the operation. 2. Removal of the posterior lobe, with as little injury to the anterior lobe as possible, is invariably followed by a marked increase in the water intake and water output. This condition is apparently permanent, for it can still be observed 6 months or more after the operation. 3. Ablation of the anterior lobe, leaving only the pars nervosa and intermediate portion intact, including the stalk attachment to the brain, produces a slight decrease in the water intake for about a week after the operation. At the end of this time the water consumption returns to the normal level. 4. Hypophysectomized rats implanted with anterior lobes of the rat hypophysis show no disturbance in water metabolism. The water intake and output of animals so treated, even over a period of 20 days, never rise above those observed in the normal animal.

8482 C

A Method for Determination of Total Pigment in Bile Which Is Applicable to "Biliverdin Biles."

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Methods for quantitation of bile pigments, with the exception of the methods of Hooper and Whipple,[1] Beccari,[2] Kerppola and Leikola,[3] and Peterman and Cooley[6] have been confined to modifications of the van den Bergh method[4] for the determination of bilirubin. That the pigment in the bile of carnivora is present chiefly as bilirubin and that of herbivora as biliverdin[5] is a conception based partly upon physical appearances of the respective bile and partly upon specific tests for these particular constituents. Modifications of the van den Bergh method are satisfactory for clinical identification or quantitation of bilirubin; however, such methods are without value when used for the quantitation of pigment in bile in which the pigment is present in the form of oxidized bilirubin

[1] Hooper and Whipple, *Am. J. Physiol.*, 1916, **40**, 332.

[2] Beccari, *Boll. Soc. Ital. Biol. Sper.*, 1928, **8**, 332.

[3] Kerppola and Leikola, *Skan. Arch. Physiol.*, 1929, **55**, 70.

[4] McNee, *Quart. J. Med.*, 1923, **16**, 390.

[5] Hawk and Bergeim, Practical Physiological Chemistry, Philadelphia, 1927, 10th edition, p. 329.

derivatives. Furthermore, Müller and Engel[6] have shown spectrophotometrically that the diazo reaction of the van den Bergh test detects only 30 to 60% of the bilirubin present in a solution, while Beccari[7] has reported that a number of compounds other than bilirubin, and present both in bile and serum, gave color reactions which were indistinguishable from that given by bilirubin.

In attempting to use the method of Hooper and Whipple[1] for the determination of total pigment, it was found that the method was uncertain and has objectionable features which have been pointed out by Peterman and Cooley.[8] These investigators have shown that the blue-green color taken as an end-point in the Hooper-Whipple and their own methods is a mixture of unoxidized bilirubin and bilicyanin, the combination of which constitutes the green pigment of bile heretofore considered as an individual pigment, "biliverdin." Thus, in colorimetric determinations, using a blue filter disc, only that portion of bilirubin which has been oxidized to bilicyanin is estimated. In order that the blue-green color be obtained always it is necessary that the amount of oxidizing reagent used and the time required to develop maximum color intensity be varied according to the amount and character of the pigment present. In our hands, the amount of nitric acid suggested by Hooper and Whipple proved sufficient to oxidize the pigment in a number of samples of dogs' bile to a greenish-yellow color within 12 hours instead of 24 hours. Thus, it was necessary to adapt the method to each sample of bile before it could be applied with any degree of reliability. Once adapted to a sample of bile, our experimental error with this method of analysis was from 5 to 10%.[9] Further, we desired to assay the total pigment in bird and rabbit bile, to which the Hooper-Whipple and Peterman-Cooley methods are not applicable.

Attempts to obtain a method applicable to both bilirubin and "biliverdin" bile resulted in the following method. The procedure is based upon the oxidation of the major pigments to yellow pigments which are soluble and relatively stable. Maximum intensity of the orange-yellow coloration is obtained in 16 hours, fading gradually to pale-yellow within 36 hours. The procedure is as follows: One-half or one cc. of bile is pipetted (attention being given to uniform drainage) into a 15 cc. volumetric tube and mixed with 10 cc. of a 1:1 glacial acetic acid-95% alcohol mixture. One cc. of freshly

[6] Müller and Engel, *Klin. Wochnschr.*, 1930, **9**, 2305.

[7] Beccari, *Klin. Wochnschr.*, **5**, 671.

[8] Peterman and Cooley, *J. Lab. and Clin. Med.*, 1933, **19**, 723, 743.

[9] Scribhishaj, Hawkins and Whipple, *Am. J. Physiol.*, 1931, **96**, 449.

prepared 2% *ammonium persulphate* solution is added and the volume made up to 15 cc. with acid-alcohol reagent. After shaking to insure thorough mixing of the contents, the tubes are stoppered and allowed to stand in a constant temperature room (25 to 30°C.) for from 16 to 24 hours when they are compared directly in a colorimeter against a bilirubin standard which has been treated in exactly the same manner. Determinations are made in duplicate. Temperatures higher than 30° are inadvisable since the products of oxidation are unstable with heat, and although the reaction can be greatly hastened by heating such practice is conducive to inaccurate results. Since sun light is known to influence the oxidation of bilirubin in acid solution, the oxidation should be carried out in a dark room. A chloroform solution of Eastman's bilirubin is used as standard, assuming the preparation to be 100% pure. Five cc. of this solution, containing from 1 to 2 mg. bilirubin, are pipetted into a volumetric tube and mixed to volume with the acid-alcohol reagent and one cc. 2% ammonium persulphate. A permanent standard has advantages over the bilirubin standard and may be obtained by matching the oxidized bilirubin standard against the following solution: 36 gm. $FeCl_3$, 40 gm. $CoCl_2$, 5 cc. conc. HCl, and 1 cc. 0.05 M $KMnO_4$. This is made up to 2 liters, allowed to stand one day, and filtered.

The selection of the glacial acetic acid-95% alcohol solvent and ammonium persulphate as oxidizing reagent was the result of an extensive study of the oxidative reactions of pigments in bile with a number of the commonly used oxidizing reagents. From our studies we concluded that, under the chosen conditions, the oxidation of bilirubin occurred uniformly through graded steps of color change. The oxidation products appear more and more stable as each successive stage is reached; thus, in ammonium persulphate we have an oxidizing reagent that may be present in excess, within limits, without the marked acceleration of the oxidative reactions obtained by excess amounts of more labile oxidizing reagents.

We found it convenient to read the permanent standard against the standard bilirubin solution at 16, 18, 20, and 22 hours and in this way established a bilirubin equivalent for each corresponding time interval. Within 8 hours after the addition of the oxidizing reagent to the acid-alcohol solution of bile an orange-red color develops, and at 16 hours the solution is deep orange-yellow. Between 18 and 22 hours the color change is scarcely perceptible but at 26 hours there is an accelerated transition of color from orange-yellow to pale-yellow. Eighteen or 20 hours have been selected as standard

for the time of color comparison with the permanent standard solution. At this time the coloration is in the middle of the plateau for this stage of color change and the values obtained on a sample of bile when read against a bilirubin standard are the same at 18 and 20 hours, indicating that the standard and the sample are being oxidized at the same rate.

An excess of oxidizing reagent, that is 1 cc. of a 2% solution, is specified because it was found to give more uniform comparative results when applied to a wide variety of bile samples. Chicken and pigeon bile, and some samples of dog bile, when treated by this procedure using a 1% solution of oxidizing reagent show the presence of a bluish pigment when the reading in a colorimeter is taken. This pigment, probably bilicyanin, is readily oxidized to orange-yellow when 2% persulphate is employed, yet the oxidizing reaction is not increased to the extent that the oxidation is carried out of the orange-yellow color phase within 22 hours.

Although a white precipitate forms, there is no apparent adsorption of pigment and the precipitate is filtered off preparatory to colorimeter readings.

In order to test the accuracy of the method, the recovery of added bilirubin to dog bile was investigated with the typical results shown in Table I. The bilirubin was added in chloroform solution. Control determinations on samples of bile to which pigment-free chloroform was added showed that this solvent has no effect on the oxidation or the colorimetric readings.

The method proved accurate to (±)0.02 mg. pigment, thus reducing the experimental error to less than 2%.

Total pigment in bile, as determined by the above procedure, was compared with the bilirubin content as shown by the van den Bergh

TABLE I.

Bile Sample	cc. Bile	Mg. Added Bilirubin	Mg. Pigment Determined	Mg. Pigment Calculated	Mg. Error	% Error
A	0.5	—	1.240	—	—	—
	0.5	0.104	1.363	1.344	0.019	+1.33
	0.5	0.208	1.430	1.448	0.018	—1.25
	0.5	0.416	1.640	1.656	0.016	—0.91
B	0.5	—	1.000	—	—	—
	0.5	0.104	1.110	1.104	0.006	+0.54
	0.5	0.208	1.190	1.208	0.018	—1.50
	0.5	0.416	1.430	1.416	0.014	+0.98
C	1.0	—	2.000	—	—	—
	1.0	0.080	2.060	2.080	0.020	—0.97
	1.0	0.160	2.144	2.160	0.016	—0.79
	1.0	0.240	2.272	2.240	0.032	+1.42

TABLE II.

Day	Mg. Total Pigment	Mg. Bilirubin van den Bergh	% Bilirubin van den Bergh
Dog X			
1	178.4	77.4	43.3
3	169.9	94.3	55.5
4	299.0	162.1	54.3
5	101.0	43.9	43.2
Dog XV			
1	594.0	284.0	47.8
2	362.8	184.0	50.7
3	155.5	85.0	54.6
4	276.5	145.0	52.4

The above values are in terms of milligrams of pigment per 100 cc. of bile.

method with results in Table II. The bile for these comparative tests was obtained daily from 2 total bile fistula dogs. It is interesting to note that the van den Bergh values when compared with total pigment figures agree favorably with percentages reported by Müller and Engel.[6]

The method has been used rather extensively during the past 18 months for assaying pigment in the bile of birds and mammals.

8483 P

Influence of Water Administration upon Oxygen Consumption Rate in Shock.

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The production of water intoxication in human beings is difficult. However, instances of such intoxication have been reported,[1-4] and it has been the subject of experimental investigation in lower animals.[5, 6, 7] Recent studies[8, 9] upon the metabolic effects of water

[1] Larson, E. E., Rowntree, L. G., and Weir, J. F., *Arch. Int. Med.*, 1922, **29**, 306.

[2] Miller, J. L., and Williams, J. L., *Am. J. Med. Sc.*, 1921, **161**, 327.

[3] Trousseau, A., Lectures on Clinical Medicine, London, 1867.

[4] Helwig, F. C., Schutz, C. B., and Curry, D. E., *J. Am. Med. Assn.*, 1935, **104**, 1569.

[5] Rowntree, L. G., *J. Pharmacol. and Exp. Therap.*, 1926, **29**, 135.

[6] Misawa, H., *Jap. J. Med. Sc. Tr. VIII*, 1927, **1**, 355.

[7] Kylin, E., *Z. f. d. ges. exp. Med.*, 1928, **63**, 606.

[8] Davis, H. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 242.

[9] Davis, H. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 245.