trogen without any stabilizer, coupled with sonication, seems to produce relatively stable storage conditions so that one can anticipate the quantity of virus employed for prospective testing. Previously, one had to interpolate a decline in infectivity secondary to inactivation of —70° in the presence of stabilizers such as sorbitol.

Cytomegalovirus was isolated from three fresh urine specimens (Table II). All three specimens yielded virus from aliquots frozen for periods up to 4.5 months. The interval between inoculation and observance of cytopathic effect in both the fresh and frozen samples was similar, indicating that quantities of virus in the urines remained relatively constant over the interval tested.

Summary. The relative stability of cytomegalovirus stored in liquid nitrogen for a period of 3 years is reported.

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Studies on Experimental Bacteremia and Pyelonephritis in the Marmoset (*Callithrix jacchus*)* (33356)

HEONIR ROCHA, EDSON DA SILVA TELES, AND EDILSON BRITO
(Introduced by D. Kaye)

Department of Medicine and Department of Pathology, Faculty of Medicine, University of Bahia, Salvador, Bahia, Brazil

There have been few reports concerned with the use of marmosets in experimental infections (1–4). These animals have been studied in relation to maintenance and care in the laboratory (5, 6), for use in the isolation of viruses (7), and for naturally occurring parasitic infections (8). Aspects of normal pathology (9), as well as base line microbiologic studies (1) also have been documented.

The present study was undertaken to determine the tissue distribution and fate of *Escherichia coli* after infection by the intravenous route or inoculation into the urinary bladder, and to evaluate the susceptibility of these animals to urinary tract infection.

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Materials and Methods. Animals. Normal marmosets (*Callithrix jacchus*) of both sexes were used in this study. Animals were housed in groups of two or three and fed water and a diet consisting of bread and fresh fruits (banana, papaya, pineapple) *ad libitum*. A total of 50 animals were used. Forty were inoculated with bacteria, 20 in the femoral vein and 20 into the lumen of the urinary bladder. Ten served as normal controls.

Bacteria. A strain of *E. coli* and a strain of *Proteus mirabilis* recovered from urine specimens of patients with urinary tract infection were employed. Stock cultures were maintained by storing aliquots of an 18-hr culture in trypticase soy broth (Biological Baltimore Laboratories) at —20°.

Inocula were prepared by subculturing an aliquot of the stock culture in trypticase soy broth at 37° for 4 hr. One-half ml of a 1:10 dilution of the culture in saline solution was

TABLE I. Distribution of *E. coli* in Tissues of *C. jacchus* after Intravenous Inoculation of 10^7 Organisms.

Time after injection	*E. coli* (no./g of tissue or ml)					
	Blood	Spleen	Liver	Lung	Kidney	Urine (bladder)
30 min	2500	100,000	149,000	1000	5000	0
	1600	56,000	54,000	1000	510	0
60 min	1420	36,000	48,000	1000	1000	0
	280	3000	199,000	1040	940	0
4 hr	96,000	370,000	500,000	9000	23,000	0
	9000	350,000	490,000	20,000	17,000	0
12 hr	5000	470	400	110	210	0
	70	2000	1000	1100	20	0
	21,000	13,000	18,000	16,000	120	0
1 day	0	0	2100	100	0	0
	0	1700	560	29,000	0	0
	1000	67,000	39,000	18,000	40	0
2 days	0	0	120	100	0	0
	0	50	430	0	0	0
4 days	0	0	280	0	0	0
	0	0	0	0	0	0
5 days	0	0	0	0	0	0
	0	0	0	0	0	0
6 days	0	0	0	0	0	0
	0	0	0	0	0	0

then injected into the femoral vein or into the lumen of the bladder. To inject into the femoral vein, animals were lightly anesthetized with ether and the vein was exposed at the femoral triangle. The injections were made through 25-gauge needles. For bladder injections, a small suprapubic midline incision was made and a 25-gauge needle was inserted through the wall of the urinary bladder. After inoculation of bacteria, the incision was closed with 000 silk.

Bacterial enumeration. Animals were sacrificed at intervals after inoculation and 1 ml of blood was taken from the exposed heart. Urine was collected from the exposed bladder, the whole right kidney, half of the left kidney, the spleen, a fragment of liver, and the lungs were weighed separately and homogenized in 1:10 dilutions in sterile distilled water. Urine, blood, and the tissue homogenates were serially diluted in saline solution and 1 ml of each dilution was plated in trypticase soy agar pour plates and incubated at 37° for 48 hr to permit enumeration of the organisms present. The number of bacteria inoculated was also determined by the same technique.

The urinary bladder and half of the left kidney were fixed in 10% formalin and then sectioned and stained with hematoxylin and eosin.

Criteria for diagnosis of pyelonephritis. The criteria for diagnosis of pyelonephritis were the presence of more than 100,000 organisms/g of kidney tissue and the presence of abcesses on histologic examination. All animals with more than 100,000 bacteria/g of kidney had renal abcesses on histologic examination.

Results. Distribution of E. coli *in tissues of normal animals.* Kidneys and urine specimens were sterile in the 10 control animals which were not inoculated with bacteria. As shown in Table I, by 30 min after in-

TABLE II. Ascending Pyelonephritis in *C. jacchus* after Inoculation of 10^7 *E. coli* into the Bladder.

Animals	Time after injection (days)	Bacteria (no./g or ml)			
		Rt. kidney	Lt. kidney	Urine	Blood
1	1–3	100	5000	>1,000,000	0
2	1–3	0	0	89,000	0
3	1–3	1000	0	24,000	0
4	7	3000	42,000	>1,000,000	0
5	7	>1,000,000	0	>1,000,000	0
6	7	180,000	50	76,000	0
7	7	0	0	120,000	0
8	7	1000	0	290,000	0
9	7	>1,000,000	220,000	>1,000,000	0
10	7	>1,000,000	>1,000,000	>1,000,000	0

travenous inoculation of 10^7 *E. coli* in 20 marmosets, there were relatively few viable organisms remaining in the blood as compared to the large numbers isolated from the liver and spleen. Also, relatively few bacteria were detected in lungs and kidneys. After 24 hr there was a progressive decline in the number of viable *E. coli* in all tissues with complete disappearance of bacteria after 4 days. At no time were *E. coli* isolated from urine. Histological studies of the kidneys of control animals, as well as those challenged by the intravenous inoculation of *E. coli*, revealed only occasional small foci of round cell infiltration in the cortical zone.

Pyelonephritis following inoculation of bacteria into the lumen of the bladder. Four of 10 animals exhibited pyelonephritis within 7 days after injection of 10^7 *E. coli* into the bladder (Table II). Two out of 10 animals were also infected after a comparable dose of *P. mirabilis* (Table III). It is of interest that all animals had bacteria in the urine at time of sacrifice (Tables II and III). No organisms were recovered from the blood of animals inoculated in the urinary bladder.

Discussion. These studies demonstrate that *C. jacchus* is resistant to infection resulting from intravenous inoculation of large numbers of the *E. coli* strain used. The distribution and apparent clearance of the organisms from blood and organs was similar to results reported in rats (10) and rabbits (11).

No instance of spontaneous urinary tract infection was detected in normal animals. Also, after intravenous inoculation of a large number (10^7 organisms) of *E. coli*, no bacteria could be isolated from the urine. It is of

TABLE III. Ascending Pyelonephritis in *C. jacchus* after Inoculation of 10^7 *P. mirabilis* into the Bladder.

Animals	Time after injection (days)	Bacteria (no./g or ml)			
		Rt. kidney	Lt. kidney	Urine	Blood
1	1–3	0	0	19,000	0
2	1–3	0	0	6000	0
3	1–3	0	0	320,000	0
4	7	>1,000,000	>1,000,000	>1,000,000	0
5	7	0	0	>1,000,000	0
6	7	230	0	>1,000,000	0
7	7	67,000	11,000	180,000	0
8	7	>1,000,000	400,000	>1,000,000	0
9	7	0	100	>1,000,000	0
10	7	59,000	0	350,000	0

interest to note that Deinhardt *et al.* (9) studied 93 marmosets (*Sanguinis fusciollis* and *Sanguinis nigricollis*) which died in the laboratory or were sacrificed for various experimental purposes, and described pyelonephritis in three and acute cystitis in one. In fact, pyelonephritis was the main cause of death in one instance. However, they did not culture the urine and/or kidneys of animals considered to have pyelonephritis and cystitis, making it difficult to evaluate their diagnosis of bacterial infection of the urinary tract.

Small foci of round cell infiltration were occasionally observed in the present study even in the kidneys of normal control animals. Deinhardt *et al.* (9) frequently found "chronic focal infiltrations" in the kidneys, and considered them to be associated with microfilarial infection.

It is of interest that the great majority of animals inoculated with *E. coli* or *P. mirabilis* showed significant bacteriuria several days after bladder inoculation. Pyelonephritis developed in 6 of the 20 animals, indicating that marmosets are susceptible to ascending urinary tract infection. This relatively high rate of retrograde infection suggests the existence of vesicoureteral reflux.

Summary. Experiments were undertaken in marmosets *(Callithrix jacchus)* to determine the tissue distribution of *E. coli* after intravenous inoculation and to investigate the susceptibility of the urinary tract of these animals to hematogenous or ascending infection. After intravenous inoculation of 10^7 *E. coli*, bacteria were rapidly cleared from the blood. High titers of bacteria were observed initially in liver and spleen but there was a rapid and progressive decline in number of organisms to complete sterility after 4 days. Pyelonephritis or bacteriuria did not result from hematogenous challenge. However, after inoculation of similar numbers of either *E. coli* or *P. mirabilis* into the lumen of the bladder, the majority of animals developed bacteriuria and 6 of 20 animals acquired pyelonephritis.

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Thyroiditis in Rats Injected Subcutaneously with 3-Methylcholanthrene (33357)

MELVIN D. REUBER AND E. LEE GLOVER (Introduced by W. E. Heston)

Laboratory of Biology, National Cancer Institute, Bethesda, Maryland 20014; and Department of Zoology, University of Maryland, College Park, Maryland 20740

Thyroiditis has been produced in the rat by immunization with homologous or heterologous thyroid extract or with thyroglobulin in Freund's adjuvant (1). The lesion has been described in rats with carbon tetrachloride-induced cirrhosis of the liver (2) and in rats ingesting 3-methylcholanthrene (MCA) in the diet (3). The purpose of the present paper is to describe thyroiditis in rats given a single s.c. injection of MCA.