a water-acetone solution. They crystallize in space group $P2_1/a$ with $a = 12.928$, $b = 13.354$, $c = 7.976$ Å, and $\beta = 90.01°$. Intensities of 3171 reflections to $2\theta = 55°$ were measured as for DPH. The crystal structure was solved by the symbolic addition procedure, and all of the nonhydrogen atoms were located in the first E map. After refinement of the atomic positions and anisotropic thermal parameters, the discrepancy index R was found equal to 0.039.

Perspective drawings of DPH and DAP molecules are shown in Figs. 1 and 2, respectively. The two phenyl groups of DPH lie at angles of 114° and 113° to the hydantoin ring, and at an angle of 90° to each other. The phenyl group in DAP makes an angle of 124° with the chlorophenyl ring. Scale models constructed to fit the observed atomic positions (Fig. 3) reveal a high degree of similarity in the conformational structures of the two anticonvulsants. Similarities in the space-filling features of the two molecules are especially striking in two aspects: (i) the arrangement of the two phenyl rings in each molecule with respect to each other, and (ii) the similar positioning in each molecule of two electron-donating groups. When the DPH and DAP molecules are superimposed so that the two phenyl rings of DPH approximately match the orientation of the phenyl and chlorophenyl rings of DAP, a ketonic oxygen of DPH and the ketonic oxygen of DAP occupy the same position in space as do the second ketonic oxygen of DPH and a trigonal nitrogen of DAP.

The role of DAP as a most important antianxiety agent and at the same time as one of the most important anticonvulsant drugs in experimental epilepsy seemingly contradicts the general rule that desynchronizing drugs are also good antiepileptics, while synchronizing drugs may have a facilitating role on seizure discharge (*6*). It may be that the various therapeutic activities of the benzodiazepines and other drugs that act upon the central nervous system are functions of two distinct molecular features—chemical composition and molecular shape. Either one or both of these features may be the determining factor in the effectiveness of drugs in the treatment of different pathological conditions. It seems clear from our structural results that the anticonvulsant properties of DAP and DPH are a consequence, to a large degree, of the three-dimensional molecular configuration of these compounds, particularly the orientational relation between the bulky rings and the electron-donating functional groups. How the steric configuration influences the exact mechanism of seizure suppression is not known. Two possible structure-dependent modes of action are that (i) the drugs may inhibit enzyme reactions that facilitate transfer of electrical energy in the brain or activate other enzyme systems that hinder such transfer, or that (ii) they may interact with membrane walls and alter the diffusion characteristics of brain cells, thus radically affecting the concentrations of sodium and potassium ions inside and outside cell membranes. An analogous mode of behavior has been independently proposed for the action of DDT (*7*).

The search for antiepilepsy agents has heretofore been directed along purely chemical lines. We suggest that a conformational approach to this problem, that is, the choosing of experimental medications on the basis of their conformational rather than chemical similarities to existing drugs, may be useful in developing new and more effective therapies. The conformational similarities between **1** and **2** also suggest that this approach may be valuable in identifying receptor sites of anticonvulsant drugs and thus elucidating the mechanism of seizures. The biological relevance of our findings can best be assessed from evidence on the question of whether other compounds with diphenylhydantoin-diazepam steric conformations always have antiepileptic activity.

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Plasmalemmal and Subsurface Complexes in Human Leukemic Cells: Membrane Bonding by Zipperlike Junctions

Abstract. *After a brief exposure to agents that provoke phagocytosis, monocytic cells from patients with acute leukemia exhibit pentalaminar and septate membranous complexes. These structures connect plasma membranes of adjacent cells and join surfaces of approximating pseudopodia on the same cell; they also appear to be present in cortical areas of the cytoplasm.*

In the course of studies of the ultrastructure and function of leukemic cells during chemotherapy, junctional surface specializations were elicited in monocytic cells from several patients with acute monocytic and monomyelocytic leukemia. To our knowledge such specializations in hematopoietic cells have not been reported (*1*). However, intercellular junctions with a multiplicity of fine-structured subunits are present in normal, differentiated, solid tissues of vertebrate and nonvertebrate species (*2*) and have been associated with functions of selective permeability (*3*), electrical coupling (*4*), cell adhesion, and contact inhibition (*5*). Intercellular adhesion sites have also been demonstrated in studies of dissociated normal cells and cells transformed by viruses and carcinogens (*6*).

Our experiments were designed to test phagocytic competence. Specimens of peripheral blood were obtained at various times during treatment and were aspirated into heparin-rinsed plastic syringes (*7*). Chemotherapeutic agents varied from patient to patient. In most instances specimens were obtained when patients were in regression, just before a new course of treatment was initiated. After gravity sedimentation of erythrocytes, the cell-rich plasma was diluted (1:1) with Hanks base medium containing 10 percent calf serum. Samples (4 ml) containing 1×10^6 to 5×10^6 cells were incubated for 15 minutes at 37°C. To each tube, 0.2-ml suspensions of thorium dioxide or *Pseudomonas aeruginosa* (*8*) were added and incubation was continued for 15 or 20 minutes. Controls were set up

simultaneously. Specimens were then immediately centrifuged at 0°C for 8 minutes at 800 rev/min. The resultant pellets were fixed for electron microscopy in 2.5 percent cacodylate-buffered glutaraldehyde and 2 percent OsO_4 in cacodylate-sucrose buffer or in buffered OsO_4 only. Some samples were stained in the block with aqueous uranyl acetate before dehydration in graded alcohols. Further processing for study was by conventional methods. Sections were examined in a Siemens Elmiskop 1A microscope.

The predominant cells in these preparations (Fig. 1) exhibited the ultrastructural criteria for mature and immature monocytes (*9*). Functionally, their responsiveness to the colloidal particles and bacteria was indicated by exhuberant arborizations of the cortical cytoplasm, multiple extrusions and invaginations of the plasmalemma, and the presence of ingested test material in endocytic vacuoles and secondary lysosomes.

Well-defined zipperlike structural bonds were observed in these responsive cells. They were associated with reciprocal interdigitations of the plasmalemmal extrusions (Fig. 2) and areas of intimate contact between surface membranes of the same or adjoining cells (Figs. 1 and 3).

These junctional complexes have some morphological features in common with sites of adhesion in other systems, but differ significantly in some respects. For example, they are considerably larger, measuring over all, cytoplasm to cytoplasm, 240 to 320 Å. Such dimensions may be related to requirements for the association of usually free independent cells, or may possibly be due to the nature of interacting micelles of the glycocalyx (*10*).

The specializations consist of facing, rigidly parallel membranes of two cell surfaces separated by a 150 to 200 Å electron-lucent space. In transverse section, this central space is hemisected by a moderately dense lamina, 40 to 70 Å wide, thus presenting a quintuple-layered image (Fig. 4). In sections from blocks stained with uranyl acetate the junctions are visible as two confronting trilaminar unit membranes that are separated by perpendicular ladderlike intercepts 30 to 50 Å in diameter, evenly spaced at 90- to 100-Å intervals (Fig. 2); in some micrographs these appear to penetrate the subjacent cytoplasm; in other views the septa are interrupted by a median dense lamina that is globular at the points of intersection (Figs. 2 and 4). In fortuitous sections shearing planar contact areas, arrays of hexagonal subunits with center to center spacing of 90 to 110 Å span the extracellular space between adjoining surfaces or appear as honeycombed patches on the cell surface (Fig. 4).

In most cases, cell to cell junctions linked monocytic cells, although those joined were not always at the same stage of maturation. However, we have occasionally observed a small mononuclear cell with the characteristic "hand mirror" configuration assumed by motile lymphocytes (*11*) bonded at the smaller pole to the surface of a large monocyte (Fig. 1).

Test particles trapped between adjacent fingerlike projections on the same cell were internalized by the development of identical junctions at the distal ends of these extrusions. Sequential stages in the formation of endocytic vacuoles by this mechanism were easily identified (Fig. 3). Some of the subsurface complexes are strikingly similar to the recently described "granules" in Langerhans cells, dermal histiocytes (*12*), and lymph node macrophages (*13*) and may represent degraded vestiges of collapsed phagocytic vacuoles.

Junction formation did not appear to be random since it occurred in both bacteria and Thorotrast preparations and in more than one time-separated sample from the same patient; it did not occur in patient controls; it did not occur indiscriminately at all contacting membranous surfaces; and it appeared to be selective for monocytic cells in three of seven patients with acute monocytic or monomyelocytic leukemia. It did not occur in specimens from 15 patients with other types of acute leukemia. Negative findings in normal controls are regarded as inconclusive until they are confirmed by experiments designed to increase the number of normal monocytes in test preparations.

Although morphological evidence had

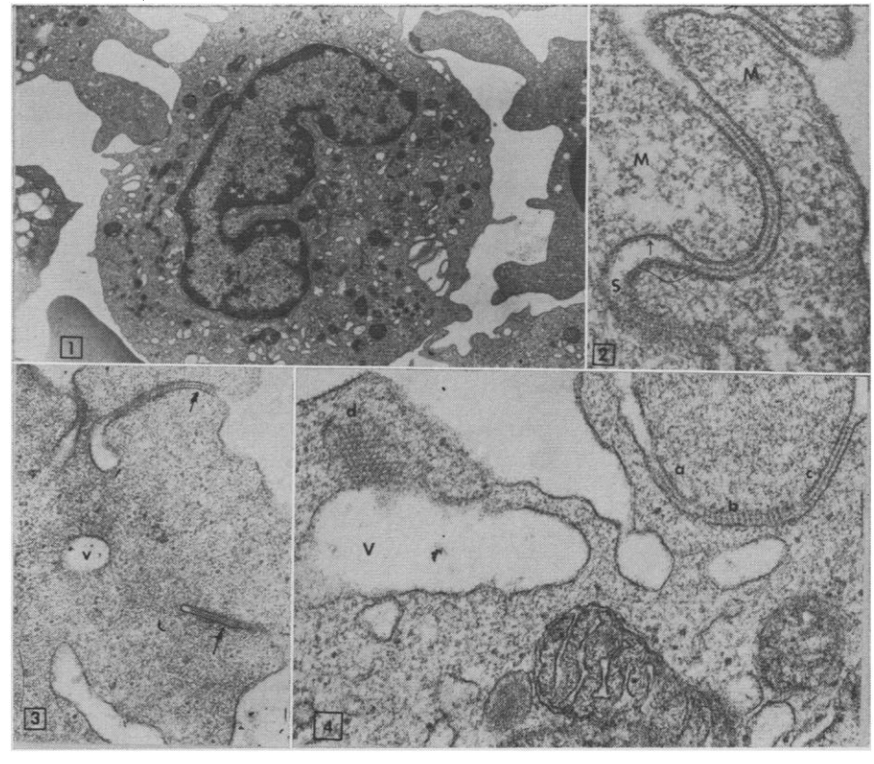

Fig. 1. Two small mononuclear cells in contact with a leukemic monocyte by means of very large cytoplasmic extrusions (thorium dioxide preparation, ×8000). Fig. 2. Junctions at interdigitating surfaces of two monocytic cells (*M*). The trilaminar structure of the unit membranes (arrows) is clearly delineated in this section from a uranyl acetate–stained block. There is no fusion of the outer leaflets of the plasmalemmae. In some areas (*s*) septa appear to penetrate the subjacent cytoplasm (*Pseudomonas* preparation, ×97,000). Fig. 3. Adhesion sites between approximating pseudopodia on the same cell. Phagocytic vacuoles (*v*) appear to be formed in these cells by this mechanism (thorium dioxide preparation, ×31,000). Fig. 4. Pentalaminar (*a*) and septate (*b, c*) aspects of a junction between two cells. The central dense lamina appears to be globular at points of contact with the septa (*c*). Cross-hatching (*d*) reflects the planar surface of an adhesion site. Vacuole containing thorium dioxide (*v*) (×71,000).

been lacking, the dynamic adaptive properties of the monocyte surface membrane could be inferred from biological experiments demonstrating the ability of these cells to engulf particulates, fuse with other macrophages to form giant cells, process and transfer antigenic information to immunologically competent cells (*14*), and bind erythrocytes coated with immunoglobulin at specific receptor sites (*15*). It seems probable that the induction or unmasking of unique, highly specialized, structural complexes in these leukemic cells is relevant to the inherent properties of normal monocytes. Our findings may be related to neoplastic modifications of the plasma membrane or its extraneous coat (*10*). It is interesting that in contrast to these hematopoietic cells, neoplastic solid tissues generally lose contact inhibition (*5*) and specialized adhesion sites are diminished or absent.

Mechanisms of membrane bonding are little understood and the significance of the junctions in these cases, under these experimental conditions, is as yet unclear. Their existence in vivo must be considered, however, since there is a high incidence of infection with *Pseudomonas aeruginosa* or similar organisms in leukemia patients.

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Potassium–Adenosine Triphosphate Complex: Formation Constant Measured with Ion-Selective Electrodes

Abstract. *Valinomycin and glass electrodes were used to measure activity of potassium ions in equilibrium with adenosine triphosphate. The thermodynamic formation constant of the complex is approximately 25 times greater than indirect measurements predict.*

The development of valinomycin-based, potassium-selective membrane electrodes (*1*) makes possible the direct measurement of ion-association processes that involve the potassium ion in aqueous media and permits extension of our work (*2*) on sodium complexes of biological interest. The potassium–adenosine triphosphate (ATP) complex is of particular interest because of its role in energy exchange (*3*, *4*). Because of the lack of an appropriate analytical method, previous measurements of the stability of this complex have been carried out by indirect methods. Both Melchior (*5*) and Smith and Alberty (*6*) estimate the formation constant K_f for K^+–ATP as ~ 10 ($\mu \sim 0.20M$) with *p*H titration, whereas O'Sullivan and Perrin (*7*) arrive at $K_f \sim 14$ ($\mu \sim 0.1M$) from the effect of the potassium complex upon the stability of Mg–ATP^{2-}. Our experiments, in which ion-selective membrane electrodes are used to measure K^+ activity directly, show these values to be in error.

We used both the new valinomycin-type liquid-membrane electrode (*1*) and an NAS 27-4 cation-sensitive glass electrode (Corning model 476220) as indicator electrodes versus a double-junction reference electrode to measure potassium activity in various experiments carried out under conditions of varying ionic strength. Because the $pK_a = 6.95$ for the reaction

$$H\,ATP^{3-} \leftrightarrows ATP^{4-} + H^+$$

we conducted our experiments in solutions of *p*H 9.0 to 9.5 where we assume all of the ligand to be accounted for by the species ATP^{4-} and $KATP^{3-}$. Under these conditions both indicating electrodes gave excellent Nernstian calibration curves to K^+ concentrations of 10^{-1} to $< 10^{-5}M$. Formation constants were calculated by standard methods with the aid of a CDC 6400 computer from the potentiometric data.

On the basis of more than 100 determinations taken in 12 separate experiments with the valinomycin and glass electrodes we arrive at values of 219 ± 24 (standard deviation) and $218 \pm 20\,M^{-1}$, respectively, for K_f of the $KATP^{3-}$ complex at $25.0° \pm 0.1°C$. Thus, our values for the formation constant are about 25 times larger than the earlier estimates. Since the association of ATP with K^+ is usually neglected in biochemical calculations (*3*), the unexpectedly high value for K_f revealed by our direct measurements requires a reconsideration of the role of $KATP^{3-}$ in bioenergetics and ionic mobilities (*4*).

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