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Isolation of Intranuclear Inclusion Producing Agents from Infants with Illnesses Resembling Cytomegalic Inclusion Disease.*† (22841)

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On morphologic grounds various authors have suggested that the syndrome termed "generalized cytomegalic inclusion disease" by Wyatt *et al.*(1) or simply "inclusion disease" by Smith and Vellios(2) is viral in etiology and is a generalized form of salivary gland inclusion disease. The demonstration by Fetterman(3) that the infant with inclusion disease may excrete inclusion-bearing cells in the urine has resulted in evidence suggesting that the generalized process may be more common than hitherto suspected and may not have a fatal outcome(4). Information as to the nature of the causative agent is now being elaborated. Smith investigated the salivary gland viruses of rodents(5). From mouse salivary gland tissue an intranuclear inclusion producing virus was isolated in cultures of mouse tissues. Then, employing cultures of human myometrial cells, Smith obtained a cytopathic virus from human salivary gland material and recently isolated a similar agent at autopsy from kidney tissues of an infant with inclusion disease(6). Related viruses also have been recovered from adenoid tissues of children by Rowe and his coworkers(7). Our interest in the problem arose from the concurrent isolation of an agent from human liver biopsy material as has been noted briefly(7,8).

In this communication we are reporting the isolation of cytopathic intranuclear inclusion producing agents from 3 infants during life; each was ill with a syndrome resembling cytomegalic inclusion disease. One virus, derived from liver biopsy material, has been maintained for 20 months in serial passage. From the second child virus was obtained on 3 occasions from the urine, and once from liver biopsy material. Recently, an agent has been isolated from the urine of a third patient.

Materials and methods. Tissue cultures: Roller tube cultures of various human and animal tissues were utilized. The nutrient fluid at first consisted of bovine amniotic fluid (90%), beef embryo extract (5%), horse serum (5%), antibiotics, soybean trypsin-inhibitor (omitted in kidney cell cultures), and phenol red as previously employed(9); for the past 12 months Hanks'(10) balanced salt solution (45%) has been included and the amniotic fluid reduced proportionately. Changes of nutrient fluid were made at 3- to 5-day intervals. For histologic studies, tissues were planted on coverslips or prepared by the collodion technic of Cheatham(11); fixation was with Zenker-acetic or Bouin's and preparations were stained with hematoxylin and eosin. For serial cultivation, inocula consisting of 0.2 ml of coarsely ground tissue removed from the preceding set of cultures were employed. Control cultures were similarly inoculated and maintained in par-

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allel. Titrations of viral content of fluids from infected cultures were performed on materials centrifuged at 3000 rpm for 15′; 10-fold dilutions in medium were prepared and groups of 4 cultures of foreskin inoculated with 0.2 ml amounts of the supernate. Such cultures were observed for a minimum of 28 days. The serologic technics employed are described below.

Cases studied and handling of specimens. Case #1: K.D. entered Children's Medical Center on 1/11/55 at age of 3 months for investigation of microcephaly. Examination revealed: (1) jaundice, (2) hepatosplenomegaly, (3) periventricular cerebral calcification, (4) bilateral optic atrophy and one area of chorioretinitis. Urine contained a few white cells; no inclusion bodies could be demonstrated. On 1/20/55 a liver biopsy was performed. Congenital toxoplasmosis was considered in the differential diagnosis and through the courtesy of Dr. W. D. Winter, Jr. a portion of the biopsy as a 20% suspension in physiologic saline was furnished for possible isolation of *Toxoplasma in vitro*. This was inoculated in 0.2 ml amounts into cultures of human embryonic skin-muscle tissue and a cytopathic agent (Davis strain) recovered. Histopathological examination of liver biopsy showed diffuse alteration of architecture with extensive bile stasis. Many degenerate hepatic cells and scattered multinucleate giant cells were present. There were focal areas of erythropoiesis and numerous mononuclear inflammatory cells in the portal areas. Later, additional sections were examined and rare large cells of cytomegalic type with intranuclear inclusions were found. Through the courtesy of Dr. Carl M. Haas, K.D. was seen on 8/9/55; the patient was well nourished but had hepatosplenomegaly; there was clinical evidence of cerebral damage. *Case #2:* B.K. was born 5/3/56. Petechiae, jaundice, and hepatosplenomegaly were noted 8 hours after birth. Roentgenologic studies showed no abnormalities, and there was no evidence of Rh or major blood group incompatibility. In the stained sediment of urine collected on 5th and 8th days of life, large epithelial cells were observed that contained multiple round homogeneous brightly eosinophilic cytoplasmic inclusions; no intranuclear inclusions were seen. Through the courtesy of the Pediatric Service, U.S. Naval Hospital, Chelsea, and in particular of Drs. R. S. Wicksman, T. Delaney, and R. Kluge, materials were collected for study. On 14th day of life urine, spinal fluid, and blood were obtained, transported in ice bath, and inoculated into tissue cultures within 2 hours of collection. The urine was centrifuged 20 min. at 2500 rpm (Internat. #2; head 240), and supernatant inoculated in 0.1 ml to 1 ml amounts into cultures of human foreskin and of human kidney cells. The blood clot was ground with equivalent volume of culture medium and similarly inoculated in 0.4 ml quantities, and the spinal fluid was used as received. Only the foreskin cultures inoculated with urine developed specific cytopathic changes (Kerr agent). On 25th day of life a liver biopsy was performed, and a 10% suspension of tissue in medium prepared; 2 foreskin cultures were inoculated and these subsequently developed focal degeneration. Histologic examination of the biopsy showed preservation of lobular pattern, but loss of cell cord pattern with formation of many large multinucleate giant cells. Rare foci of liver cell necrobiosis were present. A diffuse inflammatory reaction consisting of mononuclear cells, eosinophils, and polymorphonuclear cells was present throughout the parenchyma and in the portal triads. Bile stasis was present within liver cells. No cells with intranuclear inclusions were seen. The picture was identical with that of "neonatal hepatitis" described by Craig and Landing (12). Two foreskin cultures inoculated with urine collected on 36th day of life developed characteristic cytopathic changes. At age of 2 months, B.K. appeared normal except for persistence of hepatosplenomegaly. Yet, virus was again recovered from urine collected on 91st day of life; each of 4 cultures inoculated with 0.1-0.2 ml of urine supernatant showed characteristic focal changes. *Case #3:* (History and materials supplied through kindness of Drs. Hattie E. Alexander and Katherine Sprunt, Babies Hospital, New York.) E. Esp., born 5/9/56, had hepatosplenomegaly and periventricular cerebral

FIG. 1. Culture of human embryonic skin-muscle tissue 34 days after inoculation with Davis liver biopsy material showing 2 focal lesions with prominent pigment granules. × 135.

calcification by roentgenologic examination at 6 weeks of age. Subsequently, progressive chorioretinitis developed. Cytomegalic inclusion cells were demonstrated in the urine on several occasions. On 8/13/56, urine forwarded in melting ice was inoculated into 6 foreskin cultures; specific changes developed in all (Esp. strain).

Results. a. *Isolation of cytopathic agents.* (1) *Davis strain from liver biopsy material:*‡ On 12th day after inoculation of cultures of human embryonic skin-muscle tissue, focal collections of 2-10 swollen, rounded, translucent cells were observed in sheets of fibroblastic outgrowth. In 48 hours the foci had increased in size and a few rounded cells had become irregularly oval or leaf-shaped; the latter were noted to contain refractile, greenish-brown granules that varied considerably in size. Certain of the involved cells also contained masses of smaller, more regular, and less distinct non-pigmented granules. At this stage, degeneration was not a prominent feature, although rare large cells appeared to be undergoing dissolution, releasing granular debris. In one culture progression of cytopathic changes was observed for additional 30 days. The number of foci slowly increased until there were 1 or 2 per low power field. The individual focus, however, increased slowly in size, and tended to remain lenticular in outline. The central area of each often contained brownish granular debris as well as scattered swollen cells (Fig. 1). Two control cultures showed no unusual changes.

(2) *Kerr strains from urine and from liver*

‡ *Special groups for serologic studies*: The circumstances of isolation of the Davis strain suggested investigation of children with toxoplasma-like syndromes but with negative Sabin-Feldman dye test findings. Specimens of serum from patients with these criteria were kindly supplied by Dr. H. A. Feldman. Selected patients, with mental deficiency, have been studied at the Wrentham State School through the courtesy of Drs. K. V. Quinn and D. H. Jolly. We are indebted to various physicians for supplying us with specimens of serum, especially Drs. A. M. Margileth, G. H. Fetterman, A. E. McElfresh, M. Birdsong, R. B. Lawson, and S. W. Wright.

FIG. 2. Culture of human foreskin 20 days after inoculation with urine from baby Kerr showing early focal lesions. × 130.

biopsy material: Focal lesions were first observed in foreskin cultures inoculated with various urine specimens from B.K. after 12, 6, and 9 days respectively, and on 10th day in cultures receiving liver inoculum. Appearance and type of progression of cytopathic changes closely resembled that seen in cultures inoculated with the Davis liver material. (Fig. 2). Again, control cultures failed to develop similar changes.

(3) *Esp. strain from urine*: In the 6 cultures of foreskin tissue inoculated with urine, rounding of scattered fibroblasts was first noted on 5th day after inoculation. From such cells slowly progressive focal lesions developed that contained much dark granular pigment and were similar to those induced by the Davis agent.

b. *Serial propagation of agents*: Serial propagation of the Davis strain was accomplished in cultures of foreskin or of human embryonic skin-muscle tissue employing inocula consisting of ground infected tissue material. Two lines of the Davis agent were established from original cultures. "A" line was passed at intervals varying from 5 to 101 days and has been maintained in culture for 20 passages for 494 days. The "B" line has been maintained in culture continuously 544 days, during which 15 passages were made; in this instance subculture was performed at intervals varying from 8 to 113 days. In both lines, evidence of adaptation to *in vitro* conditions was apparent. Virus was not demonstrable in fluid removed from the original cultures. In the 3rd passage (cumulate period 134 days in culture), virus was present in low titer in the centrifuged fluid as assayed in foreskin cultures. The titer of virus in fluid removed on 25th day after inoculation from the 10th passage (314 cumulate days) was $10^{-2.5}$ and in that removed on 14th day from 13th passage (392 cumulate days) was $10^{-4.6}$. Focal lesions appeared in the original subculture tubes after intervals ranging from 7 to 18 days and persisted as such. In subsequent passages the initial focal lesions were usually followed by generalization of the cytopathogenic process after a varying period of incubation. In the later passages, when large inocula were used, the prepatent period shortened markedly, and the initial cytopathic changes often were generalized rather than focal. For example, in the 17th passage, a diffuse rounding of fibroblasts was noted 24 hours after inoculation. Yet, in this and subsequent passages when inocula approaching the limiting dilution were employed, initial lesions characteristically were of a focal nature and only appeared after a 15- to 28-day latent period. Such focal lesions were, however, usually followed after a few days of incubation, by a generalized involvement of susceptible cells. Two strains recovered from urine of B.K. have been propagated for cumulate periods to 103 days and

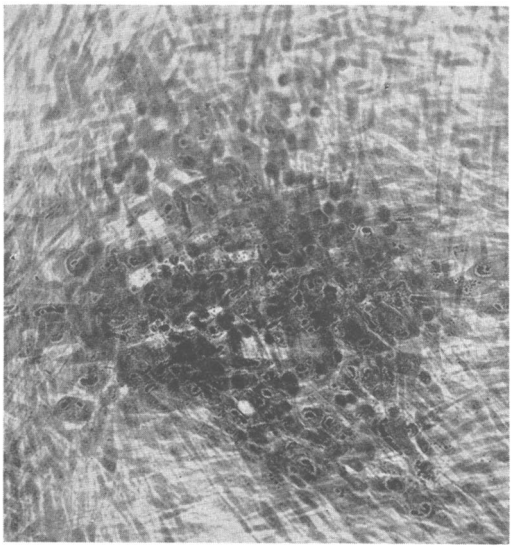

FIG. 3. Stained preparation from foreskin culture 20 days after infection with Davis agent (3rd T.C. pass.) showing discrete focus surrounded by unaltered fibroblasts. (H + E × 145.)

for 2 and 3 subcultures. The focal cytopathic changes resemble those of the early passages of the Davis agent. The Esp. strain has been subcultured once with similar results.

Through the courtesy of Dr. Rowe, the Ad. 169 virus recovered from pharyngeal tonsillar tissue(7) was furnished as 1st passage material. This agent was propagated in cultures of foreskin tissue for 8 passages during 435 days. The morphological changes were similar to those observed with the Davis strain.

c. *Some biologic properties of the agents*: (1) *Cytopathic changes in stained preparations*: Examination of stained materials from foreskin cultures infected with Davis virus revealed a marked alteration of nuclear structures and also changes in cytoplasm of infected cells (Fig. 3 and 4). The earliest nuclear changes noted consisted of the presence in the nucleus of 1 to 4 minute granular bodies that stained amphophilic or weakly eosinophilic. In nuclei in which slightly larger inclusions were present, there was loosening and coarsening of the chromatin reticulum with apparent coalescence of fragments thereof with nucleolar remnants that persisted as one or more prominent bodies near the nuclear membrane. The developing intranuclear inclusions often showed struc-

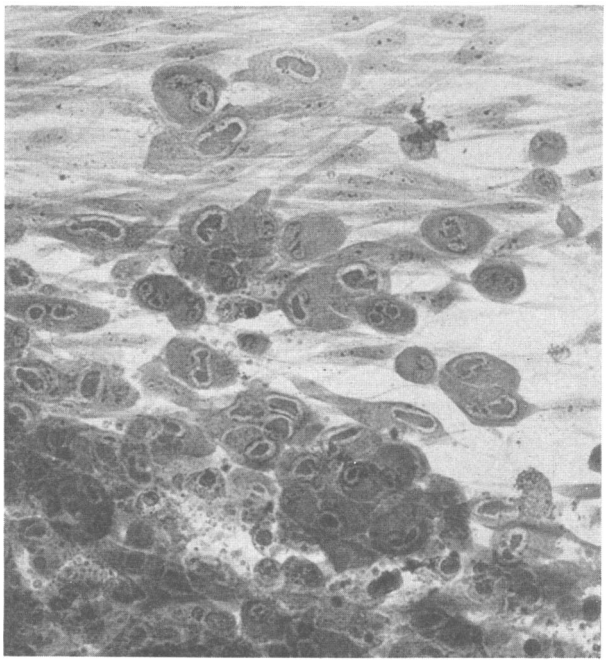

FIG. 4. Stained preparation from foreskin culture 20 days after infection with Davis agent (3rd T.C. pass.); edge of focal lesion with central degeneration and infected cells at periphery (H + E × 300).

tural differentiation with a narrow, deeper staining marginal zone and occasionally a discrete central body. Some of the larger inclusions appeared to be composed of masses of small particles of this nature. At maximum size the swollen nucleus was occupied by 1 or more large granular, moderately eosinophilic inclusions separated from the marginated nuclear chromatin by a clear zone. In degenerating cells, the inclusion occasionally resembled a honeycomb-like reticulum. Cytoplasmic changes were noted in involved cells in the focal lesions, but were more obvious in preparations wherein generalized spread had occurred. Examination of material from higher passages shortly after inoculation indicated that early rounding of fibroblasts was accompanied by cytoplasmic changes that preceded the appearance of intranuclear bodies. In a paranuclear position, and frequently lying in an indentation of the nucleus, there usually was an oval zone where the cytoplasm stained lighter, more eosinophilic, and appeared to be composed of a mass of fine granules. Infected cells apparently about to undergo dissolution often were noted to contain one to several rather large, round, hyaline amphophilic to eosinophilic cytoplasmic inclusions; in areas of cell destruction these bodies could be seen free in the cellular debris. Stained preparations from cultures infected with the Kerr and Esp. strains showed morphologic changes indistinguishable from those observed with the Davis virus.

(2) *Cytopathic range. Human tissues*: After *in vitro* infection with the Davis virus, focal or generalized cytopathic changes developed in fibroblasts derived from human foreskin, from human embryonic skin-muscle or lung tissue, and in a cultured line of fibroblasts obtained from Microbiological Associates. Similar changes occurred in cultures of human myometrial cells and in the outgrowth from human testicular tissue. The relative sensitivity of certain of these tissues as indicators of virus is suggested by the results of titrations summarized in Table I. The inoculation of cultures of human epithelial cells of various types was not immediately followed by the development of overt cytopathic changes. However, in stained preparations, changes could be demonstrated. In foreskin, the marginal cells in plaques of squamous epithelium in contact with fibroblastic foci often showed changes with intranuclear inclusions; destruction of involved squamous cells occurred at a comparatively slow rate. Variable results were obtained in cultures prepared from trypsinized human kidney cells. In one experiment, where the use of a ground tissue inoculum permitted implantation of infected cells, stained preparations showed limited involvement of cells that probably were of renal epithelial origin. More convincing evidence was afforded by an experiment inoculated with centrifuged infected fluids (Davis, 8th passage) in which staining at the 35th day of incubation revealed isolated, enlarged, rounded cells with intranuclear inclusions; these cells appeared to be of renal epithelial origin. Yet, on using 13th passage material (titer $10^{-4.6}$ in foreskin) as the inoculum in a subsequent experiment, no evidence of infection could be found in several preparations fixed and stained after 16 days. In a single experiment employing HeLa cells, again no lesions were observed in the cultures, although rare cells with suggestive nuclear changes were found on examination of stained slides prepared on the 27th day of incubation.

TABLE I. Infectivity Titers for Different Human Tissues of Pooled, Centrifuged Fluids from Davis Cultures.

Fluid material	Days stored in CO_2 box	Tissue utilized in titration	Titer
13th pass., 14 day	0	Foreskin	$10^{-4.6}$
	11	"	$10^{-3.7}$
	11	Emb. S-M*	$10^{-4.5}$
16th pass., 17th day	4	"	$10^{-3.8}$
	42	Foreskin	$10^{-2.7}$
	42	Testis	$10^{-1.5}$

* Embryonic skin-muscle tissue.

Non-human tissues: Cultures of mouse and of chick embryonic tissues, of monkey kidney and of rabbit testis were inoculated with Davis material. No evidence of growth of the agent in these tissues was noted.

(3) *Other properties*: No evidence of ill-

ness followed intravenous inoculation of rabbits with the Davis agent and intraperitoneal and intracerebral inoculation of newborn mice with the Davis, the Kerr and the Esp. strains. Heating of Davis material in the form of cell-free fluid of known titer ($10^{-2.0}$) for 30 minutes at 56°C resulted in complete loss of infectivity. In 2 experiments, the Davis agent from the higher passages showed a 10-fold decrease in titer on freezing and thawing when tested before and after storage in a dry ice chest. Once frozen at –50°C, infectivity appeared to be well maintained. Filtration of high passage Davis virus through a sintered glass filter that retained *Serratia marcescens* was accomplished in the cold room employing a pressure of 27 mm of Hg for 15 minutes; characteristic cytopathic changes developed in cultures inoculated with the filtrate.

d. *Serologic tests*: With the availability of pools of Davis virus of known titer, a reproducible neutralization procedure became feasible. The amount of virus employed per culture in different experiments was varied as indicated and was calculated to take into account the slightly different sensitivities of the two tissues, human embryonic skin-muscle and foreskin, used in the experiments. Sera were inactivated (56°C/30 min.), and diluted with culture medium. Aliquots were then mixed with dilutions of virus, and the serum-virus mixtures kept for one hour at 5°C. Three cultures were inoculated with each serum-virus dilution using 0.2 ml amounts, 2 with each serum dilution alone, and in each experiment 3 cultures received virus alone. In screening tests, a final serum dilution of 1:10 and approximately 100 ID_{50} of virus were used; the actual amounts of virus employed in the screening studies ranged from 25 to 273 ID_{50}. All cultures were observed for a 28-30 day period. A serum was recorded as "positive" only if all 3 cultures in the group failed to develop cytopathic changes during the 28-day observation period; conversely, the appearance of specific viral activity at any time during the period in all three tubes was arbitrarily recorded as "negative." Cytopathic change in a single culture of a group was termed "? positive" and the converse "? negative." Employing this technic, serum specimens from the Davis and Kerr babies and from their mothers were examined for neutralizing antibodies to the Davis virus (Table II). Utilizing 50 ID_{50} of Davis virus, serum from the Esp. baby was positive at a 1:100 dilution.

TABLE II. Neutralization of Davis Virus by Sera from Davis and Kerr Cases.

Patient	Serum collected at age:	Virus employed in test	Results at serum dilutions
Baby D	6 mo	1250 ID_{50}	Pos. 1:100
		125 "	Pos. 1:500; Neg. 1:1000
	11 "	125 "	Pos. 1:100; Neg. 1:500
Mrs. D	19 yr	1250 "	Neg. 1:10
(Mother, 11 mo post-partum)		125 "	Pos. 1:10; Neg. 1:50
Baby K	9 days	231 "	Pos. 1:10
	26 "	25 "	Pos. 1:10; ? Pos. 1:100
Mrs. K	18 yr	25 "	Pos. 1:10
(Mother, 9 days post-partum)			

Screening neutralization tests were performed on sera from 7 additional clinical cases of cytomegalic disease. Specimens from 4 patients (ages 23 days to 3 10/12 yrs.) were positive, and three were negative or questionably so. Sera were tested from mothers of 2 of the infants in the negative group; both contained neutralizing antibodies. The case well documented by Margileth(4) gave results of this type; sera from his patient, collected at 6 weeks and at 23 months of age, were negative; those collected 4 weeks and 23 months post-partum from the mother were positive.

Sera were supplied by Dr. Rowe from 2 children whose adenoid tissues had yielded an inclusion-producing virus(7); both were negative in the screening test. Sera from 4 children, with toxoplasma-like syndromes but with negative dye test titers on examination by Dr. Feldman, were also negative for Davis neutralizing antibodies. Materials from 19 children at the Wrentham State School (ages 1 to 12), selected on the basis of the presence of "idiopathic" brain damage, were similarly

TABLE III. Results of Neutralization Tests on Sera from Unselected Individuals.

Age group (yr)	Total in group	Results: Pos.	?Pos.	?Neg.	Neg.
0- 5	28	2	1	3	22
6-18	12	1	1	0	10
19-36	25	5	1	3	16
37+	23	6	1	5	11

studied; 17 were negative, 1 ?-negative, and 1 was positive.

Additional sera were examined from healthy individuals, and from patients with a variety of illnesses. The findings are summarized in Table III. Three separate lots of human gamma globulin prepared from blood collected in Massachusetts by the Massachusetts Division of Biologic Laboratories were investigated; all were positive or "? positive" when tested in a dilution of 1:100 with Davis virus.

e. *Relationship of the Davis, Kerr, and Esp. agents to other viruses*: The Davis, Kerr, and Esp. agents did not induce the rapidly spreading cytopathic changes observed with strains of herpes simplex virus *in vitro* and also failed to produce illness in newborn mice. Furthermore, six convalescent sera from patients with herpes simplex infections failed to neutralize the Davis agent.

While the focal cytopathic changes produced by the Davis, Kerr, and Esp. viruses superficially resembled those associated with varicella-zoster viruses, consistent differences were apparent. The foci produced by the former agents were more prominent than those seen with varicella virus. Pigment deposition of the type here described has not been observed in varicella cultures. In the course of the cultivation of 21 strains of varicella virus and of 4 from patients with herpes zoster, at no time has generalized spread of the cytopathic process been observed, and virus has not been demonstrable in the cell-free fluids from infected cultures. In addition, no neutralizing antibodies to the Davis agent were found in six sera known to contain varicella antibodies.

Discussion. The morphologic changes induced *in vitro* by the Davis, Kerr, and Esp. agents suggest the relationship of these viruses to those isolated by Smith(6) and by Rowe and his coworkers(7), a concept further supported by the serologic studies of the latter investigators. It would appear that a new group of human viruses, perhaps ubiquitous in distribution, is being defined. If these agents are common associates of man, their etiologic role in entities such as the cytomegalic syndrome may be difficult to define. Considerations of this nature, together with knowledge of the occasional variable behavior of the agent *in vitro,* led us to the neutralization procedure employing a known viral inoculum and a prolonged observation period. It is possible that this method failed to detect low levels of antibody. For example, if the criterion of delay in appearance of cytopathogenicity had been employed as the index of neutralization, 6 of the 7 sera from cytomegalic cases would have been positive. However, the results of our serologic studies in general parallel those obtained by Rowe, and the finding of neutralizing antibody in pooled human gamma globulin further suggests that specific antibody is commonplace in the adult population.

The etiological import of the isolation of these agents during life can not be defined at present. The recovery of the two viruses from liver biopsy material perhaps is significant when considered in the light of recent experience. We have unsuccessfully attempted isolation of virus *in vitro* from 8 liver biopsy specimens and 6 specimens of liver obtained post-mortem; these specimens derived from a variety of pathological entities, and included one fatal case of "cytomegalic disease." The continued excretion of virus in the urine for 91 days by the Kerr infant is of considerable interest. Also, the diffuse process induced *in vitro* at isolation of the Esp. agent suggests that the urine of this patient contained relatively large amounts of virus. The demonstration of viruria, therefore, may acquire value as a diagnostic procedure. Yet it is to be noted that we have failed to isolate virus from the urine of one patient who was excreting apparently typical "cytomegalic" cells.

Summary. Viruses that produce similar

cytopathic changes *in vitro* characterized by presence of intranuclear inclusions have been isolated from 3 infants during life. The first derived from a liver biopsy from a 3-months-old child (Davis) with microcephaly, persistent jaundice, and hepatosplenomegaly; cytomegalic cells were demonstrated in the liver specimen. From a second child, virus was isolated from a liver biopsy, and on three occasions between the 14th and 91st day of life from the urine. This infant had jaundice and hepatosplenomegaly. More recently, an agent was recovered from the urine of a third infant, who evidenced hepatosplenomegaly, cerebral calcification and chorioretinitis. The Davis strain has been propagated in human fibroblasts for 20 passages during an elapsed period of 494 days. Neutralization tests with the Davis agent have indicated that neutralizing antibodies occur frequently in children with cytomegalic disease but also are not uncommon in normal adults. The agents are apparently related to those recovered in other laboratories from a human salivary gland, from a fatal case of cytomegalic disease, and from spontaneously degenerating tissue cultures of human pharyngeal tonsillar tissue.

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Anticonvulsant Properties of *l*-Glutamine and *l*-Asparagine in Mice and Rats.* (22842)

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Because of the considerable experimental and clinical interest in *l*-glutamine and *l*-asparagine as potential therapeutic agents in epilepsy, based largely on the laboratory and clinical studies of Tower(1), it was thought important to screen these agents for anticonvulsant activity in mice and rats by a battery of laboratory tests. The results obtained provide the basis for this report.

Methods. Adult male mice (CF #1 strain) from the Carworth farm and adult male albino rats from the Sprague-Dawley farm were used as experimental animals. They were maintained on Purina Laboratory Chow and, except for the experiments in rats, were allowed free access to food and water until removed from their cages for testing. The *l*-glutamine and *l*-asparagine were administered either orally or intraperitoneally in aqueous solution or suspended in 10% acacia mucilage. Both compounds were tested *in mice* after *acute* administration by a battery of six anticonvulsant assay procedures: maximal electroshock seizure pattern (MES); maximal Metrazol seizure pattern (MMS); minimal electroshock seizure threshold (MET);

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