sisting for as long as 80 minutes, are produced by 5 to 10 mg. The E.C.G. remains normal with no change in A.V. or ventricular conduction time but there is a fall in the potential of the P wave and a rise in the T wave.

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A Method of Separating Small Quantities of the Coproporphyrin Isomers 1 and 3.

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Quantitative separation of the naturally occurring coproporphyrin isomers (1 and 3) has hitherto been impossible. Crystallization has usually permitted identification of the porphyrin predominating in any given mixture, such as obtained for instance from urine and feces.[1-4] This, however, has required that relatively large amounts of porphyrin be available. The present investigation was undertaken with the purpose of finding a means by which mixtures consisting of as little as 5-10 γ of total coproporphyrin could be resolved quantitatively.

We have found that the methyl esters of coproporphyrins 1 and 3 are quantitatively adsorbed on Brockmann's Al_2O_3* under the conditions noted in the following. The ester of coproporphyrin 3 may be eluted quantitatively with 35% acetone in water while that of copro-1 remains adsorbed, and is later removed by elution with pure acetone. The various steps in the procedure are as follows: (1) Esterification of the total, free porphyrin mixture in methyl alcohol saturated with HCl gas. (2) Dilution with equal volumes of distilled water, followed by neutralization of the HCl with a saturated aqueous solution of sodium acetate, which is added drop by drop with constant stirring until the solution no longer turns Congo paper blue. Ten percent NH_4OH is then added drop by drop until the mixture becomes pink to phenol red. (A few drops of an aqueous

1 Watson, C. J., *J. Clin. Invest.*, 1935, **14**, 106.
2 Watson, C. J., *J. Clin. Invest.*, 1936, **15**, 327.
3 Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.
4 Watson, C. J., *J. Clin. Invest.*, 1937, **16**, 383.
*Merck and Company, Inc.

solution of the latter indicator having been added to the entire mixture.) (3) The faintly alkaline solution is at once run through the column of Brockmann's Al_2O_3, designated "a" in the accompanying diagram (Fig. 1). The column is next washed with 15-20 cc of distilled water. (4) The copro-3 ester is then removed by repeated washing with 35% acetone, as long as any red fluorescence is visible at b (Fig. 1). The total copro-3 fraction is collected in the lower suction flask and removed, after which elution with pure acetone is carried out in the same way. Relatively large amounts of 35% acetone are necessary for the copro-3 fraction. (5) The amount of porphyrin in each of the final solutions is then measured fluori-

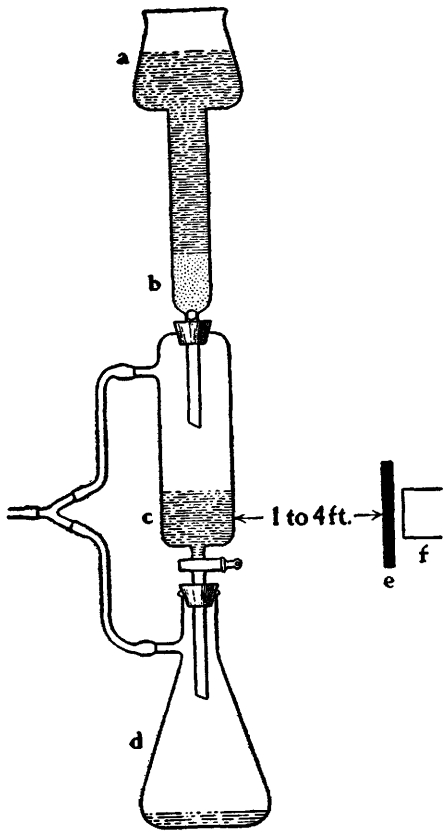

FIG. 1.

Apparatus for adsorption and elution. *a.* Fluid from which porphyrins are to be removed. *b.* Column of Brockmann's Al_2O_3 (a cotton wad is inserted in the neck of the tube just below the Al_2O_3). *c.* Fluid to be inspected for red fluorescence in UV light. *d.* Receiving suction flask. *e.* Corning red purple ultra filter No. 587. *f.* Carbon arc lamp.

metrically. In the present study a Zeiss stufenphotometer† has been used, and comparisons have been made with standard solutions of copro-3 ester in 35% acetone and copro-1 ester in pure acetone. The limit of error is within ±3%. The intensity of fluorescence is about twice that of the free porphyrin in 1% HCl. The chief objection to the measurement of red fluorescence with the stufenphotometer[5] is that the eye fatigues rather rapidly and more than a few readings cannot be taken at any one time. Other methods of measurement are being investigated.

A summary of data obtained in a number of recovery experiments carried out with the above described method is given in Table I.

It is not possible as yet to report data on the application of the above method to natural material. We have ascertained that considerable purification of the free coproporphyrin is necessary, preliminary to esterification and subsequent separation of the isomers. Investigation is now in progress to determine as simple a method of purification as possible, which will still be generally applicable.

The Al_2O_3-acetone procedure is of much value in separating small amounts of copro-esters 1 and 3 for purposes of melting point determination and observation of crystal habitus. The data in Table II is evidence of the specificity of 35% acetone in eluting the copro-3. Extensive purification, consisting of repeated fractionation between ether and 1% HCl in the usual way[1] was carried out in each of these instances.

TABLE I.
Recovery of Copro-1 and 3 Esters from Various Mixtures by Al_2O_3—Acetone Method.

No.	Amount Copro-1 used in γ	Amount Copro-1 recovered in γ	% recovery	Amount Copro-3 used in γ	Amount Copro-3 recovered in γ	% recovery	% Copro-1 in mixture
1	8.8	8.97	102	0.0	0.0	—	100
2	10.0	10.2	102	0.0	0.0	—	100
3	30.0	30.9	103	10.0	10.1	101	75
4	20.0	18.0	90	20.0	20.8	104	50
5	5.3	5.19	96	14.5	14.2	98	27
6	10.0	9.5	95	30.0	30.0	100	25
7	1.8	1.48	82	8.4	8.07	96	18
8	5.0	5.05	101	35.0	32.55	93	12.5
9	0.0	0.0	—	18.0	18.18	101	0.0

† The light source was a small, high pressure mercury arc lamp ("Mico" type) firmly attached to the front of the photometer. The light was filtered through a heat resisting red purple ultra filter, Corning No. 587.

[5] Fikentscher, R., and Franke, K., *Klin. Wchnschr.*, 1934, 922.

TABLE II.

Crystallization of Coproporphyrin Esters After Separation by Means of Al_2O_3-Acetone Method.

	Copro-1 ester			Copro-3 ester			
No.	Amt in γ (stufenphotometer)	M.P. in °C	Crystal habitus	Amt in γ (stufenphotometer)	M.P.* in °C	Crystal habitus	Remarks
1	40.3 (11%)	Not crystallized		349 (89%)	131	Prisms	Collected urines from 3 patients receiving sulfanilamide
2	265.0 (47%)	236-40	Fine curving needles	302 (53%)	150-60	Straight prisms and rosettes	Undulant fever; sulfanilamide; toxic reaction with jaundice
3	88.0 (16%)	Not crystallized		434 (84%)	152	Straight prisms and rosettes	Rheumatoid arthritis; gold therapy
4	175.0 (25%)	235	Rosettes of fine curving needles	534 (75%)	157-61	Straight prisms and rosettes	Hodgkin's disease under x-ray therapy
5	Relative amount only (43%)	242-4	Fine curving needles	Relative amount only (57%)	137-40	Straight prisms and rosettes	Rheumatic fever

*Copro-3 methyl ester exhibits dimorphism in melting point, *i. e.*, 135°C, 144°C, 167-70°C.[6]

[6] Fischer, H., and Orth, *Die Chemie des Pyrrols*, Bd. II, erste Hälfte. Akad. Verlagsgesellsch., Leipzig, 1937.