Ferric chloride hexahydrate initiated polymerization at 25°, but polymerization was incomplete, and the polymers had relatively low molecular weights.

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REFERENCES

1. Eley, D. D., and Pepper, D. C., Trans. Faraday Soc., *43*, 112 (1947).
2. Eley, D. D., and Richards, A. W., Trans. Faraday Soc., *45*, 425 (1949).
3. Gast, L. E., Schneider, Wilma J., O'Donnell, J. L., Cowan, J. C., and Teeter, H. M., J. Am. Oil Chemists' Soc., *35*, 347 (1958).
4. Gast, L. E., Schneider, Wilma J., and Teeter, H. M., J. Am. Oil Chemists' Soc., *34*, 307 (1957).
5. Pepper, D. C., Sci. Proc. Roy. Dublin Soc., *25*, No. 19, 239 (1951).
6. Schneider, Wilma J., Gast, L. E., Melvin, E. H., Glass, C. A., and Teeter, H. M., J. Am. Oil Chemists' Soc., *34*, 244 (1957).
7. Shostakavskii, M. F., Mikhantie, B. I., and Ovchinnikova, N. N., Inst. Org. Chem., Acad. Sci. USSR, Moscow, 1056 (1953), C. A. *49*, 2299 (1955).
8. Teeter, H. M., Dufek, E. J., Coleman, C. B., Glass, C. A., Melvin, E. H., and Cowan, J. C., J. Am. Oil Chemists' Soc., *33*, 399 (1956).
9. Teeter, H. M., Gast, L. E., and Cowan, J. C., Ind. Eng. Chem., *50*, 1703–04 (1958).
10. Watanabe, W. H., and Conlon, L. E., U. S. Patent 2,760,990 (August 28, 1956).

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A Review of Some Physiological Effects of Gossypol and Cottonseed Pigment Glands[1]

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THE EARLIEST recorded statement on the harmful effect of cottonseed is attributed to Voelker in England in 1859 (1). Since that time many materials have been blamed for the adverse physiological effects noted after feeding cottonseed. In 1886 Longmore (2) isolated a crude pigment from cottonseed oil "foots," and Marchlewski (3) in 1899 extracted, purified, and gave the name "gossypol" to a yellow pigment which he had obtained from cottonseed "foots." These latter two investigators were interested in the pigmented material as a dye and made no mention of physiological activity. The preliminary note by Withers and Carruth (4) in 1915 entitled, "Gossypol, a Toxic Substance in Cottonseed," was their first report of separation from cottonseed kernels of a substance which appeared to be identical with the material separated from crude cottonseed oil and named by Marchlewski in 1899. Withers and Carruth found their material to be toxic to rabbits and published three additional papers (5–7), all bearing titles similar to the first one. These publications and subsequent work primarily by Schwartze (8–10), who made a positive correlation between the toxicity of raw cottonseed and gossypol content, led to the general belief that the toxicity of cottonseed can be attributed solely to its gossypol content.

With the availability of cottonseed pigment glands, separated from cottonseed kernels by a flotation process (11, 12), and of pure gossypol (13, 14) it became possible to evaluate their toxicity by determining the oral median lethal dose, *i.e.*, the oral LD_{50} value. It was found that three different samples of untreated cottonseed pigment glands containing 40.0, 37.6, and 33.7% gossypol, respectively, were more toxic to the rat than pure gossypol itself. These early findings were reported for us by Boatner in 1947 (15). From that time to the present, studies have been made on a large series of samples of gossypol and of untreated, fractionated, treated, stored, and detoxified cottonseed pigment glands (16–22).

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In Table I are shown a series of 11 different samples of untreated cottonseed pigment glands, varying in acute oral toxicity in the rat (LD_{50} value) from 925 to 2170 mg./kg. body weight. It should be recognized that, while cottonseed pigment glands are toxic, they show a rather wide range of toxicity not referable to their analyzed gossypol content.

TABLE I

Toxicity and Gossypol Content of Untreated Cottonseed Pigment Glands [a]

Pigment glands	Acute oral LD_{50} in the rat	Gossypol content [b]
	mg./kg.	%
1	925	(40.0)
2	1060	(36.9[4])
3	1140	37.8[2]
4	1345	34.3[2]
5	1350	(33.5[3])
6	1430	32.5
7	1635	30.3[3]
8	1775	(33.0)
9	1845	34.1[3]
10	2000	33.2
11	2170	28.6[2]

[a] Eagle *et al.* (1948, 1950, 1952).
[b] The figures in parentheses denote analytical results by the antimony chloride method of Boatner *et al.* (1947, 1948); all other analyses by method of Pons and Guthrie (1949); the exponent denotes the number of analyses averaged.

Table II shows a series of 10 different samples of gossypol, the LD_{50} values of which in the rat were determined when administered in water or in oil or in each. It may be seen that gossypol is less toxic, whether administered in oil or in water, than even the least toxic of the 11 samples of untreated cottonseed pigment glands tested.

In 1947 several papers (23, 24) were presented in which it was stated that gossypol was an appetite depressant, that intestinal irritation and other toxic manifestations previously ascribed to gossypol are not found with a pure preparation of gossypol in reasonable doses, and that purified gossypol has no generally toxic properties. The widespread publicity relative to the possible use of gossypol in the treatment of obesity in man necessitated that we determine the effect of small daily doses on the body weight and food consumption of the dog.

Four litter-mate dogs were given 19 doses of 0, 50, 100, and 200 mg. of gossypol per kg. of body weight

TABLE II

Toxicological Evaluations of "Pure" Gossypol [a]

Gossypol sample	Acute oral LD_{50} administered in water	Value in rat administered in oil
	mg./kg.	*mg./kg.*
1	2400	2315
2	2480	2250
3	2600	.....
4	2800	.....
5	2800	2315
6	3340	.....
7	> 600	.....
8	>1600	.....
9	.....	2400
10	.....	2450
Σ	2630	2325

[a] Eagle *et al.* (1950 and unpublished observations).

over a period of 37 days. Quite suddenly, on the fourth and fifth days after the last dose, three of the experimental dogs were found dead. This alarming result prompted us to publish an immediate warning note (25) with the suggestion that the use of gossypol in human subjects be withheld until more data on its pharmacology and toxicology are available.

The surviving control dog and the three new ones were given smaller dose levels of gossypol (5, 10, 15, and 30 mg./kg.) by stomach tube. Except for the dog receiving the smallest dose level, all the dogs in the second test died. All of the six dogs that died in the two experiments had manifested lassitude, diarrhea, anorexia, and weight loss. Vomiting occurred only at the three highest dose levels. At autopsy there were such findings as hemorrhagic intestines, hydrothorax, edema of the lungs, excessive fluid in the peritoneal cavity, hydropericardium, congestion of the splanchnic organs, etc. (18).

In an effort to find components of cottonseed pigment glands which were more toxic than the original glands, some highly toxic pigment glands (LD_{50} 925 mg./kg.) were mixed with acetone or water and subjected to various fractionation procedures (19). It was found that the acetone-soluble, water-soluble fraction had an LD_{50} value of 700 mg./kg., making it the most toxic material ever extracted from cottonseed despite the fact that its gossypol content was only 58%. A fraction which was soluble in acetone but insoluble in water and light petroleum naphtha was half as toxic (LD_{50} 1815) as the original glands even though the gossypol content had increased from 40% in the original glands to 90% in this less toxic fraction.

Greatest detoxification of cottonseed pigment glands occurred when they were exhaustively extracted with acetone, and no LD_{50} value could be obtained in either of two different samples so treated (LD_{50}>6,000 mg./kg.).

Heating of the cottonseed pigment glands for 1 hr. at 103 or 105°C. had little effect on their toxicity, but heating in the presence of water for 1 hr. at 102 or 105°C. caused very marked decreases in toxicity. The residual toxicity of treated cottonseed pigment glands bore no apparent relation to their analyzed gossypol content (19).

Long-term storage of cottonseed pigment glands at 2 to 10°C. for even as long as 9½ years had little effect on their acute oral toxicity or their analyzed gossypol content (22).

In 1952 a series of water-soluble gossypol combination products were studied for their acute oral toxicity and effect on body weight of the rat (21). As may be seen in Table III, all five samples of the gossypol combination products were very much less toxic to rats than cottonseed pigment glands, and four of the five were even less toxic than gossypol. The toxicity of the individual samples of gossypol-combination products was not proportional to their gossypol content.

A comparison of the effect of the vehicle (water or soybean oil) on the acute oral toxicity of cottonseed pigment glands or gossypol is shown in Table IV.

TABLE III

Toxicological Evaluation of Some Gossypol Combination Products [a]

Gossypol-combination product	Acute oral LD_{50} in the rat	Gossypol content [b]
	mg./kg.	%
Gossypol-glycine (9:1)	2355	68.6[4]
Gossypol-glycine (1:1)	>6000	35.2[12]
Gossypol-glycine (1:9)	>6000	7.2[4]
Gossypol-peanut protein	3220	31.1[2]
Gossypol-dextrose	3725	45.3[12]

[a] Eagle and Bialek (1952).
[b] Analyses by method of Pons and Guthrie (1949).

Cottonseed pigment glands appear to be only slightly less toxic when administered in oil, compared to administration in water. In the case of gossypol the reverse occurs for gossypol is slightly more toxic when administered in oil than in water.

Thirty to 40 years ago the effects of feeding gossypol to experimental animals involved the use of gossypol-acetate prepared from cottonseed kernels by the method of Carruth (7). Some of these gossypol-feeding studies have already been mentioned (8–10). The modern era of biological studies on the toxic factor(s) in cottonseed was made possible in the late 1940's with the availability of cottonseed pigment glands (11, 12) and pure gossypol (13, 14).

In 1947 Groschke, Rubin, and Bird (26) published a research note in Poultry Science, in which they reported on the growth of a group of chicks fed a ration containing 0.79% cottonseed pigment glands compared with rations containing no added pigment glands. A definite weight suppression was caused by the cottonseed pigment glands. These pigment glands were reported by Eagle *et al.* (16) as having an LD_{50} value of 925 mg./kg., the most toxic sample of all the intact, untreated pigment glands studied by the latter even to the present time.

In 1948 Boatner *et al.* (27) reported some tests, in one of which a level of 0.13% gossypol was added to a ration containing screw-pressed soybean meal, lead-

TABLE IV

Effect of Vehicle on Toxicity of Cottonseed Pigment Glands and "Pure" Gossypol [a]

Sample No.	Description	Gossypol content [b]	Acute oral LD_{50} value in rat	
			in water	in oil
		%	*mg./kg.*	*mg./kg.*
1	Untreated CPG—Stored 9 yrs. 7 mos.	36.8[5]	1100	1370
2	Dry heated CPG—Stored 8 yrs. 3 mos.	35.1[6]	1310	1390
3	Untreated CPG—Stored 9 yrs.	29.7[5]	1480	1790
4	Untreated CPG—New	30.3[3]	1635	1820
5	Untreated CPG—Stored 4 yrs. 9 mos.	27.0[1]	1965	1940
6	Wet heated CPG—Stored 9 yrs. 7 mos.	38.4[4]	2470	2400
7	"Pure" gossypol	*ca.* 100.0	2800	2315
8	"Pure" gossypol	*ca.* 100.0	2480	2250
9	"Pure" gossypol	*ca.* 100.0	2400	2315

[a] Eagle and Davies (1958 and unpublished observations).
[b] Gossypol analyses by method of Pons and Guthrie (1949).

ing to relatively little retardation of chick growth. In another test a level of 0.65% cottonseed pigment glands caused greater weight retardation, and a poor correlation was found between the nutritional values of the various cottonseed products and their contents of gossypol and gossypurpurin. The cottonseed pigment glands used in their study were found by Eagle *et al.* (16) to have an LD_{50} value of 1,060 mg./kg.

In 1950 Lillie and Bird (28) reported on the effect of oral administration of pure gossypol and of cottonseed pigment glands on mortality and growth of chicks given 5, 10, 20, and 40 mg. of gossypol/100 g. chick/day. The gossypol was supplied by capsule either as pure gossypol or as gossypol supplied in cottonseed pigment glands. They obtained growth depression which was directly proportional to gossypol intake regardless of whether the gossypol was supplied as such or in the form of cottonseed pigment glands. These results were in conflict with those of Boatner *et al.* (27), and Lillie and Bird suggested that pure gossypol might be detoxified when incorporated in mixed feed. Eagle *et al.* (19) studied the toxicity of the pigment glands used by Lillie and Bird and found that these atypical pigment glands, which had been prepared from seed that had been defatted with hexane prior to the removal of the cottonseed pigment glands, had an LD_{50} value of 1,775 mg./kg. when new in October 1947 and an LD_{50} value of 2,290 mg./kg. after storage at 7°C. till March 1949, when they were returned to the Southern Regional Research Laboratory for re-analysis. The conflicting results obtained by Lillie and Bird can be explained on the basis of their use of an unusual sample of cottonseed pigment glands which was much less toxic than the samples used by the other investigators cited previously (26, 27).

In 1950 Eagle and Bialek studied the effect of 16 different intubated doses of gossypol (varying between 50 and 2,400 mg./kg.) on the body weight of rats and found that body-weight losses were proportional to the amount of "pure" gossypol administered (20).

Ambrose and Robbins (29) noted in 1951 that, when they fed two different samples of cottonseed pigment glands to rats at a level of 0.256% in the diet, one sample caused no inhibition of growth and the other sample caused definite inhibition of growth. Although they reported no LD_{50} values, they doubted that the difference noted could be ascribed to the gossypol content and attributed it to the difference in toxicity of the cottonseed pigment glands used. Eagle *et al.* (19) had already reported LD_{50} values of 1,140 and 1,490 mg./kg. for the samples of cottonseed pigment glands used by Ambrose and Robbins.

In 1952 Eagle and Bialek (21) reported four experiments in which they studied the effects of feeding various levels of "pure" gossypol in the diets of rats and concluded from the 10 different levels of gossypol tested that the body-weight depression caused by gossypol itself is proportional to the amount of this material added to the diet. It was noted however that the greater mortality and body-weight depressions caused by adding various levels of cottonseed pigment glands to the diet cannot be attributed solely to their gossypol content.

In 1955 Couch, Chang, and Lyman (30) studied the effect of gossypol supplied by cottonseed pigment glands incorporated in the rations of chicks. They concluded that when the free gossypol content of the total ration was 0.06% or less (supplied by cottonseed pigment glands), there was no detrimental effect on growth rate. These authors, who prepared their own cottonseed pigment glands and reported no LD_{50} data on them, apparently considered all physiological activity caused by cottonseed pigment glands as being caused by gossypol alone.

In the same year Heywang and Bird (31) described body-weight effects in chicks fed rations containing different levels of free gossypol supplied by "pure" gossypol. They concluded that the free gossypol content of the ration should not be greater than 0.016% when fed to White Leghorns or greater than 0.02% when fed to New Hampshire chicks.

In 1957 Eagle and Davies (32) reported a study in which a constant level of gossypol (0.1%) was supplied to various rat diets by using six different samples of cottonseed pigment glands and three different samples of "pure" gossypol. These pigment gland samples had been evaluated toxicologically, and their gossypol content had been tested independently in at least two and, in some cases, three different laboratories. Despite a constant contribution of 0.1% gossypol from a single source to each diet, the different pigment gland samples varied in their body weight-depressing effect on rats. But all six of these samples caused greater depressions in body weight than did any of the three samples of gossypol studied. Furthermore, despite the same free gossypol level in every experimental diet, the efficiencies of food utilization were less for all six groups fed the two different pigment glands than they were for the three groups fed different samples of "pure" gossypol.

An early report of detoxification of cottonseed was that of Withers and Ray in 1912 (33). They extracted cottonseed with gasoline, mixed the residue with aqueous sodium hydroxide plus alcohol, and boiled all of it on a water bath for two hours. The mass was filtered and dried and fed to six rabbits. After 39 days of feeding they reported that the rabbits were in good condition but had lost an average of 134 g. in weight.

A year later Withers and Brewster (34) fed ferric ammonium, citrate-treated cottonseed meal to rabbits and reported that iron was an antidote to cottonseed meal toxicity. They believed that gossypol and iron formed an insoluble complex, preventing the gossypol from being absorbed from the gastro-intestinal tract.

In 1949 Eagle administered cottonseed pigment glands by stomach tube to rats and determined the LD_{50} value when given in water and when given in 2% ferrous sulfate solution. It was found that cottonseed pigment glands, which were fairly toxic to the rat when administered in water, were markedly detoxified when administered in 2% ferrous sulfate solution. In the latter vehicle even doses as high as 3 to 6 times the LD_{50} value were no longer fatal (17).

In 1949–50 Eagle (35, 36) screened 28 other chemical agents for their ability to detoxify cottonseed pigment glands when the latter were administered in 2% aqueous solutions of the material being tested for detoxifying action. It was found that many agents decreased the toxicity of cottonseed pigment glands so that levels in excess of the previously determined LD_{50} value could be administered without harmful effect. Some of these reagents were alcoholic sodium hydroxide, sodium hypochlorite, disodium phosphate,

trisodium phosphate, sodium chloride, sodium alkaline pyrophosphate, sodium hypochlorite, ferrous sulfate plus NaCl, ammonium carbonate, sodium hydroxide, potassium hydroxide, calcium hydroxide, sodium sesquicarbonate, and sodium carbonate peroxide. The experience with detoxification of cottonseed pigment glands led to experiments in which deliberately chosen highly toxic cottonseed meals or flakes were treated with these reagents and fed to rats and/or chicks. The results of these detoxification investigations have been reported by Eagle *et al.* (32, 37, 38) and reconfirm the statement that the toxicity of cottonseed products cannot be accounted for solely on the basis of analyzed gossypol content (16, 19, 21).

Summary

1. Untreated cottonseed pigment glands vary widely in their acute oral toxicity in the rat, but this toxicity is not proportional to their analyzed gossypol content.

2. Pure gossypol is toxic to the rat but much less so than any untreated cottonseed pigment glands tested.

3. Repeated doses of gossypol at levels of 10–200 mg./kg./day were fatal to the dog.

4. The acetone-soluble, water-soluble fraction of a sample of cottonseed pigment glands proved to be the most toxic (LD_{50} 700 mg./kg.) material ever isolated from cottonseed.

5. One fraction, despite a gossypol content of 90%, was only half as toxic as the original pigment glands which contained only 40% gossypol.

6. The toxic factor(s) of cottonseed pigment glands were not extracted by petroleum naphthas or tetrachloroethylene, were partially extracted by ethanol, and were completely extracted by diethyl ether and acetone.

7. Gossypol combination products were considerably less toxic than cottonseed pigment glands and in four out of five cases were much less toxic than gossypol.

8. The order of decreasing sensitivity to cottonseed pigment glands in various animal species was:

guinea pig > rabbit > mouse > rat

9. Long-term storage of cottonseed pigment glands for even as long as 9½ years had little effect on their acute oral toxicity or their analyzed gossypol content.

10. Cottonseed pigment glands were slightly less toxic when administered in oil than when they were administered in water. Gossypol, on the other hand, was slightly more toxic when given in oil than when given in water.

11. Pure gossypol fed at various dose levels in the diets of experimental animals caused body-weight depression in proportion to the amount fed.

12. Cottonseed pigment glands fed to experimental animals depressed body weight considerably more than could be explained on the basis of their gossypol content.

13. Feeding constant levels of gossypol (0.1%) supplied by each of six different samples of cottonseed pigment glands caused varying body-weight depressions, but all six samples caused greater body-weight depression than did any of the three samples of gossypol similarly fed.

14. Cottonseed pigment glands are well detoxified when administered in 2% ferrous sulfate solution. A list of 14 other reagents which cause varying degrees of detoxification of cottonseed pigment glands is given.

REFERENCES

1. Voelker, A., cited by Crawford, A. C., in J. Pharmacol. Exper. Therap., *1*, 519–548 (1910).
2. Longmore, J., J. Soc. Chem. Ind., *5*, 200–206 (1886).
3. Marchlewski, L., J. prakt. chem., *60*, 80–90 (1899).
4. Withers, W. A., and Carruth, F. E., Science, *41*, 324 (1915).
5. Withers, W. A., and Carruth, F. E., J. Agr. Res., *5*, 261–288 (1915).
6. Withers, W. A., and Carruth, F. E., J. Agr. Res., *12*, 83–102 (1918).
7. Carruth, F. E., J. Am. Chem. Soc., *40*, 647–663 (1918).
8. Schwartze, E. W., and Alsberg, C. L., J. Agr. Res., *25*, 285–295 (1923).
9. Schwartze, E. W., and Alsberg, C. L., J. Agr. Res., *28*, 191–198 (1924).
10. Schwartze, E. W., J. Oil and Fat Ind., *3*, 173–178 (1926).
11. Boatner, Charlotte H., and Hall, Catherine M., Oil and Soap, *23*, 123 (1946).
12. Vix, H. L. E., Spadaro, J. J., Westbrook, R. D., Crovetto, A. J., Pollard, E. F., and Gastrock, E. A., J. Am. Oil Chemists' Soc., *24*, 228 (1947).
13. Boatner, Charlotte H., O'Connor, R. T., Curet, M. C., and Samuels, C. S., J. Am. Chem. Soc., *69*, 1268–1273 (1947).
14. Castillon, Leah E., Hall, Catherine M., and Boatner, Charlotte H., J. Am. Oil Chemists' Soc., *25*, 233–236 (1948).
15. Boatner, Charlotte H., abstracts of papers, Am. Oil Chemists' Soc., p. 6, May 21, 1947.
16. Eagle, Edward, Castillon, Leah E., Hall, Catherine M., and Boatner, Charlotte H., Arch. Biochem., *18*, 271–277 (1948).
17. Eagle, Edward, Proc. Soc. Exper. Biol. and Med., *72*, 444–446 (1949).
18. Eagle, Edward, Arch. Biochem., *26*, 68–71 (1950).
19. Eagle, Edward, Hall, Catherine M., Castillon, Leah E., and Miller, Charlotte B., J. Am. Oil Chemists' Soc., *27*, 300–303 (1950).
20. Eagle, Edward, and Bialek, H. F., Food Research, *15*, 232–236 (1950).
21. Eagle, Edward, and Bialek, H. F., Food Research, *17*, 543–549 (1952).
22. Eagle, Edward, and Davies, D. L., J. Am. Oil Chemists' Soc., *35*, 36–37 (1958).
23. Zucker, T. F., and Zucker, L. M., Abstracts of Papers, Am. Chem. Soc., p. 47B, April 1947.
24. Zucker, T. F., and Zucker, L. B., abstracts of papers, Am. Oil Chemists' Soc., p. 5, May 1947.
25. Eagle, Edward, Science, *109*, 361 (1949).
26. Groschke, A. C., Rubin, M., and Bird, H. R., Poultry Sci., *26*, 310–312 (1947).
27. Boatner, Charlotte H., Altschul, A. M., Irving, G. W. Jr., Pollard, E. F., and Schaefer, H. C., Poultry Sci., *27*, 315–328 (1948).
28. Lillie, R. J., and Bird, H. R., Poultry Sci., *29*, 390–393 (1950).
29. Ambrose, A. M., and Robbins, D. J., J. Nutr., *43*, 357–370 (1951).
30. Couch, J. R., Chang, W. Y., and Lyman, C. M., Poultry Sci., *34*, 178–183 (1955).
31. Heywang, B. W., and Bird, H. R., Poultry Sci., *34*, 1239–1247 (1955).
32. Eagle, Edward, and Davies, D. L., J. Am. Oil Chemists' Soc., *34*, 454–459 (1957).
33. Withers, W. A., and Ray, B. J., Science, *36*, 31–32 (1912).
34. Withers, W. A., and Brewster, J. F., J. Biol. Chem., *15*, 161–166 (1913).
35. Eagle, Edward, and Norris, F. A., U. S. Patent 2,740,718, April 13, 1956.
36. Eagle, Edward, unpublished observations.
37. Eagle, Edward, Bialek, H. F., Davies, D. L., and Bremer, J. W., J. Am. Oil Chemists' Soc., *31*, 121–124 (1954).
38. Eagle, Edward, Bialek, H. F., Davies, D. L., and Bremer, J. W., J. Am. Oil Chemists' Soc., *33*, 15–21 (1956).

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