Distribution of the 7α-Hydroxylating Activity in Rat Liver Homogenate Bile Acids and Steroids 35

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The formation of cholic acid from desoxycholic acid by 7α-hydroxylation has been observed in the rat *in vivo* as well as in liver slices and homogenates [1-4]. This reaction was further studied with fractionated homogenates [4]. It was found, that a considerable part of the hydroxylating capacity was present in the particle free supernatant (centrifugation for 60 min, 100 000 × g).

The conclusions reached in this publication and the statement referring to this in a review article [5] have, however, to be corrected and modified in certain respects. When taurodesoxycholic acid is incubated with particle-free supernatant we have found that the yield of more polar products with the approximate mobility of taurocholic acid varies with the ATP * preparation used. Without added ATP no reaction products are observed (Table 1).

When Papst crystalline ATP (Pabst Lab. Milwaukee 3, Wisc.) is added, the yield is low but the product is practically pure taurocholic acid. With impure preparations of ATP (ATP-Ba-salt, Schwarz Lab. Inc., Mount Vernon, N. Y. Lot Nr ATB 5301; crude Barium salt from muscle) a large increase in the formation of polar compounds with approximately the same mobility of the filter paper in the system used (Butanol / 3 % acetic acid [6]) is found; see Table 1. With a slight modification in the conditions, the taurocholic acid can be clearly distinguished from the other metabolite.

As our chemical identifications accidently were done on runs with pure ATP yielding taurocholic acid we overlooked the presence of other products formed in the presence of impure ATP preparations.

The curve in Fig. 1 in paper [4] as well as Tables 1 and 3 thus represent taurocholic acid + unknown metabolites as these experiments were done with Schwarz ATP.

However, in the presence of microsomes (= particles spun down between 15 min, 20 000 × g and 60 min, 100 000 × g) plus supernatant, the product without or with pure ATP yielded essentially pure taurocholic acid, whereas again impure ATP led to an additional formation of the other metabolites.

Mitochondria or microsomes alone or with added ATP do not yield any taurocholic acid nor any other metabolite from taurodesoxycholic acid.

It therefore is clear that the microsomes and the particle free supernatant in com-

* Abbrevation: ATP = Adenosine triphosphate.

Table 1. Conversion of 150 μg taurodesoxycholic acid-24-^{14}C in rat liver homogenate fractions. 1ml incubation medium [4] corresponding to 200 mg liver fresh weight. Incubation 2 hrs, 37°, pH = 7.4, air. Amounts in μg.

	Microsomes + supernatant (centrifuged homogenate, upper layer after 15 min, 20 000 × g)			Supernatant (upper layer 60 min, 100 000 × g)		
	without ATP	with pure ATP	with impure ATP	without ATP	with pure ATP	with impure ATP
Taurocholic acid formed	68	97	97	<1	15	3
Unknown, more polar subst. formed	<1	7	43	<1	2	78

bination are responsible for the main part of the 7α-hydroxylating capacity. Only a minor part is found in the supernatant alone in the presence of pure ATP.

Impure ATP preparations contain a factor causing the formation of relatively large amounts of other polar products from taurodesoxycholic acid *in vitro*. In *in vivo* experiment with bile fistula rats practically the only product formed from desoxycholic acid is cholic acid.

Further details will be published shortly.

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Infrared Absorption Spectra of some Salts of DL-2-Phosphoglyceric Acid

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In a previous study [1], the strength of interaction between D,L-2-phosphoglyceric acid (PGA), the substrate of the enzyme enolase, and metal ions activating this enzyme was determined. Analogously to the concept of metal-ion activation of peptidases developed by Smith and his associates [2], it was suggested that the metal ion furnishes one of the points of interaction between enzyme and substrate. Thus, knowledge of the nature of the complexes between PGA and activating ions is important in interpreting the mechanism of the enzymic reaction. Studies by Rosenberg [3] have shown that the formation of metal chelates of amino acids results in characteristic changes in infrared absorption which can be correlated with the type of bond involved. In attempts to obtain similiar information about some metal-ion complexes of PGA, their infrared absorption spectra have been recorded.

PGA was synthesized according to Kiessling [4] and purified as described by Warburg and Christian [5]. To form a metal complex, a 0.02 M solution of the neutral Na^+ salt of PGA, containing an equimolar amount of metal chloride, was allowed to stand for about 1 h at room temperature. Then, absolute ethanol was added until a turbidity formed. (With the pure Na^+ salt, 64 vol. % ethanol was required, while the Mn^{++}, Zn^{++} and Ni^{++} complexes precipitated at concentrations of 13, 11 and 17 %, respectively.) After the addition of ethanol, the sample was placed in a refrigerator (4°) for 12 h. The precipitate formed was centrifuged off and dried in a vacuum dessicator over P_2O_5. Analysis showed the metal and acid to be present in a 1:1 ratio, as in solution [1]. A Perkin-Elmer model 21 recording spectrophotometer equipped with a NaCl prism was used for the absorption measurements. The substances were examined as pressed KBr discs prepared according to Schiedt and Reinwein [6].

Some typical results are shown in Figs. 1–3, which give the spectra of the Na^+, Zn^{++} and Ni^{++} salts of PGA. The absorption spectrum of each substance was recorded twice; the second time the sample was cooled to −170° by the use of a cell described by Rosenberg [3]. As seen in the figures, the use of the low temperature resulted in considerably better resolution as compared to room temperature.

The infrared absorption spectra of PGA and its salts are dominated by bands arising from the absorption by two highly polar groups, namely carboxyl and phosphate. The carboxyl group is characterized by its antisymmetrical vibration (ν|C(=O)O). It has been established by numerous investigations summarized by Bellamy [7] that this vibration of the ionized carboxyl group has a frequency of 1 600–1 590 cm^{-1} (about 6.3 μ). The Na^+ salt of PGA can be regarded as a normal salt, and it exhibits, as expected, a broad and intense band at approximately 1 590 cm^{-1}. If the carboxyl group were to form a bond of more covalent character with Zn^{++} and Ni^{++}, a shift of this band to higher wave num-