crystals to contain ICRF-154 (Fig. 1b), not bimolane, and indicates that ICRF-154 may be the active agent in test results attributed to bimolane.

Two samples of bimolane, one (sample NA) synthesized in China and the other (NB) in the United States, were received from the Drug Synthesis and Chemistry Branch, National Cancer Institute. Crystals were obtained from both samples by solvent evaporation in dimethyl sulfoxide, in 95 percent ethanol, and in mixtures of acetone and water; all crystals gave identical x-ray diffraction patterns. Crystal data are as follows: monoclinic, $a = 5.633$ Å, $b = 16.786$ Å, $c = 7.044$ Å, β (angle between a and c) = 122.37°, and space group $P2_1/c$, with two molecules in the unit cell (one-half molecule composing the asymmetric unit). Density considerations indicated that the crystals could not contain molecules the size of bimolane, and we undertook a structure determination to ascertain the composition of the crystals.

X-ray diffraction data were collected on an automated four-circle diffractometer with Cu radiation; there were 713 observed maxima [intensity $(I) > 3\sigma(I)$] in the range $0 < 2\theta$ (dispersion angle) < 130°. The structure was solved by direct phasing procedures and refined by anisotropic full-matrix least squares to a final discrepancy index of 0.046. The results are shown in Fig. 2: the molecular structure is that of 1,2-bis(3,5-dioxopiperazin-1-yl)ethane (ICRF-154), a structural component of bimolane and a substance which has been shown previously to possess antitumor activity (*4*).

It was important to determine whether the ICRF-154 crystals were a consequence of bimolane decomposition during the crystallization procedures or whether the presence of ICRF-154 is an inherent property of the samples. The latter possibility is suggested by the identity of the crystals obtained from various solvent systems under different conditions and by melting point data (213° to 217°C for sample NA and 198° to 203°C for sample NB, compared to 236° to 239°C quoted on the product information sheet from NCI). Subsequently, 300-MHz nuclear magnetic resonance data obtained for these samples and others by an independent contractor were interpreted as indicating that all samples contained 33 to 40 percent impurities (*5*).

Evaluation of bimolane for anticancer activity by the NCI tumor panel indicated high activity against B16 melanoma and L1210 leukemia and moderate activity against P388 leukemia. On being notified of our structural results, NCI ascertained that ICRF-154 had been tested previously (NSC 129942) in similar systems, and had demonstrated activity very similar to that of bimolane in the B16 melanoma and L1210 and P388 leukemia tests (results in all cases were within the range of experimental error) (*5*).

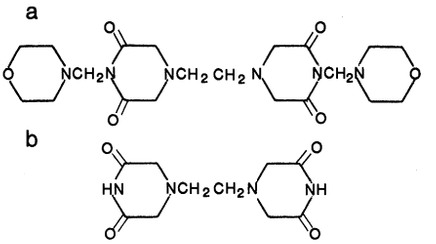

Fig. 1. Molecular structure of bimolane (a) and of ICRF-154 (b).

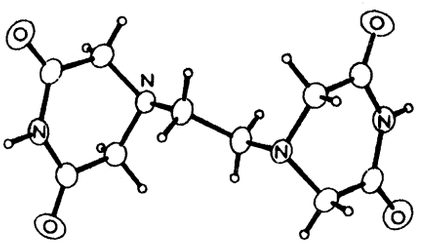

Fig. 2. Molecular structure of ICRF-154 crystallized from bimolane samples. Unlabeled atoms are C and H.

Thus the structural, chemical, and biological data strongly suggest that bimolane is inherently unstable and that the antitumor activity ascribed to it may be attributable to ICRF-154. In addition, since bimolane has been reported to be effective in the treatment of psoriasis and uveitis (*1*), it may be useful to test ICRF-154 for chemotherapeutic activity against these conditions.

The conformational characteristics and molecular parameters of ICRF-154 are similar to those observed in other diketopiperazines we have structurally elucidated (*3*). Structural parameters for the compound are available (*6*).

N. Camerman
A. Hempel
Department of Biochemistry, University of Toronto, Toronto, Canada M5S 1A8
A. Camerman
Departments of Medicine and *Pharmacology, University of Washington, Seattle 98195*

References and Notes

1. Y.-F. Ren, H.-L Shu, T.-M. Zhang, C. Lin, paper presented at the 182nd National Meeting of the American Chemical Society, New York, 24 to 28 August 1981.
2. T. H. Maugh II, *Science* **213**, 1239 (1981).
3. A. Hempel, N. Camerman, A. Camerman, *J. Am. Chem. Soc.* **104**, 3453 (1982); *ibid.*, p. 3456; *ibid.* **105**, 2350 (1983).
4. A. M. Creighton, K. Hellmann, S. Whitecross, *Nature (London)* **222**, 384 (1969).
5. K. Paul, private communication.
6. Tables of atomic coordinates and thermal coefficients, molecular parameters, and observed and calculated structure factors may be obtained from the authors.
7. Supported by the Medical Research Council of Canada and by the U.S. Public Health Service (grant CA 15879).

13 February 1984; accepted 27 June 1984

Cell Surface Changes Associated with Cellular Immune Reactions in *Drosophila*

Abstract. *In* Drosophila melanogaster *a temperature-induced change in immune competence accompanies cell surface alterations that cause its blood cells to adhere and to encapsulate a parasite. At 29°C the blood cells of the tumorous-lethal* (Tum^l) *mutant show a high degree of immune competence and encapsulate the eggs of the parasitic wasp* Leptopilina heterotoma. *At 21°C the blood cells are essentially immune incompetent. High percentages of lectin binding cells were found under conditions which potentiated cellular encapsulation responses. Some immune reactive blood cells did not bind lectin. The low percentages of lectin binding cells in susceptible hosts suggest that developing parasites alter the cell surface of the blood cells of immune reactive hosts.*

Unlike vertebrates, insects and other invertebrates lack immunoglobulin-like recognition molecules and yet manifest a high degree of specificity in their immune responses against a diversity of nonself components (*1*). Unfortunately, little is understood of the mechanisms of nonself recognition in insects. This kind of information, which is available for only a few insect species, is important since many disease causing agents that debilitate humans live for various periods of time in the body cavities of their insect vectors. For these and other endoparasites to develop unmolested within potentially immunologically hostile environments, they must be able to evade detection or actively suppress the defense mechanisms of their hosts (*2*). A basic understanding of how hosts identify nonself components requires knowledge of the types of immune competent

cells and of the mechanisms that regulate their production and differentiation under various pathological conditions.

In larvae of *Drosophila melanogaster*, the lamellocytes, and to a lesser degree the plasmatocytes from which they differentiate, are the immune reactive blood cells (hemocytes) which effectively encapsulate objects too large to be phagocytized by individual cells (*3, 4*). During encapsulation large numbers of lamellocytes are produced which adhere to one another and to foreign surfaces to form compact, multilayered capsules that melanize. In larvae of the tumorous lethal (*Tum*^{l}) mutant the aberrant blood cell–forming organs (lymph glands) fail to respond to normal endocrine regulation and consequently produce large numbers of lamellocytes that function abnormally in circulation and encapsulate various endogenous tissues to form melanotic tumors (*5*). The mutation is temperature-sensitive, with maximum gene expression at 29°C. Investigations of the cellular immune capabilities of *Tum*^{l} larvae against the parasite *Leptopilina heterotoma* showed that, despite the presence of sufficient numbers of lamellocytes at the time of infection, the blood cells were highly immune reactive only at 29°C (approximately 70 percent parasite encapsulation), and virtually immune incompetent at 21°C (<5 percent parasite encapsulation) (*6*).

Since temperature induces a change in the immune competence of *Tum*^{l} hemocytes, the plasma membranes of the cells participating in capsule formation may be modified to facilitate their adhesion to one another and to foreign objects such as parasites. Conversely, blood cells not involved in encapsulation may lack the specific surface properties of cells that adhere to form hemocytic capsules. To test the hypothesis that encapsulating hemocytes acquire altered surfaces in their differentiation, we studied the lectin binding properties of *Tum*^{l} blood cells at 21° and 29°C, and in both parasitized and nonparasitized larvae. Lectins, which bind specifically to certain sugar moieties exposed on cell surfaces, have been extensively used to study modifications in the structure and function of cell surface membranes (*7*). The binding of lectins to cell surfaces can be visualized when a fluorescent label is used.

Both control *Basc* (Müller 5) and hemizygous *Tum*^{l} hemocytes were treated with wheat germ agglutinin (WGA) conjugated with fluorescein isothiocyanate (FITC). A single drop of hemolymph from each larva was collected on a glass microscope slide, and the cells were immediately fixed with a drop of acetone. When dry, a drop of FITC-WGA solution (200 μg of protein per milliliter of 0.9 percent saline) was applied to the cells. After 2 minutes, the treated cells were rinsed by immersing the slides several times in distilled water. The cells were then located and examined with phase and episcopic fluorescence microscopy. Two categories of hemocytes were distinguished; cells with intense granular fluorescence over their surfaces were considered positive, while those with pale fluorescence or without fluorescing surface granules were classified as negative (Figs. 1 to 4).

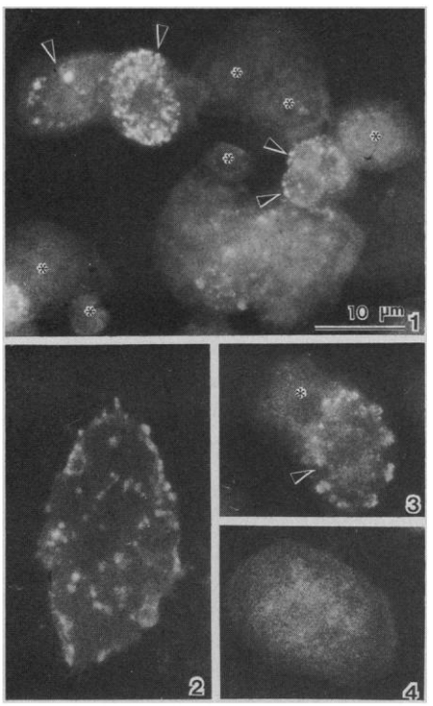

Fig. 1 to 4. Photomicrographs of *Tum*^{l} hemocytes treated with WGA conjugated with fluorescein isothiocynate and examined with a Nikon fluorescent microscope. Fig. 1. Negative cells with pale fluorescence and lacking fluorescing surface granules are indicated by an asterisk. Cells classified as positive exhibit a granular fluorescence on their surfaces (arrows). Fig. 2. Lamellocyte showing WGA binding. Fig. 3. Two plasmatocytes, one showing WGA binding (arrow), the other lacks this property (asterisk). Fig. 4. Lamellocyte lacking WGA binding activity.

Hemocytes of *Basc* larvae showed no WGA binding activity, whereas both the plasmatocytes and lamellocytes of *Tum*^{l} larvae showed binding. In larvae reared continuously at 21°C, the percentages of lectin binding cells gradually increased from 18 percent in the first instar larvae (52 hours old) to 25 percent in the third instar (Fig. 5A). At 29°C, the percentages of lectin binding cells at each instar were more than twice as high as those at 21°C, ($P < 0.001$; two-factor analysis of variance). At 29°C, the percentages of WGA binding cells increased during early larval development, but decreased slightly in the third instar. This decrease in the percentage of WGA binding hemocytes in third instar larvae coincides with the development of melanotic tumors, a process that removes cells from circulation. When melanotic tumors were collected from early third instar larvae and treated with WGA, 20 to 30 percent of the cells in the outer layers of these encapsulated masses exhibited WGA binding.

The rapidly fading fluorescence of the WGA-treated hemocytes precluded our making accurate differential determinations of the ratio of fluorescing plasmatocytes to fluorescing lamellocytes. However, cell counts from several representative photomicrographs showed that the lamellocytes constituted 30 percent

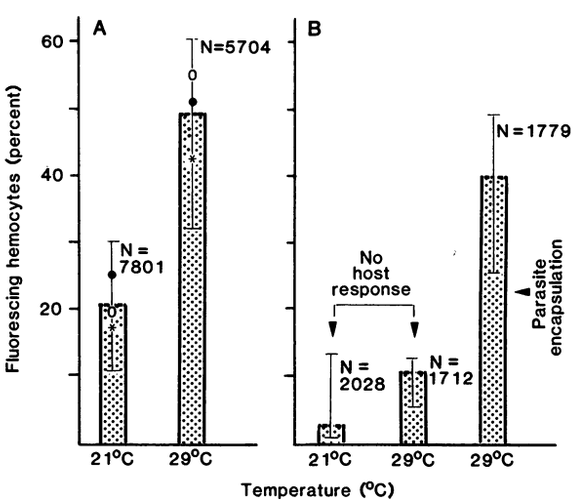

Fig. 5. Percentages of hemizygous *Tum*^{l} hemocytes binding to WGA conjugated with fluorescein isothiocyanate. (A) Lectin binding cells from nonparasitized first (*), second (○) and third (●) instar larvae reared continuously at 21°C, and from larvae shifted 24 ± 3 hours after *Drosophila melanogaster* oviposition to 29°C. *N*, total number of cells counted from 30 larvae, ten at each of the three larval instars. Ages of larvae in hours at 21° and 29°C for each instar are (*) 52, 48; (○) 72, 60; and (●) 96, 72. (B) Lectin binding cells from host larvae 37 hours after parasitization by *L. heterotoma*. Host age when parasitized was 52 ± 3 hours. *N*, is the total number of cells counted from ten larvae. Bars on each figure represent ranges of cell counts.

of the fluorescing cell populations at 21°C, and 60 percent at 29°C.

The lectin binding properties of both immune reactive and nonreactive hemocytes were studied by shifting half of the *Tum*^{l} larvae parasitized by *L. heterotoma* to 29°C, a condition that ensured a high frequency of encapsulation responses, and maintaining the others at 21°C, a condition which resulted in a low frequency of encapsulated parasites. In immune competent hosts that successfully encapsulated *L. heterotoma*, the percentages of WGA binding hemocytes were significantly higher ($P < 0.001$; *t*-test) than in susceptible hosts that, at either 21° or 29°C, exhibited no encapsulation response (Fig. 5B). Parasite eggs removed from host larvae during the early stages of encapsulation (24 hours after infection) and treated with WGA showed approximately equal numbers of fluorescing and nonfluorescing cells in the outer layer of the developing capsule. In fully formed capsules (48 to 60 hours after infection) no WGA binding hemocytes were observed in the outer layer of the capsule. This suggests either that WGA binding hemocytes are not involved in the terminal stages of capsule formation or that the heavy deposits of melanin obscure their WGA binding activity.

Parasites can develop in an immune competent host if they are not recognized as foreign (for example, molecular mimicry or antigen sharing), or if they produce substances that suppress the host's immune system. Past studies have shown that *L. heterotoma* utilizes the latter mechanism and actively inhibits the blood cell encapsulation responses of *Drosophila* by preventing the differentiation of plasmatocytes to lamellocytes (*3*, *8*). It is believed that the substance which suppresses blood cell differentiation is introduced into the host by the female during oviposition. The low percentages of WGA binding hemocytes found in susceptible *Tum*^{l} hosts may reflect a decrease in the percentage of lamellocytes resulting from parasite suppression of the immune response. However, it is possible that, in addition to blocking the formation of lamellocytes, the parasite also suppresses or modifies those cell surface properties that characterize the hemocytes of immune reactive hosts.

In summary, our studies correlate high percentages of WGA binding hemocytes in circulation with encapsulation reactions that attend parasite encapsulation and melanotic tumor formation in the *Tum*^{l} mutant. The data suggest that the temperature-induced increase in the immune competence of *Tum*^{l} hemocytes at 29°C is due to certain cells acquiring specific surface modifications, identified by WGA binding, which augment their adhesion to one another and to foreign surfaces. These findings support the work of Rizki and Rizki who showed that the percentages of WGA binding hemocytes in other *Drosophila* mutants increased at tumor permissive conditions, and also when heterospecific implants were used to provoke encapsulation responses (*9*). They suggested that aberrant tissues stimulate the differentiation of WGA binding cells, which then adhere to one another and to the tissue surfaces.

Since WGA binds to specific carbohydrate moieties, this lectin does not identify all of the cell surface alterations that may occur on the same or on different immune reactive cells. Moreover, changes in cell surface carbohydrates that are identified by this lectin may be ancillary manifestations in the attainment of immune competence, and not directly responsible for potentiating the adhesion of capsule-forming hemocytes. The fact that many of the hemocytes participating in the development of cellular capsules in *Tum*^{l} do not bind to WGA suggests there are at least two populations of immune reactive cells. The immune competence of one group of hemocytes is manifested by cell surface features which have a high affinity for WGA, while the other, equally competent population lacks this property. It is also possible that cells that do not bind WGA participate in encapsulation reactions only by adhering to WGA binding cells, or to foreign surfaces that become coated with a product believed to be elaborated from the latter cells (*8*).

ANTHONY J. NAPPI
MICHAEL SILVERS

Department of Biology,
Loyola University of Chicago,
Chicago, Illinois 60626

References and Notes

1. S. Ratner and S. B. Vinson, *Am. Zool.* **23**, 185 (1983); N. A. Ratcliffe and A. F. Rowley, in *Insect Hemocytes, Development, Forms, Functions, and Techniques*, A. P. Gupta, Ed. (Cambridge Univ. Press, London, 1979), pp. 331–414; A. M. Lackie, *Parasitology* **80**, 393 (1980).
2. R. F. Whitcomb, M. Shapiro, R. R. Granados, in *Physiology of Insecta*, M. Rockstein, Ed. (Academic Press, New York, 1974), vol. 4; A. J. Nappi, in *Invertebrate Immunity, Mechanisms of Invertebrate Vector-Parasite Relations*, K. Maramorosch and R. Shope, Eds. (Academic Press, New York, 1975) pp. 293–326; D. B. Stoltz and S. B. Vinson, *Adv. Virus Res.* **24**, 125 (1979).
3. I. Walker, *Rev. Suisse Zool.* **66**, 569 (1959); A. J. Nappi and F. A. Streams, *J. Insect Physiol.* **15**, 1551 (1969).
4. A. J. Nappi, *Parasitology* **83**, 319 (1981); T. M. Rizki, *J. Invertebr. Pathol.* **12**, 339 (1969); ——— and R. M. Rizki, *Entwicklungsmech. Org. Arch.* **189**, 207 (1980).
5. W. R. Hanratty and J. S. Ryerse, *Dev. Biol.* **83**, 238 (1981); M. Silvers and W. R. Hanratty, *J. Invertebr. Pathol.*, in press.
6. A. J. Nappi, J. Kmiecik, M. Silvers, *J. Invertebr. Pathol.*, in press.
7. H. Lis and N. Sharon, in *The Antigens*, M. Sela, Ed. (Academic Press, New York, 1977), pp. 429–529.
8. F. A. Streams and L. Greenberg, *J. Invertebr. Pathol.* **13**, 371 (1969); A. J. Nappi, *Parasitology* **70**, 189 (1975); *J. Insect Physiol.* **23**, 809 (1977).
9. T. M. Rizki and R. M. Rizki, *Science* **220**, 73 (1983).
10. We thank T. M. and R. M. Rizki for helpful discussions, and D. Bishop and W. Tabachnick for reading the manuscript and providing useful suggestions. We thank W. Hanratty for providing the initial stock of the fly mutant, and K. Bakker for the strain of the parasite used in the study. Supported in part by the National Science Foundation, National Institutes of Health, and Loyola University Research Foundation.

19 March 1984; accepted 15 June 1984

Alzheimer's Disease: Cell-Specific Pathology Isolates the Hippocampal Formation

Abstract. *Examination of temporal lobe structures from Alzheimer patients reveals a specific cellular pattern of pathology of the subiculum of the hippocampal formation and layers II and IV of the entorhinal cortex. The affected cells are precisely those that interconnect the hippocampal formation with the association cortices, basal forebrain, thalamus, and hypothalamus, structures crucial to memory. This focal pattern of pathology isolates the hippocampal formation from much of its input and output and probably contributes to the memory disorder in Alzheimer patients.*

The role of the hippocampus and related structures of the ventromedial temporal lobe in learning and memory has been examined in both man and the experimental animal (*1*). Bilateral destruction of these areas is associated with a profound and lasting memory impairment that affects learning in all modalities (*2*).

Memory impairment is an early, prominent manifestation of Alzheimer's disease, and the hippocampal formation and parahippocampal gyrus are among the brain areas most consistently and heavily implicated in its pathology (*3*). Pathologic alterations (*4*) and neurochemical deficits (*5*) have also been observed in association cortices, as well as in subcortical structures such as the amygdala (*6*), locus coeruleus (*7*), and the cholinergic