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Ejaculatory Response Induced by Potassium Chloride in Small Mammals. (22492)

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Artificial ejaculation in laboratory animals has been produced by electrical stimulation and by drug action, the latter consisting of a hypnotic followed by a stimulant. No clear description of the properties of the secretions obtained has been reported, nor has either method been described as capable of producing a complete ejaculation containing sperm as well as secretions from the accessory organs of reproduction. A high incidence of ejaculation occurs in the final convulsions of a dying animal. Loewe(1) has termed this phenomenon terminal ejaculation in contrast to intravital ejaculation which neither coincides with death nor is followed by death after a short interval of time. Potassium chloride has been found to produce an intravital ejaculation in mice following intraperitoneal injection of sub-lethal doses. This investigation was undertaken to determine the mechanism by which the ejaculations were induced and the nature of the secretions.

Methods. The animal species employed were the albino mouse, albino rat (Wistar), hamster, guinea pig, pigeon, and ground squirrel. All animals used were adult males. The ground squirrels were trapped by snaring in the vicinity of Lafayette, Ind., and were kept in captivity about 6 months prior to their use. Other animals were obtained from breeders. Chemical agents were administered by intraperitoneal injection into the lower quadrant of the abdomen. Ejaculation time was measured from the time of injection until the first appearance of seminal fluid at the penis. Weights of ejaculates were made by collection on weighed glass microscope slides. Fructose analyses were made by the colorimetric method of Roe as modified by Mann(2). Seminal vesicles were removed through a midline incision in the abdomen cephalad the base of the penis. Isolated seminal vesicles were used to test the *in vitro* action of chemical agents by the method described by Stone and Loew(3). A seminal vesicle, placed in a bath of oxygenated Locke's solution to which

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TABLE I. Ejaculatory Response of Different Animal Species to Intraperitoneal Injections of Potassium Chloride.

Species	No. tested	KCl dose range, mg/kg	No. ejac.	% ejac.	Effective dose range, mg/kg
Mouse	69	50–1000	41	68.3	100– 800
Rat	12	50– 600	10	83.3	100– 600
Hamster	11	25– 600	5	45.4	400– 600
Ground squirrel	6	175–1200	4	66.7	800–1200
Guinea pig	6	50– 150	4	66.7	100– 150
Pigeon	7	600	0	0	
Cat	1	300	0	0	

1.0% dextrose had been added, was attached to a lever writing on a slowly moving kymograph. Chemical test agents were added directly to the bath.

Results. Intraperitoneal injections of KCl in mice evoked a series of symptoms which were, in order of their appearance: 1) signs of discomfort, 2) 3 to 5 stretching movements with extension of the hind legs, and 3) arching of the back (opisthotonus). All symptoms occurred within a period of 30 seconds to one minute during which time the animal ejaculated. Often a coagulated plug followed the ejaculation. The most effective dosage of KCl for the mouse was 400 mg/kg although positive results were obtained by doses ranging from 100 to 800 mg/kg. Different dilutions of KCl were tried from 2.5 to 5.0%, the best effect being produced with 400 mg/kg of 3.5%. Other animal species showing positive results with KCl injections were rat, hamster, ground squirrel, and guinea pig (Table I). The cat and pigeon were negative. The ground squirrel required dosage from 800 to 1200 mg/kg to be effective but tolerated all doses. The hamster required 400 to 600 mg/kg KCl.

Epinephrine, nor-epinephrine, and acetylcholine also induced ejaculation in mice; however, ejaculation caused by acetylcholine more nearly resembled that produced by KCl than did that produced by epinephrine inasmuch as the ejaculate was more forceful, of larger quantity and more frequently followed by plugs. Drugs which block autonomic action such as atropine and Priscoline successfully abolished ejaculations normally caused by acetylcholine and epinephrine but did not block KCl. Deep ether anesthesia blocked KCl ejaculation while light ether anesthesia did not. Often ejaculation followed recovery from anesthesia by ether during which KCl had been given. Both acetylcholine and epinephrine stimulated excised seminal vesicles *in vitro* in much weaker concentrations than those inducing ejaculation *in vivo*. KCl even in massive doses did not act directly on the smooth muscle of the seminal vesicles. Chemical analyses of the ejaculates showed the presence of fructose but without correlation between ejaculate weight and fructose content. After removal of the seminal vesicles no ejaculatory responses were obtained with repeated tests up to 30 days. Upon postmortem examination, the animals showed complete absence of seminal vesicles but with normal appearing prostate gland. KCl-induced ejaculation exhibits an all-or-nothing response as any dose that is effective produced a maximal response.

Discussion. These experiments have demonstrated that artificial ejaculation can be induced in mice by the intraperitoneal injection of sublethal doses of potassium chloride with no apparent harm to the animals even on repeated daily injections. This response has also been obtained in other rodent laboratory and one wild species. Attempts to standardize quantitatively the ejaculatory response by measurement of ejaculation time or weight were not successful. Because the response was obtained only with compounds which contained the potassium ion the effect may possibly be attributed to the reported actions of this ion on nerves. Potassium ions alter the resting potential of nerve membrane(4) and injection of potassium into fluid perfusing ganglia causes a discharge in both pre- and

post-ganglionic fibers as well as liberation of acetylcholine(5). Acetylcholine or epinephrine injections also induced ejaculation in mice but pretreatment with atropine and Priscoline prevented, respectively, the ejaculations induced by these drugs but not those induced by KCl. Ejaculations induced by acetylcholine were qualitatively and quantitatively better than those induced by epinephrine, suggesting predominance of parasympathetic control of the efferent ejaculatory pathway.

It is assumed that KCl produces only ejaculation of the fluid present in the seminal vesicles because no sperm were ever found in the ejaculates. Repeated daily application of KCl apparently does not injure the animals so it is believed that it is a safe means of inducing ejaculation of seminal fluid without sacrificing the animals for collecting fluid for bio-assay studies.

Summary. Intraperitoneal injection of KCl has been demonstrated to induce intravital ejaculation in mice, rats, guinea pigs, hamsters and ground squirrels, but not in pigeons or cats. The response is an all-or-nothing response regardless of the size of the dose. A dose of 400 mg/kg of 3.5% KCl was found to be the most effective in mice. Only potassium-containing salts were effective in producing ejaculation. It was not possible to standardize the ejaculatory response by measuring ejaculatory time or by weighing the ejaculate. Extirpation of the seminal vesicles, bilateral castration, and analysis of the fructose content of the semen indicated the seminal vesicles as the source of the fluid. No sperm were found in the fluid at any time. Repeated daily injections of KCl cause ejaculation without apparent harm to the animals.

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Uterine Growth Stimulating and Testicular Growth Suppressing Activities of 17α-Ethinylandrostane-3β,17β-diol, Its Δ^5-Analog and Derivatives. (22493)

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Comparative studies of biological activity of 17α-ethinylandrostane-3β,17β-diol and its Δ^5-analog(1) have been concerned largely with androgenic and luteoid potency as related to other androstane and pregnane derivatives (2-5). Our observations have indicated that these steroids are characterized by predominantly estrogenic activity (growth promotion of infantile rat uterus and inducement of vaginal cornification in ovariectomized rats). The present report is a structure-activity relationship survey of ethinylandrostanediol, ethinyl-Δ^5-androstenediol, and esters of each, with respect to relative activity in stimulation of uterine growth (UGSt) and suppression of testicular growth (TGSp) in immature intact rats. Observations on the UGSt and TGSp activities of estradiol-17β, ethinylestradiol and Δ^5-androstenediol are included for reference purposes. Definitive endocrinological studies on certain compounds of the series will be reported separately.

Materials and methods. Immature male or female Sprague-Dawley rats weighing 35-40 g were used. Unless noted otherwise all test substances were dissolved in cottonseed oil containing 10% ethanol v/v and administered subcutaneously in 0.2 ml of vehicle. The uterine growth tests were performed essentially in accordance with procedures outlined by