untreated normal mice, and (iv) 5 untreated osteopetrotic mice. Mice were housed in a constant-temperature room and fed a modified McCollum's I diet (*4*) both dry and wet.

During the observation period the body weight of each animal was measured twice a week; a tibial amputation was made at 2, 4, or 6 weeks after onset; and radiograms were made every 4 to 6 weeks. At autopsy several tissues were prepared for histologic examination, including bones (tibia, femur, and mandible) and lymphatic organs (spleen, lymph nodes, and thymus).

The normal bone marrow and spleen cell infusions restored capacity to resorb bone and calcified cartilage in all of the irradiated osteopetrotic recipients. The younger the recipient, the more rapid the rate of removal of skeletal matrix. Histologically, the earliest changes were detected 2 weeks after onset when an increase in mononuclear leukocytes was noted extravascularly along the vascular channels in the proximal third of the tibia. In the younger group of experimental mice, most of the excess bone and cartilage was removed from the long bones during the third and fourth weeks after onset. Figure 1F illustrates the proximal tibia of a grey-lethal mouse 30 days of age, which at 10 days of age received an infusion of normal spleen cells. The medullary cavity is fully expanded and filled with active hematopoietic tissue; the flare along the medial border is pronounced and the cortex is moderately well developed. These signs of recovery are appreciated more readily through comparison of the proximal tibia of the experimental mouse (Fig. 1F) with those of the untreated osteopetrotic (Fig. 1E) and normal (Fig. 1D) controls.

Mutants treated at the older ages required disproportionately more time to remove all of the excess matrix. Thus, the juvenile group of mutants required 6 to 9 weeks to make a complete recovery and the adult group required 12 to 24 weeks. The slower recovery rate of the older mutants may be accounted for by the increased extent and density of the spongiosa and the fact that fewer growth centers participated in the recovery. Radiologically, the first signs of recovery in the adult group of mutants were noted 6 weeks after onset, when well-defined zones of radiolucence appeared under the growth plates at the following sites: proximal humerus, iliac crest, distal femur, and proximal tibia. As the abnormal accumulations of calcified cartilage and bone were resorbed these zones of radiolucence extended along the length of the diaphysis until the expansion of the medullary cavity was complete. Detection of the subepiphyseal radiolucent zones in radiograms is facilitated by comparing the proximal tibia and distal femur of the experimental animal (Fig. 1C) with those of the untreated osteopetrotic (Fig. 1B) and normal (Fig. 1A) controls.

Examination of the skeletal and lymphoid tissues of the controls helped to establish that the mutants were immunologically competent and that neither radiologic damage nor graft-versus-host (GVH) disease played a direct role in the restoration of the capacity to resorb skeletal tissues. Osteopetrotic mice which received normal bone marrow or spleen cells in the absence of radiation never developed bone resorptive activity, which indicates that the donor cells had been destroyed. Irradiated normal mice receiving spleen or marrow cell infusions from normal littermates recovered uneventfully without manifesting the osteoporotic changes of GVH disease (*5*). In all of the control and experimental mice histologic examination of spleen and lymph nodes revealed the presence of well-developed thymus-dependent zones. Finally, unlike the mice with GVH disease, which are subject to progressive wasting, the experimental animals of the present study maintained about the same rate of body weight gain and skeletal growth after irradiation and transplantation as before.

The reversal of osteopetrosis in mice through bone marrow transplantation provides a new rationale for treatment of Albers-Schönberg marble bone disease in man.

DONALD G. WALKER
Department of Anatomy, Johns Hopkins University, Baltimore, Maryland 21205

References

1. D. G. Walker, *Endocrinology* **91**, 916 (1972).
2. K. Toyama, H. Moutier, H. Lamendin, *C. R. Acad. Sci.* **278**, 115 (1974).
3. D. G. Walker, *Science* **180**, 875 (1973).
4. ———, *Endocrinology* **89**, 1389 (1971).
5. L. Berek *et al.*, *Experientia (Basel)* **26**, 92 (1970).

2 January 1975; revised 4 August 1975

Spleen Cells Transmit Osteopetrosis in Mice

Abstract. *Osteopetrosis was induced in lethally irradiated, normal mice of grey-lethal and microphthalmic stocks by cell infusions prepared from the spleens of osteopetrotic littermates. Failure of skeletal remodeling became evident within a few weeks after transplantation as calcified cartilage and bone accumulated excessively in the active metaphyses of the long bones. The massive lesions produced were extensively infiltrated with abnormal osteoclasts.*

Bone resorption has been restored in osteopetrotic mice through parabiotic union of mutants and normal littermates and by the intravenous infusion of normal marrow and spleen cells into lethally irradiated mutants (*1*). Recovery from osteopetrosis was thought to be mediated by cells which had migrated via the bloodstream from their sites of origin in the hematopoietic tissues to the ossification centers, where they gave rise to a new population of competent osteoclasts or to cells that stimulate osteoclastic activity (*2*). The objective of the experiment reported here was to determine if the same mechanism would function in the reciprocal situation, with the osteopetrotic specimen serving as donor and the irradiated normal littermate as recipient in spleen cell transplantations. The osteopetrotic spleen cell infusions restored hematopoiesis but arrested skeletal remodeling, thus providing decisive evidence that bone resorption is under the direct control of the hematopoietic centers.

Weanling mice of grey-lethal (*gl/gl*) and microphthalmic (*mi/mi*) stock were distributed into four groups, 20 mice per group, identified as follows: (i) experimental splenic transplants, lethally irradiated normal mice that received spleen cell infusions from their osteopetrotic littermates; (ii) control splenic transplants, lethally irradiated normal mice that received spleen cell infusions from other normal littermates; (iii) untreated osteopetrotic controls; and (iv) untreated normal controls. Females and males were represented nearly equally in the above groups, and in most instances donors were of the same sex as recipients. About two-thirds of the mice in each group were obtained from microphthalmic stocks and one third from grey-lethal stocks.

Within 2 hours before infusion of cells, recipients were exposed to whole body irradiation at a dosage of 900 rads from a cobalt-60 source. Without anticoagulant, the thoroughly dispersed cells were administered via the transverse facial vein. As determined by a standard leukocyte counting procedure, the number of nucleated cells per injection ranged from 20 million to 40 million. For the first week after irradiation tetracycline and milk were given by stomach tube once a day. Thereafter the antibiotic was added to the drinking water and McCollum's diet I (*3*) was provided as a dry powder and wet mash. To monitor skeletal changes a tibia was obtained by hind limb amputation at 1 or 2 months af-

ter onset of the experiment, and radiograms were made at monthly intervals. Three animals per group were killed and autopsied at 2, 4, and 6 weeks after onset and seven to nine animals per group at 12 weeks after onset. A few of the irradiated animals died before the scheduled times. At autopsy the lymphatic organs, skull, and long bones were prepared for light microscopy. In addition, small samples of long bones were prepared for electron microscopic and acid phosphatase histochemical analysis (*4*).

The first sign of remodeling failure noted 2 weeks after onset was the excessive accumulation of calcified cartilage and bone along the epiphyseo-diaphyseal junctions of the major long bones of the experimental animals. In radiograms these accumulations formed conspicuous radiopaque bands under the growth plates of the proximal humerus, iliac crest, distal femur, and proximal tibia (Fig. 1A). The more rapid the growth rate of the ossification center, the more massive the lesion produced. There was no evidence that any of the matrix deposited during the observation period had been resorbed in the experimental animals. Histologic study of the proximal tibial metaphysis at varying times after transplantation revealed that the spongiosa not only increased in extent, especially longitudinally, but also became progressively more dense because of continued bone matrix deposition over most of the trabecular surfaces (Fig. 1E). Radiographically and histologically, the lesions induced experimentally closely resembled those of the osteopetrotic controls (Fig. 1, C and G). The osteoclast population, which was large and extensively distributed throughout the constricted vascular channels of the experimentally induced lesions, possessed the cytologic and histochemical characteristics of the osteoclasts of the osteopetrotic controls, including an abundant organelle-poor cytoplasm, lack of ruffled borders, and deficiency or lack of acid phosphatase activity. The medullary cavities seen in the experimental mouse long bones (Fig. 1A, circles) existed at the time of transplantation and represent levels of the diaphyses where osteogenic activity is minimal or absent. According to measurements made on serial radiograms, the medullary cavities of the experimental mice did not continue to expand in pace with long bone growth as they did in the normal control (Fig. 1D). The arrest of medullary cavity expansion was attributed to failure of bone resorption.

Irradiated controls which received infusions of normal spleen cells showed no evidence of resorption failure radiologically (Fig. 1B) or histologically (Fig. 1F). Within a few weeks after irradiation, the bone marrow was fully reconstituted, and osteogenic, osteolytic, and growth plate functions which were temporarily arrested by irradiation returned within a week to normal levels of activity.

Histological examination of the lymphatic organs of all the experimental and control animals revealed thymus glands of normal appearance and well-differentiated thymus-dependent zones in the spleens and lymph nodes. These findings and the fact that body weight was maintained in the animals receiving transplants help to rule out the possibility that graft-versus-host disease played a role in the inducement of osteopetrosis. In contrast to the osteopetrotic changes observed in this study, graft-versus-host disease is associated with osteoporotic lesions (*5*).

The reciprocal transplantation of bone marrow and spleen cells in mice of osteopetrotic stocks has revealed that the hematopoietic tissues produce migratory cells essential to hard tissue removal. If these cells originate from a genetically defective source such as the spleen of grey-lethal or microphthalmic mutants, the result is osteopetrosis. However, if the mutant's original source of these cells is replaced (through irradiation and transplantation) or supplemented (through establishment of cross-circulation) by a source provided by a normal littermate, bone resorption will be restored and signs of osteopetrosis will disappear.

Additional work is necessary to determine whether or not the migratory cells are precursors of osteoclasts or the source of humoral substances such as osteoclast activating factor (*6*) or prostaglandins (*7*) which may regulate resorptive activities of bone resorbing cells.

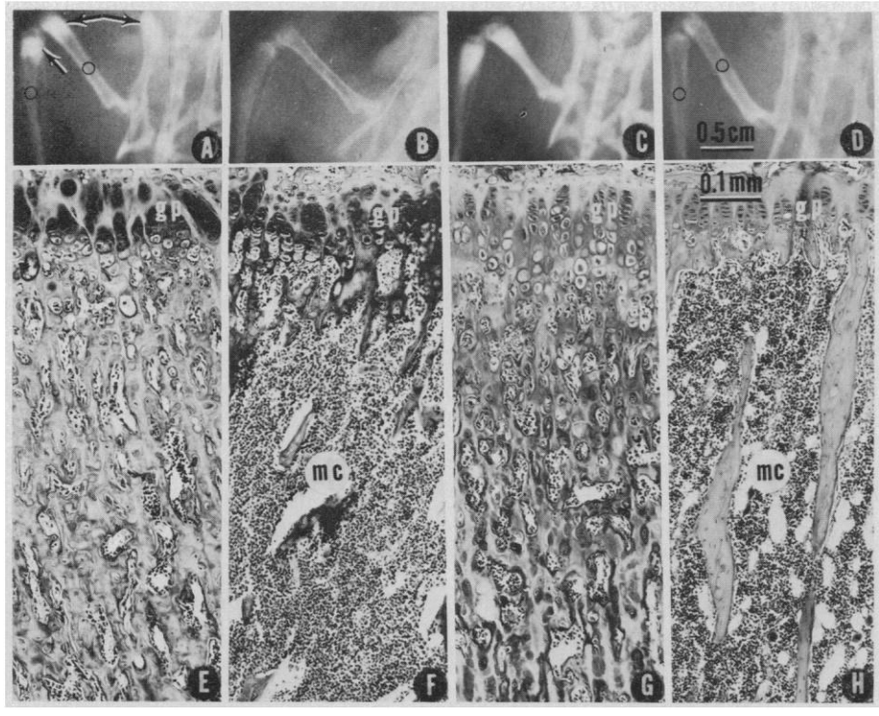

Fig. 1. (A to D) Radiograms (× 2) of experimental splenic transplant (A), control splenic transplant (B), osteopetrotic control (C), and normal control (D). At 21 days of age the experimental animal received lethal irradiation followed by an intravenous infusion of 35 million spleen cells obtained from an osteopetrotic littermate. Continued deposition of calcified cartilage and bone in the absence of resorption produced conspicuous radiopaque lesions (A, arrows) which resemble those of the osteopetrotic control (C). The presence of medullary cavities (circles) identified the portion of the long bones existing at the onset of the experiment. Unlike the medullary cavities of the long bones of the normal control (D), those of the experimental mouse did not continue to expand and hence are shorter than normal. (E to H) Photomicrographs (× 100) of the proximal tibial diaphysis, including growth plate (*gp*) of experimental (E), transplant control (F), osteopetrotic control (G), and normal control (H) mice. Histologically, the lesions of the experimental (E) and osteopetrotic control (G) consist of an abnormally dense primary spongiosa within the constricted vascular channels, in which there are numerous abnormal osteoclasts (not identified here). The transplant control preparation (F), like that of the normal control (G), possesses a fully expanded medullary cavity (*mc*) and only a few short trabeculae.

DONALD G. WALKER
Department of Anatomy,
Johns Hopkins University,
Baltimore, Maryland 21205

References and Notes

1. D. G. Walker, *Endocrinology* **91**, 916 (19 → *Science* **180**, 875 (19 → D. W. H. Barnes, J. F. Loutit, J. M. Sansom, *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **188**, 501 (1975); D. G. Walker, *J. Exp. Med.* **142**, 651 (1975).
2. D. G. Walker, *Clin. Orthop. Related Res.* **97**, 158 (1973).

3. McCollum's diet I, slightly modified, expressed on the basis of dry weight, includes whole wheat, 70 percent; casein, 16 percent; whole milk powder, 11 percent; iodized NaCl, 0.75 percent; $CaCO_3$, 1.7 percent; and a vitamin concentrate in an amount to give 1.5 USP units of vitamin D and 7.5 units of vitamin A per gram of diet.
4. B. T. Barka and P. J. Anderson, *J. Histochem. Cytochem.* **10**, 741 (1962).
5. L. Berek, Z. Banos, P. Anderlik, I. Szeri, K. Aszódi, *Experientia (Basel)* **26**, 92 (1970).
6. J. E. Horton, L. G. Raisz, H. A. Simmons, J. S. Oppenheim, S. E. Mergenhagen, *Science* **177**, 793 (1972).
7. L. G. Raisz, A. L. Sandberg, J. M. Goodson, H. A. Simmons, S. Mergenhagen, *ibid.* **185**, 789 (1974).
8. I thank S. Dobbs for histologic and animal husbandry work, C. Reather for photomicrography, and S. C. Marks, Jr., for the acid phosphatase histochemical analyses. Supported by grant AM 12491 from the National Institute of Arthritis and Metabolic Diseases.

2 January 1975; revised 4 August 1975

Hepatic Peroxisome Proliferation: Induction by Two Novel Compounds Structurally Unrelated to Clofibrate

Abstract. *Two hypolipidemic compounds [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid, and 2-chloro-5-(3,5-dimethylpiperidinosulfonyl)benzoic acid (tibric acid) greatly increased the number of peroxisomes (microbodies) in liver cells of rats and mice. This augmented peroxisome population was accompanied by significant elevation of liver catalase activity. These two hypolipidemic peroxisome proliferators are structurally different from ethyl* α-p-*chlorophenoxyisobutyrate (clofibrate) and other hypolipidemic, aryloxyisobutyrate derivatives which cause hepatic peroxisome proliferation. Induction of peroxisome proliferation by these structurally unrelated hypolipidemic compounds suggests a possible relation between hepatic peroxisome proliferation and hypolipidemia.*

Peroxisomes (microbodies), cytoplasmic constituents characterized morphologically by a single limiting membrane and a finely granular or homogeneous matrix, have recently been recognized as ubiquitous structures in animal and plant cells (*1*). In liver and kidney cells these organelles possess catalase and several H_2O_2-generating oxidases (*2, 3*), but their precise functional significance remains unknown. Nearly 10 years ago, de Duve and Baudhuin (*2*) suggested that hepatic and renal peroxisomes may participate in gluconeogenesis, while Allen and Beard (*4*) proposed that these organelles, by degrading H_2O_2, protect cells from random peroxidation. Although no satisfactory evidence supports these hypotheses, protection against heavy concentrations of H_2O_2 may be one of the principal properties of these catalase-containing organelles present in almost every cell type (*5*). Based on the close morphologic association of peroxisomes and lipid droplets in liver cells, Novikoff and Shin (*6*) postulated that peroxisomes might play a role in lipid metabolism. The observation that a potent hypolipidemic drug, clofibrate (ethyl-α-*p*-chlorophenoxyisobutyrate, CPIB), induced peroxisome proliferation in liver cells of rats and mice (*7, 8*) also suggested a relation between hepatic peroxisome proliferation and hypolipidemia. However, additional studies (*9*) favored the hypolipidemia and peroxisome proliferation of clofibrate to be independent properties.

Recently, a number of potent hypolipidemic analogs of clofibrate were shown to induce profound proliferation of peroxisomes in liver cells (*10, 11*). Whether this indicates a relationship or accidentally related properties of structurally related compounds could not be decided (*11*). Up to now, all known peroxisome proliferators possessed hypolipidemic properties; and all are closely related to clofibrate (Fig. 1, compounds **1** to **5**). We now report that [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14,643) and 2-chloro-5-(3,5-dimethylpiperidinosulfonyl)benzoic acid (tibric acid; CP-18,524) (Fig. 1, compounds **6** and **7**), two new hypolipidemic compounds with chemical structure distinctly different from clofibrate or its analogs, also produce marked proliferation of hepatic peroxisomes in rats and mice. The stimulation of hepatic peroxisome proliferation by these structurally unrelated hypolipidemic compounds suggests that the peroxisome proliferative and hypolipidemic responses are interrelated.

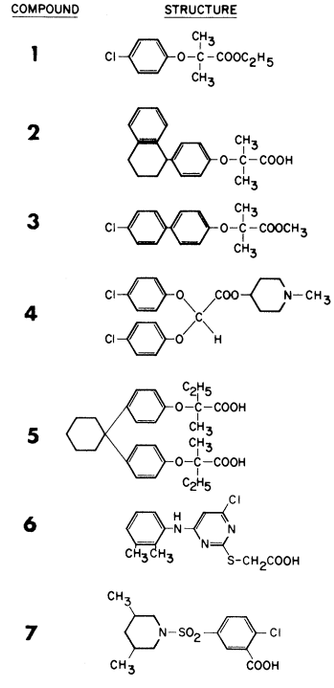

Fig. 1. Chemical structures of peroxisome proliferators. Compounds **1** to **5** are aryloxyisobutyrate derivatives: **1**, ethyl-α-*p*-chlorophenoxyisobutyrate (clofibrate; CPIB); **2**, 2-methyl-2-[*p*-(1,2,3,4 - tetrahydro - 1 - naphthyl)phenoxy]propionic acid (nafenopin); **3**, methyl-2-[4-(*p*-chlorophenyl)phenoxy]2-methyl propionate (methyl clofenapate); **4**, 1-methyl-4-piperidylbis(*p*-chlorophenoxy) acetate (SaH 42-348); **5**, 1,1-bis[4′-(1′′-carboxy-1′′-methylpropoxy) - phenyl]cyclohexane (S-8527). Compound **6**, [4-chloro-6-(2,3,-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14,643) and compound **7**, 2-chloro-5-(3,5-dimethylpiperidinosulfonyl)benzoic acid (tibric acid; CP-18,524), are not clofibrate analogs, but are as effective as aryloxyisobutyrate derivatives (compounds **1** to **5**) in inducing peroxisome proliferation and hypolipidemia.

Inbred male F-344 rats (Simonson Laboratories Inc., Gilroy, California), 125 to 150 g, and male Swiss Webster mice (supplied by a local dealer), 20 to 25 g, were housed in individual cages. The drugs, Wy-14,643 and tibric acid, were added to ground Purina laboratory chow at a level of 0.125 percent or 0.25 percent (by weight), which was always available to the animals. Liver biopsies were obtained from animals treated with these compounds for 1 to 4 weeks; after fixation for 2 hours in 2 percent OsO_4 buffered with *S*-collidine to *p*H 7.4, they were processed for electron microscopic examination (*11*). Portions of liver fixed in neutral buffered formalin were processed for light microscopy. For cytochemical localization of peroxisome catalase, samples of liver were fixed in 2.5 percent glutaraldehyde buffered with 0.1*M* sodium cacodylate, *p*H 7.4, for 4 hours. After fixation, the tissues were washed overnight in the cacodylate buffer. Sections (40 μm thick), prepared with a tissue chopper, were incubated for 30 to 45 minutes at 37°C in alkaline 3,3′-diaminobenzidine (DAB) medium (*12*). After incubation, the tissue was postfixed with OsO_4 and processed for electron microscopy.

Both compounds produced a significant ($P < .001$) increase in liver weights of rats and mice at 0.125 percent as well as 0.25 percent dose levels after 1 to 2 weeks of treatment (Table 1). Examination of these livers by light microscopy revealed enlarged hepatocytes containing abundant acidophilic granular cytoplasm. By electron microscopy, the liver cells showed a profound increase in the number of peroxisome profiles together with proliferation of smooth endoplasmic reticulum (Fig. 2). These organelles were numerous at 2 weeks and displayed considerable vari-