succumbed to tetany in less than 24 hours, whereas at lower levels withdrawal of PTH did not generally result in fatal attacks of tetany by this time(3). Another group of animals was injected until day 14 with PTH. Young were separated from mothers for 10 hours and milk yield was determined. Survival time of mothers was considerably prolonged and milk yield on day 15 obtained by same technic was significantly higher in animals not yet affected by tetany. Increased survival time may be explained by gradual inhibition of lactation and corresponding reduction in blood Ca removal during 10 hour separation period, whereas in previous group, young nursed continuously. More difficult to explain is the mean increase in milk yield on day 15 as compared to day 14, 29 hours after last injection of PTH. The increase was observed even in animals which then died in tetany a few hours later.

Summary. Thyroparathyroidectomy was performed in 44 lactating rats on day 7 postpartum. Successful replacement therapy was started immediately after surgery with optimal level of T4 (3 μg/100 g/day), 2 x 40 USP units PTH/day (corresponding to 29-30 USP units/100 g/day) and 1 USP unit OXT for complete milk removal on days 14-18. Litter weight and milk yield on day 14 reached levels of normal animals given optimal amounts of T4 (3 μg/100 g/day) and 1 USP unit OXT for milk yield test. Discontinuation of PTH on day 13 resulted, within 6-18 hours, in 10 fatal attacks of tetany in a group of 12. Withdrawal of PTH on day 14 coincided with 10 hour isolation period for milk yield test. A group of 15 such animals survived considerably longer than previous group, due, it is believed, to gradual inhibition of lactation and reduction in blood Ca removed during separation period. In 11 rats which succumbed to tetany later, milk yield was significantly higher on day 15 as compared to day 14, 29 hours after last PTH injection. Nine rats which lactated normally without exhibiting signs of tetany during 4 day period after withdrawal of PTH, were considered to have (accessory) parathyroid tissue. It is tentatively proposed to consider 29-30 USP units of PTH/100 g/day as estimate of parathyroid secretion rate in this strain of intensively lactating rats under beneficial influence of optimal T4 level.

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Non-Obstructive *Escherichia coli* Pyelonephritis in the Rat.* (25747)

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Pyelonephritis has not resulted from intravenous injection of coliform organisms in the experimental animal, in most reported studies, unless preceded by renal injury(1,2,3,4). A "virulent" strain of *E. coli* previously described(5) will, however, produce renal infection in some normal rats when injected intravenously. Pyelonephritis produced by *E. coli* tends to run a self-limited course of only a few weeks even though damage and inflammation are said to persist after infection has terminated(6). The course of pyelonephritis in the animal has been assessed by postmortem cultural and histological methods and, in some instances, by infrequent urine samples obtained by laparotomy and needle aspiration of bladder(3,6,7). "Clean-voided" urine collections with quantitative urine cultures have come into widespread use for evaluation of

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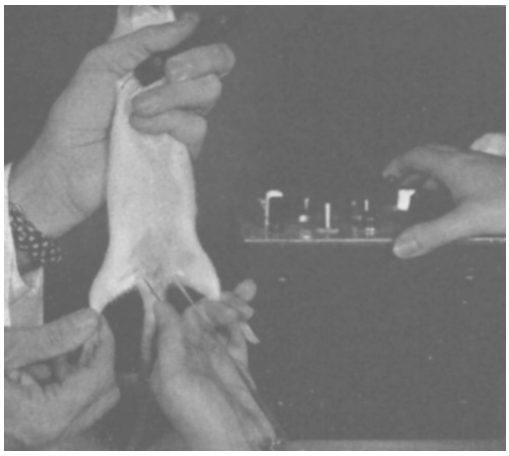

FIG. 1. Method of collecting urine specimens.

urinary infection in the human, but their use in the experimental animal has not been reported. The present investigation studies the course of non-obstructive pyelonephritis in the rat utilizing strain of *E. coli* mentioned above and "clean-voided" urine specimens.

Methods. Twenty-nine white, female Sprague-Dawley strain rats, weighing 100-140 g, were used. During control period each was found to have normal blood pressure and negative urine by microscopic examination and culture. Twenty-one experimental animals received approximately *100 million bacteria intravenously initially* and 12, which failed to develop bacteriuria of significant degree (>100,000/ml), received an additional similar injection 4 weeks later. Eight rats served as uninjected controls. Urine collections were made at 2-week intervals. Specimens were obtained in sterile test tubes after removal of lower abdominal fur with clippers and thorough cleansing of urethral papilla with 70% alcohol. Application of 300 volts by means of electrical stimulator† resulted in passage of urine in most instances, if animal had not been previously disturbed. On occasions when a specimen was not obtained, an attempt was usually successful on the following day. Method of collection is illustrated in Fig. 1. Culture of area surrounding urethral papilla, after above preparation, was carried out in all animals on one occasion as a control measure and while yielding *Staphylococcus aureus* or *albus* in a few animals, did not yield Gram negative bacteria. Urine specimens were studied microscopically, tested for protein, and cultured in thioglycollate broth and on eosin methylene blue, desoxycholate and blood agars. When growth resulted, bacteria were subcultured in citrate broth and on triple sugar iron agar for further identification. When volume of urine sufficed, serial dilutions of urine in desoxycholate pour plates were incubated and colony counts made. Determination of weight and blood pressure were also made every 2 weeks. Urine osmolarity was determined cryoscopically using Fiske osmometer and technic described by Hollander(8). Preparation of intravenous inoculum, methods for cultural and histological study of kidneys at time of sacrifice, and method of blood pressure determination have been described(5). At time of sacrifice blood was also obtained from the aorta for determination of blood urea nitrogen, and the bladder excised and placed in formalin for histological study.

Results. Significant results are shown in Table I. Of the 21 rats injected, one died of pneumonia after 17 days, and 7 failed to show cultural or histological evidence of urinary infection. The 8 uninjected controls were likewise normal throughout. Therefore, the data for these 16 animals are not presented in the Table. There was no significant difference in body weight, blood urea nitrogen, blood pressure or urine osmolarity between experimentals and controls. This is not surprising since marked renal destruction was not produced. Illustrations of microscopic lesions encountered are shown in Fig. 2. All lesions were focal and many were minimal. Microscopic evidence of cystitis was not found, but pyelitis and papillitis were encountered in 3 rats.

Of 13 experimental rats included in Table I, 3 had histological evidence of pyelonephritis, but urine cultures were only transiently positive early in the study. Of 10 with positive cultures of voided urine just prior to sacrifice, it was possible to obtain bladder urine by needle aspiration at time of sacrifice in 7 and all were positive for *E. coli.* Quantitative cultures of homogenized halves of each kidney

† Model 340 Stimulator, Harvard Apparatus Co., Dover, Mass.

were positive in only 2 of the 13, but broth culture of this material was positive in 5. Six had small, depressed cortical scars and 9 had evidence of pyelonephritis histologically.

Pyuria was present in the majority at some time during the 28-week period, but was neither striking nor persistent. Persistent proteinuria of significant degree occurred in only one (#15), and this rat had the most extensive disease.

Discussion. Persistent bacteriuria over a 7-month period is surprising in the light of the experience of others, but may be a feature of the strain of *E. coli* used which is unique in producing pyelonephritis in some normal rats. Presence of large numbers of bacteria in urine when they are no longer demonstrable in the kidney has been reported by other workers (7). The inconsistency between urine and kidney cultures may also be due to the fact that halves of 2 kidneys which were homogenized for culture may contain the only lesions, since they were focal, and similarly for the halves fixed for microscopic sections. Since it has been recently found that more severe infection in a larger percentage of animals can be produced after 2 or more "passages" of bacteria through rats with ligated ureters, further studies of the effect of nonobstructive *E. coli* pyelonephritis on blood pressure and renal function are planned.

TABLE I. Summary of Bacteriologic and Pathologic Findings at Time of Sacrifice (28 Wk).

Rat No.	Bacteriuria* colony count 10^5/ml in voided spec.	Bladder urine culture at sacrifice	Positive kidney culture	Pyelonephritis	
				Gross	Microscopic
1	+	+	+	+	0
2	+	+	+	0	+
5†	+	—‡	—‡	+	+
8†	0	0	0	0	+
10†	+	+	+	0	+
11	+	—	—	+	0
12	+	+	+	+	0
13†	0	0	0	0	+
15	+	—	—	+	+
18	+	+	0	0	+
20†	0	0	0	0	+
21	+	+	+	+	+
22	+	+	0	0	0

* Included on basis of 2 specimens prior to sacrifice, though most during study period were positive.
† Received second inj. of 100 million *E. coli*.
‡ Not obtained.

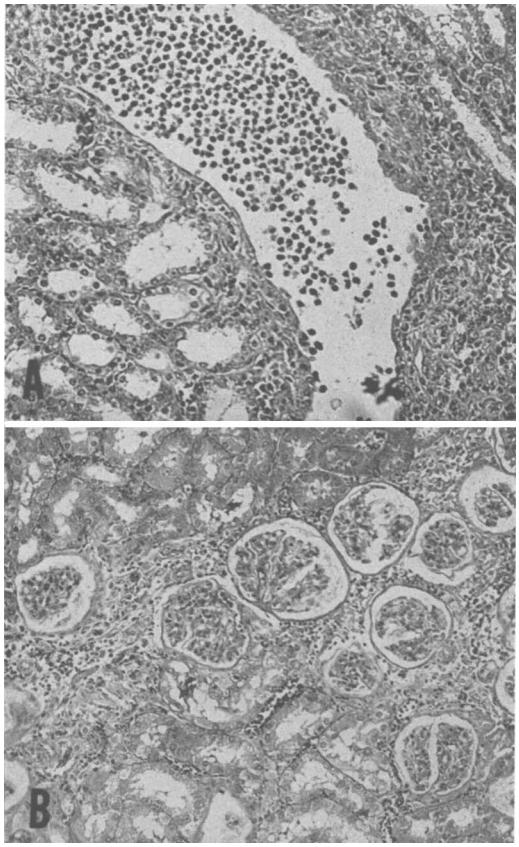

FIG. 2. Illustrations of microscopic lesions encountered. A. Chronic inflammation in wall of renal pelvis. Lumen contains polymorphonuclear leukocytes (×200). B. Scar in renal cortex of Rat 15 (×150). Hematoxylin and eosin.

Summary. An experimental model for study of non-obstructive *E. coli* pyelonephritis in the rat and successfully employing use of "clean-voided" urine specimens for quantitative culture has been described. It appears that some strains of *E. coli* may persist for long periods in the urinary tract and that minute lesions may be responsible for bacteriuria.

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Response of Plasma NEFA Levels to Epinephrine Infusions in Normal and Obese Women.* (25748)

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The physiologic and pharmacologic behavior of adipose tissue within the human body may well be a major etiologic factor in human obesity. It has been demonstrated that epinephrine markedly enhances release of non-esterified fatty acids (NEFA) by adipose tissue both *in vitro*(1) and *in vivo*(2,3). There is much evidence that nor-epinephrine, secreted by post-ganglionic sympathetic fibers, and epinephrine derived from the adrenal medulla are important regulators of fatty acid release from tissue fat depots(4). The present study was undertaken to test the hypothesis that obese subjects might exhibit a gross quantitative difference from normal individuals in response of serum NEFA levels to infused epinephrine.

Methods. Ten obese and 9 control female subjects were selected from out-patient department or hospital staff. All were free from complicating disease, had no personal or family history of diabetes, and were normally active on an ambulatory basis. Each was instructed to eat freely on day prior to infusion, but no food was allowed after 7 p. m. At 8 a. m. the following morning (13 hr. fast) a control blood sample was drawn at time of insertion of the indwelling needle. Isotonic saline was infused for 30 minutes (control period), whereupon a second sample was obtained. After control period, epinephrine bitartrate was added to infusion bottle to produce an infusion rate of 0.01 μg/lb/min (as epinephrine equivalent) and the infusion continued 30 minutes. At termination of the infusion (while epinephrine was still being infused) a third sample was taken. All blood samples were added to pre-chilled oxalate tubes, put in packed ice, centrifuged at 4°C, and immediately extracted and titrated for NEFA by Dole's method(3). An occasional sample was frozen at −15° for 1-2 days before

TABLE I. Response of Plasma NEFA Levels to Epinephrine Infusion* in Normal and Obese Women.

		NEFA conc. (μEq/l)			Glucose conc. (mg %)		
Age	Wt	C_1†	C_2‡	Epin.§	C_1	C_2	Epin.
		Normal women					
23	110	392	507	1209	87	90	108
17	115	586	682	1045	91	86	99
23	123	739	721	1243		75	81
23	129	564	691	1061	89	89	106
28	130	605	722	913	80	83	90
37	130	485	376	664	83	80	84
22	133	603	588	1102	101	96	119
25	135	715	885	1875	89	80	91
23	140	374	456	900	97	96	109
‖ 26	127	562	625	1112	90	86	99
		Obese women					
22	144	694	900	1780	93	90	125
21	185	608	681	1108	100	94	125
40	188	555	748	1676			
30	190	429	424	755	85	86	106
20	198	366	466	805	90	90	119
20	210	391	355	753	96	96	134
25	215	590	648	1242			
31	235	535	583	1055	91	93	111
20	300	676	746	807	88	84	127
39	337	885	980	1173	94	92	117
‖ 27	220	573	653	1115	92	91	121

* Infusion rate = 0.01 μg epinephrine/lb/min., 30 min.
† Initial control value.
‡ 30 min. " " .
§ At end of epinephrine infusion.
‖ Mean value.

* This study carried out during tenure of traineeship. Supported by Grant by Nat. Inst. of Arthritis and Metabolic Diseases, U. S. Public Health Service.