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A Simple Method for the Production of Anuria in Mice.* (31726)

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Anuric animals are presently prepared by bilateral nepherectomy (BLN), a time-consuming and difficult procedure which involves major surgery. The purpose of this report is to describe a rapid and simple means of preparing mice whose kidneys do not elaborate urine. Anuria is produced without major surgery by simple penile ligation (PL). PL-induced anuria is compared to BLN-induced anuria in respect to completeness of the anuric state, survival time, and digitoxin toxicity.

Methods. Groups of 10-20 male Swiss Webster-type mice weighing 25-40 g were anesthetized with ether and bilaterally nephrectomized using the dorsal approach as described by Farris and Griffith(1). Other mice were anesthetized for equal time periods during which the penis was extruded by gentle pressure on both sides of the mons pubis and ligated with 000 silk suture materials. The penis was the retracted by lifting the skin on both sides of the mons pubis. BLN and PL sham operated animals were prepared as controls. Digitoxin (K & K Laboratories, lot 53375) was administered intraperitoneally in 0.1 ml of 47.5% ethanol in dosages of 1.25 and 2.5 mg/kg to BLN, BLN-sham, PL, and PL-sham mice 2 hours after surgery; lethality was determined 12 hours after digitoxin treatment. Phenolsulfophthalein (PSP), 1 mg/kg, was administered intravenously. Urine collected from the bladder by aspiration 2 hours after BSP administration was treated with 9 volumes of 0.1 N NaOH and the absorbency determined on a Bausch and Lomb Spectronic 20 spectrophotometer at 560 mμ (2). Bladders of mice which succumbed during experiments were examined soon after death; survivors were sacrificed and bladders examined at termination of experiments. Median time to death (LT50) was calculated by the Litchfield method(3). Digitoxin lethality data were treated by the binomial expansion method(4) and the PSP data by Students "t" test. The $P_{0.05}$ level of significance was used.

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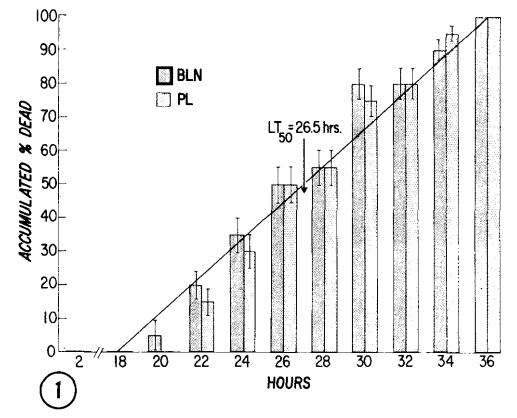

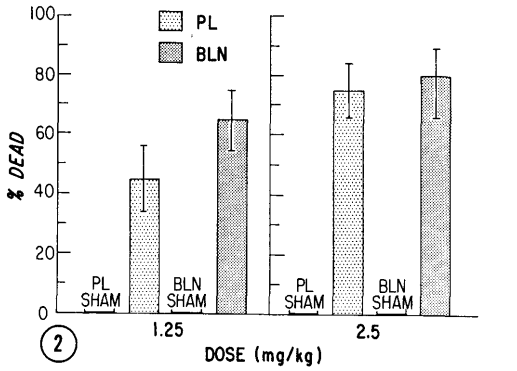

FIG. 1. Survival time of bilaterally nephrectomized and penile ligated mice. The LT50 and regression line for the 2 groups are identical. None of the 20 mice in each group died in the first 18 hr after operation.

FIG. 2. Enhancement of acute lethality of intraperitoneal digitoxin at 2 dosages in bilaterally nephrectomized and penile ligated mice compared to respective sham operated animals.

Results. PL and BLN mice exhibited essentially identical survival patterns over a 36 hour period (Fig. 1). The LT50 for each group was 26.5 hours (95% confidence limits: 24.7-29.3 hours) and the slope function for the common regression line was 1.17. None of the PL-sham or BLN-sham operated animals died during the observation period.

Production of anuria upon completion of BLN was self evident. However, the following experiment demonstrated (1) that some time must elapse before PL animals ceased to produce urine, and (2) that a 2-hour period was adequate to effect anuria. Two PL groups were prepared. PSP was administered to one group co-incident (0 hr) with PL preparation and to the other group 2 hours later. Urine PSP levels were determined after a 2-hour collection period. The 0-hour group excreted PSP (absorbency = 0.25 ± 0.04 O.D. units); the 2-hour group excreted very little PSP (absorbency = 0.03 ± 0.01 O.D. units). The difference in mean absorbency values is highly significant (P 0.005).

The foregoing experiment indicated that PL produced anuria, and that PL animals survive the anuric state no better than the BLN animals survive. Accordingly, the following experiment was undertaken to determine if a toxic substance, digitoxin, would (1) effect an enhanced lethality in PL mice and (2) if the lethality to PL mice was not significantly different than that effected in BLN mice. BLN, BLN-sham, PL, and PL-sham mice were prepared. Two hours later, the mice were treated with either 1.25 or 2.5 mg/kg of digitoxin. Lethality was determined 12 hours after drug treatment. Results are given in Fig. 2. None of the sham operated animals died. Within doses the lethality of digitoxin to PL animals is not significantly different from the lethality to BLN animals indicating both types of anuric animals are equally affected by this toxic agent.

Discussion and conclusions. Evidence that PL-induced anuria is not effected by the ligation is found in the PSP experiment which shows that PL mice treated immediately after ligation with PSP excrete PSP in a normal fashion. Impairment and cessation of kidney function is believed to be effected by penile ligation by production of a classical "stop-flow"(5) situation: urine collects in the bladder to the limit of distention of that organ and then fills the ureters and renal pelves. Stop-flow is achieved by 2 hours as shown by the PSP experiment.

Digitoxin was selected as the test drug for the toxicity comparison because it is primarily excreted *via* the urine in mice. The enhanced toxicity in PL and BLN mice is further evidence that anuria produced by PL and BLN are similar.

Anuria produced by PL appears to be indistinguishable from that produced by BLN in respect to survival time after preparation. In the later hours (*e.g.*, 18-36 hr) the appearance of the animals is not different; however,

in the early hours (*e.g.*, 0-8), PL animals appear normally active while BLN animals are somewhat depressed which may be attributed to the effects of major surgery. The PL procedure offers several advantages besides sparing the mice from surgery. In this laboratory a skilled operator can prepare about 8 BLN animals in 1 hour. On the other hand, a relatively inexperienced operator can prepare 8 PL animals in 10 minutes. The economy of PL preparation in terms of time and required skill of the operator, therefore, argues strongly for use of this method when an anuric mouse is needed as a research animal.

Summary. Penile ligation (PL) of mice effected a state of anuria which is indistinguishable from anuria effected by bilaterial nephrectomy (BLN) with respect to survival of anuric mice and enhanced lethality when treated with digitoxin. PL-induced anuria probably results from production of a urine stop-flow situation as shown by PSP excretion studies. The PL operation is simpler and faster than the BLN operation and is recommended for production of anuric mice as a research tool.

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Metabolic Effects of Isoproterenol and Propranolol in Normal Subjects Before, During and After Triiodothyronine-Induced Hypermetabolism.* (31727)

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The precise mechanism of the metabolic and circulatory abnormalities of patients with spontaneous or drug-induced hyperthyroidism are unknown. Previous clinical and experimental observations(2-4) have supported the hypothesis that a hyperactive sympathetic nervous system may be responsible for the changes associated with hyperthyroidism. Among the metabolic abnormalities reported in individuals with hyperthyroidism are elevated levels of plasma free fatty acids (FFA) and blood glucose obtained in the fasting state(5). The FFA mobilizing effects of thyroid hormone could be mediated through stimulation of beta adrenergic receptors. It is also known that epinephrine, norepinephrine and isoproterenol increase plasma FFA concentrations by enhancing mobilization from adipose tissue(6,7). Adrenergic blocking agents inhibit the augmented FFA release from adipose tissue caused by catecholamines (8).

This study was designed to evaluate the effects of beta adrenergic receptor stimulation by isoproterenol and beta receptor blockade by propranolol on levels of plasma FFA and blood glucose of normal subjects before, during and after a period of triiodothyronine-induced hypermetabolism. We also wished to test the hypothesis that triiodothyronine may augment the metabolic responses to graded doses of isoproterenol.

Materials and methods. Eight normal men

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