phosphates are determined by the photometric method.

In testing for total phosphate, 7 ml. of concentrated sulfuric acid were used because it provides a suitable volume of digestion medium for a 1-gram sample of sugar and results in an acidity (1.2*N*) which eliminates silicate interference.

When testing for inorganic phosphate in the presence of organic phosphates as in the case for sugar products, the acid concentration of the test solution, along with its temperature and standing time, is of the utmost importance. Gee, Domingues, and Deitz (*5*) used an acidity of about 0.25*N* for their inorganic phosphate test. Spencer and Meade's method for inorganic phosphate (*12*) employs solutions of about 0.38*N*. The molybdivanadophosphate inorganic test can also be carried out at an acidity of 0.25*N* with good accuracy, provided silicates are absent. The calibration curves obtained using 0.25*N* and 1.2*N* acidity almost coincide.

To obtain an estimate of the extent of hydrolysis of sugar phosphates to inorganic phosphate using 1.2*N* acidity, the author selected the three compounds listed in Table I as representative sugar phosphates. Employing the currently described method, he then determined the inorganic phosphate content of each of these compounds four times using acidity of 0.25*N* and of 1.20*N*. From the literature it appears that negligible hydrolysis of sugar phosphates occurs at 0.25*N*. Therefore, the difference between the inorganic phosphate content found at these two acidities was taken as a measure of the extent of hydrolysis of these compounds due to the higher acidity in Table I.

Correction for Initial Yellow Color of Some Sugar Solutions. If a colored solution such as a raw sugar is to be tested for inorganic phosphate, consideration must be given to the fact that raw sugar solutions themselves are initially yellow in color. This can be accomplished by measuring the absorbance of 100 ml. of the raw sugar solution containing 7 ml. of dilute (1 to 1) sulfuric acid after setting the colorimeter at zero absorbance using distilled water at the same acidity. If this absorbance value is subtracted from the absorbance value obtained by adding vanadate and molybdate to a similar raw sugar solution, the corrected absorbance value is obtained.

Precision of Total Phosphate and Inorganic Orthophosphate Methods. To evaluate the precision of the total phosphate method 28 samples of various sugar products were analyzed in triplicate. As can be readily calculated from Table II, the standard deviation of total phosphate method is ±0.010 mg. The precision of the inorganic orthophosphate method was evaluated by analyzing 27 samples of various colored sugar products and six colorless products. The standard deviation for colored products was found to be ±0.015 mg., while that for colorless samples was calculated to be ±0.005 mg.

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GOSSYPOL EXTRACTANTS

Oral Toxicity to Poultry of a Commercial Octylamine

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Several processes are being investigated in the Cottonseed Products Research Laboratory for removing gossypol from cottonseed meats with the aid of aliphatic amines during the ordinary extraction of oil from meats. Primary *n*-octylamine is the preferred amine so far. In all of the processes varying amounts of residue amine remain in the meal. The amounts are estimated to range from less than 0.1% up to about 0.4% of the weight of the meal.

Knowledge of the general level of toxicity of *n*-octylamine was sought in order to determine whether complete removal of the residual amine from meal was necessary. If such were the case, the favored process would have to be substantially changed and a definite reorientation of research plans would be necessary.

This is a report on a preliminary investigation into the toxicity of a commercial primary *n*-octylamine, Armeen 8D (Armour & Co.) (*3, 4*). This amine has been used in much of the process development so far.

A considerable amount of toxicological data on aliphatic amines appears in the literature. With one exception (*6*), however, none of it refers specifically to octylamine. Most acute toxicological data appear to have been summarized in the "Handbook of Toxicology" (*7*). Some data from this handbook which are useful to the present investigation are reproduced in Table I.

Acute oral toxicity tests have been carried out on several acetate salts of aliphatic amines (*2*). The compounds tested were Armour's Armac C, Armac T, Armac TD, and Armac HT. The descriptions of these compounds have been given (*2*). They are manufactured from amines containing from 8 to 18 carbon atoms.

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Two hundred broiler-type chickens, including both sexes, were for 9 weeks fed rations containing different levels of commercial primary *n*-octylamine. Levels of amine were 0.036, 0.072, 0.144, and 0.36% of the rations. A control ration containing no amine, but otherwise similar to the other four, was also fed. No definitely toxic effects were observed. Consistent lesions were produced only by force feeding of amine at levels above those which the chickens would voluntarily accept in the ration. Many of these birds died. Prominent changes included hardening and peeling of the crop lining, inflammation of the intestine, edema and congestion of the lungs, and swelling of the anterior lobes of the kidneys.

Table I. Acute Oral Toxicities of Primary Aliphatic Amines[a]

No.	Compound	Animal	Dosage,[b] LD_{50}, Mg./Kg.
851	Ethylamine	Rat	400
1141	Isopropylamine	Rat	820
320	*n*-Butylamine	Rat	500
1027	Hexylamine	Rat	670
153	Aniline[c]	GP[d]	2500
	Aniline	Cat	100–200
	Aniline	Dog	500

[a] Data from "Handbook of Toxicology" (7).
[b] Milligrams of chemical administered per kilogram of body weight.
[c] The dosage values given for aniline are lethal doses—i.e., *LD* rather than LD_{50}. These data are included for comparative purposes.
[d] Guinea pig.

Table II. Composition of Rations Fed to Birds in Feeding Test

Ingredient	Grams[a]	Per Cent
Ground yellow corn		30.0
Ground milo		19.0
Solvent process soybean meal, 45% protein		36.0
Dehydrated alfalfa meal, 17% protein		2.5
Condensed fish solubles		2.5
Dried whey		2.5
Soybean oil		2.5
Defluorinated rock phosphate		2.0
Oyster shell flour		1.5
Salt		0.5
Manganese sulfate, 70% (11.4 grams)		
Vitamin mix		1.0
Riboflavin	0.2	
Niacin	1.3	
Calcium pantothenate	0.5	
Choline chloride	160.0	
Vitamin B_{12}	1.2	
Vitamin A	40.0	
Vitamin D_3	3.0	
Vitamin K	0.2	
Methionine	22.5	
Aureomycin	2.5	
BHT[b]	11.3	
Ground milo	210.9	
Total	453.6	100.0

Protein, %	22.5
Calories/lb.	930
Calories/% protein	41.3

[a] Grams/lb.
[b] Butylated hydroxytoluene.

LD_{50} toxicity for Armac C was 600 to 800 mg. per kg. in rabbits. LD_{50} toxicities for the other three compounds were greater than 2000 mg. per kg. in rats. The compounds were administered in water solution by stomach tube. Surviving animals were observed for 5 days, by which time maximum mortality had been reached.

A modified subacute toxicity test was carried out on rabbits with Armac C and with Armac HT. One dose per day, 5 days per week for 37 days, was fed by stomach tube. The size of the dose was one third the LD_{50} as determined in the acute toxicity test. For both materials, the only observed effects of this treatment were slight weight losses.

Subacute and chronic toxicity tests have been carried out with octadecylamine (Armour's Armeen 18D) (*3*). No toxic effects of any kind were observed when octadecylamine was fed to rats for 2 years at levels of up to 0.05%. However rats fed this amine at a level of 0.3% of their diet for a shorter time developed an accumulation of histiocytes with pale or foamy cytoplasm in the mucosa of the small intestine and mesenteric lymph nodes. Focal granulomas were seen in the livers of some of these animals. Anorexia, weight loss, and increased mortality also occurred.

Dogs were fed octadecylamine at levels of 0.6 and 3.0 mg. per kg. per day for 1 year without toxic effects. One of three additional dogs receiving 15.0 mg. per kg. per day died after 22 weeks. The other two dogs gained less weight than did the controls. They also showed slight changes in the mucosa of the small intestine. Although the death of one dog may not have been caused by the amine, octadecylamine fed to dogs at a level of 15 mg. per kg. per day did not appear to be completely innocuous.

Experimental

Poultry feeds are the principal market toward which the low gossypol meal being developed is pointed. A complete evaluation of the toxicity of octylamine was not considered to be appropriate until the technical and economic successes of the processes under development were closer to realization. For these reasons, a practical feeding test on chickens could be expected to yield the most useful information. This test could be considered to be in the nature of a subacute toxicity test.

Rations to be fed were to be alike except for the quantities of amine they contained. The composition of the diet used is known to affect the results of toxicity studies (*8*). Therefore the basal ration was to be as complete as possible in all known nutritional requirements in order for symptoms of amine toxicity not to be confused with symptoms of nutritional deficiency.

To incorporate amine into a ration, a solution of amine in *n*-hexane was added to soybean meal before the meal was mixed into the ration. Sufficient commercial (Phillips Petroleum Co.) *n*-hexane was used to wet the meal thoroughly. After being mixed, the mixture was spread on wrapping paper on the floor and left until the *n*-hexane had evaporated. Using *n*-hexane as carrier associated the amine intimately with the soybean meal in a manner similar to the intimate association of amine and meal in amine-treated cottonseed meal.

The quantities of amine added to soybean meal by this procedure were 0.1, 0.2, 0.4, and 1.0% of the weight of the meal. The Armeen 8D used was a distilled grade of a commercial primary amine having the following analysis (*2*, *4*, *5*).

Primary amine content	89.2%
Hexylamine (approximate % of primary amine by weight)	3
Octylamine (approximate % of primary amine by weight)	90
Decylamine (approximate % of primary amine by weight)	7
Secondary amine	3.9
Moisture	2.0
Iodine value	1 5

Nitriles were other impurities.

Principal impurities in the amine beside water were secondary amines and nitriles.

Most of the process development work has been carried out with the technical, undistilled grade of amine designated Armeen 8. Because toxic effects of amine might be confused with toxic effects of impurities, the purer grade was used for the toxicity studies. This of course did not allow the potential toxicity of the impurities in the technical grade to be evaluated.

Eighty-pound batches of each ration were mixed at one time. This size of batch required 28.8 pounds of soybean meal. About 27 pounds of meal at a time were treated with amine for each batch. Feeding of each newly mixed batch of feed to the chickens was usually started within 2 days after mixing. Four batches of each ration were consumed during the test.

The composition of the basal ration is shown in Table II. The ration numbers and corresponding amine contents in the soybean meal and in the complete ration are as follows: No. 1: 0, 0; No. 2: 0.1, 0.036; No. 3: 0.2, 0.072; No. 4: 0.4, 0.144; and No. 5: 1.0, 0.36.

Sexed, 1-day-old chicks were procured from a commercial hatchery. The chicks were of a commercial broiler strain designated Peterson cross. They were vaccinated for Newcastle disease. All were then fed for 7 days on No. 1 ration.

At 8 days of age, 100 birds were selected at random from each sex, were wing banded, weighed, and distributed to 10 cages, 10 of each sex in each cage. Cages were Oakes No. 8 wire brooder cages, five per battery. Batteries were arranged side by side in the same position relative to walls, windows, radiator, etc.

One of the two cages for testing each ration was selected from each battery. Relative positions of the two cages were selected either at extremes of top and bottom of the batteries, or in the middle. This was done as an attempt to minimize the possible effects of cage position on growth.

Feeding of test rations was started as soon as the birds were placed in the test cages on the 8th day. The birds usually were weighed once each week thereafter, throughout the test. During much of the test careful feed consumption data were kept. Paper envelopes were installed under feed troughs on brooder cages to decrease the feed loss and thus to improve the reliability of calculated feed utilization.

At 30 days of age, the birds were transferred to larger wire growing cages which contained eight cages in two stacks per battery. Two batteries, side by side, were used. The birds in the two brooder cages were divided into three groups of 13 or 14 birds each and each group was placed in a separate growing cage. Thus the birds in each ration were redistributed among three cages instead of two. The birds were maintained in these growing cages until the end of the test when the birds were 9 weeks old.

Several birds were sacrified for post-mortem examination at the end of the feeding test. Eleven birds on ration 1, five on ration 4, and 10 on ration 5 were sacrified.

Because no signs of any toxic effects had been observed during the feeding test, some forced feedings were made at higher levels of amine to see whether any symptoms of toxicity could be induced. Birds from ration 1 were used.

By the same technique used to add amine to soybean meal, enough amine was added to some mixed ration to raise the amine content to 4.89% of the weight of the ration. This ration mixture was ground so that about 95% would pass a 60-mesh screen. When this feed was mixed with two and a half times as much water the resulting slurry could be easily poured down a $^1/_8$-inch inside diameter rubber tube placed down a chicken's throat with the lower end of the tube about $^3/_4$ inch above the opening into its crop. A funnel was attached to the upper end of the tube. A satisfactory dose size was found to be 20 grams of meal mixed with 50 ml. of water, followed by another 10 to 25 ml. of water, as required, to rinse out the beaker and funnel. This intubation was necessary, because the birds would not voluntarily eat the feed containing 4.89% of amine and having a strong amine odor.

Seven birds were fed this mixture twice daily until death or until they were sacrified for post-mortem examination.

Some of the above feed was mixed with additional *n*-hexane and amine to give six different amine contents ranging from 7.5 to 20%. One dose of each feed was given to a different bird, and the birds were observed for 5 days before sacrifice. These birds were fasted overnight without feed, but with water before they were dosed.

Because none of the birds in the above group died with single doses of amine mixed in feed, four birds were fed with amine emulsified in water and without feed. The emulsifier was Hercules cellulose gum equivalent to 0.5% of the weight of the water.

Post-mortem examination for gross lesions were made on all force-fed birds (those in Table VI), with the exception of birds 3C and 4P.

Results and Discussion

Evaluation of the effects of amine in the rations were made by observation of

Table III. Mean Weights of Birds on Different Rations during Feeding Test

Age of Birds, Weeks	Ration No. 1	2	3	4	5	Rank[a]
	Mean Weights of Pullets, G.					
1	74	74	76	75	74	
2	153	156	153	157	148	4, 2, 3, 1, 5
3	259	264	259	266	240	4, 2, 3, 1, 5
4	413	419	414	414	383	2, 3, 4, 1, 5
6	766	768	768	762	718	2, 3, 1, 4, 5
7	965	969	975	956	908	3, 2, 1, 4, 5
8	1153	1169	1183	1151	1106	3, 2, 1, 4, 5
9	1316	1343	1363	1323	1280	3, 2, 4, 1, 5
	Mean Weights of Cockerels, G.					
1	75	77	75	76	74	
2	157	163	163	162	151	2, 3, 4, 1, 5
3	268	284	283	278	256	2, 3, 4, 1, 5
4	439	460	461	447	420	3, 2, 4, 1, 5
6	855	886	891	869	833	3, 2, 4, 1, 5
7	1101	1139	1136	1105	1069	2, 3, 4, 1, 5
8	1344	1390	1384	1370	1306	2, 3, 4, 1, 5
9	1564	1615	1597	1582	1519	2, 3, 4, 1, 5

[a] Ranked in order of decreasing mean weight.

Table IV. Analysis of Variance of Feeding Test Data by Sex for 8 Weeks of Age

Source of Variation	Degrees of Freedom	Mean Square	F[a]
Pullets			
Between rations	4	18,000	1.5
Between cages within rations	10	8,044	
Between birds within cages within rations (error)	87	12,037	
Cockerels			
Between rations	4	22,395	1.39
Between cages within rations	10	4,938	
Between birds within cages within rations (error)	83	16,183	

[a] To be significant at the 5% or 1% probability levels, F should be as great as 2.48 or 3.56, respectively.

the external appearance of the birds, plotting of growth curves and statistical analysis of the weight data, and by post-mortem examination of randomly selected birds.

Birds on different rations did not show any differences in external appearance such as degree of feathering, crippled feet, etc.

Data on total weights of birds at the end of 8 weeks of age were chosen for statistical analysis. Because of errors in sexing the chicks and in selecting the birds from two cages to be put into three cages at 30 days of age, different numbers of each sex in each cage resulted.

Table V. Daily Intake of Amine by Birds for Two 1-Week Periods during Feeding Test

Ration No.	Average Bird Weight, G.[a]	Amine Consumption, Mg./Kg./Day[b]
2-Week-Old Birds at Beginning of Period		
2	216	47
3	198	103
4	216	200
5	198	452
7-Week-Old Birds at Beginning of Period		
2	1158	25
3	1156	52
4	1153	93
5	1088	280

[a] The average weight during the feeding period is given. These weights are not comparable among rations, because of different numbers of cockerels and pullets per ration.

[b] Consumption is given in terms of milligrams per kilogram of body weight per day.

For this reason statistical analysis of the data was carried out on each sex separately. This method of analysis did not allow possible interactions between sex and ration to be evaluated. However, on plotted growth curves, the response by both sexes to the different rations was much the same, indicating that probably no sex-ration interaction existed.

Mean weight data are presented in Table III. The statistical analysis is summarized in Table IV.

The statistical analysis showed that the mean weights by ration were not significantly different from each other within sexes. In other words, differences in mean weight as great as those shown would be expected to occur by chance alone about 15 to 25% of the time. Therefore, while the amine content of the rations may have affected growth, the data did not prove that it did.

In spite of the lack of significance between mean weights, the consistency of the relative rank of the weights from week to week, as shown in Table III, would seem to have some meaning. In particular, ration 5 was lowest in weight for both sexes beginning with 2 weeks of age and running until the end of the test.

Feed consumption data showed no evidence that birds on any of the rations consumed more feed or utilized it more efficiently in making gains than birds on any other ration.

Table V contains data on amine intake by birds during the feeding test, while Table VI contains amine intake data on birds fed by intubation. Comparing these tables with Table I, the highest level of daily intake with ration 5 in Table V is seen to be of about the same order of magnitude as the toxic levels in Table I, while all doses in Table VI are as high or higher than toxic levels in Table I.

Although no real attempt was made to determine lethal dosages for chickens, the results with birds 14C and 15C in Table VI suggest that the lethal dose may be of the same order of magnitude as the values given in Table I for other amines.

Apparently when amine is mixed with feed, toxicity is greatly reduced. This may be the reason no deaths occurred among birds during the feeding test. Also in the latter test, daily amine intake was spread out rather than being in one dose.

In all of the tests involving forced feeding, changes in the behavior of the birds were noted. The first change was a lethargy and drowsiness which appeared within 20 minutes or less, depending upon the amount of amine administered and whether it was mixed with feed. A bird so affected would sit with its eyes closed until it was handled or otherwise disturbed. Then it would open its eyes, but would make no efforts to escape as normal birds always did. The depth of lethargy was also roughly dependent upon the amount of amine in the dose. Birds in this state had to be pushed to make them walk and then they walked uncertainly and slowly. Slavering always occurred among birds which were force-fed amine. These were the principal outward changes both in birds which did and did not die.

Before a bird died it might regurgitate and have muscular spasms. Some of the deaths appeared to have been caused by obstruction of the trachea

Table VI. Forced Feeding of High Levels of Amine

Bird No.[a]	Bird Weights, G.		Amine in Feed, %	No. of Doses Recd.[b]	Rate of Intake of Amine, Mg./Kg./Day[c]	Total Intake of Amine		Days Observed
	Initial	Final				G./kg.	G.	
1C	1891	1720[d]	4.89	3	1100	1.6	2.9	1[e]
2C	1837	1704[d]	4.89	4	1100	2.2	3.9	$1^1/_2$[e]
3C	1968	1900[d]	4.89	3[f]	1500	2.3	4.4	$1^1/_2$[e]
4P	1728	1672[d]	4.89	3[f]	1700	2.5	4.3	15
5C	1888	1618	4.89	9	1100	5.1	8.9	4[e]
6C	2006	1870	4.89	16	1100	8.1	15.7	10
7P	1776	1954	4.89	16	1100	8.4	15.7	10
8P	1494	1547	7.5	1[g]	1000	1.0	1.5	5
9P	1683	1603	10.0	1[g]	1200	1.2	2.0	5
10P	1744	1762	12.5	1[g]	1400	1.4	2.5	5
11C	1648	1468	15.0	1[g]	1800	1.8	3.0	5
12C	1721	1461	17.5	1[g]	2000	2.0	3.5	5
13C	1899	1542	20.0	1[g]	2100	2.1	4.0	5
14C	1804	1576	[h]	1	600	0.6	1.0	5
15C	1878	...	[h]	1	1100	1.1	2.0	5 hr.[e]
16C	1930	...	[h]	1	2100	2.1	4.0	30 min.[e]
17C	2051	...	[h]	1	9800	9.8	20.0	10 min.[e]

[a] C or P indicates bird was cockerel or pullet.

[b] When more than one dose was administered, doses were given two per day, except on weekends when none were given.

[c] Expressed in milligrams intake per day per kilogram of body weight, based on average body weight during period of administration.

[d] Weight after $1^1/_2$ days, at time when last dose of amine was administered.

[e] This bird died.

[f] This bird received doses containing 30 grams instead of 20 grams of amine-feed mixture per dose.

[g] Birds fasted 16 hours with access to water before dose was administered.

[h] Amine was emulsified with water, but was not mixed with feed for dosage.

with regurgitated feed in birds which were too stupefied by the amine to fight against strangulation. All of the birds fed amine without feed shook violently for a time whether or not they died later.

Post-mortem examination of 11 birds on ration 1 revealed no significant gross lesions. In one bird, 15 to 20 red plaques were found in the small intestine. The plaques varied from 3 to 8 mm. in their longest dimension, had discrete borders, and were rectangular to ovoid in shape. From a close visual examination it was judged that the reddening resulted from capillary dilatation in the mucosa. The plaques were not associated with Peyer's patches (lymphatic tissue).

Three out of five birds examined from the ration 4 group had intestinal plaques similar to those seen in one control bird. Two of these plus one without plaques showed diffuse hyperemia (inflammation) of the upper one third to two thirds of the intestinal tract.

No lesions were found in three birds out of the 10 examined from the group fed ration 5. Reddened plaques, ranging from questionable to distinct, were found in the upper one to two thirds of the intestinal tracts of five birds. One with plaques and two of those without showed a marked diffuse hyperemia of the upper half of the intestinal tract.

The presence of reddish plaques in chickens receiving various amounts of *n*-octylamine cannot be ascribed to that compound, because such plaques were also found in the intestine of one bird which was fed only on the control ration. Hyperemia of the intestine may have been caused by amine; however, the effect was not observed in all birds from the same ration.

Hardening of the crop mucosa and enteritis (inflammation of the intestine) were consistent lesions in birds force-fed meal containing 4.89% of octylamine. In three crops out of the five examined, some areas of hardened mucosa were desquamating. Enteritis ranged from moderate catarrhal to a marked diffuse hyperemic inflammation of the upper half of the intestinal tract. The birds which died, Nos. 1C, 2C, and 5C (Table VI), had edema of the lungs. No. 5C also had hemorrhagic plaques in the lower trachea, congestion of the bronchi, and cloudiness of the pericardial sac.

Birds Nos. 8P through 13C (Table VI) all had hardening or desquamation or both of the crop mucosa. Upper intestinal changes in birds 9P through 13C included slight catarrhal enteritis, scattered petechiation, reddened plaques, and diffuse hyperemia. Healing was evident. If this group had been examined earlier than 5 days after the single dose, the lesions probably would have been more prominent.

The principal lesions found in birds force-fed an emulsion of *n*-octylamine involved the crop, upper intestines, and the respiratory system. Hardening of the crop mucosa and traces of a fibrinous pseudomembrane in the small intestine were found in No. 14C, killed 5 days after a single dose. The birds which died after a single dose, Nos. 15C, 16C, and 17C, all had swelling of the anterior lobes of the kidney and hardening and desquamation of the crop mucosa. No. 15C had a severe catarrhal enteritis with desquamation of villi. The lower trachea, bronchi, and lungs were hyperemic. The coronary vessels were congested. No. 16C had a severe catarrhal enteritis and moderate diffuse hyperemia of the upper intestine. Scattered red plaques were present in the duodenum. The lungs were congested and edematous. No. 17C showed swelling of the villi in the upper intestine. The surface appeared chalky. The pharynx and esophagus were necrotic. A marked diffuse hyperemia was present in the proventriculus and anterior portion of the gizzard.

The results of this investigation indicate that *n*-octylamine is not definitely toxic to poultry when it is fed in a practical ration unless the concentration of amine is greater than 0.36% of the ration. Concentrations of 5% of amine are toxic.

The subacute toxicity of *n*-octylamine for poultry appears to be considerably less than the toxicity of octadecylamine for dogs. The lowest level fed to poultry in this test was about twice the highest level fed to dogs in the Armour investigation. Some toxic effects were noted in the latter.

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