the 3 tumors. The tumor in which particles were most plentiful had 5 to 10 particles per thin section while the tumor with the least had an average of one particle for every 10 to 20 thin sections examined. Also observed were particles which appeared to be budding out from the plasma membranes of the chloroma cells (Fig. 2).

The electron dense particles, as seen in thin sections, are approximately 70 mμ in diameter. Incomplete particles budding from plasma membranes are slightly larger. All of the particles have an outer limiting membrane and some have an inner limiting membrane while others do not. This may be a function of the degree of maturation of the particles(8). The particles have a core of variable density. Some of the particles appear hexagonal in shape.

Negative stain preparations of the chloromas have not been convincing as a variety of particles of widely differing dimensions are seen. Similar preparations of spleen from animals with Shay chloroleukemia are more convincing. What are interpreted to be fragments of cytoplasm contain many particles (Fig. 3). Some of these particles have tail-like processes. The length of the tails is variable but the diameter is quite constant. The particles seen in these negative stain preparations are of comparable shape and dimension to those seen in thin sections.

Summary. An electron microscopic study of non-inbred Wistar rats with acute myelogenous leukemia has revealed the presence of intercellular particles with the morphologic features usually ascribed to type C virus particles(9). These particles are approximately 70 mμ in diameter, hexagonal in shape, and may have tail-like structures. Their distribution is similar to that reported for the murine leukemia viruses. Although attempts to isolate leukemogenic viruses from rats with spontaneous leukemia or chemically induced leukemia have been unsuccessful, this study provides morphologic evidence for the existence of such particles in leukemic rats.

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Further Studies on the Hemolytic Properties of Arboviruses.* (29875)

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In a previous report on the hemolytic properties of eastern and western equine encephalomyelitis viruses propagated in tissue culture, it was noted that other serogroup A arboviruses grown in a similar manner also possessed this property(1). The present report is concerned with the study of the hemolysins of these other serogroup A viruses as well as representatives of B, C, Bunyamwera and California serogroups(2,3,4,5,6,7). It appears that hemolytic activity is a com-

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TABLE I. Demonstration of Hemolytic Activity of Group A and B Arboviruses.

Virus	Strain	Source	Ha[1]	He[2]
Group A				
WEE	RI	MB	4096	3809
WEE	Florida	MB	4096	443
EEE	Mass	MB	14500	1830
EEE	"	CETC	80	128
EEE	Pheasant	MB	360	356
EEE	"	CETC	180	223
Sindbis	Ar 339	MB	360	4
"	" "	CETC	90	7
Mayaro	Tr 4675	MB	128	18
"	" "	DETC	128	15
Chikungunya	Ross	MB	1024	2017
"	"	DETC	45	57
Middelburg	SA Ar 749	MB	360	140
"	" " "	DETC	180	234
Semliki	Original	MB	2900	946
"	"	CETC	45	31
Group B				
St. Louis	Hubbard	MB	8192	<2
Murray Valley	Original	MB	11590	<2
" "	"	DETC	45	<2
Dengue 2	Tr 1751	MB	512	<2
Ilheus	Original	MB	19490	<2
Jap B	Nakayama	MB	23170	23
Powassan	Byers	MB	4096	<2
Yellow Fever	17D	MB	512	<2
Uganda S	Original	MB	2048	<2
Zika	MR 766	MB	1450	<2
Bussaquara	Be An 4073	MB	128	<2
West Nile	Ar 248	MB	4096	<2
Ntaya	Original	MB	256	<2

[1] Average of highest dilution or half dilution producing complete or partial agglutination.
[2] Average of highest dilution producing 25% lysis of a standardized 1% CRBC suspension.
Abbreviations: MB, mouse brain; CETC, chick embryo tissue culture; DETC, duck embryo tissue culture.

mon biological property shared by most arboviruses and independent of their antigenic structure.

Materials and methods. The preparation of tissue cultures and of hemagglutinating antigens from infective tissue culture fluids by treatment with Genetron, have been described previously(1). The sucrose-acetone method of Clarke and Casals(8) was used to extract hemagglutinating antigens from infected mouse brains. Hemagglutinating antigens for the group C viruses were prepared by acetone precipitation from sera of infected suckling mice.

Details of techniques for assay of hemagglutination (Ha) and hemolysis (He) activity have been described(1). Somewhat different control blanks were employed in the present study when mouse brain extracts were assayed for He activity. Serial dilutions of the test mouse brain extract plus an equal volume of adjusting diluent, which was used to suspend the test red blood cells, served to correct for any coloration in the extract. An additional control blank consisted of equal volumes of borate saline diluent and a standardized 1% suspension of red blood cells. This corrected for any non-specific lysis of the test red cells. Red blood cells from one-day-old chicks (CRBC) were employed in the assays for both Ha and He.

All group A arboviruses in the stock collection were assayed for He and Ha activity using antigens prepared from both tissue culture and mouse brain sources. In general only mouse brain or serum antigens were prepared for viruses of other serogroups. As a conventional procedure every virus antigen tested was frozen and thawed at least 3 times prior to assay. Hemolysis assays were conducted at that pH which previously had been found to be optimal for maximal Ha activity.

TABLE II. Enhancement of Hemolytic Activity of Certain Group B Viruses.

Virus*	Exp No.	Reciprocal of titers: Conventional method Ha[1]	Conventional method He[2]	Alternative method Ha	Alternative method He	Alternative procedure†
Japanese B		13780	52	11590	145	(1, 2, 5)
Powassan	1	2460	<2	4096	2	(1, 2, 5)
	2	1450	7	1450	16	(1, 2, 5)
St. Louis	1	2900	<2	2900	6	(1, 5)
	2	2048	<2	2048	5	(1, 5)
Ilheus	1	6890	<2	6890	19	(1, 2, 5)
	2	N.D.	N.D.	5800	46	(2, 4)
Dengue II	1	1024	<2	1450	±2‡	(1, 2, 5)
	2	3440	<2	3440	3	(1, 2)
	3	1024	<2	1024	±2‡	(1)
Zika		720	<2	720	±2	(1, 2)
Murray Valley		2048	<2	2900	<2	(1, 2, 3, 4)
West Nile		2900	<2	9096	<2	(1, 2, 3)
Bussuquara		64	<2	64	<2	(1, 2)
Ntaya		430	<2	512	<2	(1, 2, 3, 5)
Yellow Fever		512	<2	512	<2	(1, 2, 3, 5)
Uganda 5		2440	<2	2048	<2	(1, 2, 3, 5)

* All virus antigens were infant mouse brain preparations.
† See methods.
‡ A 1:2 dilution producing <25% but >16% lysis.
N.D. = Not done.
1, 2 See footnote of Table I.

In those cases in which maximal Ha activity was manifested at more than one pH value, hemolysis assays were conducted at several pH's.

For the purpose of increasing the hemolytic titers of certain viral antigens alternative procedures were employed as follows:

1. *4° thaw.* The frozen antigen (kept at —60°C) was gradually thawed by storage at 4°C for 18-48 hours before assay.

2. *Slow, then complete thaw*(1). The stored antigen was transferred from —60°C to a freezer at —20°C for a period of 18-48 hours; it was then removed and completely thawed at 37°C before assay.

3. *Assay at various pH's.* Assays for He activity were carried out at pH values which were not necessarily optimal for maximal Ha activity.

4. *Rapid freeze-thaw—10 cycles*(1). The antigen was subjected to alternate freezing in an acetone-dry ice bath and thawing at 37°C for 10 complete cycles before assay.

5. *Overnight assay.* The reaction mixture was incubated one hour in a 37°C water bath. The settled cells were resuspended by vigorous shaking, and then the tubes were held at 4°C overnight.

In addition to the above procedures, the assay method was also slightly modified by increasing the incubation time from one to two hours and by shaking the tubes vigorously every 20 minutes for the first 80 minutes of the incubation period. All tests were carried out in duplicate.

Results. The results presented in Table I indicate that all the group A arboviruses contained in this laboratory's collection possessed hemolytic activity regardless of whether the antigen had been prepared from a mouse brain or a tissue culture source. In contrast, only Japanese B virus of the group B viruses showed significant hemolytic activity. It should also be noted that the level of Ha activity of the group B viruses was comparable to, if not greater than, that of the group A viruses.

Since a hemolysin could be demonstrated for at least one group B virus it was possible that other viruses of this group also possessed this property, but that a variety of different methods might be necessary to dem-

TABLE III. Hemolytic Activity of Arboviruses from Serogroups Other Than A and B.

Group member	Virus	Source	Conventional Ha[1]	Conventional He[2]	Alternative Ha	Alternative He	Alternative* procedures
Group C	Marituba	Infant mouse serum	1024	89	1024	90	(1, 2, 5)
	Apeu	" " "	32	8	32	7	(1)
	Murutucu	" " "	32	±5†	32	±5†	(1)
Bunyamwera	Cache Valley	" " "	64	>8	64	>16	(1, 2, 5)
	Cache Valley	" " "	90	56	90	124	(1, 2)
	Bunyamwera	" " "	2048	2775	1450	6554	(1, 2, 5)
California	California	" " "	180	110	180	127	(1, 2, 5)

* See methods.
† ± = A 1:5 dilution producing <25% but >16% lysis.
1, 2 See footnote of Table I.

onstrate this. New antigen extracts for the serogroup B viruses were prepared by extraction with sucrose-acetone and all antigens were treated using a series of alternative methods. The results of these experiments are shown in Table II. Six of the 12 group B arboviruses tested possessed He activity. It was also noted that one of the Powassan Ha antigens showed hemolytic activity without processing by any of the alternative methods, whereas a hemolysin could not be demonstrated for the other Powassan preparation when treated by conventional methods. The results obtained with the Dengue 2 antigens were also rather variable, although none of the preparations showed hemolytic activity by conventional methods.

Since certain of the group B viruses did possess hemolytic activity, it was of interest to determine if this was a general biological property of arboviruses of other groups. Hemagglutinating antigens were prepared from viruses of 3 other serogroups. These were group Bunyamwera(6,7) (Cache Valley, Bunyamwera), group C(3,4,5) (Marituba, Murutucu, Apeu), and a representative of the California group(7) (California). Assay procedures utilized for the group B viruses were employed for these viruses. Antigens for the group C viruses were prepared from infected infant mouse serum by the acetone-extraction(2). The results shown in Table III indicate that all 6 arboviruses of the various groups possessed hemolytic activity. In addition, it can be seen that the conventional assay procedures were sufficient to demonstrate hemolytic activity by most of these viruses and that the alternative procedures were not necessary as in the case of the group B viruses.

Discussion. The testing of 25 distinct arboviruses comprising 5 different categories (groups A, B, C, Bunyamwera, and California) revealed that 19 of 25 agents tested possessed a hemolysin for one-day chick red blood cells. However, hemolysins could be demonstrated for 4 group B viruses only after modification of the procedure in processing the antigen or conducting the assay. It is probable that the methods employed for demonstrating He activity by the group B viruses are still not the methods of choice. This may also account for the variable results obtained with the different Dengue 2 antigen preparrations.

A few of the group A viruses and both the Bunyamwera group viruses showed He activity which exceeded their Ha activity. A similar finding was noted in the study of the hemolytic properties of the Mass strain of eastern equine encephalomyelitis previously (1). Thus, it is quite possible that hemolysins may be a general biological property of arboviruses.

Summary. A total of 25 arboviruses representing 5 different serogroups (A, B, C, Bunyamwera and California) were studied and found to possess hemolytic activity. With the exception of Japanese B virus, special treatment of viral antigens was necessary to demonstrate hemolytic activity by the group B viruses. The hemolytic properties appeared to be a common property of the arboviruses.

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Blood Supply to Hepatic V2 Carcinoma Implants as Measured by Radioactive Microspheres.‡ (29876)

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If intravascular introduction of chemotherapeutic or radioactive materials is to be effective in tumor therapy, the source of blood nourishing metastatic tumors in the liver must be known. Utilizing angiographic techniques in living patients, Bierman(1) has shown that malignant metastatic hepatic deposits derive their blood supply from the hepatic artery. Gans(2) has reached the same conclusion from injection corrosion studies of human livers with metastatic tumors obtained at autopsy. Since V2 carcinoma is used as an experimental model to evaluate effect on tumor growth of agents introduced *via* the vascular route, it is important that the source of blood vessels to this tumor be known.

Materials and methods. Mature virgin female New Zealand rabbits were utilized. A suspension of V2 carcinoma cells obtained fresh from donor rabbits was prepared by mincing in a tissue grinder, adding Tis-U-Sol* at 5°C and diluting to a concentration of 500,000 cells per millilitre. The tumor strain was maintained by injection of tumor cells into the thighs of new donor rabbits each week. Within 2 hours of the time of tumor harvest from the donor, laparotomy under pentobarbital† anesthesia, (25 mg/kg) was carried out in each animal to be studied and one ml of tumor suspension was injected into the appendiceal vein which was then ligated. Fourteen to 19 days later laparotomy was again performed and the livers of rabbits inspected. Those with gross hepatic implants of V2 carcinoma were utilized for microsphere injection and those without obvious tumors were discarded. Forty-two rabbits with obvious metastatic tumors comprised the present study.

Ceramic or plastic microspheres 15 ($\pm$ 3) μ in diameter containing Scandium-46 in tracer doses, 2 microcuries/mg of spheres, were suspended in a 15% solution of low molecular weight dextran giving 2 mg spheres per millilitre dextran. The specific gravity of plastic spheres was 1.5 and that of ceramic spheres 3.0. Occasional clumps of spheres in the dextran were dispersed by exposure to ultrasonic vibration. The mixture was also shaken and inverted immediately prior to injection of a standard 1.5 ml volume.

Hepatic artery injection of microspheres was accomplished using a 25 gauge needle at the end of a polyethylene tube (P.E. 50), and portal vein injection was accomplished by insertion of a 22 gauge needle into a mesenteric vein. Plastic disposable syringes were utilized for all injections.

Three groups of animals were studied. All animals of a group received the initial injection of tumor cells on the same day and from the same donor, and all rabbits of a

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* Tis-U-Sol, Baxter Laboratories, Inc., Morton Grove, Ill.

† Halatal-Jensen-Salsbery Co., Minneapolis, Minn.