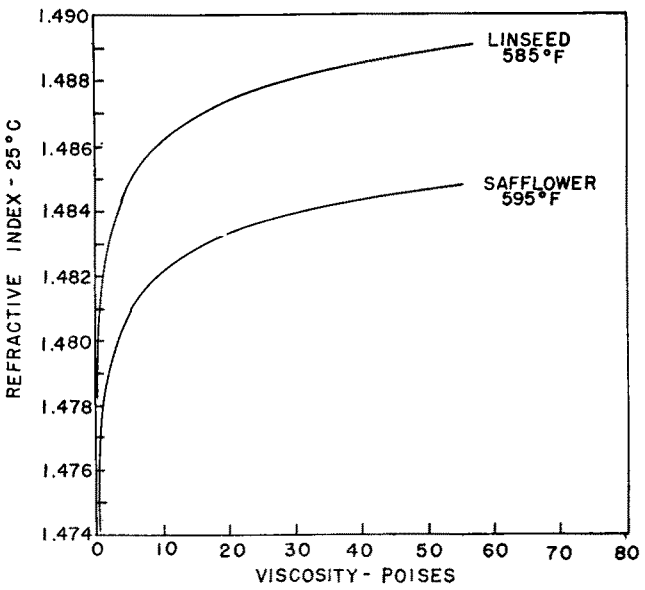

FIG. 5.

to note that in the viscosity range of 10 to 50 poises the polymerization rate of safflower closely approaches that of linseed. This rate continues until a viscosity of at least 80 poises is attained. Polymerization studies beyond 80 poises (Z_4-Z_5) should be of considerable interest. Thus it is evident that with the aid of a 10° increase in polymerization temperature, safflower oil may be bodied to a viscosity of about 50 poises (Z_4-Z_5) in approximately the same time as linseed and hence the same average rate.

The iodine value versus viscosity curves for the oils are shown in Figure 4. The curves are quite similar in shape except that in the range of 20-60 poises the iodine value of safflower oil drops at a faster rate than either linseed or soya. It should be noted that the curve for safflower is a composite curve and includes data from cooks at three temperatures. The 595°F. data is indicated by a circle, the 585°F. by a dotted circle, and the 575°F. by a triangle. The plotted points indicate that the composite curve is a good mean curve for all three temperatures. The refractive index versus viscosity curves (Fig. 5) are again about what one would expect.

One of the outstanding properties of safflower oil is its ability to bleach under heat treatment. The final constants of the oils given in Table I show this quite clearly. It might be well to mention here that the bodied safflower dries at a rate equal to or slightly better than bodied linseed but under certain conditions retains a slight degree of "after-tack" for a longer period of time.

Summary

It can be stated that the polymerization rate of safflower oil is sufficiently rapid to warrant its use on a commercial scale. Furthermore, with the proper choice of polymerization temperature, safflower can be bodied at the same rate as linseed. The increasing rate at which safflower bodies in the high viscosity ranges invites further investigation. In these higher viscosity ranges the iodine value continues to drop whereas in the same range the iodine numbers of other oils show a tendency to approach an asymptotic value.

Safflower heat bleaches considerably better than linseed and is also equal to or better than soya in this respect.

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The Effect of Screw-Press and Hydraulic-Press Processing Conditions on Pigment Glands in Cottonseed[1,2]

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WHEN cottonseed is processed by hydraulic-press procedures, many of the pigment glands are ruptured by the action of heat, moisture, and mechanical forces, and gossypol and related pigments escape from the ruptured glands. These liberated pigments react with the surrounding tissues to form "bound" gossypol which is considered to be physiologically inactive when ingested by farm animals (3, 5, 8, 13, 14). Although the by-product meal therefore contains a much smaller amount of unbound or free gossypol than the original kernels (5, 8, 14), the content is often too high to permit unrestricted feeding to non-ruminants, such as swine and poultry.

The effect of moisture and heat on pigment glands was determined by Boatner and her coworkers (4), who conducted laboratory tests in which cottonseed samples containing 7.6% and 41% moisture were heated at 241°F. for varying periods of time. In the material containing 7.6% moisture nearly all of the glands remained intact except after long exposure to heat whereas some of the glands in the moistened seed were ruptured even before heating and most of the remainder were ruptured by cooking. Likewise Williams *et al.* (12) noted that dry-cooked cottonseed meats contained very few ruptured glands whereas about 60% of the pigment glands were ruptured in meats which had been cooked with 20% of added

[1] Report of a study carried on under the Research and Marketing Act of 1946.
[2] Presented at the 42nd Annual Meeting of the American Oil Chemists' Society, May 3, 1951, New Orleans, La.
[3] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE I

Analytical Results[1] on Cottonseed Process Fractions

Test No.	Type of		Process fraction	Cooking conditions			Approx. pressure on cake (p.s.i.)	Lipids (%)	Gossypol		Recoverable pigment gland content (%)	Microscopic appearance of process fractions
	Processing	Preperation		Temp. (°F.)	Time (Min.)	Moisture addition			Free (%)	Total (%)		
1	Commercial hydraulic press	Flaked with 5-high rolls	Meats before cooking	—	—	Steam in 5-stack cooker	—	30.3	0.82	N.D.	0.99	All glands intact
			Meats after cooking	224	45		—	34.1	0.13	N.D.	0.09	Few glands intact
			Meal	—	—		2,000	5.8	0.15	0.81	0.04	Few glands intact
2	Commercial hydraulic press	Flaked with 5-high rolls	Meats before cooking	—	—	Steam in 5-stack cooker	—	27.6	0.62	0.78	1.07	All glands intact
			Meats after cooking	231	48		—	28.4	0.05	0.72	Trace	Few glands intact
			Meal	—	—		2,000	5.7	0.05	0.91	Trace	Few glands intact
3	Commercial screw press	None—meats left whole	Meats before cooking	—	—	None in 4-stack cooker	—	29.4	0.83	1.00	0.66	All glands intact
			Meats after cooking	184	25		—	28.9	0.73	1.03	0.66	All glands intact
			Meal	—	—		20,000	3.0	0.02	0.80	0.00	No glands visible
4	Laboratory hydraulic press	Flaked on pair of smooth rolls	Meats before cooking	—	—	None in closed vessel	—	35.4	0.94	0.81	1.62	All glands intact
			Meats after cooking	185	60		—	36.1	0.86	0.72	1.46	Most glands intact
			Meal	—	—		2,000	27.4	0.89	0.82	0.50	Glands whole but deformed
			Meal	—	—		20,000	21.5	0.95	0.88	0.33	Glands whole but deformed
5	Laboratory hydraulic press	Flaked on pair of smooth rolls	Meats before cooking	—	—	Steam at 15 p.s.i. in autoclave	—	35.4	0.94	0.81	1.62	All glands intact
			Meats after cooking	248	25		—	35.5	0.39	0.72	0.68	Most glands whole[2]
			Meal	—	—		2,000	14.7	0.36	0.91	0.11	Most glands whole[2] but deformed
			Meal	—	—		20,000	17.7	0.46	0.98	0.11	Most glands whole[2] but deformed
6	Laboratory hydraulic press	Flaked on pair of smooth rolls	Meats before cooking	—	—	Steam at 15 p.s.i. in autoclave	—	30.1	0.92	0.93	0.76	All glands intact
			Meats after cooking	248	25		—	29.6	0.45	0.75	0.57	Most glands whole[2]
			Meal	—	—		2,000	18.1	0.41	0.83	0.23	Most glands whole[2] but deformed
			Meal	—	—		20,000	16.8	0.46	0.82	0.33	Most glands whole[2] but deformed

[1]Data are given on a moisture-free basis. [2]Glands surrounded by diffusion veils of pigments. N.D. signifies "Not Determined."

water. It was also observed that the free gossypol content of the cooked meats was inversely proportional to the amount of water added before cooking.

Many investigators have sought to establish conditions for controlling processing variables so as to obtain maximum lowering of the free gossypol content of the cottonseed meal (3, 6, 8, 9, 13), but in most cases when the proposed controlled processing conditions were effective in reducing free gossypol content, they also reduced the nutritive value of the meal by heat damage to the proteins. Recently however a particular set of conditions for screw-press processing, representing a modification of both the cooking and pressing stages, was found to yield a meal having a low free gossypol content and a high protein solubility (7). In this procedure the meats were cooked at low temperatures without addition of moisture, and the major reduction in free gossypol took place in the screw press.

The present study was carried out in an attempt to determine what processing conditions were responsible for the effectiveness of this modified processing procedure in producing a high quality meal. For this purpose comparative assays for free gossypol, total gossypol, and intact pigment glands were made on commercial process fractions from hydraulic press and screw press mills and on appropriate samples prepared in laboratory-scale cooking and pressing tests.

Experimental

Free and Total Gossypol Determinations. Samples were analyzed for free gossypol by the method of Pons and Guthrie (10) and for total gossypol by the method of Pons, Hoffpauir, and O'Connor (11). In these methods "free gossypol" is defined as gossypol and gossypol-like compounds which dissolve in 70% acetone under specified conditions; "total gossypol" as free gossypol plus the gossypol that is extracted by aqueous acetone after hydrolysis of the meal by oxalic acid under specified conditions; and "bound" gossypol as total gossypol minus free gossypol. After the gossypol has been extracted, it is determined by measuring the amount of color produced when p-anisidine is added.

Estimation of Pigment Gland Content. To allow evaluation of the effectiveness of a given processing

operation in causing gland rupture, a method for semi-quantitatively separating the unbroken pigment glands from the meal and hull particles was developed by modifying the qualitative procedure of Boatner and Hall (2). The modified procedure is as follows:

Place 25 grams of ground cottonseed, rolled meats, flakes, or meal in a Waring blendor[4] with 150 ml. of solvent mixture (carbon tetrachloride-hexane in a volume ratio of approximately 7:3) having a specific gravity of 1.380. Run at high speed for 3 minutes, quantitatively transfer the slurry to a 250-ml. centrifuge bottle and centrifuge for 5 minutes at 2,000 r.p.m. Decant the supernatant slurry of fine meal particles and pigment glands into a 250-ml. separatory funnel and let stand 10 min. to allow the glands to float to the surface. Withdraw the meal suspension until only about 5-10 ml. of the upper portion containing the glands remains in the funnel, being careful not to discard any glands. Add 50 ml. of fresh solvent mixture to the funnel, shake, and then run the suspension onto a 100-mesh sieve to screen out coarse meal particles. Wash the material on the sieve with an additional 50-ml. portion of solvent to remove all pigment glands. Return the combined suspensions to the funnel, let stand 5 min., draw off to a residual volume of 5 ml., and discard the lower gland-free portion. Again add 50 ml. of solvent mixture to the gland fraction, agitate, allow the glands to float to the surface, and draw off the lower liquid (which at this point, should contain only traces of meal). Wash the gland fraction into a tared 50-ml. beaker, using about 25 ml. of hexane. Allow the glands to settle and decant the clear supernatant liquid. Wash the glands again with hexane to remove final traces of carbon tetrachloride and decant the solvent. Allow the glands to dry in air until no odor of solvent remains. Weigh and express as a percentage of the weight of original sample taken.

Examine the gland fraction under a microscope. If meal particles appear to make up more than about 10% of the fraction, estimate the percentage purity by counting the relative numbers of glands and meal particles in several representative subsamples. Correct the previously obtained weight of the gland fraction to compensate for impurities,

It is considered that this procedure yields a reasonably reliable estimate of the weight of intact or unruptured pigment glands in a sample with a probable precision of ± 5-10%. The solvent mixture used does not readily attack glands having unbroken walls since the gland walls are apparently impermeable to the solvents used. If however the walls are broken, the contents of the gland pigments are dissolved readily. The foregoing method therefore fails to recover either dissolved pigments or fragments of broken glands.

Microscopic Tests. Microscopic examinations of each sample were made by standard techniques to observe the effects of the processing variables on the physical appearance and relative abundance of the pigment glands present.

To illustrate the effect of dry-cooking and of screw pressing on the pigment glands in cottonseed meats, photomicrographs were made of free-hand sections of meats both before and after cooking in the modified screw pressing procedure and of the surface of a portion of the cake from the screw press. These photomicrographs are presented in Figures 1, 2, and 3.

Results

Commercial Hydraulic-Press Operations. Chemical analyses and results of the microscopic examinations of the uncooked and cooked meats and the meal from two commercial hydraulic-press mills are shown in Table I. The data indicate that the cooking operation caused a marked reduction in both the free gossypol content and in the amount of recoverable (intact)

[4]The use of trade names in this article is for identification and implies no endorsement of the manufacturer of the product.

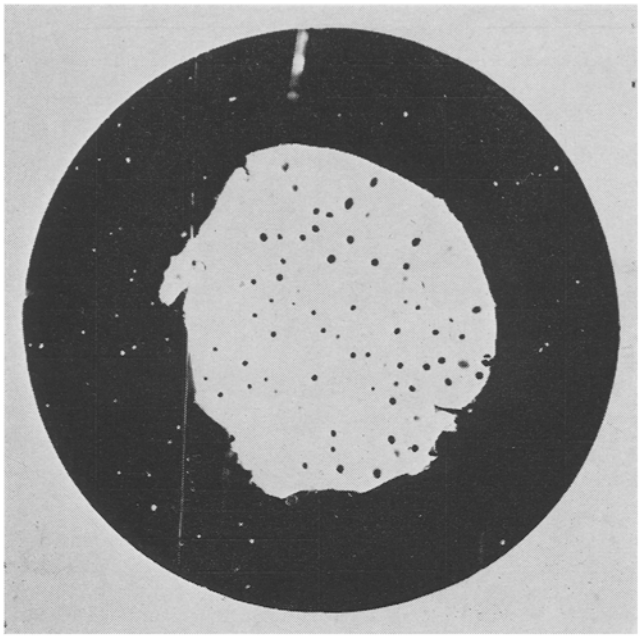

FIG. 1. Section of an uncooked meat, Test 3, showing intact pigment glands.

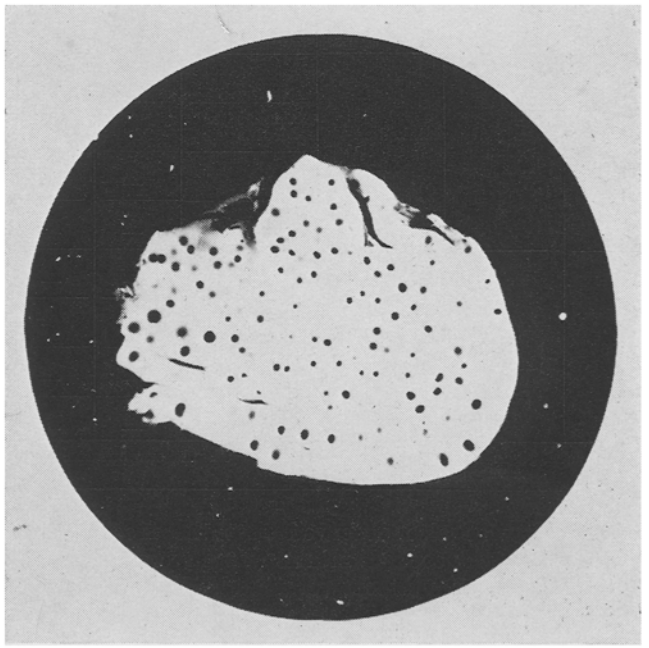

FIG. 2. Section of a cooked meat, Test 3, with pigment glands still intact.

glands, while the pressing operation produced little, if any, further effect.

In Test No. 1 cooking at 224°F. with added moisture reduced the free gossypol content in the meats from 0.82 to 0.13% and the recoverable gland content from 0.99 to 0.09%. In Test No. 2 cooking at 231°F. with moisture added caused a reduction in free gossypol from 0.62 to 0.05%. The pronounced reduction in recoverable gland content is considered to indicate that most of the glands were ruptured during the cooking. This was substantiated by microscopic examination which showed that few intact pigment glands remained in the cooked meats. The proportionally similar reduction in free gossypol is addi-

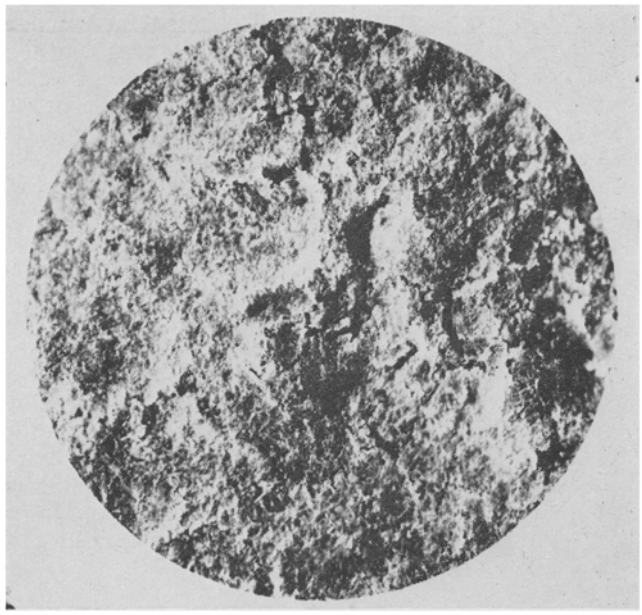

FIG. 3. Section of screw-press cake, showing no evidence of intact pigment glands. If present, pigment glands would have been visible in light areas.

tional evidence that the glands were ruptured so that the gossypol escaped and reacted with the constituents of the meats, probably in the manner suggested by earlier workers (3, 4, 5, 12). It will be further noted that the actual magnitude of the changes in either free gossypol content or recoverable pigment gland content upon pressing was small. This however should not be considered as proof that hydraulic pressing could not have brought about marked changes if the free gossypol and intact gland contents of the cooked meats had been high prior to pressing.

Commercial Screw-Press Operations. The results with commercial screw-press mill samples, prepared by the modified procedure previously mentioned (7), were in marked contrast to those from commercial hydraulic-press mills. The data for Test No. 3 indicate that the dry cooking treatment given the whole meats had no marked effect on the percentage of free gossypol or of intact pigment glands, which agrees with observations by others (4, 12), while passage through the screw press reduced the free gossypol content sharply and also ruptured all the glands. No intact glands could be recovered. The maximum temperature attained by the meats during cooking was 184°F., which is much lower than in ordinary commercial operations. In other tests at higher cooking temperatures only slight changes in either free gossypol or pigment gland contents occurred during cooking.

As a further check on the validity of the findings on the press cake, additional samples of meal from the same mill prepared under various pressing conditions, as described elsewhere (7), were examined. The results are not tabulated here, but in every instance the samples showed very low contents of free gossypol and no pigment glands could be recovered.

Photomicrographs of the uncooked meats, cooked meats, and press cake (Figures 1, 2, and 3) from Test No. 3 show that the pigment glands were not visibly ruptured by cooking but were completely disintegrated by pressing.

These results emphasize that, under the conditions used in this particular screw-press mill, the noted reduction in free gossypol and destruction of pigment glands occurred almost solely during passage of the meats through the press.

Laboratory Studies. For Test No. 4 cottonseed which had been stored for two years was hulled, and the whole meats flaked to a thickness of 0.010 inch. These flaked meats containing 8.4% moisture were cooked in a closed vessel without addition of moisture. The cooked meats were pressed while hot, using a Carver[4] laboratory-type hydraulic press. The press cylinder and piston were heated to the same temperature as the meats before charging and, during pressing, were wrapped in insulating felt. Two levels of pressure were used, 2,000 pounds and 20,000 pounds per sq. in. on the cake. The lower pressure is comparable to that used in commercial hydraulic-press mills and the higher pressure is comparable to the pressure estimated to be attained in a commercial screw press (1). The results recorded in Table I show that neither of the pressing treatments appreciably lowered the free gossypol content of the meal. Microscopic examination of the press cake indicated that the pressure caused deformation of some of the glands but no visible evidence of gland rupture could be noted. The results of the gland separation tests show however that a high proportion of the glands were either ruptured or else their walls were weakened sufficiently for the inert solvent mixture used in the tests to penetrate the walls and dissolve their contents.

The ability of the glands to withstand such a high pressure without showing visible signs of wall rupture suggests that the resistance of pigment glands to rupture during the application of direct pressure is due to their being embedded in a matrix of protein tissues and oil which supports the semi-rigid gland walls and aids in preventing excessive deformation.

In Test No. 5 flaked meats identical with those used in Test No. 4 were autoclaved at a steam pressure of 15 pounds per sq. in. The cooked meats were pressed in the same way as those in Test No. 4. The moist cooking treatment markedly lowered both the free gossypol content and the recoverable gland content of the meats. Neither pressing treatment caused much change in free gossypol but both sharply reduced the recoverable gland content. Microscopic examinations of the cooked meats and the press cakes showed that some diffusion of pigments from the glands had occurred, which suggests that the gland walls had either been completely broken or else had been rendered permeable to the gossypol pigments. Further, in the press cakes prepared at both levels of pressure, although the glands did not appear to have been broken apart, many had been deformed by being pressed against tissue aggregates and hull particles. The reduction in recoverable gland content caused by cooking and by pressing also indicated that in a high proportion of the glands the walls had been broken so that penetration by the solvent mixture occurred.

In Test No. 6 meats from seed which had been stored only four months were flaked, cooked, and pressed exactly as in Test No. 5. It will be noted that the results show trends similar to those in Test No. 5.

In other tests not recorded here it has been noted that when flaked meats from another lot of newly harvested cottonseed were subjected to wet cooking

in an autoclave and pressed under the same conditions used in Tests Nos. 4, 5, and 6, a high proportion of the glands was completely disintegrated by hydraulic pressing. Further studies on the relationship between source of seed, time elapsed after harvesting, and the susceptibility of the pigment glands to mechanical rupture are contemplated.

In the laboratory experiments with the hydraulic press, even at pressures of 20,000 pounds per sq. in., it was found that some glands still remained intact while in the commercial screw-press meal substantially all of the glands were ruptured. Evidently the screw press develops forces other than direct pressure, perhaps shearing forces, that bring about the immediate disintegration of the glands and spreading of the contents onto the meal and into the oil.

Summary

A procedure is given for estimating the amount of intact pigment glands in cottonseed kernels and meal.

The amounts of intact pigment glands, free gossypol, and total gossypol in uncooked meats, cooked meats, and press cake samples from commercial hydraulic-press mills, a screw-press mill, and laboratory-scale tests were determined. It was observed that when meats were cooked with adequate moisture present, as is common in hydraulic-press mills, most of the free gossypol was converted to bound gossypol during the cooking operation and further conversion during the pressing stage was of slight magnitude. The trend of the change in percentage of recoverable glands was roughly parallel to that of the free gossypol. By contrast, in the screw-press mill where cooking was carried out without the addition of moisture, little change in either component occurred during cooking, but both were reduced to very low levels during passage of the cooked meats through the screw press.

In laboratory tests cooked cottonseed meats were subjected to hydraulic pressures at levels of 2,000 and 20,000 pounds per sq. in. of cake surface. When meats were cooked at low moisture content, no significant change in either free gossypol or recoverable glands occurred during cooking, but hydraulic pressing at both 2,000 and 20,000 pounds per sq. in. reduced the percentage of recoverable glands. No corresponding decrease in free gossypol during pressing could be found. Wet cooking of meats decreased the percentages of free gossypol and intact glands and, although hydraulic pressing failed to further reduce the free gossypol, the percentage of recoverable glands was sharply reduced by pressing at both levels of pressure.

It is suggested that the high degree of effectiveness of the screw press in rupturing and disintegrating pigment glands in cottonseed meats is due to the development of shearing forces in combination with direct or compressive type pressure. It is believed that a shearing action is more effective for this purpose than compressive force of similar magnitude.

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Pigments of Cottonseed. IV. Gossypurpurin, a Purple Pigment Related to Gossypol[1]

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GOSSYPURPURIN, a naturally occurring, purple-colored pigment of cottonseed, exhibits a characteristic absorption spectrum in chloroform, with maxima in the visible wave length region at 565-568 mμ and 530-532 mμ. This pigment was first isolated from the red crystals (so-called "red gossypol") obtained from chloroform extracts of cottonseed kernels (2, 3, 8). It has also been obtained by treating an ethereal extract of cottonseed kernels with dilute ammonium hydroxide (2, 6). In this procedure the treatment was believed to have effected a conversion of gossypol in the ethereal solution to gossypurpurin. Gossypurpurin was not obtained readily or in large amount in either of the above cases. Except for its absorption spectrum in chloroform solution (6), no information concerning this pigment has heretofore been available. There follows a report of the conversion of gossypol to gossypurpurin via diaminogossypol and of the isolation of the native pigment from cottonseed pigment glands (5, 10). The properties of the isolated gossypurpurin are compared with those of the artificial product, and some observations are reported on the structure and relationship of this pigment to gossypol.

[1] Presented before the 4th Southwest Regional Meeting of the American Chemical Society, Shreveport, La., December 9-10, 1948.
[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Experimental

Preparation of Diaminogossypol. Gaseous ammonia was passed into a warm solution containing 1.5 g. pure gossypol in 75 ml. chloroform. The solution was refluxed 45 minutes, during which time the addition of ammonia was continued. At the end of this period heating was discontinued and the chloroform solution