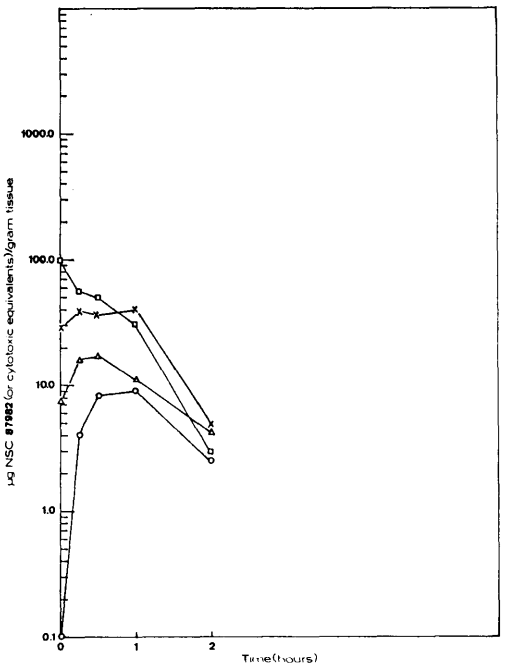

FIG. 3. Concentrations of methyl 5 (or 4)-(3, 3-dimethyl-1-triazeno) imidazole-4 (or 5)-carboxylate (NSC 87982) detected in various tissues of mice by microbiological assay following single LD_{10} (198 mg/kg) intraperitoneal dose of drug. Each point represents the levels of NSC 87982 detected in the pooled tissues from 10 mice. □ = Liver, × = Kidney, △ = Lung, ○ = Brain.

azeno)-imidazole-4(or 5)-carboxylate (NSC 87982). As little as 0.75 μg of NSC 87982/ml of sample can be detected. The applicability of this assay has been demonstrated for the estimation of concentrations of this drug in various tissues of mice.

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Hamster Hyperimmune Ascites as a Source of Antibody. (32241)

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Ascitic fluid from immunized hamsters as a source of high titer antibody has been reported(1,2). Ascites from immunized mice are extensively used as a source of antibody (3-6) and have been examined in some detail. Hamsters offer several advantages over mice; they are larger, easier to handle, and offer a possibility of greater volumes of ascites per animal. Where the hamster is the animal of choice for virus isolation and propagation, the use of hamster ascitic fluid offers a source of large volumes of homologous antibody.

Success in eliciting ascites production in hamsters has not been uniform in this laboratory. Numerous personal communications have indicated that other investigators have had the same problems. This report is concerned with factors affecting ascites production, antibody content of Syrian hamster ascites and possible causes of observed phenomena due to extensive cross reactions be-

tween hamsters and human plasma proteins. Also presented for comparison are some experimental results from fluids obtained from the Korean hamster and characterization of fluids from both.

Materials and methods. Animals. In the early experiments, young adult Syrian (*Mesocricetus auratus*) hamsters, 30 to 60 days old, were used and found to be poor producers of ascitic fluid. Later experiments showed that adult hamsters, 4 to 5 months old, gave the best fluid production. Korean (*Cricetulus triton*) hamsters were from a small colony established at the 406th Medical Laboratory. These were approximately 5 months of age.

Preparation of hyperimmune ascitic fluids. The technique for immunization for protein and virus antigen was slightly different and will be further described later. In general, the antigens were emulsified with Freund's complete adjuvant and injected by the intraperitoneal route. Onset of ascites, if produced, was 14-21 days after the immunization course was begun. After tapping, the ascites were allowed to clot for 24 hours at 4°C, centrifuged at 900 $\times$ *g*, and the clear supernatant ascitic fluid used directly for antibody studies. Occasionally, the lipid content required high speed centrifugal clarification.

Preparation of protein antigen and protein determination. 250 ml of fresh serum from a single donor was broken down into aliquots of 10 cc each and frozen at —70°C until used. Determination of concentration of protein solutions by the Biuret technique of Gornal *et al*(7) was used with human serum albumin as the standard. The sera and ascitic fluids were further characterized by paper electrophoresis using the Beckman B procedure with barbital buffer at pH 8.6. The strips were stained with Bromphenol Blue (B-4) and read in a Beckman analytrol with a B-5 cam.

Preparation of virus antigen. Tacaribe Virus, TR 11574 LTV 3990, grown in suckling mouse brain was passed twice in vero monkey kidney tissue culture and used as cell suspension in Minimum Essential Medium.

Immunoelectrophoresis and immunodiffusion. A modification of the technique of Williams and Grabar(8), by Usui(9) on lantern slides using 1.5% Ionagar #2, and Veronal buffer pH 8.2 $\mu = 0.05$ was used, along with a modification of Ouchterlony's technique(10) for immunodiffusion on lantern slides. Antigens used were the immunizing serum, a "normal" pool from the 406th Medical Laboratory Serology Department, normal hamster serum and ascites, horse, rabbit and goat anti-human serums, and specific purified antigens supplied by the Serology Department, Tokyo University. After washing in normal saline, the slides were dried and stained with the Ponceau S method of Consden and Kohn as described in Crowle(11).

Passive hemagglutination. A microtiter modification of the method of Feeley *et al* (12) was used utilizing formalized sheep cells coated with human γG globulin,* albumin,* and B_1C† globulin.

In each instance PBS pH 7.2 stabilized with 0.1% BSA F$\overline{V}$ was used as the diluent and 2-fold serial dilutions were made, beginning with an initial 1:10 dilution.

Complement fixation. Complement fixation determinations were done on the ascitic fluids from Tacaribe immunized and non-immunized control hamsters, utilizing the microtechnique of Sever(14) and mouse brain antigen (20% Borate KCl suspension) of suckling mouse brain infected with Tacaribe virus harvested on the seventh day as described by Wiebenga (1) and Mettler(15).

Results. Preparation of hyperimmune ascitic fluid to human serum. Twelve hamsters 3 to 5 months old, were immunized with human serum. Initial immunization was performed by injecting .25 ml of whole human serum (18.0 mg protein) mixed with .25 ml of Freund's complete adjuvant, intraperitoneally. A booster injection of complete adjuvant plus saline was given on the 14th day and adjuvant plus serum on the 26th day. An additional booster of .25 ml adjuvant plus .25 ml serum was given on the 42nd day. Most of the animals produced immune ascitic fluid between the 14th and 21st day. A total of 704 ml of fluid was harvested from several

* Purified γ globulin and albumin, courtesy of Dr. T. Matuhasi, Dept. of Serology, Tokyo University.

† Purified B_1C globulin, courtesy of Dr. N. Tamura, Dept. of Serology, Tokyo University.

TABLE I. Protein Composition of Ascitic Fluids and Sera from Immunized and Nonimmunized Hamsters.

	Immunized Syrian		Unimmunized Syrian control		Immunized Korean		P value*
	Serum	Ascites	Serum	Ascites	Serum	Ascites	
Total protein, g/100 ml	9.0 ± 2	3.44 ± 1.2	7.4 ± 1.2	2.7 ± .6	9.1 ± 3.6	6.8 ± 2.7	$P < .05$
% Albumin	34 ± 3.7	35.1 ± 5.8	54 ± 9.3	58.2 ± 4.6	42.5 ± 5.4	39.6 ± 5.5	$P < .001$
% α_1 Globulin	5.5 ± .56	6.6 ± 2.1	9.2 ± 3	8.0 ± 1.4	4.5 ± 1.2	12.5 ± 2	$P < .001$
% α_2 Globulin	4.7 ± 1.5	8.8 ± 4.4	12 ± 3	12 ± 3.3	12.7 ± 2.4	11.5 ± 2.6	$P < .001$
% β Globulin	22 ± 2.7	21.1 ± 5.8	12.2 ± 3	12.3 ± 2.5	26.5 ± 3.1	21.6 ± 5.5	$P < .001$
% γ Globulin	34 ± 4.2	28.6 ± 6.3	11.2 ± 5.3	9.5 ± 4.2	13.8 ± 2.2	16.0 ± 7.6	$P < .001$

* P value result of t test of values of immunized and Unimmunized Syrian hamster ascites protein values.

taps and following separation from the very light fibrin clot, 692 ml of clear ascitic fluid was obtained. This averaged 57 ml per starting animal, and 69 ml per hamster producing ascites. Protein composition of hamster sera and ascitic fluids is shown in Table I.

Eight of the ten producing animals proved to have extremely anti-complementary ascitic fluid and serum. These animals produced strong antibodies to the human B_1C globulin fraction (Fig. 1c and Table II) and some inactivated 2 full units of guinea pig complement up to a dilution of 1:1280. This probably constitutes antibody to complement as described by Muller-Eberhard(16) or a complement fixing cross-reaction with guinea pig serum components, although there was no cross-reaction by immunoelectrophoresis. The other 2 animals produced very low titer antibodies to serum components. Two control animals, immunized with normal saline plus complete adjuvant by the same schedule as above, produced no detectable antibody. Those animals with high levels of serum antibody also produced, as expected, high titer ascitic fluid antibody.

When used as a developing agent in immunoelectrophoresis against human serum, representative Syrian hamster ascitic fluid showed 5 to 12 distinct lines varying from animal to animal. It should be noted that none of the animals produced any detectable precipitin antibody to albumin (Fig. 1), or to γ globulin.

Unimmunized and immunized hamster ascites and serum show a component similar, if not identical, with human albumin components along with 6 additional cross-reacting components in the α and β areas when developed with horse antihuman serum. This similarity may have prevented formation of precipitin antibody to human albumin in the

TABLE II. Passive Hemagglutination Titers* of Syrian and Korean Hamsters Immunized with Human Serum.

Hamsters	B_1C globulin		G globulin		Albumin	
	Serum	Ascites	Serum	Ascites	Serum	Ascites
Syrian 1	20480	40960	0‡	0	0	0
2	2560	1280	0	0	0	0
5	10240	5120	0	0	0	0
6	6400	6400	0	0	0	0
9†	10240	5120	0	0	0	0
10†	20480	10240	0	0	0	0
Korean 2†	2560	2560	20480	2560	1280	40
3†	640	2560	160	1280	160	40
Nonimmunized controls	0	0	0	0	0	0

* Titers expressed as the reciprocal.
† Ascites drawn 9-21 days before compared sera obtained.
‡ Titers of 0 were found to be negative at 1:10 and were retested undiluted to confirm 0 titer.

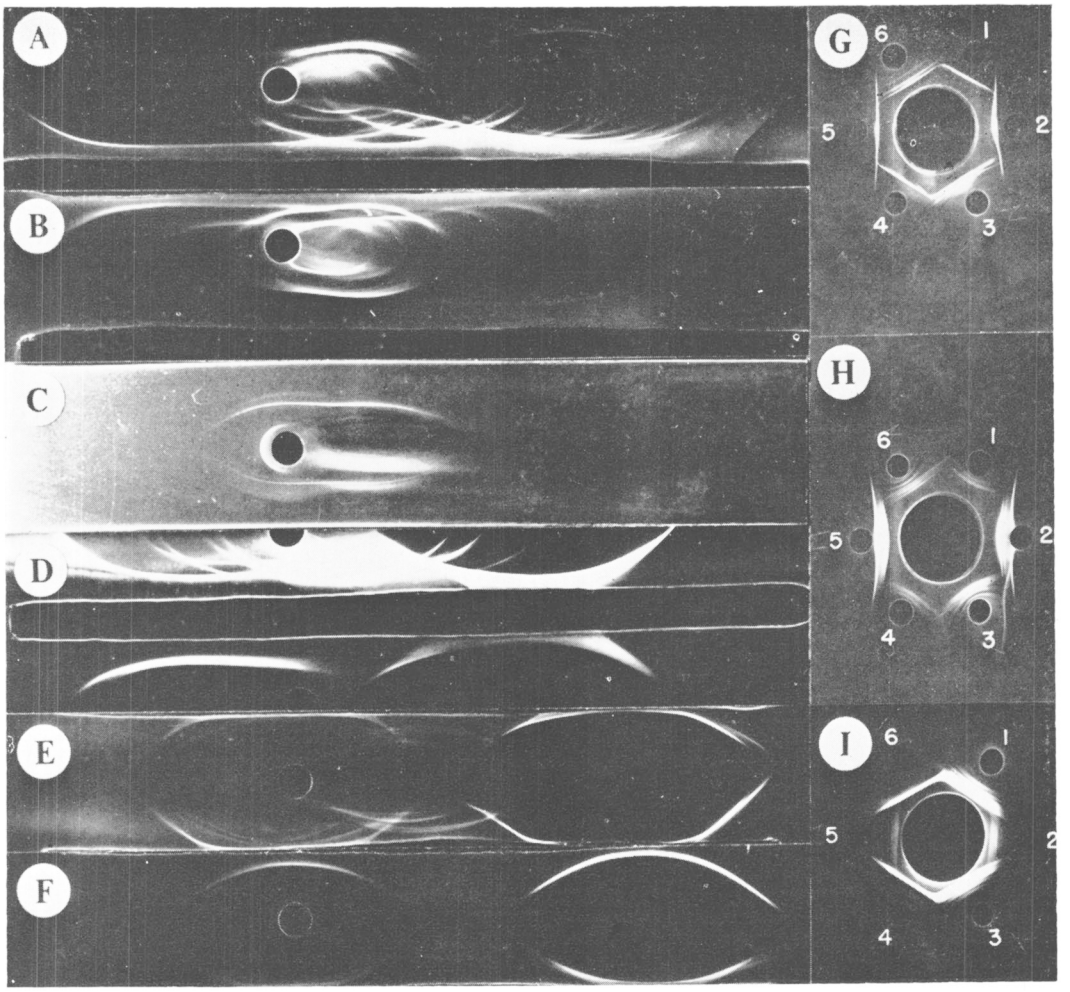

FIG. 1. Immunoelectrophoretic and immunodiffusion characterization of hamster ascites antibodies and antigens.

(Ag) Antigen, (Ab) Antibody, (SHA) Syrian Hamster Ascites, (KHA) Korean Hamster Ascites, (NHS) Normal Human Serum, (HAH) Horse Anti-Human Serum, (SHAH) Syrian Hamster Anti-Human Ascites, (RAH) Rabbit Anti-Human Serum, (KHAH) Korean Hamster Anti-Human Ascites, (BWAGH) "Behringwerke" Anti-Golden Hamster Serum.

a. Syrian hamster anti-human ascites pattern Ag NHS Ab SHA. Lower, AB HAH; b. Korean hamster anti-human ascites pattern Ag NHS Ab KHA; c. Anti B_1C pattern: Upper, AB – Rabbit anti B_1C preparation. Lower, Ab – SHAH Ag-fresh NHS; d. Rabbit anti-human cross-reactions with SHA: Upper, Ag NHS. Lower, Ag SHA Ab RAH; e. Horse anti-human cross reactions with SHA; f. Horse anti-human cross reactions with KHA; g. Immunodiffusion cross reactions with albumin. 1-4 KHA, 2-5 NHS albumin, 3-6 SHA albumin, center well HAH; h. Immunodiffuusion cross reactions with γ globulin. 1-4 KHA, 2-5 NHS γ glob., 3-6 SHA γ glob., center well RAH; i. BWAGH cross reactions with NHS albumin (2-5), SHA albumin (1-3) and KHA (4-6).

hamsters. When hamster serum or ascites were developed with some rabbit antihuman sera, both albumin and γ globulin were precipitated. Fig. 1 illustrates the similarity of human and hamster albumin and γ globulin by immunoelectrophoresis and immunodiffusion. These show 8 cross-reacting components common to both human and hamster serum, confirmed by absorption studies and differential staining.

Korean hamsters. The Korean hamsters were immunized, using an identical procedure to that of the Syrian hamsters. Only 2 of the 5 starting animals produced ascites in small quantities. A total of 63 ml of ascites was

obtained from the 2 producing animals. Antibody content of the Korean hamster ascites was markedly different from the Syrian hamsters. Antibodies were produced to both γ globulin and albumin as well as B_1C and other components of human serum. Immunoelectrophoresis of human serum with Korean hamster ascitic fluids as the developing agent produced 8-14 precipitin lines from most lots of fluid obtained (Fig. 1). Only the lipoprotein components of Korean hamster ascitic fluid cross-react extensively with a horse antihuman serum which precipitates Syrian hamster albumin; however, Korean hamster albumin is precipitated in visible amounts. A high titer rabbit antihuman serum which precipitated both albumin and γ globulin also only precipitated lipoprotein components in Korean hamster ascites and serum. Protein composition of Korean hamster sera and ascitic fluid is shown with Syrian hamster fluid components in Table I.

A further attempt was made to quantitate the relative titers of sera and ascites from individual hamsters by passive hemagglutination of formalized sheep cells sensitized with γG globulin, B_1C globulin and albumin. Table II depicts the serum and ascites titers to paired sera and ascites by passive hemagglutination.

Preparation of Tacaribe hyperimmune ascitic fluid. Ten young male hamsters, 30 to 45 days of age, were started and 10 completed the course. Immunization was performed by injecting 0.25 ml of the viral antigen emulsified with 0.25 ml of Freund's complete adjuvant intraperitoneally. An identical booster injection was given on day 14. On day 28 an injection of 0.25 normal saline emulsified in complete adjuvant was given. On day 36 an attempt was made to boost the titer utilizing 0.2 ml of the virus suspension IM, and on day 48 a 0.2 ml injection of adjuvant was given intraperitoneally to stimulate ascites production. Control animals received the same immunization schedule with normal saline replacing the virus suspension. 254 ml of ascitic fluid were produced by these young hamsters. An average of 42 ml from each of the producing hamsters and 25 ml each for the 10 hamsters in the test series. Note the great difference between ascitic fluid production in under 90-day-old hamsters and over 90-day-old hamsters used in the anti-serum protein procedure. Of the 6 producing hamsters in this series, 2 produced approximately 8 ml each and the other 4 produced 59 ml each. Three additional animals, 4 to 5 months old at the start of the immunization schedule, were immunized in the same manner with virus and produced ascites volumes comparable with the older animals immunized with human serum.

A fall of one or two 2-fold dilutions was observed when early fluid harvests were compared with later fluid harvests. This agrees with the findings of Wiebenga(1). The fluid was not anti-complementary, as was the case with whole serum immunized animals, and reacted at a level of $>1:1280$ with suckling mouse brain BOKCl Tacaribe antigen using 2 units of complement. No unusual cross-reaction with other arbovirus was noted with complement fixation tests when tested with SLE, WEE, EEE, JBE and Dengue 4.

Discussion. Investigators have considered the difficulties of obtaining adequate supplies of antibodies from small rodents, *i.e.,* mice and hamsters. Hamsters as a host animal for virus growth and production of antibodies have been employed by two investigators (1,2); both used adjuvant to stimulate production of ascites.

This study indicates that the age of the hamsters used has an effect on the production of ascites. It was found that animals older than 90 days produced significantly greater amounts of fluid when stimulated with antigen and complete Freund's adjuvant. Indeed, animals over 90 days of age in one experiment produced an average of 57 ml of ascites per animal, while in a similar experiment with animals under 90 days of age, 25 ml of fluid per animal was produced over a similar period. Ninety-two percent of the older animals produced ascites while only 50% of the younger animals produced ascites.

Hamsters have been used to produce antibodies to several arboviruses and a mixture of protein (human serum). Hyperimmune ascites to human serum were found to be extremely anti-complementary, while those to virus were

found to be free of anti-complementary components. Hamsters apparently make a potent anti-C′ when immunized with fresh human serum. Rivanol fractionation does not affect the anti-complementary character. The antibody obtained at 14 days was not 2-mercaptoethanol sensitive.

Serum and ascites from Syrian hamsters were found to contain at least 8 protein fractions similar to human serum proteins when developed with high titer horse, rabbit and goat antihuman sera. The two most important were found with albumin and γG globulin fraction. These possibly blocked production of antibody to albumin and γG globulin in the hamsters.

More "normal" antibody production was found in Korean hamsters immunized with the same antigens. The Korean hamster *Cricetulus triton* is quite unlike the Syrian hamster *Mesocricetus auratus* and more closely resembles the cotton rat or a large field mouse; however, it is classified as a hamster, and was included here for purposes of comparison. The Korean hamster produced antibodies to albumin, γG globulin and to several α and β globulins. It did not possess many of the cross-reacting antigens found in Syrian hamster sera and ascites.

In general antibody titers of the ascitic fluids were comparable to those of the sera; however, the ascitic fluid yield was 20-30 times that of serum.

Summary. Hamsters offer several likely aspects in their use as producers of antibodies. (1) They produce large volumes of ascitic fluid containing high titer antibody. (2) They provide a source of antibody free of anti γG globulin and, therefore, rich in non γ antibody specificity. (3) The hamster provides suitable system for preparation of homologous antibody to viruses which either require the hamster as a host or for which the hamster is used to isolate and/or maintian the virus. An interesting facet of this study was the finding of extensive cross-reactions between human and hamster plasma proteins.

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Differentiation of Pathological Conditions by Visual Evaluation of Serum Protein Electrophoretic Patterns. (32242)

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A clear electrophoretic pattern of serum proteins which can be inspected visually is a useful aid in the diagnosis of disease. Among the various electrophoretic methods used for fractionation of serum proteins, agar gel electrophoresis is distinguished for the clear