toin, 513-86-0; isobutyl cyanide, 625-28-5; 2-methyl-2-butenal, 1115-11-3; 3-methylbutanol, 123-51-3; (*E*)-2-pentenal, 1576-87-0; 2-methylbutanol, 137-32-6; pentanol, 71-41-0; (*Z*)-3-hexenal, 6789-80-6; hexanal, 66-25-1; furfural, 98-01-1; (*E*)-2-hexenal, 6728-26-3; (*Z*)-3-hexenol, 928-96-1; 3-methylbutyric acid, 503-74-2; 6-methyl-5-hepten-2-one, 110-93-0; 6-methyl-5-hepten-2-ol, 1569-60-4; hexanoic acid, 142-62-1; 2-pentylfuran, 3777-69-3; phenylacetaldehyde, 122-78-1; 2-isobutylthiazole, 18640-74-9; 6-methyl-3,5-heptadien-2-one, 1604-28-0; linalool, 78-70-6; 2-phenylethanol, 60-12-8; phenylacetonitrile, 140-29-4; methyl salicylate, 119-36-8; α-terpineol, 98-55-5; β-cyclocitral, 432-25-7; neral, 106-26-3; geranial, 141-27-5; eugenol, 97-53-0; β-damascenone, 23726-93-4; geranylacetone, 3796-70-1; β-ionone, 79-77-6; pseudoionone, 141-10-6; hexanol, 111-27-3; 1-nitro-3-methylbutane, 627-67-8; methional, 3268-49-3; 2-acetylfuran, 1192-62-7; benzaldehyde, 100-52-7; (*E*)-2-heptenal, 18829-55-5.

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Water-Soluble Fluorescent Compounds in Rat Tissue Fed Cottonseed Flour Supplemented with Vitamin E

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The presence and accumulation of water-soluble fluorescent compounds in the tissue of vitamin E deficient rats and in rats fed gossypol were determined. Sixteen rats were divided into four groups and were fed a control diet, a vitamin E deficient diet, a cottonseed diet, or a cottonseed plus an excess of vitamin E diet for 8 weeks. Results show that more pigment accumulated in muscle, liver, and testes of rats fed the cottonseed and vitamin E deficient diet than in those of rats fed the control diet. There was no significant difference in heart pigment levels among rats fed the four diets.

The accumulation of lipofuscinic pigment found in animal tissues has been related to aging and vitamin E deficiencies. The presence of these pigments is considered to be a consequence of the autoxidation of intracellular compounds. Apparently, the dietary deficiency of antioxidants, such as vitamin E and selenium, results in the intracellular accumulation of similar autofluorescent pigments in some tissues. Most of these pigments were believed to be due to the Schiff base type compounds, with the greatest fluorescence at 435 nm called "solvent-

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Table I. Composition of Experimental Diets (g/100 g of Diet)

	diet 1	diet 2	diet 3	diet 4
casein[a]	11.23	11.23	5.62	5.62
cottonseed flour[b]			25.50	25.50
sucrose	20.10	20.10	20.10	20.10
dextrose	19.00	19.00	16.97	16.97
dextrin[c]	25.00	25.00	15.63	15.63
lard	8.00	14.00	5.18	5.18
corn oil	6.00		6.00	6.00
mineral mix[d,e]	4.00	4.00	3.00	3.00
vitamin mix[f]	2.00	2.00[g]	2.00	2.00
fiber cellulose type[e]	4.67	4.67		
vitamin E				0.10

[a] Sigma Chemical Co., St. Louis, MO. [b] Albamex, S.A., Mexico City. [c] Productos de Maíz, S.A., Mexico City. [d] Roger and Harper mineral mix. [e] Tecklad test diet, Madison, MI. [f] Vitamin diet fortification mixture, Nutritional Biochemical, Cleveland, OH. [g] Vitamin mixture prepared according to *f* but without vitamin E.

soluble lipofuscinic pigments" (Weglicki et al., 1968; Reichel, 1968; Tonna, 1975; Csallany et al., 1977; Kruk and Enesco, 1981; Katz et al., 1984). Recently, Manwaring and Csallany (1981)found more than four water-soluble fluorescent compounds in mouse tissue, which may be related to vitamin E deficiency. The effects in animals produced by gossypol, a compound present in cottonseed, resembles vitamin E deficiency: infertility, a decrease in the number and motility of spermatozoa, and low LDH activity in the testes (Chen and Thacker, 1982; Machlin et al., 1980; Tso and Lee, 1982). Lipofuscinic pigment accumulation was also found in animals treated with gossypol (Hahn et al., 1981). These findings and the fact that both compounds, gossypol and vitamin E, have antioxidant effects suggest a possible competitive action in the chain reaction occurring in the peroxidizing systems of the tissues in which vitamin E is generally recognized as a natural biological antioxidant. The present study was undertaken to determine whether gossypol from cottonseed, vitamin E deficiency, or gossypol with excess vitamin E could stimulate the accumulation of water-soluble fluorescent compounds.

MATERIALS AND METHODS

The animals used in this study were 16 Sprague–Dawley male rats 12–14 weeks old with an average weight of 300 g. They were housed in individual cages and were given food and water ad libitum. The rats were divided into four groups of four animals each: group 1, control diet (casein); group 2, vitamin E free diet; group 3, cottonseed flour balanced diet; group 4, cottonseed flour balanced diet supplemented with excess vitamin E. Table I shows the detailed compositions of the different experimental diets. In order to compare the distribution of water-soluble fluorescent compounds, the rats in each group were sacrificed after 8 weeks of treatment. Liver, leg muscle, heart, and testes were immediately removed for spectrofluorometric determination of fluorescent compounds. Water-soluble fluorescent

Table II. Levels of Water-Soluble Autofluorescent Compounds in Rat Tissue at 270/310 nm (Excitation/Emission Maxima) (RFU/g of Tissue)[a]

diet	muscle	heart	liver	testes
		Peak 1		
1	0.000 ± 0.000	0.035 ± 0.009	0.073 ± 0.016	0.046 ± 0.013
2	0.036 ± 0.004*	0.038 ± 0.018	0.093 ± 0.046	0.030 ± 0.004
3	0.016 ± 0.002*	0.048 ± 0.011	0.116 ± 0.046	0.050 ± 0.039
4	0.012 ± 0.001*	0.067 ± 0.045	0.093 ± 0.020	0.021 ± 0.009*
		Peak 2		
1	0.064 ± 0.021	0.067 ± 0.027	0.345 ± 0.050	0.057 ± 0.006
2	0.117 ± 0.096	0.141 ± 0.075	0.346 ± 0.093	0.051 ± 0.007
3	0.111 ± 0.016	0.161 ± 0.079	0.353 ± 0.070	0.060 ± 0.011
4	0.096 ± 0.009	0.215 ± 0.102	0.340 ± 0.020	0.046 ± 0.002*
		Peak 3		
1	0.011 ± 0.012	0.086 ± 0.022	0.120 ± 0.030	0.039 ± 0.006
2	0.055 ± 0.013*	0.063 ± 0.034	0.126 ± 0.040	0.046 ± 0.011
3	0.032 ± 0.024	0.080 ± 0.031	0.130 ± 0.060	0.110 ± 0.058
4	0.029 ± 0.007	0.114 ± 0.059	0.147 ± 0.020	0.037 ± 0.011

[a] Asterisk indicates significant difference from control diet (Student's *t*-test, $P < 0.01$).

Table III. Levels of Water-Soluble Autofluorescent Compounds in Rat Tissue at 275/350 nm (Excitation/Emission Maxima) (RFU/g of Tissue)[a]

diet	muscle	heart	liver	testes
		Peak 1		
1	0.290 ± 0.103	1.280 ± 0.577	2.133 ± 0.320	0.492 ± 0.079
2	0.240 ± 0.066	1.017 ± 0.431	2.233 ± 0.080	0.350 ± 0.030
3	0.280 ± 0.109	1.427 ± 0.802	2.343 ± 0.051	0.452 ± 0.113
4	0.300 ± 0.033	1.167 ± 0.396	1.453 ± 0.036*	0.297 ± 0.510*
		Peak 2		
1	0.000 ± 0.000	0.850 ± 0.151	0.061 ± 0.028	0.402 ± 0.113
2	0.312 ± 0.041*	0.625 ± 0.219	0.145 ± 0.125	0.400 ± 0.070
3	0.340 ± 0.155*	0.866 ± 0.202	0.358 ± 0.151*	0.380 ± 0.015
4	0.370 ± 0.081*	0.570 ± 0.143	0.280 ± 0.156*	0.275 ± 0.025
		Peak 3		
1	1.172 ± 0.368	5.700 ± 0.803	7.286 ± 2.500	1.940 ± 0.438
2	1.110 ± 0.208	4.232 ± 2.121	10.266 ± 2.900	2.620 ± 0.280
3	0.846 ± 0.625	5.220 ± 1.782	11.433 ± 3.000	3.575 ± 1.502*
4	1.460 ± 0.477	5.977 ± 1.939	23.888 ± 6.018*	2.377 ± 1.502

[a] Asterisk indicates significant difference from control diet (Student's *t*-test, $P < 0.01$).

Table IV. Levels of Water-Soluble Autofluorescence Compounds in Rat Tissue Peak 1 at 320/380 nm (Excitation/Emission Maxima) (RFU/g of Tissue)[a]

diet	muscle	heart	liver	testes
1	0.662 ± 0.102	0.772 ± 0.173	2.616 ± 0.930	1.630 ± 0.735
2	0.505 ± 0.071	0.922 ± 0.107	13.516 ± 1.860*	1.895 ± 0.825
3	0.281 ± 0.046*	0.825 ± 0.354	8.476 ± 4.000*	0.766 ± 0.405
4	0.432 ± 0.156	0.702 ± 0.055	15.473 ± 8.150*	0.713 ± 0.295

[a] Asterisks indicate significant difference from control diet (Student's t-test, $P < 0.01$).

emission spectra were analyzed according to the Manwaring and Csallany method. Briefly, 1 g of tissue was weighed and homogenized in 20 mL of 2:1 chloroform–methanol (v/v) at room temperature with an Elvejhem–Potter homogenizer. The homogenate was poured into a separator funnel and washed twice with 50 mL of water. The water layers were pooled, washed four times with 25 mL of 2:1 chloroform–methanol, and then lyophilized. The dried water extracts were dissolved in 300 μL of water and applied to 1.5 × 10 cm medium particle size Sephadex G-25 column (Pharmacia Fine Chemicals, Piscataway, NJ) and eluted at 1 mL/6 min. Fifty, 2-mL fractions of the column eluate were collected.

Quantitative fluorescence measurements were made in an Amicon/Bowman Roto spectrofluorometer (American Instrument Co., Silver Spring, MD) with a 3-mm slit. Excitation and emission spectra were determined on every fraction collected from Sephadex G-25 column eluate, at 270/310, 275/350, and 320/380 nm (excitation/emission (nanometers)). The instrument was calibrated to read 100 relative fluorescence units against a quinine sulfate solution (1 μg/mL) (0.1 N H_2SO_4) at an excitation wavelength of 365 nm and an emission wavelength of 435 nm. The total fluorescence of each eluate peak was measured and calculated as relative fluorescence units (RFU) per gram of wet tissue. Statistical comparisons of the mean of the total fluorescent values of each eluate peak were analyzed by the Student's t-test (Zar, 1974) to determine any significant differences between the control group and the experimental groups.

RESULTS AND DISCUSSION

The accumulation of water-soluble fluorescent pigments differed from one tissue to another, with liver showing the highest accumulation. The pigments in all tissues exhibited the same spectral characteristics and the same elution positions as Manwaring and Csallany (1981), reported in mouse tissue.

Table II shows the relative fluorescent units (RFU) per gram of tissue when the column eluates were read at 270-nm excitation and 310-nm emission: Three peaks were detected in the fractions collected. In muscle there were no water-soluble compounds (peak 1) in normal tissue and a slight increase in fluorescence in all experimental groups. The muscle peak 1 fluorescence was greater in the vitamin E deficient diet (group 2) than in either of the cottonseed diets (groups 3 and 4). Muscle peak 3 also showed a slight increase in fluorescence for the rats in group 2. In heart and liver, no significant differences were found between groups in any of the peaks. Relative fluorescence units from the fractions read at 275/350 are shown in Table III. Control muscle peak 2 contained no detectable fluorescence, while all three experimental diets groups had statistically significant fluorescence in muscle peak 2. In the liver of animals fed cottonseed diets, peaks 2 and 3 had higher fluorescence levels than the control group; however, peak 1 was lower in group 4. In testes, only peak 3 of group 3 had fluorescence significantly higher than the control level. No differences were found in the hearts of any of the groups, which agrees with the findings of Manwaring and Csallany (1981). Table IV shows relative fluorescence units per gram of tissue of water-soluble autofluorescent compounds when the eluate fractions were read at 320/380. Only one peak was detected. Liver tissue from all three experimental diet groups contained a significant increase in fluorescent compounds compared to the control group. The concentration of water-soluble fluorescent compounds was decreased in muscle of rats fed the cottonseed diet (group 3). Neither heart nor testes from any of the experimental diet groups differed from the control group.

In conclusion, these findings suggest that both the presence of gossypol and the absence of vitamin E stimulate the accumulation of water-soluble autofluorescent compounds in muscle and in liver. In testes, the addition of vitamin E to the cottonseed diet seemed to reduce the level of autofluorescent compounds from the control levels, although neither gossypol nor the absence of vitamin E alone had a stimulating effect overall. The one exception was the stimulation of the gossypol diet in peak 3 at 275/350. Heart was the only tissue unaffected by any of the experimental diets.

Since the addition of vitamin E to cottonseed diet did not reverse the effects of the cottonseed diet, it is likely that the sites of interference in the lipoperoxidation mechanisms are different for vitamin E and gossypol.

Registry No. Gossypol, 303-45-7; vitamin E, 1406-18-4.

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