mg/ml of alginic acid sulfate were stabilized on continued incubation and a significant increase in hyaluronic acid levels of broths containing hyaluronidase inhibitor was obtained. The curves (Fig. 1), are typical of those obtained with the mucopolysaccharide and are readily reproducible with every batch of material.

Depolymerization of purified hyaluronic acid by hyaluronidase. Depolymerization of partially purified streptococcal hyaluronic acid by purified bovine testicular hyaluronidase (1550 USP units/mg) was studied using turbidimetric assay method described previously(14). A turbidity value of 100 scale divisions on the Klett-Summerson colorimeter was produced by interaction of 0.2 mg of polysaccharide and acidified horse serum. A linear correlation was found between enzyme concentration and depolymerization of the polysaccharide substrate (Fig. 2).

Summary. 1. Hyaluronic acid was released from cells of a Group A *Streptococcus pyogenes* when the latter was grown in medium in which all constituents except casein hydrolysate were chemically defined. With this medium it has been possible to produce high yields of streptococcal hyaluronic acid following 16 to 18 hour incubation at 37°C. 2. Stability of hyaluronic acid in growing cultures of *S. pyogenes* following addition of a hyaluronidase inhibitor, alginic acid sulfate, to the medium, together with a significant increase in hyaluronic acid levels of the broths is further evidence that strains of streptococci which produce hyaluronic acid also produce hyaluronidase. 3. Isolation and partial purification of the bacterial mucopolysaccharide are described.

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Induction and Maintenance of Lactation in Rats by Electrical Stimulation of Uterine Cervix.*† (25166)

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Electrical stimulation of the uterine cervix can induce pseudopregnancy in sexually mature rats(1,2) and hasten sexual maturation in prepubertal rats(3). Presumably, these are elicited by nervous stimuli through sympathetic and hypothalamic pathways, resulting in release of FSH, LH and luteotropin by the anterior pituitary(2,3). There is also evidence that mechanical stimulation of the uterine cervix may elicit oxytocin and possibly vasopressin release from the posterior pituitary(4,5). These findings, as well as our recent observation that epinephrine, acetylcholine or serotonin can initiate and maintain

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lactation in rats(6,7), has prompted us to determine whether electrical stimulation of the rat uterine cervix can liberate sufficient prolactin from the anterior pituitary to initiate or maintain lactation. It was also of interest to see whether similar effects on lactation could be elicited by oxytocin or vasopressin.

Methods. Mature virgin female rats (Carworth) weighing between 200-250 g were injected subcutaneously with 10 μg estradiol daily for 10 days to produce mammary development. This treatment has previously been shown to induce limited but definite lobulo alveolar growth(7). For the next 5 days, groups of 5 rats each were treated as follows: controls, physiological saline once daily; 1 IU oxytocin‡ twice daily; 1 IU vasopressin‡ twice daily; electrical stimulation of uterine cervix twice daily; *ibid,* 3 times daily; 70 mg morphine sulfate/kg BW twice daily, followed 20-30 minutes later by cervical stimulation. Electrical stimulation was conveyed to the cervix by platinum electrodes which were insulated except for the tips. A low current was supplied by an Electrodyne Stimulator set at a frequency of 20 cycles/second for 30 seconds. The current was just detectable on our finger tips and usually produced only momentary struggling by the rats. Post-partum rats were used to determine effects of cervical stimulation on maintenance of lactation. Litters were removed on 4th day after parturition and groups of 5 mother rats each were treated for 10 days as follows: controls, physiological saline; electrical stimulation of cervix twice daily; 1 IU oxytocin twice daily; 1 IU vasopressin twice daily. All rats were killed on day after last treatment, and the right inguinal mammary glands were removed for histological examination. The glands were fixed in Bouin's fluid, sectioned at 6 μ and stained with eosin and hematoxylin. Each gland was examined microscopically for degree of secretion and mammary development. Ovaries, uterus, adrenals and thymus glands were also removed and weighed.

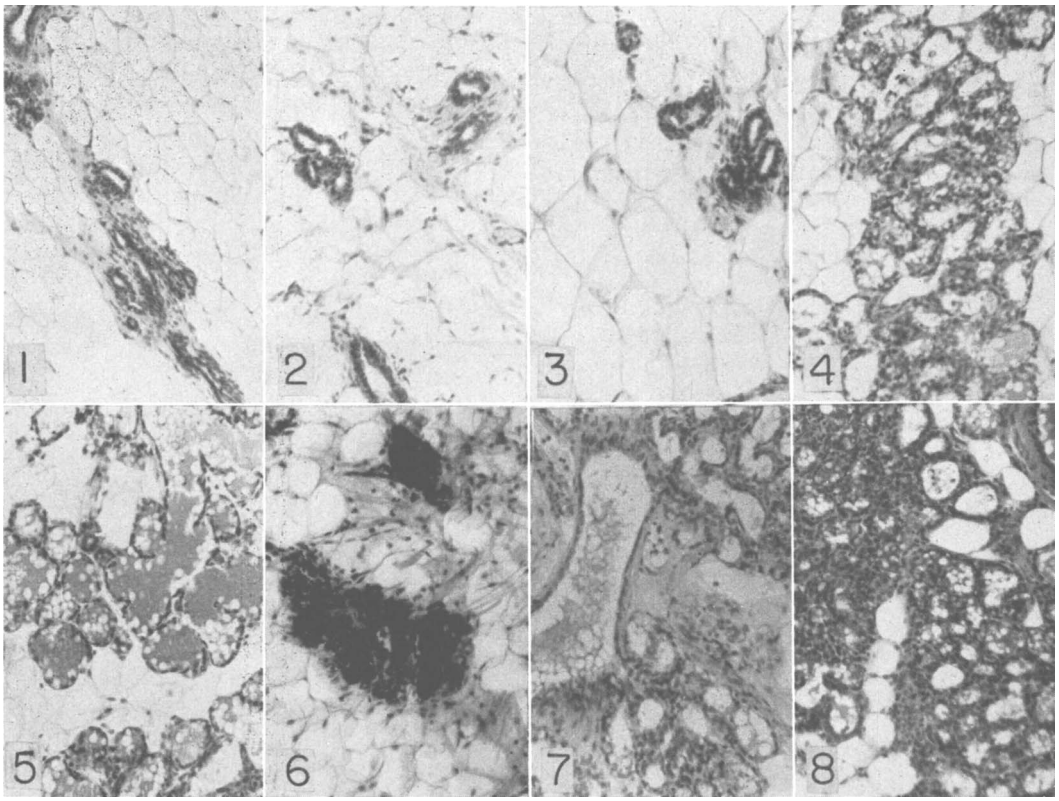
Results. In virgin rats, injections of saline (Fig. 1), oxytocin (Fig. 2) or vasopressin (Fig. 3) resulted in mammary regression to practically a bare duct system and no secretion. In sharp contrast, 4 out of 5 rats in each of the 2 groups electrically stimulated 2 or 3 times daily, showed definite secretion and intact lobulo alveolar structure (Fig. 4). The 5 rats given morphine sulfate prior to electrical stimulation all showed intense mammary secretion (Fig. 5), equivalent to that usually seen in lactating rats after parturition.

In postpartum rats, controls injected with saline for 10 days after litter removal showed pronounced mammary involution and no secretion (Fig. 6), while animals electrically stimulated (Fig. 7) or given oxytocin (Fig. 8) showed moderate secretory activity and retardation of mammary involution. Vasopressin was only slightly effective in these respects. None of the treatments produced significant changes in weights of ovaries, uterus, adrenals or thymus glands, as compared with saline-injected controls.

Discussion. These results demonstrate that electrical stimulation of rat uterine cervix can initiate milk secretion after development of mammary glands with estradiol, and can maintain secretory function and retard mammary involution in postpartum rats after litter removal. These effects are believed to be mediated through release of prolactin from the anterior pituitary *via* the autonomic nervous system and hypothalamus. Evidence for participation of the autonomic nervous system is provided by the following: (a) Interruption of sympathetic pathways during electrical stimulation of the uterine cervix prevents pseudopregnancy(2) or hastening of sexual maturation(3) in rats. (b) Dibenamine or atropine injections may inhibit prolactin liberation from the AP of lactating rats(8). (c) Epinephrine, acetylcholine or serotonin can initiate or maintain lactation in rats with developed mammary glands(6,7).

Although morphine has been reported to inhibit ACTH release(9), it did not prevent and appeared to enhance mammary response to electrical stimulation of the cervix. This cannot be considered conclusive however, since further work has indicated that the dose of morphine sulfate used failed to evoke charac-

‡ Oxytocin and vasopressin were supplied through courtesy of Dr. D. A. McGinty, Parke, Davis and Co.



FIGS. 1-5. Mammary sections from virgin rats given estradiol for 10 days, followed for 5 days by (1) saline, (2) oxytocin, (3) vasopressin, (4) electrical stimulation of uterine cervix 3 times daily, (5) morphine sulfate and electrical stimulation of uterine cervix 2 times daily.

FIGS. 6-8. From postpartum rats, treated for 10 days after litter removal with (6) saline, (7) electrical stimulation of uterine cervix, (8) oxytocin.

teristic symptoms in the rat after the first 3 days of injection.§ Apparently the lactation-inducing effects of electrical stimulation of the cervix were not mediated to any significant extent through ovaries or adrenals, since neither the weights of these organs nor of the uterus and thymus were altered. However, in view of previous demonstrations that manipulation of the uterine cervix stimulates liberation of gonadotropic hormones(1-3), the possibility cannot be excluded that slight alterations occurred in ovarian or adrenal cortical activity, not detectable by changes in organ weight.

Neither oxytocin nor vasopressin initiated lactation in these rats. Oxytocin has previously been shown to be incapable of initiating lactation in rabbits with developed mammary glands(10,11). Mammary secretion was maintained in postpartum rats by oxytocin and to a much lesser extent by vasopressin, confirming previous reports which formed the basis for the claim that oxytocin stimulates prolactin release from the anterior pituitary (11-13). These workers also believe that oxytocin is responsible for releasing prolactin when the nipples are stimulated by suckling during lactation. However, simultaneous release of oxytocin and prolactin, which occurs during stimulation of uterine cervix or nipples, may be effected by independent mechanisms, rather than by assuming that oxytocin is responsible for prolactin liberation. This is supported by observations that (a) suckling by litters results in rapid discharge of pituitary prolactin content in mother rats(14,15), whereas injections of oxytocin or posterior

§ We have recently found that injections of morphine sulfate alone can initiate lactation in estrogen-primed rats.

pituitary extract fail to induce a similar decrease in pituitary prolactin in the rat, guinea pig or rabbit(15,16), (b) injections of oxytocin and prolactin together into postpartum rats after litter removal are more effective in maintaining lactation than either hormone alone(17), (c) injections of reserpine into lactating rats inhibits oxytocin release but stimulates prolactin discharge from the pituitary(18).

Our recent work suggests that the effectiveness of oxytocin in maintaining mammary secretion in postpartum rats after litter removal depends upon removal of accumulated secretion from the alveolar lumina and smaller ducts, thereby permitting further synthesis of milk by the alveoli(19). Histological preparations of mammary tissue from postpartum rats after litter removal have shown that a single injection of oxytocin, following treatment with prolactin for 5 or 10 days, produces ejection of accumulated secretion from the alveolar lumina and smaller ducts within 10 minutes. The secretory material is believed to enter the larger ducts and numerous stromal tissue spaces, from which it can gradually be reabsorbed into the circulation or perhaps be partially reutilized for milk synthesis. Details of these observations will be published.

Passage of the fetus through the uterine cervix at parturition may provide a stimulus for prolactin release and initiation of lactation at this time. This could supplement the stimulus to prolactin secretion believed to come from estrogen at the end of pregnancy(10,11,16). However, cervical stimulation by fetal passage is probably not essential for lactation, since prolongation of gestation by progesterone(20, 21) or removal of the entire uterus during latter part of pregnancy(16) do not prevent initiation of milk secretion. We have previously suggested that stimuli arising from mammary glands at end of gestation may also evoke prolactin release and help to initiate lactation(22, 23). This problem is under further investigation.

Summary. 1. The uterine cervix of virgin, sexually mature rats was stimulated electrically 2 or 3 times daily for 5 days, following daily injections of 10 μg estradiol for 10 days to develop the mammary glands. Milk secretion was initiated in 8 out of 10 rats by this treatment, whereas 5 saline-injected controls showed no secretion and mammary involution. Twice daily injections of 70 mg morphine sulfate/kg BW prior to uterine stimulation did not inhibit lactation in 5 rats, and it was actually more intense in these animals. Twice daily injections of 1 IU oxytocin or 1 IU vasopressin for 5 days failed to initiate mammary secretion, showing that these hormones are not responsible for prolactin release in the cervical-stimulated rats. 2. Following litter removal on 4th postpartum day, injections of saline into mother rats for 10 days resulted in cessation of lactation and pronounced mammary involution. Twice daily electrical stimulation of the uterine cervix or injections of oxytocin for 10 days maintained secretory activity and retarded mammary involution, whereas vasopressin was much less effective in these respects. 3. It is concluded that electrical stimulation of the uterine cervix initiates lactation through sympathetic and hypothalamic pathways, resulting in prolactin release from the anterior pituitary. Oxytocin and to a lesser extent, vasopressin, are believed to maintain mammary secretion in postpartum, non-suckled rats by ejecting accumulated secretory material from the alveolar lumina and smaller ducts into the larger ducts and stromal tissue spaces, where it can be readily absorbed into the circulation.

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Metabolism of Sulfobromophthalein in Hepatectomized and Hepatectomized-Nephrectomized Dog.* (25167)

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Sulfobromophthalein (BSP), because it is preferentially removed by the liver, has been used extensively as a liver function test in clinical medicine. Recent studies have shown that BSP is metabolized by the liver(1-5) and excreted into the bile, in part at least, as a mercaptide with cysteine and possibly with a compound containing cysteine, glycine and glutamic acid(6). Previous studies of BSP metabolites were carried out in normal human subjects and animals(2-4), in isolated perfused livers(3) and in patients with liver disease(7). In this investigation the ability of tissues other than the liver to metabolize BSP was studied in hepatectomized and hepatectomized-nephrectomized dogs.

Methods. Two normal male dogs, weighing 16 and 23 kg respectively, were hepatectomized under pentobarbital anesthesia. The liver, isolated by the method of Dakin *et al.* (8), was removed, along with a short segment of the inferior vena cava. The resulting gap in the remaining inferior vena cava was bridged by a plastic catheter. Animals were maintained by continuous infusion of 20% glucose. The blood volume was restored when necessary by transfusion of dog blood. Control samples of urine and blood were collected. The animals were then given commercial BSP intravenously (5 mg/kg of body weight). Blood samples, taken at timed intervals thereafter, were analyzed for total BSP and BSP conjugates. Urine, collected from each animal during a 2 hr period after injection of BSP, was also analyzed for total and conjugated dye. Two additional dogs were hepatectomized and also nephrectomized. Fifteen to 30 min. later, control blood samples were obtained. Commercial BSP was then administered intravenously (5 mg/kg of body weight). Blood specimens were taken at timed intervals and analyzed for total and conjugated BSP. Concentrations of total BSP in serum and urine were determined by the method of Gaebler(9). To quantitate the proportion of conjugate, measured aliquots of serum and concentrated urine were extracted with acetone (1:3 v/v). The resulting supernatants were dried under vacuum. The material was taken up in small aliquots of water and chromatographed on Whatman 3 MM pa-

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