Identification of Rapeseed Oil in Olive Oil by Urea Fractionation*

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Olive oil is sometimes adulterated with cheaper vegetable oils. It is difficult to detect the adulteration of olive oil with oils like rapeseed by the conventional tests, such as refractive index, saponification value, and iodine value, especially when the adulterant is present in amounts less than 30%.

However, rapeseed oil contains approximately 35 to 55% of erucic acid, and this acid does not occur in olive oil. The various methods that have been suggested to detect rapeseed oil are based upon the separation and identification of erucic acid. This has been achieved by lead salt precipitation (4), magnesium salt precipitation (8), chromatography (2, 6), permanganate oxidation (1), and low pressure distillation of the methyl esters. Although such methods are effective, they are slow and tedious.

It is known that urea forms adducts with fatty acids by a spiral configuration around the hydrocarbon chain. The short-chain fatty acids do not form adducts as readily as long-chain fatty acids. Further, unsaturated fatty acids do not form adducts as readily as saturated fatty acids of the same chain length. The urea adduct technique has been used to detect butterfat adulteration by separating longer-chain and more saturated fatty acids from the shorter-chain and more unsaturated fatty acids (3, 7). This technique has also been used to detect the adulteration of mustard oil with linseed oil (5). It is the purpose of this paper to report a method, based on the urea adduct technique, by which to detect adulteration of olive oil with rapeseed oil.

METHOD

Reagents

a. *Alcoholic Potassium Hydroxide Solutions.*—(1) Dissolve 50 g KOH in 1 liter of 95% ethyl alcohol. (2) Dissolve 15 g KOH in 1 liter of redistilled 95% ethyl alcohol (the solution should remain clear).

b. *Hydrochloric Acid.*—0.1*N*.

Determination

Weigh 10 g of the oil into a 250 ml Erlenmeyer flask and saponify with 50 ml alcoholic KOH solution, **a**(1), for 30 min. on a hot plate, using an air condenser. Cool the mixture and dilute with 100 ml water. Add 50 ml HCl (1+1) and extract the fatty acids with 50 ml ether in a separatory funnel. Wash the ether extract 3 or 4 times with 25 ml portions of water until free from HCl. Dry the ether extract over 10 g anhydrous Na_2SO_4 for 15 min. Transfer the extract to a beaker, evaporate the ether in a hot water bath, and finally dry the fatty acids at 100°C in an air oven for 1 hr.

Weigh 8 g of the fatty acids into a 250 ml Erlenmeyer flask. Dissolve 30 g urea in 200 ml absolute methanol by heating on a hot plate, and add to the flask containing the fatty acids. Mix contents well, stopper flask, and hold in a water bath at 25°C for 1 hr. Filter the mixture through a Büchner funnel, using Whatman No. 1 filter paper, and wash flask and precipitate once with 25 ml ether. Return precipitate to flask, add 50 ml water, and heat to liberate fatty

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acids. Extract fatty acids with 50 ml ether and wash ether extract twice with 25 ml portions of water. Dry fatty acids as previously described.

Weigh 2 g of the fatty acids obtained from the urea adduct into a 250 ml flask, and add 7.5 g urea and 50 ml methanol. Heat on a hot plate until the solution is clear. Stopper flask and hold in a water bath at 25°C for 1 hr. Filter precipitate through a Büchner funnel, and wash the flask and precipitate once with 25 ml ether. Transfer precipitate to flask, add 50 ml water, and heat on hot plate to separate fatty acids. Extract fatty acids with 50 ml ether and wash ether extract twice with 25 ml water. Dry fatty acids as previously described.

Accurately weigh 0.5–0.7 g fatty acids obtained by foregoing procedure into a 1 ml weighing cup and transfer to a 250 ml Erlenmeyer flask. Add 25 ml alcoholic KOH solution, **a**(2), and gently reflux on hot plate for 30 min., using air condenser. Cool flask slightly and titrate with 0.1*N* HCl (phenolphthalein) until pink color just disappears. Carry out a titration on a blank, using 25 ml alcoholic KOH, **a**(2), in same manner. Calculate saponification value and molecular weight of fatty acids as follows: Saponification Value = 5.61 × (titration of blank − titration of sample)/wt sample. Molecular Weight = 56.1 × 1000/saponification value.

Results and Discussion

The type and amount of fatty acids that are precipitated by the urea adduct technique depend upon the concentration of urea in methanol and the temperature of precipitation. Erucic acid, a major constituent of rapeseed oil, has a high molecular weight, and despite the fact that it is unsaturated, can be precipitated along with the saturated fatty acids by using the suitable amounts of urea and methanol. Subsequent determination of the molecular weight of the fatty acids obtained from the adduct will indicate the presence or absence of rapeseed oil in olive oil.

The temperature for the adduct formation was kept at 25°C in all the experiments. The effect of urea concentration on the fatty acid fractionation was studied by varying the amount of urea in the mixture. The results obtained are shown in Table 1.

It is seen that 10% urea in methanol gave no significant amount of precipitate. When the concentration of urea was increased to 15%, most of the saturated fatty acids, along with a small amount of oleic acid, were precipitated. When the urea concentration was about 20%, increasing amounts of oleic acid were precipitated, as seen from the iodine values of the fatty acids obtained from the adduct. When the olive oil contained 10% rapeseed oil, the increase in the molecular weight of the fatty acids obtained was not great; this indicated that the proportion of erucic acid in the precipitate was small. Therefore a second precipitation, using 15% urea solution in methanol, was carried out. This improved the sensitivity and reliability of the procedure.

Table 1. Effect of urea concentration upon fractionation of fatty acids[a]

Urea Concentration, %	Fatty Acids Recovery, g	Iodine Value, Hanus	Mol. Weight Fatty Acids
	Olive Oil		
10.0	—	—	—
12.5	0.7	56.8	277.5
15.0	1.2	58.7	278.4
20.0	2.4	72.0	281.6
	90% Olive + 10% Rapeseed Oil		
10.0	—	—	—
12.5	0.8	57.9	281.9
15.0	1.3	62.8	282.3
20.0	2.6	77.3	284.3

[a] 4 g fatty acids + 100 ml MeOH + urea as shown; held a 25°C for 1 hour.

The relationship between the molecular weight of the fatty acids obtained after two precipitations, using 15% urea solution, and the amount of rapeseed oil in olive oil is shown in Fig. 1. The molecular weight of the fatty acids was found to increase significantly when the olive oil contained as little as 10% rapeseed oil. In addition, a straight line relationship was observed between the molecular weight of the fatty acids and the amount of rapeseed oil present in olive oil. The slope of the line will depend upon the erucic acid content of rapeseed oil. These data refer to rapeseed oil containing approximately 40% erucic acid.

The molecular weights of the double urea-

fractionated fatty acids from 145 authentic samples of olive oil packed in ten different countries were determined; the results are summarized in Table 2. The values range from 269.1 to 275.6 with an average value of 272.4.

The reproducibility of the method was studied by carrying out triplicate analyses on four successive days upon olive oil and olive containing 40% rapeseed oil. Averages of triplicates were found to be in good agreement (Table 3), and there was no significant variation in the results when the analyses were carried out on different days.

Three olive oil samples were submitted for collaborative analysis to 2 analysts in each of 6 different laboratories. Sample A was pure olive oil and Samples B and C contained 20% and 40% rapeseed oil, respectively. Each collaborator analyzed the samples on three consecutive days in different weeks. The differences in the averages obtained by three analyses in the first week and second week were not significant. The average of six molecular weight determinations on each sample is shown in Table 4.

There was no significant difference in the results of the first 9 collaborators. The results of Collaborator No. 10 were found to be consistently about 2% low, and those of Collaborators 11 and 12 were found to be consistently about 3% and 2% high, respectively, on all samples. These differences may be ascribed to variations in the normality of the acid used. If the results of the last three collaborators are omitted, the average of the 6 analyses by each of the remaining collaborators falls within 0.7% of the mean molecular weight.

Table 2. Molecular weights of fatty acids obtained by urea method from authentic olive oils

Country of Origin or Packing	No. of Samples	Molecular Weights of Urea Fractionated Fatty Acids
Italy	30	269.3–275.3
France	17	269.1–275.3
Spain	28	269.7–275.5
Greece	16	270.5–275.4
Canada	20	269.3–275.4
Tunisia	15	269.2–275.3
Lebanon	10	271.9–275.6
N. Africa	5	269.6–271.8
U.S.A.	3	269.8–274.5
Portugal	1	270.5

Total: 145. Average: 272.4. S.D. ±1.71
95% confidence limits: 269.0–275.8
99% confidence limits: 267.9–276.9

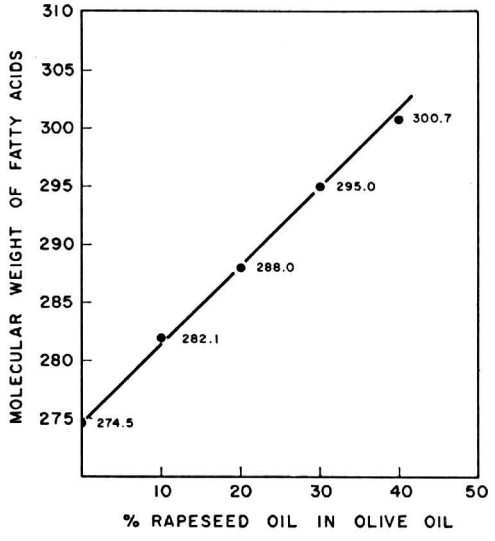

Fig. 1. Relationship between molecular weight of the fatty acids obtained and the amount of rapeseed oil in olive oil.

Table 3. Reproducibility of urea fractionation method

Day	Molecular Weight of Urea Fractionated Fatty Acids: Pure Olive Oil			Av.	60% Olive–40% Rapeseed			Av.
1	274.9	274.6	274.7	274.7	298.0	297.5	298.4	298.0
2	274.3	274.7	274.5	274.5	299.1	298.9	298.4	298.8
3	275.3	274.2	275.7	275.1	298.2	300.5	299.3	299.3
4	274.7	274.0	274.0	274.2	299.5	299.5	299.6	299.5

Av.: 274.6; Std. Dev: ±0.50
95% confidence limits: 273.5–275.7
99% confidence limits: 273.1–276.1

Av.: 298.9; Std. Dev.: ±0.84
95% confidence limits: 297.1–300.7
99% confidence limits: 296.3–301.5

Table 4. Collaborative analysis of olive oil samples

Collaborator No.	Average Molecular Weight of Fatty Acids Obtained by Urea Method		
	Sample A[a]	Sample B[b]	Sample C[c]
1	272.1	284.8	295.4
2	273.6	286.8	297.7
3	275.0	287.9	298.3
4	274.7	288.2	298.9
5	273.0	284.5	298.6
6	272.9	284.8	296.0
7	274.6	287.8	298.5
8	271.9	285.5	295.5
9	273.4	284.7	295.4
Average (1–9)	273.5	286.1	297.1
10	268.9	281.8	295.1
11	280.9	294.0	304.6
12	278.1	290.7	302.4

[a] Sample A: 100% olive oil+0% rapeseed oil.
[b] Sample B: 80% olive oil+20% rapeseed oil.
[c] Sample C: 60% olive oil+40% rapeseed oil.

In 1957 the urea adduct method was applied to a survey of 160 samples of Canadian market oils labelled as olive oils. Table 5 shows the chemical characteristics and the molecular weight of the fatty acids obtained by the urea adduct method on samples of five different brands. The sensitivity of the urea method is indicated from the fact that the first 4 samples (Brand A) were found to be adulterated with rapeseed oil even though 2 of these samples were within the range of the requirements under the Food and Drugs Act for saponification value, refractive index, and iodine value. Samples 1, 4, 5, and 8 were further examined in detail by fractional distillation of their methyl esters in order to confirm the reliability of the urea method. Erucic acid was isolated from samples 1 and 4 and the amounts determined corresponded with approximately 25% and 45% of rapeseed oil. These amounts of rapeseed oil agree closely with those obtained from Fig. 1 on the same samples by the urea adduct technique. No erucic acid was isolated from samples 5 and 8; this proved the absence of rapeseed oil and thus confirmed the results obtained by the urea method. The analysis of the commercial samples of olive oil indicates that the urea method is reliable and can be used to detect the adulteration of olive oil with rapeseed oil.

Summary

A relatively rapid and reliable method based on the fractionation of olive oil fatty acids from a solution of urea in methanol is described. The molecular weights of the fatty acids obtained by two urea fractionations from 145 authentic samples of olive oil were found to range from 269.1 to 275.6 with an average value of 272.4. Olive oils showing a value of the molecular weights of the fatty acids in excess of 280 may be considered as adulterated with an oil containing

Table 5. Analysis of olive oil samples sold in Canada

Brand	Sample No.	Sap. Value	Refractive Index at 20°C	Iodine Value	Mol. Weight (Urea Method)	Estimated[a] Rapeseed Oil, %	Erucic[b] Acid, %	Estimated[a] Rapeseed % (Fractiona Distillation Method)
A	1	188.7	1.4700	91.7	291.2	24	10.2	25
A	2	188.8	1.4700	91.8	292.9	27	—	—
A	3	186.8	1.4702	93.6	295.4	31	—	—
A	4	185.6	1.4709	95.7	301.6	40	16.8	45
B	5	190.7	1.4692	83.8	275.1	0	0.0	0
B	6	192.0	1.4686	82.1	272.9	0	—	—
B	7	192.8	1.4691	83.0	272.8	0	—	—
C	8	191.3	1.4696	86.8	270.8	0	0.0	0
D	9	191.8	1.4688	82.4	272.6	0	—	0
E	10	193.4	1.4690	84.3	270.0	0	—	—
Food and Drug Limit Limits:		185–195	1.4680–1.4700	77–94				

[a] Assuming erucic acid content of rapeseed oil to be 40%.
[b] Erucic acid isolated by fractional distillation of the methyl esters.

erucic acid glycerides. The urea adduct method has been employed in Canada to detect the adulteration of olive oil with rapeseed oil.

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Gas Chromatography of Aromatic Amines and Nitro Compounds*

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In the course of an investigation of the composition of commercial samples of FD&C Red No. 1 (Ponceau 3 R), it was necessary to analyze complex mixtures of C_7 through C_{10} aromatic amines obtained by reduction of the dye. Infrared spectrophotometry was used extensively in this study; however, this technique does not give satisfactory results for all mixtures.

As part of this investigation it was necessary to do the following: (*1*) Determine which of the theoretically possible isomeric nitro compounds were produced from nitration of the various hydrocarbons; (*2*) determine the relative amounts of each isomer present in the mixture of nitro compounds obtained by the nitration reaction; (*3*) analyze various fractions of the mixtures of nitro compounds in order to monitor purification processes such as fractional distillation, crystallization, etc.

In many cases such mixtures cannot satisfactorily be analyzed by infrared spectrophotometry because of the close similarity of the spectra of some of the isomeric nitro compounds.

Numerous reports indicate that gas chromatography is an excellent technique for the analysis of complex mixtures; this technique, when applicable, is a simple, fairly rapid procedure well suited for routine analyses. Therefore, it was decided to investigate the possibility of using gas chromatography for the analysis of the mixtures mentioned above.

Several reports of the gas chromatography of amines have appeared in the literature (1–3). James (3), in determining the relative retention volumes of various amines on paraffin wax, lubrol MO, and benzyl diphenyl, noted that the xylidines are retained for at least thirty minutes at a column temperature of 137°C. Since we were interested in the amines up to isoduridine, it was obvious that we would have to employ much higher temperatures if all the amines were to be determined in a reasonable length of time. For this reason our

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search for a liquid phase was limited to one that would be stable up to at least 200°C.

Apparatus and Procedures

The gas chromatography apparatus used was assembled in this laboratory and is of conventional design. The detector is a Gow-Mac model NRL thermal conductivity cell whose response is fed to a Varian Associates model G-10, 0–10 mv, graphic recorder.

The chromatographic column is an 8′×¼″ o.d. copper tube packed with 20 grams of Firebrick C-22 (30–60 mesh) impregnated with 8 grams of DC 710 Silicone Fluid. The column was packed by vibration with a Burgess Vibrograver (4).

Analyses are made at a flow rate of helium of about 20 ml/minute (inlet) and a column temperature of 200°–210°C. The sample inlet is heated to about 260°C by a heating tape in order to flash-volatilize the samples. Samples of 0.005 to 0.025 ml are injected onto the column through a rubber serum bottle cap with a Hamilton Co. microsyringe of 0.05 ml capacity.

The areas under the curves are used for quantitative determination (5). In this work, areas were determined with an Instron Automatic Integrator (Model A-30). When the concentration of the component to be determined is less than 1%, the signal from the thermal conductivity cell is amplified by a factor of 20 by a Kin Tel Model 111A D.C. amplifier before being fed to the recorder. When this amplifier is used it is not possible to determine the peak areas with the integrator; therefore the method of "peak height times half-band width" (5) is used to calculate concentration.

Results and Discussion

Apiezon M, Polyethylene Glycol "600," di-octyl phthalate, tricresyl phosphate, and a variety of silicones were among the many liquid phases tested and found to be unsatisfactory for this work. Good separations were obtained with Apiezon M, but the column was easily overloaded. The other liquids would not give a stable zero line at 200°C, probably because of the observed "bleeding" of the liquid from the column. The DC 710 Silicone Fluid was selected as the liquid phase because it was as efficient as any phase tried and the column described would handle samples of up to 0.025 ml with little loss of resolution. This liquid phase gives a very stable zero line; however, it is evident that the liquid does slowly bleed from the column, since a column loses much of its resolving power after several weeks at 200°C. Columns that have been operated at 200°C for 56 hours will no longer separate the ortho-xylidines from the meta-xylidines.

Table 1 lists the various compounds encountered in this study and shows the retention time of each. The retention times are corrected to these standard conditions: Column temperature, 200°C; flow rate, 20 ml/minute. In routine analyses, it is not necessary to control the temperature and flow rate precisely from day to day. The retention times will vary with the temperature and flow rate, but the relative retention times remain constant over a fairly wide range of temperatures and flow rates. We have found that essentially the same resolution is obtained at temperatures of 185° to 220°C and flow rates of 13 to 28 ml/minute.

To obtain an accurate determination of a component of a mixture it is necessary that its retention time differ by at least two minutes from that of any other component of the mixture. If the retention times differ by one minute, a good approximation of the concentration of each component can be made by noting the position of the shoulder on the peak. If the retention times differ by less than one minute, only the total concentration of these components can be determined.

In general it is noted that the amines are separated into isomeric groups. Usually, therefore, a mixture consisting of homologous aromatic amines can be separated easily into groups of isomeric compounds. In some cases the isomeric amines can also be separated; for example, mesidine is well separated from pseudocumidine and 1-amino-2,3,4-trimethylbenzene. As a rule, very closely related isomers such as m-xylidine and p-xylidine are not completely separated.

Table 2 shows the results of several analyses of known mixtures of aromatic amines very similar to the mixtures obtained by reduction of commercial samples of FD&C

Table 1. Retention times of amine- and nitro-substituted hydrocarbons

Parent Hydrocarbon	Substitution	Retention Times, min. Amine	Nitro
Toluene	1-X, 2-CH_3	8	11½
	1-X, 3-CH_3	8	—
	1-X, 4-CH_3	8	14½
Ethyl benzene	1-X, 2-C_2H_5	10½	14½
	1-X, 4-C_2H_5	11	—
Meta-xylene	1-X, 2,6-Di-CH_3	11½	11½
	1-X, 2,4-Di-CH_3	11½	—
	1-X, 3,5-Di-CH_3	11½	—
Para-xylene	1-X, 2,5-Di-CH_3	11½	18½
Ortho-xylene	1-X, 2,3-Di-CH_3	13½	19
	1-X, 3,4-Di-CH_3	13½	—
Mesitylene	1-X, 2,4,6-Tri-CH_3	15½	18
Pseudocumene	1-X, 2,3,6-Tri-CH_3	19½	21
	1-X, 2,4,5-Tri-CH_3	19½	33
	1-X, 2,3,5-Tri-CH_3	16½	28½
Hemimellitene	1-X, 2,3,4-Tri-CH_3	23½	36½
	1-X, 3,4,5-Tri-CH_3	23	45
Para-ethyltoluene	1-X, 2-CH_3, 5-C_2H_5	15	24
	1-X, 2-C_2H_5, 5-CH_3	14	22½
Meta-ethyltoluene	1-X, 2-CH_3, 6-C_2H_5	13½	16½
	1-X, 2-CH_3, 4-C_2H_5	16	26
	1-X, 2-C_2H_5, 4-CH_3	15	22½
Ortho-ethyltoluene	1-X, 2-CH_3, 3-C_2H_5	17	26
	1-X, 2-C_2H_5, 3-CH_3	14	24
	1-X, 3-CH_3, 4-C_2H_5	16	34½
	1-X, 3-C_2H_5, 4-CH_3	16	35
Tetra-methylbenzene	1-X, 2,4,5,6-Tetra-CH_3	32	
	1-X, 2,3,5,6-Tetra-CH_3	32	

Table 2. Gas chromatographic separation of known mixtures

Component	Added, %	Found, % 1	2	3
#1				
Meta-xylidine	51.5	55	54	51
Ortho-xylidine	16.4	16	15	17
Mesidine	1.2	1.2	1.1	1.0
Pseudocumidine	28	26	27	27
Isoduridine	2.8	2.9	2.3	2.9
#2				
Meta-xylidine	55.4	58	56	57
Ortho-xylidine	10.7	8.8	9.9	11
Mesidine	0.46	0.4	0.4	0.3
Pseudocumidine	30.9	29	30	28
Isoduridine	3.1	3.4	3.4	3.6
#3				
Meta-xylidine	56.1	58	57	56
Ortho-xylidine	10.3	9.5	10	10
Mesidine	0.32	0.3	0.4	0.3
Pseudocumidine	30.2	29	29	29
Isoduridine	3.0	3.4	3.2	3.2

Table 3. Separation of amines derived from commercial samples

Component	Sample A, %		Sample B, %		Sample C, %	
	1	2	1	2	1	2
Meta- and para-xylidines	64	65	74	71	60	61
Ortho-xylidine	3.4	2.7	0.0	0.0	3.8	3.3
Mesidine	0.2	0.2	0.1	0.1	0.1	0.1
Pseudocumidine	32	33	25	26	34	33
Isoduridine	1.1	1.0	0.5	0.3	1.7	1.4

Red No. 1. The results appear to be satisfactory. Table 3 shows the results of several analyses of samples of amines obtained by reduction of commercial samples of FD&C Red No. 1.

When the resolution of the amines by gas chromatography was not possible, we have had some success in separating amine mixtures into groups on a preparative scale (1″ o.d.) gas chromatography column and then analyzing these simplified mixtures by infrared spectrophotometry. A description of the construction and operation of this large column is given in *This Journal*, (p. 753).

The retention times of the nitro compounds are influenced to a considerable extent by the relative positions of the nitro and alkyl groups. In all cases in which an alkyl group is ortho to the nitro group, the retention time of the compound is considerably less than that of the corresponding meta- or para-alkyl nitro compound. This effect is enhanced strongly, of course, in the 2,6-dialkyl nitrobenzenes. These results are readily explained by intramolecular hydrogen bonding. It is interesting to note, in the case of the methyl-ethyl-nitrobenzenes, that the isomer with an ethyl group ortho to the nitro group has a lower retention time than the isomer with the same positions substituted but with the methyl group ortho to the nitro group. Apparently the methylene group is more effective than the methyl group in forming intramolecular hydrogen bonds. The hydrogen bond effect aids greatly in the separation of isomeric nitro compounds, as shown in Table 1.

Gas chromatography proved very useful for the determination of the yields of the isomeric compounds produced in nitrations of hydrocarbons and for monitoring the fractions from fractional distillation of the crude products. In several cases the presence of two compounds in a fraction was indicated by gas chromatography although only one had been indicated by other methods of analysis. In each case further work proved conclusively that two compounds were indeed present.

In addition to the compounds listed in Table 1, some work has been done on the determination of N-substituted aromatic amines. The N-methylamino alkyl benzenes are easily separated from the parent primary amines and from the next higher homologous amines by the technique described above.

Preliminary work indicates that the DC 710 Silicone Fluid used in this study is useful as the liquid phase for gas chromatography of a wide variety of aromatic compounds with boiling points from 160° to 300°C.

Summary

A gas chromatographic method for the analyses of complex mixtures of aromatic amines has been developed. This technique readily separates the members of a homologous series of amines in the C_7 to C_{10} range, and, in several cases, separates isomeric compounds. The method has been successfully applied to the analysis of amines obtained by reduction of commercial samples of FD&C Red No. 1.

The same technique is applicable to the corresponding nitro compounds and has been used with success in the analysis of mixtures of isomeric aromatic nitro compounds obtained from the nitration of C_9 hydrocarbons.

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A New Procedure for the Collection of Fractions in Gas Chromatography*

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Various methods for the detection of fractions from gas chromatography have appeared in the literature (1–4). The fact that almost all detectors give strictly quantitative and not qualitative data has made the trapping of fractions and their subsequent characterization necessary. The methods of collecting fractions have been discussed and the difficulties examined by a number of authors (5–8).

This paper describes a method for quantitatively collecting fractions from a gas chromatograph. The system is based on mixing the vapor emerging from the column with vapors of a suitable solvent, and then condensing the gaseous mixture of eluted material and solvent.

The fractions emerging from the condenser are liquid and may be collected in the type of equipment commonly used in liquid chromatography. The collected fractions can then be analyzed by a suitable method, such as ultraviolet spectrophotometry, which gives both qualitative and quantitative data.

The method has been used for both analytical and preparative scale columns, and these applications are described separately.

Applications to Analytical Scale Columns

Apparatus

The apparatus, with the exception of the collection system, is of conventional design and was assembled in this laboratory.

The collection system consists of a vapor generator, controlled by a variable transformer, connected by $\frac{1}{4}$" o.d. copper tubing and a tee joint to the end of the chromatographic column and to a condenser as shown in Fig. 1. The condenser was made by wrapping $\frac{1}{4}$" copper tubing with a spiral of $\frac{1}{8}$" copper tubing and soldering in place.

The solvent vapors are heated approximately to the temperature of the column by a heating tape wrapped around the tubing that connects the generator to the column.

We have used a fraction collector operated by a time switch to collect the condensate. Throughout the work described here, a time of $\frac{1}{2}$ minute per fraction was used. The rate of vaporization of the solvent was adjusted to give about 5 ml per fraction.

Experimental

A known mixture of aromatic amines containing 60% *m*-xylidine, 10% *o*-xylidine, and 30% pseudocumidine was prepared, and 0.005 ml of this prepared mixture was chromatographed on a column of DC-710 silicone fluid on C-22 firebrick. The column temperature was 200°C and the flow rate was 20 ml/min. of helium. Water was used in the generator.

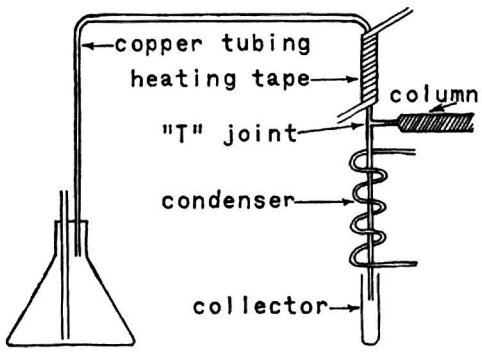

Fig. 1. Diagram of apparatus for solvent collection.

The collected fractions were analyzed by ultraviolet spectrophotometry on a Cary Model 11 spectrophotometer equipped for automatic operation. The results are shown in Fig. 2.

One part of the mixture was then diluted with twenty parts of ethanol, and 0.005 ml of this solution was chromatographed under

* Presented at the Seventy-second Annual Meeting of the Association of Official Agricultural Chemists, Oct. 13–15, 1958, Washington, D.C.

identical conditions. The fractions were analyzed, using a Cary Model 14 spectrophotometer with a 10 cm cell. The results are shown in Fig. 3. Next 0.02 ml of a mixture containing 1% isoduridine, 0.3% toluidine, 39% pseudocumidine, and 60% *m*-xylidine was chromatographed under the same conditions. The fractions were again analyzed with the Cary Model 14 spectrophotometer and a 10 cm cell. The results are shown in Fig. 4.

Application to Preparative Scale Columns

Apparatus

The preheater consists of 8 feet of ¼″ o.d. tubing. The tubing was coiled and wrapped with a heating tape. In use the preheater was maintained at a temperature about 50°C higher than that of the column.

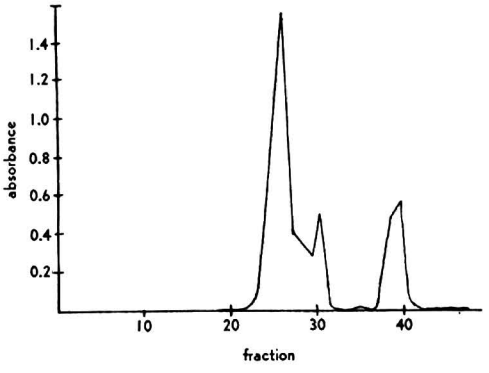

Fig. 2. Absorbance vs. fraction number; 0.005 ml sample of amine mixture. Analytical column.

The sample chamber is similar to that described by Kirkland (8). A section of brass tubing 1″ in diameter and 5″ long was connected to a piece of tubing ¼″ in diameter and 3″ long to make a tee. The 1″ tubing was packed with ⅛″ diameter steel balls, and connected to the column and the preheater with Swagelok (C) fittings. A rubber serum bottle cap was placed on the end of the ¼″ tubing so that the sample could be added with a hypodermic syringe and needle. In use the chamber is heated to about 50–100°C above the temperature of the column with a heating tape.

The heating jacket consists of a 10 foot length of 1½″ diameter pipe wrapped with

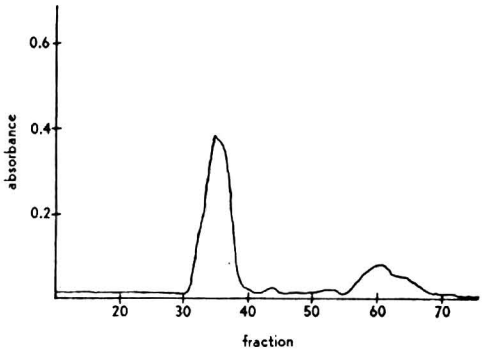

Fig. 3. Absorbance vs. fraction number; 0.00025 ml sample of amine mixture. Analytical column.

resistance wire and lagged with asbestos (10). The temperature is controlled by the use of a variable transformer.

The chromatographic column was prepared by packing a 10 foot length of 1″ diameter aluminum tubing with C-22 firebrick carefully size-graded to 30–60 mesh and impregnated with the liquid phase. Packing was accomplished by holding a Burgess Vibro-graver against the column. The entire length of the column was enclosed in the heating jacket. The collection system is identical to that used with the analytical scale column.

Experimental

Figure 5 shows the results of the separation of 200 mg of a known mixture of aromatic amines on the large column under the

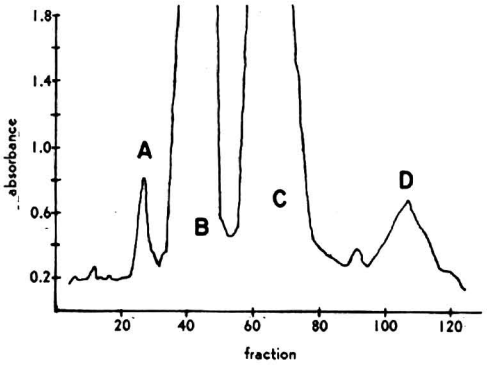

Fig. 4. Absorbance vs. fraction number; 0.02 ml sample of amine mixture. A: Toluidine. B: Xylidines. C: Pseudocumidine. D. Isoduridine.

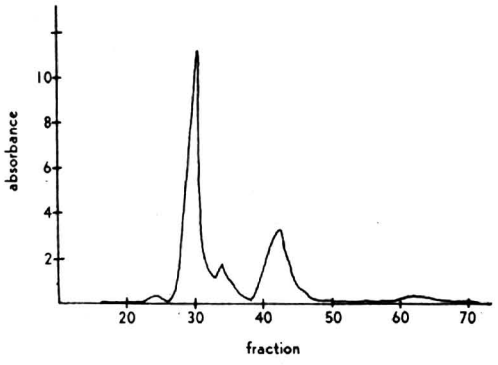

Fig. 5. Absorbance vs. fraction number; 200 mg sample of amine mixture. Preparative column.

following conditions: Column temperature, 195°C; flow rate, 1 liter/minute (N_2); liquid phase, DC-710 silicone fluid (40% on C-22 firebrick) (9); solvent, water. Figure 6 shows the separation of 500 mg of a similar mixture under the same conditions.

Pure samples of 1-nitro-2-methyl-4-ethylbenzene and 1-nitro-2-ethyl-4-methylbenzene were prepared by chromatographing 1 gram of a mixture of the two compounds[1] under the same conditions, except that ethanol was used as the solvent. Aliquot portions of the collected fractions were diluted and analyzed by ultraviolet spectrophotometry to obtain the curve shown in Fig. 7. The indicated fractions were then combined, diluted with water, and extracted with ether to isolate the pure components.

Discussion

Figure 3 shows the experimental limit of sensitivity in the detection of pseudocumidine. This limit is about 75 micrograms. Theoretically, the sensitivity should be much greater if the 200 mμ peak of absorption is used. Practically, however, the background absorption, because of minute contamination, limits the use of this peak.

It should be emphasized that the sensitivity can be increased appreciably by concentrating the fractions. This concentration takes place automatically in the case of lower boiling components because of their smaller band width. The same effect could be accomplished by lengthening the time per fraction and decreasing the rate of solvent vaporization. This would, however, also decrease the effective resolution.

One difficulty encountered in the application of this collection system to large samples is mixing of the fractions due to hold-up in the condenser. It was found that this problem could be eliminated in either of two ways: (1) increasing the rate of solvent vaporization to insure complete flushing of the condenser; (2) using smaller diameter tubing ($\frac{1}{8}$" o.d.) for the condenser.

Figures 6 and 7 show the effect of overloading the large column. Since the $\frac{1}{4}$" o.d. column will accommodate samples up to 0.025 ml, the large column would be expected to give equal resolution on samples up to 400 mg if all conditions were the same. Experimentally it was found that the large column gives comparable resolution on samples up to about 300 mg. For larger samples, of course, resolution must be sacrificed.

Recovery of the fractions of known mixtures has been better than 95% in all cases tested.

In the work described we have used ultraviolet spectrophotometry exclusively for the analysis of fractions. It is obvious that other methods of analysis could be used. Ultraviolet spectrophotometry was particularly suited to the analyses described here because of the sensitivity of the method and also because qualitative information could often be gained by visual examination of the

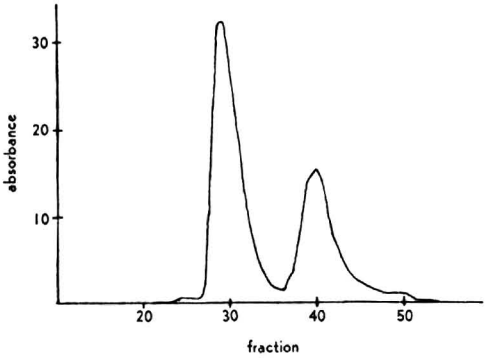

Fig. 6. Absorbance vs. fraction number; 500 mg sample of amine mixture. Preparative column.

[1] This mixture was obtained by vacuum distillation of the reaction products of nitration of *m*-ethyltoluene. These two compounds cannot be readily separated by distillation.

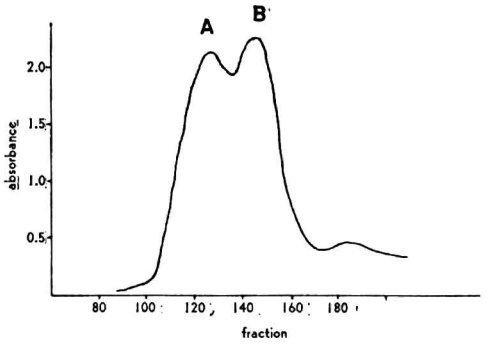

Fig. 7. Absorbance vs. fraction number; 1.0 gram of mixture of nitro compounds. Preparative column. A: 2-Ethyl-4-methyl nitrobenzene. B: 2-Methyl-4-ethyl nitrobenzene.

spectra. It is also obvious that solvents other than those used here can be used for the collection.

Conclusions

A method for quantitative collection and determination of fractions eluted from gas chromatographs has been described. The sensitivity of detection is comparable to that of thermal conductivity methods, and quite often the fractions are qualitatively identified.

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Some Micro-Crystal Tests for Colchicine

By CHARLES C. FULTON (Division of Microbiology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D.C.)

Colchicine, a very poisonous alkaloid, is official in the United States Pharmacopeia. It is used as a specific for gout and also has been frequently used in horticulture to double chromosomes. Occasions may therefore arise when microquantities of this alkaloid must be identified. For that matter, good microcrystal tests might well be used for identifying colchicine in any quantity, as is already true of a number of other alkaloids. Up to this time, however, no satisfactory microcrystal tests for cholchicine have been known.

Amelink (1) reported that colchicine forms only amorphous precipitates. The Behrens-Kley text (2) includes colchicine in a list of alkaloids giving amorphous or no precipitates, or at most forming drops, with all known reagents. This has been the general experience among analysts; however, researchers have found a few indications of crystallization. Barth (3), who listed a number of amorphous precipitates, said that, although picric acid by itself did not precipitate colchicine, the addition of some concentrated HCl threw out crystals which could be distinguished from those of the reagent alone. Rosenthaler (4) credits Klein

and Pollauf (no reference given) with obtaining crystals by using phosphotungstic acid and concentrated HCl (no proportions stated); they also obtained crystals, which could be distinguished from crystals of the reagent, by using just enough platinum thiocyanate to unite with the colchicine and slowly drying the test drop. Brühl (5) stated that when a colchicine solution containing some HCl is mixed with an excess of gold chloride solution, a precipitate that will crystallize in time is produced. Amelink, Behrens, Stephenson, and others who have used gold chloride for microcrystal tests do not confirm Brühl; they do not report having obtained any crystals at all with gold chloride. Stephenson (6) listed "no tests" (all precipitates amorphous) for colchicine in his summary; however, in his detailed descriptions he said that a few fine needles sometimes formed from the amorphous precipitate produced by iodine-KI solution (1:5 in 100 ml) with a 1:50 solution of the alkaloid.

Nevertheless, good crystal tests can readily be obtained with colchicine, if the right reagents are used, and Brühl and Stephenson were on the right track. Three types of such tests are described in this paper: the chloraurate and the bromaurate from strong H_2SO_4 or H_3PO_4, and the iodine-KI precipitate from neutral or faintly basic aqueous solution. The last of these three tests seems to be a barbiturate type of reaction that is little known or understood. Colchicine contains a -CO-group adjacent to the -NH- group; in barbiturates, of course, a -CO- group is on each side of both -NH- groups.

Colchicine is easily hydrolyzed to colchiceine (e.g., by heating in dilute acid). It merely loses a methyl group in another part of the molecule, at some distance from the -NH- group. The possible bearing of this reaction on the acid reactions given in this paper has not been studied.

Test Procedures and Crystals Formed

1. Chloraurate Crystallization

Chloraurate crystallization occurs readily in a medium of sulfuric acid (1+1) or syrupy phosphoric acid. Absorption of appreciable water from the air must be prevented; if too much water is present it should be removed by some drying agent. The reagent concentrations refer to weight in grams of $HAuCl_4 \cdot 4H_2O$ crystals per ml of solution.

(a) Add a drop of 1:20 $HAuCl_4$ in H_2SO_4 (1+1) to a little colchicine thinly scattered on a slide, and put a cover glass over the drop. Crystals form within 10 minutes; they are mainly brown, dense rosettes of needles, quite birefringent. At higher colchicine concentration some crystals will grow out into pointed plates. With more dilute reagent (1:60) these pointed plates will sometimes form attractive rosettes and show spangle-colors with crossed nicols, but crystallization does not take place as readily.

(b) Dissolve a little colchicine in a droplet of H_3PO_4 (2+1) on a cavity slide, add a drop of 1:20 $HAuCl_4$ in syrupy H_3PO_4, and cover with an ordinary slide. Within about 10 minutes rosettes of birefringent needles are formed; crystallization becomes complete on standing. Or stir the colchicine into a drop of 1:60 $HAuCl_4$ in syrupy H_3PO_4, and cover.

(c) To a drop of aqueous solution of colchicine on a slide, add a drop of 1:20 $HAuCl_4$ in (2+1) or (1+1) H_3PO_4. Pour a little concentrated H_2SO_4 into a small watch glass, set the slide on it, and invert a petri dish over both. Crystal formation should begin within 2 or 3 hours; after standing overnight the whole precipitate is crystalline. Birefringent threads form dense rosettes, the larger ones somewhat irregularly developed, with branching threads.

2. Bromaurate Crystallization

Bromaurate crystallization takes place more slowly than does chloraurate, but may be achieved by the same method described in 1(c). To prepare the reagent use 1 g $HAuCl_4 \cdot 4H_2O$ crystals, 1.5 ml HBr (40%), and enough (3+1) or (1+1) H_3PO_4 to make 25 ml. Add one drop of this reagent to an aqueous drop of colchicine, place over some concentrated H_2SO_4 to absorb the moisture, cover, and let stand overnight. The crystals are fine, branching needles. Grains and round black spots are also formed. Some of the reagent $HAuBr_4$ may crystallize out in long orange-red rods. If the drop is then

permitted to absorb a little moisture from the air, the reagent crystals redissolve and the colchicine crystals remain. They are birefringent but are not especially bright, with crossed nicols.

3. Iodine-KI Crystallization

Dissolve a little colchicine alkaloid in a drop of water (not acidified). If a solution is used and it is already acid, add a slight excess of solid $NaHCO_3$. Prepare a reagent solution containing 10 g iodine and 50 g KI made to 100 ml with water, and add 1 drop of this reagent. Precipitation is immediate and crystals form promptly in needles and plates which have very little color and are only slightly birefringent. A fairly high content of potassium ion in the test drop is even more essential to this reaction than exceptionally high iodine.

Acknowledgment

The study was carried out on a gift sample of colchicine from the Lilly Research Laboratories.

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A Gravimetric Modification of the Wilson Method for the Determination of Phosphorus*

By C. H. PERRIN (Canada Packers Limited, Toronto, Ontario, Canada)

The primary objective of this research was to develop a referee method of the highest possible accuracy for the determination of phosphorus in such materials as fertilizers, animal feeds, and foodstuffs. Speed and economy, while desirable, were relatively unimportant considerations. Therefore, it was decided that all critical measurements of solutions would be made by weight rather than by volume, and whenever justified, unusually large amounts of sample and of reagents would be used. The plan also provided for a high proportion of parallel determinations on standard samples which were as similar as possible to the unknown samples.

Three methods looked promising: the A.O.A.C. gravimetric method (1), the differential spectrophotometric method based upon the molybdovanadophosphate color complex (5), and the Wilson method (3, 7, 8) wherein the phosphate is precipitated as quinolinium phosphomolybdate. At first the differential spectrophotometric method was favored because it gave higher precision than the A.O.A.C. gravimetric method or the Wilson method.

A disadvantage of the Wilson method for work of the highest accuracy is its dependence on an alkalimetric measurement of the precipitate. Wilson's attempts to develop a gravimetric method were unsuccessful, apparently because the precipitate tenaciously retained traces of water. However, when it was observed that the quinolium phosphomolybdate could be ignited at 550°C to phosphomolybdic anhydride with a P_2O_5 content of only 3.947%, it appeared possible to develop a method even more precise than the spectrophotometric method.

* Presented at the Seventy-second Annual Meeting of the Association of Official Agricultural Chemists, Oct. 13–15, 1958, at Washington, D.C.

Later it was discovered that a more advantageous weighing form corresponding to the formula $(C_9H_7N)_3H_3[PO_4 \cdot 12MoO_3]$ resulted from simply drying the precipitate at 200–250°C. With a molecular weight of 2212.89, this compound is 3.2074% P_2O_5 or 1.3998% P. Additional advantages of this weighing form over the phosphomolybdic anhydride are that it requires no muffle furnace and it is less hygroscopic. Besides, in the case of the ignition method, it was observed that the weight of the precipitate passed through a minimum before becoming constant. Duval (4) explains this phenomenon as a transient reduction of the molybdenum followed by air oxidation. Therefore the risk of error appears less likely in the oven drying procedure than in the ignition procedure.

The referee procedure presented here approaches the volumetric methods in speed, and, partly because of the low percentage of phosphorus in the precipitate, exceeds in precision methods based upon magnesia precipitation. Other advantages of the proposed method over one or more of the standard methods for phosphorus are:

1. Relatively little interference occurs in the presence of such ions as sulfate, fluoride, iron, silicate, manganese, copper, etc.
2. The solubility of the precipitate is very low.
3. Only very simple apparatus is required.
4. No standard solutions or difficult end points are involved.
5. The precipitating solutions are stable when stored as directed.
6. Careful control of the concentration of orthophosphate ion is unnecessary.

A routine procedure is also presented. This differs from the referee procedure chiefly in the use of volumetric glassware and smaller amounts of reagents.

METHOD

Reagents

a. *Citric-Molybdate Solution.*—Stir 54 g reagent grade MoO_3[1] with 200 ml H_2O. Add 11 g reagent grade NaOH and stir while heating until the MoO_3 dissolves. Dissolve 60 g pure citric acid in about 250 ml H_2O and add 140 ml concentrated HCl. Pour the molybdate solution into the acid solution, stirring throughout the addition. Cool, filter, and dilute to 1 liter. (This solution may be slightly green or blue, and the color deepens on exposure to light.) If necessary, add drop by drop a dilute (0.5–1%) solution of $KBrO_3$ until the green color becomes pale. Store in the dark in a polyethylene bottle (glass is not suitable). Keep cap on bottle when not in use.

b. *Quinoline Solution.*—Place 60 ml concentrated HCl and 300 or 400 ml H_2O into a 1 liter beaker and warm to 70 or 80°C. Pour 50 ml pure synthetic quinoline (free from reducing agents) in a thin stream into the dilute acid with stirring. When the quinoline is dissolved, cool, dilute to 1 liter, and filter. Store in a polyethylene bottle.

Apparatus

a. *All-Polyethylene Wash Bottle.*—4 oz. capacity. (Supplied by S. H. Ansell & Sons, Boston, Mass.) For convenience, scratch lines on outside of bottle corresponding to 10 ml divisions.

b. *Flask.*—500 ml. With ground glass stopper.

c. *Gooch Crucible.*—Coors No. 4.

d. *Glass Fiber Filter Paper.*—2.4 cm circles. (Supplied by Reeve Angel & Co.)

e. *Balance.*—1 kg capacity, accurate to 50 mg or better, for obtaining the weight of solution.

f. *Forced Draft Oven.*—Capable of maintaining at least 250°C.

g. *Desiccator.*—Containing freshly dried activated alumina.

Preparation of Sample Solution

Clean, thoroughly dry, and cool flask, **b**, and obtain its tare on balance, **e**. Quickly and accurately weigh out a 2 g sample and transfer to the flask.

1. For organic materials alone or in mixtures, add 30 ml HNO_3 and boil gently 30–45 minutes. Cool somewhat and add 10 ml 70–72% $HClO_4$. Boil very gently, adjusting flame as necessary, until solution is colorless or nearly so and *dense white fumes* appear.

[1] Fine powder, supplied by Mallinckrodt Chemical Works, is preferred by the author.

(*Caution*: It is *dangerous* to boil to dryness.) Cool and add approximately 400 ml H_2O. Replace stopper (previously weighed with flask) and mix thoroughly. Wipe outside of flask and weigh again on balance. Calculate the percentage of sample in solution. Filter through a dry filter into a dry flask and stopper flask.

2. For materials containing a small percentage of organic matter, add 30 ml HNO_3 and 5 ml HCl and boil until the organic matter is destroyed. Cool, dilute, mix, and weigh as above. Calculate the percentage of sample in solution. Filter through a dry filter into a dry flask, and stopper flask.

3. For salts completely soluble in water, dissolve, dilute, mix, and weigh as above. Filter through a dry filter into a dry flask and stopper flask.

Determination

Using an analytical balance, and a weight buret or the polyethylene wash bottle, **a**, weigh a portion of the solution containing not more than 60 mg P_2O_5, preferably not less than 40 mg, into a 500 ml Erlenmeyer flask. If the polyethylene bottle is used, make certain that the spout is not full of solution during weighing, since a rise in temperature may cause dripping. Add 200 ml H_2O. If the solution is acid, make just alkaline to methyl red or methyl purple with a fresh solution of reagent grade NaOH. Then make just acid by the dropwise addition of dilute HCl.

At this stage, there must be no precipitate (dissolve any remaining precipitate by dropwise addition of more of the dilute HCl). Add 100 ml of the citric-molybdate solution and boil gently for 3 minutes. The solution must be free of precipitate at this point. Immediately add, from a buret, 50 ml of the quinoline solution. This solution should be added dropwise for the first few ml, then in a slow stream with constant swirling to ensure a precipitate of maximum particle size. Let stand 5 minutes with occasional swirling; then cool to room temperature in running water. Prepare crucible and filter, **c** and **d**, dry in oven at 250°C, cool to *room temperature* in desiccator, **g**, and weigh. Filter and wash precipitate with water at room temperature. The last trace of precipitate may be removed from the flask by use of a stream from the wash bottle. Dry to constant weight (30 minutes is usually sufficient) in the oven at 250°C,[2] and cool to *room temperature* in desiccator. The precipitate contains 3.2074% P_2O_5. Deduct a blank[3] and calculate $\%P_2O_5$ in sample.

Routine Procedure

Reagents and Apparatus

Same as described in referee method, except, under *Apparatus*, delete **a**, **b**, and **e**.

Under *Preparation of Sample Solution*, instead of weighing the solution, merely dilute to volume in a 250 ml volumetric flask.

Determination

Using a calibrated pipet, place an aliquot of sample solution containing not more than 25 mg P_2O_5 into a 500 ml Erlenmeyer flask. Add 100 ml H_2O; then add 25 ml of the citric-molybdate solution and boil gently for 3 minutes. The solution must be free of precipitate at this point. Immediately add 12.5 ml of the quinoline solution from a buret. This solution should be added dropwise for the first few ml, then in a slow stream with constant swirling throughout to ensure a precipitate of maximum particle size. Let stand 5 minutes with occasional swirling; then cool to room temperature in running water. Prepare crucible and filter, **c** and **d**, dry in oven at 250°C, cool to *room temperature* in desiccator, **g**, and weigh. Filter and wash precipitate with water at room temperature. The last trace of the precipitate may be removed from the flask by use of a stream from the wash bottle. Dry to constant weight (about 15 minutes) in the oven at 250°C[2], and cool to room temperature in the desiccator. The precipitate contains 3.2074% P_2O_5. Deduct blank[2] and calculate $\%P_2O_5$ in sample.

Discussion

Table 1 presents a number of analyses made during the early part of this investigation when the phosphorus was weighed as

[2] The precipitate may be dried to constant weight at 200°C, but a somewhat longer period of time will be required.

[3] The blank is determined by running through the procedure with water instead of an aliquot of sample solution. Blank should not exceed 2 mg.

Table 1. Total P_2O_5 analyses of fertilizers and phosphate salts

Sample	Theoretical % P_2O_5	Sample Solution Number	Av. % P_2O_5	Range	No. Determinations
Precipitation of Quinoline Phosphomolybdate Followed by Ignition at 550°C					
Single crystal monoammonium phosphate from NBS	61.701	1	61.575	.01	2
		2	61.667	.06	8
		3	61.675	.01	4
NBS phosphate rock 56-A	32.90 (certificate)	1	32.910	.02	2
		2	32.912	.03	4
		3	32.900	.06	5
NBS phosphate rock 56-B	31.55[a] (certificate)	1	31.637	.01	4
		2	31.620		1
		3	31.655	.03	2
Monopotassium phosphate (reagent grade)	52.153	1	51.957	.02	3
		2	51.949	.08	7
Monopotassium phosphate (recrystallized reagent)		1	52.073	.01	3
		2	52.095	.03	4
3-9-9 fertilizer	9.19 (differential spectrophotometry)	1	9.19		1
		2	9.19		1
0-20-0 fertilizer	21.75 (differential spectrophotometry)	1	21.74 21.76	.02	2
Triple superphosphate (National Plant Food Institute 1957 Collaborative Sample)	47.66 (differential spectrophotometry)	1	47.670	.02	3
		2	47.690		1
Precipitation of Quinoline Phosphomolybdate Followed by Drying at 250°C					
Single crystal monoammonium phosphate from NBS	61.701	1 and 2	61.705	0.03	10 (Std Dev. 0.012)
Ammonium potassium phosphate	56.526	1	56.511		1
NBS phosphate rock 56-A	32.90 (certificate)	1	32.915	0.03	4 (Std Dev. 0.01)
NBS phosphate rock 56-B	31.55[a] (certificate)	1	31.645	0.01	2
		2	31.685	0.03	2
Monopotassium phosphate (recrystallized reagent)	52.153		52.128[b]	0.02	4 (Std Dev. 0.01)
Triple superphosphate (National Plant Food Institute 1957 Collaborative Sample)	47.66 (differential spectrophotometry)	1	47.665	0.01	2
		2	47.658	0.01	2
3-18-18 fertilizer	18.73[c]	1	18.79		1
3-18-18 fertilizer	19.04[c] 18.91[d]	1	18.88		1
5-17-0 fertilizer	17.69[c]	1	17.66		1
8-20-20 fertilizer (laboratory sample)	20.85 (calculated value)		20.86[b]		1

[a] Brabson and Wilhide (2) favor 31.70 as the true value for this rock.
[b] Direct analysis of powder.
[c] "Magruder" check sample average analysis.
[d] Differential spectrophotometric average analysis.

phosphomolybdic anhydride. The data suggest that slightly greater accuracy results when the precipitate is dried rather than ignited.

The single crystal of monoammonium phosphate was obtained from the U. S. National Bureau of Standards. It was grown for piezo-electric research, and is thought to approach ultimate purity. Perrin (6), using an all-glass distillation apparatus, found that this specimen contained substantially the theoretical percentage of nitrogen. The Bureau also provided samples of phosphate rock, Nos. 56-A and 56-B.

The data in Table 1 were obtained over a period of several months. During a single day, no more than two analyses were performed on a specific sample.

The sample of triple superphosphate was issued to 27 laboratories in a study organized by the National Plant Food Institute. The average value reported, using the volumetric molybdate method, was 48.12% P_2O_5. In the present study of standard samples, both the proposed method and the differential spectrophotometric method of Gee and Deitz (5) gave results of 47.7% P_2O_5 (at a moisture content agreeing closely with the reported average) and it was concluded that the difference represented the bias in the volumetric method. Another example of this bias, even in the absence of sulfates, is indicated in the certificate issued with the NBS Phosphate Rock 56-A.

The 8-20-20 fertilizer listed in the table was made up from non-hygroscopic reagents of high purity which were ground to pass 60-mesh, dried, weighed out to formula on a semi-micro balance, and then very thoroughly mixed. The components were ammonium sulfate, potassium nitrate, acetanilide, monopotassium phosphate, sodium chloride, and calcium carbonate. The nitrogen assay of this sample was 8.54% against a calculated 8.57%. The potash assay was 23.13% against a calculated 23.16%.

The 5-17-10 fertilizer sample was also analyzed by the routine procedure described above. The average of 4 analyses agreed well with the referee method at 17.64% with a range of 0.05%.

Since the Wilson method has been investigated thoroughly with respect to interfering ions and applicability to the analysis of a variety of fertilizers (3, 7, 8) it was felt that very little additional work was necessary to show that the proposed modification is equally versatile. However, to an aliquot of the solution of the NBS single crystal monoammonium phosphate sample containing approximately 100 mg of monoammonium phosphate, 100 mg each of dried sodium borate, cupric sulfate, manganese sulfate, and magnesium chloride was added. By the 550° ignition procedure the result was 61.70° P_2O_5. The experiment was repeated with the A.O.A.C. molybdate solution (1), and a value of 63.0% P_2O_5 was obtained. On the pure solution of monoammonium phosphate, use of the A.O.A.C. molybdate solution gave a result of 61.8% P_2O_5.

Further experiments with the proposed method showed that as much as 1 g of ammonium sulfate could be added to aliquots of phosphate rock and monopotassium phosphate solution without significant error. With a 2 g addition, however, a warning opalescence of the solution was evident and a positive error of 0.3% P_2O_5 resulted. However, the Wilson method is enormously less sensitive to interference from ammonium sulfate than volumetric methods based upon ammonium phosphomolybdate.

The attractive possibility of using the proposed method for the direct determination of available P_2O_5 is now being investigated and encouraging results have been obtained.

Summary

The gravimetric modification of the Wilson method is recommended as a referee procedure for investigations in which the exact phosphorus content of the sample must be established. Such studies should include parallel determinations on standard samples of similar nature.

A routine procedure employing the usual volumetric glassware appears to offer advantages over present methods.

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Chemical Indices of Decomposition in Cod*

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Introduction

Decomposition in raw fish and other raw seafood is most readily detected by organoleptic means. Those experienced in the examination of fish have little difficulty in determining the presence of decomposition by the physical senses, particularly the sense of smell.

Decomposition of fish is principally a progressive proteolysis of the muscle tissue brought about by the action of microorganisms and, to a lesser degree, by autolytic enzymes. Changes take place in the physical appearance of the raw fish as proteolysis proceeds. It has been found convenient to describe this progressive deterioration by dividing it into five stages or classses of the raw fish. These more or less empirical designations run the gamut from perfectly fresh fish just taken from the water to fish in an advanced state of rottenness, and may be defined as follows:

Class 0—The first stage after death. The eyes are bright and fresh, the gills are bright and red, and the flesh is bright and firm except when mechanical damage has caused softening. The raw flesh has a translucent appearance. There is practically no odor.

Class 1—The second stage after death, characterized primarily by an almost complete lack of odor or only a slight "fishy" odor. The eyes are slightly dull, the flesh is somewhat soft and lacking in resiliency, and the gills are red and slightly slimy. In this stage the diffusion of hemolyzed blood may appear in small areas, especially beneath the integument of the head, within the body cavity, or along the backbone.

Class 2—The third stage, in which the eyes are definitely dull, and gills are lighter in color and quite slimy. The flesh is moderately soft and the infiltration of hemolyzed blood into the tissues has appeared. The flesh around the body cavity and near the backbone will be slightly reddened or "feverish," of a shade different from the shade of blood. Usually an odor is present which, while not really intense, is persistent and readily perceptible to the experienced examiner, who recognizes it as indicative of decomposition.

Class 3—In this stage the eyes are sunken, the flesh is quite soft and mushy, and the gills are very slimy and yellowish-gray.

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Reddening may permeate all of the flesh. The odor is similar to that found in the third stage (Class 2) but is intense enough to be readily noticeable and is indicative of decomposition so advanced as to be distinct and unmistakable.

Class 4—This stage may best be described as putrid, since the odor is extremely foul and other characteristics of decomposition, as just described, are present to an even more advanced degree.

The development of chemical methods for detecting the use of spoiled raw material by examining the canned fishery products has long been a subject of study by this Administration.

The desirability of more than one chemical index of decomposition in canned fish or any other manufactured food is self-evident. Deteriorative changes in the raw material, whether caused by autolysis or microbial growth, are known to be extremely complex. Even in a single raw material, they may vary widely with the initial microbial flora, the conditions of exposure and storage, and other factors. The degradation products may be volatile or nonvolatile. Those which are volatile may contribute to the smell of decomposed raw material. If a chemical index also contributes to the sensory evidence of decomposition, especially smell, so much the better. In any event the index must show a good correlation with sensory criteria in the raw material and (preferably) in the finished food as well. A single chemical index may "miss some of the bad ones" at times; it must *not* "hit some of the good ones." Obviously, the chances of missing some really decomposed material will be materially lessened if more than one chemical index is available.

Previous papers (1–4) dealt with the determination of volatile fatty acids in canned salmon, tuna, herring roe, and sardines, as one of the chemical indices of decomposition. Results by the distillation procedure used were reported in terms of "volatile acid numbers" (titration of 200 ml of distillate obtained from a clarified water extract of 25 g of well-mixed canned fish, multiplied by 4 to convert results to the basis of 100 g of fish). No attempt was made at that time to determine the individual volatile acids, except that formic acid was determined in the volatile acid fraction by the mercuric chloride reduction method and reported separately.

Later a partition chromatographic procedure for separating a mixture of the volatile fatty acids was adopted as first action in 1949 (5). The results obtained by this procedure for an authentic pack of canned tuna have been published (6).

Geiger (7, 8) had suggested histamine as an index of decomposition in some fishery products. The biological procedure he proposed and which Williams studied (9, 10) has been adopted as first action (11). Sager and Horwitz (12) have proposed a chemical procedure, now under study, for the determination of histamine. Volatile acids, histamine, and succinic acids have been determined in an authentic pack of Atlantic little tuna (13) prepared on the East Coast of the United States, and in authentic packs of skipjack, yellowfin, albacore, and bluefin tuna, prepared on the West Coast (14).

The determination of basic nitrogen compounds, although widely used in assessing the quality of raw fish, is not very satisfactory for the detection of decomposed raw material in canned products, since some of these compounds are liberated in processing. Dyer (15) has proposed the determination of trimethylamine in raw pelagic (oceanic) fish; at the present time his method is being studied by the Association of Official Agricultural Chemists. Another paper (16) describes a method for the determination of alcohol in canned tuna, raw cod, and eggs. A procedure described in the section on *Examination of Packs* has been utilized for the determination of volatile bases and volatile amines.

Thus eight methods which showed promise of being useful were available for the detection of decomposed raw pelagic fish. The next step in the investigation was to prepare authentic packs of fish showing progressive decomposition.

Preparation of Packs

The following authentic packs of cod, haddock, and ocean perch were prepared at Boston during the summer of 1957. Each pack was repeated with fish from a different

source to give a "first pack" and a "second pack."

1. Commercial icing procedure was followed on gutted cod and haddock and on ungutted perch. The fish were obtained directly from the boats. None had been out of the water more than 24 hours and some had not yet gone into rigor. A layer of ice was placed on the bottom of a fish box, followed by alternate layers of fish and ice until the box was filled. Then the box was well capped with ice. Each morning the temperatures of the air and the fish were taken. Fillets were cut daily for organoleptic examination and then frozen for later chemical examination.

2. A replication of No. 1, using another lot of fish.

3. Same as for No. 1 except that the fish were uniced.

4. A replication of No. 3.

5. Castell, *et al.* (17) reported that when fish were exposed on deck during the warm summer weather, the fillets softened and spoilage was accelerated. To simulate conditions that sometimes exist on the boats because of delay in getting the fish off the deck and into ice storage, ungutted cod in prime condition were allowed to stand overnight uniced. The temperature of the fish when received was 48°F (room temperature, 76°F). The next morning the temperature of the fish was 60°F (room temperature 76°F). The fish were then gutted and iced down as described for No. 1.

Examination of Packs

The following methods were used for the examination of these packs (references are to *Official Methods of Analysis*, 8th Ed. (1955), Association of Official Agricultural Chemists, unless otherwise specified):

(1) *Volatile Acid Number:* **18.18.**

(2) *Formic Acid:* **18.19.**

(3) *Acetic Acid:* **18.20.**

(4) *Succinic Acid: This Journal*, **39**, 773 (1956).

(5) *Alcohol as Ethyl Alcohol: Ibid.*, **41**, 776 (1958).

(6) *Trimethylamine: Ibid.*, **41**, 000 (1958).

(7) *Histamine: Ibid.*, **39**, 91 (1956).

(8) *Volatile Bases and Volatile Amines:* A new empirical method was used for this determination. Details are as follows:

Preparation of Solution

Comminute sample by passing 3 times through a food chopper, mixing after each grinding. Weigh 20 g into a 250 ml centrifuge bottle and pipet 100 ml H_2O into the bottle. Shake vigorously for about 2 min., centrifuge, and decant.

Determination

Volatile Bases.—Pipet 25 ml decanted material into the distilling flask of the assembly described in *This Journal*, **41**, 763 (1958), and add 125 ml distilled H_2O. To prevent foaming add about $\frac{1}{4}$ cc white beeswax, U.S.P. Use a 200 ml Erlenmeyer flask containing about 10 ml H_2O as receiver. Fit the condenser with an adapter to conduct the distillate below the surface of the water in the receiver. Heat the $Ca(OH)_2$ suspension to incipient boiling and the sample in the distilling flask to full boiling before connecting the steam inlet tube. At this point remove the flame from the distilling flask until the steam is passing freely through the sample. (These precautions are necessary to prevent the material from being sucked back into the steam boiler.) Steam distill about 100 ml distillate, and titrate this distillate with 0.01*N* HCl. Transfer titrated material to a 200 ml volumetric flask, dilute to mark, shake, and designate as Solution A.

Calculation.—(Titration in ml 0.01*N* HCl /4.17 g) $\times 100 =$ ml 0.01*N* volatile bases per 100 g fish.

(Volatile acids will be retained in the flask containing the $Ca(OH)_2$ suspension, while aldehydes, ketones, alcohols, and volatile bases, including amines, will be in the distillate.)

Volatile Reducing Substances (Amines).—Pipet another 25 ml portion of the prepared solution into the distilling flask of the assembly, add 125 ml H_2O, make acid to phenol red indicator paper with H_2SO_4 (1+1), add beeswax, and steam distill as before. Collect distillate in a 100–110 ml volumetric flask containing 5–10 ml H_2O until the volume is about 90–100 ml, dilute to 110 ml, and shake. Designate this as Solution B. (Volatile bases, including amines, will be retained in the distilling flask; volatile acids will be trapped in the flask containing the $Ca(OH)_2$ suspension; aldehydes,

ketones, and alcohols, if present, will be in the distillate.)

Oxidation of Solution A

Transfer 25–100 ml Solution A (depending upon the probable concentration of reducing substances) to a 300 ml Erlenmeyer flask, add enough distilled H_2O to give a volume of about 100 ml, and then add 10 ml 10% NaOH solution and 25 ml standard 0.05*N* $KMnO_4$. Place a short-stem funnel in neck of flask, heat to about 60°C, and place Erlenmeyer flask in boiling water bath for 20 min. Cool, and add 10 ml 6*N* H_2SO_4 and 10 ml 20% KI solution. Titrate with standard 0.05*N* $Na_2S_2O_3$, adding starch indicator when most of the iodine has been consumed. Subtract the titer from 25.0 ml to determine ml 0.05*N* $KMnO_4$ used. If the oxidation mixture turns green during the heating period, or if more than about 6 ml $KMnO_4$ is used in the oxidation, repeat with a smaller aliquot of the distillate, Solution A.

Calculations.—1.00 ml of Solution A represents 0.0208 g of sample. 100×ml 0.05*N* $KMnO_4$/(0.0208×ml Solution A taken) = ml 0.05*N* $KMnO_4$ consumed per 100 g fish.

Oxidation of Solution B

Transfer 25–100 ml Solution B to a 300 ml Erlenmeyer flask and proceed as in *Oxidation of Solution A*.

Calculations.—1.00 ml of Solution B represents 0.038 g of sample. 100×ml 0.05*N* $KMnO_4$/(0.038×ml Solution B taken) = ml 0.05*N* $KMnO_4$ consumed per 100 g fish. Ml 0.05*N* $KMnO_4$ per 100 g consumed in oxidation of Solution A − ml consumed in oxidation of Solution B = ml due to oxidation of volatile amines. (Other volatile bases, chiefly ammonia, are not oxidized by 0.05*N* $KMnO_4$.)

Results

Table 1 presents data on the determination of volatile bases and volatile amines in several samples of raw cod. Duplicate determinations are satisfactory.

The results on 5 progressive decomposition packs of the raw cod are given in Table 2, which also shows the types of packs and storage conditions. In each of the packs, Class 0 contained small quantities of volatile acids (no determinable quantity of formic acid), succinic acid, alcohol, volatile bases, volatile amines, and trimethylamine (TMA), but even after the fish were held under the various conditions described, there was no significant increase in any of these indices so long as the fish remained in Class 0.

The preparation of the progressive decomposition packs involved filleting the fish, grading each fillet, and packing by grade into individual boxes. There was necessarily some delay in placing the packaged fillets in the plate freezer. After a period of frozen storage the fillets were again classified organoleptically before being analyzed chemically. The results in Table 2 marked with asterisks represent fish which on later examination were found to have gone from the Class 1 designation recorded for them at the time of filleting to Class 2, as a result of the delay between filleting and freezing. These data have been grouped and retabulated at the end of Table 2. The change in class of these particular fillets illustrates a commercial problem that can arise with fish kept under ice for periods of 7 to 14 days. Such fish may decompose rapidly during delays in processing, particularly in warm weather.

Table 1. Volatile bases and volatile amines in raw cod

Sample No.	Volatile Bases, ml 0.01*N* $KMnO_4$/100 g	Volatile Amines, m 0.01*N* $KMnO_4$/100 g
1	54	45
	56	40
2	97	163
	101	178
3	60	117
	61	127
4	103	241
	108	234
5	98	271
	118	251
6	285	814
	300	783
7	284	658
	308	726
8	300	675
	318	708

Table 2. Studies of progressive decomposition in cod[a]

Fish No.	Age in Days	Organoleptic Class	Fish Temp., °F	Air Temp., °F	Volatile Acids			Succinic Acid, mg/100 g	Alcohol as Ethyl, mg/100 g	Volatile Bases, ml 0.01*N*/100 g	Volatile Amines, ml 0.05*N* $KMnO_4$ per 100 g	Trimethylamine (TMA), mg N/100 g
					Volatile Acid No. "VAN"	Formic, mg/100 g	Acetic, mg/100 g					
Fish Heavily Iced: First Pack												
1	0	0	47	71	6	0.0	1.6	6.0	3.2	26	20	1.5
2					6	0.0	0.0	4.6	3.2	29	14	1.5
3					5	0.0	0.9	4.5	2.6	38	53	1.6
4					2	0.0	0.9	5.4	3.3	41	55	0.7
5					3	0.0	0.8	4.2	3.2	30	33	0.7
1	1	0	34	71	4	0.0	1.0	4.0	7.4	36	87	0.5
2					4	0.0	1.0	—	4.4	32	61	1.5
3					5	0.0	1.2	4.2	4.1	32	52	1.6
1	2	0	34	71	4	0.0	1.2	1.3	5.8	35	88	1.2
2					3	0.0	1.6	3.9	4.8	36	71	0.9
3					6	0.0	1.6	2.4	4.9	25	59	0.6
1	3	0	33	71	5	0.0	1.7	2.5	4.6	34	29	1.4
2					4	0.0	2.4	1.8	4.3	53	112	3.3
3					6	0.0	2.3	2.5	4.3	40	99	2.1
1	4	1	33	71	4	0.0	2.3	2.7	3.5	43	30	2.4
2					5	0.0	2.3	2.5	4.3	37	32	2.9
3					4	0.0	2.2	2.9	5.8	43	83	1.0
4					6	0.0	2.3	1.3	3.9	37	63	4.3
5					6	0.0	2.1	2.1	4.0	40	57	3.3
1	5	1	33	71	11	trace	6.7	3.8	5.0	34	140	6.8
2					5	trace	2.5	2.9	4.3	30	10	1.3
3					5	trace	3.0	3.6	3.2	30	57	2.2
1	6	1	33	71	6	0.0	1.7	1.6	4.2	56	110	1.5
2					5	0.0	2.8	2.1	3.6	67	81	1.6
3					7	0.0	3.0	2.4	4.5	30	46	3.8
1*	7	1	33	71	37	trace	28.0	0.9	10.7	103	336	23.0
2					19	trace	12.1	5.2	7.6	61	151	13.1
3*					32	trace	18.0	9.6	8.7	78	207	17.7
1	8	1	33	71	4	trace	2.0	2.0	3.7	46	64	4.4
2					4	trace	1.8	2.1	4.1	58	76	1.6
3					5	0.0	2.1	1.2	3.7	53	65	3.9
1	9	1	33	71	11	trace	8.0	0.8	3.7	37	90	6.3
2					10	trace	9.3	1.3	5.0	33	81	6.0
3					12	0.0	8.3	1.3	5.2	54	135	8.4
1	10	1	33	71	7	0.0	3.7	1.3	4.5	44	43	1.0
2					7	0.0	2.6	1.0	3.9	32	75	3.6
1*	11	1	33	73	39	trace	30.0	2.2	10.4	96	433	16.3
2*					33	trace	25.7	1.4	9.3	78	462	11.9
3*					30	trace	22.2	1.5	10.3	107	492	11.4
1*	12	1	33	74	66	trace	56.0	8.0	13.1	181	600	37.9
2*					40	trace	31.6	2.1	9.7	129	385	22.5
1	12	2	33	74	113	15.6	95.0	16.4	20.1	216	657	50.6
2					147	35.5	109.0	15.3	23.9	176	565	51.7
1	12	3	33	74	189	50.5	133.6	27.2	41.6	229	761	73.1
1*	13	1	33	73	52	8.2	33.7	4.7	12.7	128	563	24.5
2*					32	5.7	25.4	3.4	21.9	104	541	14.8

(*Continued on p. 768*)

Table 2. Continued

Fish No.	Age in Days	Organoleptic Class	Fish Temp., °F	Air Temp., °F	Volatile Acid No. "VAN"	Volatile Acids		Succinic Acid mg/100 g	Alcohol as Ethyl, mg/100 g	Volatile Bases, ml 0.01N/100 g	Volatile Amines, ml 0.05N $KMnO_4$ per 100 g	Trimethylamine (TMA), mg N/100 g
						Formic, mg/100 g	Acetic, mg/100 g					
1	13	2	33	73	50	6.2	40.0	7.6	11.0	141	550	26.3
1	13	3	33	73	174	—	122.4	11.2	36.2	247	954	64.7
2					171	39.3	121.4	18.3	21.9	272	1544	58.7
3					207	54.0	149.6	17.1	29.0	277	1674	91.9
1	14	2	33	74	71	13.0	55.6	8.0	15.5	132	462	59.6
2					51	9.1	35.1	3.2	9.9	112	279	19.9
3					108	25.2	79.3	6.5	17.2	200	566	57.6
1*	14	1	33	74	63	6.0	52.3	9.8	15.1	179	539	9.4
1	14	3	33	74	199	54.9	151.2	23.2	33.9	257	821	81.2
1	15	2	33	74	74	20.0	52.9	4.2	18.9	144	358	41.4
2					132	36.1	95.9	16.3	27.6	200	615	67.7
3					97	24.0	74.0	9.7	22.0	163	528	52.9
1	15	3	33	74	110	18.5	86.8	12.4	16.7	241	695	65.8
1	16	2	33	76	168	37.5	112.4	22.2	27.8	223	689	77.9
2					152	35.5	103.8	18.4	23.4	236	726	73.1
3					112	16.2	90.4	15.7	19.0	185	648	51.1
4					229	69.3	162.0	18.1	37.8	237	772	89.0
5					110	32.2	71.2	8.1	21.6	185	561	52.9
1	16	3	33	76	162	32.8	127.5	20.2	25.5	261	809	78.4
2					193	42.0	142.6	40.8	21.5	207	619	74.7
3					113	32.7	83.1	16.4	19.3	181	563	56.9
4					188	62.8	128.8	34.7	25.9	246	936	68.3
5					132	24.9	98.0	26.1	21.3	224	552	70.6
Fish Heavily Iced: Second Pack												
1	0	0	45	71	5	0.0	2.2	5.2	6.5	62	21	6.7
2					4	0.0	1.7	6.3	7.1	50	37	3.5
3					6	0.0	1.7	4.3	6.4	47	35	6.5
4					5	0.0	4.3	6.1	6.2	55	54	4.7
5					7	0.0	2.7	4.8	6.5	50	36	3.7
1	2	0	33	71	10	0.0	5.2	0.7	6.6	49	57	1.5
2					9	0.0	4.6	3.1	6.2	47	114	7.9
3					10	0.0	4.5	2.7	6.5	30	102	6.7
1	4	1	33	71	12	0.0	7.6	2.0	9.0	54	102	9.5
2					9	0.0	4.8	1.8	8.1	52	83	7.8
1	6	1	33	74	8	0.0	3.7	1.5	6.9	42	81	5.2
2					8	0.0	4.6	4.1	6.7	43	80	5.3
3					10	0.0	5.0	1.1	6.7	53	88	5.1
1*	8	1	33	74	50	8.3	39.7	1.4	14.0	127	403	25.2
2					28	trace	20.6	2.8	7.0	78	203	12.8
3					24	trace	19.1	3.0	6.5	77	189	11.1
1*	10	1	33	76	57	trace	52.4	6.7	11.8	177	530	40.3
2*					55	trace	49.2	6.2	9.0	176	509	30.6
1	11	1	33	75	23	trace	17.7	1.8	7.2	72	223	18.1
2					14	trace	10.4	2.0	5.9	44	122	12.1
3*					83	9.8	73.3	12.2	16.3	225	717	53.5
1	12	1	33	76	22	trace	17.5	1.0	7.8	76	198	15.6
2*					64	5.0	58.5	6.2	12.9	199	543	40.9
3*					58	trace	50.2	5.5	12.9	217	553	42.1

(*Continued on p. 769*)

Table 2. Continued

Fish No.	Age in Days	Organoleptic Class	Fish Temp., °F	Air Temp., °F	Volatile Acid No. "VAN"	Volatile Acids: Formic, mg/100 g	Volatile Acids: Acetic, mg/100 g	Succinic Acid, mg/100 g	Alcohol as Ethyl, mg/100 g	Volatile Bases, ml 0.01*N*/100 g	Volatile Amines, ml 0.05*N* $KMnO_4$ per 100 g	Trimethylamine (TMA), mg N/100 g
1	13	2	33	77	47	6.3	40.0	6.1	11.5	118	318	32.8
2					73	13.8	55.2	9.3	20.8	169	434	42.6
3					63	12.5	47.6	5.1	19.6	126	300	25.9
1	14	2	33	75	86	10.1	73.5	11.1	23.3	195	445	49.3
2					106	5.7	85.2	16.7	15.7	245	708	72.5
3					61	trace	53.2	4.3	10.5	219	599	48.2
4					106	7.3	95.3	14.4	15.7	314	777	78.0
5					91	12.0	71.8	13.7	15.2	224	613	65.3
1	14	3	33	75	121	13.8	104.3	21.6	20.8	245	696	81.4
2					127	14.1	111.8	25.1	19.0	254	773	74.3
3					152	36.7	117.1	18.1	25.8	229	724	78.4
4					98	17.9	77.9	15.5	21.3	212	635	48.9
5					155	35.4	122.8	21.5	26.9	271	700	79.3
					Fish Uniced: First Pack							
1	0	0	45	71	5	0.0	2.2	5.2	6.5	62	21	6.7
2					4	0.0	1.7	6.3	7.1	50	37	3.5
3					6	0.0	1.7	4.3	6.4	47	35	6.5
4					5	0.0	4.3	6.1	6.2	55	54	4.7
5					7	0.0	2.7	4.8	6.5	50	36	3.7
1	1	1	66	71	16	trace	11.3	2.7	7.5	91	182	8.7
2					11	trace	8.8	2.5	4.9	79	99	—
3					16	trace	7.4	4.1	7.6	79	126	7.6
4					10	trace	4.3	3.4	8.6	94	167	9.3
5					18	trace	12.3	2.9	8.3	100	193	12.4
1	2	2	63	71	125	15.9	108.2	12.4	19.4	287	858	61.4
2					112	12.0	96.4	13.1	15.2	289	819	62.1
3					118	18.7	94.9	8.9	16.8	307	855	63.3
4					154	33.8	125.1	16.7	17.1	260	834	89.0
5					169	38.0	132.0	12.7	19.1	271	779	88.7
1	2	3	63	71	139	23.6	118.2	13.1	17.7	337	948	76.6
2					132	12.7	115.8	19.1	14.3	306	909	75.4
3					161	19.3	145.0	16.2	12.9	365	973	93.1
4					144	12.5	128.1	14.1	12.9	360	1097	90.4
5					137	16.9	121.5	15.8	14.6	295	978	85.1
					Fish Uniced: Second Pack							
1	0	0	48	76	3	0.0	2.1	1.6	6.3	48	57	4.1
2					4	0.0	1.5	3.1	2.7	54	57	6.7
3					4	0.0	1.8	5.1	7.6	34	57	9.0
4					3	0.0	1.7	2.1	6.8	61	44	5.1
5					6	0.0	2.0	5.7	2.2	55	43	12.0
1	1	1	66	71	21	trace	14.2	3.7	3.0	99	170	19.1
2					10	trace	5.9	3.1	5.8	59	76	7.9
3					8	trace	3.7	5.2	4.2	40	56	8.0
4					9	trace	3.8	4.1	4.4	68	48	6.8
5					7	trace	2.4	3.3	4.4	71	43	5.6
1	2	2	63	71	71	9.3	57.5	9.3	9.8	268	642	75.4
1	2	3	63	71	122	10.4	110.0	16.3	14.6	342	842	74.1
2					148	26.2	115.6	24.2	21.3	337	791	83.4
3					173	40.3	135.7	22.5	18.0	338	817	83.7
4					127	11.7	113.6	17.4	14.1	336	918	76.8
5					66	5.0	55.2	6.0	11.5	273	685	44.9

(*Continued on p. 770*)

Table 2. Continued

Fish No.	Age in Days	Organoleptic Class	Fish Temp., °F	Air Temp., °F	Volatile Acid No. "VAN"	Volatile Acids: Formic, mg/100 g	Volatile Acids: Acetic, mg/100 g	Succinic Acid, mg/100 g	Alcohol as Ethyl, mg/100 g	Volatile Bases, ml 0.01N/100 g	Volatile Amines, ml 005N $KMnO_4$ per 100 g	Trimethylamine (TMA), mg N/100 g
Held Ungutted 18 Hours at 76°F, Gutted and Iced												
1	0	0	48	76	3	0.0	2.1	1.6	6.3	48	57	4.1
2					4	0.0	1.5	3.1	2.7	54	57	6.7
3					4	0.0	1.8	5.1	7.6	34	57	9.0
4					3	0.0	1.7	2.1	6.8	61	44	5.1
5					6	0.0	2.0	5.7	2.2	55	43	12.0
1	1	1	60	76	21	trace	14.2	3.7	3.0	99	170	19.1
2					10	trace	5.9	3.1	5.8	59	76	7.9
3					8	trace	3.7	5.2	4.2	40	56	8.0
4					9	trace	3.8	4.1	4.4	68	48	6.8
5					7	trace	2.4	3.3	4.4	71	43	5.6
1	2	1	33	77	8	trace	4.4	2.0	10.4	46	95	4.2
2					7	trace	4.3	1.4	9.3	35	105	6.9
1	3	1	33	75	9	0.0	4.4	1.8	10.4	55	101	10.7
2					8	0.0	4.0	1.8	8.6	58	104	6.7
1	4	1	33	73	19	0.0	13.7	1.3	6.3	70	160	14.7
2					21	trace	14.6	2.5	8.1	79	178	19.0
3					12	trace	6.9	1.7	6.3	76	105	9.5
1	5	1	43	74	10	trace	3.2	2.4	6.1	43	82	7.5
2					7	trace	3.0	1.5	5.5	42	47	9.6
3					10	trace	3.9	1.2	4.6	59	74	12.6
1	6	1	46	73	14	0.0	8.3	1.6	7.0	61	122	8.9
2					28	trace	22.0	2.3	6.9	105	238	24.8
3					39	trace	31.9	5.9	7.4	108	261	27.4
1*	7	1	44	74	88	6.5	73.7	11.4	15.7	269	757	79.8
2*					42	trace	34.6	2.4	12.0	168	487	44.0
3*					31	trace	26.1	2.2	10.9	145	371	29.8
1	7	2	44	74	93	trace	84.8	8.5	13.5	293	798	75.7
2					110	13.0	93.5	16.9	17.8	323	779	83.5
3					101	7.2	92.8	12.2	7.3	245	754	78.2
1*	8	1	52	74	34	0.0	28.3	4.0	12.0	207	527	42.2
2*					47	trace	38.4	3.5	9.8	228	633	44.5
1	10	2	58	75	104	5.7	93.2	14.7	—	276	—	94.1
2					115	20.5	89.4	15.5	—	235	—	79.4
3					98	12.0	82.7	13.9	—	287	—	87.1
1	11	3	60	73	90	6.7	82.4	10.9	8.9	282	904	75.5
2					180	49.2	127.0	24.1	20.9	300	779	78.2
3					146	33.6	105.5	21.9	15.9	290	742	73.9
4					171	45.8	124.8	40.7	24.5	296	692	86.0
5					187	40.0	139.1	28.3	18.8	309	689	85.8
Grouping of All Asterisk Samples												
	7	1	33	71	37	trace	28.0	0.9	10.7	103	336	23.0
	7	1	33	71	32	trace	18.0	9.6	8.7	78	207	17.7
	11	1	33	73	39	trace	30.0	2.2	10.4	96	433	16.3
	11	1	33	73	33	trace	25.7	1.4	9.3	78	462	11.9
	11	1	33	73	30	trace	22.2	1.5	10.3	107	492	11.4
	12	1	33	74	66	trace	56.0	8.0	13.1	181	600	37.9
	12	1	33	74	40	trace	31.6	2.1	9.7	129	385	22.5
	13	1	33	73	52	8.2	33.7	4.7	12.7	128	563	24.5
	13	1	33	73	32	5.7	25.4	3.4	21.9	104	541	14.8

(*Continued on p. 771*)

Table 2. Continued

Fish No.	Age in Days	Organoleptic Class	Fish Temp. °F	Air Temp., °F	Volatile Acids			Succinic Acid, mg/100 g	Alcohol as Ethyl, mg/100 g	Volatile Bases, ml 0.01N/100 g	Volatile Amines, ml 0.05N $KMnO_4$ per 100 g	Trimethylamine (TMA), mg N/100 g
					Volatile Acid No. "VAN"	Formic, mg/100 g	Acetic, mg/100 g					
	14	1	33	74	63	6.0	52.3	9.8	15.1	179	539	9.4
	8	1	33	74	50	8.3	39.7	1.4	14.0	127	403	25.2
	10	1	33	76	57	trace	52.4	6.7	11.8	177	530	40.3
	10	1	33	76	55	trace	49.2	6.2	9.0	176	509	30.6
	11	1	33	75	83	9.8	73.3	12.2	16.3	225	717	53.5
	12	1	33	76	64	5.0	58.5	6.2	12.9	199	543	40.9
	12	1	33	76	58	trace	50.2	5.5	12.9	217	553	42.1
	7	1	44	74	88	6.5	73.7	11.4	15.7	269	757	79.8
	7	1	44	74	42	trace	34.6	2.4	12.0	168	487	44.0
	7	1	44	74	31	trace	26.1	2.2	10.9	145	371	29.8

[a] * = Frozen fillets organoleptically Class 2 at time of analysis.

A search of the literature has revealed no data on the day-by-day changes in chemical indices in cod held under varying conditions. In the first heavily iced pack, therefore, fillets were cut daily for freezing and subsequent analysis. The heavily iced fish reached Class 1 in 4 days and could be kept up to periods of 12 days, in some cases, and still be satisfactory organoleptically and chemically. There was no day-by-day significant increase in any of the indices until the fish reached that stage at which they were no longer fit for human consumption, when there was a several-fold increase in most of the indices. Formic acid was not found until the fish had reached Class 2, decomposed.

No histamine was detected in any of the samples except for a few samples of fish in advanced stages of decomposition, which showed only traces of histamine. The quantities of aldehydes and ketones found varied so much that this determination was considered useless for detecting decomposed fish.

Decomposition in uniced fish proceeded rapidly. The chemical indices were in the same ranges as those found for the heavily iced fish in each decomposition class.

The ungutted fish allowed to stand at air temperature of 76°F overnight, and then gutted and iced down, reached Class 2 and 3 (decomposed) in 10 to 11 days. Beginning on the fifth day this pack was thinly iced, as can be seen from the fish temperature on this and each succeeding day of holding; the final fish temperature was 60°F.

The data obtained on the day-by-day study of progressive decomposition of the first heavily iced pack are graphically presented in Fig. 1. The ranges for each index determined, showing the organoleptic class and the days of holding, are represented by the vertical bars; the average is indicated by the horizontal bars. It can be seen that there is no significant change in any of the indices until the fish reached Class 2 (decomposed).

The averages of each of the indices for each organoleptic classification and each type of handling are graphically presented in Fig. 2. Although there are some variations, there is no overlapping of any of the averages between Classes 1 and 2.

Boat Spoilage and "Bilgy" Fish

In addition to the packs previously described, several samples of fish of organoleptic classification 1, 2, and 3 were obtained from rejects. These fish had been rejected by the State Inspector, and although some of them were passable organoleptically, the owner of the fish did not attempt to segregate them.

"Bilgy" fish are sometimes encountered in the industry. Although the physical appearance of the fish may not always indicate decomposition, the odor of the fillet is extremely repulsive. As a further part of our investigation, bilgy fish were prepared at the suggestion of A. S. McFarlane (18). Pen boards were obtained from the bottom of the hold of a trawler that had just finished unloading. The boards were very slimy. Cod in good condition were strapped between two of these boards, and the boards were placed in a cold room at 30–33°F. After four days the boards were removed from the cold

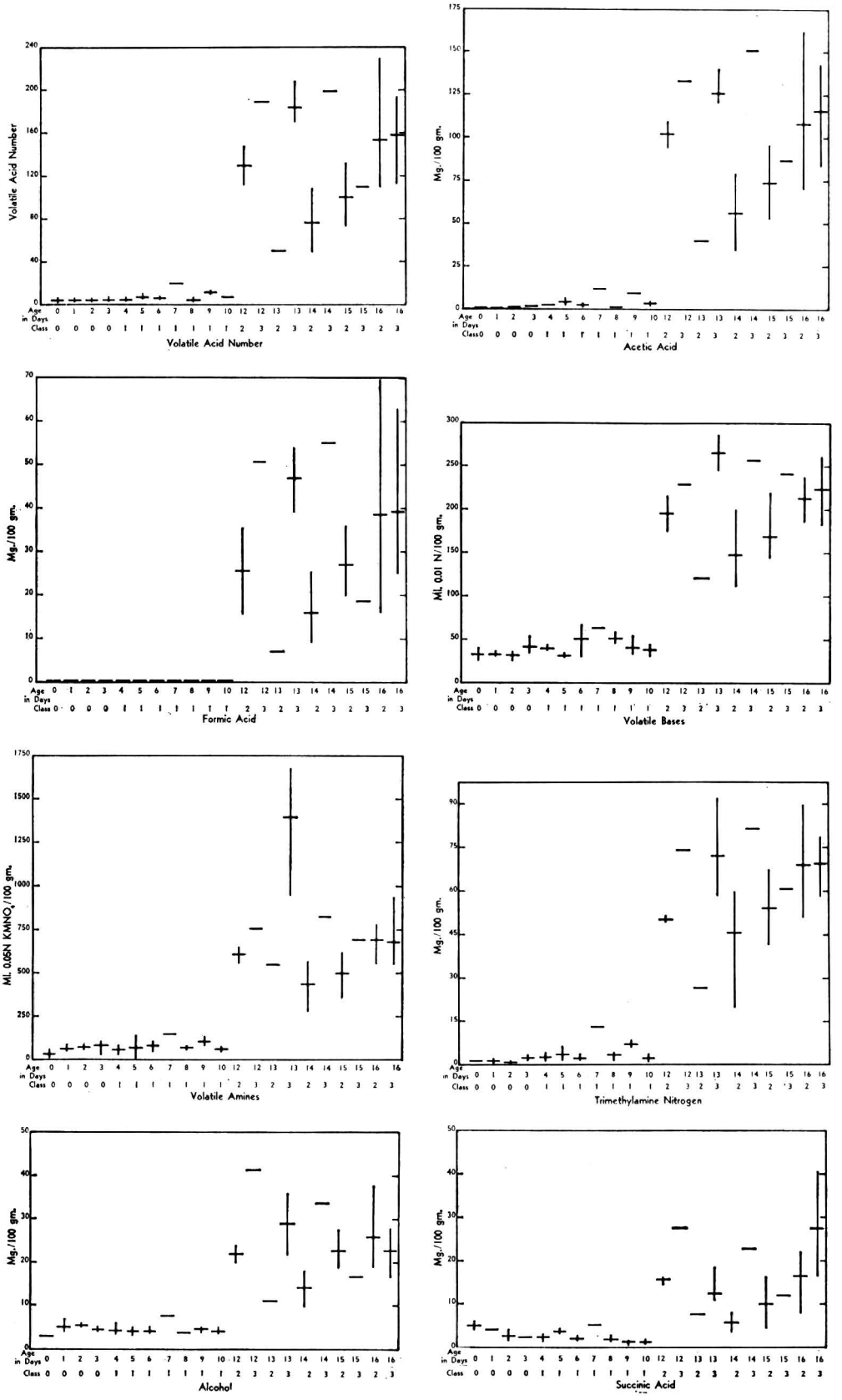

Fig. 1. Day-by-day study of progressive decomposition in heavily iced cod.

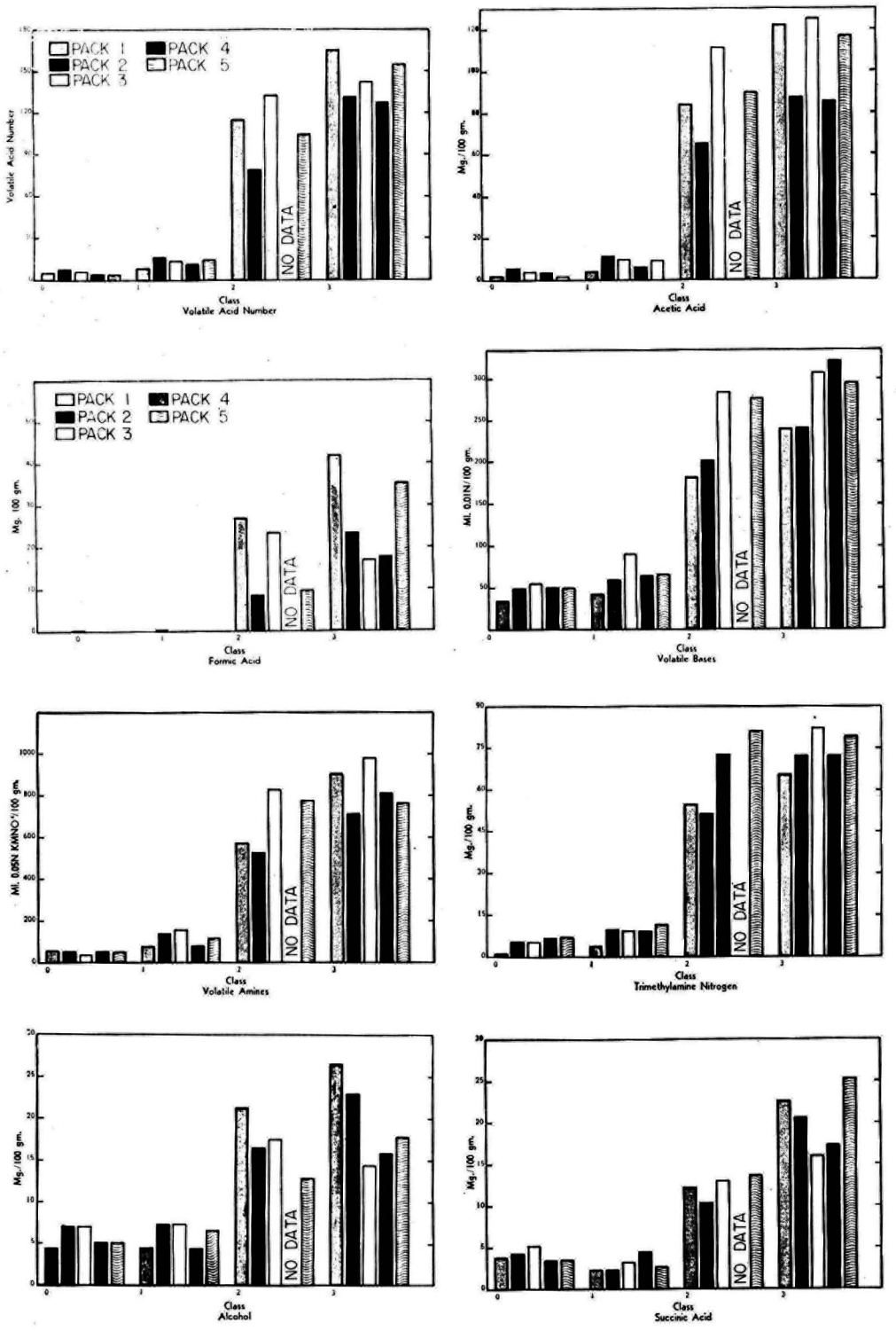

Fig. 2. Average values for indices of decomposition in cod according to organoleptic classification and type of handling. Left to right for each class: Packs 1, 2, 3, 4, 5, respectively.

Table 3. Boat spoilage and "bilgy" fish

Fish No.	Class	Volatile Acid No. (VAN)	Volatile Acids Formic, mg/100 g	Volatile Acids Acetic, mg/100 g	Propionic, mg/100 g	Succinic Acid, mg/100 g	Alcohol as Ethyl, mg/100 g	Volatile Bases, mg 0.01*N*/100 g	Volatile Amines, ml 0.05*N* $KMnO_4$/100 g	Trimethylamine (TMA), mg N/100 g
Boat Spoilage										
1	1	6	trace	2.6	0.0	4.0	9.3	113	75	7.4
2		10	trace	4.2	0.0	6.0	7.2	91	92	12.8
3		9	trace	3.6	0.0	2.9	6.3	108	88	12.0
4		8	trace	2.6	0.0	1.6	6.1	64	83	19.3
5		18	trace	14.0	0.0	2.8	6.9	94	68	20.6
6		31	trace	22.5	0.0	8.2	7.3	114	197	30.4
7		10	trace	5.9	0.0	2.2	8.2	78	80	12.1
1	2	53	9.4	42.9	0.0	10.5	12.7	186	475	35.9
2		45	5.8	35.4	0.0	10.6	11.3	181	439	35.7
1	3	129	28.3	90.0	4.5	—	18.6	265	586	48.1
2		64	11.9	44.8	0.0	10.7	10.0	211	386	26.8
3		147	40.0	103.4	4.0	18.3	23.0	207	483	62.8
4		38	8.3	25.5	0.0	5.1	7.6	84	162	15.8
5		40	8.6	27.8	0.0	9.8	8.1	163	215	25.2
6		166	46.7	125.6	0.0	26.4	21.6	293	503	90.6
7		83	18.7	62.0	0.0	14.4	15.4	185	458	47.2
8		64	10.6	44.3	4.9	8.5	24.5	188	797	22.4
9		35	6.7	24.4	0.0	7.1	20.8	120	686	15.8
10		157	16.9	144.8	0.0	20.5	12.7	329	1135	108.0
11		153	15.1	143.5	0.0	22.0	18.8	576	1316	95.4
12		19	5.2	10.1	0.0	3.6	15.4	162	345	14.8
"Bilgy" Fish										
1		64	6.2	44.7	5.0	8.1	—	186	624	30.5
2		27	trace	15.8	0.0	4.5	—	96	357	19.0
3		19	trace	12.6	0.0	3.6	11.0	129	379	17.2
4		55	7.2	42.0	5.0	6.0	11.5	182	567	36.9
5		57	trace	40.7	6.7	6.7	16.5	143	490	22.0
6		46	6.5	37.6	0.0	6.6	18.1	146	576	29.4

room and the fish examined. The eyes were slightly dull, but the flesh was still firm. Physically, the fish appeared to be in a satisfactory condition, but when filleted they were bilgy.

Table 3 presents data on fish spoiled on the boat and bilgy fish. Included in this table is a column headed **Propionic Acid.** No determinable quantities of propionic acid were found in the progressive decomposition packs reported in Table 2 and Fig. 1. In the boat spoilage packs, however, some of the Class 3 fish had bilgy odors as well as the usual odors of decomposition, and propionic acid was found in some (but not all) of the fish with these characteristics. The data on Classes 1 and 2 are very similar to those found in the progressive studies. Some of the indices of the Class 3 fish were somewhat low in comparison to the progressive studies but none of these fish would have been found acceptable on the basis of chemical analysis, since they all contained well-defined amounts of formic acid.

Generally speaking, bilgy fish appeared to be satisfactory with regard to physical condition. Very little information has appeared in the literature as to chemical methods for detecting bilgy fish, probably because their odor is so offensive that no other means of examination has been thought necessary. Castell and Triggs (19), in their studies on haddock, state:

"During the winter and early spring, fish from six to eight days on ice, and more particularly those at the bottom of the pens or those pressed tightly against the pen boards or shelves, develop a very offensive odour suggestive of pig manure or bilge water and described by fisherman as 'bilgy' fish. Such fish usually have a comparatively low pH and there is no apparent relationship be-

tween the trimethylamine value and the presence or intensity of the odour. . . . This particular type of spoilage is characterized by the following: it develops under an anaerobic environment; it is associated with heavy contamination from fish feces; the spoilage products are acidic and the vile odours are almost completely neutralized with alkali."

Whether these comments and findings apply equally well to cod is not known.

The data given in Table 3 show that several of the indices being studied will detect bilgy fish: TMA and volatile amines are produced in rather large quantities, and volatile bases in most cases are high.

Discussion

Any chemical index of decomposition in fish products, to be of value, must be correlated with the organoleptic condition of the raw fish. Since the type and extent of decomposition depend on bacterial inoculation of the fish, it would be expected that the quantities of any chemical index found would vary, even in the same decomposition class. This variation in chemical indices is acceptable as long as none of the indices condemn satisfactory fish. Conversely, it is possible that fish found to be decomposed on organoleptic examination may show one or more low chemical indices that would indicate it to be passable. It is believed, however, that this would rarely happen when all the chemical indices discussed in this paper are determined and taken into consideration in the interpretation of the analysis of any particular sample of fish.

There is very little information in the literature on the determination of any of these indices in cod with the exception of TMA. Shewan and Liston (20) in their studies on objective and subjective assessments of fish quality, and Shewan and Jones (21) in their studies of chemical changes occurring in cod muscle during chill storage, report data for TMA similar to those presented in this paper.

The maximum values found for each of the indices for Class 1 and the minimum values for Class 2 are presented below in Columns 1 and 2. All values are in mg per 100 g except volatile acid number (VAN) which is expressed in ml 0.01*N* alkali per 100 g. "Trace" in the case of formic acid indicates less than a determinable quantity in the reaction mixture, i.e. about 5 mg per 100 g.

Index	Maximum, Class 1	Minimum, Class 2
VAN	39	47
Formic	Trace	Trace
Acetic	31.9	35.1
Succinic	5.9	3.2
Alcohol	10.4	7.3
Volatile bases	108	112
Volatile amines	261	279
TMA	27.4	19.9

A study of the individual analyses leads to the conclusion that volatile acid number (VAN), formic and acetic acids, volatile bases, volatile amines, and trimethylamine (TMA) show the highest degree of correlation with organoleptic judgment, while succinic acid and alcohol show less correlation.

Acknowledgment

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Determination of Alcohol in Fish and Egg Products*

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The development of chemical methods for detecting the use of spoiled raw material by analyzing the processed food products has often been a subject of study by this Administration. In the search for such chemical methods it was found that a determination of steam-volatile acids, individually and as a group, afforded a means for evaluating spoilage both in fish used for canning and in eggs.

Lang, *et al.* (1) have proposed a determination of what they called "volatile reducing substances" for estimating spoilage in fish. In this method, washed air at room temperature is passed through the liquid pressed from fish. The air is then passed through a standard solution of alkaline $KMnO_4$. The quantity of $KMnO_4$ reduced is only a partial measure of the volatile reducing substances aerated from the fish juice, since, for complete oxidation with alkaline $KMnO_4$, it is necessary to carry out the reaction at the temperature of boiling water. These volatile reducing substances are probably a mixture of several end products of decomposition, such as volatile acids, amines, aldehydes and ketones, and alcohols. The authors did not attempt to separate and determine the individual constituents of the aerated material.

Karel, *et al.* (2) studied the quantitative aspects of this method as applied to three specific volatile substances: acetone, ethyl alcohol, and trimethylamine. They also showed that for a quantitative determination of organic volatiles, sample size should be so chosen that not more than 20% of the $KMnO_4$ is reduced.

Holaday (3) proposed a method for the determination of alcohol in fish as a measure of decomposition and concluded that the major portion of the alcohol present in decomposed fish was ethyl alcohol. The method he employed was based on that of Friedmann and Klaas (4). Their method requires a double distillation and involves a controlled alkaline $KMnO_4$ oxidation following the removal of interfering substances. It is claimed to be quite specific for alcohols as a group. They state that while the oxidation of ethyl alcohol does not proceed quantitatively to CO_2 and H_2O under the conditions chosen, the yield of other products is low. Hence the use of an empirically determined factor is justified.

In our study of a method for determining alcohol in eggs and fish products, an apparatus was designed to eliminate the necessity for a double distillation. Aldehydes and ketones are removed with 2,4-dinitrophenylhydrazine prior to distillation. The distillation is carried out in acid solution; therefore volatile bases, including amines, are not distilled. Volatile acids are trapped in alkali and do not appear in the distillate. Descriptions of the apparatus and method follow:

METHOD

Apparatus

Steam Distillation Assembly.—Consists of the volatile acid steam distillation assembly (Fig. 31, p. 312, *Official Methods of Analysis*, 8th Ed., A.O.A.C.) with another 500 ml. flask inserted between the steam boiler and the distillation flask. The additional flask has the side arm bent upwards to make connection with the inlet tube of the distillation

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flask. In this assembly the new flask is made the distillation flask and the other flask (which was the distillation flask of the volatile acid assembly) contains 150 ml of a suspension of $Ca(OH)_2$ (15 g in 1000 ml) to trap volatile acids that may be distilled. Methyl orange indicator in the flask will show when the $Ca(OH)_2$ has been consumed. A 110 ml volumetric flask containing 5–10 ml H_2O serves as the receiver, and an adapter from the condenser extends below the surface of the H_2O.

Reagents

a. *Sulfuric Acid.*—1.0*N* and 6*N* solutions.

b. *Phosphotungstic Acid Solution.*—20% w/v.

c. *2,4-Dinitrophenylhydrazine Solution.*—6.0 g reagent, 88 ml H_2O, 135 ml $HClO_4$; heat on steam bath to dissolve.

d. *Sodium Hydroxide Solution.*—10%.

e. *Potassium Permanganate Solution.*—0.05*N*. Standardize against potassium oxalate.

f. *Sodium Thiosulfate.*—0.05*N*.

g. *Starch Indicator Solution.*—2.64(d), *Official Methods of Analysis*, 8th Ed.

h. *Potassium Iodide Solution.*—20%.

Preparation of Sample Solution

a. *Fish.*—Comminute sample (include entire contents with canned products) by passing 3 times through food chopper, mixing after each grinding. Weigh 25 g comminuted material into tared 500 ml wide-mouth Erlenmeyer flask, add about 150 ml H_2O, stopper flask, and shake vigorously about 1 minute to obtain thorough suspension of material. Add 15 ml 1*N* H_2SO_4, mix, precipitate proteins with 20% phosphotungstic acid solution (20 ml is usually enough), make to 300 g with H_2O, shake vigorously about 1 minute, and filter through folded 24 cm rapid paper.

b. *Liquid or Frozen Eggs.*—Weigh 80 g into a tared 500 ml wide-mouth Erlenmeyer flask, add about 150 ml H_2O, and shake vigorously.

c. *Dried Eggs.*—Weigh 25 g into a 250 ml beaker, and make into smooth paste with H_2O, using heavy stirring rod. Transfer mixture to tared 500 ml Erlenmeyer flask, using about 200 ml H_2O.

Add 25 ml 1*N* H_2SO_4 to mixture obtained with either liquid or frozen eggs, or dried eggs, and shake about 1 minute. Add 40 ml 20% phosphotungstic acid solution, dilute to 350 g with H_2O, and shake again about 1 minute. Filter through folded 24 cm rapid paper.

Determination

Pipet 150 ml filtrate into 200 ml volumetric flask, add 1 ml of the 2,4-dinitrophenylhydrazine solution (more if yellow color does not persist), and warm on steam bath for 5 minutes. Cool, dilute to mark with H_2O, add about 500 mg of filter-aid, mix, and filter through folded paper. Transfer 150 ml filtrate to distillation flask of the distillation assembly and distill until volume in receiver is about 90–100 ml. Dilute to 110 ml with H_2O and shake.

Transfer from 10 to 100 ml of filtrate, depending on the expected alcohol content, to a 300 ml Erlenmeyer flask, add H_2O if necessary to adjust the volume to about 100 ml, and then add 10 ml 10% NaOH solution and 25 ml of the 0.05*N* $KMnO_4$ solution. Place a short-stem funnel in neck of flask, heat to about 60°C, and place flask in boiling water bath for 20 minutes. Cool, add 10 ml 6*N* H_2SO_4 and 10 ml of the 20% KI solution, and titrate with the 0.05*N* $Na_2S_2O_3$ solution, adding starch indicator when most of the iodine has been consumed. If the oxidation mixture turns green during the heating period, or if more than approximately 6 ml $KMnO_4$ is reduced, discard and take a smaller aliquot of the distillate.

Calculations

$$25 - \text{ml } 0.05N\ Na_2S_2O_3 = \text{ml } 0.05N\ KMnO_4 \text{ used}$$

$$\text{ml } 0.05N\ KMnO_4 \text{ used} \times 0.23 = \text{mg of alcohol in aliquot}$$

Results and Discussion

The factor of 0.23 mg alcohol per ml of 0.05*N* $KMnO_4$ was established in the following manner: 95% alcohol was refluxed for 6 hours in the presence of CaO, then distilled, and the middle portion of distillate used; varying quantities of this alcohol were oxidized as specified in the method (Table 1). The data produce a straight line up to

Table 1. Data establishing oxidation factor of 0.23

Alcohol Present, mg (A)	Net Titration,[a] ml 0.05 *N* $KMnO_4$ (B)	Oxidation Factor (A/B)	
0.210	0.89	0.236	
0.420	2.09	0.201	
0.630	2.90	0.217	
0.840	3.72	0.226	
1.050	4.58	0.229	
1.260	5.29	0.238	
1.470	5.72	0.257	Av. 0.229
1.680	6.33[b]	0.265	
1.890	6.95[b]	0.272	

[a] The small reagent blank was considered negligible.
[b] More than 6 ml $KMnO_4$ reduced.

Table 2. Recovery of alcohol added to canned tuna

Alcohol in Aliquot, mg	Net Titration, ml 0.05*N* $KMnO_4$	Corrected for Original, ml	Alcohol Found, mg[a]
Original	1.13[b]	—	—
0.143	1.63	0.50	0.115
0.285	2.04	0.91	0.209
0.855	4.58	3.45	0.794
Original	1.08[b]	—	—
0.428	2.80	1.72	0.396
0.570	3.31	2.23	0.513
0.712	3.92	2.84	0.653

[a] Ml×0.23.
[b] Average of two determinations.

approximately a net reduction of 6 ml of $KMnO_4$; therefore the aliquot should be chosen so that no more than 6 ml of 0.05*N* $KMnO_4$ is reduced.

In order to test the accuracy of the method, varying quantities of alcohol were added to canned tuna. It was necessary to use two 7-ounce cans of tuna to obtain enough material. Therefore in Table 2 two blanks are shown as "original."

In a previous paper (5) data were presented on the determination of seven chemical indices of decomposition in twelve progressive decomposition packs of tuna. Alcohol was also determined in these packs. The data are presented in Table 3.

A small quantity of material was determined as alcohol in Classes 1 and 2. However, the quantity in Classes 3 and 4 is several fold greater than that in Class 2. The average alcohol content for each class for each species is shown graphically in Fig. 1.

In the previous paper (5) the effect of the pre-cook on indices of decomposition was discussed. It was shown that there is some loss in some of the indices during the steaming process. The effect of the pre-cook on alcohol was studied at the same time, and it was found that about one-half of the alcohol produced during decomposition is lost

Table 3. Progressive decomposition studies on tuna: determination of alcohol (mg/100 g)

Organoleptic Class[a]	Skipjack Pack 1	Skipjack Pack 2	Skipjack Pack 3	Skipjack Pack 4	Yellowfin Pack 1	Yellowfin Pack 2	Yellowfin Pack 3	Albacore Pack 1	Albacore Pack 2	Albacore Pack 3	Bluefin Pack 1	Bluefin Pack 2
1	2	4	2	2	1	1	3	3	2	—	1	5
	1	4	2	1	2	1	1	3	3	—	1	6
	1	4	3	1	1	1	2	—	3	—	1	3
	1	4	3	1	1	1	3	—	3	—	2	3
	1	4	2	2	1	2	2	3	3	—	1	3
2	2	4	2	5	3	2	1	3	4	2	3	4
	2	5	2	3	4	2	2	3	4	2	4	3
	1	5	3	3	3	2	2	3	4	2	2	3
	1	6	2	3	2	2	2	4	3	—	3	3
	1		2	3	3	2	2	4	2	—	3	3
3	17	9	9	15	7	12	12	4	6	12	10	7
	14	11	5	11	7	9	9	4	7	12	7	7
	12	11	7	9	7	9	10	4	7	11	7	11
	19	13	8	10	10	10	9	5	6	12	15	13
	15	15	7	11	6	6	9	5	6	13	8	7
4	14	18	12	7	10	—	—	11	—	14	11	11
	11	13	8	9	11	—	—	10	—	14	9	10
	12	13	13	10	12	—	—	10	—	12	12	10
	11	14	10	11	14	—	—	10	—	12	10	12
	13	16	12	10	9	—	—	10	—	—	11	12

[a] See reference (5).

Table 4. Determination of alcohol in raw cod

Sample No.	Organoleptic Class	Alcohol, mg/100 g
1	0	2.5
		1.8
2	1	2.4
		3.6
3	1	7.8
		6.4
4	1	6.5
		7.3
5	1	7.2
		7.6
6	2	13.1
		13.8
7	3	24.8
		24.3
8	3	18.3
		19.3

during the steaming process. However, the quantity remaining in decomposed fish is so large that the loss is not enough to vitiate the usefulness of alcohol as a measure of decomposition.

In Table 4 data are given on the application of the method to raw cod.

Alcohol in Eggs

In a previous paper (6) bacteriological and chemical results were given on two authentic packs of frozen eggs. Alcohol has also been determined in these packs. The data are presented in Tables 5 and 6. For the sake of completeness the composition of each of the packs in terms of the kind of eggs used in their preparation is repeated. To compare the alcohol data with the bacteriological and chemical data previously obtained, the reader may refer to this paper (6).

Table 5. Alcohol in authentic frozen eggs[a]

No.	Description	Smell Test[b]	Alcohol, mg/100 g
A1	Current receipts promptly frozen	P	2.0
A2	Current receipts promptly frozen	P	1.0
A3	Current receipts promptly frozen	P	1.9
A4	Current receipts promptly frozen	P	2.4
A5	Current receipts promptly frozen	P	2.1
A6	Same as A5, after 24 hr at 75–85°F	R	12.5
A7	Eggs of "off" odor and tray drippings	R	41.9
A8	Eggs of "off" odor and tray drippings	R	47.6
A9	Eggs of "off" odor and tray drippings	R	36.7
A10	Eggs of "off" odor and tray drippings	R	14.0
A11	Eggs of "off" odor and tray drippings	R	27.6
A13	Incubator rejects ("18-day clears")	I	2.0
A14	Incubator rejects ("18-day clears")	I	1.8
A15	Incubator rejects ("18-day clears")	I	1.5
A16	Incubator rejects ("18-day clears")	I	1.3
A17	Cracks, chicks, leakers	P	2.4
A18	Cracks, chicks, leakers	P	1.9
A20	Current receipts+8% mixed rots	R	11.1
A21	Current receipts+16% mixed rots	R	12.1
A22	Current receipts+24% mixed rots	R	12.4
A23	Current receipts+32% mixed rots	R	24.8
A24	Current receipts+40% mixed rots	R	27.9
A25	Current receipt+0.1% black rot	P	1.7
A26	Current receipt+0.5%	R	5.2
A27	Current receipt+1.0%	R	—
A28	Current receipt+2.0%	R	3.7
A29	Current receipts+12% moldy	R	24.4
A30	Current receipts+20% moldy	R	29.7
A31	Current receipts+25% moldy	R	12.7
A32	Current receipts+30% moldy	R	not run
A33	Current receipts+35% moldy	R	8.2
A34	Current receipts+40% moldy	R	11.2
A35	Current receipts+50% moldy	R	1.6
A37	100% blood rings	R	2.2
A38	"Off" odor eggs and drippings+32% mixed rots	R	13.0
A39	"Off" odor eggs and drippings+1.0% black rots	R	10.0

[a] See Table 1, Reference (6).
[b] P = Passable; R = Rejects; I = Passable by smell test but illegal (see *Federal Register*, 23, 6834 (Sept. 5, 1958)).

Table 6. Alcohol in authentic frozen eggs[a]

No.	Description	Smell Test[b]	Alcohol, mg/100 g
B1	Current receipts, promptly frozen	P	2.0
B2	Current receipts, promptly frozen	P	0.6
B3	Current receipts, promptly frozen, held 8 hr at 70°F	P	1.1
B4	Current receipts, promptly frozen, held 16 hr at 68°F	P	0.7
B5	Current receipts, promptly frozen, held 24 hr at 72°F	P	0.7
B6	Current receipts, promptly frozen, held 4 hr at 68°F and 15 hr at 38°F	P	1.2
B7	Current receipts, promptly frozen, held 15 hr at 70°F and 9 hr at 46°F	P	0.6
B8	Trades, U. S. "C" grade	P	1.7
B9	Trades, U. S. "C" grade, held 16 hr at 70°F	R	10.0
B10	Trades, U. S. "C" grade, held 7 hr at 68° and 12 hr at 40°F	P	0.9
B11	Cracks, chicks, and leakers	P	0.6
B12	Cracks, chicks, and leakers, held 7 hr at 70°F	P	1.3
B13	Cracks, chicks, and leakers, held 7 hr at 68° and 12 hr at 40°F	R	8.9
B14	Incubator rejects	I	1.7
B15	Incubator rejects, held 8 hr at 68°F	I	1.6
B16	Current receipts +5% stuck yolks	P	0.9
B17	Current receipts +10% stuck yolks	P	0.9
B18	Current receipts +5% stuck yolks, held 8 hr at 69°F	R	5.1
B19	Current receipts +10% addled eggs	P	0.9
B20	Current receipts +3% addled eggs, held 16 hr at 70°F	P	0.5
B21	Current receipts +5% addled eggs, held 8 hr at 68° and 12 hr at 40°F	R	5.1
B22	Current receipts +1% mixed rots	P	1.4
B23	Current receipts +5% mixed rots	R	2.1
B24	Current receipts +10% mixed rots	R	1.4
B25	Current receipts +5% mixed rots, held 8 hr at 68° and 12 hr at 40°F	R	1.8
B26	Current receipts +5% white rots	R	0.7
B27	Current receipts +10% white rots	R	1.0
B28	Current receipts +2% white rots, held 8 hr at 48° and 12 hr at 40°F	R	3.9
B29	Current receipts +1% black rots	R	0.9
B30	Current receipts +3% black rots	R	1.1
B31	Current receipts +10% bloody eggs, held 8 hr at 68° and 12 hr at 40°F	R	1.1
B32	Bloody eggs, 100%	P	0.9
B33	Current receipts +1% musty	R	2.0
B34	Current receipts +3% musty	R	2.0
B35	Current receipts +10% moldy	R	1.6
B36	Current receipts +5% moldy, held 8 hr at 70°	R	2.5
B37	Dirties, 100%	P	1.4
B38	Current receipts +10% pseudomonas, held 6 hr at 70°F	R	1.9
B39	Cracks, chicks, and leakers, 100%, held 16 hr at 71°F	R	23.4
B40	Current receipts, 75%; trades, 10%; leakers, 15%; held 16 hr at 71°F	R	11.8

[a] See Table 2, Reference (b).
[b] P = Passable; R = Rejects; I = Passable by smell test but illegal (see *Federal Register*, 23, 6834 (Sept. 5, 1958)).

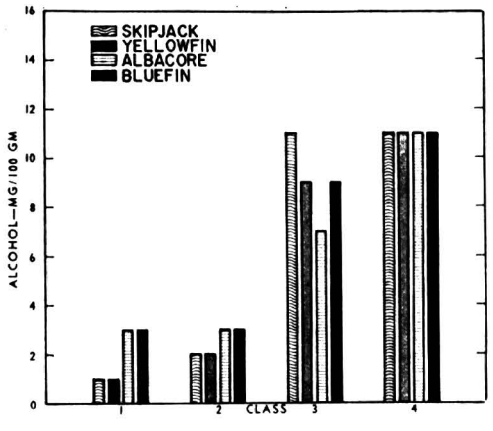

Fig. 1. Alcohol in tuna. Average for each class for each species. Left to right for each class: skipjack, yellowfin, albacore, bluefin.

The alcohol content in passable eggs was low, varying from 0.5 to 2.4 mg per 100 g. Eggs known to be of reject quality generally contained much larger quantities of alcohol, though some did not.

A sufficient quantity of concentrated alcohol distillate was isolated from tuna by fractional distillation. The 3,5-dinitrobenzoate of the alcohol was prepared by the procedure of Shriner and Fuson (7). The paper chromatogram was prepared according to the procedure of Randall, Keller, and Kirchner (8); 5 microliters of a 0.1*N* solution of the 3,5-dinitrobenzoate crystals in benzene was spotted on the paper and the paper was treated with 5% soybean oil dissolved in ethyl ether as the immobile phase. The mobile phase was 5% dioxane. The chromo-

genic agent was a 0.5% solution of 1-naphthylamine in ethyl alcohol oversprayed with a 10% solution of KOH. An orange spot indicated alcohol. With tuna, only one spot was obtained, which corresponded to that given by crystals of 3,5-dinitrobenzoate prepared from ethyl alcohol (R_F 0.32.) Crystals of 3,5-dinitrobenzoate were prepared from methyl, propyl, and isopropyl alcohols; all of their benzoates showed different R_F values, and none corresponded to the benzoate prepared from the alcohol isolated from tuna.

Summary

A procedure is proposed for the determination of alcohol in fish and egg products. Data obtained on authentic material indicate that the determination is a useful tool for detecting spoiled raw material, and will also detect the use of spoiled raw material in the processed food product.

Acknowledgment

George Yip, of the Division of Food, Food and Drug Administration, identified the alcohol in tuna as ethyl alcohol. Thanks are due L. M. Beacham and William Horwitz, Division of Food, for review of the manuscript.

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Separation and Identification of Chlorinated Organic Pesticides by Paper Chromatography. XI. A Study of 114 Pesticide Chemicals: Technical Grades Produced in 1957 and Reference Standards

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This report gives the R_F values for 213 lots of 114 different pesticide chemicals, chiefly those of the chlorinated organic type. The chromatograms were developed with two solvent systems, one aqueous and one nonaqueous, sprayed with a chromogenic agent which consisted of a solution of silver nitrate and 2-phenoxyethanol in acetone, and then exposed to ultraviolet light. Procedures followed are given below:

METHODS

Apparatus

(a) *Chromatographic Tank and Accessories.*—See *This Journal*, **36**, 1187 (1953); **40**, 999 (1957).

(b) *Dipping Tank and Accessories.*—A narrow stainless steel tank, similar in construction to the chromatographic tank, except for dimensions. The inside dimensions are $8\frac{1}{2}$" long$\times 8\frac{1}{2}$" deep and $\frac{1}{4}$ to $\frac{3}{16}$" wide (*This Journal*, **41**, 481 (1958). Accessories: (1) Stainless steel inverted U-shaped cover, 9" long$\times\frac{1}{2}$" wide (flat) $\times\frac{1}{4}$" sides; (2) elongated S-shaped glass siphon, each of the three arms about 9" long and 1" apart, with outlet tube, about 2" long, sealed to middle arm and bent slightly away from arm which is inserted in tank when emptying; (3) a metal binder clip 2" wide (available at stationery stores); and (4) a grooved 2×4" wood base to hold tank in a level, upright position.

(c) *Ultraviolet Lamps.*—(1) "Black

Light," ultraviolet lamp with filter, manufactured by the Vogel Luminescence Corp., San Francisco, Calif. View all chromatograms under "Black Light" in a dark room for fluorescent and/or quenched areas before and after application of the chromogenic agent. (2) The source of ultraviolet light used to expose chromatograms of pesticides sprayed with the silver nitrate: 2-phenoxyethanol chromogenic agent is equipped with two 36″ 30 watt germicidal tubes mounted about 5″ above the chromatograms and enclosed in a reflecting shield of sheet aluminum. Eight chromatograms can be exposed simultaneously to the light.

(d) *Chromatographic Paper.*—Whatman No. 1 Filter Paper for Chromatography, 8×8″ sheets. *Caution:* Do not handle paper with bare hands until chromatogram is completed; use clean cotton or rubber gloves. Occasionally, the paper can be handled advantageously by means of a 2″ binder clip.

Since the silver reactants in the paper interfere with the detection of those pesticides which migrate to the upper part of the chromatogram, particularly with the aqueous system, wash all papers with water. This is conveniently done by an adaptation of the continuous procedure of ascending paper chromatography (*This Journal*, **40**, 1025 (1957)).[1] Use two spacers, instead of one, to form the slits in the three-piece glass cover (*ibid.*, p. 1013); set two glass troughs upon inverted 30 ml crucibles placed at either end on bottom of chromatographic tank, and fill troughs with water. Insert 4 sheets of 8×8″ paper through each slit and hold paper in position by attaching 2 or 3 regular stainless steel clips to edge of paper which extends above cover. Let papers "develop" 24 hours or longer; then airdry overnight or longer before removing from cover. Remove airdry papers from cover with binder clip or with gloves, never with bare hands.

Reagents

(a) *Solutions of Pesticides.—Solution B:* Dissolve 0.1 g solid or viscous substances or 0.1 ml liquid substances in separate ⅋ 10 ml volumetric flasks with ethyl acetate (Eastman #300), dilute with ethyl acetate to 10 ml, and mix. *Solution A:* Transfer 1 ml solution B to separate ⅋ test tubes, 150×17 mm, add 9 ml ethyl acetate, and mix. One microliter of solution A contains 1 microgram or 1 microliter, and solution B, 10 micrograms or 10 microliters, respectively, of the pesticides. Dissolve all samples of pesticides in ethyl acetate, except the following (see Table 1): Alanap, 1, 1.A, 1.B, 1.1, dissolve in methanol; Butoxypolypropyl glycol, 11, 11.1, in ethanol; Chloranil (Spergon®), 18, 18.A, 18.B, 18.1, in benzene; and Tetraiodoethylene, 142, 142.1, in dimethylformamide. For salts, e.g., monosodium cyanamide, 23.S1; sodium 2,4-D, 29.S1; Endothol Disodium, 49, 49.1, 49.2; sodium naphthalene acetate, 96.S1; sodium orthophenylphenate, 103.S1; sodium pentachlorophenate, 112.S1; SES, 131; SES-T, 132; prepare solution B by dissolving 0.1 g solids or 0.1 ml liquids of each in 1 ml water, dilute to 10 ml with ethanol, and mix; except dissolve 23.S1 in 2 ml water, and 131 in 3 ml water before diluting to 10 ml with ethanol. For solution A dilute 1 ml solution B with 9 ml ethanol, except for 23.S1 dilute 1 ml solution B with 1 ml water and 8 ml ethanol. Disregard any volume change when mixing water and ethanol in preparation of solutions A from solutions B. (The purpose of the dilution with ethanol is to decrease drying time, especially where multiple 0.001 ml portions of the solution are applied to the same spot.)

(b) *Aqueous Solvent System.—Immobile:* Dilute 25 ml refined soybean oil to 500 ml with ethyl ether, A.C.S., and mix. *Mobile:* Dilute 25 ml water to 100 ml with 2-methoxyethanol (methyl Cellosolve®, ethylene glycol monomethyl ether), and mix.

(c) *Nonaqueous Solvent System.—Immobile:* Dilute 50 ml 2-phenoxyethanol (#P4861 Eastman Organic Chemicals or #5699 Matheson, Coleman, and Bell) to 500 ml with ethyl ether, and mix. *Mobile:* 2,2,4-trimethylpentane, 99 mole minimum (Phillips Petroleum Co., Bartlesville, Okla.)

(d) *Chromogenic Agent.*—Dissolve 1.7 g $AgNO_3$ in 5 ml water, add 10 ml 2-phenoxyethanol (for chromatograms developed with nonaqueous system) or 20 ml (with aqueous system), dilute to 200 ml with acetone, an-

[1] Some chemists prefer to wash the papers with water in a pan by decantation.

alytical grade, and mix (should solution show tendency to darken, add 1–5 drops of 30% H_2O_2, and mix. Even with addition of the peroxide, the solution may darken on standing. The darkening, however, apparently does not interfere with its chromogenic function).

Procedure

With a hard pencil rule the starting line 1″ from bottom edge of an even number of papers (one for each solvent system) and place eleven dots along the line at 0.6″ intervals beginning 1″ from either side edge. Starting at one side, identify the dots on the papers to be spotted with solution A as 2x, 5x, and with solution B as 10x, 20x, 50x. Leave the middle dot blank and mark the remaining dots similarly. (x equals 1 microgram, solids, or 1 microliter, liquids, which is equivalent to the amount of substance in 0.001 ml of solution A.) Then either one sample in duplicate or two different samples may be chromatographed on each paper.

Wash papers with water as described above and airdry; then spot papers in pairs (one for each solvent system) with 0.001 ml portion of solutions A and B, superimposing two or five portions on dots marked 2x, 5x or 20x, 50x, respectively, with adequate drying between successive spottings.

Using a glass funnel, fill dipping tank to within $\frac{1}{8}$″ of rim with the immobile solvent (5% refined soybean oil in ethyl ether is used in the aqueous system, (**b**) under Reagents) and cover. Attach binder clip to the bottom edge of one duplicate paper, invert paper, remove cover from dipping tank, insert paper into dipping tank up to the starting line, remove paper, cover tank, and clip upper edge to glass rod in hood. Repeat the dipping process with another paper from a different pair of duplicate papers. As soon as two papers are thus dipped and clipped to rod in hood, add 50 ml of the mobile solvent (25 ml water diluted to 100 ml with 2-methoxyethanol, for the aqueous system (**b**)), to each trough, clip the two papers to separate rods, transfer them simultaneously to the chromatographic tank for development, and seal glass cover with cellophane or other suitable tape. Development time is about $4\frac{1}{4}$ hours.

By means of the glass siphon drain solvent from the dipping tank to a glass-stoppered flask; cleanse and dry tank.

Add 50 ml of the mobile solvent, 2,2,4-trimethylpentane, for the nonaqueous system (**c**) to each trough in the chromatographic tank, and cover. Repeat the process for the second of each pair of papers by dipping them separately into the immobile solvent (10% 2-phenoxyethanol in ethyl ether used in the nonaqueous system (**c**)), and transfer the two papers immediately to the chromatographic tank for development. Cover, and seal tank. Development time is about $1\frac{1}{4}$ hours. Drain, cleanse, and dry dipping tank as before.

When the solvent front approaches within about $\frac{1}{2}$″ of top edge of papers (for both systems) remove cover, lift papers separately from tank, mark solvent front with soft pencil, and hang the papers from glass rods in hood until the mobile solvent has volatilized (nonaqueous system), or overnight (aqueous system). View papers under ultraviolet light ("Black Light") and mark with soft pencil any fluorescent or quenched areas.

Spray the papers with the proper chromogenic agent (**d**), and immediately expose them to strong ultraviolet light—first the back side, then the front side—until the spots are fully defined. Again view chromatograms under "Black Light." Determine the R_F values of the spots (or streaks).

Chromatograms can be stored without undue darkening if excess silver is washed from the papers. To do so, suspend rod with paper over a sink or pan, carefully wet paper with a *very* gentle stream of water from a wash bottle, and continue to wash with a back-and-forth movement. Play the water at an 80–85° angle until adequately washed (about 500 ml). Let chromatograms "dry" overnight before assembling them in piles; chromatograms can be ruined by transfer of spots from paper to paper if stacked too soon.

Discussion

Dipping Tank.—Reproducible work with an immobile solvent requires its uniform spread in the chromatographic paper. The inherent advantages of a dipping rather than a spraying technique have been dis-

cussed (*This Journal*, **41**, 481 (1958)), and need not be repeated here. The dipping tank and accessories are described under *Apparatus*, (**b**).

Chromatographic Paper.—Before use, all papers were washed with water by the adaptation of the continuous procedure of ascending paper chromatography, *This Journal*, **40**, 1025 (1957). While this procedure does not remove the interfering silver reactants from the paper as does washing by decantation in a pan, it does cause them to migrate to the upper edge of the paper above the normal mobile solvent front. The procedure requires less effort than washing by decantation and, of course, eliminates tearing the wet paper by handling. It was found that the continuous procedure is less effective during periods of high humidity than during periods of low humidity. This defect is remedied somewhat by setting the tank in a warm, well-ventilated place. The continuous procedure is less effective when four papers per cover slit, instead of only one, are washed.

Theoretically, more effective washing, i.e., better migration of interfering substances to the upper edge of the paper, should be accomplished by the mobile solvent itself. This was tried with the aqueous 2-methoxyethanol solvent (25 ml water to 100 ml with 2-methoxyethanol). However, for the 24-hour washing period this solvent was less effective than water because of its relatively slower ascent of the paper.

Solvent Systems.—Numerous solvent systems may be used in the separation and identification of pesticide chemicals, particularly of the chlorinated organic group, provided the system consists of both immobile and mobile solvents. The writer has classified the solvents into two groups, one of which has been called the aqueous or oil group, and the other the nonaqueous or paraffin group (*This Journal*, **40**, 1009, 1010 (1957). For the aqueous or oil group, various percentages, v/v, of refined soybean oil in ethyl ether are employed for the immobile solvent. Various percentages, v/v, of water in acetic acid, acetone, acetonitrile, dioxane, ethanol, methanol, 2-methoxyethanol, pyridine, or combinations of these, if compatible, may be employed as the mobile solvent. There is no apparent reason why a mineral oil or any refined vegetable or animal oil, except possibly castor oil, could not replace refined soybean oil, provided the oils are liquid at the development temperature and are insoluble in, or can be equilibrated with, the mobile solvent.

For the nonaqueous or paraffin group, various percentages in ethyl ether, v/v, of the following may be employed as the immobile solvent: acetic anhydride, dimethylacetamide, dimethylcyanamide, dimethylformamide, dimethylfuran, dimethylhydrogenphosphite, dimethylphthalate, dioxane, fenchone, formamide, glycerol, glycols, 2,2-iminodiethanol, isopropylbenzene, isopropylformate, 1-methoxy-2-propanol, 1-methylnaphthalene, 2-phenoxyethanol, N-isopropylbenzenesulfonamide, or trimethylphosphate (no known commercial source) or any combinations of them, if compatible (*This Journal*, **40**, 1029 (1957)). If they are insoluble in ethyl ether, mixtures of ethanol and ethyl ether, or acetone, may be employed.

At present, the preferred immobile solvents are acetic anhydride, dimethylacetamide, dimethylcyanamide, dimethylformamide, and 2-phenoxyethanol. The mobile solvents are the volatile liquids which belong to the straight chain, branched chain, and cycloparaffins, such as the petroleum ethers, the gasolines (lead-free), the hexanes, heptane, the octanes, the kerosenes, and the light mineral oils obtained from the petroleum industry. These mobile solvents are equilibrated with the immobile solvents when necessary.

The aqueous and nonaqueous solvent systems selected for this work are those preferred by associates who are applying ascending paper chromatography to pesticide residues isolated from foods and food products.

Conservation of Immobile Solvent.—The dipping tank holds about 300 ml of immobile solvent mixture, and about 8 papers can be dipped before it is necessary to refill the dipping tank up to about $\frac{1}{8}$″ of the rim. Papers are dipped as rapidly as possible, and the tank is covered between dippings. To conserve the immobile solvent the remainder is siphoned out (not poured) into a glass-

stoppered flask, and the dipping tank is immediately cleansed, especially if a semidrying oil such as soybean oil has been used. Concentration of immobile solvent in the vehicle (usually ethyl ether) can be checked by gravimetric, hydrometric, or refractive index methods by comparing with concentrations obtained from a freshly prepared solution, and adjusting by adding a calculated amount of ethyl ether. This adjustment procedure, however, is seldom necessary, and it is usually simpler to make up fresh immobile solvent mixtures.

Chromogenic Agent.—The original chromogenic agent consisted of silver nitrate and 2-phenoxyethanol, dissolved in water with just enough ethanol to keep the 2-phenoxyethanol in solution (*This Journal*, **39**, 981 (1956)). It was not entirely satisfactory in that it did not always wet the chromatogram quickly; this resulted in overspraying. It was also hard to tell when the water had evaporated sufficiently for exposure of the chromatogram to the strong ultraviolet light.

Paul A. Mills suggested that acetone could replace water as the vehicle and thus eliminate some of these troubles. The changes in composition of reagent (**d**) have improved it materially in that it quickly wets the chromatogram, it is more sensitive than the original agent, and the chromatogram can be exposed to the ultraviolet light immediately after spraying.

If the chromogenic agent is improperly sprayed on the chromatogram it may striate, and the striation effect gives the impression of more spots than are actually present. The striation is apparently caused by holding the nozzle of the sprayer too close to the paper; thus a "stream" of the agent hits the paper instead of a spray or mist. This defect seldom occurs when the nozzle is held far enough away from the paper.

General Remarks.—To obtain R_F values for the 213 pesticide chemicals, each paper was spotted so as to give two values (for averaging) for each amount of pesticide in the selected range (2, 5, 10, 20, 50 mmg (solids) or microliters (liquids); cf. *This Journal*, **40**, 294 (1957)). Experience has shown that this range usually gives adequate information about the sensitivity of a substance to the chromogenic agent, and the purity of the substance (provided the impurities are detectable by the agent).

For the aqueous system the procedure directs that the papers be dipped into the immobile solvent before transferring the mobile solvent to the troughs. The directions reverse this order for the nonaqueous system. The reason is that for the aqueous system, the oil of the immobile solvent is non-volatile, but the two components of the more volatile mobile solvent may change in composition if the solvent is allowed to stand in the tank for variable periods. On the other hand, for the nonaqueous system, the one-component mobile solvent does not change in composition but the 2-phenoxyethanol in the immobile solvent is more or less volatile. Of course, all steps should be completed in minimum time.

At present there seems to be no way to eliminate the step of clipping the papers to a glass rod in the hood between dipping and transfer to the tank.

Further, since it requires about 4¼ hours to develop papers with the aqueous solvent, it is directed that the chromatograms be airdried overnight before application of the chromogenic agent. The reason is that by the time the components of the mobile solvent volatilize from the papers, particularly during periods of high humidity, there is not enough time in the regular workday to expose the chromatograms to the ultraviolet light after spraying with the chromogenic agent. Fortunately, the non-volatile oil of the immobile solvent retards losses of the more volatile chromatographed substances.

The procedure directs that all chromatograms be viewed under the "Black Light," both before and after applying the chromogenic agent, to check for possible fluorescent and/or quenched areas. Although such information is not included in Table 2, it should be standard practice for all chromatographic work.

Presentation of Data

Sample Numbering.—In Tables 1 and 2 the whole numbers represent samples of individual pesticides which have been selected as reference standards for regulatory work. Sample numbers which include a letter,

A, B, etc., following the decimal of the number, are additional lots of material which will be used in alphabetical order for the same purpose when the previously lettered samples become exhausted. Samples which have a numeral, 1, 2, 3, etc., following the decimal of the number, are technical or commercial grades, usually from different manufacturers. Samples which have the letter E and a numeral, following the decimal of the number, are technical grade esters, usually from different manufacturers. Samples which have the letter S and a numeral, following the decimal of the number, are salts, usually inorganic, of technical grade products.

Table 1.—By means of the sample numbers, Table 1 serves as an index to Table 2. Table 1 lists the sample numbers, the common or trade name, if any, and the chemical name.

Table 2.—Table 2 gives the R_F values of 213 samples of 114 different pesticide chemicals for two different solvent systems (as detected by the chromogenic agent). The tabulation lists the sample numbers, the temperature at which the chromatograms were developed, the order of intensity of the spots, and the R_F values for 2, 5, 10, 20, and 50 microgram (solids) or microliter (liquids) quantities of the pesticides. Only one temperature is given, since both systems were run concurrently. The aqueous system requires about four times as long to develop the 8×8″ chromatogram as the nonaqueous system. (The nonaqueous system can be started after the aqueous system is under way.) For the nonaqueous system, and for BHC (#8) and DDT (#31), not only the order of intensity of the spots but also the identity of the responsible isomer is given.

The dash (—) in Table 2 indicates no spot, or a spot so weak as to have little significance. The letter "R" signifies a residual spot. If the "R" spot is heavy and unaccompanied by migrated spots, it probably represents the parent substance chromatographed. If it is a light (weak) spot, accompanied by a migrated spot or spots, the "R" spot probably represents impurities.

X–.50 or .50–x, or .25–.57 indicates a streak; the x denotes an indefinite lower or upper edge, and .40–.67 signifies a spot so large that it is essentially a streak, particularly if it represents the larger quantities (20 or 50 mmg or microliters) of the substance. The R_F values represent only those produced by the chromogenic agent and not those visible by daylight or under the "Black Light."

The chromogenic agent is satisfactory for the chlorinated pesticides but is unsatisfactory for some of the non-chlorinated ones such as the dinitro compounds. These may carry their own identity in the form of a distinctively colored spot. Others, such as Alanap®, may fluoresce under the "Black Light." The chromatograms of all samples reported here, however, were sprayed with the chromogenic agent described, since one of the objects of the work was to ascertain whether the non-chlorinated pesticides interfered with the detection of the chlorinated pesticides. The color and/or fluorescence were usually destroyed by the chromogenic agent or by ultraviolet light. In such

Table 1. Index to Table 2

Sample Numbers	Common or Tradename	Chemical Name
1, 1.A, 1.B, 1.1	Alanap®	N-1-Naphthylphthalamic acid
2.1	Aldrin	1,2,3,4,10,10-Hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene
3	Allethrin	*dl*-2-Allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one ester of *dl-cis* and *trans* chrysanthemum monocarboxylic acid
5, 5.A, 5.1	Aramite	2-(*p-tert*-Butylphenoxy)-1-methylethyl-2-chloroethylsulfite
8, 8.1, 8.2, 8.3	BHC	1,2,3,4,5,6-Hexachlorocyclohexane, several isomers
8.4	BHC	1,2,3,4,5,6-Hexachlorocyclohexane, several isomers, fortified with gamma
8.5	BHC	1,2,3,4,5,6-Hexachlorocyclohexane, several isomers, low in gamma
8.6	BHC	1,2,3,4,5,6-Hexachlorocyclohexane, several isomers, high in gamma

(*Continued on p. 787*)

Table 1. Continued

Sample Numbers	Common or Tradename	Chemical Name
10, 10.1	Bulan®	1,1-bis(*p*-chlorophenyl)-2-nitro-butane
11		Butoxypolypropylene glycol
11.1	Crag Fly Repellent®	Butoxypolypropylene glycol
17, 17.A, 17.1	Captan	N-Trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide
18, 18.A, 18.B, 18.1	Chloranil (Spergon®)	2,3,5,6-Tetrachloro-1,4-benzoquinone
19	Chlorbenside (Mitox®)	*p*-Chlorobenzyl-*p*-chlorophenylsulfide
20, 20.A, 20.1, 20.2	Chlordane	1,2,4,5,6,7,8,8-Octachloro-2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene
21, 21.A, 21.1	Chlorobenzilate	Ethyl-4,4′-dichlorobenzilate
22	CIPC	Isopropyl-N-(3-chlorophenyl)-carbamate
23.S1		Monosodium cyanamide
29, 29.A, 29.1, 29.2, 29.3	2,4-D acid	2,4-Dichlorophenoxyacetic acid
29.S1	2,4-D, sodium salt	Sodium salt of 2,4-D acid
29.E1, 29.E2, 29.E3	2,4-D acid: BE	Butyl ester of 2,4-D acid
29.E4	2,4-D acid: BEE	Butoxyethanol ester of 2,4-D acid
29.E5	2,4-D acid: Mixed BE	Mixed butyl esters of 2,4-D acid
29.E6	2,4-D acid: BEPE	Butoxyethoxypropyl ester of 2,4-D acid
29.E7	2,4-D acid: Iso BE	Isobutyl ester of 2,4-D acid
29.E8	2,4-D acid: Iso B and BE	Isobutyl and Butyl esters of 2,4-D acid
29.E9	2,4-D acid: 2-EHE	2-Ethylhexyl ester of 2,4-D acid
29.E10, 29.E11, 29.E12, 29.E13	2,4-D acid: Iso OE	Isooctyl ester of 2,4-D acid
29.E14, 29.E15	2,4-D acid: Iso PE	Isopropyl ester of 2,4-D acid
30	Dalapon	2,2-Dichloropropionic acid
30.S1	Dowpon®	Sodium salt of 2,2-Dichloropropionic acid
31, 31.1, 31.2, 31.3, 31.4, 31.5, 31.6, 31.7, 31.8, 31.9	DDT	Dichlorodiphenyltrichloroethane
32, 32.A, 32.B, 32.1, 32.2	Dichlone (Phygon®)	2,3-Dichloro-1,4-naphthoquinone
33	Dichloral urea	bis(1-hydroxy-2,2,2-trichloroethyl) urea
34.1	Dieldrin	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene
35	Dilan®	2 parts Bulan and 1 part Prolan (see Bulan, No. 10 and Prolan, No. 116)
36	DNAP	2,4-Dinitro-6-*sec.*-amylphenol
37, 37.1	DNOSBP	4,6-Dinitro-*ortho*-*sec.*-butylphenol
37.S1		Triethanolamine salt of DNOSBP
38, 38.1	DNOC	4,6-Dinitro-*ortho*-cresol
38.S1		Sodium salt of DNOC
39	DNOCHP	4,6-Dinitro-*ortho*-cyclohexylphenol
39.S1		Dicyclohexylamine salt of DNOCHP
41, 41.A, 41.1	Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethyl urea
42, 42.1	DMC (Dimite®)	4,4′-Dichloro-1-methylbenzhydrol
43, 43.A, 43.1	Duraset®	N-*meta*-Tolylphthalamic acid
44	Dyrene®	2,4-Dichloro-6-(*ortho*-chloroaniline)-*s*-triazine
49, 49.1, 49.2	Endothal®, Disodium	Disodium-3,6-endoxohexahydrophthalate
50, 50.A, 50.B, 50.1	Endrin	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene
51, 51.A, 51.1	EPN	O-ethyl-O-*p*-nitrophenyl phenylphosphonothioate
52	Ethide®	1,1-Dichloro-1-nitroethane
58	Glyodin	2-Heptadecyl-2-imidazoline acetate
62, 62.A, 62.1	Heptachlor	3*a*,4,5,6,7,8,8-Heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene
63	Heptachlor epoxide	1,4,5,6,7,8,8-Heptachloro-2,3-*epoxy*-2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene
65, 65.1	IPC	N-Phenylisopropyl carbamate
68, 68.A, 68.1	Karathane®	2-(1-Methylheptyl)-4,6-dinitrophenyl crotonate
69, 69.A, 69.1	Kelthane®	1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol

(*Continued on p. 788*)

Table 1. Continued

Sample Numbers	Common or Tradename	Chemical Name
75	Lethane-60®	2-Thiocyanoethyl laurate
76	Lethane-384®	2-(2-Butoxyethoxy)-ethylthiocyanate
77, 77.A	Lindane	1,2,3,4,5,6-Hexachlorocyclohexane, gamma isomer
83	Malathion	S-(1,2-Dicarbethoxyethyl)-*O,O*-dimethylphosphorothioate
84, 84.A, 84.1	Maleic hydrazide	1,2-Dihydropyridazine-3,6-dione
85, 85.1, 85.2	MCP	4-Chloro-2-methylphenoxyacetic acid
85.3	Weedar MCP®	4-Chloro-2-methylphenoxyacetic acid
85.4	Weedar-64®	4-Chloro-2-methylphenoxyacetic acid
86, 86.A		2-Mercaptobenzothiazole
87, 87.1	Methoxychlor	1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl)ethane
88, 88.A, 88.1, 88.2	Methyl parathion	*O,O*-Dimethyl-*O*-*p*-nitrophenylphosphorothioate
90, 90.A	Monuron	3-(*p*-Chlorophenyl)-1,1-dimethyl urea
90.1	"Karmex" W®	3-(*p*-Chlorophenyl)-1,1-dimethyl urea
96		Naphthaleneacetic acid
96.E1		Methyl ester of naphthaleneacetic acid
96.S1		Sodium salt of naphthaleneacetic acid
97, 97.A	Neburon	1-*n*-Butyl-3-(3,4-dichlorophenyl)-1-methylurea
103, 103.A		*ortho*-Phenylphenol
103.S1	Dowicide® A	Sodium *ortho*-Phenylphenate
104	Ovex	*p*-Chlorophenyl-*p*-chlorobenzenesulfonate
110, 110.A	Parathion	*O,O*-Diethyl-*O*-*p*-nitrophenylphosphorothioate
110.1	Thiophos®	*O,O*-Diethyl-*O*-*p*-nitrophenylphosphorothioate
110.2	Niran®	*O,O*-Diethyl-*O*-*p*-nitrophenylphosphorothioate
111, 111.1	PCNB (Tritisan®)	Pentachloronitrobenzene
112		Pentachlorophenol
112.1	Penta	Pentachlorophenol
112.2	Santobrite Powder®	Pentachlorophenol
112.3	Dowicide® 7	Pentachlorophenol 83%; other chlorophenols 12%
112.S1	Dowicide® G	Sodium pentachlorophenate 75%; other sodium chlorophenates 13%
113, 113.A, 113.1	Perthane®	1,1-bis(*p*-ethylphenyl)-2,2-dichloroethane
114, 114.A	Phenothiazine	Dibenzo-1,4-thiazine
115, 115.A, 115.1	Piperonyl butoxide	3,4-Methylenedioxy-6-propylbenzyl-*n*-butyldiethyleneglycol ether
116, 116.1	Prolan®	1,1-bis(*p*-chlorophenyl)-2-nitropropane
118	Piperonyl cyclonene	3-*n*-Hexyl-5-(3,4-methylenedioxyphenyl)-2-cyclohexenone
122	Randox®	2-Chloro-*N,N*-diallylacetamide
131	SES	Sodium 2,4-dichlorophenoxyethyl sulfate
132	SES-T	Sodium 2,4,5-trichlorophenoxyethyl sulfate
133, 133.1	Sevin®	N-Methyl-1-naphthyl carbamate
134, 134.A, 134.1, 134.2	Sulphenone	*p*-Chlorophenylphenylsulfone
138	Tedion®	2,4,5,4′-Tetrachlorodiphenylsulfone
139.1	Thiodan®	6,7,8,9,10,10-Hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
140, 140.1, 140.2, 140.3, 140.4	2,4,5-T acid	2,4,5-Trichlorophenoxyacetic acid
140.E1, 140.E2	2,4,5-T acid: BE	Butyl ester of 2,4,5-T acid
140.E3	2,4,5-T acid: BEE	Butoxyethanol ester of 2,4,5-T acid
140.E4	2,4,5-T acid: BEPE	Butoxyethoxypropyl ester of 2,4,5-T acid
140.E5	2,4,5-T acid: 2-EHE	2-Ethylhexyl ester of 2,4,5-T acid
140.E6	2,4,5-T acid: Iso OE	Isooctyl ester of 2,4,5-T acid
140.E7	2,4,5-T acid: Iso PE	Isopropyl ester of 2,4,5-T acid
141, 141.A, 141.B, 141.1, 141.2	TDE(Rhothane®) DDD	1,1-Dichloro-2,2-bis(*p*-chlorophenyl)-ethane
142, 142.1		Tetraiodoethylene
143		2,4,5-Trichlorophenoxypropionic acid
144	TCNB (Tecnazine®)	2,3,5,6-Tetrachloronitrobenzene
146	Toxaphene	Chlorinated camphene, 67–69% chlorine
151, 151.A	Vegadex®	2-Chloroallyldiethyldithiocarbamate
155	Warfarin®	3-(1-Acetonylbenzene)-4-hydroxycoumarin

Table 2. R_F values of samples of pesticides for two different solvent systems with silver nitrate: 2-phenoxyethanol in acetone as chromogenic agent

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
1	25	1	—	—	.92	.92	.92	1	R	R	R	R	R
1.A	25	1	—	—	.93	.93	.93	1	R	R	R	R	R
1.B	25	1	—	—	.92	.92	.92	1	R	R	R	R	R
1.1	25	1	—	—	.92	.92	.92	1	R	R	R	R	R
2.1	21	3	—	.02	.03	.03	.03	2	R	R	R	R	R
		2	.18	.18	.18	.18	.19	1	.92	.92	.92	.92	.92
		1	.35	.35	.36	.36	.37						
3	21	2	—	—	—	—	.53	1	—	.58	.58	.57	.57
		1	—	—	—	.67	.67						
5	22		—	—	—	—	—	—	—	—	—	—	—
5.A	21		—	—	—	—	—	—	—	—	—	—	—
5.1	21	—	—	—	—	—	—	—	—	—	—	—	—
8[a]	22	3	—	—	—	.46	.45	5	—	—	—	—	R
		1	.57	.56	.56	.56	.55	2B	.06	.07	.07	.07	.07
		2	.70	.70	.70	.70	.70	3D	.14	.14	.15	.15	.15
								4E	—	—	.23	.23	.24
								2G	.45	.45	.45	.45	.43
								1A	.54	.54	.54	.54	.53
8.1	22	1	.54	.54	.54	.54	.53	6	—	—	—	—	R
		3	—	.60	.60	.60	.59	3B	.08	.08	.08	.09	.09
		2	.67	.66	.67	.67	.68	4D	.15	.15	.15	.15	.16
								5E	—	—	—	—	.25
								2G	.47	.47	.47	.47	.47
								1A	.58	.58	.58	.57	.57
8.2	22	1	.56	.56	.54	.54	.53	5	—	—	—	—	R
		3	—	.61	.60	.60	.60	3B	.08	.07	.08	.08	.09
		2	.68	.68	.69	.69	.69	4D	.15	.15	.15	.16	.17
								2G	.47	.48	.47	.48	.48
								1A	.58	.58	.58	.58	.59

[a] B = Beta isomer; D = Delta isomer; E = Epsilon isomer; G = Gamma isomer; A = Alpha isomer.

(*Continued on p. 790*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
8.3	22	1	.55	.55	.55	.54	.53	5	—	—	—	—	R
		3	—	—	.61	.61	.61	3B	.05	.06	.06	.06	.05
		2	.68	.68	.68	.68	.69	2D	.14	.14	.14	.14	.14
								4E	—	—	—	—	.24
								2G	.46	.46	.46	.46	.46
								1A	.57	.57	.57	.57	.57
8.4	22	3	.44	.43	.43	.43	.43	7	—	—	—	R	R
		1	.59	.58	.58	.57	.58	4B	—	—	.07	.07	.08
		2	.68	.68	.68	.68	.69	2D	.13	.13	.13	.14	.14
								5E	—	—	—	.23	.23
								1G	.44	.44	.44	.44	.44
								3A	—	.55	.54	.55	.55
								6	—	—	—	—	.69
8.5	22	3	—	—	—	—	.42	5	—	—	—	—	R
		1	.56	.56	.56	.55	.55	2B	.07	.07	.07	.06	.07
		2	.69	.69	.69	.69	.68	4D	.13	.13	.13	.13	.14
								3G	.44	.43	.44	.44	.43
								1A	.53	.53	.53	.53	.54
8.6	22	4	.39	.37	.36	.37	.37	5	R	R	R	R	R
		3	.46	.45	.45	.44	.44	3B	.07	.06	.06	.06	.06
		1	.61	.61	.60	.60	.59	2D	.15	.15	.14	.14	.15
		2	.69	.68	.68	.69	.68	4E	—	—	.24	.24	.24
								1G	.45	.45	.45	.45	.46
								2A	.53	.54	.55	.56	.57
								6	—	—	—	.67	.67
								7	—	—	—	—	.77
10	20	1	.58	.58	.58	.58	.58	1	.27	.27	.27	.27	.26
10.1	21	1	—	.53–.71	.51–.71	.50–.71	.47–.71	1	—	.19–.34	.19–.36	.18–.36	.15–.37
11	21		—	—	—	—	—	1	R	R	R	R	R
11.1	21		—	—	—	—	—	1	R	R	R	R	R
17	22	1	.82	.82	.80	.63–.87	.43–.87	1	.07	.07	.07	.00–.13	.00–.13

(*Continued on p. 791*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
17.A	20	1	.87	.86	.86	.73–.91	.58–.91	1	.04	.04	.04	.00–.09	.00–.09
17.1	20	1	.84	.83	.84	.65–.90	.42–.89	1	.06	.06	.06	.00–.09	.00–.10
18	23	1	.79	.79	.57–.85	.33–.85	.00–.85	2	R	R	R	R	R
								1	.07	.07	.07	.00–.13	.00–.13
18.A	21	1	.72	.73	.73	.57–.86	.41–.84	2	R	R	R	R	R
								1	.09	.09	.09	.08	.08
18.B	23	1	.77	.77	.52–.82	.22–.82	.00–.82	2	R	R	R	R	R
								1	.11	.11	.00–.17	.00–.17	.00–.17
18.1	23	1	.77	.77	.55–.84	.38–.84	.00–.84	2	R	R	R	R	R
								1	.08	.08	.08	.00–.12	.00–.12
19	21	3	R	R	R	R	R	2	R	R	R	R	R
		2	.12	.12	.11	.11	.10	1	.65	.66	.65	.54–.71	.44–.71
		1	.37	.36	.36	.35	.34						
20	21	1	.10–.57	.10–.57	.10–.57	.08–.57	.08–.57	2	R	R	R	R	R
								1	.80–1.0	.72–1.0	.63–1.0	.47–1.0	.35–1.0
20.A	21	1	.12–.60	.11–.61	.11–.61	.10–.62	.08–.62	2	R	R	R	R	R
								1	.84–.10	.79–1.0	.71–1.0	.54–1.0	.33–1.0
20.1	21	1	.12–.55	.10–.55	.08–.58	.08–.59	.06–.52	2	R	R	R	R	R
								1	.84–1.0	.78–1.0	.68–1.0	.54–1.0	.35–1.0
20.2	21	1	.13–.57	.11–.58	.11–.58	.11–.57	.10–.60	2	R	R	R	R	R
								1	.82–1.0	.80–1.0	.80–1.0	.66–1.0	.48–1.0
21	21	2	—	—	.34	.33	.34	2	R	R	R	R	R
		3	—	—	—	.55	.55	1	.40	.40	.39	.39	.38
		1	.79	.79	.78	.79	.79						
21.A	20	2	—	—	.29	.29	.29	2	—	R	R	R	R
		3	—	—	—	.48	.48	1	.34	.35	.32	.32	.31
		1	.76	.76	.76	.75	.75						

(*Continued on p. 792*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
21.1	21	2	—	—	.29	.29	.29	2	—	—	R	R	R
		3	—	—	—	.58	.58	1	.37	.37	.37	.37	.36
		1	.76	.76	.76	.77	.77						
22	21	1	.87	.86	.86	.86	.87	1	.19	.19	.18	.17	.17
23.S1	21	Na	—	—	.36–.56	.38–.58	.33–.60	1	R	R	R	R	R
		1	.89	.89	.88	.88	.87						
29	17	1	.91	.91	.91	.92	.92	1	R	R	R	R	R
29.A	17	1	.90	.90	.90	.90	.91	1	R	R	R	R	R
29.1	20	1	.88	.87	.87	.87	.88	1	R	R	R	R	R
29.2	20	1	.87	.87	.87	.87	.87	1	R	R	R	R	R
29.3	20	1	.85	.85	.85	.86	.86	1	R	R	R	R	R
29.S1	20	Na	.68	.68	.68	.68	.68	1	R	R	R	R	R
		1	.86	.86	.86	.86	.86						
29.E1	20	1	.58	.57	.57	.57	.55	2	R	R	R	R	R
		2	.93	.93	.93	.93	.93	1	.53	.54	.52	.52	.52
29.E2	20	1	.57	.57	.58	.58	.58	2	R	R	R	R	R
		3	—	—	.74	.74	.73	1	.54	.54	.54	.54	.53
		2	—	.91	.91	.91	.91						
29.E3	21	3	—	—	.24	.23	.23	2	—	R	R	R	R
		1	.59	.59	.58	.58	.57	1	.59	.58	.58	.57	.57
		2	.95	.95	.95	.94	.94						
29.E4	20	1	.64	.64	.64	.64	.64	2	R	R	R	R	R
		2	.90	.91	.91	.91	.91	1	.35	.35	.35	.34	.34
29.E5	21	4	—	—	.22	.22	.22	2	R	R	R	R	R
		1	.60	.60	.59	.59	.58	1	.60	.60	.60	.60	.60
		3	—	—	.71	.71	.71						
		2	—	—	.92	.92	.92						

(*Continued on p. 793*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
29.E6	21	3	—	.25	.25	.25	.27	2	R	R	R	R	R
		1	.66	.64	.65	.64	.65	1	.36	.36	.34	.34	.33
		2	.92	.92	.92	.92	.92						
29.E7	21	1	.57	.57	.58	.57	.57	2	—	—	R	R	R
		3	—	—	—	.73	.73	1	.56	.57	.57	.57	.57
		2	—	—	.89	.89	.89						
29.E8	20	2	—	—	.25	.25	.25	2	R	R	R	R	R
		1	.58	.58	.58	.57	.57	1	.56	.56	.54	.54	.53
		3	—	—	—	—	.75						
29.E9	20	1	.24	.23	.24	.23	.22	2	R	R	R	R	R
		3	—	—	.60	.59	.59	1	.78	.78	.78	.78	.78
		2	.94	.95	.95	.95	.94						
29.E10	21	3	—	—	.07	.07	.07	2	R	R	R	R	R
		1	.26	.27	.27	.26	.26	1	.88	.88	.88	.88	.88
		2	—	.93	.93	.93	.93						
29.E11	21	1	.28	.28	.28	.27	.25	2	—	R	R	R	R
		3	—	—	—	.70	.71	1	.82	.83	.83	.83	.83
		2	—	—	.92	.92	.92						
29.E12	21	1	.29	.28	.27	.27	.26	2	R	R	R	R	R
		2	—	—	—	—	.93	1	.79	.79	.79	.79	.78
29.E13	21	1	.24	.24	.24	.23	.23	2	R	R	R	R	R
		2	.92	.91	.90	.90	.90	1	.81	.80	.81	.79	.79
29.E14	21	3	—	—	—	.29	.29	2	R	R	R	R	R
		1	.75	.74	.74	.74	.74	1	.60	.59	.59	.60	.60
		2	—	.92	.92	.92	.92						
29.E15	21	1	.69	.67	.67	.66	.67	2	—	R	R	R	R
		2	—	—	.91	.91	.91	1	.56	.57	.58	.58	.56
30	20	1	.92	.91	.91	.90	.90	1	.00–.04	.00–.05	.00–.07	.00–.08	.00–.09

(*Continued on p. 794*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
30.S1	17	Na	.73	.72	.72	.73	.73	1	R	R	R	R	R
		1	.92	.91	.91	.91	.91						
31	20	1	.31	.31	.31	.31	.31	3	—	—	—	R	R
								1 p,p′[b]	.63	.62	.62	.62	.61
								2 o,p[c]	.76	.76	.76	.75	.76
31.1	20	1	.32	.33	.34	.34	.34	5	R	R	R	R	R
		2	—	—	.49	.49	.49	4	—	—	.10	.10	.09
		2	—	—	.65	.65	.65	3	—	—	.24	.23	.23
		3	—	—	.90	.90	.90	1 p,p′	.66	.67	.67	.39–.75	.37–.75
								2 o,p	.80	.80	.80	.80	.80
31.2	20	1	.32	.32	.32	.32	.32	5	R	R	R	R	R
		2	—	—	.47	.48	.48	4	—	.07	.08	.08	.08
		2	—	—	.65	.65	.64	3	—	.20	.20	.20	.20
		3	—	—	.91	.92	.91	1 p,p′	.64	.62	.63	.35–.72	.29–.72
								2 o,p	.74	.75	.76	.76	.76
31.3	20	1	.32	.32	.32	.33	.33	5	R	R	R	R	R
		2	—	—	.47	.47	.47	4	—	—	.11	.10	.09
		2	—	—	.64	.64	.64	3	—	.22	.22	.22	.21
		3	—	—	.90	.91	.91	1 p,p′	.67	.68	.67	.38–.73	.33–.73
								2 o,p	.78	.79	.78	.78	.78
31.4	20	1	.33	.32	.32	.32	.32	5	R	R	R	R	R
		2	—	—	.45	.45	.45	4	—	—	—	.10	.11
		2	—	—	.64	.64	.64	3	—	.21	.22	.23	.22
		3	—	—	—	—	.91	1 p,p′	.66	.66	.66	.48–.75	.33–.75
								2 o,p	.79	.79	.78	.79	.80
31.5	20	1	.34	.32	.34	.34	.32	4	—	—	—	—	R
		2	—	—	.48	.48	.48	3	—	—	—	—	.20
								1 p,p′	.63	.63	.63	.34–.72	.26–.71
								2 o,p	.76	.76	.77	.76	.77
31.6	20	1	.33	.33	.34	.34	.34	3	—	—	—	—	.22
		2	—	—	.50	.50	.50	1 p,p′	.67	.68	.67	.36–.76	.26–.76
								2 o,p	.81	.80	.80	.80	.81

[b] p,p′: para, para isomer.
[c] o,p: ortho, para isomer.

(*Continued on p. 795*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
31.7	20	1	.33	.33	.33	.33	.33	3	—	—	—	—	.19
		2	.48	.48	.48	.48	.48	1 p,p′	.60	.60	.60	.34–.69	.29–.68
								2 o,p	.72	.72	.73	.72	.73
31.8	20	1	.32	.32	.31	.31	.30	5	—	—	—	R	R
		2	—	—	.46	.46	.45	4	—	—	.10	.11	.11
		3	—	—	—	.63	.63	3	—	—	.23	.23	.22
		4	—	—	—	.88	.89	1 p,p′	.69	.69	.68	40.–.75	.33–.76
								2 o,p	.80	.81	.81	.81	.81
31.9	23	1	.35	.35	.34	.33	.33	1 p,p′	.63	.63	.64	.48–.71	.40–.71
		2	—	—	.48	.48	.48	2 o,p	.76	.75	.76	.76	.76
32	25	1	.68	.68	.67	.48–.73	.24–.74	2	R	R	R	R	R
								1	.13	.12	.11	.10	.00–.17
32.A	25	1	.68	.67	.65	.50–.73	.27–.73	2	R	R	R	R	R
								1	.11	.11	.11	.11	.00–.17
32.B	25	1	.67	.67	.66	.46–.71	.18–.72	2	R	R	R	R	R
								1	.14	.14	.14	.13	.00–.20
32.1	25	1	.64	.63	.63	.47–.69	.26–.69	2	R	R	R	R	R
								1	.13	.12	.11	.11	.00–.18
32.2	25	1	.66	.65	.65	.50–.71	.27–.71	2	R	R	R	R	R
								1	.09	.09	.08	.08	.00–.14
33	21	1	.96	.96	.96	.96	.96	1	R	R	R	R	R
34.1	20	2	—	—	.03	.03	.03	3	—	R	R	R	R
		1	.42	.42	.42	.43	.41	1	.64	.64	.64	.64	.64
								2	—	—	.94	.94	.94
35	21	1	—	—	.52–.76	.54–.77	.48–.78	1	—	—	.26–.48	.25–.48	.27–.48
36	21	1	.97	.97	.97	.80–1.0	.57–1.0	3	—	—	R	R	R
								2	—	.17	.18	.19	.20
								1	.39	.39	.39	.39	.39

(*Continued on p. 796*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
37	23	2	—	—	.00–.14	.00–.24	.00–.43	2	—	—	R	R	R
		1	.97	.97	.89–1.0	.78–1.0	.54–1.0	1	.33	.33	.33	.33	.33
37.1	23	2	—	—	.00–.14	.00–.22	.00–.45	3	—	—	R	R	R
		1	.97	.97	.88–1.0	.78–1.0	.51–1.0	2	—	—	.16	.16	.16
								1	.38	.38	.38	.38	.38
37.S1	23	1	.97	.97	.97	.97	.97	2	R	R	R	R	R
								1	.32	.32	.32	x–.41	x–.41
38	20	1	.93	.92	.92	.92	.91	1	.10	.10	.10	.10	.10
38.1	20	1	.89	.88	.87	.87	.86	1	.09	.09	.10	.11	.11
38.S1	22	1	.97	.97	.97	.97	.97	1	.08	.08	.08	.08	.08
39	21	2	—	—	.00–.14	.00–.22	.00–.37	2	R	R	R	R	R
		1	.93	.93	.93	.66–.96	.37–.96	3	—	—	.11	.11	.11
								1	.28	.29	.29	.28	.28
39.S1	21	1	.89	.88	.90	.89	.90	2	R	R	R	R	R
								1	.00–.10	.00–.10	.00–.09	.00–.08	.00–.08
41	17	1	.96	.96	.96	.96	.96	1	R	R	R	R	R
41.A	17	1	.96	.96	.96	.96	.96	1	R	R	R	R	R
41.1	17	1	.96	.96	.96	.96	.96	1	R	R	R	R	R
42	21	1	.80	.80	.81	.80	.81	1	.17	.16	.16	.15	.15
42.1	21	1	.84	.83	.83	.82	.82	3	—	—	—	R	R
		2	—	—	.96	.96	.96	2	.04	.04	.04	.04	.04
								1	.15	.15	.16	.15	.15
43	23	1	—	—	.91	.91	.91	1	R	R	R	R	R
43.A	23	1	—	—	.93	.93	.93	1	R	R	R	R	R

(*Continued on p. 797*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
43.1	23	1	—	—	.91	.91	.91	1	R	R	R	R	R
44	17	1	.88	.88	.88	.88	.87	2	R	R	R	R	R
								1	.15	.14	.13	.13	.13
49	21	1	—	—	.38	.40	.38	1	R	R	R	R	R
		2	—	—	.43–.66	.43–.66	.45–.65						
		3	—	—	.72	.73	.72						
49.1	21	5	R	R	R	R	R	1	R	R	R	R	R
		2	.00–.09	.00–.11	.00–.25	.00–.32	.00–.33						
		4	—	—	—	.44	.45						
		3	x–.65	x–.67	x–.73	x–.73	x–.73						
		1	—	.77	.76	.76	.75						
49.2	21	2	R	R	R	R	R	1	R	R	R	R	R
		1	.31	.33	.30	.29	.24						
		3	x–.63	x–.67	x–.75	x–.77	x–.83						
50	21	1	.42	.41	.40	.39	.39	2	—	—	—	.17	.16
		2	—	—	.72	.72	.71	1	.65	.64	.64	.64	.64
50.A	21	1	.42	.41	.41	.41	.41	2	—	—	.13	.15	.15
		2	—	—	.70	.71	.71	1	.63	.63	.63	.63	.64
50.B	21	1	.43	.44	.44	.44	.42	2	—	—	.21	.21	.21
		2	—	—	.72	.72	.72	1	.73	.72	.72	.73	.73
50.1	21	4	—	—	.33	.33	.33	4	R	R	R	R	R
		1	.46	.48	.48	.48	.48	2	—	—	.22	.22	.22
		2	—	—	.78	.78	.78	1	.74	.74	.73	.73	.73
		3	—	—	.91	.91	.91	3	—	—	—	.95	.95
51	20	1	.72	.72	.72	.72	.72	1	.23	.23	.22	.23	.22
51.A	20	1	.72	.72	.71	.71	.71	1	.20	.21	.21	.21	.21
51.1	22	2	—	—	.14	.14	.14	3	—	—	—	R	R
		1	.63	.63	.63	.63	.63	1	—	—	.23	.23	.23
								2	—	—	—	.82	.82

(*Continued on p. 798*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
52	20		—	—	—	—	—	1	—	—	R	R	R
58	21		—	—	—	—	—		—	—	—	—	—
62	25	1	.31	.31	.31	.31	.31	1	.94	.94	.94	.94	.94
62.A	21	1	.33	.33	.32	.31	.30	2	—	R	R	R	R
								1	.98	.97	.88–1.0	.84–1.0	.77–1.0
62.1	21	3	—	—	—	.06	.07	3	—	R	R	R	R
		1	.35	.35	.35	.35	.35	2	—	.86	.86	.85	.82
		2	.42	.42	.44	.45	.46	1	.95	.95	.95	.95	.95
		4	—	—	.92	.92	.92						
63	25	1	.39	.39	.38	.37	.38	1	.78	.77	.77	.77	.77
65	21		—	—	—	—	—		—	—	—	—	—
65.1	23		—	—	—	—	—		—	—	—	—	—
68	20	2	—	—	—	R	R	2	—	R	R	R	R
		1	.25	.25	.25	.25	.25	1	—	.59	.59	.59	.59
68.A	20	2	—	—	—	R	R	2	R	R	R	R	R
		1	.22	.22	.22	.22	.22	1	—	.58	.58	.57	.57
68.1	20	2	—	—	—	R	R	2	R	R	R	R	R
		1	.24	.24	.24	.24	.24	1	—	.58	.59	.59	.59
69	21	2	—	—	.19	.19	.19	2	—	R	R	R	R
		1	.72	.71	.71	.70	.70	3	—	.00–.07	.00–.07	.00–.07	.00–.07
								1	.34	.31	.19–.42	.15–.42	.12–.48
								4	—	—	—	.56	.56
								5	—	—	—	—	.93
69.A	21	2	.21	.21	.20	.20	.19	2	R	R	R	R	R
		1	.69	.69	.69	.69	.69	4	—	.00–.06	.00–.06	.00–.06	.00–.06
								1	.30	.32	.17–.42	.17–.43	.06–.43
								5	—	—	—	.52	.52
								3	—	—	.91	.90	.91

(*Continued on p. 799*)

Sample No.	Temperature, °C	Aqueous System: Order of Intensity of Spots	Aqueous System: Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Nonaqueous system: Order of Intensity of Spots	Nonaqueous system: Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
69.1	22	4	.14	.15	.15	.15	.15	4	R	R	R	R	R
		5	—	.23	.23	.23	.23	1	.19	.19	.19	.19	.19
		3	.35	.35	.35	.36	.36	3	—	.40	.40	.40	.40
		6	—	—	.48	.48	.48	2	.84	.84	.84	.84	.84
		1	.63	.63	.64	.64	.64						
		2	.75	.75	.76	.76	.76						
75	23	4	R	R	R	R	R	2	—	R	R	R	R
		3	—	—	.09	.09	.09	1	—	—	.62	.62	.62
		2	—	.19	.19	.19	.19	3	—	—	1.0	1.0	1.0
		1	—	.33	.33	.33	.33						
		5	—	—	.50	.50	.50						
		6	—	—	.65	.65	.65						
76	23	2	—	—	—	.36	.36	2	—	—	R	R	R
		1	—	.45	.45	.45	.45	1	.30	.30	.30	.30	.30
		2	—	—	—	.58	.58						
		3	—	—	—	—	.81						
77	22	1	.60	.60	.60	.60	.60	1	.44	.44	.43	.44	.42
77.A	23	1	.65	.65	.65	.65	.65	1	.41	.41	.41	.42	.42
83	20	1	.89	.89	.89	.89	.89	1	.16	.16	.16	.17	.17
		2	.97	.97	.97	.97	.97						
84	21	1	.78	.78	.78	.79	.79	1	R	R	R	R	R
84.A	20	1	.77	.76	.76	.76	.77	1	R	R	R	R	R
84.1	20	1	.78	.78	.77	.77	.76	1	R	R	R	R	R
85	21	1	.88	.88	.88	.88	.88	1	R	R	R	R	R
85.1	20	1	.88	.88	.88	.88	.88	1	R	R	R	R	R
85.2	20	1	.86	.86	.86	.87	.87	1	R	R	R	R	R
85.3	22	1	.94	.93	.93	.93	.93	1	R	R	R	R	R
85.4	22	1	.92	.93	.93	.93	.93	1	R	R	R	R	R

(*Continued on p. 800*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
86	20	2	—	—	.00–.95	.00–.95	.00–.95	1	R	R	R	R	R
		1	.90	.90	.90	.90	.90						
86.A	20	2	—	—	.00–.93	.00–.93	.00–.93	1	R	R	R	R	R
		1	.89	.89	.89	.89	.89						
87	20	1	.68	.68	.67	.68	.67	2	—	—	—	R	R
								1	.30	.30	.30	.18–.38	.13–.41
87.1	21	1	.65	.64	.64	.63	.62	2	—	—	R	R	R
		3	—	.75	.75	.74	.74	1	.44	.42	.29–.52	.24–.55	.20–.55
		2	.90	.90	.90	.90	.90	3	—	—	—	—	.71
88	20	1	—	.88	.88	.88	.88	2	—	—	R	R	R
								1	.09	.09	.09	.09	.09
88.A	20	1	—	—	.85	.85	.85	2	—	—	R	R	R
								1	.12	.12	.12	.12	.12
88.1	20	1	.86	.87	.87	.88	.88	2	R	R	R	R	R
		2	—	.95	.95	.95	.95	1	.10	.10	.10	.10	.10
88.2	20	1	—	.88	.88	.88	.87	2	R	R	R	R	R
		2	—	—	.95	.95	.95	1	.10	.10	.10	.10	.10
90	21	1	.91	.91	.91	.91	.91	1	R	R	R	R	R
90.A	21	1	.90	.90	.90	.90	.90	1	R	R	R	R	R
90.1	21	1	.92	.92	.92	.92	.92	1	R	R	R	R	R
96	21	1	—	—	.89	.89	.90	1	.00–.07	.00–.07	.00–.07	.00–.07	.00–.07
96.E1	20		—	—	—	—	—		—	—	—	—	—
96.S1	21	Na	.72	.72	.71	.70	.69	1	R	R	R	R	R
		2	—	—	—	.79	.79						
		1	.88	.88	.88	.87	.87						
97	20	1	.90	.91	.91	.91	.91	1	.00–.05	.00–.06	.00–.07	.00–.08	.00–.09

(*Continued on p. 801*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
97.A	20	1	.90	.91	.91	.91	.91	1	.00–.07	.00–.08	.00–.08	.00–.09	.00–.09
103	21	1	—	.88	.89	.90	.90	1	.12	.12	.13	.13	.13
103.A	21	1	—	.89	.89	.89	.90	2	—	—	.05	.05	.05
								1	.13	.13	.13	.14	.14
103.S1	21	Na	—	.73	.73	.72	.72	1	R	R	R	R	R
		1	—	—	.90	.90	.90	2	—	.11	.12	.12	.12
104	21	1	.72	.73	.74	.74	.75	2	R	R	R	R	R
		2	.82	.82	.83	.84	.84	1	.30	.30	.31	.00–.38	.00–.40
110	20	1	—	—	.74	.74	.73	1	.23	.24	.24	.23	.23
110.A	20	1	—	—	.71	.72	.71	1	.26	.26	.26	.26	.26
110.1	20	1	—	.75	.75	.75	.75	1	.22	.22	.22	.22	.21
		2	—	—	.95	.95	.95						
110.2	20	2	—	—	.62	.62	.62	1	—	.19	.19	.19	.19
		1	—	—	.73	.73	.73						
		3	—	—	.93	.93	.93						
111	22	2	—	.18	.18	.18	.18	3	—	R	R	R	R
		1	.32	.33	.33	.33	.32	1	.61	.61	.61	.61	.61
								2	—	—	.90	.90	.91
111.1	22	2	—	—	.15	.15	.15	2	R	R	R	R	R
		1	.32	.32	.32	.32	.31	1	.63	.63	.63	.63	.63
								3	—	—	—	.93	.93
112	22	1	.89	.89	.88	.87	.86	1	.04	.04	.04	.04	.04
								1	.13	.14	.14	.15	.15
112.1	23	2	.75	.74	.73	.73	.51–.78	1	.03	.03	.03	.03	.03
		1	.93	.93	.92	.91	.91	1	.09	.10	.11	.11	.11

(*Continued on p. 802*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
112.2	23	3	—	—	R	R	R	1	R	R	R	R	R
		4	—	—	—	.50	.50	1	.10	.09	.09	.09	.09
		2	.76	.76	.75	.75	.75						
		1	.94	.94	.94	.94	.93						
112.3	20	3	—	—	—	—	.36–.95	1	R	R	R	R	R
		2	.73	.72	.72	.70	.70	1	.12	.13	.13	.14	.15
		1	.90	.90	.89	.88	.87						
112.S1	20	2	—	—	—	.59	.59	1	R	R	R	R	R
		Na	.70	.70	.70	.70	.70	1	.11	.11	.11	.11	.11
		1	.89	.88	.88	.88	.87						
113	23	2	—	—	.16	.16	.16	1	—	.93	.93	.93	.93
		1	.25	.25	.25	.25	.25						
113.A	23	1	.22	.22	.22	.22	.22	1	—	.90	.90	.90	.90
113.1	23	3	—	—	—	R	R	2	—	—	—	R	R
		1	.14	.14	.14	.14	.14	1	.90	.90	.90	.90	.66–.98
		1	.23	.23	.23	.23	.23						
		2	—	.45	.44	.44	.44						
114	22	1	.85	.86	.87	.87	.87	2	R	R	R	R	R
								1	.10	.10	.10	.10	.11
114.A	21	1	.84	.85	.85	.85	.85	2	R	R	R	R	R
								1	.09	.10	.10	.10	.11
115	20	1	—	.52	.52	.52	.52	1	—	—	.55	.55	.55
115.A	20	1	—	—	.50	.50	.50	1	—	—	.50	.50	.50
115.1	20	1	—	—	.55	.55	.55	1	—	—	.52	.52	.52
116	22	1	.69	.69	.69	.69	.69	1	.19	.19	.19	.19	.19
116.1	22	2	—	—	.44	.44	.44	2	—	—	—	R	R
		3	—	—	—	—	.55	1	.21	.21	.21	.21	.21
		1	.72	.72	.72	.72	.72						

(*Continued on p. 803*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids)					Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids)				
			2	5	10	20	50		2	5	10	20	50
118	20	1	.80	.80	.80	.80	.80	2	R	R	R	R	R
								1	—	—	.07	.07	.07
122	20	1	.95	.95	.95	.95	.95	2	R	R	R	R	R
								1	.14	.14	.14	.14	.14
131	20	Na	.70	.70	.70	.70	.71	1	R	R	R	R	R
		1	.93	.91	.91	.91	.90						
132	20	Na	.69	.69	.68	.68	.68	1	R	R	R	R	R
		1	.91	.90	.90	.89	.88						
133	22	1	—	—	.95	.95	.95	1	—	—	R	R	R
133.1	22	1	—	—	.95	.95	.95	1	—	—	R	R	R
134	23	2	—	—	—	—	R	1	.12	.12	.12	.12	.12
		1	.89	.89	.89	.89	.89						
134.A	23	2	—	—	—	R	R	1	.12	.12	.12	.12	.12
		1	.89	.89	.90	.90	.90						
134.1	23	1	.88	.88	.86	.86	.87	1	.09	.09	.08	.08	.08
134.2	23	2	—	.79	.79	.79	.79	1	.12	.12	.12	.12	.11
		1	.87	.87	.87	.87	.87						
138	26	1	.49	.49	.48	.48	.47	1	.16	.16	.15	.15	.15
139.1	25	4	—	—	.08	.08	.08	3	R	R	R	R	R
		1	.34	.35	.35	.34	.34	2	.36	.36	.35	.35	.35
		2	.53	.54	.54	.54	.54	1	.85	.85	.85	.85	.85
		3	.89	.89	.89	.89	.89						
140	20	1	.88	.88	.88	.88	.88	1	R	R	R	R	R
140.1	20	1	.87	.86	.86	.86	.87	1	R	R	R	R	R
140.2	20	1	.87	.87	.87	.88	.89	1	R	R	R	R	R
140.3	20	1	.87	.87	.87	.87	.87	1	R	R	R	R	R

(*Continued on p. 804*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
140.4	20	1	.87	.87	.86	.86	.87	1	R	R	R	R	R
140.E1	21	5	—	.10	.11	.11	.11	2	R	R	R	R	R
		1	.45	.45	.45	.44	.44	3	—	—	.11	.11	.11
		3	.60	.60	.60	.60	.60	1	.63	.63	.63	.63	.31–.74
		4	—	.72	.72	.71	.71						
		2	.93	.93	.92	.92	.92						
140.E2	21	4	—	—	.11	.10	.10	2	R	R	R	R	R
		1	.46	.45	.46	.46	.45	1	.67	.67	.67	.67	.33–.77
		2	—	.71	.70	.70	.70						
		3	—	.93	.93	.93	.93						
140.E3	21	4	—	—	.14	.14	.14	2	R	R	R	R	R
		1	.57	.56	.56	.55	.55	1	.48	.48	.47	.47	.17–.59
		3	—	.77	.77	.77	.77						
		2	.94	.94	.93	.93	.93						
140.E4	20	3	—	.15	.15	.16	.16	2	R	R	R	R	R
		1	.49	.49	.48	.48	.48	1	.47	.48	.47	.47	.23–.58
		4	—	—	.69	.69	.69	3	—	—	.67	.67	.67
		2	.87	.87	.87	.86	.86	4	—	—	—	—	.90
140.E5	21	4	—	—	.03	.03	.03	2	R	R	R	R	R
		1	.14	.14	.14	.14	.14	3	.11	.11	.11	.11	.11
		3	—	—	.31	.31	.31	1	.87	.87	.87	.87	.60–.96
		2	.92	.92	.92	.92	.92						
140.E6	21	3	—	—	.04	.04	.04	2	R	R	R	R	R
		1	.20	.18	.19	.19	.18	3	—	—	.13	.13	.13
		2	—	—	.38	.38	.38	1	.89	.89	.87	.87	.55–.97
		4	—	—	.94	.94	.94						
140.E7	20	1	.60	.59	.59	.58	.58	2	R	R	R	R	R
		2	.77	.77	.77	.77	.77	3	—	—	.35	.35	.35
		2	.88	.88	.88	.88	.88	1	.62	.62	.62	.62	.37–.72
141	23	1	.48	.48	.47	.48	.48	1	.54	.54	.54	.54	.37–.63

(*Continued on p. 805*)

Sample No.	Temperature, °C	Order of Intensity of Spots	Aqueous System — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50	Order of Intensity of Spots	Nonaqueous system — Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
141.A	23	1	.48	.47	.48	.46	.47	1	.53	.54	.41–.60	.37–.61	.32–.63
141.B	23	1	.48	.49	.48	.47	.49	1	.59	.59	.46–.69	.43–.69	.32–.69
141.1	23	3	—	—	.32	.32	.32	1	.48	.48	.54	.39–.65	.33–.67
		1	.48	.48	.51	.50	.50						
		2	.61	.62	.64	.63	.64						
141.2	21	2	.31	.29	.29	.28	.28	4	—	—	R	R	R
		1	.49	.47	.47	.47	.47	1	.46	.45	.45	.45	.45
		3	—	—	.61	.61	.61	2	—	—	—	.55	.55
								3	—	—	—	.70	.70
142	23	1	.78	.79	.79	.79	.79	1	R	R	R	R	R
								2	—	—	.15–.35	.10–.35	.10–.35
142.1	23	1	.79	.79	.79	.79	.79	1	R	R	R	R	R
								2	.29	.29	.20–.36	.18–.36	.14–.37
143	21	1	.89	.88	.89	.89	.88	1	.00–.07	.00–.07	.00–.07	.00–.07	.00–.07
144	22	2	—	—	.27	.26	.25	2	—	—	—	R	R
		1	.43	.44	.43	.43	.42	1	.68	.68	.68	.68	.68
146	22	1	.05–.48	.05–.57	.05–.67	.05–.76	.05–.93	2	R	R	R	R	R
								1	.36–.92	.21–.92	.13–.92	.06–.92	.00–.92
151	20	1	.71	.71	.70	.70	.70	1	—	.49	.49	.49	.49
151.A	20	1	.69	.69	.69	.69	.68	1	.49	.49	.49	.49	.49
155	21	1	—	—	—	—	—	1	R	R	R	R	R

cases the dash (—), which shows no spot, was used in Table 2 instead of the R_F value of the color or the fluorescence.

Among some of the samples, e.g., BHC (#8), DDT (#31), the same order of intensity was assigned to two of the separated spots since there was little or no significant visible difference between them.

Experience has shown that many unknown pesticide substances are definitely identified when they are separately chromatographed by aqueous and nonaqueous solvent systems, simultaneously and with knowns on the same paper. One system may separate possible isomers although the other system may not. For example, the nonaqueous system separates the isomers of BHC (#8) and DDT (#31), but the aqueous system does not. Again, because of differences in polarity, one system may give a low or high R_F value and the other system reverse the order (note the numerous examples in Table 2, or particularly *This Journal*, **39**, 980 (1956)).

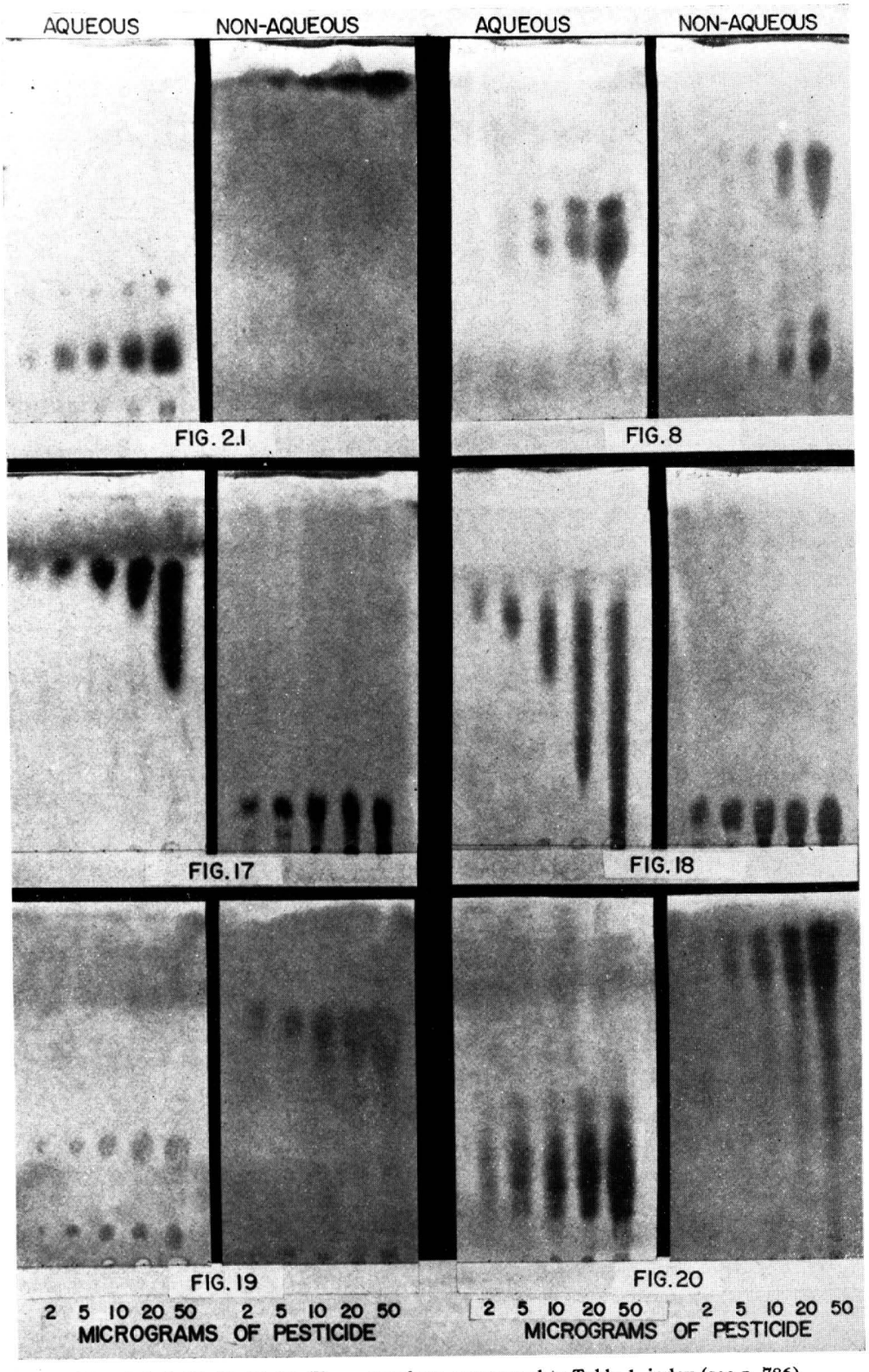
Chromatograms in which significant spots were produced by the chromogenic agent used here are reproduced in the figures accompanying this article. The figure number corresponds to the key number which identifies the compound in Table 1. Pertinent comments are made in the legends of the figures.

Summary

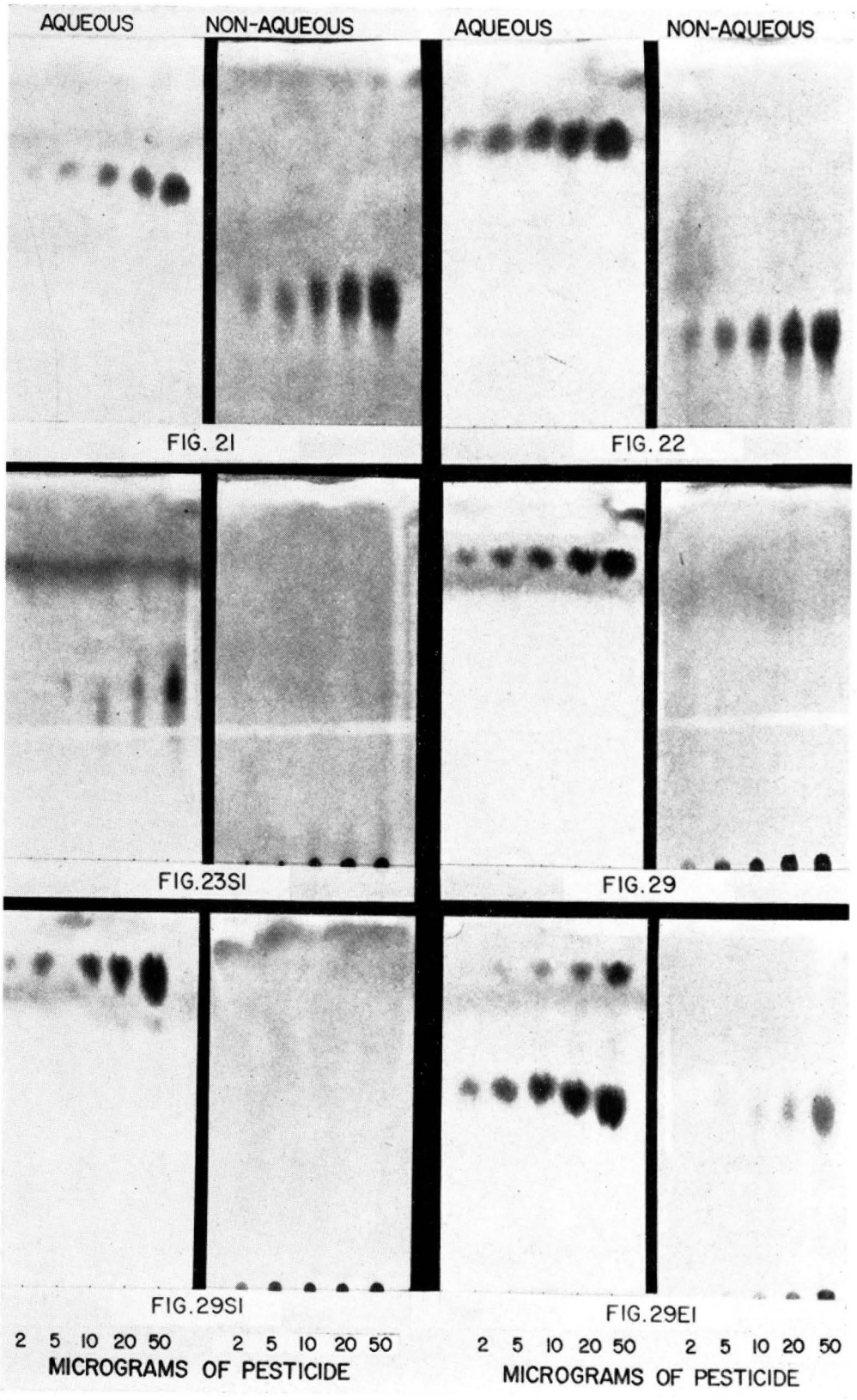
R_F values for technical grade pesticides produced during the 1957 season and for analytical or reference grade samples of some 213 lots of 114 different pesticide chemicals are given. The pesticides were chromatographed with two systems: an aqueous and a nonaqueous. The immobile solvent was applied to the paper, which had been previously washed with water, air-dried, and spotted with a solution of the pesticide, by dipping rather than by spraying. The dipping procedure eliminates many variables inherent in the previously used spraying technique.

The chromogenic agent consists of silver nitrate and 2-phenoxyethanol in acetone. Spots are delineated by immediate exposure to strong ultraviolet light.

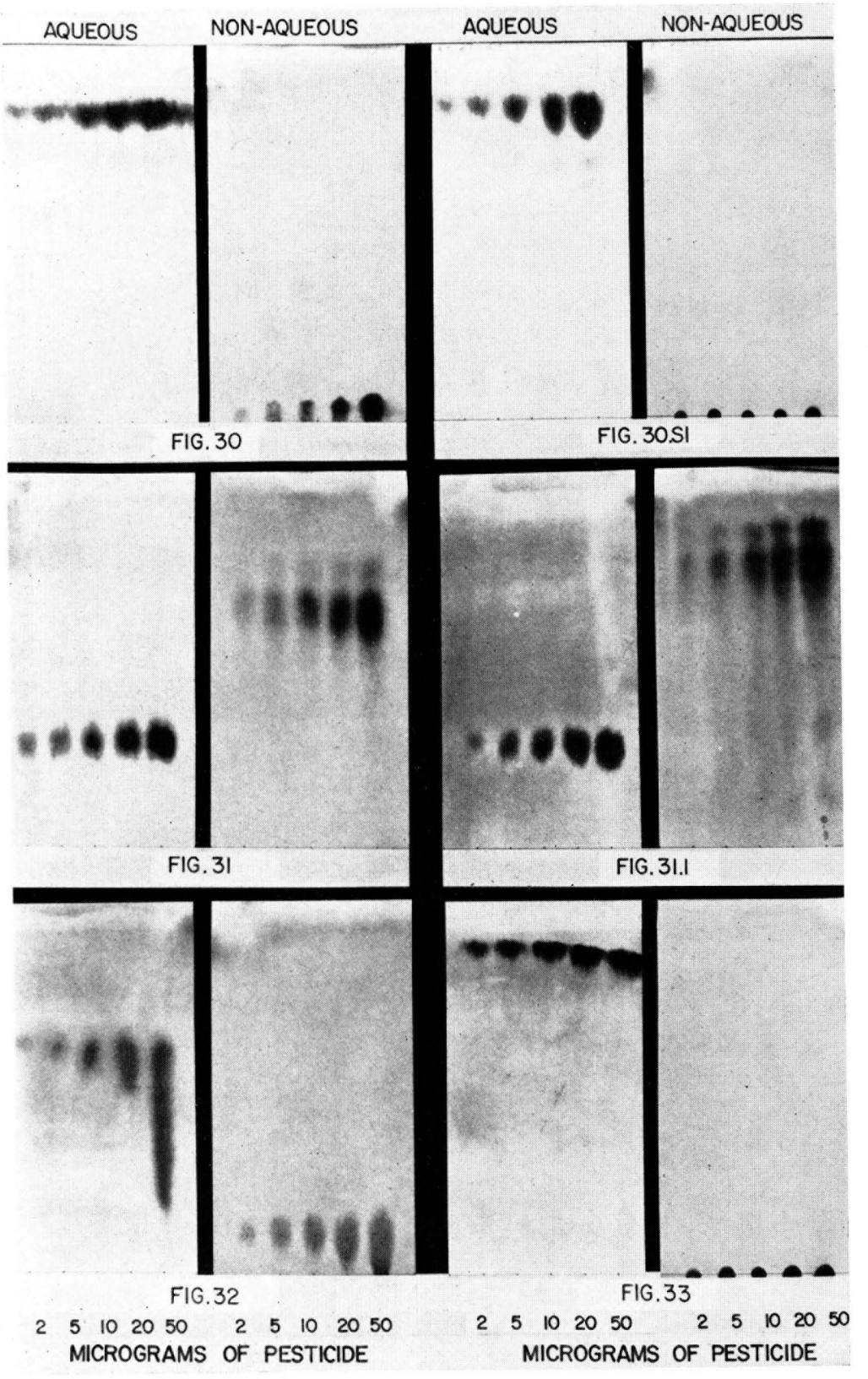
Figures 2.1 through 151. The figures illustrate the chromatograms of 60 pesticides selected from the 214 samples described in Tables 1 and 2. They are not the same chromatograms from which the R_F values of Table 2 were derived; these latter had been stored, unwashed, and had darkened too much for reproduction. The illustrated chromatograms were prepared by the described procedure, but in addition, were washed with water (*This Journal*, **39**, 486 (1956)); this obviated storage darkening to a great extent. Each figure includes two chromatograms, one developed with the aqueous, the other with the nonaqueous solvent system, for 2, 5, 10, 20, and 50 mmg (or microliters) of pesticide. Solvent fronts are not marked on the reproductions but are visible in many of the figures. R_F values may vary slightly from those recorded in Table 2 (because of temperature, etc.) and the order of intensity, and even the number of the spots, may vary. One reason for this is the difficulty of weighing out similar samples of some impure preparations which comprise a semi-crystalline solid phase and a sirupy liquid phase. Considerable detail is lost in reproduction, e.g., faint spots, and spots which may fluoresce vividly under ultraviolet light, do not appear. On the other hand, chromatograms which are recorded as "streaks" in Table 2 may appear as a series of more or less discrete spots after the washing and reproducing process. Residual spots may sometimes show distinctly, and in other cases may be barely noticeable. Sometimes, with the nonaqueous system, slight movement of the 2-phenoxyethanol may cause an irregular spot to form at the lower edge of the chromatogram (samples 18, 20, 69.1, 83, 88, 97, 143, 146). Some substances (samples 17, 18, 32) streak badly when the paper is overloaded, while others (samples 8, 19, 31, 31.1, 51, 87, 112, 131, 138, 140.E1, 141, 143) form oversized spots.



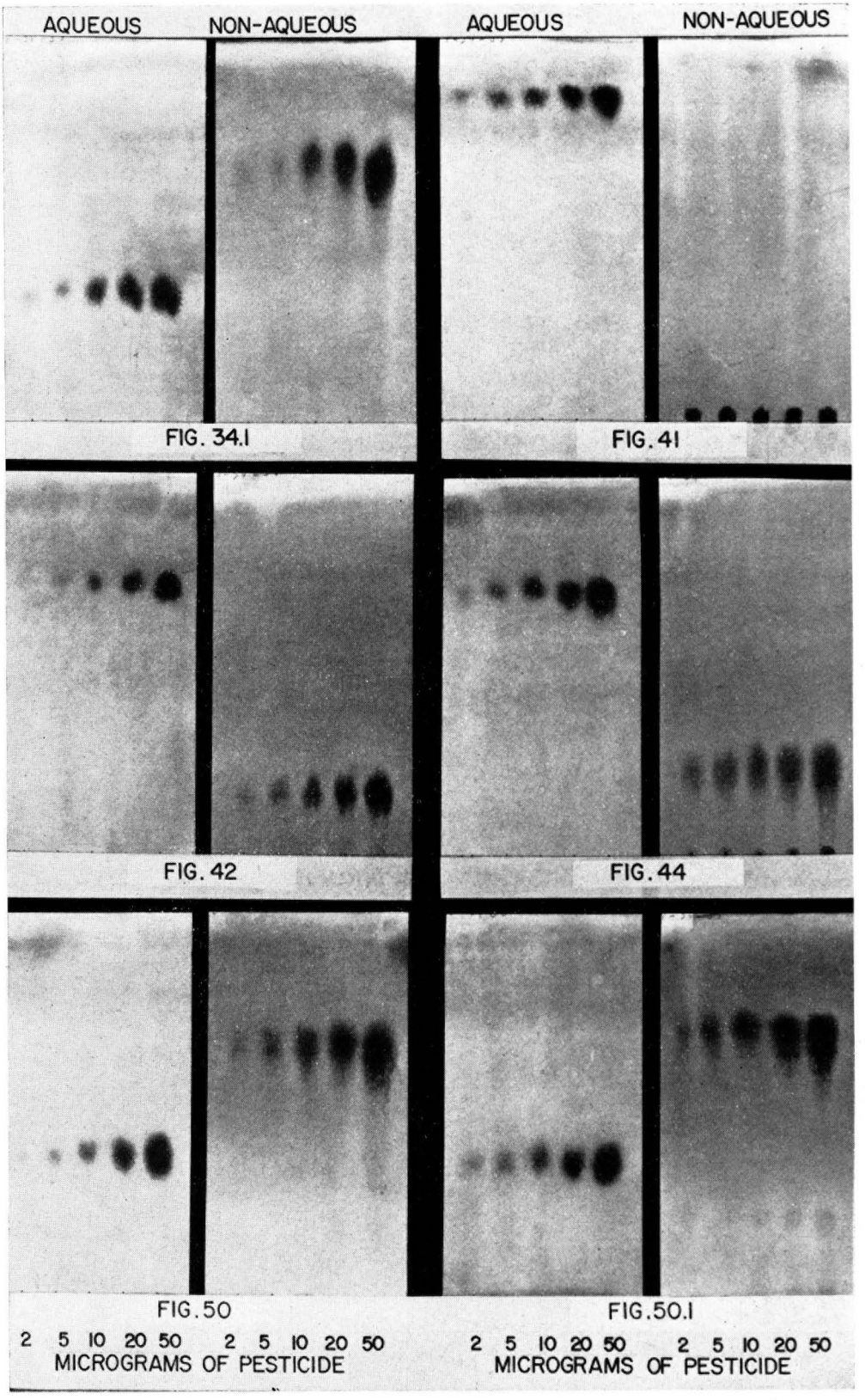
Figs. 2.1, 8, 17, 18, 19, 20. Figure numbers correspond to Table 1, index (see p. 786).



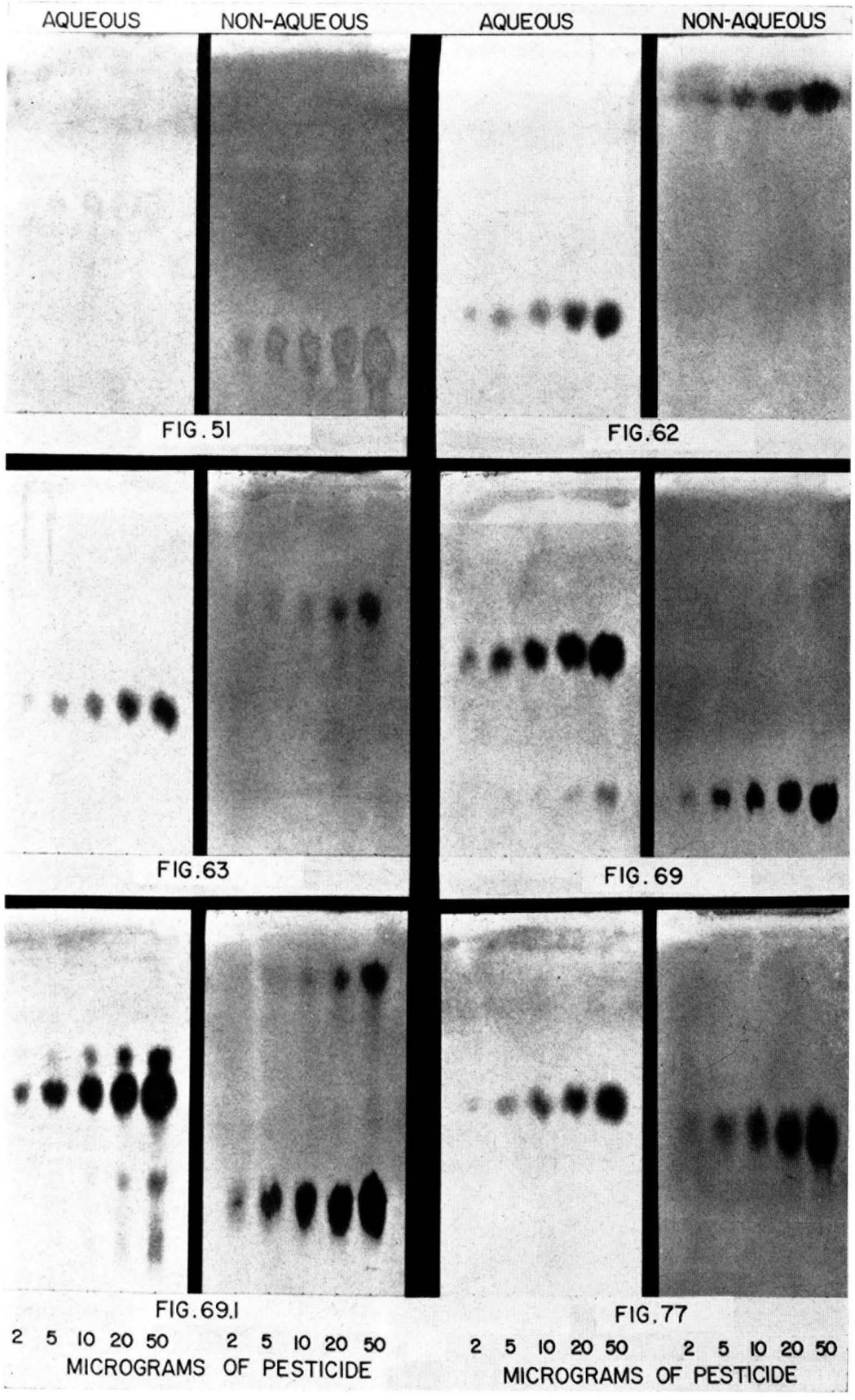
Figs. 21, 22, 23S1, 29, 29S1, 29E1. Figure numbers correspond to Table 1, index (see p. 786).



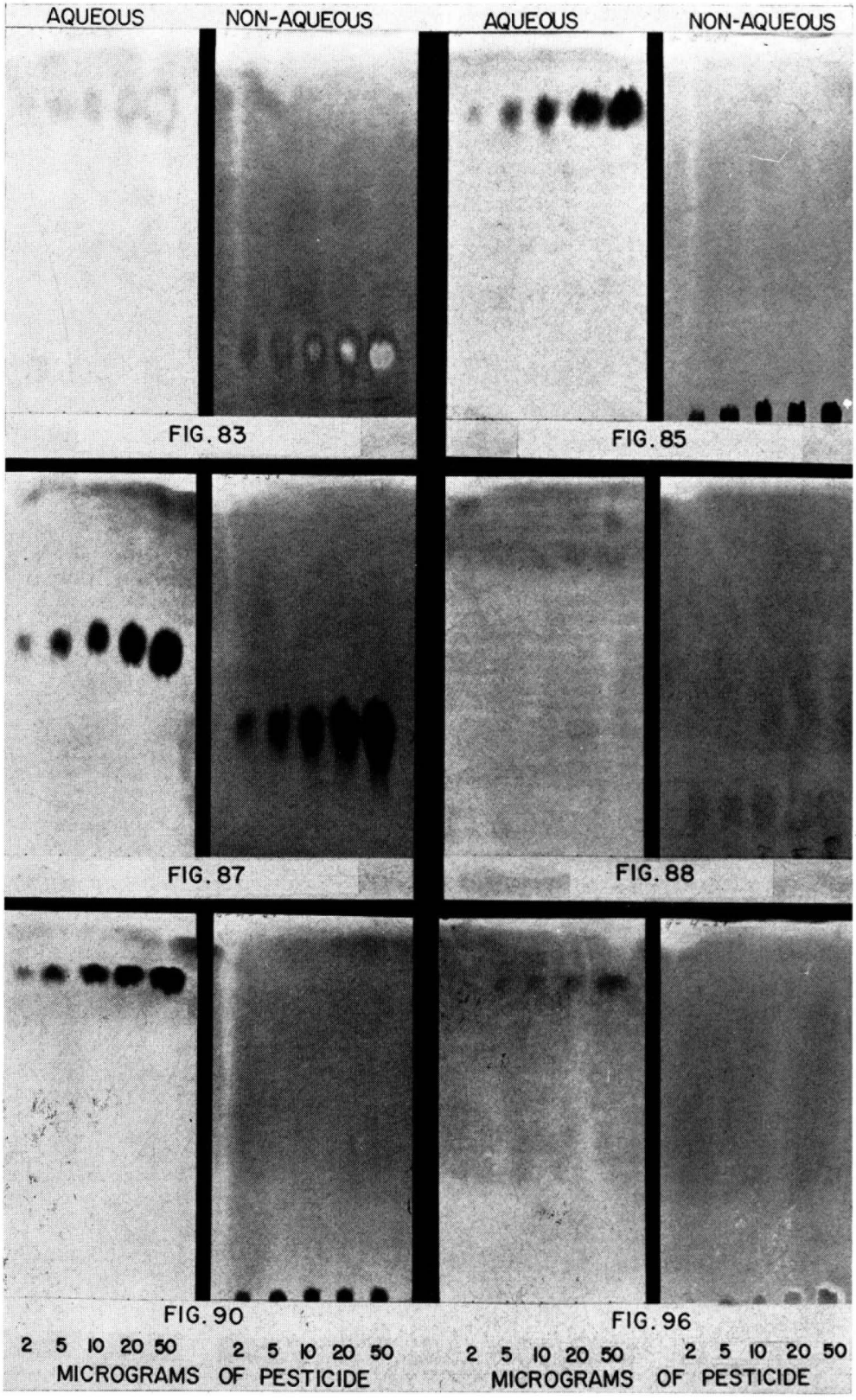
Figs. 30, 30.S1, 31, 31.1, 32, 33. Figure numbers correspond to Table 1, index (see p. 786).



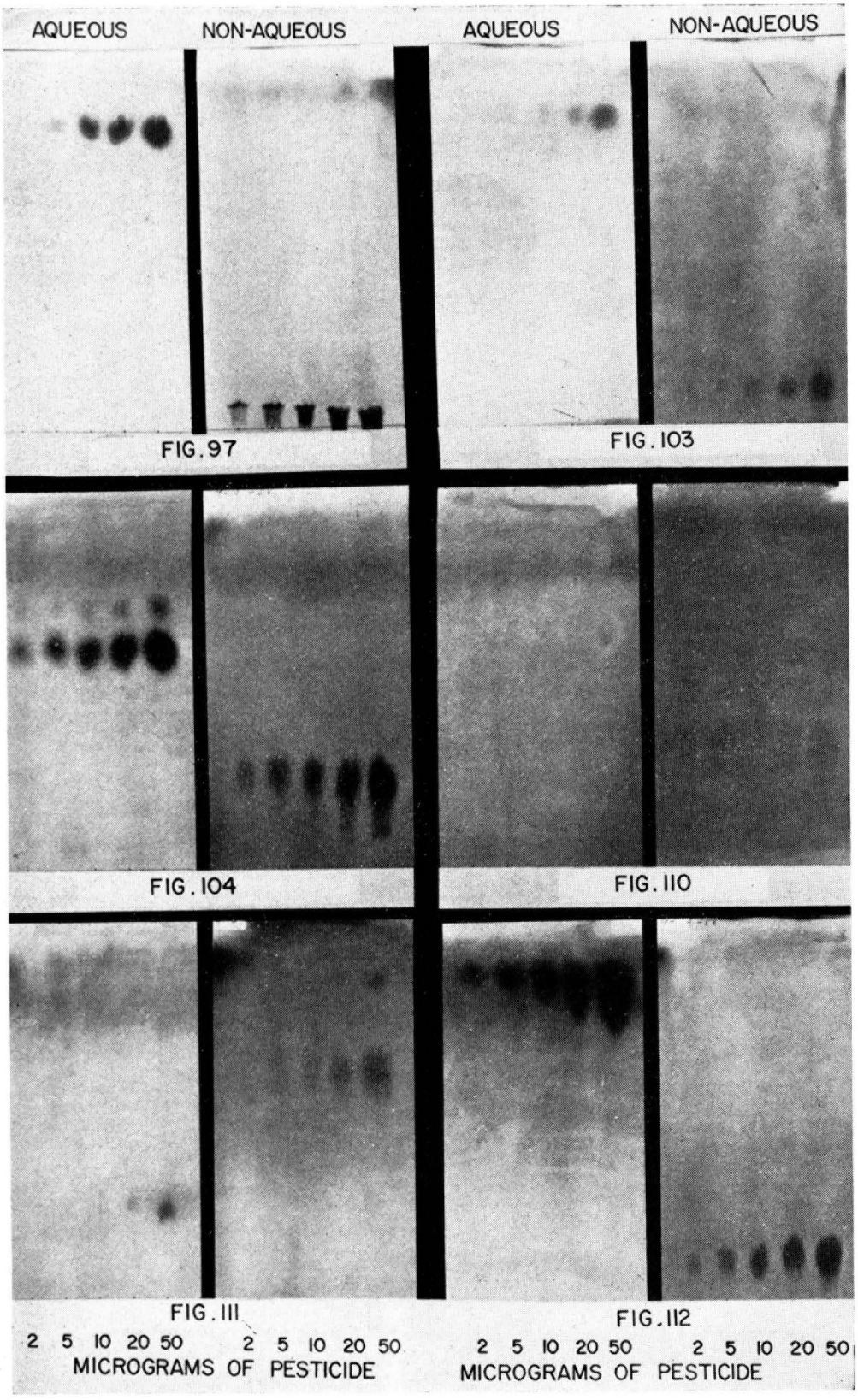
Figs. 34.1, 41, 42, 44, 50, 50.1. Figure numbers correspond to Table 1, index (see p. 786).



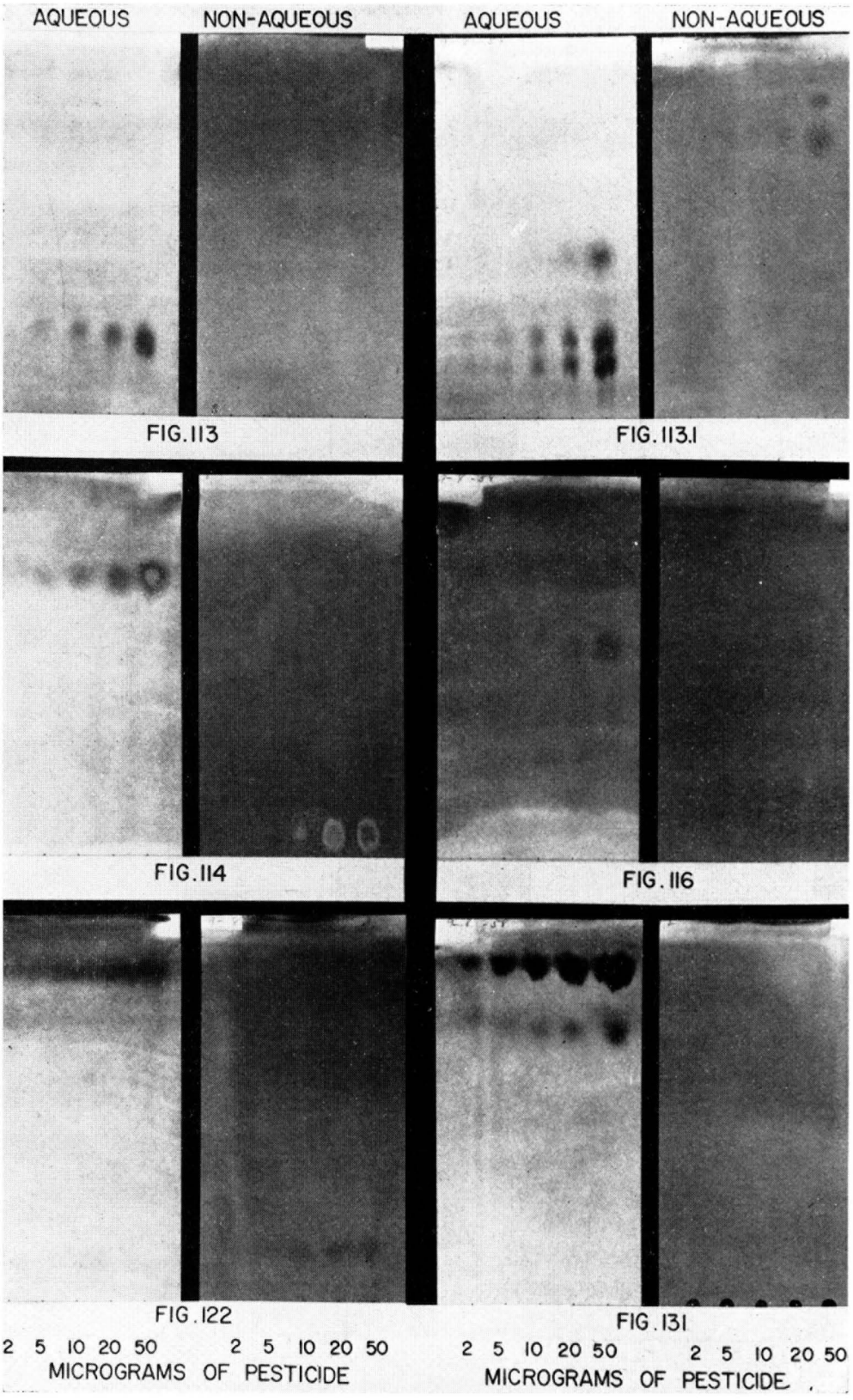
Figs. 51, 62, 63, 69, 69.1, 77. Figure numbers correspond to Table 1, index (see p. 786).



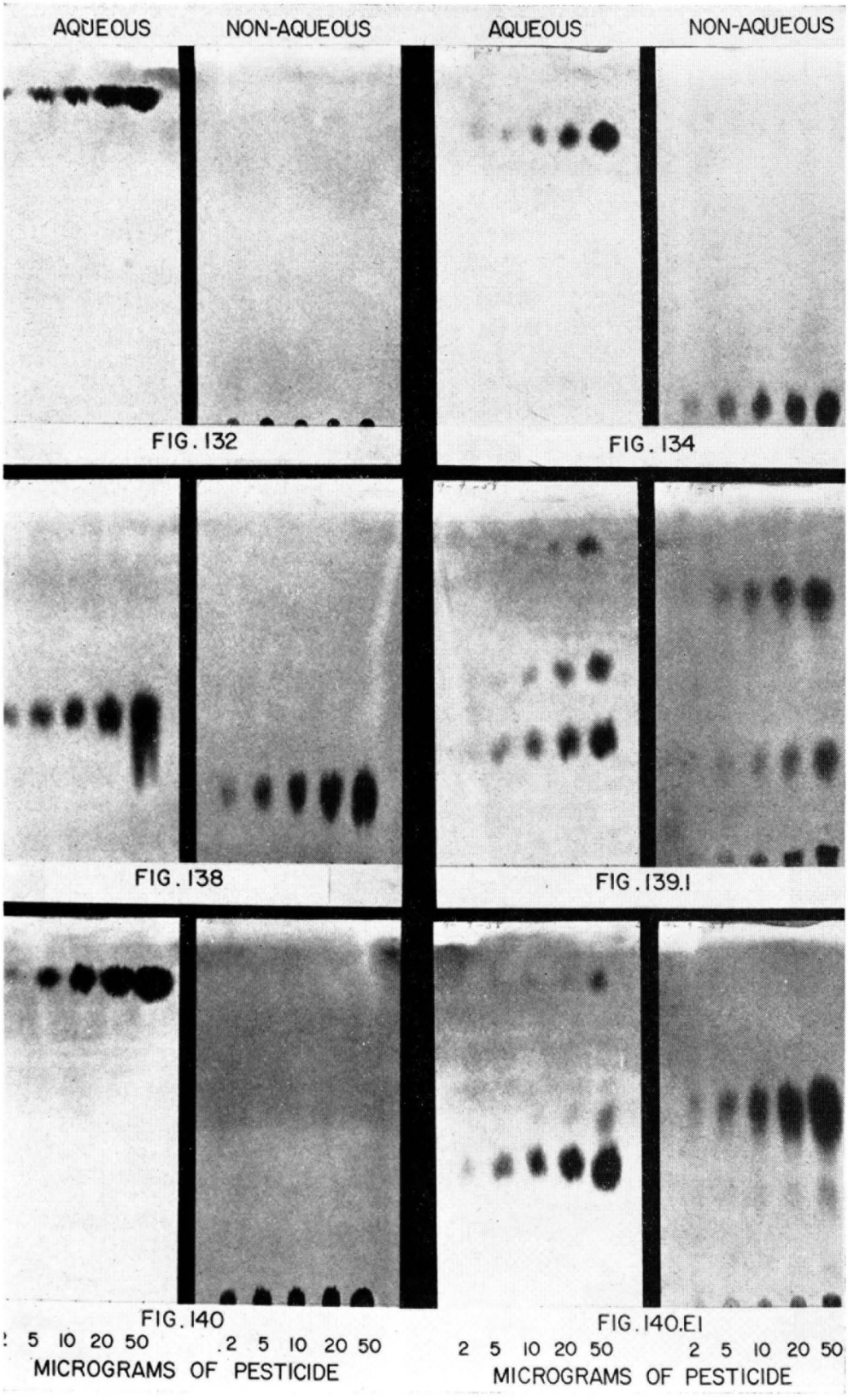
Figs. 83, 85, 87, 88, 90, 96. Figure numbers correspond to Table 1, index (see p. 786).



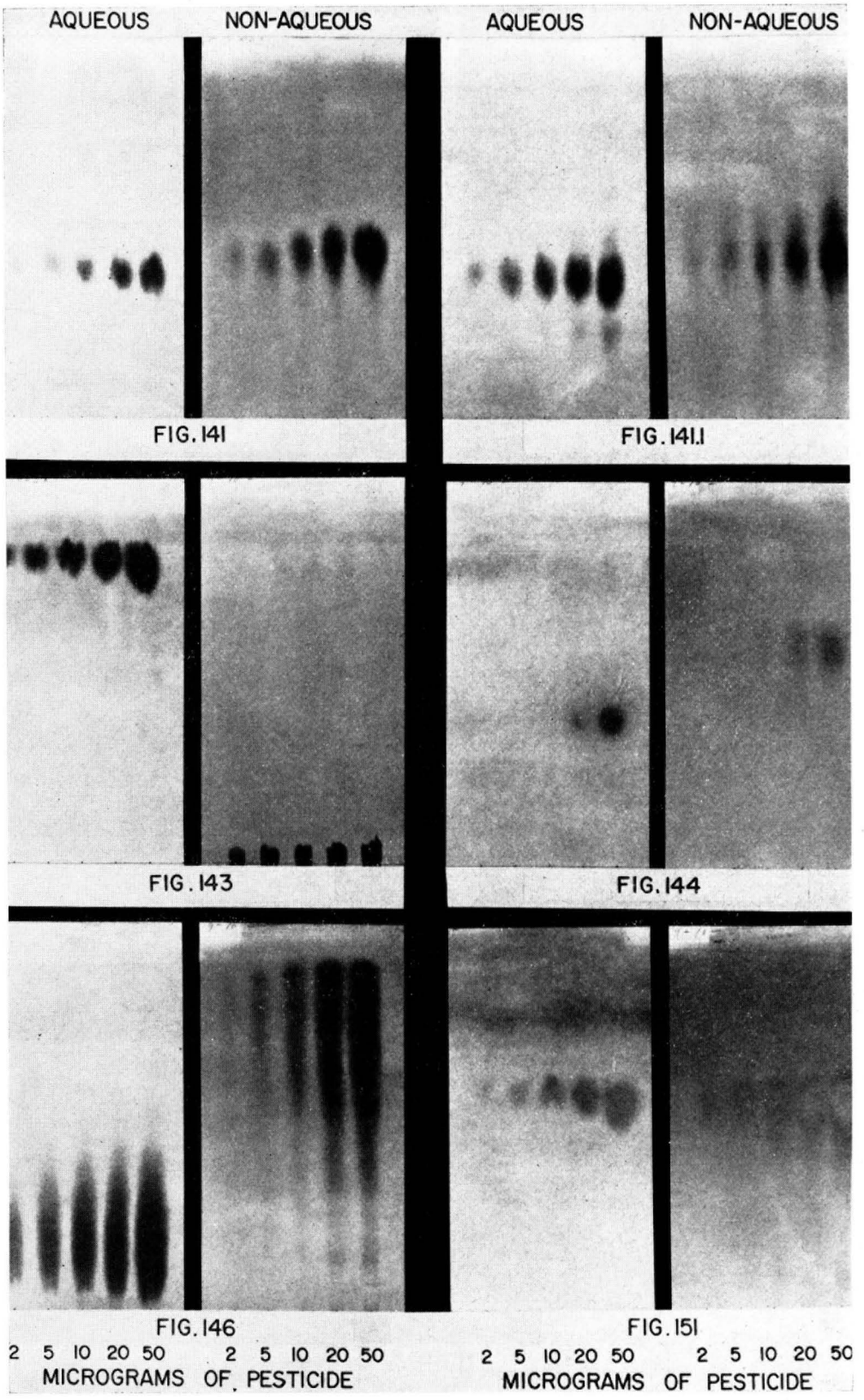
Figs. 97, 103, 104, 110, 111, 112. Figure numbers correspond to Table 1, index (see p. 786).



Figs. 113, 113.1, 114, 116, 122, 131. Figure numbers correspond to Table 1, index (see p. 786).



Figs. 132, 134, 138, 139.1, 140, 140.E1. Figure numbers correspond to Table 1, index (see p. 786).



Figs. 141, 141.1, 143, 144, 146, 151. Figure numbers correspond to Table 1, index (see p. 786).

The Isolation of Oil-Soluble Coal-Tar Colors from Foods*

By EVELYN MARK and G. G. McKEOWN (Food and Drug Laboratory, Department of National Health and Welfare, Ottawa, Ontario, Canada)

In both qualitative and quantitative analyses of oil-soluble coal-tar colors in foods, the foremost problem is to achieve a reasonably effective separation of the color from accompanying fats and oils. The colors normally occur in low concentrations of the order of 5 to 50 ppm. Of the methods in use, the A.O.A.C. procedure (1), which is based on extraction of the colors from petroleum ether by a strongly acidic solution, is possibly the most effective, although Espoy and Barnett (2) recently reported low color recoveries due presumably to decomposition. Newburger (3) has shown that alkaline saponification is of value for analysis of concentrated commercial color solutions. Column chromatography has been studied by several investigators (4–8), but we found this technique to be somewhat time-consuming and unreliable when applied directly to foods.

Two reports in the literature indicated that N,N-dimethylformamide might be of value as a partition solvent for food color work. Burchfield and Storrs (9), in their work on insecticides, reported that when corn oil is partitioned between dimethylformamide and hexane, the oil is largely taken up by the hydrocarbon phase. Campbell, Cathcart, and Giles (10) in their studies on hydrogen bond formation reported complex formation between dimethylformamide and azo compounds.

This paper describes the isolation of common types of oil-soluble azo colors from corn oil by solvent partition with N,N-dimethylformamide and petroleum ether. The colors were recovered unchanged in good yields, and removal of oil amounted to 99.8%.

METHOD

Dissolve 10 g oil in 50 ml petroleum ether, filter if necessary, and transfer to a separator. Extract with three 20 ml portions of N,N-dimethylformamide (DMF), discarding petroleum ether layer. Extract combined DMF solution with four 25 ml portions of petroleum ether, back-extracting each time with 5 ml DMF. Discard petroleum ether extracts. Dilute combined DMF solution with an equal volume of H_2O and extract with one 30 ml and one 10 ml portion of $CHCl_3$ (centrifuge if necessary to separate phases). Discard aqueous DMF layer. Wash combined $CHCl_3$ solution with water to remove dissolved DMF. Evaporate $CHCl_3$ solution to dryness under reduced pressure at room temperature. (Chromatographic or spectrophotometric examination may begin at this stage if residual fat content is low enough.) Dissolve residue in 25 ml DMF, transfer to separator, add 25 ml H_2O, and extract with three to five 25 ml portions of petroleum ether. Discard aqueous DMF layer, wash combined petroleum ether solution with H_2O to remove dissolved DMF, and evaporate petroleum ether solution under reduced pressure at room temperature. Examine residue by chromatography or spectrophotometry for presence of coal-tar colors.

Discussion

Corn oil, selected as a typical food lipid, was partitioned between petroleum ether and N,N-dimethylformamide (1 g and 5 g oil between 50 ml volumes of each solvent). The dimethylformamide layer was drawn off, diluted with water, and extracted with chloroform to determine the lipid content. The amount of oil found in the dimethylformamide layer was 105 mg or 10.5% with a 1 g sample, and 530 mg or 10.6% with a 5 g sample.

A study was made of the distribution between dimethylformamide and petroleum ether of 8 azo colors reported to be in use for food manufacture (11). The results are listed in Table 1. The indicated quantities of colors were partitioned between 25 ml portions of

* This article is based on a paper presented at the 41st Annual Conference and Exhibition of The Chemical Institute of Canada, Toronto, May 26–28, 1958.

Table 1. Distribution of azo color between N,N-dimethylformamide and petroleum ether

Name	Color Index No. (1924)	% Color in DMF 10 mg Color in 50 ml Solvents	20 mg Color in 50 ml Solvents	Av. %
Yellow AB	22	99.2	99.3	99
Yellow OB	61	99.4	99.3	99
Sudan I	24	95.0	95.0	95
Orange SS	—	93.0	91.6	92
Oil Red XO	73	91.0	89.5	90
Sudan R	113	97.6	98.3	98
Sudan III	248	97.7	98.2	98
Sudan IV	258	96.0	94.9	95

each solvent, and color concentrations were determined spectrophotometrically. From the results it would appear that the azo group is responsible for solubility in dimethylformamide. Substitution on the aromatic moieties favors solubility in the hydrocarbon phase.

One mg samples of three colors, representing the classes azonaphthylamine, azonaphthol, and disazonaphthol, were dissolved in 10 g corn oil and analyzed in triplicate by the proposed procedure. Recoveries were determined spectrophotometrically. Lipid residues contained in the chloroform extracts and the final petroleum ether extracts were determined on duplicate blank runs. The results are listed in Table 2.

Fractionation of the corn oil undoubtedly takes place during the separation. Under no circumstances could all traces be removed. The chloroform extracts from 10 g of oil contained 100 mg fatty material or more, i.e., 99.0% removal. For some purposes this is sufficient. However, for very low concentrations of colors or with complex mixtures, further purification is desirable. The second partition between dimethylformamide and petroleum ether reduced the lipid residue to approximately 20 mg without any significant loss of color. Thus 99.8% of the oil was removed.

The azonaphthol colors were found to be very stable under the conditions of isolation but the naphthylamines (Yellow AB and OB) were not. In preliminary runs, substantial color losses were observed. A study of Yellow OB isolation revealed that no color loss occurred during removal of corn oil from dimethylformamide, or during extraction by chloroform, but when the chloroform extracts were evaporated at elevated temperatures, the color was partially decomposed by reaction with some constituents of the oil. With rapid evaporation at reduced pressure and room temperature, no marked loss of color was observed.

Official samples received by this laboratory were analyzed by this method and were identified by a procedure based on partition chromatography, which will be described at a later date. Satisfactory results were obtained.

Table 2. Separation of azo colors from corn oil

Color Solution (1 mg Dye in 10 g Oil)	Recovery of Color, %	Av. Recovery, %
Yellow OB	82.7	84.1
	86.6	
	83.2	
Orange SS	88.8	89.6
	91.1	
	89.0	
Sudan III	93.5	91.2
	90.5	
	89.6	

Lipid Residues, Av.:
Chloroform extracts: 124 mg
Petroleum ether extracts: 19 mg

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Twelve-Month Survey of WIA in Commercial Cream

By FRED HILLIG* and WILLIAM WEISS† (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D.C.)

Earlier studies have shown that when cream decomposes there is frequently a concurrent increase in its content of water-insoluble fatty acid (WIA) and butyric acid (1). These studies have required extensive field and laboratory investigations to correlate organoleptic classification of cream, first with its WIA content, and then with the WIA content of the butter produced from that cream. To establish such correlations, each can of cream used in each churning was examined organoleptically and classified. Cans for each churn selected at random in each classification were analyzed for WIA, as were also the mixed vat cream and the finished butter. Because the official method—the only method then available for WIA—was time-consuming, limited manpower and laboratory facilities severely restricted the extent of these earlier studies.

The development of the rapid method for the determination of WIA (2), which adheres to the original principles of isolation and titration, made possible a survey, over a period of one year, of WIA in cream separated at the farm. This survey was made (*1*) to secure additional information on the relationship between organoleptic class and WIA content of cream, and (*2*) to measure seasonal effects on the WIA content of cream.

The survey was begun by the Food and Drug Administration in August 1954 and continued for an entire calendar year. St. Louis, Minneapolis, Kansas City, Cincinnati, and Denver Districts of the Food and Drug Administration, having inspectors with considerable training and experience in cream grading, participated in the survey. The territories of these Districts embrace some of the principal butter-manufacturing areas of the country and include areas where decomposition in commercial cream is known to be a problem. The distribution of the samples by states is given in Table 1.

Cream from 2,825 cans, examined in 85 different plants located in 13 states, was covered in the survey. Twelve of these plants were visited twice and two were visited three times. Creams graded as first grade and as second grade by the creamery were examined. Chemists accompanied the inspectors during the visits to the plants, and when feasible, performed the rapid WIA determinations in the plants. The inspectors graded each can by taste and took the WIA sample as soon thereafter as possible. The grading system used was the same as that reported previously and is repeated here.

Class 1: Passable. Sweet or clean sour cream that is free from objectionable odors or flavors, other than slight weed or feed flavors.

Class 2: Borderline. Cream containing odors or flavors not normal to sweet or clean sour cream, which cannot be classified as definitely decomposed.

Class 3: Reject. Cream which exhibits cheesiness, putridity, or other characteristics of decomposition.

The inspectors assisted the chemists in the WIA determinations so as to keep the grading and the WIA examination approximately simultaneous.

If determinations by the rapid method could not be performed in the plant, the

Table 1. Distribution of samples by states

State	No. of Creameries Visited	No. of Samples
Missouri	16	595
Iowa	28	628
Colorado	7	204
Illinois	16	454
Kansas	7	189
Idaho	4	98
Nebraska	3	90
New Mexico	2	55
Indiana	8	221
Kentucky	2	48
Texas	2	63
Oklahoma	3	90
Ohio	3	90
Totals:	101	2,825

* Division of Food.
† Bureau of Program Planning and Appraisal.

butter was churned out of the cream sample in the flask (first step of the rapid method) and the flask immediately placed on ice (if it was to be brought to the laboratory within an hour or two) or on dry ice (if the time interval was longer), and the determination was completed in the laboratory. In most cases, however, the determinations were completed in the plants.

Sodium ethylate was standardized separately for each set of WIA determinations. Creamery fat tests on the individual cans of cream were used to calculate the WIA to 100 grams of fat. If a fat value was not available from the firm's records, a separate sample was taken for fat determination in the laboratory by the Babcock or Roese-Gottlieb method.

Table 2. Average WIA found for each class for each month (WIA determined by rapid method)

Month	Class 1	Class 2	Class 3
January	290	395	710
February	250	350	810
March	265	415	795
April	180	395	650
May	255	360	530
June	190	375	—
July	325	375	705
August	270	365	630
September	325	365	670
October	245	370	605
November	195	385	650
December	235	425	845
Average	252	381	691

The average WIA values found for each class for each month of the year are listed in Table 2 and are presented graphically in Fig. 1.

WIA in Class 1 cream has a tendency to be slightly higher during July, August, and September. Class 2 cream shows little variation from month to month. Class 2 cream, as was to be expected, contained more WIA than Class 1. Class 3 cream was materially higher in WIA than Classes 1 and 2. During December, January, February, and March, the colder months of the year, Class 3 cream showed larger average quantities of WIA than did the Class 3 cream produced during the other eight months of the year.

Data in Table 3 gives the percentage of cans of cream in stated ranges of WIA in the three organoleptic classes. Figure 2 presents the same data graphically.

The data show that 96% of the determinations of WIA in Class 1 cream, 81% in Class 2, and 39% in Class 3 fall below 500 mg. The peak for Class 1 is in the range

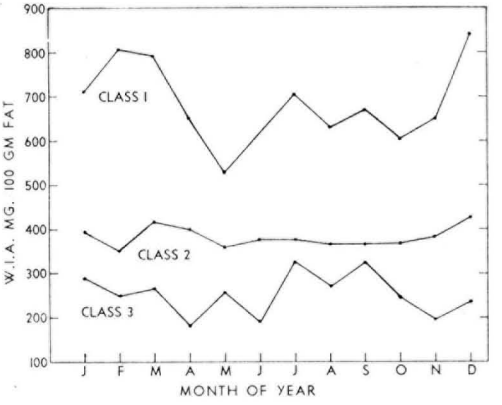

Fig. 1. Average WIA found for each class for each month.

100–200 mg; for Class 2, 200–400 mg; and for Class 3, 400–500 mg. Very few determinations of WIA in Classes 1 and 2 were found above 1,000 mg, but in Class 3, 17.8% of the determinations were above 1,000 mg.

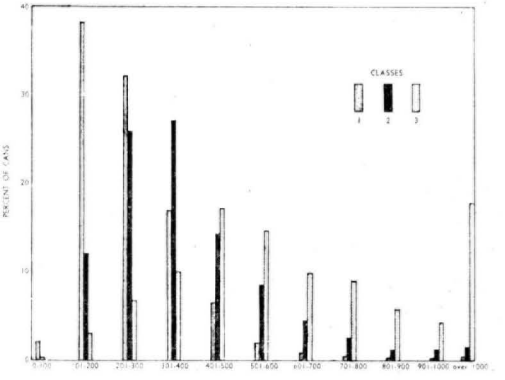

Fig. 2. Per cent of cans in stated ranges of WIA by organoleptic class. Left to right: Class 1, 2, 3, respectively.

To compare data obtained by the rapid method with those obtained by the official method (3), results determined by both the rapid and official methods in this investigation on 69 vats of cream were used. These

data are shown in Fig. 3. The line which best fits the data, calculated by the least squares method, is: WIA (rapid method) $= 58.7 + 1.007 \times$ WIA (official method).

This line is also shown in Fig. 3. The standard deviation about this line for WIA in single vats by the rapid method is 72.0. The equation indicates that the rapid method gives values for WIA that are, on the average, 59 mg per 100 grams fat higher than WIA as determined by the official method. This holds true for any level of WIA within the range of WIA encountered in this investigation.

Table 3. Per cent of cans in stated ranges of WIA by organoleptic class

WIA by Rapid Method, mg/100 g fat	Class 1 (989 cans)	Class 2 (1262 cans)	Class 3 (574 cans)
0–100	2.1	0.2	0.0
101–200	38.3	12.4	3.0
201–300	32.2	26.7	6.6
301–400	16.8	27.3	12.2
401–500	6.5	14.1	17.2
501–600	1.8	8.5	14.6
601–700	0.8	4.4	9.8
701–800	0.5	2.5	8.9
801–900	0.2	1.2	5.7
901–1000	0.3	1.2	4.2
1001 and over	0.5	1.5	17.8

The data in Table 3, and in Fig. 2, indicate that a few cans of cream were graded as passable which had a high WIA content. The probable explanation is that decomposed cream can be masked by the addition of fresh cream to the can and thus be graded by the inspector as passable (4). Cream is often collected on the farm by the daily addition of fresh cream to the holding can. Also, station cream may represent a composite of several producers' output, some of which may be decomposed, with the organoleptic characteristics of decomposition being hidden by the addition of sound cream from another producer.

The composition of a churn of butter is determined by the average of all cans of cream going into the churn. Therefore, if an occasional can of cream with high WIA content, graded by the inspector as passable by organoleptic examination, should get into a vat of cream, the average WIA of the entire churn would not be greatly influenced by its presence.

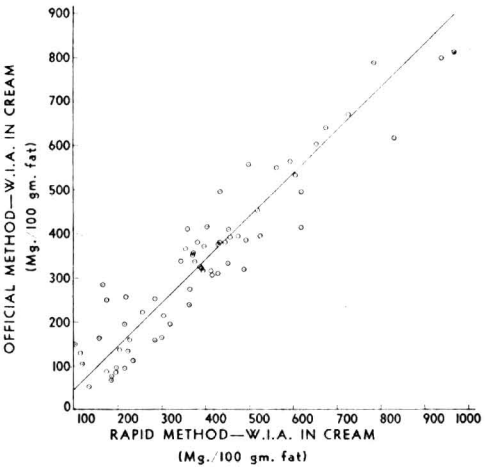

Fig. 3. Comparison of results by rapid and official methods.

Summary

WIA (by the rapid method) has been determined in 2,825 cans of graded commercial cream obtained in 85 different plants in 13 states. These states include some of the principal butter-manufacturing areas in the United States, where decomposition in commercial cream is known to be a problem.

Acknowledgment

Thanks are due J. L. Trawick and other members of the staff of the Bureau of Program Planning and Appraisal for their part in helping to set up the investigation, to L. L. Warden of the Bureau of Field Administration for his assistance in monitoring the investigation, and to the numerous inspectors and chemists in the Food and Drug District laboratories who participated in the investigation. Thanks are also due Jerome Eisner, Division of Food, Food and Drug Administration, for preparing the illustrations, and Lowrie M. Beacham and William Horwitz, also of Division of Food, for review of the manuscript.

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Carbohydrate Studies of the Lima Bean

By KENNETH T. WILLIAMS and ARTHUR BEVENUE (Western Regional Research Laboratory,* Albany, Calif.)

In the investigation of analytical techniques (15) for isolating and identifying the hemicelluloses of vegetables, the lima bean, *Phaseolus lunatus* (commercial grade, large, dry California lima), presented an interesting deviation in the character of its mannan and araban components. The mannan was easily and completely removed from the bean by extraction with water; the araban was relatively insoluble in water and only partially soluble in strong alkali. Since a search of the available literature failed to disclose any information on the polysaccharides present in the lima bean, the experimental conditions used and the compositional data obtained are reported in this paper.

Experimental

A quantity of dried, commercial grade California lima beans was soaked for several hours in distilled water at room temperature (25°C). The seed coat of each bean was removed by hand. (Hereafter in this report, *bean* will refer to the seed minus the seed coat.) The beans and the seed coats were dried separately in a current of warm air (30–40°C) and then ground to pass a 60-mesh sieve. Data on compositions of the two fractions, as determined by standard procedures (1), are given in Table 1. The seed coat represented approximately 7% of the whole bean. Soaking the whole beans in water for the short period of time produced a leaching loss of bean solids of less than 0.5%.

The bean meal was extracted with a solvent mixture of 95% ethanol-benzene (32–68 v/v) until it was free of lipid-like material. This extraction removed about 2% of the bean solids.

Free Sugars.—The lipid-free bean meal was repeatedly soaked and washed with copious amounts of 80% ethanol, which removed about 15% of the bean solids. The alcoholic extracts were combined and concentrated (at a temperature below 50°C) to a suitable volume for chemical analysis and paper chromatography. Paper chromatography (14) revealed stachyose, raffinose, and sucrose as the only free sugars present in the bean. Confirmation of the identities of these sugars was obtained by chromatographically examining their hydrolysis products formed with either invertase or acid.

Water-soluble Polysaccharides.—The bean residue from the alcoholic extraction step was extracted with distilled water (1 part bean to 8 parts water) at room temperature (25°C). Several ppm of phenyl mercuric nitrate was added to inhibit the growth of microorganisms. The meal was agitated in water for 24 hours and then separated from the extract by centrifugation. The residue was resuspended in water, agitated for 1 hour, and again centrifuged to separate the extract from the residue. The residue was washed with 80%, 95%, and absolute ethanol and then dried in a current of air at room temperature. The ethanol washings were discarded. The water extracts were combined, filtered through Celite Analytical Filter-Aid,[1] and concentrated at a temperature below 50°C. A crystal-clear filtrate could not be obtained.

Approximately 25% of the bean solids and 50% of the bean proteins (N×6.25) were water soluble.

Unless otherwise specifically stated, all carbohydrate fractions obtained from the bean were hydrolyzed with 1*N* hydrochloric acid in a sealed tube at 100°C and examined for sugars by paper chromatography. In most instances, the hydrolysis time was 2 hours; however, shorter periods of time were occasionally used to observe any differences in the sugar pattern that might occur because of possible reversion products or sugar destruction. Examination of the acid-hydrolyzed, water-soluble bean solids for

* Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] Mention of specific products does not imply endorsement by the Department of Agriculture over others of a similar nature not mentioned.

carbohydrates showed mannose to be the predominant sugar, with galactose, glucose, arabinose, and xylose also present. The mannose was confirmed by the formation of crystalline D-mannose phenylhydrazone (13).

In another experiment 450 g (dry basis) of bean meal was agitated in 3 l of distilled water (containing a few ppm of phenylmercuric nitrate) at room temperature for 24 hours. The residue was removed from the extract by centrifugation and again agitated in 1.5 l of water for 2 hours, and the final residue was removed by centrifugation. The *p*H of the combined water extracts was 7.1;

Table 1. General composition of lima bean[a]

	Bean[b]	Seed Coat
Protein (N×6.25), %	25.66	5.98
Crude fat (ether extract), %	1.26	0.58
Crude fiber, %	1.65	49.42
Ash, %	4.75	2.51
Phosphorus, %	0.44	0.17
Reducing sugars, %[c]	<0.10	0.34
Total sugars, %[c]	2.40	0.48

[a] Moisture-free basis.
[b] Refers to the seed minus the seed coat.
[c] As glucose.

this *p*H was adjusted to 5.5 with 1*N* hydrochloric acid, and the resultant flocculent precipitate was removed by centrifugation. The precipitate weighed 9.3 g and contained 87% protein. The carbohydrate portion of the precipitate was composed of mannose, galactose, glucose, and arabinose. During overnight storage of the extract (*p*H 5.5) at 10°C a precipitate formed; it was also removed by centrifugation. This precipitate weighed 11.0 g and contained 88% protein, together with carbohydrate material composed entirely of mannose. By lowering the *p*H of the extract to 2.5 a third precipitate (6.6 g) was obtained; it contained 75% protein plus carbohydrate material composed of mannose, glucose, and arabinose.

After the removal of the three precipitates the water extract having a *p*H of 2.5 was divided into three equal fractions. One fraction was heated on the steam bath for 1 hour and the precipitate that formed was removed by centrifugation. The precipitate, 6 g (18 g when calculated on the basis of the entire extract), contained 80% protein. The carbohydrate portion of the precipitate was primarily mannose with only traces of galactose, glucose, and arabinose. The centrifugate contained galactose, glucose, and arabinose.

Enough ethanol was added to the second fraction to make a final alcohol concentration of 90%. The precipitate obtained weighed 7 g; it contained 74% protein, plus mannose, glucose, and arabinose. The supernatant contained galactose and glucose.

A precipitate of 6 g was obtained from the third fraction by the addition of trichloroacetic acid. This precipitate contained 83% protein and the carbohydrate was exclusively mannose.

In supplementary experiments the water extract was analyzed by various techniques in an effort to isolate the mannose polymer from the protein component. The addition of Fehling's solution (2) to the water extract did not precipitate the mannan. Dimethyl sulfoxide (8) was used both as a possible precipitating agent for the water-soluble polysaccharide and as an extractant applied directly to the bean meal. Neither procedure isolated the mannan.

Cetyltrimethylammonium bromide in the presence of a borate buffer (*p*H 8.5; 1%) was used in an effort to precipitate the mannan in the form of a borate complex (3). Precipitation was negligible, and both the precipitate and the solution contained mannan and protein.

Gradient fractionation of the solids in the water extract was tried with both ethanol and ammonium sulfate. Precipitations were made at 10, 20, 30, 40, 50, 60, and 90% ethanol concentrations. Each precipitate was removed by centrifugation and the ethanol concentration of the extract was then increased by the addition of absolute ethanol. At each ethanol concentration level, up to and including 50%, the precipitate contained protein, and its acid hydrolysate showed the presence of mannose plus trace amounts of galactose, glucose, and arabinose. Ethanol concentrations greater than 50% produced only small amounts of precipitate that contained protein but no mannose.

Ammonium sulfate was used at 10, 25, 40, 50, 60, 70, 80, 90, and 100% saturation.

Each precipitate was removed by centrifugation and the solid salt was added to raise the sulfate concentration to the subsequent higher level. No markedly different fraction could be obtained by using this procedure, as all fractions contained protein and, after acid hydrolysis, all contained mannose with trace amounts of galactose, glucose, and arabinose.

Exploratory work with paper electrophoresis techniques indicated that the water extract of the bean contained a mixture of proteins, suggesting that considerable investigatory work with this technique would be necessary to bring about their separation before the relationship of the mannan to the protein component could be studied.

In another series of experiments, various reagents of the type ordinarily used to deproteinize plant extracts were added to separate portions of the water extract of the bean. The addition of barium chloride produced a precipitate consisting primarily of protein plus a small amount of mannan. The further addition of sodium hydroxide and zinc sulfate (10) to this extract produced a precipitate containing both protein and mannan.

Zinc sulfate was added to a portion of the water extract and adjusted to *p*H 8 with 0.5*N* sodium hydroxide. In another instance, barium hydroxide was added to a part of the water extract, followed by the addition of zinc sulfate. In each case the precipitate (removed by centrifugation) was made into a thick slurry with water and then treated with glacial acetic acid. Enough ethanol was added to each of the mixtures to make a final ethanol concentration of 60%. The ethanol-insoluble fractions were removed by centrifugation, washed repeatedly with 60% ethanol, air-dried, and subsequently examined for protein and polysaccharide content. Basic lead acetate was also used as a precipitating agent. In every case, the deproteinizing reagents precipitated various amounts of protein, and every precipitate contained the mannan component.

Starch.—Two methods were tested for the removal of starch from the water-insoluble fraction of the bean meal. A portion of the bean residue was extracted with a 33% chloral solution (v/v) for 1 hour at 80°C (4, 12), and the hot extract was separated from the residue by centrifugation. The residue was washed twice with hot chloral solution. The washings were combined with the original extracts and poured into acetone. The acetone precipitate was separated by centrifugation, and was washed twice with acetone and finally with ethyl ether. The bean residue from the chloral extraction was washed thoroughly with acetone and ethyl ether to remove all traces of chloral.

Table 2. Extraction of starch from the lima bean

Method	% Starch[a]	% Protein in Starch	Sugars in the "Starch"
1st amylase extraction	70	2.9	All contained maltose, glucose, and trace amounts of two aldose polymers
2nd amylase extraction	3	8.3	
3rd amylase extraction	2	20.8	
Total	75		
Chloral	86	13.7	Glucose, arabinose, trace amounts of an aldose polymer, and xylose[b]

[a] The per cent of starch was calculated on the water-insoluble solids of the bean.
[b] After 1*N* HCl hydrolysis.

Another portion of the bean residue was made into a slurry with water (1 part bean, 50 parts water) and heated to gelatinize the starch. Salivary amylase plus a few drops of chloroform was added to the slurry and the mixture was agitated for 24 hours at 30°C (5). The residue was removed by centrifugation and the procedure was repeated two more times, or a total of 72 hours' amylase treatment. The centrifugate, which was almost crystal clear, was evaporated to "dryness" at 60°C in a forced-air oven. The residue, after amylase treatment, was washed thoroughly with water and then ethanol, and air-dried.

Salivary amylase solubilized 75% of the water-insoluble bean residue, whereas chloral solubilized 86% of the bean residue (Table 2). The solids removed by amylase were examined by paper chromatography before and after acid hydrolysis; the chloral "starch" (after acid hydrolysis) was similarly examined.

Table 3. Typical data obtained from KOH extracts of 1 g bean residue[a]

Extractant[b]	Precipitate, g[c] pH 5	Precipitate, g[c] 80% EtOH	% Protein in Precipitate pH 5	% Protein in Precipitate 80% EtOH	Sugars in Acid-hydrolyzed Precipitates pH 5	Sugars in Acid-hydrolyzed Precipitates 80% EtOH
1% KOH	0.31	0.10	80.6	75.0	Traces of glucose, mannose, arabinose	Large amount of arabinose, traces of galactose, glucose, xylose
4% KOH	0.05	0.03	86.9	4.7	Trace of arabinose	Large amount of arabinose, glucose, trace of galactose
24% KOH[d]	0.00	0.30	—	0.5	—	Large amount of arabinose, traces of galactose, glucose, xylose

[a] One g of bean residue, free of lipids, sugars, water-solubles, and starch (amylase-treated).
[b] Alkaline extractions performed in sequence as shown.
[c] Alkaline extract adjusted to *p*H 5 with acetic acid and any precipitate formed removed before adding ethanol to the extract.
[d] The residue from the 24% KOH extraction, *alpha*-cellulose, weighed 0.24 g, contained no protein, and was composed entirely of arabinose and glucose in the weight ratio of ca 1:1.

Chromatograms of the amylase-extracted solids, before acid hydrolysis, showed trace amounts of two aldose polymers, a large amount of maltose, and glucose. The acid-hydrolyzed material contained glucose only. The chromatograms of the acid-hydrolyzed chloral "starch" showed glucose, arabinose, trace amounts of an aldose polymer, and xylose (Table 2).

Polysaccharides Soluble in Potassium Hydroxide.—The bean residue from the amylase-treated material was extracted at room temperature for 24 hours with 1% KOH solution (1 part bean in 50 parts KOH solution), under nitrogen, with constant agitation. The insoluble material was removed by centrifugation and washed several times with 1% KOH. The extract, combined with the washings, was adjusted to *p*H 5 with acetic acid. The *p*H 5 precipitate was removed by centrifugation, washed with a 2% acetic acid solution followed by washing with increased concentrations of ethanol, and air-dried. After the removal of the precipitate (*p*H 5), ethanol was added until the concentration was 80%. The ethanol precipitate was removed by centrifugation and washed thoroughly with 80%, 95%, and absolute ethanol, respectively, and air-dried. The bean residue from the 1% KOH treatment was extracted with 4% KOH and then with 24% KOH, and the above procedure was repeated with each concentration of KOH. No precipitate at *p*H 5 was obtained with the 24% KOH extract.

The final bean residue from the 24% KOH extraction ("*alpha*-cellulose," by definition) was washed first with KOH solution and then with a 2% acetic acid solution and 80%, 95%, and absolute ethanol, respectively, and air-dried.

The *p*H 5 precipitates of the KOH extracts were primarily protein with trace amounts of glucose, mannose, and arabinose, indicating that no polysaccharides were fractionated at this *p*H level (Table 3).

All of the ethanol precipitates contained large amounts of arabinose with trace amounts of galactose, glucose, and xylose. The glucose content of the ethanol precipitate from the 4% KOH extract was higher than that of ethanol precipitates of the other two KOH concentrations.

alpha-Cellulose.—A portion of the residue of the bean remaining after extracting with 24% KOH, considered to be *alpha*-cellulose by definition, was heated with 1*N* hydrochloric acid in a sealed tube in a boiling water bath. Samples were withdrawn for examination by paper chromatography after 0.5, 1, and 2 hours' hydrolysis. All of the chromatograms showed the presence of large quantities of arabinose plus glucose, and trace amounts of galactose and xylose. Because of incomplete hydrolysis (a considerable amount of insoluble residue was apparent) another portion of the *alpha*-cellulose fraction was treated with 72% sulfuric acid for 20 hours at 10°C. The mixture was then diluted to 3% acid concentration and heated for 2 hours in a boiling water bath. The *alpha*-cellulose was completely solubilized.

The hydrolysate was divided into two portions. One was treated with barium carbonate, centrifuged, and then treated with a batch mixture of ion-exchange resins, Amberlite IR-120 (H^+) and Duolite A-4 (OH^-), for 2 hours. The hydrolysate was filtered and the filtrate was concentrated to an amount suitable for paper chromatography. Chromatography again showed the presence of glucose, arabinose, and trace amounts of galactose and xylose. The other portion of the sulfuric acid hydrolysate was divided into two parts. One part was treated with glucose oxidase to remove the glucose component present in the extract (16). The other portion was neutralized with sodium carbonate. The two fractions, so treated, were then examined for reducing sugars by the micro Somogyi copper procedure (1). Standard sugar curves of both glucose and arabinose were prepared by the Somogyi procedure. The reducing sugar data obtained before and after glucose oxidase treatment are sufficient for calculating the amounts of glucose and arabinose present in the *alpha*-cellulose. Repeated experiments indicated that the *alpha*-cellulose was composed of glucose and arabinose in the weight ratio of ca 1:1.

Seed Coat.—The removal of the seed coat effected the mechanical separation of a material considerably different in composition from the remainder of the bean (Table 1). The free sugars were primarily glucose and fructose with a trace of raffinose. Acid hydrolysis of the seed coat showed the polysaccharide pattern to be similar, at least qualitatively, to that of the seed, namely, galactose, glucose, mannose, arabinose, and xylose.

Discussion

Although stachyose, raffinose, and sucrose are not usually found together in the same plant material, their occurrence is a characteristic of the *Leguminosae* (7). However, it is rather startling to find these three fructose-containing sugars in a plant material that also contains a considerable amount of starch but no detectable amounts of free fructose or glucose. This, of course, could also be a characteristic of the bean, since it is the storage organ of the plant.

The ready solubility in water of the mannan component is unusual in that mannans, in general, require an alkaline medium for solubilization; a few exceptions have been reported, such as the salep mannan (11) and yeast mannan (6, 11). Concomitant precipitation of mannan and protein was invariably consistent with a wide variety of reagents ordinarily used for the removal of proteins from plant extracts. Heat treatment produced a precipitate of mannan and protein. It is well known that heat coagulation is a fundamental procedure for the preparation of proteins. Gradient precipitation procedures, with either ethanol or ammonium sulfate, did not separate the mannan from the protein. Fehling's solution, commonly used in the isolation and purification of mannans, did not precipitate the lima bean mannan. All of the experimental data indicate a close association between the mannan and protein components. In fact, although irrefutable proof is not given, the data strongly suggest the presence of a mannan-protein complex in the lima bean.

The araban polymers in the lima bean must exist in a wide variation of size or structure (perhaps both factors are present), because of their indiscriminate range of solubility. They are present in the water extract, in the bean residue remaining after extraction with 24% KOH, and in all of the extracts obtained between the two extremes of the fractionation procedure. Usually, the "*alpha*-cellulose" component of a plant material is primarily composed of glucose, with perhaps trace amounts of other sugars. However, araban accounts for approximately one-half of the weight of the lima bean *alpha*-cellulose.

The importance of including a water-extraction step prior to extracting a plant material with alkaline reagents is certainly apparent from the results of this investigation. If this procedure had been omitted, the mannan fraction, which was completely water-soluble, would have been present in the alkaline-soluble fractions. This is an example of why it is unwise to follow a fixed or established pattern of analysis. Because carbohydrate polymers vary so widely in their solubility characteristics, a prelimi-

nary investigation of a plant material should always be made, so that the appropriate analytical procedure can then be followed.

Starch was the only carbohydrate removed from the lima bean by the action of salivary amylase. This starch contained 3.6% protein, which may have been due primarily to water solubility of the proteins, since the lima bean proteins were extremely water-soluble and not subject to a definite line of demarcation in their solubility characteristics. Chloral removed appreciable amounts of araban in addition to the starch; the chloral "starch" contained 13.7% protein. Although chloral may remove only starch when applied to some plant materials (9), it is obvious that it cannot be applied indiscriminately to all types of plant materials.

The lima bean cannot be easily fractionated into its component parts because of the wide and overlapping solubility characteristics of both the protein and araban components, and because of the apparent close association of the mannan with a part of the protein fraction. The composition of the "*alpha*-cellulose" fraction is contrary to the usual definition of this component. All of the observations illustrate that the lima bean has unique characteristics, which may, of course, be true of other members of the *Leguminosae*.

Summary

1. The free sugars found in the dehulled lima bean (*Phaseolus lunatus*) were sucrose, raffinose, and stachyose.

2. The mannan component was removed easily and completely from the bean by extraction with water. Concomitant precipitation of mannan and water-soluble protein was invariably consistent with a wide variety of reagents ordinarily used for the removal of proteins from plant extracts; this suggests the presence of a mannan-protein complex in the lima bean.

3. The araban was relatively insoluble in water and only partially soluble in strong alkali. The "*alpha*-cellulose" fraction was approximately one-half arabinose.

4. The free sugars of the seed coat were primarily glucose and fructose with a trace of raffinose. An acid hydrolysis showed the polysaccharides to be composed of galactose, glucose, mannose, arabinose, and xylose.

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Identification of Stored Products Insects by the Micromorphology of the Exoskeleton. IX. Head, Thorax, and Abdomen

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I. Introduction

A number of investigations have been published (1–9) relating to identification of fragments of insects which frequently infest cereal grains, grain products, and stored products in general. This study is a part of the series and presents information characterizing the head, thorax, and abdomen of sixteen groups of adult beetles. The emphasis of this study, in contrast to the previous investigations, is on the identification of nondiscrete fragments that cannot be identified as sclerites. Most of the beetles studied can be recognized when the sclerites are studied intact, and some information is presented in this regard, but in many food products comminution breaks the body sclerites of contaminating insects into fragments so nondescript that it is difficult, if not impossible, to determine the body region from which the fragment originated. Therefore, it is necessary to incorporate the varying characteristics of all major body sclerites into one comprehensive key to the identification of species. The key is based on the identification of small fragments without characteristic shape. Study of these minute fragments has shown that many of them retain distinguishing characteristics in cuticular pattern, setal origin, structure, and distribution in spite of the modification in the cuticular pattern and setal structure which occurs from one anatomical region to another within a single species. Basic characteristics may be retained, but in modified form. The key may also be used as an adjunct or for further reference in the study of larger structural units. (Some larger body-region characteristics are given in Part II.) Also, comparative size can be determined by checking magnifications of the photomicrographs.

This study is divided into Part I: an introduction; Part II: a discussion of the gross morphology of the head, thorax,[1] and abdomen, and salient characteristics of differentiation of the sclerites; Part III: descriptions of individual species; Part IV: a key; and Part V: line drawings and photomicrographs grouped in plates by species and alphabetically arranged by common name. Included in Part V, the plates, are general outlines of the larger and more significant structures and photomicrographs of the cuticular surface and setal structure, so that a basis for comparison of all types of fragments is available.

There are several types of characteristics relating to the nature of the insects and their specific morphology which must be considered in a study of this type One is the size of the fragments encountered. If the fragments of insects found in food products are large, it may be possible to recognize their origin. Fragments of this type can be identified by comparison with the descriptions in Parts II and III; the key, Part IV; and plates, Part V.[2]

There will be isolated fragments which bear no characteristic marking, do not fit into the classification key, and cannot be confirmed by the photomicrographs or species descriptions.

Cuticular Pattern

Since this paper is primarily concerned with the identification of fragments by cuticular and setal morphology, a brief description of these features is necessary.

Cuticular pattern denotes the design or pattern, including ridging, which may be observed in the cuticle. This may be due to

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[1] The authors would like to acknowledge work on the thorax contributed by Earl R. Bloomingdale of the Chicago District of the Food and Drug Administration.

[2] Investigations to establish the differential identification of the intact mesonotum and head capsule of stored products insect contaminants are being conducted at the present time and will be presented in two subsequent reports.

a surface sculpturing, an arrangement of the deeper layers, or a combination of the two. In some species there occur independently, in the cuticle, highly refractile areas. These may be punctures or other cuticular structures; for convenience, they are termed "inclusions". Some may be modified setal bases without a limiting margin; others do not appear to be associated with setae. No attempt is made to determine the histologic origin of these cuticular structures, although they are distinguishing criteria and will be referred to in further descriptions.

Origin of Setae

The origin of setae on many insects is quite distinctive . Most species are characterized by having the setae arise from cuticular structure such as an area which may be a translucent well-defined depression, a puncture, a ridge or protuberance, or perhaps modifications of these. The marginal area, or rim about the setae, its characteristic size, shape, completeness and distinctness, and the number of setal punctures or occurrence of setae without limiting margins are all valuable factors in identification.

Structure of Setae

The structure of the seta, if present, is often distinctive. Setae may be characterized as fan-like, club-shaped, barbed, forked, fringed, blunt, or flattened. Descriptions and illustrations may be found in discussions of the various species and are in the photomicrographs.

Distribution of Setae

The distribution of the setae and their origin from within a characteristic perimeter, or their origin unassociated with a cuticular structure, aids in species recognition. To use density as a characteristic, a setal count number is included in the key and refers particularly to identification of head fragments. The setal count is determined by counting the number of setae or bases in an area of 0.01 square mm.[3]

[3] The area of 0.01 square mm is the area of four squares of the Howard Mold Count grid when the microscope is adjusted as in *Official Methods of Analysis*, A.O.A.C., 8th Ed. (1955), 35.1(k)(1), and used at 440× magnification.

II. Gross Morphology of the Head, Thorax, and Abdomen with Distinctive Characteristics of the Larger Sclerites

A. The Head

The mature insect head is a capsule in which most obvious evidence of segmentation is lost. Antennae and mouthparts were described in the second article of this series (*This Journal*, **39**, 879 (1956)). If the insect is subjected to grinding, as in the manufacture of various food products, the head capsule does not usually shatter into discrete areas of sclerites but into fragments nondescript in shape. For this reason, general terminology such as dorsal, ventral, and lateral areas is used rather than delineating specific and variable anatomical sclerites.

Most of the species studied have relatively large compound eyes consisting of dark, hexagonal facets, positioned in a characteristic location on the head. Several simple eyes, the ocelli, usually are situated between the compound eyes.

Reasonably intact head capsules may be encountered in examination of food products. Some of the stored product contaminants may be identified specifically by considering gross appearances, such as size, shape, unique structure, sutures, and eye placement, and structure. Following this the often obvious finer characteristics of unique setal structure and distribution and of cuticular pattern can be used for identification.

1. *Size of Head Capsule as a Species Characteristic.*—Of the species covered by this paper, the large size of the Cadelle Beetle, Mealworm, and Larder Beetle are readily apparent and distinctive , as is the small size of the Flat Grain Beetle head capsule.

2. *Shape of the Head Capsule as a Species Characteristic.*—The heads of the Drugstore Beetle, Cigarette Beetle, Flat Grain Beetle, Cadelle Beetle, Mealworm, and Rust-Red and Confused Flour Beetles are nearly spherical, whereas the Square-necked Grain Beetle, Foreign Grain Beetle, Sawtoothed Grain Beetle, Buffalo Carpet Beetle, and the Dried-fruit and Larder Beetles to a lesser extent , are characterized by nearly squared or rectangular head

capsules. The Weevil is unusual in possessing a snout (see Plate 31, Figs. a, b, e) while the expanded clypeus of the male Broad-horned Flour Beetle is diagnostic (see Plate 3, Fig. a).

3. *Sutures on the Head Capsule as a Species Characteristic.*—The following insects may be identified by characteristic sutures or markings on the head capsule: the Lesser Grain Borer dorsal suture extends anteriorly $\frac{1}{3}$ to $\frac{1}{2}$ the length of the head from the center of the posterior margin (see Plate 26, Fig. a). The ventral head surface of this species bears a Y-shaped suture as illustrated in Plate 26, Fig. d. The Flat Grain Beetle has a vertical suture on the midline of the dorsal head surface (see Plate 19, Fig. a).

4. *Gular Modifications as a Species Characteristic.*—The Drug-store Beetle and Cigarette Beetle, and to a lesser degree the Black Carpet and Buffalo Carpet Beetles, may be differentiated by a broad gular nonsclerotized area which separates the ventral mid-section of the head into two lateral cheeks or gena (see Plates 1, 5, 9, 17).

5. *Eye Structure of the Rust-Red and Confused Flour Beetles.*—The comparative shape of the eye and number of facets is a distinguishing characteristic between the Rust-Red and Confused Flour Beetles (see Plates 11 and 12). The Confused Flour Beetle has only a small portion of the eye visible when viewed dorsally, while the Rust Red Flour Beetle has exposed, dorsally, a large surface of the compound eye and several lightly pigmented ommatidea.

6. *Other Structures as Species Characteristics.*—Other unusual and distinctive identifying structures are the horizontal paired ridges on the Broad-horned Flour Beetle which are mesal and adjacent to the eyes (see Plate 3, Fig. a), and the bald central spots on the dorsal head surfaces of the Black Carpet and Buffalo Carpet Beetles (see Plates 1 and 5, Figs. a).

7. *Setal Appearance as a Species Characteristic.*—Perhaps the most striking characteristic of a number of stored grain insects is the over-all appearance of the setae, their distribution, and the cuticular pattern and its variations. The head capsule may bear all types to be found. Some species may be identified by their highly characteristic types. Examples are the club-shaped setae of the Rice and Granary Weevils (see Plate 32, Fig. a) and the large forked setae of the Cadelle (see Plate 8, Fig. e). The modification of setae to form scales in the Buffalo Carpet Beetle leaves no doubt of its identity. The setae of the Larder Beetle, Dried-Fruit Beetle, Drug-store Beetle, Cigarette Beetle, and Black Carpet Beetle are so numerous and often coarse that this group may be readily separated from all others which bear fewer, finer setae.

8. *Setal Origin as a Species Characteristic.*—Setal origin is a helpful factor in determining the identity of contaminants of stored products. A few of the unusual and differentiating features are set forth here. The setae of the Cigarette Beetle always arise from an area readily visible and identifiable as a setal base. On the Lesser Grain Borer the setae appear on small protuberances, as is the case with some of the Mealworm and Drug-store Beetle setae (see Plates 18, 27, and 28). The presence of a triad of setal punctures in some areas of the Mealworm head capsule is diagnostic (see Plate 28, Fig. c). Fragments of Sawtoothed Grain Beetle, Foreign Grain Beetle, and Square-necked Grain Beetle may bear two closely adjacent punctures although only one seta may be observed. Complete descriptions of these markings and structures are included in the discussion of individual species and illustrated in the photomicrographs.

9. *Cuticular Pattern as a Species Characteristic.*—The cuticular structure of the weevil is unique in its uniformly spaced scalloped or longitudinal striations (see Plate 31, Figs. d, f). The Larder Beetle cuticle appears "rippled" when observed at a focus slightly below the setal base (see Plate 24, Fig. f.). The Lesser Grain Borer may be distinguished by two types of patterns: one, a cell-like honeycomb arrangement; the other a "veined" pattern (see Plate 26).

B. The Thorax

The thorax of any insect is the middle body region that bears the wings and legs. There are three thoracic segments: the pro-

thorax, mesothorax, and metathorax. Each of these is typically divided into three groups of sclerites, although in the prothorax the sutures are often fused and the structure so modified that these areas, per se, are not readily distinguishable. The dorsal sclerite is the notum (or tergum) which may be subdivided into smaller sclerites, the identities of which are not necessary in this study. The primary lateral sclerite is the pleuron. This is usually divided dorso-ventrally into anterior and posterior sclerites which commonly bridge the wing and leg. The primary ventral sclerite is the sternum, which may be divided into two smaller sclerites. Only those anatomical details pertinent to identification are discussed. Small, nondescript sclerites in most cases are difficult to identify; therefore, emphasis has been placed on shape of the larger sclerites. Many of these small sclerites retain the setal and cuticular characteristics of the species and can be identified by use of the key. Some of these structures are included to illustrate the over-all makeup of a species rather than differentiate on the basis of comparative morphology.

The Prothorax

The size of the prothorax usually follows the over-all dimensions of the species. Comparative data and size may be obtained by checking the magnification given on individual plates. It may be observed from these photomicrographs that in most species the prothorax appears rectangular when viewed dorsally and becomes rounded and compressed as it extends laterally and ventrally. The sternal area may be smaller than that of the notum, and in many species the posterior ventral margin is modified to form a mesal projection, the poststernum, which in many cases forms the coxal cavity. The setal and cuticular configuration on the prothorax are roughly comparable to those on the head, although there may be a lessening in the number and coarseness of the setae on the sternum and pleura.

Shape of Prothorax as a Species Characteristic.—(a) There are a number of species in which the outline of the prothorax is similar to the generalized description above and that shown in Plate 16, Fig. b, and Plate 20, Fig. b. In this group are the Cadelle Beetle, Square-necked Grain Beetle, Broad-horned Flour Beetle, and Dried-fruit Beetle. The Mealworm notum is of the same typical outline, but the ventral sternite and lateral pleuron are modified by posterior and lateral projections as shown on Plate 29, Figs. c, d.

(b) The Flat Grain Beetle (see Plate 20), Rust-Red and Confused Flour Beetles, and the Weevil dorsal and ventral (notal and sternal) sclerites are rectangular, which places them in a group very similar to those named above, although the poststernum is only rudimentary. The Foreign Grain Beetle prothorax is similar, although the ventral mesal articulation (poststernum) is shortened (see Plate 22, Figs. a, b).

(c) Of greater value in differentiation are those species in which modification of the prothorax has produced distinctive characteristics. Most classic is the Sawtoothed Grain Beetle, whose saw-edged lateral margins are the basis for the common name of this pest (see Plate 34, Fig. a). The Lesser Grain Borer presents a heavily sclerotized roughened prothorax, as is described and illustrated (see Plate 27, Fig. b), while the Drug-store and Cigarette Beetle group (and to lesser extent the Buffalo Carpet and Black Carpet Beetles) are distinguished by a narrow, fragile sternum, as illustrated in Plate 10, Fig. c.

The Mesothorax

The structure of the mesothorax of each species studied is distinctive. The mesothoracic sclerites serve for attachment of the elytra and have many modifications for the functioning and support of the wings. Size, shape, and cuticular and setal arrangement remain important differential characteristics. However, in contrast to the prothorax, the principal identifiable setal and cuticular structures are found on the mesosternites and areas of the pleura in some species.

The nota (or terga) of most species studied are sclerotized, similar in shape, and generally present a triangular scutellar area between bases of the elytra; an example is shown on Plate 4, Fig. g.

The mesopleura may appear among the larger sclerites that are found in insect-contaminated food products. A typical shape is that shown on Plate 4, Fig. f. The shape is not distinctive in most species, and identification may best be made by use of the key on cuticular pattern and setal structure.

The Mesosternite

The mesothoracic sternite in most species is a large structure and may be distinctively shaped. A characteristic mesosternite in which three posterior projections are evident is shown on Plate 4, Fig. c. These projections are formed by the mesal poststernal process and lateral sternal projections, rim the coxal cavities, and are used for attachment of muscles of the coxa and the metasternum.

1. *Posterior and/or Lateral Projections as a Species Characteristic.*—Species exhibiting three posterior projections are: the Foreign Grain Beetle (see Plate 22, Fig. d), Square-necked Grain Beetle (see Plate 36, Fig. b), Lesser Grain Borer (see Plate 27, Fig. c), Rust-Red and Confused Flour Beetles (see Plate 13, Fig. d), Buffalo Carpet and Drug-store Beetles (see Plate 18, Fig. c), Mealworm (see Plate 30, Fig. a), and the Saw-toothed Grain Beetle (see Plate 34, Fig. g). The Larder and Black Carpet Beetles (see Plate 2, Fig. b) each have a modified T-shaped mesosternite.

2. *Diagnostic Modifications of 1 (above).*—(a) The Larder Beetle mesosternite may be distinguished from that of the Black Carpet Beetle by the large size of the Larder Beetle sclerite.

(b) Extension of the median poststernum in the Square-necked and Foreign Grain Beetles is differentiated by a distinct indentation near the posterior margin of the projection (see Plate 22, Fig. d; Plate 36, Fig. b).

(c) The Broad-horned Flour Beetle poststernal processes are modified distinctively at the tip which divides to form an open-end socket for articulation with the metathorax.

3. *Mesosternum Reduced to Function as Connecting Sclerite as a Species Characteristic.*—Groups in which the mesosternum is reduced in size and appears primarily as a connecting bridge are the Dried-Fruit Beetle (see Plate 16, Fig. c), Cigarette Beetle (see Plate 10, Fig. e), and the Cadelle and Buffalo Carpet Beetles (see Plate 6, Fig. d).

4. *Distinctive Mesosterna as a Characteristic of Other Species.*—(a) The Flat Grain Beetle Mesosternum is distinctive in that the poststernum is broad and flattened as illustrated in Plate 20, Figs. c, d.

(b) The weevil mesosternum may be identified by little poststernal development, a relatively constricted anterior-posterior dimension, and a shortened poststernum that is squared off acutely.

The Metathorax

The outlines of the metathoracic sclerites possess a general similarity. The shape of the notum (or metatergum) follows that illustrated in Plate 22, Fig. e. No species distinction is attempted in this paper on the whole sclerites of the metatergite because of their similarity in appearance, comparative fragility, and low survival after food processing. They are covered by the elytra and remain as lightly sclerotized membranes without the cuticular or setal characteristics found on heavily sclerotized unprotected exoskeletons.

The larger sclerites of the pleura are similar to that illustrated in Plate 27, Fig. e. In most species studied these pleura are similar in outline, and no attempt is made to differentiate on the basis of gross appearance. However, cuticular and setal characteristics are used in the key and discussed in the species descriptions.

The Metasternum

The following descriptions and grouping of species which have like areas of the metasternite are presented to assist in basic separation. However, this sclerite usually carries typical cuticular arrangement that follows the key for final identification. The metasternum forms a broad, shield-like structure which in most species is like that shown on Plate 20, Fig. c. It has fewer modifications than the wing-bearing terga and pleurites and is usually characterized by increased size and some modification in areas of articulation with the mesosternum and the first abdominal sternite. Thus

again, size, shape, unique structures, and cuticular and setal configuration are the basis for species identification. Morphologic nomenclature in this study is purposely general: however, the identification of several structures on the metasternite is necessary for description and recognition of the species. One of these structures is the median sternal groove or suture, which is typified by the extent to which it appears medially down the anterior-posterior axis of the sternite: entire, partial, or absent. Also, the modification of the anterior median margin and the posterior median margin of the sclerites is often distinctive. Thus gross identification of some groups may be made if the median portion of the sternite is observed or if the median anterior or posterior areas are present.

1. *Median Sternal Groove as a Species Characteristic.*—The following separation of a number of species may be made on the basis of the median sternal groove which often is accompanied by a prominent internal ridge or apodeme.

(a) The Sawtoothed and Dried-fruit, Beetles' median groove extends the entire length of the mestasternite.

(b) The groove on the Mealworm, Square-necked Grain Beetle, Foreign Grain Beetle, and Larder Beetle is almost the entire length of the sternite, usually terminating just posterior to the central anterior projection (see Plate 30, Fig. e).

(c) In the Drug-store Beetle, Rust Red and Confused Flour Beetles, and Flat Grain Beetle the median groove originates on the posterior margin of the metasternite and terminates midway up the sclerite (see Plate 14, Fig. c), while the Broad-horned Flour Beetle suture may extend even further anteriorly but terminates short of the anterior margin (see Plate 4, Fig. e).

2. *Median Sternal Groove and Antecostal Suture as a Species Characteristic.*—The Rice and Granary Weevils and Buffalo Carpet Beetle are the only species studied in which there occur both a portion of the median sternal groove and an antecostal suture. In the Weevil these bisect the posterior sternellum which in turn is notched by a short median groove to form small sclerites between the coxae (see Plate 32, Fig. f). The Buffalo Carpet median sternal groove arises on the anterior margin of the sternum and terminates above the antecostal suture.

3. *Absence of Distinct Sternal Groove as a Species Characteristic.*—The Lesser Grain Borer, Cadelle Beetle and Cigarette Beetle are distinguished by the absence of a prominent groove, although the Cadelle Beetle does have a slight separation of posterior median projections and a groove-like depression in the surrounding area. The Cigarette Beetle is readily separated from the group by a narrow anterior-posterior depth and the presence of an antecostal suture which crosses the sternum transversely (see Plate 10, Fig. g); the Cadelle is distinguished by the pincer-like posterior projections and typical shape (see Plate 8, Fig. f); and the Lesser Grain Borer is typical in the extreme simplicity and lack of differentiation of the entire sclerite (see Plate 27, Fig. d).

The further differentiation which is observed on the margins of the metasternite has been mentioned briefly above. Although structural differentiation on the sternite is not great, the species studied may also be grouped under broad characteristics of shape and projection of the anterior and posterior median margins.

4. *Typical Anterior Median Projection (Spina) as a Species Characteristic.*—The most typical appearance on the anterior margin is a shape like that illustrated in Plate 8, Fig. f. This is generally characteristic of the Lesser Grain Borer, Foreign Grain Beetle, Square-necked Beetle, Mealworm, Drug-store Beetle, Sawtoothed Grain Beetle, Cadelle Beetle, Rust-Red and Confused Flour Beetles, Black Carpet Beetle, Broadhorned Flour Beetle, and Weevils.

5. *Other Types of Anterior Median Projections (Spina) as a Species Characteristic.*—(a) The Flat Grain Beetle and Dried-fruit Beetle anterior median projection is flattened (see Plate 20, Fig. c).

(b) The Larder Beetle spina forms paired knobs as illustrated on Plate 25, Fig. c.

(c) The Buffalo Carpet Beetle's metasternite median antecostal margins are not indented as are most of the metasterna studied, and the mesal anterior tip is narrowly notched by the median sternal groove.

6. *Absence of an Anterior Median Projection (Spina) as a Species Characteristic.*—The metasternite of the Cigarette Beetle is illustrated on Plate 10, Fig. g. It possesses a true antecostal suture which, with the lack of a spina, produces a depression and uniform extension of the eusternum that is unique among the species studied.

7. *Modifications of the Posterior Margin of the Metasternite as a Species Characteristic.*—Posterior modifications of the metasternite are primarily in the areas of the median sternal groove and points of articulation and attachment with the coxa. These usually occur as knobbed projections, often forking from the sternal groove.

(a) Species in which these projections are branched and yet close to the midline of the sclerite are the Foreign Grain Beetle, Square-necked Grain Beetle, Mealworm, Dried-fruit Beetle, and Broad-horned Flour Beetle.

(b) The Sawtoothed Grain Beetle, Rust-Red and Confused Flour Beetles, Drugstore Beetle, and Flat Grain Beetle follow the pattern of 7a (above) although the projections are more widely spaced and not as prominent (see Plates 14, 18, 20, and 34).

(c) The Cigarette Beetle and Larder Beetle display unusual rounded socket effects on the posterior margins as shown in Plate 25, Fig. d.

(d) On the central projection of the Lesser Grain Borer the indentation is small and appears almost as a single articulating knob.

(e) The Black Carpet, Buffalo Carpet, and Cadelle Beetles, and the Weevil, exhibit either a pincer-like posterior median tip, or the paired sternella on the antecostal suture and median sternal groove as discussed in paragraphs 2 and 4 above. In the Buffalo Carpet Beetle the groove re-occurs on the posterior projection of the sternella, dividing it into paired, pincer-like sclerites (see Plate 6, Fig. e).

C. Abdomen

General Considerations

The number of abdominal segments in the beetles varies from five to eight; in the species studied only five abdominal segments are present (Weevils four). All segments are similar in shape, the breadth dimension being greater than the length; those of the visceral region are usually simple and differ only slightly from one another. Distinguishing features occur in the first sternite and in the terminal area. The terga and sterna overlap posteriorly, and many of the first abdominal sternites are overlapped by the metathorax.

The coxal cavity may extend posteriorly for different distances into the first addominal sternite, but the first sternite is never entirely divided, and the posterior edge extends completely across the body. It is broadly joined to the thorax and offers some diagnostic landmarks for species identification. For this reason the bulk of this section is devoted primarily to the characteristics of the first abdominal sternite, the cuticular and setal configuration of most segments, and in some instances to the distinctive characteristics of the terminal segments.

First Abdominal Sternite

Identification of an entire first abdominal sclerite is possible by studying the shape, size, and cuticular pattern and setal arrangement. If the sternite is fragmented, identification of the species is more difficult. The key may be used for successful identification of most of these fragments. The most striking feature of this sternite is an extension on the median anterior margin (the spina). In addition there is often a depressed shelf on the anterior margin (see Plate 2, Fig. c), which lies beneath the overlapping metasterna. The outline of the median projection may be repeated on the underlying sclerite, or there may be a change in the subsurface shape near the anterior projection which gives a characteristic appearance to the sterna of various species.

The species studied may be divided into three groups on the basis of shape of the median anterior projection.

1. *Median Anterior Projection Flattened.*—In this group are the Flat Grain Beetle (see Plate 20, Fig. e) and Dried-Fruit Beetle (see Plate 16, Fig. h) in which the projections are wide and definitely flattened on top. These species can be easily distinguished by size differences. The Rice and

Granary Weevil first abdominal sternite is also flattened (see Plate 32, Fig. c), although a small knob arises from the center of the plateau. The Weevil sterna is, of course, marked by the species-characteristic setae and cross-hatching.

2. *Median Anterior Projection Sharply Tapered: Ascending Slopes Concave.*—This group is characterized by a pointed anterior median projection, one in which the ascending slope to the tip is concave. It is composed of the Broad-horned Flour Beetle, Lesser Grain Borer, Square-necked Grain Beetle, the Tribolia (Rust-Red and Confused Flour Beetles), Larder Beetle, Black Carpet Beetle, and Cadelle Beetle (see Plate 2, Fig. c).

(a) The presence of a distinct rounded indentation on each side of the base of the projection may be used for separation of the Black Carpet Beetle (see Plate 2, Fig. c) and Larder Beetle (see Plate 25, Fig. e). The Larder Beetle may be identified by its large size and other intrinsic setal and setal membrane differences.

(b) A suture line appearing on each side of the projection and extending into the mid-portion of the sternite with a continuation of the projection outline (see Plate 37, Fig. d) is characteristic only of the Square-necked Grain Beetle.

(c) A very pointed tip, large size, and duplication in outline of the structure by the precostal shelf (see Plate 8, Fig. h) are characteristics by which the Cadelle Beetle first abdominal sternite may be recognized.

(d) The following differences may be used to separate the Lesser Grain Borer, Rust-Red and Confused Flour Beetles, and Broad-horned Flour Beetle. First there are several distinguishing characteristics between the Lesser Grain Borer (see Plate 27, Fig. f) and the Rust-Red and Confused Flour Beetles (see Plate 14, Fig. e). The precostal suture of the Lesser Grain Borer extends almost straight across the top of the sclerite, while the precosta of the Tribolium species studied is depressed centrally at the median projection, forming two oval lateral sclerites beneath the coxae. The first abdominal sclerite of the Lesser Grain Borer is distinctive in that sclerotization outlining the tip appears heavier and darker (even knob-like) than that of the Tribolia and has a definite ventral tilt. The median projection of the Tribolia and the anterior-posterior depth of the sternite is greater than that of the Lesser Grain Borer. The key should be utilized for identity following study of segments on the basis of these observations.

The subsurface sculpturing may be used to separate the Broadhorned Flour Beetle from the Lesser Grain Borer and Tribolia. In the Broad-horned Flour Beetle the subsurface sculpturing follows the outline of the anterior projection and in the others it does not. The median projection of the first abdominal sternite of the Broad-horned Flour Beetle is pointed although the lateral slopes of the projection are not distinctly concave (see Plate 4, Fig. h). Both the Broad-horned Flour Beetle and Lesser Grain Borer projections, in contrast to the Rust-Red and Confused Flour Beetles, appear rimmed because of thickening of the margin of the cuticle, but the Broad-horned rim curls ventrally along the edge of the projection, forming a ridge. It may be distinguished by lack of the overall ventral tilt of the projection which is quite prominent in the Lesser Grain Borer. These characteristics, in addition to cuticular and setal structure outlined in the key, will serve for identification of these species.

3. *Median Anterior Projection Rounded; Ascending Slopes Convex.*—In this group the projections on the anterior median surface of the first abdominal sternite have essentially convex slopes and a broad, curved, anterior surface. The species which exhibit this are the Sawtoothed Grain Beetle, Foreign Grain Beetle, Drug-store Beetle, Cigarette Beetle, Mealworm, and Buffalo Carpet Beetle.

There are a number of distinctive characters by which these species may be separated and identified.

(a) The most obvious characteristic is size. The Mealworm (see Plate 30, Fig. h), although very typical in general outline, is readily separated by its large size.

(b) The Sawtoothed Grain Beetle (see Plate 34, Fig. i) and Foreign Grain Beetle (see Plate 22, Fig. g) are distinguished from the other groups by subsurface sculpturing

which appears as a band across the tips of the median anterior projection. The Foreign Grain Beetle may be identified by a smaller tip located centrally on the subsurface projection, and by a suture which extends in a convex slope from the outlines of the projection. The Sawtoothed Grain Beetle does not possess these latter structures.

(c) The Drug-store Beetle, Cigarette Beetle, and Buffalo Carpet Beetle, although very similar, present distinguishing characteristics. The Drug-store Beetle projection is usually a pronounced knob rising from an essentially straight anterior margin (see Plate 18, Fig. h); the posterior margin of this sclerite forms a direct line bisecting the abdomen transversely. The transverse margins of the Cigarette Beetle are more rounded, and the antecostal suture slopes posteriorly from each side to the central projection which rises only slightly above the anterior margin of the sclerite. The configuration of the posterior margin follows this pattern by dipping posteriorly in the center (see Plate 10, Fig. h).

(d) The Buffalo Carpet Beetle may be distinguished by a lateral notch on each side of the median anterior projection which continues as a distinct ridge or suture (see Plate 6, Fig. h) almost to the posterior margin of the sclerite.

III. Species Descriptions

Black Carpet Beetle (*Attegenus piceus*). **Plate 1 and 2.**

Head capsule is dark brown. Setae on the dorsal surface are dark and long, about 0.04–0.07 mm long and about 0.03 mm apart; near the base of these setae may be one or more small holes. Setal count is 14–18. These setae arise only from a setal base. Setae on the ventral and lateral surfaces are shorter, about 0.02–0.05 mm long and about 0.02 mm apart. Setal count on these surfaces is 18–26, and the setae appear to rise from the top of a darkened ridge or mound. There may be a small hole near the base of these setae. There is no distinctive cuticular pattern, other than the pronounced ridging on the head capsule. Eye is large and oval with small facets, about 0.01 mm in diameter.

Thorax is characterized by a cape-like pronotum, a T-shaped mesosternite, and a rather typical broad metasternite. All of these structures are dark reddish brown and are thickly covered with dark, spiny setae. The differential characteristics of each segment are described in the introductory discussion on the appropriate structure and illustrated in Plates 1 and 2.

Abdomen is oval; five segments are visible ventrally. The first and second abdominal sclerites are the widest horizontally, and the first bears setae typical of all the abdominal sternites, in addition to the usual modifications found on that structure.

The first abdominal sternite is approximately 2 mm wide and is profusely clothed with large black setae which arise from a simple setal pit adjacent to the rim of a semi-circular area. Posterior margin of the sternite is straight, and coxal cavities are shallow with a definite oval indentation at the base of the median projection. The median anterior projection is sharply pointed, and the angle of slope is convex.

The range of setal variation is not great. The large dark setae are rather uniform throughout most segments. The base of the seta is consistently large. However, surrounding the seta is an area which varies in modification from simple undistinguished cuticle to a completely or partially outlined surface. The setal socket may be undistinguished except for a surrounding aura, or may be set in pronounced cuticular ridges. These are described in detail in the applicable sections of the key, and are shown on Plates 1 and 2.

Broad-horned Flour Beetle (*Gnathocerus cornutus*). **Plates 3 and 4.**

The head of this beetle is an orange-yellow. On the anterior-lateral margins of the head of the male is the expanded clypeus which appears as paired flanges. This structure is not found on the female. Both are illustrated in Plate 3.

The setae of the dorsal surface of both male and female are short, about 0.01–0.02 mm long and about 0.02 mm apart. On the anterior portion of the dorsal surface the setae arise from a circularly outlined area, whose margins may be indistinct, or from a

setal base only. The setal count is 16–26. The setae on the posterior portion of the dorsal surface appear to arise from the edge of an oval-outlined area whose margin opposite the setal base is indistinct or lacking (see Plate 3, Fig. f). Scattered between the setae are small inclusions about the size of setal sockets. The ventral and lateral surfaces of the male and female are similar in shape. The setae on these surfaces are somewhat longer, about 0.03 mm, and further apart, about 0.03 mm. They appear to arise from the top of irregular humped ridges on the ventral surface (see Plate 3, Fig. d), and from behind elongated protuberances or pedestals on the lateral surface (see Plate 3, Fig. e). Setal count is 16–36; count is highest on the lateral surface. There is a honeycomb cuticular pattern over the entire dorsal surface. The ventral surface shows a honeycomb pattern on the anterior portion and an irregular sculptured cuticular pattern posteriorly. Lateral surface also shows an irregular sculptured cuticular pattern. The eyes are "bow-shaped" and have somewhat large facets, 0.025 mm in diameter.

The general outline of the thoracic segments follow, in general, that typical of the majority studied. Differential characteristics are discussed in Part IIB, and photomicrographs are included in Plate 4.

There are five red-brown abdominal segments that overlap and taper posteriorly to an oval, rounded segment. First abdominal segment is about 1 mm wide and bears species-typical setae and setal bases. This segment is similar to that of the Confused and Rust-Red Flour Beetles. Their differentiation is discussed in Part IIC and illustrated in Plate 4, Fig. h, and in the key. The median projection of the abdominal sternite tapers with a convex angle to an apex. It is heavily sclerotized and the suture rim curls ventrally along the anterior margin. The setae arise from the margin of an incompletely rimmed cuticular area.

Cuticle and setae, as in all the insects studied, vary somewhat from one segment to another. Because of modifications, the head generally bears the greatest variety of patterns (as described above), while the abdomen is more consistent in morphology. The prothorax and mesosternite bear the transitional types; the metasternite cuticle and setae more closely resemble those of the abdomen.

Buffalo Carpet Beetle (*Anthrenus scrophulariae*). **Plates 5 and 6.**

Head is dark red-brown and large, measuring about 0.8 mm long and 0.7 mm wide. Dorsal surface is uniformly and completely covered with setae modified to form scales or feather-like structures. These are about 0.2 mm long and arise from a distinct setal puncture which may be completely or partially circled. The ventral surface of the head bears evenly spaced, medium brown, long spike-like setae. The bases are moderately large and are embedded (without indentation) in cuticular ridges (see Plate 5, Fig. b). The lateral head surface does not have as numerous setae as are on the dorsum and ventrum, but those present resemble the cuticular and setal structure of the ventral surface.

In the thorax the pronotum has a cape-like triangular shape similar to the Black Carpet Beetle. It is uniformly covered with feathery or scale-like setae. The sternal surface of the prothorax is small and is distinguished principally by a central ventral sclerotization which forms a thickened ridge down the center (see arrow, Plate 6, Fig. b).

The pleura and tergum of the mesothorax have few characteristics that distinguish the species, other than the unique setae. The mesosternite is typical of this family and forms a triangular bridge across the ventral surface of the mesothorax (see Plate 6, Fig. d). The metasternite is discussed in Part IIB and is illustrated on Plate 6, Fig. e. Principle characteristics are the shape of the median anterior and posterior margins and the median sternal groove. The typical setae are diagnostic.

Abdomen is five-segmented; all segments are similar in shape and cuticular morphology with the exception of the first and the fifth. First abdominal sternite is about 2.8 mm wide and has a slightly convex posterior margin. The coxal cavities are shallow, with an indentation next to the base of the median projection (see Plate 6, Fig. g). On either side of the median projection there are

strengthening ridges or sutures which extend from the bottom of the small indentations in the coxal cavity and diverge laterally almost to trisect the sternite.

Fifth abdominal sternite is modified by a thickened lateral ridge or groove which extends down each side of the sternites. This terminates abruptly in a small knob just short of the mid-line of the apical margin, leaving an indentation on the posterior surface of the abdomen. On this segment occur setae similar to those found on the ventral surface of the head.

Setae are distinctive because of their unusual scale-like shape. However, many of these scales are knocked off the fragment; therefore it may be necessary to use the setal bases for identification. In addition to the types previously described, there occur on some dermestids very long barbed setae. These are numerous but do not arise from the exterior surface of the exoskeleton, and may be found independent of a fragment. They may be 1 mm long; some are shown on the surface of the prothorax, Plate 6, Figs. a and c.

Cadelle Beetle (*Tenebroides mauritanicus*). **Plates 7 and 8.**

Head capsule of this large beetle is dark reddish brown. There is an area of darker pigmentation on the posterior area of the dorsal surface. Setae on the dorsal surface are short, about 0.01 mm long, and are thorn-like in appearance. They are far apart, about 0.07 mm, and have a count of 2–5; they arise from the center of a circular or oval area whose margins may be indistinct. Very numerous short spines occur between the setae. Large round inclusions are scattered over the dorsal surface; each has a small cleft in the margin (see Plate 7, Fig. d). Ventral surface is similar to the dorsal except that setae arise from a definite margin (see Plate 7, Fig. c). The setae on the lateral surface arise from the edge of a larger oval, circumscribed area whose margin opposite the setal base is indistinct; these setae are longer, about 0.02 mm long (see Plate 7, Fig. g). Cuticular pattern on head capsule is usually indistinct; it may have a "cross-hatch" appearance. Beneath the eyes on the lateral surface is a honeycomb pattern. A long trough originates on the ventral surface directly above the eyes and leads to the antennal sockets. This trough displays a straight ridged cuticular pattern and has no setae, although just above it are three very long setae, about 0.2 mm long. Eyes are shaped like half-moons and are fairly small; facets are about 0.025 mm in diameter.

Thoracic sclerites are typical; differential features of anterior and posterior margins and distinguishing projections are discussed in Part IIB and are illustrated in Plate 8. Setal pattern has a rather uniform appearance, even though there are various types: the large forked seta arising from a partially circumscribed area, small setae with no outline, and large, emarginated inclusions. Further descriptions may be found in the key.

Abdomen is five-segmented; all sternites except the first abdominal sternite are similar in outline and in cuticular and setal morphology. First sternite is about 3 mm wide; posterior margin is straight, and coxal cavities are of medium depth. Median anterior projection is a distinctive elongate formation with sharply-pointed tip (see Plate 8, Fig. h). Setae are similar to those found on the head and thorax; they have a rather consistent pattern of large forked setae arising from an incompletely rimmed area or very minute setae with no encircling margin. Inclusions may be found throughout the cuticle.

Cigarette Beetle (*Lasioderma serricorne*). **Plates 9 and 10.**

Head capsule is light reddish yellow. Setae on the dorsal surface and most of the lateral and ventral surfaces are about 0.02–0.04 mm long and about 0.01 mm apart; count is 60–90. These setae do not arise from a unit but from a setal base only (see Plate 9, Fig. b). Setae on the posterior area of the ventral surface are less than 0.01 mm long and about 0.02 mm apart. Fragments of this sclerite are difficult to identify because they are similar to those from the same area of the Drug-store Beetle. The only cuticular pattern on the head is a honeycomb on the anterior area of the ventral surface (see Plate 9, Fig. f). Eyes are oval, with facets about 0.015 mm diameter.

The thoracic structures of the Cigarette Beetle reflect the rounded, humped appearance of the whole adult. Sterna are greatly reduced, while the dorsum of the prothorax is expanded to form a semicircle about the body. The narrow bridge of the prothoracic sterna is indicated by an arrow on Plate 10, Fig. c. Mesosternite, tergite, and pleuron are considerably reduced and altered, as shown in Plate 10, Figs. d, e, f. Metasternite exhibits the ventral reduction in size; it is the only metasternite which has a very narrow anterior-posterior dimension.

The pattern of reduction is retained in the five-segmented abdomen and the differential morphology discussed in Part IIC and illustrated in Plate 10, Fig. h.

First abdominal sternite is similar to that of the Drug-store Beetle but is slightly larger, 1.5 mm wide. Coxal cavities are shallow; median projection is heavily sclerotized for about $\frac{1}{3}$ its depth. Setae are sparse, are concentrated mesally, and arise from simple setal pits.

Setal variety and cuticular pattern are limited; in most fragments the setae originate from setal bases with no surrounding margin. On the metasternite the seta appears with an incompleted concentric rim (see Plate 10, Fig. g).

Confused and Rust-Red Flour Beetles (*Tribolium confusum or T. castaneum*). Plates 11, 12, 13, 14.

Head capsules of these species are quite similar and are orange-yellow. Short setae on the dorsal surface are about 0.01 mm long and about 0.02 mm apart. These setae arise from the center of a concentrically rimmed area whose margins may be indistinct. Setal count is 20–28. There are small inclusions about 0.04 mm apart between the setae (see Plate 12, Fig. d.) Setae on the ventral and lateral surfaces are similar in size but farther apart, about 0.03 mm; they appear to arise from curved indentations (see Plate 12, Fig. f). Cuticular pattern is distinctly but lightly honeycombed over most of the surface of the head. Eyes are "bow-shaped," and wrap around the head capsule. The Rust-Red Flour Beetle's eyes are larger and extend farther around the head capsule than those of the Confused Flour Beetle. Facets on the Confused Flour Beetle are clearly visible dorsally (see Plate 11, Fig. g); facets are large, about 0.025 mm in diameter.

Thoracic sclerites are typical; description of gross morphology and differentiation from related groups is discussed in Part IIB. Further differentiation may be made by use of cuticular and setal characteristics presented in the key. Photomicrographs of the principle sclerites are shown on Plates 13 and 14.

Abdomen of the Confused and Rust-Red Flour Beetles are five-segmented; all the segments, except the first and fifth, are similar in general outline. First abdominal sternite of the two species is similar and bears more distinctive modifications than does the fifth; it is reddish brown and about 1 mm wide. Coxal cavities are moderately deep, and the slope to the median-sternal projection is convex; the posterior margin is almost straight. This sternite is very similar to that of the Broad-horned Flour Beetle but has less sclerotization on its anterior margin. Differentiation is presented in Part IIC. Setae near the center and adjacent to the median projection arise from simple setal pits; setae near the lateral margins arise near the center of an incompletely outlined surface.

The fifth abdominal sternite has a knob on the lateral margin of the sclerite just posterior to the overlapping fourth abdominal sternite. The third and fourth sternites have projections also, but the cuticle appears projected laterally because of the posterior overlapping of the sternites, in contrast to the fifth on which the knobs occur independently of the articulation point on the posterior margin of the fourth. It is possible that this outgrowth is the result of reduction in abdominal segmentation and remains as the only visible evidence of a former sixth segment.

Setae, setal bases, and cuticular pattern of these species are simple and rather consistent throughout all segments. The cuticle has a light mosaic or honeycomb pattern, which appears more prominently in certain areas of the head. There are large, emarginated, refractile inclusions, discussed in other sec-

tions of this study, scattered in the cuticle. The setae and surrounding cuticle retain a basic pattern of symmetry throughout most fragments, and the setae themselves, short and rigid, form a definite, yet simple, arrangement. Further details for identification are presented in the key and on Plates 11, 12, 13, and 14.

Dried-Fruit Beetle (*Carpophilus hemipterus*). **Plates 15 and 16.**

Head capsule is light to dark dull brown or grey. Setae on the anterior portion of the dorsal surface are broad and long, about 0.06–0.07 mm long, and about 0.04 mm apart; setal count is 8–12. Setae arise from the edge of a circularly outlined area whose margin opposite the setal base is indistinct. There may be a minute puncture in this cuticular area, and small holes about the size of a setal base are scattered between the setae (see Plate 15, Fig. c; Plate 16, Figs. a, e). Setae on the posterior portion of the dorsal surface are shorter, about 0.02–0.03 mm long and about 0.03 mm apart; setal count is 12–18. They appear from the edge of a large oval or irregularly circumscribed area whose margin opposite the setal base is lacking or indistinct (see Plate 15, Fig. d). Setae on the ventral and lateral surfaces vary greatly in size; some on the anterior portion of the ventral surface are 0.2 mm long, and others are only 0.02 mm long. These setae arise from the edge of a large irregular area whose outlined margin is incomplete (see Plate 15, Fig. g). The only distinct cuticular pattern on the head capsule of this insect is on the lateral surface under the eye where there is a honeycombed pattern, and on the anterior portion of the ventral surface where there is an irregular sculptured pattern. Eyes are small and round; facets are about 0.015 mm in diameter.

The thoracic segments have been discussed in the differential study of the various species, Part IIB. They and their cuticular detail are illustrated on Plate 16 and described in the appropriate sections of the key.

The five abdominal segments of the Dried-Fruit Beetle are slightly modified. The first abdominal sternite is a light yellow or yellowish brown sclerite about 1.8 mm wide. The median anterior projection is plateau-like and shows no subsurface structures. The coxal cavities are shallow and the posterior margin straight. The sternite is uniformly covered with long setae arising from an incompletely outlined cuticular area. The second and third abdominal segments are narrow anterior-posteriorly and telescope over the succeeding segment. The fourth segment widens, and the fifth is one-half deep as wide. The fifth segment is joined laterally to the dorsal sclerite which is not covered by the elytra. The terga extends posteriorly beyond the sterna. At the base of the sterna is a modified sixth segment covering an invagination cavity in which the genitalia are retracted.

The cuticular pattern of the Dried-Fruit Beetle is undistinguished except for inclusions, which are frequent on the head and thorax but less frequent on the abdominal segments, and occasional mosaic or honeycomb patterns. The depressions surrounding the setae vary from being completely rimmed with a central setal base to incompletely rimmed with an eccentric setal base. The setae on the dorsal exposed abdominal sclerites are the heaviest found. Most of the setae of this species are broad-based long setae, as illustrated in Plates 15 and 16. The differential characteristics of the various setae used for separation from other species are detailed in the accompanying key.

Drug-store Beetle (*Stegobium paniceum*). **Plates 17 and 18.**

Head capsule is reddish brown. There are two different types of setae on the dorsal surface; the longer of these thin setae are about 0.07–0.08 mm long and about 0.04 mm apart. Setal count is 10–16. When the microscope is focused slightly below the setal bases, these setae appear to arise from the side of a small darkened protuberance. The shorter setae are about 0.01–0.02 mm long and about 0.01 mm apart; setal count is 70–100 (see Plate 17, Fig. c); these setae appear to arise only from a setal base. The setae on the ventral and lateral surfaces are about 0.02 mm long and about 0.02–0.03 mm apart; they rise from the base of smooth semicircular slits (see Plate 17, Fig. e). Setal

count is 16–26. Further descriptive detail may be found in the key. Head capsule does not show any cuticular pattern; eyes are oval; facets are 0.015 mm in diameter.

The thoracic structure follows the general pattern of other closely related species in that the dorsal areas of the prothorax are large, and the ventral sternite is greatly reduced. The meso- and metasternite are not modified so extensively, and present a typical sternite structure discussed and differentiated in Part IIB. The setal morphology and cuticular pattern on the thorax follow the variety of types and bases which occur on the head capsule, although there is less modification and variety found on any one fragment. The prothorax may exhibit an expanded base "rosette" type of cuticular area about the setae, which is described in the key and seen on Plate 18, Fig. b, but is not found on the mesosternite and metasternite. Fragments of these sclerites are covered with many fine setae with no circular outlines and fewer large setae which arise from cuticular areas with incomplete outlines.

Abdomen is five-segmented and light reddish-brown. Only the first abdominal sternite is modified and distinctive; other sterna bear typical setae and non-distinguished cuticle that may be differentiated by use of the key. The first abdominal sternite is about 1 mm wide. The median projection appears to he heavily sclerotized for about $\frac{3}{4}$ its depth; there is an inconspicuous subsurface formation which bisects the medial projection laterally. The coxal cavities are rather shallow, and the posterior margin is almost straight except for curving near the lateral edges. The setae arise from a simple setal pit without a surrounding unit. Discussion of differential morphology may be found in Part IIB and is illustrated on Plates 17 and 18.

Flat Grain Beetle (*Laemophloeus pusillus*). **Plates 19 and 20.**

Head capsule of this small beetle is light orange-yellow. Dorsal surface is covered with long, thin setae about 0.03–0.04 mm long and about 0.02 mm apart; these setae arise from the edge of a small oval-rimmed cuticular area whose margin opposite the setae is indistinct (see Plate 19, Fig. b). On the ventral surface only a few setae arise from rimmed cuticular areas; most of the setae arise from cuticle circled by indistinct margins, from an unadorned setal base, or from small depressions. Scattered at random over most of the surface of the head capsule and between the setae are small inclusions about the size of the setal bases, no cuticular pattern except on the lateral surface directly under the eye; here the cuticular pattern is a mixture of honeycomb and ridged pattern (see Plate 19, Fig. f). Several prominent sutures on this head capsule may be used for differentiation, as discussed in Part IIA. Eyes are round and small, facets are about 0.01 mm in diameter.

Thorax is much smaller than that of other species, although the general morphology is similar to several. Little modification from the typical is seen in these sclerites; differential characteristics and morphology are presented in Part IIB and illustrated on Plate 20. Cuticular pattern is undistinguished except by the presence of inclusions and the long setae which arise primarily from incompletely rimmed cuticular areas. Angle of projection of setae from the metasternite is unusual: the setae that occur along the lateral areas extend laterally rather than posteriorly (see Plate 20, Fig. c).

Abdomen is distinguished primarily by the structure of the first abdominal sternite. The cuticular and setal morphology on all segments is uniform. First abdominal sternite is 0.5 mm wide and straw-colored; it has a subsurface formation that bisects the plateau-like median projection and lies parallel to the anterior margin (see Plate 20, Fig. e). Coxal cavities are indented; posterior margin is almost straight or only slightly concave. Setae are long and fine, and usually rise from simple setal bases. Differentiation of this sternite and cuticular and setal descriptions are presented in Part IIC and in the key.

Foreign Grain Beetle (*Cathartus advena*). **Plates 21, 22, 23.**

Head capsule of this small beetle is light orange-yellow. Setae on the anterior portion of the dorsal surface are long and thin, about 0.03–0.04 mm long; setae on the posterior

portion of the dorsal surface are shorter, about 0.01 mm long; setae on the ventral surface are about 0.02–0.04 mm long. All setae are about 0.02 mm apart; setal count is 20–28. These setae, with the exception of those on the posterior portion of the dorsal surface which rise from the top of straight ridges (see Plate 21, Fig. d), arise directly from a setal base. Very close to each seta, about the diameter of a setal base distant, is a small puncture. Over most of the dorsal surface there is a honeycomb pattern augmented by a scattering of irregularly-shaped, slightly depressed or translucent areas (see Plate 21, Fig. b). Cuticular pattern on the anterior portion of the ventral surface is an irregularly sculptured pattern. Posterior portions of the ventral and lateral surfaces are devoid of setae. Eyes are round and fairly large with facets about 0.02 mm.

Thorax and abdomen belong to the typical majority studied. Differential characters are discussed in Part IIB and C and illustrated on Plates 22 and 23. It may be difficult to separate this species from the Square-necked Grain Beetle; in some instances the absolute distinction cannot be made.

The most typical cuticular pattern found on most fragments is the pair of setal bases and the lightly rimmed cuticle surrounding them. Other patterns, principally on the head capsule, do not appear to any extent on the body regions. Details of differentiation of fragments, when possible, are presented in the key.

Larder Beetle (*Dermestes lardarius*). **Plates 24 and 25.**

One of the largest dermestids infesting stored products. Head capsule is dark brown; dorsal surface is covered with long, dark, broad setae about 0.07–0.12 mm long. Setae are about 0.02–0.03 mm apart; setal count is 12–16. Setae arise from the center of a circularly outlined area of cuticle whose margins are indistinct (see Plate 24, Fig. e, f). Setae on the ventral and lateral surfaces are shorter, about 0.03–0.06 mm long, and about 0.02–0.04 mm apart; setal count has a wide range, 8–24. Some setae about 0.10 mm long occur on the extreme anterior area of the ventral surface. When the microscope is focused slightly below the base of the setae, the setae on the ventral and lateral surfaces appear to arise from small darkened areas or ridges (see Plate 24, Fig. g). A "rippled" cuticular pattern is also seen over the entire head capsule surface when the microscope is focused in the above manner (see Plate 24, Fig. f). On the dorsal surface this cuticular pattern has a slightly more cellular or honeycomb appearance. Eyes are round and large, with facets about 0.015 mm in diameter.

Thoracic segments are large in proportion to the size of the whole insect. Prothorax is reduced ventrally (common in most dermestids) as shown on Plate 25, Fig. a. Mesosternite is similar in shape to that of the Black Carpet Beetle (see Plate 2, Fig. b). The anterior margin is depressed, and there is a median ridge projecting ventrally which extends to the base of the antecostal suture. The antecostal suture and apodome is U-shaped, separating the lateral poststernal arms and the sternellum from the eusternum. Metasternite of the Larder Beetle is differentiated in some detail in Part IIB. Cuticular and setal morphology is rather consistent on these structures, of the types described on the head capsule, although the setae may be larger.

The abdomen is distinguished particularly by the first abdominal sternite which is dark reddish-brown and about 4 mm wide; differential characteristics of the abdominal spina are discussed in Part IIC. The remaining four sternites are similar in cuticular and setal morphology and in general outline; they overlap posteriorly and decrease in size toward the terminal segment. Cuticular and setal variation is limited and conforms to the above descriptions, the key, and the illustrations on Plate 25, Figs. b, c, d, e.

Lesser Grain Borer (*Rhyzopertha dominica*). **Plates 26 and 27.**

Head capsule is orange-yellow to brown; over most of it are small setae about 0.01 mm long and about 0.02–0.03 mm apart. On the extreme anterior area of the head capsule there are some long setae 0.02–0.20 mm long, about 0.01–0.02 mm apart. These setae arise only from a setal base; setal

count is 6–12. There is a very characteristic cuticular pattern made up of rounded irregular areas crossed by several ridges resulting from intercuticular arrangement (see Plate 26) over most of the ventral surface and a portion of the anterior area of the dorsal and lateral surfaces. Posterior portion of the dorsal and lateral surfaces exhibits a heavily "veined" cuticular pattern (see Plate 26, Fig. b). Both the ventral and dorsal surfaces exhibit prominent sutures which are discussed in Part IIA. Eyes are round, with facets about 0.01 mm in diameter.

Dorsal surface of thorax is heavily sclerotized, with ridged cuticle. Prothorax dorsal area is large and rounded, extending over the deflected head; anterior margin is scalloped by ridges, shown on Plate 27, Fig. b. Thoracic sternites are quite undifferentiated and are discussed in Part IIB. Cuticular arrangement on the sternal areas is not as unique as on the head and prothorax; it may be seen on Plate 27 and is described in the key.

First abdominal sternite is the only grossly distinctive segment of five abdominal segments. Medial anterior projection is rather heavily sclerotized and is knob-like at the tip with a subsurface apex. The entire structure is very simple and is differentiated as shown in Part IIB and illustrated on Plate 27, Fig. f.

Mealworm (*Tenebrio molitor or T. obscurus*). **Plates 28, 29, 30.**

One of the largest beetles infesting stored products. Head capsule is orange-brown to dark brown. Dorsal surface and anterior area of the ventral surface are covered with thin, small setae about 0.01 mm long and about 0.05 mm apart; setal count is 3–6. Setae arise from the center of a circularly rimmed cuticle area with indistinct margins. Near the base of the setae are several small punctures (see Plate 28, Fig. c). Small inclusions about the size of setal bases are scattered between these setal areas. Setae on the ventral and lateral surfaces are longer, about 0.02–0.03 mm long and about 0.04 mm apart; setal count is 3–6. These setae appear to rise from the apex of sclerotized protuberances, mounds, or ridges (see Plate 28, Fig. b, f). Setae around the eyes are heavy and thornlike. Most of the surface of the head capsule has a honeycomb cuticular pattern. Gular area has no setae, but there are groups of small spines scattered over this area. Eye is "bow-shaped"; facets are large, about 0.04 mm in diameter.

Thorax and abdomen are dark, reddish-brown, and similar (except for large size) to the majority of the species studied. Type of cuticular and setal morphology found on each sclerite is uniform and consistent, as found on head capsule. The fragments are illustrated on Plates 28, 29, and 30, and differentiated in Parts IIB, IIC, and the key.

Rice and Granary Weevils (*Sitophilus oryzae and S. granarius*). **Plates 31 and 32.**

Head capsules are dark reddish brown. Both head capsules are similar in size and in their characteristic snouted shape (see Plate 31, Figs. a, b, e). Head capsules are covered with small tasseled or club-like setae about 0.04–0.05 mm apart; there are a few much longer setae, about 0.04 mm long, around the eyes on the dorsal surface; setal count is 5–12. Pronounced cuticular pattern of uniformly spaced ridges which occasionally converge, or equidistant scalloped ridges (see Plate 31, Figs. c, d, f), extends over the entire head capsule. Eyes are "teardrop" in shape and wrap almost completely around the head capsule; facets are small, about 0.01 mm in diameter.

Principle thoracic sclerites are large. They are discussed in Part IIB. The mesally bisected meta-poststernum is unusual; it has a small hooked projection extending laterally, shown in the fragment, Plate 32, Fig. f. Cuticle on the thoracic segments retains the characteristic ridged and cross-hatched patterns.

Abdomen is distinctive in possessing only four discernible segments; first and fourth are the largest, second and third are very narrow undifferentiated sclerites. The first sternite bears the distinctive knobbed spina, discussed in Part IIB and illustrated on Plate 32, Figs. c, e. Sternite of the fourth segment is characterized primarily by large size. Tergum of the fourth segment is distinctive; this area is not completely covered by the elytra and thus is sclerotized and

bears the typical cuticular and setal patterns. A central, relatively broad depression extending the anterior-posterior length further differentiates this sclerite. Cuticular and setal development is not extensive on this depression; but very large typically tasseled setae do occur near the posterior margin of the tergum.

Sawtoothed Grain Beetle (*Oryzaephilus surinamensis*). **Plates 33 and 34.**

Head capsule is dark reddish brown. Setae are long, about 0.04–0.05 mm, on all but the extreme posterior portions, and are about 0.04 mm apart; setal count is 12–20. Setae appear to arise from the edge of an irregularly outlined area of cuticle with margins equally distinct throughout. Margin is usually slightly indented at the setal base, and the area may contain one or two small holes (see Plate 33, Fig. b; Plate 34, Fig. b, d). Setae on the extreme posterior portions are about 0.02 mm long and about 0.03 mm apart; setal count is 16–24; setae appear to rise from the top of straight ridges. No setae are on the posterior portion of the ventral surface. There is a honeycomb cuticular pattern over the whole head capsule. Eyes are small and round, with facets about 0.02 mm in diameter.

Thoracic sclerites show typical morphology of the majority of species. The major exception is the lateral surface of the prothorax which is characterized by saw-edged protuberances (see Plate 34, Fig. a). Differentiation of the various sclerites is presented in Parts IIB and C; illustrations are on Plates 34.

Five very similar segments are on the abdomen. The principle modifications occur on the first and fifth sternites; first sternite is illustrated on Plate 34 and differentiated in Part IIC. Fifth sternite is a simple structure, more oval than rectangular; it closely resembles a football. Cuticular and setal morphology, shown on Plate 34, Figs. i and j, is uniform throughout the abdomen.

Square-Necked Grain Beetle (*Cathartus quadricollis*). **Plates 35, 36, 37.**

As members of the same genus, the Square-necked Grain Beetle is very similar to the Foreign Grain Beetle. The Square-necked Grain Beetle is slightly larger, about 2–3 mm long. Head is orange-brown; the setae are long and thin, about 0.04–0.08 mm over the dorsal and ventral surfaces and about 0.02–0.04 mm apart. Setal base is visible and usually accompanied by an adjacent puncture approximately the diameter of a setae distant on most of the head surface, except on the posterior dorsal area; here the prothorax overlaps anteriorly, and the setae appear from ridges. No setae are on the ventral and lateral cuticular areas overlapped by the prothorax. Eyes are prominent, project laterally, and extend around the head onto the ventral surface; facets are about 0.02 mm in diameter.

Thoracic sclerites show significant variation in morphology from the Foreign Grain Beetle; differentiation is presented in Part IIB and is illustrated on Plates 36 and 37. Cuticle of Square-necked Grain Beetle may be honeycombed in some thoracic areas and also exhibit irregularly edged translucent areas, but general observations indicate that partially concentric outlines occur more frequently on the thoracic sclerites of this species than the Foreign Grain Beetle.

Abdomen is five-segmented and undistinguished except for the first abdominal sternite, the differentiation of which is discussed in Part IIC and illustrated on Plate 37. Over-all cuticular patterns and setal morphology are as described above, illustrated in Plates 35, 36, and 37, and distinguished in the key.

IV. Key of Cuticular and Setal Characteristics

1	Seta or setal scar present	2
1′	No seta or setal scar present	3
2	Setae originate from area of cuticle circularly outlined, smoothly or irregularly, partially or completely	4
2′	Setae originate from area of cuticle not circularly outlined either smoothly or irregularly, partially or completely	5

3	Pronounced cuticular pattern	30
3′	No pronounced cuticular pattern	Unidentifiable
4	Outline completely encircles setal base	6
4′	Outline does not completely encircle setal base; setal base occurs near margin or arc	7
5	Setal base on pedestal or rounded cuticular protuberance	8
5′	Setal base in groove or ridges	9
5″	Setal base in undistinguished cuticle	31
6	Setal base in center of circularly rimmed area	10
6′	Setal base near margin of circularly rimmed area	11
7	Cuticle heavily striated or cross-hatched; setae distally enlarged or may be forked	22
7′	Cuticle not heavily striated	12
8	Dot-like, very refractile setal bases predominantly on large rounded protuberances. Some protuberances may appear ridged or rasp-like. Dark cuticle with numerous inclusions scattered throughout. Cuticle in areas of few setae has pebbled appearance. Setal count 6–12 (see Plate 26, Fig. b, and Plate 27, Figs. a and b). *Lesser Grain Borer*	
8′	Protuberances small, few refractile inclusions, dark cuticle with uniform pattern, no heavy ridging. Setal count 3–6 (see Plate 28, Fig. b, and Plate 29, Fig. e). *Mealworm*	
8″	Pedestal bears dark, rounded setal base with short, broad-based setae; cuticle light. Pedestals may give irregular ridged effect. Setal count 16–36 (see Plate 3, Figs. d, e). *Broad-horned Flour Beetle*	
8‴	Small protuberances with irregular refractile margins bearing long setae. Focusing beneath surface of cuticle, the protuberance and base appear "rosette" shaped. Other small numerous setae occur with or without refractile setal bases; no circular margin. Setal count 10–16 on large setae, about 100 on smaller (see Plate 18, Fig. b). *Drug-store Beetle*	
9	Large setal bases located on distinct ridges (dark cuticle) with few marked depressions for setal base. Setae are long and thick. Setal count 18–26 (see Plate 1, Figs. d, f). *Black Carpet Beetle;* or ventral surface of head capsule of *Buffalo Carpet Beetle*	
9′	Setal bases in ridge with angular indentation for base. Setae very large, flat, tapered at tip. Setal count 8–24 (see Plate 24, Figs. f, g). *Larder Beetle*	
9″	Setae based in ridges, moderately heavy. Some fan or scale-like setae may be visible. *Buffalo Carpet Beetle*, ventral surface	
10	More than one setal base or puncture within circumscribed area	13
10′	Only one setal base or puncture within circumscribed area	14
11	Circular margin smooth and even	15
11′	Circular margin irregular with paired enclosed punctures in addition to other cuticular inclusions not associated with setae or setal bases. Setal count 12–24 (see Plate 33, Fig. b; Plate 34, Fig. b, d). *Sawtoothed Grain Beetle*	
12	Inclusions in cuticle (rounded, refractile areas, may be non-structural) are scattered among encompassed setal bases; inclusion may be uniform and distinct or irregular and infrequent	16

12′ No inclusions in cuticle other than encompassed setal bases and setae 17

13 Cuticle forms parallel ridges, may be heavily cross-hatched. More than one setae may appear, giving tasseled effect. Setal count 5–12 (see Plate 32, Figs. a, e).
Rice or Granary Weevil

13′ Cuticle thick and usually dark, has fine-grained cross-hatch. Three or more punctures may be present within circular area with one or more fine setae. Setal count 3–6 (see Plate 28, Fig. c; Plate 29, Figs. f, g, h; Plate 30, Figs. d, f, i).
Mealworm

13″ Cuticle not ridged or dark. Fine honeycomb pattern apparent. Three punctures may appear within circumscribed area. Large, emarginated inclusions present. Setal count 20–28 (see Plate 12, Fig. d).
Confused or Rust-Red Flour Beetle

14 Cuticle forms parallel ridges, may be heavily cross-hatched. Setae may be distally enlarged or plume-like. Setal count 5–12 (see Plate 32, Figs. a, e).
Rice or Granary Weevil

14′ Dark setal base outlined by smooth round margin, may give "bulls-eye" appearance. Cuticle moderately light. Setae are large, flattened, uniformly and distinctly spaced. Setal count 8–12 (see Plate 15, Fig. a; Plate 16, Figs. a, e).
Dried-Fruit Beetle

14″ Setal base large but not distinctly outlined. Cuticle dark, may be heavily ridged. Setae large, dark, uniform, and spike-like. Setal count 14–18 (see Plate 1, Figs. a, b, c).
Black Carpet Beetle

14‴ Cuticle not heavily ridged. Setal bases large. Setae very large, flattened, taper to a point. Setal count 10–20 (see Plate 24, Figs. e, f; Plate 25, Fig. b).
Larder Beetle

15 Numerous inclusions scattered in cuticle independent of encompassed setal bases and setae. Margin outlining setal base is egg-shaped. Setal count 30–40 (see Plate 19, Figs. b, d).
Flat Grain Beetle

15′ Few, if any, inclusions occur independently in the cuticle 18

16 Predominately uni-punctured setal bases within encircled area 19

16′ Predominately more than one puncture within encircled area 20

17 Circumference about setal base is simple elliptical U-shaped or V-shaped arc, partially complete; setae thorn-like 21

17′ Circumference about setal base appears circular, almost complete, with heavy setal puncture on margin or adjacent to it. Setae are scaly or fan-like (see Plate 5, Fig. e; Plate 6, Figs, c, f, g).
Buffalo Carpet Beetle

18 Cuticle dark, setal base large and distinct, may have surrounding aura. Setae are large and fan-like or scale-like (see Plate 5, Fig. f; Plate 6, Fig. c).
Buffalo Carpet Beetle

18′ Cuticle moderately light; setal base distinct (sometimes dark) with no surrounding aura. Margin around base smooth and even. Setae large and flat, taper to a point. Setal count 8–12 (see Plate 15, Fig. c; Plate 16, Figs. a, e).
Dried-Fruit Beetle

19 Inclusions in cuticle are indistinct. Two distinct types of setae bases and setae arise from undistinguished cuticle. In areas where semi-circular margin of irregular and varying size surrounds large setae, the small setae, having no margin and with or without a refractile base, are less numerous than in areas where the long setae originate from a protuberance (see 8‴). Setal count: large,

10–16; small, about 100 (see Plate 17, Figs. c, e; Plate 18, Figs. b, f, g, h, i).
Drug-store Beetle

19′ Inclusions in cuticle distinct, uniform in size, and usually occur in regular pattern 23

20 Paired setal punctures; only one setae may be observed 24

20′ More than two setal punctures or setae present. Three predominate. Cuticle is dark with fine cross-hatching which may give a textured look to surface or, less commonly, a mosaic pattern. Setal count 3–6 (see Plate 28, Fig. c; Plate 29, Figs. f, g, h).
Mealworm

21 Setal puncture appears deep, almost bulb-like, often extends beneath the encompassing margin. Margin may be indented in area of setal origin. Setae are not much longer than diameter of circumscribed area. Setal count 16–26 (see Plate 3, Fig. f; Plate 4, Figs. d, i).
Broad-horned Flour Beetle

21′ Setal punctures not as deep; setae predominately longer than diameter of circumscribed arc 25

22 Cuticle heavily striated and/or cross-hatched. More than one setal puncture may occur centrally. Setae tassel-shaped. Setal count 5–12 (see Plate 31, Figs. d, f; Plate 32, Fig. e).
Rice or Granary Weevil

22′ Cuticle dark, granular, finely cross-hatched. Many small setal bases and inclusions amidst large setae which may be pronged or forked within semi-circular margins. Over-all pattern rather uniform. Large setae, count 2–5 (see Plate 7, Figs. c. d, f, g; Plate 8, Figs. a, c, e, g).
Cadelle Beetle

23 Setal base deep, may appear bulb-shaped beneath the semi-circular margin. Some short setae occur without marginal arc. Most setae limited in length to the diameter of surrounding arc. Setal count 16–36 (see Plate 3, Figs. f, h; Plate 4, Figs. a, d, i).
Broad-horned Flour Beetle

23′ Setal bases not deep 29

24 Margin about paired punctures usually distinct but irregular. Long setae. Setal count 12–24 (see Plate 33, Figs. a, b, c; Plate 34, Figs. b, d, j).
Sawthoothed Grain Beetle

24′ Margin around paired punctures usually indistinct. Cuticle may show pebbled surface pattern. Setae somewhat shorter. Setal count 20–28 (see Plate 21, Figs. b, f; Plate 23, Figs. a, c, d).
Foreign Grain Beetle
(Or see Plate 35, Figs, b, c; Plate 36, Fig. c; Plate 37, Figs. b, c.)
Square-necked Grain Beetle

25 More than one puncture in cuticle in area of setal origin. One may be smaller than the other 24

25′ Only one puncture in cuticle in area of setal origin 26

26 Dark cuticle; very large dark setae 27

26′ Light cuticle; setae not so large, dark or heavy 28

27 Considerable ridging of dark cuticle, sometimes resulting in an ill-defined margin. Setal bases are heavy and may have lighter aura about them; setae are uniformly large and spike-like. Setal count 18–26 (see Plate 1, Fig. c; Plate 2, Fig. d, e, f).
Black Carpet Beetle

27′ Ridging not heavy. Some striation evident. Setal base small in proportion to

size of large setae. Setal count 10–20 (see Plate 24, Figs. c, e, f; Plate 25, Figs. b, c, d).
Larder Beetle

28 Margin about setal base well-defined; setae are uniformly spaced, broad, and of even length. Cuticle moderately light. Setal count 8–18 (see Plate 15, Figs. c, d, g; Plate 16, Figs. a, i).
Dried-Fruit Beetle

28′ Circumferential arc may be ill-defined. Cuticle undistinguished. Setal bases are irregularly spaced. Setae are numerous and of uneven length. Setal count 60–90 (see Plate 9, Fig. c; Plate 10, Figs. b, g, h).
Cigarette Beetle

29 Margins about setal base distinct. Setae are uniformly spaced, longer than diameter of surrounding arc, broad at the base, and tapered at the tip. Inclusions are infrequent. Setal count 8–18 (see plate 15, Figs. c, d, g; Plate 16, Figs. a, c, d, f, g, h, i).
Dried-Fruit Beetle

29′ Margins about setal base are moderately distinct and uniform in appearance. Inclusions in cuticle are frequent, large, and of uniform occurrence; high magnification reveals an emargination in the outline. Setae are rigid in appearance and project perpendicularly from the base of the arc about them; the length varies on different body regions. A mosaic pattern can be observed in the cuticle. Setae count 20–28 (see Plate 11, Figs. b, d, f; Plate 12, Figs. b, d, f; Plate 14, Figs. b, d, f).
Rust-Red or Confused Flour Beetle

29″ Setal bases haphazard in appearance: some are well defined; others indistinct. Numerous very small refractile inclusions. Setae are fine, longer than diameter of marginal arc, and appear flexible in their projection from the base of the margin. Cuticle is light in color. All structures comparatively small. Setal count 30–40 (see Plate 20, Figs. b, c, d).
Flat Grain Beetle

30 Cuticular pattern gives irregular pebbled look (see Plate 26, Figs. a, b, d, e, f).
Lesser Grain Borer

30′ Longitudinal striations, usually parallel, may be scalloped at the top; may be cross-hatched (see Plate 31, Fig. f).
Rice or Granary Weevil

30″ Fine cross-hatching with many scattered inclusions giving scotch grain leather effect (see Plate 7, Fig. f).
Cadelle Beetle

31 Setal base area widely spaced with punctures grouped in pairs. No inclusions. Setae are long. Setal count 20–28 (see Plate 23, Figs. a, d; Plate 35, Figs. b, c; Plate 36, Figs. b, c; Plate 37, Figs. b, c).
Foreign Grain Beetle; or *Square-necked Grain Beetle*

31′ Setal bases not characterized by paired punctures 32

32 Setal bases numerous. Cuticle moderately light. Setal long, of irregular length. Setal count 60–90 (see Plate 10, Fig. b).
Cigarette Beetle

32′ Cuticle dark. Setal base large; may have aura surrounding it. Setae spike-like. Possibly ridging. Setal count 14–26 (see Plate 1, Fig. c; Plate 2, Figs. d, e, f).
Black Carpet Beetle

32″ Cuticle dark. Setal base small in proportion to size of large blade-like setae. Some striation may be evident. Setal count 8–24 (see Plate 24, Figs. e, f; and Plate 25, Figs. b, c, d, e).
Larder Beetle

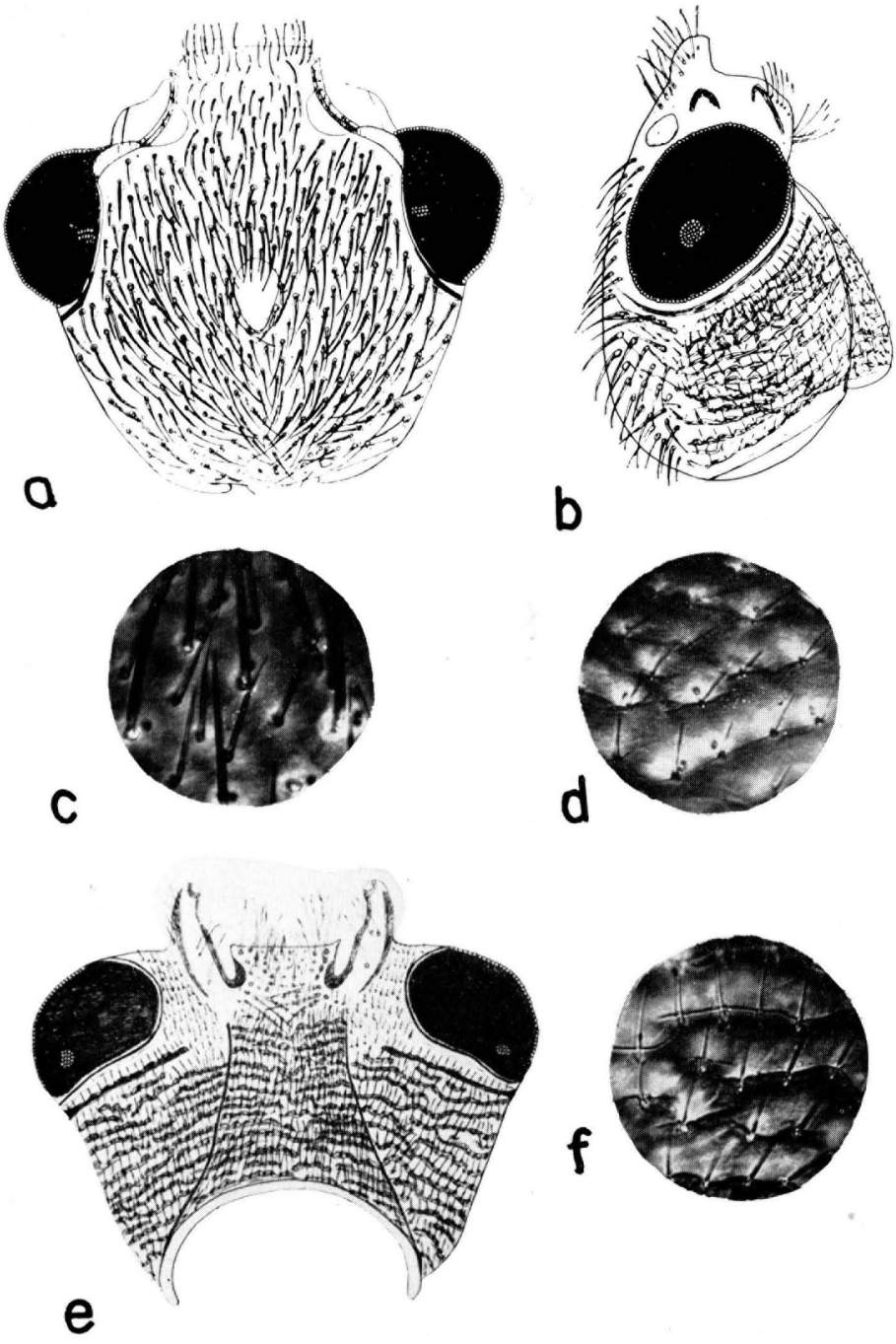

Plate 1. Black Carpet Beetle (*Attagenus piceus*), Head.

Fig. a: Dorsal view (75×). Fig. b: Lateral view (75×). Fig. c: Setae, dorsal surface (300×). Fig. d: Setae and cuticular ridging (300×). Fig. e: Ventral view (75×). Fig. f: Setae and cuticular ridging, ventral surface (300×).

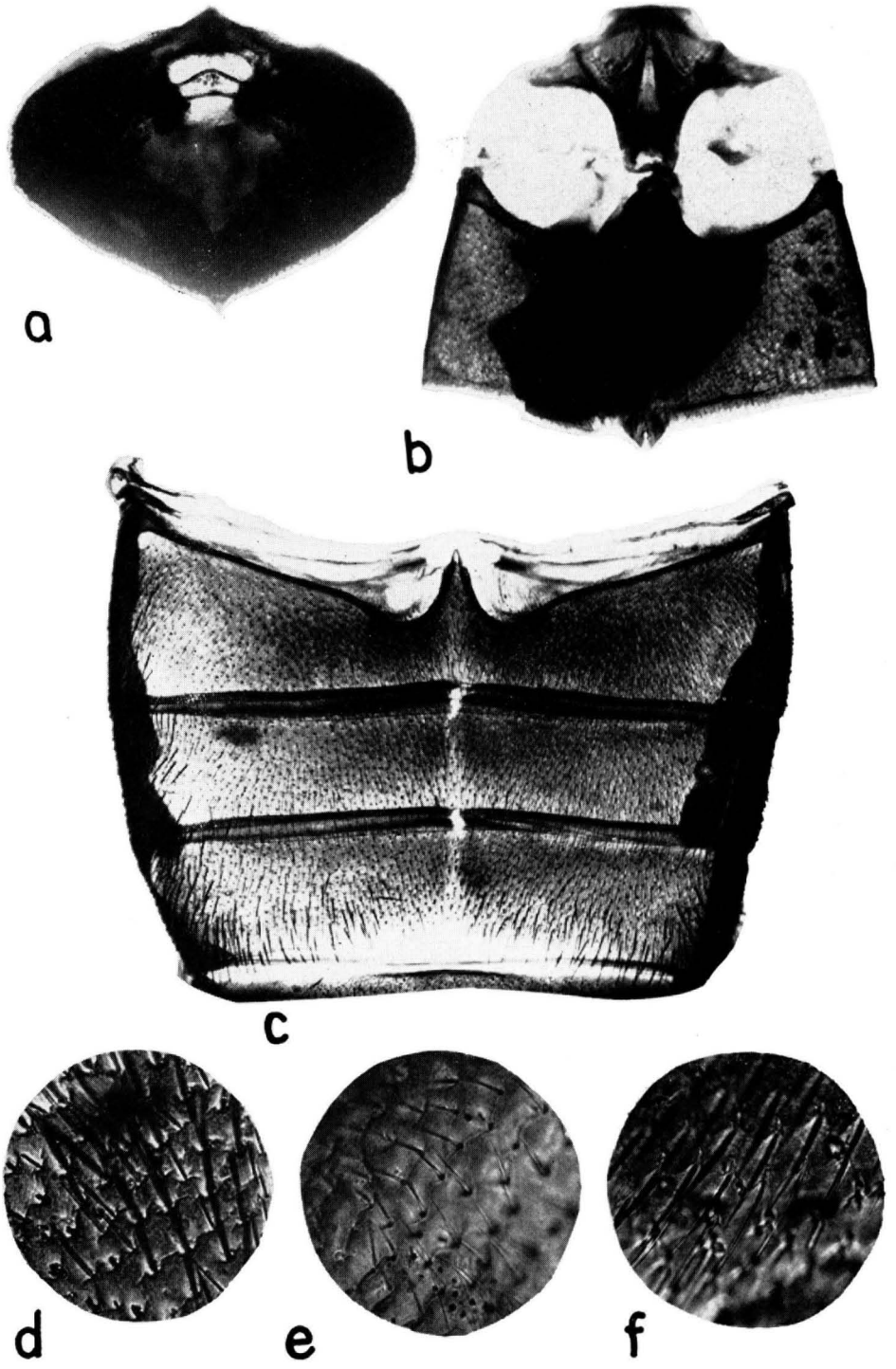

Plate 2. Black Carpet Beetle (continued).

Fig. a: Prothorax, ventro-posterior view (25×). Fig. b: Mesosternite and metasternite (40×). Fig. c: First, second, and third abdominal sternites (40×). Fig. d: Prothoracic setae and cuticular ridging (210×). Fig. e: Metapleuron setae and cuticle (210×). Fig. f: First abdominal sternite setae (210×).

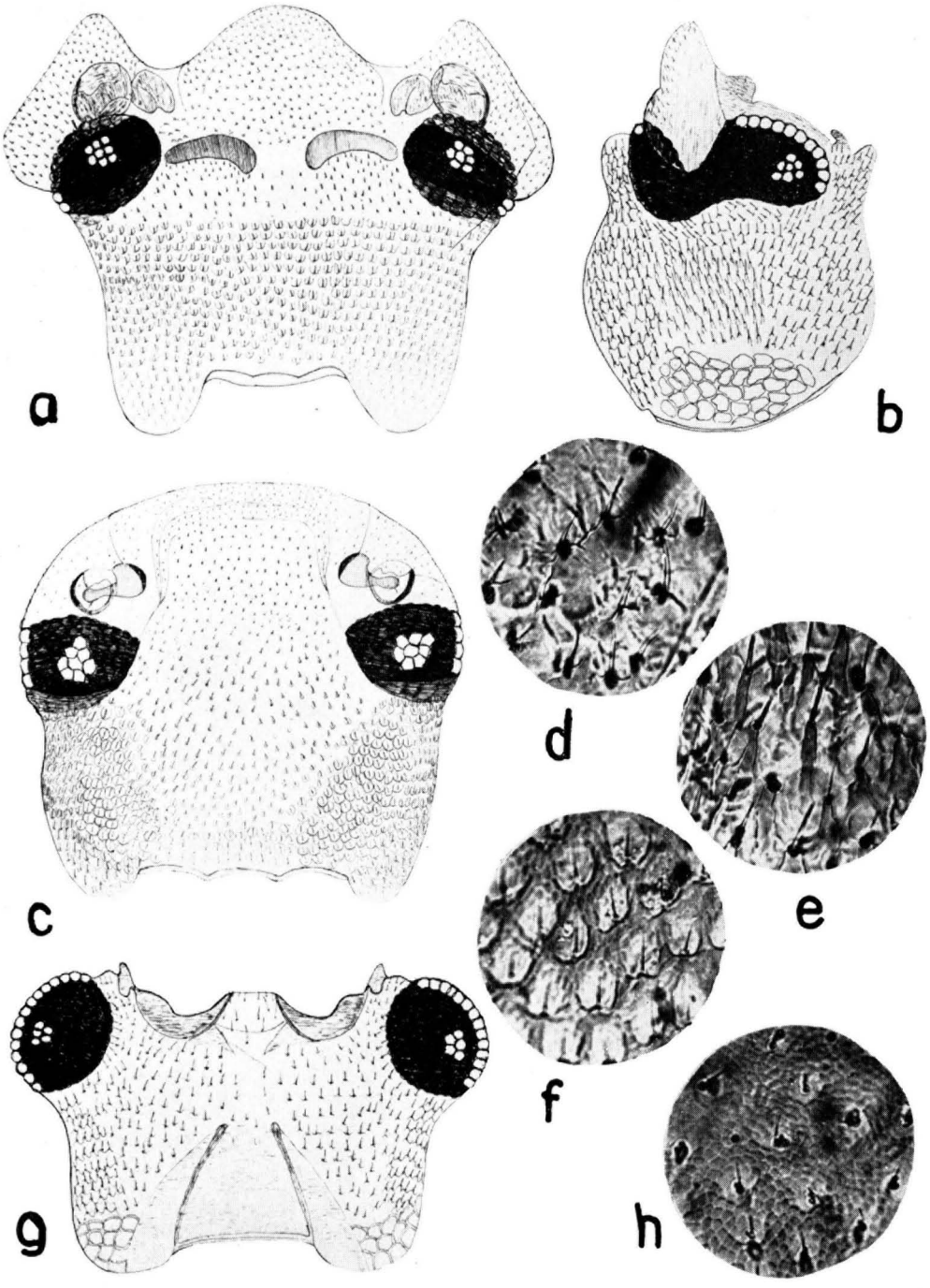

Plate 3. Broad-horned Flour Beetle (*Gnathocerus cornutus*), Head.

Fig. a: Male, dorsal view (75×). Fig. b: Lateral view (75×). Fig. c: Female, ventral view (75×). Figs. d, e, f: Setae and cuticle from dorsal and lateral surfaces (300×). Fig. g: Ventral view (75×). Fig. h: Ventral view, setae and cuticle (300×).

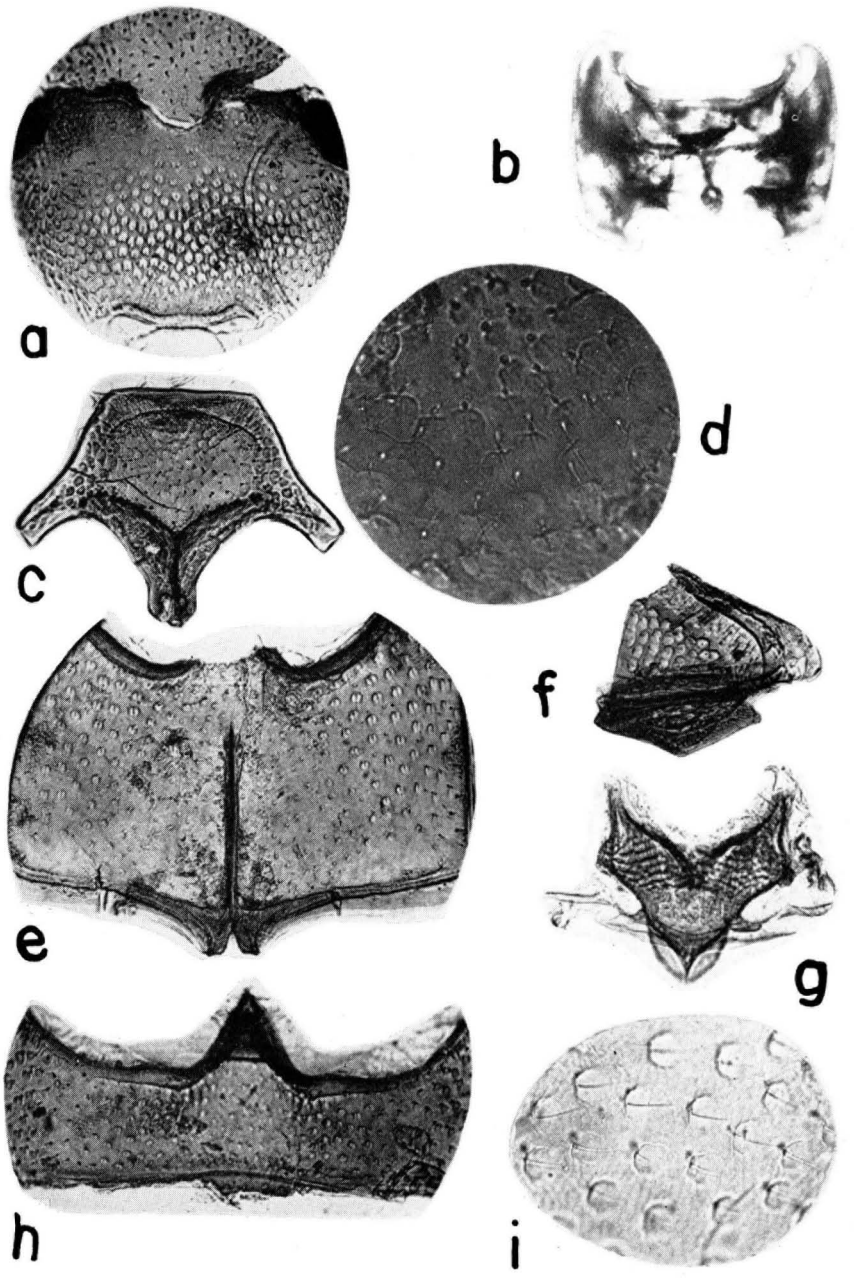

Plate 4. Broad-horned Flour Beetle (continued).

Fig. a: Head capsule fragment (50×). Fig. b: Prothorax, ventral view (25×). Fig. c: Mesosternite (50×). Fig. d: Mesosternite, setae (210×). Fig. e: Metasternite (50×). Fig. f: Mesopleuron (50×). Fig. g: Mesotergite (50×). Fig. h: First abdominal sternite (50×). Fig. i: First abdominal sternite setae (210×).

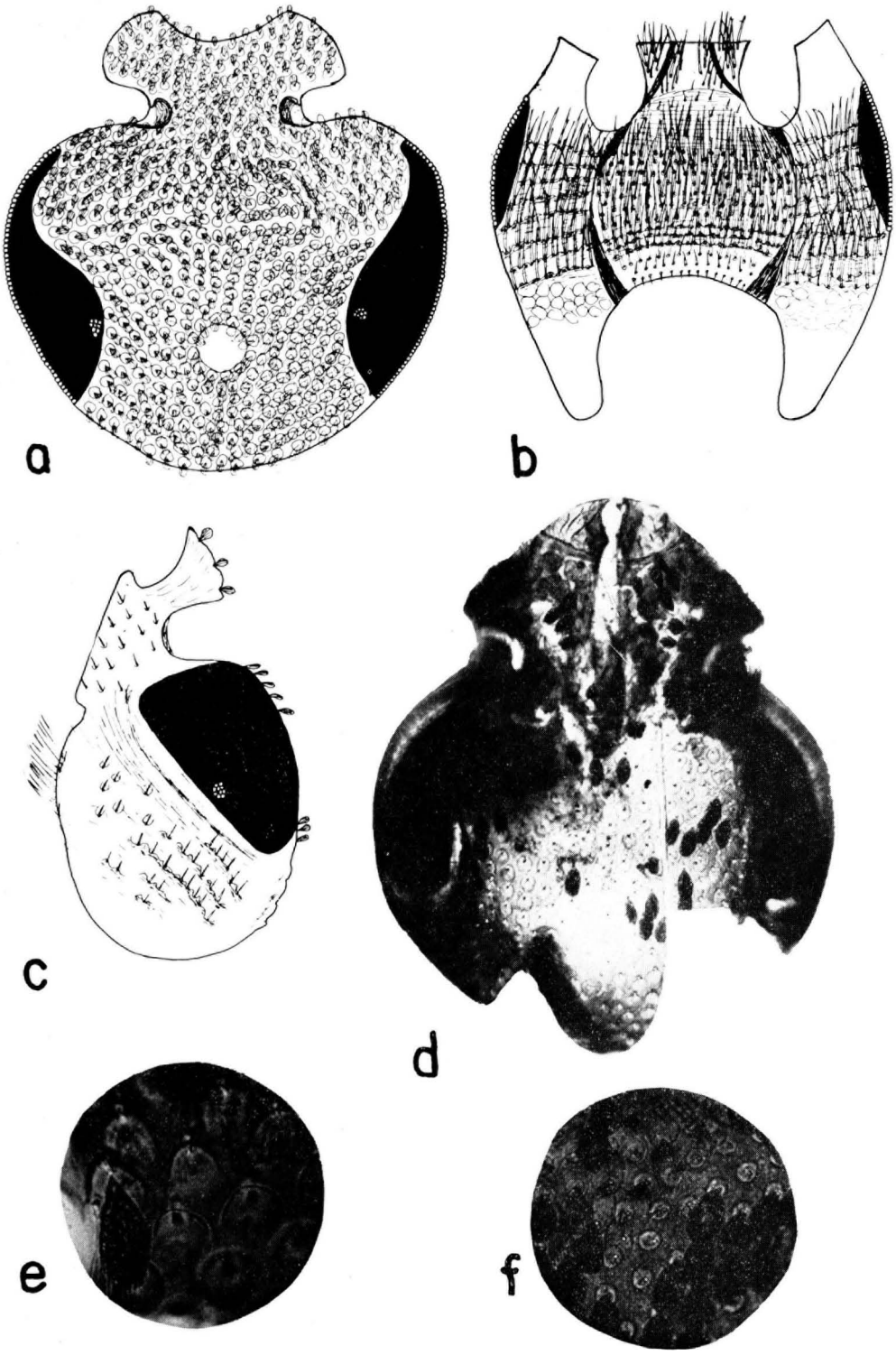

Plate 5. Buffalo Carpet Beetle (*Anthrenus scrophulariae*), Head.

Fig. a: Dorsal view (80×). Fig. b: Ventral view (80×). Fig. c: Lateral view (80×). Fig. d: Dorsal view (100×). Figs. e, f: Setae and cuticle (210×).

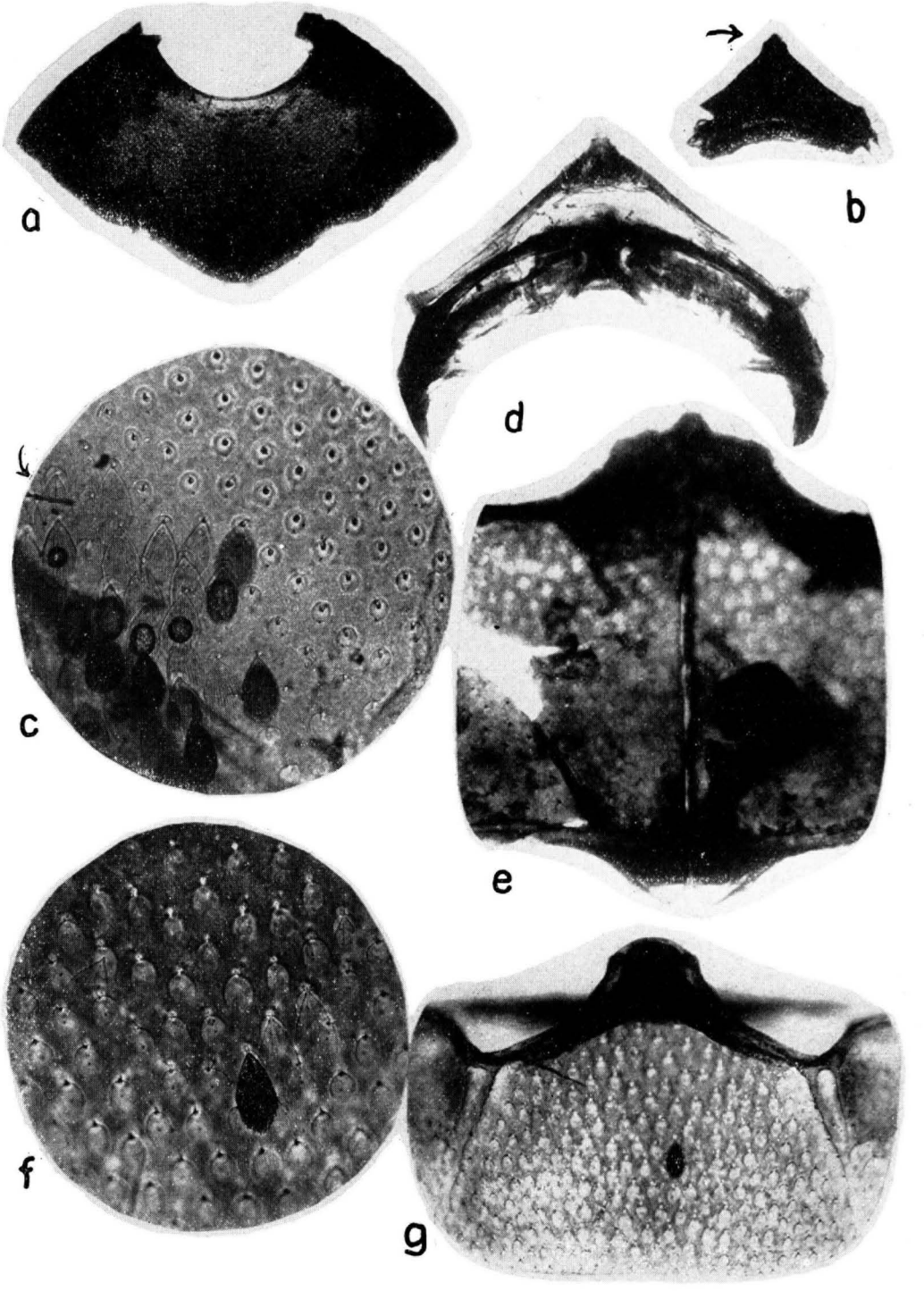

Plate 6. Buffalo Carpet Beetle (continued).

Fig. a: Prothorax, dorsal view (25×). Fig. b: Prothorax, ventral view (25×). Fig. c: Prothoracic setae and cuticular pattern (100×). Fig. d: Mesosternite (60×). Fig. e: Metasternite (60×). Fig. f: First abdominal sternite setae (100×). Fig. g: First abdominal sternite (60×).

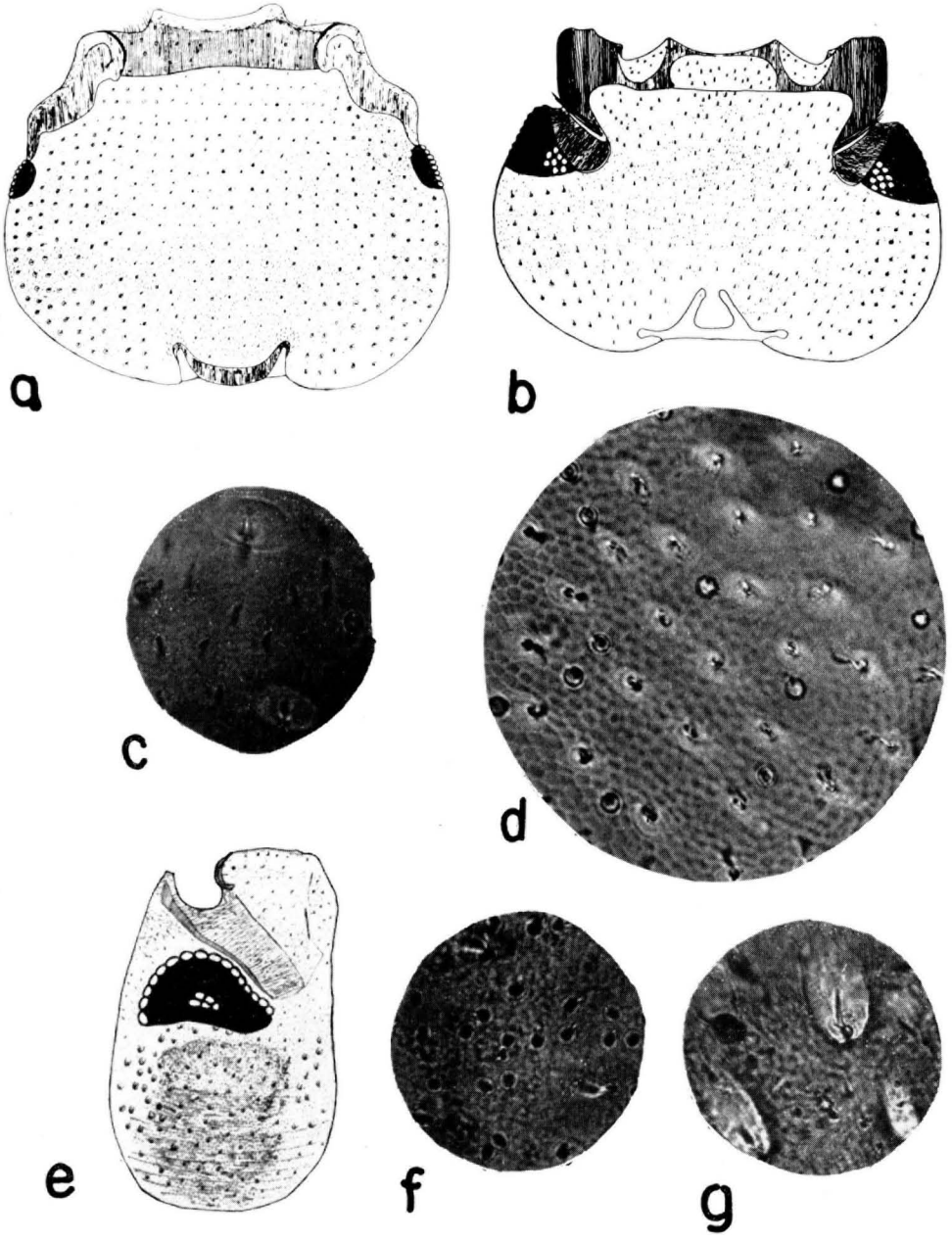

Plate 7. Cadelle Beetle (*Tenebroides mauritanicus*), Head.

Fig. a: Dorsal view (40×). Fig. b: Ventral view (40×). Figs. c, f, g: Setae and cuticle (300×). Fig. d: Setae and cuticle (210×). Fig. e: Lateral view (40×).

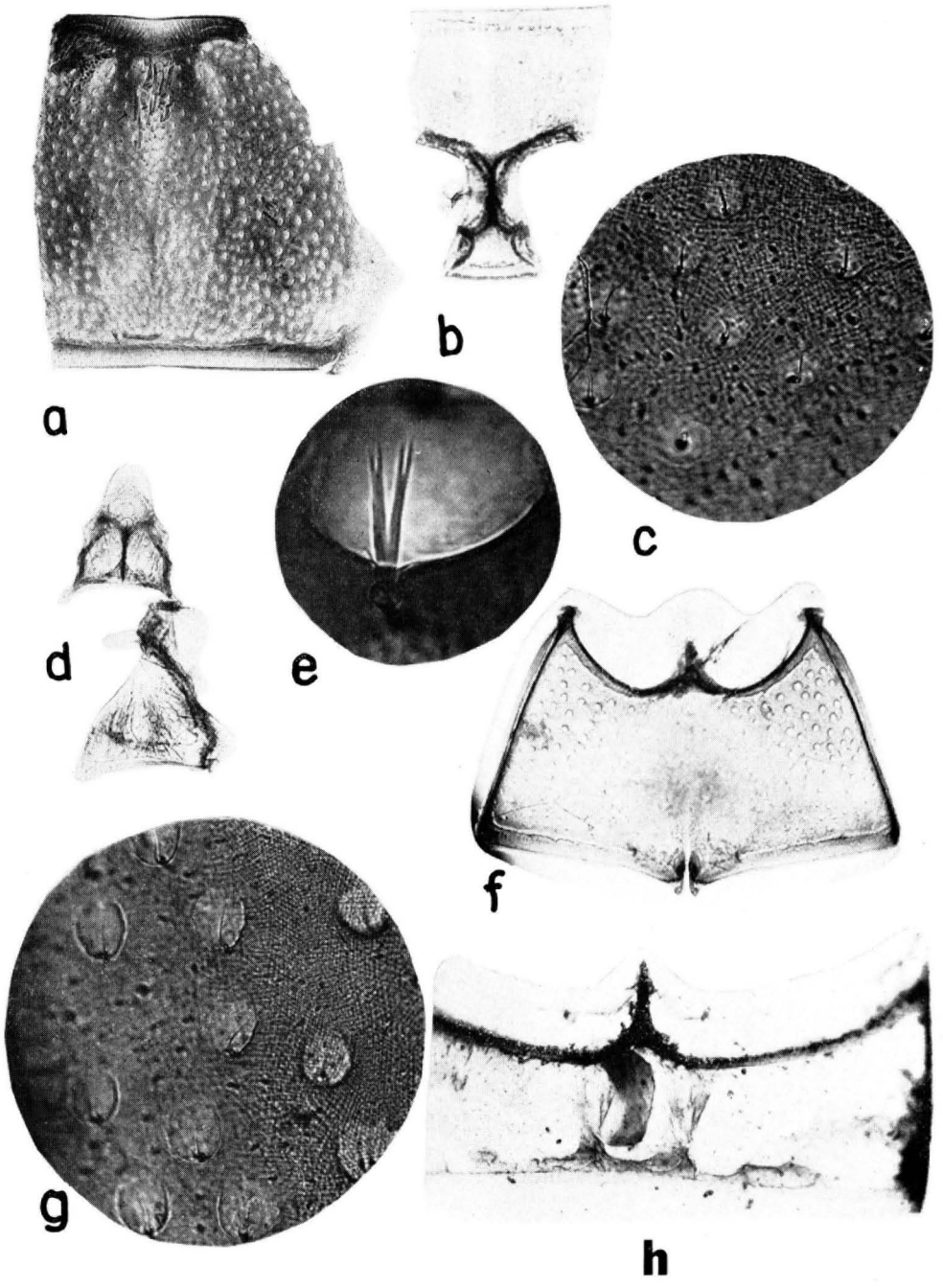

Plate 8. Cadelle Beetle (continued).

Fig. a: Prothorax, dorsal view (25×). Fig. b: Prothorax, ventral view (25×). Fig. c: Prothoracic setae (210×). Fig. d: Mesotergite and mesopleuron (25×). Fig. e: Seta from mesopleuron (430×). Fig. f: Metasternite (25×). Fig. g: First abdominal sternite setae (210×). Fig. h: First abdominal sternite (40×).

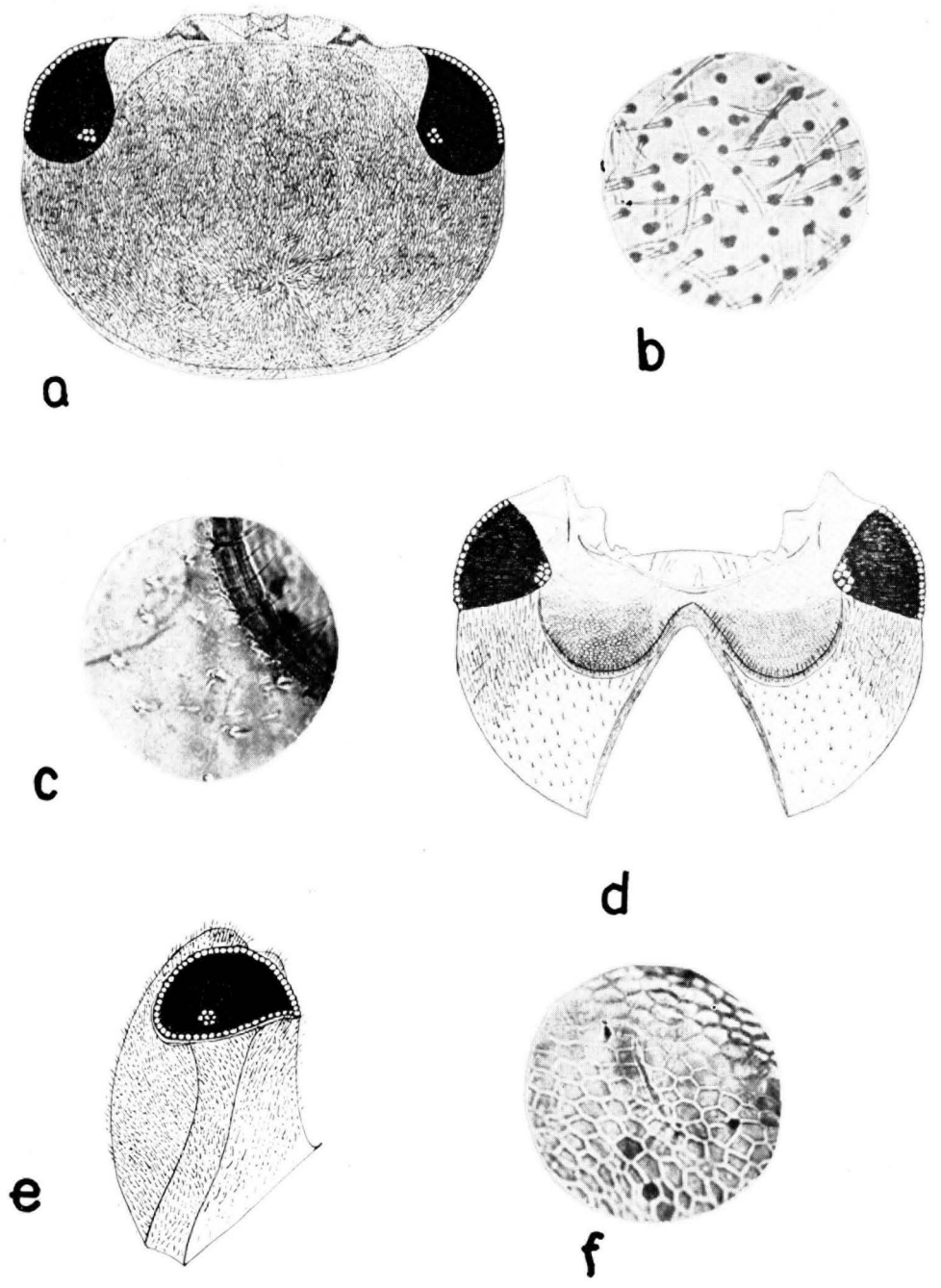

Plate 9. Cigarette Beetle (*Lasoiderma serricorne*), Head.

Fig. a: Dorsal view (75×). Fig. b: Dorsal view, setae (300×). Fig. c: Ventral view, setae (330×). Fig. d: Ventral view (75×). Fig. e: Lateral view (75×). Fig. f: Lateral view, cuticular mosaic (300×).

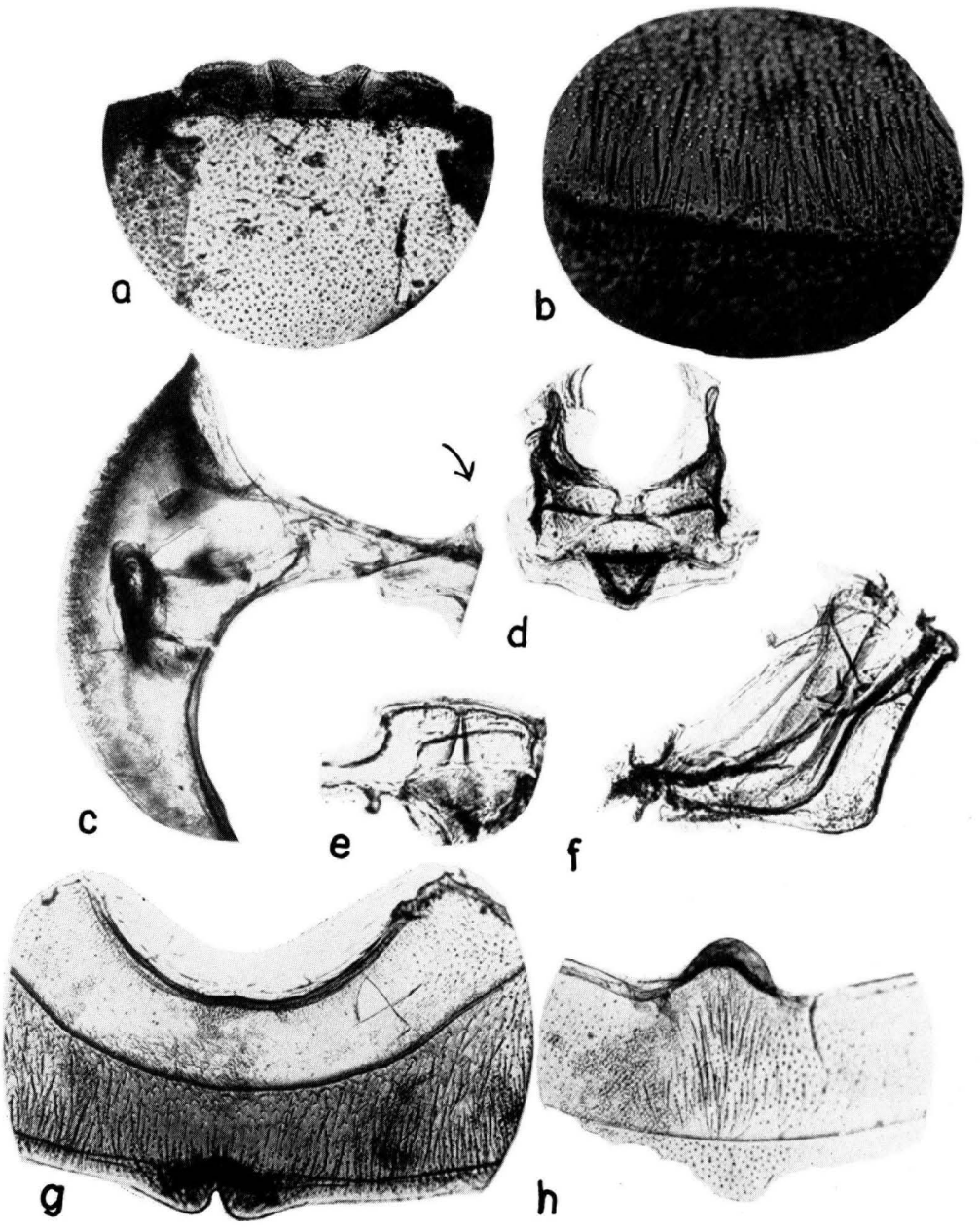

Plate 10. Cigarette Beetle (continued).

Fig. a: Head capsule (60×). Fig. b: Prothorax setae (125×). Fig. c: Prothorax, anterior view (60×). Fig. d: Mesotergite (60×). Fig. e: Mesosternite (60×). Fig. f: Mesopleuron (60×). Fig. g: Metasternite (60×). Fig. h: Central projection, first abdominal sternite (60×).

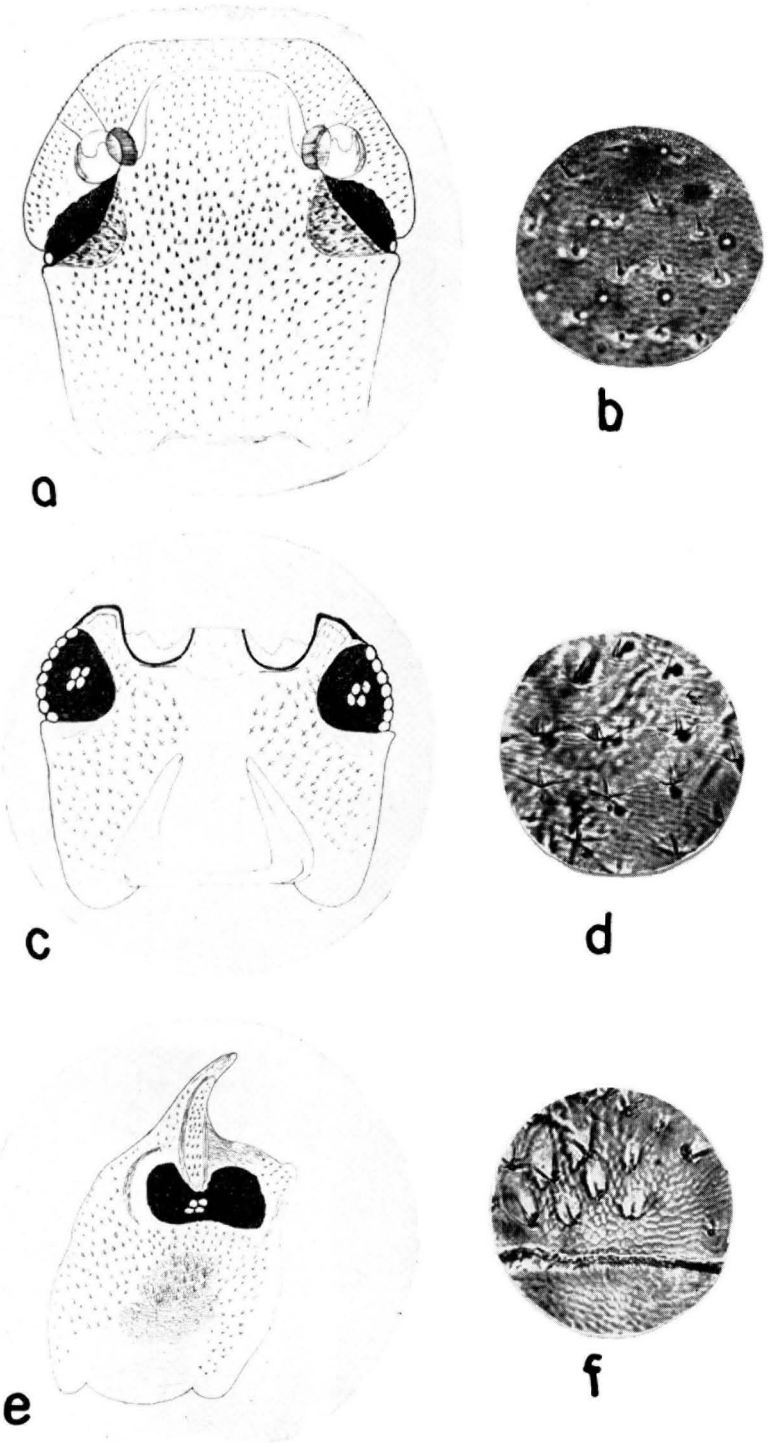

Plate 11. Confused Flour Beetle (*Tribolium confusum*), Head.

Fig. a: Dorsal view (100×). Fig. b: Dorsal view, setae and cuticle (430×). Fig. c: Ventral view (100×). Fig. d: Ventral view, setae and cuticle (430×). Fig. e: Lateral view (100×). Fig. f: Lateral view, setae and cuticle (430×).

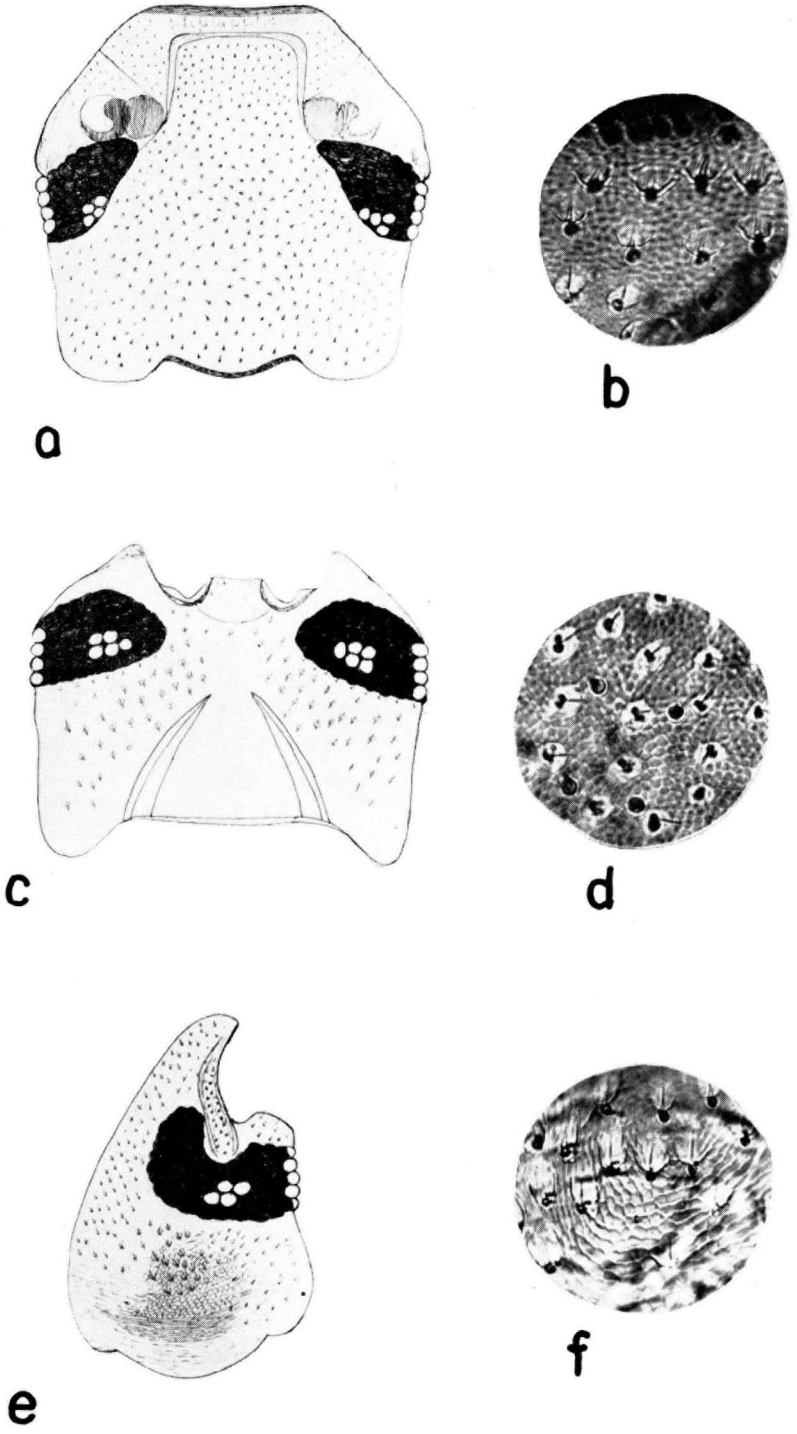

Plate 12. Rust Red Flour Beetle (*Tribolium castaneum*), Head.

Fig. a: Dorsal view (100×). Fig. b: Dorsal view, setae and cuticle (430×). Fig. c: Ventral view (100×). Fig. d: Ventral view, setae and cuticle (430×). Fig. e: Lateral view (100×). Fig. f: Lateral view, setae and cuticle (430×).

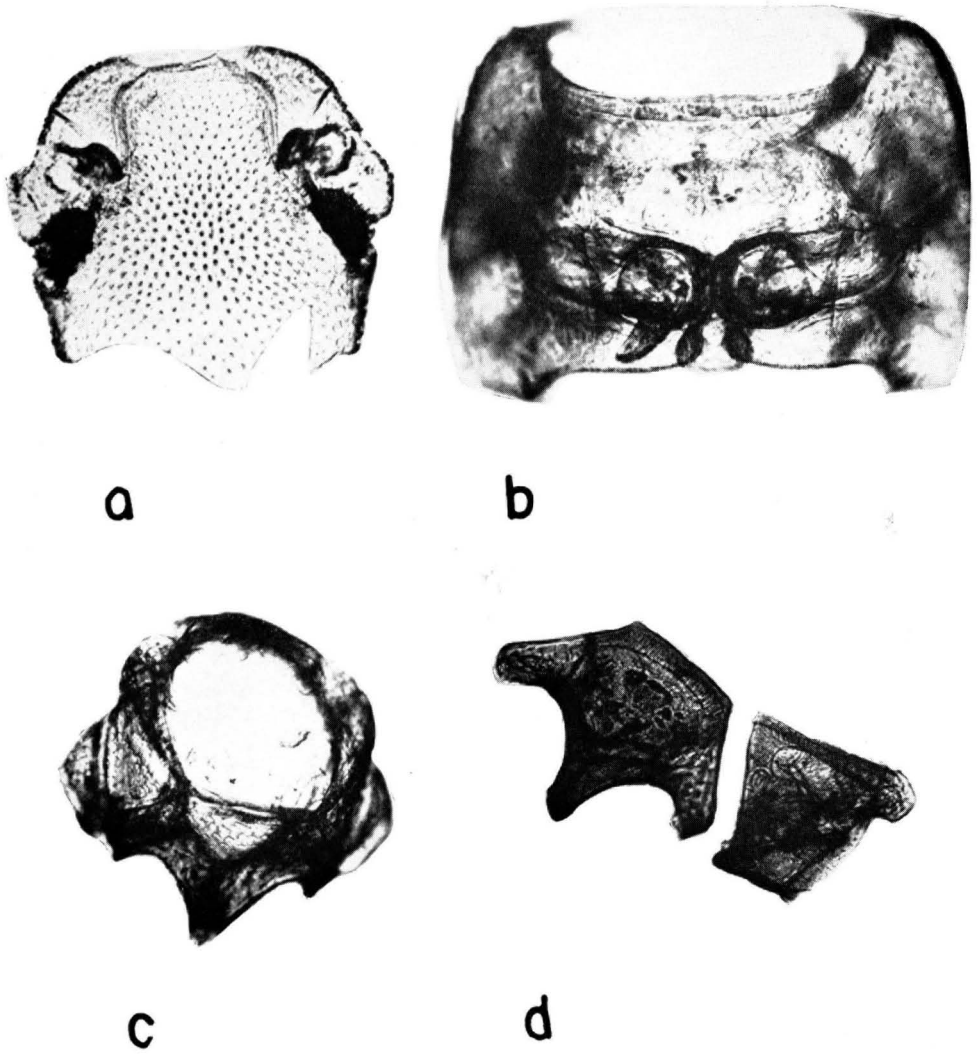

Plate 13. Confused and Rust Red Flour Beetles (continued).

Fig. a: Confused Flour Beetle, Head capsule (50×). Fig. b: Confused Flour Beetle, Prothorax, ventral view (50×). Fig. c: Rust Red Flour Beetle, Mesothorax (50×). Fig. d: Confused Flour Beetle, Mesosternite and Mesopleuron (50×).

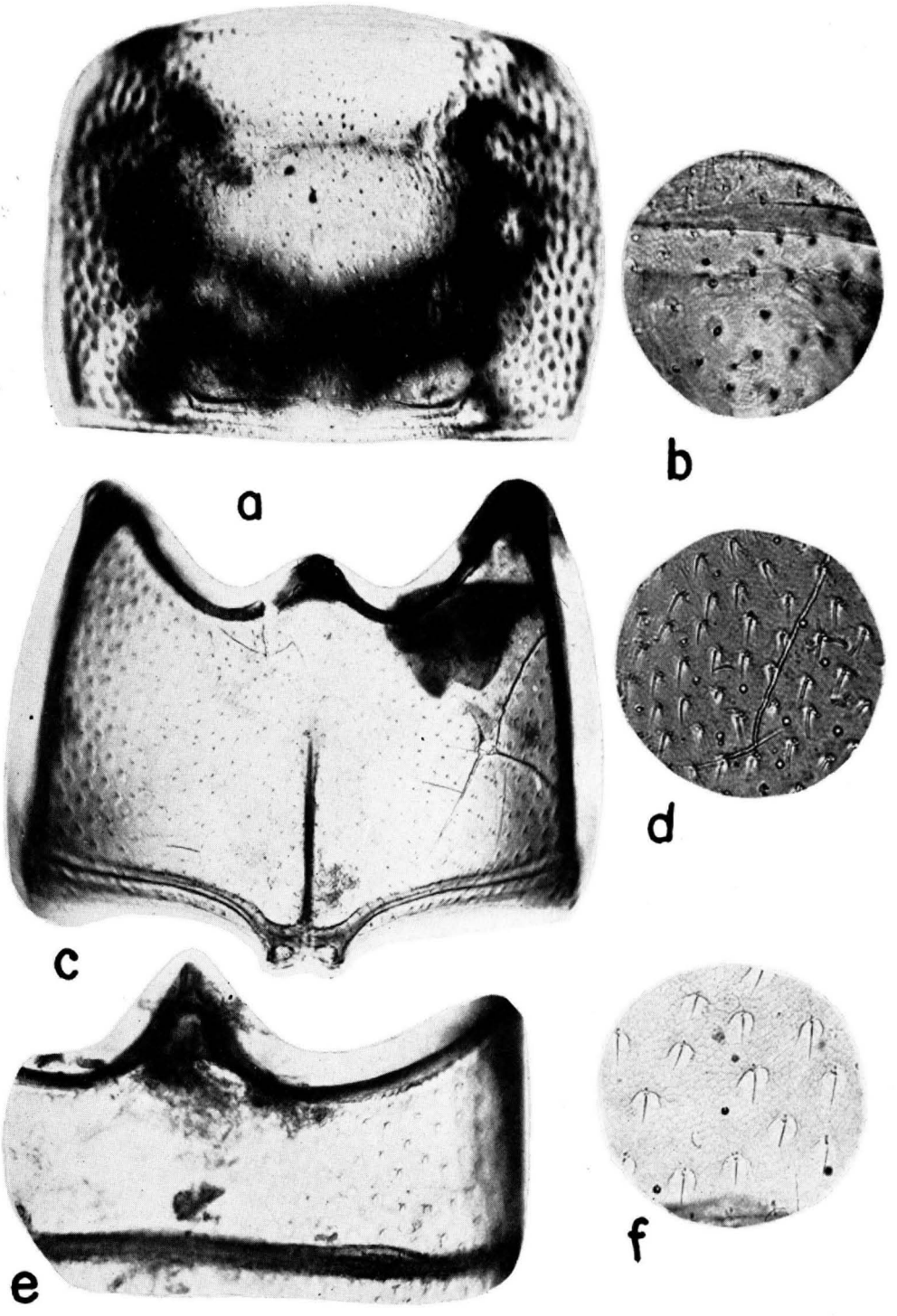

Plate 14. Confused and Rust Red Flour Beetles (continued).

Fig. a: Confused Flour Beetle, Prothorax, dorsal view (50×). Fig. b: Confused Flour Beetle, Prothoracic setae (210×). Fig. c: Metasternite (50×). Fig. d: Metasternite (210×). Fig. e: Rust Red Flour Beetle, first abdominal sternite (100×). Fig. f: Rust Red Flour Beetle, first abdominal sternite setae (210×).

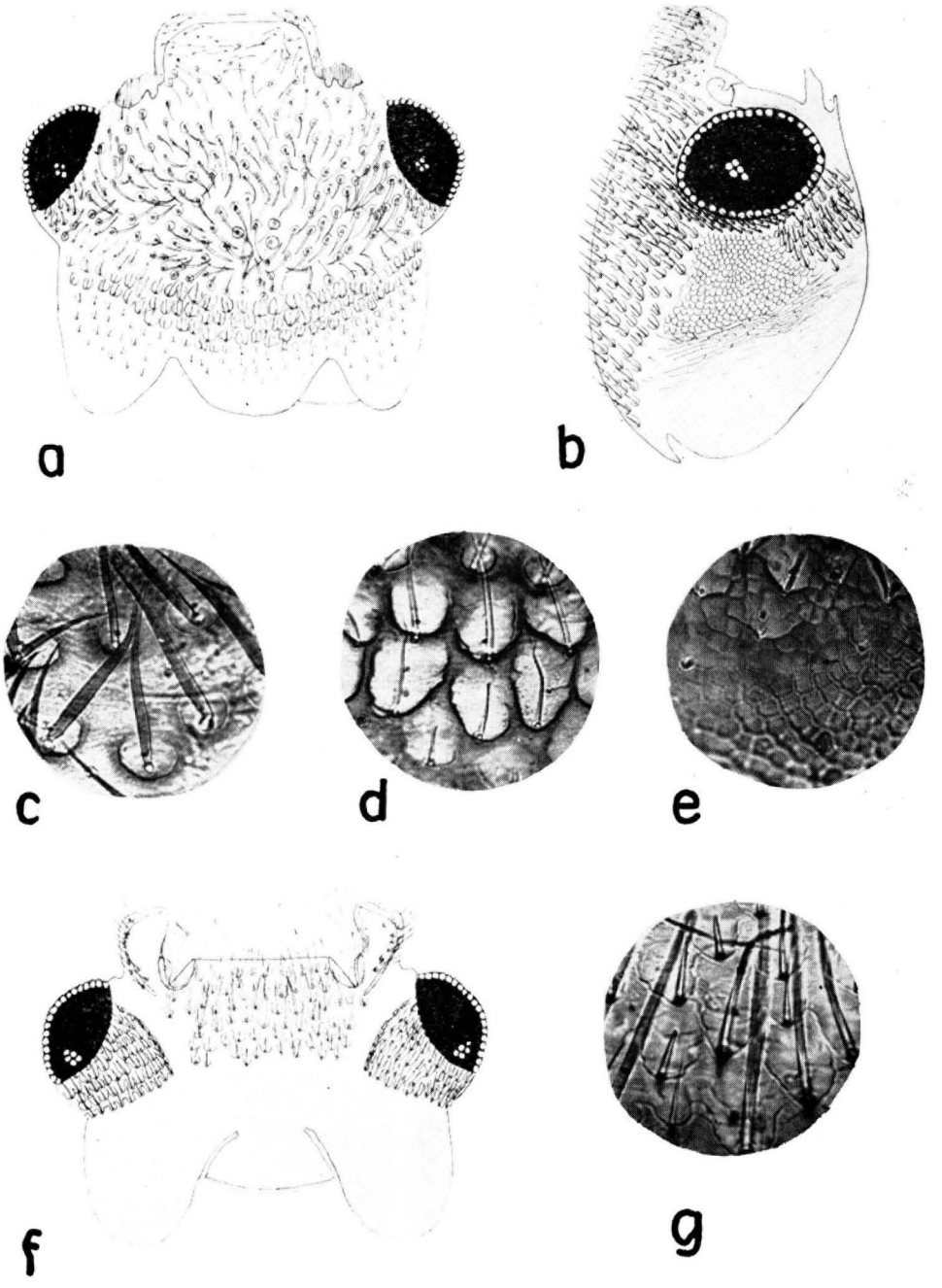

Plate 15. Dried-fruit Beetle (*Carpophilus hemipterus*), Head.

Fig. a: Dorsal view (75×). Fig. b: Lateral view (75×). Figs. c, d, e: Setae and cuticle (300×). Fig. f: Ventral view (75×). Fig. g: Setae and cuticular pattern (300×).

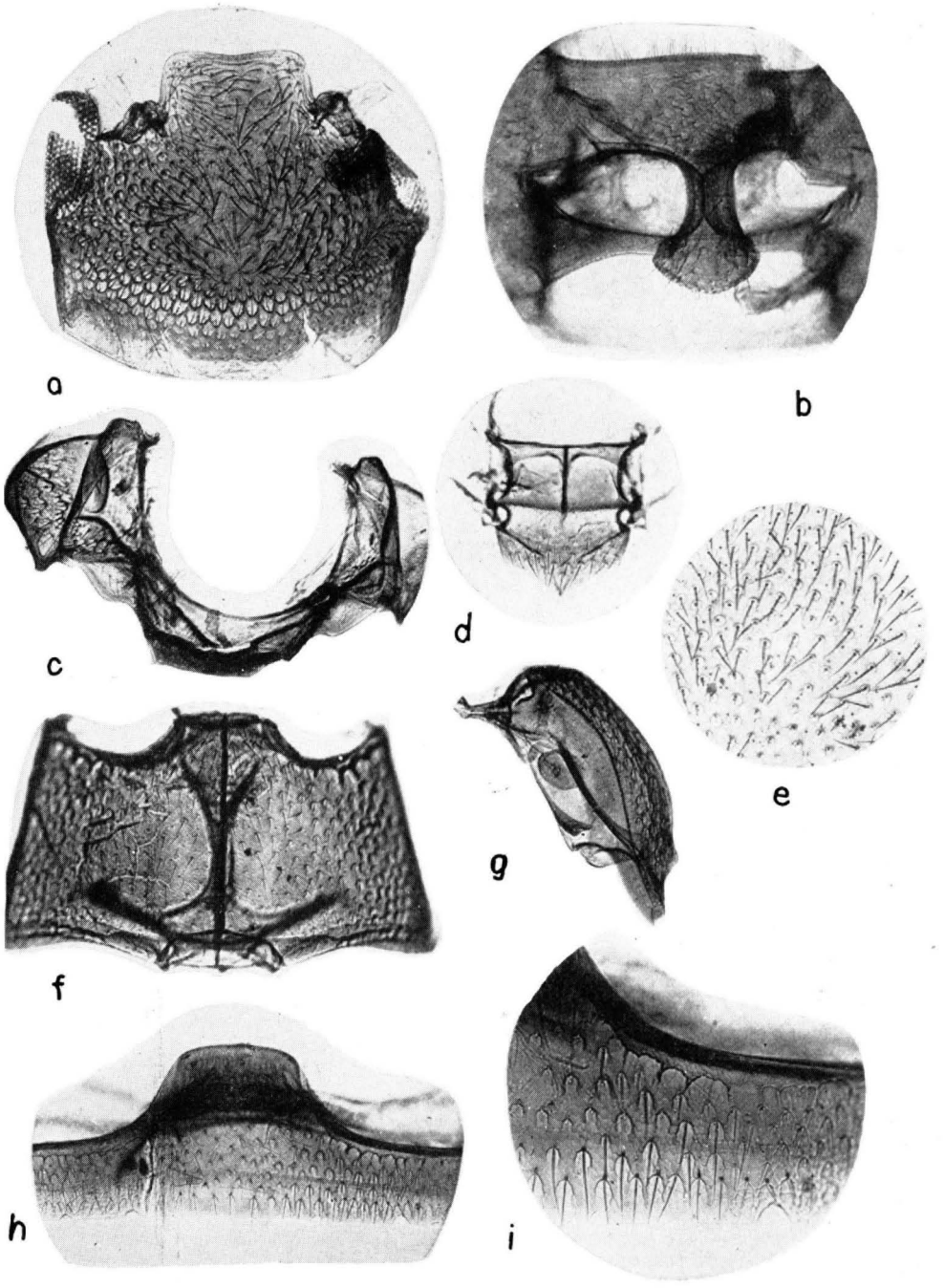

Plate 16. Dried-fruit Beetle (continued).

Fig. a: Head capsule (37×). Fig. b: Prothorax, ventral view (37×). Fig. c: Mesothorax, anterior view (37×). Fig. d: Mesotergite (37×). Fig. e: Prothorax, setae (80×). Fig. f: Metasternite (37×). Fig. g: Metapleuron (37×). Fig. h: First abdominal sternite (37×). Fig. i: First abdominal sternite setae (80×).

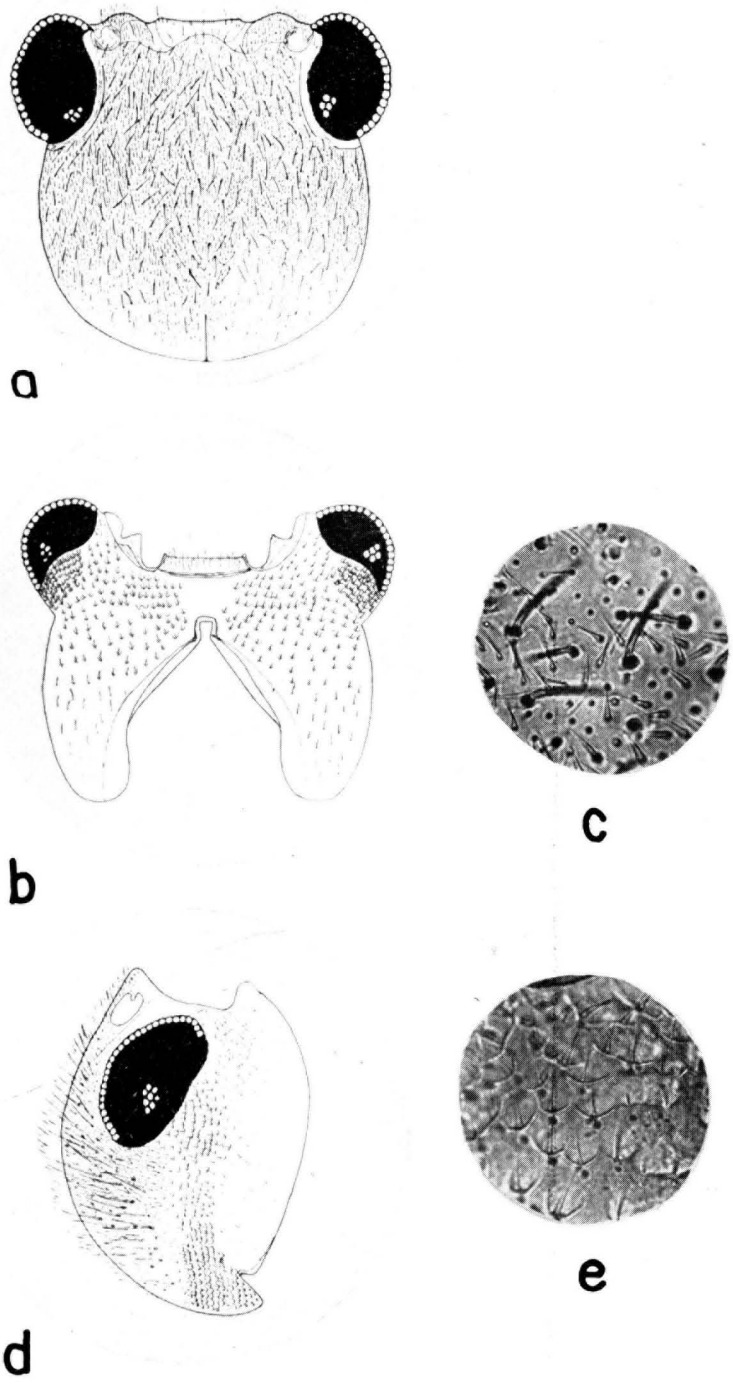

Plate 17. Drug-store Beetle (*Stegobium paniceum*), Head.

Fig. a: Dorsal view (75×). Fig. b: Ventral view (75×). Fig. c: Ventral view, setae (300×). Fig. d: Lateral view (75×). Fig. e: Lateral view, setae and cuticle (300×).

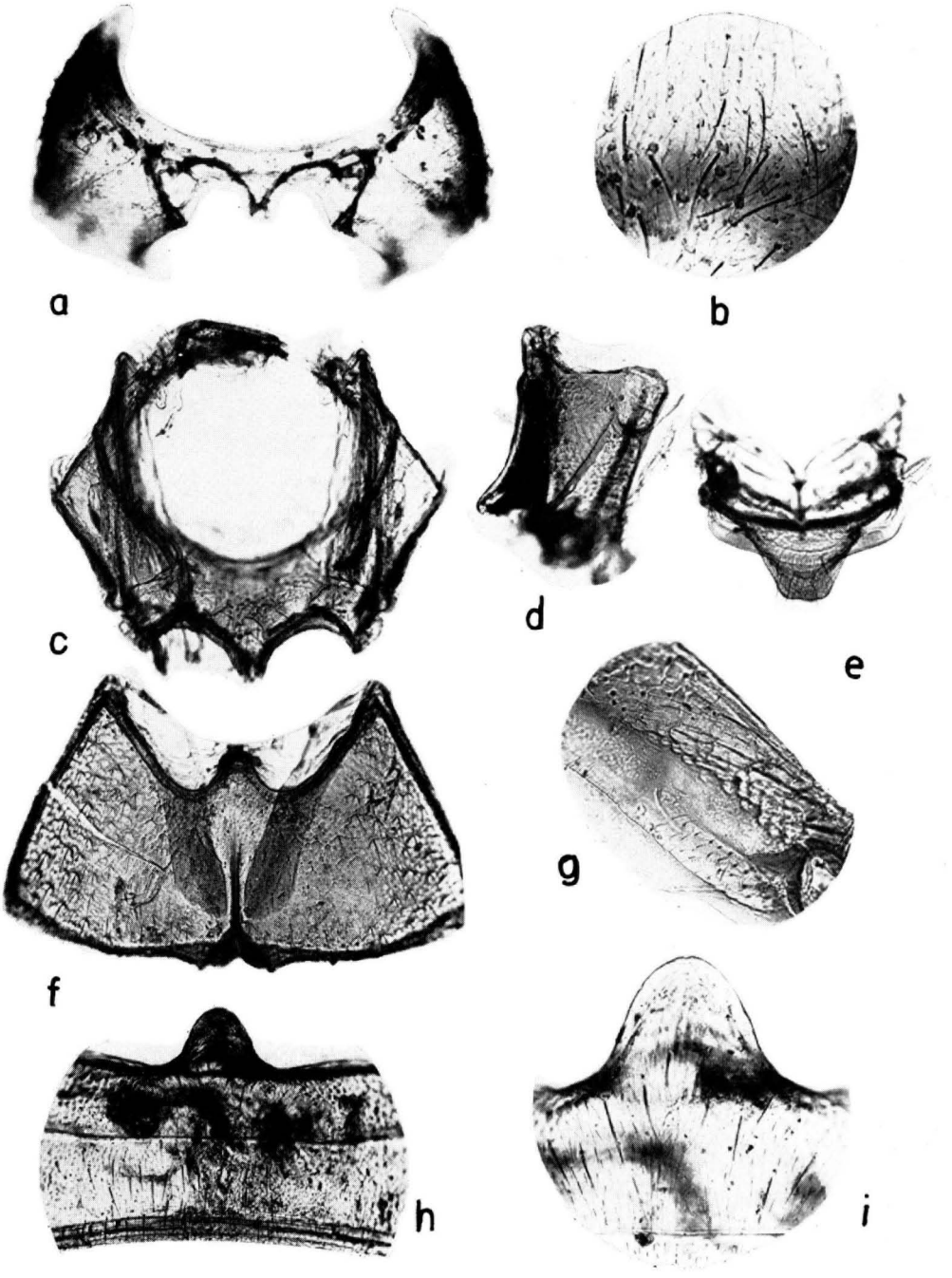

Plate 18. Drug-store Beetle (continued).

Fig. a: Prothorax, ventral view (60×). Fig. b: Prothorax, ventral view setae (120×). Fig. c: Mesothorax, ventral view (60×). Fig. d: Mesopleuron (60×). Fig. e: Mesotergite (60×). Fig. f: Metasternite (60×). Fig. g: Metapleuron (60×). Fig. h: First abdominal sternite, median projection (60×). Fig. i: First abdominal sternite setae (120×).

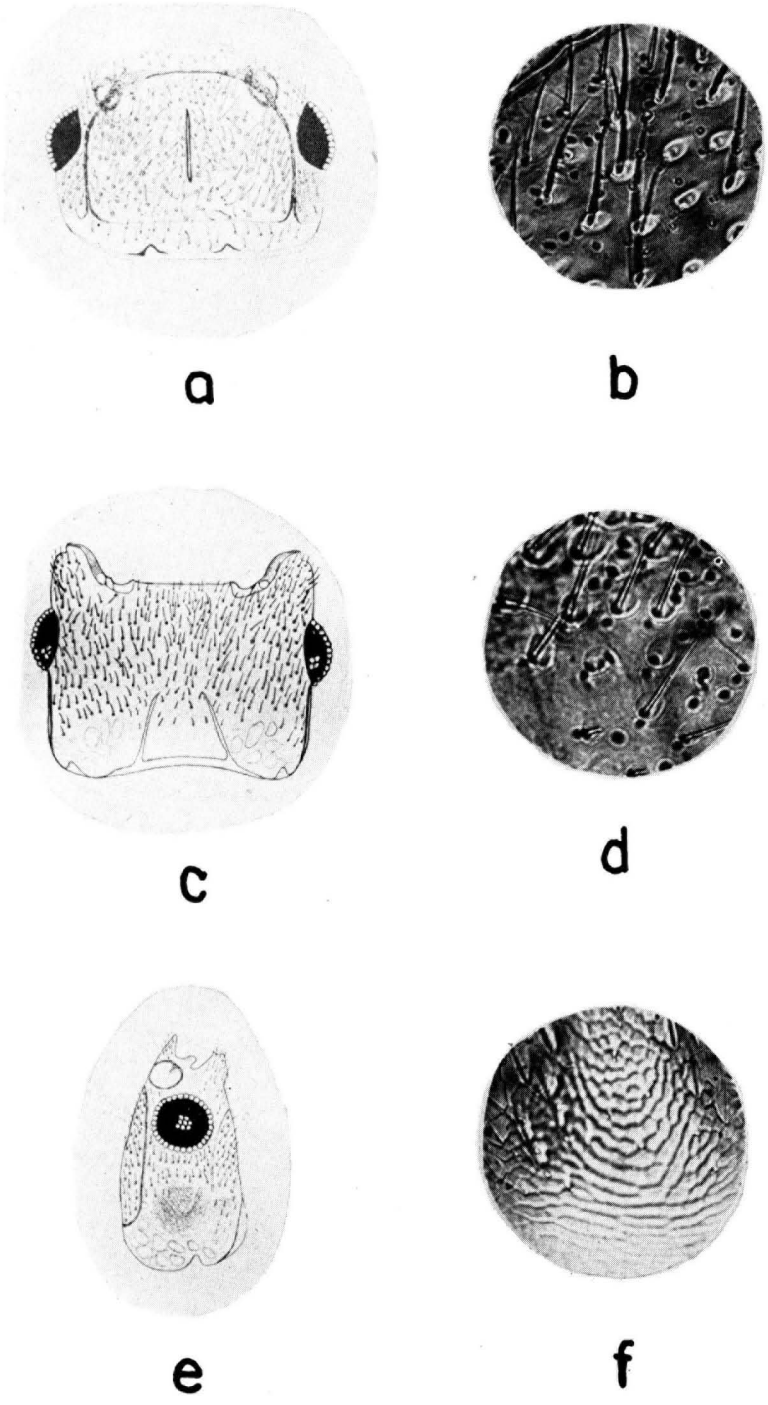

Plate 19. Flat Grain Beetle (*Laemophloeus pusillus*), Head.

Fig. a: Dorsal view (75×). Fig. b: Dorsal view, setae and cuticle (300×). Fig. c: Ventral view (75×). Fig. d: Ventral view, setae and cuticle (300×). Fig. e: Lateral view (75×). Fig. f: Lateral view, setae and cuticular pattern (300×).

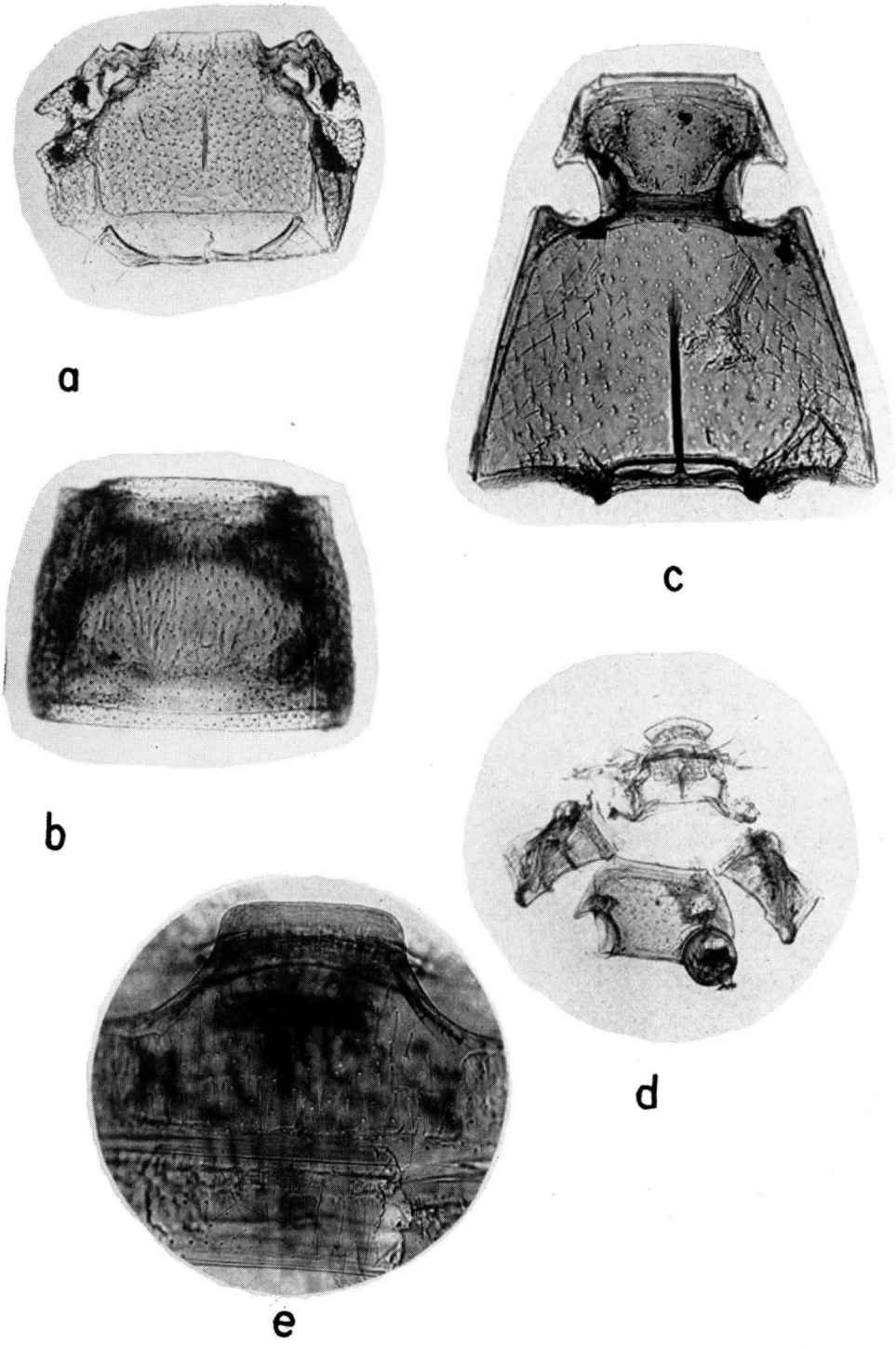

Plate 20. Flat Grain Beetle (continued).

Fig. a: Head capsule (70×). Fig. b: Prothorax, dorsal view (70×). Fig. c: Mesosternite attached to metasternite (70×). Fig. d: Mesothorax fragmented into tergite, pleura, and sternite (70×). Fig. e: First abdominal sternite, median projection showing setae (150×).

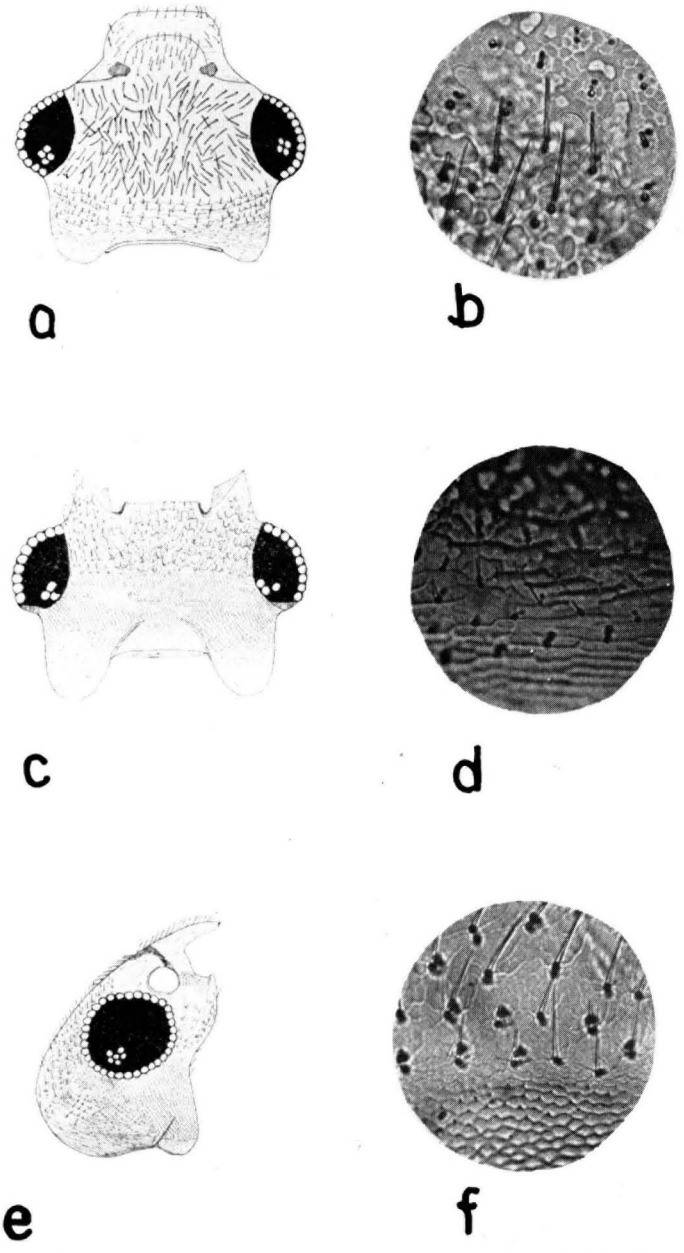

Plate 21. Foreign Grain Beetle (*Cathartus advena*), Head.

Fig. a: Dorsal view (75×). Fig. b: Setae, dorsal surface (300×). Fig. c: Ventral view (75×). Fig. d: Setae and cuticular pattern, ventral surface (300×). Fig. e: Lateral view (75×). Fig. f: Setae and cuticular pattern (300×).

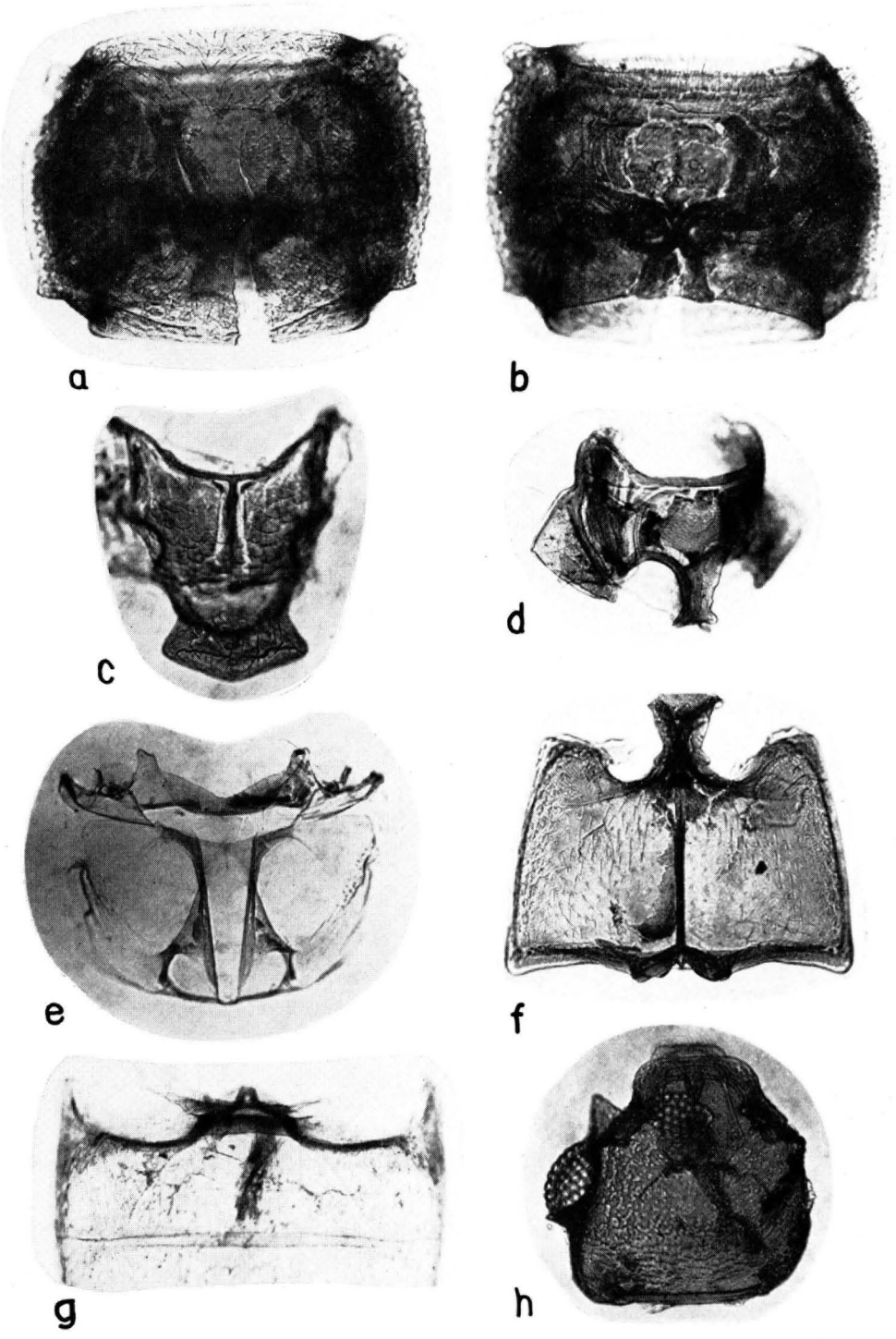

Plate 22. Foreign Grain Beetle (continued).

Fig. a: Prothorax, dorsal surface (60×). Fig. b: Prothorax, ventral surface (60×). Fig. c: Mesotergite (120×). Fig. d: Mesosternite and portion of pleuron (60×). Fig. e: Metatergite (60×). Fig. f: Metasternite (60×). Fig. g: First abdominal sternite (60×). Fig. h: Head capsule (60×).

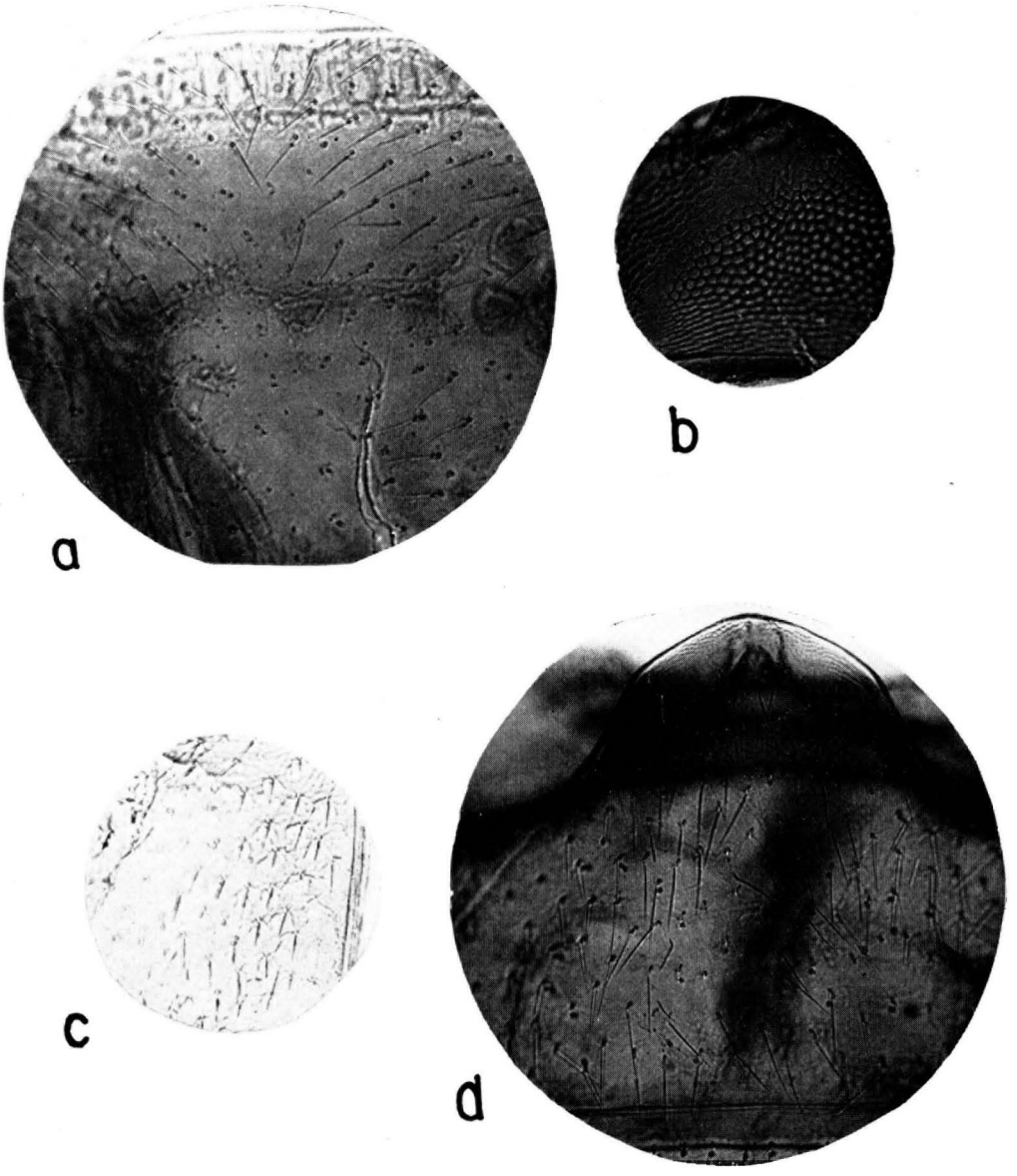

Plate 23. Foreign Grain Beetle (continued).

Fig. a: Prothoracic setae, dorsal surface (210×). Fig. b: Mesosternite, cuticular pattern (210×). Fig. c: Metasternite, setae (210×). Fig. d: First abdominal sternite setae (210×).

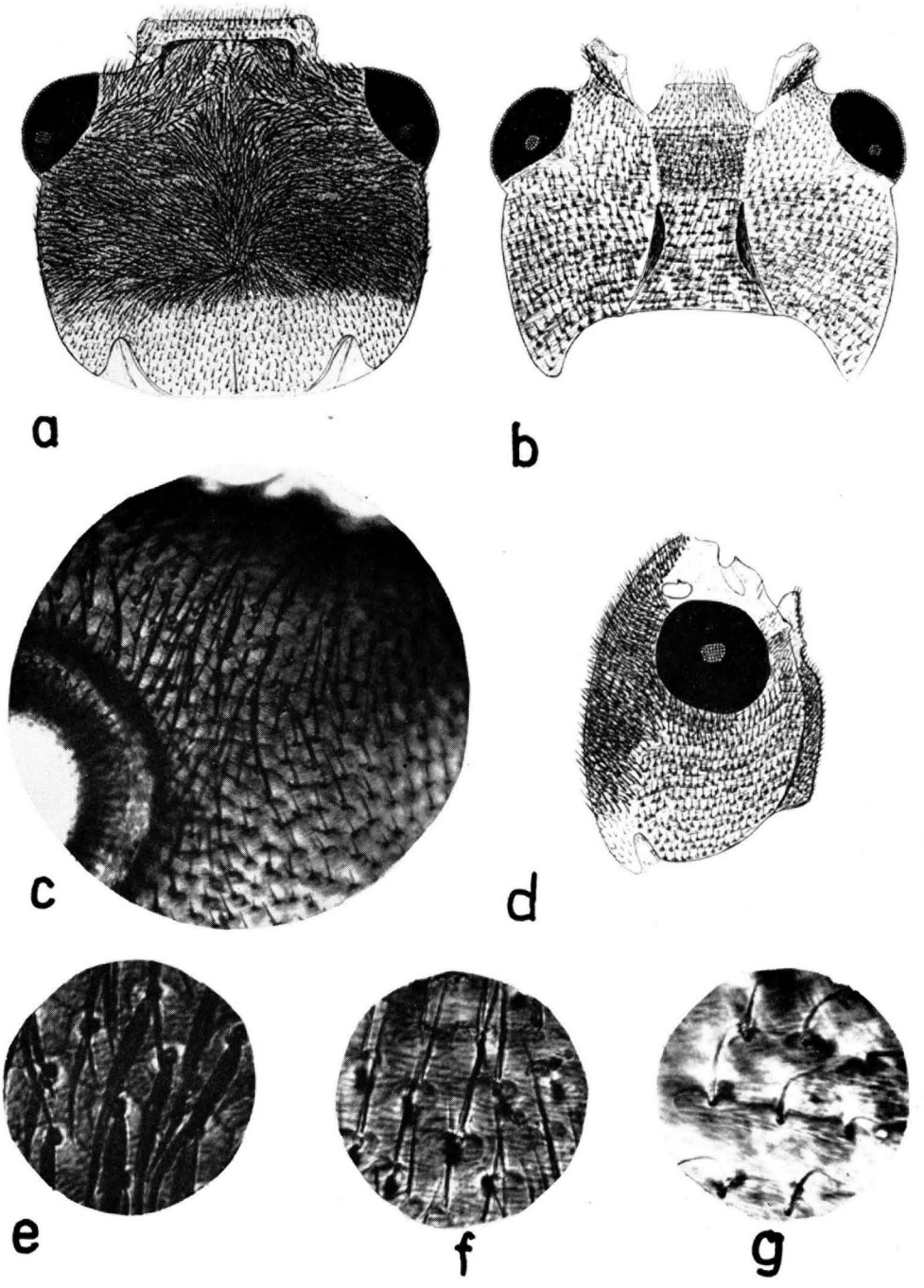

Plate 24. Larder Beetle (*Dermestes lardarius*), Head.

Fig. a: Dorsal view (40×). Fig. b: Ventral view (40×). Fig. c: Setae and cuticular ridging, dorsal surface (300×). Fig. d: Lateral view (40×). Figs. e, f, g: Setae and cuticular patterns (300×).

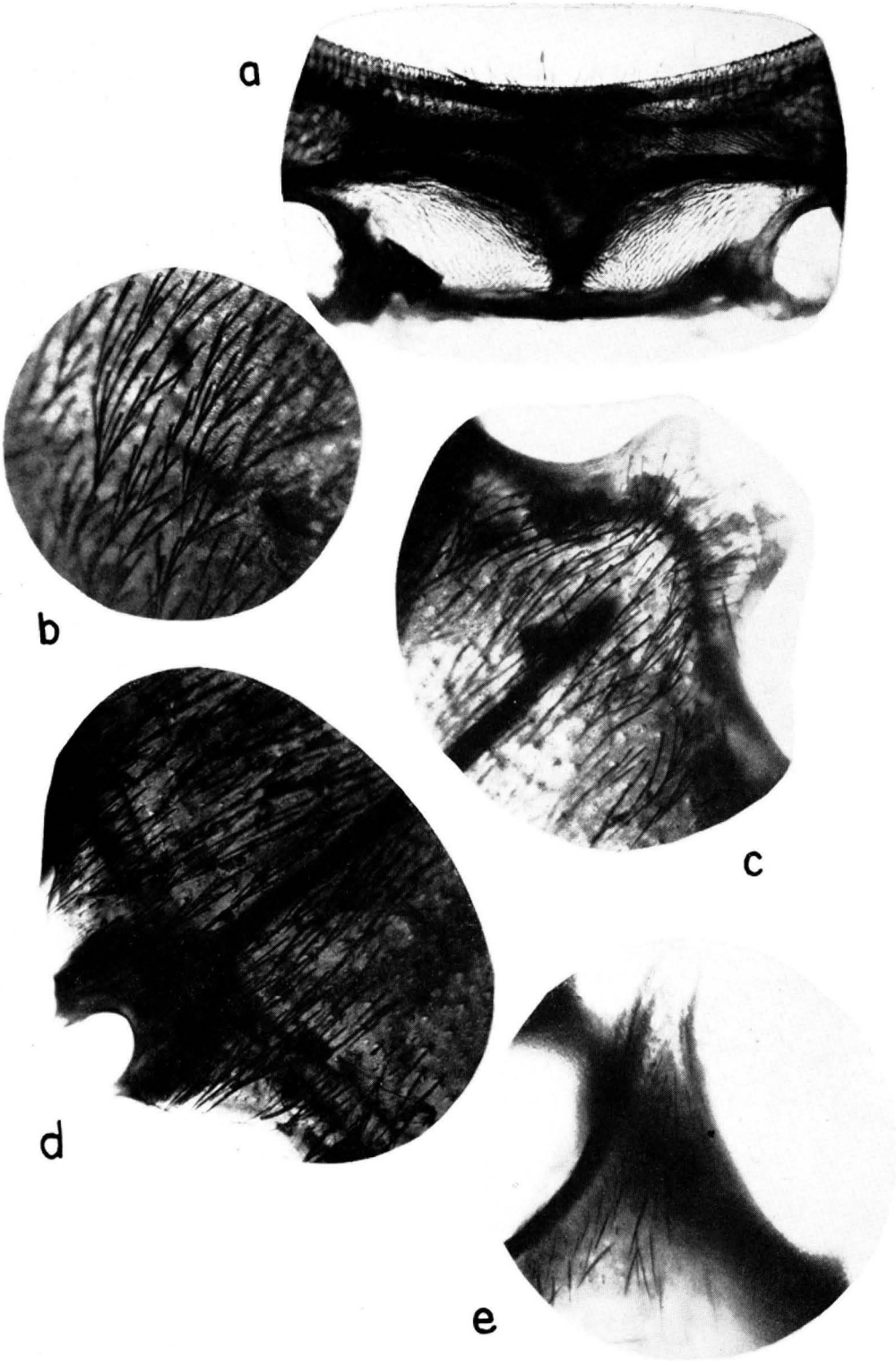

Plate 25. Larder Beetle (continued).

Fig. a: Prothorax, ventral surface (70×). Fig. b: Prothoracic setae (70×). Fig. c: Metasternite, anterior median projection (70×). Fig. d: Metasternite, posterior median projection (70×). Fig. e: First abdominal sternite, median anterior projection (70×).

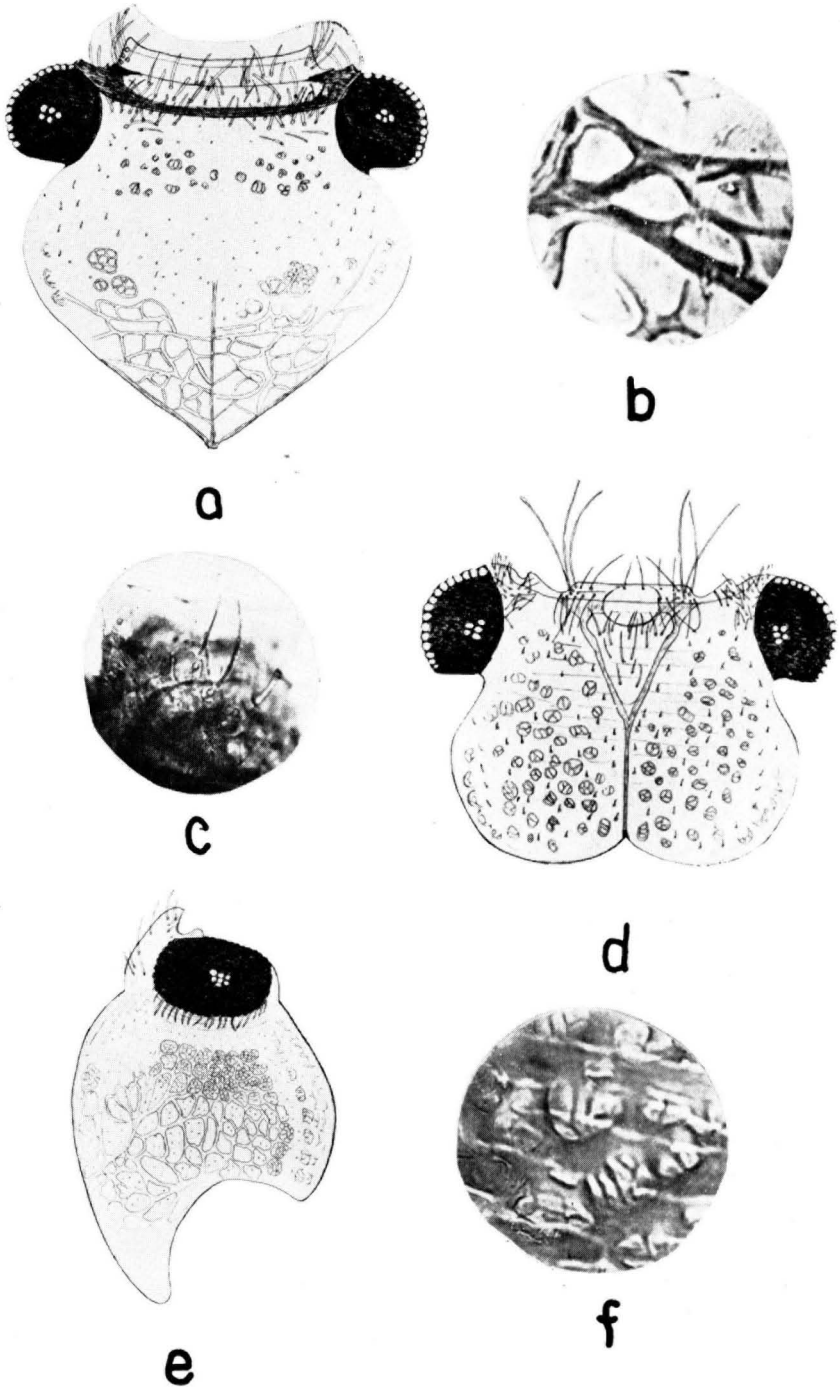

Plate 26. Lesser Grain Borer (*Rhyzopertha dominica*), Head.

Fig. a: Dorsal view (75×). Fig. b: Cuticular pattern, dorsal surface (300×). Fig. c: Setae in labral area (210×). Fig. d: Ventral view (75×). Fig. e: Lateral view (75×). Fig. f: Cuticular pattern, lateral surface (300×).

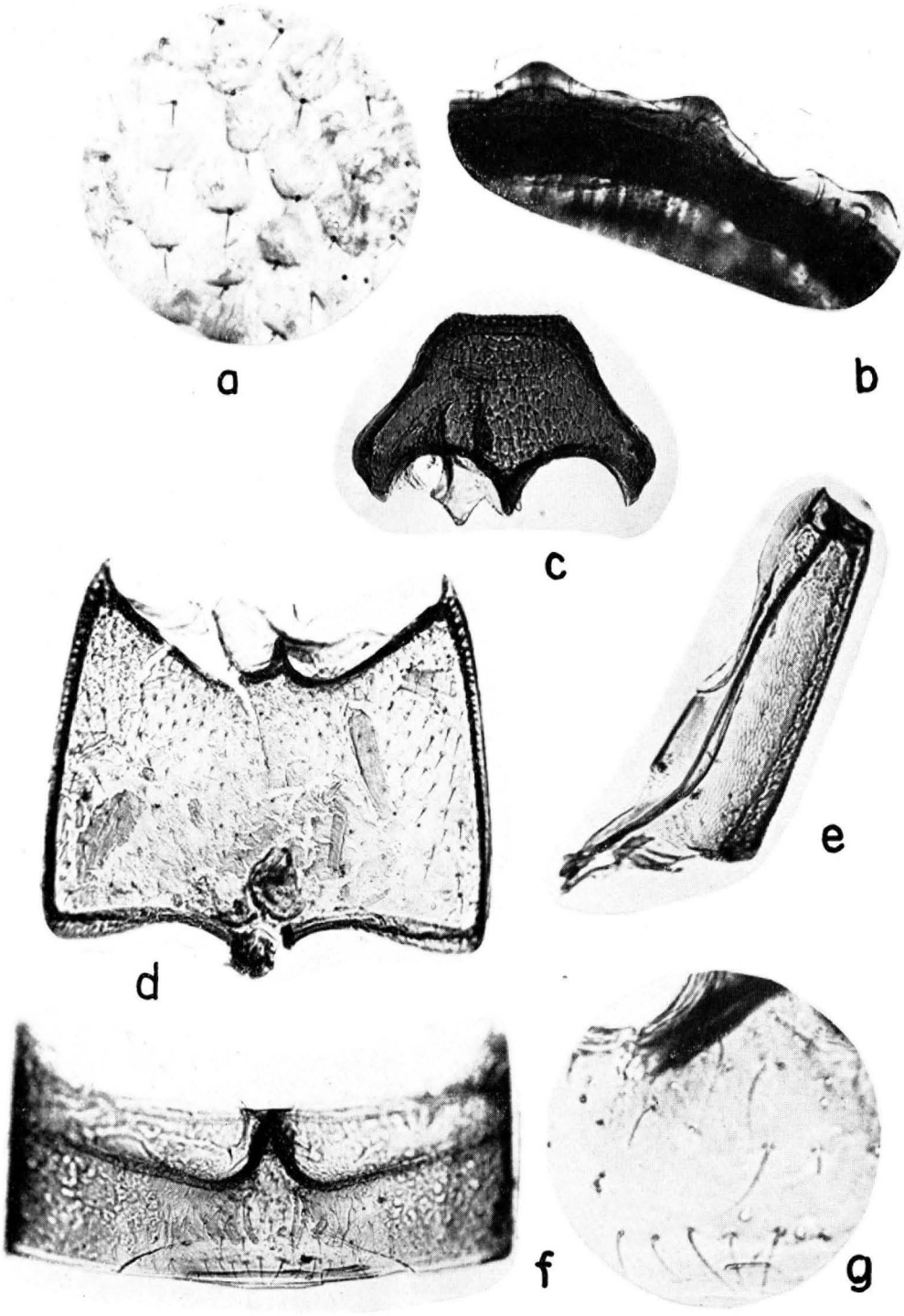

Plate 27. Lesser Grain Borer (continued).

Fig. a: Prothoracic setae (150×). Fig. b: Prothoracic ridging (150×). Fig. c: Mesosternite (70×). Fig. d: Metasternite (70×). Fig. e: Metapleuron (70×). Fig. f: First abdominal sternite (70×). Fig. g: First abdominal sternite setae (150×).

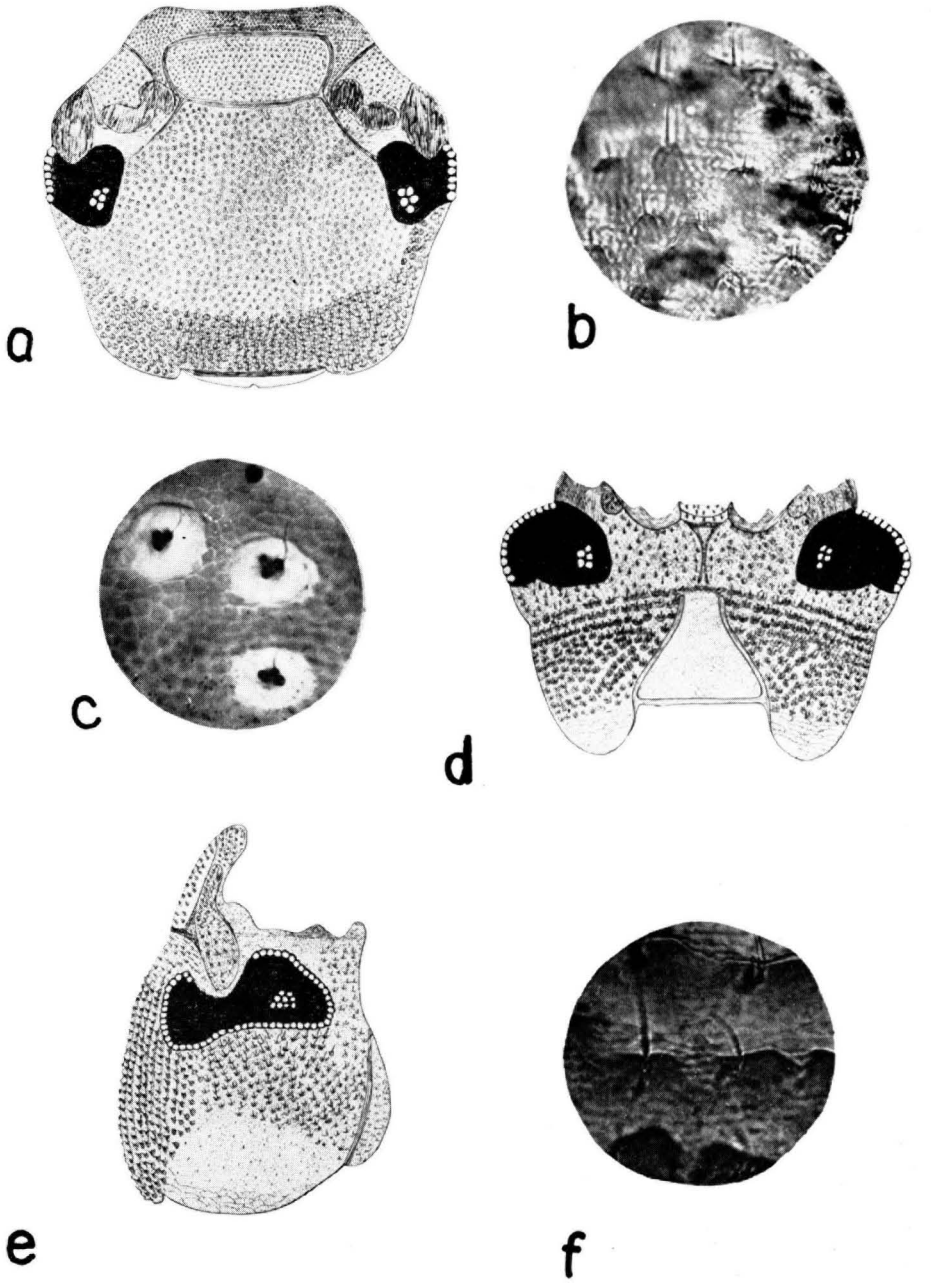

Plate 28. Mealworm (*Tenebrio molitor or T. obscurus*), Head.

Fig. a: Dorsal view (20×). Fig. b: Setae and cuticular protuberances, dorsal surface (300×). Fig. c: Triad of punctures associated with seta in cuticular network, ventral surface (300×). Fig. d: Ventral view (20×). Fig. e: Lateral view (20×). Fig. f: Setae in cuticular ridging, lateral surface (210×).

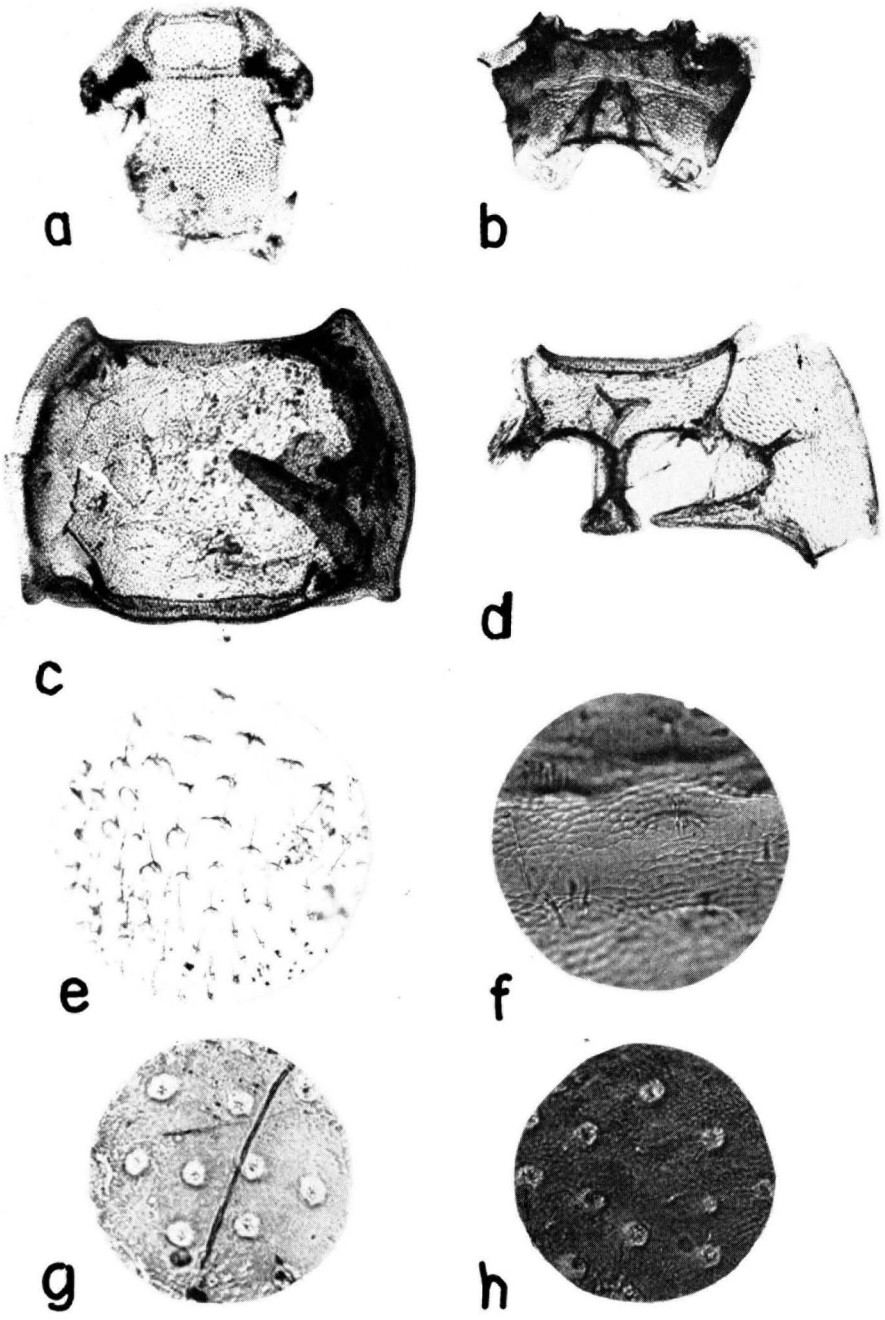

Plate 29. Mealworm (continued).

Fig. a: Head capsule, dorsal surface (10×). Fig. b: Head capsule, ventral surface (10×). Fig. c: Prothorax, dorsal view (10×). Fig. d: Prothorax, ventral view (10×). Fig. e: Prothorax, dorsal surface setae and cuticular protuberances (210×). Fig. f: Prothorax, lateral surface, setae, and cuticular ridging (210×). Figs. g, h: Prothorax, setae bases, and circumferential margins (210×).

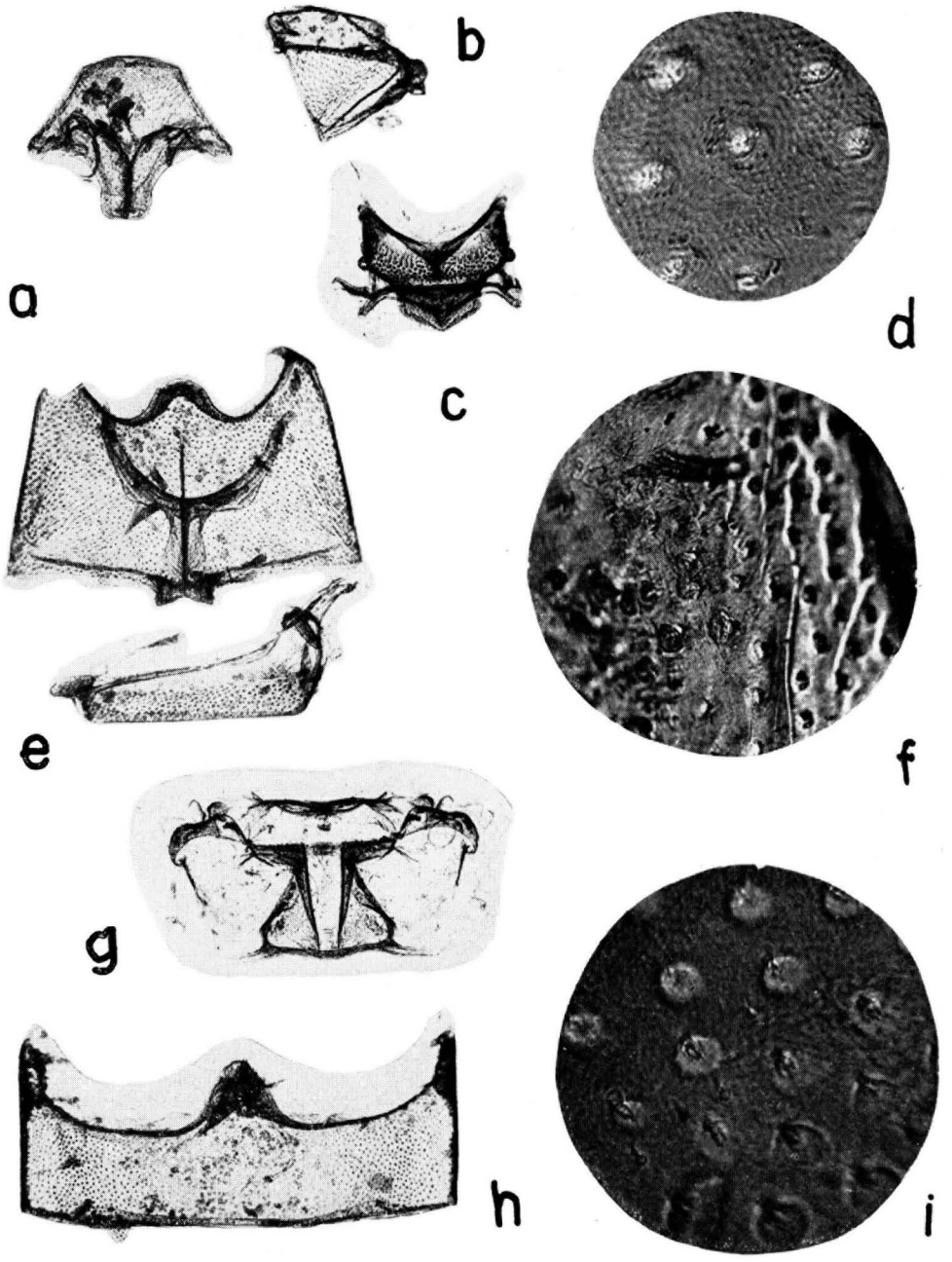

Plate 30. Mealworm (continued).

Fig. a: Mesosternite (10×). Fig. b: Mesopleuron (10×). Fig. c: Mesotergite (10×). Fig. d: Mesosternite cuticle (100×). Fig. e: Metasternite and metapleuron (10×). Fig. f: Metasternite cuticle (100×). Fig. g: Metatergite (10×). Fig. h: First abdominal sternite (10×). Fig. i: First abdominal sternite setae and cuticular pattern (210×).

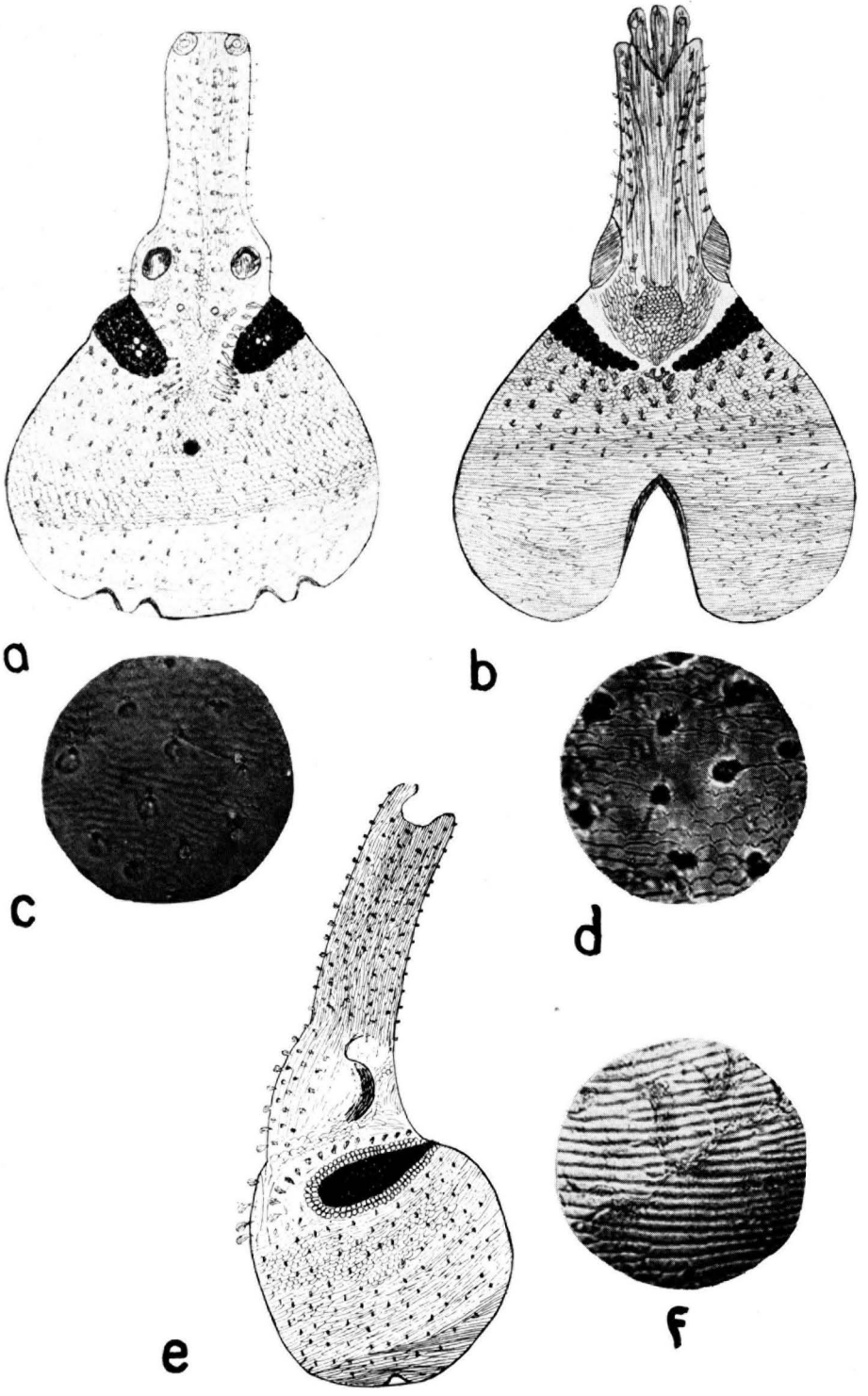

Plate 31. Rice Weevil (*Sitophilus oryza*), Head.

Fig. a: Dorsal view (75×). Fig. b: Ventral view (75×). Fig. c: Setae, dorsal surface (300×). Fig. d: Setae and cuticular ridging, ventral surface (300×). Fig. e: Lateral view (75×). Fig. f: Cuticular striations (210×).

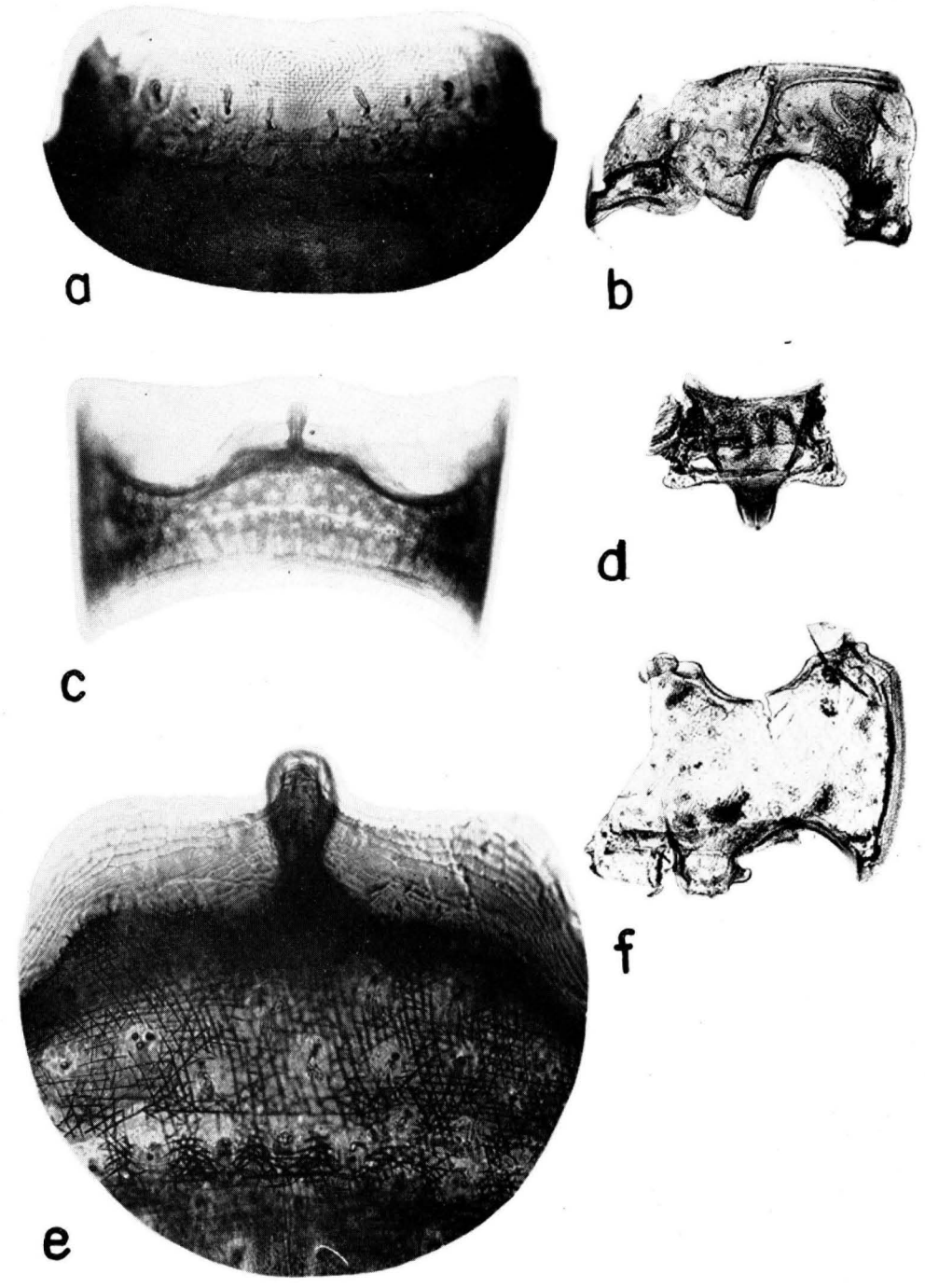

Plate 32. Rice and Granary Weevil (*Sitophilus oryza or S. granarium*) (continued).

Fig. a: Prothorax, anterior margin (100×). Fig. b: Mesosternite and pleural fragment (40×). Fig. c: First abdominal sternite (40×). Fig. d: Mesotergite (40×). Fig. e: First abdominal sternite setae and cuticular pattern (210×). Fig. f: Metasternite fragment (40×).

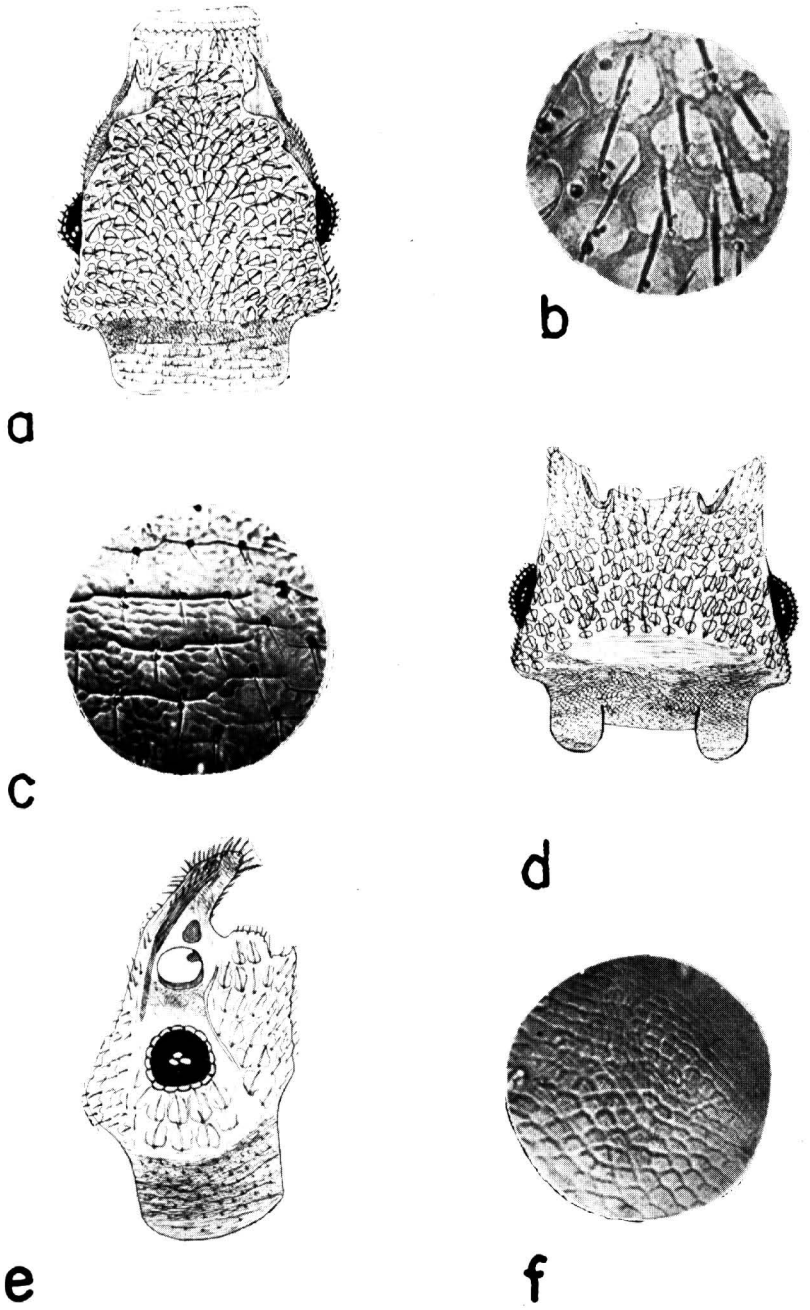

Plate 33. Sawtoothed Grain Beetle (*Oryzaephilus surinamensis*), Head.

Fig. a: Dorsal view (75×). Fig. b: Setae and cuticular pattern, dorsal surface (300×). Fig. c: Setae and cuticular ridging, ventral surface (300×). Fig. d: Ventral view (75×). Fig. e: Lateral view (75×). Fig. f: Cuticular pattern, lateral surface (300×).

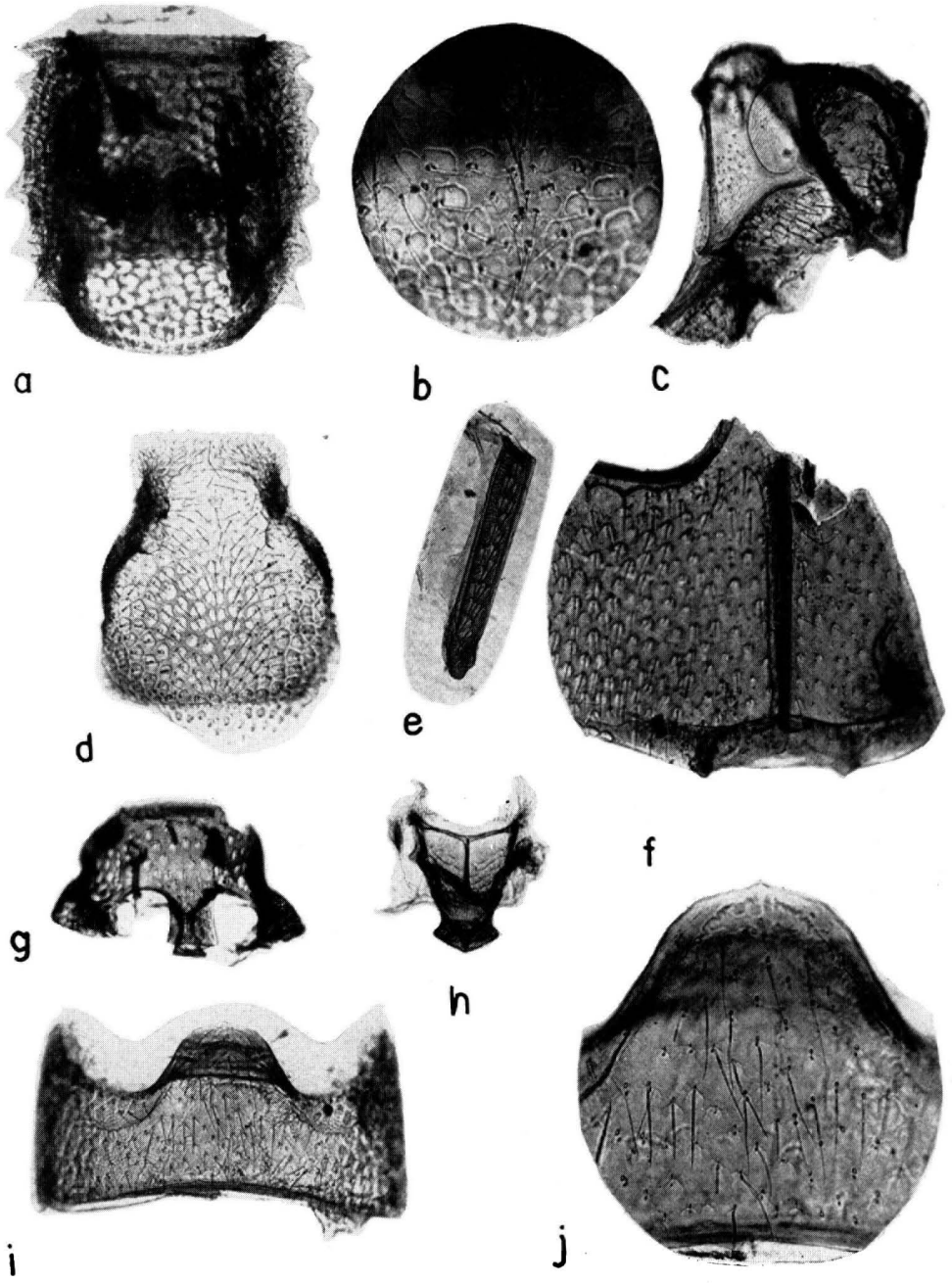

Plate 34. Sawtoothed Grain Beetle (continued).

Fig. a: Prothorax, dorsal view (50×). Fig. b: Prothorax, setae, and cuticle, dorsal surface (100×). Fig. c: Mesosternite and pleuron (50×). Fig. d: Head capsule (50×). Fig. e: Metapleuron (50×). Fig. f: Metasternite (50×). Fig. g: Mesosternite (50×). Fig. h: Mesotergite (50×). Fig. i: First abdominal sternite (50×). Fig. j: First abdominal sternite setae and cuticular pattern (100×).

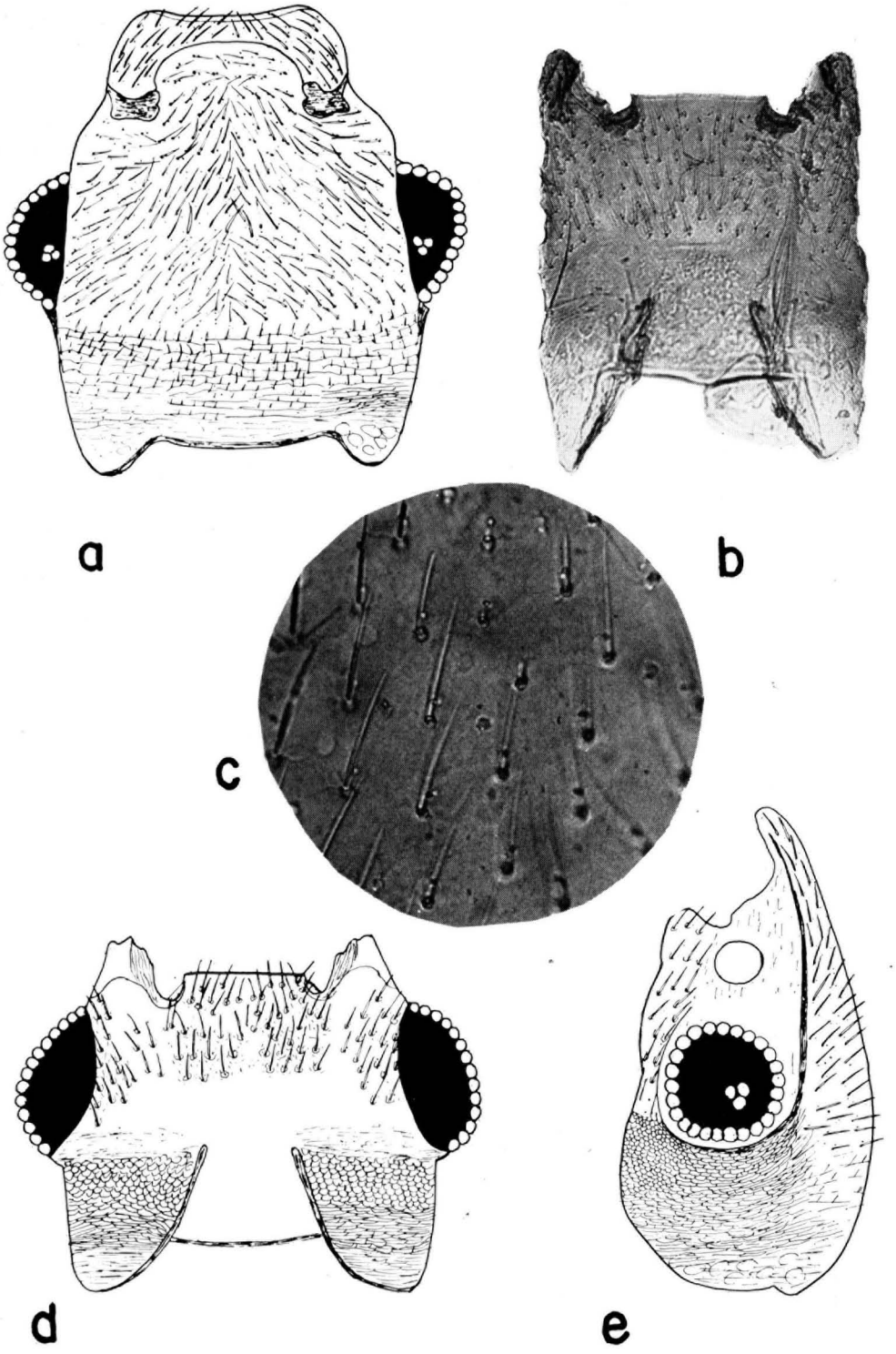

Plate 35. Square-necked Grain Beetle (*Cathartus quadricollis*), Head.

Fig. a: Dorsal view (100×). Fig. b: Ventral view (100×). Fig. c: Setae, dorsal surface (430×). Fig. d: Ventral view (100×). Fig. e: Lateral view (100×).

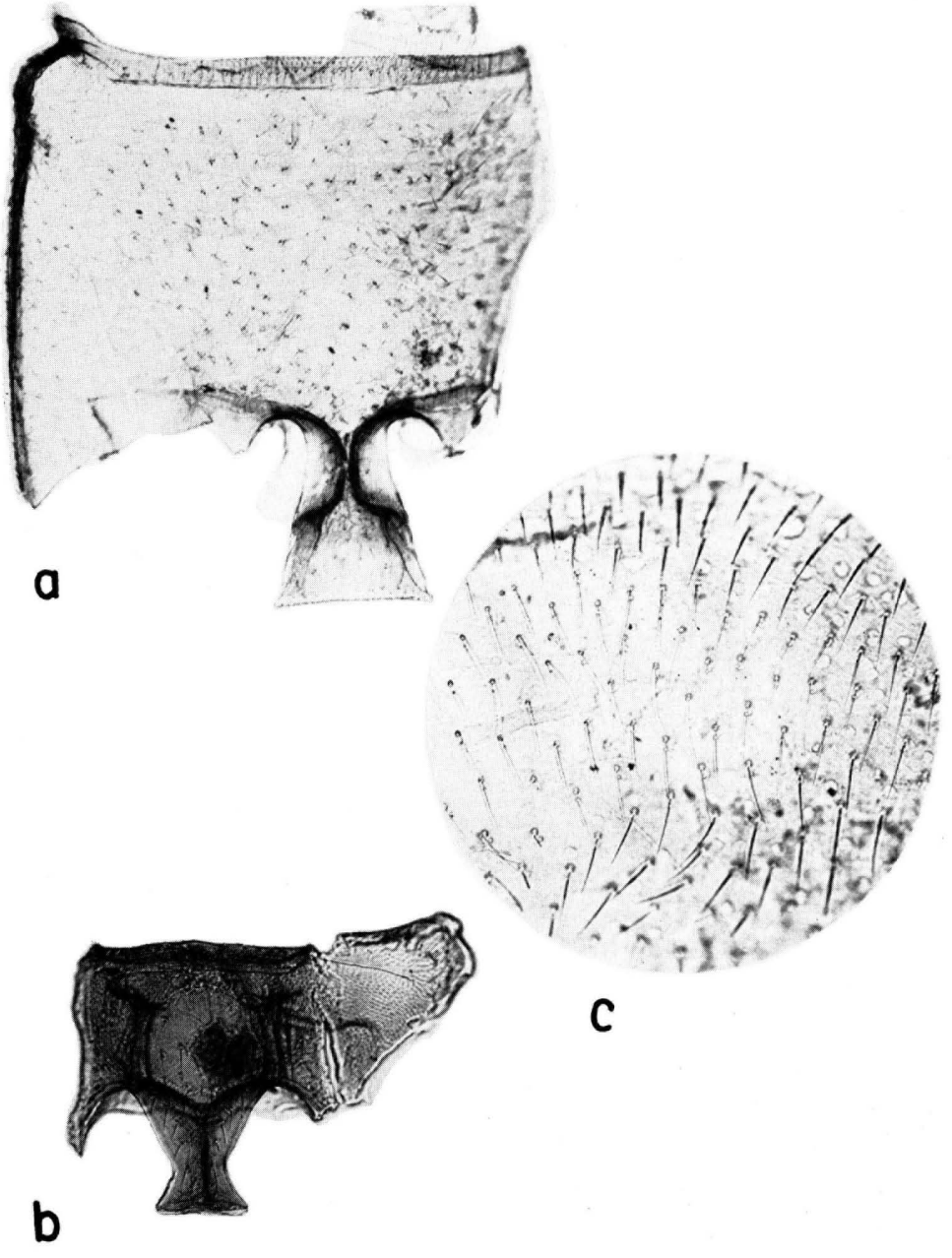

Plate 36. Square-necked Grain Beetle (continued).

Fig. a: Prothorax, ventral view (100×). Fig. b: Mesosternite and fragment of pleuron (100×). Fig. c: Prothoracic setae, dorsal surface (210×).

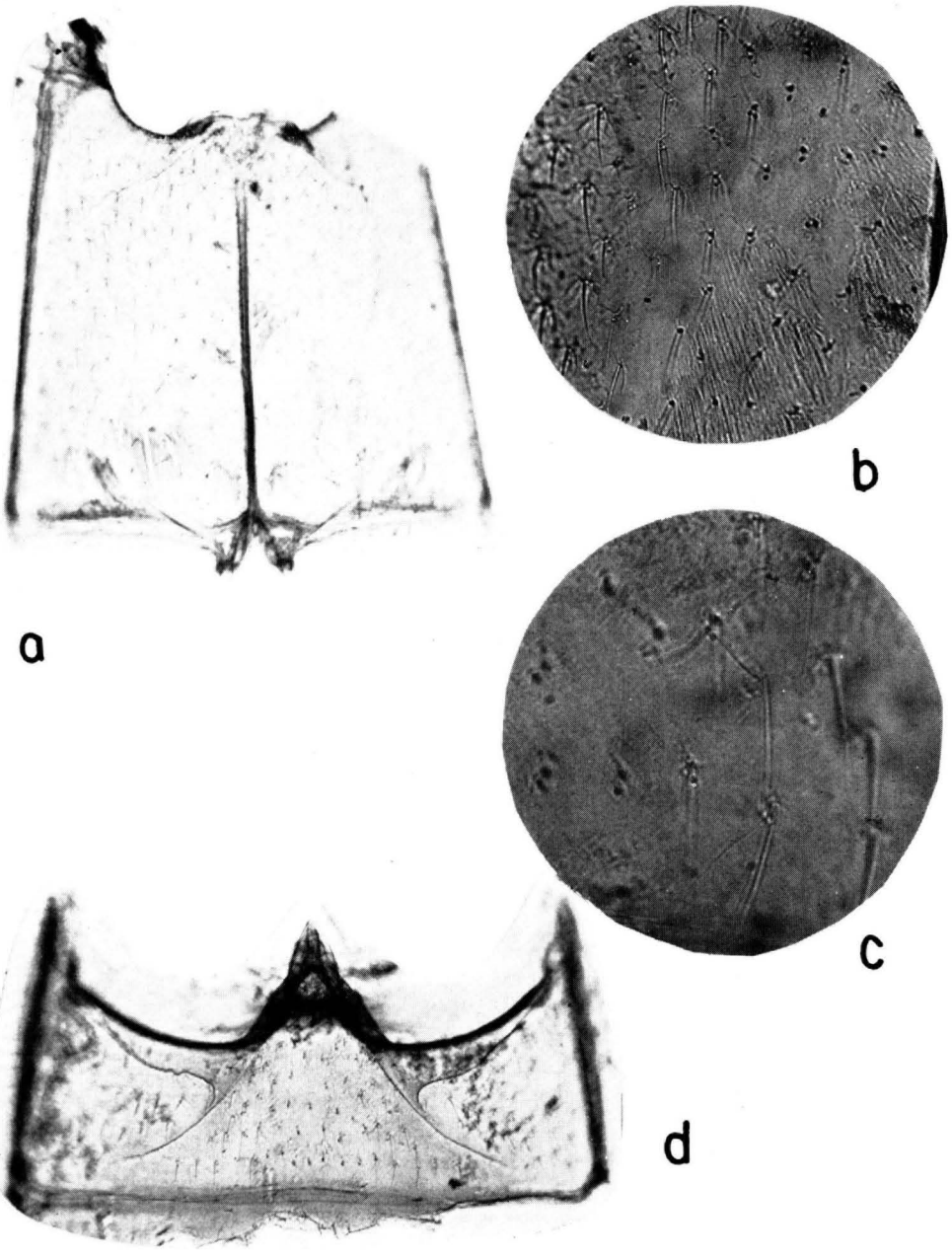

Plate 37. Square-necked Grain Beetle (continued).

Fig. a: Metasternite (100×). Fig. b: Metasternite setae and cuticle (210×). Fig. c: First abdominal sternite setae (410×). Fig. d: First abdominal sternite (100×).

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Cockroaches and Their Fragments as Food Contaminants*

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The identification of insect parts, or fragments, in food products by their micromorphological characteristics has been investigated extensively (1–7). Previous publications have reported studies of the storage or cereal insect group. When insects infest cereal grains that are subjected to grinding, the contamination appears in the finished flour as relatively large numbers of microscopic fragments. The character and condition of these fragments have been contrasted with insect filth incorporated during post-milling contamination (2).

Food sanitation must also deal with other forms of insects that inhabit food processing establishments. Cockroaches, unlike many of the storage insects—beetles and moths, for example—infest foods under such conditions that contamination of the finished food product does not usually occur in large numbers of microscopic fragments.

Cockroach infestation is generally associated with grossly insanitary conditions, especially conditions that have remained unchecked for a long time. The habits of roaches are as characteristic as their structures. Their secretive habit of hiding during the day under debris of all kinds in dark, warm, damp areas makes it difficult to detect their presence. Generally these concealed quarters are somewhat removed from the immediate product zone, especially where processing equipment is in operation, and the roaches move into the exposed foods or manufacturing equipment during their night prowlings when plant operations have ceased. Hence, the contamination most frequently occurs as small (immature) whole or mangled forms that have become entrapped in the food material, or as large body parts or similar parts that have been isolated from partially decomposed or moisture-soaked specimens after subsequent processing and handling, or which readily break from fragile cast skins. Cockroaches have the peculiar habit of devouring the old skin after it has been cast. Since their excreta pellets are deposited indiscriminately, some fragments, not always identifiable, may stem from this source. Because of the peculiar habits of roaches, and because contamination usually occurs at a point in the food establishment or in a stage of the technological process at which the insect is not subjected to severe comminution, the identification of roach material recovered from foods can usually

* No. X in a series of related studies.

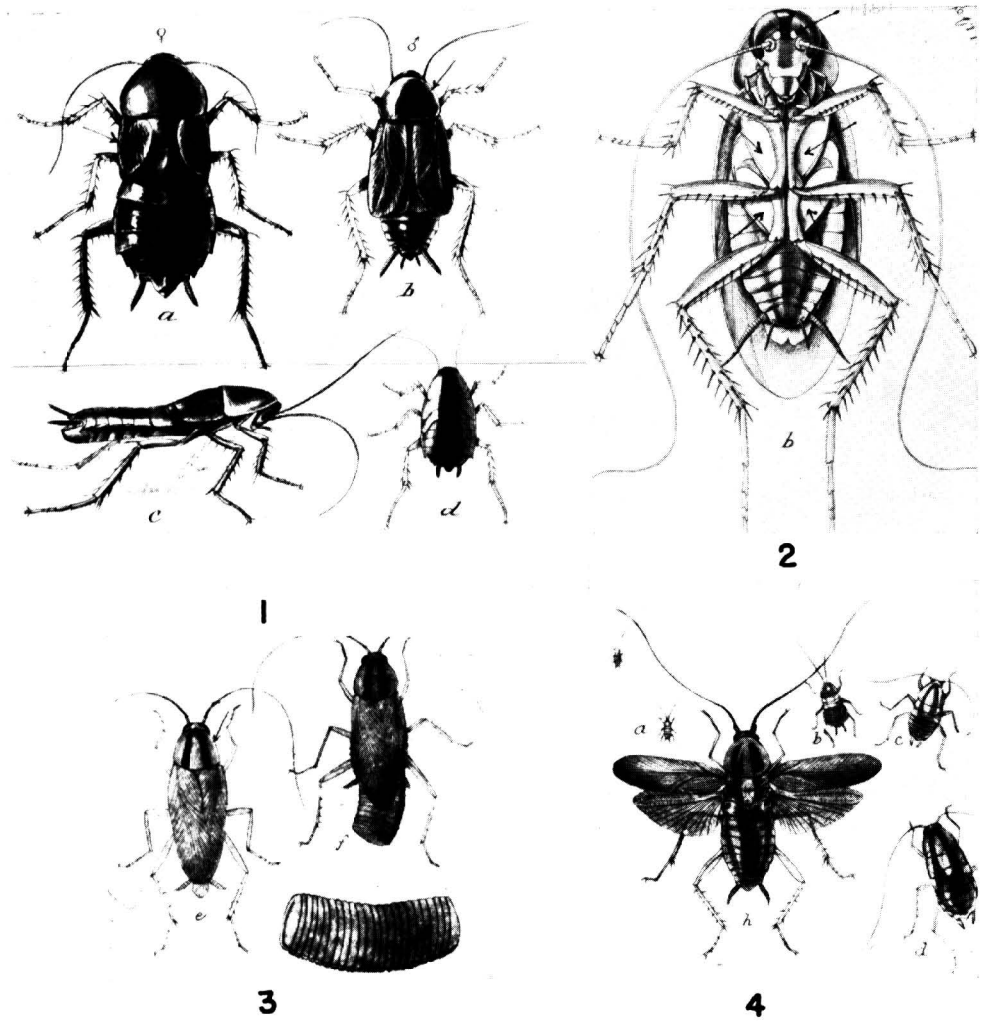


Fig. 1. Oriental cockroach. a. Mature female with shortened wing pads. b. Mature male with abdomen partly exposed. c. Side view showing dorso-ventro flattening. d. Immature (nymph) form.
Fig. 2. American cockroach, ventral view. (Note position of the head, long antennae, and enlargement of the basal leg structures, or coxae.)
Fig. 3. German cockroach. Note the paired stripes on the pronotum, characteristic of this species, and the egg capsule carried by the female (f) and the enlarged capsule below it.
Fig. 4. German cockroach: Various stages of the immature form and the adult with fully developed wings. These are only slightly less than natural size.

be based on rather gross morphological regions, structures, or areas involving primarily a "macro" rather than a micro-morphological determination.

Inspection of food manufacturing establishments has shown that the most prevalent species of roach infestation are the German (*Blatella germanica*), Oriental (*Blatta orientalis*), and American (*Pereplaneta americana*). Of the three species, the German Roach is the most widely distributed geographically and the most commonly encountered in food establishments. On the basis of fragments and mangled specimens, especially those of immature forms, it was found impractical and frequently impossible to differentiate the three species. The illustrations and discussion presented here are representative, for the most part, of cockroaches as a group.

This paper presents a general discussion of cockroaches that commonly occur in food establishments, and briefly describes, with illustrations, the types of cockroach parts that have been, or are likely to be, recovered from the contaminated product.

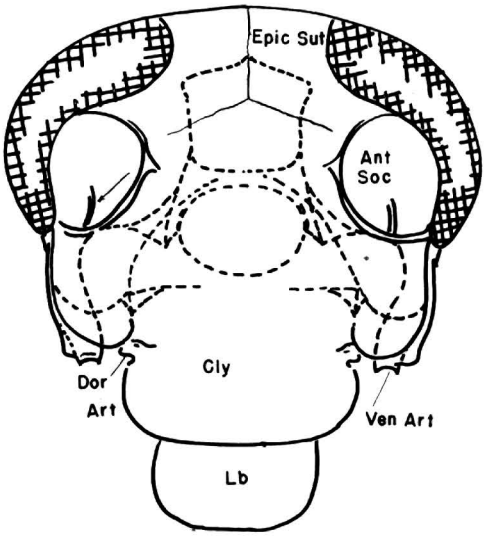


Fig. 5. Cockroach head (drawing). Dotted lines indicate structures below, solid lines above. Identification: Lb, labrum; Cly, clypeus; Dor Art, dorsal mandibular articulation; Ven Art, ventral mandibular articulation; Epic Sut, epicranial sutures; Ant Soc, antennal socket. The antennae and mouthparts are not shown.

General Characteristics

Roaches are characteristically depressed (flattened) dorso-ventrally (see Fig. 1c, side view), smooth bodied, and chestnut brown to black in color. The head is decidedly withdrawn beneath the conspicuous, shield-like pronotum (Figs. 1c and 2) and assumes a nearly horizontal position with the mouth parts directed posteriorly. The many-segmented antennae are extremely long and slender (Fig. 2), and the relatively long and bristly legs are fitted for rapid running. Because the body is flattened, the large basal leg segments (coxae) occupy most of the ventral surface (Fig. 2; see arrows). In most mature (adult) forms, two pairs of wings are present, the outer pair thick and leathery, the inner pair membranous. The wings of the mature female Oriental roach are vestigial and appear as ovate, lateral pads (Fig.

Fig. 6. Clypeus attached to part of the head cuticle. Note dorsal mandibular articulation at arrow.

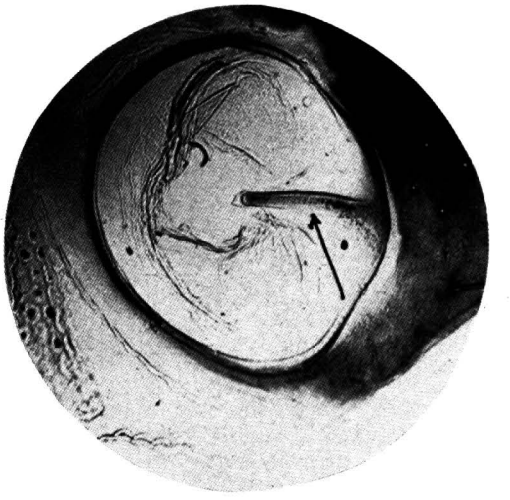


Fig. 7. Isolated antennal socket. Note rod-like structure at arrow.

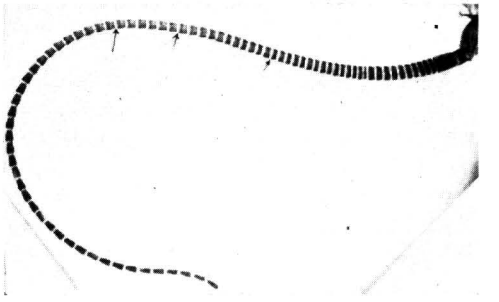


Fig. 8. Intact antenna. Note membranous articulations at arrows.

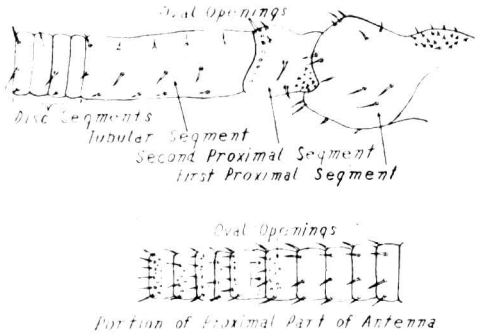


Fig. 9. Basal segments of antennae. Note hourglass shape of second segment, emargination along one side of first segment, and patch of minute spines on the two basal segments.

1a; see arrow). Immature forms (nymphs) resemble the adults except in size and in the degree of wing development. Early nymphs (Fig. 4a, b, c, and d) have no wings but later instars develop small lateral pads that gradually develop into adult structures.

Structural and Microscopic Characters

The fragment structures and characters illustrated and described below are those likely to occur in food products produced under conditions that permit infestation by cockroaches. The structures illustrated are also basic to an interpretation and identification of certain fragments or components breaking from them.

Head Capsule (Fig. 5): See head in Fig. 2. Like the body, the head is generally compressed dorso-ventrally and is somewhat triangular or pear-shaped; compound eyes are large and elongate, and occupy much of the head region. The clypeus (Figs. 5 and 6) extends broadly beyond the paired, hook-shaped, dorsal mandibular articulations (Fig. 6; see arrow). The socket for the reception of the antennal base is distinguished by the presence of a rod-like structure (antennefer), extending from the rim to the center of the socket (Figs. 5 and 7; see arrow). Between the bases of the antennae on the head is a pair of shallow, depressed areas that mark the attachment of internal muscles (see arrow, Fig. 2).

Head Appendages: These structures, like those of most insects that contaminate foods, readily break from the body. They are the most readily recognized of the discrete insect structures and usually provide an array of characteristic landmarks, especially as an intact structural fragment, or in a fragmented form.

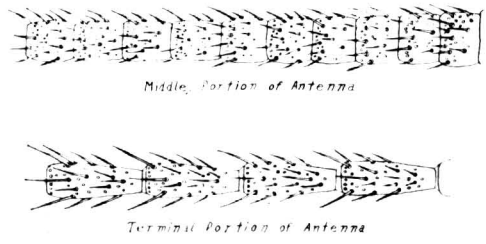


Fig. 10. Middle and terminal segments of antennae.

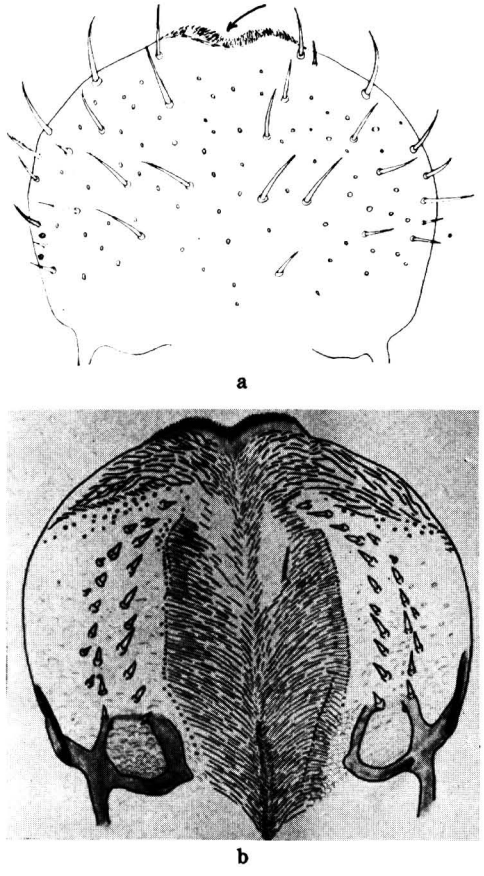
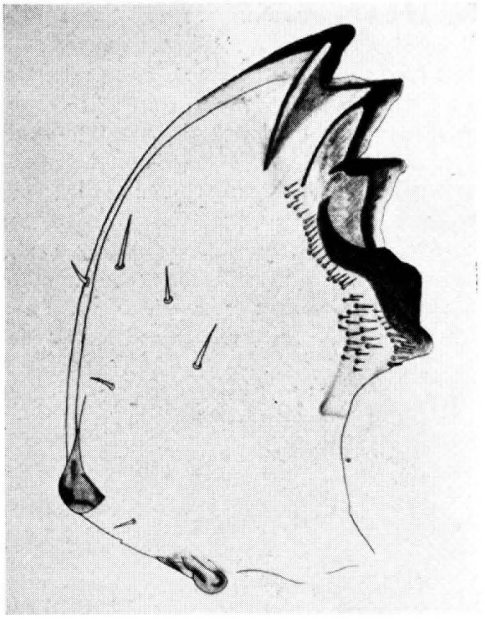


Fig. 11. Labrum. a. Dorsal view. b. Ventral view.

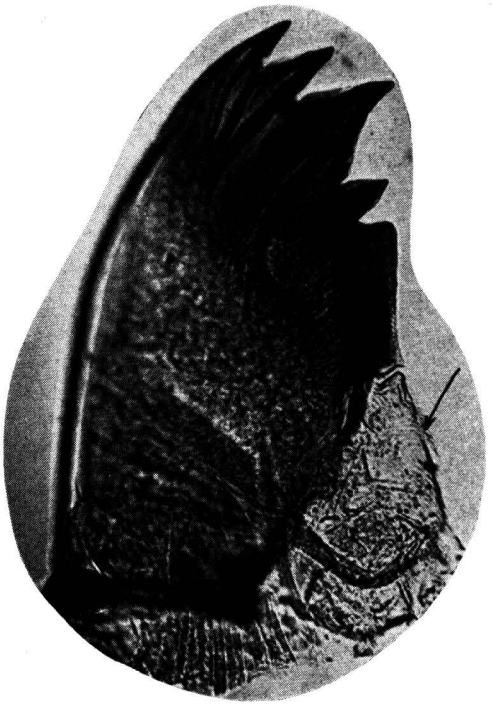
Antennae (Fig. 8): The intact structure is not likely to be found as an isolated fragment, but may be essentially intact on



a

b

Fig. 12. Mandibles. a. Right. b. Left. c. Left with membranous appendage at arrow. Note row of short setae along base of teeth.



c

whole or mangled roaches (especially small nymphs) present as contaminants. The many-segmented structure tapers very slightly and gradually to the apex. The basal segments (Fig. 9) are wide and short, the middle segments (Fig. 10) nearly square, and the distal segments elongate (Fig. 10). Most segments have minute, oval pores (high magnification). Setae are profuse and become more abundant as the segments lengthen. Especially noticeable are the setae at the distal end of most segments, which are arranged more or less in rows. Individual segments are connected by a delicate translucent membrane (Fig. 8; see

Fig. 13. Isolated intact maxilla. Note that mesal lacinia is partly superimposed on lateral galea.

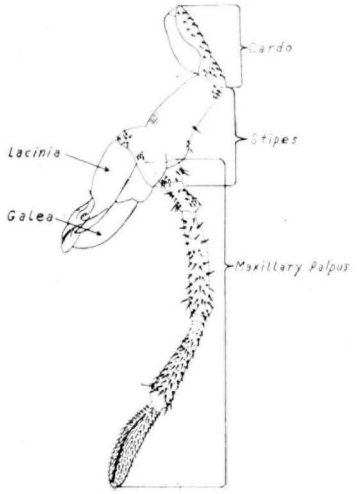


Fig. 14. Drawing of maxilla with components labeled.

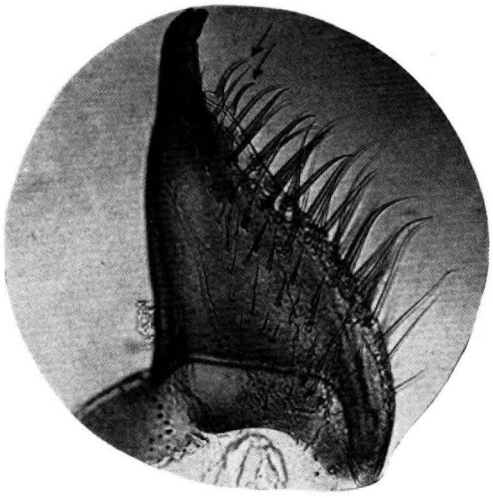


Fig. 16. Lacinia of German cockroach.

arrows). Such segments, or groups of them, are common structural fragments resulting from roach contamination. Most setae on the antennae are typical; they have a slight general curvature except for the very tip, which curves back to form a minute hook. (See Fig. 10.)

Labrum (Figs. 5 and 11a and b): This is a broad, flat lobe; it is distinctly indented at the narrowed apex, which is clothed with a dense brush of small hairs (see arrows). The dorsal surface (Fig. 11a) is simple; it is equipped with only scattered small setae and minute oval pores. The ventral membranous surface (epipharyngeal) is more highly ornamented (Fig. 11b); its most distinctive features are the paired, curved

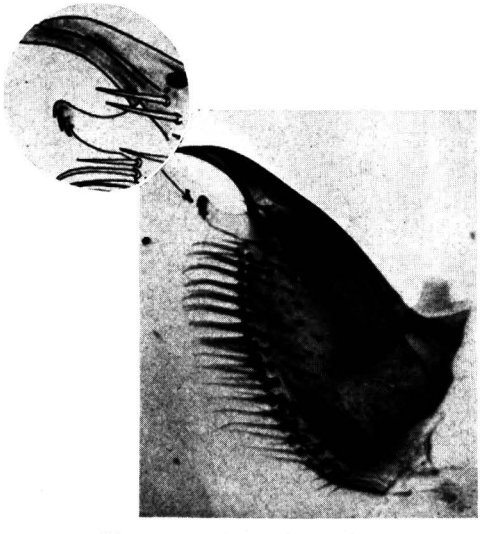


Fig. 15. Lacinia of maxilla.

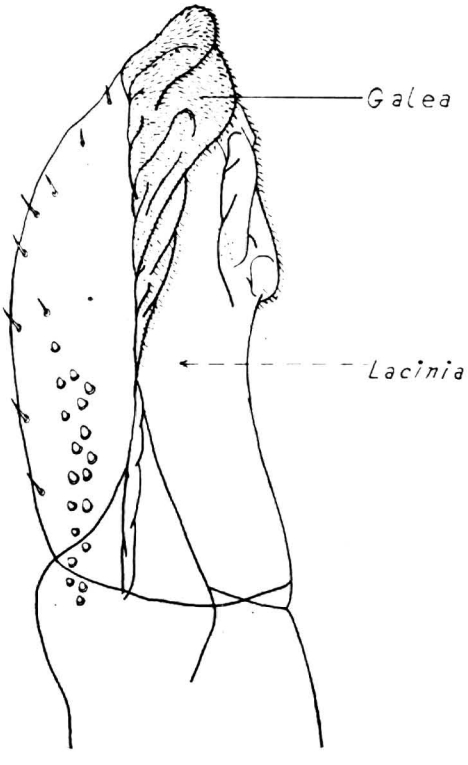


Fig. 17. Drawing of galea of maxilla.

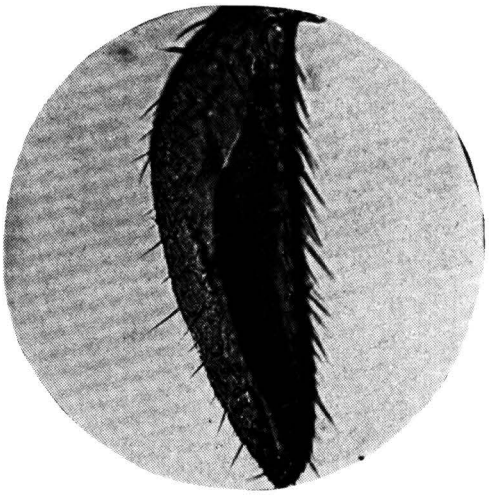


Fig. 18. Terminal segment of maxillary palpus.

rows of stubby setae, the thickened, pigmented rings at the proximo-lateral corners, and the central area of fine hairs or striations.

Mandibles (Fig. 12a, b, and c): The toothed lobes of the opposing right and left mandibles differ distinctly. A translucent membranous appendage occupies the mar-

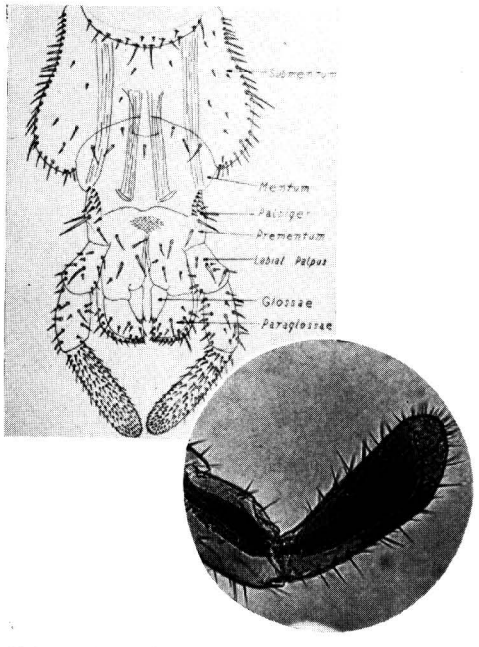


Fig. 19. Drawing of intact labium with components labeled. Note insert of terminal palpal segment.

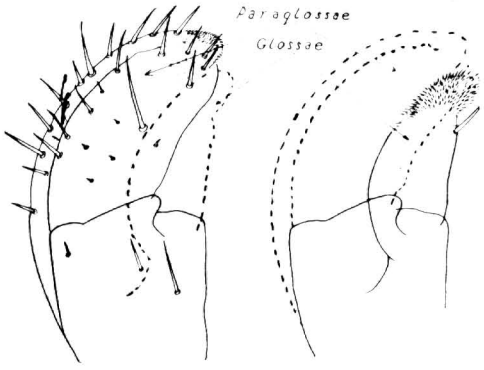
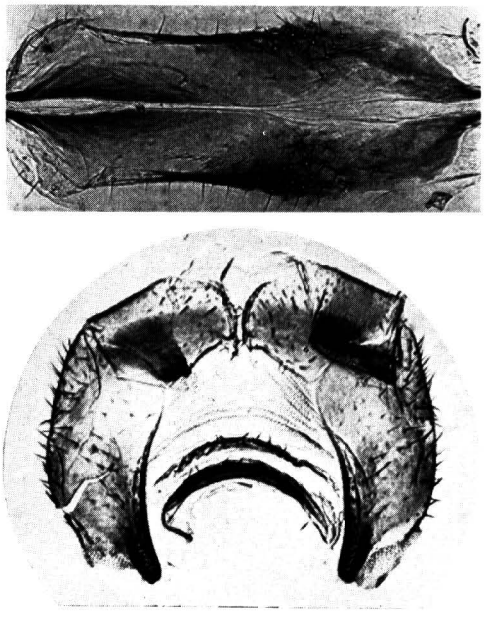


Fig. 20. Enlargement of glossae and paraglossae.

gin between the molar tooth and the base of the mandible (Fig. 12c; see arrow). Often this appendage becomes dried and shriveled or is absent from the isolated mandible.

Maxillae: This paired appendage is composed of several segments. In general form (Figs. 13 and 14) it resembles the maxillae of other insects (beetles). The most distinctive feature is the characteristically shaped mesal lacinia (Fig. 15) which terminates apically in a pair of hardened, curved teeth and a somewhat membranous finger-like



Figs. 21 and 22. Neck sclerites. The articulating membrane (see Fig. 22, bottom) is profusely clothed with microspines.

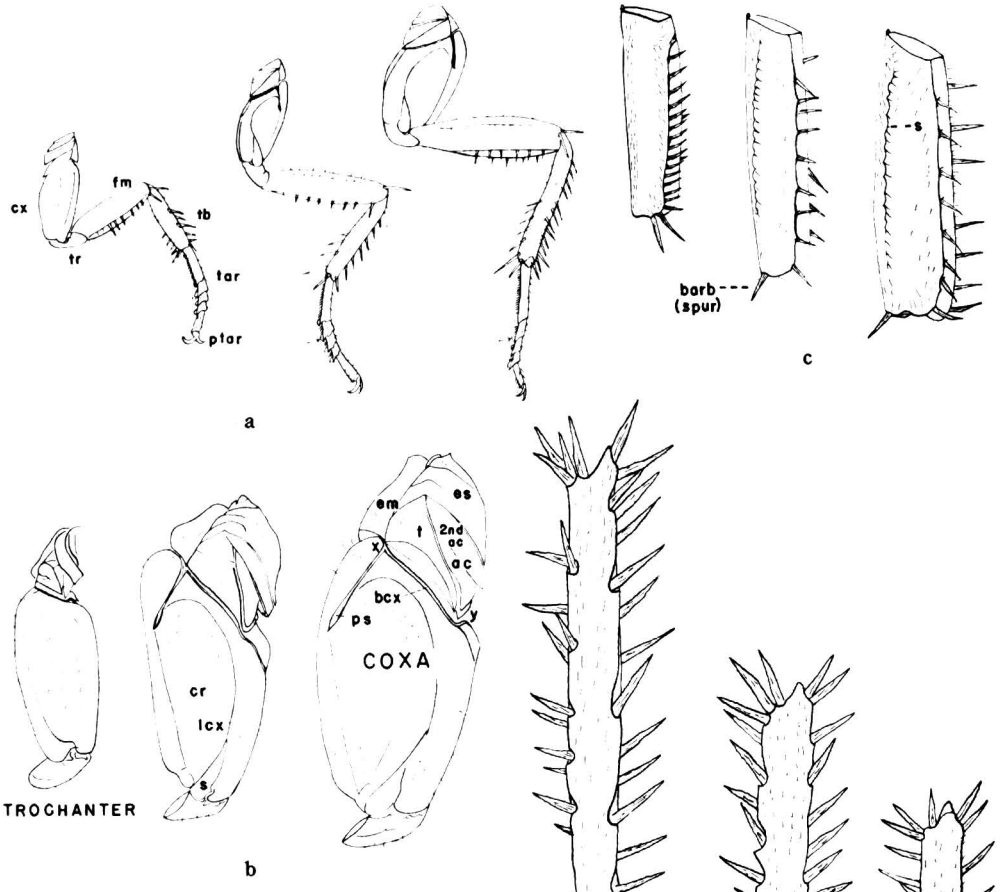


Fig. 23. a. Drawing of intact legs. From left to right: front, middle, hind. Identification: cx, coxa; tr, trochanter; fm, femur; tar, tarsus; ptar, pretarsus. b. Coxae, From left to right: front, middle, and hind coxae. Identification: x and y are points of articulation to the thoracic sclerites; ps, pleural suture; cr, oval depressed area; lcx, semicircular suture; bcx, basicoxal suture; s, shield. c. Femora. From left to right: front, middle, hind. Note shape and double row of stout setae along one side. s = suture. d. Tibiae. From left to right: front, middle, hind. Note size and arrangement of setae (barbs).

appendage with three short, hooked teeth (Fig. 15; see arrow). The maxillae of the German roach do not have this triple-toothed appendage; in its place is a pair of slender, curved, setae-like structures arising from a membranous raised base (Fig. 16; see arrows).

The lateral galea (Figs. 14 and 17) is roughly rectangular. It is densely clothed with fine hairs distally and stubby, cone-shaped setae along the lateral-basal half.

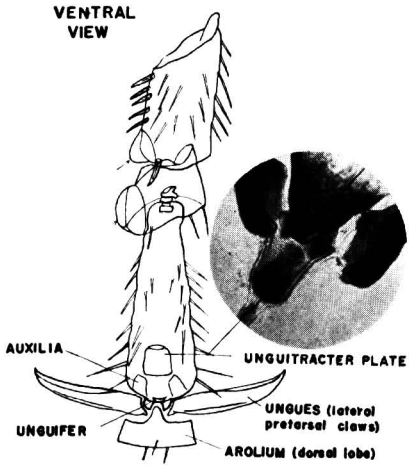


Fig. 24. Tarsal segments. Note arrows.

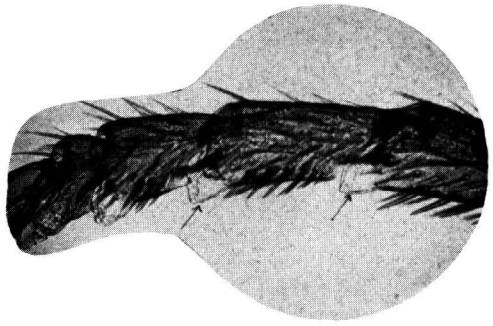


Fig. 25. Drawing of terminal tarsal segment with parts labeled. Note insert showing surface pattern of unguitracter plate.

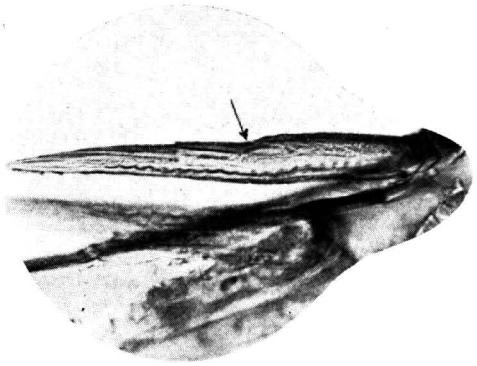


Fig. 27. Large setae from tibia showing "step" at arrow, and irregular polygons of surface cuticle.

The 5-segmented palpus (Fig. 14) is heavily clothed with setae which become denser distally. A shallow groove extends the full length of the terminal segment (Fig. 18) and appears as a translucent membranous area when viewed under the widefield microscope.

Labium: (See Figs. 19 and 20, which show labeled parts.) Like the antennae and maxillary palpi, the setae of the labial palpal segments become more profuse distally.

Thoracic Region: As mentioned in the general discussion, the oval, shield-like pronotum (Figs. 1 and 3; see arrows) is the conspicuous portion that conceals the head. It is useful in identifying cockroaches that are recovered essentially intact, but is not a good source of identifiable fragments.

Neck Sclerites (Figs. 21 and 22): The intact structures are unique. They occur in the articulating membrane between the head and thorax.

Legs: (Fig. 23a. See Figs. 23b, c, and d for enlargement of individual leg segments.) Experience has shown that the segments of the tarsus are recovered from contaminated foods more frequently than any other identifiable fragment. The leg shape and detailed morphology of all species are similar

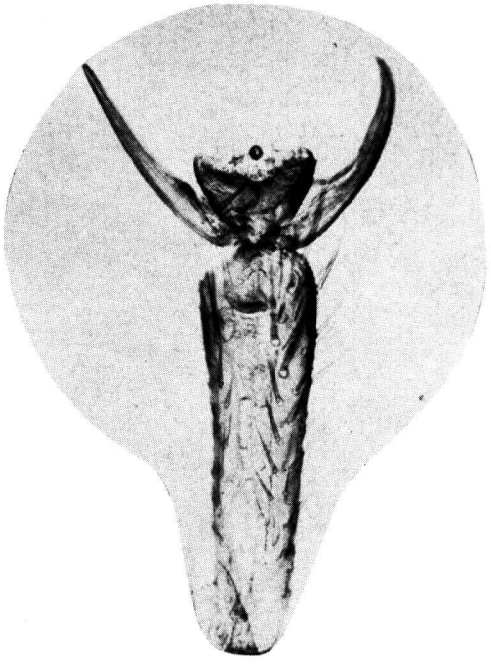


Fig. 26. Last tarsal segment with claws.

Fig. 28. Large seta from tibia showing serrations.

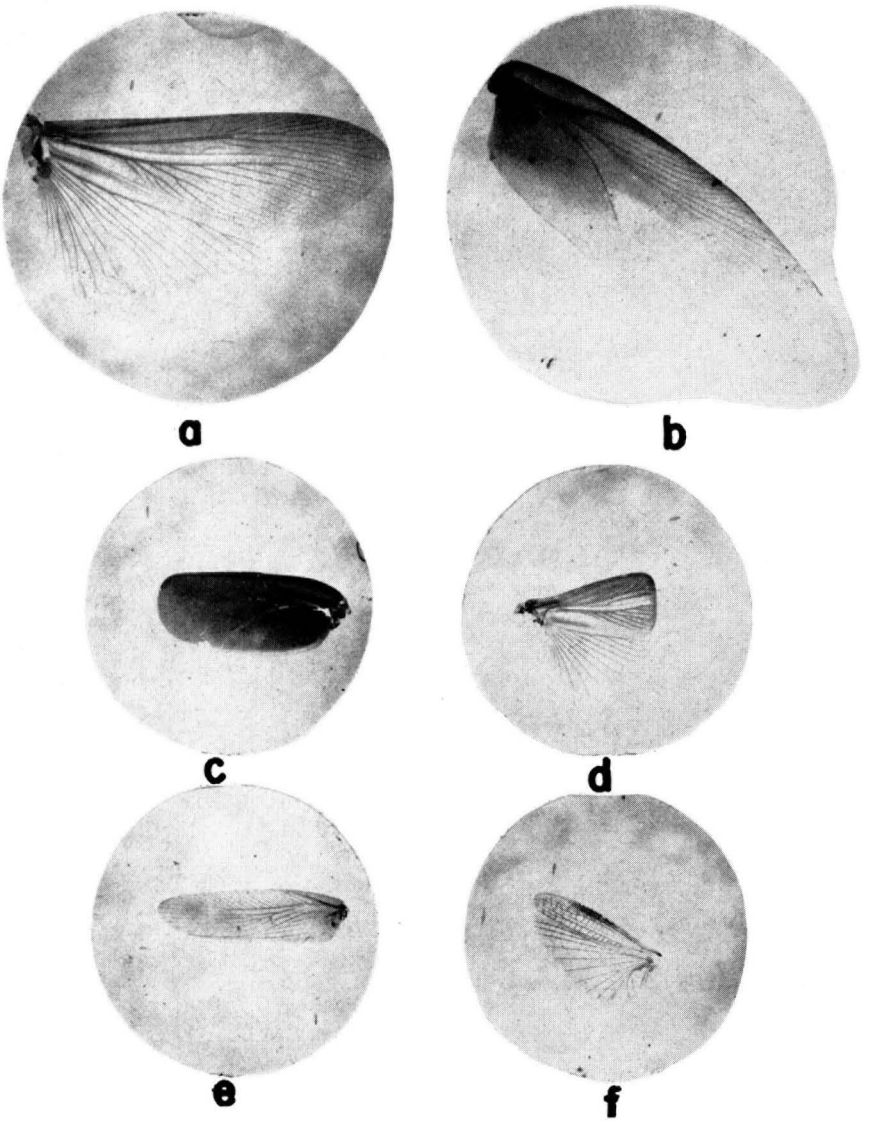


Fig. 29. Outer and inner wings of adult cockroaches. a. American (inner wing). b. American (outer wing). c. Oriental (outer wing). d. Oriental (inner wing). e. German (outer wing). f. German (inner wing).

and characteristic. Although the legs are bulky, the cuticle is delicate, thin, and pliable, in contrast to the thick, brittle, horny cuticle of many beetle exoskeletons.

The most useful and positive landmarks for identification are the translucent, glossy projections (pulvilli) beneath the distal end of the tarsal segments (Figs. 23a, 24 and 25; see arrows), the complex morphology of the last tarsal segment with its paired claws (Figs. 24 and 26), and many of the large setae that are borne by most of the leg segments. The most prominent and distinctive setae are those with a vague to distinct "step" about midway along one side (Fig. 27; see arrow) and varying degrees of serrations along part or all of one side (Fig. 28). Under high magnification most of these setae appear striated or ridged, perhaps because of the irregular and elongate polygons of the surface cuticle (Fig. 27).

Cockroach Wings (Fig. 29a, b, c, d, e, and

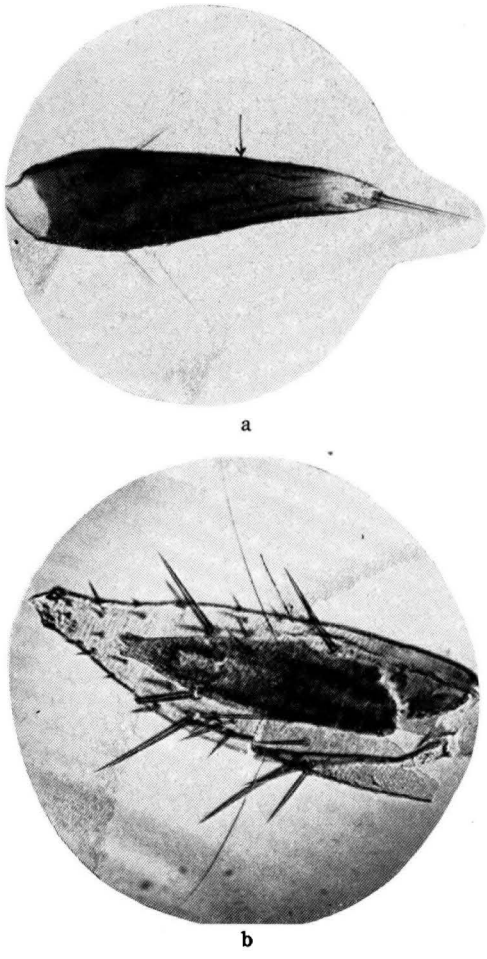


Fig. 30 a and b. Intact cerci of cockroach nymphs.

f): The wings reach their characteristic form and venation in the adult stage. Unless a significant portion of either the fore-wing (leathery tegmina) or the hind-wing (membranous) is available as a fragment, positive identification is doubtful.

Abdomen: In terms of isolated parts and nondescript fragments, the abdomen provides few features for positive identification. The paired cerci projecting from the tip (see caudal segmented appendages, Fig. 4h) are distinctive structures. They appear to vary somewhat in form among the nymphal instars (Figs. 30a and b) and differ strongly between nymphs and adults. The adult cerci (Fig. 31) are distinctly segmented, with prominent joints between the segments. The

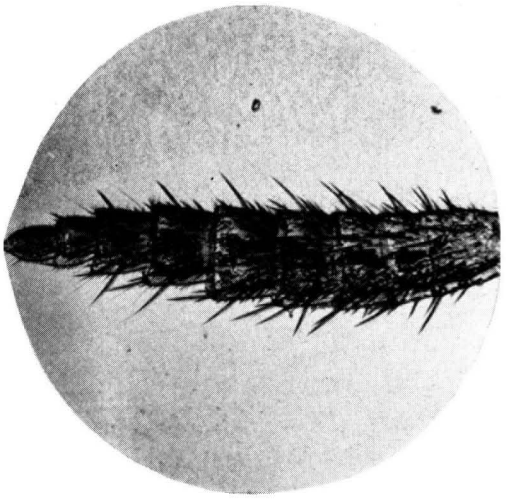


Fig. 31. Adult cockroach cercus.

nymphal cerci are cone-shaped, with a smooth surface and without the jointed segmentation of the adult cerci; even at high magnification the lines of segmentation are represented only by fine fractures encircling the cone (Fig. 30a and b; see arrow). Early nymphs appear to be 3-segmented, and although the fine lines of segmentation are vague, the cerci readily fragment into their component segments (Fig. 32). In cross sec-

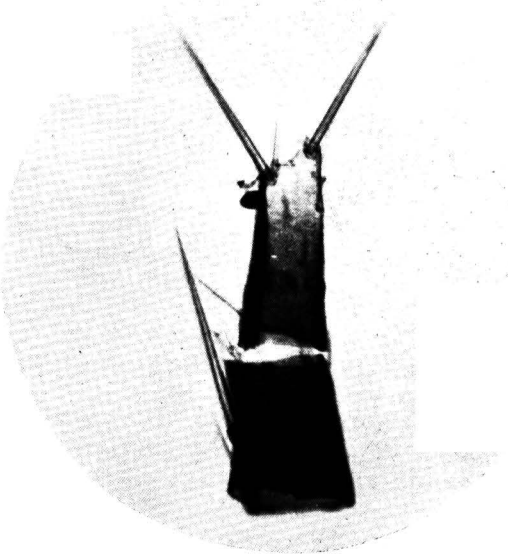


Fig. 32. Fragmented cercus of nymphs. Fragmentation is along fine, vague lines.

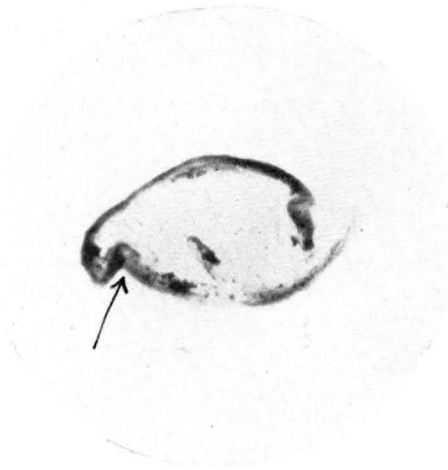
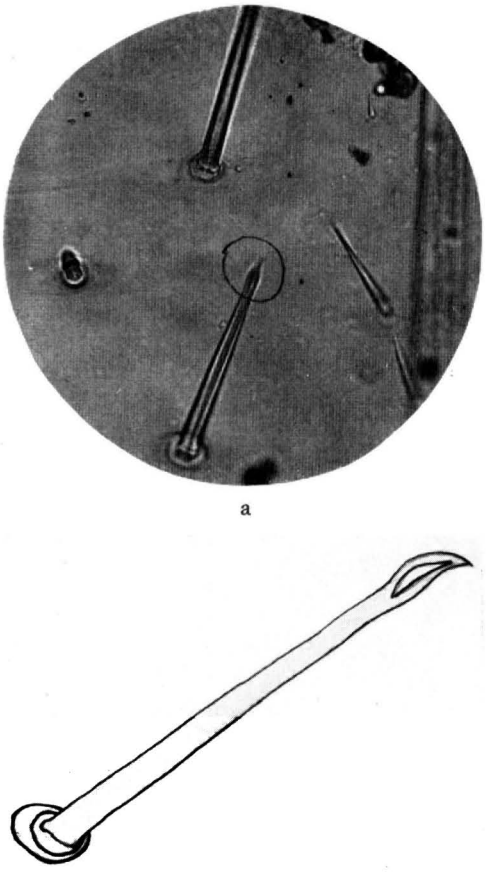


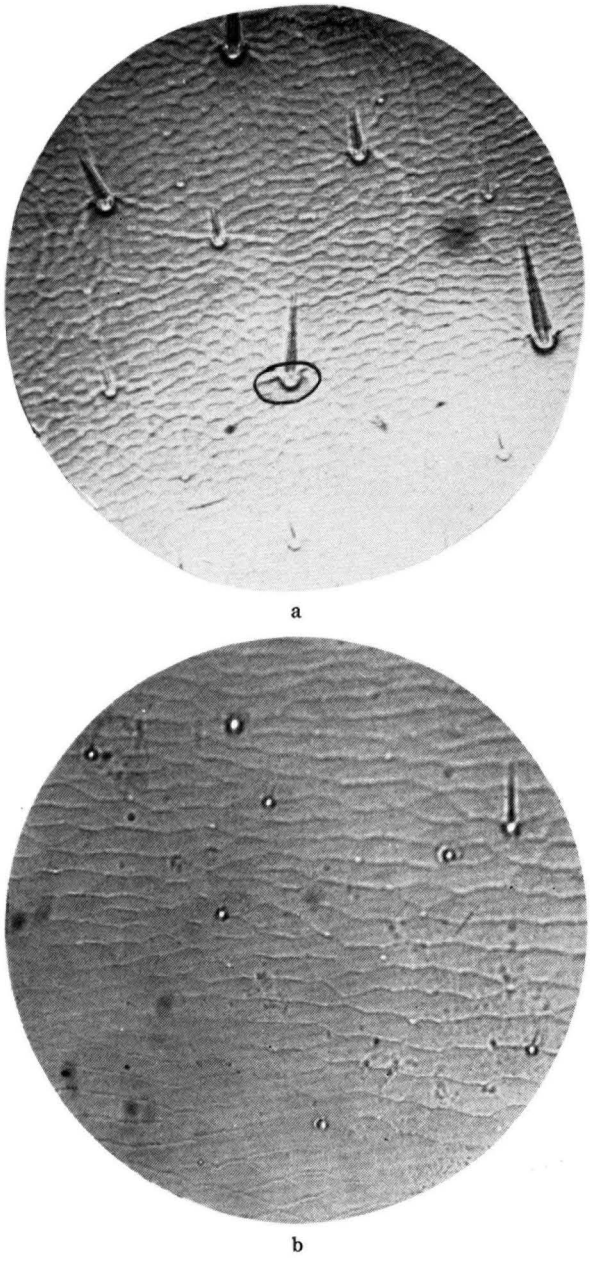
Fig. 33. Cross section of adult cercal segment.



a

b

Fig. 35. Setae from abdominal cuticle. a. Photomicrograph. Note enlarged tip in circle, which looks like the flame on a tapered candle. b. Drawing.



a

b

Fig. 34. Surface pattern of cuticle. a. Showing more distinct polygon cells with close sides and enclosure around setal base (see circle). b. Showing irregular polygons with sides open.

tion (Fig. 33) the segments of the adult cerci are somewhat flattened along one side; the upper and lower halves are jointed by a "membranous" connection in the form of a "clasp," similar to the seam of a metal can (see arrow).

Surface Pattern: At high magnifications the surface cuticle is seen to be reticulately ridged in honey-comb fashion (Fig. 34a and b). The ridges enclose four, five, or six-sided polygonal areas, some of which have a minute pore in one angle. In some areas the polygon may be elongated or the sides of the polygons may be open so as to give a ridged, striated, rasp, or scale-like appearance (Fig. 34b). Setae are widely scattered and small (excluding some of the head and leg appendages). The base or the scar remaining after the setae are broken off is surrounded by a characteristic formation (Fig. 34a; see circle). Some setae from the abdomen, although minute, can be distinguished under high magnification by the enlarged tip (see Fig. 35, circle).

The general cuticle of the adults, and especially of the nymphs, is smooth, rather glassy, relatively thin, and pliable, in contrast to the usually thick, roughened, brittle exoskeleton of many beetles.

Acknowledgment

The following members of the Food and Drug Administration contributed work to these studies: Mary C. Harrigan, Boston District; William Cox, Atlanta District; and Edward Steagall, Los Angeles District.

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NOTES

Note on Determination of Crude Fiber

By ABDUL H. HALLAB (Feed and Fertilizer Laboratory, Louisiana Experiment Station Baton Rouge, La.)

Difficulty with the final filtration is often a complicating factor in the determination of crude fiber. We have found that the following procedure consistently permits rapid, trouble-free filtration:

Place a 30 mm fiber glass disk[1] supported by a Witte plate in a 100 mm diameter borosilicate glass funnel attached to a flask or manifold to which suction may be applied. Add enough asbestos slurry to form a mat approximately 5 mm thick; then apply suction to form the mat. Proceed with filtration, wash with water and alcohol, and continue suction for 10 minutes. Quantitatively transfer asbestos mat and crude fiber to a porcelain crucible for drying and ignition.

The fiber glass disk becomes clogged and should be discarded after four or five filtrations. The asbestos can be reused after suspending it in water, decanting the finer fibers, and adding enough fresh asbestos to provide the original quantity.

[1] Disks are cut from sheets obtained from: Skuttle Manufacturing Co., 150 West Summit St., Milford, Mich.

Blood Withdrawal and Processing Preparatory to Plasma and Cell Cholinesterase Determinations in Rats

By ERNEST C. HAGAN, PAUL M. JENNER, WILLIAM I. JONES, and O. GARTH FITZHUGH (Division of Pharmacology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D.C.)

In an attempt to follow a significant percentage of individual rats through an entire experimental period, and still obtain sufficient quantities of blood for frequent assays, the technique of repeated cardiac puncture was instituted. Previously a certain number of rats were sacrificed at intervals for blood cholinesterase determinations. As a possible aid to survival, the quantities of blood taken for assay were decreased from a previous 4–5 ml to 1 ml.[1] Particulars are outlined in the following paragraph:

One ml of rat blood is withdrawn by cardiac puncture, using syringe equipped with 22 gauge 1″ needle, and rinsed with 0.1% heparin solution. Needle is removed; then blood is carefully squirted into a 10×75 mm culture test tube containing 0.1 ml of the heparin solution. After mild agitation to insure thorough mixing of blood and anticoagulant, the mixture is centrifuged. Plasma is then thoroughly aspirated from the cells, the cells are washed 3 times with physiological saline (0.9%), and 0.2 ml of plasma or cells is diluted to 5 ml with distilled water. Next 2 ml of each enzyme preparation is mixed with 2 ml of its appropriate buffer, and a 2 ml aliquot is carried through the cholinesterase assay procedure.[2]

[1] Blood withdrawal has been repeated on the same rats on alternate days for 14 days with loss of only 20% of animals.

[2] Frawley, J. P., Hagan, E. C., and Fitzhugh, O. G., *J. Pharmacol. Exptl. Therap.*, **105**, 156 (1952).

Microdetermination of the Saponification Number

By FRANK A. LEE (New York State Agricultural Experiment Station,* Cornell University, Geneva, N. Y.)

The necessity of working with limited amounts of material has resulted in the development of many microanalytical methods. Micro and semimicro methods are available for the determination of the saponification number of oils and fats (1–6), but none of these methods were completely satisfactory for samples to be tested in this laboratory. The present A.O.A.C. method (7) would be satisfactory if it could be modified to use micro quantitites of material. This would require a change in the refluxing procedure.

Rupp and Lehmann (8) proposed a macro method in which the melted fat or oil is heated with potassium hydroxide solution in a stoppered flask in boiling water. The present modification employs the heating step of the Rupp and Lehmann procedure except that the temperature of the bath is 75°C, which is just a little below the boiling point of the solutions used. In other respects, the method is like that of the A.O.A.C. (7).

Micro-Kjeldahl Pyrex flasks[1] were used for the saponification. They hold about 18 ml and can be fitted with ₹ 19 reagent bottle stoppers. Other flasks of comparable size

* Approved for publication by the Director of the New York State Agricultural Experiment Station as Journal Paper No. 1119. The author acknowledges with thanks the assistance of Joanne Whitcombe and Sandra Braverman. The assistance of L. Lewin at the start of the work is also acknowledged with thanks.

[1] Microgram-Kjeldahl flasks were obtained from Microchemical Specialties Co., Berkeley, Calif.

Table 1. Saponification numbers of oils[a]

Material	Microsaponification Numbers			Macrosaponification Numbers		
	Av.	Min.	Max.	Av.	Min.	Max.
Olive oil	190.3 (6)	187.7	192.3	189.2 (2)	189.0	189.4
Purified soybean oil	190.4 (8)	188.0	192.5	191.3 (3)	191.0	191.7
Peanut oil	189.6 (4)	188.6	190.9	190.4 (3)	190.1	190.9
Linseed oil	191.8 (2)	190.8	192.7	188.5 (3)	188.4	188.7
Expressed almond oil	188.4 (3)	186.8	190.2	189.5 (2)	189.3	189.7

[a] Figures in parentheses indicate the number of replications.

fitted with ground glass stoppers should work equally well. A Fisher microburet calibrated to 0.01 ml was used for the titrations, and 1% phenolphthalein in 95% alcohol was used as indicator.

Alcoholic potassium hydroxide solution was prepared as follows (7): 1200 ml alcohol in a distilling flask was refluxed for 30 minutes with 10 g KOH and 6 g granulated Al. The alcohol was distilled and 1 liter was collected after discarding the first 50 ml; 40 g KOH was dissolved in this liter of alcohol and the solution was stored in a glass-stoppered bottle.

Standard 0.1*N* HCl was used to titrate the alkali remaining after the saponification of the macro samples. For titration of the micro samples 0.05*N* HCl was employed because this solution was found to give closer checks.

Procedure.—About 40 mg samples of the oil or fatty material are accurately weighed into the micro Kjeldahl flasks. To each sample is added 0.5 ml of the alcoholic potassium hydroxide solution by pipet (the pipet is allowed to drain exactly 30 seconds). Reagent blanks are run at the same time. The flasks are immersed to the bottom of the stoppers in a constant temperature bath at 75°C and are closed with the stoppers after the contents have warmed to the temperature of the bath (2–3 min.). The flasks are then held in the bath for 50 minutes. Slight shaking is desirable during the heating period; this can be effected by mechanical means. After the heating period the tubes are removed from the bath, a drop of phenolphthalein indicator is added to each, and the excess alkali is titrated, while warm, with standard acid. The flasks are heated again just before the end point is reached. The titer of the blanks minus that of the sample gives the amount of alkali consumed by the saponification of the fatty material.

Check macro determinations were run on the oils by the A.O.A.C. method. Calculations, as directed by the A.O.A.C., were employed for both the macro and the micro methods. The results are shown in Table 1.

While the micromethod described is based on the 40 mg samples, smaller samples can be used if desired.

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BOOK REVIEWS

Topics in Microbial Chemistry. By F. M. Strong. John Wiley and Sons, Inc., New York, 1958. xi+166 pp. Illustrated. Price $5.00.

The topics considered in this book are antimycin, coenzyme A, and kinetin. Dr. Strong presented this material at the inauguration of a new series of Squibb Lectures given at the Rutgers University Institute of Microbiology. He is eminently qualified to discuss these materials, since he is personally responsible for, or acquainted with, most of the basic developments.

The monograph on antimycin furnishes the only published review of this toxic antifungal antibiotic. Produced by a species of Streptomyces, antimycin has been crystallized, separated into several fractions, characterized chemically, physically, and biologically, and tentatively assigned a structural formula. Because of its specific action in blocking hydrogen transport in aerobic organisms, antimycin is an almost indispensable reagent of the enzymologist.

In the second paper the purification and chemistry of coenzyme A are summarized. While this bound form of pantothenic acid is obtained from a number of natural sources, including liver and cultures of *Streptomyces fradiae*, dried brewer's yeast is the only practical source.

Finally, the author strays a little from microbial chemistry to discuss kinetin, a newly discovered plant hormone. This purine derivative differs from the auxins which regulate cell enlargement in that it affects cell division. It has been crystallized from the milk and meat of coconuts and from samples of desoxyribonucleic acid which have been aged or autoclaved for an hour at pH 4.2. However, while kinetin stimulates cell division of plant tissue cultures as well as seedlings and cuttings, it has no stimulatory effect on cultures of various microorganisms.

All three of the substances considered in this book are physiologically active in extremely small quantities. Investigators of microbial products should find, in the detailed descriptions of studies in this book, much profitable information on the use of modern experimental techniques.

WILLIAM W. WRIGHT

Dairy Handbook and Dictionary. By J. H. Frandsen. J. H. Frandsen, Publisher, 92 High St., Amherst, Mass., 1958. Illus. Price $9.75.

The reviewer recommends this book unreservedly and believes it should be available to everyone interested in this broad field. It is divided into three sections: the Handbook proper, a reference section, and the Dictionary.

The Handbook is complete in itself; it contains few references to other work. The various chapters not only cover production and processing of milk and its products, but also deal with such topics as breeding, feeding, and housing of dairy cattle. Each dairy product is treated in a separate chapter, and each chapter is written by an authority in the field. Since this section covers almost all dairy products, the individual chapters are, of necessity, rather brief. Some recent developments are treated, for example, bulk tank shipments and simplified short-time methods for making cheddar cheese from pasteurized milk. This reviewer believes the chapter on butter making should include a description of the rather recently introduced continuous processes, now widely used abroad as well as in the United States.

The reference section contains formulas and tables for use in the laboratory, such as Federal and State standards for the composition of some milk products. The section includes a great deal of other very useful information, all of which cannot be mentioned in this brief review.

A unique and especially valuable part of the book is the Dictionary. In this portion, the author has compiled and defined a large number of words and terms he came across

in his many years of university teaching. The value of this section to the student, the teacher, the laboratory worker—in fact, to all those in dairy work—cannot be emphasized too strongly.

FRED HILLIG

The Lynn Index. A Bibliography of Phytochemistry, Monograph II. Edited by John W. Schermerhorn and Maynard W. Quimby. Massachusetts College of Pharmacy, Boston, Mass., 1958. 39 pp. Price $1.00.

This is the second of a series of monographs providing annotated bibliographic references for plant species which have been investigated to determine chemical constituents of economic importance. The families *Bombacaceae, Elaeocarpaceae, Gonystylaceae, Malvaceae, Sterculiaceae,* and *Tiliaceae,* of the order *Malvales,* are discussed. Three of these families include genera providing the important fibers, kapok, cotton, and jute. An extensive bibliography of analytical data on the seed oil and other parts of these fiber-producing plants completes this portion of the study. This monograph also treats of the phytochemistry of other plants of considerable economic importance, namely, the cocoa bean, cola nut, and karaya gum plants of the family *Sterculiaceae.*

The editors have undertaken a considerable task in organizing the extensive bibliographic file of the late Dr. Eldin V. Lynn, which extends into the 1954 coverage of the chemical investigations of natural products. Pharmacognosists, biochemists, and chemurgic scientists will be grateful to the editors for undertaking the task of organizing this worthwhile and thorough bibliography of phytochemistry.

WILLIAM V. EISENBERG

Literature of Agricultural Research. By J. Richard Blanchard and Harold Ostvold. University of California Press, Berkeley and Los Angeles, 1958. ix+231 pp. Index. Price $5.00.

This work is one of the finest compilations of literature sources that this reviewer has seen. It should be welcomed by all engaged in research work in the field of agriculture and related sciences. Literature sources have been arranged under six broad headings: general agriculture, plant sciences, animal sciences, physical sciences, food and nutrition, and social sciences. Subdivisions within each heading or section include reference works that fall into the following categories: bibliographies of bibliographies and general works, abstracting journals, bibliographies and indexes, encyclopedias, dictionaries, directories, handbooks, yearbooks, history and biography, geography, abbreviations, periodical lists, societies and organizations, tables, and miscellaneous categories.

As an example of how each major section is treated, the section on plant sciences is subdivided into botany, horticulture and agronomy, plant breeding, plant pathology, and forestry and forest products. Each subdivision is introduced by a discussion of the subject matter covered and a review of major literature sources in the field. Where desirable, a subdivision treats special phases separately. Thus the botany subdivision includes sections on floras and economic botany.

The style of presentation is commendable. An excellent index and the system of numbering each reference for cross indexing enables the searcher to survey desirable references speedily. This reference source is an essential addition to the bookshelf of the professional worker in the field of agriculture and the related sciences.

WILLIAM V. EISENBERG

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