that expected for this age(1). Following 2 weeks of steroid therapy the following changes were observed: a) 32 exhibited decreased H/C values of 5% or greater. In 29 of these the losses did not exceed 30%, in 3, decreases of −43%, −63% and −69% were obtained (the latter due to the individual mentioned above): b) 6 had changes of less than 5%;† c) 3 had increased values (+23%, +10%, +34%). It is of additional interest in the case of the latter that pretreatment H/C values were .027, .020, .023 respectively, values expected for aged individuals(1). The latter 2 H/C values were the lowest pretreatment values in the series. The significance of the increased values following corticoid treatment is not clear and has not been observed in rats(2,3). The mean percent change in H/C values for 35 subjects (exclusive of the 3 who exhibited exceptionally large decreases and the 3 who exhibited increases) was −12.9% ±1.5%.‡

Discussion. In general H/C of dermal punch biopsies decreased following corticoid therapy. This had been previously observed in the rat following starvation and administration of cortisone(2). Since the dose of corticoids administered to patients was based upon clinical requirements, the effect of graded doses upon H/C could not be determined. The wide range in response of H/C seemed to be due to individual variation and not to choice of steroid employed. There appeared to be no correlation between clinical response of patient to the corticoid and decrease in H/C. Since the decrease in H/C in the rat following cortisone administration precedes a large decrease in body nitrogen, the change in ratio in the human might likewise presage other metabolic events. This suggests employment of this technic as an adjunct in tests used for the screening of new steroid therapeutic agents prior to clinical application. Decrease in H/C occurs in the rat in other connective tissue-rich organs in addition to the skin(3). The findings suggest that in man the composition of the ground substance of lung, blood vessels and bone is affected as well.

Summary. Dermal punch biopsies were obtained from 41 subjects prior to and following 2 weeks of daily medication with prednisone, methyl prednisilone or triamcinalone. The hexosamine-collagen ratio (H/C) was determined. In 32 subjects decrease in H/C values of 5% or greater were observed. The mean change in H/C in 35 patients following corticoid treatment was −12.9% ±1.5%.

Analysis of skin punches for hexosamine and collagen was carried out by George Bonorris.

† This classification was established because in previous experience obtained with multiple biopsies from rats and dogs taken concurrently or on different days from the same region of skin, values of H/C did not exceed ±5% of the mean. It was considered clinically unjustifiable to obtain more than one punch at a time in human subjects and consequently this same technical error is assumed.

‡ Standard error.

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Local Action of Oxytocin on Mammary Glands of Postpartum Rats After Litter Removal.*† (25432)

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Petersen(1) first suggested that oxytocin may stimulate release of prolactin by the anterior hypophysis and thereby initiate lacta-

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tion. This was challenged by Meites and Turner(2,3) who reported that oxytocin does not induce prolactin discharge or initiate lactation in laboratory animals. Both oxytocin and prolactin are released by the suckling stimulus in lactating rats (see 4), as indicated by ejection of milk and decrease in pituitary prolactin content. There is no evidence, however, that release of prolactin following suckling is dependent on stimulation by oxytocin (4,5). In contrast to its inability to initiate lactation, oxytocin has recently been shown by Benson and Folley(6) and Benson *et al.* (7) to maintain mammary secretion in postpartum rats after litter removal. This was confirmed in our laboratory(8,9) and by McCann *et al.*(10). Inasmuch as prolactin also maintains mammary secretion in such rats (see 4), Benson and Folley(6) and McCann *et al.*(10) reaffirmed that the effects of oxytocin were mediated through inducing prolactin release. Absence of any direct evidence for the latter viewpoint has led us to examine the possibility that the action of oxytocin in maintaining mammary secretion in postpartum rats after litter removal is due to its well-established ability to induce ejection of milk from the alveoli into the larger ducts.

Methods. Five groups of 5 rats each (Carworth strain), weighing 200-250 g, were bred and on 4th day after parturition their litters were removed. Mammary secretion was subsequently maintained by injecting them subcutaneously as follows: Group 1, 1 mg prolactin‡ daily for 5 days; Group 2, 2 mg prolactin daily for 5 days; Group 3, 2 mg prolactin daily for 9 days; Groups 4 and 5, 1 IU oxytocin§ twice daily for 5 days. About 16 hours after last injection, a biopsy was taken from the right inguinal mammary gland of each rat under light ether anesthesia, and 30 minutes later Groups 1-4 received an intraperitoneal injection of 2 IU oxytocin and Group 5 an intraperitoneal injection of .85% saline. Ten minutes later, all rats except Group 2 were killed and remainder of right inguinal mammary gland was removed. From Group 2, biopsies were taken from right inguinal mammary gland at 10 minutes and 1, 2, 4 and 8 hours after oxytocin injection. Mammary tissues were sectioned and stained by standard histological methods, and examined microscopically.

Results. More mammary secretion was seen in Groups 1 and 2 after 5 days injection with prolactin than in Group 3 after 9 days of similar treatment. About the same amount of milk was seen in mammary alveoli of rats injected with oxytocin for 5 days (Groups 4 and 5) as in Groups 1 and 2, but the mammary ducts were better filled with secretion in the former rats. Epithelial cells lining most of the alveoli of prolactin and oxytocin-injected rats were flattened by the secretion, and blood capillaries surrounding alveoli appeared closed.

Gross and histological appearance of the mammary glands 10 minutes after intraperitoneal injection of oxytocin was profoundly different from biopsies taken previously. When fresh mammary glands were first removed and viewed macroscopically through transmitted light, the ducts leading to the nipples were widely distended with milky secretion, in contrast to the very thin ducts in mammary glands prior to oxytocin injection. Microscopically, the mammary alveoli of oxytocin-injected rats were largely empty of secretion and were shrunken or collapsed, while the ducts were heavily swollen with secretion. In many sections, the distended ducts appeared to take up as much or more space than the alveoli in the mammary parenchyma. Some alveoli had ruptured and the secretion had apparently escaped into the stromal tissue spaces. In Group 5, injected with saline, the alveoli remained filled with secretion and could not be distinguished from control mammary biopsies taken prior to saline administration. Representative mammary sections are shown in Fig. 1-4.

In Group 2, somewhat greater emptying of alveoli appeared to occur after one hour than 10 minutes after intraperitoneal injection of oxytocin. Subsequently, little change was observed until the 8th hour, when a moderate amount of secretion had reappeared in the alveoli and the ducts were less distended. Ap-

‡ Prolactin (1 mg = 20 IU) was kindly donated by Endocrinology Study Section, NIH.

§ Oxytocin was supplied through courtesy of Dr. D. A. McGinty, Parke, Davis and Co.

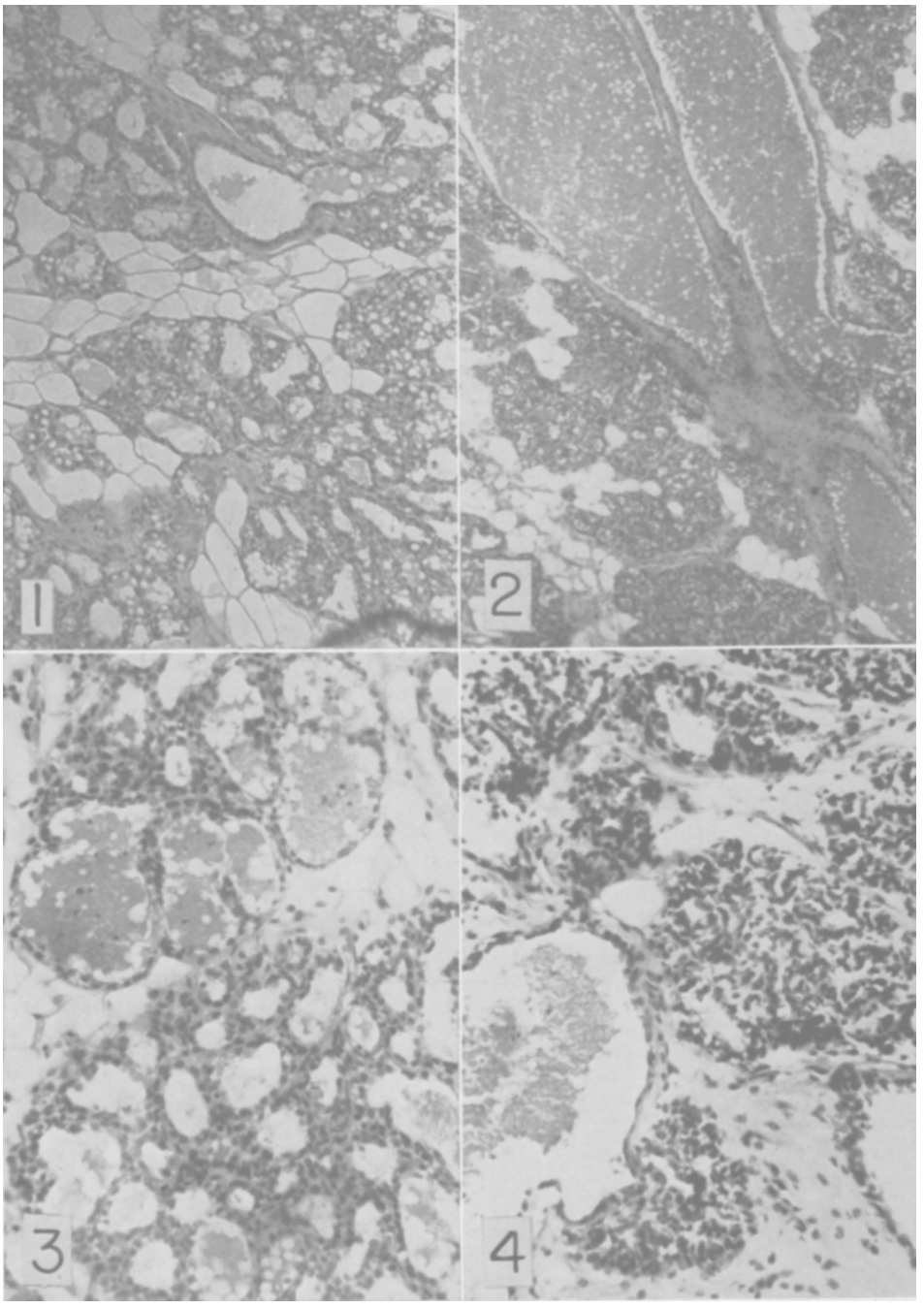

FIG. 1-4. Histological sections of mammary glands from postpartum rats after litter removal: (1) From rat given 2 mg prolactin daily for 5 days. × 45. (2) From same mammary gland 10 min. after intraper. inj. of 2 IU oxytocin. Note shrunken alveoli with relatively little secretion in contrast to engorgement of ducts. × 45. (3) From rat given 1 IU oxytocin twice daily for 5 days. Note distended alveoli with flattened epithelial cells. × 135. (4) From same mammary gland 10 min. after intraper. inj. of 2 IU oxytocin. Note collapsed alveoli and milk in ducts. × 135.

parently, more than 8 hours was required to refill the alveoli after oxytocin administration. Linzell(11) observed that alveolar diameter in mammary glands of mice increased 10-fold 6 hours after milk removal by suckling litters. No comparable increase in alveolar diameter was apparent in the mammary gland of unsuckled rats after oxytocin administration.

Discussion. These results show that in postpartum rats after litter removal, oxytocin induces ejection of milk into the larger duct, just as it does in rats from which milk is normally removed by litters. It is probable that secretion from ruptured alveoli is ejected into stromal tissue spaces, although no such material was detected microscopically and may have been lost during staining procedures. Selye(12) did observe milk in the stroma of suckled rats from which milk could not be removed because the main ducts had been cut. Emptying of alveoli is believed to reduce the pressure against epithelial cells and the blood capillaries surrounding them, permitting more synthesis of milk under stimulus of circulating prolactin and other hormones.

The fate of ejected secretion in ducts is not clear. It is probable that some is reabsorbed into the circulation, and some may flow back into the alveoli. Linzell(11) observed the latter phenomenon following oxytocin injections in anesthetized mice from which milk was prevented from leaving the nipples. Rate of milk secretion by mammary glands of postpartum rats treated with prolactin or oxytocin after litter removal, is obviously much less than if milk is removed. Continuation of mammary secretion in non-suckled rats is believed to depend on establishing an equilibrium between rate of milk produced and that reabsorbed into the circulation.

Benson and Folley(6) do not believe that the action of oxytocin in maintaining mammary secretion in postpartum rats after litter removal is a direct one on the mammary gland, since they were unable to observe milk secretion following oxytocin injections into hypophysectomized rats. However, milk secretion cannot be maintained without stimulation from prolactin and ACTH(4). Recently, we reported that when oxytocin and prolactin were injected together into postpartum rats after litter removal, mammary secretion was maintained at a higher level and for a longer period of time than when prolactin or oxytocin were given alone(9,13). If oxytocin acted through increasing prolactin secretion, then oxytocin injections should have been ineffective when prolactin was also administered, and vice versa. Cortisol was found further to supplement the actions of these 2 hormones and permitted lactation to continue for 75 days. This suggests that the action of oxytocin in maintaining mammary secretion in postpartum rats after litter removal is a direct one on the mammary gland, and is not mediated through prolactin or other hormones.

Summary. 1. Since no convincing evidence exists that the action of oxytocin in maintaining mammary secretion in postpartum rats after litter removal is due to prolactin release, attempts were made to determine whether its effects were exerted directly on the mammary gland. After maintaining milk secretion in 25 such rats for 5 or 9 days by daily injections of prolactin or twice-daily injections of oxytocin, a mammary biopsy was taken from each rat. They were then given an intraperitoneal injection of 2 IU oxytocin or physiological saline, and 10 minutes later all except 5 rats were killed and the remainder of the previously biopsied mammary gland was removed. Mammary biopsies from the remaining 5 rats were taken after 10 minutes and also 1, 2, 4 and 8 hours later. 2. Histological examination revealed that prior to intraperitoneal oxytocin administration, most mammary alveoli contained considerable secretion and the ducts were relatively thin, whereas 10 minutes after oxytocin the alveoli appeared shrunken or collapsed with little or no secretion, and the ducts were widely distended with milk. No notable increase in alveolar filling followed until the 8th hour after oxytocin injection. Saline had no effect on mammary alveoli and they remained filled with secretion. 3. Ejection of milk from the alveoli into larger ducts by oxytocin is believed to remove the pressure on epithelial cells which secrete milk and on blood capillaries surrounding them, permitting synthesis

of more milk by circulating prolactin and other hormones. Milk ejected into ducts is believed to be gradually reabsorbed into the circulation, and some may flow back into the alveoli. These local effects of oxytocin on the mammary gland are believed to account for its ability to maintain mammary secretion in postpartum rats after litter removal.

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A Modified Bile Return System in the Dog.* (25433)

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Steatorrhea in bile fistula dogs was reduced substantially following hourly return of the dogs' own bile to the duodenum(1). The steatorrhea was greater when bile was returned every 4 to 8 hours, even though total cholic acid returned was the same. Thus, it appears that bile must be in the intestine nearly continuously to effect normal fat absorption. However, complete correction of the steatorrhea of bile deprivation by continuous return of bile has not been demonstrated. A modified Rous-McMaster bile fistula preparation(2) is described in this report, permitting free entry of bile to the duodenum, and restoration of normal fecal fat levels following bile deprivation.

Methods. Duodenal and gall bladder cannulas were prepared for implantation with Tygon tubing (1/16″ wall, 1/4″ and 5/16″ O.D. respectively, overall length approximately 12″ each). Two Tygon collars (Fig. 1; a,b) and an abdominal wall brace (c,d) were cemented on each cannula with Tygon paint. The cannulas were then coated with a silicone preparation (Siliclad, Clay Adams, Inc.) to minimize bile sedimentation on the inner wall. Sterilization was accomplished by soaking in benzalkonium chloride (Zephiran, Winthrop Stearns, Inc.). Clogging of the duodenal cannula tip with intestinal contents was obviated by placing 1½″ slits bilaterally in the intraduodenal segment (e). The surgical procedure was carried out aseptically on mongrel female dogs weighing 9 to 18 kg with due regard for the requirements of proper animal care. The common duct was ligated and sectioned. The cannulas were anchored with a double row of purse string sutures and brought through stab incisions in the right abdominal wall (Fig. 1). The bile cannula extended into the base of the gall bladder about ½″ (f), in a line with the cystic duct. A male luer fitting (g) and a stethoscope fitting (h) were inserted. During bile collection periods the duodenal cannula was capped and bile was drained into a rubber balloon. During bile return periods the 2 cannulas were connected to reestablish the enterohepatic biliary circuit. A many-tailed binder covered the cannulas. A balloon bag was pinned to the binder during collection periods. 30-50 ml of isotonic saline was in-

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