boxyethyl-5-(46)carboxyfluorescein (BCECF) and suspended in RPMI containing 1% FBS (2×10^5 cells per 0.2 ml per well) were allowed to adhere to EC monolayers for 10 min at 37°C. Wells were then filled with buffer, and the plates were sealed, inverted, and centrifuged (250*g* for 5 min at 22°C).
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Human Immunodeficiency Virus Infection of Human-PBL-SCID Mice

DONALD E. MOSIER, RICHARD J. GULIZIA, STEPHEN M. BAIRD, DARCY B. WILSON,* DEBORAH H. SPECTOR, STEPHEN A. SPECTOR

Severe combined immunodeficient (SCID) mice reconstituted with human peripheral blood leukocytes (hu-PBL-SCID mice) have inducible human immune function and may be useful as a small animal model for acquired immunodeficiency syndrome (AIDS) research. Hu-PBL-SCID mice infected with human immunodeficiency virus–1 (HIV-1) contained virus that was recoverable by culture from the peritoneal cavity, spleen, peripheral blood, and lymph nodes for up to 16 weeks after infection; viral sequences were also detected by in situ hybridization and by amplification with the polymerase chain reaction (PCR). Mice could be infected with multiple strains of HIV-1, including LAV-1/Bru, IIIB, MN, SF2, and SF13. HIV-1 infection affected the concentration of human immunoglobulin and the number of $CD4^+$ T cells in the mice. These results support the use of the hu-PBL-SCID mouse for studies of the pathogenesis and treatment of AIDS.

THE NEED FOR BETTER SMALL ANIMAL models for research into the causes and prevention of AIDS has prompted the development of two experimental systems (*1, 2*) that involve the transplantation of human lymphoid cells or tissue into mice with the SCID mutation (*3*). When adult human peripheral blood leukocytes (PBLs) are transferred to SCID mice, thus creating hu-PBL-SCID mice, the human T and B lymphocytes survive, human immunoglobulin is produced, and secondary antibody responses can be elicited (*1, 4*). Implantation of human fetal lymphoid tissue into SCID mice creates SCID-hu mice (*2*); such mice support HIV-1 RNA transcription and lesser expression of viral proteins (*5*). We have recovered HIV-1 from the peritoneal cells, lymphoid organs, and blood of hu-PBL-SCID mice infected with either cell-free or cell-associated virus for up to 16 weeks after infection. HIV-1 infection with high virus loads led to an initial increase, then a reduction in human immunoglobulin production in hu-PBL-SCID mice and changes in the number of $CD4^+$ T cells in many mice. We conclude that HIV-1 is infectious in the hu-PBL-SCID model, and that infection results in changes in human lymphocyte function consistent with those seen in HIV-1–infected individuals.

Hu-PBL-SCID mice were generated by reconstituting adult SCID mice by intraperitoneal injection of 1×10^7 to 4×10^7 PBLs from donors that were negative for antibodies to Epstein-Barr virus (EBV) capsid antigens. [EBV-seronegative donors were used to prevent the possible development of EBV-associated B cell lymphoproliferative disease (*1, 6*) and to eliminate one potential cofactor in HIV-1 pathogenesis.] Mice were challenged by intraperitoneal injection (*7*) either with 10^1 to 10^5 median tissue culture infectious doses ($TCID_{50}$) of cell-free virus (144 mice) or with virus-infected autologous T lymphoblasts (27 mice) (*8*) and evaluated for HIV-1 infection by (i) culture of hu-PBL-SCID lymphoid tissue with fresh human PBLs (*9*), (ii) in situ cytohybridization with a full-length cDNA probe for the IIIB strain of HIV-1 ($HIV\text{-}1_{IIIB}$) (*10*), and (iii) amplification of HIV-1 *gag* sequences by the polymerase chain reaction (PCR)

D. E. Mosier, R. J. Gulizia, D. B. Wilson, Division of Immunology, Medical Biology Institute, La Jolla, CA 92037.
S. M. Baird, Veterans Administration Medical Center, La Jolla, CA 92161, and Department of Pathology, University of California at San Diego, La Jolla, CA 92093.
D. H. Spector, Department of Biology, University of California at San Diego, La Jolla, CA 92093.
S. A. Spector, Department of Pediatrics, University of California at San Diego, La Jolla, CA 92093.
*Present address: La Jolla Institute of Experimental Medicine, La Jolla, CA 92037.

(*11*). The percentage of HIV-1–injected hu-PBL-SCID mice from which virus could be isolated by culture in eight separate experiments was calculated (Fig. 1). Although virtually all hu-PBL-SCID mice were infected with HIV-1 at 3 to 4 weeks after virus inoculation, the percentage of mice from which virus could be recovered declined significantly ($P < 0.005$, $n = 45$) between 6 and 8 weeks after injection with cell-free HIV-1; mice infected with autologous cells showed a similar but smaller decrease. Thus, all hu-PBL-SCID mice were susceptible to HIV-1 infection, and a substantial fraction of animals (33 to 50%) established persistent infection for the 16-week period examined in these experiments. In a separate control experiment, unreconstituted SCID mice (that is, mice with no human cells) injected with HIV-1 showed no evidence of infection (*12*), demonstrating that the presence of the engrafted human cells is essential for HIV-1 infection. In addition, HIV-1–infected and uninfected hu-PBL-SCID mice were housed together for up to 6 months, and no evidence of horizontal transmission of HIV-1 between mice was observed.

The site from which virus was isolated most frequently was peritoneal lavage cells, followed by spleen cells (85% of virus-positive mice), mouse peripheral blood (33%), and peripheral (excluding mesenteric) lymph nodes (10%). Virus replication was confirmed both by in situ hybridization and by PCR amplification of spleen samples (below) from many of the mice. Plasma samples from 32 hu-PBL-SCID mice from which it was possible to isolate HIV-1 were examined for the presence of p24 core antigen (*13*) or antibodies to HIV-1 antigens by immunoblotting (*14*). Plasma p24 antigen was detected in 21% of culture-positive animals, and no antibodies to HIV-1_{IIIB} were detected in any of the mice. Detection of p24 antigen in the plasma was correlated with early detection (<1 week of culture) of p24 in PBL cocultures.

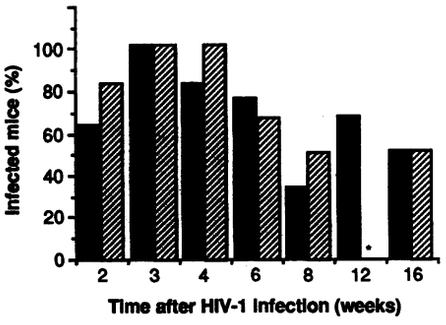

Fig. 1. Percentage of hu-PBL-SCID mice infected with HIV-1 at different times after intraperitoneal injection of 10^4 to 10^5 $TCID_{50}$ of free virus (either HIV-$1_{LAV\text{-}1/Bru}$ or HIV-1_{IIIB}) or 5×10^6 to 7×10^6 HIV-$1_{LAV\text{-}1/Bru}$–infected syngeneic T cell blasts exposed 3 to 4 days previously to HIV-1 (*8*). Virus recovery was assessed as described (*9*). Mice were considered infected if HIV-1 was isolated from any tissue examined. Data are compiled from eight separate experiments, and the number of mice evaluated at each time point ranges from 8 at 16 weeks after infection to 85 at 4 weeks after infection. In two of the pooled experiments, mice were infected at 8 weeks after PBL reconstitution; in the remaining six experiments, HIV-1 infection was at 2 weeks after PBL transfer. At this high titer of virus, there was no difference in the percentage of mice infected at the two time points. Mice were killed for virus isolation, so that data were collected on different mice at each time point. Filled columns, mice infected with cell-free virus; striped columns, mice infected with T lymphoblasts. No data were available for mice infected with T lymphoblasts at 12 weeks after infection.

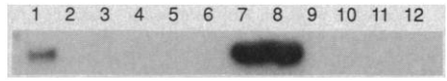

Fig. 2. Analysis of PCR-amplified HIV-1 sequences from infected hu-PBL-SCID mouse tissues. PCR was performed as described (*11*) with HIV-1 *gag*–specific primers SK 38/39 and SK 19 probe (Cetus, Emeryville, California). An autoradiogram of the liquid hybridization product after polyacrylamide gel electrophoresis is shown. Lane 1, HIV-1 DNA (1 fg); lane 2, DNA from uninfected hu-PBL-SCID mouse spleen; lanes 3 to 6 and 9 to 12, DNA from lymph node or spleen of HIV-1–infected but culture-negative hu-PBL-SCID mice; lanes 7 and 8, DNA from lymph node and spleen, respectively, from an individual hu-PBL-SCID mouse that gave positive results in the coculture assay after 10 (lymph node) and 3 (spleen) days of culture.

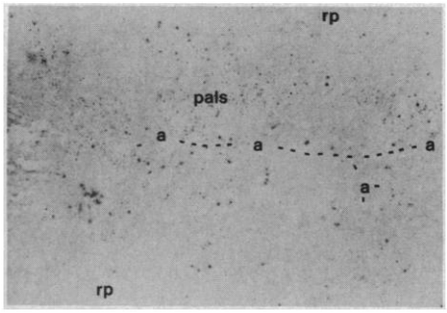

Fig. 3. In situ hybridization of a section of spleen taken from an HIV-1–infected hu-PBL-SCID mouse 4 weeks after infection with cell-associated HIV-$1_{LAV\text{-}1/Bru}$. Frozen sections were mounted and hybridized as described (*10*). Original magnification was ×100. The same probe yielded no signal above background when applied to sections of uninfected hu-PBL-SCID mouse spleen. A central arteriole (a; dashed line) indicates the area of a lymphoid sheath sectioned longitudinally. The area normally occupied by the periarteriolar lymphoid sheath (pals) is indicated, as are the more peripheral red pulp (rp) zones.

As a more sensitive measure of the virus, HIV-1 DNA sequences in spleen and lymph node samples from culture-positive and culture-negative animals were amplified by PCR at 6 to 8 weeks after infection. Amplification of viral *gag* sequences gave a positive signal on culture-positive samples and no signal on culture-negative samples (Fig. 2), indicating that the isolation of virus by coculture was highly efficient. On the basis of the sensitivity of PCR (*11*), it appears that PCR-negative animals contained less than ten copies of proviral DNA in the sampled hu-PBL-SCID spleen; thus, culture-negative animals in our studies do not represent mice with latent HIV-1 infection.

Analysis of spleens from HIV-1–infected hu-PBL-SCID mice by in situ (*15*) hybridization with a full-length viral probe, pHXB-2D (*10*), showed that many cells were positive; these cells were somewhat more concentrated in areas corresponding to the periarteriolar lymphoid sheath (Fig. 3). Our data suggest that HIV-1 replicates in the human cells and that a significant fraction of engrafted human cells are infected. The possibility of infection of $CD4^-$ human cells by pseudotypes or phenotypic mixtures of xenotropic murine leukemia virus and HIV-1 has been raised (*16*). However, we have observed no evidence for aberrant spread of HIV-1 in hu-PBL-SCID mice, and HIV-1 recovered from such mice only grew in $CD4^+$ human T cells (*17*).

To determine the minimal dose of HIV-1_{IIIB} required for infection, we injected hu-PBL-SCID mice intraperitoneally with 10^1 to 10^4 $TCID_{50}$ at either 2 or 8 weeks after PBL reconstitution (Fig. 4). As little as 10 $TCID_{50}$ of virus was sufficient to infect hu-PBL-SCID mice if the virus was inoculated at 2 weeks after PBL reconstitution; however, 10^4 $TCID_{50}$ was the minimal infectious dose if virus inoculation was delayed until 8 weeks after reconstitution. Other strains of virus [HIV-1_{MN} and viral stocks derived from molecular clones of HIV-1_{SF2} and HIV-1_{SF13} (*18*)] were capable of infecting hu-PBL-SCID mice when 10^2 to 10^3 $TCID_{50}$ of virus was injected 2 weeks after PBL reconstitution.

A potential advantage of the hu-PBL-SCID model is the ability to determine if HIV-1 infection alters the function of the human lymphoid graft. Therefore, hu-PBL-SCID mice were reconstituted with 2×10^7 PBLs and infected 8 weeks later with 10^5 $TCID_{50}$ of HIV-1_{IIIB}. At biweekly intervals after HIV-1 infection, total human serum immunoglobulin concentrations of four animals (and of four uninfected controls) were determined from a small sample of blood (*1*). HIV-1 infection resulted in an initial 200 to 300% increase in human immunoglobulin concentration which was followed by a decrease (Fig. 5A). These changes in human B cell function were reproducible

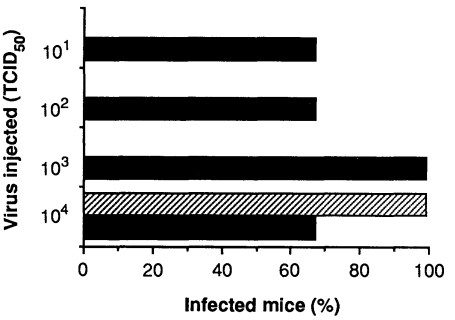

Fig. 4. Minimum infectious dose of HIV-1 required to infect hu-PBL-SCID mice. Mice were intraperitoneally injected with titered stock of HIV-1_{IIIB} at 2 (filled bars) or 8 (striped bar) weeks after reconstitution with PBLs (2×10^7) from EBV-seronegative donors. Infectious titer was confirmed by in vitro titration of virus on the day of HIV-1 inoculation into mice. Four weeks after HIV injection, recovery of virus was assessed as described (*9, 11*) in three or four mice per group. No difference between the groups in estimated virus recovery was evident as judged by equivalent time intervals between coculture initiation and first detection of p24 antigen and equal intensity of hybridization signals of the PCR products. A repeat experiment with 24 hu-PBL-SCID mice gave similar results.

after injection of high concentrations of virus, but infection of hu-PBL-SCID mice with 10^2 to 10^4 $TCID_{50}$ of HIV-1_{IIIB} resulted in smaller increases in immunoglobulin, and only a fraction of HIV-1–infected mice showed the later decrease. The fate of the small number of $CD4^+$ human T cells in the spleens of hu-PBL-SCID mice was also examined 8 weeks after infection with 10^4 $TCID_{50}$ of HIV-1_{IIIB} (*4, 19*) (Fig. 5B). Changes in the number of $CD4^+$ T cells correlated with human immunoglobulin concentrations, with many mice having low immunoglobulin concentrations and no detectable $CD4^+$ cells.

These results suggest that HIV-1 infection can impair the immunological function of hu-PBL-SCID mice. HIV-1 infection may lead to the depletion of human T cells that regulate immunoglobulin synthesis. Alternatively, HIV-1 infection may affect T and B cell function by more indirect means, such as by altering human cytokine concentrations or enhancing the frequency of opportunistic infection, although no evidence for the latter possibility was noted. These effects, which appear to vary from animal to animal, are more profound at higher initial virus doses. Thus, these immunological consequences of HIV-1 infection of hu-PBL-SCID mice mimic human disease to some extent (*20*), but the pace of these changes is accelerated in mice compared to humans.

We conclude from our data that (i) hu-PBL-SCID mice are susceptible to infection with HIV-1, and virus can be detected not only by PCR and in situ hybridization, but also by cocultivation of hu-PBL-SCID lymphoid tissue with fresh human phytohemagglutinin A (PHA)–stimulated lymphoblasts; (ii) both cell-free virus and HIV-1–infected T lymphoblasts can efficiently transmit infection to hu-PBL-SCID mice; and (iii) human immune function as reflected in human immunoglobulin concentrations and recovery of $CD4^+$ T cells are altered in infected animals. Our inability to recover virus from every injected hu-PBL-SCID mouse may have multiple explanations. Because more virus was required to infect hu-PBL-SCID mice at 8 weeks compared to 2 weeks after reconstitution, the number of HIV-1–infectable human cells may decline with time. The loss of $CD4^+$ T cells in some infected mice suggests that a direct cytopathic effect of HIV-1 infection may lead to a severe depletion of human target cells for HIV-1 infection. This relatively simple small animal model system may be useful for studies of acute pathogenic effects of HIV-1 in vivo, for therapeutic trials of agents designed to interfere with HIV-1 infection, and for evaluation of candidate vaccines (*21*).

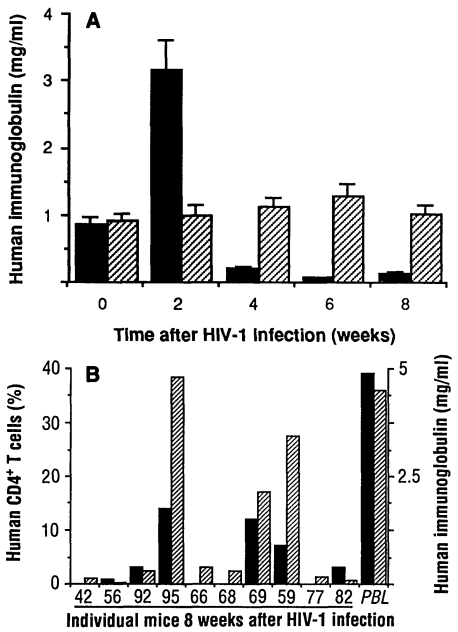

Fig. 5. (**A**) Alterations in human immunoglobulin concentrations in HIV-1–infected hu-PBL-SCID mice. Total human immunoglobulin M (IgM) + IgA was determined by an enzyme-linked immunosorbant assay (*1*); separate experiments have shown that ~80% of the human immunoglobulin is IgG. Hu-PBL-SCID mice were reconstituted with PBLs (2×10^7) and infected with 10^5 $TCID_{50}$ of HIV-1_{IIIB} at 8 weeks after reconstitution. Filled columns, infected mice; striped columns, uninfected controls in the same experiments. Values represent the mean ± SEM of three or four replicate values. (**B**) Alterations in $CD4^+$ T cell percentages (filled columns) in the spleens and human immunoglobulin concentrations (striped columns) of individual hu-PBL-SCID mice infected 8 weeks earlier with 10^4 ($TCID_{50}$) of HIV-1_{IIIB}. The $CD4^+$ T cell numbers were determined by staining with fluorescein-labeled monoclonal antibodies to CD4 as described (*19*). Individual mice are shown by number code; *PBL* indicates normal $CD4^+$ cell number from a PBL sample and serum immunoglobulin concentration from the same donor. Note that mice with persistently high human immunoglobulin concentrations had increased numbers of $CD4^+$ T cells (14% maximum compared to 2 to 3% in uninfected control mice). In contrast, most mice with depressed human immunoglobulin concentrations had no detectable $CD4^+$ T cells, although some mice (for example, 82 and 92) had $CD4^+$ T cell numbers in the same range as controls.

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6. The relation between the number of PBLs from EBV-seropositive donors injected and the fraction of hu-PBL-SCID mice that develop B cell lymphomas is linear. From the Poisson distribution, it can be estimated that one tumor precursor is contained in 40×10^6 PBLs.
7. All hu-PBL-SCID mice were housed in microisolator caging and maintained in a sterile environment. Mice were infected with HIV-1 in a Biosafety Level 3 containment facility, and additional precautions were taken to prevent accidental release of mice. All virus-infected animals were manipulated under halothane-induced anesthesia.
8. PBLs were prepared by Ficoll-Hypaque density separation from the same donor that was used to reconstitute the hu-PBL-SCID mice and were stimulated in RPMI 1640 medium containing 10% fetal bovine serum (FBS), phytohemagglutinin A (PHA) (5 μg/ml), and 5% native interleukin-2 (IL-2) (Cellular Products, Buffalo, NY) for 4 days. The resulting T cell blasts were infected with 2×10^6 $TCID_{50}$ of the HIV-$1_{LAV\text{-}1/Bru}$ strain for an additional 3 to 4 days and then injected intraperitoneally into hu-PBL-SCID mice.
9. For virus isolation, fresh peritoneal lavage cells or cell suspensions were prepared from spleens or lymph nodes of virus-infected hu-PBL-SCID mice. These cells (0.5×10^6 to 2×10^6) and fresh PHA-stimulated human PBLs (2×10^6) from unrelated donors were cocultured in RPMI 1640 medium containing 20% FBS, PHA (2.5 μg/ml), and recombinant human IL-2 (5 unit/ml). Culture supernatants were sampled for p24 antigen twice weekly, and cultures were replenished with PBLs (2×10^6) in medium containing PHA and IL-2 at weekly intervals.
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12. Fifteen unreconstituted SCID mice were injected with 10^5 $TCID_{50}$ of HIV-$1_{LAV\text{-}1/Bru}$ and evaluated for virus infection at 4, 6, and 8 weeks after infection. No virus was recovered by culture, nor were viral genomes detected by PCR.
13. HIV-1 core p24 antigen was quantitated by the Abbott or Coulter enzyme-linked immunosorbant procedure. Positive PBL cultures eventually yielded

greater than 500 pg of p24 antigen per milliliter in all cases. Plasma concentrations of p24 antigen as low as 5 to 10 pg per milliliter can be detected in this assay.
14. Antibodies to HIV-1 were assessed by diluting the pooled plasma samples from HIV-1–positive hu-PBL-SCID mice tenfold and applying the samples to Dupont immunoblot strips.
15. In situ hybridization of hu-PBL-SCID mice with the Blur-8 probe [P. L. Deininger, D. J. Jolly, C. M. Rubin, T. Friedmann, C. W. Schmid, *J. Mol. Biol.* **151**, 17 (1981)] for human *Alu* sequences showed a concentration of labeled cells in the peripheral area of the periarteriolar "lymphoid" sheath (a misnomer in SCID mice, in which these areas are normally devoid of lymphocytes) and fewer labeled cells scattered through the red pulp. Examination of these tissues by hematoxylin and eosin staining revealed the presence of lymphoid cells in the same areas beginning about 4 weeks after human PBL injection into SCID mice. Thus, small numbers of human lymphoid cells migrate from the peritoneal cavity to the spleen at some interval after injection.
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The Assimilation of Elements Ingested by Marine Copepods

JOHN R. REINFELDER AND NICHOLAS S. FISHER*

The efficiency with which a variety of ingested elements (Ag, Am, C, Cd, P, S, Se, and Zn) were assimilated in marine calanoid copepods fed uniformly radiolabeled diatoms ranged from 0.9% for Am to 97.1% for Se. Assimilation efficiencies were directly related to the cytoplasmic content of the diatoms. This relation indicates that the animals obtained nearly all their nutrition from this source. The results suggest that these zooplankton, which have short gut residence times, have developed a gut lining and digestive strategy that provides for assimilation of only soluble material. Because the fraction of total cellular protein in the cytoplasm of the diatoms increased markedly with culture age, copepods feeding on senescent cells should obtain more protein than those feeding on rapidly dividing cells. Elements that are appreciably incorporated into algal cytoplasm and assimilated in zooplankton should be recycled in surface waters and have longer oceanic residence times than elements bound to cell surfaces.

THE PACKAGING OF ELEMENTS INTO rapidly sinking particles in the ocean is predominantly controlled by biological processes, including the production of zooplankton fecal pellets, which concentrates and encapsulates unassimilated elements associated with small suspended food particles (phytoplankton, microzooplankton) (*1*). Elements that are largely unassimilated in organisms are therefore likely to have short residence times in surface waters, whereas those that are assimilated should be recycled with organic matter (*2*). To better understand the cycling and vertical distribution of elements in the ocean, we have conducted a series of experiments to measure the efficiency with which marine copepods, important components of many zooplankton communities, assimilate a variety of essential and nonessential elements ingested in their food.

Experiments were conducted with monospecific assemblages of *Acartia tonsa, Acartia hudsonica*, or *Temora longicornis*. These calanoid copepods were collected with a 63-μm mesh according to availability from coastal waters off Long Island, New York, just before the experiments were performed. Adults were separated by pipette from other particulates and were fed uniformly radiolabeled diatoms, *Thalassiosira pseudonana* (clone 3H). The diatoms were grown axenically in modified f/2 medium prepared with sterile filtered seawater (*3*) to which the radionuclides ^{110m}Ag, ^{241}Am, ^{14}C, ^{109}Cd, ^{32}P, ^{35}S, ^{75}Se, and ^{65}Zn were added in trace amounts, individually or in pairs (*4*). Cells were taken from cultures after at least 3 days (≥six generations) of exposure to the radiotracers. We fed both diatoms that were actively growing (log-phase cells) and senescent (stationary-phase) cells to the copepods. Feeding suspensions were 200 ml and contained 1×10^5 cells per milliliter (2.2 mg of dry weight or 6.1×10^9 μm^3 $liter^{-1}$) and 20 to 47 animals. Immediately before the feedings, some of the labeled diatoms were lysed by resuspension in deionized water (pH 8.0) and fractionated by centrifugation to yield three pelletized fractions (pellets 1, 2, and 3) and a final supernatant fraction (*5*), each of which was assayed for radioactivity.

Assimilation efficiencies of ingested elements in the copepods were measured in two ways. For C and Se, a radiotracer ratio method was followed in which ^{51}Cr was used as an inert tracer of bulk ingested material in conjunction with ^{14}C (*6*), and ^{241}Am was used as an inert tracer in conjunction with ^{75}Se (*7*). For the other elements, we calculated assimilation efficiencies by dividing the amount of radiotracer retained by the animals after gut evacuation (*8*) by the amount ingested. We monitored the grazing activity of the animals by detecting changes in cell density with the use of in vivo chlorophyll a fluorescence (*9*). The radioactivity per diatom cell (*10*) stayed essentially constant during the 6-hour feedings. The gamma-emitting isotopes were measured with a Pharmacia-Wallac LKB gamma counter equipped with a well-type NaI(Tl) crystal; the beta emitters (^{14}C, ^{32}P, and ^{35}S) were measured with an LKB Rack Beta liquid scintillation counter (*11*).

The cellular fractionation of the radiotracers in the diatoms (Table 1) varied with the element; for example, only 10.2% of the total cellular ^{75}Se and 93.0% of the total cellular ^{241}Am were contained in pellet 1. Generally, the proportions of the nonessential elements (Ag, Am, and Cd) in the cytoplasmic fractions (that is, in pellets 2

Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794.

*To whom correspondence should be addressed.