

a 14-year-old male with acute myelogenous leukemia, and patient 064 was a 16-year-old male with severe aplastic anemia. Both patients received high-dose chemotherapy and 1000 rads of total body irradiation (3). Nucleated donor bone marrow cells (2.5×10^8 and 3.8×10^8 per kilogram body weight, respectively) were injected intravenously within 24 hours of irradiation. Both patients promptly showed evidence of hematologic engraftment, demonstrated by the complete conversion to female (46,XX) karyotype of peripheral blood lymphocytes and bone marrow cells, red blood cell antigens, and isoenzymes of blood and bone marrow cells (4). Patient 051 developed severe chronic graft-versus-host disease (GVHD) from which he died 1½ years after the transplant. Patient 064 developed acute GVHD on day 30 and died 104 days after the transplant.

At the time of autopsy, hepatic macrophages from both patients and from both a male and female control were obtained by touch preparations of liver sections on glass slides. Kupffer cells were easily identified by their typical morphological features with Giemsa stain and confirmed by α -naphthyl butyrate esterase cytochemistry, Prussian blue stain for iron, and the presence of ingested particles and bile pigment (5) (Fig. 1). We examined 100 morphologically typical macrophages for the presence or absence of a fluorescent Y body after staining with quinacrine mustard, or for sex chromatin (Barr body) (6, 7).

Fluorescent Y bodies were seen in 16 percent of the macrophages from normal male liver, and no sex chromatin was observed. Sex chromatin was identified in 21 percent of the macrophages from female control liver similar to the 20 percent or more typically seen in buccal smear specimens. No Y bodies were observed. Hepatic macrophages from both patients showed sex chromatin in 16 percent of the cells, and no fluorescent Y body was seen in more than 100 macrophages screened. Since the techniques we used do not depend on identifying dividing Kupffer cells, our results are probably representative of the entire hepatic macrophage population.

Our studies indicate that human hepatic macrophages are repopulated from donor-derived precursors after allogeneic bone marrow transplantation. Although GVHD and inflammatory stimuli in the patients may have influenced mononuclear phagocyte migration, our findings are similar to those in alveolar macrophages, which are also repopulated with bone marrow-derived donor cells in rodents, dogs, and humans (8). Similar-

Table 1. Hepatic macrophages from two males who received bone marrow transplants from females were compared with those from a normal male and female for Y body fluorescence and sex chromatin.

Cell source	Time after transplant (days)	Percentage of cells with	
		Y body	Sex (X) chromatin
Normal male		16	0
Normal female		0	21
Patient 051	559	0	16
Patient 064	104	0	16

ly, peritoneal macrophages in rodents are derived from donor bone marrow cells after bone marrow transplantation. These studies provide evidence for a bone marrow origin of the hepatic macrophage in humans, and support the concept that allogeneic bone marrow transplantation results in a repopulation of the tissue macrophage system with bone marrow-derived donor cells.

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Emission of Maternal Pheromone

I would like to resolve the ambiguity in a report by Moltz and Leidahl (1) in which they stated that hepatic bile may be involved in the appearance of maternal pheromone in the rat. Two points should be made about the significance of their report. The first concerns the proposed "prolactin-hepatic interaction . . . underlying synthesis of the pheromone." They argue in their discussion that ". . . the chemistry of the cecum may be altered so that fecal material comes to contain the pheromone" when a change occurs in bile over the course of lactation. Synthesis of the attractant has been shown to depend on the action of cecal microorganisms (2) in the absence of elevated prolactin or bile activity, for cecotrophe is attractive to pups whether it is taken from the cecum of males, virgin females, mothers that emit the pheromone, or those that do not (2, 3). Moreover, maternal cecal material is no more attractive than that of males, although it is somewhat more attractive than virgin female cecotrophe (2, 4). It is therefore unlikely that bile plays a major causal role in pheromone synthesis.

Bile may, however, affect pheromone

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emission, and the second point concerns its relative importance in this process. Pheromone emission occurs in mothers when rising food intake induces large amounts of cecotrophe to be defecated but not totally reingested (2, 5). Emission can be suppressed by limiting the amount of food that mothers are allowed to ingest, and it can be stimulated by inducing mothers to ingest increased quantities of food by means of dietary manipulation (4). Emission can also be blocked by suppression of prolactin, a procedure that suppresses maternal food intake (2, 3). The involvement of bile in pheromone emission could be primary if it increased food intake or decreased cecotrophe reingestion, but it might play a less critical, subordinate role.

The elevated caloric intake that is characteristic of pheromone-emitting mothers has been shown to stimulate bile acid activity, and a linear relationship exists between bile acid output and amount defecated by rats (6). This laxative action of bile has long been known (6, 7), and it seems likely that Moltz and Leidahl have provided additional supporting evidence in this regard—measuring

the odor produced by the anal excreta, rather than its bulk.

Within this framework, it is possible to reinterpret the conclusions reached by Moltz and Leidahl. It is not surprising that a laxative agent, when infused into the gut of males, stimulates cecotrophe defecation and thereby forces the attractant into the environment. I would expect that if the anal excreta had been measured in these males a significant increase would have been observed. That all bile does not stimulate pheromone emission might well be because bile is capable of facilitating defecation by exogenous administration only when taken from rats that are eating enough food to stimulate a significant increase in bile acid activity. Day 21 mothers eat great quantities of food (3) and produce bile capable of stimulating cecotrophe defecation in males (1). Conversely, day 5 and prolactin-suppressed mothers eat relatively little (3) and produce bile that does not stimulate the male gut sufficiently to emit cecotrophe (1). It is also possible that prolactin could stimulate the laxative action of bile directly, without an increase in food intake (8), and such a mechanism might complement the one involving the increase in food consumption. Females induced to be maternal by continuous exposure to pups emitted the odor, while similarly behaving males did not, perhaps because pups did not induce prolactin release in males or because prolactin did not stimulate either increased bile activity or increased food consumption by males.

The role of bile in maternal pheromone emission thus can be considered to be subordinate to the primary mechanisms normally controlling pheromone emission in mothers, its effects being due to a facilitatory action on gut clearance.

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Leon has shown that material taken directly from the ceca of adult male rats normally contains the pheromone (1). That the anal excreta of these same males, however, do not contain the pheromone, and so do not attract the young, suggests one thing to me and another to Leon. What it suggests to Leon is that, in some as yet undetermined order, the male always excretes both pheromone- and nonpheromone-containing feces and, in the usual practice of coprophagia, preferentially consumes the former. What is left, the hard boli that are typically found in cage pans, does not attract the young since such boli do not have the pheromone. The lactating female, in Leon's view, also excretes both pheromone- and nonpheromone-containing feces. However, since the lactating female overeats (the well-established phenomenon of lactational hyperphagia), she produces, and consequently excretes, more pheromone-containing feces than she can consume. What she leaves of such material, the young find highly attractive.

My conceptualization of pheromone "dynamics" is different. I do not think that hyperphagia is a precondition for pheromonal emission since virgin females, induced to behave maternally through continuous association with young, emit the pheromone and yet show no evidence of hyperphagia (2). Rather, I think that whether or not the pheromone actually appears in anal excreta depends on events taking place within the liver, events that enable the pheromone, which as Leon points out is always present in the cecum, to survive passage through the colon. This, of course, raises the question of what is normally lost in the colon and what change occurs in the liver of pheromone-emitting animals to overcome such a loss. Since a full answer, not written in response to Leon, is currently in press (3), only an outline need be given here.

My colleague and I think that what is lost in colonic passage is deoxycholic acid, a secondary bile salt. In other words, we think that the pheromone is deoxycholic acid or, more likely, an intestinal effluent having deoxycholic acid as an essential moiety. Moreover, we conceive of the sustained production of prolactin by the maternally behaving female (4), and the progressive increase in the binding of that hormone by her liver (5), as resulting in the synthesis of greater-than-normal amounts of deoxycholic acid, thereby enabling a critical fraction to escape reabsorption from the colon and consequently to show up at the anus. It is this fraction, now contained in what is actually excreted, that we think of as the pheromone. That the male characteristically forms fewer hepatic prolactin receptors than the female (6), and so probably experiences little or no change in the output of deoxycholic acid, explains to us why the male, although capable of releasing the pheromone in response to injected bile, cannot do so endogenously. In other words, to us it was not the laxativeness of the bile administered, reported in the study by Moltz and Leidahl (7), that was critical, but rather that such bile, drawn as it was from pheromone-emitting females, contained a high concentration of deoxycholic acid. As a result, we suspect that our injected males were provided with an essential resource for pheromonal emission, one they normally command in only insufficient quantities.

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