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THE EFFECT OF VARIOUS ANAESTHETIC TECHNIQUES ON THE FLOW RATE, CONSTITUENTS AND ENZYMIC COMPOSITION OF RAT BILE

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Abstract—Bile composition and flow rate were measured over a period of 5 days in rats subjected to biliary drainage. In the first 24 hr bile flow fell due to the reduction in the pool of bile salts but remained approximately constant thereafter. There was an increased secretion of β-glucuronidase, acid phosphatase and acid ribonuclease in bile from normal rats during the period studied. Ether, halothane and pentobarbital were administered on the third day after the operations establishing the biliary fistulae. Ether and, to a lesser extent, halothane produced a transient fall in bile flow and in the secretion of bile salt; however, this treatment produced sharp rises in the activities of β-glucuronidase and acid phosphatase in the bile. Pentobarbital did not produce the changes seen following exposure to ether or halothane. Since exposure of rats to ether (but not pentobarbital) is known to result in high serum ACTH levels the effects of ACTH on bile flow and composition were studied. ACTH had little effect on bile flow or bile salt secretion, but did produce an increase in the activity of β-glucuronidase and to a smaller degree in the activity of acid phosphatase in bile. It is postulated that the fall in the bile production observed after exposure to ether or halothane results from direct actions of these volatile anaesthetics on the liver whilst the release of the acid hydrolases into the bile may be at least partially caused through an effect mediated by ACTH.

One frequently used procedure when studying the metabolism of a drug is to follow the excretion of the drug and its metabolites in the bile. Such experiments are frequently performed with anaesthetized animals, yet the effects of the commonly used anaesthetics on bile flow rate and composition are not well documented. The following experiments were designed to study this problem by the use of rats that had recovered from the anaesthetic given at the time of establishing a biliary fistula. Since prolonged biliary drainage interferes with the enterohepatic circulation of some of the bile components, the bile flow rate and bile constituents were measured daily for a period of 5 days.

Biochemical studies on the excretion of drugs and metabolites in rat bile are often performed after anaesthetizing the animals by simple inhalational procedures that do not allow close control of the exposure to the anaesthetic agent, nor are the tissue levels of the anaesthetic usually determined. In the experiments reported here, using simple methods for anaesthesia similar to those widely used in biochemical studies, we show that the choice of anaesthetic can greatly affect the composition of bile subsequently collected and analysed.

Among the components of bile studied in this investigation were four enzymes. The occurrence of a variety of enzyme activities in bile is well established; the enzymes present in normal rat bile include alkaline phosphatase and a number of acid hydrolases [1, 2]. Pronounced increases in the activities of some of these enzymes in bile following exposure of the rat to volatile anaesthetics have been briefly reported in an earlier publication [3], and a detailed analysis of this phenomenon is given here.

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MATERIALS AND METHODS

Female Wistar rats (body wt 180–230 g) were fed a standard diet (Diet 41B modified; Oxo Ltd., S.E.1.) and provided with drinking water *ad lib.* Cannulation of the common bile duct was performed whilst the rat was anaesthetized with sodium pentobarbital, the cannula passing under the skin to emerge in the middle of the back. Bile was collected overnight in a glass container as described by Van Zyl [4].

Samples of bile were collected immediately following the operation and on the four subsequent days when the rats were fully conscious. Bile collection from unanaesthetized rats was accomplished by placing the rat into a narrow cage that prevented the rat from turning round, whilst allowing some motion forwards and backwards. There was a long narrow slit in the roof of the cage through which emerged flexible nylon tubing (0.63 mm outside dia) that was connected to the indwelling biliary cannula. All bile samples were collected in small pre-weighed tubes cooled in ice. The weight of bile secreted in 1-hr periods was determined and a number of biliary components were assayed as follows:

(1) *β-Glucuronidase*, using a modification of the procedure of Gianetto and DeDuve. [5] The incubation vol was 0.225 ml and contained 25 μl of bile; incubation was for 1 hr at 37°.

(2) *Acid phosphatase*, using a modification of the procedure of King.[6] The incubation was for 1 hr at 37° in a total vol of 0.625 ml; the substrate was disodium phenyl phosphate (5 mM) and the volume of bile used was 25 μl.

(3) *Acid ribonuclease*, using a modification of the procedure of Slater [7].

(4) *β-Galactosidase*, measured by the procedure of Sellinger *et al.* [8] using as substrate *o*-nitrophenyl-β-D-galactoside 25 μl samples of bile and an incubation time at 37° of 1 hr. The buffer used was 0.1 M acetate buffer pH 5.

(5) *5'-Nucleotidase*, using the method of Belfield and Goldberg [9] where any alkaline phosphatase activity (which is low in rat bile [1]) is directed to the hydrolysis of β-glycerophosphate.

(6) *3-Hydroxy bile salts*, assayed by a modification of the procedure of Talalay [10] as described by Slater and Delaney [11].

(7) *Bilirubin*, using a modification of the method of Malloy and Evelyn [12] as described by Slater and Delaney [11].

(8) *Sodium and potassium ions*, measured using a Perkin–Elmer atomic absorption spectrophotometer, Model 303.

Where appropriate, rats were exposed to an anaesthetic on the 3rd day following the operation to insert the biliary cannula. The procedure used was to collect two 30-min bile samples before the administration of the anaesthetic, two samples during the 1-hr period of light anaesthesia and a further two samples when the rat had regained consciousness. Two volatile anaesthetics, diethyl ether and halothane (2-bromo-2-chloro-1:1:1-trifluoroethane, Fluothane, I.C.I) were used. These were administered by soaking a cotton wool pad with the anaesthetic and placing it in a face mask. The level of these anaesthetics in blood and in the liver was estimated by gas chromatography as described by Butler and Freeman [13] using a Perkin Elmer F.11 gas chromatograph. Extraction of the anaesthetic from arterial blood into *n*-heptane followed the procedure of Butler and Freeman [13], while liver samples were homogenized in ice-cold *n*-heptane using an Ultraturrex homogenizer and the debris removed by centrifuging at 0°. Sodium pentobarbital (dissolved in physiological saline) (May & Baker Ltd.) was given by i.p. injection at 40 mg/kg body wt.

Crystalline porcine ACTH (Corticotrophin, Organon) was injected s.c. at 300 μg/kg body wt dissolved in physiological saline.

RESULTS

Bile flow rate and biliary constituents in the normal rat. One-hr samples of bile were collected immediately following the operation to insert a biliary cannula and from the conscious, unrestrained rats each day on the second to the fifth days following the operation; the results obtained following analysis of the bile samples are given in Tables 1 and 2. The bile flow rate had fallen considerably by the second day and continued at this new rate until a further fall occurred on the fifth day. The secretion of bile salt also fell considerably during the first 24 hr but then showed a smaller rise during the subsequent days. The concentration of sodium ions in the bile had increased slightly by the second day and then remained at this level while potassium-ion concentration increased gradually over the 5-day period. The ratio of sodium ion/potassium ion concentration in bile showed a marked drop over the period studied ranging from approx 25 at the beginning of the experiment to approx 19 on the 5th day. There was a tendency for the excretion of bilirubin to decrease during the 5-day period but this trend was not statistically significant.

The activity in the bile of the acid hydrolases β-glucuronidase, acid phosphatase and acid ribonuclease increased from the second to the fifth day. 5'-Nucleotidase activity was not significantly altered (Table 2).

The activity of β-glucuronidase in normal rat bile was completely destroyed by heating the bile samples at 100° for 5 min; moreover, no activity could be detected in bile samples deproteinized by treatment with an equal vol of 6% (v/v) perchloric acid followed by precipitation of the excess perchlorate ions with potassium carbonate. The enzyme activity was strongly inhibited by Saccharo-1:4 lactone; the percentage inhibitions corresponding to Saccharo-lactone concentrations of 1,10 and 100 μM were 14, 74

Table 1. Bile flow rate and constituent levels over 5 consecutive days

Parameter	Days 1	2	3	4	5
Bile flow ml/hr	0.96 ± 0.05	0.52 ± 0.03	0.51 ± 0.05	0.50 ± 0.03	0.43 ± 0.07
Bile salt μmoles/hr	20.7 ± 1.8	4.7 ± 0.5	6.3 ± 0.6	8.0 ± 2.5	8.7 ± 3.1
Sodium *m/e* per l.	138 ± 4	152 ± 2	147 ± 2	151 ± 2	148 ± 5
Potassium *m/e* per l.	5.5 ± 0.2	6.0 ± 0.1	6.7 ± 0.1	8.1 ± 0.8	7.8 ± 0.8
Bilirubin Total μg/hr	54.2 ± 9.6	42.0 ± 2.3	51.1 ± 5.6	38.5 ± 7.7	40.5 ± 7.1
Bilirubin direct μg/hr reacting	46.2 ± 3.2	38.7 ± 1.9	39.0 ± 5.4	32.6 ± 7.9	34.8 ± 5.6

A 1-hr bile sample was taken on the establishment of a biliary fistula (day 1) under pentobarbital anaesthesia and on the four following days in the conscious rat. All the data are means from 6 animals ± S.E.M.; for further details see the text.

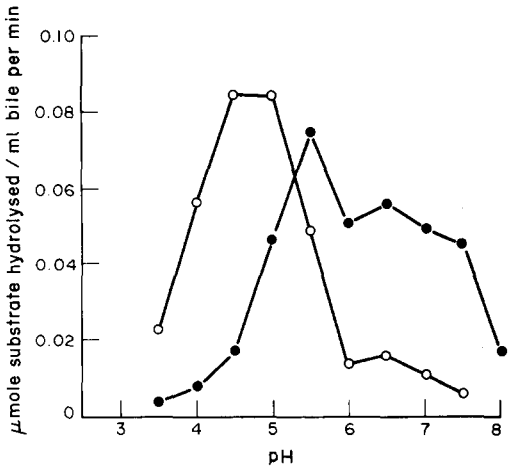

Fig. 1. The pH dependency of β-glucuronidase (●) and acid phosphatase (○) in rat bile. The incubation procedures are as described in the text; the buffer systems used were acetate buffer for the range pH 3.5–5.5 and Tris–HCl buffer for the range pH 6.0–8.0.

and 100 per cent respectively. The pH dependence of β-glucuronidase activity in normal rat bile was as shown in Fig. 1; the pH optimum was approx 5.5.

The activity of acid phosphatase in normal rat bile was destroyed by heating and by deproteinization as described above for β-glucuronidase. The pH-dependency of acid phosphatase activity is shown in Fig. 1, where it can be seen that the maximum activity can be demonstrated at approx pH 5.0. Acid phosphatase activity in rat bile was strongly inhibited by tartrate ions (98 per cent inhibition with 2 mM) and by fluoride ions (100 per cent inhibition with 2 mM).

Effects of ether, halothane and pentobarbital on bile flow rate and composition. From data given in Tables 1 and 2 it was concluded that the experiments with the anaesthetics would be best undertaken on the third day after the operation since by that time a new steady state in bile flow rate and composition had been attained and the original anaesthetic given when preparing the biliary fistula would have been completely metabolized and excreted [14].

During the administration of ether the level of the anaesthetic was considerably higher in the blood than in the liver. This difference narrowed as both levels decreased in the hour following the withdrawal of the anaesthetic when the rats were conscious (Table 3). The peak concentration of halothane in the blood of anaesthetized animals was approximately one third of the level attained during ether administration. However, liver halothane levels were higher than those observed in the blood and were in the same range as the ether levels during anaesthesia (Table 3). On removal of the anaesthetic, the halothane was rapidly lost from both the blood and liver although the level in the liver remained the greater of the two.

Ether anaesthesia on the third day after the operation to insert a biliary cannula produced a striking fall in bile flow rate with a parallel decrease in bile salt secretion and bilirubin excretion compared to the control values. The same pattern was observed after the administration of halothane, but to a smaller degree. In contrast to the effects of ether and halothane there was no significant effect on the bile flow rate or on the excretion of bilirubin or bile salt following pentobarbital injection, although the excretion of bilirubin and bile salt secretion rate tended to be somewhat less than the control levels (Fig. 2).

The activities of β-glucuronidase and acid phosphatase in bile before and after the administration of the anaesthetic agents are shown in Fig. 3. Both ether and halothane produced marked increase in the enzyme activities within 1 hr. This increase continued for a further half-hour and then began to decline.

Table 2. Activities of enzymes in bile over 4 consecutive days

Enzyme	Days 2	3	4	5
Acid phosphatase	9.9 ± 0.6	12.3 ± 1.9	17.0 ± 3.1	17.6 ± 6.4
β-Glucuronidase	0.71 ± 0.18	1.53 ± 0.38	1.54 ± 0.40	1.36 ± 0.34
Acid ribonuclease	0.70 ± 0.22	1.08 ± 0.26	1.20 ± 0.12	3.56 ± 1.44
5′-Nucleotidase	10.4 ± 2.1	12.2 ± 1.2	12.5 ± 0.6	10.1 ± 2.5

Units: Acid phosphatase, μmoles phosphate liberated/ml bile/hr incubation at 37°; β-glucuronidase, μmoles phenolphthalein liberated/ml bile/hr incubation at 37°; acid ribonuclease, change in $E_{260\,nm}$/ml bile/hr incubation at 37°; 5′-nucleotidase, μmoles phosphate liberated/ml bile/hr incubation at 37°.

One-hour bile samples were taken from the conscious rats on the second to the fifth days after the operation. The results are expressed as the means of six animals ± S.E.M.; for other details see the text.

Table 3. Concentration of halothane and ether in blood and liver

Anaesthetic	Tissue	Time (hr) 0.5	1.0	1.5	2.0
Diethyl ether	Blood	91.5 ± 5.2	82.1 ± 3.7	28.7 ± 4.2	18.6 ± 1.4
	liver	54.2 ± 2.4	65.3 ± 8.8	24.4 ± 5.1	13.6 ± 0.8
Halothane	Blood	30.4 ± 3.3	24.4 ± 3.5	3.5 ± 0.4	2.2 ± 0.2
	liver	51.2 ± 3.1	49.6 ± 5.7	8.7 ± 0.6	5.8 ± 1.0

The anaesthetics were administered over the period of the first hour: the rats were conscious in the following hour. The results are expressed as mg per 100 g of tissue and are given as the mean ± S.E.M. of three rats in each group.

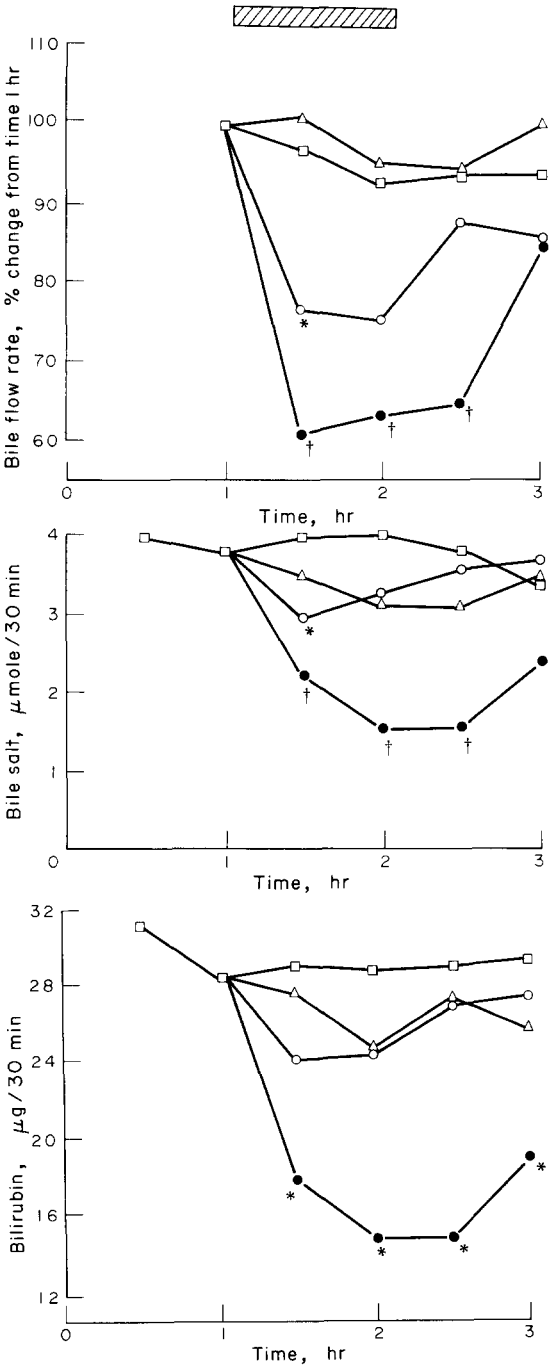

Fig. 2. The effect of ether (●——●), halothane (○——○) and pentobarbital (△——△) induced anaesthesia on bile flow, bile salt secretion and bilirubin excretion compared with control ratio (□——□). The anaesthetic was given in the hatched interval of time. The results given are the means of six animals in the control group and of four animals in the remaining groups. † $P < 0.02$, * $P < 0.05$, compared with control value.

Again the effect of ether was greater than that of halothane. There was a small decrease in activity of both enzymes following the administration of pentobarbital. The same pattern of changes in enzyme activities was seen in other experiments where bile was analysed immediately after the operation to insert a biliary cannula. When the operation was performed (as normally done) under pentobarbital anaesthesia, the acid hydrolase activities in bile were low and showed no tendency for increase over the 3-hr period following cannulation. When the operation was done with the rat under ether anaesthesia, there were marked rises in acid phosphatase and β-glucuronidase activities (Fig. 4) but no increase in the activity of 5′-nucleotidase. Estimations of β-glucuronidase and acid phosphatase on the same samples of bile showed that the changes in enzyme activities were closely correlated (Fig. 5); in other experiments (not included here) similar changes with time after exposure to ether were observed with β-galactosidase and β-glucuronidase. No alteration was observed in the activity of 5′-nucleotidase or in the concentration of sodium or potassium in the bile following the administration of ether, halothane or pentobarbital to rats 3 days after the operation to insert a biliary cannula.

Effect of ACTH on bile flow and constituents. A well known effect of ether anaesthesia is to produce high levels of serum ACTH in rats [15, 16]. It has also been reported that the injection of pentobarbital does not lead to an increase in serum ACTH [17] but can lead to an inhibition of its release [18]. These facts suggested the possibility that ACTH may be involved in some of the bile changes effected by ether (and halothane) anaesthesia.

Following injection s.c. of corticotrophin the bile flow rate at first rose slightly and then decreased. The

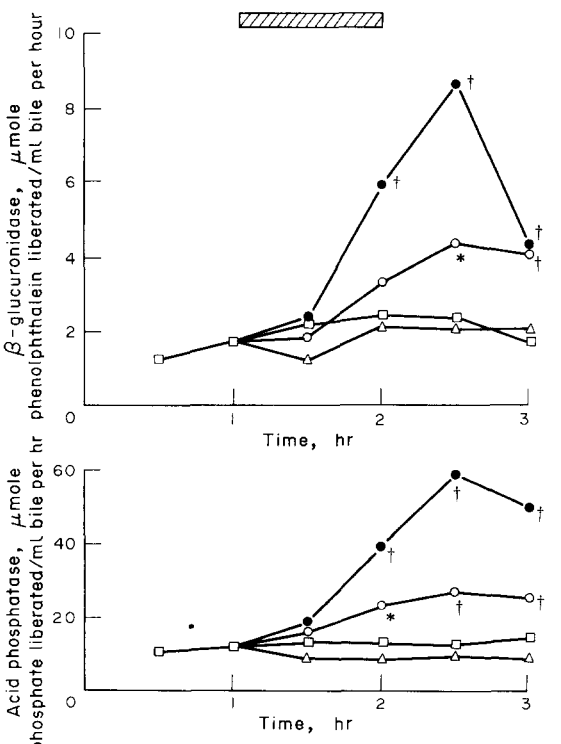

Fig. 3. The effect of ether (●——●), halothane (○——○) and pentobarbital (△——△) induced anaesthesia on β-glucuronidase and acid phosphatase activity in the bile compared to the control group (□——□). The anaesthetic was given in the hatched interval of time. The results are the means of six animals in the control group and of four animals in the remaining groups. † $P < 0.02$, * $P < 0.05$, compared with control values.

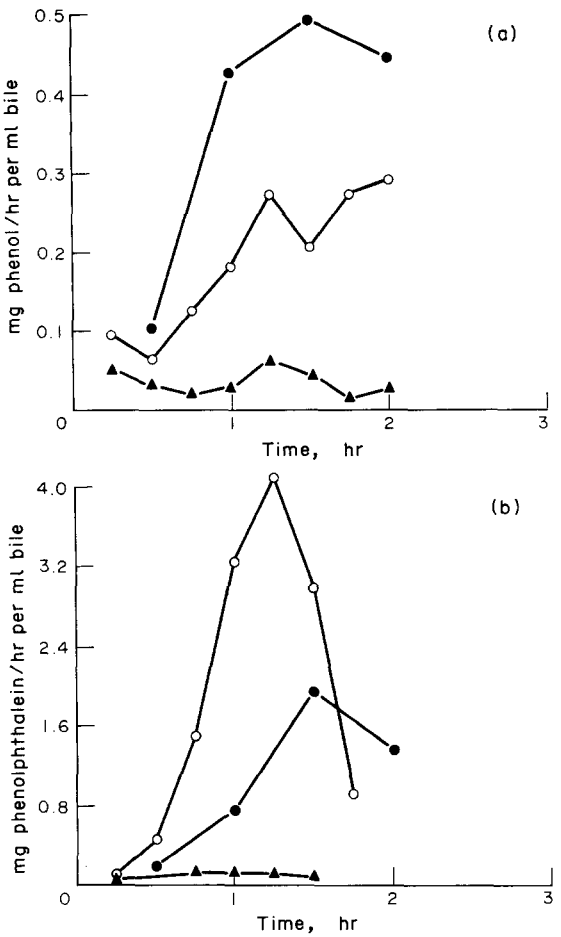

Fig. 4. Acid phosphatase (a) and β-glucuronidase (b) activities in rat bile samples collected immediately after the operation to insert a biliary cannula with the rats under ether (●——●), halothane (○——○) or pentobarbital (△——△) anaesthesia. The number of rats used in each case were (a) ether, 3; halothane, 2; pentobarbital, 3; (b) ether, 4; halothane, 1; pentobarbital, 2. For details of assay methods see text.

pattern of bilirubin excretion followed that of the bile flow rate. Bile salt secretion was unchanged (Fig. 6). The activity of β-glucuronidase in the bile rose steadily for 90 min after the administration of ACTH and then began to fall, while acid phosphatase showed only a slight rise in activity (Fig. 6). There was no effect on 5′-nucleotidase activity or on the concentration of sodium or potassium in the bile.

The effects of ether on serum ACTH concentration under the conditions used in the saddle rat procedure were checked using a sensitive cytochemical procedure for ACTH estimation [19]. (These estimations were performed in collaboration with Dr. J. Chayen, Dr. L. Bitensky, and Mr. D. Chambers of the Kennedy Institute, Hammersmith.) Rats that had been operated upon 3 days previously for the attachment of a glass saddle were exposed to ether for a 1-hr period as described in experiments outlined above. Blood samples were taken before and after anaesthesia and ACTH estimations performed. ACTH concentrations in pg/ml serum were 20 immediately before ether anaesthesia, 205 and 70 after 30 and 60 min of exposure to ether respectively and 19 after a further hour in which the animal was conscious following 60 min of anaesthesia. The level in a normal untreated rat was found to be 19 pg/ml.

Bacterial contamination. Samples of bile from rats bearing the glass saddles were taken for microbiological testing to check whether the acid hydrolase activities found were due to microbial contamination. Plating out was done on nutrient agar. (We are grateful to Mrs. V. Phillips for assistance with this aspect of the study.) Although occasional samples showed contamination this was in no way related to the rapid change in activities seen after ether, or related to the enzyme activities found in bile from unanaesthetized rats.

DISCUSSION

Bile secretion in normal rats subject to biliary drainage. It has been demonstrated that under normal conditions only 10 per cent of the bile salt pool is synthesized *de novo* from cholesterol each day, the remainder being reabsorbed from the gut [20]. The interruption of this enterohepatic circulation by the establishment of a biliary fistula leads to a rapid depletion of the bile salt pool. Since bile flow is partially dependent on the secretion of bile salts, the sudden drop in the bile salt pool results in a fall of the bile flow rate, as seen in the first 24 hr following biliary drainage. The removal of the block on the feedback mechanism which controls the rate of synthesis of bile salts from cholesterol [21] allows both an increase in bile salt synthesis and in its output in the bile over the subsequent days as shown in Table 1. The capacity to excrete bilirubin, however, appears to be unaffected by the changes in bile flow and bile salt secretion that follow the initiation of biliary drainage.

Bile flow in the rat is known to be partially dependent on a bile salt-independent mechanism (for refs see [22]). The rise in the concentration of sodium and potassium in the bile may reflect an increasing ionic contribution from this fraction in response to the decreased bile salt concentration.

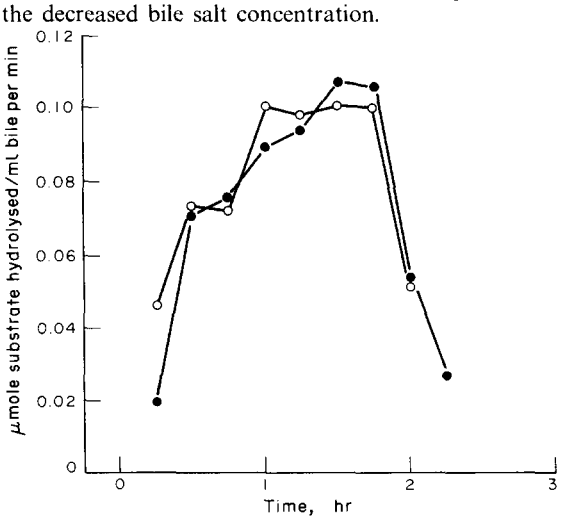

Fig. 5. Secretion of β-glucuronidase (●) and acid phosphatase (○) in pooled rat bile samples collected from three rats that had been anaesthetized with ether prior to the operations to insert biliary cannulae. β-glucuronidase was estimated at pH 5.5 and acid phosphatase at pH 4.75. For other details see the text.

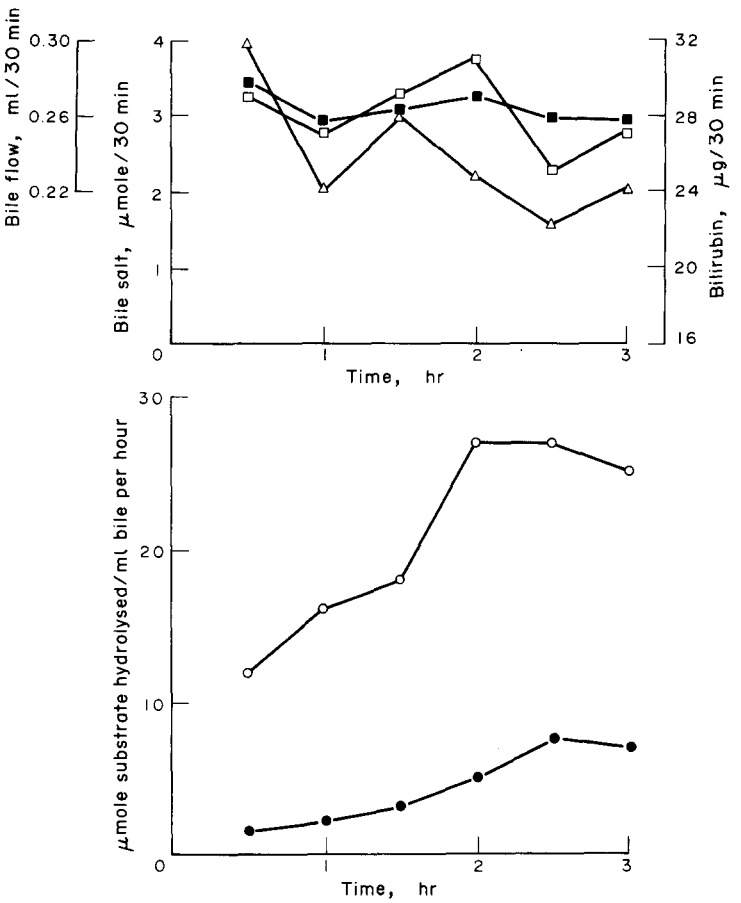

Fig. 6. Effect of ACTH (30 i.u./kg) injected s.c. after 1 hr on bile flow (△——△), bile salt secretion (■——■), bilirubin excretion (□——□), β-glucuronidase (●——●) and acid phosphatase activity (○——○) in the bile. The results are expressed as the means of four individual experiments.

The occurrence of various acid hydrolases in rat bile has been previously reported [1, 3, 23]; the intracellular origin of these enzymes released into the bile is not easy, however, to ascertain. Both acid phosphatase and β-glucuronidase occur in rat liver in more than one intracellular compartment: β-glucuronidase in the lysosomes and in the endoplasmic reticulum; acid phosphatase in the lysosomes and cytosol [24–26]. β-Glucuronidase activity in the lysosomes and endoplasmic reticulum appears to involve several different proteins that are controlled through separate genetic loci [25]; the various forms, however, exhibit similar kinetic properties and pH dependence [27, 28]. Lysosomal acid phosphatase can be distinguished from its soluble form present in cytosol in that the lysosomal enzyme is strongly inhibited by tartrate and fluoride ions [26]. These facts taken together with the data given in the text suggest strongly that the β-glucuronidase and acid phosphatase enzymes present in bile are largely lysosomal in origin. This suggestion is supported by the occurrence of other lysosomal acid hydrolases in bile (acid ribonuclease and β-galactosidase), by the close association between the activities of acid phosphatase and β-glucuronidase when measured in the same samples of bile collected at various times after cannulation (Fig. 5), and by the normal pericanalicular location of lysosomes in rat liver [29].

The origin of the 5′-nucleotidase activity in the bile may well be from the canalicular microvilli, since histochemical studies have clearly demonstrated its presence at this site [30].

The demonstration of bacterial contamination in some of the bile samples is not an uncommon occurrence. The presence of micro-organisms in human bile has been extensively reported [31–34].

Effect of anaesthetics on bile secretion. Samples of blood and liver taken from rats anaesthetized by the application of ether or halothane to a simple face mask contained concentrations of these anaesthetics that were within a relatively narrow range of values at each of the periods of time studied. The differing physico-chemical properties of ether and halothane produce different concentrations of the two anaesthetics in blood during anaesthesia; the values obtained here are in close agreement with previously published values [35, 36]. The high oil:water partition coefficient of halothane has a distinct influence on its tissue distribution: tissues rich in lipid (i.e. the liver) concentrate the anaesthetic agent relative to blood [36]. Ether has a much lower oil:water partition coefficient than halothane, and during the period of anaesthesia the concentration of ether in the blood exceeded that in the liver. Using the values for anaesthetic concentrations given in Table 3 it is possible to calculate the partial pressures of ether and halo-

thane in the samples tested. The values obtained are ether 2.1 per cent and halothane 1.3 per cent of one atmosphere pressure respectively. The normally quoted values for the loss of the righting reflex in the mouse are 3.2 per cent for ether and 1.0 per cent for halothane. Assuming that similar values apply to the rat then we may conclude that the depth of ether anaesthesia used in this study was less than obtained with halothane.

The most striking actions of ether and, to a less extent, halothane reported in this study, are on the bile flow rate and the activities of the acid hydrolases in the bile. The fall in the bile flow rate and in bilirubin excretion confirms a result noted by Ostrow *et al.* [37], who administered ether following cannulation of the bile duct under pentobarbital anaesthesia. There is also a fall in bile production in the isolated perfused rat liver after ether [37, 38], halothane [39] and also chloroform [40], demonstrating therefore that these volatile anaesthetics exert a direct action on the liver in this respect.

The rise in activity of the acid hydrolases in the bile has been briefly reported following the administration of ether [1, 3] and halothane [3]. If ether (or halothane) is given a second time within 6 hr of the original exposure, there is only a small increase in acid hydrolase activity in the bile; a response equivalent to the primary one is only found after a lag period of 17 hr [41]. This suggests that there is a small pool of acid hydrolases that are in a relatively labile state and that may be secreted into the bile in response to ether or halothane, but the pool needs several hours for replenishment. Another marked feature is that the stimulus of the volatile anaesthetics does not produce an immediate result, the response beginning after 30 min (Fig. 3) have lapsed and continuing for some time after the animal has regained consciousness. This latter event is not simply a reflection of the outflow time for a material secreted into the biliary canaliculi to pass out of the liver, along the cannula and into the collecting tube for the volumes of the rat biliary tree [42] and of the collecting cannula are small in comparison to bile flow. The rise in acid hydrolase activities in bile following exposure to volatile anaesthetics appears probably to result from an increased release of these enzymes from lysosomal sites within the liver rather than the removal of some inhibitory agent normally present.

This speculation is consistent with the observed data that a number of acid hydrolases show closely similar patterns of change after volatile anaesthetics have been given. Moreover, attempts using the chromatographic procedure of Matsushiro [43] to demonstrate changes in the biliary excretion of saccharo-1,4-lactone, a component of normal rat bile [43] and a strong inhibitor of β-glucuronidase [44], have been unsuccessful (B. Cooper and T. F. Slater, unpbulished data). It appears likely therefore that the increased enzyme levels observed in bile after ether or halothane administration results from increased secretion of such enzyme proteins rather than from a decrease in some endogenous inhibitor. The values reported here for the levels of acid hydrolases in the bile of rats recovering from the operation to insert a biliary cannula are considerably higher than those found in bile immediately after operation under pentobarbital anaesthesia (see Fig. 4 and Table 2). Using data presented here and previously reported for liver acid hydrolase activity in female rats of similar age and strain [45] it is possible to make an estimate of the proportions of liver acid hydrolases secreted into bile in 24 hr. On the third day after operation the proportion for β-glucuronidase is approx 15 per cent, while for acid phosphatase and acid ribonuclease the corresponding values were 15 and 4 per cent respectively (Table 4). It must be stressed, however, that these proportions should be viewed as approximate values and more accurate comparisons using data on liver and bile composition from the same animals are in progress. The injection of pentobarbital had very little overall effect on bile production. The slight increase in the flow rate may be due to the presence of pentobarbital and its metabolites in the bile [46]. The effect of chronic dosing with phenobarbital on bile flow rate and composition has been discussed by Slater and Delaney [11].

ACTH and bile flow. The facts that ether is known to produce high serum ACTH levels [15, 16] while pentobarbital inhibits its release [18] when compared with similar effects on the secretion of the acid hydrolases prompted the investigation of ACTH as a possible intermediary effector on this labile pool of enzymes.

The injection of ACTH at a dose designed to give maximum stimulation on the adrenal cortex had little effect on the bile flow rate or the secretion of bile salt. This dissociation of bile flow rate and ACTH concentration is compatible with the data obtained by using the isolated perfused rat liver technique where a fall in bile flow rate after ether [37] and halothane [39] was reported and where increased ACTH concentration does not occur. The data reported here for the lack of effect of administered ACTH on bile flow and bile salt excretion, together with the previously reported effects of ether [37] and halothane [39] on the isolated perfused rat liver, strongly suggest that these effects produced by the volatile anaesthetics reflect a direct action on the liver rather than an indirect mediation through ACTH release.

ACTH and enzymes in the bile. The large increase in the activity of acid phosphatase and to a smaller

Table 4. Comparison of total acid hydrolase activities in rat liver with the daily secretion of the acid hydrolases in bile

Enzyme	Total activity in liver (units/hr/ whole liver)	Total activity in bile (units/hr/day)
β-Glucuronidase	124	18.7
Acid phosphatase	1067	150
Acid ribonuclease	336	13

Units: β-Glucuronidase, μmoles phenolphthalein liberated; acid phosphatase, phosphate released calculated as μmoles of inorganic phosphate; acid ribonuclease, increase in $E_{260\,nm}$.

The data for rat liver are taken from Slater and Greenbaum [45]. The data for bile are taken from Tables 1 and 2 for the 3rd day after the operation to insert a biliary cannula.

degree that of β-glucuronidase following ACTH indicates a possible involvement of ACTH in the release of acid hydrolases into the bile. The 5'-nucleotidase activity in the bile is not affected, so not all biliary enzymes are influenced in this way. It is interesting to note that the time course of the release of the acid hydrolases after ACTH is comparable to that seen after ether and halothane. A possible explanation for this effect of ACTH may be found in the role ACTH normally plays. ACTH stimulates the adrenal cortex to synthesize and to release a number of steroid molecules collectively referred to as the glucocorticoids, mineralocorticoids and sex hormones. Several of these steroids have the ability to increase the permeability of lysosomal membranes [47] while others, for example, cortisol, are known to stabilize the membranes [48, 49]. Thus it can be postulated that one contributory factor in the release of these acid hydrolases (probably from the peribiliary lysosomes) may be one or several of the adrenal steroids released by ACTH. A final point to be considered is that the high levels of β-glucuronidase released after ether or halothane anaesthesia may alter the excretion of drugs which are conjugated with glucuronide, although Pryor and Slater [3] have found that there was no change in the percentage of free/conjugated bilirubin and in the bile during the period of increased β-glucuronidase activity.

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