pulse echo ultrasound and, to an even greater extent, with x-ray mammography, extensive clinical experience and careful analytic studies correlating breast characteristics with image properties can lead to very useful diagnostic procedures. Many who have worked with pulse echo ultrasound consider it to be not only useful, but even the method of choice for diagnosing benign breast conditions and imaging young dense breasts (*7*). It probably is not far behind x-ray mammography in the general diagnosis of malignancy, but diagnostic accuracy of pulse echo imaging lags somewhat where relatively fatty breasts are concerned (*8*). As in the two examples shown here, however, the speed-of-sound images are most revealing in fatty breasts. It appears that the combination of UCT and pulse echo ultrasound is usually superior to either imaging technique alone.

We expect this diagnostic improvement to stimulate the development of clinical prototype pulse echo-UCT units capable of searching the breast for occult masses in a clinically acceptable examination time. Considerable improvement in image quality can be expected over the examples shown here. The signal-to-noise ratio in the pulse echo electronics of our system can be improved significantly, and progress is being made in the development of UCT attenuation and speed of sound techniques that will give higher resolution and a much more accurate quantitative representation of the bulk attenuation coefficient of the imaged tissues.

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The Hagfish Slime Gland: A Model System for Studying the Biology of Mucus

Abstract. *The hagfish slime gland may provide a model system for studying certain aspects of the biology of mucus. Mucus is obtained in nonhydrated form by electrically stimulating the anesthetized hagfish and the secretions are stirred into ammonium sulfate. Centrifugation and filtration are then used to isolate the two major secretory products, mucous vesicles and threads. Specific advantages of the model and potential applications for research are discussed.*

The epidermally derived slime glands of the hagfish are primarily responsible for its capacity to produce copious quantities of mucus (*1*). In the Pacific hagfish *Eptatretus stouti* (*2*) there are approximately 150 of these glands (Fig. 1A) evenly spaced in two linear rows along its ventrolateral sides. Each slime gland is connected to the epidermal surface by a short duct, and the location of a gland is readily identified by its grossly visible pore on the epidermal surface (Fig. 1, A and C). The glands contain two large and morphologically distinct cell types: gland thread cells and gland mucous cells (Fig. 1B). The gland thread cell is filled with a long, coiled, proteinaceous thread (*3*); the gland mucous cell is filled with mucus-containing vesicles. The entire gland is surrounded by a connective tissue capsule, and outside the capsule are skeletal muscle fibers.

In this report we (i) propose that the hagfish slime gland may provide a model system for studying the biology of mucus, (ii) describe methods of secretory product acquisition, manipulation, and separation, (iii) point out some characteristics of the secretory products, and (iv) discuss possible research applications of the model.

Using a modification of an electrical stimulation technique suggested by Ferry (*4*), we collected gram quantities of the two gland cell types and their products. Anesthetized hagfish (*3*) were draped over a beaker covered with absorbent paper and blotted dry. An electrical stimulator was used to administer a mild electric shock (*5*) to the skin adjacent to a slime gland pore (Fig. 1C). This caused the skeletal muscle cells outside the slime gland capsule to contract, expelling the contents of the gland onto the epidermal surface, where they formed a large white drop (Fig. 1, C and D). The glandular exudates were then harvested with a spatula (Fig. 1D) for processing.

One of many advantages to obtaining the glandular contents by this procedure is that hydration of the glandular exudates, which occurs during normal secretion, is circumvented. The extent to which these exudates are hydrated during normal secretion in an aqueous environment is indicated by stirring the electrically obtained exudates into seawater (Fig. 1, E to G) (*6*).

Although stirring the exudates into seawater provides useful information on mucus hydration, the extensive dilution and resultant high viscosity of the mucus makes biochemical characterization of the cellular components of the mucous

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and thread cells more difficult. We have observed, however, that if electrically obtained slime gland exudates are stirred into 1*M* $(NH_4)_2SO_4$ solution, the hydration phenomenon does not occur. The threads of the thread cells retain their tightly coiled configuration, and, although the mucous cells break open, the mucous vesicles discharged are stabilized (*7*).

In a typical isolation, glandular exudates are stirred into 1*M* $(NH_4)_2SO_4$ containing 60 percent sucrose (weight to volume). The sucrose helps keep the vesicles in suspension as the thread cells are removed by low-speed centrifugation. The supernatant containing the stabilized mucous vesicles is passed through 60- and 41-μm nylon mesh (*8*). Since the thread cells measure approximately 80 by 150 μm and the stabilized vesicles are 3 to 5 μm in diameter, the vesicles pass through the mesh and any residual thread cells are filtered out (*9*). Subsequent lowering of the sucrose concentration of the vesicle-containing filtrate by dilution with 1*M* $(NH_4)_2SO_4$ permits removal of the stabilized vesicles by a second centrifugation (*10*). Scanning electron micrographs of the separated products (coiled threads and stabilized mucous vesicles) are shown in Fig. 1, H and I.

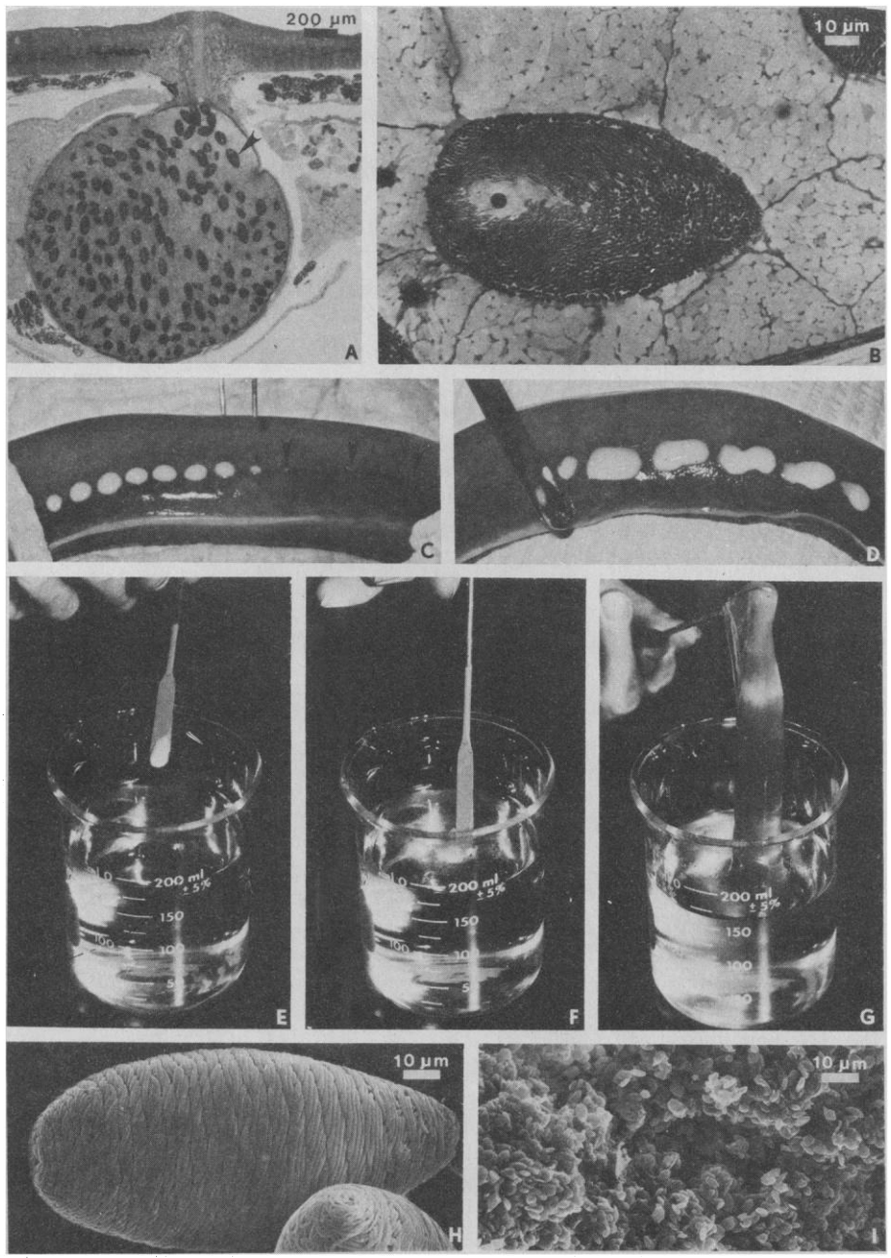

Fig. 1. (A) Photomicrograph of a 1-μm-thick cross section through an Epon-embedded slime gland. The darkly stained cells (arrow) are thread cells and the lighter cells are mucous cells. (B) High magnification view showing the long fibrous protein thread in a thread cell and mucus-containing vesicles in mucous cells. (C) Electrical stimulation of an anesthetized hagfish. The cellular contents of the glands are being exuded onto the epidermal surface. Eight glands have been stimulated; the pores of nonstimulated glands (arrows) can be seen on the right. (D) The cellular exudates are readily removed with a spatula. (E to G) Sequence of photographs taken at 5-second intervals, showing a spatula bearing a small amount of exudate (E) being stirred in seawater (F) and then lifted out of the water (G). (H) Scanning electron micrograph of an isolated thread cell. (I) Scanning electron micrograph of isolated mucous vesicles.

As long as the electrically obtained cellular exudates are kept in 1*M* $(NH_4)_2SO_4$, the vesicles remain stabilized and the threads remain coiled. Once the stabilized vesicles are separated from the thread cells, the amount of vesicle material present in a suspension of stabilized vesicles can be determined turbidimetrically (*11*) and adjusted to a standard dilution or suspension. Finally, if the $(NH_4)_2SO_4$ solution is diluted or removed by dialysis (as against water or seawater), the vesicles hydrate to form a clear, viscous mucus and the threads uncoil to form a tangled network of fibers.

The hagfish slime gland thus provides us with a system for studying the biology of mucus. Using this system, we can minimize the possibility of exogenous contaminants in the collected mucus, control the desired degree of mucus dilution or hydration, work with nonviscous preparations until viscous preparations are desired, and seek to characterize the enzyme systems responsible for the formation of the gland cell products. We are confident that data obtained in one laboratory will be reproducible in other laboratories.

This system may be of use in research on (i) mucus structure and chemical composition, (ii) factors involved in the hydration and gelation of mucus, (iii) effects of *p*H, ionic concentration, and temperature on the physical properties of mucus, (iv) interactions of mucus with fibrous proteins, and (v) biochemical analysis of the synthetic pathways of mucous and fibrous proteins.

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5. We used a Grass 56 stimulator to deliver 80-Hz, 24-V, 0.8-msec shocks.
6. Although we do not routinely stir the harvested slime gland cells back into seawater, this procedure clearly demonstrates that hydration phenomena must be considered when studying the formation and properties of mucus.
7. We prefer the term stabilized to intact because the degree of integrity of the vesicle membranes is not clear; nevertheless, the vesicle contents remain stable and do not appear to interact with water or other components of either of the gland cell types.
8. We used Nitex Swiss nylon monofilament screening fabric (Tetko HC 3-60 and HC 3-41).
9. If thread cells are desired, they can be collected by backwashing the mesh with 1*M* $(NH_4)_2SO_4$.
10. We have found that initial centrifugation at 1000*g* for 10 minutes is sufficient to remove the thread cells. Dilution of the sucrose to 30 percent by adding an equal volume of 1*M* $(NH_4)_2SO_4$ permits pelleting of the vesicles at 3000*g* for 15 minutes. All isolation procedures are performed at 4°C.
11. Freshly harvested vesicles are suspended in 1*M* $(NH_4)_2SO_4$, appropriately diluted; the optical density is read at 350 nm. An optical density of 0.5 at 350 nm is equivalent to 0.11 mg/ml (dried weight).

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Reduction in Oocyte Number Following Prenatal Exposure to a Diet High in Galactose

Abstract. *When pregnant rats were fed a 50 percent galactose diet there was a striking reduction in oocyte number in the offspring. The most prominent effects were noted after exposure to galactose during the premeiotic stages of oogenesis. Prenatal exposure to galactose or its metabolites may contribute to the premature ovarian failure characteristic of human galactosemia.*

In classical galactosemia a congenital deficiency of galactose 1-phosphate uridyltransferase results in the accumulation of galactose 1-phosphate, galactose, and galactitol in various tissues and body fluids. Dietary galactose is a substantial source of galactose 1-phosphate; however, even without galactose intake some galactose 1-phosphate can still be synthesized from uridine diphosphate glucose (*1*). The disease is characterized by mental retardation, cataracts, hepatosplenomegaly, and renal tubular dysfunction. Recent clinical observations suggest a frequent association between galactosemia and premature ovarian failure, even when a galactose-restricted diet is started early in infancy (*2*). Endocrinologic studies indicate hypergonadotropic hypogonadism. Hypoplastic uteri and streak ovaries have been observed in some cases. Galactosemic women with ovarian failure have normal karyotypes, elevated levels of bioactive gonadotropins even before puberty, and an absence of antiovarian antibodies (*2, 3*). The etiology of premature ovarian failure in galactosemia is not clear.

Since a 30 to 50 percent galactose diet produces cataracts, liver abnormalities, and aminoaciduria in rats, these animals can be used to model the human galactosemia syndrome (*4*). We report here experiments on rats to explore the effects on oocyte and follicle number resulting from prenatal or early postnatal exposure to a diet high in galactose.

Sprague-Dawley rats were divided into several groups for exposure to a 50 percent galactose diet (Zeigler Bros., Inc.). One group was exposed to galactose prenatally from the third day after conception until birth. Another group was exposed postnatally: the diet was given to lactating mothers after they gave birth and was continued until weaning at approximately 1 month of age (a subgroup received the galactose diet from 1 month of age until 2 months). A third group was treated with galactose both pre- and postnatally. Control animals received a normal diet.

Body and organ weights were determined after the groups were treated and killed. Galactose and galactose 1-phosphate concentrations were determined by a fluorometric method (*5*) for blood samples taken from animals maintained on the galactose or control diet for at least 4 days. Ovaries removed from rats at weaning or at 2 months of age were fixed in Bouin's medium for 24 hours and then placed in 70 percent ethanol. The oocytes and follicles were classified and counted in stained serial sections by light microscopic examination. Our classification scheme was modified from that of

Table 1. Effect of galactose on body and organ weight and oocyte number. Weights were measured at the end of each treatment period and are for six or more animals per group. Oocyte counts were performed on ovaries from animals killed at weaning (groups 2 and 3) or at 2 months of age (group 4) and are for three to five animals per group. Values are means ± standard errors.

Treatment	Weight (g)				Number of oocytes per ovary		
	Body	Liver	Kidney	Brain	Small	Medium	Large
1. Prenatal (day 3 post-conception to birth)							
Control	4.18 ± 0.04	0.29 ± 0.01		0.15 ± 0.04			
Galactose	2.86 ± 0.10*	0.19 ± 0.05*		0.11 ± 0.01*			
2. Pre- and postnatal (day 3 postconception to weaning)							
Control	53.34 ± 2.86	2.16 ± 0.16	0.58 ± 0.03	1.29 ± 0.03	6480 ± 880	980 ± 157	175 ± 42
Galactose	18.03 ± 2.09*	0.71 ± 0.11*	0.27 ± 0.03*	0.87 ± 0.04*	1372 ± 294†	668 ± 82	84 ± 31
3. Postnatal (birth to weaning)							
Control	36.76 ± 0.89	1.59 ± 0.02	0.22 ± 0.01	1.11 ± 0.05	7517 ± 633	974 ± 157	197 ± 43
Galactose	12.12 ± 0.25*	0.43 ± 0.05*	0.09 ± 0.01*	0.89 ± 0.01*	7288 ± 317	1000 ± 187	120 ± 43
4. Postnatal (1 to 2 months)							
Control	211.5 ± 5.24	8.06 ± 0.32	1.80 ± 0.07	1.61 ± 0.06	3805 ± 195	650 ± 36	265 ± 11
Galactose	120.4 ± 2.55*	6.56 ± 0.29*	1.51 ± 0.08*	1.30 ± 0.08*	4760 ± 522	496 ± 40*	172 ± 26*

*Significantly different from corresponding control value at $P < .05$ (Student's *t*-test). †$P < .0001$.

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