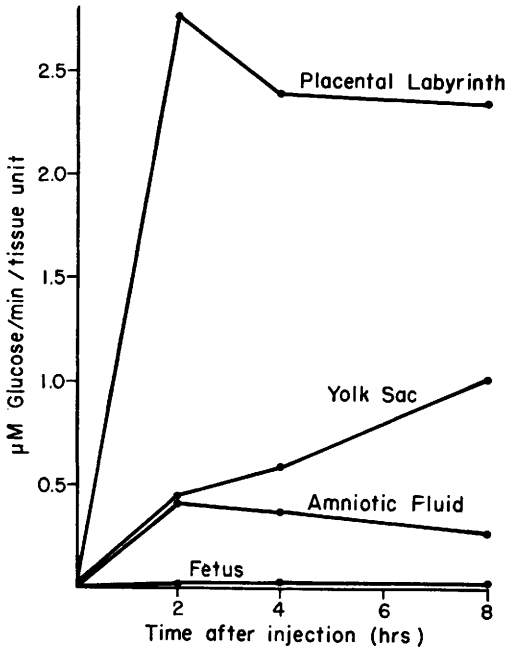

FIG. 1. Invertase activity in fetal and placental tissues at varying time intervals after injection on day 13 of pregnancy. Each point represents a mean value of tissues from 5 injected rats. Tissue unit is a single fetus or part of the placenta. 0 time represents control values.

multiple intravenous injections of the enzyme on days 14 and 15 of pregnancy. Single injections of the enzyme were given on day 13 of pregnancy and samples of conceptuses were taken over an 8-hour time interval. Enzyme analyses showed a low but consistent amount of invertase in the fetus, a gradual decrease in enzyme concentration in the amniotic fluid over the time period studied, a gradual increase in the yolk sac and a relatively stable concentration in the placental labyrinth. Invertase was present in the 12-day fetus 2 hours after injection.

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A Micro Method for Performing Parainfluenza Virus Neutralization Tests.* (31327)

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Tissue culture neutralization tests for the parainfluenza viruses have a variety of applications. These include identification of viral isolates, studies on antigenic relationships between viral strains, determination of the immunogenicity of viral vaccines and serologic diagnosis of human infections (although hemagglutination inhibition (HI) or complement fixation (CF) tests are usually employed for this purpose). Also, neutralization tests are useful in monitoring for antibodies to the parainfluenza viruses in sera of laboratory animals intended for experimental use, or for production of viral immune sera or complement.

Neutralization tests for this group of viruses are generally performed in tube cultures of monkey kidney cells; after inoculation of serum-virus mixtures, the cultures are incubated for an appropriate length of time and the presence of un-neutralized virus determined by testing for hemadsorption of

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guinea pig erythrocytes(1). This procedure requires large numbers of tube cultures and does not lend itself readily to large scale use. Thus, neutralization tests are usually reserved for identification of isolates and for studies on antigenic relationships between viral strains, while antibody assays are performed by HI or CF tests.

A microneutralization technic has been developed in this laboratory for use with human and animal parainfluenza viruses. The procedure is conducted in cups in disposable microplates, and it is less cumbersome and time-consuming to perform and read than conventional tests conducted in tube cell cultures. This report describes the technic and its use for antibody assay and virus identification.

Materials and methods. Virus strains. Parainfluenza virus strains employed for the microneutralization technic were the HA-2 strain of type 1 (hominis), the HVJ strain of type 1 (muris), the Greer strain of type 2 (hominis), the SV-5 strain of type 2 (simiae), the HA-1 strain of type 3 (hominis), the SF-4 strain of type 3 (bovis) and the M-25 strain of type 4.

Hemagglutination inhibition and complement fixation tests. These *in vitro* serologic tests for the parainfluenza viruses were performed in the microtiter system by our standard procedures(2).

Isolation of parainfluenza viruses. Viruses were recovered in primary or secondary rhesus monkey kidney cell cultures from throat washings of patients with respiratory illness. The isolation procedures are described in detail elsewhere(1,3).

Tube neutralization tests. Conventional tissue culture neutralization tests for the parainfluenza viruses were conducted in tube cultures of primary or secondary monkey kidney cells; un-neutralized virus was detected by hemadsorption of guinea pig erythrocytes(1).

Microneutralization tests. Tests were conducted using a rhesus monkey kidney cell system in disposable plastic microplates (Linbro model IS-MRC-96†); plates were soaked in 95% ethyl alcohol for 2 hours and sterilized by ultraviolet irradiation before use. Serum dilutions, virus dilutions and the cell suspension were all prepared in a medium consisting of 90% Leibovitz medium No. 15 (4) and 10% fetal bovine serum. Sera were inactivated at 56°C for 30 minutes and 2-fold dilutions were prepared in cups using 0.025 ml diluting loops. To each serum dilution was added 0.025 ml of a parainfluenza virus type diluted to contain 100 TCD_{50} of virus, as determined by a previous titration. Serum-virus mixtures were incubated for one hour at room temperature, and to each cup was added 0.025 ml of a suspension of monkey kidney cells (dispersed from primary cell cultures) containing 200,000 cells per ml. A titration of each test virus was performed in every run to establish that the test dose contained approximately 100 viral TCD_{50}. The plates were sealed with 3¼ inch Paklon tape‡ and set to incubate at 36°C for 6 days.

After the appropriate incubation period the tape seals were removed and the medium in each cup was carefully aspirated, using a blunt 23 gauge needle. In order to remove nonspecific inhibitors of hemagglutination which might be present in the test sera, each cup was carefully rinsed with 0.05 ml of physiological saline, which was then aspirated. To each cup was added 0.05 ml of a 0.5% suspension of guinea pig erythrocytes prepared in physiological saline. The plates were sealed with tape and the tests for parainfluenza virus types 1, 2 and 3 were incubated at 4°C for 30 minutes, while those for parainfluenza virus type 4 were incubated at room temperature for 30 minutes. (In virus identification tests, the mixtures were incubated first at 4°C and then at room temperature if the isolates were not identifiable as virus types 1, 2, or 3.)

The tape seals were checked to make certain that all cups were sealed; the plates were then inverted, permitting unadsorbed erythrocytes to flow from the cell sheet while erythrocytes adsorbed to virus-infected cells remained attached. Hemadsorption was de-

† Linbro Chemical Co., Inc., New Haven, Conn.

‡ Minnesota Mining & Manufacturing Co., St. Paul, Minn.

TABLE I. Homotypic Neutralizing Antibody Responses of Patients from Whom Parainfluenza Viruses Were Isolated.

Infecting virus type	No. of patients	Neutralizing antibody response to infecting virus (No. of patients)		
		≥4× titer rise	No titer rise, antibody present	Antibody absent (titer <1:8)
Para. 1	14	9	3	2
" 2	3	3		
" 3	5	4	1	
Totals	22	16	4	2

TABLE II. Comparison of Homotypic Neutralizing and HI Antibody Responses in Patients with Parainfluenza Virus Isolations.

Virus type isolated	Homotypic neutralizing antibody response	No. patients	Homotypic HI antibody response		
			≥4× titer rise	No titer rise, antibody present	Antibody absent (<1:8)
Para. 1	≥4× rise	9	9		
	No rise, Ab. present	3	2		1
	Ab. absent	2			2
" 2	≥4× rise	3	3		
	No rise, Ab. present	0			
	Ab. absent	0			
" 3	≥4× rise	4	3	1	
	No rise, Ab. present	1		1	
	Ab. absent	0			
	Totals	22	17	2	3

tected microscopically, with the plates inverted, using a standard light microscope. Viral neutralization was evidenced by inhibition of hemadsorption.

Results. Neutralizing antibody responses of patients infected with parainfluenza viruses. To evaluate the microneutralization test as a diagnostic procedure, acute- and convalescent-phase sera from patients yielding a parainfluenza virus were assayed for neutralizing antibodies to the 4 human parainfluenza virus types. Table I summarizes the homotypic neutralizing antibody responses of 22 patients from whom virus was recovered. Significant increases in homotypic neutralizing antibody were demonstrable for 16 of the 22 patients, 4 showed stationary antibody levels for their infecting virus type, and 2 patients (both with type 1 virus isolations) failed to show homotypic neutralizing antibody (titer <1:8).

Table II compares the homotypic neutralizing and HI antibody responses of the 22 patients, and it is seen that, for this small group of individuals, the two tests were comparable in diagnostic value. Sixteen of the patients showed significant rises in homotypic neutralizing antibody, and 17 showed significant increases in homotypic HI antibody. One patient from whom parainfluenza type 1 virus was recovered showed a stationary titer of neutralizing antibody but failed to show homotypic HI antibody. The 2 patients who failed to show homotypic neutralizing antibody for parainfluenza type 1 virus also had no demonstrable HI antibody. Significant antibody increases were demonstrated by both tests with sera from patients infected with parainfluenza type 2 or 3 viruses, with the exception of one patient with a type 3 virus infection, who showed a 4-fold increase in neutralizing antibody but a stationary HI antibody titer.

The heterotypic antibody responses of the patients with virus isolations are summarized in Table III, which compares neutralizing antibody responses to HI antibody responses for each parainfluenza virus heterotype. (CF rather than HI tests were employed for para-

TABLE III. Comparison of Heterotypic Antibody Responses Detected by Neutralization and by HI Tests (CF Tests for Parainfluenza Type 4).

Infecting virus type	Heterotypic test virus type	Neutralizing antibody response	No.	HI (or CF) antibody response to indicated viral heterotype		
				Ab.—*	Ab.+*	≧4× rise
1 (14 patients)	2	Ab.—	14	14	0	0
		Ab.+	0	0	0	0
		≧4× rise	0	0	0	0
	3	Ab.—	10	8	0	2
		Ab.+	2	0	2	0
		≧4× rise	2	0	1	1
	4	Ab.—	9	9	0	0
		Ab.+	4	2	2	0
		≧4× rise	1	0	1	0
2 (3 patients)	1	Ab.—	2	2	0	0
		Ab.+	1	0	1	0
		≧4× rise	0	0	0	0
	3	Ab.—	1	1	0	0
		Ab.+	1	0	1	0
		≧4× rise	1	0	0	1
	4	Ab.—	3	3	0	0
		Ab.+	0	0	0	0
		≧4× rise	0	0	0	0
3 (5 patients)	1	Ab.—	3	3	0	0
		Ab.+	1	1	0	0
		≧4× rise	1	0	1	0
	2	Ab.—	5	5	0	0
		Ab.+	0	0	0	0
		≧4× rise	0	0	0	0
	4	Ab.—	4	4	0	0
		Ab.+	1	1	0	0
		≧4× rise	0	0	0	0

* Ab.— = antibody absent (<1:8); Ab.+ = antibody present but no significant titer rise.

influenza type 4.) In general, there was good agreement between heterotypic antibody responses seen in neutralization and HI (or CF) tests. In a few instances neutralizing antibody, but not CF antibody, was demonstrable for parainfluenza type 4; this probably reflects a longer persistence of neutralizing antibody.

Microneutralization tests with animal strains of parainfluenza viruses. The Sendai (murine) strain of parainfluenza virus type 1, the SV-5 (simian) strain of parainfluenza virus type 2 and the SF-4 (bovine) strain of parainfluenza virus type 3 were employed in microneutralization tests with sera from a variety of species. Results of these tests are summarized in Table IV.

Of 17 mouse sera examined for the presence of neutralizing antibody for Sendai virus, 3 were positive and these 3 also contained HI antibodies to the viral strain. Paired sera from 16 humans with parainfluenza virus isolations (11 with type 1 isolations, 3 with type 2 isolations and 2 with type 3 isolations) were tested against the Sendai virus, and paired sera of 3 of the individuals infected with parainfluenza type 1 virus showed significant increases in neutralizing antibody to the Sendai strain. None of the other human sera examined showed antibody.

TABLE IV. Results of Microneutralization Tests with Animal Strains of Parainfluenza Viruses.

Test virus	Serum species	No. sera examined	No. sera showing neutralizing antibody
Sendai	Mouse	17	3
	Human	16 (pairs)	3
SV-5	Monkey	12	3
	Guinea pig	12	11
	Rabbit	12	0
	Hamster	10	0
	Human	16 (pairs)	2
SF-4	Bovine	8	7
	Human	12 (pairs)	4

In tests with the SV-5 strain of parainfluenza type 2 virus it is seen that 3 of the 12 monkeys and 11 of the 12 guinea pigs examined showed antibody. None of the rabbit or hamster sera tested showed neutralizing antibody to this parainfluenza virus strain. Of the 16 paired human sera examined (10 from type 1 infections, 3 from type 2 infections and 3 from type 3 infections), 2 showed neutralizing antibody to the virus, with one pair showing a significant increase in titer. Both of these patients were infected with parainfluenza type 2 virus.

Tests with the SF-4 strain of parainfluenza type 3 virus were conducted on 8 bovine sera, and neutralizing antibody was detected in 7. Of the 12 human serum pairs tested from patients with parainfluenza virus isolations (5 from type 1 infections, 2 from type 2 infections and 5 from type 3 infections), 4 showed antibody, 2 of them significant rises. All of these patients showing antibody to the bovine strain were infected with the human strain of parainfluenza virus type 3.

Identification of parainfluenza virus isolates by the microneutralization test. The possibility has been explored of using the microneutralization technic for identification of viral isolates. Isolates representing first or second monkey kidney passage material were tested at dilutions of $1 \times 10^{0.5}$ and $1 \times 10^{-1.0}$ against 1:16 and 1:32 dilutions of parainfluenza virus immune sera with homologous titers of 1:256 or greater. Each isolate was also tested against normal rabbit serum. In Table V it is seen that slightly over one-half of the isolates tested could be identified by this method. The major drawback was the fact that at the low passage levels tested, isolates frequently did not have high enough titers to produce clear-cut hemadsorption in the micro test; this was particularly true of parainfluenza type 4 virus strains.

Discussion. These studies have demonstrated the feasibility of performing neutralization tests for the human and animal parainfluenza viruses by a micro method. The test is less cumbersome to perform and read than conventional tube neutralization tests, and is also far more economical in terms of cell cultures and reagents. It is likely that the technic would prove applicable to use with influenza viruses and other myxoviruses in addition to the parainfluenza group.

TABLE V. Identification of Parainfluenza Virus Isolates by the Microneutralization Technic.

Parainfluenza virus type*	No. tested	Results in microneutralization system		
		Positive†	Negative	No hemadsorption
1	4	2	0	2
2	6	3	0	3
3	5	5	0	0
4	5	1	0	4
SV-5	1	1	0	0
Total	21	12	0	9

* Based upon conventional tube neutralization tests.

† Identified as same type indicated in conventional neutralization test.

Although neutralization tests are rarely required for routine serologic diagnosis of parainfluenza virus infections in man, they are useful for studying immunologic relationships between virus strains, and they also have application in vaccine immunogenicity studies.

In monitoring laboratory animal colonies for parainfluenza virus infections, neutralization tests may be used for confirming any equivocal results obtained in HI tests. The sensitivity of the technic also makes it highly useful for detecting pre-existing antibodies in the sera of animals intended for use in the production of complement or viral immune sera.

The use of the microneutralization test for identification of parainfluenza virus isolates was limited by the fact that the isolates, representing first or second passage material, frequently were so low in titer that hemadsorption was not demonstrable in the system. However, in instances in which the isolates had sufficiently high titers, the results obtained in the microneutralization tests were in complete agreement with those obtained in conventional neutralization tests. The micro technic might prove to be a useful method for screening viral isolates, leaving only those of low titer to be identified in tube tests.

Summary. A micro technic has been devised for performing neutralization tests with the human and animal strains of parainfluenza viruses. Tests are conducted in a rhesus

monkey kidney cell system in disposable microplates; virus is detected by the hemadsorption technic and results are read with a standard light microscope. The microneutralization technic was shown to be a highly reliable method for demonstrating significant neutralizing antibody titer rises with sera from patients from whom parainfluenza viruses were isolated and also for detecting antibody to the animal strains in the sera of laboratory animals. The test may also be used for identification of viral isolates, but this application is limited by the fact that certain isolates do not possess sufficiently high titers to produce clear-cut hemadsorption in the micro system.

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Effect of Superior Vena Cava Constriction and Obstruction of Thoracic Duct Lymph Flow on the Liver of the Dog.* (31328)

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"Cardiac cirrhosis" of the liver follows long-standing venous congestion(1,2); part of this extensive hepatic fibrosis may be due to an associated lymph stasis. There is evidence that lymph stasis is present when there is hepatic venous stasis. Dumont *et al*(3) have shown that "venting" the thoracic duct in patients with intractable congestive heart failure results in striking clinical improvement. Ascites, produced by constriction of the superior vena cava, is similarly relieved in dogs when the thoracic duct is shunted to the esophagus(4). These studies have clearly demonstrated that hepatic venous congestion is associated with lymph stasis due to inability of the thoracic duct to handle the added lymph flow. It is known that chronic impairment of lymph flow predisposes to fibrosis in the affected organ(5,6). Thoracic duct lymph pressure is elevated in experimental constrictive pericarditis(7), and it is in this entity, which is undoubtedly associated with marked hepatic lymph stasis as well as venous blood stasis, that "cardiac cirrhosis" is the most severe.

The present studies were initiated to determine whether chronic constriction of the superior vena cava combined with resection of a segment of the thoracic duct can lead to discernible alteration in liver histology in the dog. There is good evidence that elevation of central venous pressure interferes with drainage of thoracic duct lymph(8).

Material and methods. Mongrel dogs were anesthetized with intravenous sodium pentobarbital (25 mg/kg). Using sterile precautions, the chest was opened through a left lateral incision in the third or fourth interspace. Artificial respiration was maintained *via* tracheal intubation with a mixture of 95% oxygen and 5% carbon dioxide. Prior to opening the chest, a wedge liver biopsy

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