Tamm and his associates, interferes with the replication of both DNA- and RNA-viruses (10).

The fact that its biologic effect can be so easily assayed *in vitro* via inhibition of VV plaque formation supplies an additional laboratory tool for elucidation of the biochemical mechanism of action of 6-MP.

Additional important parameters remain to be fully evaluated, *i.e.*, the phase during virus-host cell interaction at which 6-MP operates, the ability of 6-MP to interrupt *in vitro* virus infection once well established, and finally and most crucially the response of virus infection *in vivo* to 6-MP therapy. These areas are being investigated.

Summary. *In vitro* experiments with 6-mercaptopurine have demonstrated that concentrations of the purine analog as low as 1 μg/ml effectively disrupt the multiplication of both DNA-viruses (vaccinia, herpes simplex) and RNA-viruses (measles, polio) in established strain Lass cells.

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Sudden Unexpected Death in Infancy. Isolations of ECHO Type 7 Virus. (29211)

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Sudden and unexpected death in infancy (crib death) has become a public health problem of considerable magnitude. The almost classic symptomatology has been adequately described by several investigators(1, 2), where an apparently well baby with or without mild respiratory difficulties is found dead in its crib. Viral etiology was suggested as early as 20 years ago(3); recent reviews (4,5), however, have shown a paucity of isolations from autopsy material. The following paper presents studies on isolation of viruses from 7 of 10 crib deaths, obtained randomly within a 12-month period from the metropolitan Phoenix area.

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Materials and methods. Tissue cultures. Preparation and history of human embryonic epithelial lung cultures (HEL) have been reported elsewhere(6). For use in these studies, approximately 200,000 cells per ml suspended in 20% calf serum-M199 were dispensed into screw-cap roller tubes. Confluent sheets of cells were observed within 18 to 24 hours, at which time the medium was replaced with 2% calf serum-M199 for maintenance. Primary renal cultures obtained from the grivet (*Cercopithecus aethiops*) were prepared according to methods described for preparation of rhesus monkey kidney cell cultures(7). Kidney cell roller cultures were usually ready to use in 5 to 7 days, at which time fresh 2% calf serum-M199 was substituted for maintenance medium.

Prototype enterovirus serums were ob-

tained from Microbiological Associates, Bethesda, Md. ECHO 7 prototype virus was obtained through the courtesy of Dr. Herbert Wenner.

Post mortem specimens. Specimens were obtained from 6 infants under one year of age in which the pathologist reported only interstitial pneumonitis. In 4 instances death was attributed to other factors and these were included as controls. Approximately 1- to 5-g specimens of brain, lung, liver, and stool were obtained from each case; an attempt was made in each instance to obtain blood. The specimens were removed with clean but not sterile instruments and individually dispensed into small widemouth sample bottles. The interval of time between death of the infant and receipt of the specimens in our laboratory ranged from 2 to 8 hours. Usually the specimens were harvested immediately upon arrival and stored at −60°C. Infrequently specimens arrived at inopportune times and were stored immediately as obtained. The routine processing of specimens was as follows:

When tissues were processed, each was washed carefully in approximately 5 volumes of M199 using double strength antibiotic mixtures. They were then minced with scissors, ground in a mortar, and suspended to approximately 20% by weight in M199. Stool suspensions were prepared at approximately 10% by weight in M199; whereas, whole blood was used as collected. All specimens were clarified at 3,000 r.p.m. for 10 minutes. Prior to assay for virus, each specimen was centrifuged at 10,000 r.p.m. in a refrigerated centrifuge.

Procedural. Antibiotics were used routinely in all specimen and cell preparations at concentrations of 100 units penicillin per ml and 100 μg streptomycin per ml. All initial isolation attempts were made in HEL cultures. Those specimens showing no virus were similarly tested in grivet renal cells.

For each specimen a 0.1-ml inoculum in each of 2 tissue culture tubes was used. All tubes were incubated in roller drums at 37°C. Cultures were observed daily for 7 to 10 days, and those showing cytopathogenicity (CPE) or cellular toxicity after appropriate time intervals were removed, frozen, and thawed 3 times in a dry ice-alcohol bath and stored at −60°C. At 10 days, all remaining tubes were harvested similarly and held until a second passage could be initiated. Second and succeeding passages (through 5 or more) were identical with the first except that inoculum was increased to 0.2 ml per each of 2 tubes.

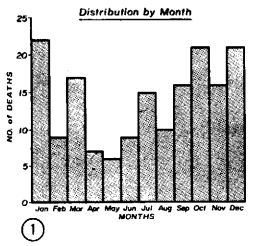
Neutralization tests. Viral isolates were identified using serum pools according to methods of Schmidt *et al*(8). The viruses (100 TCD_{50}) and serums were mixed, 0.2 ml plus 0.2 ml, and incubated at room temperature for 1 hour. Each of 2 tissue culture tubes then received 0.1-ml aliquots, followed by daily observation for characteristic neutralization patterns. Tentative identifications were confirmed with individual type-specific serum.

Hemagglutination and hemagglutination-inhibition studies. Hemagglutination (HA) and, where appropriate, hemagglutination-inhibition (HAI) studies were performed on all isolates using methods described by Lahelle(9).

Suckling mice. Aliquots of each specimen were inoculated IP (0.02 ml) and IC (0.01 ml) into 1- to 3-day-old suckling mice with subsequent blind passage at 7 days post inoculation. All passages were in duplicate—2 litters of mice, each with 12 to 18 pups, per specimen.

Epidemiological features. Maricopa County, which includes the city of Phoenix and its suburbs, experienced a phenomenal burst of population from 1950 to 1962, increasing from approximately 330,000 to approximately 730,000. The county medical examiner's office‡ provided data compiled from October 1951 through December 1962, showing 169 infant deaths necessitating autopsy or a pathologist's verdict as to cause of death. Fig. 1 shows the distribution of infant deaths by month for this period. Peak deaths were recorded for January, October, and December with an apparent tailing off in the late spring and early summer months. Fig. 2

‡ The excellent cooperation of county medical examiner Dr. D. J. Condon and his staff is gratefully acknowledged.



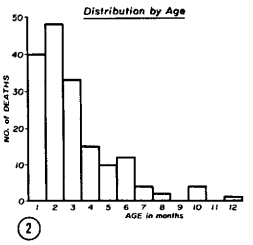


FIG. 1. Distribution of infant deaths by month. Phoenix, Ariz., Oct. 1951 through Dec. 1962.

FIG. 2. Crib deaths by age. Phoenix, Ariz., Oct. 1951 through Dec. 1962.

shows the number of crib deaths by age for the period October 1951 through December 1962. As shown, 48 of the infants, or approximately 28%, were at or near 2 months of age at time of death. A rapid diminution is then apparent, with only one death reported at an age of approximately 12 months. Age distribution as well as peak months for infant deaths were essentially the same as reported by Coe(5) on Hennepin Co., Minn. During 1961 and 1962, there were 21 and 19 cases reported, respectively, 10 of which were included in the present study. None of the families or infants in the study was acquainted with or, to the best of available knowledge, had had contact with another. Similarly, there was no indication of a common source of infection although a particularly high incidence of crib deaths was apparent in certain geographical areas of the county characterized by a high influx of transient laborers.

Results. Pathologists' findings. In none of the cases were the anatomic findings considered adequate to explain the deaths of these infants. A consistent finding in each instance was subepicardial and subpleural petechial to purpuric hemorrhage. Generally there was pulmonary congestion and edema with microscopical evidence of peribronchiolar thickening of the alveolar septae and infiltration with mononuclear cells. The reports on 6 infants showed only interstitial pneumonitis. Two infants had indications of broncho-pneumonia. One infant was reported to have a carcinoma of the lung and, finally, one infant showed indications of complicating enteritis.

Virus studies. Viral agents were isolated from 7 of the 10 study subjects. Table I shows the individual organs tested from each infant, the pathologists' findings, and the results at attempts to isolate viruses. ECHO 7 virus was isolated from stools in 3 instances and from livers, lungs, and brains of each of 2 individuals. ECHO 22 virus was isolated from a single stool specimen. Polio 1 virus was isolated from both liver and stool obtained from a single infant. Polio 2 virus was obtained from the stool of 1 infant.

Hemagglutination studies with ECHO 7 viral isolates were of interest in that an apparent enhancement was observed from 1 to 2 passages through primary grivet kidney cells. A similar and more marked effect was observed for these isolates obtained in HEL cultures. The initial isolate after 2 or more passages in HEL would have no HA activity, whereas subculture to primary grivet kidney enhanced this activity (titer 1:8 to 1:16). A second passage (but rarely a third) in the latter cells showed enhanced HA titers (1:32). Prototype ECHO 7 virus obtained from CDC laboratories in Atlanta behaved similarly but

TABLE I. Viral Agents Isolated from Infants Dying Suddenly and Unexpectedly. Phoenix, Arizona, 1961-1962.

Specimen No.	Pathology*	Stool	Liver	Lung	Brain	Blood
10	Interstitial pneumonitis	ECHO 7	ECHO 7	ECHO 7	N.S.†	Neg.‡
45	*Idem*	" 7	" 7	" 7	Neg.	N.S.
09	"	" 22	Neg.	Neg.	ECHO 7	Neg.
04	"	" 7	"	"	Neg.	"
35	"	Neg.	"	"	ECHO 7	N.S.
69	"	"	"	"	Neg.	Neg.
87	Enteritis	Polio 2	"	"	N.S.	N.S.
61	Broncho-pneumonia	" 1	Polio 1	"	Neg.	Neg.
91	Carcinoma of lung	Neg.	Neg.	"	"	"
27	Broncho-pneumonia	"	"	"	"	"

* Principal pathological finding. † N.S.—No specimen. ‡ Neg.—No virus detected.

consistently showed higher titers (1:256).

Mice. Aliquots of the various materials tested in suckling mice were consistently negative for viruses.

Discussion. The studies reported here were oriented to the recovery of enteroviruses—viruses infectious for a primary or a stable cell line or for suckling mice. ECHO 7 virus was isolated from 9 of 27 specimens obtained from infants in which interstitial pneumonitis was the only apparent pathological finding.

Credulity of the isolates from these organs is, of course, speculative. However, the usual precautions were observed and special attempts were made to harvest the specimens from individual cases when they were received. In addition, several attempts to reisolate viruses from these specimens were successful and subsequent serological studies confirmed their identity.

The isolation of an enterovirus in 2 instances from the brain and not in the stool of one infant is of more than academic interest. Gold *et al*(10) reported on the recovery of Coxsackie A4 from the central nervous system, and Kleinman and his associates (11) listed ECHO 7 virus as a possible factor in aseptic meningitis.

No attempts were made to contact or to obtain specimens from the families of these infants, so that no data are available concerning the source or transmission of these viruses. Mention should be made, however, that an "intensive family survey" carried on in our laboratories during this time revealed the presence of ECHO 7 virus within the area.

The number of infants dying "suddenly" from year to year in the Phoenix area was somewhat constant, in contrast to the great increase of population. A first impression is that the transient population represents a more steady influx of individuals and that this group might contribute the majority of infant deaths.

The contention often has been made that the label "crib death" or "sudden and unexpected death" is inadequate for describing infant deaths of unknown origin. The classification for this entity is buried in statistical data under "mechanical trauma" or "asphyxia," which perhaps belies its true significance as a public health problem. New methodology or a reevaluation of present techniques may uncover additional information of value in support of an infectious process. The authors contend, and this is not to be construed as an original thought, that many agents could be involved and that the significance of any one might depend on the susceptibility of the individual infant, the microbe(s) present in the area at the time, and physiological factors as yet little understood. We believe that many more studies should be made in an attempt to lessen the numbers of unexplained infant deaths.

Summary. Viral isolations were made from 7 of 10 infants dying a sudden and unexpected death. Forty-five specimens, including brain, lung, liver, whole blood, and stool, were included for the study. Nine of 12 isolations were identified as ECHO 7 virus—2 isolations from the brain, 2 from the lung, 2 from the liver, and 3 from stool. Two of the remaining isolates were identified as polio 1 virus and polio 2 virus, respectively, the former obtained from both liver and stool in one individual and the latter from the stool only of a second infant. The final isolate was ECHO 22 virus obtained from a single stool specimen.

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