10–12%; crisping by evaporative cooling to a temperature of about 130°F.; and a moisture content of about 8–10%. Conditions suitable for extraction are: slurring for 40 min., hexane-meats ratio of 1.2–1.4 to 1, three washes, cake thickness of 2 in., vacuum of 3–6 in. of mercury, and temperature of slurrying and washes of about 140°F.

b) *Decorticated Seed.* Conditions recommended would be the same as for undecorticated seed except that, prior to extraction, the crisped material would be rerolled in 1-pair-high flaking rolls set at a clearance of about 0.002–.003 in.

Acknowledgment

Appreciation is expressed to John A. Kneeland, Pacific Vegetable Oil Corporation, for furnishing the safflower seed used in the investigation and to Stanley Koltun for his able assistance in conducting part of the experimental work.

REFERENCES

1. Kromer, G. W., Fats and Oils Situation, Agr. Market Service, U.S.D.A., *FOS-190*, 37–40 (May 1958).
2. Purdy, R. H., Cummings, L. O., Claassen, C. E., and Kneeland, J. A., J. Am. Oil Chemists' Soc., *36*, No. 9, 26, 28, 30 (1959).
3. Anon., Chem. Eng. News, *37*, No. 16, 31–32 (Apr. 20, 1959).
4. Haines, H. W. Jr., Perry, G. C., and Gastrock, E. A., Ind. Eng. Chem., *49*, 920–929 (1957).
5. Furman, L. J., Amadori, M. E., and Guzman, F. O., J. Am. Oil Chemists' Soc., *36*, 454–457 (1959).
6. Gastrock, E. A., Spadaro, J. J., Gardner, H. K., Knoepfler, N. B., and Molaison, L. J., Oil Mill Gaz., *59*, No. 2, 40–41 (1954).
7. Graci, A. V. Jr., D'Aquin, E. L., Paredes, M. L., and Vix, H. L. E., J. Am. Oil Chemists' Soc., *33*, 645–650 (1956).
8. King, W. H., Knoepfler, N. B., Hoffpauir, C. L., and McCourtney, E. J., J. Am. Oil Chemists' Soc., *34*, 27–30 (1957).
9. Graci, A. V. Jr., Spadaro, J. J., Paredes, M. L., D'Aquin, E. L., and Vix, H. L. E., J. Am. Oil Chemists' Soc., *32*, 129–31 (1955).
10. Knoepfler, N. B., Spadaro, J. J., McCourtney, E. J., and Vix, H. L. E., J. Am. Oil Chemists' Soc., *33*, 272–276 (1956).
11. Pominski, Joseph, Knoepfler, N. B., Graci, A. V. Jr., Molaison, L. J., Kulkarni, B. S., and Vix, H. L. E., J. Am. Oil Chemists' Soc., *32*, 361–364 (1955).

[Received June 6, 1960]

Effects of Oxidized Soybean Oil on the Vitamin A Nutrition of the Rat[1,2,3]

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Three lots of cold-pressed soybean oil were treated with bubbling oxygen for 70, 80, and 180 hrs. at 70°C. and fed to rats at a level of 18% in diets which were nutritionally adequate but devoid of vitamin A. Untreated soybean oil was fed in similar control diets. Subgroups of 15 weanling rats each were given graded injections of vitamin A acetate intramuscularly each week.

Diarrhea developed in the rats fed the diets containing oxidized oil. This condition soon subsided in the groups receiving vitamin A injections but not in the vitamin A-free group. Diarrhea was not noted in the rats receiving the untreated soybean oil, without respect to the amount of vitamin A they received.

The rats on the vitamin A-free diets developed deficiency more rapidly when the diet contained oxidized rather than the untreated oil. The food efficiencies of the groups fed the oxidized oils were lower than the controls. The intestines of the groups receiving the oxidized oils were distended with fluid and were hemorrhagic. Enlarged kidneys were noted in the vitamin A-deficient control as well as in test rats.

The retroperitoneal lipids of the groups on the oxidized oil were less unsaturated, had lower refractive indices, higher peroxide values, and higher carbonyl values than comparable groups fed the control oil.

Vitamin A deficiency decreased the unsaturation of the kidney and liver lipids but increased that of the retroperitoneal lipids. Injections of increasing amounts of vitamin A produced increases in the unsaturation of the body lipids.

The kidney lipids of the groups on the oxidized oil diets were less unsaturated and contained more peroxidic compounds than the controls. Vitamin A deficiency increased the peroxidic compounds in the kidney and liver lipids, even in rats fed the control oil. The liver lipids of the groups fed oxidized oil were less unsaturated, lower in vitamin A content, and higher in peroxide compounds than the controls.

The vitamin A content of the whole blood varied in relation to the amounts injected. The content of tocopherol in the tissues were not affected significantly by the oxidized oil in the diet.

The evidence indicates that severely oxidized oil may destroy vitamin A in the tissue of the rat, thereby hastening the development of deficiency on vitamin A-free diets, reducing the storage of injected vitamin A, and increasing the vitamin A requirement.

These effects are with abused oil and should not be interpreted to mean that the mildly oxidized oils and fats, such as those in the diets of human beings in this country are toxic.

A NUMBER of feeding studies have been conducted with oxidized or heated oils and fats in recent years. Depending on the type of lipid, the extent of treatment, and the level of feeding in the diet of experimental animals, investigators have observed diverse effects, ranging from no observed reaction (1), to slight decrease in body weight (2), to death of the experimental animals (3). The effects of these treated lipids may result from destruction of dietary nutrients, interference with diet absorption, interference with nutrient metabolism, and/or direct toxic reaction with the tissues.

In an unpublished study conducted in these laboratories in 1952 soybean oil was treated at 350°F. for 8 hrs. and fed at a 15% level in a nutritionally-complete diet to rats. The animals grew poorly, developed severe diarrhea, and died within three weeks. This experiment was repeated, but this time the heated oil was freshly incorporated into the diet each day, and the diet not consumed within 24 hrs. was discarded. These rats grew fairly well, they developed a mild diarrhea which cured spontaneously within several days, and most survived the 35-day experiment. Assay of the nutrients present in the diet after it had been stored at room temperature for 14 days revealed that major proportions of the fat-soluble vitamins (vitamins A, D, K, and E), and significant amounts of the water-soluble vitamins had been destroyed. A control diet containing the untreated oil which had been stored under the same conditions showed very small losses in these vitamins. It was apparent that

[1] Read in part at the 36th Fall meeting, American Oil Chemists' Society, Chicago, October 20–22, 1958.
[2] Assisted by a grant from the Roche Anniversary, Fund, Hoffmann-LaRoche Inc.
[3] Contribution No. 414 from the Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge, Mass.

TABLE I
Characteristics of Soybean Oils Used in Feeding Experiments[4]

Experiment	Untreated (control) Soybean Oil			Oxidized Soybean Oil			
	I	II	III	I	II	IIIa[a]	IIIb[a]
Iodine No. (Wijs)	127	127	127	113	116	120	100
Peroxide No. (meq./kg.)	<1	1	1	133	128	99	286
Carbonyl-value (Henick)							
Saturated	......	0.5	......	......	16.6	......	......
Unsaturated	......	2.7	......	......	24.3	......	......
Refractive index (25°C.)	1.4735	1.4725	1.4730	1.4748	1.4740	1.4739	1.4779
Saponification value	190	......	......	187	......	......	......
Relative viscosity[b]	......	......	61	......	......	66	994

[a] Two batches were used, oxidized for (1) 80 hours and (2) 180 hours, respectively.
[b] Cannon and Fenske viscometer.

the heated oil contained chemically reactive fractions which destroyed the nutrients in the diet during storage, and the "toxicity" of the heated oil fed in the diet was due in large part to malnutrition.

Several investigators have demonstrated the presence of the oxidation products of lipids in both normal and pathological tissues by both histological (4, 5) and chemical (6,7) procedures. However better techniques must be developed for the identification and quantitation of these products of fatty acid oxidation in animal tissues before rapid progress can be made on this important problem.

This is a report of three studies of the effects of oxidized and untreated soybean oils on the rates of depletion of vitamin A in weanling rats. Diets devoid of vitamin A, but otherwise nutritionally adequate, were fed, and graded levels of vitamin A acetate were given by intramuscular injection. It was found that the oxidized oil hastened the depletion of vitamin A and interfered with its retention in the tissues.

Experimental Procedure

Five gallons of cold-pressed soybean oil[4] were mixed and separated into two equal parts. One part was sealed in a can after treatment with nitrogen and placed at 0°C. for later use as the control oil. Three-liter portions of the remaining oil were heated at 70°C. for 70 hrs. while cleaned dry oxygen was bubbled into it through a sintered glass tube until the peroxide value had risen to 110 to 135 me./kg. These batches of oxidized oil were then pooled, sealed, and placed at 0°C. until used in the feeding study.

For the second and third experiments 2-gal. batches of soybean oil were similarly treated with heat and oxygen during 80 hrs. and 180 hrs., respectively, and the untreated and treated samples were placed in 0°C. storage. A sample was taken just before each batch was placed in storage and submitted to analysis (Table I). In each of the three experiments a group of weanling albino rats (Charles River strain), was placed on a stock diet (8) for two days.

In Series I the rats were divided into eight groups of 15 rats each, with equal distribution of large and small animals. Four groups were then placed on the control diet while four were given the diet containing the oxidized oil (Table II). The rats in each subgroup received, respectively, 0, 700, 1400, and 7000 I.U. of vitamin A acetate intramuscularly every seven days. In each case the vitamin A was contained in 0.1 ml. of an emulsion of sterile water and Tween 80 (9:1). The control rats were given a placebo injection of 0.1 ml. of vitamin A-free emulsion. Diets were freshly prepared each week and refrigerated until offered to the rats *ad libitum*. The body weight of each rat and the food intake of each group were recorded on a weekly basis. Each rat was observed frequency for gross evidences of vitamin A deficiency or other abnormalities.

Similar procedures were followed in Experiments II and III. However the rats in Experiment II were given 0, 500, 1500, 3000, or 7000 I.U. weekly, and those in Experiment III were given 0, 300, 1500, or 6000 I.U. of vitamin A by intramuscular injection. The consistency of the test diet fed to the rats in Experiment III was improved by replacing 6.5% of the starch in the diet in Experiment II by an equal weight of celluflour.

When the vitamin A syndrome (body weight loss, xerophthalmia, etc.) was clearly evident in the groups receiving no vitamin A, all rats in each series were decapitated. Liver, kidneys, retroperitoneal tissue, and blood were sampled from each rat at autopsy, placed immediately in carbon dioxide ice, and stored at −40°C. until analyzed.

The retroperitoneal tissue was macerated with 5 parts of sodium sulfate, and the lipid was extracted with low-boiling petroleum ether. This extract was filtered through sodium sulfate, and the solvent was removed under vacuum. These lipids were analyzed immediately. The other tissues were macerated with an ethanol:ether mixture (3:1) and the mixture was extracted with low-boiling petroleum ether. Peroxidic oxygen was determined by the Lea method (9), modified by placing the lipid and iodide in separate beakers within the reaction flasks, and sweeping with N_2 for 5 min. before sealing and mixing. The iodine numbers were determined with Wijs' solution (10), using 0.1 to 0.2-g. samples, and a 25-min. reaction time. The relative amounts of carbonyl compounds were esti-

TABLE II
Composition of Experimental Diets[5]

	Test Diets		Control Diets	
	Expt. I, II	Expt. III	Expt. I, II	Expt. III
Casein (Labco)	30.0	30.0	30.0	30.0
Cystine	0.3	0.3	0.3	0.3
Cornstarch	45.2	38.7	45.2	38.7
Soybean oil (cold-pressed)	2.0	2.0	20.0	20.0
Oxidized soybean oil	18.0	18.0	0.0	0.0
Salt mixture (Hubbell)[a]	4.0	4.0	4.0	4.0
Vitamin mix[b]	0.5	0.5	0.5	0.5
Celluflour	0.0	6.5	0.0	6.5
Total	100.0	100.0	100.0	100.0

[a] Salt mixture: Hubbell *et al.*, J. Nutrition, *14*, 273 (1937).
[b] Vitamin mix (mgm./100 g. diet): thiamine 0.4; riboflavin 0.5; pyridoxine 0.4; niacin 2.0; calcium pantothenate 2.0; p-amino benzoic acid 10.0; inositol 200.0; biotin 0.003; choline chloride 300.0; menadione 0.3; folic acid 0.25; *alpha*-tocopherol 10.0; ascorbic acid 10.0; and vitamin D 125 USP units/100 g. diet.

[5] The authors wish to thank Hoffmann-LaRoche Inc., Nutley, N. J., for supplying the vitamins used in this study.

[4] Kindly donated by Miami Valley Laboratories, Procter and Gamble Company, Cincinnati, O.

mated by Henick's procedure (11), making suitable corrections for the saturated and unsaturated compounds present. Tocopherol was estimated by the dipyridyl (12) method; the phosphomolybdic acid (13) method was tried, but the results were too high. Vitamin A was determined by the antimony trichloride method according to Moore (14). Analyses of variance were made by the method of two variables of classification-repeated measurements as outlined by Dixon and Massey (15).

Observations and Discussion

Feeding Studies. All those groups which had received extensively oxidized oils in the diet developed diarrhea. This condition subsided within a week in those animals which were receiving vitamin A injections but not in the vitamin-A free controls. Diarrhea was noted in none of the rats which were ingesting the control oils, whether or not they received vitamin A. Thus the diarrhea was induced by the oxidized oils and was alleviated by the vitamin A injections.

The average body weight gains of the groups fed the oxidized oils were lower than of the groups fed the control oils (Table III). This effect was significant at the 95% level in Experiment I but not in Experiments II and III. The groups fed the oxidized oils and receiving no vitamin A developed the vitamin syndrome sooner than comparable rats fed the control oils. Since the development of the vitamin A

TABLE III
Data on Growth and Food Efficiences

Experiment	I.U. vitamin A injected—rat weekly	Oil fed [a]	Average body weight (g.)[b]				Average gain in body weight		Over-all "Food Efficiency" [c]
			Initial	(S.D.)	Final	(S.D.)		(S.D.)	
I 54 days	0	c	56	5.2	261	25.2	198	38.8	....
		o	56	8.5	213	41.5	155	39.0	....
	700	c	56	8.2	332	30.5	275	32.5	....
		o	56	8.5	286	25.2	230	24.8	....
	1400	c	56	9.8	334	22.8	287	26.2	....
		o	56	7.8	273	18.4	217	21.4	....
	7000	c	56	6.4	351	24.5	295	26.9	....
		o	56	7.7	280	30.3	224	33.3	....
II 74 days	0	c	49	9.1	282	38.8	238	70.0	41
		o	49	9.1	262	73.0 [e]	212 [e]	55.1	40
	500	c	49	6.9	393	48.5	339	45.0	45
		o	49	6.2	381	31.6	329	44.9	41
	1500	c	49	8.6	374	39.6	327	45.9	42
		o	49	8.1	372	38.8	322	34.4	40
	3000	c	49	9.0	403	46.5	360	50.3	45
		o	49	7.2	371	23.2	322	23.0	41
	7000	c	49	8.7	384	28.5	335	34.0	44
		o	49	6.4	384	33.8	336	34.5	42
III[d,f] 65 days	0	c	57	3.2	281	50.2	224	58.1	31
		o	56	4.8	258	53.0	201	59.1	27
		p	57	4.2	.... [f]				
	300	c	56	5.0	367	39.7	311	40.1	38
		o	56	6.1	351	30.1	301	35.1	35
		p	57	5.1	.... [f]				
	1500	c	58	2.2	390	33.5	331	29.9	42
		o	56	3.1	378	25.2	318	29.3	40
		p	57	6.2	.... [f]				
	6000	c	56	2.9	384	37.5	327	41.4	40
		o	56	6.2	382	30.0	324	26.4	38
		p	57	4.2	.... [f]	....	....	....	....

[a] Oil fed: c = control, untreated soybean oil; o = oxidized soybean oil; p = soybean oil oxidized for 180 hrs.
[b] (S.D.), Standard Deviation.
[c] "Food Efficiency": (g. gain in body weight/g. food ingested) × 100. No data for Experiment 1.
[d] 6.5% Celluflour included in diet at the expense of carbohydrate.
[e] One animal died; four moribund animals sacrificed at 66 days.
[f] All animals died; half of the animals in Experiment Series III receiving diet of soybean oil oxidized for 180 hrs. died within 15 days.

TABLE IV
Retroperitoneal Lipid Data

Experiment	I.U. vitamin A injected/rat weekly	Oil fed [a]	Iodine number	Refractive index @ 25°C.	Peroxides me./kg.	Saponification number	Tocopherol mcgm./g. fat		Total relative carbonyls
								(S.D.)[b]	
I Pooled tissues	0	c	92	1.4678	2.7	182	....	....	....
		o	86	1.4668	11.5	184	....	....	....
	700	c	103	1.4685	3.0	181	....	....	....
		o	89	1.4672	4.7	181	....	....	....
	1400	c	107	....	2.8	....	....	....	....
		o	91	1.4691	10.7	183	....	....	....
	7000	c	110	1.4689	2.4	186	....	....	....
		o	92	1.4686	5.1	190	....	....	....
II Pooled tissues	0	c	100	1.4694	0.3	....	14	....	2.3
		o	96	1.4691	9.6	....	12	....	4.4
	500	c	103	1.4699	0.5	....	22	....	2.1
		o	97	1.4693	4.0	....	12	....	7.0
	1500	c	103	1.4692	0.3	....	14	....	1.6
		o	98	1.4695	5.6	....	17	....	9.0
	3000	c	105	1.4700	0.3	....	17	....	1.0
		o	100	1.4698	5.8	....	23	....	9.3
	7000	c	106	1.4701	0.6	....	13	....	1.0
		o	100	1.4699	4.8	....	15	....	9.2
III Individual tissues	0	c	101	1.4685	1.8	....	52	17	....
		o	96	1.4668	2.3	....	29	3	....
	300	c	106	1.4673	0.6	....	71	7	....
		o	101	1.4666	0.8	....	25	4	....
	1500	c	106	1.4673	0.7	....	26	8	....
		o	101	1.4661	1.4	....	15	6	....
	6000	c	114	1.4676	0.7	....	33	7	....
		o	106	1.4686	0.6	....	22	5	....

[a] Oil fed: c = control, untreated soybean oil; o = oxidized soybean oil.
[b] (S.D.), Standard Deviation.

symptoms was dependent upon the rate of depletion of tissue stores of this vitamin, it may be deduced that the oxidized oils hastened the depletion of tissue stores of vitamin A.

The oxidized (180 hr.) oil fed in Experiment III was so toxic that half of the rats died during the first two weeks. Death was not prevented by vitamin A injections.

The food efficiencies (g. of body weight gain per g. of diet intake) of the groups fed the oxidized oils were generally slightly lower than those of comparable groups fed the control oils. This was likely caused in part by a lower digestibility and lower caloric value of the oxidized fat.

Autopsy Findings. The only abnormalities noted at autopsy were distension with fluids and minor hemorrhages in the intestines in the groups fed the oxidized oils. The organ weight/body weight ratios indicated that the kidneys of the rats with vitamin A deficiency were enlarged, whether or not the oil in the diet was oxidized.

Tissue Analysis. The retroperitoneal lipid samples from the groups fed the oxidized oils had consistently lower iodine numbers and refractive indices and had higher peroxide values and carbonyl values than the lipids from comparable groups fed untreated soybean oils (Table IV).

The iodine numbers of the retroperitoneal lipids were directly proportional to the amount of vitamin A received. Vitamin A deficiency appears to decrease the unsaturation of the petroleum ether-soluble retroperitoneal fats and to increase the susceptibility of these fats to oxidation. This may be related to increased tissue storage of peroxidic compounds derived from food sources. This relationship deserves further study.

The renal lipids (Table V) from the groups fed oxidized oil were lower in iodine value and higher in peroxide content than comparable controls. Vitamin A deficiency increased the peroxidic and carbonyl compounds in kidney lipids, especially in the groups fed oxidized oils.

The liver lipids (Table VI) from the groups fed oxidized oil were lower in iodine value and vitamin A content and slightly higher in peroxide content than liver lipids from comparable groups fed the control oils. Vitamin A deficiency increased the peroxide value of liver lipids. On a liver-weight basis the vitamin A content of the livers varied directly with the amount of vitamin A injected. The unsaturation of the liver lipids increased during vitamin A deficiency, in confirmation of Leutskii and Lyubovitch (16) and others. A contrary trend was noted in the retroperitoneal lipids, indicating that various tissue fats are not similarly affected by vitamin A deficiency. Agnew and Meyer (17), Lowe and Morton (18), and others have reported that vitamin A is not directly involved in the metabolism of tissue fatty acids. It has been suggested that the increase in the unsaturation of liver lipids is caused by a general deficiency of nutrients rather than by vitamin A deficiency alone.

The vitamin A content of the whole blood varied in relation to the amount of vitamin A injected. The blood-clotting times and the pyruvic acid content were not increased significantly in vitamin A deficiency.

TABLE V
Pooled Renal Lipid Data

Experiment	I.U. vitamin A injected /rat weekly	Oil [a] fed	Iodine number	Peroxides me./kg.	Total relative carbonyls	Tocopherol mcgm./g. lipid	Vitamin A I.U./g. tissue
I	0	c	97	14	....	....	2
		o	87	26	....	....	2
	700	c	110	11	....	....	21
		o	96	18	....	....	20
	1400	c	106	9	....	....	27
		o	99	18	....	....	27
	7000	c	111	10	....	....	30
		o	103	17	....	....	31
II	0	c	92	....	1.2	23	....
		o	87	....	2.1	20	....
	500	c	85	....	1.2	26	....
		o	85	....	1.9	16	....
	1500	c	89	....	1.3	18	....
		o	85	....	1.5	15	....
	3000	c	93	....	1.1	21	....
		o	88	....	1.9	13	....
	7000	c	97	....	1.0	21	....
		o	92	....	1.5	20	....

[a] Oil fed: c = control untreated soybean oil: o = oxidized soybean oil.

TABLE VI
Liver Lipid Data

Experiment	I.U. vitamin A injected/rat weekly	Oil fed [a]	Iodine number	Peroxides me./kg.	I.U. vitamin A [c] /g. tissue		Extracted liquid % [d]	
						(S.D.) [b]		(S.D.)
I	0	c	124	16	....	....	....	....
		o	86	25	....	....	....	....
	700	c	107	11	8	....	....	....
		o	89	14	3	....	....	....
	1400	c	110	12	335	....	....	....
		o	91	17	300	....	....	....
	7000	c	114	13	2200	....	....	....
		o	92	13	2050	....	....	....
II	0	c	116	....	....	....	....	....
		o	115	....	....	....	....	....
	500	c	108	....	51	....	....	....
		o	107	....	7	....	....	....
	1500	c	108	....	360	....	....	....
		o	108	....	40	....	....	....
	3000	c	107	....	1135	....	....	....
		o	108	....	725	....	....	....
	7000	c	107	....	2500	....	....	....
		o	110	....	2050	....	....	....
III	0	c	....	....	....	....	3.1	0.4
		o	....	....	....	....	3.0	0.2
	300	c	....	....	4	1	4.6	1.0
		o	....	....	4	1	3.2	0.2
	1500	c	....	....	446	39	4.8	0.9
		o	....	....	391	20	4.2	1.0
	6000	c	....	....	2580	413	4.1	0.9
		o	....	....	1732	225	3.3	0.4

[a] Oil fed: c = control, untreated soybean oil: o = oxidized soybean oil.
[b] (S.D.), Standard Deviation.
[c] Weanling rats (0 day of experiment) 136 (S.D. 36) I.U. vitamin A g. tissue.
[d] Weanling rats (0 day of experiment) 2.4 (S.D. 0.7)% lipid extracted from liver.

Tissue tocopherol content was not affected adversely by the oxidized oil. This may be due in part to the high tocopherol content (10 mg. %) of the diet or due to interference from ubiquinone in the assay for tocopherol. Bacq and Alexander (19) reported no change in tissue tocopherol in rats receiving whole body radiation and noted an increase in tocopherol as the fat reserves were mobilized. Though Alfin-Slater (2) found that tocopherol was helpful in relieving the "toxic" symptoms in rats fed fried fats, Machlin *et al.* (20) reported that tocopherol did not reduce the mortality or pathology of highly-oxidized fats fed to chickens.

It should be pointed out that the oils used in this research were much more severely oxidized than the fats and oils used in human dietaries in the United States. Melnick (21) and Keane *et al.* (1) have shown that commercial food fats that have been used in commercial frying are not toxic.

REFERENCES

1. Keane, K.W., Jacobson, G.A., and Krieger, C.H., J. Nutrition, 68, 57 (1959).
2. Alfin-Slater, Roslyn B., Auerbach, S., and Aftergood, L., Paper 29, 32nd Fall Meeting, Am. Oil Chemists' Soc., Chicago, October 20–22, 1958.
3. Matuso, N., "Toxicity of Fish Oils," I–VIII, Seikagaku, 29, 792 (1958).
4. Aaes-Jorgensen, E., Abst. and Commun., 1st Int. Congress Biochem., London, 399/1 (1949).
5. Prange, O., Abst. and Commun., 1st Int. Congress Biochem., London, 399/1 (1949).
6. Glavind, J., and Hartmann, S., Experientia, 7, 464 (1952).
7. Kibrick, A.C., Safier, L.B., and Skupp, S.J., Proceed. Soc. Expt. Biol. and Med., 101, 137 (1959).
8. Sherman, H.C., and Campbell, H.L., J. Biol. Chem., 60, 5 (1924).
9. Lea, C.H., J. Sci. Food and Agric., 3, 586 (1952).
10. Methods of Analysis, A.O.A.C., 8th ed., Sect. 26.18, p. 464. A.O.A.C., Washington, D.C., 1955.
11. Henick, A.S., J. Am. Oil Chemists' Soc., 31, 88 (1954).
12. Quaife, M.L., and Harris, P.L., Anal. Chem., 20, 1221 (1948).
13. Rosenkrantz, H., J. Biol. Chem., 224, 165 (1957).
14. Moore, T., "Vitamin A," Appendix P., London, Elsevier Publishing Company (1957).
15. Dixon, W.J., and Massey, F.J., "Introduction to Statistical Analysis," 2nd ed., ch. 10, New York, McGraw-Hill Book Co. (1957).
16. Leutskii, K.M., and Lyubovitch, E., Doklady Akad. Nauk. SSSR, 96, 341 (1954).
17. Agnew, L., and Meyer, J., Nature, 177, 1255 (1956).
18. Lowe, E.R., and Morton, R.A., "Vitamins and Hormones," Vol. XIV, p. 129 (ed., Harris, R.S., Marrian, G.F., and Thimann, K. V.) New York, Academic Press (1956).
19. Bacq, Z., and Alexander, P., "Fundamentals of Radiobiology," p. 236, London, Butterworths Scientific Publishers (1955).
20. Machlin, L.J., Gordon, R.S., Meisky, K.A., and Maddy, K.H., J. Poultry Science, 38, 579 (1959).
21. Melnick, Daniel, J. Am. Oil Chemists' Soc., 34, 578 (1957); 35, 271 (1958).

[Received June 27, 1960]

Spontaneous Conversion of Gossypol to Anhydrogossypol

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An alkali-fast pigment is produced at room temperature when gossypol is dissolved in ethyl acetate. This pigment has been isolated from ethyl acetate as an orange crystalline material and identified as anhydrogossypol. The identity was established by identity of the infrared spectra with that of authentic anhydrogossypol; by elementary composition; by mixture-melting point behavior with authentic anhydrogossypol; and by the identity of the aniline derivatives produced from the orange crystalline product from ethyl acetate and authentic anhydrogossypol.

GOSSYPOL (2,2′-bi-1,6,7,-trihydroxy-3-methyl-5-isopropyl-8-aldehydronaphthyl) occurs in specialized structures in the seeds of species of the genus *Gossypium* (3). It is present in the seeds of upland cotton, *G. hirsutum,* to the extent of 0.4–1.7% (6). This yellow pigment is extracted along with the oil in the commercial processing of cottonseed and is found in solution in the oil.

There is strong evidence that gossypol so extracted is responsible for the undesirable fixed-red coloration that occurs in about 25% of the domestically-produced cottonseed oil (1). For example, it was shown by Berardi and Frampton that a large part of the gossypol added to either crude or refined and bleached cottonseed oil disappears in a relatively short period of time, and in the case of refined and bleached oil to which gossypol is added there is a concomitant increase in fixed-red coloration with an increasing disappearance of gossypol. Apparently the initial reaction in the fixation of gossypol in refined and bleached cottonseed oil is of the second order with respect to gossypol (5). However none of the gossypol reaction products have been isolated from cottonseed oil.

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Because of the experimental difficulties encountered in studying the series of reactions which gossypol undergoes in solution in glyceride oils, some experimentation has been carried out with simpler systems; one of the observations is that gossypol cannot be recovered quantitatively from ethyl acetate solution if the solution is permitted to age. Several of the colored reaction products that occur in aged ethyl acetate solutions of gossypol can be resolved chromatographically on a cellulose column. One of these products is anhydrogossypol.

Experimental

An ethyl acetate solution of gossypol (0.5 g./100 ml.) was permitted to age in the dark at room temperature (25–27°) for a month. The unreacted gossypol was removed from this solution by scrubbing it with 0.5 N aqueous NaOH which contained a small quantity of dithionite. The ethyl acetate solution was then washed with water, dried over anhydrous Na_2SO_4, and reduced to a volume of about 5 ml. by evaporation on a water bath. A small quantity of powdered cellulose was added to this concentrate, and after the residual ethyl acetate had evaporated, the dried powder was placed on the top of a chromatographic column composed of powdered cellulose. The yellow component eluted when the chromatogram was developed with petroleum ether was rechromatographed on a second cellulose column. Orange crystals, m.p. 224–225°, separated when the eluent from this second column was concentrated on a water bath, yield, 10%. They were recrystallized from toluene, m.p. 225–226°. *Anal.*: C, 74.47; H, 5.50; mol. wt. (ebullioscopic method in ethanol), 443. Calcd. for