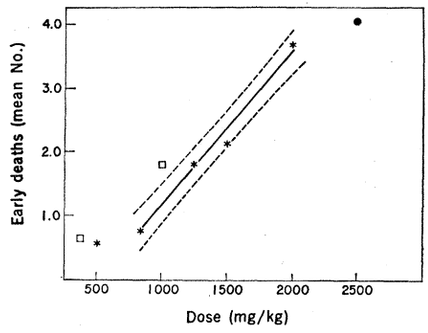

Fig. 2. Dose-response relationship for numbers of early fetal deaths per pregnancy in females mated in the 2nd week after treatment of males with trimethylphosphate. Numbers of early deaths are mean values per pregnant female. Mean (—) and 95 percent confidence interval (----) for linear response of four highest doses of five dosages administered by single intraperitoneal injection. Superimposed points from other experiments showing general agreement. □ Single intraperitoneal injection at two dosages. • Gavage on five successive days at 500 mg/kg each (cumulative dose of 2500 mg/kg).

ance of dose as a function of weeks after TMP administration was performed with matrix inversion techniques.

Trimethylphosphate generally was not toxic at the doses tested. One male that received five oral doses of 1000 mg/kg did die (Table 1). Pregnancy rates in females mated with test and control males did not differ consistently; however, the incidence of pregnancy was generally reduced at the highest total dosages. Weekly means of total implants per pregnant control ranged from 10.5 to 12.6. After injection of 200 and 1000 mg/kg, reduction in numbers of total implants during the first 3 weeks of mating was significant and related to dosage (Table 2); however, when TMP was injected over a wider range of dosages, 500 to 2000 mg/kg, no distinct reductions related to dosage were observed, although lower numbers of total implants were apparent in the 2nd week. After gavage with higher total doses, reduction in numbers of implants in the first 3 weeks of mating was significant and related to dosage (Fig. 1, Table 2); at the highest dosage, implants were also reduced at the 5th week. This is also apparent from the alternate criterion of proportion of females with reduced numbers of total implants (Fig. 1). At the higher total oral doses, losses before implantation were consistent and related to dosage in the first 3 weeks of mating (Fig. 1).

Mean numbers of early fetal deaths per pregnant control ranged from 0 to 0.68 with a mean of 0.33 (Table 1). A highly significant increase in early deaths occurred during the first 3 weeks of mating for all experiments (Table 2). This is manifested as a dosage-dependent increase in early fetal deaths occurring in the second mating week at all dosages, except the highest oral dose where effects were noted earlier (Fig. 1); absence of early fetal deaths at the 2nd week of mating for the latter dosage probably reflected reduced pregnancies (Table 1) and losses before implantation (Fig. 1). The dose-response regression for early deaths in the second mating week was linear over the four highest doses injected intraperitoneally, with a slope of 1.2 (± 0.1 early deaths per 500 mg); the effects of TMP appear cumulative and depend on total dose administered irrespective of route (Fig. 2).

In these experiments, single parenteral and repeated oral administration of less than toxic concentrations of TMP to male mice produced both antifertility and mutagenicity dependent on dosage. Antifertility effects, as manifested by reduced pregnancies, were less clearly defined and evident only at highest total dose. Mutagenic effects, as manifested by increased numbers of early fetal deaths and by losses before implantation, were restricted to matings during postmeiotic stages of spermatogenesis. As previously reported for mice treated with the mutagens tris(1-aziridinyl)phosphine oxide (TEPA) and tris(2-methyl-1-aziridinyl)phosphine oxide (METEPA), the time and dose-response relations for both losses before implantation and for increased numbers of early fetal deaths are similar. At high dosages, early deaths occurred in females with reduced number of total implants and in those with normal numbers of implants (*9*). Our results indicate a higher degree of mutagenic sensitivity of mice to TMP than suggested by previous data (*6*); in both studies, however, TMP appeared equally active after oral or parenteral administration.

Evaluation of potential human genetic hazards due to TMP requires data, presently generally unavailable, on precise conditions of its industrial use, including concentration in fuels. Additional information is also required on the concentration of unreacted TMP, and of any biologically active pyrolysis products, in automobile exhaust.

SAMUEL S. EPSTEIN, WILLA BASS
ELSIE ARNOLD, YVONNE BISHOP
Laboratories of Environmental Toxicology and Carcinogenesis, Children's Cancer Research Foundation, Inc., Department of Pathology, Harvard Medical School, and *Harvard School of Public Health, Boston, Massachusetts*

References and Notes

1. Ethyl Corporation, Technical publication ICC 4, *Ethyl ignition control compound 4.* A gasoline additive for control of spark plug fouling, surface ignition, and rumble; J. B. Hinkamps and J. A. Warren, *Ind. Eng. Chem.* **50**, 251 (1968).
2. J. H. Billman, A. Radike, B. W. Mundy, *J. Amer. Chem. Soc.* **64**, 2977 (1942); A. D. F. Toy, *ibid.* **66**, 499 (1944); F. W. Jones, G. O. Osborne, G. J. Sutherland, R. D. Topsom, J. Vaughan, *Chem. Commun.* **1**, 18 (1966).
3. A. D. F. Toy, *J. Amer. Chem. Soc.* **71**, 2268 (1949).
4. M. R. Gumbmann, W. E. Gagne, S. W. Williams, *Toxicol. Appl. Pharmacol.* **12**, 360 (1968).
5. W. B. Deichmann and S. Witherup, *J. Pharm. Exp. Ther.* **88**, 338 (1946).
6. H. Jackson and A. R. Jones, *Nature* **220**, 591 (1968).
7. A. R. Jones and H. Jackson, *Brit. J. Pharmacol.* **37**, 531 (1969).
8. G. Kolmark, *Compt. Rend. Trav. Lab. Carlsb. Ser. Physiol.* **26**, 205 (1956).
9. A. J. Bateman, *Nature* **210**, 205 (1966); U. H. Ehling, R. B. Cummin, H. V. Malling, *Mutat. Res.* **5**, 417 (1968); S. S. Epstein and H. Shafner, *Nature* **219**, 385 (1968); G. Rohrborn, *Humangenetik* **6**, 345 (1968); S. S. Epstein, S. R. Joshi, E. Arnold, E. C. Page, Y. Bishop, *Nature* **225**, 1260 (1970); S. S. Epstein, E. Arnold, K. Steinberg, D. MackIntosh, H. Shafner, Y. Bishop, *Toxicol. Appl. Pharmacol.*, in press.
10. Supported by NIH grants C-6516 and FR-05526 and by National Air Pollution Control Administration contract PH-86-66-169.

2 March 1970

Aerial Vision: Unique Adaptation in an Intertidal Fish

Abstract. Mnierpes macrocephalus, *a clinid fish of rocky shores of the eastern tropical Pacific, makes frequent terrestrial sojourns. The normal fish eye is myopic in air because of curvature of the cornea. This is overcome in* Mnierpes *by the presence of two flattened corneal surfaces.*

In aquatic vision, fishes rely solely on the movement of the round crystalline lens for visual accommodation. The cornea does not function in image formation because its refractive index is the same as that of water. In air, fish vision becomes myopic because the refractive power of the curved cornea

is in turn added to that of the lens (*1*). Previously reported adaptations for aerial vision in amphibious fishes have demonstrated the role of modified lens shape. The lens of the mudskipper, *Periophthalmus*, is slightly flattened to correct for the convergent refraction of its strongly curved cornea in air. The four-eyed fish, *Anableps*, utilizes a pyriform lens positioned to focus simultaneous images from both the aquatic and the aerial focal planes (*2*).

The clinid fish *Mnierpes macrocephalus* (Teleostei, Perciformes) inhabits the surf-swept rocky shores of the tropical east Pacific and relies heavily on aerial vision for feeding and orientation in its habitat (*3*). The eye of *Mnierpes* has two flattened corneal surfaces (Fig. 1). The utilization of a flat cornea to facilitate acute aerial vision, as found in the eye of *Mnierpes*, represents a previously unknown adaptation in fishes.

The flattened surfaces are supported at the center of the eye by a narrow, lightly pigmented vertical thickening of the cornea (Fig. 2). The dorsal and ventral margins of the cornea are heavily pigmented and, along with the opaque vertical thickening, reduce the aperture of the eye to the anterior and posterior corneal windows. A flattened cornea allows accommodation in air, as demonstrated in the flying fish *Cypselurus heterurus*, in which the cornea is pyramidal so that the fish looks out through three somewhat flattened surfaces (*4*). This adaptation avoids the myopic condition normally produced by a fish's cornea in air. The moveable lens would focus the image in the usual way.

There is a groove between two nodules on the ventral margin of each corneal window (Fig. 1). We have observed that this functions in draining water from the corneal surface when the fish emerges. Dissection and histological sections show that the retinal fissure is appropriately positioned directly opposite the vertical corneal bar and that a falciform process is absent, retinal nourishment being facilitated by hyaloid venation.

The eye of *Mnierpes* is very similar to that of the related Galápagos endemic form, *Dialommus fuscus*. Breder and Gresser (*5*) described the eye of *Dialommus* and pointed out the heavy corneal pigmentation and division of the eye by a vertical bar, but failed to note flattened corneal surfaces. Walls (*2*) pointed out that if the corneal aperture is reduced by pigmentation, the horizontal visual field will be maximized by two openings. However, it seems unlikely that the most important function of the corneal pigmentation is to shield the retina from excess light; rather, it screens the curved parts of the cornea that would produce an unfocused image in air.

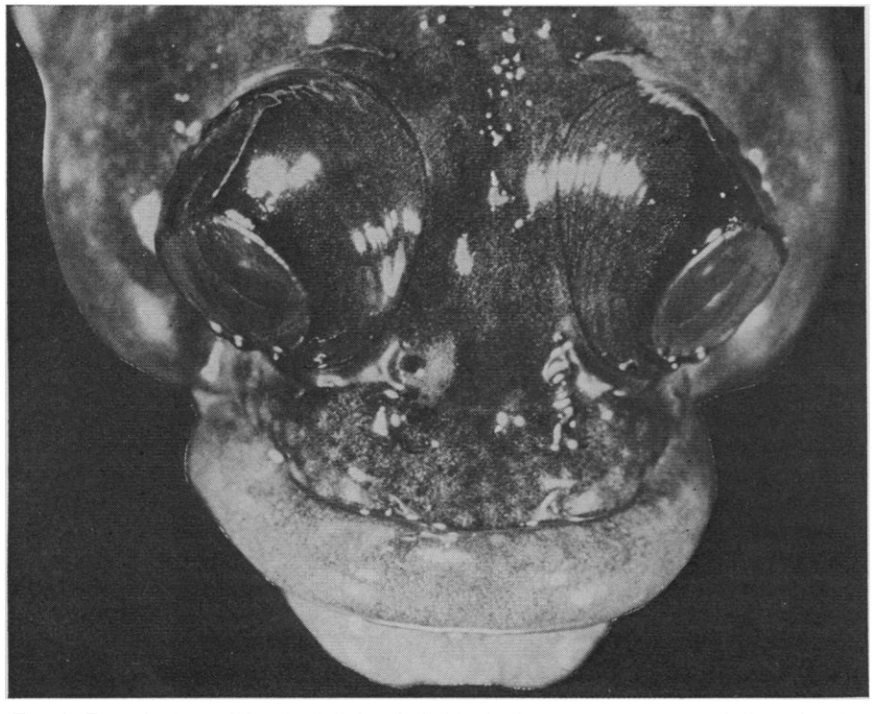

Fig. 1. Dorsal view of head of living individual of *Mnierpes macrocephalus*, showing flattened corneal areas and nodules on ventral margin of eye.

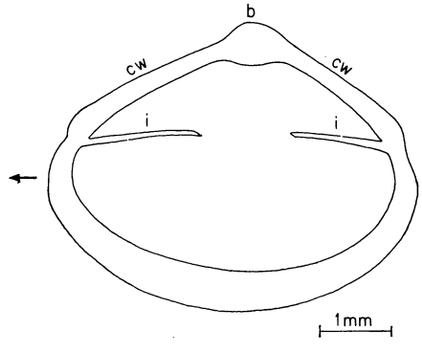

Fig. 2. Diagrammatic representation of a median horizontal section through the eye of *Mnierpes macrocephalus* (lens removed). Abbreviations: *b*, median pigmented bar; *cw*, corneal window; and *i*, iris.

Munk (*6*) has redescribed the eye of *Dialommus*. He found that the eye is foveate, both rods and cones are present, there is no falciform process, and hyaloid venation is present. His specimen was preserved and he was unable to determine whether the cornea had flattened surfaces but suggested that they were probably present. We have examined the eyes of preserved specimens of *Dialommus* and find that they are very similar to those of preserved *Mnierpes*. This strongly suggests that *Dialommus* shares the adaptation we have demonstrated for *Mnierpes*.

The mudskipper, *Periophthalmus*, utilizes a flattened lens to avoid myopia when in air; however, in water it probably is hypermetropic (*2*). This is not a serious limitation for *Periophthalmus*, which is active during periods of low tide and spends high tide in burrows (*7*) or remains above the water most of the time (*8*). However, *Mnierpes* lives at the air-water interface and moves continually from one medium to the other, and its observed acute vision in both air and water (*3*) suggests that the flattened corneal surfaces allow emmetropia in both media.

JEFFREY B. GRAHAM
RICHARD H. ROSENBLATT
Scripps Institution of Oceanography, La Jolla, California 92037

References

1. J. R. Brett, in *The Physiology of Fishes*, M. E. Brown, Ed. (Academic Press, New York, 1957), vol. 2, pp. 144–146.
2. G. L. Walls, *The Vertebrate Eye and Its*

Adaptive Radiation (Hafner, New York, 1963), pp. 429–436.
3. J. B. Graham, *Mar. Biol.*, in press.
4. E. R. Baylor, *Nature* **214**, 307 (1967).
5. C. M. Breder and E. B. Gresser, *Zoologica* **24**, 239 (1939).
6. O. Munk, *Vidensk. Medd. Naturhist. Foren. Kjobenhavn* **132**, 7 (1969).
7. R. C. Stebbins and M. Kalk, *Copeia* **1961**(1), 18 (1961).
8. M. S. Gordon, J. Boëtius, D. H. Evans, L. C. Oglesby, *ibid.* **1968**(4), 853 (1968).

22 December 1969; revised 17 February 1970

Formation of Virus-like Particles by Bone Cells in Mice with a High Incidence of Spontaneous Leukemia

Abstract. *Bone samples from potentially leukemic and leukemic mice revealed numerous 90- to 110-nanometer particles morphologically identical to murine leukemia virus. Particles were observed budding from plasma membranes of osteocytes and osteoblasts but were most numerous in osteocyte lacunae. Particles were not observed in bone samples from mice which rarely develop leukemia.*

Electron microscopy of lymphopoietic tissues from potentially leukemic and leukemic mice reveals budding and extracellular particles which have been identified as leukemogenic viruses (MLV) (*1*). Similar particles can also be formed by apparently normal epithelial cells (*2*). Budding of virus from connective tissue cells, however, is rare (*3*). During examination of apparently normal bone from leukemic mice, we observed large numbers of particles budding from osteocytes and osteoblasts. Similar production of virus-like particles by bone cells occurs in chickens with osteopetrosis induced by avian leukosis virus (*4*). However, virus production in osteopetrosis was confined to the periosteum and was not observed in osteocytes of the subperiosteal matrix. Virus particles have also been reported in mouse osteosarcomas (*5*).

Ten AKR and ten C3H/Fg female mice, 3- to 36-weeks old were used in this study. These strains have an 80 to 90 percent incidence of spontaneous lymphocytic leukemia which usually develops between 7 and 9 months of age (*6*). Comparable samples from five C57/Bl, five L CS/Fg, five A/Jax, and five Ha/ICR female mice were also examined. Mice of these strains have a low incidence of leukemia (*6*). Portions of skull, sternum, vertebra, metaphyseal bone, bone marrow, thymuses, and spleen were removed under ether anesthesia and prepared by standard procedures for electron microscopic examination following fixation in 6 percent glutaraldehyde (*7*). Bone samples were not decalcified. Samples of the same materials were fixed in 10 percent formaldehyde, and bone samples were decalcified in 10 percent buffered ethylenediaminetetraacetic acid. Paraffin sections stained with hematoxylin and eosin were examined by light microscopy.

Numerous particles morphologically identical to MLV were associated with osteocytes and osteoblasts of every C3H/Fg and AKR mouse (Fig. 1, A and C). More particles were associated with osteocytes than with osteoblasts; they appeared to originate from both cell types by budding from plasma membranes (Fig. 1B). No evidence of virus production by cartilage cells, osteoclasts, or fibroblasts of the periosteum was observed. Particles were not observed in thymuses or spleens of 3- to 5-week-old mice. Although present in small numbers in older mice, large numbers of particles were not observed in lymphopoietic tissues except in obviously leukemic mice. No virus-like particles were observed in bone or lymphopoietic tissues from the four strains of mice having a low incidence of leukemia.

Particles resembling MLV have been reported in newborn and in embryonic AKR mice (*8*), but Dirksen and Cailleau (*9*) did not find similar particles in lymphopoietic tissues of AKR mice less than 10 weeks old. Our study confirms that MLV particles are rare in lymphopoietic tissue of mice of this age and demonstrates that particles morphologically identical to MLV are numerous in bone; this suggests that bone cells contribute significantly to the viremia that precedes the onset of leukemia in these mice. However, we have not determined whether the particles produced by the bone cells are leukemogenic. Although we saw no alterations in bone morphology in this study, female AKR mice, protected from leukemia by thymectomy and surviving beyond 18 months of age, have an 87.5 percent incidence of osteomas. Nonthymectomized AKR mice, which rarely reach this age, exhibit a similar incidence of osteomas (*10*).

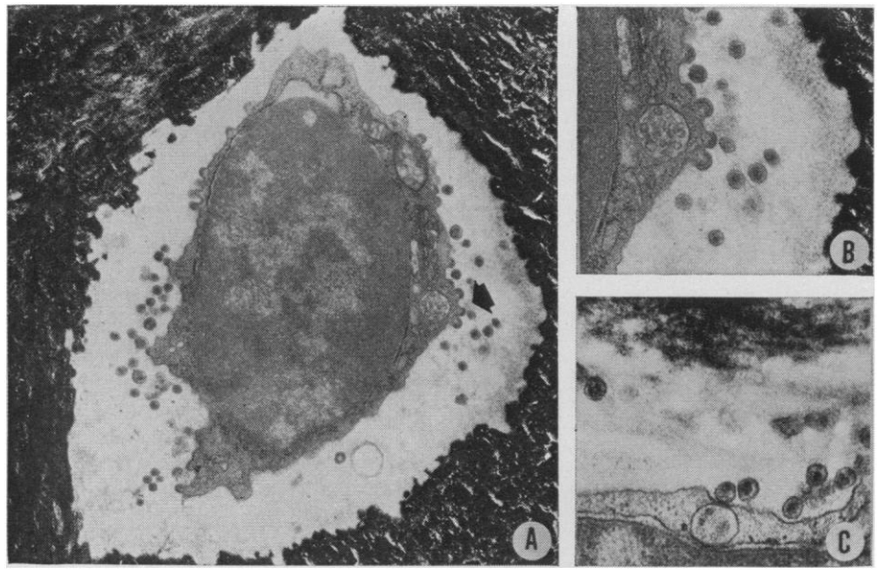

Fig. 1. (A) Osteocyte from undecalcified metaphyseal bone of a 5-week-old AKR mouse showing budding particles (arrow) and particles in the lacuna (× 13,000). (B) Enlargement of budding particles (× 25,000). (C) C-type particles between an osteoblast and the mineralized bone matrix in undecalcified bone of a 6-week-old C3H/Fg mouse (× 31,000).

BRIAN H. SCHOFIELD
Department of Orthopaedic Surgery, Johns Hopkins Hospital, Baltimore, Maryland 21205
CHARLES P. BARRETT
Department of Anatomy, University of Maryland School of Medicine, Baltimore 21201
STEPHEN B. DOTY
Johns Hopkins Hospital
FRANK H. J. FIGGE
University of Maryland
ROBERT A. ROBINSON
Johns Hopkins Hospital