lent beat without fibrillation or other signs of cardiac arrhythmia.

To date the technic of total cardiopulmonary by-pass with retroperfusion of the coronary sinus has been applied to 8 patients(4). The first case was done over a year ago(3) and the patient is well at this time. The clinical lesions for which we have used retroperfusion were instances of aortic stenosis, and regurgitation, aortic-pulmonary window, ruptured sinus of Valsalva, and complete transposition of the great vessels. The longest clinical retroperfusion was 32 minutes, and in every patient a strong beat was maintained throughout the procedure with no instance of fibrillation during or after the retroperfusion. These clinical observations substantiate the anatomical fact that the human heart is more completely perfused than the dog's by a retrograde flow through the coronary sinus. On the basis of these experimental studies and the clinical observations mentioned above, the combination of retroperfusion and potassium asystole appears valuable for precise, direct vision reparative procedures upon the aortic valve or coronary arteries in a quiescent operative field.

Conclusions. 1. Only one of 10 dogs submitted to 15 to 30 minutes of potassium citrate produced asystole was a long-term survival (90% mortality). 2. Ten dogs were submitted to 30 minutes of retroperfusion of the coronary sinus, and 4 died from cardiac causes, a 40% mortality. 3. Ten additional dogs were subjected to combination of 30 minutes retroperfusion and potassium citrate provoked asystole. Two of the 10 died from cardiac complications, mortality 20%. 4. Certain aspects of cardiac metabolism in the retroperfused heart with and without potassium citrate induced asystole have been studied in 10 dogs. There appears to be an additionally reduced degree of metabolism under these latter circumstances.

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Failure to Transmit Human Nonbacterial Gastroenteritis to Cats.* (23054)

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Existence of acute infectious nonbacterial gastroenteritis of presumed virus etiology has been documented by epidemiologic observations(1-3) and by transmission of the disease to human volunteers(1,4,5) although no etiologic agent has been established in tissue culture or laboratory animals. Studies in collaboration with Gordon and Dorrance(4) demonstrated, by feeding stool supernates to volunteers, that there are at least 2 different types of such gastroenteritis. Because Yamamoto *et al.*(6) had reported transmission of human diarrheal disease to cats by feeding 2 to 5 ml of filtered supernatant of aqueous stool, aliquots of the same inocula used in human experiments with Gordon and Dorrance(4) were given to cats. The present re-

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port was prompted by recent editorial reference(7) to these cat experiments of Japanese workers, and records failure of inocula infectious for man to induce disease in cats.

Materials and methods. Two inocula were used. *Marcy inoculum* was obtained from an induced case (T.H.) of afebrile gastroenteritis on 7/3/50 and processed(1) by Gordon on 8/27/51. The watery stools from which the inoculum was prepared were collected after 6th human passage of the Marcy agent. Seven ml of inoculum induced disease in 14 of 16 volunteers in experiments done between December 1951 and June 1952(4). *FS inoculum* was processed from the stool of volunteer (Case E.E.) with induced febrile gastroenteritis, representing the first human passage(4). Two to 10 ml of the inoculum caused disease in 5 of 15 volunteers(4). Both inocula were stored at −70°C during 11 months between human and cat experiments. *Tryptose phosphate broth* was used as control inoculum. *Animals.* Cats were collected from semi-rural areas and housed in separate cages removed from general animal quarters. They were held for 8 or more days prior to inoculation. These measures were taken to avoid infectious feline enteritis or panleucopenia(8,9). On receipt, the cats' nails were clipped, each was weighed and bled 10 ml by intracardiac puncture, and total leucocyte count was done. Subsequently, for 6 to 12 days prior to inoculation, daily rectal temperatures, number and character of stools passed and gross food intake were recorded. Stools were counted and characterized as formed, soft, or loose by changing the drop pans each morning and afternoon. Under intraperitoneal nembutal anesthesia, 5 ml of appropriate inoculum was introduced by stomach tube. Observations of weight, appetite, stools, leucocyte count (weekly), and rectal temperature (now recorded twice daily) were continued.

Results. Exp. 1. On 12/2/52, 5 cats (1 male and 4 females; Group A, nos. 1, 3, 5, 7, 9) were given tryptose broth, and 5 cats (2 males and 3 females, Group B, nos. 2, 4, 6, 8, 10) were given FS inoculum. Observations made before and after administration of these

TABLE I. Intragastric Administration to Cats of Stool Supernates from Humans with Nonbacterial Gastroenteritis.

Animal		Before inoculation					After first† inoculum					After second† inoculum				
		Stools*					Stools*					Stools*				
Group	No.	F	S	L	Wt, kg	WBC × 1000	F	S	L	Wt, kg	WBC × 1000	F	S	L	Wt, kg	WBC × 1000
A	1	11/12	2/12	19/12	2.6	14.2	4/6	1/6	9/6	2.6	8.6	5/6	5/6	0/6	2.8	nd
	3	14/12	10/12	8/12	2.7	14.4	2/6	5/6	4/6	3.1	10.9	3/6	4/6	5/6	3.0	12.0
	5	1/11	11/11	8/11	3.3	17.2	3/6	2/6	6/6	3.6	15.6	2/6	5/6	1/6	3.5	12.5
	7	9/12	6/12	10/12	2.6	23.3	2/6	6/6	4/6	2.8	18.0	4/6	5/6	0/6	2.8	22.7
	9	4/ 6	0/ 6	0/ 6	2.1	17.6	5/6	1/6	0/6	2.2	15.5	5/6	1/6	0/6	1.9	14.8
B	2	9/12	8/12	14/12	2.2	17.0	3/6	5/6	9/6	2.2	10.2	4/6	2/6	4/6	2.4	8.6
	4	11/12	5/12	11/12	2.4	14.8	3/6	3/6	8/6	2.5	17.3	0/6	11/6	6/6	2.6	17.7
	6	9/11	3/11	9/11	3.6	19.6	2/6	2/6	8/6	3.9	22.7	1/6	6/6	3/6	3.8	21.3
	8	5/ 6	0/ 6	0/ 6	2.6	22.0	4/6	2/6	2/6	2.8	13.2	5/6	5/6	0/6	2.5	nd
	10	2/ 6	3/ 6	4/ 6	2.5	19.1	1/6	4/6	4/6	2.0	17.7					

* Numerator = No. of stools. Denominator = No. of days observed. F = Formed; S = Soft; L = Loose.
† Inocula: A: First = broth; second = Marcy. B: First = FS; second = broth.

materials are summarized in Table I. No febrile responses were apparent in either group. During control period, it was noted that animal No. 10 ate poorly and exhibited weight loss of 0.4 kg in 7 days. FS inoculum provoked no change in number and character of stools, but because of continued anorexia and weight loss, this cat was not given a second inoculum.

Exp. 2. Since no apparent disease was produced, on 12/16/52, 2 weeks later, the cats were again bled and intubated. Group A was given Marcy inoculum; group B was given broth. Animal no. 7 showed an increase in temperature from baseline of about 102°F to 105°F 3 days after inoculation. None of the other 4 cats given Marcy supernate showed a febrile response. None developed diarrhea (Table I). With the exception previously noted, all animals maintained or gained weight. Leucopenia did not occur. Thus, there were no signs suggestive of infectious gastroenteritis of cats(8.9).

Discussion. Since simultaneous human volunteer experiments were not done, the question may be raised as to whether the material used was still infective for man at the time of inoculation into cats. Aside from difficulty of execution, such simultaneous human experiments were considered to be unnecessary because available data indicated that the agents survived storage at −70°C. Prior to the experiments mentioned(4), the fecal source of Marcy agent had been stored for 18 months; the processed inoculum had been stored for 6 months. Further, with aliquots of this same inoculum Gordon, Patterson, and Whitney(10) later induced disease in human volunteers on 4 occasions 3 to 5 months after the cat experiments. These investigators(10) achieved a 66% attach rate in man with a dose of 3.5 ml, 1.5 ml less than the amount given to the cats. As regards the FS agent, the original inoculum had induced disease in man after storage for 4 months. The material given the cats (first passage inoculum) had induced disease in man after 8 months storage. These data justify the assumption that the FS agent was still viable at the time of the cat experiments.

Yamamoto *et al.*(6) provoked diarrhea in all 6 cats given human stool supernate. The cats became ill 9 to 13 days after inoculation, and 5 died 1 to 8 days after onset. Filtered aqueous cat excreta were then used to induce diarrhea and death on 2 subsequent passages in cats. It is of interest that an uninoculated cat, living with those first inoculated, developed diarrhea and died. Excreta from this animal were used to initiate a second passage series. Filtered, twice diluted cat stool supernate from each series was fed in 3 ml and 5 ml amounts, respectively, to 2 human volunteers. This material, which in amounts of 1.5 ml and 1.7 ml produced a fatal disease in cats, caused no reaction in human subjects. The investigators noted that several cats had died in area of epidemic of human gastroenteritis under study, and acknowledged the possibility of "other incidental disease." However, they favored the idea that the same disease "prevailed among cats while it spread among men"(6).

The clinical description of the illness supposedly induced in cats by Yamamoto *et al.* (6) closely resembles that of infectious gastroenteritis of cats (feline distemper; feline panleucopenia). This disease has an incubation period of 6 to 14 days, and is characterized by depression, weakness, vomiting, profuse diarrhea, fever, and coma in 3 to 4 days (8.9). It is possible, therefore, that the Japanese investigators were dealing with a naturally occurring feline disease.

In the experiments here recorded 2 human gastroenteritis inocula of demonstrated infectiousness failed to produce disease in healthy cats.

Summary. Aliquots of supernates prepared from stools of volunteers with induced afebrile (Marcy) and febrile (FS) nonbacterial gastroenteritis and capable of inducing human disease were administered by stomach tube to cats. No recognizable disease was produced.

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Effect of Ultrasonics on Thromboplastinase-Labile Component and Toxicity of Injected Thromboplastin. (23055)

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The effects of heat on the thromboplastinase-labile component of tissue thromboplastins have been previously reported(1). Loss of thromboplastic potency due to heating of tissue suspensions and loss due to thromboplastinase are apparently distinct phenomena. Further, heat inactivated thromboplastin retained all of its *in vivo* toxicity for rabbits while thromboplastinase inactivated suspensions were non-toxic(2). Axelrod (3) recently reported on loss of potency of commercial thromboplastin following ultrasonic treatment. It was of interest to determine what effect ultrasonics would have both on the TPase-labile component of human brain thromboplastin and on the lethal effect upon intravenous injection of such thromboplastic suspensions into experimental animals.

Materials and methods. Thromboplastinase (TPase) was prepared as previously described(4). 2 mg crude TPase powder/ml water was employed as standard enzyme concentration. Heat inactivated enzyme (100° for 10 min) served as a control. *Thromboplastin (TP).* Thromboplastic suspensions were freshly prepared 1 hr before use by extracting 0.5 g acetone dehydrated human brain powder with 10 ml 0.9% saline at 50°C for 15 min and then centrifuging at 3000 rpm for 5 min. The supernatant was decanted and refrigerated prior to use. *Clotting time determinations (CT).* A modification(4) of the Quick one-stage test was used for assay of clotting potency. Duplicate determinations were made. *Thromboplastin dilution curves* were constructed as previously described(4). *Ultrasonic treatment.* Raytheon Model DF-101 200 watt sonic oscillator was used with a frequency of 10 kc. 50 ml samples of thromboplastic suspensions were treated at one time and aliquots were removed at intervals for assay of potency, preparation of dilution curves, treatment with TPase and injection into animals. *TPase assay.* The activity of TPase was followed by change in clotting potency and liberation of organic phosphorus after incubation at 37°C for 60 min(1). 10 ml of thromboplastic suspension and 1.0 ml TPase solution (2 mg) was used as a standard incubation mixture. *Experimental animals* employed were male albino rabbits weighing 2-2½ kg. Preparation of suspensions for intravenous injection and technics used have been described(2).

Results. The loss in clotting potency of human brain thromboplastic suspensions due to ultrasonic treatment is shown in Fig. 1. It can be seen that there was a progressive loss in potency over a time course. When dilution curves were plotted for aliquots removed at intervals (Fig. 2), it was evident that most of the activity had been destroyed quite rapidly. After 30 min of ultrasonic treatment clotting time was 19 sec. On the