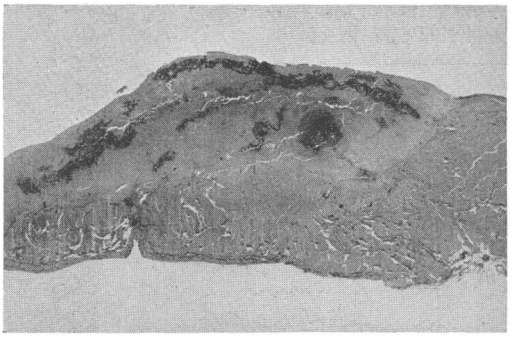

FIG. 2. Cross section of dog #5 heart showing extensive subendocardial hemorrhage.

45 minutes. These complications have not been reported previously.

Conclusions. 1. Subendocardial hemorrhage and intracardiac thrombosis are found when catheters remain in the heart for over 45 minutes. 2. No such lesions could be attributed to "high pressure" intracardiac injections of ordinarily used contrast agents. 3. Disturbed pressure relationships probably due to asynchronous ventricular extrasystoles are frequently seen with injection into the heart, superior cava or pulmonary artery. 4. Such electrical, contractile and pressure disturbances were not noted with injection into the inferior cava or aorta.

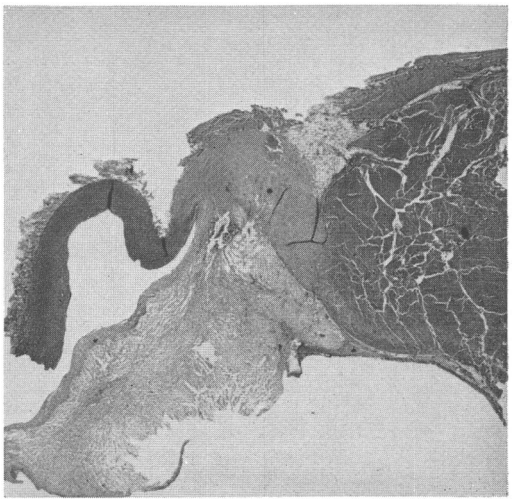

FIG. 3. Cross section of dog #5 heart in region of aortic valves showing a moderate sized thrombus.

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Growth of Several Arthropod-Borne Viruses in Tissue Culture. (23458)

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Diagnostic isolation and identification of arthropod-borne viruses, an antigenically related group with widely variable host range, is difficult for the small laboratory when infections by several types occur in the same area. A single host system susceptible to a majority of the group, conveniently available, easily manipulated, and sensitive to small amounts of virus, is desirable. Tissue culture systems for this group have not been extensively investigated. The group A viruses, eastern (EEE), western (WEE), and Venezuelan (VEE) equine encephalitis viruses have a wide host range and no particular difficulty has been experienced in infecting chick embryo fibroblasts and HeLa cells. Except for West Nile virus, the group B viruses do not produce cytopathic (CP) changes with regularity in the above tissue culture systems (1,2). Reports are lacking on the infection of other tissue culture systems with the group B viruses except for St. Louis encephalitis which produced CP effects on 2 chemically induced rodent tumors and 2 tumors of human origin(3), and Japanese B encephalitis (JBE) and West Nile (WN) which has recently been reported to cause CP effects in Detroit-6 cells (4).

TABLE I. Results of Comparative Titrations of Several Arthropod-Borne Viruses in Mice and Hamster Kidney Cell Culture.

Virus	Strain	Laboratory passage history at start of exp.	Virus derived from infected mouse brain or chick embryo		Virus derived from infected hamster kidney cultures	
			LD_{50} in mice	CPD_{50} in hamster cultures	LD_{50} in mice	CPD_{50} in hamster cultures
EEE	AR-167	*M_2E_1	8.5†	8.5	6.6	7.8
	BCC 6	E_1	9.0	8.0		
WEE	L_2-34a	M_1E_1	7.5	7.3	5.2	7.0
	C 6	E_1	7.5	7.0		
VEE		‡$GP_{13}E_2$	10.2	8.2	6.8	6.8
SLE	904	M_8	8.2	8.5	4.2	4.6
	Hubbard	M_{103}	7.5	7.0		
JBE	Nakayama	M_{97}	6.3	6.5	4.6	4.6
	Ohmori	M_3			4.5	5.2
WN		M_{28}	6.6	8.1	<2.6	3.2
MVE		E_2M_{11}			3.8	3.8
Ilheus		M_{27}			5.8	5.5

* M_2E_1 = Two mouse brain passages followed by one chick embryo passage. † Log_{10}.
‡ GP = Guinea pig brain passage.

The work to be described was an investigation of several tissue culture systems as regards the cytopathic changes produced by arthropod-borne viruses, and a comparison of the sensitivity of tissue culture method with the mouse intracerebral inoculation test.

Materials and methods. Cell suspensions from hamster and guinea pig kidneys, and chick embryos were prepared by overnight trypsinization at refrigerator temperature. Those of dog kidney were trypsinized at 37°C in 30 minute cycles. After 2 washings in Hanks' balanced salt solution, the cells were suspended in growth medium in concentration of 300,000 to 500,000 cells/ml and distributed in 1 ml amounts into glass tubes with bakelite screw caps. These were then incubated in a slanted stationary position at 36°C for 4 to 7 days until ready for use. Kidney cell cultures were grown and maintained in 3 to 5% calf serum, 0.5% lactalbumin hydrolysate in Hanks' balanced salt solution. The medium used for chick embryo fibroblasts consisted of 5% chick embryo extract and 10% calf serum in Hanks' solution. Antibiotics in the proportion of 500 units of penicillin, 250 μg of streptomycin sulfate and 25 units of mycostatin per ml were added to all mediums. Dilutions of virus were made in the respective growth mediums and 0.1 ml volume inoculated per tube. Mice used were 3 weeks old, of the CFW strain, and were inoculated intracerebrally with volumes of 0.03 ml. The viruses used are indicated in Table I.

Results. Dog kidney cells. Cytopathic effects were obtained irregularly in dog kidney cells infected with EEE virus. In comparative titrations in dog kidney cells and intracerebrally in mice the CP changes in the tissue cultures, when they occurred, were always at dilutions more concentrated than those necessary to kill mice. No CP changes were obtained in dog kidney cells infected with St. Louis encephalitis (SLE) virus. Since dog kidney tissue cultures did not satisfy the requirements sought for in a host system with these 2 viruses, no systematic exploration of their sensitivity to the other viruses was made.

Guinea pig kidney and chick embryo cells. Guinea pig kidney and chick embryo cultures and mice were inoculated with 10,000 to 100,000 mouse LD_{50} units of the various viruses. CP changes resulting in complete degeneration of the cells were seen on the second day after inoculation with EEE, WEE, and VEE in both guinea pig kidney and chick embryo cultures. Incomplete degeneration of guinea pig kidney cultures (50%-75% of the cells destroyed) was seen by the 6th day with West

Nile (WN) and Ilheus viruses. No degeneration was seen in the guinea pig cultures by the 6th day with SLE, JBE, or Murray Valley encephalitis (MVE) viruses at which time the fluids were harvested and inoculated into mice. Partial degeneration of the chick embryo cells was seen on the 6th day in those tubes inoculated with WN virus. No changes were seen by the 7th day in the chick embryo cultures inoculated with SLE, JBE, MVE, or Ilheus viruses at which time the fluids were harvested and inoculated into mice. Although all fluids contained virus neither type of culture showed a sufficiently broad spectrum for susceptibility accompanied by CP effects. Therefore, no effort was made to prove or disprove actual increases in virus titer.

Hamster kidney cells. Hamster kidney cultures were inoculated with 10^{-2} or 10^{-3} dilutions of the various viruses. CP changes amounting to complete destruction were produced in these cells in 2 days by EEE, WEE, VEE, and Ilheus viruses. Similar degeneration was seen in 2 to 3 days in cultures inoculated with concentrated dilutions of SLE, JBE, and MVE viruses. West Nile virus produced degeneration in 5 days when a 10^{-3} dilution was used for the inoculum. Uninoculated cultures held during the same period showed no detectable change.

Comparative titrations were made intracerebrally in mice and in hamster kidney tissue cultures using 5 mice and 5 tubes per 10-fold dilution. Appreciably higher titers in mice were found with EEE strain BCC6 and VEE, while the titer of WN was higher in hamster kidney cultures.

Fluids from the tissue culture tubes around the range of the cytopathogenic endpoint were individually inoculated into mice for the detection of virus. In each case virus was recovered from those tubes showing CP effects but not from those which appeared normal, indicating that the infectious dose and cytopathogenic dose were identical. The fluids were harvested from the highest dilutions causing cytopathogenesis in the preceding titrations and a second passage made in hamster kidney tissue cultures. The fluids from these second tissue culture passages were then titrated in mice and in hamster kidney tissue cultures except for a few instances where fluids from the first tissue culture passage were used. The results are shown in Table I.

In general, virus derived from infected tissue culture did not display as high titer as that derived from infected mouse brain. This is probably a reflection of the difference in number of cells per unit volume which were available for infection.

With EEE, WEE, SLE, WN, and the Ohmori strain of JBE the virus derived from infected hamster kidney cultures titered higher in hamster kidney cultures than in mice. The titers in the 2 host systems were the same for VEE, the Nakayama strain of JBE and MVE. With Ilheus virus titers of the first hamster kidney passage material were slightly higher in mice than in tissue cultures.

Using approximately 50 to 100 tissue culture doses of virus, neutralization tests were performed with EEE, WEE, VEE, SLE, and JBE. Equal amounts of the immune serum and virus, and also normal serum and virus, were combined and allowed to stand at room temperature for one hour before inoculation of the tissue cultures. The CP effect was neutralized by the immune serum in each instance except for VEE in which a partial degeneration of the cells took place 2 days after the control tubes were completely degenerated.

Since the passage history of each virus apparently influenced its respective comparative titer, it was thought desirable to attempt direct isolation of virus in hamster kidney tissue culture, comparing this method with intracerebral inoculation of mice and intra-amniotic inoculation of chick embryos. Thirty pheasant and horse brains suspected of being infected with EEE were available for the purpose. Seventeen of these yielded no isolate in any of the 3 host systems. Eleven yielded EEE virus by each of the 3 isolation methods. EEE virus was isolated from one specimen in hamster kidney tissue cultures and in chick embryos but not in mice. Another specimen yielded EEE virus only by chick embryo inoculation, 1 of 6 embryos inoculated succumbing to infection.

To compare the usefulness of hamster kidney cells for isolation of WEE virus, several

tissues from a naturally infected bird, a purple grackle, were inoculated into mice, tissue cultures, and chick embryos. Four mosquito suspensions (*Culex tarsalis*) from which WEE virus had been isolated in 1955 were obtained from Dr. Preston Holden. These arrived in a thawed condition. However it was felt that this fact would allow a more severe test of the sensitivity of the various hosts than if they had remained continuously frozen.

WEE virus was isolated from all of 6 different grackle tissues by inoculation of chick embryos, from 4 of the 6 tissues by inoculation of tissue cultures, and from 3 of the 6 tissues by inoculation of mice.

One of the mosquito suspensions yielded WEE virus by each of the 3 isolation methods. A second suspension yielded virus only in chick embryos, 1 of 6 embryos inoculated dying specifically. No virus could be isolated from the remaining 2 mosquito suspensions by any of the 3 methods.

Heavy brain suspensions produced incomplete degeneration of the hamster kidney cell cultures whether or not virus was present in such suspensions. It was necessary, therefore, to subinoculate into a second set of cultures in order to observe specific typical cytopathogenesis. Mosquito suspensions did not prove toxic to hamster kidney cells and cytopathogenesis progressed in a typical manner when these suspensions contained virus.

Discussion. Hamster kidney tissue cultures proved susceptible to infection with a wide range of arthropod-borne viruses and responded with cytopathogenesis. Those strains of SLE and JBE which had a low number of mouse brain passages titered higher in hamster kidney cultures than in mice, while the reverse was true of those strains having many mouse brain passages. It has been observed in this laboratory that after continued intracerebral passage most of these arthropod-borne viruses lose much of their ability to infect mice by peripheral routes. Thus, laboratory adapted strains and viruses as they are encountered under natural conditions may differ in their relative infectivities for a neural or a visceral tissue. The experience reported here with EEE and WEE viruses indicates that a visceral tissue is more sensitive than brain tissue to "wild" forms of these viruses. The same probably holds true of the SLE-JBE group of viruses in view of the differences obtained with strains of high and low laboratory passage history.

TABLE II. Time Required to Produce Evidence of Infection by Several Arthropod-Borne Viruses in Tissue Cultures and in Mice.

Virus	Strain	Dilution	CP effect in tissue cultures (days)	Death in mice (days)
EEE	BCC 6	10^{-7}	2–3	3– 4
WEE	C 6	10^{-6}	3–4	5– 8
VEE		10^{-7}	2–3	4– 6
SLE	904	"	5–7	6– 8
JBE	Ohmori	10^{-4}	4	8–11
WN		10^{-3}	5	6
MVE		"	5	6– 8
Ilheus		10^{-4}	4	6– 7

Advantages of hamster kidney cultures over mice include in addition to greater sensitivity, less cost, less space for maintenance, and more rapid results. Complete destruction of cultures preceded death of mice receiving equal virus inocula by 1 to 7 days (Table II).

Chick embryos when inoculated by the amniotic route were slightly more sensitive indicators of EEE and WEE virus than were hamster kidney tissue cultures. However, in those cases where the quantity of material available for isolation attempts would limit the choice to a single host system, hamster kidney tissue cultures would be more advantageous than either mice or chick embryos since they display a high sensitivity to all the major arthropod-borne encephalitis viruses which might be encountered in this country.

Summary. Cytopathogenesis was observed in hamster kidney tissue cultures infected with eastern equine, western equine, Venezuelan equine, St. Louis, Japanese B, and Murray Valley encephalitis viruses and by Ilheus and West Nile viruses. The sensitivity of these tissue cultures was equal or superior to that of mice. Guinea pig kidney and chick embryo cultures reacted with complete cytopathic effects only with EEE, WEE, and VEE viruses.

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Increased Prothrombin Consumption Following Total Body X-Irradiation in Man. (23459)

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In a preliminary investigation of the hematological effects of a single exposure to therapeutic total body x-irradiation in cancer patients, an increase in prothrombin consumption during blood coagulation was observed. The average of postirradiation serum prothrombin time values was significantly elevated over the pretreatment average. The incidence of individual serum prothrombin time prolongations was also significantly increased after irradiation. In addition, it was found that in duplicate tests of serum prothrombin time in which the only known variable was the alternate use of prothrombin-free plasma (Cappel)†, and of purified bovine fibrinogen (Warner-Chilcott)‡, the latter reagent resulted in a significantly more sensitive test in the elevated range.

Materials and methods. Forty-one patients having neoplastic lesions with metastases requiring the use of the total body x-ray therapy according to the Radiation Therapy Committee of M. D. Anderson Hospital were studied. All were ambulatory and, apart from their primary disease, were in general good health and not cachectic. X-ray dosages, delivered in a single total body exposure in each case, (400 kv; 200 cm focal skin distance; 4.1 mm copper half value layer) ranged from 25 r through 150 r in 25 r steps. Dosage in each case depended on medical requirements of the patient. Complete hematological studies were performed daily in a 3- to 5-day baseline period immediately prior to therapy. Similar tests were performed immediately postirradiation and through a 10-day follow-up period before any further radiation therapy was given. Only platelet counts, clotting time, and plasma and serum prothrombin time tests are considered in this report. *Platelet counts* were performed in the Neubauer counting chamber with Rees-Ecker diluent (1). Clotting time tests were made by the 3 tube modified Lee-White procedure(2). Plasma prothrombin time was determined by the Link-Shapiro modification(3) of Quick's one-stage method, using Simplastin (Warner-Chilcott). Serum prothrombin tests were done by a previously reported modification (4,5) of the Quick one-stage method using Simplastin (Warner-Chilcott) and Fibrinogen (Warner-Chilcott). When no end-point was reached within 3 minutes, a reading of 180-plus seconds was recorded. Prothrombin-free plasma (Cappel) was used in place of fibrinogen in duplicate tests in 33 cases.

Results. There was no significant alteration from baseline levels in platelet count, clotting time, or plasma prothrombin time. Also, there was no evidence of relationship of these test results to the serum prothrombin time when the latter was prolonged above average. There was no significant relationship of serum prothrombin time changes to air dose or to integral dose in megagram-roentgens.

Serum prothrombin time. A. *Comparison of pre- and postirradiation averages.* It will be seen in Table I that the posttreatment serum prothrombin time averages, determined by the fibrinogen technic, increased over pretreatment times at the .001 significance level.

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