

conjugated with taurine^{2,3}. Only 1—2 % of the labelled cholic acid is present as glycine conjugate and none was found free. Chromatographic separation of the labelled bile acid products excreted in feces of the rats treated with antibiotics¹ 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.

1. Bergström, S. and Norman, A. *Proc. Soc. Exptl. Biol. Med.* **83** (1953) 71.
2. Bergström, S., Rottenberg, M. and Sjövall, J. *Hoppe-Seylers Z. physiol. Chem.* **295** (1953) 278.
3. Norman, A. *Acta Physiol. Scand. In press.*

Quantitative Determination of Bile Acids on Paper Chromatograms

Sten Eriksson and Jan Sjövall

*Department of Physiological Chemistry,
University of Lund, Lund, Sweden*

Bile acids like other steroids give characteristic ultraviolet spectra when dissolved in strong sulfuric acid¹. Kier² 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 absorption 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 absorption maximum at 390 m μ the conjugated desoxycholic acid has to be heated 60 minutes at 60° whereas free desoxycholic acid gets a maximum at 385 m μ after 15 minutes at 60°.

Cholic, taurocholic and glycocholic acid had absorption maxima at 320 m μ after heating for 15 minutes at 60° in 65 % sulfuric acid.

On further heating a second maximum appeared at 389 m μ but after about 150 minutes at 60° the maxima decreased.

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

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 Sjövall⁴ 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 \pm 6 % for each of the acids.

1. Bernstein, S. and Lenhard, R. H. *J. Org. Chem.* **18** (1953) 1146.
2. Kier, L. C. *J. Lab. Clin. Med.* **40** (1952) 755.
3. Mosbach, E. H., Kalinsky, H. J., Halpern, E. and Kendall, F. E. *Personal communication.*
4. Sjövall, J. *Acta Chem. Scand.* **8** (1954) 339.

A Paper-chromatographic Method for the Estimation of Peptidase Activity

Torsten Ekstrand

Centrallaboratoriet, Astra, Södertälje, Sweden

The activity of proteolytic enzymes is usually determined by titrimetric methods. The well known Linderström-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.

An amino acid-free peptidase preparation from a bacillus, closely related to but not identical with, *Bacillus brevis*, was found to split glycylglycine (GG), LGG and a number of other di- and tripeptides. If GG was incubated at pH 7.4 with this enzyme preparation the reaction could be followed titrimetrically. Tests from the incubation mixture, as well as reference tests of glycine and GG were simultaneously placed on a Whatman No. 1 paper and chromatographed with butanol — acetic acid. It was found that the colour strength of the spots, after developing with ninhydrin, run parallel with the titration values, but that the glycine spot could be detected on the paper on a very early stage of the incubation when the titration values had not altered perceptibly. It is also easily possible to follow the reaction at very short intervals, as the incubation can be made in a capillary pipette, from which drops can be placed on the paper with an interval, if desired, of only a few seconds.

If, in the manner outlined here, LGG was incubated with the same enzyme preparation, only leucine and GG could be detected on the paper but no glycine. Apparently the liberated leucine inactivated the GG-splitting capacity of the enzyme preparation. This was confirmed by incubating GG together with varying amounts of leucine, when it was found that also very small quantities of leucine reduced the velocity of the splitting of GG.

The same enzyme preparation as well as guinea pig liver homogenate has been studied by this method also with other di- and tripeptides. In every case it was found that the ninhydrin spots of the split products could be detected before the titration values had altered and that the colour strength of the spots was proportional to the titration values.

The Conversion of [1-¹⁴C] Cetyl Alcohol into Palmitic Acid in the Intestinal Mucosa of the Rat

Rolf Blomstrand and
Jürg A. Rumpf

Department of Physiological Chemistry,
University of Lund, Lund, Sweden

The mechanisms involved in the intestinal absorption of the higher aliphatic alcohols are still very little known.

Stetten and Schoenheimer¹ feeding deuterium labeled cetyl acetate to rats found that

the cetyl alcohol was well absorbed, and they were also able to isolate deuterated fatty acids from the carcass.

In order to investigate whether cetyl alcohol is converted into palmitic acid already during the passage through the intestinal mucosa, 0.5 ml of a 5 % solution of [1-¹⁴C] cetyl alcohol in olive oil has been administered to rats with a thoracic duct fistula.

The amount of isotope in the fecal lipids was determined and the amount of isotope absorbed was calculated. The lymph fat was subjected to chromatography on silicic acid according to Borgström². A method was worked out for separating free cetyl alcohol from neutral fat.

From 63 to 96 % of the fed activity was absorbed. These results confirm earlier investigations that cetyl alcohol is well absorbed in the rat^{1,3,4}.

In four experiments from 31 to 64 % of the absorbed activity was recovered in the lymph lipids. The major portion of the activity in the lymph lipids was recovered in the neutral fat. After saponification and recrystallisation with inactive palmitic acid it was found that all the activity in the neutral fat fatty acids could be accounted for as palmitic acid. The oxidation of the cetyl alcohol must largely have taken place during its passage through the intestinal mucosa.

About 15 % of the absorbed cetyl alcohol passed unchanged through the intestinal mucosa and could be isolated as free cetyl alcohol in the lymph lipids. The remainder of the activity in the lymph lipids was present in the phospholipid fatty acids. The proportions of the activity between neutral fat and phospholipids were those characteristic for palmitic acid.

From the results of this investigation it is thus apparent that there is an intense metabolic activity in the intestinal mucosal cells. During the absorption of cetyl alcohol most of this compound is oxidized to palmitic acid that is subsequently incorporated into glycerides, phospholipids and cholesterol ester.

A full report of this work will be published in *Acta Physiologica Scandinavica*.

1. Stetten, D. Jr. and Schoenheimer, R. J. *Biol. Chem.* **133** (1940) 347.
2. Borgström, B. *Acta Physiol. Scand.* **25** (1952) 101.
3. Carter, C. L. and Malcolm, I. *Biochem. J. (London)* **21** (1927) 484.
4. Channon, H. J. and Collinson, G. A. *Biochem. J. (London)* **22** (1928) 391.