Table 2. Effect of starvation, prednisone administration, streptozotocin diabetes, and species differences on the specific binding of [^{125}I]insulin to isolated adiposites. Fat cells obtained from the same or similar animals described in Table 1. The adiposites were incubated for 40 minutes in 0.2 to 0.3 ml of buffer (Krebs-Ringer-bicarbonate) containing 2 percent (weight to volume) albumin at ambient temperature. Specific binding of [^{125}I]insulin was determined (*3*). Separate experiments, each with separate control cells, are presented. The concentrations of [^{125}I]insulin in the incubation medium ranged from $2 \times 10^{-10}M$ to $3 \times 10^{-9}M$; representative concentrations are given in italics in the table. The specific binding assay was repeated at least two times for each species or insulin-resistant state studied, and in all cases the results were qualitatively identical. Standard deviations in these experiments averaged ± 9.3 percent.

Animal	Specific binding of [^{125}I]Insulin [(mole/cell) × 10^{20})]	
	8.1×10^{-11}M	
Control rat	0.2	
Starved rat	0.2	
	6.3×10^{-10}M	*2.5×10^{-9}M*
Control rat	1.2	4.6
Prednisone-treated rat	1.0	4.6
	3.0×10^{-10}M	*1.5×10^{-9}M*
Control rat	0.4	1.3
Streptozotocin-diabetic rat	0.4	1.4
	2.2×10^{-10}M	*9.0×10^{-10}M*
Control rat	0.8	1.4
Mouse	1.1	3.0
Hamster	1.4	3.1
	2.2×10^{-10}M	*1.1×10^{-9}M*
Control rat	1.1	1.8
Guinea pig	1.9	4.2
Rabbit	1.7	2.8

were either starved (*5*), were treated with prednisone (*6*), or were diabetic (*7*), and cells obtained from certain species such as hamsters, guinea pigs, and rabbits (*8*), exhibit a very poor enhancement of the rate of glucose oxidation in response to insulin (Table 1). Under the conditions of these experiments, the rate of glucose transport across the cell membrane appears to be the limiting step in the oxidative pathway (*9*). Cell size alone is probably not responsible for diminished insulin responses of fat cells as no significant difference is observed in cells with an average diameter of 70 μm compared to cells with a diameter of 32 μm (Table 1) (*10*).

Despite the diminished metabolic effects of insulin in fat cells from rats in these "resistant" states, no significant diminution in the specific binding of [^{125}I]insulin to these fat cells was detectable (Table 2). There was no reduction in the total insulin-binding capacity of the cells, as determined by measurements which use saturating concentrations (10^{-9} to $10^{-10}M$) of [^{125}I]-insulin. The specific binding of insulin to the fat cells of mice, guinea pigs, hamsters, and rabbits was greater than the binding to fat cells of rats. There also appeared to be no reduction in the affinity of the complex of insulin and cell in these conditions as no differences in binding were observed at concentrations of [^{125}I]insulin that were below saturation. The apparent absence of a defect in affinity during formation of the complex indicates that binding equilibrium had been achieved in all measurements and that differences in the rates of association of insulin and cell cannot explain the differences in metabolic responses.

This indicates that, in the insulin-resistant states studied here (*10*), no serious defect exists in the binding function of the insulin receptor. The resistance to insulin observed in these conditions must therefore result from changes in processes which occur after the initial interaction of insulin and cell. Our results also demonstrate that the metabolic differences in insulin response are not explained by possible differences in the capacity of these cells to inactivate insulin; this would have been detected as a decrease in insulin binding.

It is unlikely that fat cells from various species or from rats with altered states of metabolism are more susceptible to damage by proteolytic enzymes to which they may be exposed during cellular isolation (*12*); the primary effect of digesting fat cells with various proteases is a decrease in the affinity of the cell receptor for insulin (*13*). Also a similar resistance to insulin has been observed in studies with other species with adipose tissue slices rather than with isolated fat cells (*14*). It is possible, however, that different metabolic states may affect the susceptibility of the fat cells to other enzymes, such as neuraminidases; the action of such enzymes on the fat cell surface can result in abolition of the insulin response without significantly affecting the interaction of insulin and receptor (*15*). It has recently been demonstrated that insulin can directly modulate the activity of adenylate cyclase (*16*); the effects of insulin on this enzyme in insulin-resistant metabolic states has yet to be determined.

G. VANN BENNETT
PEDRO CUATRECASAS
Departments of Medicine and *Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

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Crystal Structure of Ethylene Di-11-bromoundecanoate

Abstract. *Ethylene di-11-bromoundecanoate, $C_{24}H_{44}Br_2O_4$, was synthesized as a model for the hydrophobic moiety of saturated phospholipids. The crystals are monoclinic, space group* $P2_1/a$, *with two molecules per unit cell. Unlike folded configurations proposed for phospholipids in biological membranes, the hydrocarbon chains of this diester are fully extended in the crystalline state.*

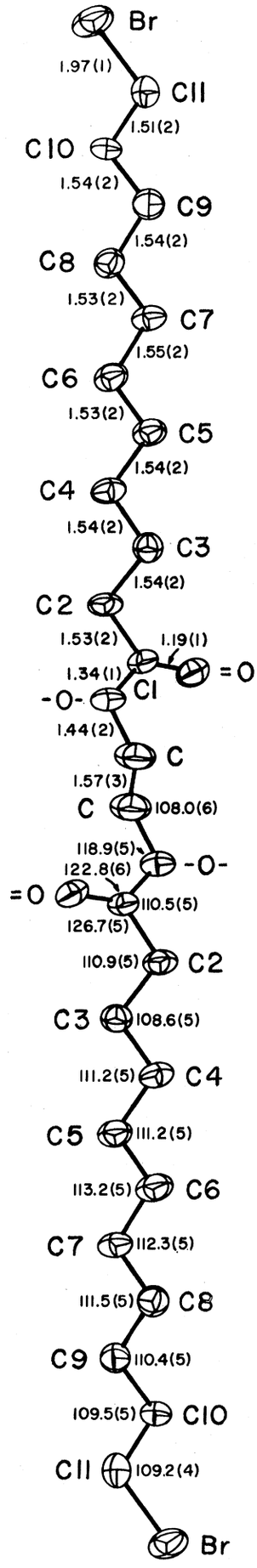
Several analyses of low-angle x-ray data from biological membranes and oriented lipid bilayers (*1*) reaffirm many of the earlier x-ray studies (*2*), which proposed membrane lipid packings based on the Danielli-Davson model (*3*). This model, which implies a polar interaction between phospholipid and protein, predicates a folding of the phospholipid hydrocarbon chains

away, from the polar surfaces, probably as envisioned by packing models proposed by Finean and Vandenheuvel (*4*).

The nature of lipid-protein interactions in membranes is still not known (*5*). The equivocal evidence for hydrophobic interactions between these membrane components as well as the possibilities for alternative membrane structural models have been reviewed (*6*). However, calculations which indicate that there is insufficient surface area for the volume of lipid extracted from cell membranes (*7*) have cast doubt on the ubiquity of the lipid bilayer as a structural model for all membrane types. In order to arrive at the preferred packing modalities for phospholipids in biological membranes, it is desirable to examine their orientation in the crystalline state. However, for several reasons (*8*), no crystal structure of a phospholipid has been reported to date.

Since bromine has a van der Waals radius close to that of a methyl group and has been incorporated successfully for isomorphous heavy atom derivatives of fatty acids (*9*), the brominated diester, ethylene di-11-bromoundecanoate, was synthesized as a model for the nonpolar moiety of saturated phospholipids. Ethylene glycol was treated with 11-bromoundecanoyl chloride in the presence of pyridine in anhydrous CCl_4 (*10*). Platelike crystals of the diester, which were grown by slow evaporation of the solvent in the cold, melt between 58.5° and 59.5°C on a hot stage.

The monoclinic space group is $P2_1/a$ and the unit cell has the parameters: a=5.57 ± 0.05 Å, b=7.32 ± 0.01 Å, c=32.3 ± 0.2 Å, and $\beta = 94.1 \pm 0.2°$. From a density measurement of the crystal by the neutral buoyancy method in aqueous KI solution (d=1.39 g/ml) and the unit cell dimensions, two molecules were found per unit cell, indicating that one-half the molecule is the asymmetric unit. Due to the short c^* reciprocal spacing, peak height intensity data were manually collected on a quarter circle diffractometer (G.E. XRD-6, Ni-filtered CuKα radiation). Three crystals were needed to collect a full set of intensity data because of the well-known decay of ω-brominated organic compounds exposed to x-rays (*9*). "Standard" reflections were measured periodically to facilitate corrections for intensity decline and to correlate the data from the separate crystals. Of the possible 2491 reflections measured, 1953 were designated observed.



Intensity data were initially phased by the use of the positions of the Br atoms in the unit cell located on a Patterson map. The center of symmetry at the origin was assumed to coincide with the molecular center of symmetry at the C-C bond in the ethylene moiety. Subsequent electron density maps increasingly revealed more of the chain structure as apparent "light" atom positions were included in the phasing. This operation was first carried out in projection, locating the x,z coordinates of the atoms, and then in three dimensions. The final block diagonal least-squares anisotropic refinement on all nonhydrogen atoms, where hydrogen positions were generated on the assumption that the C-H bond distance is 1.0 Å and the C-C-H bond angle is 109°, gave an R value (*11*) of 0.15. In this final refinement, 49 observed reflections were excluded because of the possible errors due to angle setting or to absorption of x-rays by the crystals. In view of the irregular geometry of the crystal plates no correction was made for absorption.

As the thermal ellipsoid drawing (*12*) in Fig. 1 illustrates, the molecule assumes an extended configuration in the crystalline state, much like a hydrocarbon (*13*). After correction for thermal librations (*14*), all valence parameters in the molecule (Fig. 1) are normal with the average corrected chain C-C bond distance being 1.54 ± 0.01 Å and the average corrected chain C-C-C bond angle being 110.9 ± 1.3°. The standard deviations of the valence parameters in Fig. 1 were calculated assuming isotropic errors (*15*). Although subcell diffraction patterns are not seen on the Weissenberg films, the hydrocarbon chains pack in the common $O_\perp$ orthorhombic subcell (*16*) with cell parameters: $a_s = 4.97 \pm 0.05$Å, $b_s = 7.32 \pm 0.00$ Å, $c_s = 2.53 \pm 0.02$ Å, $\alpha_s = 89.9 \pm 0.1°$, $\beta_s = 90.2 \pm 0.3°$, $\gamma_s = 90.2 \pm 1.3°$. The average volume per methylene group is 23.0 Å^3. The Br-Br end-plane packing distances are 3.68, 4.04, 4.10, and 4.50 Å. The chain axes intersect the bromine end plane at an angle of 64.1°. Rigid body analysis (*17*) reveals essentially iso-

Fig. 1. The molecular structure of ethylene di-11-bromoundecanoate. Three-digit numbers are bond lengths in angstroms; four-digit numbers are bond angles in degrees. Standard deviations of these valence parameters are given in parentheses. Bond lengths and angles are corrected for thermal librations.

tropic translational librations, but a prominent rotational libration of 8° around the chain axis.

The chain orientation in this molecule is consistent with the previously reported crystal structures of two 1,3-diglycerides (*18*) and the α,β chain conformation in three triglyceride crystal structures (*19*) where the hydrocarbon chains are extended. This result is a possibility which was excluded in early interpretations of x-ray powder spacings from glycerides (*20*).

From work on the structure of *rac*-glycerol 1,2-(di-11-bromoundecanoate)-3-(*p*-toluenesulfonate) (*21*), it appears that the short, more rotund shape of a phospholipid polar head group is important in determining the packing arrangement. Yet, the presence of extended chain packing, as demonstrated by this diester, suggests another possible stable phospholipid acyl chain configuration for biological membranes, especially if nonpolar lipid-protein interactions are significant.

DOUGLAS L. DORSET*
Department of Biological Sciences, State University of New York at Albany, Albany 12203

ALBERT HYBL
Department of Biophysics, University of Maryland School of Medicine, Baltimore 21201

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$$R = \frac{\Sigma||F_{obs}| - |F_{calc}||}{\Sigma|F_{obs}|}$$

where F is the structure factor.
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* Present address: Electron Optics Laboratory, Biophysics Department, Roswell Park Memorial Institute, Buffalo, N.Y. 14203.

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Reversible Inhibition of Chloroplast Movement by Cytochalasin B in the Green Alga Mougeotia

Abstract. *Light-oriented chloroplast movement is reversibly inhibited by cytochalasin B. The photoperception is not influenced by this inhibitor. These results support the assumption that contractile protein fibrils are essential for this intracellular movement.*

In the filamentous alga *Mougeotia*, the single large plate-shaped chloroplast performs orientation movements with respect to light. In blue or white light of high intensity, the chloroplast orients its edge to the light, whereas in white light of low or medium intensity or in red light it orients its face to the light (*1*). We now discuss the latter response, which is mediated mainly by phytochrome ("low intensity movement").

Compared with our knowledge about the nature of the photoreceptor and some details about its localization (*1*), much less is known about the mechanism of movement. Microtubules that have been demonstrated in the cytoplasm (*2*) appear not to be involved in the movement of the chloroplast, but fibril structures have been found and are assumed to be involved in the movement (*3*).

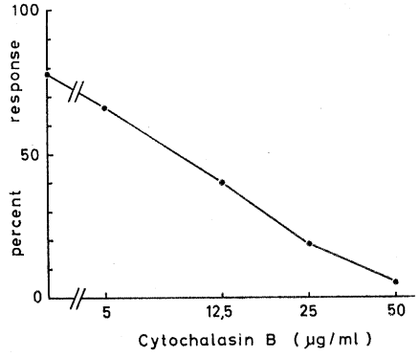

Fig. 1. Light-induced chloroplast movement as influenced by cytochalasin B. On the ordinate, the percentage of cells is given in which orientation of the chloroplast is found 40 minutes after a pulse of red light.

Cytochalasin has been reported to be a very specific inhibitor of contractile protein fibrils (*4*). Therefore we have investigated the effect of this substance on chloroplast movement. The *Mougeotia* cell is particularly suitable for investigating possible roles of protein fibrils in the mechanism of chloroplast movements. If the movement is induced by a short exposure to light (even less than 1 minute), it proceeds during a period of complete darkness following this light (*1*); moreover, the effect of such a short light induction can be stored by the cell for some time if the response is blocked (*5*). We therefore can separate experimentally the effect of an inhibitor on the photochemical reaction from that on the response.

Preparations of *Mougeotia* were first treated by appropriate irradiation so as to be oriented in profile position (*6*). Before the inductive irradiation was applied, the "MXS" culture medium (*7*) was removed and replaced by medium containing cytochalasin B (Serva) in increasing concentrations (0, 5, 12.5, 25, and 50 μg/ml). Fifteen minutes later, all samples were subjected to unpolarized red light for 60 seconds; this pulse was equivalent to the physiological effect of 1500 erg/cm² sec of 683-nm light (*8*). Immedi-