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# Ampicillin inhibits the movement of biliary secretory vesicles in rat hepatocytes

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**A number of biliary secretory processes are inhibited by administration of ampicillin to isolated perfused rat livers. Reduction in output was observed for phospholipid, cholesterol, the endogenous protein rat serum albumin and the exogenous protein bovine serum albumin, whilst secretion of bile salts was virtually unaffected. All of the affected materials are secreted by processes involving vesicles which are brought to the appropriate pole of the hepatocyte, and the observed inhibitory effects of ampicillin may, therefore, possibly be due to a blockage in the transport of these substances. The effects of ampicillin were much less marked on materials secreted at the sinusoidal pole of the cell.**

## Introduction

Various reports have shown that organic anions such as bilirubin [1], iodipamide [2], sulphobromophthalein [3] and sodium valproate [4] inhibit biliary cholesterol and phospholipid secretion without affecting bile salt secretion. The movement of cholesterol and phospholipid across hepatocytes is probably via vesicles [5,6] which are brought to the appropriate pole of the hepatocyte via microtubules. Recently, we have shown that the antiepileptic drug, sodium valproate, not only decreases the output of cholesterol and phospholipid but also a number of proteins which are secreted or transcytosed via vesicular movement [7].

Apstein and Russo [8] have shown that ampicillin, another organic anion, reduced output of cholesterol and phospholipid into bile. The present study was conducted to extend the initial observations of Apstein and Russo [8] to include other components secreted by the liver and, hence, to determine whether ampicillin has a wider effect on hepatic function, especially in relation to vesicle movement, as does sodium valproate [7].

## Materials and Methods

*Materials.* Antisera to rat and bovine serum albumins were purchased from Nordic Immunological Laboratories, Maidenhead, Berks., U.K. Sagatal was obtained from May and Baker, Dagenham, Essex, U.K. Cannulation tubing PP10 was manufactured by Portex, Hythe, Kent, U.K. and heparin was made by Weddel Pharmaceuticals, London, U.K. Ampicillin and all other fine chemicals were obtained from Sigma Chemical Co., Poole, Dorset, U.K.

*Treatment of isolated perfused rat livers with ampicillin.* Male Wistar rats, weighing 250–300 g, were used throughout. These had been maintained on a standard laboratory diet under a constant light/dark cycle. The bile ducts of animals under pentobarbitone (Sagatal) anaesthesia were cannulated with PP10 tubing and their livers were then

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isolated in situ [9]. Liver anoxia was minimised (5–10 s) by commencing perfusion immediately, at a constant flow rate of 16 ml · $min^{-1}$, with 150 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) [10]; this buffer also contained 2 mM $CaCl_2$, 5 mM glucose, 1% (w/v) bovine serum albumin, a physiological amino acid mixture [11] and 20% (v/v) of packed human red blood cells. This solution was recycled, gassed continuously with $O_2/CO_2$ (19 : 1, v/v) and maintained at 37 ± 0.5 °C within a thermostatically controlled cabinet similar to that recommended by Collins and Skibba [12].

10 mM taurocholate was immediately infused at a rate of 432 nmol/min (as used by Apstein and Russo [8]). After the first 60 min of isolation 148.4 mg/100 g body weight of ampicillin was added (equivalent to the higher dose used by Apstein and Russo [8]), dissolved in 1 ml 0.9% sterile saline, into the circulating perfusion fluid. Controls received an equal volume of saline only. Bile and perfusion fluid samples were collected in pre-weighed tubes on ice. The volume of bile was determined gravimetrically, a density of 1 g/ml being assumed.

The health of the livers was monitored by analysing the extent of leakage into the perfusate of the cytosolic hepatocyte enzyme aspartate aminotransferase (EC 2.6.1.1). In no case was the leakage of this enzyme increased by any ampicillin treatment.

*Specific determinations.* Aspartate aminotransferase was assayed using kits supplied by the

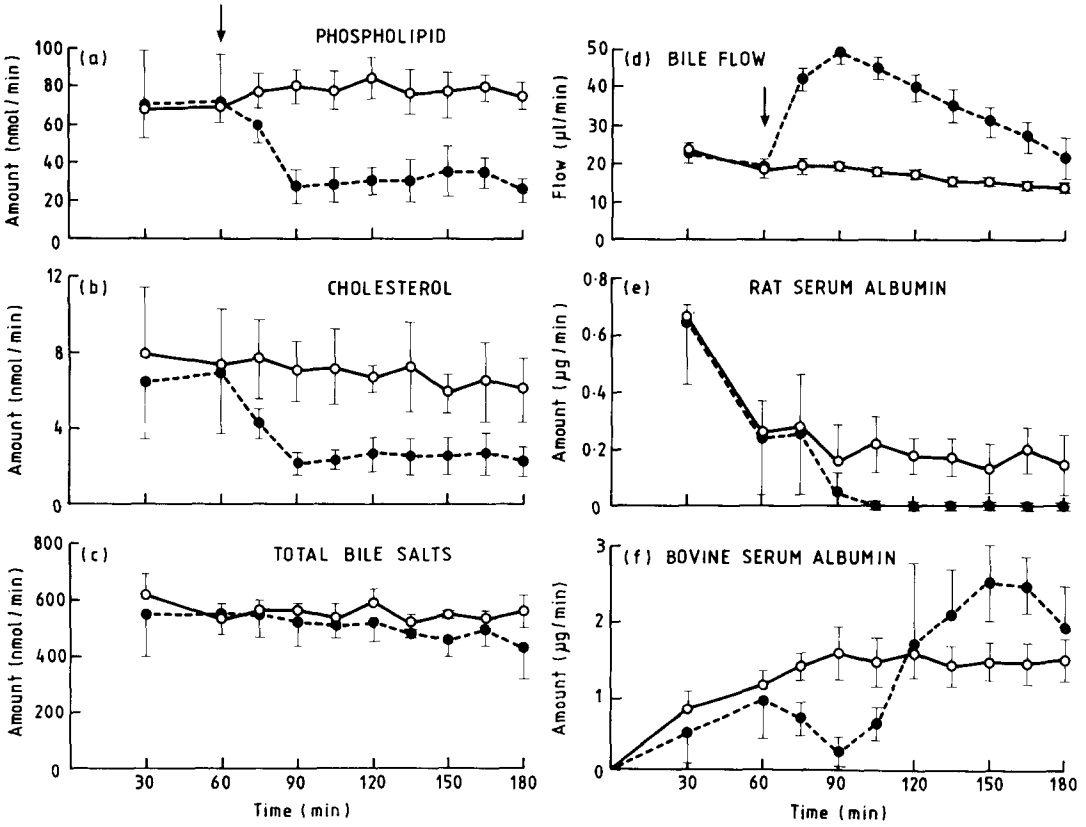


Fig. 1. Effect of ampicillin in the isolated perfused rat liver on biliary output of: (a) phospholipids, (b) cholesterol, (c) bile salts, (d) bile flow, (e) rat serum albumin and (f) bovine serum albumin. For details of animal preparation and assay procedures see Materials and Methods. Ampicillin (148.4 mg/100 g body weight dissolved in 1 ml 0.9% sterile saline) was added to the recirculating perfusion fluid 60 min into the liver isolation (denoted by an arrow). Controls received saline only. The values on the graphs are plotted to represent the end of each collection period, and are the means for five experiments (in both controls and treated) ±S.E. Symbols: ○———○, control livers; ●— — —●, livers treated with ampicillin.

Boehringer Corp., based on the method of Bergmeyer et al. [13].

Rat and bovine serum albumins in bile or perfusion fluid were determined by quantitative radial immunodiffusion by the method of Mancini et al. [14] with specific antisera. Authentic rat and bovine serum albumins were used for standardisation.

Phospholipid present in bile was determined by the method of Bartlett [15] after lipid extraction by the method of Bligh and Dyer [16].

Bile salt concentrations were determined with hydroxysteroid dehydrogenase (EC 1.1.1.50) as described by Coleman et al. [17].

Triacylglycerols were measured using the Peridochrom test kit from the Boehringer Corp., the assay was based on the method of Wahlefeld [18].

Cholesterol was analysed as trimethylsilyl ether derivatives by a slight modification [19] of the method of Vanlerenberghe and Cassaigne [20]. This method was used because of the small quantity of cholesterol present in bile and in order to prevent loss of the parent compound on the column; also, low cholesterol concentrations cannot be detected satisfactorially by other methods.

## Results

The effects of ampicillin upon a number of secretory parameters in the isolated livers were seen shortly after its administration and, in most cases, a maximal effect was observed within 30 min.

*Biliary secretion*

Bile flow more than doubled and subsequently returned towards control levels (Fig. 1d). Phospholipid and cholesterol secretion into bile (Fig. 1, a and b) decreased by approx. 50% and subsequently stayed at the lower level throughout the remainder of the perfusion despite bile salt output being unaffected by ampicillin administration (Fig. 1c).

Rat serum albumin output into bile was completely inhibited (within 45 min) after ampicillin administration (Fig. 1e). Biliary output of bovine serum albumin (an exogenous protein derived from the perfusion fluid) was reduced by approx. 70% at 30 min after ampicillin administration. This

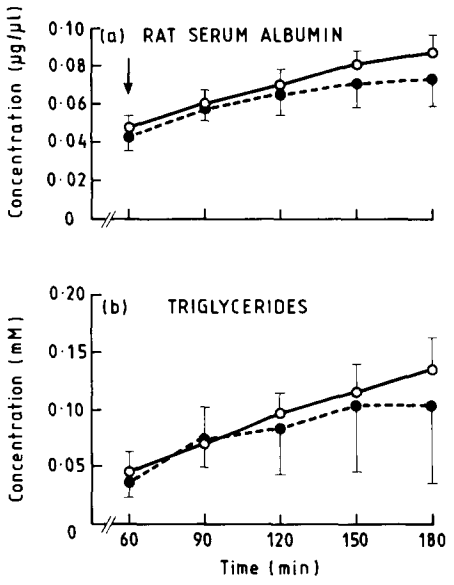


Fig. 2. Effect of ampicillin on the output of (a) rat serum albumin and (b) triacylglycerols into perfusion fluid. For details see the Materials and Methods and the legend to Fig. 1. Rat serum albumin and triacylglycerol output are given as concentrations, since the volume of the perfusion fluid remained constant throughout the experiment.

then recovered after 60 min and was subsequently greater than the controls (Fig. 1f).

*Sinusoidal secretion*

Secretory processes at the sinusoidal pole of the hepatocyte appeared to be much less affected by ampicillin as shown by measurements of rat serum albumin and triacylglycerols in the perfusate (Fig. 2, a and b). It can be seen from the figures that there is a general inhibitory trend in output from ampicillin-treated livers but this difference is not statistically significant largely due, especially in the case of triacylglycerol secretion, to the variability in individual responses.

## Discussion

Rat and bovine serum albumins [21], phospholipids, cholesterol [5,6] and triacylglycerols (as very-low-density lipoprotein [22,23]), all are transported within and across the hepatocytes in vesicles (see Ref. 24); these are brought to the appropriate pole of the hepatocyte via microtubules. Rat serum albumin is synthesised endogenously, secreted at

the sinusoidal pole and can then enter bile by vesicle-mediated transcytosis across the hepatocyte or, to a slight extent, paracellularly through the tight junctions; exogenous proteins such as bovine serum albumin can also enter bile by the same two routes (see Ref. 24). The lipids, phospholipids and cholesterol, appearing in bile are secreted at the biliary pole of the hepatocyte, via vesicle-mediated transfer, and are related to secretion of bile salts even though the bile salts themselves are probably transported across the hepatocyte as discrete molecules rather than in vesicles (see Ref. 24 for a review).

We have observed in this study that not only does ampicillin decrease the output of cholesterol and phospholipid into bile, as has been shown by Apstein and Russo [8], but it also partially inhibits the secretion of proteins such as rat and bovine serum albumins. The phenomena whereby organic anions affect secretion of cholesterol and phospholipid but not of bile salts, have been documented with iodipamide [2,25], sulphobromophthalein [3], bilirubin [1,26], sodium valproate [4], sulphated glycolithocholic acid [27] and other sulphated bile acids [28,29] and also ampicillin [8]. We have recently studied this effect in greater detail using both sodium valproate and sodium octanoate (caprylic acid: sodium salt) in the isolated perfused rat liver [7]. We observed that not only were cholesterol and phospholipid output affected but also a range of other materials including various lipids and proteins at both poles of the hepatocyte. The present inhibitory effects of ampicillin on biliary phospholipid, cholesterol, rat and bovine serum albumin secretion complement and amplify these other studies. Sinusoidal secretion of rat serum albumin and triacylglycerol in ampicillin-treated livers showed no significant decrease from that of controls, and in this respect the response to ampicillin was much less than that to sodium valproate; there was, however, a general but not statistically significant inhibitory trend.

The immediate choleresis produced by ampicillin, which was also noticed with the other anions, is probably an osmotic effect caused by the biliary secretion of the drug. An anomalous feature in the results is the subsequent increase in bovine serum albumin into bile after the initial decrease; the reason for this is not yet apparent.

All the secretory materials measured, with the exception of bile salts, are transported across the hepatocyte via vesicle-mediated transport. Since organic anions affect secretion very rapidly, it is unlikely that the reductions in output are due to inhibition of synthesis of the various materials. We have suggested [7] that the organic anions may be acting on the movement of vesicles within the hepatocyte, possibly by a common mechanism, but to different degrees for different materials. The nature of this mechanism, e.g., disruption of energy supply, the cytoskeleton, or second messenger levels, has yet to be identified.

Ampicillin is predominantly excreted via the kidney in man, but is also excreted hepatically [31–33], except in cases of obstructive biliary tract disease [34]. It is not generally hepatotoxic in the whole animal or in humans [30]: this is probably due to the smaller concentrations which will reach the liver in a whole animal compared to those in the isolated liver (see also Ref. 7).

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## References

1 Apstein, M.D. (1984) Gastroenterology 87, 634–638.
2 Apstein, M.D. and Robins, S.J. (1982) Gastroenterology 83, 1120–1126.
3 Shaffer, E.A. and Preshaw, R.M. (1981) Am. J. Physiol. 240, G85–89.
4 Jezequel, A.M., Bonazzi, P., Novelli, G., Venturini, C. and Orlandi, F. (1984) Hepatology 4, 1159–1166.
5 Barnwell, S.G., Lowe, P.J. and Coleman, R. (1984) Biochem. J. 220, 723–731.
6 Lowe, P.J., Barnwell, S.G. and Coleman, R. (1984) Biochem. J. 222, 631–637.
7 Bellringer, M.E., Rahman, K. and Coleman, R. (1988) Biochem. J. 249, 513–519.
8 Apstein, M.D. and Russo, A.R. (1985) Dig. Dis. Sci. 30, 253–256.
9 Hems, R., Ross, B.D., Berry, M.N. and Krebs, H.A. (1966) Biochem. J. 101, 248–292.
10 Krebs, H.A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66.
11 Barnwell, S.G., Godfrey, P.P., Lowe, P.J. and Coleman, R. (1983) Biochem. J. 210, 549–554.

12 Collins, F.G. and Skibba, J.L. (1980) J. Surg. Res. 28, 65–70.
13 Bergmeyer, H.U., Scheibe, P. and Wahlefeld, A.W. (1978) Clin. Chem. 24, 58–73.
14 Mancini, G., Carbonara, A.O. and Heremans, J.F. (1965) Immunochemistry 2, 235–254.
15 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
16 Bligh, E.G. and Dyer, W.I. (1959) Can. J. Biochem. Physiol. 37, 911–919.
17 Coleman, R., Iqbal, S., Godfrey, P.P. and Billington, D. (1979) Biochem. J. 178, 201–208.
18 Wahlefeld, A.W. (1974) Methods of Enzymatic Analysis. (Bergmeyer, H.U., ed.), p. 1831. 2nd Edn. Verlag Chemie, Weinheim.
19 Rahman, K. and Coleman, R. (1986) Biochem. J. 237, 301–304.
20 Vanlerenberghe, J. and Cassaigne, R. (1968) Rev. Fr. Etud. Clin. Biol. 8, 541–544.
21 Kloppel, T.M., Brown, W.R. and Reichen, J. (1986) Hepatology 6, 587–594.
22 Orci, L., Le Marchand, Y., Singh, A., Assimacopoulos-Jeannet, F., Rouiller, C. and Jeanrenaud, B. (1973) Nature 244, 30–32.
23 Le Marchand, Y., Singh, A., Assimacopoulos-Jeannet, F., Orci, L., Rouiller, C. and Jeanrenaud, B. (1973) J. Biol. Chem. 248, 6862–6870.
24 Coleman, R. (1987) Biochem. J. 244, 249–261.
25 Apstein, M.D. and Robins, S.J. (1981) Gastroenterology 80, 1326.
26 Apstein, M.D. (1982) Hepatology 2, 143.
27 Kuipers, F., Havinga, R. and Vonk, R.J. (1987) Clin. Sci. 68, 127–134.
28 Mathis, U., Karlaganis, G. and Preisig, R. (1983) Gastroenterology 85, 674–681.
29 Yousef, I.M., Barnwell, S.G., Tuchweber, B., Weber, A. and Roy, C.C. (1987) Hepatology 7, 535–542.
30 Galante, D., Esposito, S., Barba, D. and Ruffilli, M.P. (1987) J. Antimicrob. Chemother. 19, 527–532.
31 Bear, D.M., Turck, M. and Petersdorf, R.G. (1970) Med. Clins. N. Am. 54, 1145–1159.
32 Goodman, L.S. and Gilman, A. (1985) in The Pharmacological Basis of Therapeutics. 7th Edn., Ch. 50, pp. 1115–1149, Macmillan, New York.
33 Katzung, B.G. (1987) in Basic and Clinical Pharmacology. 3rd Edn., Ch. 43, pp. 516–526, Lange Medical Publications, Los Altos.
34 Mortimer, P.R., Mackie, D.B. and Haynes, S. (1969) Br. Med. J. 3, 88–89.