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Simulation of the Effects of Leaf Senescence on Membranes by Treatment with Paraquat

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ABSTRACT

Chloroplast and microsomal membranes from the primary leaf of bean acquired increasing proportions of gel phase lipid as the tissue senesced. The lipid-phase transition temperature for microsomes rose from about 25 to 43 C and that for chloroplasts rose from below −30 C to about 52 C within 5 weeks of planting. This was accompanied by large increases (2- to 4-fold) in the sterol to phospholipid ratio of the membranes, which reflected breakdown of phospholipid. Changes in fatty acid saturation were of insufficient magnitude to account for the rise in transition temperature. All of these senescence-related changes in chloroplast and microsomal membranes were also induced by treating young, 2-week-old-plants with 10 milligrams per liter paraquat. Within 48 hours of treatment, the transition temperature rose from 25 to 57 C for microsomes and from below −30 to 24 C for chloroplasts. The membranes sustained only small changes in fatty acid saturation, comparable to those incurred during natural senescence, and there was a selective loss of phospholipid, resulting in augmented sterol to phospholipid ratios. Malondialdehyde, a product of lipid peroxidation, rose by 2- to 3-fold in both senescing and paraquat-treated leaves. Paraquat is known to form cation radicals that react with O_2 to produce O_2^- and has been implicated as an agent of lipid peroxidation. Accordingly, these observations suggest that membrane deterioration during natural senescence may be due in part to free radical damage.

The precise sequence of events governing senescence of cells remains speculative. Nevertheless, a disruption of biochemical homeostasis involving both qualitative and quantitative changes in synthesis and degradation of cellular components is certainly characteristic of the process (11, 14). Although there is a net decline in cell polymers and enzyme function, lytic processes and catabolism are precisely regulated, apparently controlled by *de novo* synthesis of digestive enzymes and by programmed release of hydrolases from membrane-bound compartments within the cell (14). Prior to actual lysis, marked changes in membrane permeability and structure occur among the various organelles in a fairly well-ordered sequence (1). Whether this loss of membrane integrity is the primary cause or merely the result of senescence has not been resolved (10, 14), but there is now little doubt that membrane alterations inimical to normal function intensify as senescence proceeds (1, 20). In particular, recent work has shown that membrane phospholipids undergo a progressive transition from the liquid-crystalline to the gel phase (15, 19, 20), and this change in physical state of lipids appears to be closely correlated with loss of membrane function (16–18, 29, 30). Although the causes of the degradative changes associated with the onset of membrane senescence have not been identified, within genetic limits, senescence *in toto* may be governed by the intensity of environmental assaults that strike at the biochemical level (13). Free radicals arising from metabolism or from the environment have been implicated as one probable source of senescence-related membrane damage, including lipid peroxidation and Chl destruction (13, 27).

Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) is a potent herbicide that produces marked changes in cell ultrastructure including disruption of membranes (2, 9). Its phytotoxicity is dependent on formation of the highly reactive superoxide radical (O_2^-) that arises when the partially reduced dipyridal cation is reoxidized by molecular oxygen (6, 22). Indeed, spin trapping techniques have revealed that the addition of 1 mM paraquat to illuminated chloroplast suspensions enhances the production of O_2^- by up to 10-fold (8). In the present study, we have examined the ability of paraquat to simulate the effects of senescence on membranes.

MATERIALS AND METHODS

Growth Conditions and Fractionation Procedures. *Phaseolus vulgaris* L. (cv. Kinghorn) was grown under greenhouse conditions in a mixture of sand, peat moss, and soil (1:1:2) at a mean temperature of 26 C with a 16-h photoperiod. Primary leaves that served as control tissue were harvested after 2 weeks growth, and senescent primary leaves were obtained after 5 weeks. Two-week-old plants were treated with paraquat applied as an aqueous spray at concentrations of 10 or 50 mg l^{-1} in distilled H_2O, and the primary leaves were harvested at intervals of 12, 24, and 48 h after treatment.

Microsomal membranes and a partially purified fraction of chloroplasts were isolated from the leaves essentially according to procedures previously described (18). For each preparation, 100 g leaf tissue were homogenized with a Sorvall Omni-Mixer in 50 ml cold 0.3 M sucrose 50 mM $NaHCO_3$ (pH 7.5). After filtering through cheesecloth, a 200g × 10-min supernatant was centrifuged at 2,000g for 10 min to yield partially purified chloroplasts. Microsomes were isolated from a 10,000g × 20-min supernatant by centrifugation at 150,000g for 2 h through 1.8 M sucrose, a procedure that is designed to separate the membranes from free ribosomes (17). Each gradient tube contained 9 ml supernatant overlaying 3 ml sucrose. The microsomal membranes collected at the interface were removed with a syringe, diluted with 3 volumes homogenizing buffer, and pelleted by centrifugation at 165,000g for 1 h.

Lipid and Chl Analysis. Total lipids were extracted from the isolated membranes according to the procedure of Nichols (23). Fatty acid methyl esters were prepared from the total lipid extract as described by Morrison and Smith (21). A volume of lipid extract containing at least 1 μmol phospholipid phosphorus was evaporated to dryness under N_2. The sample was dissolved in 1 ml 14% BF_3 in methanol (J. T. Baker), sealed under N_2, and tested at 90 C for 90 min.

The methyl esters were extracted with 2 ml pentane-H_2O (2:1, v/v) and identified by flame ionization-gas chromatography using

a stainless steel column (183 × 0.64 cm) packed with 5% EGSS-X in Supelcoport (100–120 mesh) and maintained at 170 C. Heptadecanoic acid (17:0) was used as an internal standard.

Free sterols in the lipid extracts were quantified as described by Grunwald (7). After precipitation with digitonin, the sterols were silylated in equal volumes of acetonitrile and *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (Chromatographic Specialties). The sterol derivatives then were analyzed by GLC on a glass column (182.9 × 0.64 cm) packed with 3% OV-17 on Chromosorb W (100-120 mesh) at 260 C. Cholestane was used as an internal standard. The sterols were identified on the basis of their retention times relative to known standards. Relative weight responses to cholestane were determined for cholesterol, campesterol, stigmasterol, and β-sitosterol using authentic standards. The relative weight responses for the other sterols for which standards could not be obtained were assumed to be 1.

Lipid phosphate was determined by the method of Fiske and Subbarow as described by Dittmer and Wells (5). Levels of Chl *a* and *b* were determined spectrophotometrically by extracting 0.1-ml aliquots of homogenate with 10 ml 90% (v/v) acetone and reading the *A* of the extracts at 645, 652, and 663 nm, respectively (12). Malondialdehyde levels in homogenates were assayed using a modified thiobarbituric acid test (3).

X-ray Diffraction. Microsomal and chloroplast membranes were prepared for x-ray diffraction as described previously (17, 20). Wide-angle diffraction patterns were recorded at various temperatures using CuKα radiation from a point-focused x-ray tube (type PW 2103/01) on a Philips (type 1030) camera under conditions in which the samples retain 40 to 75% moisture with respect to final dry weight (17). The lipid-phase transition temperature was determined to within 1 C.

RESULTS

Treatment of bean leaves with paraquat induced an increase in the lipid phase transition temperature of chloroplast and microsomal membranes comparable in magnitude to that incurred for both types of membrane during natural senescence (Fig. 1). The transition temperature is defined as the highest temperature at which gel-phase lipid can be detected (17) and, for microsomes, this rose from about 25 C for young 2-week-old tissue to 43 C by week 5, at which time the leaves were extensively senescent and beginning to abscise. Treatment of 2-week-old plants with either 10 or 50 mg l^{-1} paraquat caused a similar time-dependent increase

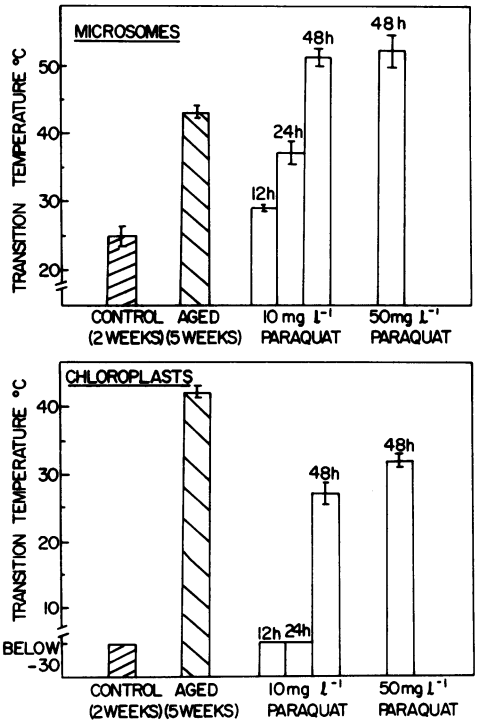

FIG. 1. Comparison of the effects of senescence and paraquat treatment on the lipid-phase transition temperature of microsomal and chloroplast membranes from the primary leaves of *P. vulgaris*. Duration of paraquat treatment and standard errors of the means are indicated; $n = 3$.

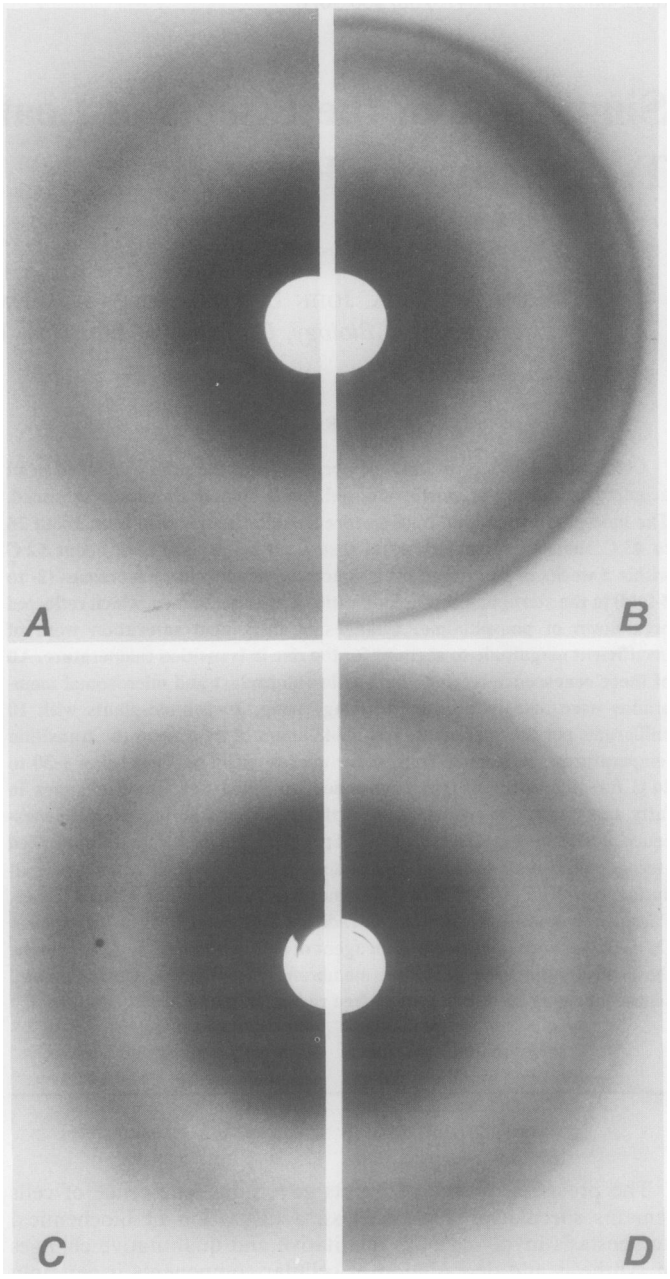

FIG. 2. Wide-angle x-ray diffraction patterns recorded at 26 C for isolated membrane fractions from the primary leaves of *P. vulgaris*. A and C, patterns for microsomal and chloroplast membranes respectively, isolated from 2-week-old control leaves and featuring (from outside to inside) two broad diffuse bands centered at Bragg spacings of 4.6 and about 10 Å, respectively. B and D, patterns for microsomal and chloroplast membranes, respectively, isolated from 2-week-old leaves treated for 48 h with 10 mg l^{-1} of paraquat and featuring (from outside to inside) a sharp band centered at a Bragg spacing of 4.15 Å and two broad diffuse bands centered at Bragg spacings of 4.6 and about 10 Å.

Table I. *Comparison of Effects of Senescence and Paraquat Treatment on Fatty Acid Composition of Microsomal and Chloroplast Membranes from Primary Leaves of P. vulgaris*

Values for fatty acid composition are means of three experiments and those for unsaturated to saturated (U/S) fatty acid ratios are means of five experiments. Standard errors of the means are shown. Treated plants were sprayed with paraquat at 2 weeks of age and the primary leaves were harvested 48 h later.

Membrane Fraction	Fatty Acid	Mole % Fatty Acid		
		Control, 2 weeks old	Aged, 5 weeks old	Paraquat, 10 mg l^{-1}
Chloroplasts	16:0	16.39 ± 0.34	21.87 ± 0.99	23.18 ± 0.44
	16:1	10.26 ± 0.06	2.17 ± 0.29	7.60 ± 0.04
	18:0	3.12 ± 0.22	3.62 ± 0.46	4.79 ± 0.05
	18:1	1.34 ± 0.24	5.95 ± 0.32	2.14 ± 0.28
	18:2	6.31 ± 0.28	7.45 ± 0.21	8.84 ± 0.37
	18:3	62.55 ± 1.12	58.80 ± 1.59	53.42 ± 0.69
	U/S	4.30 ± 0.56	2.87 ± 0.08	3.03 ± 0.07
Microsomes	16:0	29.20 ± 0.55	21.17 ± 1.21	35.47 ± 0.54
	16:1	5.72 ± 0.30	1.45 ± 0.14	8.11 ± 0.13
	18:0	7.14 ± 0.38	4.45 ± 0.51	7.37 ± 0.18
	18:1	2.61 ± 0.21	5.10 ± 0.34	2.71 ± 0.06
	18:2	18.52 ± 0.02	10.52 ± 0.45	15.27 ± 0.09
	18:3	36.77 ± 1.19	51.22 ± 1.79	31.16 ± 0.44
	U/S	2.03 ± 0.08	1.95 ± 0.08	1.54 ± 0.04

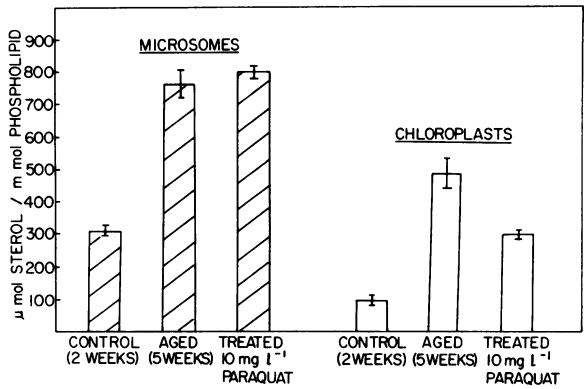

FIG. 3. Comparison of the effects of senescence and treatment for 48 h with 10 mg l^{-1} paraquat on the sterol to phospholipid ratio of microsomal and chloroplast membranes from the primary leaves of *P. vulgaris*. Standard errors of the means are indicated; $n = 3$.

in microsomal transition temperature to a high of about 51 C within 48 h (Fig. 1). Analogous results also were obtained for chloroplast membranes. Again, there was a large increase in transition temperature during natural senescence, from below −30 C at 2 weeks to 42 C by week 5, and treatment with 10 or 50 mg l^{-1} paraquat raised the transition temperature to 27 and 32 C, respectively, within 48 h (Fig. 1).

The formation of crystalline (gel phase) lipid which accompanies natural senescence of these membranes can be simulated by treatment of young leaves with paraquat. This is confirmed by diffraction patterns recorded at 26 C, the mean growth temperature for these experiments. Wide-angle patterns for microsomal and chloroplast membranes from 2-week-old control leaves featured only the broad, diffuse lipid reflection, centered at a Bragg spacing of 4.6 Å, which derives from liquid-crystalline (fluid) lipid (Fig. 2, A and C). By contrast, corresponding patterns for chloroplast and microsomal membranes isolated from paraquat-treated leaves displayed the broad 4.6-Å liquid-crystalline reflection as well as a sharp ring at 4.15 Å representing gel-phase lipid (Fig. 2, B and D). Thus, the older senescent membranes, as well as those isolated from the paraquat-treated leaves, contained a mixture of liquid-crystalline and gel-phase lipid in their matrices.

The formation of gel-phase lipid in microsomal membranes during senescence was not accompanied by any significant change in the unsaturated to saturated fatty acid ratio. Treatment with 10 mg l^{-1} paraquat appeared to cause a slight reduction in the degree of unsaturation, but the decrease was not statistically significant (Table I). For chloroplasts, the unsaturated to saturated fatty-acid ratio decreased by about 20% between weeks 2 and 5 as the leaves senesced, and treatment of 2-week-old leaves with paraquat caused a similar reduction in the proportion of unsaturated fatty acids (Table I). Thus, the changes in fatty acid saturation induced by the herbicide were not pronounced and closely paralleled those incurred during natural senescence of the membranes. Indeed, for both types of membrane, senescence and paraquat treatment caused relatively minor changes in the relative proportions of fatty acids. During senescence, the largest change occurred in the chloroplast membranes where the proportion of linolenic acid decreased from 62% for 2-week-old leaves to 58% by week 5. A similar change was noted following treatment with the herbicide. However, in each case, there was a compensatory increment in palmitic acid (Table I).

Far more striking was a dramatic increase in the sterol to phospholipid ratio observed in chloroplast and microsomal membranes during natural senescence and after treatment of young leaves with paraquat. For microsomes, the ratio rose by 2.3-fold during senescence and by 2.5-fold following paraquat treatment. The changes for chloroplasts were even more pronounced; senescence caused a 5-fold increment in relative sterol concentration and paraquat caused a 3-fold increase (Fig. 3). Since chloroplasts are rich in glycolipids, sterol levels were also determined relative to fatty acid rather than phospholipid concentrations. Again, the same trend was evident. Within 48 h after treatment with 10 mg l^{-1} paraquat, the relative sterol concentration rose from 12 ± 1 to 44 ± 8 (± SE) and from 115 ± 5 to 290 ± 17 (± SE) μmol sterol/mmol fatty acid for chloroplasts and microsomes, respectively.

β-Sitosterol and stigmasterol collectively comprise more than half of the total sterols in each membrane system. Others that were detectable included campesterol, isofucosterol, cholesterol, and two unidentified sterols with retention times greater than that for isofucosterol (Table II). Each of the sterols, in both microsomes and chloroplast membranes, showed increased concentration relative to fatty acids upon treatment with paraquat (Table III). For chloroplasts, the increments were of comparable magnitude for the four major sterols but, for microsomes, the isofucosterol to fatty acid ratio increased by 6-fold, whereas the corresponding ratios for campesterol, stigmasterol, and β-sitosterol rose by only 1.4-fold. Cholesterol and the two unidentified sterols also showed comparatively large increments relative to fatty acid for both types of membrane. However, total sterol levels in the isolated membrane fractions expressed on a per unit dry weight of tissue did not change with advancing senescence or as a result of paraquat treatment, whereas phospholipid levels declined steeply (Table IV). Thus, it is clear that the increased sterol to phospholipid ratios reflect loss of phospholipid. This was also apparent from measurements of the fatty acid content of the isolated fractions. For example, after treatment for 48 h with 10 mg l^{-1} paraquat, chloroplast fatty acids decreased from 25.98 ± 2.14 to 7.24 ± 0.85 (± SE) μmol/g dry weight of leaf, and microsomal fatty acids decreased from 9.82 ± 0.18 to 2.64 ± 0.03 (± SE) μmol/g dry weight of leaf. Accordingly, in the case of chloroplasts, the increased sterol to fatty acid ratio may also reflect loss of glycolipid.

Levels of Chl *a* and *b* in the leaves declined by upwards of 50% within 48 h after treatment with either 10 or 50 mg l^{-1} paraquat (Table V). Between 2 and 5 weeks, the total Chl titer of these leaves decreased by about 70% as a result of natural senescence

Table II. *Comparison of Effects of Senescence and Paraquat Treatment on Sterol Composition of Microsomal and Chloroplast Membranes from Primary Leaf of P. vulgaris*

Values are means of three to four experiments; standard errors of the means are shown. Treated plants were sprayed with paraquat at 2 weeks of age and the primary leaves were harvested 48 h later.

Membrane Fraction	Sterol	Mole % Sterol		
		Control, 2 weeks old	Aged, 5 weeks old	Paraquat, 10 mg l^{-1}
Chloroplasts	Cholesterol	0.63 ± 0.07	0.80 ± 0.17	0.87 ± 0.15
	Campesterol	5.10 ± 0.13	6.70 ± 0.35	4.79 ± 0.17
	Stigmasterol	29.74 ± 0.42	41.52 ± 0.36	31.54 ± 1.57
	β-Sitosterol	32.76 ± 0.80	42.02 ± 0.71	28.64 ± 1.39
	Isofucosterol	20.07 ± 0.78	2.67 ± 0.68	21.24 ± 1.26
	Unknown 1	11.19 ± 0.66	1.55 ± 0.39	11.62 ± 0.29
	Unknown 2	0.48 ± 0.29	1.05 ± 0.32	1.89 ± 0.79
Microsomes	Cholesterol	0.39 ± 0.13	1.05 ± 0.16	0.67 ± 0.16
	Campesterol	6.37 ± 0.36	6.75 ± 0.22	3.42 ± 0.22
	Stigmasterol	34.45 ± 2.38	39.90 ± 0.56	19.41 ± 1.74
	β-Sitosterol	37.34 ± 1.96	44.35 ± 0.27	22.76 ± 0.83
	Isofucosterol	12.39 ± 0.03	3.72 ± 0.49	29.33 ± 1.12
	Unknown 1	6.09 ± 0.21	2.35 ± 0.28	20.41 ± 0.86
	Unknown 2	2.79 ± 0.22	1.75 ± 0.01	3.97 ± 0.61

Table III. *Changes in Sterol to Fatty Acid Ratio in Chloroplast and Microsomal Membranes from Primary Leaf of P. vulgaris following Treatment with Paraquat*

Values are means of three experiments; standard errors of the means are shown. Treated plants were sprayed with 10 mg l^{-1} paraquat at 2 weeks of age and the primary leaves were harvested 48 h later.

Sterol	Sterol: Fatty Acid			
	Control leaves		Treated leaves	
	Chloroplasts	Microsomes	Chloroplasts	Microsomes
	μmol/mmol			
Cholesterol	0.07 ± 0.01	0.45 ± 0.16	0.32 ± 0.04	1.72 ± 0.39
Campesterol	0.57 ± 0.04	7.23 ± 0.75	1.87 ± 0.19	9.08 ± 0.89
Stigmasterol	3.32 ± 0.21	38.18 ± 4.20	12.47 ± 1.69	51.08 ± 6.68
β-Sitosterol	3.67 ± 0.30	41.24 ± 4.14	11.07 ± 1.17	60.21 ± 4.26
Isofucosterol	2.22 ± 0.05	13.06 ± 2.40	8.30 ± 1.01	77.06 ± 1.53
Unknown 1	1.24 ± 0.03	6.30 ± 1.93	4.25 ± 0.41	53.56 ± 0.39
Unknown 2	0.06 ± 0.03	3.22 ± 1.48	0.73 ± 0.34	10.30 ± 1.31

Table IV. *Changes in the Sterol and Phospholipid Levels of Microsomal and Chloroplast Fractions from Primary Leaf of P. vulgaris during Senescence and following Treatment with Paraquat*

Values are means of three experiments; standard errors of the means are shown. Treated plants were sprayed with paraquat at 2 weeks of age and the primary leaves were harvested 48 h later.

Treatment	Sterol		Phospholipid	
	Chloroplasts	Microsomes	Chloroplasts	Microsomes
	μmol/g dry wt leaf			
Senescence				
2-week-old	0.39 ± 0.16	0.71 ± 0.12	3.34 ± 0.82	2.99 ± 0.81
3-week-old	0.37 ± 0.14	0.68 ± 0.02	2.01 ± 0.41	1.34 ± 0.31
4-week-old	0.35 ± 0.12	0.51 ± 0.07	1.16 ± 0.42	0.86 ± 0.09
5-week-old	0.35 ± 0.02	0.73 ± 0.02	0.63 ± 0.15	0.86 ± 0.06
Paraquat treatment				
Control	0.39 ± 0.16	0.71 ± 0.12	3.25 ± 0.16	2.68 ± 0.28
10 mg l^{-1}	0.38 ± 0.04	0.71 ± 0.02	1.42 ± 0.13	0.97 ± 0.01

(18). In addition, levels of malondialdehyde, a product of lipid peroxidation, increased by 2- to 4-fold relative to protein during natural senescence and as a result of paraquat treatment (Table VI). When these values were expressed relative to dry weight of tissue, the increases ranged from 2- to 3-fold.

DISCUSSION

It has been previously reported for several plant tissues that, as senescence intensifies, increasing proportions of the membrane lipids become crystalline at physiological temperature (17, 18, 20,

Table V. *Effect of Paraquat Treatment on Chl Content of Primary Leaves from P. vulgaris*

Treated plants were sprayed with paraquat and primary leaves were harvested after 12, 24, and 48 h. Standard errors of the means are indicated; $n = 3$.

Treatment	Leaf Age, Post-2 Weeks	Chl *a*	Chl *b*	Total Chl
	h		*mg/g fresh wt*	
Control	0	0.76 ± 0.04	0.20 ± 0.01	0.96 ± 0.06
	24	0.81 ± 0.06	0.25 ± 0.02	1.06 ± 0.06
	48	0.79 ± 0.04	0.23 ± 0.02	1.02 ± 0.04
Paraquat	12	0.69 ± 0.01	0.16 ± 0.01	0.85 ± 0.02
10 mg l^{-1}	24	0.59 ± 0.04	0.14 ± 0.01	0.73 ± 0.05
	48	0.49 ± 0.02	0.12 ± 0.01	0.61 ± 0.02
Paraquat 50 mg l^{-1}	48	0.48 ± 0.04	0.12 ± 0.01	0.60 ± 0.05

Table VI. *Changes in Malondialdehyde Levels of Primary Leaves from P. vulgaris following Treatment with Paraquat and during Senescence*

Treated plants were sprayed with 10 mg l^{-1} paraquat. Values are means of three experiments; standard errors of the means are shown.

Treatment	Malondialdehyde Content
	nmol/mg protein
Senescence	
2-week-old	2.03 ± 0.33
3-week-old	2.55 ± 0.39
4-week-old	6.97 ± 0.70
5-week-old	8.04 ± 0.60
Paraquat Treatment	
Control	2.88 ± 0.42
1 h after tretment	3.89 ± 0.18
2 h after treatment	4.36 ± 0.19
5 h after treatment	5.58 ± 0.26
8 h after treatment	6.29 ± 0.37
11 h after treatment	7.31 ± 0.09
15 h after treatment	7.85 ± 0.17
20 h after treatment	7.83 ± 0.62
24 h after treatment	7.54 ± 0.20
28 h after treatment	7.54 ± 0.20
32 h after treatment	7.39 ± 0.30
36 h after treatment	7.25 ± 0.90
40 h after treatment	7.24 ± 0.50
44 h after treatment	7.12 ± 0.21
48 h after treatment	6.95 ± 0.11

30). It is now clear that, for chloroplast and microsomal membranes from primary leaves of bean, this alteration in lipid phase behavior can be simulated by treating young leaf tissue with paraquat. Indeed, treatment of 2-week-old leaves with as little as 10 mg l^{-1} paraquat caused the lipid-phase transition temperature for both types of membrane to rise within 48 h to values comparable to those obtained for membranes from extensively senescent 5-week-old tissue. In all of these experiments, the transition temperatures were thermally reversible. The membrane lipid was exclusively liquid-crystalline above the transition point but, as the temperature was progressively lowered below the transition, increasing proportions of gel-phase lipid formed. Once the transition temperature had risen above the growing temperature, as it did during natural senescence and as a result of paraquat treatment, liquid-crystalline as well as gel-phase lipid domains were present in the membrane matrices. This mixture of lipid phases is known to render lipid bilayers leaky (25, 31) and thus could conceivably account for the loss of photosynthetic capability and tissue leakiness that characterize leaf senescence.

Crude, rather than highly purified, chloroplast preparations were used in the present study because of a previous report that the stringent gradient centrifugation required for purification excludes senescent chloroplasts containing crystalline membrane lipid (18). A microsomal fraction is by nature heterogenous, consisting of small fragments of membrane derived primarily from cytoplasmic membranes, but also from the plasmalemma. The microsomal and chloroplast membranes displayed distinctly different transition temperatures during all phases of the study, indicating that there was little cross-contamination during their isolation.

In addition to the physical changes noted, treatment with paraquat also induced chemical changes in the membrane lipids that paralleled those incurred during natural senescence. In particular, there was a marked increase in the concentration of free sterols in the membranes relative to either phospholipid or fatty acids, which was attributable to loss of glycerolipid. Parallel changes were also observed in the fatty acid composition of chloroplast and microsomal membranes during natural senescence and following treatment with the herbicide. These alterations did not significantly affect the degree of lipid saturation in microsomes and caused only a small reduction in the unsaturated to saturated fatty-acid ratio in chloroplasts. These observations are consistent with previous reports (15, 17, 19, 30) that the formation of gel-phase lipid in senescing membranes cannot be attributed to an increase in fatty-acid saturation but, rather, is imposed on the phospholipids by neutral lipids other than the free sterols, which accumulate with age (19). Paraquat also induced breakdown of Chl to approximately the same degree as occurred during senescence.

Paraquat is known to form cation radicals which react with O_2 to produce the superoxide radical O_2^- (6, 8, 22). Moreover, the presence of O_2 accentuates the sensitivity of plants and animals to paraquat (22), and it seems that O_2^- generated by cyclic redox processes may well be the agent of paraquat toxicity. Inasmuch as paraquat treatment so closely simulates the effects of natural senescence on membranes, it seems reasonable to propose that much of the membrane deterioration incurred during senescence is due to free radical damage, in particular lipid peroxidation, since paraquat has been implicated as a peroxidative agent (4). This is supported by the observation that malondialdehyde levels rose during natural senescence of the leaves and as a result of paraquat treatment. Moreover, *in vitro* experiments in which isolated membranes were exposed to ozone have demonstrated that formation of gel-phase lipid is a consequence of lipid peroxidation (26). Accordingly, it is perhaps surprising to note that there was no decrease in the degree of unsaturation in microsomes with advancing senescence or as a result of paraquat-treatment and only a small decrement for the chloroplast membranes. However, recent evidence indicates that de-esterification of fatty acids is a major consequence of O_2^--mediated lipid deterioration (24), and saturated and unsaturated fatty acids should be equally susceptible. This, together with enhanced phospholipase activity (28), could result in extensive dismantling of phospholipid and could logically account for the large increase in the sterol to fatty acid ratio observed during senescence and following paraquat treatment.

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