conjugated with taurine [2,3]. Only 1—2 % of the labelled cholic acid is present as glycin conjugate and none was found free. Chromatographic separation of the labelled bile acid products excreted in feces of the rats treated with antibiotics [1] showed that the major part is excreted as taurocholic acid (70—80 %), 5—10 % being presumably excreted as glycocholic acid. About 10 % of the labelled compound was, however, found at the place where free cholic acid occurred. None of the bile acid metabolites found in normal rat feces were observed after administration of antibiotics. This indicates that most of the peptide bonds of the conjugated bile acids are split by the intestinal microorganisms and these also cause further modifications of the bile acid molecules.

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Quantitative Determination of Bile Acids on Paper Chromatograms

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Bile acids like other steroids give characteristic ultraviolet spectra when dissolved in strong sulfuric acid [1]. Kier [2] has developed a method for the simultaneous determination of cholic acid and desoxycholic acid using 65 % sulfuric acid. This method has been improved by Mosbach *et al.*[3]

We have studied the absorbtion spectra of free and conjugated bile acids in 96 % and 65 % sulfuric acid in order to develop a method for the determination of individual bile acids after separation by paper chromatography. It was found that tauro- and glycodesoxycholic acid did not behave like free desoxycholic acid when heated in 65 % sulfuric acid. To get a main absorbtion maximum at 390 mμ the conjugated desoxycholic acid has to be heated 60 minutes at 60° whereas free desoxycholic acid gets a maximum at 385 mμ after 15 minutes at 60°.

Cholic, taurocholic and glycocholic acid had absorbtion maxima at 320 mμ after heating for 15 minutes at 60° in 65 % sulfuric acid. On further heating a second maximum appeared at 389 mμ but after about 150 minutes at 60° the maxima decreased.

In 96 % sulfuric acid cholic acid has its main absorbtion maximum at 389 mμ whereas conjugated and free desoxycholic acid and chenodesoxycholic acid have their maxima at 310 mμ.

We have used these methods for the quantitative determination of bile acids after paper chromatography. The acids were run on 1.5 cm wide strips of Whatman 3 MM filter paper. The strips had to be washed with ethanol and 10 % acetic acid before use. After chromatography according to Sjövall [4] the spots were located by spraying with phosphomolybdic acid in ethanol. The spots on the other strips including one blank were cut out and eluted with ethanol into small test tubes. The ethanol was evaporated at 100° C and the acids were determined by using the reactions in 65 % sulfuric acid. Nine determinations of mixtures of cholic acid and desoxycholic acid (0.02 mg of each acid) gave a standard deviation of about ± 6 % for each of the acids.

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A Paper-chromatographic Method for the Estimation of Peptidase Activity

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The activity of proteolytic enzymes is usually determined by titrimetric methods. The well known Linderstrøm-Lang and Holter method, for instance, is based upon the acidimetric titration, in acetone, of the amino groups liberated from the peptides after the enzymatic cleavage. By this method it is possible to determine the reaction velocity of an enzymatic cleavage of, for instance, D,L-leucylglycylglycine (LGG), but nothing can be said about the order of splitting of the peptide linkages. As we were interested to know if, by certain peptidase preparations, leucine or glycine was initially liberated, we have used a semi-quantitative paper chromatographic method.