Biochem. J. (1982) **208**, 153–157
Printed in Great Britain

Effects of colchicine and vinblastine on output of proteins into bile

Philip P. GODFREY, Linda LEMBRA and Roger COLEMAN
Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

(*Received 18 January 1982/Accepted 7 July 1982*)

The microtubule poisons colchicine and vinblastine caused a reduction in the biliary output of total protein, compared with controls, in bile-fistula rats. The various protein components of bile showed patterns of change in output differing from one another. Alkaline phosphodiesterase I, a 'plasma-membrane' enzyme, showed a decline in output during the first hour after drug administration. Immunoglobulin A output did not decline until after the first hour. In contrast with these reductions, the biliary output of (rat) plasma albumin into bile was increased. At no time was there any evidence (as measured by lactate dehydrogenase release into bile) for any cytolytic damage. These results are discussed in relation to current theories on the output of proteins into bile and the repair of the plasma membrane in the bile canaliculus.

A considerable number of proteins have now been identified in mammalian bile. Proteins derived from serum contribute a substantial proportion of the total, but most of these occur at lower concentrations and in different relative proportions to their contributions in serum (Mullock *et al.*, 1978). A number of hepatocyte enzymes have also been detected; these appear to be derived largely from the plasma membrane and lysosomes with other characteristic intracellular enzymes being present in much lower relative concentration (Holdsworth & Coleman, 1975; Coleman *et al.*, 1979; La Russo & Fowler, 1979; Godfrey *et al.*, 1981).

Of the serum proteins present in rat bile, two (albumin and immunoglobulin A) are present at relatively high concentration. Immunoglobulin A, in polymeric form, bound to secretory component (Le Maitre-Coelho *et al.*, 1977; Mullock & Hinton, 1981) and also to the haptoglobin–haemoglobin complex (Hinton *et al.*, 1980) are transferred in the hepatocyte from blood to bile via a system of endocytotic vesicles (Mullock *et al.*, 1979, 1980*a*).

Treatment with the microtubule poisons colchicine, vinblastine or vincristine inhibits the secretion into blood of serum proteins and very-low-density lipoprotein (Orci *et al.*, 1973; Le Marchand *et al.*, 1974; Stein *et al.*, 1974; Feldman *et al.*, 1975; Redman *et al.*, 1975).

In the present study, microtubule poisons are investigated for their effects on the output of a number of proteins into bile.

Materials and methods

Male Wistar rats, weighing approx. 300g, were used throughout. These had been maintained on a standard laboratory diet and under a constant light cycle. Bile-duct cannulations were performed while each rat was under pentobarbital anaesthesia. After cannulation, bile was collected for 60min, then colchicine (20μmol/100g body wt.), vinblastine (2.5mg/rat or 4.0mg/rat) or 0.9% NaCl was administered into a jugular vein: each drug was dissolved in 1.0ml of sterile iso-osmotic NaCl. Bile was then collected for a further 3h; the volume of fluid lost in bile was replaced by intravenous infusion of iso-osmotic sterile NaCl. Bile was collected in ice and, for most experiments, was stored at −20°C until assayed. At the end of the experiment the animal was killed and samples of liver were removed and homogenized in 9vol. of 0.14M-NaCl/15mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4, by using a tightly fitting Potter–Elvehjem homogenizer. The homogenate was filtered through coarse nylon mesh and was frozen at −20°C until required; storage at −20°C did not affect the activities of either homogenate or biliary enzymes.

Antisera to rat albumin and rat immunoglobulin A were obtained from Nordic Immunological Laboratories, Maidenhead, Berks., U.K. Vinblastine sulphate (Velbe) was from Eli Lilly, Basingstoke, Hants., U.K. Colchicine and other fine chemicals were from Sigma (London) Chemical Co., Poole, Dorset, U.K.

Samples were assayed for phosphodiesterase I (EC 3.1.4.1) and lactate dehydrogenase (EC 1.1.1.27) as described previously (Godfrey *et al.*, 1981). Protein was estimated by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Rat albumin and rat immunoglobulin A were determined by quantitative immunodiffusion by the method of Mancini *et al.* (1965).

0306-3283/82/100153-05$01.50/1

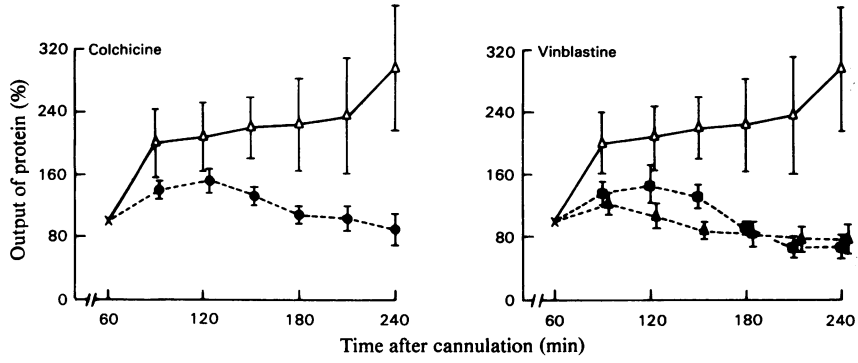

Fig. 1. *Effects of colchicine or vinblastine on biliary output of protein*
Biliary output is defined as the amount of the component in the bile sample divided by collection time. The bile duct was cannulated at time zero and bile was collected for 60 min. Symbols: ●, colchicine (20 μmol/100 g body wt.); ■, vinblastine (2.5 mg/rat); ▲, vinblastine (4.0 mg/rat); △, control saline. All treatments were administered intravenously in 1 ml of saline. Bile was collected for a further 3 h. Results are means ± S.E.M. ($n = 5$ for treated animals; $n = 7$ for controls) and are expressed as percentages of the output at the time of introduction of the drug. Initial (100%) values for protein (μg/min): colchicine series, 76 ± 17; vinblastine (2.5 mg) series, 129 ± 38; vinblastine (4.0 mg) series, 97 ± 23; saline controls, 82 ± 21.

Table 1. *Volume of bile secreted by control and treated rats*
Results are means ± S.E.M. ($n = 7$ for controls; $n = 5$ for treated animals). The drug, or iso-osmotic NaCl, was administered at 60 min. Colchicine was administered at 20 μmol/100 g body wt.

Time of collection (min)	Bile secretion (μl/min)			
	Controls	Colchicine	Vinblastine (2.5 mg/rat)	Vinblastine (4.0 mg/rat)
0–20	20.5 ± 1.5	20.6 ± 1.8	23.1 ± 2.1	24.1 ± 0.8
20–40	18.5 ± 1.2	19.5 ± 1.9	21.6 ± 1.0	19.8 ± 0.95
40–60	18.9 ± 1.0	19.4 ± 1.3	21.0 ± 1.0	20.1 ± 0.8
60–90	19.7 ± 1.5	22.1 ± 2.4	25.8 ± 1.3	28.1 ± 1.2
90–120	21.1 ± 1.7	23.0 ± 1.4	23.6 ± 1.0	25.8 ± 1.2
120–150	20.8 ± 1.2	22.5 ± 1.5	26.6 ± 1.1	24.7 ± 1.2
150–180	21.0 ± 1.1	21.2 ± 1.1	24.4 ± 1.0	21.8 ± 0.7
180–210	21.2 ± 1.7	19.7 ± 0.9	22.3 ± 1.2	21.0 ± 0.6
210–240	22.2 ± 1.9	17.4 ± 1.0	22.4 ± 0.9	19.8 ± 0.5

Results and discussion

In control rats the volume of bile secreted remained relatively constant (Table 1). However, an initial choleresis was observed after treatment with both drugs. This slowly decreased, such that by completion of the experiment, bile flow in the rats dosed with colchicine and higher amounts of vinblastine was less than in controls (Table 1). Outputs of bile salts and phospholipid were not significantly affected, in the time-scale of these experiments (results not shown); this agrees with the results *in vivo* of Stein *et al.* (1974) and Dubin *et al.* (1980).

Total protein output in control biles increased considerably after 60 min (from 4.2 to 8.4 mg/ml) and continued to rise for the rest of the experiment. Biliary protein output in treated rats also increased, to a smaller extent, just after the drugs were administered, but then slowly declined (Fig. 1).

Biliary activity of the cytosol marker enzyme, lactate dehydrogenase, was 0–3.0 μmol/h per ml (about 0.001% of the liver activity per hour) in both treated and control animals (results not shown); this low output is similar to that obtained in previous studies (Godfrey *et al.*, 1981), and indicates that no cytolytic damage is caused to the hepatocytes by either colchicine or vinblastine.

Serum proteins

Endocytotic vesicles are thought to carry the immunoglobulin secretory component and haptoglobin–haemoglobin complexes from the sinusoidal membrane to the bile canalicular pole of the hepatocyte (Birbeck *et al.*, 1979; Mullock *et al.*,

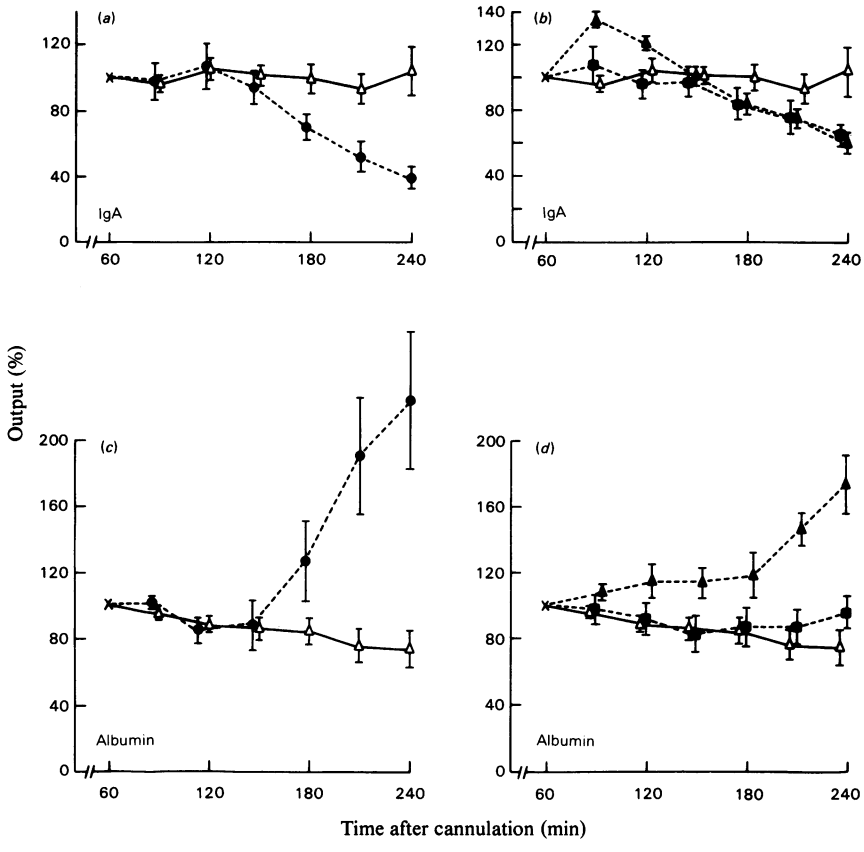

Fig. 2. *Effects of colchicine (a and c) and vinblastine (b and d) on biliary output of immunoglobulin A (IgA) and albumin*
Methods and symbols are as described in the legend to Fig. 1. 100% values for immunoglobulin A (arbitrary units/min): colchicine series, 275 ± 40; vinblastine (2.5 mg) series, 263 ± 30; vinblastine (4.0 mg) series, 317 ± 46; saline controls, 265 ± 39. 100% values for rat plasma albumin (arbitrary units/min): colchicine series, 205 ± 40; vinblastine (2.5 mg) series, 461 ± 65; vinblastine (4.0 mg) series, 247 ± 43; saline controls, 341 ± 74.

1979, 1980*a*; Hinton *et al.*, 1980). Colchicine and, to a lesser extent, vinblastine inhibit the secretion of immunoglobulin A into bile (Fig. 2), commencing at about 90 min after administration of the drugs. This is a similar time scale to that seen in colchicine inhibition of insulin secretion by pancreas (Dustin, 1978) and for inhibition of biliary lipid secretion by the isolated perfused rat liver (Gregory *et al.*, 1978).

The results show a general similarity to the results of other studies on inhibition of protein and lipid secretion into blood (Orci *et al.*, 1973; Stein *et al.*, 1974; Redman *et al.*, 1975; Morland *et al.*, 1981) and fluid endocytosis (Ose *et al.*, 1980) by liver cells. Mullock *et al.* (1980*b*) have suggested that the microtubule poisons might inhibit both movement of the secretory component from the Golgi complex to the sinusoidal membrane and movement of endocytotic vesicles from the sinusoidal membrane to the canaliculus. The decrease in endocytotic vesicle movement may account for the decline in protein output in treated rats.

In animals given colchicine and the higher dose of vinblastine, output of the other serum protein, albumin, increased considerably during the period that immunoglobulin A declined (Fig. 2). The normal output of albumin into blood is by a system of vesicles that deliver albumin from the Golgi apparatus to the sinusoidal membrane; this transport can be disrupted by microtubule poisons (Le Marchand *et al.*, 1974; Stein *et al.*, 1974; Redman *et al.*, 1975). One explanation for the increased biliary output of albumin after colchicine or vinblastine treatment may be that some of these vesicles may mistakenly discharge into bile, especially in view of the close proximity (Evans *et al.*, 1980) of the Golgi apparatus to the canalicular membrane.

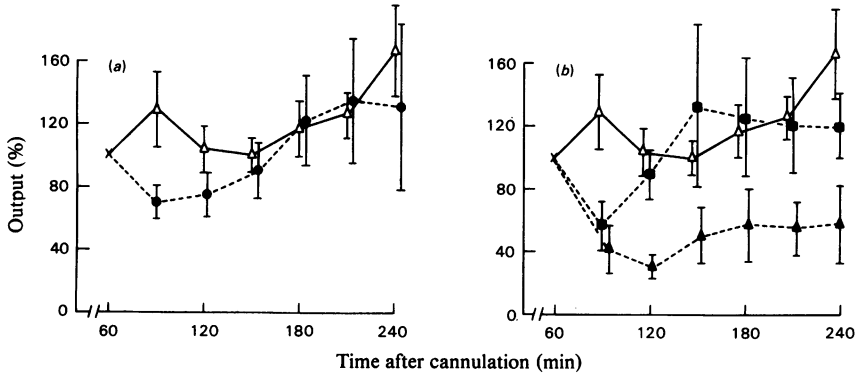

Fig. 3. *Effects of colchicine (a) and vinblastine (b) on biliary output of alkaline phosphodiesterase I*
Methods and symbols are as described in the legend to Fig. 1. 100% values for alkaline phosphodiesterase I output (μmol of substrate hydrolysed/min): colchicine series, 0.62 ± 0.13; vinblastine (2.5 mg) series, 1.91 ± 0.62; vinblastine (4.0 mg) series, 1.39 ± 0.36; saline controls, 0.88 ± 0.24.

The normal entry of albumin into bile, however, has been suggested to be by diffusion across the tight junctions (Dive *et al.*, 1974; Dive & Heremans, 1974; Mullock & Hinton, 1981); a further possibility, therefore, for the increase in biliary albumin could be that the microtubule poisons have in some way brought about an increase in the permeability of the tight junctions.

Plasma-membrane enzymes

There is a rapid and immediate decline in biliary output of phosphodiesterase I after administration of both colchicine and vinblastine (Fig. 3); this decrease is maintained in the rats given the higher dose of vinblastine. This rapid decline could be due to a direct effect on the enzyme, unrelated to any effect on the microtubule system (see Stein & Stein, 1977; Dustin, 1978; Brady *et al.*, 1980), but it is unlikely that two drugs of dissimilar structure would have the same inhibitory effect on alkaline phosphodiesterase I. In addition, Leoni *et al.* (1980) have shown that several plasma-membrane enzymes are insensitive to colchicine *in vitro*.

Another possibility is an effect of the two drugs on the shedding process of the plasma-membrane enzyme (by vesiculation or solubilization; see Godfrey *et al.*, 1981) and, in this context, colchicine has been shown to have an inhibitory effect on the shedding of membrane immunoglobulin D from spleen cells (Emerson & Cone, 1980).

The most probable effect, however, is upon the movement of vesicles repairing the plasma membrane. Recent experiments have shown delayed incorporation of newly synthesized glycoproteins into the canalicular membrane when compared with the sinusoidal membrane. This has led to the suggestion that some canalicular membrane glycoprotein may be derived from the sinusoidal membrane via endocytotic vesicles (Buchsel *et al.*, 1980; Evans *et al.*, 1980; Evans, 1981). Disruption of the transport of these vesicles by colchicine or vinblastine may account for some of the decline in biliary phosphodiesterase I activity, though the speed of this effect relative to the influence of these drugs on immunoglobulin A output indicates that these repair vesicles may be of a separate class from those carrying immunoglobulin A.

Differential timing of drug effects

The different response times and directions of response of the various protein output systems serve to illustrate the wide variety of mechanisms necessary to elaborate the protein output profile of bile.

P. P. G. is in receipt of a University of Birmingham Demonstratorship. We thank the M.R.C. for financial support.

References

Birbeck, M. S. C., Cartwright, P., Hall, J. G., Orlans, E. & Peppard, J. (1979) *Immunology* **37**, 477–482

Brady, S. T., Crothers, S. D., Nosal, S. & McClure, W. D. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5909–5913

Buchsel, R., Berger, D. & Reutter, W. (1980) *FEBS Lett.* **113**, 95–98

Coleman, R., Iqbal, S., Godfrey, P. P. & Billington, D. (1979) *Biochem. J.* **178**, 201–208

Dive, C. & Heremans, J. F. (1974) *Eur. J. Clin. Invest.* **4**, 235–239

Dive, C., Nadalini, R. A., Vaeriman, J.-P. & Heremans, J. F. (1974) *Eur. J. Clin. Invest.* **4**, 240–246

Dubin, M., Maurice, M., Feldman, G. & Erlinger, S. (1980) *Gastroenterology* **79**, 646–654
Dustin, P. (1978) *Microtubules*, Springer-Verlag, Berlin
Emerson, S. G. & Cone, R. E. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6582–6586
Evans, W. H. (1981) *Hepatology* **1**, 452–457
Evans, W. H., Flint, N. A. & Vischer, P. (1980) *Biochem. J.* **192**, 903–910
Feldman, G., Maurice, M., Sapin, C. & Benhamov, J. P. (1975) *J. Cell Biol.* **67**, 237–242
Godfrey, P. P., Warner, M. J. & Coleman, R. (1981) *Biochem. J.* **196**, 11–16
Gregory, D. H., Vlahcevic, Z. R., Prugh, M. F. & Swell, L. (1978) *Gastroenterology* **74**, 93–100
Hinton, R. H., Dobrota, M. & Mullock, B. M. (1980) *FEBS Lett.* **112**, 247–250
Holdsworth, G. & Coleman, R. (1975) *Biochim. Biophys. Acta* **389**, 47–50
La Russo, N. F. & Fowler, S. (1979) *J. Clin. Invest.* **64**, 948–954
Le Maitre-Coelho, I., Jackson, G. D. F. & Vaerman, J.-P. (1977) *Eur. J. Immunol.* **7**, 588–590
Le Marchand, Y., Patzelt, C., Jeannet, F. A., Coten, E. G. & Jeanrenaud, B. (1974) *J. Clin. Invest.* **53**, 1512–1520
Leoni, S., Spagnuolo, S., Conti-Devergiliis, L., Baldini, P., Incerpi, S. & Luly, P. (1980) *Biochim. Biophys. Acta* **596**, 451–455
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Mancini, G., Carbonara, A. O. & Hereman, J. F. (1965) *Immunochemistry* **2**, 235–245
Morland, J., Rothschild, M. A., Oratz, M., Mongelli, J., Donor, D. & Schreiber, S. S. (1981) *Gastroenterology* **80**, 159–165
Mullock, B. M. & Hinton, R. H. (1981) *Trends Biochem. Sci.* **6**, 188–191
Mullock, B. M., Dobrota, M. & Hinton, R. H. (1978) *Biochim. Biophys. Acta* **543**, 497–507
Mullock, B. M., Hinton, R. H., Dobrota, M., Peppard, S. & Orlans, T. (1979) *Biochim. Biophys. Acta* **587**, 381–391
Mullock, B. M., Hinton, R. H., Dobrota, M., Peppard, J. & Orlans, T. (1980*a*) *Biochem. J.* **190**, 819–826
Mullock, B. M., Jones, R. S., Peppard, J. & Hinton, R. H. (1980*b*) *FEBS Lett.* **120**, 278–282
Orci, L., Le Marchand, Y., Singh, A., Jeannet, F. A., Rouiller, C. & Jeanrenaud, B. (1973) *Nature (London)* **244**, 30–32
Ose, L., Ose, T., Reinersten, R. & Berg, T. (1980) *Exp. Cell Res.* **126**, 109–119
Redman, C. M., Bannerjee, D., Howell, K. & Palade, G. E. (1975) *J. Cell Biol.* **66**, 42–59
Stein, O. & Stein, Y. (1977) in *Membrane Alterations as a Basis for Liver Injury* (Popper, H., Bianchi, L. & Reutter, W., eds.), pp. 257–266, MTP Press, Lancaster, U.K.
Stein, O., Sanger, L. & Stein, Y. (1974) *J. Cell Biol.* **62**, 90–103