i.p. or s.c., but not by oral route, and the component polyribonucleotides are also inactive. The activity of the double-stranded complex in prolonging survival is not due to *in vitro* cytotoxic or cytostatic activity. It is proposed that the activity of poly I:poly C is due to the induction in the host on interferon which then slows the multiplication of the L-1210 cells *in vivo*.

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Host Specificity and Serologic Disparity of African Swine Fever Virus and Amphibian Polyhedral Cytoplasmic Viruses (33504)

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African swine fever virus (ASFV) and an amphibian-pathogenic virus, designated frog virus 3 (FV3), display remarkable similarity when examined by electron microscopy (1–4). Both viruses contain DNA, multiply in the cytoplasm, have a similar mode of egression from infected cells, and are inactivated by lipid solvents (1, 4, 5). The number of subunits that comprise the capsids of either virus, however, has not yet been ascertained.

The similarity of these agents prompted studies to determine if ASFV and the polyhedral cytoplasmic viruses observed in frogs (2), bullfrogs (6), and newts (7) have a similar host range or antigenic relationship. All of the isolates from amphibia appear to be serologically related (8, 9). Burton *et al.* (10) reported that an amphibian may serve as a host for a virus that is pathogenic for a mammal (Western equine encephalitis). Thus, we investigated the susceptibility to ASFV of an amphibian host known to be susceptible to the polyhedral cytoplasmic viruses isolated from amphibia (6, 9). No reservoir in nature, other than the swine, is known for ASFV. These studies also permitted serologic analysis of these morphologically similar agents. Knowledge relevant to animal-host reservoirs or serologic relationships would have epidemiologic significance and taxonomic interest as these two viruses, as well as the DNA containing, polyhedral, lymphocystis virus of fish and Tipula and Sericesthis iridescent viruses of insects do not fit into the present virus classification scheme.

Materials and Methods. Animals. Tamworth pigs, weighing approximately 60 lb were used throughout. Toads, *Bufo fowleri,* approximately 2.5 cm long, were from the shore of Lake Erie. The toads were maintained in tilted, stainless steel cages containing sand, 1–4 cm deep, and some tap water at

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[3] Toads were supplied by Dr. H. F. Clark, State University of New York, Buffalo, New York.

TABLE I. Host Specificity of African Swine Fever Virus (ASFV) and an Amphibian Polyhedral Cytoplasmic Virus (FV1).

Host	No.	Inoculum and dose	Observation period (days)	Results	Challenge results[a]
Toad	6	ASFV (3000 pig LD_{100})	8	Normal; killed at 8 DPI and homogenized as 20% suspension for pig inoculation	—
	7	ASFV (3000 pig LD_{100})	42	Normal; killed at 42 DPI	—
	12	PBS (0.05 ml dilution)	43	Normal; killed at 42 DPI	—
	8	FV1 (5×10^4 pfu)	42	7 died by 12 DPI	—
Pig	2	ASFV (3000 pig LD_{100})	13	Died; disease signs	—
	2	FV1 (7×10^7 pfu)	37	No signs	Died
	1	Control	37	No signs	Died
	2	Toad homogenate (20%)	37	No signs	Died
	1	Control	37	No signs	Died

[a] Pigs were given challenge inoculation with 3000 pig LD_{100} of ASFV. All died within 10 days.

one end. Temperature was 22–26°. The number of animals used, history, and responses are presented in Table I.

Viruses. African swine fever, $Lisbon_{57}$ isolate, cell-culture-adapted virus was used in the virus neutralization (VN) and hemadsorption inhibition tests in swine cell culture. Cytopathogenic effect (CPE) and hemadsorption (HAd) were observed in pig kidney (PK_{13}) cell line and leukocyte cultures, respectively.

Frog virus 1 (FV1), originally isolated from *Rana pipiens* kidney culture, was passaged 5 times in fathead minnow cells (11), and once in chick embryo fibroblasts before pig inoculation with 7×10^7 plaque forming units (pfu).

Frog virus 3 (FV3), which is antigenically indistinguishable from FV1 (8, 9, 12) was serially passaged in fathead minnow cells.

Cell cultures. Cultures of PK_{13} cell line were propagated in 4-oz prescription bottles for VN tests. The medium used consisted of Earle's salt solution with 0.5% lactalbumin hydrolysate, 0.1% yeast extract, 0.1% bovine albumin and 5% bovine serum. One hundred units/ml of penicillin and 100 μg/ml each of streptomycin and kanamycin were added.

Swine leukocyte cultures were made according to the method of Hess and DeTray (13). The cell suspension was distributed into Leighton tubes in 1-ml amounts and inoculated after approximately 72 hr incubation at 37°.

Antiserum. African swine fever antiserums were from pigs that survived ASFV infection. Tengani and Lisbon isolates were used in the complement fixation (CF) tests, and Dakar and Rome isolate antiserums were used in the VN tests. All 4 of these antiserums were used in agar gel diffusion precipitin (AGDP) tests. Serums from normal pigs were used as controls in all serologic tests. All serums were stored at —20° and heated to 56° for 30 min before use.

Rabbit FV3 antiserum has been described elsewhere (9). The antiserum reduced plaque formation of FV1 and FV3 to the same extent and produced lines of identity in the AGDP test.

African swine fever virus neutralization. Twofold dilutions of serum were tested with 10 and 100 tissue culture infective doses 50% ($TCID_{50}$) of virus. All serum–virus mixtures were maintained in a water bath for 1 hr at 37° before inoculation onto PK_{13} or swine leukocyte cultures. Prescription bottles containing confluent layers of PK_{13} cell and Leighton tubes containing leukocyte cultures were each inoculated with 0.2 ml of serum–virus mixtures. Pig $kidney_{13}$ cells were examined for CPE and leukocyte cultures for HAd. Inhibition of CPE and HAd were used rather than plaque inhibition because ASFV

TABLE II. Serologic Assays of African Swine Fever Virus (ASFV) and an Amphibian Polyhedral Cytoplasmic Virus (FV3).

Antiserum	FV3[a]	ASFV[a]
Rabbit anti-FV3	1) Precipitating antibody (8)	1) No precipitating antibody with undiluted serum
	2) 90% plaque reduction (25)	2) No inhibition of hemadsorption (<10)
		3) No inhibition of CPE (<10)
		4) No complement fixation (25)
Swine anti-ASFV	1) No precipitating antibody with undiluted serum	1) Precipitating antibody (32)
	2) No plaque reduction (2)	2) Inhibition of hemadsorption (>80)
		3) Positive complement fixation (>100)

[a] Number in parentheses indicates reciprocal of highest serum dilution at which reaction was observed.

does not produce plaques by present methods.

Complement fixation. The CF tests were described previously (14). Antigens for CF and AGDP tests were prepared from ASFV-infected PK_{13} cells as described by Hess *et al.* (15).

Agar gel diffusion precipitin. The method described by Malmquist (16) was used for AGDP tests, using agar gel (1.5%) in borate buffer (pH 8.4).

Frog virus 3 plaque reduction. Equal volumes of fourfold dilutions of FV3 antiserum or ASFV antiserum, beginning at 1:2, were added to FV3 diluted to contain approximately 2000–4000 pfu/ml; 2 hr at 37° was allowed for neutralization. Unneutralized virus was determined by plaque assay on fathead minnow monolayers as previously described (9).

Results. Serologic assays. The results of CF, VN, and AGDP tests are summarized in Table II. Antiserum to ASFV at various dilutions reacted with its homologous antigen in three tests: CF, VN, and AGDP. The use of undiluted and slightly diluted FV3 antiserum failed to react in any of these tests. When ASFV antiserum was used in AGDP and plaque neutralization tests with FV3 antigen, precipitation or neutralization could not be detected with undiluted serum whereas homologous antiserum had both precipitating and neutralizing activity at dilutions of 1:32 and 1:30, respectively.

Host specificity. Results of reciprocal tests to determine if ASFV is pathogenic for toads, or if FV3 is pathogenic for domestic swine are presented in Table I. No deaths occurred when 13 toads were given intraperitoneal injections of 3000 pig lethal doses 100% (LD_{100}) of virulent ASFV. Six of the 13 toads were killed 8 days postinoculation (DPI) and homogenized in an Omnimix blendor as a 20% suspension in phosphate buffer saline (PBS). The homogenate was inoculated by intramuscular route into 2 pigs. These pigs appeared clinically normal throughout 37 days' observation, suggesting that the toads excreted or destroyed the ASFV rapidly. The remaining 7 toads survived for 42 days when the experiment was terminated. Twelve toads given PBS by injection survived throughout the test.

To ascertain that the ASFV preparation was infectious, 2 pigs were inoculated intramuscularly at the same time as the toads. Both pigs died after either acute ASF disease signs or gross lesions were observed within 13 days. African swine fever virus was detected in blood samples taken at the peak of their temperature rise and in spleens collected at necropsy.

A preparation of FV1 containing approximately 5×10^4 pfu injected intraperitoneally killed 7 of 8 toads within 12 days. A 1000-fold greater dose of virus, 7×10^7 pfu, did not elicit any disease signs in 2 pigs observed for 37 days.

The susceptibility to ASFV of the 2 pigs previously inoculated with FV1 was also in-

vestigated to determine if the FV1 conferred resistance or immunity to ASFV challenge exposure. Each pig was given challenge inoculation intramuscularly with 3000 pig LD_{100} of ASFV. Pigs not previously given FV1 were also included. As indicated in Table I all of these pigs died of ASF between 7 and 10 DPI with signs and gross lesions. The brief period from inoculation to death (10 days or less) reflects lack of acquired resistance to ASFV by FV1 inoculation. Furthermore, serum samples taken from pigs given FV1 at 12, 24, and 37 DPI did not contain precipitating antibody to ASFV.

Discussion. Viruses having common characteristics of a regular hexagonal profile in thin section, DNA content, lipid solvent sensitivity and a cytoplasmic site of synthesis have been isolated from fish (17, 18), amphibia (6, 7, 19), and mammals (1, 5, 20). The wide distribution in nature of viruses with these properties and the finding by Burton *et al.* (10), that a virus pathogenic for a mammal (Western equine encephalitis) can be sustained in nature in an amphibian host (the frog) prompted this investigation to explore the possibility that ASFV might also have an amphibian host.

The recent availability of a susceptible host (*B. fowleri*) for the polyhedral cytoplasmic viruses of amphibia (6, 9) permitted investigation of reciprocal host range. No evidence of cross species pathogenicity could be detected. Furthermore, serologic analysis of the viruses also demonstrated dissimilarity.

Further studies with ASFV, FV3, or morphologically related viruses, however, may provide valuable epidemiologic and taxonomic knowledge. It is noteworthy that a 1:10 dilution of serum from toads surviving infection with FV1 and FV3 (Came, unpublished data) do not contain detectable neutralizing antibody to FV3 as evidenced by plaque reduction tests. This observation is similar to the findings by De Boer *et al.* (21), that serum from swine surviving ASF do not contain detectable neutralizing activity to that agent and may represent another similarity between ASFV and the polyhedral cytoplasmic viruses from amphibia.

Preliminary reciprocal neutralization studies by Midlige (18) and by Came (unpublished data), employing FV3 and lymphocystis virus antiserums also demonstrated no heterologous neutralization.

Summary. Serologic tests designed to demonstrate a relationship between polyhedral cytoplasmic viruses from amphibia and African swine fever virus indicated no cross-reactivity between the agents using techniques that revealed clean homologous activity. A 3000 pig LD_{100} of ASFV injected into toads susceptible to FV1 and FV3 did not cause death. Large doses of FV1 (greater than 1000 toad LD_{50}) neither produced disease signs nor stimulated the production of antibodies against ASFV in swine. The data indicate that cytoplasmic frog viruses and ASFV, which have many common properties, are not serologically related and do not share common host range.

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Mechanism of Excessive Postprandial Lipemia in the Diabetic Rat* (33505)

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The dog (1), rabbit (2), and rat (3) made diabetic by the administration of alloxan, exhibit a rise in concentration not only of plasma glucose but also of plasma triglyceride (PTG). The reasons for the rise of PTG were not completely ascertained, although it was discovered that the diabetic rat lacks lipoprotein lipase both in its blood (4), and in its adipose tissue (5), a defect remedied or markedly ameliorated by the administration of insulin (6). Recently Bierman *et al.* (7) reported that the alloxanized rat appeared to become hyperlipemic *only* if fed excess fat but these authors did not attempt to measure either the intestinal absorption, synthesis, and discharge of triglyceride (TG) or the rate of disappearance of intravenously administered TG from the plasma of their diabetic rats.

For the past several years we studied the disposition of exogenously derived TG in the normal rat (8–10), and we thought it of interest also to study the lipid dynamics of the diabetic rat. The results of these studies are described herein. They indicate that the diabetic rat exhibits a mildly elevated fasting PTG and a markedly elevated postprandial PTG, both of which appear due to the relative inability of this type of animal to rid its blood of fat.

Methods. A. Determination of pre- and postprandial PTG values of treated and untreated diabetic rats. Forty mature male rats, weighing an average of 290 g, (Long Evans strain) were given 40 mg of alloxan monohydrate/kg of weight by intravenous injection after being starved for 72 hr. One week later, 18 of the 35 surviving rats, after prior starvation for 15 hr, were given 3 ml of cottonseed oil by oral intubation. The remaining 17 rats received 0.5 units of regular insulin (Iletin, Lilly) by subcutaneous injection prior to the administration of the cottonseed oil. Twenty normal rats (controls) also were given the oil. Blood samples obtained before, and 6 hr after the oil feeding were analyzed for their PTG content according to the method of Van Handel and Zilversmit (11) and their glucose content according to the method of Marks (12) using uranyl acetate[1] precipitation.

B. Determination of intestinal absorption of TG in treated and untreated diabetic rats. Five rats made diabetic by the administration of alloxan 1 week previously, were starved for 15 hr and then given by oral intubation, 3 ml of cottonseed oil to which was added tripalmitin-^{14}C sufficient to supply 500,000 cpm/3 ml dose. The animals were bled before and 6 hr after the feeding. The initial sample was analyzed for its PTG and glucose content and the 6 hr sample was

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[1] The reagents were supplied as a "Biochemica-test" by C. F. Boehringer & Soehne.