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 α chain conformation of $PGF_{2\alpha}$ relative to PGE_2 are necessary to maintain the close proximity of the α and ω chains. The α 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 ω chains are interpreted as fine-tuning mechanisms to maximize the α and ω 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 α face of the prostaglandin. If binding occurs primarily in two complementary steps—a nondifferentiating contact between the α 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 ω chain. Methylation at C(15) does not disturb either binding at the α face of the molecule or the disposition of the O(9) hydroxyl relative to the α face because the 15-methyl points up and away from the α face; similarly, replacement of carbons C(18) through C(20) by a phenyl ring has little effect on the α 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 9α-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 9α-methyl analog would be very similar in conformation to $PGF_{2\alpha}$ yet lack the hydrogen-bonding capacity of the 9α-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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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 ω 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

marked(80 percent) between 1- and 27-month-old animals.

In previous morphometric studies in this laboratory, hepatic fine-structural parameters were compared in virgin and retired breeder male Sprague-Dawley rats between 3 and 16 months of age (*7*). In contrast to the data of Pieri *et al.* (*6*), our results showed that the surface areas of both the RER and the SER continued to increase in these animals through 16 months of age. An analysis of several morphometric studies of the rat liver suggests that differences in animal strain or sex or in the mode of data expression cannot account for the conflict between our results (*7*) and those of Pieri *et al.* (*6*). Furthermore, the data from both laboratories are difficult to reconcile with those from numerous studies of age-dependent changes in certain hepatic functions, especially those thought to be associated with the endoplasmic reticulum.

To clarify these discrepancies, the hepatic fine structure in male Fischer 344 rats, obtained from the colony of the National Institute on Aging maintained at Charles River Breeding Laboratories, Wilmington, Massachusetts, was evaluated by quantitative electron microscopy. Groups of six rats each at ages of 1, 10, 20, and 30 months (survivorship in the colony, 100, 100, 96, and 21 percent, respectively) were fasted overnight and anesthetized, and their livers were prepared for electron microscopy by perfusion via the hepatic portal vein with 2.7 percent glutaraldehyde and 0.8 percent paraformaldehyde in 0.2*M* sodium bicarbonate buffer (*8*). After postfixation in 1 percent osmium tetroxide, dehydration, and embedding in Epon (*9*), the tissue was subjected to extensive morphometric analysis by methods previously described (*10, 11*). Organelle volumes and membrane surface areas were estimated in electron micrographs at final magnifications of × 13,800 and × 45,600, respectively.

Since Loud (*10*) and others (*12*) reported significant differences in the amounts or distributions of several liver cell organelles and inclusions depending on lobular orientation, both centrolobular and portal zone hepatocytes were examined. The Golgi apparatus, which has been implicated in hepatocyte albumin, lipoprotein, and bile acid synthesis or secretion (*13*), was included in the analysis. In addition to estimating the surface area of the Golgi membranes, we measured the volume of an arbitrarily defined "Golgi-rich area" (*12, 14*). The morphometric data are expressed in two ways: (i) as relative surface areas or volumes (square meters per cubic centimeter of hepatocyte ground substance or cubic centimeters per cubic centimeter of intralobular liver tissue) and (ii) as specific surface areas or volumes (square micrometers or cubic micrometers per average mononuclear hepatocyte).

The volumes of the hepatocytes and their constituent ground substance compartments were of interest since the morphometric data were expressed relative to both of these reference volumes. Hepatocyte volume densities did not change significantly as a function of age of the animal, although the volume of the average mononuclear liver cell increased by 63 and 31 percent in the centrolobular and portal zones, respectively, between 1 and 20 months of age (Table 1). However, average liver cell volume decreased significantly between 20 and 30 months, especially in the portal zone, where this value approached that found in the 1-month-old rats. With the exception of the youngest age group, the centrolobular hepatocytes were significantly larger than their respective portal zone cells (Table 1).

The volume density of the hepatocyte ground substance compartment increased slightly (10 percent) in the centrolobular zones of animals between 1 and 10 months of age but returned to the initial level by 20 months. On the other hand, there were no changes in this parameter in the portal zones of these animals until they reached 30 months of age. Between the ages of 20 and 30 months, the relative volume of this hepatocellular component increased by 9 percent in both the central and portal zones of the liver lobule (Table 1). The changes in the specific volume of the ground substance reflected the age-related alterations in average liver cell size. The fact that the specific volumes of the hepatocytes and the ground substance decreased and the relative volume of the latter constituent increased in animals between 20 and 30 months of age

Table 1. Surface density measurements of the total endoplasmic reticulum (TER), SER, and RER; relative and specific values for the Golgi membranes and the Golgi-rich area; and volume densities and specific volumes for the hepatocytes and the ground substance. Each value is the mean ± standard error of the mean. Units are: volume density, cubic centimeters per cubic centimeter of intralobular liver tissue; specific volume, cubic micrometers per average mononuclear hepatocyte; surface density, square meters per cubic centimeter of hepatocyte ground substance; and specific surface area, square micrometers per average mononuclear hepatocyte.

Age and lobular orientation	Hepatocytes		Ground substance		TER surface density	RER surface density	SER surface density	Golgi membrane		Golgi-rich area	
	Volume density	Specific volume	Volume density	Specific volume				Surface density	Specific surface area	Volume density	Specific volume
1 Month											
Central	0.85 ± 0.01	5242 ± 414	0.59 ± 0.01	3618 ± 62	12.42 ± 0.73	4.32 ± 0.35	8.10 ± 0.32	0.46 ± 0.09	1664 ± 326	0.017 ± 0.001	105 ± 6
Portal	0.82 ± 0.01	4742 ± 899	0.55 ± 0.01	3185 ± 52	12.50 ± 0.64	4.57 ± 0.32	7.93 ± 0.32	0.49 ± 0.11	1561 ± 350	0.016 ± 0.001	93 ± 6
10 Months											
Central	0.88 ± 0.01	8614 ± 895	0.65 ± 0.01	6333 ± 117	14.86 ± 0.85	3.86 ± 0.29	11.00 ± 0.56	0.58 ± 0.15	3672 ± 950	0.011 ± 0.001	108 ± 10
Portal	0.88 ± 0.01	7032 ± 539	0.56 ± 0.01	4450 ± 64	14.78 ± 0.93	4.23 ± 0.37	10.55 ± 0.56	0.72 ± 0.10	3200 ± 444	0.015 ± 0.001	120 ± 8
20 Months											
Central	0.89 ± 0.01	8563 ± 829	0.57 ± 0.01	5455 ± 96	14.00 ± 0.89	3.00 ± 0.32	11.00 ± 0.57	0.52 ± 0.10	2836 ± 545	0.012 ± 0.002	115 ± 19
Portal	0.86 ± 0.02	6226 ± 274	0.55 ± 0.01	3963 ± 65	12.62 ± 0.79	3.78 ± 0.33	8.84 ± 0.46	0.69 ± 0.10	2732 ± 396	0.013 ± 0.001	94 ± 7
30 Months											
Central	0.87 ± 0.01	6998 ± 564	0.62 ± 0.01	4988 ± 97	10.98 ± 0.53	4.11 ± 0.24	6.87 ± 0.29	0.38 ± 0.11	1893 ± 548	0.014 ± 0.001	113 ± 8
Portal	0.85 ± 0.01	4787 ± 508	0.60 ± 0.01	3402 ± 51	10.54 ± 0.42	4.27 ± 0.22	6.27 ± 0.20	0.77 ± 0.14	2617 ± 476	0.018 ± 0.001	100 ± 6

suggests loss of hepatocellular organelles, dilution of the liver cell cytoplasm by the ground substance, or both.

No obvious age-related changes in the amounts or distributions of the RER, SER, or Golgi membranes were detected by qualitative examination. However, morphometric analysis revealed several significant differences in the surface area of the endoplasmic reticulum among animals of different ages. The surface density of the total endoplasmic reticulum increased in rats between 1 and 10 months of age (~ 20 percent) but subsequently decreased until the levels measured in the 30-month-old rats were 13 and 19 percent below those found in the central and portal zones, respectively, of 1-month-old animals (Table 1). The relative surface area or surface density of the SER followed a similar age-dependent pattern, but there were no significant differences in the surface densities of the RER across these age groups. Previously reported lobular differences in the distributions of both the RER and the SER (*12*) were confirmed in this study. The centrolobular zone contained more total endoplasmic reticulum and SER, whereas the portal zone tissue contained more RER. There was no net change in either the surface density of the Golgi membranes or the relative volume of the Golgi-rich area as a function of age of the animal (Table 1).

The age-related changes in the specific surface areas (membrane surface area per average liver cell) of the total endoplasmic reticulum and the SER were even more dramatic than the differences in the surface densities of these parameters (Fig. 1). The total endoplasmic reticulum in the centrolobular cells more than doubled in surface area in animals between 1 and 10 months of age (Student's t-test, $P < .001$). Although the increase was less marked in the portal zone hepatocytes (65 percent, $P < .001$), the estimates for both zones of the liver lobule in the 30-month-old animals were similar to those in the 1-month-old rats, demonstrating a significant age-dependent loss of intracellular membrane. The estimates of the RER and SER specific surface areas confirmed our previous conclusion that the overall loss of endoplasmic reticulum membrane was attributable to a reduction in the amount of SER (Table 1, Fig. 1).

The surface area of the RER increased significantly between 1 and 10 months of age in the centrolobular hepatocytes (127 percent, $P < .001$) and this difference was still apparent between the 1- and the 30-month-old animals (90 percent, $P < .001$). Across the entire age span the centrolobular liver cells contained considerably more SER than did the respective portal zone hepatocytes, but the suspected lobular gradient in the distribution of RER was not confirmed by the specific surface area data. The fluctuations in the specific surface areas of the Golgi membranes and in the specific volumes of the Golgi-rich areas failed to produce significant net differences in these parameters as a function of age of the animal (Table 1).

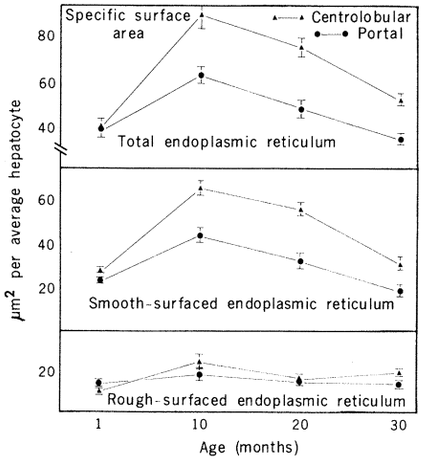

Fig. 1. Specific surface areas of total endoplasmic reticulum, SER, and RER. The values are expressed as the mean ± standard error of the mean. Note the significant loss of membrane surface area of the total endoplasmic reticulum and the SER as a function of age. The absence of any significant change in the amount of RER does not correspond to the reported losses of functional capacities associated with this membrane system.

The fact that the estimates of the specific surface area reflect the surface density measurements suggests that the loss of SER membrane is a real age-dependent change. This reduction in the amount of SER supports previous biochemical studies showing age-related losses in a number of hepatic functions associated with this membrane system, most notably drug, steroid, and cholesterol metabolism (*2*, *3*). Although the hepatic capacity to synthesize proteins is reported to decline during senescence (*1*), the absence of a significant reduction in the amount of RER, an important component in the maintenance of this parameter, suggests that certain age-related hepatic functional changes may reflect impaired cellular control mechanisms, such as transcription or translation. On the other hand, age-related changes in the composition of these intracellular membranes may result in the formation of a functionally inefficient membrane system (*15*).

These new morphometric data on hepatocellular membranes conflict with those recently reported by Pieri *et al.* (*6*). In our opinion, differences in animal strain and in the method of data expression cannot account for the extreme disagreement between these two sets of data. Pieri *et al.* included the Golgi membranes with the SER in their stereological evaluation, whereas we estimated the amount of Golgi membranes both as a separate entity and as part of an arbitrarily defined Golgi-rich area. The contribution of the Golgi profiles to the estimates of SER membrane surface areas is insignificant (Table 1). Furthermore, both studies employed essentially identical morphometry procedures, and neither our data nor those of Pieri *et al.* were corrected for possible systematic errors introduced by the Holmes effect, angle, specific membrane configurations, and tissue compression during sectioning. Since (i) our sample size was considerably larger than that of Pieri *et al.* and (ii) the animals we used were obtained from a carefully controlled colony maintained solely for aging studies, we believe that our data correctly reflect the age-dependent changes in these hepatic fine-structural parameters.

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Combustion of Several 2,4,5-Trichlorophenoxy Compounds: Formation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Abstract. *Grass and paper coated with several compounds containing the 2,4,5-trichlorophenoxy moiety have been subjected to combustion. By using compounds that had been purified to achieve low background amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) together with an efficient cleanup and analysis of the residue, it was possible to detect as little as 0.001 microgram of TCDD in the combustion products of 0.5 gram of the 2,4,5-trichlorophenoxy material. Small self-supported fires converted about 10^{-6} of the 2,4,5-trichlorophenoxy material to TCDD.*

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has been used for the control of vegetation for more than 25 years. A contaminant of 2,4,5-T, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been reported to persist and accumulate in the environment (*1*, *2*). It has also been suggested that the combustion or burning of vegetation sprayed with 2,4,5-T could result in the formation of TCDD (*2*). To accurately assess the potential hazards, if any, from the continued use of herbicides containing the 2,4,5-trichlorophenoxy linkage in areas that might be subject to burning, we conducted a series of combustion experiments. In each case we attempted to take advantage of the most appropriate technology and analytical methodology to separate any TCDD formed as a result of the combustion of the 2,4,5-trichlorophenoxy compound from that present as an impurity in the herbicides themselves. We used a multiple cleanup procedure followed by gas chromatography–mass spectrometry (GC–MS) for the determination of TCDD as a trace impurity in the herbicide and its formulations (*3*).

At a symposium on various aspects of chlorinated dibenzo-*p*-dioxins (*4*), several references were made to the formation of chlorinated dioxins by thermal condensations or rearrangements (*5*, *6*). Langer (*5*) reported that, in controlled heating experiments, 0.13 percent of 2,4,5-T was converted to TCDD when the potassium salt was heated at temperatures up to 400°C for up to 43 hours. Buu Hoi *et al.* (*2*) reported much higher amounts of TCDD formed when several 2,4,5-trichlorophenoxy species were pyrolyzed. However, some question remains regarding this latter work since the mass spectrum taken to indicate TCDD is not consistent with other published mass spectra (*7*). Nilsson *et al.* (*8*) have described pyrolysis experiments on chlorinated phenoxyphenols which resulted in the formation of up to 6 percent dioxin condensation product.

In our work, we have used conditions as "natural" as feasible: a cellulosic combustible material burning at its own rate with good air feed; an amount of the 2,4,5-trichlorophenoxy moiety applied to the surface at a concentration of 12 pounds of active ingredient per acre (13.5 kg/ha); an inert collection system to avoid the catalytic effects of walls and surfaces; and an analytical scheme that would permit examination of all the combustion products with high sensitivity and specificity. The combustion takes place in an open-bottom 500-ml flask with the combustible sample contained in a quartz mesh basket; a 15-cm petri dish is mounted below the basket to catch any small fragments of ash.

The combustion flask is connected to a series of four gas-absorber traps by means of glass ball-and-socket joints. The entire apparatus is constructed on the center line of the ball joints, so that any section or sections of the apparatus can be inverted by simple rotation. Gas trap A is cooled with an ice bath, and all other gas traps are cooled with Dry Ice. To attain good collection efficiency, each trap is filled with smooth glass beads (3 mm). Air is drawn through the system with a vacuum pump connected at the outlet of trap D. In the analysis we used low-resolution GC–MS with a cleanup procedure described by Hummel and Shadoff (*9*).

The 2,4,5-trichlorophenoxy materials used for the combustion were obtained either from regular production or from the Ag-Organics Research Laboratory of Dow Chemical Company. Each was purified to reduce the residual TCDD content by the cleanup described in (*3*) (ion exchange or silica gel), and then the matrix was recovered. Samples of grass from a field that had been treated with Esteron 245 (a registered Dow formulation of 2,4,5-T esters), at 12 pounds of 2,4,5-T equivalent per acre, were obtained immediately after spraying and also 1 week later. The Esteron 245 was not purified in the manner described above. These grass samples were frozen, ground, blended, and stored in polyethylene bags prior to analysis. The herbicides were dissolved in methanol or acetone and applied uniformly over the surface of laboratory filter paper (Whatman No. 1, acid-washed). A 5-g portion of grass was air-dried at a slight vacuum (500 torr) at 50°C for 1 hour, and the

Table 1. Combustion of material containing 2,4,5-trichlorophenoxy species.

Sample	TCDD produced* (μg)	TCDD conversion† (% by weight)
0.41 g of sodium 2,4,5-trichlorophenoxy acetate on 1350 cm² of paper	0.05	1.2×10^{-5}
0.125 g of 2,4,5-T on 450 cm² of paper	0.03	2.4×10^{-5}
0.46 g of butyl 2,4,5-trichlorophenoxy acetate on 1350 cm² of paper	0.2	4.3×10^{-5}
0.32 g of sodium 2,4,5-trichlorophenate on 1350 cm² of paper	0.2	3×10^{-5}
0.10 g of 2,4,5-trichlorophenol on 450 cm² of paper	0.05	5×10^{-5}
0.15 g of butyl 2,4,5-trichlorophenoxy acetate on 450 cm² of paper	0.05	3.3×10^{-5}

*After correction for the TCDD content of the trichlorophenoxy starting material: 0.1 part per billion (ppb), 2 ppb, and 6 ppb for 2,4,5-T, trichlorophenol, and butyl 2,4,5-trichlorophenoxy acetate, respectively. †Expressed as the percentage conversion of the 2,4,5-trichlorophenoxy species to TCDD.