ward, T. G., and Parrot, R. H., Amer. J. Hyg. **61**, 197 (1955).

2. Ginsberg, H. S., J. Immunol. **77**, 271 (1956).

3. Ginsberg, H. S., Virology **18**, 312 (1962).

4. Pereira, H. G. and Kelly, B., Nature **180**, 615 (1959).

5. Betts, A. O., Jennings, A. R., and Lamont, P. H., Nature **193**, 45 (1962).

6. Sinha, S. K., Fleming, L. W., and Scholes, S., J. Amer. Vet. Ass. **136**, 481 (1960).

7. Pereira, H. G., Allison, A. C., and Niven, J. S. F., Nature **196**, 244 (1962).

8. Busch, H., Fujiwara, E., and Keer, L. M., Cancer Res. **20**, 50 (1960).

9. Trentin, J. J., Yabe, Y., and Taylor, G., Science **137**, 835 (1962).

10. Huebner, R. J., Rowe, W. P., and Lane, W. T., Proc. Nat. Acad. Sci. U. S. **48**, 2051 (1962).

11. Larson, V. M., Girardi, A. J., Hilleman, M. R., and Zwicky, R. E., Proc. Soc. Exp. Biol. Med. **118**, 15 (1964).

12. Rabson, A. S., Kirschstein, R. L., and Paul, J., J. Natl. Cancer Inst. **32**, 77 (1964).

13. Reed, L. J. and Muench, H. A., Amer. J. Hyg. **27**, 493 (1938).

Received Oct. 15, 1969. P.S.E.B.M., 1970, Vol. 133.