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THE INFLUENCE OF CONJUGATION OF CHOLIC ACID ON ITS UPTAKE AND SECRETION: HEPATIC EXTRACTION OF TAUROCHOLATE AND CHOLATE IN THE DOG

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SUMMARY

1. Sodium taurocholate or cholate was administered systemically at a constant rate of about 2·9 μmole/min.kg body wt. to anaesthetized dogs in which the common bile duct had been cannulated. In steady-state conditions blood was sampled from systemic and hepatic veins and the fraction of bile salt removed in a single passage through the liver was determined. Total hepatic blood flow was estimated by application of the Fick principle.

2. The hepatic extraction fraction for synthetic taurocholate in ten experiments was 92 % ± 5 % (S.D.) over the blood flow range encountered (1·1–2·8 ml./min.g liver). The extraction of cholate extensively conjugated in the liver before excretion into bile was 79 % ± 8 % (S.D.) (twenty-one observations, thirteen experiments). In circumstances of similar hepatic blood flow the extraction of cholate transferred to bile in the free form (after acute taurine depletion) was significantly less than that of either synthetic taurocholate or cholate which could be actively conjugated before excretion. These results, which are discussed and criticized, support previous work on the advantage of conjugation in the transfer of cholic acid from blood to bile.

3. The hepatic clearance of bile salt decreases with increasing administration rate, but the values obtained may be influenced by changes in hepatic blood flow. With regard to taurocholate an increase in total hepatic flow was observed when its administration rate exceeded about 5 μmole/min.kg body wt.

4. The secretory maximum for glycocholate, a bile salt not normally found in dog bile, was of the same order as that for taurocholate.

INTRODUCTION

In the early phase of a constant intravenous infusion of cholic acid in the anaesthetized dog in which the common bile duct is cannulated most of the administered cholate is first conjugated with taurine before excretion into bile. The small fraction of cholate which escapes conjugation with taurine appears in bile in the free (or unchanged) form. However, after a few hours of such cholic acid infusion a state of acute taurine depletion is produced during which most of the administered cholate appears free in bile; this condition can be reversed by the administration of taurine. In a previous study (O'Máille, Richards & Short, 1965*a*), it was found that conjugation of cholic acid with taurine afforded certain advantages in the transfer of this substance from blood to bile. This was inferred from the findings that (*a*) the maximal excretory rate of synthetic taurocholate, or of cholate actively conjugated in the liver before excretion into bile, was much greater than that of cholate secreted in the free form; and (*b*) the hepatic clearance of synthetic taurocholate or of actively conjugated cholate was greater than that of cholate transferred from blood to bile without change. The present experiments were carried out to extend our information on the influence of conjugation on cholic acid transport by determining the hepatic extraction (the fraction of a test substance removed from the blood in a single passage through the liver) of (*a*) synthetic taurocholate, (*b*) cholic acid actively conjugated with taurine in the liver before excretion, and (*c*) cholic acid transferred to bile in the unconjugated form. These studies also give us an estimate of the total liver blood flow and this factor is discussed in relation to changes in hepatic extraction and clearance of bile salt. A brief report of some of these findings has already been published (O'Máille, Richards & Short, 1965 *b*).

METHODS

Adult mongrel dogs which had been fasting for 16 hr were anaesthetized with pentobarbitone (Nembutal, Abbot Laboratories). Some animals were premedicated with diethylthiambutene (Themalon, Burroughs Wellcome and Co.). The abdomen was opened by a median incision, the common bile duct cannulated and the cystic duct ligated. In some experiments a catheter was inserted into a radicle of the splenic vein for the purpose of infusions into the portal system. When urine was to be collected both ureters were catheterized intra-abdominally. Hepatic venous blood was obtained from a polythene catheter (i.d. 1 mm) directly inserted through the wall of the left common hepatic vein (see Shoemaker, Walker, van Itallie & Moore, 1959). The vein was first punctured with a No. 14 needle (which was bent at a convenient angle near its tip), the catheter passed through and the needle withdrawn. A small clip was placed on the catheter at its entrance through the vein wall to prevent it slipping into the vein more then the desired distance (1–2 cm). In later experiments 'angiocaths' (Desert Angiocath, 16 ga., 0·044 in. (0·112 cm) i.d., 2¼ in. (5·7 cm), Bard-Davol Ltd.) were found to be more convenient. A 'stop' in the form of a

tight rubber ring was placed about 1 cm from the end of the catheter which entered the vein. It was found unnecessary to take any measures to prevent either catheter from slipping out of the vein. In twenty dogs (9·5–25·2 kg body wt., mean wt. 14 kg) the distance between the puncture site in the left common hepatic vein and the inferior vena cava was 3–6 cm, mean 4·2 cm. To test if the catheter were sampling caval as well as hepatic venous blood a rapid injection of bromsulphthalein was given into the femoral vein at the end of each experiment and blood withdrawn through the hepatic venous catheter over the succeeding 12 sec. These blood samples were free of bromsulphthalein in almost all instances. The left common hepatic vein drains about 40 % of the total liver mass and blood sampled from it has been found to be representative of hepatic venous blood in general (Shoemaker, 1960, 1964).

Bile salt solutions were administered systemically (either the right femoral or one of the external jugular veins was used) and taurine solutions portally by means of constant infusion pumps. Systemic blood samples were withdrawn from the left femoral vein and both these and the hepatic venous samples were transferred to tubes containing 2–3 drops of heparin solution (1000 i.u./ml., Evans Medical) and gently shaken. Bile and urine were collected in graduated tubes. Cholic acid (B.D.H.) and pure preparations of synthetic sodium taurocholate and glycocholate (L. Light and Co.) were used. The ways in which solutions of these substances and of taurine were made up have been described in previous papers (O'Máille *et al.* 1965*a*; O'Máille, Richards & Short, 1966).

Analyses

The methods used for the determination of bile salts in bile and blood have been described previously (O'Máille *et al.* 1965*a*). The following modifications were adopted:

Bile. Following chromatography the taurocholate spots were eluted with distilled water instead of phosphate buffer.

Plasma. Analyses were according to the modification of the original method described at the end of the 'Methods' section of O'Máille *et al.* (1965*a*). In the later experiments 1 extra ml. of acetic acid was added to both test and blank samples in the final stage of the analysis to provide sufficient fluid to fill the 2 cm cuvettes of an E.E.L. 'Spectra' photometer. Each sample was read against its own blank at 600 mμ. Control blood samples were taken before bile salt infusion and the readings given by these (which were largely due to non-bile acid chromogen) were subtracted from the remainder of the experimental readings; the net readings were used in the calculation of the plasma cholate concentration due to bile salt administration. In this series of experiments standard plasma samples designed to cover the expected range of bile salt concentrations were prepared on every occasion, usually from the experimental animals' own plasma. The method gives the plasma concentration of total cholate (i.e. conjugated plus free cholate).

Uptake of bile salt by red cells

Plasma, rather than whole blood, was used for the above analyses on the assumption that bile salt is effectively confined to this fraction of the blood. To find out if the red cells remove any bile salt, *in vitro* tests were carried out in which a measured quantity of taurocholate or cholate was added to (*a*) whole blood, and (*b*) a volume of plasma equal to the plasma content of the whole blood sample as determined from the haematocrit value. The whole blood samples, which were left standing (with occasional agitation) at room temperature for periods up to 280 min, were centrifuged and the plasma was analysed according to the above method. The recovery of taurocholate from whole blood was only slightly less than that from plasma so that for practical purposes this bile salt is confined to plasma. However, in the blood concentration range 0·10–0·16 mM, with respect to sodium cholate, 22–26 % of the total blood cholate content was taken up by the red cells (haematocrit range of samples, 49–54 %). No correction for this uptake of cholate by the red cells was made in these experiments for the following reasons. The plasma cholate concentration of samples used

for analysis in the *in vivo* experiments was in equilibrium with the red cells. On the reasonable assumption that the distribution of cholate between cells and plasma is similar throughout the range of concentrations obtained the same correction factor would be applied to systemic and hepatic venous samples so that the extraction fraction as calculated below would not be affected. Not applying a correction factor leads to over-estimation of the hepatic blood flows and clearances determined during cholate infusion, but the relative values obtained in these experiments should not be altered.

Blood transfusion

In most instances the blood removed for standards and samples from the experimental animal was replaced with fresh blood, which was obtained as required from an anaesthetized donor dog kept in the laboratory during the experiment.

RESULTS

General design of experiments

In all experiments bile salt was administered systemically at constant rate, and blood samples were taken when over-all steady-state conditions were attained (i.e. when the output rate of bile salt in bile equalled the infusion rate). In these circumstances $E = (F-H)/F$, where E is the hepatic extraction and F and H are the concentrations of bile salt in blood plasma entering and leaving the liver, respectively. The extraction fraction may be expressed as a percentage by multiplying by 100. In steady-state conditions the concentration of bile salt in a peripheral vessel, e.g. the femoral vein is equal to that of blood entering the liver. The total hepatic blood flow (B) was calculated by application of the Fick principle and was given by

$$B = \frac{Q}{F-H} \times \frac{1}{\text{liver wt.}} \times \frac{100}{100-\text{haematocrit}},$$

where Q was the administration rate of bile salt which in turn equalled the excretory rate (less the very low control output of bile salt), since extrahepatic loss or removal of bile salt was negligible at the rates employed in these experiments. If Q is in μmole/min, F and H in mM and the liver weight in g, blood flow has the units ml./min.g liver. In the rest of this paper when the word 'extraction' is used without further qualification the meaning intended is that of 'extraction fraction' as defined above.

Blood sampling procedure. In the earlier cholate-infusion experiments blood was sampled during steady-state conditions once in each phase (before taurine depletion, after taurine depletion and again after the administration of taurine), enough blood being withdrawn for duplicate or triplicate analyses. In later experiments blood samples were taken during each phase at from 15 to 35 min intervals for periods up to 120 min.

Hepatic extraction of cholate during continuous infusion. In the first group of experiments to be described cholic acid was infused throughout at a

constant rate of about 2·9 μmole/min.kg body wt. Extraction and blood flow studies were carried out at three phases of the experiment: (*a*) 60–105 min from the beginning of the infusion when most (> 87 %) of the administered cholate was being conjugated with endogenous taurine before excretion, (*b*) after 300–500 min. when most of the infused cholate was appearing free in bile (following depletion of the taurine 'pool'), and (*c*) when extensive conjugation was restored by taurine infusion, sampling during this phase taking place from 60 to 240 min after the start of the

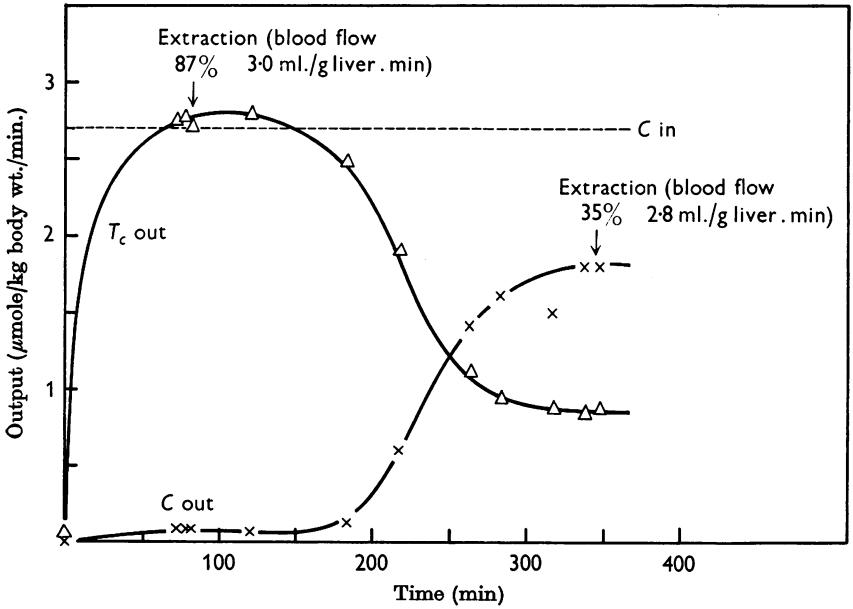

Fig. 1. Sodium cholate was administered systemically throughout the experiment at a constant rate of 2·71 μmole/kg body wt./min. - - - - - - - *C* in; Δ—Δ T_c out, output rate of taurocholate in bile; × — × *C* out, output rate of free cholate in bile. Systemic and hepatic venous samples were removed at the times indicated by the arrows, and the hepatic extraction fractions and blood flows calculated as described in the text. Note that the hepatic extraction of cholate which was extensively conjugated before excretion into bile was much greater than that of cholate transferred to bile largely in the free form, in circumstances of approximately similar hepatic blood flows. (Liver wt. = 3·05 % of body wt.; haematocrit = 51 %.)

taurine administration. As in the previous study (O'Máille *et al.* 1965*a*) taurine was given portally as a priming dose (usually about 0·5 m-mole/kg body wt.) followed by constant infusion at a rate 3–6 times (molar) the corresponding cholate administration rate.

Since hepatic extraction may vary with blood flow rate it is necessary to take this factor into account when assessing any changes in cholate extraction that may occur in the various phases of an experiment. The

simplest circumstances in which to compare cholate extraction during any two phases of an experiment is when blood flow rate is the same in both phases. This was approximately the situation in the experiment illustrated in Fig. 1. The times of blood sampling are indicated by the arrows. Here the cholate extraction during the phase of extensive conjugation was 87 % as against 35 % when most of the infused cholate was being excreted free in bile. Blood flow was approximately the same in both phases so that the hepatic clearance (extraction fraction × blood flow) changed in the ratio of the extractions (clearance first phase 2·6 ml./min.g liver, second phase 1·0 ml./min.g liver). However, this experiment is one of a minority with respect to blood flow constancy. In the larger group the variation most commonly seen was an increase in the blood flow rate during the taurine-depleted phase (when free cholate secretion predominated), which tended to revert to a lower value after restoration of conjugation by taurine administration. We will return to a consideration of these when the next group of experiments has been described.

Comparison of hepatic extraction of synthetic taurocholate and actively conjugated cholate

The extractions of taurocholate and of actively conjugated cholate were compared in the *same* dog in four experiments. In these (one of which is illustrated in Fig. 2) taurocholate was first administered at a constant rate similar to that used for cholate in the above series. Systemic and hepatic venous blood samples were withdrawn when steady-state conditions obtained (60–100 min later). The infusion was then stopped and 130–185 min allowed for the removal of taurocholate from the animal. When this had been achieved a constant infusion of cholate (sometimes with a supplementary portal infusion of taurine) at approximately the same rate was begun and again blood was sampled when steady conditions prevailed. In the instance depicted in Fig. 2 the extraction of taurocholate was 100 %, that of cholate extensively conjugated (11 % of the administration rate was appearing in bile as free cholate) was 71 %. In general, taking all experiments into consideration, the hepatic extraction of synthetic taurocholate, when its administration rate was in the region of 2·9 μmole/min.kg body wt., was 92 % ± 5 % (S.D.) (ten experiments) over the blood flow range encountered, 1·1–2·8 ml./min.g liver. The hepatic extraction of cholate extensively conjugated before excretion (< 13 % of infusion rate appearing free in bile) was 79 % ± 8 % (S.D.) (twenty-one observations, thirteen experiments). The data from these experiments are gathered together in Fig. 3 which is a plot of clearance ($E \times B$) against blood flow (B). The slopes of the lines joining the points in each of these two groups are the respective extraction fractions; in Fig. 3 the lines drawn represent the

mean extractions. The experiments of this group are further discussed below.

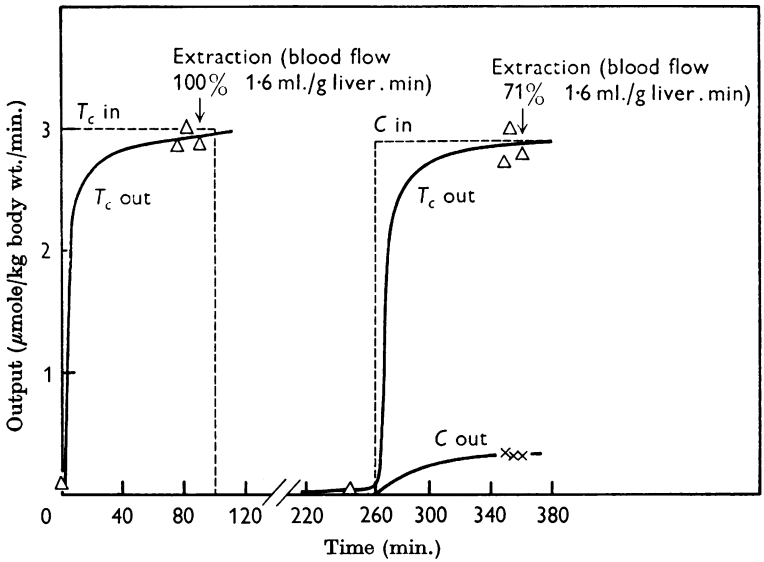

Fig. 2. To show the plan of experiments in which the hepatic extractions of taurocholate and actively conjugated cholate were compared in the same dog. Symbols and notation as in Fig. 1. Taurocholate was first infused at a constant rate of 2·98 μmole/kg body wt./min for 100 min. The infusion was then stopped and 165 min allowed for the removal of taurocholate from the animal. Sodium cholate was then infused systemically at a constant rate of 2·91 μmole/kg body wt./min together with a supplementary intraportal infusion of taurine at 5·9 μmole/kg body wt./min for 115 min. Systemic and hepatic venous blood samples were taken at the times indicated by the arrows. (Liver wt. = 2·92% of body wt.; haematocrit = 46·5%.)

The relation between hepatic extraction, blood flow and clearance

It has already been pointed out that changes in hepatic blood flow from one phase of an experiment to another, which occurred in the majority of cases in the first group described, complicate the use of the extraction fraction as a comparative index of the liver's performance in removing substances from the blood. Brauer, Leong, McElroy & Holloway (1956), using [^{32}P] Cr PO_4 have investigated the relation between total hepatic blood flow rate and extraction in the isolated perfused rat liver, and have shown that in general, as perfusion rate was increased the extraction ratio of radio colloid fell. The hepatic clearance (C) of a substance from the blood reflects the combined effects of changes in extraction and blood flow rate and is given by $C = B \times E$. The advantage of a plot of the type shown in Fig. 3 (clearance *v.* blood flow) in circumstances of variable blood flow

is that one can see at a glance, for any given blood flow rate, the differences in clearance (and hence extraction) between cholate transferred to bile with and without previous conjugation, and synthetic taurocholate. The entire data for this plot are drawn from twenty-three experiments (ten with synthetic taurocholate and thirteen with cholate). It can be seen (Fig. 3) that in general for a given blood flow rate the hepatic clearance (and therefore extraction) of taurocholate and cholate extensively conjugated before excretion (< 13 % appearing free in bile) was greater than that of cholate transferred from blood to bile without undergoing conjugation in transit (> 65 % of infusion rate appearing as free cholate in

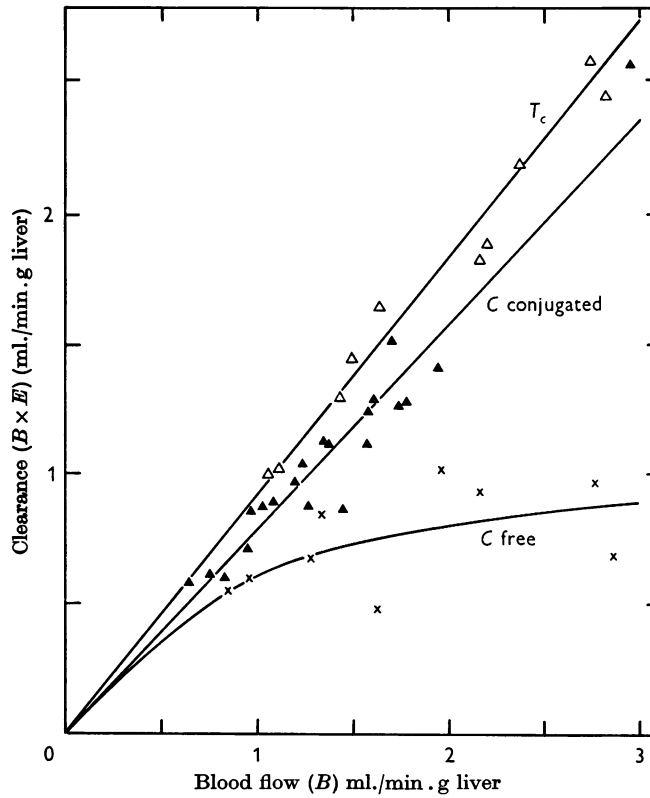

Fig. 3. Collected data from twenty-three experiments (which are described in detail in the text) to show the relation between hepatic clearance and blood flow for taurocholate (Δ—Δ T_c), cholic acid which was first actively conjugated in the liver before excretion to bile (▲—▲ *C* conjugated) and for cholate largely transferred to bile in the free form (×—× *C* free). It can be seen that, in general, for a given hepatic blood flow rate the clearance and hence the extraction of taurocholate or cholate actively conjugated before excretion is greater than that of cholate transferred to bile unchanged. (Liver wt. = 2·73 % body wt.; haematocrit = 46·3 %: means for twenty dogs.)

bile). It should be pointed out that since these experiments were performed on whole animals the blood flows depicted in Fig. 3 represent (*a*) variations from animal to animal, and (*b*) variations in the same animal from one phase of the experiment to another. The points for cholate extensively conjugated before excretion include both those obtained during the phase before taurine depletion (when endogenous taurine was being used for conjugation) and those after the restoration of conjugation by taurine administration following on the phase of taurine depletion.

Extraction of taurocholate at different rates of administration

It had been noticed (but not commented upon) in previous experiments in which taurocholate or cholate had been administered at several constant rates in stepwise fashion (see O'Máille *et al.* 1965*a*) that the hepatic clearance of bile salt declined with increasing administration rate. However, this decline was not always steadily progressive, e.g. two adjacent, but different, rates of administration occasionally gave the same value for hepatic clearance. It was therefore decided to repeat some of those experiments with hepatic venous in addition to systemic blood sampling, to determine the hepatic extraction and blood flow at a series of steady-state rates of taurocholate administration.

In three such experiments taurocholate was administered at several constant rates (1·3–8·0 μmole/min.kg body wt.) each lasting for about 90 min. Systemic and hepatic venous blood samples were taken near the end of each stage. The results of one of these experiments are illustrated in Fig. 4, which is a plot of taurocholate administration rate against the taurocholate concentration of systemic plasma. It is evident from the curvature of the plot that the clearance (infusion rate/plasma concn.) decreases with increasing administration rate. However, if the hepatic blood flow figures for each stage (Fig. 4) are examined it will be seen that there was a progressive increase in this parameter with increasing administration rate. A well-marked increase (up to 85% over initial flow) in hepatic blood flow rate was observed in two out of three experiments when the administration rate of taurocholate exceeded about 5 μmole/min.kg body wt. The blood flow changes in the third experiment were in the same direction but less marked. The possible significance of these variations is discussed below.

Glycocholate experiments

Some preliminary steady-state experiments in which glycocholate was administered at a series of constant rates (1·6–7·8 μmole/min.kg body wt.) indicate that the hepatic clearance of this bile salt also declines with increasing administration rate. The hepatic extraction fraction for glycocholate was of the same order as that for taurocholate. The maximal rate

at which glycocholate could be excreted into bile (T_m) was determined in two experiments which gave values of 8·5 and 7·8 μmole/min.kg body wt.; these fall within the range of values obtained for the taurocholate T_m (8·6 ± 1·5 (S.D.) μmole/min.kg body wt.; nine experiments).

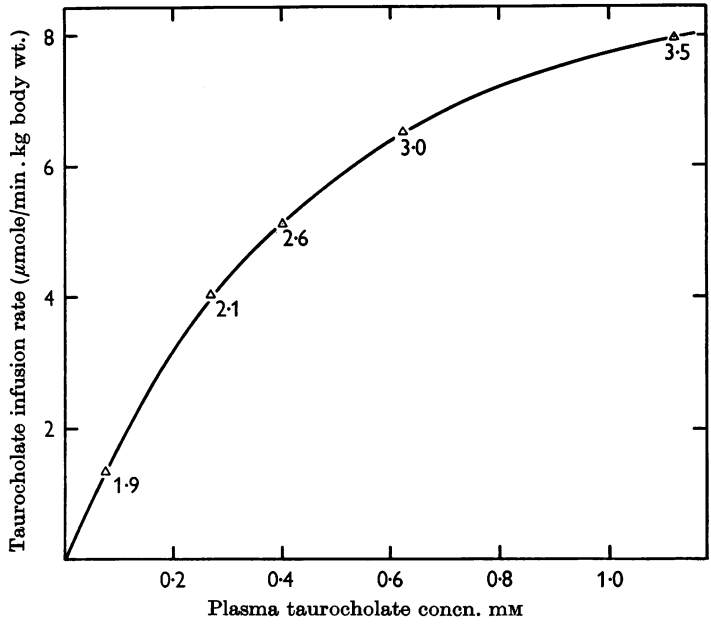

Fig. 4. Five steady-states of taurocholate administration are plotted against the plasma concentration obtaining in each stage. The figures under each point indicate the hepatic blood flow in ml./min. g liver calculated for that particular stage. The hepatic clearance (infusion rate/plasma concn.) declines with increasing administration rate, but the values obtained are likely to be modified by the progressive increase in hepatic perfusion (see text for discussion). (Liver wt. = 2·08% of body wt.; haematocrit = 48%.)

DISCUSSION

The phenomenon of acute taurine depletion and the reduced hepatic clearance of cholate which is excreted in the free form have been discussed in a previous paper (O'Máille *et al.* 1965*a*). In that study hepatic blood flow determinations were not made, so it was not possible with certainty to attribute the reduced clearance to reduced hepatic extraction. The present studies show that for a given hepatic blood flow the extraction of cholate transferred to bile in the free form is significantly less than that of either synthetic taurocholate or cholate actively conjugated before excretion.

The authors can find no account of quantitative studies on bile salt extraction of the kind reported here in the literature. However, the high

hepatic extraction of conjugated bile salt described above is in complete accord with the observations of Greene, Aldrich & Rowntree (1928) and Bollman & Mann (1936) who, after feeding large amounts of bile salts to dogs, found no significant rise in the bile acid concentration of peripheral blood. Josephson & Rydin (1936) reported large differences between the bile acid concentration of portal and systemic blood after sodium cholate or glycocholate had been injected into the intestine of the cat, rabbit and horse. Recently Portman & Shah (1962) using tracer doses of radioactive bile salts, determined the bile salt concentration of peripheral, portal and hepatic venous blood in fasted monkeys by means of an isotope dilution principle. (The portal and hepatic venous samples were obtained in the course of an acute terminal operation.) No hepatic blood flow measurements were made but on the assumption that four fifths of the total hepatic flow is through the portal vein they give the extraction ratio for taurocholate as 44–99 %; in half of their experiments the ratio was over 87 %. These authors (Portman & Shah, 1962) also noticed no consistent change in the bile salt concentration of peripheral blood, after the monkeys had been given a fat meal. Finally, Olivecrona & Sjovall (1959) have estimated the bile salt concentration of portal venous blood in the rat (this species does not have a gall-bladder) to be about 2 mg/100 ml.; in a separate study (Grundy & Sjovall, 1961) the systemic bile salt concentration of the rat was found to be approximately 0·08 mg/100 ml. Calculation from these figures gives the hepatic extraction fraction to be about 95 %. All studies, therefore, concur in the finding that at low presentation rates conjugated bile salt is almost completely removed from the blood in a single passage through the liver.

Criticism of taurine depletion experiments

The lower hepatic extraction and maximal rate of secretion of free cholate compared to taurocholate and actively conjugated cholate would, at first sight, seem to indicate simply a less-efficient transport system for free cholate arising from the difference in molecular structure between cholic and taurocholic acids. However, the following alternative possibilities must be considered:

(*a*) Since, in the course of washing out the taurine pool by means of continuous cholate administration, depletion of metabolically active taurine precursors such as cysteine, cystine and possibly methionine is likely to occur to some extent (O'Máille *et al.* 1965*a*), inferior secretory performance with free cholate might be due to metabolic ill-effects rather than a less efficient transfer system for this molecular species as against taurocholate or glycocholate.

(*b*) In the guinea-pig (Elliott, 1956*a*; Siperstein & Murray, 1956) and rat

(Bremer, 1956), the enzymic synthesis of taurocholic acid has been shown to occur according to the following reactions:

$$\text{cholic acid} + \text{CoA} \overset{\text{ATP, } Mg^{2+} \text{ or } Mn^{+}}{\rightleftharpoons} \text{cholyl CoA}, \quad (1)$$

$$\text{cholyl CoA} + \text{taurine} \longrightarrow \text{taurocholic acid} + \text{CoA}. \quad (2)$$

If conjugation in the dog liver involves similar reactions, then when taurine is available reaction 1 above would not go to equilibrium due to the rapid conversion of cholyl CoA to taurocholic acid and CoA. However, when reaction 2 is substantially reduced (following taurine depletion) cholyl CoA would accumulate in the cell until its equilibrium concentration was reached and hence a greater amount of CoA would be sequestrated as cholyl CoA. In the steady state of free cholate secretion (when the net rate of cholate entry into the liver equalled its rate of exit into the bile) the concentration of cholyl CoA would not change further with time. Inferior free cholate secretion might, therefore, be the result of (i) relative CoA deprivation (due to the increased amount bound as cholyl CoA), or (ii) competitive inhibition due to the occupying of excretory sites by cholyl CoA.

The evidence against (*a*) being the case is that secretory performance is fairly promptly restored by the administration of taurine, a procedure which would not replenish the liver content of taurine precursors. Taurine itself is an end-product of sulphur metabolism and is not known to have any cellular function other than that of acting as a conjugant for bile acids.

It is difficult to assess the importance of the possibilities mentioned in (*b*). A maximal conjugation rate of cholic acid with taurine could occasionally be demonstrated (O'Máille *et al.* 1965*a*), in which experiments very high excretory rates of bile salt (mostly taurocholate) were achieved. If reaction 2 (above) were rate limiting in these circumstances (but we have no evidence that this was so) then neither CoA deprivation nor competitive inhibition by cholyl CoA can be important factors which affect bile salt secretion, because in those experiments designed to demonstrate a maximal conjugation rate, cholyl CoA would have accumulated in the liver cell but, despite this, high rates of bile salt secretion were maintained. There are two circumstances in which the above problems would not arise. First, according to Elliott (1957) a cholyl CoA deacylase (an enzyme which catalyses the reaction cholyl CoA + $H_2O \rightarrow$ cholic acid + CoA) probably exists in liver and is most likely to be significantly operative when the conversion of cholyl CoA (in Elliott's case to cholylhydroxamic acid) is slow. If this enzyme is definitely present in liver its activity would prevent or minimize accumulation of cholyl CoA. Secondly, Elliott (1956*b*) found that when cholic acid and hydroxylamine were incubated with a *pig* liver enzyme

fraction cholylhydroxamic acid was readily formed in the absence of CoA and ATP. (The formation of cholylhydroxamic acid may be regarded as a reaction similar to taurocholic acid formation from cholic acid and taurine.) If a similar simple condensation of cholic acid and taurine takes place in the dog liver cholyl CoA accumulation should again be avoided. In either of these two circumstances the possible complications mentioned in (*b*) above, which beset interpretation of the results, would no longer exist, and the less efficient transfer of free cholate could be considered to result from different membrane kinetics as discussed previously (O'Máille *et al.* 1965*a*).

Changes in taurocholate clearance. Taurocholate is actively transferred from blood to bile by an excretory mechanism which has been shown to exhibit 'saturation' at administration rates above a certain level (Wheeler, Mancusi-Ungaro & Whitlock, 1960; O'Máille *et al.* 1965*a*). It is, therefore, to be expected that the hepatic clearance of taurocholate would decline with increasing plasma concentration (resulting from increases in administration rate) particularly at infusion rates approaching the taurocholate T_m. The observations reported above, however, indicate that these clearance changes may be modified by changes in total hepatic blood flow. Since the full plot of clearance versus blood flow is curvilinear (see Brauer, 1958), and taurocholate is a 'high-extraction substance' the effect that an increase in hepatic blood flow would be expected to produce is an increase in clearance for any given administration rate. Thus in the experiment depicted in Fig. 4 the clearance for each administration rate after the first is relatively higher than it would have been had the blood flow remained constant throughout, so that the effect of the progressive increases in blood flow is to 'linearize' the excretory rate versus plasma taurocholate concentration curve. However, such gains in clearance from increases in hepatic blood flow become smaller as infusion rates approaching that required to produce saturation of the excretory membrane are used.

Finally, with regard to the glycocholate experiments, it is of interest to point out that even though the dog lacks a glycine-conjugation mechanism for bile acids it can handle synthetic glycocholate (judging from the hepatic extraction and secretory maximum achieved) almost as well as its native taurocholate.

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REFERENCES

BOLLMAN, J. L. & MANN, F. C. (1936). The influence of the liver on the formation and destruction of bile salts. *Am. J. Physiol.* **116**, 214–224.

BRAUER, R. W. (1958). Blood flow distribution in the liver and the circulatory control of liver function. In *Liver Function*, ed. R. W. BRAUER, pp. 113–127. Amer. Inst. Biol. Sci. Washington D.C.

BRAUER, R. W., LEONG, G. F., MCELROY, R. F. E. & HOLLOWAY, R. J. (1956). Circulatory pathways in the rat liver as revealed by P^{32} chromic phosphate colloid uptake in the isolated perfused liver preparation. *Am. J. Physiol.* **184**, 593–598.

BREMER, J. (1956). Cholyl-S-CoA as an intermediate in the conjugation of cholic acid with taurine by rat liver microsomes. *Acta chem. Scand.* **10**, 56–71.

ELLIOTT, W. H. (1956*a*). The enzymic synthesis of taurocholic acid; a qualitative study. *Biochem. J.* **62**, 433–436.

ELLIOTT, W. H. (1956*b*). The enzymic activation of cholic acid by guinea-pig liver microsomes. *Biochem. J.* **62**, 427–433.

ELLIOTT, W. H. (1957). The breakdown of adenosine triphosphate accompanying cholic acid activation by guinea-pig liver microsomes. *Biochem. J.* **65**, 315–321.

GREENE, C. H., ALDRICH, M. E. & ROWNTREE, L. G. (1928). Studies in the metabolism of bile. 3. The entero hepatic circulation of the bile acids. *J. biol. Chem.* **80**, 753–760.

GRUNDY, S. M. & SJOVALL, J. (1961). Studies on bile acids in rat systemic blood. Bile acids and steroids 107. *Proc. Soc. exp. Biol. med.* **107**, 306–309.

JOSEPHSON, B. & RYDIN, A. (1936). The reabsorption of the bile acids from the intestine. *Biochem. J.* **20**, 2224–2228.

OLIVECRONA, J. & SJOVALL, J. (1959). Bile acids in rat portal blood. Bile acids and steroids 77. *Acta physiol. scand.* **46**, 284–290.

O'MÁILLE, E. R. L., RICHARDS, T. G. & SHORT, A. H. (1965*a*). Acute taurine depletion and maximal rates of hepatic conjugation and secretion of cholic acid in the dog. *J. Physiol.* **180**, 67–79.

O'MÁILLE, E. R. L., RICHARDS, T. G. & SHORT, A. H. (1965*b*). Hepatic extraction of taurocholate and cholate in the dog. *J. Physiol.* **179**, 95*P*.

O'MÁILLE, E. R. L., RICHARDS, T. G. & SHORT, A. H. (1966). Factors determining the maximal rate of organic anion secretion by the liver and further evidence on the hepatic site of action of the hormone secretin. *J. Physiol.* **186**, 424–438.

PORTMAN, O. W. & SHAH, S. (1962). The determination of concentrations of bile acids in peripheral, portal and hepatic blood of Cebus monkeys. *Archs Biochem. Biophys.* **96**, 516–523.

SHOEMAKER, W. C. (1960). Measurement of hepatic blood flow in the unanaesthetized dog by a modified bromsulphthalein method. *J. appl. Physiol.* **15**, 473–478.

SHOEMAKER, W. C. (1964). Methods and techniques for measurement of hepatic physiology and metabolism. In *The Liver, Morphology, Biochemistry, Physiology*, ed. Ch. ROUILLER, vol. 2, pp. 243–266. New York: Academic Press.

SHOEMAKER, W. C., WALKER, W. F., VAN ITALLIE, T. B. & MOORE, F. D. (1959). A method for the simultaneous catheterization of major hepatic vessels in a chronic canine preparation. *Am. J. Physiol.* **196**, 311–314.

SIPERSTEIN, M. D. & MURRAY, A. W. (1956). Enzymatic synthesis of cholyl CoA and taurocholic acid. *Science, N.Y.* **123**, 377–378.

WHEELER, H. O., MANCUSI-UNGARO, P. L. & WHITLOCK, R. T. (1960). Bile salt transport in the dog. *J. clin. Invest.* **39**, 1039–1040.