INDIVIDUAL VOLATILE ACIDS, SUCCINIC ACID, AND HISTAMINE AS INDICES OF DECOMPOSITION IN ATLANTIC "LITTLE TUNA" (*EUTHYNNUS ALLETERATUS*)

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Since the beginning of World War II, demand for canned fishery products has generally exceeded the supply, and world conditions in the foreseeable future seem to promise a continuation of this trend. Many of the areas of production are at points far removed from principal consumption centers and newer fishing grounds are at long distances from canneries. Consequent increase in costs has renewed interest in canning of tuna and tuna-like fish along the Atlantic Coast, near the more concentrated markets of the United States (1). A small amount of "little tuna" (*Euthynnus alleteratus*) has been canned on the Eastern Seaboard, and possibilities seem to exist for an extension of operations with this variety of fish.

Determination of individual volatile acids has been shown to afford a means for evaluating spoilage in fish used for canning (2–8). Succinic acid content has also been found to correlate well with the condition of raw material employed (9). Histamine* has been advanced as yet another index of the relative freshness of certain fishery products, including the more commonly encountered varieties of tuna (10–13). The applicability of these indices to evaluation of spoilage of little tuna has not heretofore been studied.

PROGRESSIVE DECOMPOSITION STUDIES

Frozen fish in good condition were selected for progressive decomposition packs. The eyes were bright and the gills were deep red in color, with no odor. Small portions of the flesh removed by incisions made an inch or two from the backbone, in the front, middle, and tail portions of the fish, had no odor. The fish were placed on the cannery floor and allowed to remain while decomposition took place. They were examined daily for general appearance and for the odor of small portions of flesh removed in the manner described. The fish were flushed daily with water to remove any slime that had been formed and to minimize dehydration. Some of the fish selected for the study were cooked immediately and canned in accordance with the regular factory practice (cleaning, addition of oil and salt, and processing). As each organoleptic stage of decomposition (8) was reached, several fish were cooked and canned in a similar manner. Each fish in each of the decomposition classes 2, 3, and 4 was canned under a separate code number. No attempt was made to distinguish individual class 1 fish in this way.

In the analysis of the first progressive decomposition pack (No. I), every can obtained from each fish (classes 2, 3, and 4) was analyzed. The purpose was to show the spread, if any, in the chemical criteria among different portions of the same fish as well as among different fish of the same organoleptic class.

The data for volatile acids were obtained by following *Official Methods of Analy-*

* Throughout this paper, the term "histamine" is used to refer to histamine-like substances.

sis, 7th Ed., **18.11–18.17**. The method for succinic acid was that of Hillig, Patterson, and MacLean (8), and for histamine the method proposed by Geiger (11) was followed.

ANALYTICAL DATA

Maximum, minimum, and average values are given in Table 1. In Class 1 fish, formic, propionic, and butyric acids were absent, the volatile a^cid numbers were low, and only small quantities of acetic and succinic

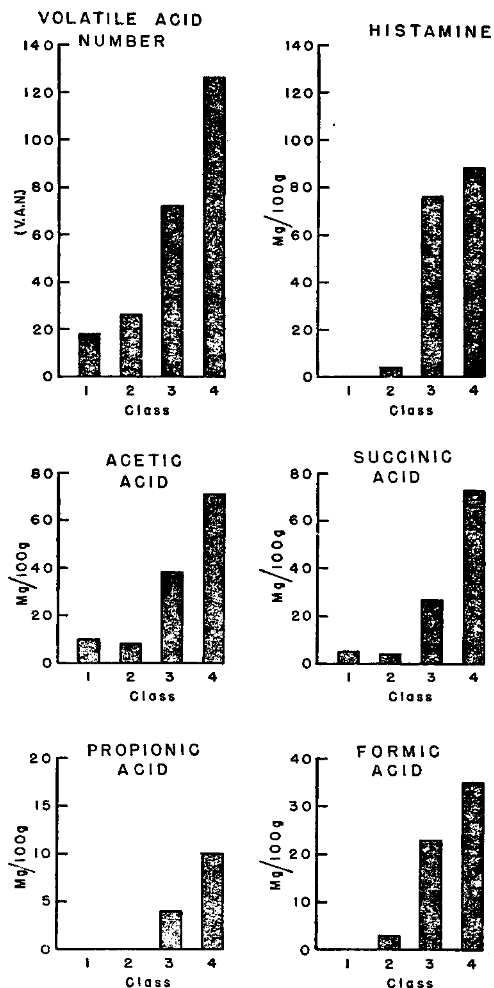

FIG. 1.—Average values for each decomposition class.

TABLE 1.—*Indices of decomposition in "little tuna," Pack No. 1*

INDEX OF DECOMPOSITION		CLASS 1	CLASS 2						CLASS 3						CLASS 4				
			A (8)[a]	B (9)	C (10)	D (11)	E (9)	AV.	F (10)	G (6)	H (5)	I (9)	J (6)	AV.	K (8)	L (7)	M (6)	N (7)	AV.
Volatile acid number	Max.	18	23	31	35	35	45		60	80	96	98	152		155	147	147	225	
	Min.	17	15	18	19	31	23		37	39	62	60	54		91	92	112	142	
	Av.	18	18	22	24	33	37	27	46	48	72	80	112	72	115	122	129	172	135
Formic acid, mg/100 g	Max.	trace	trace	trace	trace	10	11		19	17	28	39	58		41	41	47	57	
	Min.	trace	trace	trace	trace	6	6		9	9	13	23	30		19	24	29	33	
	Av.	trace	trace	trace	trace	8	8	3	3	12	20	31	42	23	30	30	38	41	35
Acetic acid, mg/100 g	Max.	14	12	17	12	8	12		31	31	51	57	84		114	77	88	88	
	Min.	6	8	8	4	5	6		15	18	34	29	46		49	52	63	51	
	Av.	10	9	11	6	7	8	8	22	23	38	44	64	38	73	66	74	69	71
Propionic acid, mg/100 g	Max.	0	0	0	0	0	0		6	4	5	5	14		15	12	16	22	
	Min.	0	0	0	0	0	0		0	0	4	0	4		0	6	9	4	
	Av.	0	0	0	0	0	0	—	3	3	4	1	10	4	5	8	12	14	10
Butyric acid, mg/100 g	Max.	0	0	0	0	0	0		0	0	5	0	8		19	22	20	72	
	Min.	0	0	0	0	0	0		0	0	0	0	0		0	0	8	43	
	Av.	0	0	0	0	0	0	—	0	0	3	0	4	1	4	14	12	54	21
Succinic acid, mg/100 g	Max.	8	12	11	3	trace	5		9	25	38	50	81		84	59	102	104	
	Min.	3	3	trace	trace	trace	trace		5	9	15	18	45		44	46	72	73	
	Av.	5	7	8	2	trace	1	4	7	15	23	31	58	27	60	53	88	91	73
Histamine, mg/100 g	Max.	Not	5	5	8	5	4		Not determined						—	120	80	180	
	Min.	deter-	4	3	4	1	3								—	25	55	125	
	Av.	mined	4	4	5	2	4	4							—	47	66	151	88

[a] Figures in parentheses = no. of cans obtained from entire fish.

acids, identified chromatographically, were found. Histamine was not determined.

Five fish were canned as Class 2. The volatile acid number and formic acid content suggest that, while decomposition was not advanced, it had progressed further in fish D and E than in the other three fish.

Five fish were canned as Class 3 and four fish as Class 4. All of the indices correlate with the advanced decomposition that had taken place in the fish. Because of the wide variation in the data obtained on different portions of the same fish, it can be assumed that the fish did not decompose uniformly. Histamine was not determined in Class 3 fish.

Average data for each decomposition class are presented in Fig. 1.

TABLE 2.—*Acids and histamine in "little tuna," Pack No. II*

INDEX OF DECOMPOSITION		CLASS 1	CLASS 2	CLASS 3	CLASS 4
Volatile acid number	Max.	33	48	123	106
	Min.	21	29	44	64
	Av.	27	35	77	85
Formic acid, mg/100 g	Max.	Trace	8	45	26
	Min.	Trace	Trace	9	18
	Av.	Trace	5	22	21
Acetic acid, mg/100 g	Max.	7	15	53	52
	Min.	2	2	14	24
	Av.	4	7	31	36
Propionic acid, mg/100 g	Max.	None	None	None	4.9
	Min.	None	None	None	Trace
	Av.	None	None	None	2
Butyric acid, mg/100 g	Max.	None	None	None	9.7
	Min.	None	None	None	Trace
	Av.	None	None	None	3
Succinic acid, mg/100 g	Max.	3	5	40	54
	Min.	1	Trace	3	16
	Av.	2	2	19	37
Histamine, mg/100 g	Max.	Trace	1	200	60
	Min.	Trace	1	15	18
	Av.	Trace	1	76	42

The histamine data for Classes 1 and 3 were taken from pack No. II. Butyric acid data are not shown in Fig. 1.

Analytical data for decomposition pack No. II are presented in Table 2. As decomposition progressed, values for the various indices increased in much the same manner as for pack No. I. In pack No. II, histamine was determined on all four classes. When decomposition reached Class 3 (which is defined as unquestionably unsuitable for human consumption), large quantities of histamine were found.

SUMMARY

Progressive decomposition studies on "little tuna" demonstrate that volatile acid number, content of formic, acetic, propionic, butyric, and succinic acids, and histamine are indices of decomposition, and that these values correlate with the progress of decomposition of the raw material from which the canned product was prepared.

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REFERENCES

(1) Jarvis, N. E., *Food Technol.*, **6**, 113 (1952).
(2) Clark, E. P., and Hillig, F., *This Journal*, **21**, 684 (1938).
(3) Hillig, F., and Clark, E. P., *ibid.*, **21**, 688 (1938).
(4) Hillig, F., *ibid.*, **22**, 116 (1939).
(5) ———, *ibid.*, **22**, 414 (1939).
(6) Clague, J. A., *Food Research*, **7**, 56 (1942).
(7) Sigurdsson, G. J., *Ind. Eng. Chem., Anal. Ed.*, **19**, 892 (1947).
(8) Hillig, F., Patterson, W. I., and MacLean, M., *This Journal*, **33**, 834 (1950).
(9) ———, ———, and ———, *ibid.*, **33**, 842 (1950).
(10) Geiger, E., Courtney, G., and Schnakenberg, G., *Arch. Biochem.*, **3**, 311 (1944).
(11) Geiger, E., *Food Research*, **9**, 293 (1944).
(12) Masao, K., and Akira, K., *Bull. Research Inst. Food Sci., Kyote University,* **10**, 83 (1952) (English summary); thru *Chem. Abstr.*, **46**, 4695c (1952).
(13) Williams, D. W., *This Journal*, **37**, 567 (1954).

REFRACTIVE INDICES OF DEXTROSE SOLUTIONS BETWEEN 15° AND 30°

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In order to continue the investigation of phase equilibria of the dextrose-water system, which is of basic importance in the preservation of fruit by freezing, a refractive index-composition table was needed for dextrose solutions between 20° and 30°. The only reliable refractive indices for dextrose solutions appear to be those determined to the fifth decimal place at 20° by Zerban and Martin (1).

In the present paper, refractive indices of dextrose solutions over a range of composition and temperatures are reported. From these indices, an equation has been derived which relates the temperature coefficients of refractive indices of these solutions to temperature and to dextrose concentration. A table of temperature corrections has been calculated from this equation by which, with the data of Zerban and Martin (1), dextrose concentrations can be calculated from refractive indices measured at any temperature between 15° and 30°. In addition, equations have been derived for the refractive indices of dextrose solutions at 25° and 30° as functions of per cent dextrose.

EXPERIMENTAL

In order to achieve the greatest accuracy with the available equipment,[1] temperature coefficients of refractive indices were first determined and fitted by an equation, which was then used to derive a table of temperature corrections. This procedure was adopted to take advantage of the insensitivity of these temperature coefficients to small errors in composition or in absolute refractive index.

Temperature coefficients were determined on each sample by measurements of refractive indices in sodium light at several temperatures. With two exceptions at 25°, at least three measurements (estimated to 0.00005) were made on each sample at each temperature.

The temperature of the circulating water was adjusted by a thermostatic mixing valve, which held it constant to less than ±0.1° at each setting (except for five of the first samples: 79.82, 60.10, 70.29, 39.96, and 10.80 per cent at 30°). With the first two samples, temperature oscillations resulting from malfunction of the mixing valve decreased from ±0.4° to ±0.2° after several readings. These temperature oscillations remained within ±0.2° on the remaining three of these five samples and did not occur at other temperatures nor with other samples.

In the range from 20° to 30°, the temperature of the circulating water was measured at the prism of the refractometer by a thermometer graduated to 0.1°. Outside of this range, a thermometer graduated to 1° was used. Both thermometers were checked against thermometers calibrated at the National Bureau of Standards.

A correction of 0.1° for each 5° difference between room temperature (27.5°) and the temperature of the circulating water was applied to each temperature reported

[1] An Abbé refractometer without Amici prisms, with a range of 1.30 to 1.71.

here. This correction was determined by a small thermocouple with one junction in the sample between the prisms and the other in the circulating water.

Dextrose solutions were prepared from redistilled water and National Bureau of Standards dextrose (Standard Sample No. 41). Where necessary, the solutions were carefully warmed on a water bath to obtain complete solution. All solutions were allowed to stand for twenty-four hours to permit attainment of mutarotatory equilibrium before refractive indices were measured.

Measurements were made at approximately 20°, 25°, and 30° on the same sample. When either temperature or sample was changed, time was allowed for temperature and refractive index to become constant before measurements were made. When indices were measured at temperatures outside of this range, evaporation and condensation were minimized by making measurements at 20° or 30° before making those at the temperature extremes.

No correction was made for the small change in refractive index of the refractometer prism with temperature; consequently such corrections are unnecessary when these data are used with similar instruments.

DATA AND CALCULATIONS

The refractive indices and temperatures measured in this investigation are reported in Tables 1, 2, and 3. The composition of each sample was obtained from its refractive index (Table 1) by utilizing Zerban and Martin's 20° table. The necessary conversions of the measured indices to the corresponding indices at 20° were made, in preliminary calculations, by temperature coefficients obtained directly from the data given in Tables 1 and 2. The derivation of Equation [1] allowed more precise corrections to be made. The final compositions thus obtained differed by only a few hundredths of a per cent from the preliminary values.

The 51 temperature coefficients of refractive indices which can be calculated from the data in Tables 1, 2, and 3, and additional coefficients calculated from published refractive indices for water (2) were used to derive Equation [1].

TABLE 1.—*Refractive indices measured at 20°C.*

n_D^t MEASURED (MEAN)[a]	TEMP., °C.	DEXTROSE CALCD FROM n_D^{20}	n_D^t MEASURED (MEAN)	TEMP., °C.	DEXTROSE CALCD FROM n_D^{20}
		per cent			*per cent*
1.34895	20.15	10.80	1.39866	21.84	40.12
1.34900	21.84	10.95	1.41785	20.07	49.80
1.36280	21.84	19.67	1.41780	21.84	49.93
1.36397	19.97	20.25	1.43938	20.07	60.10
1.38058	20.07	30.05	1.43923	22.09	60.19
1.38104	21.84	30.44	1.46222	20.12	70.29
1.39746	21.84	39.48	1.48030	22.13	78.08
1.39751	21.94	39.51	1.48500	19.87	79.82
1.39860	20.27	39.96	1.49612	21.74	84.42
1.39859	22.06	40.10			

[a] Total number of measurements, $N = 83$; $\sigma = \sqrt{d^2/(N-19)} = 3 \times 10^{-5}$, from 19 means.

TABLE 2.—*Refractive indices of dextrose solutions at 30°; comparison of measured indices and values computed from equation [2]*[a]

p % DEXTROSE	TEMP., °C.	n_D^t MEASURED (MEAN)[b]	n_D^t COMPUTED EQUATION [2][c]	DIFFERENCE $\times 10^{5}$[d]	p % DEXTROSE	TEMP., °C.	n_D^t MEASURED (MEAN)[b]	n_D^t COMPUTED EQUATION [2][c]	DIFFERENCE $\times 10^{5}$[d]
10.80	29.57	1.34784	1.34784	0	40.10	28.98	1.39746	1.39746	0
10.95	29.42	1.34807	1.34809	+2	40.12	29.25	1.39746	1.39746	0
19.67	29.37	1.36182	1.36178	−4	49.80	29.57	1.41615	1.41618	+3
20.25	29.52	1.36264	1.36270	+6	49.93	29.52	1.41646	1.41647	+1
30.05	28.93	1.37925	1.37930	+5	60.10	29.61	1.43763	1.43763	0
30.44	29.66	1.37994	1.37986	−8	60.19	29.47	1.43781	1.43784	+3
39.48	29.69	1.39618	1.39617	−1	70.29	29.22	1.46053	1.46046	−7
39.51	30.90	1.39600	1.39603	+3	78.08	30.18	1.47864	1.47871	+7
39.96	29.42	1.39707	1.39712	+5	79.82	29.28	1.48314	1.48314	0
40.10	29.42	1.39734	1.39738	+4	84.42	29.32	1.49460	1.49459	−1

[a] $\sigma = 4 \times 10^{-5}$ (N=20). Standard deviation of mean refractive index from index computed by Equation [2].
[b] Total number of measurements represented by their means, 86.
[c] Indices calculated at 30° converted to t° by Equation [1].
[d] Computed minus measured indices.

TABLE 3.—*Additional refractive indices of dextrose solutions used to calculate temperature coefficients*[a]

p % DEXTROSE	TEMP., °C.	n_D^t MEASURED (MEAN)	p % DEXTROSE	TEMP., °C.	n_D^t MEASURED (MEAN)
10.80	25.09	1.34833	78.12	22.61	1.48031[c]
	39.36	1.34635		40.98	1.47650
39.51	11.87	1.39900	78.36	11.38	1.48303
	22.05[b]	1.39748		22.13	1.48100
78.08	41.61[c]	1.39400	84.42	25.68	1.49520
	38.28	1.47701		39.65	1.49242

[a] Total number of measurements, N=71. $\sigma = 4 \times 10^{-5}$ (from means).
[b] Mean of 3 independent sets of measurements.

TABLE 4.—*Temperature coefficients of refractive indices of dextrose solutions*[a]

p % DEXTROSE	TEMPERATURE, °C.			
	17.5°	20°	25°	30°
	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$
0	8.0	8.9	10.5	12.0
10	9.5	10.3	11.8	13.2
20	11.1	11.9	13.2	14.5
30	12.7	13.5	14.7	15.8
40	14.4	15.0	16.1	17.2
50	15.9	16.5	17.5	18.4
60	17.2	17.7	18.6	19.4
70	18.2	18.7	19.4	20.1
80	18.8	19.2	19.9	20.4
85	19.0	19.4	19.9	20.4

[a] Calculated by Equation [1].

$$(\Delta n/\Delta t) \times 10^5 = 0.87 + 0.454t - 0.00281t^2 + (0.182 - 0.0024t)p + 0.00105p^2 - 0.0000138p^3, \quad [1]$$

where $(\Delta n/\Delta t)$ represents the temperature coefficient of refractive index at the mean temperature, t, for a solution containing p per cent dextrose.

Between 17° and 34°, and 0 and 84.4 per cent dextrose, the values of the temperature coefficients calculated from this equation have a standard deviation of 5×10^{-6} from the experimental data. Temperature coefficients calculated at several temperatures by Equation [1] are shown in Table 4.

The mean refractive indices listed in Table 2, corrected to 30° by Equation [1], and the refractive index of water at 30° (2), were used to derive Equation [2]:

$$n_D^{30} = 1.33194 + 1.41593 \times 10^{-3}\, p + 4.4734 \times 10^{-6}\, p^2 + 2.3938 \times 10^{-8}\, p^3 - 6.4930 \times 10^{-11}\, p^4 \quad [2]$$

which gives the refractive index at 30° for solutions containing p per cent dextrose. The standard deviation of the 20 measured mean indices from the values calculated by this equation is 4×10^{-5}. If this standard deviation had been based on the 86 individual measurements instead of their means, it would have been somewhat larger (about 5×10^{-5}).

A similar equation, [3]:

$$n_D^{25} = 1.33250 + 1.41984 \times 10^{-3}\, p + 4.5146 \times 10^{-6}\, p^2 + 2.5050 \times 10^{-8}\, p^3 - 8.3000 \times 10^{-11}\, p^4 \quad [3]$$

was derived to fit the refractive indices measured at 25°. Since no further

use was made of these indices, they are not tabulated here. The 18 mean indices used to derive Equation [3] fit it with a standard deviation of 5×10^{-5}.

Tables 5 and 6 are included to facilitate the routine calculation of per

TABLE 5.—*Refractive indices of dextrose solutions at 20°C.*[a]

p (% DEXTROSE)	n_D^{20}	$\Delta n/\Delta\%$[b]	p (% DEXTROSE)	n_D^{20}	$\Delta n/\Delta\%$[b]
		$\times10^5$			$\times10^5$
0	1.33299	143	40	1.39872	189
1	3442	144	41	1.40061	190
2	3586	145	42	0251	192
3	3731	146	43	0443	193
4	3877	147	44	0636	195
5	4024	149	45	0831	197
6	4173	149	46	1028	198
7	4322	150	47	1226	199
8	4472	151	48	1425	200
9	4623	152	49	1625	201
10	1.34775	153	50	1.41826	203
11	4928	154	51	2029	204
12	5082	155	52	2233	206
13	5237	156	53	2439	207
14	5393	158	54	2646	209
15	5551	159	55	2855	210
16	5710	160	56	3065	211
17	5870	161	57	3276	212
18	6031	162	58	3488	214
19	6193	163	59	3702	216
20	1.36356	164	60	1.43918	217
21	6520	165	61	4135	219
22	6685	167	62	4354	220
23	6852	168	63	4574	222
24	7020	169	64	4796	223
25	7189	170	65	5019	225
26	7359	171	66	5244	226
27	7530	172	67	5470	227
28	7702	174	68	5697	229
29	7876	175	69	5926	230
30	1.38051	177	70	1.46156	232
31	8228	178	71	6388	233
32	8406	179	72	6621	235
33	8585	180	73	6856	236
34	8765	181	74	7092	238
35	8946	183	75	7330	239
36	9129	184	76	7569	241
37	9313	185	77	7810	242
38	9498	186	78	8052	244
39	9684	188	79	8296	246
			80	1.48542	247
			81	8789	249
			82	9038	250
			83	9288	251
			84	9539	252
			85	9791	

[a] Computed from equation of Zerban and Martin (1), smoothed graphically.
[b] Change in refractive index for increase of 1% dextrose.

TABLE 6.—*Corrections for dextrose concentrations from refractive indices measured above and below 20°*[a]

TEMP., °C.	APPROXIMATE PER CENT DEXTROSE																	
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85
15	0.28	0.29	0.31	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.39	0.39	0.40	0.40	0.39	0.39	0.38	0.38
16	0.22	0.23	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.31	0.31	0.32	0.32	0.32	0.32	0.31	0.31	0.30
17	0.18	0.18	0.19	0.20	0.21	0.22	0.22	0.22	0.23	0.24	0.24	0.24	0.24	0.24	0.24	0.23	0.23	0.23
18	0.11	0.12	0.12	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15
19	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
20	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
21	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
22	0.13	0.13	0.14	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16
23	0.20	0.21	0.22	0.22	0.23	0.23	0.24	0.24	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.24	0.24	0.23
24	0.27	0.28	0.29	0.30	0.31	0.31	0.32	0.33	0.33	0.33	0.34	0.34	0.34	0.33	0.33	0.32	0.32	0.31
25	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.41	0.42	0.42	0.42	0.42	0.42	0.41	0.41	0.40	0.39
26	0.41	0.42	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.51	0.51	0.51	0.51	0.50	0.49	0.48	0.47
27	0.48	0.49	0.51	0.52	0.54	0.55	0.56	0.57	0.58	0.59	0.59	0.59	0.59	0.59	0.58	0.57	0.56	0.55
28	0.56	0.58	0.59	0.61	0.62	0.64	0.65	0.66	0.67	0.68	0.68	0.68	0.68	0.68	0.67	0.65	0.64	0.63
29	0.63	0.65	0.66	0.69	0.70	0.72	0.73	0.74	0.75	0.76	0.77	0.77	0.76	0.76	0.75	0.74	0.72	0.71
30	0.71	0.73	0.75	0.76	0.78	0.80	0.82	0.83	0.84	0.85	0.85	0.85	0.85	0.84	0.83	0.82	0.80	0.79

[a] Corrections are per cent dextrose. Derive apparent dextrose concentration from Table 5, and *subtract* correction for temperatures *below* 20°C.; *add* corrections for temperatures *above* 20°C.

cent dextrose from refractive indices. Table 5 was computed for intervals of 1 per cent dextrose at 20° by the equation derived by Zerban and Martin (1). The temperature corrections, in per cent dextrose, given in Table 6, were calculated from temperature coefficients calculated by Equation [1]. To use these tables for indices measured at temperatures other than 20°, the per cent dextrose is interpolated from Table 5 as if the index used had been determined at 20°, and the appropriate correction from Table 6 is applied. The correction in Table 6 is to be added to the per cent dextrose calculated from Table 5 if the temperature is above 20° and subtracted if the temperature is below 20°.

SUMMARY

Refractive indices of dextrose solutions containing up to 84.4 per cent dextrose have been measured in the range from 12° to 41°. From these data, those of Zerban and Martin at 20° (1), and published data for water (2), the following equations have been derived:

$$(\Delta n/\Delta t) \times 10^5 = 0.87 + 0.454t - 0.00281t^2 + (0.182 - 0.0024t)p + 0.00105p^2 - 0.0000138p^3 \quad [1]$$

$$(\sigma = 5 \times 10^{-6};\ N = 51;\ p = 0\text{–}85\%\ \text{dextrose};\ t = 17\text{–}34°.)$$

$$n_D^{30} = 1.33194 + 1.41593 \times 10^{-3}\,p + 4.4734 \times 10^{-6}\,p^2 + 2.3938 \times 10^{-8}\,p^3 - 6.4930 \times 10^{-11}\,p^4 \quad [2]$$

$$(\sigma = 4 \times 10^{-5};\ N = 20;\ p = 0\text{–}85\%\ \text{dextrose.})$$

$$n_D^{25} = 1.33250 + 1.41984 \times 10^{-3}\,p + 4.5146 \times 10^{-6}\,p^2 + 2.5050 \times 10^{-8}\,p^3 - 8.3000 \times 10^{-11}\,p^4 \quad [3]$$

$$(\sigma = 5 \times 10^{-5};\ N = 18;\ p = 0\text{–}85\%\ \text{dextrose.})$$

Tables are included by which dextrose percentage can readily be calculated from refractive indices measured in the range from 15 to 30°.

REFERENCES

(1) ZERBAN, F. W., and MARTIN, J., *This Journal*, **27**, 295 (1944).
(2) TILTON, L. W., and TAYLOR, J. K., *J. Research Natl. Bur. Standards*, **20**, 419 (1938) (RP 1085).

METHODS FOR EVALUATING THE RELATIVE EFFECTIVENESS OF THE DETERMINATIONS USED IN THE ANALYSIS OF MAPLE PRODUCTS

By ALBERT CHOLETTE and MARCEL JEAN (Laval University, Quebec, Canada)

Methods for the analysis (1) of the various constituents of authentic maple products yield values which range between extremes, called "limits of variation." In the case of a sirup suspected of being adulterated, analytical values are compared to the values for pure maple sirup. From the results of each determination, one concludes that the sample is or is not like pure maple sirup, and nonagreement, of course, is considered to be evidence of adulteration.

When dealing with known adulterated products, the most effective methods of analysis may confirm adulteration while the others may show values within the range of pure maple. Thus, the conductivity value of a sirup known to be adulterated may well be outside the limits of variation of the pure product although the lead number may be within the normal range. It is then obvious that no two methods of analysis are equally effective in disclosing adulteration. This has long been recognized and has induced regulatory analysts to select certain methods in preference to others. However, it appears from a survey of different laboratories that the effectiveness of the various methods is not always similarly appreciated.

This paper describes mathematical and graphical procedures whereby the relative efficacy of these methods, either singly or in combination, may be appraised. (The efficacy or reliability of a given method or combination of methods depends upon the smallest amount of adulterant or adulterants it can reveal.)

TREATMENT OF DATA

The study will differ depending on whether one adulterant or two are considered. In either case, the validity of the conclusions rests upon the following assumptions:

(*a*) The addition of an adulterant to a pure product brings about a variation in the composition.

(*b*) The analytical values of the adulterated product lie between those of the two starting products, and for a given pure product the magnitude of such a variation is proportional to the amount of adulterant added.

It follows that the constituents of a mixture can be calculated by means of their analytical values from the following equation. When, for example, the amounts of the starting products consist of 1 part pure maple product, x parts brown sugar, and y parts white sugar (possible adulterants of maple):

$$M + xB + yW = (1 + x + y)A \qquad [1]$$

where: M = analytical value of the pure product; B = analytical value of brown sugar; W = analytical value of white sugar; and A = analytical value of the mixture.

For a given method of analysis, a mixture is considered to be adulterated if its analytical value A is outside the accepted limits of variation for pure products. In the particular case of adulteration with one adulterant, the general equation reduces to the following simpler form through the disappearance of one variable:

(a) *The adulterant is brown sugar:* $M + xB = (1 + x)A$ [2]

(b) *The adulterant is white sugar:* $M + yW = (1 + y)A$ [3]

I. MIXTURES CONTAINING ONE ADULTERANT

When a single adulterant is used, equations [2] or [3] enable one to calculate the maximum amounts, m, of adulterant, W or B, which can be added to one part of pure product without being detected by a given determination. Depending on the adulterant used, the following two cases must be considered:

(a) *The constituent in the adulterant (B) is higher than that accepted for pure maple sugar (M).*—The value for m in this case is that amount which can be added to a pure product of minimum value, M_{min}, so as to result in a mixture whose A value is equal to the maximum value, M_{max}, for pure products:

$$(M)_{min} + m(B) = (1 + m)(M)_{max} \quad [4]$$

$$m = \frac{(M)_{max} - (M)_{min}}{B - (M)_{max}} \quad [4a]$$

(b) *The constituent in the adulterant (W or B) is lower than the lower limit, M_{min}, for pure maple sugar.*—The value of m is that amount of adulterant which, when added to a pure product of maximum value, M_{max}, yields a mixture with an A value equal to the minimum accepted for pure products, M_{min}.

Thus, equation [3] becomes:

$$(M)_{max} + m(B \text{ or } W) = (1 + m)(M)_{min} \quad [5]$$

or:

$$m = \frac{(M)_{max} - (M)_{min}}{(M)_{min} - B \text{ or } W} \quad [5a]$$

The analytical values used to calculate m for various constituents and m values for white sugar (W) and brown sugar (B) are given in Table 1.

It is emphasized that the reliability of the general treatment for selecting the methods best suited for the detection of adulteration depends fundamentally upon the quality of the authentic data. Thus the values given in Table 1 are subject to revision when more extensive data become available. For the present, it can be said that the values for pure products, M_{min} and M_{max} (2, 3), represent many analyses and should be acceptable to most regulatory laboratories.

The values for K_2O were determined by the analysis of 100 samples of authentically pure sirups produced in different bushes in various parts of the Province of Quebec. They were evaluated by a modified dipicrylaminate method (4). The P_2O_5 values were obtained from the analysis of 35 authentically pure sirups analyzed by a $SnCl_2$ method (5).

Because of its nature, the analytical values for white sugar may be taken as zero, or near zero. The values given for brown sugar are, in each case, the average values obtained by analysis of six different samples of brown sugar obtained in the Province. These data are admittedly meager, and are subject to later revision. For the time being, their use is mainly illustrative.

Since, by definition, m represents the maximum amount of adulterant in a mixture which cannot be detected by a given determination, it follows that the smaller

TABLE 1.—*Analytical values and "m" values for white and brown sugars*

DETERMINATION	ANALYTICAL VALUES				"m" VALUES	
	PURE PRODUCTS		WHITE SUGAR	BROWN SUGAR	WHITE SUGAR	BROWN SUGAR
	M_{min}	M_{max}	W	B		
Conductivity value	110	230	0	260	1.09	4.0
Total ash	0.61	1.68	0	1.49	1.75	∝
Alkalinity of soluble ash	4.1	12.2	0	8.8	1.97	∝
Soluble ash	0.30	1.23	0	1.15	3.10	∝
Lead number	1.74	7.50	0	2.7	3.31	∝
Insoluble ash	0.23	1.01	0	0.33	3.39	∝
Alkalinity of insol. ash	4.1	20.8	0	7.5	4.07	∝
Malic acid value	0.21	1.82	0	0.075	7.68	11.9
K_2O	0.26	0.42	0	0.59	0.62	0.94
P_2O_5	0.0002	0.0028	0.0002	0.013	∝	0.25

the value of m the smaller will be the amount of adulterant which can be detected and the greater the probability of detecting adulteration. Thus in Table 1 the methods of the Association of Official Agricultural Chemists (through malic acid value) are classified in their order of decreasing reliability for the detection of white sugar adulterations.

For brown sugar adulterations, conductivity is the most reliable index, followed by the malic acid value. Further comparisons of the values of m show that the K_2O value is much more effective than conductivity for detecting white or brown sugar adulterations. The P_2O_5 value is still more effective than that of K_2O for detecting brown sugar adulterations, but it is useless in detecting white sugar adulterations.

Equations [4*a*] and [5*a*] show that the effectiveness of a given determination is not only determined by the range between its limits of variation, $M_{max} - M_{min}$, but also by the difference indicated in the denominator of each equation. This shows how the value for each constituent of the adulterant is associated with those of pure products in the evaluation of any method for its effectiveness.

II. MIXTURES CONTAINING TWO ADULTERANTS

Equation [1] provides the means for calculating the lines for any constituent that enclose areas outside of which all points indicate mixtures which would be found to be adulterated. Four lines can be constructed from the four different conditions as shown in Fig. 1. These are:

Line B, a where the values for $M = M_{max}$ and the value for $A = M_{min}$. In the case of conductivity analysis, the value for $M_{max} = 230$ and $M_{min} = 110$ and equation [1] becomes:

$$230 + x260 + y0 = (1 + x + y)110$$
$$y = \frac{15}{11}x + \frac{12}{11}$$

Line A, a where the values for $M = M_{min}$ and $A = M_{min}$. In the case of conductivity, equation [1] becomes:

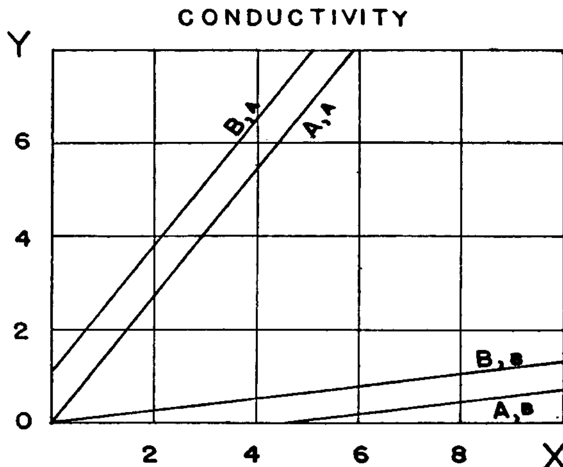


FIG. 1.—Graphical evaluation of the effectiveness of analyses.

$$110 + x260 + y0 = (1 + x + y)110$$
$$y = \frac{15}{11}x$$

Line B, b, where the values for $M = M_{max}$ and $A = M_{max}$. The equation [1] for conductivity is then:

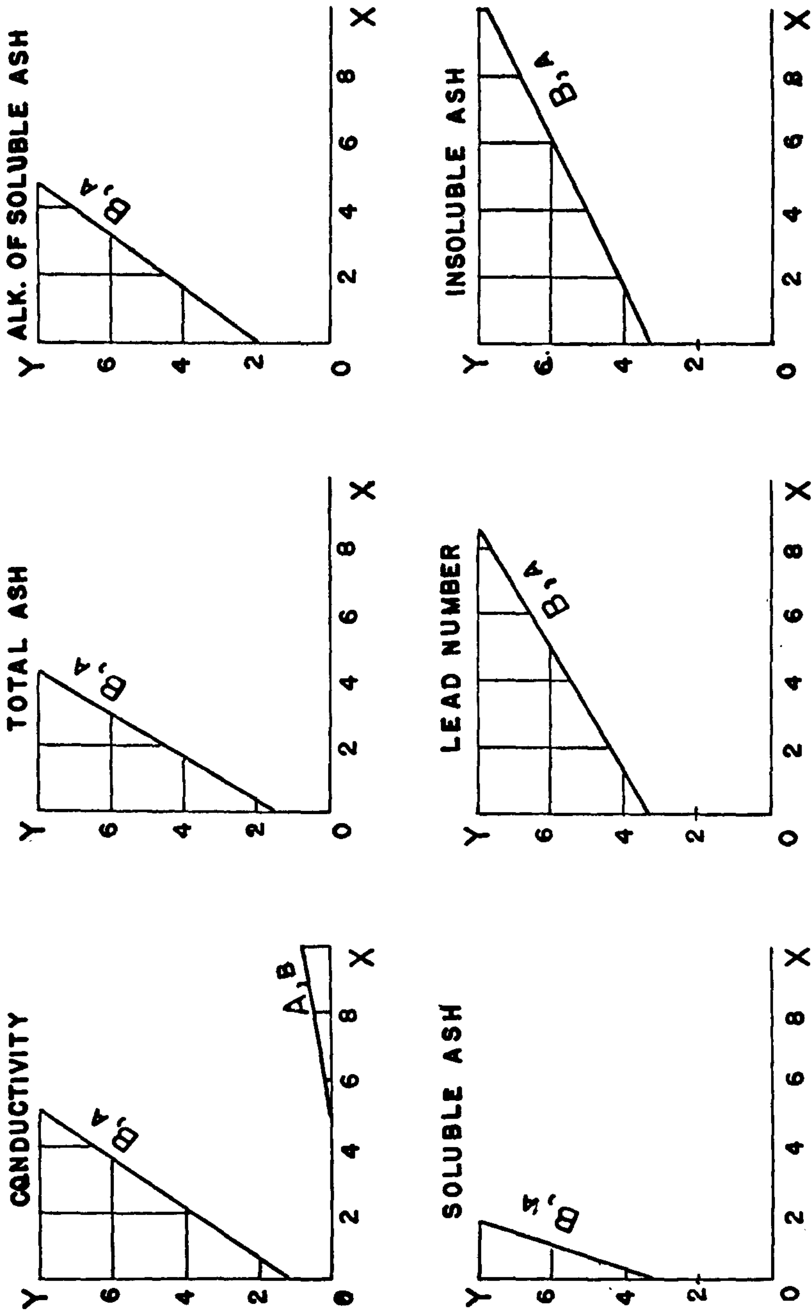
$$230 + x260 + y0 = (1 + x + y)230$$
$$y = \frac{3}{23}x$$

Line A, b, where the values for $M = M_{min}$ and $A = M_{max}$. The equation [1] for conductivity is then:

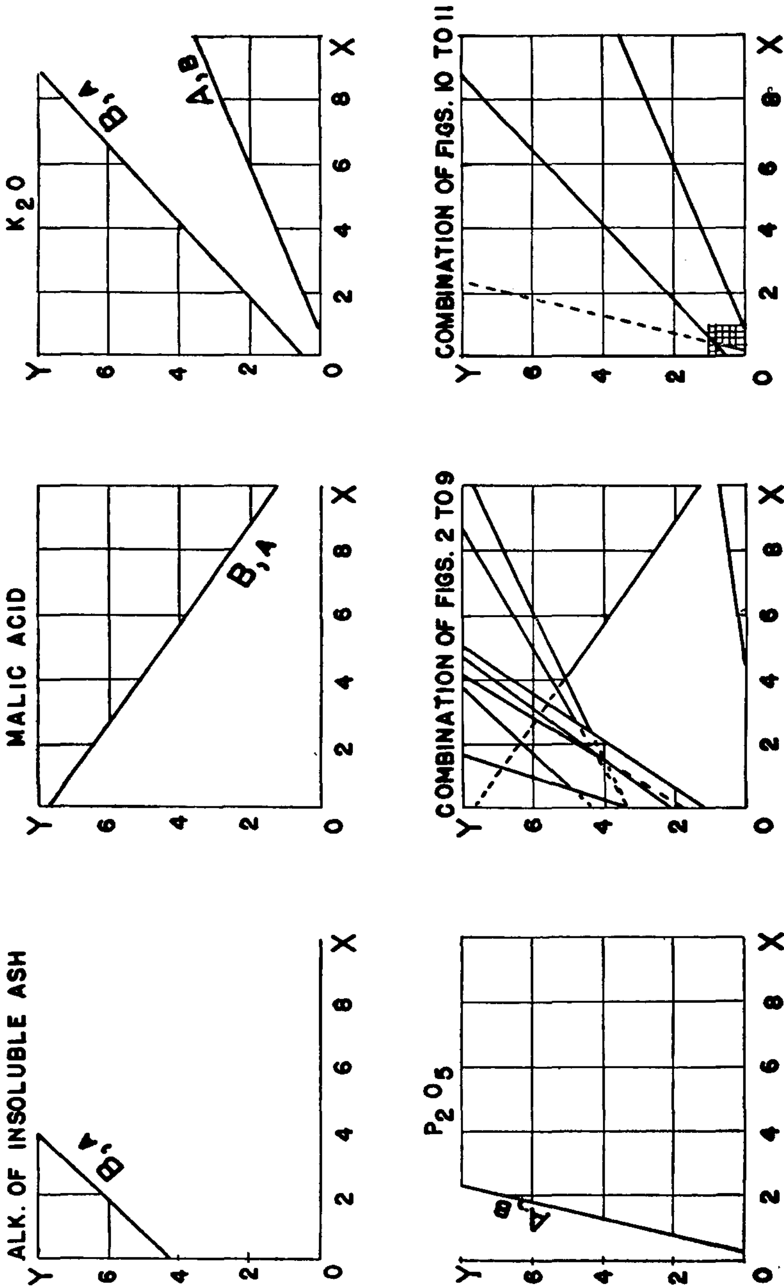
$$110 + x260 + y0 = (1 + x + y)230$$
$$y = \frac{3}{23}x - \frac{12}{23}$$

In Fig. 1, it is observed that all the mixtures defined by points in the area lying between lines A, a and B, b have A values ranging between $(M)_{min}$ and $(M)_{max}$. They cannot therefore be distinguished from a pure product. The areas lying between the lines B, a and A, a and between the lines B, b and A, b represent mixtures which may or may not have values corresponding to those of pure products. Thus, such a region cannot be used in evaluating the effectiveness of a given determination. Finally, the areas outside B, a and A, b represent mixtures whose A values are always different from those of pure products. Such mixtures would be identified as adulterated products.

In Figs. 2–11, only the areas outside lines A, b and B, a have been indicated. These represent the mixtures which would be shown to be adultered by the different determination. For a given graph, the larger the area outside the lines, the greater the effectiveness of the determination. Furthermore, as these areas are not the same and are not always similarly located, it can be seen that the effectiveness of various



FIGS. 2–7.—Graphical evaluation of the effectiveness of various analyses.



FIGS. 8–13.—Graphical evaluation of the effectiveness of various analyses.

determinations differ from each other; one may be more effective for certain mixtures as indicated by its graph, while another will be more suitable for mixtures of different proportions.

This suggests that a combination of determinations may, in many cases, give greater effectiveness than one alone. To appreciate the effectiveness of a combination of methods, one can superimpose the corresponding graphs and consider the area outside the *A*, *b* and *B*, *a* lines. Applied to the determinations of the A.O.A.C., this evaluation shows conductivity, malic acid, and insoluble ash to be the most effective combination (Fig. 12). The same evaluation shows that the combination of K_2O and P_2O_5 determinations is even more effective (Fig. 13).

The graphical method is more general and should be preferred to the other. Not only does it apply to mixtures containing two adulterants but also to those containing only one. In such cases, x or y becomes equal to zero and the value of m can be obtained directly from the graphs at the intercepts.

SUMMARY

The relative effectiveness of methods, and combinations of methods, in current use for the detection of adulteration of maple products is evaluated through the data now available. The mathematical and graphical treatment should be applicable to other products wherein the analysis is compared with those of authentics.

ACKNOWLEDGMENT

The authors are deeply grateful to Dr. E. Bois of Laval University for introducing this study in a preliminary form to the 1953 Philadelphia Conference on Maple Products. They also wish to thank him for proposing that it should be studied collaboratively by the Association of Official Agricultural Chemists.

REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Wash. 4, D. C., 1950.
(2) WOODMAN, A. G., *Food Analysis*, 2nd Ed., McGraw-Hill Book Co., Inc., New York, 1924, p. 290.
(3) SNELL, J. F., and SCOTT, J. M., *Ind. Eng. Chem.*, **6**, 216 (1914).
(4) BOIS, E., and JEAN, M., *Anal. Chem.*, **26**, 727 (1954).
(5) JEAN, M., *Thesis*, Laval University, Quebec, Canada.

METALLIC ELEMENTS IN WINE BY FLAME PHOTOMETRY*

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Flame photometry permits rapid study of the alkali and alkaline earth content of wines and provides a means for compiling sufficient data for statistical treatment of various wines from many geographical localities.

The metallic constituents of wines account for the alkalinity of the ash,

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and their oxides and carbonates for most of its weight. Changes in the concentrations of these metals as a result of manufacturing processes make possible the estimation of amelioration as permitted under Federal wine regulations (1). The concentration of the metallic elements shows no significant change due to age (2); hence, the extent of officially authorized treatments of wines and wine products can be checked by noting the change in metal concentration.

The Beckman Model DU flame spectrophotometer with oxygen-hydrogen flame permits the determination of line and oxide band type emissions (3–4), with minimum background interference. Utilization of a photomultiplier attachment (5) makes possible slit-width reductions and the detection of smaller concentrations of those metals which emit in the "blue" phototube range.

The background and wavelength of maximum emission of the alkalies and alkaline earths are often affected by other constituents present in wines. Those interferences can be minimized, and in some cases completely eliminated, by narrowing the slit width or changing wavelengths, or by proper dilution or addition techniques.

The literature contains many descriptions of flame photometric determinations of the alkalies and alkaline earths. These publications encompass a wide variety of products (6–9), flame photometers (10–16), and fuels (3, 14–17). Several investigators (18, 19) have compared the flame photometric determinations of these metals with chemical, increment, and spectrographic techniques. The results reported (20–23) show that the alkalies and alkaline earths can be determined photometrically with accuracy and reproducibility.

Interferences cause most trouble in flame photometric procedures. Certain organic materials enhance and others inhibit the light emitted by the alkalies and alkaline earths (24–26). Background and radiation interferences due to cations and anions affect the emissions of these metals (3, 20, 23, 27–34).

Some papers mention the mechanical difficulties involved with various types of jets, and suggest methods for cleaning (20, 24, 35–38).

DETERMINATION OF CALCIUM, MAGNESIUM, POTASSIUM, AND SODIUM

INSTRUMENTS AND APPARATUS

(a) *Beckman Model DU flame spectrophotometer* with oxygen-hydrogen flame and photomultiplier attachment.

(b) *Polyethylene bottles* for storing reagents and standards.

REAGENTS AND STANDARDS

(a) *Calcium chloride.*—Dissolve 25.0 g reagent grade $CaCO_3$ in a slight excess of dilute HCl and dilute to 1 l. This solution contains approximately 10,000 p.p.m. calcium. The reagent was standardized by double precipitation as calcium oxalate and weighed as calcium oxide.

(b) *Magnesium chloride.*—Weigh 10.0 g Grignard-type Mg metal turnings, dis-

solve in a slight excess of HCl, and dilute to 1 l. This solution contains approximately 10,000 p.p.m. Mg. The reagent was standardized by double precipitation as magnesium ammonium phosphate and weighed as magnesium pyrophosphate.

(c) *Potassium chloride.*—Dry reagent grade KCl at 100°C. overnight. Weigh 19.068 g and make to 1 l. This solution contains 10,000 p.p.m. K.

(d) *Sodium chloride.*—Dry reagent grade NaCl at 100°C. overnight. Weigh 25.422 g and make to 1 l. This solution contains 10,000 p.p.m. Na.

(e) *Phosphate solution.*—Dilute 73 ml reagent grade (85%) H_3PO_4 to 1 l. This solution contains approximately 100,000 p.p.m. phosphate.

(f) *Dextrose solution.*—Make 20 g reagent grade dextrose to 100 ml with distilled H_2O.

(g) *Hydrochloric acid solution.*—Dilute 84 ml reagent grade HCl to 1 l. This solution is approximately normal.

DETERMINATION

Flame spectra.—The flame spectrum of each metal, illustrated by Fig. 1, was utilized in selecting the best working wavelength. Arc lines of potassium at 768 mμ and of sodium at 589 mμ exhibit no spectral line interferences and very low background with the specified instrument settings. Background is almost negligible at the calcium arc line, but is significantly high in the vicinity of the oxide bands. The flame spectrum of magnesium exhibits molecular band maxima at 371 and 383 mμ, with considerable background at both wavelengths. The emission at 383 mμ is preferable because its background levels off before and after the wavelength of maximum brightness. Interference from the hydroxy spectrum rendered the arc line of magnesium at 285 mμ undesirable with the oxygen-hydrogen flame (3).

Readings at a point before and after the maximum emission shows the background of the sample. By subtracting the average of these readings from the maximum emission, variations in background may be minimized (38). The difference is designated as "unit rise."

Calibration curve.—The "semi-permanent" calibration curves, illustrated in Fig. 2, are prepared for 1–10 p.p.m. Ca, K, and Na and 1 to 50 p.p.m. Mg, in the presence of 1% dextrose and 100 p.p.m. phosphate. Readings are made at the following wavelengths: Ca, 410, 422.7, and 430 mμ; Mg, 376, 383, and 389 mμ; K, 740, 768, and 790 mμ; Na, 570, 589, and 610 mμ. Optimum instrument adjustments for each metal are given in Fig. 2, and the calibration curves are plotted as "Unit Rise" *vs.* p.p.m.

To determine calcium, magnesium, and sodium.—Pipet 10 ml wine into a Pt dish and evaporate to dryness at 100°C. Char and burn most of the organic material over a very low flame, transfer to a muffle furnace, and ash at 550°C. Remove the last traces of carbon by repeatedly moistening and re-ashing the sample.

Add 2 ml normal HCl to the ash and evaporate the solution to about 0.5 ml over a very low flame. Cool and transfer the contents of the dish to a 50 ml volumetric flask. Make to mark with distilled H_2O and mix thoroly.

Further dilutions are dependent on the metal concentrations under consideration. Calcium, magnesium, and sodium can generally be diluted another 5–10 times. On the assumption that the three metals can be diluted another 5 fold, pipet a 10 ml aliquot into a 50 ml volumetric flask containing 100 p.p.m. phosphate and 2.5 ml dextrose solution. Make to mark with distilled H_2O and mix thoroly.

Prepare 100 ml of aqueous standard solution containing 10 p.p.m. each of Ca, K, Na, and Mg; 100 p.p.m. phosphate; and 1 per cent dextrose.

Each sample should be read immediately after the standard and the process repeated 3–5 times.

To determine potassium.—Dilute 10 ml wine 200 times with distilled H_2O, fill

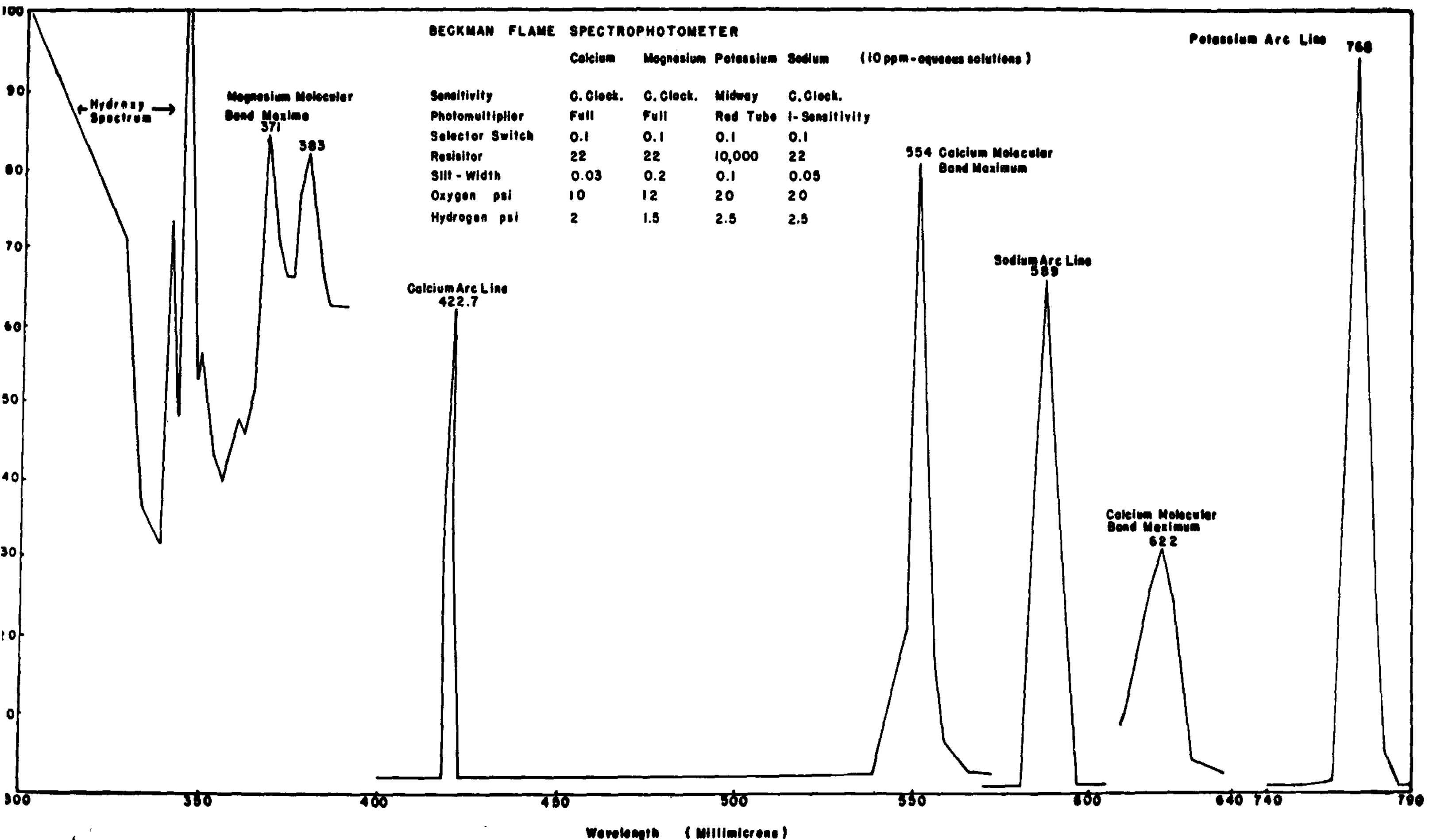

FIG. 1.—Flame spectra of calcium, magnesium, potassium, and sodium.

TABLE 1.—*Analytical data*

TYPE OF WINE	GRAVIMETRIC		FLAME PHOTOMETRIC															
							WINE+100 P.P.M. Ca, Mg, Na, K				WINE+50 P.P.M. Ca, Mg, Na, K				WINE+20 P.P.M. Ca, Mg, Na, K			
	Ca	Mg	Ca	Mg	Na	K	Ca	Mg	Na	K	Ca	Mg	Na	K	Ca	Mg	Na	K
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Blackberry	92.9	99.5	93.2	96.3	27.3	859	195.0	191.3	130.0	960	142.5	150.0	80.5	910	117.5	125.0	48.0	890
Loganberry	139.4	87.0	137.2	86.3	50.5	804	247.1	183.8	155.0	918	185.3	137.5	93.8	863	165.0	112.5	78.8	832
Currant	125.0	49.8	129.5	46.3	41.0	1052	227.9	151.8	148.8	1155	174.5	93.8	91.3	1095	149.4	65.0	61.7	1075
Apple	46.5	58.5	39.0	50.0	32.0	1125	151.8	157.5	135.0	1222	95.0	106.3	95.0	1175	66.3	76.3	52.6	1145
Vermouth	68.6	105.2	69.9	97.5	63.6	832	160.0	207.5	165.0	923	125.0	145.0	115.0	890	90.0	120.0	83.0	855

TABLE 2.—*Effect of sugar on aqueous solutions of sodium, potassium, calcium, and magnesium*[a]

PER CENT SUGAR	SODIUM, P.P.M.					POTASSIUM, P.P.M.					CALCIUM, P.P.M.					MAGNESIUM, P.P.M.				
	10	20	30	50	100	10	20	30	50	100	10	20	30	50	100	10	20	30	50	100
1	9.8	19.8	30.2	49.7	99.8	9.8	20.6	30.0	50.4	101.0	9.8	19.7	29.6	47.3	99.0	10.0	20.2	29.7	49.6	97.9
2	9.8	19.8	30.2	49.8	101.3	9.8	20.4	30.8	49.5	99.2	9.7	19.6	29.4	48.0	96.6	10.2	19.7	32.3	53.4	97.8
3	9.5	19.5	29.1	49.5	101.0	10.0	20.5	29.9	49.7	101.1	9.9	19.8	29.5	47.6	96.6	10.2	23.2	32.3	54.1	96.5
4	9.4	19.8	30.1	50.1	101.0	10.1	20.3	29.5	50.1	101.1	9.9	19.5	30.6	48.0	96.9	10.2	21.3	31.9	54.8	99.9
5	9.5	18.7	29.8	49.4	99.4	10.0	19.5	29.5	49.5	100.9	9.8	19.8	31.2	48.6	99.8	10.2	21.3	33.3	53.4	94.4
10	9.3	18.1	29.3	50.4	98.9	9.7	18.7	28.7	47.8	98.7	10.1	20.4	32.5	50.8	101.9	9.5	18.4	28.8	56.1	88.2
15	8.5	17.6	28.3	48.9	99.8	9.4	18.2	27.3	47.6	98.3	10.2	20.5	31.5	52.3	104.2	8.4	19.8	28.8	52.6	88.2
20	8.0	17.1	27.6	47.8	99.3	8.7	15.9	25.7	46.2	98.5	10.0	19.8	32.5	51.1	104.5	8.0	19.7	28.0	49.0	99.7

[a] Corrected for the presence of the alkalies and alkaline earths in sugars.

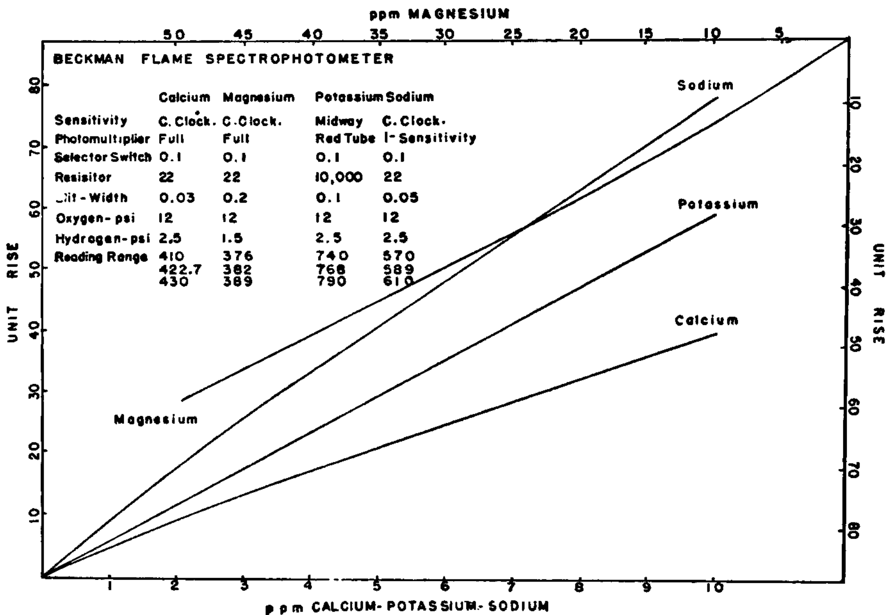

FIG. 2.—Calibration curves for calcium, magnesium, potassium, and sodium.

the sample cup, make the necessary instrument settings, and burn. Read the sample and standard 3–5 times at the wavelengths used in preparing the calibration curve.

CALCULATIONS

For standard: $T_{Max} - \left[\frac{T_b + T_a}{2}\right] = \text{Unit Rise } A$

For sample: $T_{Max} - \left[\frac{T_b + T_a}{2}\right] = \text{Unit Rise } B$

where: T_{Max} = per cent transmission at maximum emission; T_b = per cent transmission before maximum; and T_a = per cent transmission after maximum.

$$\frac{\text{Theoretical Unit Rise from Calibration Curve}}{\text{Unit Rise } A} = \text{Correction Factor}$$

Then: Unit Rise $B \times$ correction factor = corrected Unit Rise B
From calibration curve: Derive p.p.m. equivalent to corrected Unit Rise B
And: P.p.m. $\times$ dilution factor = P.p.m. in sample.

DISCUSSION

Jet Operation and Correction.—Progressive clogging and incrustation of the jet will reduce the sensitivity. Aspiration of glacial acetic acid followed by ethanol is suggested for removing carbonization due to sugars in wines. Variations in the degree of brightness exhibited by specific metals at their

wavelengths of maximum emission were noted for five new and two repaired jets. Optimum gas pressures were found to vary among jets after prolonged use and repeated cleaning. These occurrences make it imperative to read a standard with each determination.

Comparison of Flame Photometric and Chemical Procedures.—Five different wine samples were chilled and filtered several times to obtain clear, homogeneous solutions. Calcium and magnesium were determined gravimetrically by double precipitation to establish accurate comparison data. Calcium was precipitated at *p*H 6.5 as the oxalate, and magnesium was precipitated from the combined calcium filtrates as the ammonium phosphate. Calcium was weighed as calcium oxide, and magnesium was weighed as magnesium pyrophosphate. Chemical and increment data presented in Table 1 show that these alkalies and alkaline earth metals can be determined with accuracy and reproducibility by the flame technique.

INTERFERENCES

Emission of these metals is affected by organic materials and by inorganic anions and cations. Numerous flame spectrophotometric methods require the preparation of blanks simulating unknowns to allow for interferences (39). In this experimental work, the removal of these effects by dilution and addition procedures permits the preparation of standards which are applicable to any wine ash.

Organic Material.—The effects of 1 to 20 per cent sucrose on varying concentrations of calcium, magnesium, potassium, and sodium are presented in Table 2. Aqueous solutions containing different percentages of sucrose were tested for the presence of these metals, and the data were corrected accordingly. Sucrose concentrations smaller than 5 per cent had no significant effect. Difficulty in obtaining data for these metals in the presence of larger sucrose concentrations was attributed to reduced atomization because of frequent jet clogging and carbonization. The viscosity effect on the light emitted by the alkalies was noted by Berry, *et al.* (24).

The effects of varying ethanol concentrations on the light emitted by these alkalies and alkaline earths are presented in Table 3. More than 50 per cent ethanol enhanced the light emitted by potassium, while calcium, magnesium, and sodium emissions were enhanced by 5 to 50 per cent ethanol. The emissions were not reproducible in the presence of more than 50 per cent ethyl alcohol, due to changes in surface tension. Barnes, *et al.*, (40) observed the same effect.

Destruction of organic materials by ashing eliminates viscosity and surface tension effects, and reduces carbonization and incrustation of salts on the burner tip.

Cations.—All the metals presented in Table 4 were not expected to be found in wines, but due to the variety of wines and the many localities

TABLE 3.—*Effect of alcohol on sodium, potassium, calcium, and magnesium*

PER CENT ALCOHOL	SODIUM		POTASSIUM		CALCIUM		MAGNESIUM	
	P.P.M.		P.P.M.		P.P.M.		P.P.M.	
	10[a]	50[a]	10[a]	50[a]	10[a]	50[a]	10[a]	50[a]
5	11.0	54.3	10.7	55.6	10.6	50.9	11.5	49.3
10	11.5	54.9	11.0	57.1	11.5	56.2	11.9	55.1
15	11.7	57.1	11.2	55.6	12.3	58.9	12.1	60.2
20	12.3	57.7	11.1	54.7	12.5	59.8	11.8	63.2
30	12.8	58.6	10.5	52.7	12.8	63.3	11.8	63.2
50	13.7	63.9	10.4	51.8	12.9	66.8	11.8	63.5
90	16.7	68.9	16.9	75.0	23.6	112.8	6.3	77.0

[a] These values are the concentrations actually prepared.

in which they were produced, it was considered essential to study these metals. Aqueous solutions of the cations, prepared from the purest available salts, were checked for the presence of calcium, magnesium, potassium, and sodium, and the data were corrected accordingly.

Cations listed in Table 4, with the exception of lithium, had no appreciable effect on 10 to 50 p.p.m. potassium and sodium. Most metals affected the calcium and magnesium emissions.

Cations commonly found in wines are copper, iron, and manganese. Amerine and Joslyn (41) noted that wines contained less than 1 p.p.m. copper and approximately 1 to 2 p.p.m. manganese. Metallic iron in some wines was reported by Simon (2) to be less than 1 p.p.m. The iron content of many fruits and berries was reported by Jacobs (42) to be less than 10 p.p.m. Laboratory data indicate very low manganese concentration in grape wines, but much larger quantities were found in berry wines. Since all wines contained less than 100 p.p.m. copper, iron, and manganese, the study was limited to this amount. Iron concentrations greater than 2 p.p.m. and manganese concentrations greater than 10 p.p.m. inhibited the light emitted by calcium and magnesium, while copper concentrations smaller than 10 p.p.m. had no effect.

The effects of varying concentrations of calcium, magnesium, sodium, and potassium with one another are presented in Tables 5, 6, and 7. Calcium concentrations up to 100 p.p.m. were not affected by less than 200 p.p.m. potassium and 500 p.p.m. sodium. The presence of 100 p.p.m. magnesium had no effect on less than 50 p.p.m. calcium. When the sodium to magnesium ratio was greater than 1, the light emitted by magnesium was depressed. Potassium concentrations greater than 500 p.p.m. inhibited the emission of magnesium. Up to 100 p.p.m. calcium did not affect less than 10 p.p.m. magnesium, but it did slightly enhance the emission of more than 10 p.p.m. Potassium and sodium in concentrations below 1,000 p.p.m. had no effect on each other. Slight variations above 10

TABLE 4.—*Effect of cations on aqueous solutions of sodium, potassium, calcium, and magnesium*

CATION	P.P.M.	SODIUM, P.P.M.		POTASSIUM, P.P.M.		CALCIUM, P.P.M.		MAGNESIUM, P.P.M.	
		10	50	10	50	10	50	10	50
Al	100	10.0	49.5	9.9	50.0	4.4	34.8	4.9	40.9
B	100	10.0	50.0	9.9	49.7	5.6	25.0	5.8	26.5
Ba	100	9.7	48.8	9.6	50.0	10.3	50.4	9.7	50.0
Cd	100	10.0	50.0	9.9	49.7	9.2	48.6	9.2	49.3
Ca	100	10.0	50.0	10.2	50.7			9.7	57.5
Cs	100	9.5	49.7	10.3	52.0	8.5	49.4	8.1	50.0
Cr	100	9.8	50.3	10.0	50.0	4.9	31.4	6.5	35.5
Co	100	9.8	50.0	10.0	49.7	8.4	45.8		
Cu	2					10.5	50.0	10.0	49.6
	5					10.1	49.7	10.0	48.8
	10					10.0	49.5	9.1	45.5
	50					9.3	47.4	7.8	45.0
	100	9.9	50.1	10.3	50.3	8.7	44.4	6.1	46.0
Au	100	10.0	50.0	9.9	49.8	10.0	49.6	9.2	49.4
In	100	9.7	50.3	9.9	50.0	6.1	30.1	6.5	27.5
Fe	2					10.0	50.5	9.5	50.0
	5					9.3	49.0	8.8	48.7
	10					8.9	47.9	8.0	45.4
	50					6.8	41.9	9.8	48.0
	100	10.1	50.6	10.0	49.3	5.5	37.7	11.4	49.0
Li	100	8.1	47.1	9.2	48.6	8.6	44.6	5.6	37.3
Mg	100	9.7	49.6	10.0	49.3	9.9	49.2		
Mn	2					9.9	50.0	10.0	51.0
	5					10.0	50.0	9.6	52.0
	10					10.2	50.3	10.0	51.0
	50					9.7	49.0	8.6	49.0
	100	9.7	50.2	9.8	50.3	8.4	45.6	7.5	47.9
Mo	100	9.8	49.5	9.8	50.0	5.3	24.1	7.6	41.6
Nd	100	10.0	50.0	9.9	50.0	10.1	49.6	11.5	54.0
Ni	100	9.8	49.6	10.0	49.7	8.4	45.1	9.8	49.3
Pt	100	10.3	51.2	10.0	50.0	9.9	48.8	9.6	46.6
Pr	100	9.8	49.4	9.9	50.0	10.0	50.0	11.6	55.6
Rb	100	9.9	50.0	9.9	49.8				
Sm	100	9.9	49.5	9.9	50.0	10.1	49.6	11.6	55.6
Se	100	10.0	50.0	9.9	50.0	5.7	33.9	6.0	32.2
Sr	100	9.6	48.9	9.7	49.5	9.8	50.0	9.8	47.7
Tl	100	10.0	49.6	10.0	49.6	6.3	45.6	[a]	
Th	100	9.7	50.0	10.0	50.0	8.7	46.7	8.8	43.4
V	100	10.2	50.6	9.9	50.0	3.6	29.1	7.7	47.4
Zn	100	10.2	50.6	9.8	49.5	9.1	48.1	7.8	44.9
Zr	100	9.8	50.2	9.7	49.7	2.5	34.0	5.8	39.4

[a] Interference due to Tl arc line at 377.6 mμ.

TABLE 5.—*Effect of aqueous solutions of sodium and potassium on each other*

SODIUM (OR) POTASSIUM	SODIUM, P.P.M.						POTASSIUM, P.P.M.					
	10	30	50	100	500	1000	10	30	50	100	500	1000
p.p.m.												
10	9.9	29.7	50.5	100.8	504	1005	10.1	29.7	50.5	100.0	504	1005
30	9.9	29.9	50.5	100.1	502	998	9.9	30.3	49.5	100.6	502	1002
50	9.9	29.9	50.2	100.8	504	1009	10.0	30.3	50.0	99.3	507	1002
100	9.8	29.4	50.5	101.5	502	991	9.9	29.7	50.0	100.6	502	1005
500	9.8	29.4	50.7	100.8	507	1009	10.2	30.6	50.7	101.3	507	1007
1000	10.1	29.9	50.5	103.9	509	1023	10.2	30.9	51.2	103.6	510	1015

TABLE 6.—*Effect of magnesium, potassium, and sodium on calcium in aqueous solutions*

CALCIUM	CALCIUM RECOVERY, P.P.M.																	
	SODIUM ADDED, P.P.M.						POTASSIUM ADDED, P.P.M.								MAGNESIUM ADDED, P.P.M.			
	10	30	50	100	500	1000	10	30	50	100	200	300	500	1000	10	30	50	100
p.p.m.																		
3	2.9	2.9	3.0	3.0	2.9	3.1	3.0	2.9	2.9	2.9	2.7	2.7	2.9	2.8	2.9	2.8	2.8	2.9
5	5.0	5.0	5.0	5.0	5.1	5.1	4.8	4.9	4.8	4.8	4.5	4.4	4.4	4.4	5.0	4.9	4.9	4.8
10	9.8	9.9	9.9	9.8	9.4	9.5	10.0	9.8	9.9	10.0	9.0	8.6	8.4	8.4	9.9	9.9	9.7	9.7
20	19.8	19.8	19.7	19.5	19.2	18.7	19.8	19.8	20.0	20.0	19.0	18.1	17.9	18.1	20.0	19.6	19.5	19.5
30	30.3	29.8	29.5	29.8	29.9	28.6	29.8	29.8	29.8	29.8	29.3	28.3	27.2	25.3	30.3	29.4	29.0	29.3
50	49.4	49.4	49.6	49.1	48.9	48.4	48.9	48.5	48.3	48.5	48.0	47.2	46.1	43.7	49.8	48.2	48.4	47.7
100	100.2	100.4	99.8	99.1	98.6	97.6	99.6	99.1	99.4	99.6	99.6	95.3	94.1	91.9				

TABLE 7.—*Effect of sodium, potassium, and calcium on magnesium in aqueous solutions*

MAGNESIUM	MAGNESIUM RECOVERY, P.P.M.																				
	SODIUM ADDED, P.P.M.						POTASSIUM ADDED, P.P.M.							CALCIUM ADDED, P.P.M.							
	10	30	50	100	500	1000	10	50	100	200	300	500	1000	2	3	5	10	20	30	50	100
p.p.m.																					
2														2.1	2.1	1.9	2.0	2.0	2.1	1.9	1.8
3														3.0	3.1	3.2	3.2	3.3	3.1	2.9	3.1
5	4.8	4.3	4.1	3.6	3.5	3.5	5.1	5.4	5.1	5.4	5.0	5.3	3.6	5.0	5.0	5.0	4.9	5.3	5.2	5.1	4.9
10	9.4	8.5	8.2	7.3	6.4	6.9	9.9	10.9	11.0	11.1	9.9	10.5	7.9	10.1	10.0	9.6	10.1	10.3	10.4	10.2	9.7
20	20.9	19.1	17.7	17.9	14.5	11.5	19.8	19.8	19.5	19.6	19.8	19.5	16.0	19.9	20.2	19.8	21.5	22.3	22.9	23.1	23.1
30	31.2	29.0	30.3	27.5	22.5	21.9	30.3	30.3	31.4	30.5	31.4	29.7	26.6	30.5	30.2	29.9	32.9	33.2	34.5	34.8	34.1
50	48.8	50.0	49.1	48.1	40.0	38.8	48.3	49.2	50.0	50.5	51.7	48.3	40.7	50.6	52.3	53.2	52.1	53.0	54.0	55.5	57.5
100	100.6	101.9	100.0	100.6	91.5	77.9	99.5	99.8	99.0	99.5	99.0	98.5	80.0								

TABLE 8.—*Effect of anions on aqueous solutions of sodium, potassium, calcium, and magesium*

ANION		SODIUM, P.P.M.		POTASSIUM, P.P.M.		CALCIUM, P.P.M.		MAGNESIUM, P.P.M.	
		10	50	10	50	10	50	10	50
	ppm								
Acetate	500	10.0	48.8	10.0	49.6	7.9	45.4	6.5	48.1
	1000	10.1	50.0	10.2	50.4	7.9	42.7	6.1	40.6
	2000	10.1	49.3	10.4	51.0	7.6	40.5	6.1	35.0
	5000	10.3	50.5	10.3	51.0	7.5	38.5	7.0	29.1
Bicarbonate	500	10.0	50.0	10.2	49.5	8.1	39.0	7.6	48.1
	1000	9.9	51.0	10.5	50.0	8.1	27.9	6.8	42.3
	2000	9.9	48.8	10.0	50.0	7.0	20.9	7.0	34.6
	5000	9.8	50.0	10.8	50.0	6.4	20.9	6.9	32.7
Chloride	500	9.9	49.5	10.0	51.1	10.0	50.0	9.7	50.0
	1000	9.8	50.0	9.9	51.1	10.0	49.6	8.9	51.5
	2000	9.9	49.5	10.2	50.9	10.0	49.1	8.5	49.1
	5000	9.7	49.3	10.0	51.9	9.7	48.7	8.3	49.1
Nitrate	500	10.0	50.4	10.0	49.6	6.9	33.9	10.0	49.7
	1000	10.0	50.0	9.8	50.1	6.9	32.9	9.6	48.9
	2000	10.2	49.6	10.1	49.6	6.9	32.3	9.8	48.2
	5000	9.9	49.6	9.8	49.6	6.9	32.5	9.8	48.9
Phosphate	20					3.2		5.2	46.3
	50					3.2		5.2	34.7
	100					3.4		4.8	20.4
	200					3.4		4.7	17.7
	500	9.8	50.5	9.4	47.0	3.2	11.3	4.7	16.9
	1000	9.7	50.5	9.2	46.3	3.2	11.3	4.8	16.8
	2000	9.7	50.7	8.8	46.3	3.3	11.6	4.7	17.1
	5000	10.0	50.7	8.0	43.8	3.6	13.5	4.8	18.7
Sulfate	20					6.1		6.6	46.0
	50					5.5		6.2	38.9
	100					5.5		5.4	30.8
	200					5.5		5.6	28.9
	500	10.8	49.8	9.8	49.5	5.7	23.5	5.7	21.5
	1000	10.8	50.5	9.7	49.0	5.7	23.5	5.5	21.1
	2000	10.8	50.7	9.8	48.5	5.7	23.5	5.7	22.1
	5000	10.8	50.7	9.8	48.3	5.7	23.5	5.3	22.0

p.p.m. were due to an unsteady galvanometer needle caused by selector switch change.

The combined effects of the alkalies and alkaline earths were evaluated. The metals were read alone, after the addition of each separately, and in the presence of all others. No interferences were observed when calcium,

magnesium, potassium, and sodium were present in the concentrations shown in Fig. 2.

Anions.—Anion concentrations of 500 to 5,000 p.p.m. in the presence of 10 to 50 p.p.m. of the alkalies and alkaline earths were investigated and are presented in Table 8. Acetate, bicarbonate, chloride, nitrate, and sulfate anions had no appreciable effect on potassium and sodium. More than 500 p.p.m. phosphate depressed the light emitted by potassium but did not affect sodium. Light emitted by calcium and magnesium was depressed by these anions. However, more than 1,000 p.p.m. chloride inhibited calcium only slightly, and more than 500 p.p.m. decreased slightly the light emitted by magnesium.

Wines contain less than 1,000 p.p.m. phosphate and sulfate anions. Their removal by chemical methods is long and tedious, and the elimination of these interferences by dilution was not practical because small quantities of these anions caused considerable inhibition of the light emitted by calcium and magnesium. Depression of light emitted by the alkaline earths reaches a maximum in the presence of certain phosphate and sulfate concentrations. Furthermore, this decrease in the brightness of the emissions from the alkaline earths, due to the trivalent phosphate, predominated over that caused by the divalent sulfate anions. These effects may be observed from Figs. 3 and 4.

To minimize the effect of phosphate and sulfate, several complexing agents were evaluated. It was found that the interference due to these anions was reduced in the presence of dextrose. Data presented in Table 9 show that 1 per cent dextrose increases the sensitivity of the calcium and magnesium emissions in the presence of these anions to approximately 87 per cent of that obtained from an aqueous solution of these metals. The constancy of this value for 20 to 1,000 p.p.m. phosphate and sulfate

TABLE 9.—*Dextrose-phosphate effect*

10 P.P.M. CALCIUM OR 10 P.P.M. MAGNESIUM +PHOSPHATE+DEXTROSE	CALCIUM, P.P.M. RECOVERY	MAGNESIUM, P.P.M. RECOVERY
Aqueous standard solution (no dextrose or phosphate)	10.0	10.0
500 p.p.m. phosphate	3.2	4.7
500 p.p.m. sulfate	5.7	5.7
500 p.p.m. phosphate+500 p.p.m. sulfate	3.1	4.8
500 p.p.m. phosphate+500 p.p.m. sulfate +1% dextrose	8.8	8.7
20 p.p.m. phosphate+1% dextrose	8.9	8.8
50 p.p.m. phosphate+1% dextrose	8.6	8.7
100 p.p.m. phosphate+1% dextrose	8.6	8.6
500 p.p.m. phosphate+1% dextrose	8.6	8.7
1000 p.p.m. phosphate+1% dextrose	8.7	8.8

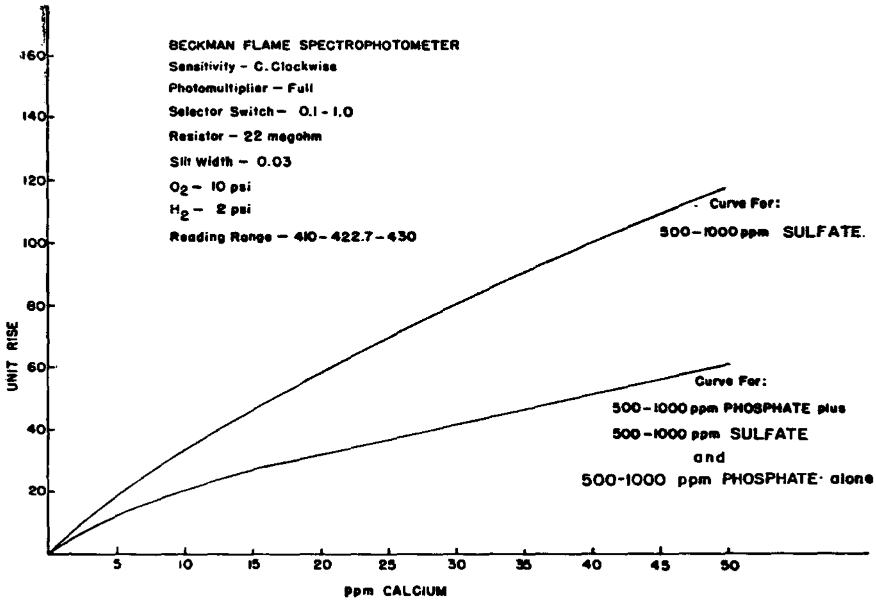

FIG. 3.—The effect of phosphate and sulfate on calcium.

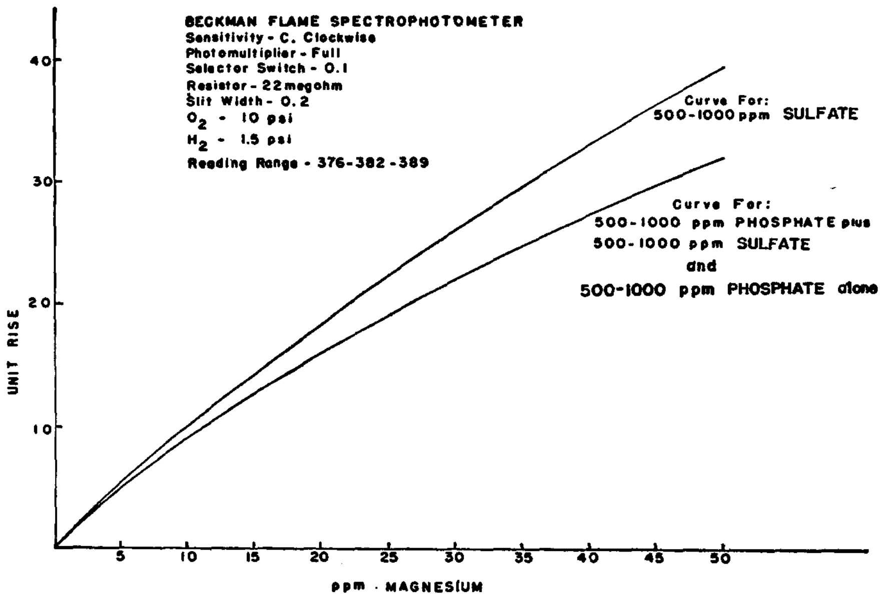

FIG. 4.—The effect of phosphate and sulfate on magnesium.

concentrations makes possible the elimination of simulated standards. This dextrose-phosphate effect is immediate, and is stable for at least two days. Investigation of the exact mechanism by which dextrose affects phosphate was not undertaken, but other materials such as glycerine failed to inhibit the phosphate effect. This led to the speculation that a complex might have been formed by dextrose and phosphate.

SUMMARY

Methods are presented for the determination of calcium, magnesium, potassium, and sodium in wines by a Beckman flame spectrophotometer with an oxygen-hydrogen flame and a photomultiplier attachment. Analytical results are compared with gravimetric procedures, and data are presented in which known increments of these metals are added to samples. Interferences due to organic material and to inorganic cations and anions are described in detail. It is shown that dextrose markedly decreases phosphate and sulfate inhibition of the calcium and magnesium emissions.

REFERENCES

(1) *Internal Revenue Service,* Regulations 7, Article XXIV.
(2) SIMON, A. L., *A Dictionary of Wine,* Longmans, Green & Co., New York, 1936, p. 260.
(3) GILBERT, P. T., JR., *Ind. Labs.* (Beckman Reprint R-56) 1952.
(4) BRODE, W. R., *Chemical Spectroscopy,* 2nd Ed., John Wiley & Sons, Inc., New York, 1943.
(5) Beckman Instruments, Inc., Bulletin 282-B, South Pasadena, Calif., 1953.
(6) DOMANGE, L., and LONGUEVALLE, S., *Ann. pharm. franc.,* **19,** 647 (1951).
(7) LEPPANEN, V., and FORSANDER, O., *Scand. J. Clin. & Lab. Invest.,* Finland, **3,** 33 (1951).
(8) WALLACE, W. M., HOLLIDAY, M., CUSHMAN, M., and ELKINGTON, J. R., *J. Lab. Clin. Med.,* **37,** No. 4, 621 (1951).
(9) GILLILAND, J. L., *Am. Soc. Testing Materials,* **No. 129** (1951).
(10) LUNDEGARDH, H., *Z. Physik,* **66,** 109 (1930).
(11) SCHMITT, L., and BREITWEISER, W., *Bodenkunde u. Pflanzenernahr.,* **9,** 750 (1938).
(12) RAUTERBERG, E., and KNIPPENBERG, E., *ibid.,* **20,** 364 (1940).
(13) HERRMANN, R., and LEDERLE, P., *ibid.,* **30,** 189 (1942).
(14) Perkin-Elmer Corp., *Instruction Manual for the Flame Photometer,* Model **18,** 1945.
(15) GILBERT, P. T., JR., HAWES, R. C., and BECKMAN, A. O., *Anal. Chem.,* **22,** 772 (1950).
(16) BIFFEN, F. M., *ibid.,* **22,** 1014 (1950).
(17) GENTINI, A., *Anales asoc. quim. y farm., Uruguay,* **44,** No. 2, 21 (1941).
(18) SMITH, R. G., CRAIG, P., BIRD, E. J., BOYLE, A. J., ISERI, L. T., JACOBSEN, S. D., and MYERS, G. B., *Am. J. Clin. Path.,* **20,** 263 (1950).
(19) WEST, P. W., FOLSE, P., and MONTGOMERY D., *Anal. Chem.,* **22,** 667 (1950).
(20) CLOSE, P., SMITH, W. E., and WATSON, M. T., JR., *ibid.,* **25,** 1022 (1953).
(21) BROWN, J. G., and LILLELAND, O., *Proc. Am. Soc. Hort. Sci.,* **48,** 341 (1946).
(22) SNYDER, J. Q., *Proc. Oklahoma Acad. Sci.,* **31,** 134 (1950).
(23) KUEMMEL, D. F., and KARL, H. L., *Anal. Chem.,* **26,** 386 (1954).
(24) BERRY, J. W., CHAPPELL, D. G., and BARNES, R. B., *ibid.,* **18,** 19 (1946).

(25) BILLS, C. E., MCDONALD, R. G., and NIEDERMEIER, W., *ibid.*, **21**, 1076 (1949).
(26) CURTIS, G. W., KNAUER, H. E., and HUNTER, L. E., *Am. Soc. Testing Materials*, Reprint No. 132 (1951).
(27) PRATT, R. F., and LARSON, N. E., *Anal. Chem.*, **21**, 1296 (1949).
(28) SEVERINGHAUS, J. W., and FERREBEE, J. W., *J. Biol. Chem.*, **187**, 621 (1950).
(29) KNIGHT, S. B., MATHIS, W. C., and GRAHAM, J. R., *Anal. Chem.*, **23**, 1704 (1951).
(30) PARKS, J. D., JOHNSON, H. O., and LYKKEN, L., *ibid.*, **20**, 822 (1948).
(31) IVANOV, D. N., *Zavodskaya Lab.*, **10**, 401 (1941).
(32) CRIMSON, J. M., *Federation Proc.*, **7**, 24 (1948).
(33) SHAPIRO, S., and HOAGLAND, H., *Am. J. Physiol.*, **153**, 428 (1948).
(34) BROWN, J. G., LILLELAND, O., and JACKSON, R. K., *Proc. Am. Soc. Hort. Sci.*, **52**, 1 (1948).
(35) AMERINE, M. A., THAUKIS, G., and MARFA, R., V-B, *Proc. Amer. Soc. Enologists*, Davis, California, p. 164 (1953).
(36) Beckman Instruments, Inc., Bulletin 193-B (1948).
(37) Beckman Instruments, Inc., Bulletin 259 (1951).
(38) PRO, M. J., and ETIENNE, A. D., *This Journal*, **36**, 1150 (1953).
(39) GILBERT, P. T., JR., Beckman Reprint R-26, National Technical Laboratories (1949).
(40) BARNES, R. B., RICHARDSON, D., BERRY, J. W., and HOOD, R. L., *Anal. Chem.*, **17**, 605 (1945).
(41) AMERINE, M. A., and JOSLYN, M. A., *Table Wines*, University of California Press, Berkeley and Los Angeles, 1951, p. 243–283.
(42) JACOBS, M. B., *The Chemistry and Technology of Food and Food Products*, Interscience Publishers, Inc., New York, 1944, p. 762–781.

IDENTIFICATION OF INSECT SETAE AS AN INDEX OF CONTAMINATION IN DAIRY PRODUCTS*

By FELIX J. SABATINO (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D.C.)

This paper describes an investigation to determine the practicability of identifying fly setae[1] found in foods, especially those found in dairy products. As the work progressed, the characteristics of other setae were investigated as a matter of general interest, and preliminary findings in this direction have been included.

The manufacture of foods under insanitary conditions or from insect-infested raw materials may result in the contamination of the finished product with insect fragments. Many of these fragments can be recovered by known techniques (1). However, the interpretation of the significance of the contamination, as related to its origin, depends upon the identification of the insect types that contributed the fragments. Some specific fragments which are commonly encountered in insect-infested cereal products have been discussed in recent articles (2–5).

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* Presented at the 68th Annual A.O.A.C. meeting, Oct. 11–13, 1954, Washington, D. C.
[1] In this paper, the term "seta" is used broadly to include all movable hair; scale; or bristle-like structures.

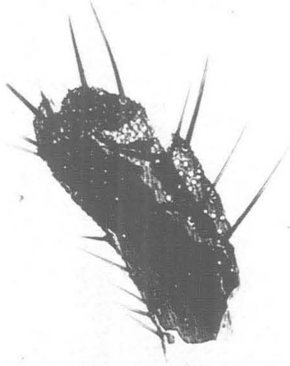

FIG. 1.—Housefly—thoracic fragment (100×).

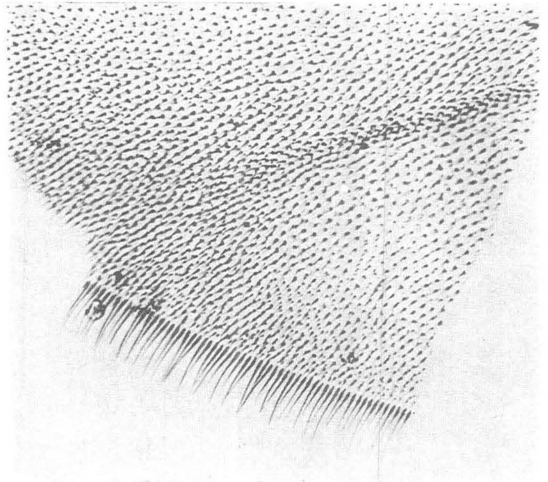

FIG. 2.—Housefly—wing fragment (100×).

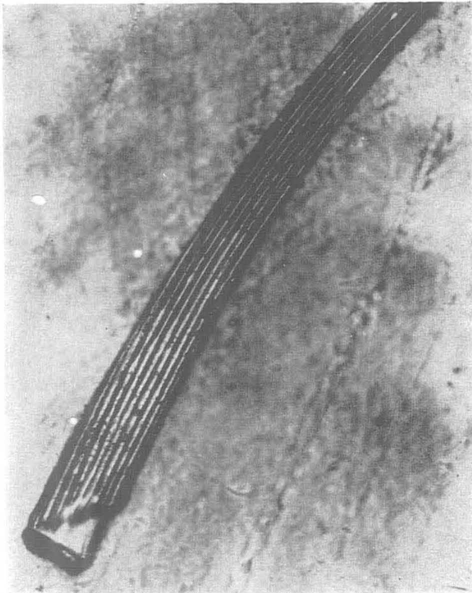

FIG. 3.—Housefly—setal impression pattern (400×).

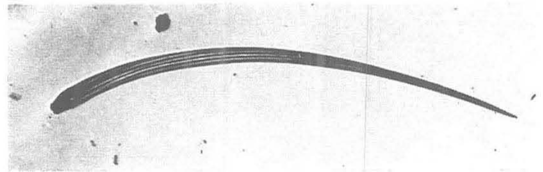

FIG. 4.—Drosophilia seta (200×).

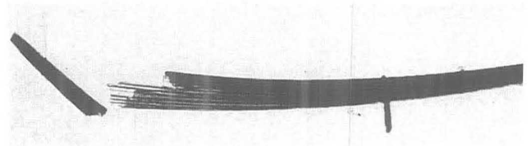

FIG. 5.—Tachinid fly—crushed seta (200×).

Fig. 6.—Housefly seta.

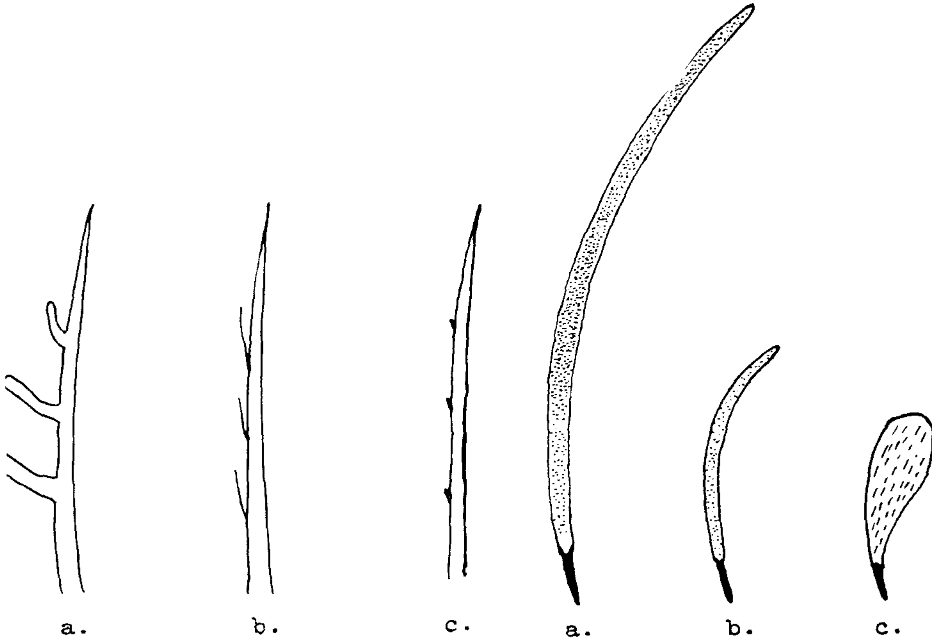

Fig. 7.—Spider's setae.

Fig. 8.—Psychodid fly—seta.

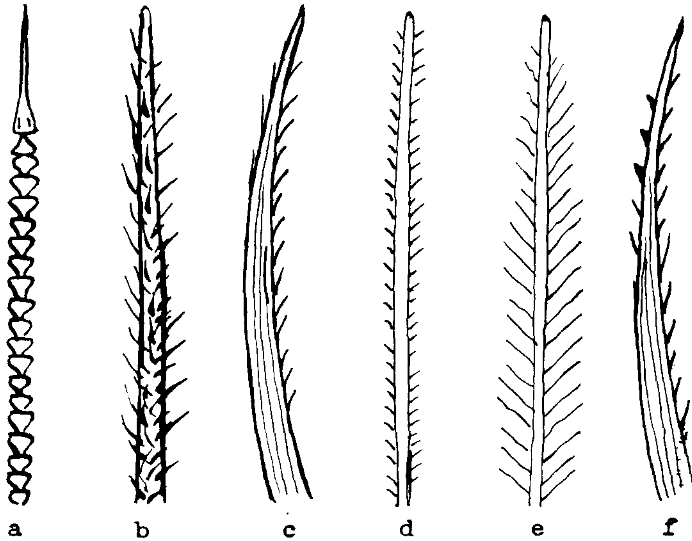

Fig. 9.—Seta—a, b, Dermestid larvae; c, Phorid fly; d, e, honey and bumble bee; f, Fungivoridae (Mycetophilidae).

In the dairy and certain other food industries, filth and insanitation are often due to flies. During manufacture, filtering and clarifying processes reduce the amount of insoluble filth and the larger filth elements may thus be entirely eliminated. However, minute and loosely attached setae, which occur in large numbers on the surface of insects, may pass through filtering and clarifying operations and appear in the finished product, where they constitute a useful index of contamination. Since insects bear setae, there has been a need for some means of distinguishing the setae from different types of insects.

Accordingly, an examination was made of the setae of representative species of several fly families as well as of other insects and allied forms (bees, ants, wasps, beetles, cockroaches, spiders), to see if fly setae could be distinguished from other insect setae, and, secondarily, whether setae of various fly families could themselves be differentiated. Since the examinations for insect fragments, including insect setae, are routinely made at low magnification (30×), this study was restricted to the examination of setae of such a size as would be found at this magnification during a routine analysis. Fig. 1 shows a portion of the housefly thorax with numerous attached setae and seta pits. Such setae are sufficiently large to be recognized as insect fragments. However, there are areas of the insect body, particularly the wing surfaces, where the setae are so minute that they would not be detected during routine examination (Fig. 2).

KEY

I. **One or both lateral[2] surfaces with minute and sparse, to large and dense, bristle-, leaf-, or feather-like projections,** or markedly segmented, discontinuous; irregular when seen in silhouette; possibly interrupted lines or checking; jagged, sawtoothed. These are due to ubiquitous insects or spiders not commonly associated with dung and/or decaying animal or vegetable matter, and which include, among other insects:

(*A*) Setae with saw-toothed edges (*Dermestids*, Fig. 9, *a*).

(*B*) Setae with feather-, leaf-, or bristle-like projections.

(1) With many bristles along top[2] as well as lateral surfaces (Fig. 9, *b*, *Dermestid* larvae).

(2) With a lack of bristles along most of top surface.

(*a*) Lateral surface medium to heavily bristled, seta tapering to point at tip (*Phoridae*, *Fungivoridae* (*Mycetophilidae*), Fig. 9 *c* and *f*).

(*b*) Lateral surface sparsely bristled or with checking (Spiders, Fig. 7, *b* and *c*).

(*c*) Lateral surface heavily feathered (projections as long as or longer than greatest width of seta), seta of relatively narrow uniform width (Bumble and honey bee, Fig. 9, *d* and *e*).

(*d*) Lateral surface bears some leaf-like projections, particularly at the distal end, in addition to bristles (*Fungivoridae* (*Mycetophilidae*), Fig. 9, *f*).

(*C*) With truncated branches (Spiders, Fig. 7, *a*).

[2] Because of the curved shape, there will be a uniform orientation with respect to the over-all curvature, and the surfaces can then be designated as "lateral" (inside and outside of the curvature) and "top" and "bottom" as it orients on the microscope slide.

II. **Both lateral surfaces smooth.** Lateral surfaces seen in silhouette form smooth, continuous arcs. These are due to flies of several families, some of which breed in dung and decayed matter; some of which are field pests:

(*A*) Furrowing present. May be visible throughout entire setae at 100×; or only at more lightly-colored tip at 200–400×; or after crushing; or by using concentrated reflected illumination.

(1) Clear to translucent, lightly-colored to brown, but no extensive black areas. Furrowing noted at 100× (Fig. 4). *Agromyzidae, Anthomyiidae, Bombylidae, Culicidae, Drosophilidae, Fungivoridae (Mycetophilidae), Ortalidae, Phoridae, Psilidae, Syrphidae, Tachinidae, Tipulidae, Trypedidae.*

(2) Setae black, furrowing present; not noted at 100× due to coloration, furrowing may be noted at 200× to 400× at tip or where broken. (Largely breeding in dung or decaying animal or vegetable matter.) Fig. 6, *Anthomyiidae* (some species are important crop pests), *Calliphoridae, Muscidae, Sarcophagidae, Tachinidae.*

(*B*) Furrowing absent. Largely not dung or decayed-matter feeders. *Bombilidae, Psychodidae, Stratiomyiidae, Syrphidae, Tabanidae, Tachinidae,* and other insects.

DISCUSSION

Furrowing, branching or feathering, and pigmentation were found to be characteristics useful in distinguishing the various type of setae.

In the examination of setae, it was noted that some possess a longitudinal surface furrowing which was rather difficult to see when dark coloration was present. Three procedures were used to establish the presence of these furrows when they were not visible with transmitted light. The use of a concentrated source of light, slightly above the specimen and at right angles to the length of the setae (in place of the more commonly used transmitted light) made the ridges visible by intense reflected illumination. Crushing the seta often produced a diagonal break, sufficiently elongated to give a thin longitudinal section, through which the light penetrated and made the grooves visible at the fracture point (Fig. 5). The furrows were also demonstrated by sandwiching the seta between a glass microscope slide and a piece of polyethylene film which, in turn, was backed up by another glass microscope slide. The entire mount was then squeezed in a small vise for 5 to 10 minutes. Fig. 3 is a photograph of an impression made by a housefly seta on the polyethylene film.

The presence of lateral projections on insect setae was used as a differentiating characteristic. Nearly all the fly setae examined were devoid of lateral projections, while certain nondipterous insects had such projections. The setae of various bees, for example, have filamentous and short, stubby projections (Fig. 9, *d* and *e*), whereas some coleoptera larvae bear segmented setae (Fig. 9, *a*). All spider setae examined were equipped with projections, and some of these setal variations are diagramed in Fig. 7. In one type, the projections are so minute and inconspicuous that they may be overlooked if only a cursory examination is made (Fig. 7, *c*).

Coloration and pigmentation was also used as a key separating character. In one group (II A-1 in the key), the coloration ranges from clear yellow to dark brown. The furrows in this group can be noted at 100× throughout the entire length of the seta. The other group (II A-2) has black coloration, and here the furrows are not noted at all at 110×. However, the tip end of the seta may appear brownish, and it is in this region that the furrows may be noted at higher magnifications (200–400×) with transmitted light.

Since one of the characteristics used in identification was the dark pigmentation, approximately 50 setae taken from milk and butter were examined to determine whether this characteristic persisted. It was found that the type of treatment received in the manufacture of butter and during its analysis (1) did not interfere with this characteristic. However, the mounting of setae in some media (Permount or acid chloral hydrate in glycerine) does have a slight bleaching or clearing effect after several months.

In addition to the above characters which form the basis of the key for the separation of the insect setae, the shape of the base appeared to be somewhat characteristic and may be used in further investigations of this type. For example, some fly-setae, particularly the larger bristle-like types, have a notch cut out of the base as shown in Fig. 6. Some setae of the syrphid flies taper to a notch, which then enlarges to a bulbous swelling. However, since this latter type of base often remains behind in the setal pit, its usefulness in the identification of setae found in food products is limited.

Since the primary purpose of this work was to provide a means of identifying setae commonly encountered as contaminants of dairy products or serving as an index of dairy product contamination, the key to setae identification has been set up so as to separate the setae from flies associated with filth and decaying vegetable matter from other insects which are primarily specific pests, pests of growing crops, or pests of stored products. Those flies which breed in dung and decayed matter have dark black setae with smooth lateral surfaces and longitudinal furrows appearing as striations along the setal surface.

Only four of the fly families investigated had setae with no apparent furrowing. Two others had two types of setae present, one with furrowing and one without, which partially accounts for the presence of a family in more than one classification. Because of the color variation in the striated setae, certain families also appear in more than one classification. Many of the flies examined had more than one type of seta, e.g., Psychodidae, which had three types of setae, all apparently non-furrowed. (See Figure 8, *a*, *b*, and *c* which shows a long seta with a bulb-shaped base and elongation resembling a thermometer; a shorter version of the above with a similar base but with the "stem" shorter; and a small scale-like structure with the same typical base.)

ACKNOWLEDGMENTS

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REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950, sections **35.9–35.21.**
(2) HARRIS, K. L., *This Journal*, **33,** 898 (1950).
(3) ———, and NICHOLSON, J. F., *Northwestern Miller*, **232,** 10 (1947).
(4) KURTZ, O. L., *et al., This Journal*, **35,** 817 (1952).
(5) KURTZ, O. L., *ibid.*, **37,** 167 (1954).

SOME OBSERVATIONS ON THE VERSENATE METHOD FOR CALCIUM AND MAGNESIUM IN AGRICULTURAL LIMING MATERIALS*

By W. M. HOFFMAN and H. SHAPIRO† (Fertilizer and Agricultural Lime Section, Soil and Water Conservation Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.)

The A.O.A.C. methods for the determination of calcium and magnesium in agricultural liming materials (1) have the disadvantage of requiring several lengthy operations, including separation of the two elements, gravimetric or volumetric measurement of the calcium, and gravimetric measurement of the magnesium. Recent investigations have indicated, on the other hand, that calcium and magnesium in certain types of liming materials (2, 5, 7, 9), as well as in water (3, 8), milk (10), soil and plant materials (6), and silicate rocks (16), can be determined rapidly and with a high degree of precision and accuracy by the so-called Versenate method. The present paper reports the results of a further study of certain phases of the Versenate method.

In its several modifications the Versenate method is based on the property of ethylenediaminetetraacetic acid (the disodium salt of which is marketed under names such as Versenate, EDTA, Sequestrene, and Complexone III, among others) to form complex combinations with the ions of many metallic elements. Thus, by controlling the *p*H and by using suitable indicators, specific metallic ions can be titrated in the presence of others with Versenate.

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Schwarzenbach and Ackermann (14) used murexide (ammonium purpurate) as the indicator in the Versenate titration of calcium, in which the disappearance of the calcium ion is signified by the color change from red to violet. Schwarzenbach and Biedermann (15) reported that the total calcium and magnesium can be determined by titration with Versenate in the presence of Eriochrome Black T (F 241), preferably at *p*H 10. The color change from red to blue signals the disappearance of the magnesium ion, which occurs subsequent to the disappearance of the calcium ion, so that the titer represents the total calcium and magnesium. For the analysis of limestone, Cheng, Kurtz, and Bray (7) made use of both indicators; the total calcium and magnesium was determined with F 241, the calcium was determined with murexide on another aliquot of equal volume, and the magnesium was calculated from the difference in the titers.

Certain other Versenate procedures are based on titrations that involve, in part, the prior separation of the calcium from the magnesium by several means which include ion exchange chromatography (5) and precipitation as the oxalate (2), tungstate (16), or sulfite (9).

Instrumental determination of the endpoint of the Versenate titrations has received considerable attention. Potentiometric and amperometric procedures have been described by Pribil and co-workers (12, 13) and by Laitinen and Sympson (11). Blaedel and Knight (4) made use of a high frequency technique, Sweetser and Bricker (17, 18) have described a spectrophotometric procedure, and Shapiro and Brannock (16) have designed a photometric instrument for automatically recording the progress of the titration.

EXPERIMENTAL

Samples and Procedures.—Samples 1 and 2 were a high-calcium burnt lime and a marl, respectively. The other samples (Nos. 3 to 8) were high-magnesium limestones from various sources (see Tables).

Solutions of the sample were prepared by the procedure outlined in the A.O.A.C. method (1), except that removal of dissolved silica, iron, and aluminum was omitted in the Versenate titrations. With the A.O.A.C. method, calcium was determined by double precipitation as the oxalate and weighing as the oxide.

Pure calcium carbonate, magnesium metal ribbon, and National Bureau of Standards standard sample No. 88 (dolomite) were used as standards in the preparation of the Versenate solution. The Versenate titrations were made at a *p*H of approximately 10.

The range and average of the reported analyses are for triplicate determinations.

Differential Determination of Magnesium.—In trials of the method of Cheng, Kurtz, and Bray (7), described above, trouble was experienced in the visual determination of the endpoints of both titrations. The color change in the calcium determination is not sharp, and much practice is necessary to determine the proper point at which to stop the titration. With F 241 the visual determination of the endpoint is easier but is still subject to some difficulty because of the color gradation near the end of the titration.

An automatic photometric titrator of the kind described by Shapiro and Bran-

with visual and photometric determination of endpoint

SAMPLE	CALCIUM OXIDE						MAGNESIUM OXIDE					
	A.O.A.C. METHOD		VERSENATE TITRATION,[a] ENDPOINT DETD:				A.O.A.C. METHOD		VERSENATE TITRATION,[b] ENDPOINT DETD:			
			VISUALLY		PHOTOMETRICALLY				VISUALLY		PHOTOMETRICALLY	
	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	67.00–67.35	67.13	67.20–67.80	67.50	67.03–67.31	67.15	2.37– 2.43	2.40	3.52– 3.63	3.56	4.65– 4.72	4.68
2	53.95–54.07	54.00	53.70–54.00	53.88	53.89–53.98	53.93	0.59– 0.62	0.60	1.54– 1.67	1.59	1.90– 1.96	1.93
3	32.45–32.60	32.54	32.40–32.61	32.53	32.27–32.35	32.31	18.59–18.66	18.62	20.44–20.64	20.54	19.19–19.30	19.25
4	41.95–42.08	42.01	42.36–42.54	42.45	41.59–41.87	41.75	8.65– 8.68	8.66	9.81– 9.90	9.86	9.06– 9.10	9.08
5	38.54–38.88	38.77	38.76–39.30	39.02	38.88–39.12	39.02	12.17–12.29	12.23	13.53–13.73	13.62	12.80–12.99	12.89
6	37.36–37.65	37.48	37.32–37.50	37.40	37.42–37.46	37.45	13.29–13.44	13.38	14.21–14.39	14.30	13.93–14.15	14.02
7	30.89–31.19	31.00	31.26–31.50	31.37	31.15–31.35	31.24	18.69–18.99	18.86	19.91–20.06	20.00	19.14–19.19	19.16
8	30.28–30.47	30.35	30.00–30.21	30.10	30.45–30.53	30.40	19.42–19.58	19.53	21.23–21.45	21.33	19.81–19.89	19.85

[a] Direct determination (7).
[b] Differential determination (7).

TABLE 2.—*Magnesium oxide by the A.O.A.C. method and by Versenate titration after removal of calcium*

SAMPLE	A.O.A.C. METHOD		VERSENATE TITRATION[a] AFTER REMOVAL OF CALCIUM WITH:							
			AMMONIUM OXALATE				SODIUM TUNGSTATE		SODIUM SULFITE	
			AMMONIA BUFFER		ACETATE BUFFER					
	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2.37– 2.43	2.40	2.38– 2.46	2.42	2.36– 2.50	2.43	2.34– 2.43	2.38	2.35– 2.38	2.36
2	0.59– 0.62	0.60	0.60– 0.64	0.62	0.63– 0.65	0.64	0.57– 0.59	0.58	0.66– 0.68	0.67
3	18.59–18.66	18.62	18.52–18.66	18.60	18.56–18.67	18.62	18.50–18.62	18.57	18.60–18.78	18.68
4	8.65– 8.68	8.66	8.58– 8.63	8.61	8.63– 8.75	8.69	8.53– 8.64	8.59	8.62– 8.65	8.64
5	12.17–12.29	12.23	12.14–12.17	12.15	12.14–12.24	12.20	12.15–12.27	12.21	12.13–12.27	12.18
6	13.29–13.44	13.38	13.32–13.40	13.35	13.30–13.38	13.34	13.31–13.36	13.34	13.43–13.51	13.47
7	18.69–18.99	18 86	18.78–18.86	18.82	18.69–18.79	18.74	18.77–18.86	18.81	18.96–19.10	19.03
8	19.42–19.58	19.53	19.40–19.61	19.50	19.40–19.51	19.46	19.36–19.51	19.43	19.45–19.61	19.52

[a] Photometric determination of endpoint.

nock (16) served very well for determining the endpoint in the Versenate titration of calcium and of calcium plus magnesium. This instrument records the change in photometric characteristics of the solution in the vicinity of the endpoint, as indicated in Figure 1.

The data of Table 1 show generally good agreement between the results for calcium by the A.O.A.C. method and those by the Versenate method with murexide as indicator, especially when the endpoint was determined photometrically. The precision of the Versenate method was improved by use of the photometer.

The Versenate results for magnesium, as determined differentially, were consistently higher (often very much higher) than those by the A.O.A.C. method (Table 1). As with calcium, the values by the two methods were generally in better agreement and the precision of the Versenate method was improved when the endpoint of the Versenate titration was determined photometrically instead of visually.

Direct Determination of Magnesium.—As previously mentioned, several workers have applied the Versenate method, with F 241 as indicator, to the direct determination of magnesium in solutions from which the calcium had been precipitated as the oxalate (2), tungstate (16), or sulfite (9). In comparative analyses, practically the same values for magnesium by the Versenate method were obtained on solutions prepared with each of these precipitants, and results were in very good agreement with those by the A.O.A.C. method (Table 2).

Analyses of solutions prepared with sodium sulfite as the precipitant showed no significant difference in the results for magnesium by the Versenate method whether the endpoint was determined visually or photometrically (Table 3). Similar agreement between the values for magnesium was obtained when sodium tungstate was used as the precipitant. Banewicz and Kenner (2) reported that the visual endpoint in the titration of magnesium after separation of calcium oxalate was indistinct and unsatisfactory, but addition of hydrogen peroxide resulted in a satisfactory and reproducible endpoint. In the present study, no difficulty was experienced in the photometric determination of the endpoint, even without the addition of hydrogen peroxide.

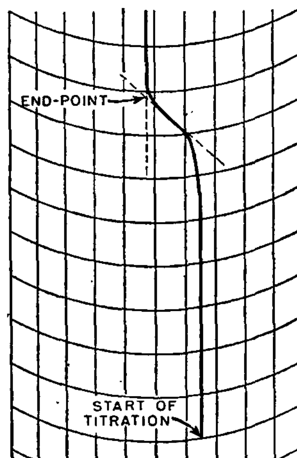

FIG. 1.—Chart of Versenate titration of calcium as recorded by automatic photometric titrator.

TABLE 3.—*Magnesium oxide by Versenate titration after separation of calcium with sodium sulfite*

SAMPLE	ENDPOINT DETERMINED			
	VISUALLY		PHOTOMETRICALLY	
	RANGE	AVERAGE	RANGE	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*
1	2.41– 2.44	2.43	2.35– 2.38	2.36
2	0.62– 0.66	0.64	0.66– 0.68	0.67
3	18.53–18.66	18.59	18.60–18.78	18.68
4	8.65– 8.72	8.69	8.62– 8.65	8.64
5	12.11–12.21	12.17	12.13–12.27	12.18
6	13.38–13.44	13.41	13.43–13.51	13.47
7	18.94–19.03	18.98	18.96–19.10	19.03
8	19.45–19.57	19.52	19.45–19.61	19.52

SUMMARY

Determinations of calcium and magnesium were made on 8 samples of agricultural liming materials by the A.O.A.C. method and by several variations of the Versenate method.

The values for calcium by direct titration with Versenate, with murexide as the indicator and with visual or photometric determination of the endpoint, compared favorably with those determined by the A.O.A.C. method.

The values for magnesium by differential titration with Versenate were higher in all cases, whether the endpoint was determined visually or photometrically, than the values determined by the A.O.A.C. method.

Direct titration of magnesium with Versenate and F 241 indicator, after separation of calcium by precipitation as the oxalate, tungstate, or sulfite, gave results that agreed very closely with those by the A.O.A.C. method.

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REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D.C., 1950, pp. 3, 32, 33.

(2) BANEWICZ, J. L., and KENNER, C. T., *Anal. Chem.*, **24**, 1186 (1952).

(3) BETZ, J. D., and NOLL, C. A., *J. Am. Water Works Assoc.*, **42**, 49 (1950).

(4) BLAEDEL, W. J., and KNIGHT, H. T., *Anal. Chem.*, **26**, 743 (1954).
(5) CAMPBELL, D. N., and KENNER, C. T., *ibid.*, **26**, 560 (1954).
(6) CHENG, K. L., and BRAY, R. H., *Soil Sci.*, **72**, 449 (1951).
(7) ———, KURTZ, T., and BRAY, R. H., *Anal. Chem.*, **24**, 1640 (1952).
(8) DIEHL, H., GOETZ, C. A., and HACH, C. G., *J. Am. Water Works Assoc.*, **42**, 40 (1950).
(9) GEHRKE, C. W., AFFSPRUNG, H. E., and LEE, Y. C., *Anal. Chem.* (in press).
(10) JENNESS, R., *ibid.*, **25**, 966 (1953).
(11) LAITINEN, H. A., and SYMPSON, R. F., *ibid.*, **26**, 556 (1954).
(12) PRIBIL, R., KORIDELA, Z., and MATYSKA, B., *Collection Czechoslov. Chem. Communs.*, **16**, 80 (1951).
(13) ———, and MATYSKA, B., *ibid.*, **16**, 139 (1951).
(14) SCHWARZENBACH, G., and ACKERMANN, H., *Helv. Chim. Acta*, **30**, 1798 (1947).
(15) ———, and BIEDERMANN, W., *ibid.*, **31**, 678 (1948).
(16) SHAPIRO, L., and BRANNOCK, W. W., *U. S. Geological Survey Circular* (in press).
(17) SWEETSER, P. B., and BRICKER, C. E., *Anal. Chem.*, **25**, 253 (1953).
(18) ———, and BRICKER, C. E., *ibid.*, **26**, 195 (1954).

ETHYLENEDIAMINE TETRAACETIC ACID AS AN ANALYTICAL REAGENT TO EVALUATE AGRICULTURAL LIMING MATERIALS*

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Many agricultural soils will produce greater yields if they are properly limed. The amount of liming material to use depends on soil characteristics and on the composition of the liming material itself. The purpose of the present paper is to outline a method of evaluating liming materials by the use of ethylenediamine tetraacetic acid (EDTA) and to suggest that a modification of the method be used in evaluating the need of soils for liming materials.

Recently, a large number of articles have appeared on the analytical use of this compound in industry, in agriculture, and in medicine. Schwarzenbach, Biedermann, and Bangerter (7) showed that the calcium content of water may be determined in a strongly alkaline solution by titrating against EDTA with ammonium purpurate (Murexide) as indicator. Its use in the determination of the hardness of water (i.e., the calcium and magnesium content) has since been developed by a number of investigators, and has become common practice. A number of methods based on the use of EDTA have been listed, together with a comprehensive bibli-

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ography (1, 2). Among the publications indicating specific uses for EDTA is that of Cheng and Bray (3) who used EDTA to determine the calcium and magnesium content of soils and plant material, and of Perkins (6) who proposed a method for determining the cation exchange capacity of soils.

EXPERIMENTAL AND METHODS

The EDTA employed in this work has been used in two ways to determine the calcium plus magnesium content of calcareous materials. The calcium and magnesium content of the calcareous materials has also been determined by A.O.A.C. methods (4) which involve both volumetric and gravimetric procedures after removal of silica and the R_2O_3 group. The neutralizing value of these calcareous materials has also been found by the A.O.A.C. method (5). The results of the quantitative determination of the silica, R_2O_3, calcium, and magnesium contents of a group of minerals are reported in Table 1. The calcium and magnesium content of these minerals, calculated to the $CaCO_3$ equivalent, are given in Table 2, together

TABLE 1.—*Analysis of a group of minerals by A.O.A.C. methods*

MINERAL	LAB. NO.	SiO_2	R_2O_3	Ca as $CaCO_3$	Mg as $MgCO_3$	Ca and Mg as $CaCO_3$
		per cent	*per cent*			
$CaCO_3$, A.R.	None	0.00	0.50	99.87	0.06	99.94
Oyster shell	31988	1.08	0.26	95.43	1.36	97.05
Oyster shell	32473	1.76	0.60	95.69	1.40	97.36
Limestone[a]	M-7540	0.66	1.84	94.93	1.91	97.20
Towanda[c]	M-7821	3.42	1.66	94.45	1.61	96.37
Phosphate rock	M-7653	2.80	1.14	94.19	2.22	96.86
Crouse[c]	M-7815	4.46	1.64	92.43	2.57	95.49
Lime and soda ash[b]	M-7577	4.34	1.86	87.75	5.28	94.05
Funston[c]	M-7818	7.66	2.20	89.87	2.31	92.62
Morill[c]	M-7738	4.74	2.50	88.74	5.42	95.20
Ft. Riley[c]	M-7817	7.02	2.60	88.97	5.42	91.76
Neva[c]	M-7819	6.62	3.10	87.64	2.49	90.61
Florence[c]	M-7816	9.43	2.10	87.13	2.99	90.69
Florence[c]	M-7739	7.62	1.82	87.01	1.85	89.21
Limestone[a]	M-7538	5.64	2.40	88.21	1.91	90.48
Limestone[a]	M-7536	6.14	3.76	85.78	2.08	88.26
Limestone[a]	M-7539	7.72	1.86	86.03	1.98	88.39
Cresswell[c]	M-7814	15.26	3.80	79.05	3.20	82.67
Stovall[c]	M-7820	32.14	3.12	62.46	2.37	65.29
Lime and soda ash[b]	M-7578	37.86	10.42	41.04	2.98	44.57
Hoosier shale	M-7740	61.46	17.44	2.70	5.30	9.01
Shale[a]	M-7654	83.24	5.30	4.94	2.52	7.95
Madison, Wis.[d]	None	0.32	0.18	55.13	41.87	105.95
Baltimore, Md.[d]	None	0.23	0.20	54.15	45.60	108.38
Wards #1[d]	None	0.41	0.31	56.42	42.88	107.45
Wards #2[d]	None	0.30	0.20	54.18	45.77	108.64

[a] Unidentified formation.
[b] Waste from water softening.
[c] Limestone.
[d] Dolomite.

TABLE 2.—*Calcium and magnesium content of a group of minerals by several methods*

	METHOD NO.					
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]
		per cent	*per cent*			
$CaCO_3$	99.94	100.04	99.80	99.75	0.04	100.08
Oyster shell	97.05	98.82	99.44	98.00	1.31	99.84
Oyster shell	97.36	97.84	97.86	96.25	1.37	97.84
Limestone[g]	97.20	96.62	97.98	96.00	1.76	96.34
Towanda[i]	96.37	95.65	95.22	93.50	1.34	96.84
Phosphate rock	96.86	95.16	95.58	93.50	1.75	93.82
Crouse[i]	95.49	95.16	94.18	91.75	2.04	94.85
Lime and soda ash[h]	94.05	94.06	94.88	89.50	4.52	92.85
Funston[i]	92.62	91.74	92.42	90.00	2.03	93.35
Morill[i]	95.20	91.26	92.94	88.25	4.94	90.85
Ft. Riley[i]	91.76	90.65	91.89	86.75	4.31	90.85
Neva[i]	90.61	89.79	89.95	87.25	2.27	89.86
Florence[i]	90.69	89.67	89.25	86.00	2.73	89.86
Florence[i]	89.21	89.55	89.95	88.00	2.34	89.86
Limestone[g]	90.48	89.06	88.72	86.50	1.86	87.86
Limestone[g]	88.26	87.48	88.37	86.25	1.74	88.36
Limestone[g]	88.39	86.86	87.86	85.50	1.98	86.36
Cresswell[i]	82.67	81.25	81.53	78.50	2.44	80.87
Stovall[i]	65.29	64.30	64.13	61.50	2.21	65.89
Lime and soda ash[h]	44.57	42.70	42.87	39.50	2.73	43.83
Hoosier shale	9.01	12.45	12.66	7.00	4.75	11.98
Shale[g]	7.95	9.27	8.25	5.50	2.31	6.99
Madison, Wis.[j]	105.95	106.15	106.50	55.00	43.26	106.50
Baltimore, Md.[j]	108.38	107.98	108.25	52.50	46.83	108.50
Wards #1[j]	107.45	107.49	107.75	56.25	43.26	107.75
Wards #2[j]	108.64	109.15	108.00	53.75	45.57	108.25

[a] 1 = Calcium and magnesium from Table 1 calculated as $CaCO_3$.
[b] 2 = $CaCO_3$ calculated from solution in HCl and titration with NaOH.
[c] 3 = Calcium and magnesium by solution of mineral in HCl and titration against EDTA with Eriochrome Black T indicator calculated as $CaCO_3$.
[d] 4 = Calcium by solution of mineral in HCl and titration against EDTA with murexide indicator calculated as $CaCO_3$.
[e] 5 = Magnesium as $MgCO_3$ by difference.
[f] 6 = Calcium and magnesium by solution in EDTA and back titration against $CaCl_2$ with Eriochrome Black indicator calculated as $CaCO_3$.
[g] Unidentified formation.
[h] Waste from water softening.
[i] Limestone.
[j] Dolomite.

with the neutralizing value of the calcareous materials, determined by boiling the mineral with an excess of standard HCl and back titrating against NaOH with phenolphthalein as indicator. The amount of acid neutralized by the calcareous material was calculated to the $CaCO_3$ equivalent.

The calcium and magnesium content of the same minerals was determined by EDTA in two ways (Table 2). One procedure was to boil the calcareous mineral with an excess of HCl, adjust the *p*H to 9.5 with NH_4OH, and buffer at *p*H 9.5 with an NH_4Cl–NH_4OH buffer. The calcium plus magnesium content was then determined by titrating against EDTA, buffered at *p*H 9.5, with Eriochrome Black

T (National Aniline No. 14175) as indicator. The EDTA was standardized by titrating against a standard calcium solution made by dissolving a known amount of $CaCO_3$ in a minimum amount of HCl. A small amount of $MgSO_4$ solution, equivalent to 0.2 ml of the EDTA solution, was used in each titration to facilitate the detection of the endpoint. The results are reported in Table 2. The calcium content was then determined by boiling the mineral in HCl, adjusting the *p*H to 12.0 with KCl and KOH, adding an excess of EDTA, and back titrating against $CaCl_2$ with murexide as indicator (Eastman 6373). The results are reported in Table 2.

The other EDTA procedure for determining the calcium and magnesium content of the minerals was direct solution of the calcium and magnesium in EDTA. The minerals were ground to pass a 60-mesh screen, an excess of EDTA was added, and the *p*H was adjusted to 9.5 with sufficient NH_4Cl–NH_4OH buffer to maintain the *p*H. (A 250 mg sample plus 50 ml of an EDTA solution containing 10 g EDTA, 2 g NH_4Cl, and 25 ml NH_4OH per liter, and 50 ml of a buffer containing 50 g NH_4Cl and 10 ml NH_4OH per liter was found to maintain the *p*H at 9.5.) The mineral plus the EDTA buffer mixture was allowed to stand for 40 hours with frequent shaking. A 25 ml aliquot was then removed and the excess EDTA was titrated against the standard calcium solution with the same indicator and precautions used in the standardization of the EDTA. It was found that, by taking an aliquot and separating the insoluble residue from the indicator, the endpoint was much easier to detect and better checks could be obtained. The addition of a small amount of KCN also aided in detecting the endpoint of the titration.

DISCUSSION

The 22 minerals analyzed for calcium and magnesium included one sample of analytical reagent grade calcium carbonate, two samples of oyster shell, 14 samples of limestone, one sample of phosphate rock, two samples of lime and soda ash residue from water softening, two shales, and four dolomites. The sum of the analytical results reported in Table 1 do not always approximate 100 per cent. In the cases where the results total more than 100 per cent, the difference might be due to calculating the calcium carbonate equivalent of the magnesium compounds or of such calcium compounds as silicates, since the silicate was also included. In the cases where the sum of the constituents adds up to less than 100 per cent, the difference may be explained by the presence of bound water in the clay material which would be lost at the temperatures of the sodium carbonate fusion.

That the several methods yield comparable results is evident from the data presented in Table 2. The most accurate analysis is probably the separate determination of calcium and magnesium by volumetric and gravimetric procedures. In the EDTA methods, 0.1 milliliter of the EDTA or calcium chloride used for titration was equivalent to 0.5 per cent of the calcium carbonate equivalent; thus perfect checks between the several methods could not be expected. It is noted that either of the EDTA methods will yield results comparable to those of the other methods for calcium and magnesium content of liming materials and related minerals.

As the sensitivity of the indicators is not limited to calcium and mag-

nesium, the *p*H must be carefully controlled throughout the determinations. In adjusting the acid solution to the desired *p*H of either 9.5 or 12.0, the indicator and EDTA may be added first; then the base is added until the desired color is obtained. Final adjustment is made by means of a glass electrode and the proper base. Experience will show the amount of buffer that may be added on a routine basis.

SUMMARY

A group of agricultural liming materials and closely related minerals were analyzed by several methods, including two procedures which employed EDTA. In one method the minerals were treated with acid to dissolve the calcium and magnesium, which were then titrated with EDTA. In the other method, the minerals were solubilized with EDTA, and the excess EDTA was titrated with calcium chloride solution. Either method gives excellent results and should prove valuable for the evaluation of liming materials. In either basic method the calcium and magnesium together may be determined by using Eriochrome Black T as an indicator; the calcium alone may be found by using murexide as indicator. The magnesium is then determined by difference. The use of EDTA in soil work can undoubtedly be extended to advantage.

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REFERENCES

(1) *Versenes*, Technical Bulletin No. 2 of the Bersworth Chemical Co., Framingham, Mass., 1950 (later edition available).
(2) *Bibliography of Ethylenediaminetetraacetic Acid*, Bersworth Chemical Co., 1953.
(3) CHENG, R. L., and BRAY, R. H., *Soil Sci.*, **72,** 449 (1951).
(4) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Wash. 4, D.C., 1950, Sections **3.11–3.13, 3.14,** and **3.15.**
(5) *Ibid.*, Sections **1.3** and **1.4.**
(6) PERKINS, A. T., *Soil Sci.*, **74,** 443 (1951).
(7) SCHWARZENBACH, G., BIEDERMANN, W., and BANGERTER, F. B., *Helv. Chim. Acta*, **29,** 811 (1946); thru *Chem. Abstr.*, **40,** 5859 (1946).

COMPOSITION AND SOLUBILITY BEHAVIOR OF A COMMERCIAL TRACE-NUTRIENT GLASS

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About a decade ago Badger and Bray (4) suggested that glasses are probably useful carriers of nutrient elements required by plants. There has since been considerable effort devoted to the development of glasses bearing one or more of the trace nutrients (11, 13, 17–20). Advocates of the use of these materials claim that as the glasses decompose only slowly under soil conditions, they provide a controlled supply of the trace elements in amounts below toxic levels. Thus, the danger of toxic concentrations occasioned by the application of very soluble compounds could be reduced. Moreover, the glass is said to minimize removal of the applied nutrients from the root zone by leaching processes and by immobilizing reactions with the soil.

Glasses carrying one or two trace nutrients have been offered on the market for separate application and also for incorporation in commercial mixtures of the major nutrients. Multi-nutrient glasses containing all of the principal known trace nutrients are now becoming commercially available. One glass of this type has been analyzed for total constituents and was examined in order to estimate the probable rates of release of its nutrients in terms of solubility in certain conventional solvents. The methods used, the results obtained, and some problems of evaluation are presented in this paper.

EXPERIMENTAL

PREPARATION OF EXTRACTS

Insofar as possible, the glass was submitted to conditions ordinarily used in the determination of phosphorus availability in fertilizers (10), and to other treatments to obtain comparable extracts in triplicate. Briefly, the procedures were as follows:

Neutral ammonium citrate solution.—A 2 g sample was placed in 200 ml of 22% neutral ammonium citrate (3), agitated continuously for 1 hr at 65°C., and sepd by centrifuging. The NH_4 citrate was removed from an aliquot portion by charring and dry-ashing at 550°C. The residue was then taken up with H_2SO_4 and HNO_3; the HNO_3 was later removed by evapn. In order to obtain clear solns free of dehydrated silica, the processed solns had to be filtered before making up to vol.

Citric acid solution.—A 2 g sample was placed in 200 ml of 2% citric acid (9) and agitated continuously for 30 mins at room temp. (31°C.). The citric acid was removed from an aliquot portion by charring on a hot plate and destroying the carbonized material with small portions of 30% H_2O_2. (An undesirable feature of this procedure is that the organic matter tends to ignite; this might cause some mechanical loss or volatilization of the elements being determined.)

Dilute hydrochloric acid solution.—The procedure (9, 14) was the same as that

used with neutral NH_4 citrate except that 0.4% HCl soln was agitated with the glass for 30 mins.

Constant boiling hydrochloric acid solution.—A 1 g sample wrapped in a filter paper was placed in 100 ml of constant boiling (20.2%) HCl at 108.8°C., and digested by refluxing the acid soln for 1 hr; the residual solid material was then sepd by filtration. The filtrate was evapd to dryness to remove the HCl, and the residue was then taken up with H_2SO_4 and HNO_3.

DETERMINATION OF ELEMENTS

The composition of the glass was established by appropriate analytical procedures in triplicate determinations. Copper, zinc, and iron were separated by precipitation with hydrogen sulfide (7); the copper and zinc were determined gravimetrically, and the iron was titrated with a standard dichromate solution. In the determination of silica, boron was removed as the methyl borate (8), and correction was made for other contaminants by a hydrofluoric acid evaporation (1). A modification of the J. Lawrence Smith method (2) was used to determine sodium and potassium. The manganese content was found by the colorimetric permanganate method, with sodium periodate as the oxidant. Molybdenum was determined colorimetrically as the thiocyanate complex (16), calcium and magnesium were determined by Versenate titration (5), and boron was separated by the classical methyl borate distillation and titrated acidimetrically.

The amounts of the constituents that entered a solution were ascertained by single determinations of the elements in each of three like extracts. Manganese and molybdenum were determined by the methods cited above; iron, spectrophotometrically as the orthophenanthroline complex; and copper and zinc, as dithizonates, by a mixed-color procedure (15). Sodium and potassium were determined by a flame photometric procedure, with compensation for interference errors by using the glass itself as the primary standard so that the extraneous ion concentrations would closely approximate those in the extracts.

DESCRIPTION OF THE GLASS

In manufacture, the glass is prepared by quenching a sensibly homogenous melt in water and grinding the granular product. Petrographic examination of the test sample proved the material to be essentially all glass. A sieve analysis showed 9.9% to be coarser than 100-mesh and 69.3% to be finer than 200-mesh. The chemical composition of the glass was found to be: Fe[1] 7.15, Mn 2.77, Cu 3.39, Zn 4.41, Mo 0.17, K 6.79, Na 4.86, B 0.57, Ca 1.87, Mg 0.10, and SiO_2 56.22%, respectively. Though Al was not determined, it is probably present also, but only in small amount.

ELEMENT RELEASE IN CONVENTIONAL SOLVENTS

The amounts of the trace elements released from the glass to the various solutions are shown in Table 1. The superior dissolving power of constant boiling hydrochloric acid was to be expected. Results obtained with other solvents fall within a fairly narrow range of 0.02 to 2.20 mg/g of glass. The elements entering solution appear to be much affected by the total amounts of each present initially in the glass. It was observed that a considerable quantity of silica dissolved in the constant boiling hydrochloric acid during analysis and lesser amounts in the other extractive media.

The efficiencies of the solvents in extraction of the various elements are

[1] Ferrous iron content was 2.78% Fe.

TABLE 1.—*Composition of extracts*

CONSTITUENTS		DISSOLVED IN:			
ELEMENT	TOTAL IN GLASS	NEUTRAL NH_4 CITRATE	CITRIC ACID	DILUTE HCL	CONSTANT-BOILING HCL
	mg/g	*mg/100 ml solvent*	*mg/100 ml solvent*	*mg/100 ml solvent*	*mg/100 ml solvent*
Fe	71.5	2.17	1.10	1.85	65.8
Mn	27.7	0.49	0.33	0.73	24.9
Cu	33.9	1.05	0.35	1.24	28.9
Zn	44.1	0.92	0.41	1.33	39.2
Mo	1.7	0.031	0.023	0.039	1.37
K	67.9	0.78	0.99	2.00	59.6
Na	48.6	1.15	1.01	1.75	46.0

given in Table 2. The average solvent efficiencies, in the order of increasing effectiveness, are as follows: citric acid, 1.4%; neutral ammonium citrate, 2.2%; dilute hydrochloric acid, 3.0%; and constant boiling hydrochloric acid, 88.4%. This range of efficiencies reflects both differences in procedure and variation in chemical properties of the solvents, such as concentration, *p*H, and capacity to form chelate compounds.

It is evident that these chemical and physical factors might cause some of the elements to be extracted preferentially. The data in columns 2, 4, and 6 of Table 2 have been treated statistically to establish probable significant differences. The order of selectivity, indicated as significant, in the extraction of trace elements by solvents of special interest are: Cu and Fe > Zn > Mn and Mo in neutral ammonium citrate; Fe > Mn, Cu, and Zn with Mo = Fe but only > Cu and Zn in citric acid; and Cu > Zn > Mn, Fe, and Mo in dilute hydrochloric acid.

The comparative uniformity of element extraction by different solvents may be observed from the columns of Table 2 headed "per cent deviation from mean." There are six instances in which these relative deviations exceeded 24%; all occurred in either the ammonium citrate or the citric acid solutions. In addition, the per cent deviation from the mean for these solutions is larger than for hydrochloric acid solutions. Thus, since the results for hydrochloric acid solutions more nearly approach proportionality in experiment, they seem to be the most suitable for evaluation of a multi-nutrient glass.

CHEMICAL NATURE OF GLASS

Glasses are formed by cooling a molten material rapidly enough to prevent appreciable crystallization. Since these amorphous substances are under-cooled liquids without definite lattice structures, they resemble crystalline solids only in rigidity. In that a glass is thermodynamically unstable, it does not possess a true solubility. Instead, equilibrium with a solvent is approached by uncharted reactions that may involve dissolu-

TABLE 2.—*Efficiencies of the solvents in extraction of the various elements from the glass*

ELEMENT	FRACTION OF TOTAL CONSTITUENT DISSOLVED BY:							
	NEUTRAL AMMONIUM CITRATE		CITRIC ACID		DILUTE HCL		CONSTANT BOILING HCL	
	DISSOLVED[a]	DEVIATION FROM MEAN	DISSOLVED[a]	DEVIATION FROM MEAN	DISSOLVED[a]	DEVIATION FROM MEAN	DISSOLVED	DEVIATION FROM MEAN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Fe	3.03	+38.4	1.54	+12.4	2.59	−12.5	92.03	+4.1
Mn	1.77	−19.2	1.19	−13.1	2.64	−10.8	89.89	+1.6
Cu	3.10	+41.6	1.03	−24.8	3.66	+23.6	85.25	−3.6
Zn	2.09	− 4.6	0.93	−32.1	3.02	+ 2.0	88.89	+0.5
Mo	1.82	−16.9	1.35	− 1.5	2.29	−22.6	80.59	−8.9
K	1.15	−47.5	1.46	+ 6.6	2.95	− 0.3	87.78	−0.7
Na	2.37	+ 8.2	2.08	+51.8	3.60	+21.6	94.65	+7.0
Mean	2.19		1.37		2.96		88.44	
Mean % deviation from mean		±25.2		±20.3		±13.3		±3.8

[a] LSD = 0.29% at the 5% level.

tion of the glass (wholly or in part), hydrolysis of the constituents, precipitation of crystalline compounds, etc. Furthermore, skeletal siliceous remains of the glass and surface coatings of newly-formed insoluble compounds are being increasingly regarded as a consequence of solvent attack. A residue of undissolved materials would tend to retain small amounts of the elements being released to solution and to reduce ion mobility at the liquid-solid interface—factors which may be associated with the observed variations in element release. Hence, the selective extraction of constituents from the test sample (Table 2) is regarded as normal behavior.

Because glass possesses characteristics typical of solutions, its composition may vary arbitrarily over a wide range. As a rule, a change in composition is accompanied by alterations in the properties of the glass; accordingly, its solubility can often be adjusted, within limits, to some preferred value. Thus the sample used in this work may be merely one of a large number of possible similar glasses. The use of glass as a carrier of trace nutrients suggests a means for studying crop response to different rates of nutrient release from a slightly soluble homogeneous material. Such studies will be of value if the attainable solution rate of the glass can be adapted to supply plants with optimal concentrations of the nutrients.

NUTRIENT RATIO

The problem of providing proper relative amounts of the nutrients in a multi-component glass can be illustrated by further consideration of the experimental results. In Table 3 the ratio amounts of the elements (Fe equal to 1.00) in the glass and in the various extracts are compared to the composition of certain nutrient culture solutions. The concentrations of elements in the culture solutions may deviate somewhat from actual needs of soils, but they still may serve as examples in the absence of estab-

TABLE 3.—*Comparison of nutrient ratios of the glass and its extracts with the composition of certain water culture formulations*

NUTRIENT ELEMENT	GLASS	NUTRIENT RATIO (FE=1.00)				P.P.M. IN NUTRIENT CULTURE SOLUTIONS	
		NEUTRAL AMMONIUM CITRATE	CITRIC ACID	DILUTE HCL	CONST. BOIL. HCL	WHEAT[a]	SOYBEAN[b]
Fe	1.00	1.00	1.00	1.00	1.00	1.00	2.00
Mn	0.39	0.23	0.30	0.39	0.38	0.50	1.00
Cu	0.47	0.48	0.32	0.67	0.44	0.02	0.02
Zn	0.62	0.42	0.37	0.72	0.60	0.05	0.05
Mo	0.02	0.014	0.021	0.021	0.021	0.05	0.01
B	0.08	—	—	—	—	0.50	0.50

[a] Used in experiments by Wynd (17).
[b] Used in experiments by Klein and Warren (11).

lished data. The ratios of elements in the extracts are essentially equivalent to those originally in the glass, despite minor significant differences in release noted above. To simplify comparison, the ratio amounts in the glass, which are representative values, may be related directly to the trace element content of culture solutions. It then is evident that if the glass breaks down at a sufficient rate to provide the amount of iron required by plants, it would also supply the approximate apparent plant demand for manganese and molybdenum. It is similarly indicated, however, that the copper and zinc released would be considerably higher (24 and 12 times as much, respectively, as in the culture solutions), whereas the relative amount of boron in the glass is significantly lower (only about 1/6 as much). Furthermore, although the amount of boron released was not determined, it would probably be only about 1/144 that present in the culture solutions, if the breakdown of the glass yielded an equivalent amount of copper. Thus, the addition of the particular test material would probably not provide a significant amount of boron unless the rates of application were fairly large, in which case excessive amounts of other elements would likely be released to the soil. It must be stressed, however, that these hypothetical conditions are described only to show the nature and importance of nutrient balance. The rate at which the glass will decompose in soils is not known and the validity of using the composition of culture solutions as an index to plant requirements is subject to question. It is probable that better values will become available in the future, but in general principle, the problem would be expected to remain the same.

CRITICAL ASPECTS OF GLASS EVALUATION

The determination of nutrient release from a glass carrier in solvents under specified conditions appears to be suitable for use in the evaluation of a glass. The values obtained would be measures of the solution rate or the relative ease with which the constituents dissolve, a function corresponding to availability.

Results of extractions made in the laboratory will have to be correlated with data obtained in vegetative tests. This general approach to method development is similar to that which has been used successfully in the evaluation of phosphorus compositions (10), with the following differences: The amounts to be determined in the case of trace elements may be less than a milligram, compared to a hundred mg or more ordinarily dealt with in phosphate availability tests. Only a small fraction of the elements in the glass is released, except under rigorous conditions. Furthermore, the measurement involves rate of solution, and it is doubtful that a point near equilibrium can be established within a reasonably short time. Thus, a general procedure for determining the efficacy of this type of material must be very precise in the physical conditions of extraction and the methods of analysis.

The total nutrient content of a glass, as well as rate of solution, is significant. These properties are related inasmuch as the total element content is an independent factor affecting rate of solution. Moreover, after placement in the soil a glass will most probably continue to break down; this process, though slow, will be essentially complete over a period of time, introducing the possibility of an eventual high concentration resulting from the accumulative release of an element. A similar question arises as to the compatibility of a glass with mixtures of the major nutrients. The glass, if incorporated in mixed fertilizers, will be exposed to the solvent action of concentrated solutions of ammonium, potassium, and/or other salts under various conditions of *p*H during use. It is probable that high concentrations attributable to such effects can be minimized adequately through careful control of the total amount of a given element actually added to a soil. This concept of the behavior of glass suggests that both agronomic and chemical studies should be made on the nature of this breakdown and the way in which it affects the rate of solution with time. Particle size, chemical durability, and total element considerations are important factors in this phase of the problem.

A complicating factor in estimating solution rate is the possibility of contamination by more readily soluble forms of the component elements. From data recently published by Myers and Barnett (12), it is shown that iron, molybdenum, manganese, and copper are sometimes added in appreciable quantities by mill-wear during the grinding of hard materials. It is improbable that significant amounts of such metals were introduced in processing the particular glass studied. However, the presence of foreign matter of this kind, not actually an integral part of the glass, should be taken into account.

The relative error in determination is somewhat greater with the trace amounts found in extracts than with larger amounts of the same elements. The precision of the analytical results reported was usually <5 per cent, though occasionally it did exceed this value. In similar work with the same material, reported by Hart (6) of the Florida Department of Agriculture, similar results were obtained. However, the data of the two laboratories cannot be compared directly because of differences in extractive techniques and analytical methods.

Some observations may be made as to the suitability of the reagents used as extractive media. The removal of ammonium citrate by charring and subsequent dry-ashing is time-consuming and involves danger of sample loss; also, the elements determined may be occluded to some extent in the dehydrated silica. The citric acid extracts present a similar problem in reagent removal. The hydrochloric acid solutions were thus the medium of choice.

SUMMARY AND CONCLUSIONS

A commercial trace-element glass has been studied from the standpoint of element release in several solvents to obtain information for use in

devising methods of evaluation. The amounts of Fe, Mn, Cu, Zn, and Mo that dissolved in neutral ammonium citrate, 2 per cent citric acid, and 0.4 per cent hydrochloric acid ranged from about 1 to 4 per cent of the respective total amounts present in the glass. When the glass was treated with constant boiling hydrochloric acid, approximately 88 per cent of these constituents dissolved. Similar behavior was exhibited by the sodium and potassium present in the glass.

Selective extraction of individual elements in a particular solvent was observed to occur. The elements dissolved with greater uniformity in hydrochloric acid than in citrate or citric acid solutions. The latter two solutions present greater difficulty in reagent removal before analysis. With respect to these factors and range of dissolving power, dilute hydrochloric acid is preferable for use in estimating the relative ease with which elements are released from a glass carrier.

The data indicate that the elements in a multi-nutrient glass must be balanced with great precision. When the composition of the particular glass studied was compared to certain water culture solutions in terms of corresponding ratio quantities of the elements, the glass was found to be about the same in iron, manganese, and molybdenum; higher in copper and zinc; and lower in boron. In extracts, similar results were obtained for these elements, except for boron which was not determined.

Other chemical aspects of the development and evaluation of trace element glass have been considered.

ACKNOWLEDGMENT

The authors are indebted to K. D. Jacob, who suggested the problem, for encouragement and counsel; to W. L. Hill, for technical advice; to J. G. Cady, for the petrographic examination of the glass; and to A. V. Breen for the sieve analysis of the glass.

REFERENCES

(1) *Am. Soc. Testing Materials, Standards,* Part **3**, 730 (1952).
(2) *Ibid.*, pp. 73–75.
(3) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Wash. 4, D.C., 1950, pp. 10–11.
(4) Badger, A. E., and Bray, R. H., *Chem. & Met. Eng.*, **52**, No. 4, 112 (1945).
(5) Cheng, K. L., Kurtz, T., and Bray, R. H., *Anal. Chem.*, **24**, 1640 (1952).
(6) Hart, Gordon, Florida Dept. of Agriculture, private communication.
(7) Hillebrand, W. F., *et al.*, *Applied Inorganic Analysis*, 2nd Ed., New York, John Wiley and Sons, Inc., 1953, pp. 58–66.
(8) *Ibid.*, pp. 752–753.
(9) Hill, W. L., Reynolds, D. S., Hendricks, S. B., and Jacob, K. D., *This Journal*, **28**, 105 (1945).
(10) Jacob, K. D., and Hill, W. L., Chapt. X in *Soil and Fertilizer Phosphorus*, Agronomy Monograph Vol. 4, New York, Academic Press, Inc., 1953, pp. 313–328.
(11) Klein, W. H., and Warren, G. F., *Plant Physiol.*, **27**, 204 (1952).
(12) Myers, A. T., and Barnett, P. R., *Am. J. Sci.*, **251**, 841 (1953).

(13) NAFTEL, J. A., and FAJANS, E. W., *This Journal*, **37**, 537 (1954).
(14) REYNOLDS, D. S., HILL, W. L., and JACOB, K. D., *ibid.*, **27**, 559 (1944).
(15) SANDELL, E. B., *Colorimetric Determination of Traces of Metals*, 2nd Ed., New York, Interscience Publishers, Inc., 1950, pp. 320–321, 620–621.
(16) *Ibid.*, p. 459.
(17) WYND, F. L., *Lloydia*, **14**, 1 (1951).
(18) ———, and BOWDEN, R. A., *ibid.*, **14**, 34 (1951).
(19) ———, and ———, *ibid.*, **14**, 55 (1951).
(20) ———, and STROMME, E. R., *ibid.*, **14**, 40 (1951).

PAPER CHROMATOGRAPHY OF SOME ORGANIC PHOSPHATE INSECTICIDES. I. NEW SPOT TEST*

By J. W. COOK (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

Application of paper chromatography is, of course, limited in part by the simplicity and sensitivity of available procedures for the location of chromatographed products. A number of techniques are usable for locating various organic phosphate insecticides, but none so far described are wholly satisfactory for Systox.[1] The present investigation concerned itself primarily with Systox, but the test developed for it is also usable for some of the other sulfur-bearing organic phosphate insecticides. This report discusses some of the limitations of the techniques found in the literature, and describes the new, simple, rapid spot test.

Gardner and Heath (1) used radioactive P^{32}-systox, and its principal isomer, in the development of their chromatographic method for the determination of isomers of Systox. March, *et al.*, (2) used both P^{32}- and S^{35}-tagged Systox and schradan in their paper chromatographic work with these compounds. After separation of the compounds by reverse phase chromatography with Silicone 550 as the stationary phase, the location of the separated compounds was accomplished by fastening the chromatogram to a flat slide, passing it under standard scaling equipment in small increments, and measuring the radioactivity of each setting. March and coworkers were able to develop pictures of the chromatograms by placing them on X-ray film and allowing one to two weeks for exposure. Some of the difficulties encountered with these techniques are the necessity of expensive tagged compounds and scaling equipment, and the length of time required to measure activity at each point on the chromatogram, or length of exposure time needed to produce the photographic picture of

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[1] The capitalized name "Systox" refers to the *technical* product of Chemagro Corporation, New York, and is their registered trade name for a mixture of O,O-diethyl,O-ethylmercaptoethyl thiophosphate (systox), and O,O-diethyl,S-ethylmercaptoethyl thiophosphate (isosystox). The names of these two individual isomers are not capitalized.

the chromatogram. Also, of course, no use can be made of this technique in analysis of insecticidal residues on food products of unknown history.

Following reverse phase chromatography, both Metcalf and March (3), and Gage (4) used the conversion of the *p*-nitrophenyl group of parathion and related compounds to the intensely yellow *p*-nitrophenate ion in order to locate them on paper chromatograms. This procedure is rapid, and is reported to be very sensitive for those compounds which have a *p*-nitrophenyl group, but of course it does not apply to such compounds as Systox, malathion, or diazinon.

Cholinesterase inhibitory power of some of the compounds can be used to obtain additional information on the location or purity of the chromatographed compounds. Chromatograms are sectioned and either eluted or the sections of paper are added directly to the enzyme preparation (2, 9). A serious difficulty with this technique is that, when it is used alone, misleading interpretation is possible, because impurities may be stronger inhibitors than the compound sought. This objection may be overcome by employing the procedure in conjunction with some of the other techniques (9).

March, *et al.*, (2) adapted the method for phosphate esters reported by Hanes and Isherwood (6), in which the phosphorus portion of the molecule is converted to phosphate and the phosphate in turn converted to phosphomolybdenum blue spots. Easily hydrolyzed compounds yield blue spots rapidly, while those more difficult to hydrolyze require considerable time. Systox and related esters are so difficult to hydrolyze that the necessary conditions also destroy the paper unless long periods of time are allowed for hydrolysis. Twenty-five to fifty micrograms of Systox give positive tests when treated with alkali for periods of twenty-four to seventy-two hours.

Gage (4) used the ultraviolet absorption of parathion to locate fractions eluted from a column, and Pfeil and Goldbach (7) employed the same technique to locate parathion eluted from paper chromatograms. This is applicable to parathion and related compounds but not to other organic phosphates.

EXPERIMENTAL

Preparatory to the chromatography of Systox, its isomers, and the active metabolite extracted from apples treated with Systox during growth, it was found that Systox could be located by a number of simple techniques not found in the literature review. For instance, it was discovered that deposits of technical Systox on paper dipped into a neutral solution of $KMnO_4$ for a few minutes, and then washed free of excess $KMnO_4$, would yield distinct brown spots. The insecticide could also be located by spraying the paper with dilute $AgNO_3$ solution with subsequent exposure to light or reducing agents, or both. These modifications were not thoroughly investigated, however.

The procedure found to be most satisfactory, and to possess advantages over those in the literature, depends on bromine absorption of Systox either from bromine vapors or from N-bromosuccinimide sprayed on the paper. The excess bromine

TABLE 1.—*Quantity of organic phosphate insecticide required for positive test*

INSECTICIDE	MICROGRAMS PER SPOT
Systox (technical)	1–2
Systox isomer (purified)	1–2
Isosystox isomer (purified)	1–2
Parathion (technical)	4
Methyl parathion (technical)	2
Chlorthion (technical)	10
Malathion (technical)	2
EPN (technical)	1–2
Diazinon (technical)	2

can be located by any of a number of standard techniques, such as spraying with dilute solutions of KI, benzidine, $AgNO_3$, fluorescein, phenol red, etc. (8). The use of N-bromosuccinimide was chosen as the brominating agent because of the ease of standardizing the quantity of bromine put onto the paper. Fluorescein was chosen as the agent to detect the excess unabsorbed bromine. When the proper amounts of bromine and fluorescein are used, the bromine is completely absorbed by a spot of Systox, which is then located by the highly fluorescent yellow-green of fluorescein on a pink background of brominated fluorescein. This technique recommends itself by reason of its simplicity and sensitivity, and because it yields chromatograms which retain their color for some time when protected from exposure to light. Also, questionable spots can be checked by the fluorescence of the fluorescein.

It has been found that technical Systox (including both principal isomers in purified form), technical parathion, technical methyl parathion, technical chlorthion, technical malathion, technical EPN, and technical diazinon all gave positive tests with this technique. Technical TEPP and purified OMPA failed to show positive tests when 40 micrograms were spotted. Table 1 records the approximate quantity of each product that will give a positive test when deposited on the paper from ether solutions. These papers were sprayed lightly with an approximately 0.002 *M* solution of recrystallized N-bromosuccinimide in washed methyl chloroform (CH_3CCl_3), since it was found that the reagent made up in that solvent is more stable than when it is prepared with any other of a number of common solvents. The solvent was allowed to evaporate and the papers were re-sprayed with a slightly alkaline, approximately 0.0003 *M* fluorescein solution in alcohol. Slightly larger quantities are needed for chromatograms because the spots usually diffuse over a larger area.

This procedure is similar in principle to that of Kirchner, *et al.*, (5) for the location of terpenes on paper chromatograms. They sprayed the whole paper with a 0.05% water solution of fluorescein and then exposed it to bromine vapor. The technique described in the present paper has an advantage in that a controlled amount of bromine is used. In this manner, one can obtain more consistent results with very small quantities of the insecticide, because if a quantity of bromine greater than is needed to brominate the insecticide spot is used, the excess will brominate the fluorescein, as in the background, and thus mask the spot.

SUMMARY

A simple and rapid technique is presented for detecting, on paper chromatograms, small quantities of some organic phosphate insecticides which contain sulfur. The technique consists of spraying the whole paper

with a solution of N-bromosuccinimide as the brominating agent and then overspraying with a solution of fluorescein. The insecticides appear as highly fluorescent yellow-green spots on a pink background of brominated fluorescein.

ACKNOWLEDGMENT

The use of N-bromosuccinimide was suggested by Joseph Levine, Division of Pharmaceutical Chemistry, Food and Drug Administration, Washington, D. C.

REFERENCES

(1) Gardner, K., and Heath, D. F., *Anal. Chem.*, **25**, 1849 (1953).
(2) March, R. B., Metcalf, R. L., and Fukuto, T. R., *Agr. & Food Chem.*, **2**, 732 (1954).
(3) Metcalf, R. L., and March, R. B., *Science*, **117**, 527 (1953).
(4) Gage, J. C., *Biochem. J.*, **54**, 426 (1953).
(5) Kirchner, J. G., Miller, J. M., and Keller, G. J., *Anal. Chem.*, **23**, 420 (1951).
(6) Hanes, C. S., and Isherwood, F. A., *Nature*, **164**, 1107 (1949).
(7) Pfeil, E., and Goldbach, H. J., *Klin. Worschr.*, **31**, 1011 (1953).
(8) Snell, F. D., and Snell, C. T., *Colorimetric Methods of Analysis*, D. Van Nostrand Co., Inc., New York, 1936.
(9) Cook, J. W., *This Journal*, **37**, 989 (1954).

PAPER CHROMATOGRAPHY OF SOME ORGANIC PHOSPHATE INSECTICIDES. II. SEPARATION AND IDENTIFICATION

By J. W. Cook (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

This report presents details of a paper chromatographic technique for separation and identification of a number of pesticidal thiophosphates by means of reverse phase chromatography, in combination with the spot test described in the preceding report (1). This procedure should be useful in identifying these compounds when they occur as unknown residues on food products.

Limited but satisfactory experience has led to a preference for paper chromatographic techniques similar to those described by Mitchell, including the use of 8×8 inch sheets of Whatman No. 1 paper, spray application of a stationary solvent (2), and a lined chromatographic chamber (3, 4). By these means the technical products, Systox ®,[1] parathion,

[1] Chemagro Corp., N. Y. See footnote, p. 984.

methyl parathion, chlorthion, malathion, EPN, and diazinon have been separated by using mineral oil as the stationary phase and (1+1+2) ethanol, acetone, and water as the mobile phase.

METHOD

APPARATUS

The lined chromatographic tank and accessories are those described by Mitchell (3, 4).

REAGENTS

(a) *Immobile solvent.*—Dilute 4 ml U.S.P. grade mineral oil to 100 ml with ethyl ether, A.C.S. grade.

(b) *Mobile solvent.*—Mix ethanol, acetone, and water (1+1+2, v/v).

(c) *N-bromosuccinimide stock soln.*—0.01 *M* soln. Dissolve 0.09 g N-bromosuccinimide in 50 ml washed methyl chloroform (wash technical methyl chloroform 5–6 times with equal volumes of H_2O). *Spray dilution.*—Dilute 5 ml stock soln to 25 ml with washed methyl chloroform.

(d) *Fluorescein stock soln.*—0.01 *M* soln. Dissolve 0.33 g fluorescein in 100 ml 0.1 *N* NaOH soln. *Spray dilution.*—Dilute 3 ml stock soln to 100 ml with ethanol.

(e) *Standards.*—Dilute 100 mg technical Systox, parathion, methyl parathion, chlorthion, malathion, EPN, or diazinon to 100 ml with ethyl ether.

(f) *Filter paper.*—Whatman No. 1, 8×8 in. sheets.

PROCEDURE

Spot paper with 0.01 ml (10 mmg) portions of standard solutions at equal intervals along a base line located about 1 in. from lower edge of paper. Hang paper from a supporting glass rod, spray uniformly with immobile solvent, and allow to dry for a few minutes. Add mobile solvent to trough and to bottom of lined tank (4). Immediately hang paper in tank with lower edge dipping in trough of mobile solvent and seal cover on tank. When solvent front has approached within 1 in. of (but has not reached) top of paper, remove it from tank and hang it to dry at room temperature. When dry, spray uniformly with N-bromosuccinimide spray soln; allow solvent to evaporate, and overspray with fluorescein spray soln. Observe finished chromatogram under both ordinary and ultraviolet light.

RESULTS AND DISCUSSION

Table 1 shows the R_F values obtained for seven pesticides chromato-

TABLE 1.—*R_F values of some organic phosphate insecticides*

COMMERCIAL INSECTICIDE	R_F
Systox	
Unknown component	0.10
Systox isomer	0.47
Isosystox isomer	0.99
EPN	0.35
Parathion	0.52
Diazinon	0.61
Chlorthion	0.70
Methyl parathion	0.85
Malathion	0.93

graphed as directed above. It can be seen that there is good distribution of R_F values. These values are sensitive to the amount of oil that is put onto the paper (a factor difficult to duplicate quantitatively) as well as to other factors. Consequently, it is essential to run standards along with unknowns on the same sheet of paper.

Systox is a systemic insecticide; neither isomer is recoverable as such from plant tissue shortly after plant treatment. The "metabolite" of Systox that is recoverable from plants does not respond to this spot test; thus it does not interfere with the location of any of the other six thiophosphates in an unknown mixture.

This procedure separated technical Systox into the systox isomer, isosystox isomer, and a third component less soluble in water than systox isomers.

SUMMARY

Seven technical organic thiophosphates have been chromatographed and all exhibited different R_F values. The separation was accomplished by reverse phase paper chromatography with mineral oil as the stationary phase and ethanol, acetone, and water (1+1+2, v/v) as the mobile phase.

REFERENCES

(1) COOK, J. W., *This Journal*, **37**, 984 (1954).
(2) MITCHELL, L. C., *ibid.*, **35**, 920 (1952).
(3) ———, *ibid.*, **36**, 1187 (1953).
(4) ———, *ibid.*, **37**, 530 (1954).

PAPER CHROMATOGRAPHY OF SOME ORGANIC PHOSPHATE INSECTICIDES. III. EFFECTS OF LIGHT ON SYSTOX AND ISOSYSTOX

By J. W. COOK (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

During chromatographic separation of a number of organo-phosphate pesticides (1), including Systox ®,[1] certain unexpected phenomena were observed, which on the basis of studies reported herein, are attributed to the production of new compounds by the effect of light on both isomers of Systox. Solubility, anticholinesterase (CHE) activity, and infrared spectra of the new compounds are compared to those of the parent compounds.

It has been observed that parathion is changed by exposure to light.

[1] Registered trademark of Chemagro Corp., N. Y. Technical Systox is composed of two isomers in the ratio of 65 parts systox to 35 parts isosystox. See footnote, p. 984.

While pure parathion is said to be devoid of anti-CHE activity *in vitro* (9), Payton (7) reported that an emulsion, when exposed to ultraviolet light, developed progressive anti-CHE activity. He suggested, as a likely possibility, the transformation of parathion to an active isomer. No prior mention of effect of light on Systox has been noted in the literature.

It is well known that a short time after plants are treated with technical Svstox, neither isomer (systox or isosystox) is recoverable from the plant tissue, but that another compound (or compounds), more water-soluble than either isomer, is recoverable from within the plant. The solubility in water of the systox isomer is reported to be about 75 p.p.m., that of the isosystox isomer about 2000 p.p.m. (4, 5). Hartley's explanation for the apparent anomaly of absorption and translocation in the watery plant juice of a substance as relatively insoluble as Systox is that the "compound is very rapidly converted into a water-soluble compound within the living plant and that it is a derivative which is translocated and is responsible for the systemic insecticidal action. . . " (3). The effect of light on solubility and other factors, herein reported, may be one of the first steps in the "metabolism" of Systox.

EXPERIMENTAL

Effect of light on R_F values.—Reverse phase paper chromatography, using mineral oil as the stationary phase and water as the mobile phase, was found satisfactory for the separation of systox and isosystox. This same system proved to be applicable for the separation of the products obtained from Systox after exposure to light. The sample being investigated was spotted in the usual manner on Whatman No. 1 paper (usually 8×8 in. sheets). The paper was sprayed with mineral oil in ether, hung in a lined stainless steel tank, as described by Mitchell (8), and developed by ascending chromatography, with H_2O as the mobile phase. With this system the amount of oil on the paper influences the R_F of the various compounds. Increasing amounts of oil depress the R_F of the less water-soluble compounds. For most of this investigation, the papers were sprayed 3 times with 4% v/v U.S.P. mineral oil in ether. This results in the R_F values shown in Table 1.

TABLE 1.—*R_F values of systox and related compounds*

Systox	0.0
Light product of systox	0.8–0.9
Isosystox	0.3
Light product of isosystox	0.8–0.9
Plant metabolite (from apples)[a]	0.8–0.9

[a] Obtained by $CHCl_3$ extraction of apples grown on trees treated with Systox.

Fig. 1 shows a series of chromatograms developed in the above manner before and after the spotted compounds were exposed to light. In this figure it can be seen that systox isomer, the "light" product of systox isomer, and isosystox isomer are located by the spot test reported in the previous paper (2) which depends on bromine absorption from N-bromosuccinimide. The "light" product of isosystox and the "metabolite" from apples do not respond to the spot test and were located by CHE inhibition. (This will be discussed in more detail later.)

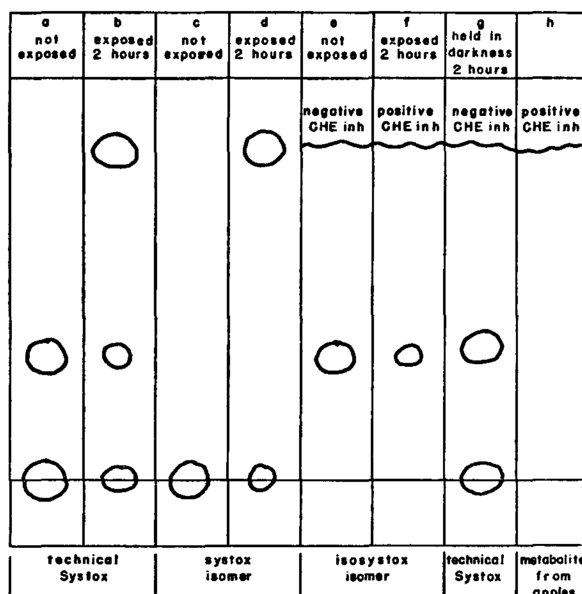

Fig. 1.—Paper chromatograms showing the effect of sunlight on systox and isosystox.

In the chromatographs identified in Fig. 1 as *a*, *c*, and *e*, the samples were deposited on the papers from ether solution, 10 mmg per spot, and the papers were immediately sprayed with oil in ether and placed in the tank for development. Chromatographs *b*, *d*, and *f* were spotted in the same manner but were exposed to sunlight for 2 hours before spraying with oil and developing. Chromatograph *g* is a duplicate of chromatographs *a* and *b* except that it was held in darkness for 2 hou-s and was then sprayed and developed along with *b*, *d*, and *f*. The one marked *h* was spotted with chloroform extract of apples containing the plant "metabolite" of Systox. It can be seen that new spots appear, or new CHE inhibition develops, toward the top of those chromatographs exposed to light; thus the "light" products of both isomers more strongly favor the water phase than either of the parent compounds. In this respect they are similar to the plant metabolite.

The effect of the light appears to be quantitative; that is, the "light" spot from systox appears to increase in size with exposure to light at about the same rate as the systox spot disappears. The spot from isosystox disappears at about the same rate as that from systox.

Ether solutions of the parent isomers have been held in clear flasks in daylight for one month without any indication of change until exposure after spotting on paper, suggesting that air is instrumental in the change. There appears to be no significant effect of light during the period of development of the chromatogram, inasmuch as all spots are distinct and uniform with no "tails."

Parathion, methylparathion, chlorthion, malathion, EPN, and diazinon were exposed to light and chromatographed in a similar manner; no new spots appeared under the conditions studied.

When Systox was added to a clump of dry soil, exposed to light for 2 hours, extracted from the soil with $CHCl_3$, chromatographed, and spotted with the test rea-

gents as before, the same "light" effect occurred, as indicated by the new spot at R_F 0.8.

Effect of light on CHE inhibition.—Pure systox isomer has low CHE-inhibiting power; the "light" compound is not greatly altered from systox in this respect, as illustrated in Fig. 2. In this experiment, four spots, each equivalent to 10 mmg of systox isomer, were put on a line 1 in. from the 4 in. edge of a 4×8 in. piece of Whatman No. 1 paper. The paper was divided lengthwise so that two spots were on each half. One half was held in darkness for 2 hours, while the other half was exposed to sunlight for 2 hours. They were then sprayed with oil and chromatographed as usual. When the front had advanced 6 in., the papers were removed from the tank, cut into 1 in. strips, and allowed to dry. One each of the unexposed and exposed 1 in. strips was then sprayed with the indicator solutions (2), while the other strips were cut into 1 in. squares and each square was eluted with 5 ml H_2O. Aliquots of the eluate were taken for CHE inhibition measurements (6). Relative CHE inhibi-

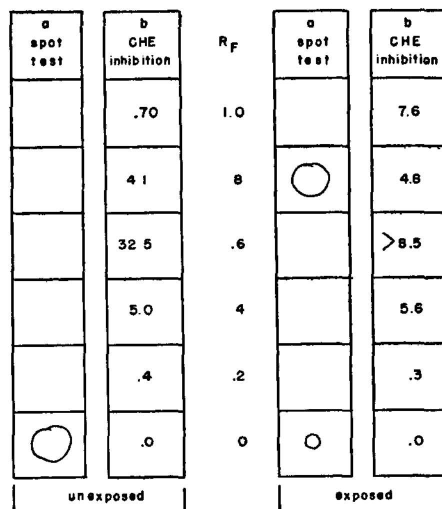

FIG. 2.—Paper chromatograms showing the effect of sunlight on CHE inhibition of systox isomer.

tion calculated in terms of micrograms of technical Systox, in each square inch of chromatograph, is indicated by the numbers in the squares designated as column *b*. It can be seen that there was no detectable change, due to exposure to light, in CHE-inhibiting value at positions occupied by systox or the light product thereof. The inhibition at R_F 0.6 found on both the exposed and unexposed papers is probably due to an impurity. The change at R_F 1.0 is unaccountable at the present time.

A similar experiment was attempted with a sample of isosystox isomer, but an impurity (possibly TEPP as judged by stability and R_F value) caused so much inhibition at R_F 0.9–1.0, both before and after exposure, that little could be deduced from this experiment as to the effect of light on isosystox.

In an alternate approach to the same objective, a sample of technical Systox which had been adopted as a standard in CHE inhibition work (6) was used. In this experiment, duplicate two-dimensional chromatographs were developed, using the same solvent (H_2O) for both directions. The initial development separated the

two isomers at different positions on the paper. One paper was exposed to light and re-developed at right angles in order to separate the "light" products from the parent compounds. The other paper was redeveloped similarly but with no exposure to light between the two developments. Ten micrograms of technical Systox were spotted at locations *A* and *B*, on each of two 8×8 in. papers, as shown in Fig. 3. The papers were sprayed with oil and chromatographed with H_2O as usual in the direction marked "1*st*." When the front had advanced 6 in., the papers were removed from the tank; one was exposed to sunlight for 2 hours and the other was held in darkness for the same period of time. At the end of this time, both papers were cut at line *x–y* and the section containing spot *B* was sprayed with indicator solutions. By this means it was possible to locate the positions of systox and isosystox

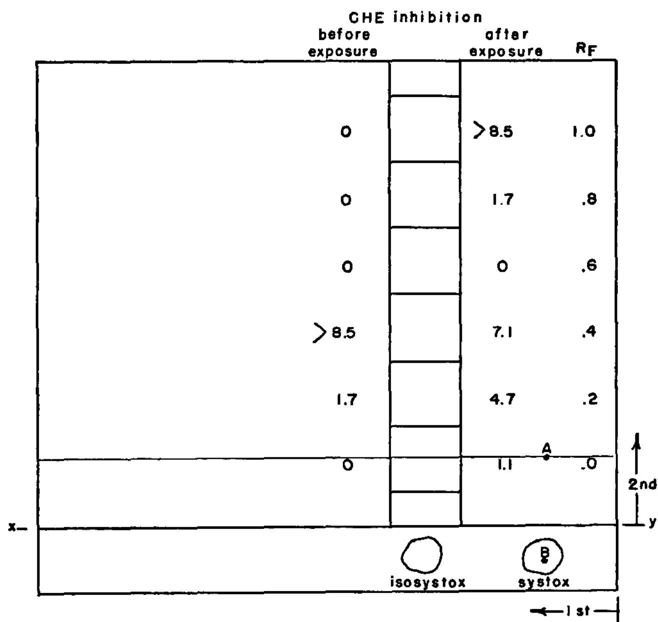

FIG. 3.—Paper chromatogram showing the effect of sunlight on CHE inhibition of isosystox isomer.

after their migration from spot *A*. The remainder of each paper was then redeveloped with H_2O in the direction marked "2*nd*." After completion of this development a 1 in. vertical strip including isosystox was cut into 1 in. squares, and CHE inhibition was determined for both the unexposed and the exposed papers. Fig. 3 shows the sections and the CHE inhibition in terms of micrograms of Systox found in the exposed and unexposed chromatograms, respectively. It can be seen that the main focus of inhibition shifted from R_F 0.4 to R_F 0.9–1.0 after irradiation.

The quality of the light which produces these effects on systox and isosystox has not been studied, except that a Hanovia black light with a C-H4 bulb and black-light filter, sunlight, and a 40-watt fluorescent lamp all produced the effect, while no detectable change took place in darkness.

Effect of light on infrared absorbance.—Infrared absorbance curves of systox,

isosystox, and the corresponding light compounds are shown in Fig. 4. Three mg of systox or isosystox in ether was applied in a streak at the bottom of an 8×8 in. piece of Whatman No. 1 paper, exposed to light for 4 hours, sprayed with mineral oil, and developed with water in the usual manner. After the chromatograms were developed, two vertical strips (each ca ½ in. wide) were cut out in different sections of each chromatogram and sprayed with indicator solutions. By this means the "light" compound of systox isomer was located on the papers. The portion of the paper containing the "light" compound was cut out, washed with a small amount of H_2O, and filtered to remove paper and globules of oil. Since the "light" compound of isosystox, unlike its parent, is not detected by the spot test, that portion of paper above the area shown by the test to contain unchanged isosystox was used for elution of the "light" compound of that isomer.

The H_2O eluates were extracted 3 times with equal volumes of $CHCl_3$ in a separatory funnel. The $CHCl_3$ layers were evaporated, leaving oily liquids as residues. The residues were each dissolved in 1 ml of CS_2, and infrared curves were obtained on a Perkin-Elmer recording spectrophotometer. As a check determination, a sample of isosystox was treated in the same manner except that it was not exposed to light. No oily liquid was recovered.

Comparison of the curves shows that the "light" compounds are different from the parents. Henglein, *et al.*, (10) published infrared curves for both systox and isosystox. They attribute the absorbance at 8 μ to P=O linkage and the absorbance at 12.25 μ to a P=S linkage. A comparison of absorbance at these points indicates that the "light" compound from systox appears to have a P=S but no P=O linkage, whereas the "light" compound from isosystox is the reverse. From these curves it would appear that the "light" compound from systox cannot be an isomer formed by a shift in position of sulfur, inasmuch as in that case there would be a P=O group and there would be no remaining P=S linkage. The "light" compound from isosystox is different from the "light" compound from systox.

An infrared curve run on the $CHCl_3$ extract of 1000 g of apples which had been sprayed with Systox during growth yielded an infrared curve which had no organic phosphate characteristics. The extract was not pure, and the curve of the impurity may have masked the curve of the "metabolite."

SUMMARY AND CONCLUSIONS

When exposed to light and air on paper the isomers of Systox are converted quite readily to other compounds, both of which are more hydrophilic than are their parents, as judged by their action when chromatographed on paper with mineral oil and water as fixed and mobile phases, respectively.

The systox isomer is not changed into a CHE inhibitor. The "light" compound from isosystox, like its parents, is a strong CHE inhibitor, but the quantitative inter-relationship of inhibiting power has not been determined.

Infrared absorbance curves indicate that the "light" compound from systox retains a P=S linkage and the "light" compound from isosystox retains a P=O linkage; the change must be in some other portion of the molecule. The curves indicate that the compounds are quite closely related to the parent compounds.

Apparent increased water-solubility of the "light" produced compounds

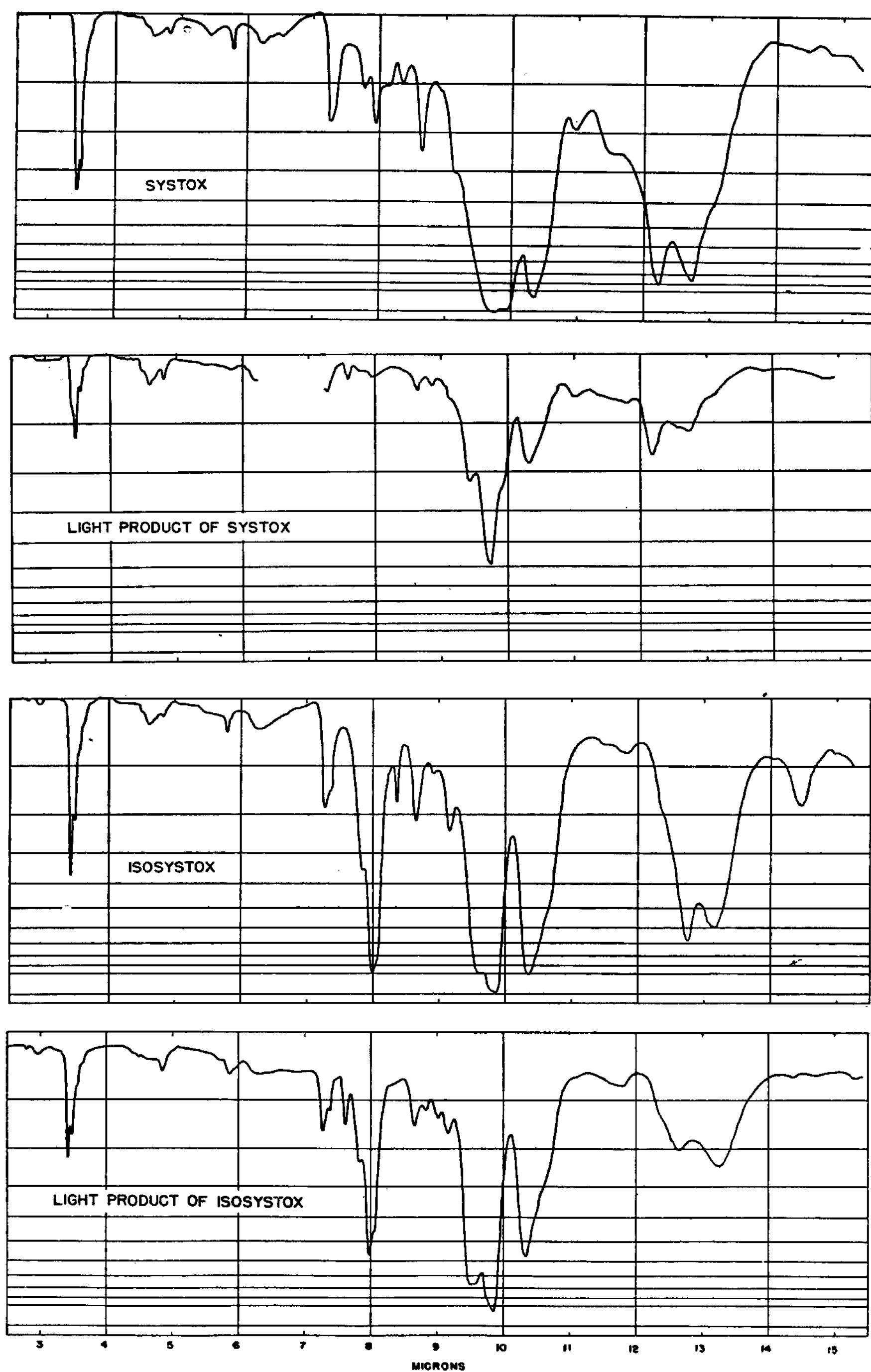

FIG. 4.—Infrared absorbance spectra of systox, isosystox, and their light products.

suggests that plant absorption would be enhanced, and that the effect of light may therefore be the first step in the unexplained metabolism of the pesticide by plant tissue.

ACKNOWLEDGMENT

The author wishes to express his appreciation to Jonas Carol, Division of Pharmaceutical Chemistry, Food and Drug Administration, for preparation of the infrared absorbance spectra.

REFERENCES

(1) Cook, J. W., *This Journal*, **37**, 987 (1954).
(2) ———, *ibid.*, **37**, 984 (1954).
(3) Hartley, G. S., *Summary of Communication to 3rd International Congress of Crop Protection*, Paris, September, 1952.
(4) *Communication from Chemagro Corp.*, New York, manufacturers of Systox.
(5) *Official Publication*, Association of Economic Poison Control Officials, 1954.
(6) Cook, J. W., *This Journal*, **37**, 561 (1954).
(7) Payton, J., *Nature*, **171**, 355 (1953).
(8) Mitchell, L. C., *This Journal*, **36**, 1187 (1953); **37**, 530 (1954).
(9) Diggle, W. M. and Gage, J. C., *Biochem. J.*, **49**, 491 (1951).
(10) Henglein, A., Schrader, G., and Mulhmann, R., *Z. Anal. Chem.*, **141**, 276 (1954).

SEPARATION AND IDENTIFICATION OF CHLORINATED ORGANIC PESTICIDES BY PAPER CHROMATOGRAPHY. VI. TECHNICAL BENZENE HEXACHLORIDE, LINDANE, TECHNICAL DDT, AND RHOTHANE

By Lloyd C. Mitchell (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare. Washington 25, D. C.)

The author has previously described techniques of paper chromatography for separation and identification of isomers of benzene hexachloride (1), and also of isomers of DDT and rhothane (also called TDE and DDD) in admixture (2). Practical application to spray residue detection requires, not necessarily the separation of all isomeric entities, but rather the disclosure and identification of commercial pesticides as they may occur in residue samples.

A paper chromatographic solvent system that may not distinguish among the individual isomers of BHC and among those of DDT, but which would separate these unrelated groups from each other and from rhothane, would suffice to differentiate among either lindane or technical BHC or both, technical DDT, and rhothane. On the other hand, a solvent system capable of distinguishing among isomers of at least BHC is obvious-

ly necessary for differentiating technical BHC from lindane, its gamma isomer. (The practicality of this step would depend upon the comparative intensity of chromatographed spots normal to the relative proportion of alpha and gamma isomers occurring, in ratio of about 5 or 6 to 1, in the technical product, and upon the influence of lindane, if present, on that comparative intensity.) In other words, a highly critical solvent system is needed for the latter distinction, while one of low discriminating capability is preferable for the former. Accordingly, it would appear improbable that a means for obtaining a single chromatogram could be devised to identify each and all of these commercial pesticides occurring in all combinations in a single residue.

A solvent system comprising a vegetable oil as the immobile solvent, and either aqueous acetone or dioxane as the mobile solvent, separates BHC, rhothane, and DDT in that order of migration, without separating from each other the isomers of either BHC or DDT.

A second solvent system employing dimethylformamide as the immobile, and either *n*-hexane (below 25°C.) or colorless mineral oil (above 25°C.), separates the four principal isomers of BHC from each other and from DDT, but does not separate rhothane from lindane. (Usually, but not always, the two isomers of DDT are also distinguished, but this is of no consequence to the present purpose.)

If technical BHC should occur together with both lindane and rhothane, joint evaluation of the two chromatograms would not reveal lindane. Also, in the event of admixture of only a relatively small proportion of lindane with technical BHC, comparative intensity of the chromatographed spots of gamma and alpha isomers may not be influenced sufficiently to disclose lindane.

With these exceptions, which are probably of minor consequence, duplicate chromatograms developed with these two solvent systems will serve, jointly, to identify all components of all combinations of these commercial pesticides.

METHOD

REAGENTS

(a) *Immobile solvents.*—(1) Refined soybean oil, 1% v/v in ethyl ether, A.C.S. (2) Dimethylformamide[1] (DMF), 20% v/v in ethyl ether, A.C.S. For use with mobile solvent 2 at temperatures below 25°C. (3) DMF, 25% v/v in ethyl ether, A.C.S. For use with mobile solvent 3 at 25°C. and above.

(b) *Mobile solvents.*—(1) Acetone, A.C.S., 75% v/v in water; or dioxane, tech.,[2] 75% v/v in water. Choice optional but should be consistent to obtain comparable R_F values. (2) *n*-Hexane, tech.[3] For use with immobile solvent 2 below 25°C. (3) Colorless mineral oil[4] (sp. gr. at 15.5°C., 0.775–0.825) plus 3% v/v DMF. For use with immobile solvent 3 at 25°C. and above.

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[1] Matheson, Coleman, and Bell, Inc., East Rutherford, N. J.
[2] Carbide and Carbon Chemicals Corp., South Charleston, W. Va.
[3] Phillips Petroleum Corp., Bartlesville, Okla.
[4] "Bayol-D," Esso Standard Oil Co., Lindon, N. J.

(c) *Chromogenic agents.*—(1) $AgNO_3$, 0.05 *N*, in water. (2) Formaldehyde soln, U.S.P., ca 37% aq. (3) KOH, 2 *N*, in water. (4) Concd HNO_3 plus 30% H_2O_2, 1+1 v/v.

(d) *Standards.*—Technical BHC, lindane, technical DDT, rhothane, 0.1 *M* soln of each; and the following combinations, 0.1 *M* in each component, in ethyl acetate, A.C.S., contained in glass-stoppered bottles: (1) Combination of 4 components; (2) combination of tech. BHC and lindane; (3) combination of tech. DDT and rhothane; and (4) combination of lindane and rhothane.

(e) *Paper.*—Whatman No. 1 filter paper, 8×8" sheets. Some batches may carry sufficient silver-reacting substances to interfere, in which case paper should be washed (3) before use with mobile solvent containing water, i.e., appropriate mobile solvent under (b) 1.

PROCEDURE[5]

Spot two sets of duplicate papers in the usual fashion (4) with 0.001 ml portions of test solns. Spray one set of duplicate papers with immobile solvent 1, and the other set with immobile solvent 2 or 3, depending on room temperature. Transfer papers to separate tanks (5) and develop with appropriate mobile solvent as indicated under "Reagents." Development requires ca 1.25 hr for *n*-hexane, 3 hr for mineral oil or aq. acetone, 5.5 hr for aq. dioxane (the solvent front must not reach top edge of paper). Remove papers from tanks, mark solvent front, and hang them from rod in hood until apparently dry. Wearing rubber gloves, spray both papers with chromogenic agents in the following sequence:

Agent 1: Allow papers to hang until apparently dry.

Agent 2: Allow papers to hang until only slightly damp.

Agent 3: Transfer papers, suspended from rod, to rack in oven at 130–133°C.; heat for 0.5 hr, cool, and return papers to hood.

Agent 4: Air-dry overnight.

Expose papers to full daylight or to unshaded sun until spots are fully defined.

DISCUSSION

In earlier work on BHC (1) the chromatograms were sprayed with normal KOH in methanol, and, after oven-heating, were hung overnight (to convert residual hydroxide to carbonate). They were then sprayed with 0.05 *N* $AgNO_3$ in (1+3) HNO_3, air-dried, and exposed to light. Excessive darkening of background sometimes tended to obscure the spots. This interference was overcome by using stronger HNO_3 (1+1) as solvent for $AgNO_3$; a modification, incidentally, which permits application of this agent without overnight delay for conversion of hydroxide to carbonate. Some of the data reported in the tables were obtained by these variations of chromogenic technique, which do not, of course, influence the R_F values in any way.

The preferred chromogenic agents and techniques described in the method presented herein are adapted, with certain modifications, from procedures devised for aldrin and dieldrin (3). By trial and error it was observed that those modifications served to improve definition of the spots.

[5] The procedure described is that developed by use of pure standard solutions. The application was not extended to actual residue samples. Such application would necessitate the devising and adaptation of a suitable sample preparation, which is under study by others.

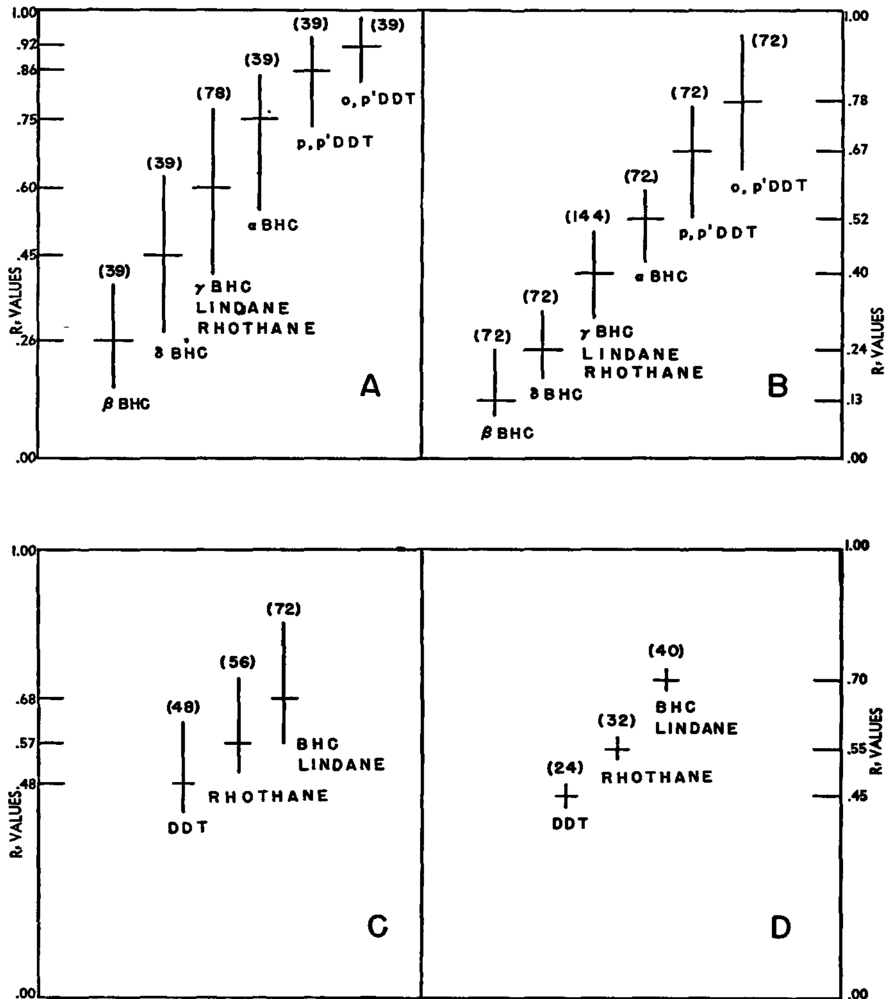


FIG. 1.—R_F of technical BHC, Lindane, technical DDT, and Rhothane. Range = vertical bar. Average = horizontal bar. Figures in parentheses = number of observations.

A: Solvent: Immobile—dimethylformamide in ethyl ether, 20+80 (v/v). Mobile—N-hexane. Temperature: 22–24°.

B: Solvent: Immobile—dimethylformamide in ethyl ether, 25+75 (v/v). Mobile—mineral oil and dimethylformamide, 97+3 (v/v). Temperature: 30–34°.

C: Solvent: Immobile—refined soybean oil in ethyl ether, 1+99 (v/v). Mobile—acetone and water, 75+25 (v/v). Temperature: 19–26°.

D: Solvent: Immobile—refined soybean oil in ethyl ether, 1+99 (v/v). Mobile—dioxane and water, 75+25 (v/v). Temperature: 29°.

In previous work with BHC (1), dimethylformamide as immobile solvent was not tried; the writer first used it in work with DDT (2). It is to be preferred for separation of the isomers of either pesticide but requires particular attention to adjustment for temperature conditions when it is employed in conjunction with hydrocarbons as mobile solvents. Below 25°C., 20% DMF affords more critical separation with *n*-hexane than with other hydrocarbons tried (petroleum ether (b.p. 30–60°C.), isohexane, mixed octanes, *n*-heptane, 2,2,4-trimethylpentane) although *n*-heptane and 2,2,4-trimethylpentane are generally satisfactory. A lower concentration of DMF at these temperatures permits excessive migration of alpha and gamma BHC and allows no room for DDT to separate; at higher concentrations of DMF the mobile solvent often ascends erratically and produces an irregular front.

At 25°C. and above, excessive migration of alpha and gamma BHC also occurs. It would be preferable to work under temperature control, but where such facilities are unavailable, increasing the concentration of immobile solvent and substituting mineral oil (with added DMF) as the mobile solvent, produced satisfactory separations.

Data on R_F values obtained with the alternate systems are given in Figs. 1A and 1B.

The complementary solvent system, for separating rhothane from lindane, provides optional mobile solvents because, although dioxane gives wider separations, its use is more time consuming; circumstances may therefore dictate a choice. Data on R_F values obtained with these solvents are given in Figs. 1C and 1D.

The wide range in R_F values, which may be due in large measure to variations in the amount of immobile solvent on the paper, might give the impression of serious overlappings and incomplete separations; however, in any single chromatogram the various components were completely resolved.

The amount and concentration of test solution suggested usually yields a spot, of somewhat more than threshold visibility, for gamma BHC when it is present in normal proportion to the technical product. The adjustment of amount or concentration spotted on the paper may sometimes aid in disclosing lindane in the presence of technical BHC.

SUMMARY

A paper chromatographic procedure is outlined for testing for components (with certain relatively unimportant exceptions noted) of admixtures of technical BHC, lindane, technical DDT, and rhothane. The procedure is designed for practical spray residue testing but has not actually been tried out on such samples. The practical application is under study by other workers.

REFERENCES

(1) Mitchell, L. C., *This Journal*, **35**, 920 (1952).
(2) ———, *ibid.*, **37**, 530 (1954).
(3) ———, and Patterson, W. I., *ibid.*, **36**, 553 (1953).
(4) ———, *ibid.*, **35**, 920 (1952); **36**, 943 (1953).
(5) ———, *ibid.*, **36**, 1187 (1953).

THE ISOLATION AND IDENTIFICATION OF BARBITURATES*

By Felix J. Sabatino (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

A number of commercial sedative preparations contain two or more barbiturates. The combinations most commonly encountered include the following: phenobarbital with pentobarbital; phenobarbital with secobarbital; phenobarbital with butabarbital and secobarbital; pentobarbital with butabarbital; and secobarbital with amobarbital (Tuinal ®). Ribley, Kennedy, Hilty, and Parke (1) have published a procedure for the assay of Tuinal ® by infrared spectrophotometry; however, their method cannot be readily adapted for the analysis of other mixtures because the equipment required is not generally available. Keppel (2) has developed a method for the analysis of Tuinal ® in which the quantity of total barbiturates is measured by ultraviolet spectrophotometry, secobarbital is next determined by bromination, and amobarbital is calculated by difference.

Neither of these methods is entirely satisfactory for the analysis of a mixture of barbiturates because neither provides for the positive identification of the individual barbiturates present in the mixture. However, several of the barbiturates have recently been separated by chromatography, and the differences in their chromatographic properties may be utilized in their identification and determination. The combination of chromatographic procedures with Keppel's method should afford a complete analysis of mixed barbiturates.

IDENTIFICATION OF BARBITURATES BY PAPER CHROMATOGRAPHY

REAGENTS

(a) *Stationary solvent.*—Sodium carbonate solution, 0.5 *M*.
(b) *Mobile solvent.*—Ethylene chloride (redistilled).
(c) *Development reagent.*—Silver nitrate, 0.05 *N*, in alcohol solution.
(d) *Standard barbiturate solutions.*—20 mg/10 cc alcohol.

APPARATUS

(a) *Filter paper.*—Whatman No. 1, 8×8″ sheets.
(b) *Stainless steel tank.*—Equipped with trough, hangers, clips, and cover plate (4) for ascending chromatography.
(c) *Chromatographic atomizer.*

* Presented at the 68th Annual A.O.A.C. meeting, Oct. 11–13, 1954, Washington, D. C.

DETERMINATION

An 8×8″ Whatman No. 1 filter paper sheet was spotted with about 1 λ (0.001 ml) of the barbituric acid solutions on a starting line 1″ from the base. The paper was mist-sprayed with 0.5 *M* Na_2CO_3 so that uniform *dampness* was achieved without soaking. The paper was immediately placed in the chromatographic tank containing ethylene chloride as the mobile solvent, and the development was allowed to progress until the mobile solvent was 4–6″ above the spotting line. The paper was then removed from the tank, the ethylene chloride was allowed to evaporate, and the sheet was placed in a 100°C. forced draft oven until it was dry. It was then sprayed with $AgNO_3$ solution and was again placed in the oven until tan spots appeared on the brown background. Table 1 shows the R_F values obtained in a typical chromatogram. When mixtures of all five barbiturates were developed from a single spot, their respective R_F values were identical with those of the individual standards in the same chromatogram. The quantity of Na_2CO_3 solution on the paper affects the time of development and R_F values; thus, standards must be developed on the same sheet with the unknowns for purposes of comparison.

TABLE 1.—*Typical R_F values of barbiturates*

SUBSTANCE	R_F VALUE
Phenobarbital	0.02
Butabarbital	0.18
Amobarbital	0.35
Pentobarbital	0.47
Secobarbital	0.56

DETERMINATION OF PHENOBARBITAL AND OTHER BARBITURATES IN MIXTURES

REAGENTS

(a) *Iso-octane–chloroform* (1+1).—Wash U.S.P. grade $CHCl_3$ three times with H_2O. Mix equal volumes of washed $CHCl_3$ with U.S.P. iso-octane, and filter.

(b) *Formamide–water* (2+1).—Shake the formamide with dry potassium carbonate for 15 minutes, filter, and redistill under vacuum (3).

(c) *Standard barbiturate solutions.*—30 mg/100 cc in alcohol.

CHROMATOGRAPHIC PROCEDURE

The chromatographic column and sample were prepared for differential elution, following the procedure of Banes (3). The tube (25×200 mm), containing a small plug of cotton, was packed with a *wash layer* consisting of 1 g Celite mixed with 1 ml H_2O; a *trap layer* consisting of 5 g Celite mixed with 5 ml (2+1) formamide–H_2O; and the *sample layer*. The latter was prepared by heating a weighed sample (containing 2–100 mg of the barbiturates with 5 ml (2+1) formamide–H_2O on a steam bath) and thoroly mixing the cooled solution with 5 g Celite. The mixture was quantitatively transferred to the prepared chromatographic tube, 150 ml (1+1) iso-octane–chloroform was allowed to percolate through the column, and the eluate was collected in a 200 ml volumetric flask. (This fraction contains any pentobarbital, secobarbital, or amobarbital present. If butabarbital is present, it must be eluted with a total of 250 ml (1+1) iso-octane–chloroform.)

Phenobarbital was then eluted from the column with 150 ml washed $CHCl_3$ and the eluate was collected in another 200 ml volumetric flask. The solutions were diluted to the mark with $CHCl_3$.

DETERMINATION

Aliquots equivalent to 1–2 mg barbiturate were transferred to 50 ml beakers and evaporated to dryness on a steam bath with the aid of a current of air. A 5 ml portion of alcohol was added to dissolve each residue, and the solutions were transferred with *water* to 100 ml volumetric flasks containing 5 ml M K_2HPO_4 and were made to volume. Aliquots of 5 ml each of the corresponding barbiturate standards (30 mg/100 ml alcohol) were similarly diluted with H_2O and 5 ml M K_2HPO_4 to 100 ml. Absorbances were measured at 240 mμ with respect to a blank, and the quantities of barbiturate present were calculated.

TABLE 2.—*Analysis of mixed barbiturates*

SAMPLE	MIXTURE	CONTAINING		RECOVERED	
		mg		*mg*	*per cent*
1	Phenobarbital	25.0		25.4	101.6
	Pentobarbital	32.6		32.8	100.6
2	Phenobarbital	77.1		77.5	100.5
	Pentobarbital	26.6	74.3	75.5[a]	101.6
	Secobarbital	12.7			
	Amobarbital	35.0			
3	Phenobarbital	57.6		59.5	103.4
	Secobarbital	24.3		24.6	101.2
4	Phenobarbital	35.0		36.7	104.8
	Amobarbital	12.7		13.2	103.5
5	Phenobarbital	29.0		29.6	101.9
	Butabarbital	22.1		21.9	99.0

[a] Gravimetrically.

DISCUSSION

Table 2 lists results obtained by the application of the column partition method to five known barbiturate mixtures containing phenobarbital. The recoveries indicate an efficient separation of phenobarbital from the other barbiturates. On the basis of the R_F values listed in Table 1, it should be feasible to separate butabarbital from the other barbiturates by column chromatography when large samples (5–100 mg) are available. For unknowns containing 1 mg or less of mixed barbiturates, paper chromatography and ultraviolet spectrophotometry would provide both a positive identification of the constituents and the determination of their total.

SUMMARY

Procedures have been described for the paper chromatographic separation of five barbiturates commonly encountered in sedative mixtures and for the quantitative isolation of phenobarbital from other barbiturates by column partition chromatography.

REFERENCES

(1) RIBLEY, A. M., KENNEDY, E. D., HILTY, W. W., and PARKE, T. V., *J. Am. Pharm. Assoc., Sci. Ed.*, **40**, 572 (1951).
(2) KEPPEL, G. E., *This Journal*, **36**, 725 (1953).
(3) BANES, D., *J. Am. Pharm. Assoc., Sci. Ed.*, in press.
(4) MITCHELL, L. C., *This Journal*, **36**, 1187 (1953).

CHLOROGENIC AND CAFFEIC ACIDS IN CERTAIN STANDARD GRADES OF U. S. TYPE 12 TOBACCO

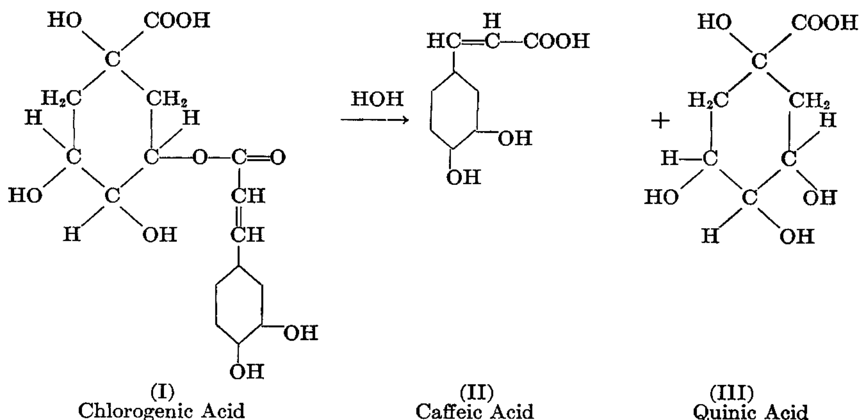
By FRANK B. WILKINSON, MAX PHILLIPS, and AUBREY M. BACOT (Standards Branch, Tobacco Division, Agricultural Marketing Service, U. S. Department of Agriculture, Washington 25, D. C.)

This investigation was undertaken as part of a general study of the Official Standard Grades for Flue-cured Tobacco (1), and the relationship of the grades to their chemical composition. In order to make a preliminary investigation of the standards as applied to U. S. Type 12 tobacco produced in Eastern North Carolina, 224 samples representing 21 standard grades of the 1949 crop were collected on several auction markets and were carefully appraised, with respect to their physical properties and characteristics, in relation to the official grade specifications. The 21 grades used in this study represent only 24 per cent of the 378 million pounds of Type 12 which were produced in 1949. To classify the whole crop, over 80 grades and subgrades were required. It is evident that the 21 grades which were investigated represent only a relatively small segment of the type and crop. Conclusions, therefore, on the relationship of chlorogenic and caffeic acids to the grades are necessarily restricted in scope.

REVIEW OF LITERATURE

Payen (2), in 1846, isolated from coffee beans a crystalline potassium-caffein double salt of an acid. This acid he designated as "chlorogenic acid" because when exposed to the air its ammoniacal solution assumed a green coloration.

Gorter (3) conducted extensive investigations of chlorogenic acid and showed that by hydrolysis it could be split into two acids, namely, caffeic acid (II) and quinic acid (III). However, the structural formula for chlorogenic acid proposed by Gorter was later disproved by Freudenberg (4), who proposed a structure which was subsequently established by Fischer and Dangschat (5). From the structural formula (I), as illustrated below, it can be seen that chlorogenic acid is the quinic acid ester of caffeic acid:



(I) Chlorogenic Acid

(II) Caffeic Acid

(III) Quinic Acid

Gorter (3, 6) and Oparin (7) found chlorogenic acid in many plants of different families, and Oparin has suggested that this acid, which readily undergoes reversible oxidation and reduction, acts as one of Palladin's (8) respiratory chromogens. According to Oparin, atmospheric oxygen oxidizes chlorogenic acid to the quinone form, which then acts as a hydrogen acceptor and is thus reduced back to the phenolic form.

Rudkin and Nelson (9) showed that the addition of small quantities of chlorogenic acid, isolated from the sweet potato, increased the oxygen uptake of, and also the carbon dioxide given off by, thin slices of the root of this plant.

Shmuk and Piatnitskii (10) isolated caffeic acid from a mixture of tobacco acids which had been hydrolyzed with a hot 10 per cent sodium hydroxide solution. They followed a procedure similar to that described by Rosenthaler (11). They also hydrolyzed tobacco with a hot 10 per cent sulfuric acid solution and obtained indirect evidence of the presence of quinic acid in the hydrolysate. The authors considered that the caffeic and quinic acids resulted from the hydrolysis of chlorogenic acid, and concluded that this acid is probably present in tobacco.

Koenig and Dörr (12) isolated pure crystalline chlorogenic acid from a low-nicotine variety of tobacco (so-called nicotine-free tobacco; "nicotinfreien Tabak").

In a previous article on the chemical composition of twelve grades of U. S. Type 11 tobacco (13), it was pointed out that all twelve grades gave a positive test for chlorogenic acid by the method of Hoepfner (14), and that caffeic acid was obtained by procedure of Rosenthaler (11). The several grades of U. S. Type 12 tobacco reported in this study, similarly, gave positive tests for chlorogenic acid by Hoepfner's method. Some of these grades were also examined for chlorogenic acid by the method of Rosenthaler, and they all afforded caffeic acid.

There is no information in the literature on the chlorogenic acid and caffeic acid contents of American or foreign tobacco types. In this paper, data are presented on the percentages of chlorogenic and caffeic acids found in several grades of U. S. Type 12 tobacco.

MATERIALS

The 21 samples used in this study were all U. S. Type 12 tobacco of the 1949 crop. Each sample contained equal proportions of tobacco produced on several different farms. The grades of each farm lot as assigned on the auction markets by Government inspectors were carefully checked and verified. Each farm lot was then re-sorted in the Tobacco Standards Laboratory in Washington, and all leaves which were not true to grade were discarded. (This re-sorting operation was done so that each lot would represent the basic Federal grade without allowing for different degrees of mixtures and irregularities, commonly found in farm-sorted tobacco, which are permitted as a tolerance in the practical application of the standards.)

The several growers' lots of the same grade were then commingled, dried for four days at room temperature, and stemmed by hand. Only the web portions of the leaves, from which the stems (midribs) had been removed completely, were used for chemical analyses. The web portion of the combined samples of each basic grade was then granulated sufficiently fine to pass through a sieve having 4 mm square openings. To eliminate most of the sand and other earthy material, each of the granulated samples was then sifted in a sieve with 0.5 mm square openings, and the material which passed through this sieve was discarded. The tobacco which did not pass through this sieve was ground in a Wiley mill equipped with a 1.0 mm sieve, thoroughly blended, placed in jars with air-tight closures, and stored where it was protected from light.

DETERMINATION OF CHLOROGENIC AND CAFFEIC ACIDS

The analytical method used was substantially that of Slotta and Neisser (15) and is based on the fact that *o*-dihydroxyphenolic compounds, such as chlorogenic and caffeic acids, can be oxidized quantitatively with an alkaline hypoiodite solution. For every mole of either chlorogenic acid or caffeic acid, ten gram atoms of iodine are required for complete oxidation. The method consists of extraction of the sample with hot water, precipitation of the acids with a solution of neutral lead acetate, and regeneration of the acids with hydrogen sulfide. The liberated chlorogenic and caffeic acids are determined in an aliquot of this solution by taking advantage of their reaction with an alkaline hypoiodite solution. A second aliquot of the solution is extracted with peroxide-free ether to remove the caffeic acid, and the chlorogenic acid is then determined in the extracted aqueous solution by the above procedure. The caffeic acid content is determined by difference.

It must be pointed out in this connection that should isochlorogenic acid (16) be present in tobacco, it would be determined as chlorogenic acid by this method. Rutin does not interfere to any apprciable extent with the determination.

ANALYTICAL PROCEDURE

A detailed description of the procedure for the determination of chlorogenic and caffeic acids follows:

Preparation of Solution A.—The sample (equivalent to 5 g of moisture-free tobacco) was transferred to a 500 ml volumetric flask, which had been calibrated to correct for volume occupied by the sample. Three hundred ml of boiling distilled

water was added to the flask, and the mixture was digested on the steam bath for 1 hour and shaken frequently. The mixture was allowed to cool to room temperature, diluted to the mark with water, mixed, and filtered through a folded filter paper; the first 25–30 ml of the filtrate was discarded. Two hundred ml of the remaining filtrate (corresponding to 2 g of moisture-free sample) was pipetted into a 250 ml centrifuge bottle. Two ml of a saturated solution of neutral lead acetate was added dropwise from a pipette, and the mixture was stirred and allowed to stand overnight. To the supernatant solution a drop of the neutral lead acetate solution was added, and if a precipitate formed, about 0.5 ml of the lead acetate solution was added, while stirring; the mixture was allowed to stand for 12 hours and was again tested for completeness of precipitation of the lead salts. If no precipitate formed, the mixture was centrifuged for 10 minutes at 1300 r.p.m. The clear supernatant solution was decanted; 50 ml of water was added to the centrifuge bottle, mixed with the precipitate of lead salts, and centrifuged for 5 minutes at 1300 r.p.m.; the clear supernatant solution was decanted again. This operation was repeated until the supernatant solution no longer gave a test for reducing sugars with Benedict's reagent. The several quantities of supernatant solutions obtained in this operation were discarded. The precipitate of lead salts in the centrifuge bottle was dispersed in about 50 ml of boiling water; the centrifuge bottle was placed in a 400 ml beaker containing ca 125 ml of boiling water and heated on the steam bath; and a stream of H_2S was passed into the suspension of lead salts for about 30 minutes. The centrifuge bottle was then stoppered and allowed to stand overnight. The reaction mixture was transferred quantitatively to a 250 ml beaker and boiled for 5 minutes to expel the excess of H_2S. The mixture was allowed to cool to room temp., and was transferred quantitatively to a 100 ml volumetric flask, diluted to mark with water, and mixed. It was filtered through a dry folded filter paper (7–8 cm in diameter) and the first 5–10 ml of the filtrate was discarded. The remainder of the filtrate was designated as "Solution A."

Preparation of Solution B.—A 50 ml aliquot of solution A (corresponding to 1 g of moisture-free sample) was measured out with a pipet and extracted for 4 hours with peroxide-free diethyl ether in a continuous liquid-liquid extraction apparatus (17, p. 228). The aqueous solution and ether in the extraction tube were transferred quantitatively to a separatory funnel and the aqueous solution was drawn off into a 250 ml beaker. After removal of the ether, the separatory funnel was washed with a stream of water from a wash bottle, and the washings were added to the 250 ml beaker containing the aqueous solution. The outer and inner tubes of the extraction apparatus were similarly washed with water, and the washings were added to the solution in the 250 ml beaker. The resulting solution was concentrated on the steam bath to 60–75 ml to remove ether and the concentrate was filtered through a filter paper (7 cm) into a 100 ml volumetric flask. The filtrate was cooled to room temperature, diluted with water to the mark, mixed, and designated as "Solution B." A stream of H_2S was passed through a small portion of solution B, and if a precipitate of PbS separated out, solutions A and B were discarded and new solutions were made from another sample. If no precipitate was obtained, the determinations were made as follows:

DETERMINATION OF CHLOROGENIC ACID

Twenty-five ml of solution B (corresponding to 0.25 g of the moisture-free sample) was pipetted into a 250 ml glass-stoppered Erlenmeyer flask; 30 ml of 0.05 *N* I solution and then 30 drops of 2 *N* NaOH solution were added. The mixture was stirred, and allowed to stand in a dark cabinet for one hour. It was then acidulated with 2.5 ml of 2 *N* sulfuric acid and the liberated iodine was titrated with 0.05 *N* $Na_2S_2O_3$ solution, using starch solution as indicator. One ml of 0.05 *N* I solution

consumed in the reaction is equivalent to 0.00177 g of chlorogenic acid. The percentage of chlorogenic acid was calculated as follows:

$$\frac{\text{ml } 0.05\ N \text{ I used} \times 0.00177 \times 100}{0.25},$$

or ml 0.05 N I $\times$ 0.708 = % chlorogenic acid in moisture-free sample.

DETERMINATION OF CAFFEIC ACID

An aliquot of 12.5 ml of solution A (corresponding to 0.25 g of moisture-free sample) was pipetted into a 250 ml glass-stoppered Erlenmeyer flask containing 12.5 ml of water, and 30 ml of 0.05 N I solution and 30 drops of 2 N NaOH solution were added. Caffeic acid was estimated, following exactly the procedure described above for the determination of chlorogenic acid. The number of ml of 0.05 N I solution required for the titration of the 25 ml of solution B was subtracted from the number of ml of 0.05 N I solution used in the titration. The difference represents the number of ml of 0.05 N I solution required for oxidation of the caffeic acid. One ml of 0.05 N I solution used in the above reaction corresponds to 0.000905 g of caffeic acid. The percentage of caffeic acid was calculated as follows:

$$\frac{\text{ml } 0.05\ N \text{ I used} \times 0.000905 \times 100}{0.25},$$

or ml 0.05 N I $\times$ 0.362 =% caffeic acid in the moisture-free sample.

The results obtained, recalculated on a moisture and sand-free basis, are presented in Table 1. The percentages of moisture and sand, used to calculate the moisture and sand-free weights, were determined by methods previously described by Phillips and Bacot (13). (It should be noted that most of the sand had been eliminated in the handling, stemming, and sieving processes.)

DISCUSSION

As a basis for discussing the relationships between the several grades listed in Table 1 and their contents of chlorogenic and caffeic acids, a brief explanation of the Federal grading system for flue-cured tobacco and the relationship between grades of different groups is presented.

Comments on the Standards.—Flue-cured tobacco is divided into four major divisions, designated as U. S. Types 11, 12, 13, and 14. The designation of each standard grade of these types consists of three symbols. The first, a capital letter, designates the group of the type; the second, a numeral, the relative quality within the group; and the third, a capital letter, the relative color of the group and quality. The names by which the groups are commonly known are: B—Leaf, H—Smoking-leaf, C—Cutters, X—Lugs, P—Primings, and N—Nondescript. The tobacco of each of the B and H groups is divided into six qualities (1 to 6); the C and X groups, into five qualities (1 to 5); and the P group into three qualities (3 to 5). The letter "L" designates the lighter color of the group which is a shade of lemon. The letter "F" designates a darker color known as orange. The symbols "GF" designate a green-orange color, while the letter "G" in N2G designates crude green. The samples used in this study did not include the red and mahogany colors which are designated by the

TABLE 1.—*Percentages of chlorogenic and caffeic acids, calculated on a moisture and sand-free basis, in certain grades of U. S. Type 12 tobacco (midribs removed)*[a]

U. S. GRADE	CHLOROGENIC ACID	CAFFEIC ACID
	(Leaf Grades)	
B2F	5.11	0.96
B3L	6.45	0.96
B4GF	6.87	1.02
B5L	7.74	0.97
N2G	6.74	0.65
Av.	6.58	0.91
	(Smoking-leaf Grades)	
H2L	6.21	0.69
H3F	5.80	0.76
H5L	6.89	0.88
Av.	6.30	0.78
	(Cutter Grades)	
C2F	5.14	0.47
C5L	4.71	0.50
C5F	5.26	0.57
Av.	5.04	0.51
	(Lug Grades)	
X3L	4.24	0.37
X3F	5.19	0.49
X5L	3.69	0.53
X5F	3.21	0.26
Av.	4.08	0.41
	(Priming Grades)	
P3L	3.29	0.38
P3F	4.04	0.70
P4L	3.95	0.41
P4F	3.20	0.51
P5F	2.93	0.20
N2L	2.31	0.23
Av.	3.29	0.41

[a] Results are the averages of at least two determinations.

letters "R" and "S."[1]

The groups, as well as the quality subdivisions of the groups, are based upon the relative degrees of certain elements of quality in tobacco, such

[1] Persons who may wish to make a further study of the Official Standard Grades for Flue-cured Tobacco may obtain copies from the Standards Branch, Tobacco Division, A.M.S., Department of Agriculture, Washington 25, D. C.

as elasticity, oil or life, body, tensile strength, porosity, maturity, and smoothness, as well as the percentages of injury and waste. The determination of the several degrees of quality, and the percentages of injury and waste, are based upon the appraisals of competent judges of the type, and are not determined by physical measurements.

Inter-group Grade Relationships.—Due to certain common group characteristics, the grades within the same group are generally more closely related than the grades of different groups. However, except in the case of Nondescript, the grades of the several groups of like quality and color can be compared. The corresponding qualities of the different groups are more closely related in the order in which the groups are listed in Table 1. For example, H5L can be compared with B5L on the one hand and C5L on the other, but B5L is not so closely related to C5L as it is to H5L. Similarly, X5F can be compared with C5F on the one hand and to P5F on the other, but C5F is not so closely related to P5F as it is to X5F.

The Nondescript group was established for the classification of tobacco of very low quality which does not meet the specifications of the lowest grade of any other group. For this reason, the Nondescript grades cannot be compared on a corresponding quality or color basis with the grades of other groups. The two Nondescript grades, N2L and N2G, have distinctly different characteristics and have very little relationship to each other. The N2L grade, a very low quality Priming, is closely related to P5F, while N2G, a very low quality crude green Leaf, is somewhat related to the B4GF grade.

Relationship of Chlorogenic Acid to Grade.—The percentages of chlorogenic acid found in the 21 samples have a very definite relationship to the grades into which the tobacco was classified. The groups are arranged in Table 1 in a natural sequence according to the general characteristics of the groups. It will be noted, from the group averages shown, that the percentages of chlorogenic acid decrease in the same order from Leaf to Primings, and that the average for the leaf grades is just double that of the priming grades. The relationship of the corresponding qualities within the several groups also follow the same trend. For example, H5L is lower than B5L, C5L is lower than H5L, X5L is lower than C5L, X5F is lower than C5F, and P5F is lower than X5F. In the B, H, and C groups the percentages of chlorogenic acid tend to increase as the quality of the grades of like color within each group decreases (except in the green leaf grades of B4GF and N2G). However, in the X and P groups, with the exception of P4L, the percentages of chlorogenic acid decrease rapidly as the quality decreases. This decrease in percentages of chlorogenic acid with a comparable decrease in quality is particularly noted in P3F, P4F, P5F, and N2L with the corresponding percentages of 4.04, 3.20, 2.93, and 2.31.

In general, the grades having more body, oil, elasticity, and tensile strength contain larger percentages of chlorogenic acid, while the grades

which have a higher degree of maturity and porosity contain smaller percentages of chlorogenic acid. These general relationships between the percentages of chlorogenic acid and these elements of quality in tobacco are very constant, except in the two green leaf grades.

Relationship of Caffeic Acid to Grade.—Caffeic acid in general follows the same relationship to grade as chlorogenic acid, but there are certain inconsistencies in the general pattern. For example, 0.70 per cent caffeic acid in P3F is relatively high and inconsistent with the percentages found in the closely related grades. Also, 0.53 per cent of this acid in X5L is relatively high in comparison with closely related grades of the X and P groups. It will be noted that while the percentages of caffeic acid are relatively small, B4GF was found to contain five times as much of this acid as P5F. Although the caffeic acid data do not bear as close relationship to the grades as the chlorogenic acid data, there is a fairly high degree of relationship between the two acids. The coefficient of correlation of the data for the two acids (Pearson's r) is 0.83.

Chlorogenic Acid as a Possible Factor in Aging Tobacco.—Before flue-cured tobacco is used in the manufacture of cigarettes or other tobacco products it is subjected to a process known as "aging." The time required for proper aging varies from one to three years, depending upon the type and grade of the tobacco. The heavier bodied tobaccos of the B group generally require a longer aging period than the thinner bodied tobacco of the X and P groups. The purpose of aging is to improve the aroma, smoking qualities, and flavor of the tobacco. Aging brings about certain chemical changes, as well as a loss of luster and a darkening of the color. Dixon and co-workers (18), who investigated the aging of American flue-cured tobacco, conclude that it is essentially a chemical process. There is a decrease in the total sugar amounting to 1.45 per cent of the dry weight of the tobacco. There is also a loss of water-soluble nitrogenous components, chiefly amino acids and nicotine. It may very well be that chlorogenic acid, because of its ability to undergo reversible oxidation and reduction, plays an important role in this process as a carrier of atmospheric oxygen. In this connection it may be pointed out that Oparin (7) has shown that certain amino acids, in the presence of chlorogenic acid, are readily oxidized by atmospheric oxygen and suffer a loss of nitrogen. The role of chlorogenic acid in the tobacco plant and in the aging of flue-cured tobacco merits further investigation.

SUMMARY

Data are presented on the contents of chlorogenic and caffeic acids in samples of 21 grades of stemmed tobacco of U. S. Type 12. The results show that a significant relationship exists between the contents of these acids and the groups and grades into which the tobacco was classified under the Federal grading system. The chlorogenic acid content was found

to range between 2.31 and 7.74 per cent while the caffeic acid content ranged from 0.20 to 1.02 per cent.

ACKNOWLEDGMENT

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REFERENCES

(1) Official Standard Grades of Flue-Cured Tobacco (U. S. Types 11, 12, 13, and 14), **17**, *Federal Register*, p. 6610 (1952); **7**, *Code of Federal Regulations*, Part 29, p. 160–169 (1953).
(2) Payen, A., *Ann.*, **60**, 286 (1846).
(3) Gorter, K., *ibid.*, **358**, 327 (1908); **359**, 217 (1908); **379**, 110 (1911).
(4) Freudenberg, K., *Ber.*, **53B**, 232 (1920).
(5) Fischer, H. O. L., and Dangschat, G., *ibid.*, **65B**, 1037 (1932).
(6) Gorter, K., *Archiv. Pharm.*, (Berlin) **247**, 184, 436 (1909).
(7) Oparin, A., *Biochem. Zeit.*, **124**, 90 (1921); **182**, 155 (1927).
(8) Palladin, W., *Ber. deut. Bot. Ges.*, **26a**, 125, 378, 389 (1908); **27**, 110 (1909); **30**, 104 (1912); *Z. Gär. Physiol.*, **1**, 91 (1912).
(9) Rudkin, G. O., and Nelson, J. M., *J. Am. Chem. Soc.*, **69**, 1470 (1947).
(10) Shmuk, A., and Piatnitskii, M., "*Sbornik Rabot po Khimii Tabaka*," No. **69**, 19, *Krasnodar* (1930).
(11) Rosenthaler, L., "*Grundzüge der Chemischen Pflanzenuntersuchung*," p. 105, Berlin, Julius Springer, 1928.
(12) Koenig, P., and Dörr, W., *Biochem. Zeit.*, **263**, 295 (1933).
(13) Phillips, M., and Bacot, A. M., *This Journal*, **36**, 504 (1953).
(14) Hoepfner, W., *Chem. Ztg.*, **56**, 991 (1932).
(15) Slotta, K. H., and Neisser, K., *Ber.*, **71**, 1616 (1938).
(16) Barnes, H. M., Feldman, J. R., and White, W. V., *J. Am. Chem. Soc.*, **72**, 4178 (1950).
(17) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Wash. 4, D. C., 1950, p. 228.
(18) Dixon, L. F., Darkis, F. R., Wolf, F. A., Hall, J. A., Jones, E. P., and Gross, P. M., *Ind. Eng. Chem.*, **28**, 180 (1936).

THE DECOMPOSITION OF AZO COLORS IN ACID SOLUTION*

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Many of the colors listed as certifiable for use in foods, drugs, and cosmetics under the Coal-Tar Color Regulations (1) are azo colors. The stability of these colors under various conditions is of interest to this laboratory.

The decomposition of azo compounds in acid solution has been a sub-

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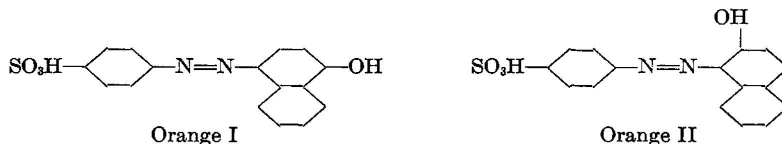
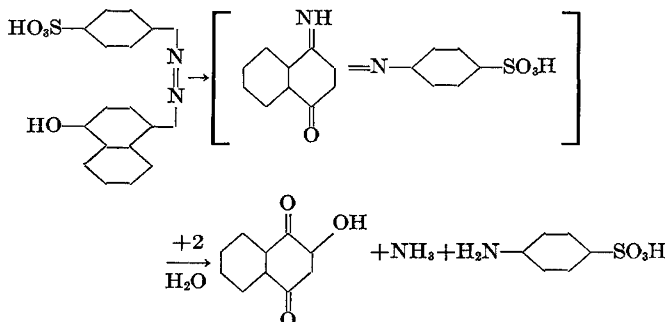
ject of investigation for many years. Bucherer, *et al.* (2–4), after refluxing Orange I in 20 per cent hydrochloric acid solution for 10 to 16 hours, were able to produce complete decomposition of the dye, as indicated by decolorization of the solution. Ammonia and sulfanilic acid were identified as the decomposition products. When these workers heated Orange I in a solution of aniline and 10 per cent hydrochloric acid, they were able to isolate a small amount of 2-phenylamino-1,4-naphthoquinone. When Orange II was treated in the same manner, 2-hydroxy-1,4-naphthoquinone was isolated. These workers did not obtain this compound from Orange I.

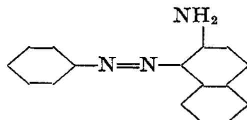


Rowe and Dangerfield (5) made similar studies and concluded that the findings of Bucherer, *et al.*, were essentially correct. Both groups of workers regarded the most probable course of the decomposition reaction as a rearrangement, followed by hydrolysis, according to the following schematic diagram:



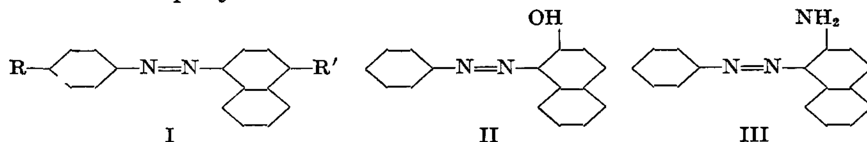
Fisher and Hepp (6) were able to produce 2-phenylamino-1,4-naphthoquinone by heating 1-amino-4-phenylazonaphthalene in 80 per cent acetic acid at 130°C. under pressure, and by refluxing 1-hydroxy-4-phenylazonaphthalene with glacial acetic acid for 8 to 10 hours.

A search of the literature failed to reveal any investigation of the decomposition of compounds which have structures typified by the formula shown below:



However, some workers (2–4) concluded that the mechanism of decomposition must be similar to that of other azo compounds.

In the course of this investigation, it was found that azo colors of the types represented by the basic formulae I and II appear to decompose in acid solution by the same mechanism, although Type I compounds react much more rapidly:



R = H, SO_3H; R′ = OH, NH_2.

Compounds of the type represented by formula III decompose by an entirely different mechanism. The decomposition of these basic types is discussed in order.

DECOMPOSITION OF TYPE I COLORS

Preliminary studies showed that solutions of Orange I decolorize under much less acidic conditions than those described by previous workers. Decomposition was found to occur to a measurable extent in solutions as weakly acid as *p*H 3.0. It was also observed that, with a relatively small initial concentration of color (100 mg/l), decomposition takes place in 1 to 2 hours' reflux time. (The ease with which this reaction occurs leads the writers to believe that similar reactions may occur on ingestion of the color by living organisms.)

Analysis of the partially decolorized solutions resulting from the decomposition of Orange I showed the presence of phenylhydrazine-*p*-sulfonic acid, 1,4-naphthoquinone, sulfanilic acid, and 2-hydroxy-1,4-naphthoquinone. When the decomposition experiments were conducted with more concentrated solutions, 2-(4-sulfophenylamino)-1,4-naphthoquinone was also isolated.

EXPERIMENTAL

Decomposition of Orange I.—Orange I (20 mg) was dissolved in 20 ml of 0.1 *N* HCl and heated to boiling. (In some experiments a yellow crystalline material collected in the lower part of the reflux condenser. This material was recovered and identified as 1,4-naphthoquinone.) The solution was refluxed until no further loss of color could be detected in the solution (ca 1–1.5 hrs). About 90% of the color had disappeared. At the end of this time, the reaction mixture was cooled to room temperature and extracted with ether. The aqueous residue was reserved for spectrophotometric examination. The ether extracts were combined and washed once with dilute HCl, and were then extracted with 1% ammonia solution until the ammoniacal extracts were colorless. The ammonia solution was acidified, warmed on a steam bath for one-half hour, and cooled. The ether solution was evaporated over 50 ml of 0.1 *N* HCl and reserved for spectrophotometric examination.

The ultraviolet absorbance curve of the original aqueous residue revealed the presence of phenylhydrazine-4-sulfonic acid and sulfanilic acid. The evaporated ether solution contained a small amount of an unidentified material. The ultraviolet absorbance curve of the acidified ammoniacal extract was predominantly that of a "naphthoquinone-type" compound (Fig. 1).

On the basis of its observed chemical and spectrophotometric properties, the material appeared to be closely related to 1,4-naphthoquinone. The ultraviolet and infrared absorbance curves of the unknown were therefore compared with those of several compounds related to 1,4-naphthoquinone; absorbance curves of the unknown were found to be identical with those of 2-hydroxy-1,4-naphthoquinone. The melting point of the isolated material was 185–186°C.; the mixed melting point of the unknown and a standard sample of the 2-hydroxy-1,4-naphthoquinone was also 185–186°C. (melting points are uncorrected).

When larger amounts of Orange I (1 g/100 ml *N* HCl) were refluxed as described previously, a water-insoluble material was obtained. This material was filtered and washed with alcohol. The ultraviolet absorbance curve of the material in acid solution indicated that it was also a naphthoquinone compound (Fig. 2). The infrared absorbance curve indicated the presence of sulfonic acid, quinoid, and either hydroxyl or amino groups, or both (Fig. 3). When 100 mg of the material was refluxed in 100 ml of 0.1 *N* HCl for 16 hours, the reaction mixture was found to contain 42 mg of 2-hydroxy-1,4-naphthoquinone, 39 mg of sulfanilic acid, and approximately 7 mg of unreacted residue.

From the chemical analysis and the spectrophotometric data, it was concluded that the insoluble material is 2-(4-sulfophenyl)-1,4-naphthoquinone ($C_{16}H_{11}O_5NS$).

Analysis.—Nitrogen: *Calcd,* 4.26%; Found, 4.17%, 4.22%. Sulfur: *Calcd,* 9.73%; *Found,* 9.42%.

Orange I was decomposed by treating it as described above, and the composition of the extracts was estimated spectrophotometrically. It was found that 100 mg of the dye yielded 6 mg of color, 11 mg of phenylhydrazine-4-sulfonic acid, 10 mg of sulfanilic acid, and 23 mg of 2-hydroxy-1,4-naphthoquinone.

In another experiment, Orange I (20 mg/100 ml *N* HCl) was refluxed for 4 hours, cooled, made alkaline with 20% NaOH, and steam distilled. The amount of ammonia found corresponded to 1 mole per mole of color initially present.

Reversibility of the Orange I decomposition.—A mixture of 20 mg of phenylhydrazine-4-sulfonic acid and 20 mg of 1,4-naphthoquinone was refluxed for 4 hours

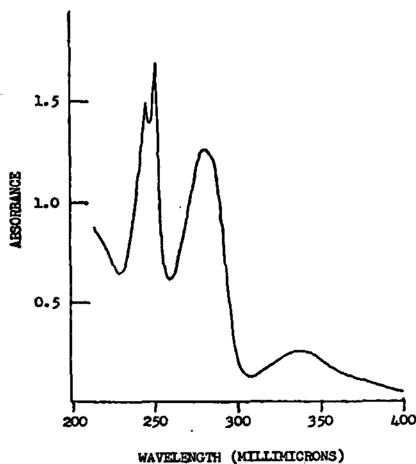


FIG. 1.—2-Hydroxy-1,4-naphthoquinone (10 mg/l in 0.1 *N* HCl solution).

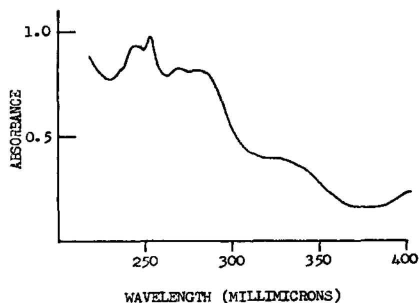


FIG. 2.—2-(4-Sulfophenylamino)-1,4-naphthoquinone (10 mg/l in 0.1 *N* HCl solution).

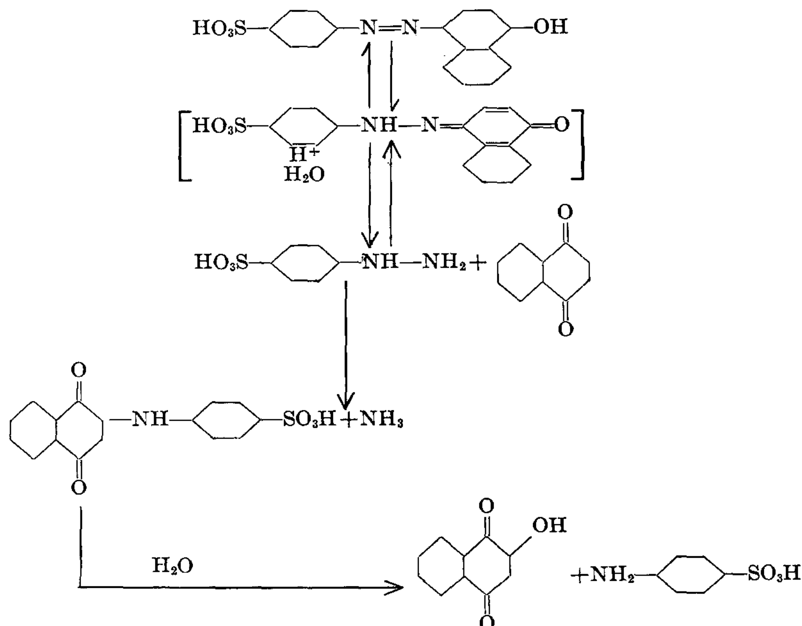
in 200 ml of 0.1 *N* HCl. The reaction product at the end of this time was examined spectrophotometrically. It was found that a mixture of 20 mg of phenylhydrazine-4-sulfonic acid and 20 mg of 1,4-naphthoquinone yielded 2 mg of Orange I, 3 mg of phenylhydrazine-4-sulfonic acid, and 7 mg of 2-hydroxy-1,4-naphthoquinone.

Decomposition of 1-hydroxy-4-phenylazonaphthalene and 1-amino-4-phenylazonaphthalene.—Weighed quantities of the dyes (20–100 mg) were refluxed in 1.0 *N* and in 0.1 *N* HCl for 2 to 3 hours. (About 90% of the color disappeared.) At the end of this time the mixtures were cooled, extracted with ether, made alkaline, and re-extracted with ether. The acidic and basic ether extracts were extracted with dilute base and dilute acid, respectively. The aqueous solutions obtained in this manner were examined spectrophotometrically.

In all cases large amounts of 2-hydroxy-1,4-naphthoquinone and aniline were separated from the reaction mixture. Attempts to isolate 1,4-naphthoquinone and 2-phenylamino-1,4-naphthoquinone or phenylhydrazine were not successful.

The proposed mechanism of Bucherer, *et al.*, can hardly account for the presence of either phenylhydrazine-4-sulfonic acid or 1,4-naphthoquinone in the reaction products from Orange I. Furthermore 2-(4-sulfophenylamino)-1,4-naphthoquinone is not a postulated intermediate of the Bucherer mechanism.

On the basis of the present experiments, the following mechanism for the decomposition of Orange I in acid solution is proposed:



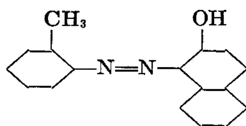
Other compounds of this structural type probably decompose in the same manner.

In order for the proposed mechanism to be valid, the dyes must exist, at least in part, as the tautomeric hydrazone form in solution. It appears to be established that a tautomeric equilibrium can exist in compounds of this type and that the state of the system will depend upon the surrounding medium. Hodgson and Marsden (7) have discussed the mobility of this tautomeric system and explain it on the basis of the influence of hydrogen bonding between the solvent and the solute molecules. Muller, Blangey, and Fierz-David (8) have offered evidence that Orange I exists in a solution as an equilibrium mixture of the azo and hydrazo forms and that the hydrazo form does undergo reversible hydrolytic cleavage. Desai and Giles (9) have also postulated a hydrolysis of the hydrazo form of the color as the initial phase of the oxidative decomposition of Orange I.

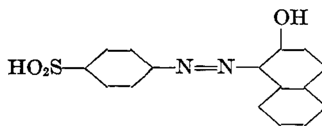
Quantitatively, the amounts of the various reaction products produced in the decompositions correspond in order of magnitude to the amounts expected from the proposed mechanism.

DECOMPOSITION OF TYPE II COLORS

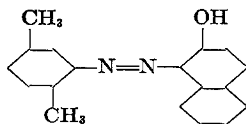
Many of the dyes certifiable for use in foods, drugs, and cosmetics may be classified chemically as 1-substituted-2-naphthol azo dyes (Type II). The structural formulas of some important dyes of this group are shown below:



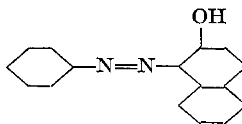
1-(2-methylphenylazo)-2-naphthol
Orange SS



1-(4-sulfophenylazo)-2-naphthol
D&C Orange No. 4, Orange II



1-(2,5-dimethylphenylazo)-2-naphthol
FD&C Red No. 32



1-phenylazo-2-naphthol
C.I. 24

When compounds of this type were refluxed in dilute acid, the amount of decomposition was considerably less than for compounds of Type I. The end products of the decomposition (and presumably the intermediate products) were the same, however.

EXPERIMENTAL

A weighed quantity of color (100 mg) was suspended in 200 ml of 0.1 *N* HCl and the solution was refluxed for 12 to 16 hours. Approximately 15% of the color is decomposed under these conditions. At the end of this time the reaction mixture was cooled to room temperature and extracted with ether. The aqueous residue was reserved for spectrophotometric examination. The ether extracts were combined

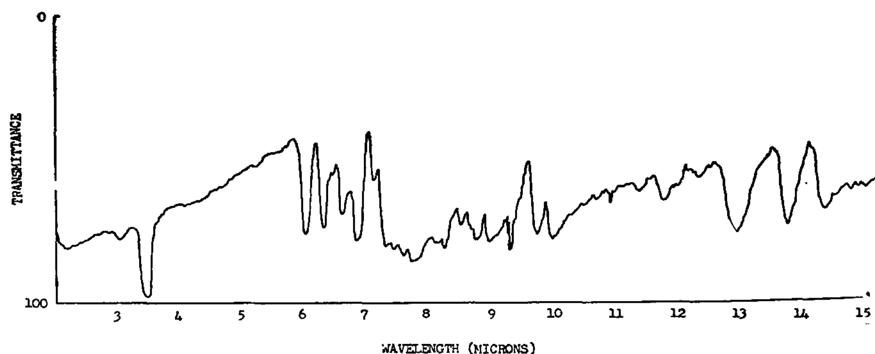


FIG. 3.—2-(4-Sulfophenylamino)-1,4-naphthoquinone (mineral oil mull).

and washed once with dilute HCl, and were then extracted with 1% ammonia solution until the ammoniacal extracts were colorless. The ammonia solution was acidified, warmed on a steam bath for one-half hour, and cooled. The ether solution was evaporated over 50 ml of 0.1 *N* HCl and reserved for spectrophotometric examination (Table 1).

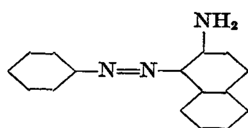
TABLE 1.—*Results of spectrophotometric examination of fractions obtained from decomposition experiments*

COLOR	MATERIAL IDENTIFIED IN FRACTION CONTAINING BASIC COMPOUNDS	MATERIAL IDENTIFIED IN FRACTION CONTAINING ACIDIC COMPOUNDS
1-(phenylazo)-2-naphthol	aniline	2-hydroxy-1,4-naphthoquinone
1-(2-methylphenylazo)-2-naphthol	*o*-toluidine	2-hydroxy-1,4-naphthoquinone
1-(4-sulfophenylazo)-2-naphthol	sulfanilic acid	2-hydroxy-1,4-naphthoquinone
1-(2,5-dimethylphenyl-azo)-2-naphthol	xylidine	2-hydroxy-1,4-naphthoquinone

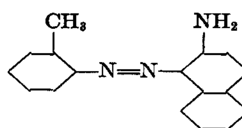
The major difference in the decomposition in acid solution of Type I and II compounds appears to be the rate of decomposition rather than the mechanism of the reaction. It is well established that the 1,2-phenylazo naphthols differ considerably in electronic configuration from their 1,4-isomers. This difference appears responsible in some manner for the differences in decomposition rates.

DECOMPOSITION OF TYPE III COLORS

Several 2-amino-1-phenylazonaphthalene dyes are commercially important colors. FD&C Yellow No. 3 and FD&C Yellow No. 4 are typical of this group of colors. Their structural formulas are shown at top of the next page:



FD&C Yellow No. 3



FD&C Yellow No. 4

If the decomposition mechanism postulated from Groups I and II colors also applies to the decomposition of these two colors, one would expect as reaction products a mixture of phenylhydrazine, 1,4-naphthoquinone, 2-phenylamino-1,4-naphthoquinone, ammonia, and 2-hydroxy-1,4-naphthoquinone from FD&C Yellow No. 3, and a mixture of 2-methylphenylhydrazine, 1,4-naphthoquinone, 2-(2-methylphenylamino)-1,4-naphthoquinone, and 2-hydroxy-1,4-naphthoquinone from FD&C Yellow No. 4.

In experiments with FD&C Yellow No. 3, the authors did not find the expected decomposition products. Instead, however, a mixture of phenol and β-naphthylamine could be isolated (in most cases quantitatively). The corresponding products, *o*-cresol and β-naphthylamine, were isolated from the acidic decomposition of FD&C Yellow No. 4.

EXPERIMENTAL

Samples of the colors (20 mg) were suspended in 5 ml of alcohol and were then diluted to approximately 50 ml with *N* HCl. The mixture was refluxed for four hours, cooled, and extracted with ether. The ether extracts were washed with several portions of water and were then extracted with dilute ammonia solution. In the case of FD&C Yellow No. 3, the absorbance curve of this fraction was identical with that of phenol, and in the case of FD&C Yellow No. 4, identical with that of *o*-cresol.

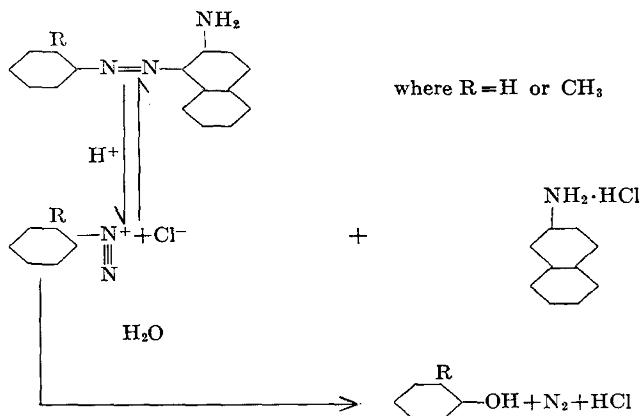
The original acidic reaction mixture was made alkaline to litmus with 30% NaOH solution, cooled, and extracted with ether. This ether extract was washed with several portions of water and extracted with 20 ml portions of 0.1 *N* HCl solution. The absorbance curves of this fraction from both FD&C Yellow No. 3 and FD&C Yellow No. 4 were identical with that of β-naphthylamine.

The acidic decomposition mechanism common to the first two groups of azo dyes discussed does not, obviously, apply to 2-amino-1-phenylazonaphthalene colors. The present experiments indicate that a reversal of the coupling reaction is the primary mechanism involved in the acidic decomposition of the two dyes studied. The diazonium compound produced is, of course, further decomposed to the corresponding phenol. This mechanism is shown schematically at the top of the next page.

This decomposition takes place with equal facility in either 1.0 *N* HCl or 0.1 *N* HCl solution.

SUMMARY

The acidic decomposition of three types of azo colors has been investigated. The 4-hydroxy and 4-amino-1-azophenylnaphthalene colors have been found to decompose when heated in relatively dilute acid solutions.



It has been established that the mechanism of decomposition involves an initial hydrolysis of the tautomeric hydrazone form of the color, followed by cleavage to yield a phenylhydrazine compound and 1,4-naphthoquinone. These two products, in turn, recombine to produce a 2-phenylamino-1,4-naphthoquinone. Hydrolysis of this adduct produces ammonia, a primary amine, and 2-hydroxy-1,4-naphthoquinone. FD&C Orange No. 1, for example, gives the following decomposition products; 4-sulfophenylhydrazine, 1,4-naphthoquinone, 2-(4-sulfophenylamino)-1-4-naphthoquinone, sulfanilic acid, 2-hydroxy-1,4-naphthoquinone, and ammonia.

The 2-hydroxy-1-phenylazonaphthalene compounds are believed to decompose according to the same mechanism, but at a much slower rate.

Compounds of the 2-amino-1-phenylazonaphthalene type are found to decompose by a simple reversal of the coupling reaction. FD&C Yellow No. 3, for example, produces phenol and β-naphthylamine as final products.

REFERENCES

(1) S.R.A., F.D.C. 3, Food and Drug Administration, Coal-Tar Color Regulations.
(2) Bucherer, H. T., and Rauch, M., *J. prakt. Chem.*, **132,** 227 (1931).
(3) ———, and Hanusch, F., *ibid.*, **132,** 274 (1931).
(4) ———, and Hayashi, M., *ibid.*, **132,** 302 (1931).
(5) Rowe, F. M., and Dangerfield, W. G., *J. Soc. Dyers Colourists*, **52,** 48 (1936).
(6) Fisher, E., and Hepp, O., *Ber.*, **25,** 2732 (1892).
(7) Hodgson, H. H., and Marsden, E., *J. Soc. Dyers Colourists*, **60,** 121 (1944).
(8) Muller, J. B., Blangey, L., and Fierz-David, H. E., *Helv. Chim. Acta*, **314,** 2579 (1952).
(9) Desai, N. F., and Giles, C. H., *J. Soc. Dyers Colourists*, **65,** 639 (1949).

NOTE

OBSERVATIONS ON THE UTILITY OF SILVER NITRATE AS A CHROMOGENIC AGENT TO LOCATE ANIONS ON PAPER CHROMATOGRAMS

By LLOYD C. MITCHELL (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

Silver-containing reagents, for indicating the location on paper chromatograms of spots due to a variety of reducing agents, have been frequently referred to in the literature. In recently reported work (1, 2), such indicators are mentioned for detecting organic acids as well. In the course of studies on halogenated pesticides (3), the writer has employed silver compounds as indicators of halides hydrolyzed *in situ* from such paper-chromatographed substances. Observations made during this work have led to the adoption of certain techniques in the use of silver-bearing indicator reagents.

Silver salts of most acids are white. To render such spots visible on paper, either the indicative silver deposit must be converted to a colored form, or the background must be treated so that it will form a contrast to the spot.

For the halogens bromine, chlorine, and iodine, this is readily accomplished, after spraying the chromatogram with silver nitrate and air-drying, by exposing the paper to direct sunlight for a few seconds or to shaded daylight for a longer period.

Fluorine may often be located if, after the treatment described above, the chromatogram is heated at 100°C. for about 15 minutes. Where the solvent system employed in developing the chromatogram does not separate the spot due to fluorine from those due to any potassium or sodium that may be present in the test solution, location of the fluorine spot is obscured.

For many acids, the air-dried, silver-treated chromatogram is oversprayed with a substance which will produce a background contrasting with the white deposit, or contrasting with the deposit modified in color by subsequent exposure to sunlight or to heat. For such overspraying, pyrogallol (0.0005 *M* in ethanol) was generally found more satisfactory than other reagents tried (catechol, resorcinol, hydroquinone, dextrose, chlorides, and bromides). Fresh solutions of pyrogallol give somewhat better contrast than those a week or two old. Examination of the finished chromatogram under ultraviolet light aids in distinguishing spots due to some acids, e.g., fumaric, malic, succinic, and tartaric acids.

Paper used in chromatography varies widely in its natural content of silver-reacting substances; with some batches it may become necessary to wash the paper prior to using it (4).

The silver reagent is made up by dissolving 170 mg $AgNO_3$ in 1 ml H_2O, adding 5 ml concentrated NH_4OH, and diluting to 200 ml with ethanol. This silver concentration is suitable for test solutions approximately 0.1 *M* in the sought-for anion, spotted on the paper in 0.001 ml volume. Half this concentration of silver gives better results with test solutions of approximately 0.01 *M* concentration, similarly spotted.

The writer has had success with this general technique on chromatograms of the halides, phosphates, sulfates, various fatty acids (saturated and unsaturated, C_8 to C_{18}), and various other carboxylic acids. It is also satisfactory with various amino acids, although ninhydrin is the more sensitive chromogenic agent for them.

REFERENCES

(1) GORE, D. N., *Chem. and Ind.*, 479 (June 23, 1951).
(2) BECKER, E., *Z. Lebens-Untersuch. u. Forsch.*, **98**, 249 (1954).

(3) Mitchell, L. C., *This Journal*, **35**, 920, 928 (1952).
(4) Mitchell, L. C., and Patterson, W. I., *ibid.*, **36**, 553 (1953).

ERRATA

This Journal, **37**, 586 (1954) — On p. 589, delete the second sentence in the paragraph beginning, "Filter the soln . . . etc.," and substitute: "Transfer as much of the ppt as possible in the process, drain flask well, and suck crucible dry (no free liquid). Rinse out flask, and wash down crucible walls, with 3 successive portions of ca 5 ml of filtered reagent (d), allowing crucible to suck dry each time. Then rinse flask and wash ppt several times with ca 2 ml portions of the acid-alcohol wash soln (e), by means of a wash bottle."

Ibid., **37**, 648 (1954) — On p. 649, equation in next to last paragraph, for "(ml H_2O/ml K.F.)," substitute: "(mg H_2O/ml K.F.)."

BOOK REVIEWS

Chemical Tissue Tests for Determining Mineral Status of Plants in the Field. By D. J. D. NICHOLAS. The Tintometer Ltd., Salisbury, England, 1953. 33 pp. Price 8*s* 6*d*.

This booklet contains a series of selected chemical methods and extraction procedures for the semi-quantitative determination of certain macro and micronutrients in fresh plant tissue.

The chemical procedures listed are largely those which have been used with success in both rapid soil and tissue testing. The author has apparently chosen these procedures to include reagents which are specific and sensitive for the determination of certain nutrients. Since no analytical separations are made to remove interfering ions or certain organic constituents, the author has made good use of such compounds as purified Darco carbon and hydroxylamine hydrochloride to remove or reduce the effect of interfering substances. Likewise glycerol and sucrose are used to help stabilize suspensions for turbidimetric measurements.

It is the reviewer's opinion that aluminon (sodium salt of aurintricarboxylic acid) and thiazol yellow might be more sensitive reagents for the estimation of aluminum and magnesium, respectively. These reagents are definitely superior to hematoxylin and titan yellow in the analyses of soil extracts. However, since no direct comparisons with fresh plant tissue are available, this is a matter of conjecture. Another question is whether the author has placed enough emphasis on a definite period or reaction time for the development of color in the phosphorus determination.

The reviewer has had no experience with the reagents used in the manganese determination, or with the procedures for the estimation of heavy metals as used in tissue tests, and makes no comment on these methods.

In general, it appears that the author has compiled, through experience, an excellent group of chemical procedures designed to give reasonably accurate and rapid results for the mineral status of fresh plant tissue. The conditions and techniques such as temperature control, time of reaction, shaking, etc., which need to be observed and standardized to carry out the tests successfully, have been clearly pointed out. In addition, directions for the preparation and storage of reagents and for making the tests are concise and easy to follow.

KIRK LAWTON

Silage Fermentation. By A. J. G. BARNETT, Academic Press Inc., New York (Butterworths Scientific Publications, London), 1954. x+208 pp. Price $5.00.

The author, an agricultural biochemist of the University of Aberdeen, states that . . . "The primary purpose of this volume is to present to the student a review of the whole question of silage, largely in relation to its interest as a subject of scientific study."

In the first chapter, hay *vs.* silage is thoroughly discussed with the arguments in favor of the latter. A. I. Virtanen, Nobel laureate, is accepted as the authority on silage. The long winters of Finland caused him to make detailed studies of the silage process as a means of overcoming the long nights; thus the A.I.V. process is often referred to.

Acids and other additives are discussed, and molasses is suggested as a substrate for lactic organisms. Virtanen found no de-amination below *p*H 4, and more respiration below *p*H 3. The process requires seventeen to twenty-one days.

Unavoidable losses (respiration, drainage, etc.) are discussed, along with avoida-

ble losses (heating, wrong silage, etc.). It seems that A.I.V. silage loses 12 per cent of calcium and more than 7 per cent of phosphorus. Of course, the loss of trace minerals may be the most serious mineral loss.

Silos are described, and methods of analysis are given in concise but thorough detail. Finally, the digestibility and nutritive value of silage are discussed.

This book covers most phases of the scientific study of silage. The student should have had a good grounding in organic chemistry in order to understand some of the discussion, which includes chlorophyll, vitamins, carbohydrate breakdown, the amino acids, and other acids. It is said that a steam distillation of ten grams of silage will yield formic, acetic, isopropylacetic, butyric, valeric, caproic, and lactic acids. The amino acids most likely to de-carboxylate are tyrosine, histidine, arginine, ornithine, lysine, and glutamic acid.

The book is written in a scholarly manner, and there are very few errors. It is rather unfortunate that the price is $5.00, for surely no agricultural graduate of a land-grant college or University should be without this book.

M. P. ETHEREDGE

Plant Growth Substances. By L. J. AUDUS. Interscience Publishers, Inc., New York, 1953. xix+465 pages. Price $6.50.

This book presents the fundamentals of the physiological processes that the auxins regulate and the biochemical reactions which are responsible for their formation in plants. The author discusses the chemistry of auxins and the effect of auxins as growth stimulators, initiators, and inhibitors. The effects of the application of these materials and methods of application are described in detail. The book concludes with a glossary of definitions, appendices on the responses of various auxin treatments of plants, and an extensive bibliography.

The subject matter is presented effectively in language that is not too technical and the book can thus be appreciated by the non-scientific layman as well as by the plant physiologist and biochemist. Much of the knowledge of plant growth substances scattered throughout the literature is assembled in this volume. Manufacturers, agricultural chemists, plant physiologists, botanists, and non-scientific laymen interested in this field will find this treatise useful and an excellent source of information on auxins.

HAROLD M. SELL

Abnormal and Pathological Plant Growth. Brookhaven Symposia in Biology No. 6. Office of Technical Services, U. S. Department of Commerce, Washington, 1954. vii+303 pages. Illus. Price $2.10.

The subject matter of this volume was presented at the sixth annual symposium held under the sponsorship of the Biology Department, Brookhaven National Laboratory, at Upton, New York, August 3 to 5, 1953. Both the full text of the papers and the discussions following each paper are given here.

Abnormal growths are induced in plants by a considerable number of different agents or factors, which vary from physical agents such as heat or radiation through a wide range of chemical and biological stimuli. These various kinds of abnormalities are in turn scrutinized and studied under such diverse disciplines as genetics, physiology, and morphology.

The following subjects are discussed: Substances involved in normal growth and differentiation of plants, the use of *in vitro* cultures in the investigation of growth and differentiation in vascular plants, abnormal plant growth, genetic tumors in *Nicotiana* hybrids, nutrition and diseased plant growths, mechanisms of crown-gall induction, studies on the origin of the crown-gall tumor cell, the experimental inhi-

bition of the growth of plant tumors, size and shape of wound-tumor virus, virus-induced abnormalities, nutritional aspects of virus-tumor growth, studies on growth and regeneration in gametophytes and sporophytes of gymnosperms, morphogenesis of the leguminous root nodule, the nature of the stimulus in the *Solidago* gall induced by the larva of *Gnorimoschema Gallaesolidaginis*, and aberrant growth in plants induced by ionizing radiation.

There are author and subject indices and numerous bibliographies.

R. C. ROARK

Bibliographie der Pflanzenschutzliteratur. 1940–1945. J. BÄRNER, Editor. Verlag Paul Parey, Berlin SW 68, Lindenstrasse 44–47 (Westberlin). Vol. I, xlviii+740 pages; Vol. II, pages 741–1308. Price 97 DM.

This bibliography is a continuation of one published in 1921 which included all of the phytopathological literature of the world for the years 1914–1919. Subsequent volumes covered the literature through 1939.

The information is classified under headings such as general, diseases and causes, diseases and host plants, and measures of plant protection, under which heading are placed all references to insecticides, fungicides, and damage by plant insecticides. There is an author index. References to a few analytical methods for pesticides such as DDT, rotenone, pyrethrins, and nicotine are included.

This work will be of value chiefly to the plant pathologist but can be consulted with profit by the chemist interested in combing the world's literature for information on pesticides.

R. C. ROARK

Index to the Literature on Spectrochemical Analysis. Part III. 1946–1950. American Society for Testing Materials, 1916 Race St., Philadelphia, 1954. Publication No. 41-C. 226 pp. Price $4.50.

This volume is third in a series of bibliographic surveys of the literature of spectrochemical analysis. It contains 1,264 references, each with a short abstract, most of which are quoted verbatim from *Chemical Abstracts*. Subject and author indices are supplied.

Part I of this series, issued in 1941, covers the period from 1920 to 1939, and Part II covers the years 1940 to 1945. The price of all three parts is $7.50.

P. A. CLIFFORD

Standard Methods for the Examination of Dairy Products, 10th Edition. Published by the American Public Health Association, 1790 Broadway, New York 19, N. Y., 1953. 345 pp. Index, 23 figs. Cloth. Price $4.75.

This manual is a compilation of accepted methods of analysis, mainly for the sanitary (bacteriological) control of dairy products, but it also contains various chemical methods commonly used by officials and industry in dairy control work.

Chapter I (80 pages) gives fairly comprehensive directions to guide users of the book in proper selection of methods and interpretations of results. Succeeding chapters present microbiological methods for milk and cream, detection of special bacterial groups or species, microbiological methods for butter, for cheese, for frozen dairy food ingredients, for frozen dairy foods, and tests for sanitization of equipment.

Other chapters deal with sediment in fluid milk, phosphatase tests for heat treated products, and chemical analysis of dairy products. Most of the chemical methods have been taken, with permission, from the 7th Edition of "Official Methods of Analysis" of the Association of Official Agricultural Chemists. Vitamin methods included in the 9th Edition are omitted.

The final chapter outlines useful screening or rapid tests which allow closer control of sanitation, composition, and treatments during processing. Proper cautions are included against their use where litigation might be required.

Notable among changes in methods are recognition of two new milk-free plating media to replace milk-containing media, substitution of improved staining procedures for those previously used in the direct microscopic method, improved methods for sediment in retail milk, introduction of a transfer syringe for 0.01 ml quantities of milk and cream for the microscopic technique, and clarification of the dilution procedures for dried milk products.

The book is conveniently cross-referenced and lists the most useful, fairly recent references in the bibliographies. Very few typographical errors were evident. However, under mold mycelia count in butter, three filaments instead of the proper two were allowed for a positive field.

E. S. WINDHAM

Official Publication of the Association of Economic Poisons Control Officials, Inc., 1954. Obtained from A. B. Heagy, Secretary-Treasurer, College Park, Md. Index. 202 pp. Price $1.00.

For the second year, the official publication of the A.E.P.C.O. contains a summarized compilation of chemical, physical, and biological properties of 150 pesticide chemicals, in addition to the yearly report of the Association and its usual affairs, such as the constitution, by-laws, officers, committees, etc., which are primarily of interest to its membership. This review will be limited to that portion of the publication devoted to pesticide chemicals, in view of the general interest that the volume will have for all workers in this field.

The number of pesticide chemicals has increased so rapidly during the past decade or so that few workers can hope to maintain acquaintance with more than a limited portion, so that a ready reference of this type is a necessity. The data presented for each pesticide chemical include, as far as possible, the common or trade name, chemical name, empirical formula, chemical and physical properties, analytical methods, toxicity data, antidotes and first-aid measures, hazards to wildlife, use precautions, residues, uses, formulations, applications, phytotoxicity, accumulation in soil, and storage and shipping directions. Much of this information is not readily available in any other single publication.

An "Index to Pesticide Chemicals" appends the compilation. However, the index does not do full justice to the text, for it does not include all the common or trade names which are cited in the various monographs. For instance, some of the monographs are headed by two or more common names, such as "Kleneg, Saneg", or in another case, "Systox, E1059"; yet only the first name listed, i.e., "Kleneg" and "Systox," is found in the index. In one instance, neither of the common or trade names is listed. For the pesticide under the common names "Penta, PCP" and the chemical name "Pentachlorophenol," only the chemical name is given in the index. Thus the index would be far more useful if it listed all the known names mentioned in the monographs, and the value of the entire compilation would be increased if a few more common or trade names of some of the compounds were included. The field of pesticide chemicals abounds in multiple names, and their omission from an index may lead to confusion on the part of the reader.

The volume contains a quantity of very useful information. The Association committee responsible for this work should be commended for their fine achievement. It is hoped that they will continue with this project and that they will be able to keep the list and data abreast of the frequent changes in their field.

J. W. COOK

The Story of Spices. By JOHN W. PARRY. Chemical Publishing Co., Inc., New York, 1953. viii+208 pp. Bibl., index. Price $4.50.

This is truly a story about spices, from the author's visualization of the practices of primitive man in seasoning his food, to the uses of spices in the modern world. The book presents nothing of the scientific phases of the subject, but the relatively small number of books on spices makes this treatise of interest to the food scientist. One gathers from the volume how man's urge to please his palate has helped to shape the destiny of nations.

H. A. LEPPER

The Optical Properties of Organic Compounds. 2nd Ed. By ALEXANDER H. WINCHELL. Academic Press, Inc., New York, 1954. xviii+487 pp. Illus., index. Price $12.00.

Those who are acquainted with the first edition of this work (published in 1943) will surely welcome the appearance of the revised edition. The present edition strives to include data on all organic compounds whose optical crystallographic properties have been described prior to October 1, 1952, a goal which has resulted in the addition of some 900 crystalline compounds to about 1600 described in the earlier edition.

The minimum requirement for the inclusion of a compound is that data providing the refractive index or indices be available. However, in most cases the descriptions of the individual substances also provide such optical-crystallographic properties as crystal system, habit, crystallographic orientation of the directions of vibration corresponding to the indices of refraction, optical character, size of the optic axial angle, extinction, dispersion, and pleochroism.

The compounds are arranged according to the Beilstein system (Beilstein's *Handbuch der Organischen Chemie*) into four main divisions: (I) Acyclic stem nuclei; (II) Isocyclic stem nuclei; (III) Heterocyclic stem nuclei; and (IV) Natural products not assigned places in the three preceding divisions. Aptly, the author deviates from Beilstein's classification in dealing with salts of organic acids formed from inorganic cations by bringing together those salts which are isomorphous and therefore can in some cases intercrystallize in any proportion to form homogeneous crystals. A complete list of citations to the original data is given.

The determinative tables and diagrams provided are considerably improved over those contained in the first edition. Wisely, in these keys the author has separated those compounds for which data are incomplete (in that the optical character is uncertain) from those whose optical-crystallographic properties with respect to principal refractive indices, optical character, and optic angle have been adequately described. The former compounds have been listed in two tables, one arranged according to the ascending value of the higher index, and the other according to the ascending value of the lower index. This arrangement is particularly satisfactory, for the author has recognized that many compounds have been described in the literature whose data may not be complete, but which still prove quite useful in the specific identification of these substances by means of this arrangement. Many of these measurements represent commonly occurring refractive indices associated with the crystal habit.

Those compounds whose data have been more adequately described have been plotted on two diagrams. Diagram 1 is based on the measurement of the principal refractive indices and the optic sign. Diagram 2 has been added to provide for those cases in which the optic angle (for biaxial substances), optic sign, and one index of refraction (N, N_O, or N_Y) can all be measured on a single crystalline fragment which has an easily recognized orientation since it produces the lowest interference

color. In the case of uniaxial substances, the measurement of the birefringence on such a fragment, estimated from the number of color bands seen in the uniaxial interference figure, is used as a basis for plotting the compounds.

WM. V. EISENBERG

Matthews Textile Fibers, Their Physical, Microscopic, and Chemical Properties. 6th Ed. Edited by HERBERT R. MAUERSBURGER. John Wiley and Sons, Inc., New York; Chapman and Hall, Ltd., London; 1954. x+1283 pp. Illus. Price $16.50.

This volume is the complete book on fiber science. Now in its sixth edition, the work brings up-to-date all essential data on the important fibers of commerce. Each of the twenty-two chapters has been written by an expert in the particular field.

About one-fourth of the pages are devoted to cotton and wool, which are discussed completely under the headings: History, Growth and Statistics, Microscopic Characteristics, and Physical and Chemical Properties. The same pattern of discussion is followed wherever possible in describing some 1,000 other fibers of the animal, plant, mineral, and synthetic groups which are covered in the text.

The chapter on cellulose is treated excellently by Professor Philip C. Sherer. All recent data on the chemical nature of this important substance are presented and evaluated.

The book is replete with data on the physical and chemical properties of fibers which can be measured by modern analytical techniques. These descriptions and constants are tabulated and arranged in graphical form for ready comparison and identification. Thus, to single out only one of these many features, in the introductory general discussion of fibers, the editor has tabulated the refractive indices and birefringence of 31 fibers determined by optical immersion methods and has related these optical properties to fiber structure and molecular orientation.

A very useful chapter on "Identification Methods and Quantitative Fiber Analysis" by Walter Kraus organizes the principle determinative data for fibers. One section covers the new dye-stain tests which offer a rapid method for fiber identification.

The volume is profusely illustrated with excellent photographs which are helpful in understanding fiber technology. Photomicrographs of practically every important fiber are included in the text.

The book also makes available an excellent chapter on the application of X-ray technique to fiber analysis. X-ray diffraction patterns are provided for both the natural and synthetic fibers.

WM. V. EISENBERG

An Introduction to Bacterial Physiology. By EVELYN L. OGINSKY and WAYNE W. UMBREIT. F. W. Freeman and Co., San Francisco, 1954. xi+416 pp. Illus., index. Price, text: $6.00; trade: $7.25.

This volume was written for the undergraduate student "to bridge the gap between general bacteriology and graduate studies in bacterial physiology." Its intent was to serve not as a reference work but as a beginner's guide.

A partial listing of the chapter headings indicates the scope of the coverage: Cytology and Cytochemistry; Growth; Nutrition; The Chemical Environment—Toxicity; The Physical Environment; Genetics; Enzymes; Energy; Dehydration and Respiration; The Metabolism of Carbohydrates; The Metabolism of Fats, Steroids, Aromatic Rings; Amino Acids and Proteins; Nucleic Acids, Purines, and Pyrimidines; Autotrophic and Photosynthetic Bacteria; Bacteriophages, Animal

and Plant Viruses, and Rickettsia; Adaptation; Mechanisms of Survival; Virulence as a Physiological Problem.

The arrangement of the subject matter is handled in an interesting manner. Difficult concepts are described in a lucid fashion, and explanations are logically unfolded without loss of essential substance. Sections are prefaced by introductory remarks and the theme is developed by the chapters which follow. At the end of each chapter is a list of references, generally to standard texts, and there are, in addition, listings of a variety of fairly recent monographs, reviews, symposia, and original publications. The section is concluded with a group of thought-provoking questions.

The subject matter is essentially that which may be found in other text books on bacterial physiology; however, the authors have sought to develop an over-all picture in simplified terms. Principles, processes, and metabolic routes are clearly described in a straightforward manner. The topics are all timely.

There are some shortcomings in the book. Reference is made to the "phenol coefficient" test of the Food and Drug Administration, giving the erroneous impression that this test is at present the official means of the F.D.A. to evaluate disinfectant effectiveness. The treatment of buffers is too scanty; also, a fuller development of the tricarboxylic acid cycle would have been most valuable. Topics devoted to the phase microscope and the electron microscope, and the chapter on Physical Environment, were worthy of a more expanded treatment.

By far the best portions of the book dealt with such subjects as the metabolism of carbohydrates, fats, steroids, aromatic rings, etc. Some noteworthy sections were the description of the oxidation-reduction balance, the Meyerhof-Embden system, amino acid transformations, and others.

This volume is primarily suited to the needs of the student and is a good source of information for the practicing bacteriologist. The subject matter is timely, and the presentation is excellent.

M. Fishbein

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