sional RCS pigment epithelial cell profile in each culture may contain one or two phagosomes (Fig. 2c). Under the same conditions, cultured normal pigment epithelium continues to phagocytize large amounts of normal (Fig. 2d) or RCS outer segment fragments.

The reduced ability of cultured RCS pigment epithelium to phagocytize outer segment material establishes that the RCS mutant gene is expressed as a defect in the phagocytic mechanism in pigment epithelial cells. This is not a general defect in phagocytosis, since RCS pigment epithelium is capable of ingesting polystyrene spheres in vitro. The envelopment of outer segments by microvillous processes of RCS pigment epithelial cells indicates that the RCS cells respond to the presence of outer segments, a phenomenon also observed in the RCS rat in vivo (*5*). The fact that RCS rat outer segment fragments are phagocytized by cultured normal rat pigment epithelium is direct evidence that the RCS mutant gene is not expressed in photoreceptor cells in a way that prevents phagocytosis of RCS outer segment material. This in vitro experimental system now makes accessible to further study the genetic defect in the phagocytic mechanism of the pigment epithelial cell which is responsible for photoreceptor cell degeneration in the RCS rat.

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11. Rod outer segments were prepared by flotation in 34 percent (by weight) sucrose [D. S. Papermaster and W. J. Dreyer, *Biochemistry* **13**, 2438 (1974)], pellets were formed by centrifugation, and these were resuspended in 0.5 ml of culture medium per original retina. A 100-μl portion of the outer segment suspension was added to the pigment epithelium explants.
12. Polystyrene (Latex) spheres (Dow Chemical) were rinsed three times in distilled water by centrifugation and resuspended to 0.04 percent (weight to volume) in culture medium, and 100-μl portions of the suspension were added to pigment epithelium explants.
13. Cultures were fixed with 2 percent osmium tetroxide in 0.2*M* sodium cacodylate buffer (*p*H 7.35) at 0°C for 30 minutes. The cultures were then rinsed with cold distilled water and dehydrated in a graded series of ethanol solutions. The explants were loosened from the plastic surface with propylene oxide and embedded in Epon 812. Thin sections cut perpendicular to the culture substratum were stained with uranyl acetate and lead citrate.
14. We thank E. F. McCarthy, Jr., for technical assistance. Supported by grants from the National Retinitis Pigmentosa Foundation, Baltimore, Md., and the George Gund Foundation, Cleveland, Ohio. Breeding pairs of RCS-$p^+$ rats were provided through National Institutes of Health research contract EY-3-2133 to R. L. Sidman.

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## Conformations of Prostaglandin $F_{2\alpha}$ and Recognition of Prostaglandins by Their Receptors

Abstract. *The conformation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been determined by x-ray diffraction techniques. Two independent conformers of $PGF_{2\alpha}$, studied as the tris(hydroxymethyl)methylamine salt, are observed to adopt the familiar "hairpin" conformation with the α and ω chains aligned roughly parallel. The conformers differ in ring conformation and at the C(17)–C(18) bond, one adopting a C(9) envelope ring conformation and a* trans *geometry at the C(17)–C(18) bond, while the other adopts a C(8) envelope ring conformation and a novel* gauche *geometry about C(17)–C(18). Comparison of the conformation of $PGF_{2\alpha}$ with that of prostaglandin $E_2$ suggests a recognition mechanism which would permit $PGF_{2\alpha}$ and prostaglandin E receptors to distinguish between the two potent prostaglandins. The recognition model explains much of the binding data for the $PGF_{2\alpha}$ receptor in the corpus luteum and predicts the existence of an interesting $PGF_{2\alpha}$ analog.*

By virtue of its diversity of action and potential for pharmacological exploitation, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is perhaps the most interesting of the classical prostaglandins. It plays an important role in luteolysis and is clinically employed in pregnancy termination and induction of labor at term. Prostaglandin $F_{2\alpha}$ and prostaglandin $E_2$ ($PGE_2$) are the most abundant classical prostaglandins. Recent binding studies (*1*) reveal that $PGF_{2\alpha}$ and $PGE_2$ have little affinity for each other's receptors, suggesting that the conformations of the two prostaglandins differ to a significant degree, even though they differ chemically only in the saturation of the C(9)–O(9) bond (Fig. 1). Having previously determined the conformation of $PGE_2$ by diffraction techniques (*2*), we now report the determination of the molecular conformations of $PGF_{2\alpha}$, and offer plausible explanations for the recognition of prostaglandins by their receptors.

Prostaglandin $F_{2\alpha}$ was crystallized as its tris(hydroxymethyl)methylamine (tris) salt from a methanol-acetonitrile solution. The crystals are triclinic, space group $P\bar{1}$, with unit cell axial lengths $a = 8.330(3)$, $b = 26.458(6)$, and $c = 6.221(2)$ Å; angles $\alpha = 99.98(3)°$, $\beta = 91.75(4)°$, and $\gamma = 97.88(3)°$; and volume 1335.6 Å$^3$. The observed density, 1.189 g/cm$^3$, measured by flotation in a chloroform-benzene mixture, implies that there are two independent molecules of $PGF_{2\alpha}$-tris salt per asymmetric unit (calculated density, 1.183 g/cm$^3$). Of 5472 independent reflections, measured in the $\theta$-$2\theta$ scan mode on an automatic diffractometer employing CuK$\bar{\alpha}$ radiation, 4820 are considered to be observed (that is, their intensities are more than twice their standard deviations). This is an unusually favorable percentage of observed data for prostaglandin crystals, reflecting the quality of the data. The structure was determined by application of a system of programs, QTAN, written by one of us (D.A.L.) and described previously (*3*). The model has been refined by using anisotropic thermal parameters for carbon, nitrogen, and oxygen atoms to a current residual $R = 0.08$.

The two conformers of $PGF_{2\alpha}$ are shown in Fig. 2. Although distinct in various ways, both display the "hairpin" conformation observed for other active prostaglandins (*4*); that is, the α [C(1) through C(7)] and ω [C(13) through C(20)] side chains of each conformer align in such a way that they run roughly parallel. To accomplish this, the ω chains twist (*5*) at the C(15) positions [torsion angles C(13)–C(14)–C(15)–C(16) are −150° and −118° for conformers A and B, respectively], effectively positioning the O(15) hydroxyl oxygens out and away from the centroids of the conformers. In a description of L-shaped prostaglandin $B_1$, DeTitta (*6*) discussed the importance of the hairpin conformation and the particular importance of the position of the C(15) hydroxyl with respect to the metabolizing enzyme 15-

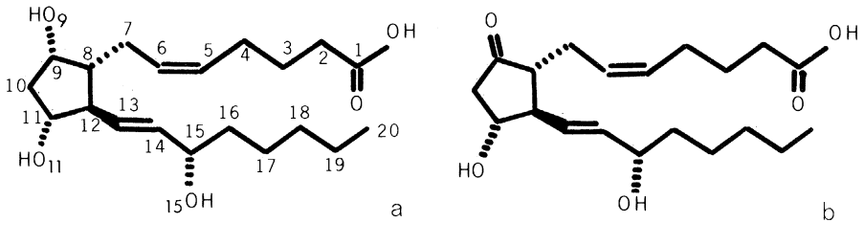


Fig. 1. Chemical formulas and atomic nomenclature for (a) $PGF_{2\alpha}$ and (b) $PGE_2$.

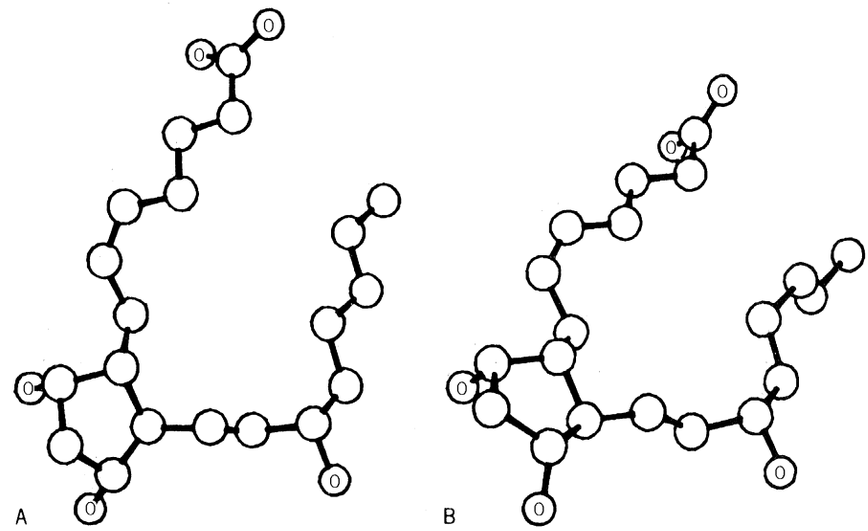


Fig. 2. The two conformers, A and B, of $PGF_{2\alpha}$. The views are the projections on the planes defined by atoms C(12), C(15), and O(15).

hydroxyprostaglandin dehydrogenase. Although not unexpected, the observation of a hairpin conformation for both independent molecules of $PGF_{2\alpha}$ supports the hypothesis that active prostaglandins adopt this overall shape.

The two conformers differ significantly in two respects. Conformer A adopts a C(9) envelope conformation with C(9) below (*7*) the plane of the remaining ring atoms; conformer B adopts a C(8) envelope conformation with C(8) above (*7*) the remaining ring atoms. While in both conformers the O(9) oxygen is in a pseudoaxial and the O(11) oxygen is in a pseudoequatorial orientation relative to the ring, the C(8) envelope conformation exaggerates the O(11) pseudoequatorial orientation relative to the C(9) envelope conformation. The conformers very closely approximate ideal C(8) and C(9) envelope conformations rather than any intermediate conformation along the cyclopentane pseudorotation pathway (*8*). Even though the conformers adopt differing ring geometries, the chain junction geometries remain identical, with the C(7)–C(8)–C(12)–C(13) torsion angle equal to +80° for both forms. The conformers differ significantly at the C(17)–C(18) bonds. In form A the conformation is the normally observed all-*trans* planar arrangement of the chain, whereas in form B we observe a *gauche* (*9*) conformation [torsion angle C(16)–C(17)–C(18)–C(19) is +172° for conformer A and +49° for conformer B]. Molecular models of conformer B show that the *gauche* twist prevents the $\alpha$ and $\omega$ chains from too close an approach to one another, yet permits the chains to retain the hairpin shape.

It is generally assumed that the prostaglandins express their diverse biological roles by interaction with specific receptor sites, initiating a series of amplifying events which culminate in a recognizable physiological response. Recent binding studies (*1*) of the luteal prostaglandin receptors reveal the specificities of the receptors for their substrates. Prostaglandin E receptors are not specific for $PGE_2$ or $PGE_1$, but $PGF_{2\alpha}$ receptors do not bind $PGF_{1\alpha}$. Prostaglandin E receptors bind PGE analogs to a certain extent but do not bind $PGF_{2\alpha}$ or other PGF analogs. Similarly, $PGF_{2\alpha}$ receptors show varying degrees of binding by PGF analogs but little binding by $PGE_2$ or other PGE analogs. Both receptors show a great deal of sensitivity to modifications of the $\alpha$ chain but much less sensitivity to $\omega$ chain modification. Epimerization (*10*) or even removal (*1*) of the C(11) hydroxyl does not abolish binding at either receptor, and methylation at the C(15) or C(16) position maintains or enhances binding at both sites. Truncation of the $\omega$ chain generally reduces binding, whereas elaboration or even aromatization of the $\omega$ chain can increase binding (*11*). Altogether the data suggest that (i) the $\omega$ chain, while vital to binding, is not the site of differentiation between prostaglandins, and (ii) binding is much less sensitive to modifications on the $\beta$ face of the molecule than to modifications on the $\alpha$ face. Differentiation between PGE and PGF is then probably controlled by the position of the O(9) oxygen relative to the plane of the cyclopentane ring (either below for $PGF_{2\alpha}$ or in the plane for $PGE_2$). We were interested to determine whether the observed conformations of $PGF_{2\alpha}$ and $PGE_2$ (*2*) could explain prostaglandin receptor specificity.

Examination of molecular models of $PGF_{2\alpha}$ and $PGE_2$ built from their observed conformations reveals distinct dispositions of the ring oxygenic functions of $PGF_{2\alpha}$ and $PGE_2$ relative to the $\alpha$ face of the molecules (Fig. 3). In $PGE_2$ the O(9) carbonyl oxygen is approximately in the plane of the cyclopentanone ring, the C(5)=C(6) double bond is below the ring plane, and the geometry of the C(8) ring junction is $-$*gauche*, $+$*gauche* [torsion angles C(6)–C(7)–C(8)–C(9) and C(6)–C(7)–C(8)–C(12) are −60° and +62°, respectively].

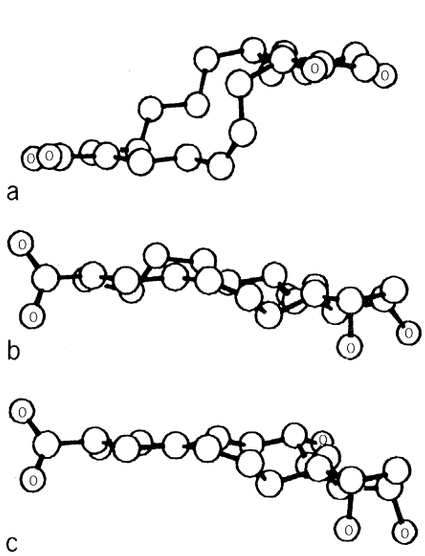


Fig. 3. Conformations of (a) $PGE_2$ and (b and c) $PGF_{2\alpha}$ (conformers A and B, respectively) as seen in the plane of the $\alpha$ face. The views were obtained by requiring the C(4)→C(1) vector to define the horizontal and the C(1)→C(20) vector to define the vertical, followed by a 90° rotation about the horizontal.

In $PGF_{2\alpha}$ the pseudoaxial O(9) oxygen is below the plane of the cyclopentane ring, the C(5)=C(6) double bond is approximately in the ring plane, and the C(8) ring junction is *+gauche, trans* (corresponding torsion angles are +57° and 179°, respectively, for both conformers). The junction conformations are not interconvertible; in either case, attempted interconversion leads to close contact between O(9) and C(6). Further changes in the $\alpha$ chain conformation of $PGF_{2\alpha}$ relative to $PGE_2$ are necessary to maintain the close proximity of the $\alpha$ and $\omega$ chains. The $\alpha$ chains of $PGF_{2\alpha}$ twist on either side of the 5,6-*cis* double bond [torsion angles C(5)=C(6)–C(7)–C(8) and C(3)–C(4)–C(5)=C(6) are +142° and +153° for conformer A and +128° and +148° for conformer B] relative to the values found for $PGE_2$ (−127° and −100°, respectively). Conformational shifts in the alkane portions of the $\omega$ chains are interpreted as fine-tuning mechanisms to maximize the $\alpha$ and $\omega$ chain proximity in both $PGE_2$ and $PGF_{2\alpha}$. The end result of this series of shifts (Fig. 3) shows the movement of the O(9) oxygen relative to the $\alpha$ face of the prostaglandin. If binding occurs primarily in two complementary steps—a nondifferentiating contact between the $\alpha$ side of the molecule with the receptor, and a specific, differentiating hydrogen bond contact between the O(9) oxygen and the proteinaceous surface of the receptor—it is clear that $PGF_{2\alpha}$ and $PGE_2$ cannot satisfy the conformational requirements of binding for both receptors simultaneously.

This model of prostaglandin recognition suggests why the binding data for $PGF_{2\alpha}$ receptors are insensitive to modification of the $\omega$ chain. Methylation at C(15) does not disturb either binding at the $\alpha$ face of the molecule or the disposition of the O(9) hydroxyl relative to the $\alpha$ face because the 15-methyl points up and away from the $\alpha$ face; similarly, replacement of carbons C(18) through C(20) by a phenyl ring has little effect on the $\alpha$ face of the molecule if we consider that three atoms of the ring occupy the C(18)–C(20) positions. The model also suggests that a particularly interesting analog of $PGF_{2\alpha}$ might be 9$\alpha$-methyl-9-deoxy-$PGF_{2\alpha}$; it could distinguish between the requirements of binding and the requirements of activity, since it is likely that the 9$\alpha$-methyl analog would be very similar in conformation to $PGF_{2\alpha}$ yet lack the hydrogen-bonding capacity of the 9$\alpha$-hydroxyl. It is possible that such an analog might bind the $PGF_{2\alpha}$ receptor quite well and yet fail to elicit a response. Such a result would show that the recognition event of importance is the formation of an O(9) . . . receptor hydrogen bond.

In summary, the conformations of $PGF_{2\alpha}$ have been described. It is hairpin-shaped, as are other active prostaglandins we have examined. The observed conformations of $PGF_{2\alpha}$ and $PGE_2$ suggest a mechanism of recognition of these prostaglandins by their receptors. These results lead us to state with confidence that the crystallographically observed conformations of prostaglandins are the biologically relevant ones at the receptor sites.

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7. Disposition above or below the ring plane is referred to Fig. 1; thus C(7) is below and C(13) is above the ring plane.
8. W. Conover and J. Fried [*Proc. Natl. Acad. Sci. U.S.A.* **71**, 2157 (1974)] measured the 270-Mhz proton magnetic resonance spectrum of $PGF_{2\alpha}$. By arbitrarily attributing the H(8)···H(12) coupling constant of 11.4 hertz to an H(8)–C(8)–C(12)–H(12) torsion angle of 180°, they deduced the torsion angles (modulo 180°) for the remaining ring resonances. They concluded that $PGF_{2\alpha}$ possesses *C*2 symmetry at C(10). Because $PGF_{2\alpha}$ probably exists as an equilibrium mixture of the C(8) and C(9) envelope conformations in solution also (Conover and Fried's $^{13}C$ relaxation measurements suggest an equilibrium mixture) their simple assumption that the observed averaged coupling constants are related to some average ring conformation does not hold.
9. In their crystallographic study of the *p*-iodophenacyl ester of 15(*S*)-methyl $PGF_{2\alpha}$, C. G. Chidester and D. J. Duchamp [in *American Crystallographic Association, Spring Meeting, Berkeley, Calif., Program and Abstracts* (American Crystallographic Association, New York, 1974), ser. 2, vol. 2, p. 34] observed two independent molecules of the prostaglandin derivative. Both conformers display the C(9) envelope observed here for conformer A. In one of the forms of the 15(*S*)-methyl $PGF_{2\alpha}$ derivative the $\omega$ chain assumes the normal all-*trans* conformation, while in the other there is a +84° torsion angle about the C(17)–C(18) bond. Both conformers display the hairpin conformation.
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## Age-Related Changes in the Hepatic Endoplasmic Reticulum: A Quantitative Analysis

Abstract. *Morphometric analysis demonstrated a twofold increase in the surface area of the hepatic endoplasmic reticulum in Fischer 344 rats between 1 and 20 months of age, followed by a significant decrease in this parameter between 20 and 30 months. These changes are attributed to the smooth-surfaced endoplasmic reticulum, since neither the rough-surfaced variety nor the Golgi membranes underwent any significant change in surface area as a function of the age of the animal.*

The intracellular membrane systems of the hepatic parenchymal cell—that is, rough-surfaced endoplasmic reticulum (RER), smooth-surfaced endoplasmic reticulum (SER), and Golgi apparatus—are intimately involved in a wide variety of functions, including protein and cholesterol synthesis and drug and lipid metabolism. There have been numerous reports of age-dependent changes in these hepatic parameters in the rat, such as decreased protein and cholesterol synthesis (*1*), reduced turnover and excretion rates of cholesterol (*2*), reduced drug-metabolizing activity (*3*), and loss of certain adaptive capacities (*4*). However, the literature describing the fine structural correlates of these age-dependent functional alterations, particularly changes in the endoplasmic reticulum membranes, is both limited and conflicting. Qualitative ultrastructural analyses revealed an overall loss of RER in the liver cells of aged rats in comparison to those of young animals (*5*). Using quantitative electron microscopic techniques or morphometry, Pieri *et al.* (*6*) observed a significant loss of RER and no change in the amount of SER in the hepatocytes of rats between the ages of 1 and 12 months. The age-related reduction in RER surface area was even more