# Terminal Formation of Urethral Plugs in Male Mice.* (27755)

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Plugs of white congealed material have been observed in the pelvic urethras of male laboratory rodents at autopsy(1-4). These plugs are similar in consistency to cooked egg white and form perfect casts of the lower urinary tract, and sometimes extend back into the bladder (Fig. 1). They often fill the urethral diverticuli, extend down the penile urethra (Fig. 1), and frequently are attached to small pedicles which extend from the openings of the seminal vesicles. Microscopically they are amorphous eosinophilic masses (H and E).

Because of a recent report(3) in which urinary obstruction in mice was attributed to these plugs, evidence is presented here to show that they are formed immediately prior to death and that their frequency of occurrence depends on the method used to kill the mice.

*Materials and methods.* Mature male white laboratory mice were used. The methods employed to kill the mice were overdoses of ether (inhaled), and sodium pentobarbital (1 ml of a saturated solution, subcutaneously).

In studying nervous pathways involved in terminal ejaculation the spinal column was cut at various places under ether anesthesia, using a small Liston bone forceps.

*Results.* Table I summarizes the findings in the urethras of male mice killed by different methods.

To determine when and how a urethral plug is formed, mice were anesthetized with ether and their pelvic urethras exposed and opened *via* a ventral abdominal incision. The animals were killed with an overdose of ether. Just as breathing stopped seminal fluid was expelled into the urethra by contraction of the seminal vesicles. This fluid congealed to form a plug within a few minutes. This procedure was repeated in several mice, with the same result.

The participation of the nervous system in the formation of plugs was demonstrated by the following experiment. Mice were anesthetized with ether and their spinal columns severed at various places. They were killed by ether overdosage and examined for urethral plugs. Results are presented in Table II.

To determine if urethral plugs form in male mice after normal ejaculation 12 sexually mature male mice that had been isolated since weaning were paired individually with females. The females were examined 3 times daily for vaginal plugs which would indicate that copulation had taken place. When a female was found with a vaginal plug, her male

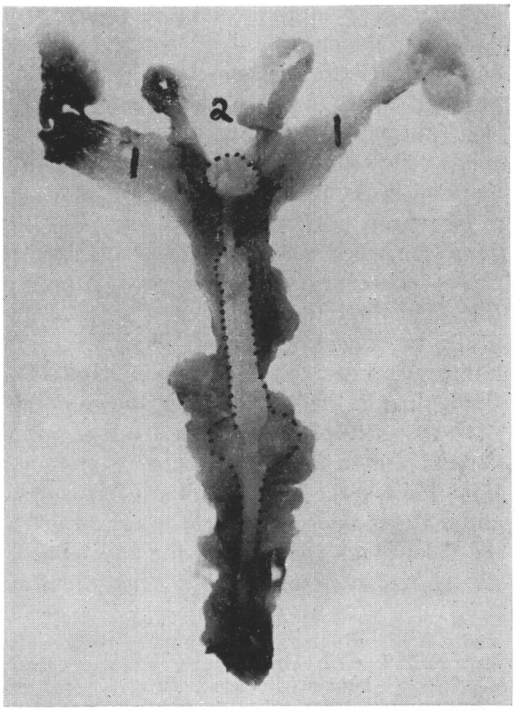


FIG. 1. Lower urinary tract of a male mouse killed by ether overdosage. The bladder and entire urethra have been opened to show a large urethral plug forming a perfect cast of the lower urinary tract. The kidneys of this animal were normal, indicating that this urinary obstruction was not present during life. The plug is outlined by dotted line. 1, seminal vesicles; 2, opened bladder.

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TABLE I. Findings in Urethras of Mature Male Mice, Isolated Individually from Weaning, and Killed with Ether, or Sodium Pentobarbital.

| Method of killing | Total No. of animals | Urethra free of plug material | Small sand-grain sized pieces of plug material at openings of seminal vesicles | Large plug present |
|---|---|---|---|---|
| Ether | 12 | 0 | 2 | 10 |
| Sodium pentobarbital | 12 | 7 | 5 | 0 |

mate was killed with sodium pentobarbital. None of these 12 males had urethral plugs, although 5 had small sand-grain size pieces of plug material at the openings of the seminal vesicles.

The significance of urethral plugs in urinary obstruction was investigated by making urethral plugs in 13 male mice by squeezing the seminal vesicles and coagulating glands through a ventral abdominal incision under ether anesthesia. The mice were allowed to recover and were killed with sodium pentobarbital 4 days later. Five mice had urinary obstruction by urethral plugs, as indicated by distended bladders and large spongy, pale kidneys which were occasionally mildly hydronephrotic. Those mice without urinary obstruction (normal bladders and kidneys) had, however, yellow-stained friable chunks of plug material in the bladder and urethra.

*Discussion.* The origin of urethral plugs from congealed seminal fluid is obvious because such plugs can be seen to form from this fluid directly and can be produced artifically by squeezing seminal fluid and coagulating gland secretion into the urethra. Urethral plugs in male mice have the same consistency, texture, and color as the normal vaginal plug in female mice soon after copulation. However, Silverstein *et al.*(3) found the chromatographic patterns of proteins extracted from plugs and seminal fluid to be different and pointed out that seminal fluid undoubtedly is altered by the secretion from the coagulating glands.

TABLE II. Findings in Urethras of Mature Male Mice Killed with Ether After Severing Spinal Column at Various Places.

| Treatment | No. animals with plugs | Total No. animals tested |
|---|---|---|
| Spinal column not severed | 4 | 6 |
| Spinal column severed in high lumbar region | 0 | 6 |

Since mice killed with ether sometimes die with a terminal convulsion, whereas those killed with sodium pentobarbital do not, it seems likely that the presence of urethral plugs in the former case is produced by a terminal, abnormal ejaculation. The seminal fluid and secretion of the coagulating glands must mix in the urethra and fail to be expelled by coordinated muscular contractions. Coagulated seminal fluid forms the plug.

Data from the experiment on spinal cord transection are interpreted to mean that impulses, probably originating in the brain, were blocked from reaching the reproductive tract *via* the pudendal nerve by spinal transection.

Urethral plugs may also occur when animals die a natural death, as a large plug was seen in a chinchilla that died of streptococcal septicemia.

It is doubtful if the small sand-grain sized particles of plug material found in the urethras of about 50% of pentobarbital-killed mice (Table I) were present during life because they were not seen in the ether-killed mice after high lumbar spinal transection.

Hydronephrosis occurs with low frequency in male mice in our colony, but urethral plugs are not a cause of this, for the following reasons: 1) plugs can be produced at will, depending on the method of killing; 2) hydronephrosis sometimes is unilateral; 3) nearly all of the mice with plugs have no hydronephrosis.

Silverstein *et al.*(3) recently concluded that urethral plugs cause urinary obstruction in inbred mice with primary polydypsia. They reasoned that even though both their polydypsic and control males uniformly had urethral plugs, only in those mice with polydypsia (and therefore a large urine volume) did such plugs

cause backing up of urine. This explanation is untenable since the presence of such plugs depends on the method of killing, and because such plugs form at time of death. Silverstein *et al.* did not find hydronephrosis in control or polydypsic female mice. Therefore, the obstructive phenomenon in their animals may well depend on anatomical differences between male and female in the lower urinary tract, but this difference is not the urethral plug, for the reasons given above.

The above facts also must be considered in experiments in which the weight of the seminal vesicles is used as an index of androgen activity. It should be kept in mind that killing animals with sodium pentobarbital prevents the loss of seminal fluid that accompanies the formation of urethral plugs.

The significance of urethral plugs was properly interpreted by Engle(2) who wrote: "Those who have worked with laboratory rodents know that a tough coagulum usually is found in the urethra of a male which is killed by a sudden blow on the head, and also after death by illuminating gas, especially if the animal died struggling."

*Summary.* The terminal formation of urethral plugs of coagulated seminal fluid in male mice depended on the method of killing. Plugs were found when mice were killed by ether inhalation, but not when killed with sodium pentobarbital injected subcutaneously. These plugs were produced by an abnormal ejaculation during a terminal convulsion which occurred in mice killed by ether, but not in sodium pentobarbital-killed mice. High lumbar cord transection prevented plug formation. Normal ejaculation did not produce urethral plugs. It was concluded that such urethral plugs are not a natural cause for urinary obstruction since they form terminally.

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## Nitrites XX. Pharmacologic Studies with 1-Ethylglyceryl Trinitrate. (27756)

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For a number of years we have been investigating the structure-activity relationships of compounds related to glyceryl trinitrate. It was shown that the number of nitrate groups in the molecule was not a salient factor in determining vasodilating potency. Furthermore the activity was dependent upon the intact molecule of the ester(1). These studies are concerned with the activity of 1-ethylglyceryl trinitrate (EGT)(1,2,3-Pentanetriol-trinitrate). This ester is identical with glyceryl trinitrate with the exception that one of the hydrogen atoms on $C^1$ has been replaced by an ethyl group.

*Methods.* Coronary blood flow was determined by the modified Langendorff(2) procedure using rabbits' hearts.

Studies were conducted on the fate of EGT and glyceryl trinitrate upon passage through the heart in a Langendorff preparation omitting the glucose from the perfusion fluid. Total ester and nitrite were determined in the perfusion fluid to which each ester had been added respectively. The same procedure was carried out by adding the respective esters to the perfusion fluid and determining the total ester and nitrite content at definite time periods in the perfusates.

The hydrolysis studies of the nitrate esters and their effect upon blood pressure were