# The Incorporation of Methyl and Carboxyl Labelled Acetate into the Purine-Bound Sugars of Nucleic Acids in Liver Slices

S. Laland and I. Smith-Kielland

*Biochemistry Department, The University, Oslo*

The incorporation of carbon-labelled acetate into ribose has been the subject of several investigations. Thus it was shown that in chicks carboxyl labelled acetate was incorporated into the purine-bound ribose of RNA [1]. Löw [2] using rats found that when given $^{13}CH_3$ $^{14}COOH$ both isotopes were present in the ribose of RNA and that the methyl carbon was incorporated to a considerable greater extent than the carboxyl. However, studies on the incorporation of labelled acetate into the purine-bound 2-deoxy-D-ribose has not been reported. The present study concerns this problem together with an investigation of the simultaneous incorporation of isotope into the purine-bound ribose. Liver slices obtained from regenerating rat liver were incubated in Krebs-Hensleit medium at pH.7.4 containing glucose together with labelled sodium acetate for 3 hours at 37° C. The nucleic acids were isolated from the liver slices by the method of Brown *et al.*[3] The purine-bound 2-deoxy-D-ribose was isolated from the DNA by a slight modification of a method described previously[4]. The sugar was finally purified by paper chromatography, eluted, estimated and counted. D-Ribose was isolated from the RNA after acid hydrolysis, followed by passage of the hydrolysate through a column of mixed resins (IR 120(H) and IR4B(OH)) and finally paper chromatography. The sugar was eluted from the chromatogram, estimated and counted. In both cases results obtained from three different chromatographic solvent systems were found to agree satisfactorily and the figures quoted in the table below represent the mean of the three figures.

| Acetate incubated | 2-Deoxy-D-ribose c/min/μmole | D-Ribose c/min/μmole |
|---|---|---|
| $^{14}CH_3COONa$ | 147 | 790 |
| $CH_3{}^{14}COONa$ | 292 | 300 |

It thus appears that in regenerating liver slices both the methyl and carboxyl carbon of acetate can be incorporated into the purine-bound 2-deoxy-D-ribose. Experiments performed with liver slices from non regenerating liver showed as expected no incorporation of carboxyl labelled acetate. Furthermore it is seen that in the case of the methyl labelled acetate the incorporation of isotope is 5 times as large in the case of the purine-bound D-ribose, as for the purine-bound 2-deoxy-D-ribose. With carboxyl-labelled acetate there is however the same degree of incorporation of isotope.

1. Bernstein, I. A. *J. Biol. Chem.* **205** (1953) 317.
2. Löw, B. *Acta Chem. Scand.* **6** (1952) 304.
3. Bendich, Aa., Russel, P. J. and Brown, G. B. *J. Biol. Chem.* **203** (1953) 306.
4. Jones, A. S. and Laland, S. G. *Acta Chem. Scand.* **8** (1954) 603.

# Studies on the Regulation of Bile Acid Formation in Bile Fistula Rats

S. Bergström, H. Danielsson and U. Gloor

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

When a bile fistula is made in a rat the excretion of bile acids increases 20—40 fold over the normal daily synthesis to 40—60 mg per day!

In order to study the influence of the bile acids in the enterohepatic circulation on the bile acid production in the liver, we have used two experimental approaches.

We have prepared fistula rats as normal but also inserted a cannula into the lower part of the severed bile duct and continuously injected 0.5 ml of saline per hour containing the synthetic bile salt investigated.

In order to follow the taurocholic acid production we have injected sodium-taurochenodesoxycholate. It is known that this substance does not give rise to cholic acid but to relatively small amounts of higher hydroxyla-

ted compounds, that can be differentiated from cholic acid by partition chromatography [2,3]. The amount of cholic acid in the hydrolysed bile was then calculated from the titration values of the cholic acid band.

It was found, that the injection of 2.5 mg of sodium-taurochenodesoxycholate per hour scarcely influenced the cholic acid production, 5 mg per hour reduced the daily production to about 4 mg, whereas 10 mg per hour caused a reduction down to the normal daily synthesis of 1—2 mg.

If the amount of bile salts in a 200 g rat is about 15 mg the bile acids have to circulate 15—20 times per day in the enterohepatic circulation to maintain a concentration in the portal blood similar to that in the last mentioned experiment.

The daily formation of 40—60 mg of bile acids corresponds to about three times the amount cholesterol present in the liver. We have therefore injected $^{14}C$-acetate intraperitoneally and followed the appearance of $^{14}C$ in the secreted bile.

In a typical experiment about 1 mg of sodium acetate ($1.9 \times 10^6$ c.p.m.) in 1.5 ml of saline was injected intraperitoneally into fistula rats at the time indicated after the operation. The percentage of the administered isotope that was recovered in the bile during the following 6 hours is shown in the second column below.

| Hours after operation | % injected isotope recovered in bile |
|---|---|
| 16 | 0.13 |
| 64 | 0.61 |
| 112 | 1.41 |

According to paper chromatography about 80 % of the isotope was contained in the bile salts.

The correlation of data of this type with the bile acid formation will be exemplified and discussed. The method has been found useful for studying the regulation of the synthesis of bile acids under different experimental conditions.

1. Eriksson, S. *Acta Chem. Scand. In press.*
2. Bergström, S., Rottenberg, M. and Sjövall, I. *Hoppe Seylers Z. physiol. Chem.* **295** (1953) 278.
3. Norman, A. *Acta Chem. Scand.* **7** (1953) 1413.

## Increase of Cholinesterase Content in Mouse Liver Homogenates after Incubation

Karl Håkon Skramstad

*The Norwegian Defence Research Establishment, Toxicological group, Kjeller, Norway*

The rate of hydrolysis of acetylcholine by mouse liver homogenates increases by about 100 % when the homogenates are left either overnight at room temperature (20° C) or two to three hours at 37° C.

Of the organs investigated (brain, kidney, plasma, erythrocytes and liver) only the liver presented a real "autoactivation". This increase in activity takes place in liver homogenates from mice of different ages and both sexes, but seems to be more progressive in the female. There seems to be a greater "activation" in liver homogenates from infantile mice than mature ones, although there are large fluctuations.

The "activation" does not disappear with dialysis and seems to be localized to the particles of the liver cell upon "high speed" centrifuging. Calcium ions have no influence, neither is the increase in activity dependent on the isotonicity of the medium.

Kinetic experiments with a selective inhibitor of "pseudo"-cholinesterase [1], Nu 683, suggest that one and the same enzyme, *viz.* a "pseudo"-cholinesterase, is responsible for the major part of the activity with acetylcholine as substrate, both prior to and after the "activation".

The increase of hydrolysis takes place with acetylcholine and butyrylcholine as substrates, while deactivation results when tributyrin and benzoylcholine are employed. With acetyl-$\beta$-methyl-choline the rate of hydrolysis is quite slow, but has a slight tendency to increase with time; too small, however, to be of any experimental significance. These experiments indicate that we are dealing with a butyrocholinesterase [2].

Benzoylcholine, often used as a specific substrate for "pseudo"-cholinesterase [3], is not hydrolysed at an increased rate after incubation. On the contrary, the hydrolysis decreases with time. Thus there might perhaps be two "pseudo"-cholinesterases in the mouse liver; one butyrocholinesterase and one "benzoylcholinesterase". As known, "benzoylcholinesterases" are occasionally found in the plasma of rabbits [4] and in the liver of guinea pigs [5,6].