to the distance between the ruffles and folds. Exaggeration of the transverse reticulae formed by the boundaries of a series of cells could produce the folds and ruffles. The morphology of the scale surfaces suggests that the setae of *P. virens* and the fold-ruffle design found in related species are alternative specializations of a reticular arrangement of cell boundaries, which is itself derived from the primitive lamellate-dentate pattern.

The behavior of the scincid species we examined is poorly known. The original description of *Lipinia (Aulacoplax) leptosoma* (*4*) indicates that *L. leptosoma* leap 1½ to 2 feet or more between leaf surfaces and climb vertical and overhanging glass surfaces. In behavioral terms, the ruffle-fold architecture appears to be functionally comparable to setae—it can establish a grip that supports the animal. This makes the divergence of *P. virens* even more problematical, for if a ruffled scale surface can establish an "adhesive" grip as do setae, and the fold-ruffle architecture exists in the more primitive members of the radiation, the adaptive significance of the shift to setae in *P. virens* is unclear.

Among lizards, at least four different fine structural designs are capable of forming an adhesive grip—three types of setae (*6*) and the fold-ruffle morphology. It is not yet possible to interpret the functional significance of the differences among the setae nor those between the setae and ruffles, but the morphological comparisons reveal a variety of interesting evolutionary patterns. Convergence in setal morphology and alternative designs for adhesion in distantly related lineages, such as anoles and the scincid species other than *P. virens*, are examples of relatively common themes in comparative morphology. Striking divergence of closely related species and the appearance of a complex specialization as an isolated species [or generic (*11*)] adaptation is less common. The *P. virens* fine structure appears to illustrate very rapid morphological change relative to the taxonomic rate of evolution. Investigation of the adaptive significance of cases such as this—illustrating striking morphological divergence at low taxonomic levels—may provide insight into the evolution of new adaptive complexes.

E. E. WILLIAMS
Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138
J. A. PETERSON
Department of Biology, University of California, Los Angeles 90024

References and Notes

1. A. Russell, in *Morphology and Biology of Reptiles*, A.d'A. Bellairs and C. B. Cox, Eds. (Academic Press, London, 1976), p. 217; *Copeia* **1979**, 1 (1979).
2. E. E. Williams, *Bull. Mus. Comp. Zool. Harv. Univ.* **129**, 463 (1963).
3. P. F. A. Maderson, *Forma Functio* **3**, 179 (1970).
4. M. A. Smith, *Rec. Indian Mus.* **39**, 216 (1937); W. C. Brown and A. Fehlman, *Occas. Pap. Nat. Hist. Mus. Stanford Univ.* **6**, 3 (1958).
5. Preparation and measurement techniques follow those of J. A. Peterson and E. E. Williams [*Bull. Mus. Comp. Zool. Harv. Univ.* **149**, 215 (1981)] and J. A. Peterson (*ibid.*, in press). The measurements in Table 1, which were taken by us, are reported as means of 12 to 20 individual measurements with the 95 percent confidence interval. The scincid specimens examined are: *P. virens* (MCZ 15037, 15029, 15034, and 135368), *P. prehensicauda* (MCZ 89127 and 83733), *P. flavipes* (MCZ 88866 and 88865), *Lipinia noctua* (MCZ 93826), *L. leptosoma* (MCZ 58091 and 58092), and *L. longiceps* (MCZ 49614) (MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, Mass.). A number of the more distantly related species in the radiation were also examined: *Lobulia elegantoides* (MCZ 90141), *L. stanleyana* (MCZ 103630), *Scincella reevsi* (MCZ 39231 and 39230), and *Mabuya longicaudata* (MCZ 104532).
6. R. Ruibal and V. Ernst, *J. Morphol.* **117**, 271 (1965). Only a few gecko species have been examined thus far [(*1*) and U. Hiller, *Z. Morphol. Tiere* **62**, 307 (1968)], but geckos appear to exhibit more variation in setal morphology than anolines. As the fine structure of more gecko species becomes known the variety of setal designs may increase significantly.
7. N. E. Stork, *Zool. J. Linn. Soc.* **68**, 173 (1980).
8. A. E. Greer, *Aust. J. Zool. Suppl. Ser.* **31**, 12 (1974); ——— and G. Raizes, *Science* **166**, 392 (1969).
9. R. Etheridge, *The Relationships of the Anoles (Reptilia: Sauria: Iguanidae): An Interpretation Based on Skeletal Morphology* (University Microfilms, Ann Arbor, Mich., 1960).
10. R. Ruibal, *Copeia* **1968**, 698 (1968).
11. The presence of setae in *P. virens* might indicate that it is a member of a lineage separate from *P. flavipes* and *P. prehensicauda*. If this is true it cannot be retained in *Prasinohaema* (type *flavipes*). A new genus for *virens* may be required.
12. We are grateful to Dr. Allen Greer for discussion of the problem and Dr. J. Berliner and Mrs. S. Beydler for providing access to a scanning electron microscope.

9 October 1981

Tumor Imaging with Radioactive Metal Chelates Conjugated to Monoclonal Antibodies

Abstract. *High-resolution gamma camera images of mouse erythroid tumors were obtained by use of leukemia cell–specific monoclonal antibodies labeled with bifunctional radioactive metal chelates. Small tumors (200 to 300 milligrams) were visible without subtraction or enhancement 1 to 5 hours after injection of antibody. Chelate-derivitized monoclonal antibodies permit targeting of a broad spectrum of radioisotopes, including those that are optimum for gamma camera imaging or positron tomography, as well as those that are tumoricidal.*

Imaging of tumor-specific antibodies labeled with radioactive isotopes has the potential to be a relatively noninvasive, yet sensitive, diagnostic procedure for visualizing otherwise undetectable tumors and metastases (*1*). In addition, the labeled tumor-specific antibodies may have tumoricidal effects (*2*). Development of techniques for preparing monoclonal antibodies (*3*), some with absolute tumor specificity (*4*), has encouraged such an approach. Although experimental tumor imaging, as well as therapy with radioactively labeled antibodies, has usually been done with immunoglobulins labeled with iodine isotopes (*1*), the iodine isotopes are not ideal for scanning. Of the three commonly available isotopic forms, only iodine-123 has the appropriate emission characteristics for imaging and a short enough half-life to be safely used diagnostically. The gamma radiation of iodine-125 is too weak for imaging. Iodine-131 has often been used (*1*), but is undesirable because of its long half-life and high-energy gamma and cytotoxic beta radiations. Iodine-131 has also been used therapeutically for large tumors (*2*), but appears ineffective in the treatment of small tumor foci or metastases (*5, 6*). Furthermore, the rapid metabolism of specifically bound radioiodinated antibodies leads to the incorporation of metabolized iodine into the thyroid and the active excretion of iodine by the stomach and urinary tract, preventing specific tumor imaging (*6, 6a*).

A more versatile method for labeling of antibodies is the use of covalently attached bifunctional radiometal chelates (*5, 7*). Chelatable radioactive metals with half-lives ranging from 1 hour to 3 days are available (*8*). Of these, gallium-67, indium-111, and technetium-99m are optimum for gamma camera imaging; gallium-68 is optimum for positron emission tomography; and scandium-47 or alpha-emitting isotopes are optimum for therapeutic effects. Khaw *et al.* (*7*) demonstrated the utility of this method with ^{111}In-labeled diethylenetriaminepentaacetic acid (DTPA) conjugated to rabbit antibodies to canine cardiac myosin Fab fragments for imaging myocardial infarcts in dogs.

We have used Rauscher murine erythroleukemia as a model system for studies of the diagnostic and therapeutic potential of monoclonal antibodies (*5, 6*). This disease, induced by the Rauscher

0036-8075/82/0319-1511$01.00/0 Copyright © 1982 AAAS

leukemia virus (RLV), is characterized by rapid, dramatic splenomegaly within 10 days after virus inoculation (*9*). The RLV envelope glycoprotein of 70,000 daltons (gp70) is expressed in large amounts on the surface of erythroleukemic cells. We prepared a monoclonal immunoglobulin G1 (IgG1) antibody, 103A, specific for the erythroleukemic gp70. This antibody selectively targets erythroleukemic cells in vivo and is also capable of curing the Rauscher erythroleukemia (*5, 6*). Sixty times as much antibody is bound to leukemic cells as to normal spleen cells within hours after injection into mice. The IgG1's used as controls [P3, produced by the P3 × 63Ag8 myeloma cells (*2*), and 263D, a nonrelevant monoclonal antibody] did not bind to normal or leukemic cells.

The effect on the biological activity of the antibody, the maximum number of chelates that can be conjugated per antibody, the biological half-life of the radioactive metal chelate on the antibody in vivo, and the technical ease of covalently attaching several different chelates to immunoglobulins were studied. The chelates included 1-(*p*-benzyldiazonium) EDTA, the *p*-hydroxybenzimidate of the 1-(*p*-benzyldiazonium)EDTA (*10*), 1-(*p*-carboxymethoxybenzyl)EDTA (*11*), and the carboxycarbonic anhydride of DTPA (*12*). Details of these procedures appear elsewhere (*13*). Only the 1-(*p*-carboxymethoxybenzyl)EDTA and the carboxycarbonic anhydride of DTPA could be conjugated to immunoglobulins in sufficient yield without large losses of biological activity, as measured by direct and indirect cell binding studies in vitro and in vivo.

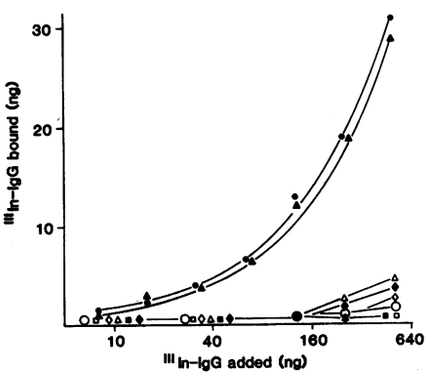

Fig. 1. Direct binding of live normal (○, □, △, ◇) and leukemic (●, ■, ▲, ◆) spleen cells to IgG's conjugated with ^{111}In-DTPA and ^{111}In-EDTA, measured in vitro. The IgG's, conjugated to bifunctional chelates and labeled as described, had the following specific activities: (●, ○) ^{111}In-103A-DTPA, 1 μCi/μg; (■, □) ^{111}In-P3-DTPA, 0.5 μ Ci/μg; (▲, △) ^{111}In-103A-EDTA, 2.6 μCi/μg; and (◆, ◇) ^{111}In-263D-EDTA, 2.7 μCi/μg. Spleen cells, isolated from normal BALB/c mice and mice made leukemic by inoculation with RLV, were washed in phosphate-buffered saline (PBS) containing 0.1 percent bovine serum albumin (BSA) and diluted to 1 × 10^8 spleen cells per ml. Serial dilutions of the ^{111}In-labeled IgG's in PBS containing 5 percent BSA were incubated at 23°C for 60 minutes with 5 × 10^6 cells in siliconized glass tubes (12 by 75 mm). Cells were then washed three times with 1 ml of PBS containing 0.1 percent BSA and the amount of bound radioactivity was determined in an LKB gamma counter.

The carboxycarbonic anhydride of DTPA was prepared as described in (*12*) and conjugated to monoclonal antibody (5 mg/ml) in a 250-fold molar excess in 100 m*M* bicarbonate, *p*H 7.5. This resulted in approximately 0.5 chelate per immunoglobulin G (IgG) molecule.

A modification of the method of Yeh *et al.* (*11*) was used for the preparation of 1-(*p*-carboxymethoxybenzyl)EDTA. The bifunctional chelate was saturated with ferric ion and coupled to immunoglobulins (5 mg/ml) at a 250-fold molar excess in 5 m*M* 2-(*N*-morpholino)ethanesulfonic acid (MES), *p*H 5.0, and 200 m*M* NaCl, with three portions of 1,3-(3-dimethylaminopropyl)carbodiimide (20-fold molar excess) added at 20 minute intervals. The reaction was terminated by fractionating the mixture on a column consisting of 1 ml of Dowex 1 × 4 (formate), 1 ml of Chelex-100, and 8 ml of Bio-Gel P10 (all obtained from Bio-Rad), equilibrated in 20 m*M* citrate, *p*H 5.0, and 400 m*M* NaCl. This coupling reaction yielded 0.9 chelate per IgG molecule. The ferric ion in the chelates was removed by dialysis against 20 m*M* citrate, 30 m*M* ascorbate, 5 m*M* EDTA, and 200 m*M* NaCl, *p*H 7.0.

After exhaustive dialysis against metal-free saline, the chelate-conjugated antibodies (2 mg/ml) were then labeled to high specific activity with 1 mCi of indium-111, gallium-67, or scandium-46, 30 percent carrier-free, in 50 m*M* citrate, *p*H 3.6. Free radiometal was removed by ion-exchange chromatography on a 5.5-ml column of Chelex-100, followed by dialysis against saline buffered with 10 m*M* MES, *p*H 6.6.

Indium-111 chelate–conjugated IgG's were tested for specificity by direct binding to normal and leukemic cells in vitro (Fig. 1). The leukemia cell–specific monoclonal antibody 103A bound to the leukemic spleen cells but not to normal spleen cells; the control immunoglobulin P3 and 263D bound to neither cell type. Immunoglobulins labeled with ^{111}In-DTPA or ^{111}In-EDTA retained their specificity and titer as compared to the same antibodies labeled with iodine-125.

The feasibility of gamma camera imaging with radioactive metals conjugated to IgG's was demonstrated by injecting them into normal and leukemic mice (Fig. 2). In leukemic mice injected with the ^{111}In-103A, the enlarged spleens were the only visible organs (Fig. 2D). In contrast, normal animals injected with the ^{111}In-103A (Fig. 2B), as well as both normal and leukemic animals injected with ^{111}In-labeled control immunoglobulins (Fig. 2, A and C), showed diffuse radioactivity in the blood without organ-specific uptake of the antibody. These mice were killed immediately after the imaging, and the amount of radioactivity per gram of tissue was measured to quantitate the images. There was 40- to 55-fold more radioactivity per gram of spleen of the leukemic mouse injected with ^{111}In-103A than there was in the spleens of the three other mice. The radioactivity in the liver of the leukemic mouse injected with 103A (seven times as much as that in the other mice) was probably the result of metabolism of the specifically bound immunoglobulin (*6a, 14*). Radioactivity in all of the other

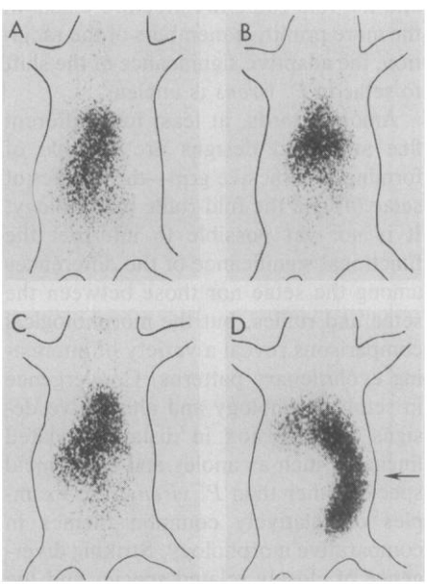

Fig. 2. Gamma camera images of normal and leukemic mice injected with ^{111}In-DTPA-103A and ^{111}In-DTPA-263D IgG's. Monoclonal antibodies conjugated to radioactive metal chelates were prepared as described in the text. Six-week-old female BALB/c mice were injected intravenously in the tail veins with 15 μg of IgG (4 μCi/μg) 5 to 6 hours before imaging; 4 × 10^4 counts were recorded at 160 keV with a pinhole collimator. Mice are shown lying on their backs, their heads at the top and the spleen on the right. (A) Normal mouse injected with ^{111}In-263D. (B) Normal mouse injected with ^{111}In-103A. (C) Leukemic mouse injected with ^{111}In-263D. (D) Leukemic mouse injected with ^{111}In-103A. Mice were made leukemic by inoculation with RLV 12 days before scanning. The leukemic spleen (arrow) contained 15 percent of the injected dose of ^{111}In-103A. Spleen weights of the four mice were, respectively, 118, 98, 652, and 339 mg.

organs of these four mice was approximately 1/2 to 1/4 as much per gram as the blood radioactivity. The specificity shown by these data indicates that the conjugated antibodies were stable and that free radiometal was not released in vivo. Studies of the half-life of the ^{111}In-DTPA–IgG's in normal mice support this conclusion (*15*).

We had determined the optimal time for tumor imaging after intravenous injection of ^{111}In-EDTA-103A (*5*). Although tumor targeting occurs immediately, more accurate imaging is obtained if sufficient time is allowed (5 to 6 hours) for extravascular distribution of the antibody and the consequent reduction of the radioactivity in the blood pool. Several parameters that must be considered for optimal tumor imaging are the half-lives of the isotopes used, the accelerated metabolism of the specifically bound antibody (*6a*, *14*), the slower rate of accumulation of nonspecific antibody (*14*) than of the targeted antibody, and the half-life of the particular monoclonal antibody used. Therefore, imaging immediately after antibody distribution is preferable.

Thus, rapid and specific tumor imaging without computer enhancement or subtraction techniques is possible with radioactive metal chelates conjugated to monoclonal antibodies. Erythroleukemic spleens of 200 to 300 mg were easily resolved, and the limits of resolution may be much lower. By analogy, it may be possible to detect small human tumors or metastases. The chelate-conjugated monoclonal antibodies are extremely versatile and can be used with a variety of isotopes of different half-lives and emission spectra to yield superior imaging and to accommodate to a particular tumor system. We have labeled both IgG's and IgM's with indium, gallium, and scandium. One advantage of the labeling with radioactive metal chelates is that if the chelate becomes unconjugated from the antibody during metabolism, the protected isotope is excreted immediately. This contrasts with the fate of the unprotected iodine isotopes (*6*, *6a*).

The chelate-conjugated monoclonal antibodies have, in addition, great therapeutic potential, since cytotoxic isotopes may also be chelated. The most powerful therapeutic isotopes are the alpha emitters because their immense energy of radiation is confined to an extremely small volume, about the size of a few cells. Theoretically, a single alpha emission in a tumor focus is likely to kill a cell (*16*) with minimum damage to normal tissues. Substitution of a short-lived alpha emitter for indium-111 in the targeting experiments (Fig. 2D) could have resulted in the delivery of several thousand rads of high linear energy transfer radiation to the leukemic spleen cells; this dose is vastly in excess of that necessary to kill every leukemic cell.

DAVID A. SCHEINBERG
METTE STRAND
Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
OTTO A. GANSOW
Department of Chemistry, Michigan State University, East Lansing 48824, and Laboratory of Chemical Physics, National Institute of Arthritis, Metabolism, Digestive Diseases, and Kidney, Bethesda, Maryland 20205

References and Notes

1. D. M. Goldenberg, Ed., *Cancer Res.* **40** (No. 8) (1980), part 2.
2. S. E. Order, J. L. Klein, D. Ettinger, P. Alderson, S. Siegelman, P. Leichner, in *ibid.*, p. 3001.
3. G. Köhler and C. Milstein, *Nature (London)* **256**, 495 (1975).
4. H. Koprowski, Z. Steplewski, D. Herlyn, M. Herlyn, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3405 (1978); M. Herlyn, Z. Steplewski, D. Herlyn, H. Koprowski, *ibid.* **76**, 1438 (1979); M.-Y. Yeh, I. Hellstrom, J. P. Brown, G. A. Warner, J. A. Hansen, K. E. Hellstrom, *ibid.*, p. 2927; M. Strand, *ibid.* **77**, 3234 (1980); W. G. Dippold, K. O. Lloyd, L. T. C. Li, H. Ikeda, H. F. Oettigen, L. J. Old, *ibid.*, p. 6114; W. W. Young, Jr., and S. Hakomori, *Science* **211**, 487 (1981).
5. D. A. Scheinberg, M. Strand, O. A. Gansow, in *Monoclonal Antibodies in Drug Development*, J. T. August, Ed. (American Society for Pharmacology and Experimental Therapeutics, Bethesda, Md., 1981).
6. D. A. Scheinberg and M. Strand, *Cancer Res.* **42**, 44 (1982).
6a. Our studies of the kinetics of the binding and catabolism of specifically bound radiolabeled monoclonal antibodies (D. A. Scheinberg and M. Strand, in preparation) come to the same conclusion as Bale *et al.* (*14*).
7. B. A. Khaw, J. T. Fallon, H. W. Strauss, E. Haber, *Science* **209**, 295 (1980).
8. The half-lives of these isotopes are scandium-47, 3.4 days; gallium-67, 2.8 days; gallium-68, 68 minutes; technetium-99m, 6 hours; and indium-111, 3.2 days.
9. F. J. Rauscher, *J. Natl. Cancer Inst.* **29**, 515 (1962).
10. An azoimidate of diethylenetriaminetetraacetic acid has been described by C. Paik, D. E. Herman, W. C. Eckelman, and R. C. Reba [*J. Radioanal. Chem.* **57**, 553 (1980)].
11. S. M. Yeh, D. G. Sherman, C. F. Meares, *Anal. Biochem.* **100**, 152 (1979).
12. G. E. Krejcarek and K. L. Tucker, *Biochem. Biophys. Res. Commun.* **77**, 581 (1977).
13. D. A. Scheinberg, thesis, Johns Hopkins University, Baltimore (1981); ———, M. Strand, O. A. Gansow, in preparation.
14. W. F. Bale, M. A. Contreras, E. D. Grady, *Cancer Res.* **40**, 2965 (1980).
15. The half-life of the ^{111}In-DTPA–IgG in the serum of mice was 60 hours. Sodium dodecyl sulfate–polyacrylamide gel electrophoretic analysis of the radioactivity in the serum after 50 hours in vivo showed no transfer of ^{111}In from the IgG to transferrin, the predominant serum metal binding protein.
16. J. A. Smit, J. A. Myburgh, R. D. Neirinckx, *Clin. Exp. Immunol.* **14**, 107 (1973).
17. Scans were made with the assistance of the staff of the Johns Hopkins Hospital Nuclear Medicine Clinic. Supported by NCI grant NOI-CP8 1052 from the hospital and by NSF grant GP10935; D.A.S. was supported by the NIH medical science training program grant GM07309.

1 September 1981

Congenital Malformations Induced by Laetrile

Abstract. *Laetrile administered orally to pregnant hamsters caused skeletal malformations in the offspring, but intravenous laetrile failed to result in embryopathic effects. Oral laetrile significantly increased in situ cyanide concentrations, while intravenous laetrile did not. Thiosulfate administration protected embryos from the teratogenic effects of oral laetrile. The embryopathic effects of oral laetrile appear to be due to cyanide released by bacterial β-glucosidase activity.*

The term laetrile refers to a class of cyanogenic glycosides that occurs naturally in the pits of certain edible fruits and berries. Recent legislative activity has legalized the use of laetrile in the treatment of cancer in at least 23 states. The National Cancer Institute is sponsoring clinical studies on the efficacy of laetrile at the University of California (Los Angeles) Comprehensive Cancer Center, the Mayo Clinic, the Memorial Sloan-Kettering Cancer Center, and the University of Arizona Health Sciences Center.

Amygdalin (D-mandelonitrile-β-D-glucoside-6-β-D-glucoside) is the most common constituent of laetrile samples, but injectable preparations from Mexican laboratories have also contained substantial amounts of unknown pyrogens, visually apparent microbial growth, sucrose, phenol, diisopropylammonium iodide, and isopropyl alcohol (*1–4*). Oral preparations contain primarily D-amygdalin (*3*). The glycone moiety of the amygdalin molecule consists of two sugars that are glycosidically attached to mandelonitrile, which is the genin or aglycone portion of the molecule and the cyanohydrin of benzaldehyde (*5*). The β-glycosidic bond can be hydrolyzed by amygdalase to yield the secondary glycoside, prunasin, and glucose. Prunasin may be further degraded by prunase to yield glucose and mandelonitrile. Amygdalin can also be hydrolyzed to mandelonitrile and glucose by bacterial β-glucosidase in the gastrointestinal lumen. The mandelonitrile decomposes in vivo to yield free cyanide and benzaldehyde (*6*). The benzaldehyde formed is a weak anesthetic agent (*7*), but it is rapidly oxi-

0036-8075/82/0319-1513$01.00/0 Copyright © 1982 AAAS