20. Differences in light-reflecting pigmentation of the organisms is also a factor in estimating relative population densities. For example, when flood lamps were used, I estimate that the silver myctophid could be seen at distances up to 7 m; the large *Nanomia*-type physonect with orange-pigmented gastrozooids was recognizable at up to 5 m; and the small *Nanomia*-type could be seen only at distances of up to 3 m. The half-angle of the cone of vision from the saucer port is about 45° in water; thus these volumes compute to 359, 131, and 28 m^3, respectively. Use of the movie light at least doubles these distances and the volumes increase eight times. I did not keep a separate record of sightings while using the regular floods and the movie light. Because of these variables, I have not attempted to reduce numbers of organisms observed to a common volume denominator.
21. Examination of specimens provided by R. L. Wisner was done by R. Capen. Descriptions and measurements of these and other bathypelagic fishes are in manuscript.
22. U.S. Natl. Defense Res. Committee Div. 6, *Summary Tech. Rept.* (1948), vol. 7. Andreeva and Chindonova (*12*) have recently considered the sheer modulus effect on bubbles, which theoretically enhances resonance at 300- to 400-m depths.
23. J. Kanwisher and A. Ebeling, *Deep-Sea Res.* **4**, 211 (1957), have questioned, on a physiological basis, whether physoclistous, swim-bladdered fishes can secrete and absorb gas rapidly enough to maintain neutral buoyancy throughout their vertical range. In this respect, note that the silver myctophids observed on their downward migrations were swimming rapidly. In contrast, their upward swimming was more leisurely, and upward migration of the main layer associated with these fish was slow, never exceeding 2 m/min and taking several hours to complete.
24. I thank R. Kientzy for skilled saucer piloting, and N. Shenton and the Westinghouse team for logistic support. R. Nubigin, W. Bunton, R. Bradley, and I. Davies assisted in operations at sea. J. Flynn aided analysis of data. G. Curl, E. Buffington, W. Batzler, G. Pribble, E. Hamilton, and G. Pickwell have given advice. Conversations with R. Backus have been helpful.

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Mammary Glands of Pregnant Rats: Development Stimulated by Licking

Abstract. *At the end of gestation, the mammary glands of pregnant rats that have been prevented from licking their ventral surfaces by neck collars are about 50-percent less developed than those of control animals. Neither the burden nor the stress effect of the collar is an alternative explanation. A considerable proportion of mammary development during pregnancy is thus caused by the female's own licking.*

Although suckling and other stimulation of the ventral surface of the rat are known to maintain lactation and cause mammary growth after parturition, there is no evidence that sensory stimulation contributes to mammary development during pregnancy (*1*). Yet by the time the female gives birth her mammary glands have increased considerably in size and have begun to produce milk (*2*).

A striking behavioral feature of the pregnant rat is intensified licking of her own ventral surface. Recently we reported that licking of the nipple lines and of the genital and pelvic regions increases markedly with the advance of pregnancy, whereas licking of the more anterior body parts, of the head, forepaws, shoulders, and upper back, tends to decline (*3*).

Since sensory stimulation is necessary for postpartum mammary function, and since self-licking is prominent in the behavior of the pregnant rat, it seems reasonable to ask whether self-licking stimulates mammary development during pregnancy. Mammary development was assessed at the end of gestation in rats that had been prevented from licking themselves throughout pregnancy and in several groups of controls that had been allowed to lick.

The main group of nulliparous pregnant rats (*4*) were prevented from licking by attachment around the neck of an 8.7-cm-wide full rubber collar so designed that the female could not extend her head beyond the collar's edge and lick her ventral surface (Fig. 1, *5*).

To control for the burden of wearing a full collar, a second group wore notched collars, equal in weight to the full collars but having 5-cm notches cut out to allow the females to reach under the collar and lick their bodies. A third group, uncollared throughout pregnancy, provided normative data on mammary development.

Because the full collar not only prevented licking but also interfered with various normal behavior patterns, interference that could cause stress (*6*), it was necessary to add another control group in which licking was allowed but in which stress was present. This fourth group therefore wore no collars but were injected twice daily with 0.25 ml of 2-percent formalin, a procedure reported to produce a stress reaction shown by enlargement of the adrenal glands, without interfering with pregnancy (*7*). A fifth group, injected with distilled water, served as controls on the formalin injection.

Samples of mammary gland were obtained after the females had been killed on the 22nd day of pregnancy, just prior to parturition. The left abdominal mammary gland was removed and fixed in 10-percent formaldehyde solution for 24 hours; a 0.5-mm piece of tissue close to the anteriormost nipple was then cut away,

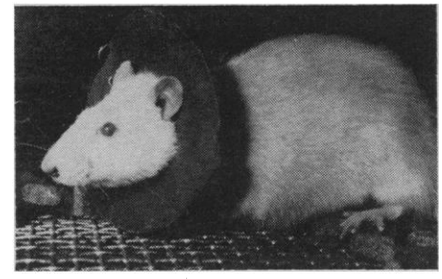

Fig. 1. A pregnant rat wearing a full collar.

dehydrated and cleared, and embedded in paraffin before being serially sectioned at 5 μ and stained with hematoxylin and eosin. One section from each gland was chosen randomly whenever the developmental level was assessed.

As a measure of mammary development, the proportion of secretory tissue to total glandular tissue was obtained by projecting the image of a section on a paper of uniform weight and by tracing the image in detail; the area representing the entire gland section was then cut out and weighed, as were the smaller areas representing secretory tissue. Secretory tissue was thus determined as a weight percentage of the total gland section. This weighing method is comparable to a standard planimeter technique (*8*); it yields results that correlate positively with ratings of the density of alveoli within the lobules, as well as with the amounts of secretion within them.

Figure 2 shows that the mammary glands of full-collared rats contained only about half as much secretory tissue as those of each control group. An analysis of variance for all groups was significant (F, 36.23; $p<.001$), as was the difference between the full-collared group and each control group, revealed by Duncan's new multiple-range test at the 1-percent level.

Since the notched-collared group did not differ significantly from the uncollared group, the burden of the collar is

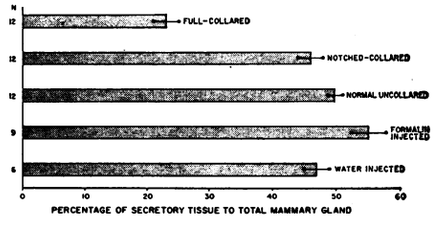

Fig. 2. Mammary development during pregnancy in the five groups of rats. Mean ± SE, •—|—•.

ruled out as an explanation of the mammary underdevelopment in the full-collared group.

The physiological irritant, formalin, far from retarding mammary development, increased it to the highest level, significantly exceeding the level of development in the group injected with distilled water. The stress effects of restraint also are thus ruled out as a cause of the underdevelopment.

We conclude that the mammary underdevelopment in the full-collared rats was caused by the prevention of self-licking and not by the method of prevention. Self-licking is thus shown to effectively stimulate mammary development during pregnancy, at a time when mammary growth and secretion were heretofore thought to be primarily under endogenous control.

Among sites that are licked on the ventral surface are the nipple areas and the genital and pelvic regions. The most likely route by which the licking stimulates the mammae, however, is by way of sensory receptors located in the nipples. This possible dependence of mammary development during pregnancy on stimulation of the nipples is consistent with what is known about the sensory conditions that maintain postpartum mammary function, and it suggests that a similar neuroendocrine mechanism and a similar hormone (or hormones) are involved. Grosvenor and Turner (*9*) implied a similar conception when they suggested that late in gestation a neural stimulus, possibly arising from the uterus, caused release of prolactin from the pituitary, which in turn caused prepartum lactation. Our results indicate that a behavioral event of pregnancy can provide the required stimulation.

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Mucopolysaccharides: N-Acetylglucosamine- and Galactose-6-Sulfates from Keratosulfate

Abstract. *Galactose-6-sulfate and* N*-acetylglucosamine-6-sulfate were obtained pure from a partial acid hydrolyzate of corneal keratosulfate by paper chromatography and electrophoretic fractionation. These sugars were also present in hydrolyzates of skeletal keratosulfate. The distribution of the sulfate groups in the various keratosulfates might depend upon their source, and this distribution probably has important biological significance.*

Keratosulfate is a sulfated mucopolysaccharide composed of equimolar amounts of D-galactose and *N*-acetyl-D-glucosamine and of variable quantities of methylpentose and sialic acid (*1*). It has been isolated from cornea (*2*), nucleus pulposus (*3*), and cartilage of mammals and lower species (*4*). In these tissues keratosulfate is covalently bound to protein by at least two different types of bonds, either by *N*-glycosyl bonds to the amide groups of asparagine and glutamine or by *O*-glycosyl bonds to the hydroxyls of serine and threonine (*1*). The *O*-glycosidic bonds are alkali-labile and are split by β-elimination (*5*). The *N*-glycosyl bonds are mainly present in keratosulfate of cornea, the *O*-glycosyl linkages in skeletal keratosulfate. The structure of corneal keratosulfate was deduced from methylation and enzymatic digestion of the sulfated and desulfated polymer as *N*-acetyllactosamine-6-sulfate, polymerized by 1,3 bonding of the alternating hexosaminyl and D-galactosyl moieties (*6*).

Since the yields of the isolated methyl ethers were rather low, the methylation of bovine cornea keratosulfate was reinvestigated. Improved methods markedly increased the yields of methyl ethers, which, in general, confirmed the proposed structure. However, the evaluation of the experiment suggested that carbon No. 6 of galactose was partly blocked, indicating either branching at this position or substitution by another group.

After searching for optimum conditions we obtained, on hydrolysis of 1.0 g of keratosulfate for 1 hour with 0.5*N* H_2SO_4 at 100°C, two sulfated monosaccharides, which were isolated by paper chromatography and electrophoresis. Fraction I (8.0 mg) was galactose-6-sulfate, the other (fraction II, 13.2 mg) *N*-acetylglucosamine-6-sulfate. In addition, a number of sulfated oligosaccharides were present.

The properties of fraction I are as follows: $[\alpha]_D$ as barium salt was +42.5°, as ammonium salt was +52.0°. In paper chromatography its migration was 10 percent slower than that of synthetic glucose-6-sulfate. On paper electrophoresis at *p*H 6 it had the same mobility as glucose-6-sulfate. Its staining with aniline phthalate and tetrazolium chloride showed that the reducing group and carbon No. 2 were free.

The ratio of sulfate to galactose was 1.06. The reducing value (Schales and Schales) was 84 percent that of galactose. On infrared spectroscopy the substance absorbed at 1240 and 820 cm^{-1}, showing the absence of a sulfate on carbon No. 4. It consumed 3 moles of periodate rapidly in unbuffered solution and then consumed slowly an additional mole. Negligible amounts of formaldehyde were released on oxidation in bicarbonate buffer (*p*H 7.5) for 24 hours (*7*).

The properties of fraction II were as follows: $[\alpha]_D$ as ammonium salt was +47.9°. Chromatographically and electrophoretically it had the same mobility as authentic *N*-acetylglucosamine-6-sulfate. It stained with aniline phthalate and gave a positive reaction for *N*-acetylhexosamine. The ninhydrin reaction was negative. On complete hydrolysis glucosamine was the only carbohydrate present. The ratio of sulfate to *N*-acetylglucosamine was 0.97, that of sulfate to glucosamine was 0.98. The reducing value (Schales and Schales) was 98 percent that of *N*-acetylglucosamine. On infrared spectroscopy the substance absorbed at 1240 and 820 cm^{-1}, indicating a primary sulfate ester group.