The great majority of "late" cases showed a normal titer, irrespective of whether the onset had been acute, subacute or insidious. However, it was occasionally observed that among the "late" cases acute exacerbations were attended by a marked rise in the antistreptolysin titer.

Of particular interest was a third group of cases in which the differentiation between rheumatoid arthritis and rheumatic fever could not be made with certainty. Many of these cases conformed to that type designated as "secondary chronic polyarthritis" in the German literature. Twenty-eight such cases were examined and the titers varied from 10,000 to 25, with a median of 166. As in typical cases, the great majority of early cases in this group showed a markedly increased titer, more particularly in those instances in which the onset occurred in an acute or subacute manner. It is of some interest that the median in this group was higher than that observed in typical cases of rheumatoid arthritis.

Controls. The antistreptolysin titers were determined on 91 controls—24 cases of osteoarthritis, 24 of non-articular rheumatism (neuritis, bursitis, myositis, etc.), 9 of gonococcal arthritis, 2 of gout and 32 miscellaneous sera. The median titer observed in this control group was 62. Titers above 125 were observed only in 5 instances.

Summary. In "early" cases of rheumatoid arthritis the antistreptolysin titer is usually increased. The increase in titer is particularly well marked in those cases in which the onset was acute or subacute.

8509 P

Determination of Cholic Acid in Bile and in Duodenal Drainage.

HENRY DOUBILET.* (Introduced by Louis Gross.)

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The Gregory-Pascoe[1] reaction was found to be specific for the determination of cholic acid. The Reinhold and Wilson[2] modification of this method was combined with the procedure of Harwood[3]

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[1] Gregory, R., and Pascoe, T. A., *J. Biol. Chem.*, 1929, **83**, 35.

[2] Reinhold, J. G., and Wilson, D. W., *J. Biol. Chem.*, 1932, **96**, 637.

[3] Harwood, R. U., *J. Lab. and Clin. Med.*, 1934, **19**, 1003.

in such a fashion as to obviate both the use of the color filter and the necessity of carrying out the reaction in the presence of alcohol. In addition it was found that the large amount of deoxycholic acid present in human bile, although not taking part in the reaction, formed a precipitate which interfered markedly with the colorimetric procedure. This difficulty was overcome by the addition of alcohol after the Gregory-Pascoe reaction had been carried out to completion.

Method. 1-2 cc. of human or canine gall bladder bile, 3-5 cc. of canine fistula bile, 10-30 cc. of human fistula bile or 20-35 cc. of duodenal drainage material are the usual quantities measured out into a 50 cc. centrifuge tube. Three cc. of 2N KOH is then added and thoroughly mixed with the bile. When small quantities of concentrated bile are used, 15-20 cc. of water is added, the presence of alkali preventing the precipitation of protein. Following this, 3 cc. of 40% zinc sulphate is added drop by drop with constant stirring. When the contents are thoroughly mixed, the supernatant fluid is separated by centrifuging and the precipitate washed 3 times with 15 cc. quantities of hot water. The 4 water washings are poured into a 100 cc. volumetric flask. The precipitate is then washed 3 times with 20 cc. portions of 95% alcohol. For the first washing cold alcohol is used, and for the subsequent washings hot alcohol. The alcohol washings are all transferred to a 100 cc. beaker and dried on a water bath. The residue, taken up with 5 cc. 2N K_2CO_3, is combined with the water washings in the 100 cc. flask, which is made up to volume.

One cc. is pipetted from the volumetric flask into a test tube (18 mm. in diameter) and the procedure of Reinhold and Wilson is then followed. One cc. of 0.9% aqueous furfural solution and 6 cc. of 16N H_2SO_4 are added and the tube heated for 8 minutes at 70°C. in a water bath. After cooling for 2 minutes the resultant blue color is compared with that of the standard. In the cases of human bile, 7 cc. of 95% alcohol is added at this point to both the unknown and to the standard and thoroughly mixed before reading. The 2 standards, which are always prepared with the reading of each batch of unknown solutions, contain 0.2 and 0.4 mg. of cholic acid per cc. The standard solutions are made up as follows: 200 mg. cholic acid (Riedel-De Haen cholic acid dried at 110°C. for 24 hours to drive off the combined alcohol, present in this preparation) is weighed into a 50 cc. beaker and 4.7 cc. N/10 NaOH added. The beaker is warmed gently on a water bath to dissolve the cholic acid and then transferred to a 100 cc. volumetric flask,

which is made up to volume. To make up the standard containing 0.2 mg. per cc., 10 cc. of this solution is made up to 100 cc. For the standard containing 0.4 mg. per cc., 20 cc. of the solution is made up to 100 cc.

As a rule no more than 4 unknowns are prepared and read at one time, since the color tends to fade rapidly.

If the color of the unknown solution does not match in the colorimeter within 5 mm. of reading of one of the standards, measured portions are removed from the 100 cc. flask and suitably diluted or concentrated. Since different samples of bile vary in their content of cholic acid, this procedure gives a high degree of flexibility to the method.

Cholic acid added to bile of known bile acid composition could be recovered to within 5% of its theoretical value.

8510 P

Differential Quantitative Analysis of Bile Acids in Bile and in Duodenal Drainage.

HENRY DOUBILET.* (Introduced by Louis Gross.)

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By the combination of 3 different methods the analysis of bile for taurocholic acid, glycocholic acid, total conjugated bile acids, cholic acid, deoxycholic acid, total bile acids, and free bile acids has been achieved. It has also been possible to analyze duodenal drainage material for cholic acid, deoxycholic acid and total bile acids.

Methods. 1. The Cuny[1] modification of the Schmidt-Dart[2] method is used to determine the taurine and glycine nitrogen percentage. The first is multiplied by 33.87 and the second by 28.29 to give the bile acids conjugated with taurine and with glycine. The sum of these 2 figures gives the total bile acids that are conjugated. 2. Cholic acid is determined by a method outlined in the previous paper.[3] 3. The total bile acids are estimated by an iron precipitation method based partly on principles devised by Harwood.[4] After

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1 Cuny, L., "Le dosage des sels biliares dans la bile et le liquide duodenal," Paris, 1930.

2 Schmidt, C. L. A., and Dart, A. E., *J. Biol. Chem.*, 1920, **45**, 415.

3 Doubilet, H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 84.

4 Harwood, R. U., *J. Lab. and Clin. Med.*, 1934, **19**, 1003.