SOME FACTORS AFFECTING THE ENTERO-HEPATIC CIRCULATION AND METABOLISM OF BILE ACIDS IN PATIENTS AND IN EXPERIMENTAL ANIMALS

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INTRODUCTION

A wide variety of Biochemical Tests have been used in assessing liver function and pathology, but there remains a need for a new approach to the problem. It might be possible by tests to differentiate liver function into its various components. Currently liver function tests include serum bilirubin, serum alkaline phosphatase activity, serum glutamic-pyruvic transaminase activity, plasma protein determination by electrophoresis and less frequently the bromsulphthalein excretion tests. Individually these tests are inconclusive and even as a group they diagnose only broad aspects of liver disease.

It has been felt for many years that a more specific breakdown of liver disease would be achieved by analysis of serum bile acids. The bile acids are exclusively synthesised in the liver and describe an entero-hepatic circulation. Disturbances in all the phases of hepatic physiology should therefore be reflected in the distribution of bile acids in the intestine, plasma and urine.

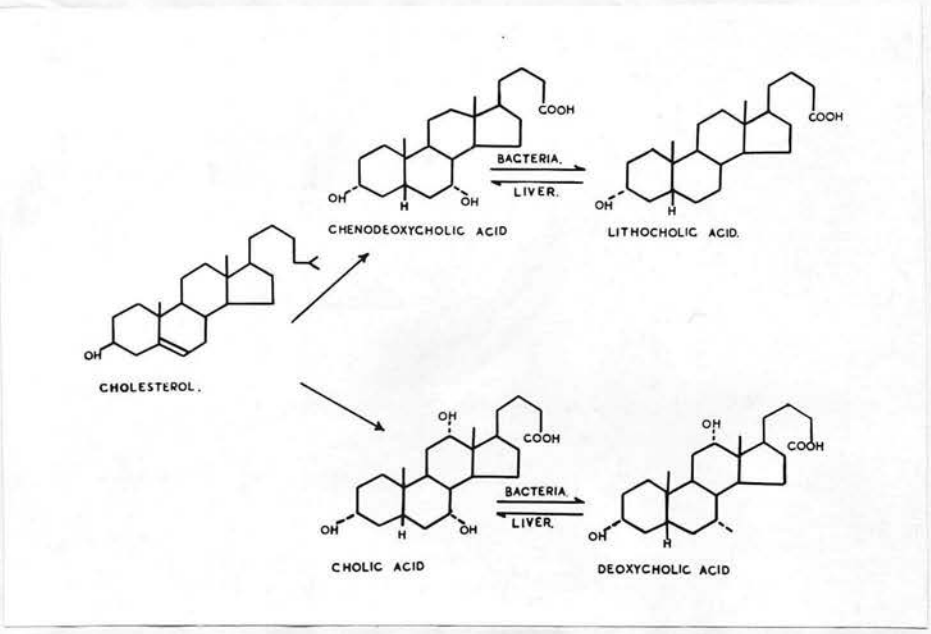

COOH
BACTERIA.
LIVER.
OH
H
OH
CHENODEOXYCHOLIC ACID
COOH
OH
H
LITHOCHOLIC ACID.
OH
CHOLESTEROL.
OH
COOH
BACTERIA.
LIVER.
OH
H
OH
CHOLIC ACID
OH
COOH
OH
H
DEOXYCHOLIC ACID

Fig. 1

Fortunately, new methods are now available and are at present being used as an adjuvant to clinical investigation.

It is proposed in this essay to describe some details of bile acid synthesis and metabolism. The method of analysing bile acids will be discussed in relation to other methods and an account given of its application to animal experiments and clinical situations.

The metabolism of bile acids

In the higher mammals cholesterol is degraded in the liver to two bile acids, cholic acid and chenodeoxycholic acid, (see Fig. 1.). Bergstrom[1] has shown that in 15 days 80 - 90% of a dose of 4-C^{14} cholesterol given intravenously appears in the faeces in an acidic fraction.

The free bile acids are conjugated with glycine and taurine by the liver microsomes and for which the presence of DPN, ATP, magnesium ions and nicotinamide and coenzyme A are required[2]. An increase in the dietary level of taurine results in an increased proportion of taurocholate. Normally the taurine to glycine ratio is 1:3.2. In the newborn baby tauroconjugates predominates but this gradually decreases over the course of the first year.

The quantity of bile acids formed and excreted is dependent upon a number of factors amongst which diet and hormonal state are of paramount importance. The concentration of bile acids in the hepatic bile of man varies from 0.4 to 2.0 gr. per 100 mls. and the total daily excretion from 5 - 10 gr. In man the proportion of bile acids in the bile is approximately 40% cholic acid, 35% chenodeoxycholic and 15% deoxycholic acid[3].

In the rat the pool size may vary from 4 - 15 mg. with a daily production of 1.5 - 4.5 mg. cholic acid[4].

The most important functions of the bile acids are -

1. to facilitate the digestion of fats by their emulsifying action

2. to activate pancreatic lipase

3. by micellar formation to aid the absorption of fatty acids, cholesterol and the fat soluble vitamins

4. they have a potent choleretic action and increase bile flow several fold.

These functions are predominantly enteric, a fact that is borne out by the distribution of bile acids. In the rat, which has no gall bladder, 70% of bile acids are in the small intestine, 25% in the large intestine

and only 5% are to be found in the liver and bile ducts[5] (Fig. 2.).

The conjugated bile acids circulate approximately ten times per day. Those bile salts which are reabsorbed from the relatively sterile small intestine, return unchanged to the liver, though there are profound changes brought about by the caecal flora to those bile acids which pass into the large intestine. Such bile acids are hydrolysed and cholic acid loses its 7α-hydroxyl groups to yield the third principal bile acid deoxycholic acid. Only 50% of bile acids passing into the caecum enter the entero-hepatic circulation within the next 24 hours. This is due to slow caecal reabsorption and bacterial activity with the production of unabsorbable and unidentifiable substances.

Those acids which are reabsorbed return to the liver in the portal vein and where possible are metabolised to parent bile acids e.g. deoxycholic acid is rehydroxylated to cholic acid, reconjugated with glycine and taurine and re-excreted into the bile.

If the bowel is sterilised the half life of cholic acid increases from 2 - 3 days to 10 days though the distribution remains the same[6].

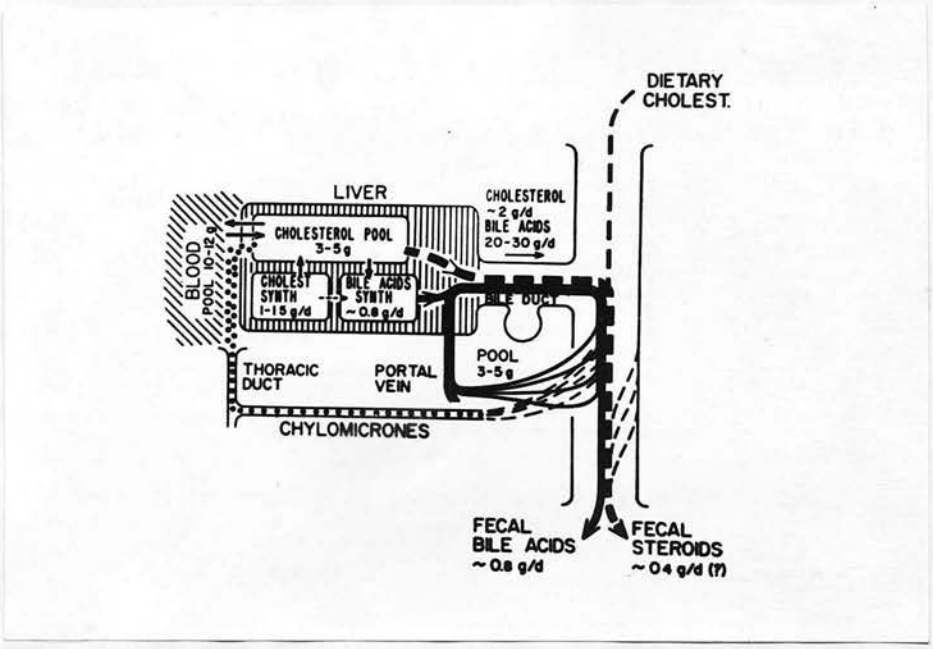

DIETARY
CHOLEST.
LIVER
CHOLESTEROL
~2 g/d
BILE ACIDS
20-30 g/d
BLOOD
POOL 10-12 g
CHOLESTEROL POOL
3-5 g
CHOLEST
SYNTH
1-1.5 g/d
BILE ACIDS
SYNTH
~0.8 g/d
BILE DUCT
POOL
3-5 g
THORACIC
DUCT
PORTAL
VEIN
CHYLOMICRONES
FECAL
BILE ACIDS
~0.8 g/d
FECAL
STEROIDS
~0.4 g/d (?)

Fig 2

In man the bile acids seem to be reabsorbed in the proximal jejunum whereas in the rat there is an increasing gradient along the small intestine with maximum absorption in the distal ileum[7].

Liver physiology can be differentiated into the following phases, synthesis, cellular metabolism, hepatic blood flow, hepatic lymph drainage, cellular uptake and storage, cellular excretion to the biliary tree and the flow to the duodenum.

Bile acid studies provide a method by which the components may be more clearly differentiated.

1. synthesis by the rate of production of cholic acid and chenodeoxycholic acid from cholesterol

2. cellular metabolism by rehydroxylation of deoxycholic acid to cholic acid

3. hepatic blood flow. Bile acids are removed almost entirely in one passage through the liver and hence blood flow theoretically can be measured. Unfortunately, portal flow is affected by the bile acids.

Porto-caval shunts will divert a portion of the entero-hepatic circulation to the peripheral circulation

and this will be reflected in the serum concentration.

4. flow to duodenum. The two most common causes of extraheptatic obstruction are by calculi and neoplasm. The bile acids, as will be shown later, fall rapidly after the relief of obstruction. This can be of diagnostic value in demonstrating relief of an obstruction and also the bile acid concentration may fluctuate during the day where the cause of obstruction is an impacted stone.

5. excretion to biliary tree. This is difficult to differentiate from an extrahepatic biliary obstruction and bile acid studies are no exception in failing to throw a clear light on this difficult differentiation.

6. cellular uptake and storage. It will be necessary to apply experiments being carried out in laboratory animals to studies in patients in which bile acids are added to the enterhepatic circulation.

7. hepatic lymph drainage. Though the lymph bile acids rise in the acute phase of common bile duct obstruction there appears to be little further information to be gained from these difficult cannulation techniques. It is interesting to compare this analysis of bile acids with other liver function tests.

Bilirubinaemia is found in all three types of jaundice. However, now that further information is available on the direct acting bilirubin it may be possible to use these measurements to give a clearer picture of hepatic dysfunction. Bilirubin which is bound to protein is conjugated in the liver. A glucoronyl transferase conjugates bilirubin with one or two glucuronide moieties[8]. These are excreted at different rates after the relief of jaundice[9]. It may be of value to measure the concentration of protein bound, free and the two types of conjugated bilirubin in various diseases of the liver.

Alkaline phosphatasaemia is found in bone diseases and also where an obstruction to biliary excretion exists. The concentration found after ligation of the common bile duct in the rat varies with the previous diet[10]. Though this may be irrelevant in man it would warrant further investigation.

An increased serum glutamic pyruvic transaminase activity does not reflect liver function per se but rather represents a reaction to acute liver damage[11]. In this respect it can be shown that the glutamic

oxaloacetic transaminase titre is greater than the glutamic pyruvi c transaminase activity in liver cells and yet the latter appears in the plasma in greater concentration after cellular damage. This phenomenon may occur because of cumulative effects secondary to a longer half life.

Extensive investigations have been made on the kinetics of bromsulphthalien test[12].

The products of bromsulphthalien metabolism have been studied chromatographically in both man and animals. These show that bromsulphthalien is conjugated possibly with glycine, glutamic acid and alanine. The conjugates may appear in the plasma and are excreted at different rates. The importance of these re-excretion rates has not been elucidated.

Former methods of estimating bile acids

1. Hay's test. This crude urine test merely expresses the presence of bile salts and is hardly specific or quantitative.

2. Spectrophotometric reaction. Methods[13] have been evolved for calculating background corrections and claims have been made for the simultaneous estimations

of at least three bile acids. Sometimes, however, these background corrections constitute 90 - 95% of the extinction.

3. Colorimetric techniques. Bile acids with sugars and concentrated sulphuric acid produce a pink colour. This Pettenkoffer reaction has been widely used in the past to estimate serum bile acids. Using this reaction in normal sera, values for trihydroxy bile acids of 0 - 10 mg. per 100 mls. and 0 - 100 mg. per 100 mls. for the dihydroxy bile acids have been found[14]. This variation is outwith reasonable limits and must be due to defects inherent in the methods. One recent method quotes recoveries of 30%[15] but generally, recoveries are in the region of 70 - 100%. Consequently the specificity of these methods must be questioned. It had been assumed that jaundiced serum contains the two parent bile acids cholic and chenodeoxycholic acid. However, in obstructive jaundice there are quantitative and qualitative differences in the bile acid pattern of bile, serum, urine and intestinal contents.

Modification of the Pettenkoffer reaction have been used by many workers who claim specificity in

cholic acid determination but the wide diversity of the results brought this reaction into disrepute. Various biological substances give the reaction for example oleic acid, cholesterol, lecithin, 7α hydroxy cholesterol and cholesteryl oleate.

Modern methods.

The most modern procedures for the estimation of bile acids in serum have included a hydrolysis step to release the bile acids from their conjugated states[16]. This has the effect of reducing the number of molecular species and also facilitates the separation of bile acids into groups dependent upon the number of hydroxyl groups. This separation can be achieved by paper[17] or partition chromatography. By these techniques the background is reduced to a constant small percentage and the use of a spectrophotometer or a modified Pettenkoffer reaction is feasible.

In our experiments we have used the method of Boyd and Reid[18]. A protein free ethanolic extract of serum is taken to dryness. The residue is dissolved in a carbonate-bicarbonate solution and the neutral

fats and sterols extracted with petrol ether.

Following alkaline hydrolysis the solution is acidified and the lipid component recovered with a chloroform-ethanol solvent. This extract is evaporated to dryness and a solution made with a known quantity of 70% acetic acid. An aliquot is exposed to the Doisy partition-coefficient chromatography system and clear separation into the mono- di and tri hydroxy substituted bile acids achieved. The efficiency of this separation is demonstrated by ultra violet absorption and thin layer chromatography. The dihydroxy acids (principally deoxycholic acid and chenodeoxycholic acid) and the tri hydroxy bile acids (cholic acid) are estimated as groups. Lithocholic acid in the monohydroxy elution gives no colour reaction. A standard of sodium taurocholate is extracted simultaneously and gives recoveries of 80 - 95%.

This method gives values for the serum dihydroxy bile acids of 0.1 mg. per 100 mls. and for the serum trihydroxy bile acids of 0.1 mg. per 100 mls. This is in agreement with isotope dilution estimations[19].

In animal experiments the small intestines are

homogenised and refluxed with ethanol and extracted as described for serum. Similarly urine samples are collected directly into 5 Normal sodium hydroxide which is made 2.5 Normal and refluxed for 5 hr. prior to further extraction and estimation.

Total and free cholesterol estimations were made by the method of Sperry and Webb[20]. The serum glutamic-pyruvic transaminase activity was determined by Reitman and Frankel's method[21].

ANIMAL EXPERIMENTS

METHOD AND MATERIALS

The experiments were carried out on Wistar strain rats, bred in the laboratory colony. For at least ten days prior to operation they were fed on a stock diet constisting of 70% wholewheat flour, 25% milk powder and 5% brewer's yeast.

Rats weighing 190 - 250 gr. were selected for the experiments.

Cholestyramine is a quaternary ammonium anion exchange resin with the basic groups attached to a styrene -divinyl benzene copolymer skeleton, which at the pH of the small intestine adsorps bile salts[22]. In the appropriate experiment a 1% (by weight) mixture with stock diet was fed until frank steatorrhoea developed.

Ether was used to anaesthetise the animals. The common bile duct was doubly ligated and divided under clean but not sterile conditions. Sham operations were carried out with the ~~same~~ Same procedure except that

the patency and continuity of the common bile duct were not impaired.

A minimum of four animals were killed at intervals post-operatively varying from half an hour to ninety-six hr.

Blood samples were obtained by cardiac puncture and the serum frozen until used. The entire small intestine from the pylorus to the ileo-caecal valve was removed and placed in alcohol. No estimation was made on the intestines of cholestyramine fed rats, as chemical extraction and biological availability of the bile salts from the small intestine are not synonymous.

RESULTS

After the bile flow from the liver to the duodenum had been arrested the serum bile acid concentrations abruptly changed, (see Fig. 3).

Half an hour after ligation the serum trihydroxy cholanic acid concentration was twenty times the initial value and the dihydroxy acids had increased by eight fold. For the remainder of the period covered by the experiment this eight fold increase was maintained, whereas, the trihydroxy acids continued to rise to a maximum concentration at eight hours. This concentration of approximately 12 mg.% was sustained for a further 8 hr. after which there was a gradual fall until 96 hr. post operatively when average values which were less than those at 30 min. were recorded.

Simultaneously there was a rapid disappearance of bile acids from the small intestine. Again, as in the serum, the dihydroxy acids fluctuate much less after the initial abrupt change in the first 30 min, (see Fig. 4).

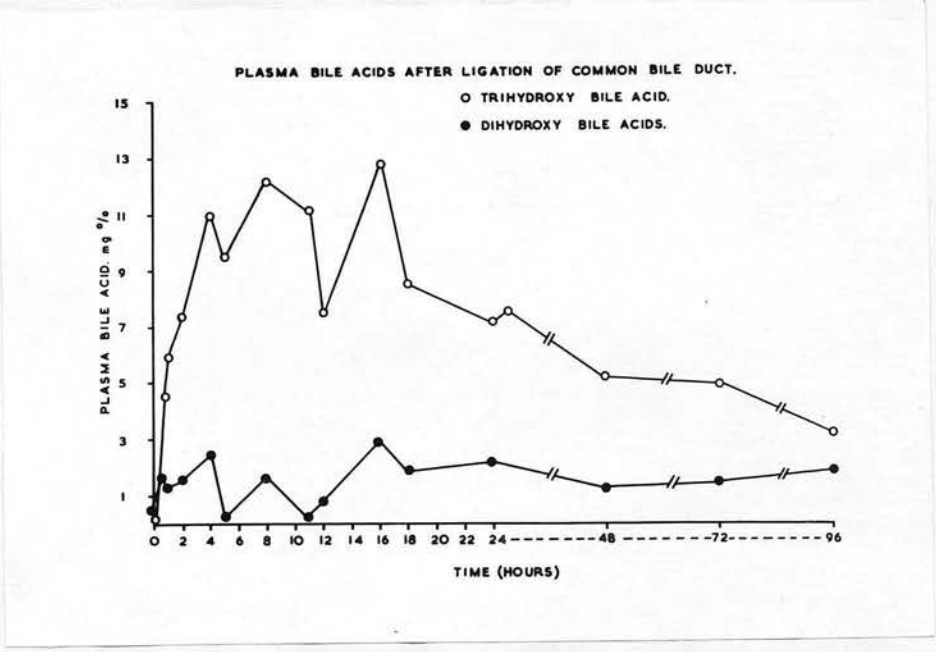

PLASMA BILE ACIDS AFTER LIGATION OF COMMON BILE DUCT.
O TRIHYDROXY BILE ACID.
● DIHYDROXY BILE ACIDS.
PLASMA BILE ACID. mg %
15
13
11
9
7
5
3
1
0 2 4 6 8 10 12 14 16 18 20 22 24 48 72 96
TIME (HOURS)

Fig 3

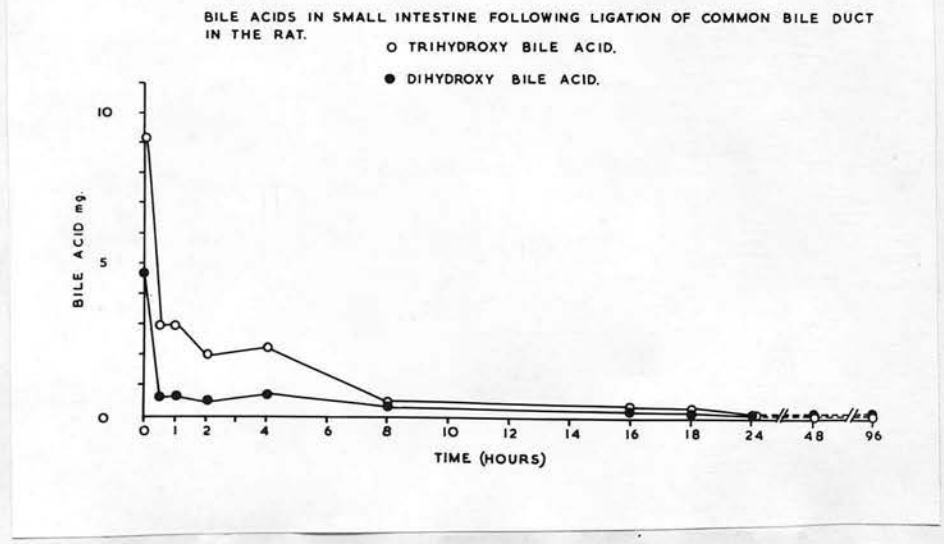

BILE ACIDS IN SMALL INTESTINE FOLLOWING LIGATION OF COMMON BILE DUCT IN THE RAT.
O TRIHYDROXY BILE ACID.
● DIHYDROXY BILE ACID.
BILE ACID mg.
10
5
0
0 1 2 4 6 8 10 12 14 16 18 24 48 96
TIME (HOURS)

Fig 4

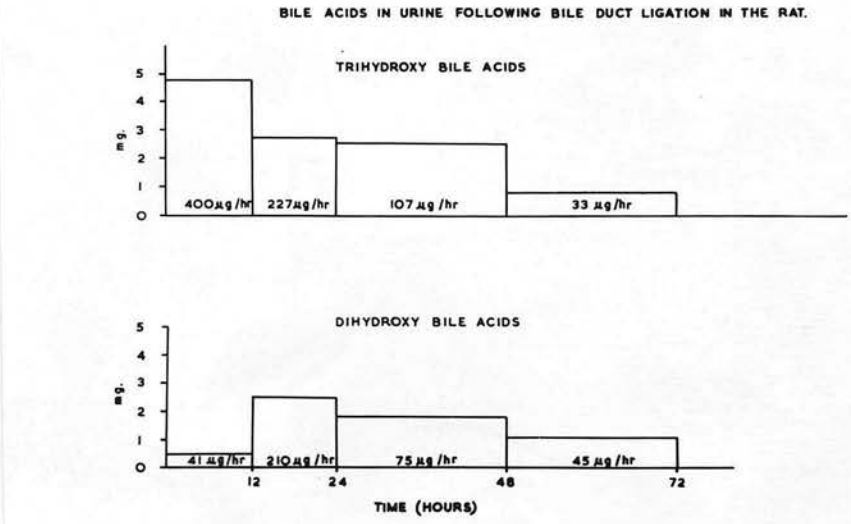

BILE ACIDS IN URINE FOLLOWING BILE DUCT LIGATION IN THE RAT.
TRIHYDROXY BILE ACIDS
mg.
5
4
3
2
1
0
400µg/hr
227µg/hr
107µg/hr
33 µg/hr
DIHYDROXY BILE ACIDS
mg.
5
4
3
2
1
0
41 µg/hr
210µg/hr
75 µg/hr
45 µg/hr
12
24
48
72
TIME (HOURS)

Fig 5

The trihydroxy acids continue to fall for a longer period. During the first 8 hr. following the operation there is a 92% fall in the measurable bile acids from the small intestine.

During the first 12 hr. 5 mg. of trihydroxy acid and 0.5 mg. of dihydroxy acid appear in the urine. During the subsequent 12 hr. the dihydroxy acids increase five fold whereas the outflow of trihydroxy acids is halved, (see Fig. 5).

Over the subsequent 48 hr. the dihydroxy acids decline to half this value and the trihydroxy acids to a mere fifth of that found during the first 12 hr.

The pattern found on these urinary bile acids also changes with time. After the first 24 hr. there are increasing quantities of more polar materials. These cannot be the 3, 6, 7 trihydroxy acids of Doisy et al.[23] as these acids are pettenkoffer negative. Thus these findings must be regarded as somewhat unsatisfactory minimum values.

During these 62 hr., 10. 9 mg. of cholic acid is excreted into the urine. This figure is almost identical with that found by Doisy for chromatophore

positive material and yet they were able to show considerable amounts of other pettenkoffer negative material present in the urine of jaundiced animals. Thin layer chromatography was carried out on the individual fractions following chromatography. Eneroth's system[24] proved to be the most satisfactory During the first 12 hr. bile acids with polarities similar to chenodeoxycholic and 3α 7β were found in the dihydroxy peak. At this same time the trihydroxy peak contained cholic acid with additional more polar materials one of which had an R_F equal to that of 3α 6β 7β and the other material was even more polar.

During the subsequent 12 hr. deoxycholic acid is readily detectable with chenodeoxycholic and more polar bile acids. This appearance of deoxycholic acid will represent the slower reabsorption and subsequent excretion of caecal metabolites. The bile acid pattern however, of the urine will be a superimposition of 6 and 7 α and β hydroxylation upon dihydroxy parent bile acids and bacterial metabolites. By the end of the first 24 hr. the trihydroxy peak contains bile acids with similar polarities to 3α 6β 7α cholic acid, 3α 6α 7α

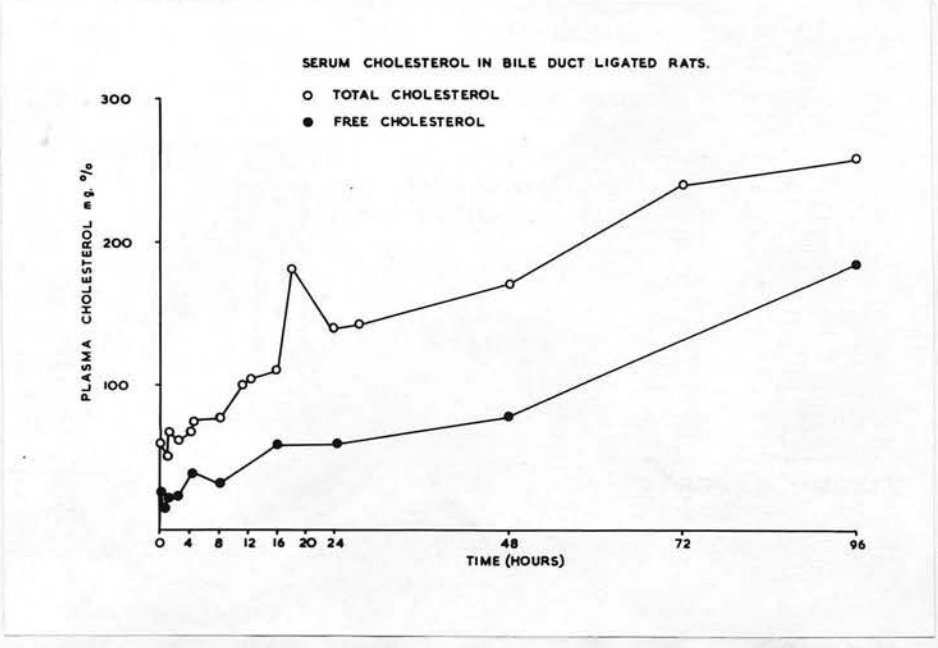

SERUM CHOLESTEROL IN BILE DUCT LIGATED RATS.
TOTAL CHOLESTEROL
FREE CHOLESTEROL
PLASMA CHOLESTEROL mg. %
300
200
100
0
4
8
12
16
20
24
48
72
96
TIME (HOURS)

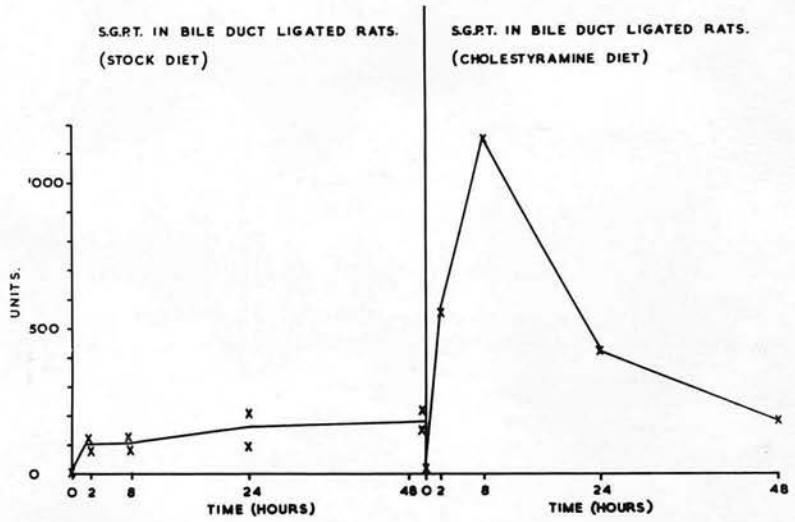
Fig 6

and 3α 7β 12α. There is material, however, which is more polar and was not identified.

The urine collected during the period 24 - 48 hr. following ligations of the common bile duct contained no detectable cholic acid only material with an R_F equivalent to 3α 6α 7α and some less polar material. It was still possible to find deoxycholic acid in this urine but no chenodeoxycholic acid was detectable. There is, however, an additional material which has an R_F similar to that of 3α 7β and also additional less polar material.

Serum taken from rats which had had their common bile ducts ligated for 8 hr. was extracted and thin layer chromatography done. It is difficult to show bile acids from normal plasma by this technique. Yet the jaundiced plasma contained bile acids with R_F equivalent to chenodeoxycholic, deoxycholic 3α 7β and 3α 6α. The trihydroxy acid peak contained material with R_F's equivalent to cholic acid 3α 6β 7β and 3α 7β 12α.

The gradual elevation of the serum total cholesterol largely as free cholesterol is in accordance with


S.G.P.T. IN BILE DUCT LIGATED RATS.
(STOCK DIET)
S.G.P.T. IN BILE DUCT LIGATED RATS.
(CHOLESTYRAMINE DIET)
UNITS.
1000
500
0
0 2 8 24 48
TIME (HOURS)
0 2 8 24 48
TIME (HOURS)

(a) (b)

Fig 7

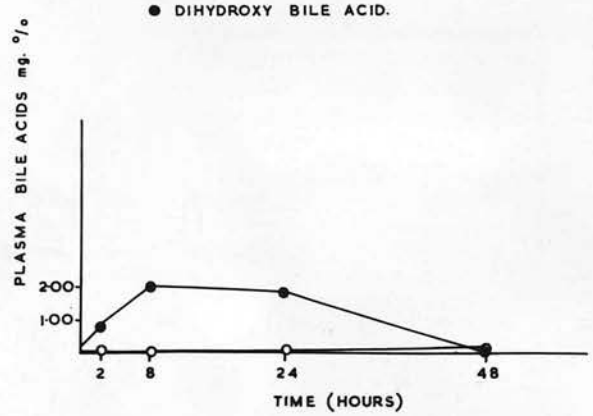

SERUM BILE ACIDS IN RATS FED CHOLESTYRAMINE
WITH COMMON BILE DUCT LIGATION.
O TRIHYDROXY BILE ACIDS.
● DIHYDROXY BILE ACID.
PLASMA BILE ACIDS mg. %
200
100
2 8 24 48
TIME (HOURS)

Fig 8

previous observations[25] (see Fig. 6). The onset of hypercholesterolaemia is relatively delayed, 11 hr. of cholestasis elapse with serum cholesterol concentrations comparable to that of sham ligated animals. On the other hand, within 2 hr. the SGPT activity is approximately 100 units and subsequently rises to twice this value, (see Fig. 7a).

Sham ligation

During these experiments the sham ligated animals showed unaccountable variation in the trihydroxy bile acid content of the small intestine. Less frequently the serum bile acid concentration was raised with no evidence of accidental obstruction. Further work is in progress to ascertain the nature of these findings.

Cholestyramine fed rats (see Fig. 8).

Evidence that the plasma elevation of bile acids is a direct consequence of the entero-hepatic circulation being diverted into the systemic circulation is given by the experiment with cholestyramine fed rats. Even after 48 hr. there were only small quantities of

trihydroxy bile acids in the plasma but the dihydroxy acids followed their usual "one step" pattern. A similar disregard for the binding powers of the resin has been noticed in a patient receiving cholestyramine to alleviate pruritis secondary to biliary cirrhosis. The coecal flora evidently can remove bile salts from the resin and consequently some of the caeco-hepatic circulation is maintained. Furthermore, any hydroxylation of deoxycholic acid and other bacterial metabolites of bile acids does not appear materially to affect the plasma concentrations of trihydroxy bile acids. If the excretion pattern is analogous with that found in untreated obstructed rat's urine then there is a rapid excretion of these dihydroxy bile acids, relative to the slower reabsorption of bile acids from the caecum.

Within 2 hr. the SGPT rises to activities which are fifty times those found in normal rats, (see Fig. 7b). The number of rats treated are too small to bear statistical analysis but 2 hr. post operatively activities more than ten times the untreated jaundiced values were registered. Therefore in the absence of measurable trihydroxy bile acids from the serum there is considerable mobilisation of

transaminase activity to the sera. This finding is contradictory if bile acids are to be implicated as an important source of damage in the obstructed liver[16].

Feeding experiment (see Fig. 10).

If cholic acid is fed to rats then after 10 days there are considerable amounts of trihydroxy acids in the serum. There is a linear relationship between the cholic acid in the diet and the observed serum concentrations. However, with a 2% supplement of cholic acid to the diet, which is 30 x the pool size estimated for rats living on the stock diet the serum level reaches 5.1 mg.%. This is only minimally in excess of the value found for rats in which the common bile ducts have been ligated for 30 min. If a cholic acid production of 3 mg./day is assumed in these rats then the liver is exposed to an additional entero-hepatic load which is 100 x greater each day than the basic production. Obviously a great strain is placed upon the clearing mechanism. If, however, 0.1% of cholic acid is added to the diet then the cholic acid pool is doubled each day with a five fold increase of the former

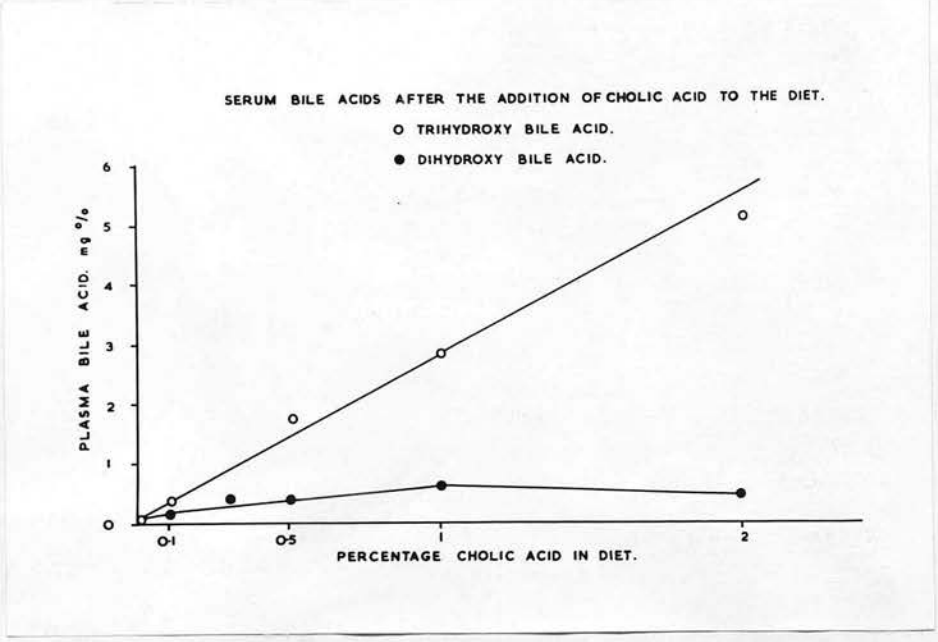

SERUM BILE ACIDS AFTER THE ADDITION OF CHOLIC ACID TO THE DIET.
O TRIHYDROXY BILE ACID.
● DIHYDROXY BILE ACID.
PLASMA BILE ACID. mg %
0
1
2
3
4
5
6
0·1
0·5
1
2
PERCENTAGE CHOLIC ACID IN DIET.

Fig. 10

production of cholic acid. Yet in these extremes the sera from rats receiving twenty times as much cholic acid supplement contained only ten times as much trihydroxy acid. The difference must be due to enhanced urinary and faecal excretion. The elevated dihydroxy acid peak will be a reflection of metabolism of the cholic acid by bacteria in the caecum.

Patient	Diagnosis	Date	Dihydroxy bile acids mgm. %	Trihydroxy bile acids mgm. %	Bilirubin mgm. %	Alkaline Phosphatase K.A. units	S.G.P.T.	Zinc Turbidity	B.S.P.
Mrs. J.S.	Obstructive Jaundice	27.12.62 3. 1.63	0.46 0.16	0.6 0.3	2.0 0.8	50 21	34 20	- -	- -
E.D.	Obstructive Jaundice	16.11.62 17.11.62 20.11.62 28.11.62	3.05 3.45 0.4 0.6	1.6 1.4 0.5 0.4	2.7 2.7 1.3 -	17 18 14 -	80 - 20 -	2 - 1 -	- - - -
R.A.	Obstructive Jaundice	20. 3.63	1.04	2.04	2.2	22	216	6	
J.McL.	Obstructive Jaundice	20. 3.63	2.6	2.6	11.5	52	27	3	
W.B.	Obstructive Jaundice	18.12.62 20.12.62 29.12.62	0.95 1.8 0.4	0.5 0.96 1.9	2.8 3.3 1.7	21 16 15	20 20 20	5 4 3	
Mrs. M.T.	Obstructive Jaundice	9. 2.62 14. 2.62 21. 2.62	0.78 0.30 0.23	0.2 0.07 0.03	2.2 0.5 0.4	18 12 9	112 20 20	4 1 3	 4%
D.L.	Cholecystitis	12.12.62 15.12.62	1.14 nil.	0.1 0.4	1.2 1.0	10 6	36 20	4 2	- -
M.McN.	Cholecystitis & myxoedema	17.11.62 20.11.62	0.7 0.7	0.7 0.6	0.8	20	29	2	- -

Fig. 11

patients with obstructive jaundice, in order to discover whether or not there is a diurnal variation in levels. Possibly an impacted stone might cause such a variation whereas a complete obstruction, such as a carcinoma of the common bile duct, or head of the pancreas, would not give rise to such fluctuations.

If Fig. 11 is examined, certain trends are amply demonstrated. There can be a rapid rise and fall of serum bile acids and conditions analogous to those found in experimental rats can be demonstrated.

Mrs. J.S. had a stone impacted in the common bile duct with a resultant rise in serum bile acids and other liver function tests. Follow relief of the obstruction, a fall in the bile acid levels preceded the fall in the other tests.

E.D. was an old lady suffering from recurrent biliary cholic with associated jaundice. The first sample of blood on 16.11.62 was taken within 16 hr. of her developing biliary colic. Her serum bile acid concentration and other liver function tests rose quite gradually. This contrasts with the situation in the rat, as in man the presence of a gall bladder prevents the direct pressure of bile upon the cannaliculi, which

pressure is responsible for the dramatic changes in concentration in the rat. By 20.11.62 the serum bile acids had fallen suggesting complete or partial patency of the duct, the residual elevation possibly being evidence of a residual biochemical lesion.

R.A. This is a single observation on a patient with an intrahepatic obstruction secondary to protracted viral hepatitis in which both groups of bile acids are markedly raised.

J. McL. who had carcinoma of the bile duct died shortly after the sample was taken.

W.B. shows great fluctuations in the bile acid pattern despite lesser changes in the other liver function tests. This obstruction was caused by an impacted stone and such observations may be of possible diagnostic value.

Mrs. M.T. had probably passed the offending stone at the time when the first sample was taken. The dihydroxy acids appear to fall more slowly than the trihydroxy acids. This is analogous to bilirubin glucuronide metabolism where bilirubin is conjugated as mono- and di-glucuronides. After relief of an obstruction the former disappear from the blood at a

slower rate than the diglucuronide. There is, as suggested, with E.D. (above) a residual lesion which is metabolic rather than mechanical. There is, however, considerable debate concerning the vascular changes following obstruction to the common bile duct. If vascular anastomoses are produced a return to the 'status quo' after relief of the obstruction, will, of necessity, be slower.

On the other hand it has been shown that bile acids, bilirubin and bromsulphthalein compete for common mechanisms when being taken up from the plasma by the liver.

At normal levels bile acids are preferentially removed, but at lower bile acid concentrations the more slowly falling bilirubin may be a more successful competitor.

D.L. In this case there is the possibility that the stone had been passed at the time when the first sample was taken and that these figures represent post-obstructive values. Once again the dihydroxy bile acid levels fall more slowly than the trihydroxy.

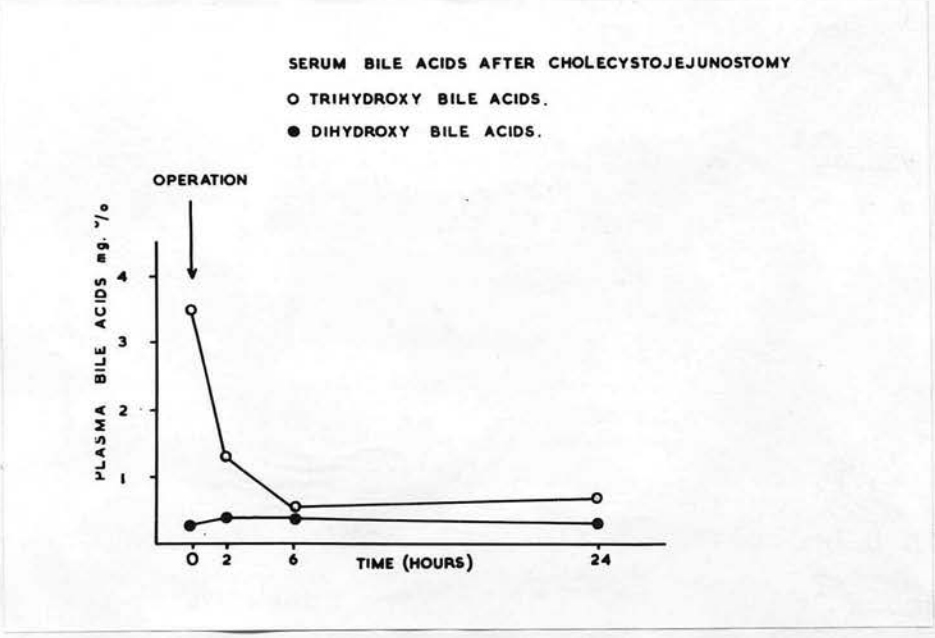

SERUM BILE ACIDS AFTER CHOLECYSTOJEJUNOSTOMY
O TRIHYDROXY BILE ACIDS.
● DIHYDROXY BILE ACIDS.
OPERATION
PLASMA BILE ACIDS mg. %
4
3
2
1
0
2
6
TIME (HOURS)
24

Fig. 12.

In myxoedematous M.McN. the bile acids remain elevated after the common bile duct was shown to be patent. It is well known that the bile acid pool size increases in hypothyroidism, but unfortunately we have yet to examine plasma levels in an uncomplicated case of this disease.

Cholecystojejunostomy (see Fig. 12)

In this patient, W.D., serial bile acid concentrations have been studied following relief of a complete obstruction due to carcinoma of the head of the pancreas. Blood was obtained before operation and at 2 hr., 6 hr. and 23 hr. post-operatively.

Within 2 hr. of the biliary flow being re-established the trihydroxy level has fallen by more than 50% and after 6 hr. only 15% of the trihydroxy bile acids remain in the blood. Again the dihydroxy bile acid changes are very slight, and there appears once more to be a resistance to a complete return to normal. In the light of these bile acid levels it is of interest, especially in relation to Mrs. A.T., discussed later, that the pruritis which troubled W.D. pre-operatively

Patient	Diagnosis	Date	Dihydroxy bile acids mgm. %	Trihydroxy bile acids mgm. %	Bilirubin mgm. %	Alkaline Phosphatase K.A. units	S.G.P.T.	Zinc Turbidity	B.S.P.
J.H.	Hepatocellular cirrhosis	1. 3.63	0.8	2.4	6.0	30	20	1	
G.W.	Hepatocellular cirrhosis	1. 3.63	0.4	0.12	0.4	9	20	4 Thymol turbidity 2	
J.H.	Hepatomegaly	11. 1.63	0.9	0.2	0.4	8	20	5	
Mrs. J.P.	Hepatocellular cirrhosis	26.12.62	0.7	0.3	0.5	33	20	7	A/G 1
Mrs. M.A.	Post-porto-caval shunt	22.12.62 24.12.62 29.12.62 5. 1.63	0.3 0.5 1.2 2.1	0.5 0.96 0.7 1.4	6.8 6.3 2.8 2.6	22 27 26 29	32 59 20 20		
C.M.	Hepatocellular cirrhosis & malignant hepatoma	10.10.62 14.10.62	1.5 0.5	2.7 2.5	9.6 11.4	34 26	34 109	3 4	

Fig. 13.

was relieved by the next day.

The other liver function tests were minimally restored in 3 days and even 7 days post-operatively they were still abnormal.

Hepato-Cellular Cirrhosis (see Fig. 13)

It is frequently difficult to obtain biochemical confirmation of hepato-cellular cirrhosis. Defects of cellular metabolism of the bile acids are commonly reflected in a raised dihydroxy bile acid peak. This is in keeping with the necessity of caecal metabolites being rehydroxylated such as d-oxycholic acid to cholic acid. The storage capacity, in cirrhotic cells, of bile acids may be diminished and saturation of the storage mechanisms rapidly occurs, with the appearance of dihydroxy acids in the plasma.

The first patient was believed to have hepato-cellular cirrhosis but the pattern was more in keeping with an obstruction. His condition rapidly deteriorated and he died. A postmortem showed the lesion to be a cholangio-carcinoma with hepatic metastases.

The next three cases show how serum bile acid estimations are invaluable as a sensitive research liver

Patient	Diagnosis	Date	Dihydroxy bile acids mgm. %	Trihydroxy bile acids mgm. %	Bilirubin	Alkaline Phosphatase K.A. units	S.G.P.T.	Zinc Turbidity	B.S.P.
Mrs. T.	Congestive failure	28.11.62	1.0	0.9	0.2	9	20	3	
Mrs. A.F.	Congestive failure	28.11.62	0.8	0.3	-	-	-	-	

Fig. 14.

function test.

In the first two cases the liver function tests were normal and only moderately deranged in the third patient.

Mrs. J.P. Confirmation of cirrhosis depended upon splenovenograms, biopsies and other non-chemical techniques, yet there is a definite increase in the dihydroxy bile acids.

Frequently following the establishment of a porto-caval shunt in hepato-cellular cirrhosis there are deranged liver function tests. This occurred in the case of Mrs. M.A. During the subsequent 2 weeks the levels slowly returned to nearly normal values. Meanwhile the serum bile acids increased in the plasma to values of 14 - 21 times the normal. This must be due to the short circuiting of the entero-hepatic cycle into the systemic circulation.

Hepatic vein hypertension represents another vascular influence on bile acid metabolism, as in congestive cardiac failure, but our series is too small to be able to draw any firm aetiological conclusions, (see Fig. 14).

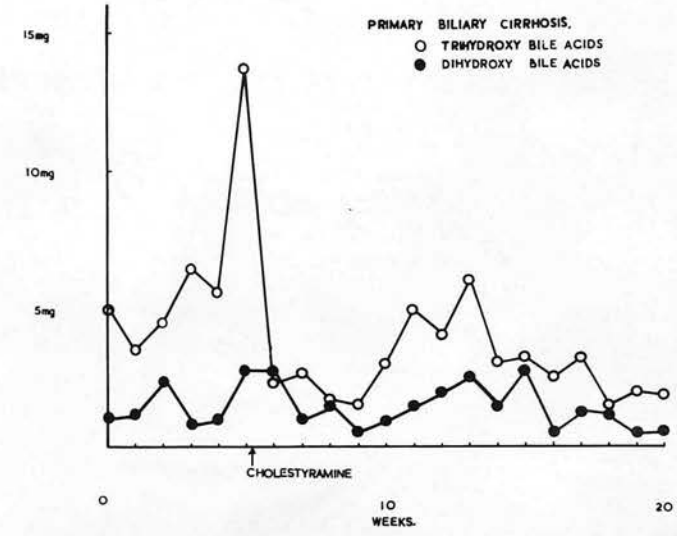

PRIMARY BILIARY CIRRHOSIS.
TRIHYDROXY BILE ACIDS
DIHYDROXY BILE ACIDS
15mg
10mg
5mg
CHOLESTYRAMINE
0
10
20
WEEKS.

Fig. 15.

Primary Biliary Cirrhosis (see Fig. 15)

Mrs. A.T. Suffers from primary biliary cirrhosis and her main complaint is intractible pruritis. It was assumed that the itch is caused by dermal deposits of bile acids secondary to the raised serum concentration. Consequently cholestyramine, a resin which adsorbs bile acids, at the pH of the small intestine, was prescribed. Her pruritis was immediately relieved by a dose of 20 g./day. It is noteworthy that the bile acids fell to lower figures over a period of weeks.

A duodenal aspirate contained extremely small amounts of bile acids, and yet the pruritis which had tormented this lady almost to distraction, was relieved for the first time in years. This relief has persisted for six months.

One must assume that there is some transferance of bile acids through the intestinal mucosa for the non-absorbable resin to have achieved this effect.

The hypercholesterolaemia follows a course parallel to the trihydroxy bile acid levels but the dihydroxy acids are relatively unaffected by the administration of cholestyramine, which must mean that

the caecal bacteria are capable of removing the bile acids from the resin and hence maintaining a diminished caeco-hepatic circulation. Experimental attempts to correlated the pruritis with the levels of non-conjugated bile acids have failed.

In these experiments the hydrolysis stage was omitted and the extraction of free bile acids continued. The hydrolysis stage in the extraction must therefore destroy some interfering substances, possibly sugars which enhance the Pettenkoffer reading as in our experiments erroneously high values were obtained.

Metabolic Changes

There have been conflicting reports of liver dysfunction in newly diagnosed untreated diabetics. There is, at present, a survey being carried out of such patients, of normal weight, with acetonuria and blood sugar concentrations in excess of 300 mg.%. Out of 17 patients in whom serum bile acids have been estimated, 7 have a dihydroxy bile acid concentration of more than 0.2 mg.% (normal $<$0.1 mg.%). The other liver function tests were normal apart from inconsistent, random abnormalities in isolated tests. This elevation

Patient	Diagnosis	Date	Dihydroxy bile acids mgm. %	Trihydroxy bile acids mgm. %
J.W.	Carcinoma of colon	4.11.62	0.6	0.6
Mrs. J.G.	Carcinoma of transverse colon	15. 1. 62	0.96	0.4
Mrs. S.	Obstruction of terminal ileum	20.12.62	.0.98	1.5

Fig. 16.

Patient	Diagnosis	Date	Dihydroxy bile acids	Trihydroxy bile acids	Bilirubin mgm. %	Alkaline Phosphatase K.A. units	S.G.P.T.	Zinc Turbidity	B.S.P.
Mrs. A.C.	Infective Hepatitis	10.12.62	0.4	0.5	1.5	15	26	5	
		15.12.62	2.8	0.6	1.3	12	26	7	
		20.12.62	0.6	0.1	0.9	8	20	4	15%
Mrs. M.M.	Infective Hepatitis	14.10.62	3.3	7.1	6.4	17	24	1	
		29.10.62	1.3	1.8	1.3	15	22	4	

Fig. 17

of dihydroxy bile acids subsides only slowly, with treatment, suggesting that though the blood sugar is stabilised there is still diminished enzyme activity in the liver and proximal tubules of the kidney.

We have also found elevated bile acids in patients with haemochromatosis in whom no other liver dysfunction was noted.

Large Bowel Obstruction (see Fig. 16.)

If the outlet to the entero-hepatic cycle is blocked as in carcinoma of the large bowel, then systemic concentrations of bile acids rise.

Viral Hepatitis (see Fig. 17.)

The two cases of infective hepatitis which we have examined showed two distinct phases. The very high bile acid levels are found in the cholestatic phase and the lower figures are associated with the cellular phase of hepatitis. These high cholestatic figures, as in primary biliary cirrhosis and hepatitis, contrast quite markedly with values in established extrahepatic obstruction. It was been shown earlier that some bile

acids in established obstructive jaundice are further metabolites of the primary bile acid chenodeoxycholic acid and are Pettenkoffer negative. In obstructive jaundice bile acids may be excreted into the biliary tree and regurgitated into the plasma via the hepatic cells. Thus there is a double passage through the cells where further metabolism e.g. 6α and 6β-hydroxylation occurs. By contrast, in obstruction, at the cellulo-canalicular junction there is no double passage merely overflow to the plasma of Pettenkoffer positive material. Consequently in both cases plasma will contain considerably raised amounts of bile acids but their full extent is only estimable in the latter case.

DISCUSSION AND SUMMARY

For many years the application of incomplete information to bile acid metabolism, in experimental animals and in clinical problems, has yielded erroneous results. More recently the use of isotopes and chromatographic separation techniques has allowed considerable progress to be made in this branch of steroid chemistry.

In our laboratory we have used such a chromatographic separation technique for quantitative analysis and thin layer chromatography for qualitative analysis of bile acids in serum, intestinal contents, urine and bile. The trihydroxy bile acid pool is principally in the small intestine and the large intestine contains much of the dihydroxy bile acid pool. The rapid fluctuations in the serum trihydroxy acids is a function of the absorptive powers of the small intestine, the less dramatic changes in the serum dihydroxy bile acids being a consequence of the slower rate of absorption from the caecum.

These variations are well demonstrated in experimental obstructive jaundice and in patients with liver diseases. Where still further metabolic changes occur as in diabetes mellitus, abnormal variations may be investigated and our present work is continuing along these lines.

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