bone may play an important role in the pathogenesis of bone loss near areas of chronic inflammation. Identification of the factor may lead to new approaches in treatment or prevention of this bone loss.

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4. Phytohemagglutinin, 10 μg/ml (Burroughs Wellcome); Ag, representing 10 μg of wet frozen weight of starting material per milliliter of saline buffered with 0.02*M* phosphate.
5. Tritiated thymidine (1 μc) (specific activity, 6.0 μc/mmole; Schwarz Biochemical) was added to each residual 0.15-ml sedimented cell culture, incubated for 4.5 hours, and harvested by trichloroacetic acid precipitation. The counts per minute of radioactivity were determined in a Packard Tri-Carb scintillation spectrometer.
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7. For samples with culture medium, supernatant from A leukocytes and from B leukocytes and supernatant from the 4-hour culture of D leukocytes, the control mixture consisted of RPMI 1640 with 20 percent autologous plasma incubated for 6 days without leukocytes, and then mixed 1 : 1 with modified BGJ. For samples with supernatants from C leukocytes, the control mixture consisted of 10 percent serum from subject C, which had been inactivated by heat at 60°C for 30 minutes in modified BGJ. The control mixture for samples with supernatants from D leukocytes consisted of 10 percent serum, 40 percent RPMI 1640, and 50 percent BGJ. The phosphate concentration was adjusted to between 3.5 and 5.0 m*M*. There was no consistent difference in Ca^{2+} or PO_3^{3-} concentration or in *p*H between test and control media.
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Histochemical Phosphorylase Activity in Regenerating Muscle Fibers from Myophosphorylase-Deficient Patients

Abstract. *Fresh frozen sections of mature skeletal muscle fibers from patients with genetically determined "absence" of skeletal muscle phosphorylase (McArdle's disease) have no histochemical phosphorylase activity. That regenerating muscle fibers, in vitro and in vivo, from such patients do have histochemical phosphorylase activity present suggests a loss of enzyme activity with fiber maturity.*

Complete absence of skeletal muscle phosphorylase (McArdle's disease) (*1*) has been considered a genetically determined defect of glycogen metabolism, which results in the absence of biochemically and histochemically determined myophosphorylase activity in skeletal muscle (*2*). Attempts at demonstrating the enzyme immunologically in affected patients have also failed (*3*), suggesting that the enzyme is absent or inactivated both as an antigen and in its enzymatic activity.

Using collagen-coated cover slips (*4*) with the Maximow chamber, we have explanted small fragments of muscle (*5*) obtained by open or needle biopsy from three adult patients with verified myophosphorylase deficiency (*6*) and ten patients with histologically normal muscle. The muscle developed in vitro from the mononuclear myoblast stage through the early multinucleated stage to form very long, cross-striated fibers containing multiple large nuclei with one or two large round nucleoli. This growth occurred outside the original explant. No differences were noted in the overall growth pattern and cellular morphology of muscle derived from affected patients as compared to normal controls. At varying developmental stages, whole mounts of the cultures were rinsed in a balanced salt solution, dried in air for 1 hour, and stained for phosphorylase by the Takeuchi technique (*7*). This technique depends on the development of a purple color when newly formed long glycogen branches are reacted with dilute iodine.

Although the fresh frozen sections of the skeletal fibers from muscle biopsies of the three patients with phosphorylase deficiency showed complete absence of phosphorylase histochemically (Fig. 1a), the early multinucleated fibers and striated myofibers newly grown in vitro from those patients' biopsies had definite phosphorylase activity (Fig. 2, c to e). The distribution and amount of deep purple stained material was similar to that in cultured muscle from histologically normal biopsies (Fig. 2, a and b). In other cultures of similar developmental stages from the patients with phosphorylase deficiency, "preformed" cellular glycogen stained with the periodic acid–Schiff reaction was abundant in the newly formed early multinucleated muscle fibers and striated myofibers. It was diastase-sensitive and similar in distribution to the phosphorylase staining, but somewhat more abundant; cultures from the control patients were similar.

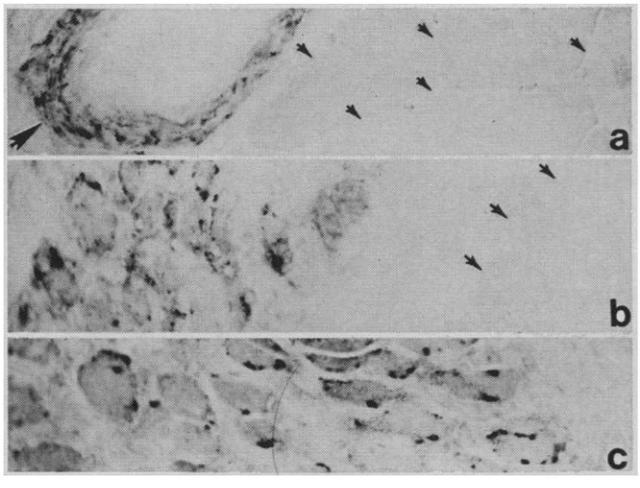
We used four types of histochemical controls. (i) A frozen section of the muscle biopsy from a phosphorylase-deficient patient and from a control patient with normal phosphorylase activity, and the tissue culture of muscle from the phosphorylase-deficient patients were stained simultaneously in the same solution. Only the biopsy from the phosphorylase-deficient patient failed to show staining of skeletal muscle fibers (although smooth muscle fibers in arterial vessels were stained), a procedure which ruled out possible technical errors. (ii) Sister cultures of similar developmental stages from the phosphorylase-deficient patients were dried in air for 1 hour and dipped into the dilute iodine without prior (histochemical) incubation; they did not show staining. (iii) Other sister cultures grown from the muscle of phosphorylase-deficient patients were incubated in the histochemical medium for phosphorylase, from which the substrate glucose-1-phosphate was omitted and then processed as usual; these cultures failed to show staining (Fig. 2f). (iv) Fresh frozen sections of muscle biopsies from a normal patient, a phosphorylase-deficient patient, and a phosphorylase-deficient patient with regenerating muscle fibers were dried in air for 1 hour, and handled as in (ii) and (iii) above; again, no staining occurred.

We considered the possibility that fibers from phosphorylase-deficient pa-

tients might have acquired the enzyme phosphorylase from the nutrient medium. This incorporation of exogenous enzyme seems to occur in fibroblasts cultured from arylsulfatase-A–deficient patients who have metachromatic leukodystrophy (*8*). To evaluate this possibility, Dr. D. L. Martin used a modification of the techniques of Sutherland (*9*), to perform biochemical assays for total phosphorylase activity on the following samples: (i) samples of fresh nutrient medium used for feeding the cultures; (ii) pooled drops of medium taken from cover slips containing growing cultures of mouse embryo spinal cord after the medium had been in contact with the culture for 2 days; (iii) nutrient medium taken from a Rose perfusion chamber containing only human muscle growing from a histologically normal biopsy specimen, after having been in contact with the culture for 2 days; and (iv) pooled drops of medium taken from cover slips containing growing mouse embryo spinal cord plus human muscle explants showing early outgrowth, harvested after having been in contact with the cultures for 2 days. In none of these samples was assayable phosphorylase activity present, although control muscle homogenates treated simultaneously as controls showed normal amounts of phosphorylase activity. These results suggest that exogenous phosphorylase was not being supplied to the growing muscle cells.

We have extended our observations in vitro to skeletal muscle regenerating in vivo. In a phosphorylase-deficient patient, we again obtained a biopsy of muscle from the site that was originally biopsied 1 week earlier. The skeletal muscle fibers of the original biopsy were histochemically phosphorylase negative (*10*). However, regenerating fibers in the second biopsy were found to be histochemically phosphorylase positive, in a pattern similar to that of the patients' cultured fibers (Fig. 1, b and c), while uninjured mature fibers in adjacent parts of the biopsy remained phosphorylase negative (Fig. 1b).

Because only a limited number of biopsies are permitted by the patients and because it has not yet been possible to establish a cell line or reproducible subcultures of the differentiated human muscle fibers, our studies have been limited to growth from primary explants. In the regenerating fibers of phosphorylase-deficient patients we describe histochemical phosphorylase activity, but have not had an adequate amount of tissue to do biochemical



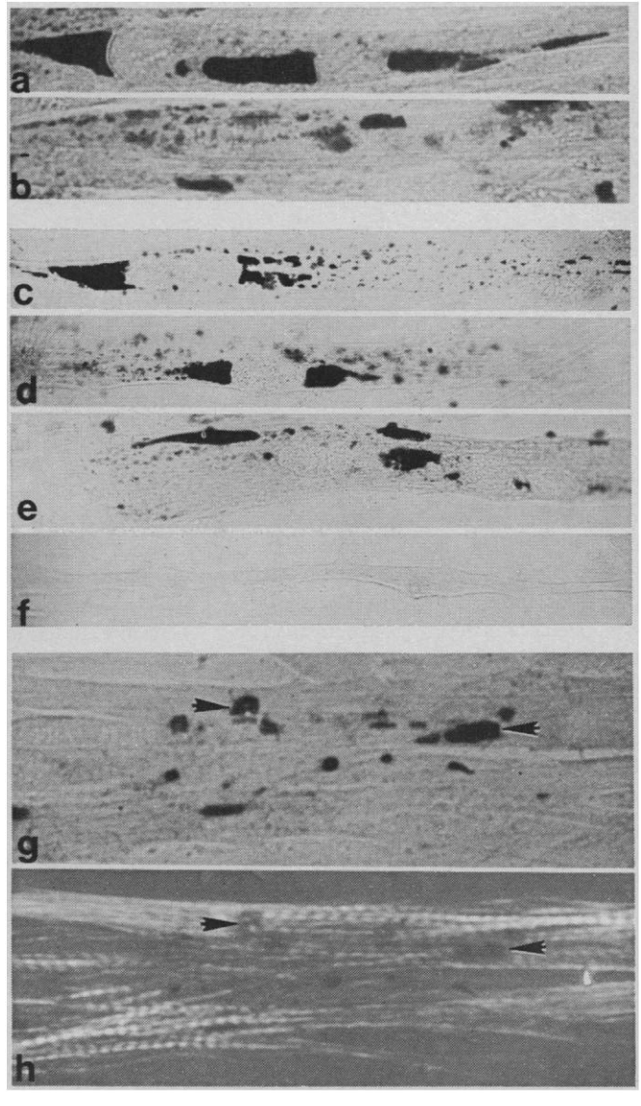


Fig. 1 (left). (a) The original biopsy of the myophosphorylase-deficient patient from which cultures were grown; skeletal muscle fibers (right, small arrows) are unstained while smooth muscle of the blood vessel wall (left, large arrow) is stained; × 300. (b and c) The second biopsy of that patient taken 7 days later shows that in (b) the small regenerating fibers (left) are stained in puddles and small lakes, while undamaged fibers (far right, arrows) are unstained; in (c) details of the puddles of staining in the small regenerating fibers are shown; × 190.
Fig. 2 (right). Human skeletal muscle fibers stained by the standard histochemical method for phosphorylase activity. (a and b) Fibers newly grown in tissue culture from a normal control show lakes (a) and puddles (b) of dark staining in the cytoplasm; 22 days in culture; × 700. (c, d, and e) Fibers newly grown in culture from a myophosphorylase-deficient patient showing lakes and puddles of dark staining in the cytoplasm, like those of normal fibers; 16 days in culture; × 400. (f) Fiber from a sister culture of the myophosphorylase-deficient patient; omission of the substrate glucose-1-phosphate results in no staining; × 190. (g and h) One field of newly grown fibers from a myophosphorylase-deficient patient, in bright-field (g) and polarized light (h); phosphorylase staining is within cross-striated muscle fibers; arrows point to identical areas of phosphorylase staining in the two pictures, 24 days in culture; × 700.

kinetic and other studies to establish that the activity is attributable to perfectly normal myophosphorylase enzyme. Nevertheless, these observations suggest that genetic coding for the development of a form of myophosphorylase activity is present in precursor cells of the regenerating skeletal muscle fibers of patients with myophosphorylase deficiency, and is expressed as enzymatic activity in regenerating fibers both in vitro and in vivo. Several hypotheses may be considered to explain the subsequent absence of phosphorylase activity (*11*, *12*) in mature fibers in vivo. (i) With muscle fiber maturity there may be loss of a specific enzyme which normally maintains phosphorylase production, survival, or activity. (ii) An abnormal specific protease may develop in the mature fibers which selectively inactivates myophosphorylase. (iii) A normally repressed "myophosphorylase repressor gene" may be derepressed, inhibiting the gene for myophosphorylase production. (iv) A normally present but inactive myophosphorylase inhibiting or destroying enzyme (or other factor) may become active, impairing myophosphorylase enzyme survival or activity.

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6. All three patients met these four diagnostic criteria: (i) A history of muscle pain and cramps provoked by exercise, often limiting exercise, cases 1 and 2 having had pigmenturia following strenuous exercise; (ii) absence of a venous lactate rise with ischemic exercise, which produced contractures in cases 1 and 3; (iii) absence of striated muscle phosphorylase activity histochemically, with phosphorylase activity being present in the smooth muscle of blood vessels; and (iv) apparently excessive muscle glycogen histochemically. In addition, patients 2 and 3 were brother and sister; biochemical assay of the first muscle biopsy from the brother showed complete absence of total phosphorylase activity.
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Mouse Leukemia: Depression of Serum Interferon Production

Abstract. *Production of circulating interferon is significantly impaired in AKR/J mice after development of lymphoblastic leukemia and in Balb/c mice with clinical signs of Friend erythroblastic leukemia. This alteration has been observed with three interferon inducers, each one known to elicit an interferon response in different cells.*

Induction of interferon in vivo is currently being explored as a possible approach to the therapy of viral diseases and also of certain forms of neoplasia (*1*). I therefore report the marked inhibition of the production of interferon observed both in Balb/c mice with Friend virus–induced erythroblastic leukemia and in AKR/J mice after development of lymphoblastic leukemia.

Friend leukemia was induced in 6- to 8-week-old Balb/b mice by intravenous injection of a 1 to 10 (weight to volume) spleen extract prepared from the fourth passage in Balb/c mice of a Friend virus suspension, routinely maintained in Swiss mice (*2*). This virus suspension caused a palpable increase of spleen size in about 10 days, with an ensuing mortality of nearly 100 percent 8 to 10 weeks after inoculation. Production of circulating interferon was measured when the size of the spleen had increased significantly, that is, usually about 3 weeks after inoculation of Friend virus (Fig. 1). Three different interferon inducers were tested, each stimulating serum interferon production in a different cell system: (i) Newcastle disease virus (NDV), a paramyxovirus that induces interferon synthesis mainly in lymphocytes (*3*); (ii) encephalomyocarditis virus (EMC), a small RNA virus that stimulates interferon production in radioresistant cells that are not derived from bone marrow stem cells [(*4*) and unpublished results]; and (iii) polyriboinosinic-polyribocytidylic acid [poly(I) · poly(C)], a synthetic polyribonucleotide that induces interferon synthesis in a radioresistant cell population derived from hemopoietic stem cells, probably macrophages [(*5*) and unpublished results]. Origin of virus strains, titers, and mode of cultivation have been published (*6*). Poly(I) · poly(C) (P-L Biochemicals Inc., Milwaukee, Wisconsin) was resuspended in Field's phosphate buffer (*7*) at a concentration of 250 μg/ml before use. Figure 1 represents individual serum interferon levels, measured at the peak of production, in Balb/c mice with and without Friend leukemia. For all three interferon inducers, a highly significant ($P < .001$) decrease of interferon levels was observed in leukemic animals. The inhibition was related to the degree of splenomegaly, as shown in experiments with poly(I) · poly(C) and EMC, in which interferon response was measured at an early and a later stage of the disease. When the average spleen weight was about twice the normal value, that is, 250 mg, serum interferon levels were already significantly lower than those of control mice ($P < .01$); at a later stage of the disease, when spleen size averaged 2000 mg, serum interferon response had come down further, to represent only 5 to 10 percent of that obtained in healthy mice.

Similar results were obtained in the case of a spontaneous leukemia, the AKR mouse lymphoblastic leukemia. Results plotted in Fig. 2 show a highly significant ($P < .001$) decrease of production of serum interferon in leukemic AKR/J mice, 6 to 9 months old, injected intravenously with NDV or poly(I) · poly(C). In the control