ical Adaptations for Breeding in Birds (Methuen, London, 1968), pp. 149–150].
3. J. Verner, *Proc. Int. Ornithol. Congr.* **13**, 299 (1963); *Evolution* **18**, 252 (1964).
4. G. H. Orians, *Am. Nat.* **103**, 589 (1969).
5. S. Martin, thesis, Oregon State University (1971); C. H. Holm, *Ecology* **54**, 356 (1973); J. G. Wittenberger, thesis, University of California at Davis (1976); R. A. Howard, Jr., thesis, Cornell University (1977); M. Carey and V. Nolan, Jr., *Science* **190**, 1296 (1975).
6. P. J. Weatherhead, thesis, Queen's University at Kingston (1976).
7. J. Verner and M. F. Willson [*Ecology* **47**, 143 (1966)] showed that polygyny was especially common in two-dimensional habitats (marshes, savannahs, grasslands) where the potential difference in quality of two adjacent sites can be more extreme than in three-dimensional habitats (forests).
8. Territorial boundaries were determined by plotting the sites of aggressive encounters between neighboring males, by a multiple flush procedure [J. A. Wiens, *Ornithol. Monogr.* **8**, 1 (1969)] and by plotting the points where playbacks of conspecific songs elicited a response from a given territorial male.
9. Secondary females do not receive any assistance from the males.
10. I compared over the three seasons the number of fledglings from each nest of a secondary female with that from each of the monogamous nests initiated on the same day. I hypothesized that the secondary nests would fledge as many or more young than the monogamous nests.
11. An analysis of variance was carried out on the daily time spent singing for bachelors, monogamists, and bigamists. For morphological attributes, correlations were calculated (r = .12 for body length; r = .35 for wing patch surface).
12. The variables were measured according to the following procedure. Numbered stakes were placed in a square grid system at 25-m intervals over the entire field. Sampling positions were located by a stratified random design, which ensures random placement of samples as well as equal sampling intensities over all sections of the field. Grid lines served as transects, and a sample location determined randomly was positioned between every pair of points along each grid line [J. A. Wiens, *Ornithol. Monogr.* **9**, 1 (1969)]. At each sampling location, two crossed stakes delineated horizontally four 90-degree sectors. The distance from the intersection of the stakes to the nearest alfalfa plant in each quadrant was measured [R. L. Dix, *J. Range Manage.* **14**, 63 (1961)]. At each of the four ends of the stakes, vertical foliage density was measured by inserting a rod vertically through the vegetation to the ground. Counts were made of the number of stems and leaves of all vegetative types intercepting the rod in 10-cm intervals above the ground. To evaluate the shading properties of the vegetation at the sample points, penetration of light (in lux) through the vegetation was measured with a photometer (Lunasix 3) 10 cm above the ground. Light readings were made on a clear day between 1130 and 1330 C.D.T.
13. The biological rationale behind the use of this variable is the following. Organisms may be said to generally select nesting sites with the least variation of daily temperature, and light provides a rapid and accurate covariant of temperature (r = .94) [W. K. Pleszczynska, thesis, University of Toronto (1977)].
14. The first eigenvector of a principal component analysis had already segregated the sample points into three groups pertaining to territories of bachelors, monogamists, and bigamists. A discriminant function was constructed.
15. The F value to enter or remove, 246, associated with light was much larger than the next largest F, 36.
16. Sixth-degree polynomials were calculated for light by a trend surface analysis.
17. Predation pressure is not the cause of the increasing nestling mortality with the advancement of the season, because an increasing proportion of this mortality is due to desiccated young. This proportion is furthermore underestimated because dead nestlings are removed by the parents whenever at least one live nestling remains in the nest.
18. I thank R. I. C. Hansell and S. T. Emlen for helpful discussions, encouragement, and constructive criticism. I also thank F. Briand and J. D. Rising for critically reading the manuscript. Many people provided valuable assistance in the field, in particular S. Pleszczynski, C. Anders, and R. Hunter. Supported by Canada Council grant 3-654-120-60 to J. D. Rising.

29 August 1977; revised 28 April 1978

Bone Marrow Origin of Hepatic Macrophages (Kupffer Cells) in Humans

Abstract. *Hepatic macrophages (Kupffer cells) from two male recipients of bone marrow transplants from females were studied for fluorescent Y body staining and sex chromatin (Barr body). After the transplant, macrophages had the sex karyotype of the donor, indicating that human hepatic macrophages originate in bone marrow.*

Transplantation studies in rodents have suggested that hepatic macrophages (Kupffer cells) are derived from a bone marrow precursor. For example, mice that received high-dose radiation and bone marrow transfusion from T6/T6 chromosome–marked donors possessed dividing hepatic macrophages of donor origin (*1*). However, other investigators have demonstrated a low but measurable proliferative capacity in hepatic macrophages, a finding that lends support to the concept of a self-sustaining population (*2*). Thus, there are two potential mechanisms for hepatic macrophage renewal, development from a bone marrow precursor and local self-replication.

To evaluate the mechanism of hepatic macrophage reconstitution in humans, we studied two male patients who had received bone marrow grafts from HLA-identical female donors. Patient 051 was

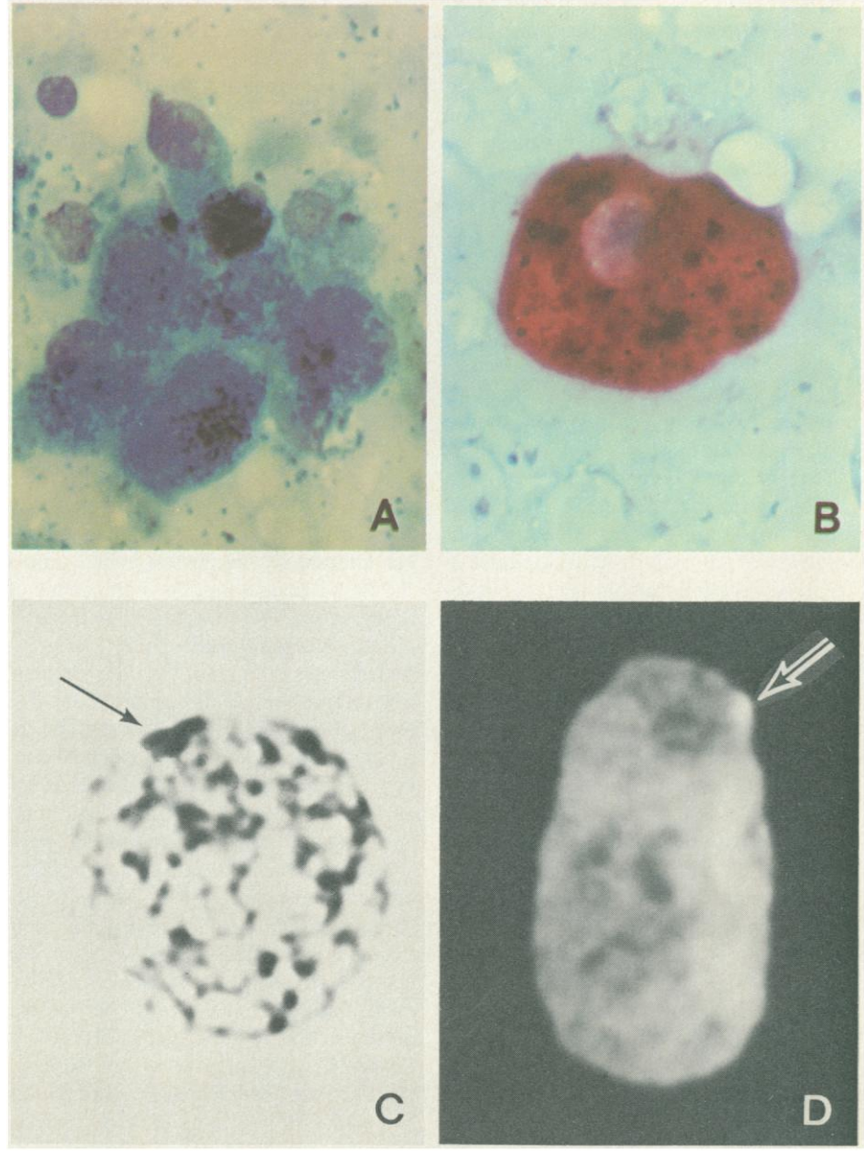

Fig. 1. Morphological studies of hepatic macrophages. (A) Giemsa stain of Kupffer cells with ingested bile pigment and hemosiderin. (B) α-Naphthyl butyrate esterase stain of a hepatic macrophage. (C) Sex chromatin in hepatic macrophage (patient 051). (D) Fluorescent Y body in normal male hepatic macrophage.

0036-8075/78/0908-0937$00.50/0

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 $1^1/_2$ 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*). Similarly, 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.

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

ROBERT PETER GALE
ROBERT S. SPARKES
DAVID W. GOLDE
Bone Marrow Transplant Unit, School of Medicine, University of California, Los Angeles 90024

References and Notes

1. J. L. Boak, G. H. Christie, W. L. Ford, J. G. Howard, *Proc. R. Soc. London, Ser. B* **169**, 307 (1968); J. G. Howard, J. L. Boak, G. H. Christie, *Ann. N.Y. Acad. Sci.* **129**, 327 (1966).
2. J. L. Edwards and R. E. Klein, *Am. J. Pathol.* **38**, 437 (1961); L. S. Kelly, B. A. Brown, E. L. Dobson, *Proc. Soc. Exp. Biol. Med.* **110**, 55 (1962); E. Wisse, *J. Ultrastruct. Res.* **46**, 449 (1974); L. S. Kelly and E. L. Dobson, *Br. J. Exp. Pathol.* **52**, 88 (1971).
3. UCLA Bone Marrow Transplant Team, *Lancet* **1976-II**, 921 (1976); *ibid.* **1977-II**, 1197 (1977).
4. M. C. Sparkes, M. L. Crist, R. S. Sparkes, R. P. Gale, S. A. Feig, *Vox Sang.* **33**, 202 (1977).
5. M. J. Willcox, D. W. Golde, M. J. Cline, *J. Histochem. Cytochem.* **24**, 979 (1976).
6. Cells on glass slides were fixed with methanol, stained for 15 minutes in a 0.5 percent solution of acrinal dissolved in buffer, rinsed four times in buffer, mounted in buffer, and sealed. They were viewed with a Zeiss Photomicroscope II equipped with an epi-illumination head, HBO 200/4 burner, fluorescein isothiocyanate filter, and a 500 reflector. The barrier filter was set at 50.
7. U. Mittwoch, in *Human Chromosome Methodology*, J. J. Yunis, Ed. (Academic Press, New York, 1974), p. 73.
8. J. J. Godleski and J. D. Brain, *J. Exp. Med.* **136**, 630 (1972); F. L. Shand and E. B. Bell, *Immunology* **22**, 549 (1972); R. van Furth, *Semin. Hematol.* **7**, 125 (1970); M. Virolainen, *J. Exp. Med.* **127**, 943 (1968); P. L. Weiden, R. Storb, M. S. Tsoi, *RES. J. Reticuloendothel. Soc.* **17**, 342 (1975); E. D. Thomas, R. E. Ramberg, G. E. Sale, R. S. Sparkes, D. W. Golde, *Science* **192**, 1016 (1976).
9. We thank Dr. H. Muller, Ms. N. Lyddane, and Ms. S. Quan for technical assistance, and the members of the University of California, Los Angeles Bone Marrow Transplant Team for their clinical care of these patients. Supported in part by grants CA-23175, HL-20675, CA-12800, and CA-15688 from the National Institutes of Health. R.P.G. is a scholar of the Leukemia Society of America.

6 March 1978; revised 10 May 1978

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 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 supportive evidence in this regard—measuring

0036-8075/78/0908-0938$00.50/0