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A Method for Quantitative Estimation of Pigment in Gallstones.*

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None of the methods known for quantitative estimation of bile pigment has been found satisfactory for gallstone pigment estimations. van den Bergh's method[1] is unsatisfactory, as the unoxidized fractions of pigments are not included; the method of Schmidt and Jones[2] is objectionable for similar reasons; Peterman and Cooley[3] carried oxidation with peroxide to a blue end-point and used a light filter when making the colorimetric reading.

The method offered here uses perchloric acid for oxidation, as the steps are better controlled and the end-products are more stable. A light filter is not necessary with this method. Bile pigments are extracted from weighed amounts (10-20 mg) of dry, well powdered stones, by refluxing with 5 to 8 cc of a mixture of equal parts of chloroform, ethyl alcohol, and glacial acetic acid, until the solvent drops colorless from the tip of the filter cone holding the powdered stone. Previous to this, cholesterol, and calcium and phosphorus are removed by washing first with warm ether and then with hydrochloric acid. Excess temperature and prolonged extraction are avoided to diminish the oxidation as far as possible. The extract is cooled to room temperature and made to a known volume, using the triple mixture. Aliquots (usually 1-2 cc) of this extract, as the intensity of the solution indicates, are measured into small test tubes and made up to a volume of 3 cc with 95% alcohol. At least one cc of the alcohol should be used.

Oxidation to a blue color is accomplished by the addition of 0.8 cc of 72% perchloric acid. Colorimetric comparison is made after 10 minutes against an aliquot from a known strength of bilirubin solution in the triple mixture, which has been oxidized at the same time and in the same manner. A standard from a 3-5 mg %

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1 van den Bergh, Hymans A. A., *Press Med.*, 1921, **29**, 441.

2 Schmidt, C. R., Jones, K. K., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 17.

3 Peterman, E. A., and Cooley, T. B., *J. Lab. and Clin. Med.*, 1934, **19**, 723, 743.

solution of Eastman's bilirubin has been found satisfactory for the depth of readable color.

Bilirubin dissolved in this triple mixture, when exposed to heat, is recovered within the range of experimental error; when previously treated with ether and hydrochloric acid and then exposed to heat, about 70% of the bilirubin is recovered. The loss is chiefly due to the differences in surface tension between the bilirubin and dilute hydrochloric acid. Gallstones do not show this difference, so we feel that little mechanical loss occurs on treating them. Chemical factors may also play a part in the recovery. Figures obtained by this method[4] do not always check within a close range, yet they give a fair idea of the total amount of pigment in stones.

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Anticatalase Activity of Sulfanilamide and Related Compounds. I. Effect of Ultraviolet Irradiation.

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The production of a violet color by irradiation of dilute solutions of sulfanilamide with ultraviolet light has been reported by Ottenberg and Fox.[1] They suggested that this colored substance, possibly produced by oxidative changes in the body, may be responsible for the cyanosis frequently observed in patients under treatment. Whether or not the colored derivative was superior to sulfanilamide in bactericidal power was not determined.

In work to be published[2, 3] it has been suggested, as a possible explanation of the retardation of growth of pneumococci by sulfanilamide, that the bacteriostatic agent involved may be—not sulfanilamide itself—but hydrogen peroxide. The latter substance was presumed to accumulate in the immediate locality of the invading coccus, following oxidation by the coccus of sufficient absorbed sulfanilamide to produce inhibition of catalase as rapidly as the latter principle enters the reaction zone. The oxidation product may be

4 Phemister, D. B., Aronsohn, H. G., and Pepinsky, R., in press.

1 Ottenberg, R., and Fox, C. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 479.

2 Locke, A., Main, E. R., and Mellon, R. R., *J. Immunol.*, in press.

3 Mellon, R. R., *Mod. Hosp.*, 1938, **51**, 53.