Liver Adenosine Triphosphate Content and Bile Flow Rate in the Rat

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The effects of a number of hepatotoxic and other agents on the ATP content of rat liver are described. Changes in the distribution of ATP between the cell sap and the large-particle fraction were determined at intervals after rats had been dosed with various substances. Ethionine produced a rapid decrease in total liver ATP but no alteration in its intracellular distribution. Carbon tetrachloride, sodium salicylate, dimethylnitrosamine, 2,4-dinitrophenol, icterogenin, sodium succinate, sodium malonate and sodium taurocholate did not significantly alter the total ATP content of liver in the periods studied but changes in intracellular distribution were found. Carbon tetrachloride, malonate and taurocholate decreased, and salicylate treatment increased, the proportion of ATP in the cell sap. Treatment with sodium phenobarbitone increased the total liver ATP and the total amount of ATP in the cell sap. The changes in ATP concentration and in the intracellular distribution of ATP are correlated with changes previously reported in bile flow (Delaney & Slater, 1969). No general correlation was found between changes in total ATP and changes in bile flow rate, but there was a relationship between changes in bile flow and in ATP content in the case of ethionine. With the exception of taurocholate and icterogenin, which possibly act on a membrane site, an approximate correlation was found between changes in bile flow and changes in the amount of ATP in the cell sap. The findings are discussed in terms of possible mechanisms for biliary secretion.

The purpose of this investigation was to study the relationships between bile flow rate in the rat and either the total liver ATP content or the concentration of ATP in an intracellular fraction of rat liver. The intracellular fraction studied (see Delaney & Slater, 1970) was free of nuclei, mitochondria and, to some extent, endoplasmic reticulum, and was similar in nucleotide content to the cell sap. This intracellular fraction will be called the cell-sap fraction.

The effects on bile flow rate and composition after the administration to rats of a number of cholestatic and choleric substances have been described by Delaney & Slater (1969). In the present paper the effects of the same substances on total liver ATP and on the ATP content of the cell-sap fraction are described and correlated with the previous results on bile flow (Delaney & Slater, 1969).

The establishment of a correlation between bile flow rate and liver ATP content or, conversely, direct evidence to the contrary, is of considerable importance to studies concerned with identifying the source of chemical energy required for the active secretion of certain biliary components.

METHODS AND MATERIALS

Female albino rats (body wt. approx. 150 g), were used in this work. Rats were obtained from Twyford Laboratories Ltd., London N.W.10, U.K. (source A) or from University College London (source B). For some experiments on the intracellular distribution of ATP, rats were also obtained from Carworth (Europe), Alconbury, Hunts., U.K. (source C). Changes in the source of supply were necessitated by factors outside our control; in each experiment, however, control and treated rats from the same source were used. The rats were fed a standard diet (modified diet 41B; Oxo Ltd., London S.E.1, U.K.) and water *ad libitum*. They were killed by cervical dislocation and samples of liver quickly removed and put immediately into liquid N₂ for ATP analyses (these are referred to as 'intact ATP' values) or into ice-cold homogenizing medium for intracellular-distribution studies. This latter procedure was described in detail by Delaney & Slater (1970). The frozen liver samples for 'intact ATP' analyses were quickly weighed in the frozen state and homogenized in ice-cold 6% (v/v) HClO₄. After neutralization with 5 M-K₂CO₃, ATP determinations were carried out by a modification of the spectrophotometric method of Lamprecht & Trautschold (1964), as described by Delaney & Slater (1970). Oxidized and reduced nicotinamide-adenine dinucleotides in liver samples were measured by the method of Slater, Sawyer & Sträuli (1964c). Student's

t test was used to evaluate the significance of differences between the means of control groups and the groups treated with the various hepatotoxic agents.

The doses and methods of administration of the substances used, with the exceptions of icterogenin and ethanol, were as described by Delaney & Slater (1969). Icterogenin was suspended in water and given by stomach tube at a dose of 20 mg/100 g body wt.; the rats were killed 18 h later. Control rats were given an equal volume of water by stomach tube. Ethanol (20%, v/v) in 0.9% NaCl was given by intraperitoneal injection; the dose was 1.5 ml of mixture/100 g body wt.

RESULTS

Whole-liver values. The mean ATP contents $\pm$ S.E.M. in $\mu\text{mol/g}$ wet wt. of liver in untreated rats from sources A and B were respectively 1.76 ± 0.06 (29) and 2.20 ± 0.11 (13); the numbers of rats used are given in parentheses. The corresponding mean values obtained from rats subjected to the various control treatments used in this study (e.g. intraperitoneal injection of 0.9% sodium chloride or water, or administration of liquid paraffin by stomach tube) were: source A, 1.85 ± 0.05 (34); source B, 2.44 ± 0.12 (11); source C, 2.03 ± 0.13 (13). There was little difference between untreated rats and rats of the same source subjected to the control procedures, but there were considerable differences in the ATP contents of liver samples obtained with rats from different sources. There were no significant differences in the liver weight/100 g body wt. of the three different sources of rat.

The effects of the administration of various substances on total liver ATP or the ATP content of the cell-sap fraction are given below. The times chosen

for analysis correspond to the periods at which maximum bile-flow variations occur (Delaney & Slater, 1969).

Table 1 shows the results obtained after the administration of ethionine to female rats. Liver ATP began to decrease as early as 1 h after dosing and continued to decrease rapidly. At 3 h after the administration of ethionine the ATP content of the liver had fallen to 25% of the control value and remained at a low value for at least a further 20 h. No significant differences were found to exist between the ATP values obtained from control rats treated with water at different times before death so that the control values were grouped together and the mean value was used.

Table 2. *Effect of dosing with carbon tetrachloride, dimethylnitrosamine or ethanol on the ATP content of female rat liver*

Values are given as μmol of ATP/g wet wt. of liver $\pm$ S.E.M., with the numbers of rats used in parentheses. The rats were obtained from source A. For other details see the Methods and Materials section.

Expt. no.	Group	Time after dosing (h)	ATP ($\mu\text{mol/g}$ wet wt.)
A	CCl ₄	1	1.52 ± 0.14 (4)
	Control	1	1.60 ± 0.09 (4)
	CCl ₄	2	2.19 ± 0.11 (4)
	Control	2	1.96 ± 0.07 (4)
B	Dimethyl-nitrosamine	2	1.51 ± 0.14 (3)
	Control	2	1.71 ± 0.06 (4)
C	Ethanol	1	1.78 ± 0.12 (4)
	Control	1	1.81 ± 0.17 (4)

Table 1. *Effect of ethionine treatment on the ATP content of female rat liver*

Values are given as μmol of ATP/g wet wt. of liver $\pm$ S.E.M., with the numbers of rats used in parentheses. Values found for control rats killed 1–24 h after being injected intraperitoneally with water are grouped together. (The numbers of control rats studied at the various times were: 1 h, one rat; 2 h, one rat; 3 h, four rats; 5 h, one rat; 8 h, one rat; 12 h, one rat; 24 h, two rats.) The rats were obtained from source A. For other details see the Methods and Materials section.

Group	Time after dosing (h)	ATP ($\mu\text{mol/g}$ wet wt.)
Controls	1–24	1.69 ± 0.09 (11)
Ethionine	1	1.02 ± 0.05 (4)
	2	0.52 ± 0.01 (3)
	3	0.40 ± 0.06 (4)
	5	0.43 ± 0.01 (3)
	8	0.43 ± 0.09 (3)
	12	0.51 ± 0.03 (3)
	24	0.71 (2)

Table 3. *Effect of dosing with 2,4-dinitrophenol on the ATP content of rat liver*

Values are given as μmol of ATP/g wet wt. of liver $\pm$ S.E.M. with the numbers of rats used in parentheses. 2,4-Dinitrophenol was administered at two sizes of dose: (a) 2.5 mg and (b) 7.5 mg/100 g body wt. The rats were obtained from source B. For other details see the Methods and Materials section.

Group	Time after dosing (min)	ATP ($\mu\text{mol/g}$ wet wt.)
(a) Dinitrophenol	15	2.13 ± 0.41 (5)
	30	2.23 ± 0.62 (3)
Control	15, 30	2.46 ± 0.46 (3)
(b) Dinitrophenol	15	2.28 (2)
	30	1.82 (2)
	60	2.15 (2)
Control	15, 30, 60	2.49 ± 0.08 (6)

The results given in Table 2 show the effects of carbon tetrachloride on liver ATP content. No significant change occurred in liver ATP during the first 2 h treatment with carbon tetrachloride. No changes in total ATP content were observed during the first 2 h of poisoning with dimethylnitrosamine or during the first hour of poisoning with ethanol (Table 2).

Table 3 shows the results obtained when 2,4-dinitrophenol was administered to rats and the liver ATP content measured. Two sizes of dose of 2,4-dinitrophenol were used; no decrease in liver ATP occurred 15–30 min after administration of the lower dose (2.5 mg of 2,4-dinitrophenol/100 g body wt.). A small decrease in ATP occurred 30 min after a dose of 7.5 mg of 2,4-dinitrophenol/100 g body wt. (Table 3).

Sodium salicylate had no effect on the ATP content of rat liver up to 1 h after dosing; sodium malonate likewise produced no appreciable change in liver ATP 30–60 min after dosing (Table 4).

Distribution of ATP. Tables 5–8 give the relevant results on the alterations in the ATP content of the cell-sap fraction after treatment with the various agents already described above.

Carbon tetrachloride decreased the percentage of ATP present in the cell-sap fraction 2 h after dosing. There was no change, however, in the amount of ATP in the cell-sap fraction when expressed as $\mu\text{mol}/\text{whole liver per } 100\text{ g body wt.}$ An extensive decrease in the percentage of ATP in the cell-sap fraction was found 30 min after treatment with sodium malonate; only 40% of the total ATP was found in the cell-sap fraction, compared with values of 63 and 66% for the sodium chloride-treated and succinate-treated groups respectively. Some recovery to the normal distribution pattern seemed to have taken place 2 h after malonate administration, when a value of 53% was obtained for the cell sap (Table 5). These changes in distribution after malonate administration were accompanied by decreases in the amount of ATP in the cell sap (Table 5, column 6).

Sodium salicylate altered the distribution pattern of ATP in the opposite direction to that reported for sodium malonate. In this case 75% of the total ATP was found in the cell-sap fraction compared with a value of 59% for the sodium chloride-treated controls. This change was accompanied by an increase in the amount of ATP in the cell sap (Table 5, column 6).

Table 6 gives the results obtained for the distribution pattern of ATP after ethionine administration, and shows that although a considerable drop in the ATP contents of homogenate and cell-sap occurred

Table 4. *Effect of dosing with sodium salicylate, sodium malonate or sodium succinate on the ATP content of rat liver*

Values are given as μmol of ATP/g wet wt. of liver $\pm$ s.e.m., with the number of rats used in parentheses. The rats for Expts. 1 and 3 were obtained from source A; for Expt. 2 the rats were obtained from source B.

Expt. no.	Group	Time after dosing (min)	ATP ($\mu\text{mol/g wet wt.}$)
1	Salicylate	30	1.74 ± 0.02 (5)
	Control	30	1.84 ± 0.13 (4)
2	Salicylate	60	2.21 ± 0.12 (4)
	Control	60	2.24 (2)
3	Malonate	60	2.11 ± 0.28 (4)
	Succinate	60	1.99 ± 0.10 (4)
	Control	60	2.18 ± 0.22 (4)

Table 5. *Effect of treatment with carbon tetrachloride, sodium malonate sodium succinate or sodium salicylate on the distribution of ATP in rat liver fractions*

Means values $\pm$ s.e.m. are given; for other details see the Methods and Materials section. Each group consisted of four rats. Rats were obtained from source A for Expt. 1, and from source C for Expts. 2 and 3. Differences from control values: * $P < 0.05$; † $P < 0.01$; ** $P < 0.001$.

Expt. no.	Group	Time after treatment (h)	Liver wt. Body wt. $\times 100$	ATP in whole homogenate ($\mu\text{mol/g wet wt.}$)	ATP in cell-sap fraction	
					($\mu\text{mol/liver per } 100\text{ g body wt.}$)	(%)
1	Control	2	4.60 ± 0.42	2.24 ± 0.18	4.82 ± 0.33	47 ± 1
	CCl_4	2	4.75 ± 0.19	2.50 ± 0.18	4.86 ± 0.37	$41 \pm 1^{**}$
2	Control	0.5	3.90 ± 0.13	1.80 ± 0.12	4.35 ± 0.17	63 ± 2
	Malonate	0.5	3.63 ± 0.16	1.93 ± 0.23	$2.67 \pm 0.46^*$	$40 \pm 1^{**}$
	Succinate	0.5	3.25 ± 0.19	2.35 ± 0.46	4.81 ± 0.69	66 ± 3
	Malonate	2.0	3.43 ± 0.13	1.95 ± 0.14	$3.55 \pm 0.05^\dagger$	$53 \pm 3^*$
3	Control	0.5	3.51 ± 0.04	2.48 ± 0.10	$5.03 \pm 0.22^*$	59 ± 2
	Salicylate	0.5	3.74 ± 0.07	2.53 ± 0.16	7.00 ± 0.56	$75 \pm 4^*$

Table 6. *Effect of ethionine on the distribution of ATP in rat liver fractions*

Mean values $\pm$ S.E.M. are given; for other details see the Methods and Materials section. Each group consisted of four rats. Rats were obtained from source A. Differences from control values: ‡ $P < 0.1$; † $P < 0.01$; ** $P < 0.001$.

Group	Time of treatment (h)	Liver wt. Body wt. $\times 100$	ATP in whole homogenate ($\mu\text{mol/g}$ wet wt.)	ATP in cell-sap fraction	
				($\mu\text{mol/liver per 100g body wt.}$)	(%)
Control	1.5	4.97 $\pm$ 0.20	1.73 $\pm$ 0.14	4.90 $\pm$ 0.45	57 $\pm$ 2
Ethionine	1.5	4.25 $\pm$ 0.11	1.07 $\pm$ 0.07	2.55 $\pm$ 0.26†	56 $\pm$ 1
Control	3.0	4.50 $\pm$ 0.09	1.89 $\pm$ 0.20	5.17 $\pm$ 0.61	61 $\pm$ 3
Ethionine	3.0	4.15 $\pm$ 0.21	0.63 $\pm$ 0.16	1.18 $\pm$ 0.17**	45 $\pm$ 6‡

Table 7. *Effect of dosing with sodium phenobarbitone on the distribution of ATP in rat liver fractions*

Sodium phenobarbitone was administered by intraperitoneal injection (10 mg/100 g body wt. in 0.9% NaCl) on each of 3 successive days; the rats were killed 22 h after the last injection. Mean values $\pm$ S.E.M. are given for four rats; for other details see the Methods and Materials section. Rats were obtained from source C. Difference from control values: ‡ $P < 0.10$.

Group	Liver wt. Body wt. $\times 100$	ATP in whole homogenate ($\mu\text{mol/g}$ wet wt.)	ATP in cell-sap fraction	
			($\mu\text{mol/liver per 100g body wt.}$)	(%)
Control	5.78 $\pm$ 0.24	1.62 $\pm$ 0.12	5.88 $\pm$ 0.46	63 $\pm$ 2
Phenobarbitone	7.40 $\pm$ 0.21	1.92 $\pm$ 0.23	8.47 $\pm$ 1.19‡	59 $\pm$ 2

Table 8. *Effect of intraperitoneal dosing with sodium taurocholate or oral dosing with icterogenin on the ATP content of rat liver*

Mean values are given $\pm$ S.E.M. The rats were obtained from source C. For other details see the Methods and Materials section. Difference from control values: ‡ $P < 0.10$.

Group	Time (h)	No. of rats	ATP in whole homogenate ($\mu\text{mol/g}$ wet wt.)	ATP in cell-sap fraction	
				($\mu\text{mol/liver per 100g body wt.}$)	(%)
Control	0.25	7	2.25 $\pm$ 0.27	7.73 $\pm$ 0.82	58 $\pm$ 4
Taurocholate	0.25	10	2.23 $\pm$ 0.18	5.42 $\pm$ 0.81†	47 $\pm$ 5
Control	18	4	1.87 $\pm$ 0.31	4.07 $\pm$ 0.44	58 $\pm$ 4
Icterogenin	18	5	2.07 $\pm$ 0.16	4.23 $\pm$ 0.24	53 $\pm$ 2

90 min after dosing, no significant change in the percentage of total ATP in the cell sap was found.

Table 7 shows the results obtained after the administration of sodium phenobarbitone. There was virtually no change in the ATP contents of the homogenate and the cell sap when expressed as $\mu\text{mol/g}$ body wt. but since the liver weights of the treated rats were much increased the actual amount of ATP present in the cell sap when expressed as $\mu\text{mol/whole liver per 100g body wt.}$ was 8.47 compared with an average value of 5.88 obtained from the control animals.

Table 8 gives the results for the ATP contents of the cell sap after dosing with sodium taurocholate or icterogenin, and shows that neither agent affected

the ATP content of whole homogenates. There was, however, less ATP in the cell sap after taurocholate than in the control group.

DISCUSSION

Total ATP content. The results given above on the effects of a number of hepatotoxins and other agents on the total ATP content of liver complement previous results on the changes in the nicotinamide-adenine dinucleotides under similar conditions (Slater, Sträuli & Sawyer, 1964a; Slater, Sawyer & Sträuli, 1964b; Slater & Sawyer, 1966a,b,c). The changes found in total ATP in liver after dosing with ethionine and carbon tetrachloride are in

accord with previous reports (Villa-Trevino, Shull & Farber, 1963; Farber, Shull, Villa-Trevino, Lombardi & Thomas, 1964; Green, Bunyan, Cawthorne & Diplock, 1969). The failure of 2,4-dinitrophenol and salicylate to produce significant decreases *in vivo* in liver ATP probably reflects their binding to plasma protein; this is discussed by Bullock, Delaney, Sawyer & Slater (1969).

The dose of malonate used in this study was that shown by Busch & Potter (1952) to cause substantial inhibition of succinate oxidation in rat liver *in vivo*. Despite this inhibition, malonate had no effect on liver ATP content over the period studied (Table 3). Chronic administration of sodium malonate (1.5–2.0 g/kg body wt. daily for 8–15 days), however, is known to cause a considerable decrease in liver ATP content (Gajdos, Gajdos-Török, Palma-Carlos & Palma-Carlos, 1966).

Liver ATP and bile flow. It is generally accepted that the secretion of bile is dependent on the continuation of metabolic reactions to provide the chemical driving force for the secretion (see Brauer, 1959; Sperber, 1959). The identities of the particular metabolic reactions involved are unknown. By analogy with other energy-requiring reactions within the liver cell, bile secretion may require the participation of ATP in enzyme reactions that control the transfer of material from the liver into the bile (for review see Bresnick & Schwartz, 1968). The hypothesis that ATP may have a direct role in the secretory mechanism is somewhat strengthened by the localization of an active adenosine triphosphatase in the membrane lining the biliary canaliculi (Wills & Epstein, 1966), which may be linked to the transport of material from the parenchymal cells into the biliary canaliculi. If such a mechanism were operative then it can be expected that sufficiently large changes in the concentration of liver ATP will be reflected by corresponding changes in bile flow rate. In addition, a conformational change in the membrane system, by altering the activity of the constituent enzymes, can be expected to have an effect on bile flow at least as profound as that caused by changes in the concentrations of required coenzymes (e.g. ATP).

It is possible that taurocholate and icterogenin exert their effects on bile flow through such an effect on the canalicular membrane. Bile salts are known to have powerful effects on membranes in general (e.g. endoplasmic reticulum; see Ernster, Siekevitz & Palade, 1962) and it seems likely that the choleretic action of these materials results from a membrane effect(s). The molecular configuration of icterogenin and the extreme stereospecificity of the inhibitory effects on bile flow on related compounds (Brown, Rimington & Sawyer, 1963) suggests that icterogenin exerts its cholestatic action by competition with some natural bile-salt-dependent

Fig. 1. Effect of intraperitoneal dosing with ethionine on bile flow rate and total liver ATP in female rats. Results for intact rats (○, □, ▲) taken from Delaney & Slater (1969) and the present paper. Symbols: ○, 1h; △, 1½h; □, 2h; ▲, 3h after ethionine *in vivo*.

reaction. We can expect such membrane-motivated effects on bile flow to be largely independent of ATP concentration and this is the case for taurocholate and icterogenin (Table 8). Clearly, in view of such arguments, a correlation between liver ATP and bile flow should be sought for by using agents that are known to be directly responsible for changes in ATP concentration. The most suitable agent so far studied in this respect is ethionine. Fig. 1 shows results relating bile flow and total ATP during the early phase of ethionine poisoning; a reasonable correlation is apparent. In addition, it is known that ethionine produces a smaller decrease in total ATP in male rats than in females (Shull, 1962); the results of Delaney & Slater (1969) show a similar sex difference in respect of bile-flow changes.

Although results on total liver ATP after ethionine administration indicate that there is a relationship between bile flow and total liver ATP this is not a general relationship that applies to any of the other agents tested (Fig. 2), a conclusion in agreement with Brauer (1965) and Schenker & Coombes (1967). The results of Tables 1–3 in combination with bile-flow results reported by Delaney & Slater (1969) are summarized in Fig. 2, which shows that eight of the ten points are dispersed along the bile-flow axis with no variation in total liver ATP. This anomalous situation is overcome if the ATP content of the cell sap is considered (Tables 5 and 6). Malonate administration decreases the bile flow and decreases the ATP content of the cell sap. No change in the intracellular distribution of ATP occurred after dosing with ethionine and a decreased bile flow is associated with a decreased ATP content of the cell sap (Table 6). Phenobarbitone resembled salicylate: it increased bile flow and, largely due to the increased liver

Fig. 2. Effects of various agents on bile flow rate and total liver ATP in the rat. The ordinate shows the percentage change in bile flow produced by the various agents, compared with the appropriate control group; the abscissa gives the corresponding percentage change in total ATP concentration. The results are taken from this paper and from Delaney & Slater (1969); the bile-flow value for icterogenin is from unpublished results of B. E. Pryor. For details of dosing and other experimental details see the Methods and Materials section. The ATP values used were $\mu\text{mol/g}$ wet wt.; the bile-flow values used were ml/h per g of liver. Symbols: ●, sodium taurocholate, 15 min after dosing by intraperitoneal injection; ▲, sodium phenobarbitone, 22 h after the last of three daily intraperitoneal injections; ○, sodium salicylate, 1 h after intraperitoneal dosing; △, dimethylnitrosamine, 1 h after intraperitoneal dosing; ▽, 2,4-dinitrophenol, 30 min after intraperitoneal dosing with 2.5 mg/100 g body wt.; □, carbon tetrachloride, 2 h after oral dosing; ▼, sodium succinate, 1 h after intraperitoneal dosing; ■, sodium malonate, 1 h after intraperitoneal dosing; ⊙, ethionine, 90 min after intraperitoneal dosing; ⊖, ethionine, 3 h after intraperitoneal dosing.

weight, it increased the ATP content of the cell sap. The results discussed above are experimental evidence in favour of the suggestions of Brauer (1965) and Schenker & Coombes (1967) that bile flow may be correlated with the ATP content of a relatively small intracellular pool.

In conclusion, it may be said that agents that alter the amount of ATP in the cell sap have a similar effect on bile flow rate. This suggests that liver ATP is directly involved in bile secretion, but the details of the mechanism(s) are likely to be difficult to unravel since extrahepatic bile is a

function not only of secretion across the canalicular membrane but also of changes in composition at the peribiliary plexus and of fluid transfer at the secretin-dependent locus (Forker, 1967; Kelly & Klopfer, 1967). These sites may, and probably do, differ in their ATP requirements. In order to obtain more precise information on the role of ATP in bile secretion by the parenchymal cells it is necessary to study the transfer processes across the canalicular membrane itself.

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