loss on standing 1 hour. At the absorbance maximum, 530 mμ, interference from milk pigments is much less than at 430 mμ. A further advantage is that the specific absorbance of the blue color at 530 mμ is somewhat greater than the corresponding nitrofuran absorbance at 430 mμ. The absorbance curves for each of these, as recorded with a Beckman DK spectrophotometer, are shown in Figure 1.

This method has been applied to the milk from cows treated with a commercial nitrofurazone preparation according to label directions. The results will be reported in a separate publication.

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FEED ADDITIVE RESIDUES

A Colorimetric Procedure for the Microdetermination of Sulfonamides in Animal Tissues

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A colorimetric procedure for the determination of microgram quantities of *p*-aminobenzenesulfonamides is presented. The sulfonamide is adsorbed, from an acid medium, on a strong cationic exchange resin in the hydrogen ion form. The column is then washed with acid and water to remove interfering compounds and the sulfonamide subsequently eluted with ammonium hydroxide. The Bratton-Marshall colorimetric procedure is used for the quantitative measurement of the sulfonamide. The average recovery of two sulfonamides from swine, calf, chicken, and turkey tissues, as well as bovine milk, ranged from 71.9 to 96.5%. The control values obtained on the analysis of unmedicated tissues (blood, muscle, liver, kidney, and fat) ranged from 0.050 to 0.181 p.p.m. of apparent sulfonamide. Thirteen different sulfonamide standards were successfully carried through this procedure. A minimum of 2.5 μg. of the sulfonamide can be accurately determined.

THE SULFONAMIDES, because of their bacteriostatic properties, have recently been proposed for use as animal feed supplements in combination with antibiotics as a prophylactic aid in increasing the growth rate in swine, improving feed efficiency, and reducing losses associated with bacterial diseases (*2*).

Because of this new aspect of sulfonamide therapy and the low treatment levels involved, interest developed in a highly sensitive tissue residue analysis procedure. This necessitated the development of a highly specific cleanup procedure which would eliminate naturally occurring interfering compounds found in the tissues and other chemotherapeutic agents present.

Existing procedures using the Bratton-Marshall colorimetric reaction for the determination of primary aromatic amines (*3*) employed no cleanup other than protein precipitation and dilution. These procedures were inadequate because of the low levels of sulfonamide present and the high control values obtained from the analysis of unmedicated tissue.

A method is presented whereby the sulfonamide is extracted from the sample with a mixture of acetone and carbon tetrachloride and then partitioned into 1*N* HCl. The aqueous acid solution is subsequently passed through a strong cationic exchange resin in the hydrogen ion form, resulting in adsorption of the sulfonamide on the resin. After being washed free of interferences with water, the compound is eluted with concentrated NH_4OH, acidified, heated to convert any acetyl compound to the free acid, and quantitatively measured by the Bratton-Marshall colorimetric procedure (*1*).

This technique has been successfully used to separate and distinguish aminobenzenesulfonamides from procaine penicillin, which also reacts to the Bratton-Marshall test and is commonly used as a feed supplement and in animal medication. The method is capable of quantitatively determining as little as 2.5 μg. of the sulfonamide.

Experimental

Reagents. Sodium nitrite, 0.1%. Ammonium sulfamate, 0.5%. *N*-(1-naphthyl)ethylenediamine dihydrochloride, 0.1%. Aqueous solutions of these three reagents should be prepared fresh daily.

Trichloroacetic acid, aqueous 50% solution.

Dowex 50W-X2 (H+) ion exchange resin, 50- to 100-mesh (J. T. Baker Co.). Transfer 1 pound of dry resin (as shipped) to a 2000-ml. glass funnel with a fused, coarse fritted disk, and add 500 ml. of 1*N* NaOH (C. P. reagent). Stir with a glass rod until well mixed and allow the mixture to filter by gravity for 5 minutes. Apply suction and remove the NaOH solution until approximately 5 mm. of liquid remains above the resin bed. At no time should the resin be allowed to become dry. Repeat the above treatment until at least 2 liters of 1*N* NaOH have been used or until the filtrate is colorless. Wash the resin with distilled water, filtering with suction, until the effluent is the same pH as the distilled water. Regenerate the resin by the addition of 500 ml. of 1*N* HCl to the funnel. Mix well with a glass rod and allow the slurry to filter by

gravity for 5 minutes. Apply suction and remove the HCl solution until approximately 5 mm. of liquid remains above the resin bed. Repeat the above HCl treatment with three additional 500-ml. portions of 1*N* HCl. Wash the resin with distilled water, filtering with suction, until the effluent is the same pH as the distilled water. Transfer the prepared resin to an amber glass bottle and store as a water slurry.

Sulfonamide Standard Solution. Weigh accurately 100 mg. of sulfonamide standard and transfer to a 100-ml. volumetric flask. Bring to volume with 1*N* HCl, mix well, and label standard solution *A*. Pipet a 10-ml. aliquot of solution *A* into a 100-ml. volumetric flask and bring to volume with 1*N* HCl. Mix well and label standard solution *B*. Pipet a 5-ml. aliquot of solution *B* into a 100-ml. volumetric flask and bring to volume with 1*N* HCl. Mix well and label standard solution *C*. Pipet a 25-ml. aliquot of solution *C* into a 50-ml. volumetric flask and bring to volume with 1*N* HCl. Mix well and label standard solution *D*. This is the working standard containing 2.5 μg. of sulfonamide per ml. of solution. Standard solutions should be prepared fresh daily.

Apparatus. Borosilicate glass chromatographic columns, 10 × 300 and 30 × 400 mm. The 10-mm. column has a fused, coarse fritted disk. The sample is supported in the 30-mm. columns by a pledget of glass wool.

Rinco rotating evaporators.

Centrifuge, International Equipment Co. Model 2-V, with International Head No. 267. This head gives a centrifugal force of 800 × gravity at 1500 r.p.m.

Analytical Procedure

Calibration Curve. Transfer aliquots of standard solution *D* containing 0 to 30 μg. of sulfonamide to 50-ml., glass-stoppered graduated cylinders and bring to equal volume with 1*N* HCl. Dilute to 30 ml. with water and mix well. Add, by pipet, 2 ml. of 0.1% sodium nitrite. Mix well and let stand 3 minutes. Add, by pipet, 2 ml. of 0.5% ammonium sulfamate. Mix well and let stand 2 minutes. Add, by pipet, 2 ml. of 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride. Mix well and let stand 1 hour. Read the absorbance of the solutions on a Beckman Model B spectrophotometer at 545 mμ in a cell with a light path of 5 cm. Plot the absorbance of the solutions versus the concentration of the sulfonamide in total micrograms present. The curve should be annotated to be based on a volume of 34 ml. before the addition of *N*-(1-naphthyl)ethylenediamine dihydrochloride.

Preparation and Extraction of Samples. Muscle, Liver, Kidney, and Fat. Pass a portion of tissue, in excess of 25 grams, through a meat grinder. Weigh a 25-gram sample of the ground tissue. Transfer it to a Waring Blendor jar containing 150 grams of anhydrous sodium sulfate and blend at medium speed for 2 minutes. Transfer the sample to a 30- × 400-mm. chromatographic column. Pass 200 ml. of a solvent mixture containing 2 parts of CCl_4 to 1 part of acetone through the sample at a rate of approximately 100 drops per minute, collecting the extract in a 500-ml. Erlenmeyer flask. Transfer the extract to a 500-ml. separatory funnel and rinse the flask with 10 ml. of the solvent mixture. Pipet 5 ml. of 1*N* HCl into the funnel and shake vigorously for 1 minute. Allow the layers to separate for 5 minutes. If an emulsion is present at the end of this time, draw off as much of the solvent layer as possible into a second 500-ml. separatory funnel. Add acetone by 1-ml. increments to the emulsion, shaking vigorously for a few seconds after each addition until the emulsion breaks. Allow the layers to separate and combine the solvent layers in the second funnel. Draw the aqueous acid layer into a 250-ml. round-bottomed flask with a 24/40 ground glass neck. Rinse the separatory funnel with 5 ml. of 1*N* HCl adding it to the acid extract.

Repeat the above extraction with another 5-ml. portion of 1*N* HCl again rinsing the separatory funnel. Combine all acid extracts and washes in the 250-ml. flask.

Evaporate the solution, using a Rinco evaporator and a hot water bath at 60° to 70° C., until no odor of solvent persists. Cool to room temperature and filter the solution through a pledget of glass wool contained in a small funnel into a 250-ml. Erlenmeyer flask. Wash the evaporation flask and glass wool with two 5-ml. portions of 1*N* HCl, combining the filtrate and washes.

Charge a 10- × 300-mm. column with the prepared ion exchange resin to a depth of 10 cm. always leaving 5 mm. of liquid above the resin bed. Pass the aqueous acid extract through the resin column. When the solution level is 5 mm. above the resin bed, rinse the flask into the column with 5 ml. of 1*N* HCl, followed by two 5-ml. portions of distilled water. Elute the sulfonamide by passing two 10-ml. portions of concentrated NH_4OH through the resin column, collecting the eluate in a clean 250-ml. Erlenmeyer flask.

Add concentrated HCl (approximately 12 ml.) until the solution is pH 1 with indicator paper. Add 3 ml. of HCl in excess. Heat the samples in a boiling water bath for 15 minutes and cool to room temperature. Transfer the solutions to 50-ml., glass-stoppered graduated cylinders and bring to equal volumes with distilled water. Add, by pipet, 2 ml. of 0.05% ammonium sulfamate, mix well, and let stand 2 minutes. Note the volume of solution at this point. Filter through Whatman No. 42 paper, or its equivalent, into 50-ml., glass-stoppered graduated cylinders, collecting 34 ml. of the filtrate. Add, by pipet, 2 ml. of *N*-(1-naphthyl)ethylenediamine dihydrochloride, mix well, and let stand 1 hour. Read the absorbance of the solutions on a Beckman Model B spectrophotometer at 545 mμ in a cell with a light path of 5 cm. Correct the absorbance reading for volume differences between the sample and the volume at which the standard curve was prepared (34 ml.). If the unknown solutions are too intensely colored to read accurately, dilutions with distilled water can be made and the appropriate factor included in the calculation of results.

Blood. Transfer a 25-ml. sample to a 4-ounce bottle fitted with a polyethylene-lined screw cap, and add 50 ml. of acetone. Shake on a mechanical shaker for 30 minutes and filter through Whatman No. 12 fluted filter paper into a 500-ml. separatory funnel. When the mixture has filtered completely, wash the bottle and solids remaining on the filter paper with two 25-ml. portions of acetone, combining the extract and washes in the separatory funnel. Add 2 ml. of concentrated HCl followed by 200 ml. of CCl_4 and shake vigorously for 1 minute. Continue with the procedure as for preparation and extraction of muscle, liver, etc., beginning with "Allow the layers to separate for 5 minutes . . . ."

Milk. Transfer 200 ml. of whole milk to a 600-ml. beaker and add 250 ml. of acetone. Stir intermittently for 15 minutes. Transfer the mixture to a centrifuge bottle with the aid of 20 ml. of acetone. Centrifuge the mixture for 15 minutes at 800 × gravity (1500 r.p.m.). Decant the supernatant liquid through glass wool into a 1000-ml. flask with a 24/40 ground glass neck. Wash the solids remaining in the centrifuge bottle by shaking with 20 ml. of acetone. Centrifuge the wash for 5 minutes at 800 × gravity and combine the wash and extract. Evaporate the solution using a Rinco evaporator and a hot water bath at 60° to 70° C. until the odor of acetone is no longer detectable. Add 6.0 ml. of a 50% solution of trichloroacetic acid and let stand 15 minutes. Transfer to a centrifuge bottle and centrifuge for 15 minutes at 800 × gravity. Decant the supernatant liquid into a 250-ml. flask. Rinse the evaporation flask into the centrifuge bottle with 20 ml. of water and centrifuge for 5 minutes at 800 × gravity. Combine the sample and the wash. Continue with the procedure as for preparation and extraction of muscle, liver, etc., beginning with "Charge a 10- × 300-mm. column . . .", washing this column with 50 ml. of water instead of 10 ml.

Recovery Determinations. The tissue

used in gathering all of the control and recovery data, Tables I through VII, was obtained from animals of known history except where otherwise noted. In an experiment involving the feeding

Table I. Recovery of Sulfamethazine from Porcine Tissues[a]

P.P.M. Added	P.P.M. Found	Recovery, %
	BLOOD	
0	0.101 ± 0.039[b]	...
0.10	0.087	87.0
0.10	0.049	49.0
0.25	0.289	115.6
0.25	0.316	126.4
0.50	0.465	93.0
0.50	0.449	89.8
1.00	0.731	73.1
1.00	0.724	72.4
2.00	1.139	60.0
2.00	1.060	53.0
5.00	3.279	65.6
5.00	3.099	62.0
	MUSCLE	
0	0.112 ± 0.077[b]	...
0.10	0.088	88.0
0.10	0.080	80.0
0.25	0.168	67.2
0.25	0.170	68.0
0.50	0.400	80.0
0.50	0.450	90.0
2.00	1.400	70.0
2.00	1.220	61.0
	LIVER	
0	0.074 ± 0.046[b]	...
0.10	0.060	60.0
0.10	0.050	50.0
0.20	0.140	70.0
0.50	0.304	60.8
0.50	0.316	63.2
1.00	0.910	91.0
1.00	0.870	87.0
2.00	1.960	98.0
2.00	1.672	83.6
5.00	4.468	89.4
5.00	4.060	81.2
	KIDNEY	
0	0.182 ± 0.046[b]	...
0.10	0.070	70.0
0.10	0.090	90.0
0.25	0.190	76.0
0.25	0.190	76.0
0.50	0.370	74.0
0.50	0.286	57.2
1.00	0.770	77.0
1.00	0.836	83.6
2.00	1.560	78.0
5.00	4.060	81.2
5.00	3.500	70.0
	FAT	
0	0.050 ± 0.048[b]	...
0.10	0.080	80.0
0.10	0.076	76.0
0.20	0.210	105.0
0.20	0.156	78.0
0.50	0.380	76.0
0.50	0.398	79.6
1.00	0.676	67.6
1.00	0.632	60.2
2.00	1.388	69.4
2.00	1.360	68.0

[a] All recoveries are corrected for the average control value.
[b] The mean and standard deviation for replicate control determinations.

Table II. Recovery of Sulfamethazine from Milk[a]

P.P.M. Added	P.P.M. Found	Recovery, %	P.P.M. Added	P.P.M. Found	Recovery, %
0	0.061 ± 0.005[b]	...	0.50	0.406	81.2
0.10	0.089	89.0	0.50	0.394	78.8
0.10	0.076	76.0	1.00	0.858	85.8
0.10	0.076	76.0	1.00	0.854	85.4
0.20	0.177	88.5	2.00	1.587	79.4
0.20	0.171	85.5	2.00	1.657	82.9

[ab] Same as in Table I.

Table III. Recovery of Sulfamethazine from Calf Tissues[a]

P.P.M. Added	P.P.M. Found	Recovery, %
	BLOOD	
0	0.166 ± 0.074[b]	...
0.10	0.141	141.0
0.10	0.134	134.0
0.25	0.279	111.6
0.25	0.289	115.6
0.50	0.370	74.0
0.50	0.390	78.0
1.00	0.771	77.1
1.00	0.758	75.8
2.00	1.52	76.0
2.00	1.64	82.0
	MUSCLE	
0	0.082 ± 0.038[b]	...
0.10	0.082	82.0
0.10	0.071	71.0
0.25	0.184	73.6
0.25	0.178	71.2
0.50	0.422	84.4
0.50	0.340	78.0
1.00	0.823	82.3
1.00	0.824	82.4
2.00	1.47	73.5
2.00	1.34	67.0
	LIVER	
0	0.096 ± 0.015[b]	...
0.10	0.114	114.0
0.10	0.100	100.0
0.25	0.245	98.0
0.25	0.229	91.6
0.50	0.357	71.4
0.50	0.438	87.6
1.00	0.632	63.2
1.00	0.712	71.2
2.00	1.39	69.5
2.00	1.48	74.0
	KIDNEY	
0	0.071 ± 0.030[b]	...
0.10	0.108	108.0
0.10	0.099	99.0
0.25	0.189	75.6
0.25	0.182	72.8
0.50	0.342	68.4
0.50	0.412	82.4
1.00	0.819	81.9
1.00	0.712	71.2
2.00	1.56	78.0
2.00	1.42	71.0
	FAT	
0	0.089 ± 0.016[b]	...
0.10	0.081	81.0
0.10	0.114	114.0
0.25	0.163	65.2
0.25	0.171	68.4
0.50	0.413	82.6
0.50	0.397	79.4
1.00	0.779	77.9
1.00	0.751	75.1
2.00	1.65	82.5
2.00	1.40	70.0

[ab] Same as in Table I.

Table IV. Recovery of Sulfaethoxypyridazine from Porcine Tissues[a]

P.P.M. Added	P.P.M. Found	Recovery, %
	BLOOD	
0	0.108 ± 0.053[b]	...
0.10	0.065	65.0
0.10	0.072	72.0
0.25	0.167	66.8
0.25	0.167	66.8
0.50	0.395	79.0
0.50	0.405	81.0
1.00	0.724	72.4
1.00	0.716	71.6
2.00	1.43	71.6
2.00	1.45	72.6
	MUSCLE	
0	0.060 ± 0.036[b]	...
0.10	0.110	111.0
0.10	0.101	101.0
0.25	0.221	88.4
0.25	0.218	87.2
0.50	0.321	64.2
0.50	0.339	67.8
1.00	0.693	69.3
1.00	0.721	72.1
2.00	1.46	73.0
2.00	1.42	71.0
	LIVER	
0	0.098 ± 0.029[b]	...
0.10	0.074	74.0
0.10	0.145	145.0
0.25	0.209	83.6
0.25	0.211	84.4
0.50	0.382	76.4
0.50	0.408	81.6
1.00	0.632	63.2
1.00	0.622	62.6
2.00	1.26	63.1
2.00	1.34	67.1
	KIDNEY	
0	0.124 ± 0.022[b]	...
0.10	0.088	88.0
0.10	0.087	87.0
0.25	0.186	74.4
0.25	0.198	79.2
0.50	0.353	70.6
0.50	0.383	76.6
1.00	0.900	90.0
1.00	0.880	88.0
2.00	1.56	78.0
2.00	1.54	77.0
	FAT	
0	0.081 ± 0.003[b]	...
0.10	0.079	79.0
0.10	0.071	71.0
0.25	0.292	116.8
0.25	0.251	100.4
0.50	0.439	87.8
0.50	0.509	101.8
1.00	0.787	78.7
1.00	0.803	80.3
2.00	1.57	78.5
2.00	1.78	89.0

[ab] Same as in Table I.

Table V. Recovery of Sulfaethoxypyridazine from Milk[a]

P.P.M. Added	P.P.M. Found	Recovery, %
0	0.061 ± 0.005[b]	...
0.10	0.089	89.0
0.10	0.076	76.0
0.10	0.076	76.0
0.20	0.177	88.5
0.20	0.171	85.5
0.50	0.406	81.2
0.50	0.394	78.8
1.00	0.858	85.8
1.00	0.854	85.4
2.00	1.587	79.4
2.00	1.657	82.9

[ab] Same as in Table I.

Table VII. Recovery of Sulfaethoxypyridazine from Turkey Tissues[a]

P.P.M. Added	P.P.M. Found	Recovery, %
	Breast Muscle	
0	0.165 ± 0.022[b]	...
0.10	0.068	68.0
0.10	0.064	64.0
0.25	0.196	78.4
0.25	0.200	80.0
0.50	0.420	84.0
0.50	0.416	83.2
1.00	0.756	75.6
1.00	0.770	77.0
2.00	1.552	77.0
2.00	1.688	84.4
	Leg Muscle	
0	0.162 ± 0.039[b]	...
0.10	0.076	76.0
0.10	0.080	80.0
0.25	0.232	92.8
0.25	0.168	67.2
0.50	0.400	80.0
0.50	0.356	72.0
1.00	0.816	81.6
1.00	0.816	81.6
2.00	1.660	83.0
2.00	1.540	77.0
	Blood	
0	0.139 ± 0.009[b]	...
0.10	0.088	88.0
0.10	0.120	120.0
0.25	0.176	70.4
0.25	0.204	81.6
0.50	0.356	71.2
0.50	0.364	72.8
1.00	0.760	76.0
1.00	0.644	64.4
2.00	1.344	67.2
2.00	1.324	66.2
	Liver	
0	0.122 ± 0.018[b]	...
0.10	0.116	116.0
0.10	0.072	72.0
0.25	0.196	78.4
0.50	0.532	106.2
1.00	0.733	73.3
1.00	0.974	97.4
2.00	1.456	72.8
2.00	1.624	81.2
	Kidney	
0	0.280 ± 0.004[b]	...
0.25	0.244	97.5
0.50	0.416	83.2
1.00	0.722	72.2
1.00	0.845	84.5
2.00	1.472	73.6
2.00	1.540	77.0

[ab] Same as in Table I.

Table VI. Recovery of Sulfaethoxypyridazine from Chicken Tissues[a]

P.P.M. Added	P.P.M. Found	Recovery, %
	Breast Muscle	
0	0.132 ± 0.029[b]	...
0.10	0.088	88.0
0.10	0.112	112.0
0.20	0.172	86.0
0.20	0.180	90.0
0.40	0.356	89.0
0.50	0.452	90.4
0.80	0.696	87.0
1.00	0.752	75.2
1.00	0.820	82.0
2.00	1.636	81.8
2.00	1.500	75.0
	Leg Muscle	
0	0.154 ± 0.018[b]	...
0.10	0.072	72.0
0.25	0.228	91.2
0.25	0.212	84.8
0.50	0.432	86.4
0.50	0.380	76.0
1.00	0.680	68.0
1.00	0.716	71.6
2.00	1.572	79.0
2.00	1.476	73.8
	Blood	
0	0.243 ± 0.021[b]	...
0.10	0.116	116.0
0.10	0.072	72.0
0.25	0.240	96.0
0.25	0.188	75.3
0.50	0.396	79.2
0.50	0.356	71.2
1.00	0.768	76.5
1.00	0.704	70.4
2.00	1.396	69.8
2.00	1.300	65.0
	Liver	
0	0.221 ± 0.016[b]	...
0.10	0.108	108.0
0.10	0.136	136.0
0.25	0.348	139.2
0.25	0.220	88.0
0.50	0.416	83.2
0.50	0.344	68.8
1.00	0.756	75.6
1.00	0.736	73.6
2.00	1.532	76.6
2.00	1.260	63.0

[ab] Same as in Table I.

of sulfamethazine in combination with procaine penicillin, the recoveries were run in the presence of procaine penicillin at levels corresponding to the level of the sulfamethazine added. The control samples were run with 0.5 p.p.m. of procaine penicillin added.

A 25-gram sample of tissue was used for all determinations of recovery and control values except with milk, where 200 ml. was used.

Discussion of Analytical Procedures

It was noted during the investigation of extraction procedures that once interfering compounds were transferred to an aqueous medium, they could not be removed by solvent partitioning. Evidently then the sample must be extracted with a water-immiscible solvent. Difficulties were encountered here because of the low solubility of sulfonamides in this type of solvent. The mixture of carbon tetrachloride and acetone afforded both water immiscibility and sulfonamide solubility.

The partitioning into aqueous solutions was studied by shaking the sulfonamide into 5, 10, and 25-ml. portions of 1*N* HCl and 1*N* NaOH. Neither the increase in volume nor the change in pH altered the extraction of the sulfonamide; however, the blank value was higher when NaOH was used, as it was when the volume of HCl was increased.

The concentrated ammonium hydroxide used in this procedure was limited to that supplied by Baker and Adamson Co. During the routine use of this procedure, ammonium hydroxide from another supplier was introduced into the laboratory with the result that incomplete color development was experienced.

The resin, as received, is not suitable for use in the procedure. Preliminary treatment is necessary, as described in the method, to remove red-colored material

Table VIII. Adsorption Characteristics of Various Primary Aromatic Amines

(+ = yes; − = no)

Compound	Structure (p-$H_2NC_6H_4X$), X =	Adsorbed from 1N HCl	Eluted with Concd. NH_4OH
p-Chloroaniline	Cl	+	−
p-Nitroaniline	NO_2	+	−
Procaine	$CO_2C_2H_4N(C_2H_5)_2$	−	..
Sulfanilic Acid	SO_3H	−	..
N^1-Dimethylsulfanilamide	$SO_2N(CH_3)_2$	+	+
Sulfacetamide	$SO_2NHCOCH_3$	+	+
Sulfabenzamide	$SO_2NHCOC_6H_5$	+	+
Sulfachloropyrazine	$SO_2NHC_4N_2$	+	+
Sulfadiazine	$SO_2NHC_4N_2H_3$	+	+
Sulfaethoxypyridazine	$SO_2NHC_4N_2H_2OC_2H_5$	+	+
Sulfaguanidine	$SO_2NHCN_2H_3$	+	+
Sulfamerazine	$SO_2NHC_4N_2H_2CH_3$	+	+
Sulfamethazine	$SO_2NHC_4N_2H(CH_3)_2$	+	+
Sulfamethoxypyridazine	$SO_2NHC_4N_2H_2OCH_3$	+	+
Sulfapyridine	$SO_2NHC_5NH_4$	+	+
Sulfaquinoxaline	$SO_2NHC_8N_2H_5$	+	+
Sulfathiazole	$SO_2NHC_3NSH_2$	+	+

soluble in alkaline solutions, which would absorb light at the wavelength at which the sulfonamide is measured.

Table VIII demonstrates the adsorption characteristics of a number of primary aromatic amine compounds which were subjected to the resin treatment as outlined in the procedure. All 13 sulfonamides follow the same adsorption and elution pattern. It is expected, then, that the methods will be effective in determining these sulfonamides.

Other compounds in the series studied behaved in different ways. Two compounds, *p*-chloro- and *p*-nitroaniline, were adsorbed from the acid medium but were not eluted with the concentrated NH_4OH. Sulfanilic acid and procaine were not adsorbed by the resin but passed through in the acid effluent.

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SEED MEAL CONSTITUENTS

Oxazolidinethiones and Volatile Isothiocyanates in Enzyme-Treated Seed Meals from 65 Species of Cruciferae

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Information is unavailable concerning the amounts and types of isothiocyanate-yielding glucosides in many species of Cruciferae seeds. Because such compounds have nutritional significance, a number of unreported species were investigated. Information about the parent thioglucosides was obtained by estimation of the oxazolidinethione and steam-volatile isothiocyanate contents of enzymatic hydrolyzates of the seed meals. Significant amounts of oxazolidinethione were found in hydrolyzates from 11 species not previously known to contain such glucosidic precursors. Oxazolidinethione (calculated as vinyl oxazolidinethione) measurements ranged from 0 to 19.3 mg. per gram of pentane–hexane-extracted meal. Total volatile isothiocyanate measurements (calculated as butenyl isothiocyanate) ranged from 0 to 21.6 mg. per gram of pentane–hexane-extracted seed meal. Probable identification of the predominant volatile isothiocyanates produced in some of the hydrolyzates was obtained by paper chromatography of their thiourea derivatives.

Many Cruciferae produce potentially valuable industrial oilseeds (*18*). The solvent-extracted seed meals from the species contain large amounts of protein (14 to 61%) having nutritionally favorable amino acid composition as determined by chemical analysis (*19*). However, Cruciferae contain thioglucosides which yield isothiocyanates with various structures (*8*). These isothiocyanates are liberated from their parent thioglucosides by enzymes normally present in crushed or ground moist seed. Isothiocyanates, other enzymatically formed products, or thioglucosides themselves may impart unpalatability or toxicity or both to the meals. The β-hydroxyisothiocyanates, after liberation from their thioglucosides, spontaneously cyclize to oxazolidinethiones (*14*), which are known to interfere with proper function of the thyroid (*1*). The literature concerning these compounds, even in very closely related species, is inadequate for predicting or assuming what types or amounts will be found in uninvestigated species.

In the present seed compositional analyses, many Cruciferae were encountered for which little or no literature information of this nature was available. Fundamental information was needed on the amount and kind of these compounds which affect the use of the seed meal as food or feed. This paper reports oxazolidinethione and volatile isothiocyanate measurements after enzyme treatment of seed meals from 65 species of Cruciferae. For comparison or because additional information was obtained, measurements on some species cited in prior literature have been included.

Experimental

Preparation of Sample and Estimation of Volatile Isothiocyanates. Seed meals were prepared by grinding the seed in a 6-inch hammer mill, with $^1/_{16}$-inch, round-hole screen, followed by extraction in a Butt apparatus with petroleum ether (pentane-hexane, b.p. 35°–57° C.). Extraction by this method appeared to avoid the conversion of thioglucoside and loss of isothiocyanate encountered by Kjaer, Conti, and Larsen (*10*) when they extracted rapeseed with ligroin and ethanol at room temperature.