References and Notes

1. Other investigators working with hagfish epidermal secretions have used the terms slime, mucus, and mucin interchangeably [W. W. Newby, *J. Morphol.* **78**, 397 (1946); R. Strahan, *Copeia* **2**, 165 (1959); A. Lehtonen, J. Kärkkäninen, E. Haati, *Acta Chem. Scand.* **20**, 14 (1966); R. H. Spitzer, S. W. Downing, E. A. Koch, *Cell Tissue Res.* **197**, 235 (1979)]. It is not known how hagfish mucus and mammalian mucus compare chemically.
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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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Table 2. Galactose and galactose 1-phosphate concentrations in maternal and fetal blood. Duplicate determinations were made for each animal and averaged. Values shown are group means and (in parentheses) ranges.

Group	Number of animals	Galactose (mg per 100 ml)	Galactose 1-phosphate (mg per 100 ml)
Controls	10	< 1.5	<1.0
Prenatal			
Mothers	4	357 (107 to 450)	14 (1.3 to 33)
Fetuses	20	387 (120 to 540)	15 (1.6 to 41)
Postnatal			
Mothers	3	44 (11 to 62)	2.0 (1.3 to 3.6)
Pups, birth to weaning	8	35 (7.5 to 101)	1.8 (1.0 to 3.0)
Pups, after weaning	12	638 (68 to 900)	

Pedersen and Peters (*6*) as follows: small follicles ranged from the smallest oocyte to an oocyte surrounded by no more than a single layer of follicular cells; medium follicles contained growing oocytes surrounded by more than one layer of follicular cells and had no antrum; and large follicles were the more mature antral follicles. Oocytes and follicles were counted without knowledge of the treatment group represented.

In the prenatal treatment group there was only a small to moderate effect on mean body and organ weight (Table 1). Weight reductions were much larger in the pre- and postnatal treatment group and in the postnatal treatment groups. Galactose treatment during the month after weaning produced less dramatic weight changes.

In animals in the pre- and postnatal treatment group there was a mean reduction of about 80 percent in the number of small follicles (Table 1). No significant decrease in the number of small follicles was noted in either postnatal treatment group, even though these groups had substantial reductions in body and organ weight. In this experiment there were no survivors in the prenatal treatment group for ovarian study. There was no significant difference between treatment and control groups in the mean number of medium and large follicles, except in ovaries of pups from the postweaning treatment group (1 to 2 months), which showed a small but significant reduction in the number of these follicles.

In general, during treatment the concentrations of galactose and galactose 1-phosphate were slightly higher in fetal blood than in maternal blood (Table 2). Lactating mothers and their pups had lower concentration of these sugars in their blood during galactose exposure than did pregnant dams and their fetuses. [This is consistent with the observations of Wells and Wells (*7*).] Control animals had concentrations below the level of detection.

Thus exposure to galactose both before and after birth substantially decreased the mean number of small follicles and body and organ weight at weaning, while treatment from birth to weaning produced a comparable decrease in body and organ weight but had no effect on oocyte number. Although blood galactose concentrations were lower in the suckling pups than in the prenatally exposed fetuses, the group exposed to galactose from 1 to 2 months of age had galactose concentrations similar to those in prenatally exposed fetuses yet manifested much less marked ovarian effects.

In the rat the migration of primordial germ cells from the yolk sac to the urogenital ridge takes place from days 8 to 13 after conception. The germ cells then undergo mitosis with the formation of oogonia, which continue further mitosis. The peak of mitosis occurs about $16^1/_2$ days after conception. The oocytes begin meiosis on about day 17 and are arrested at prophase of the first meiotic division until ovulation (*8*). In human females the corresponding processes are completed during the first two trimesters.

To define the periods in prenatal life when exposure to galactose affects oocyte number, groups of rats were fed the 50 percent galactose diet at different times during pregnancy: day 1 after conception to day 9, days 9 to 14, days 15 to 20, or the entire gestation period. Each group received the control diet when not on the galactose diet. There were no significant differences in body and organ weight at weaning between any of these groups, indicating reversibility of the moderate effects of prenatal galactose exposure on body and organ weight at birth. In the animals treated throughout gestation there was about a 50 percent reduction in the mean number of small follicles (Fig. 1). In the groups exposed to galactose from days 1 to 9 or from days 9 to 14 there was also a significant reduction. In the group treated from days 15 to 20 there was no reduction in the number of small follicles. There was no effect on follicle number in rats pair-fed a normal diet matched to the intake of the galactose diet throughout gestation. No significant difference in the mean number of medium or large follicles was observed between treatment and control groups.

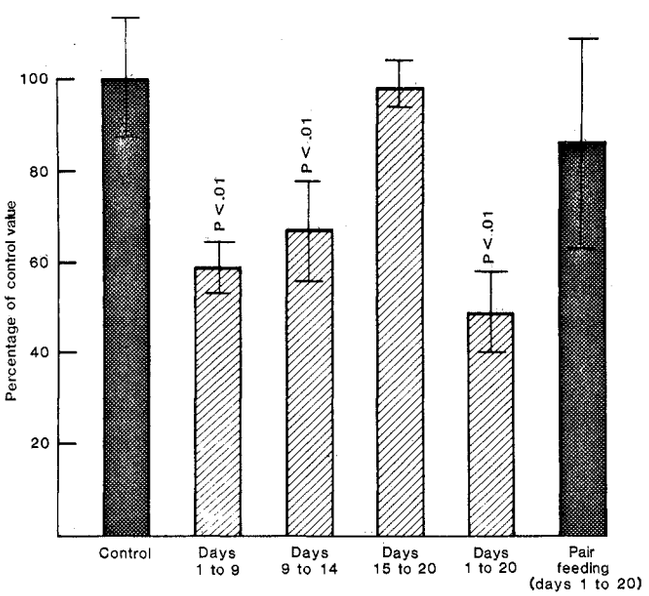

Fig. 1. Quantitation of small follicles in rats exposed to galactose during the indicated days of gestation. Oocyte counts were performed at weaning on both ovaries for at least six animals per group. Values are means ± standard errors.

Not represented in Fig. 1 are data on two surviving pups from a small group exposed to galactose from the first day after conception until weaning. The number of small follicles in one of these animals was within the range determined for the group treated with galactose throughout gestation; the other animal had very small ovaries and an almost complete absence of oocytes.

Vaginal opening, thought to be mediated by ovarian estrogens, was delayed in the animals treated with galactose from

days 1 to 9 after conception (44 ± 0.87 days, mean ± standard error) compared to the controls (38 ± 0.68 days). The delay was not observed after galactose exposure later in gestation.

We conclude that prenatal exposure to galactose or its metabolites substantially decreases oocyte number in the rat. The most striking effects were observed when exposure occurred during the premeiotic stages of oogenesis. This reduction in oocyte number might result from interference with germ cell migration, proliferation, or differentiation. The data suggest that the effects of galactose on the rat ovary are less pronounced after the initiation of meiosis. The reduction in the number of oocytes in prenatally treated rats is clearly not related simply to growth reduction secondary to galactose administration.

Prenatal galactose or its metabolites primarily reduce the number of small oocytes and follicles. The absence of effects on the medium and large follicles is unexplained but not without precedent for other ovotoxic agents. Mandl (*9*) reported that radiation reduced the number of small follicles 90 percent in the rat, and Krarup (*10*) noted oocyte destruction by postnatal exposure to polycylic aromatic compounds—without reduction in the number of medium or large follicles.

Prenatal toxic effects on oocytes or their precursors by galactose or its metabolites could be a cause of premature ovarian failure in human galactosemia. Restriction of galactose intake in pregnancies at risk for this disease might reduce or prevent fetal ovarian damage and subsequent premature ovarian failure, although galactose synthesized by the fetus or mother could still have toxic effects (*3*).

YUAN-TSONG CHEN
DONALD R. MATTISON
LIONEL FEIGENBAUM
National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205
HENRY FUKUI
National Eye Institute, National Institutes of Health
JOSEPH D. SCHULMAN
National Institute of Child Health and Human Development

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Pheromone Orientation: Role of Internal Control Mechanisms

Abstract. *Male American cockroaches walk a zigzag path upwind toward a source of female sex pheromone. Although the maximum width of the pathway is regulated by the width of an odor plume, many turns are made before the edge of a wide plume is encountered. In addition to the pheromone regulation of the insect's orientation movements, an internal mechanism appears to influence the zigzag turning pattern.*

It is generally agreed that orientation of insects toward distant sex pheromone sources is not controlled by chemotaxis, that is, by the concentration gradient of the odor, but rather by an indirect anemotactic (wind-directed) mechanism (*1*). Orientation of both walking and flying insects is never observed to be straight or direct, but occurs in irregular zigzag or sinusoidal pathways that can be characterized by two components, namely, turns (*2*) and straighter connecting portions. The mechanism controlling orientation during the straight portions is believed to entail the use of the wind direction to establish the insect's course and to correct deviations. The mechanisms that control or interact to regulate turning, however, are poorly understood.

I used a time-lapse camera system (*3*) to photograph individual male cockroaches orienting to synthetic female sex pheromone in a wind tunnel. By analysis of the insects' pathways, I compared the position of turning with the boundaries of the plume and determined the effect on the pathway of changing the width of the plume. This analysis could provide direct evidence for the pheromone regulation theory, which proposes that a turn is made only when the insect detects the decrease in odor concentration at the edge of the plume (*4*). An alternative theory is that the turning pattern is influenced or generated internally within the insect's nervous system (*5*).

An individual male was placed in a wire cage centered 0.3 m from the downwind end of a 2.5-m wind tunnel (wind speed, 22 cm/sec). After a period of 12 to 15 minutes to allow the male to adjust to its new surroundings, the side door of the cage was opened, and the male was observed for a 2-minute control period during which no pheromone was presented. Males generally ceased moving in the cage several minutes after being placed in the tunnel and usually remained motionless during the control period. A dispenser containing synthetic female sex pheromone, (±)-periplanone B (*6*), was then placed in the tunnel 2 cm above the floor and centered at the upwind end. Arousal, elicited after 0.5 to 2.0 minutes, was characterized by rapid antennal movements and locomotion. The male would then leave the cage and turn upwind, indicating a positive anemotactic polarization of the odor plume (*7*). While proceeding upwind in the narrow plume, each cockroach tended to remain within the boundaries of the plume and close to the center line of the wind tunnel (Fig. 1, B to D). Pathways in the wide plume varied considerably and included wide zigzags traversing the entire width of the plume (Fig. 1F), turns at random points within the plume (Fig. 1G), and relatively direct upwind paths characterized by a long-period sine pattern (Fig. 1G).

The coordinate positions of the insect were entered into a computer by projecting the photographic negative onto a bit-pad digitizer. Coordinates were then aligned and scaled according to reference points marked on the wind tunnel floor. Computer programs with x and y coordinates designating the distance from the source and the distance from the center axis of the plume, respectively, were used to determine the anemotactic angle (upwind angle, defined by successive data points), the turning rate (the angle made by three successive data points), the position of sign-reversal turns, and the locomotion rate (Table 1). Orientation pathways were graphically displayed superimposed on the boundaries of the plume, which were calculated from digitized photographs of white titanium tetrachloride smoke released from the same dispensers as the pheromone (*8*). The narrow plumes were similar in dimension and structure to those described previously (*9*).

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