# EFFECT OF COLCHICINE ON THE TRANSFER OF IgA ACROSS HEPATOCYTES INTO BILE IN ISOLATED PERFUSED RAT LIVERS

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## 1. Introduction

In hepatocytes a stream of endocytic vesicles moves rapidly from the sinusoidal surface to the cytoplasm in the region of the bile canaliculus. The vesicles carry polymeric IgA or the haptoglobin–haemoglobin complex if these are available in the blood [1–4]. The rapid concentration in a particular region of cytoplasm suggests that cytoskeletal elements may be involved. Accordingly we have examined the effects of the microtubule-disruptive drug, colchicine, on the transport of IgA across hepatocytes.

Transportation of IgA across hepatocytes involves at least 4 separate processes which could require participation of microtubules.

(1) Before uptake of IgA can take place, the receptor for IgA, the glycoprotein secretory component, which is synthesised in hepatocytes [5], must itself reach the sinusoidal plasma membrane [6]. Such movement of glycoproteins from the Golgi apparatus to the plasma membrane is inhibited by colchicine although glycoprotein synthesis is unaffected [7].

(2) However, if this were the only point of interference, the secretory component already on the membrane before the addition of colchicine would be expected to continue to transfer any IgA available to it, and also continue to move to the bile on its own [4]. The actual formation of endocytic vesicles is unlikely to be inhibited since in [8] the movement of the plasma membrane enzyme 5′-nucleotidase into the interior of the cell was unaffected by colchicine.

(3) The next stage in the transfer process, the rapid movement of shuttle vesicles from the sinusoidal plasma membrane to the pericanalicular cytoplasm, might well be inhibited. The secretory component which was on the sinusoidal plasma membrane at the time of colchicine addition might not transfer into bile.

(4) Finally, the release of material from the vesicles into the bile might be inhibited.

We now report that colchicine appears to inhibit both the movement of secretory component from the Golgi apparatus to the sinusoidal plasma membrane and the movement of secretory IgA and of free secretory component to the pericanalicular cytoplasm in the isolated perfused rat liver.

## 2. Materials and methods

Liver perfusion experiments were performed as in [4]. L-[4,5-$^3$H] Leucine (160 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, Bucks). Colchicine, obtained from Sigma (London) was added to the perfusion medium to give 50 $\mu$M final conc., which has been shown to be in the middle of the concentration range for maximal inhibition of plasma protein release and asialoglycoprotein uptake and degradation [7,9]. Protein synthesis and nucleotide phosphate levels are not affected [7]. Rat monoclonal polymeric IgA was prepared and labelled with $^{125}$I as in [2]. $^{125}$I in bile samples was counted directly on a PRIAS liquid scintillation counter (Packard Instruments, Downers Grove, IL). Acid-precipitable $^3$H in the perfusion medium was determined and crossed immunoelectrophoresis of bile samples

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against anti-(rat bile) carried out as in [10]. Free secretory component, which is electrophoretically faster moving than secretory IgA, and albumin in bile were estimated from the areas of the appropriate peaks on the crossed immunoelectrophoresis plates. IgA in bile samples was assayed by rocket immunoelectrophoresis [11] in gels containing an antiserum to rat IgA heavy chain [12].

## 3. Results

Rat livers perfused with media containing radioactive precursors steadily secrete labelled proteins into the medium [4]. As expected [7] the rate of this secretion was reduced after addition of colchicine to the medium (fig.1a).

The transport of $^{125}$I-labelled IgA from the perfusion medium to bile resembled that seen in the intact animal [12] with an initial lag period of ~30 min followed by a steady rise. Recovery of $^{125}$I-labelled IgA in the bile did not approach that seen in intact animals [12] presumably because the IgA level in the relatively large volume of perfusion medium remained relatively steady unlike the serum level in the intact animal. The amount of IgA added was insufficient to saturate the transport system and a further addition of IgA 3 h after the first produced a further rise in the radioactivity of bile after the characteristic 30 min lag (fig.1b).

Addition of colchicine to the medium 15 min before addition of labelled IgA greatly reduced the initial rise in biliary radioactivity and almost obliterated the rise after the second addition of IgA (fig.1b). In a similar experiment (not shown) where colchicine was added between the doses of IgA, the rise in activity in the bile after the second addition of IgA was prevented in a liver which had already shown a normal ability to transport IgA.

The amount of free secretory component per unit volume of bile from normal perfused livers rose with time (fig.2), since only negligible amounts of secretory component were reaching bile as secretory IgA. Addition of colchicine caused an immediate drop in the amounts of free secretory component appearing in the bile and, if added early in the perfusion, prevented any rise (fig.2). Levels of major serum proteins, exemplified by albumin, per unit volume of bile remained steady in the absence of colchicine. About 1 h after colchicine addition however, levels

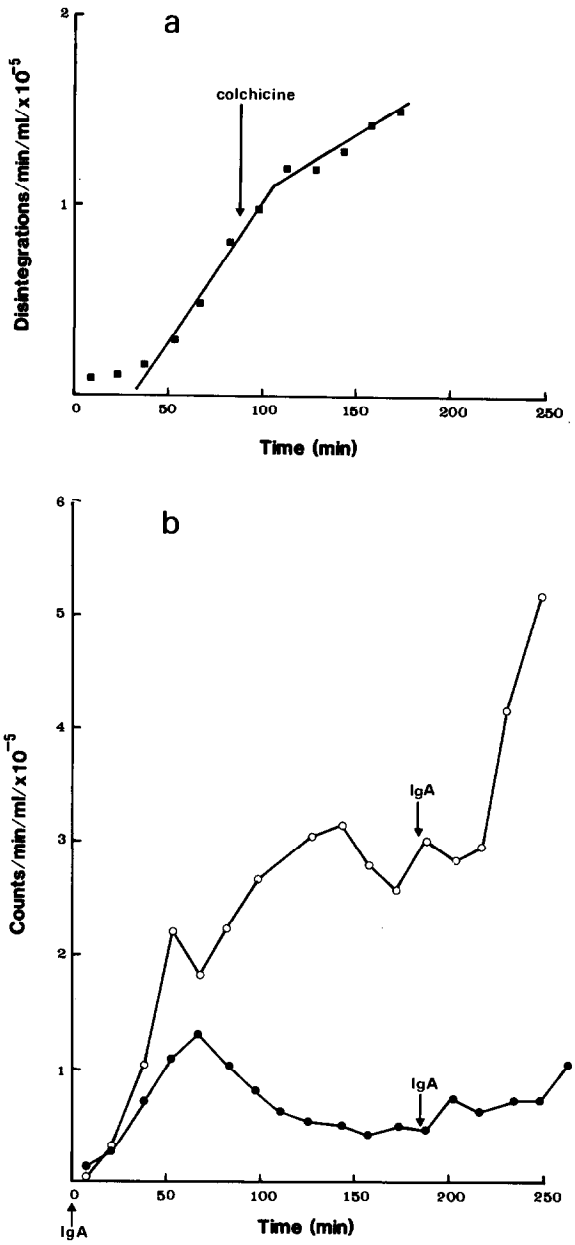


Fig.1. Effects of 50 μM colchicine following the addition of L-[4,5-$^3$H]leucine or $^{125}$I-labelled IgA to the recirculating perfusion medium supplied to isolated rat livers on the appearance of (a) acid-precipitable [$^3$H]leucine in perfusion medium and (b) $^{125}$I-labelled IgA in bile. Radioactivity is plotted against time after addition of the isotopically labelled compound to the medium. A second addition of $^{125}$I-labelled IgA was made at the times marked. Each line shown illustrates an experiment on a single liver but similar results were obtained on at least two livers in each case. (a) Colchicine was added at the time indicated; (b) (○) no colchicine, (●) colchicine added 15 min before $^{125}$I-labelled IgA.

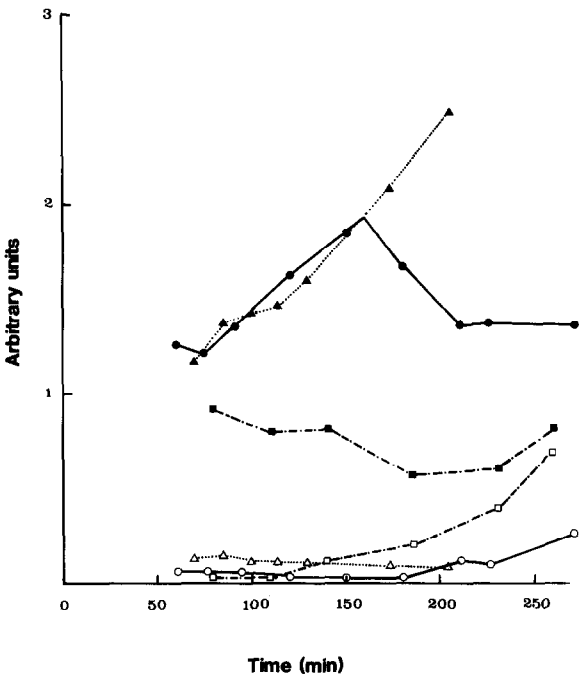


Fig.2. Effects of 50 μM colchicine on amounts of free secretory component and albumin appearing in bile from isolated rat livers. Amounts of secretory component (closed symbols) and albumin (open symbols) in arbitrary units are plotted against time after cannulation of the bile duct. Each line shown illustrates an experiment on a single liver but similar results were obtained on at least two livers in each case. (▲ . . . ▲, △ . . . △) No colchicine; (●——●, ○——○) colchicine added after 160 min; (■–.–.–■, □–.–.–□) colchicine added as soon as the liver was established in the perfusion apparatus (60 min).

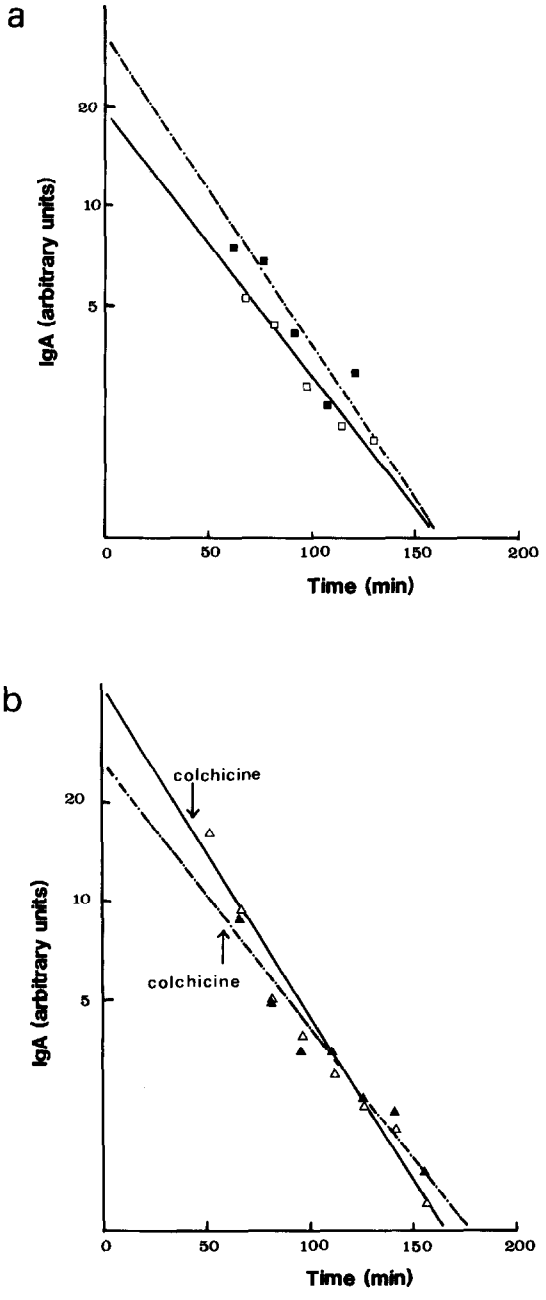


Fig.3. Effect of colchicine on amounts of IgA appearing in bile from isolated rat livers. Amounts of IgA in arbitrary units are plotted on a logarithmic scale against time after cannulation of the bile duct. (a) No colchicine; (b) colchicine added as soon as the liver was established into the perfusion apparatus: bile collection for the first point began immediately. The results of two independent experiments are illustrated in each half of the diagram. (□——□, ■. . .■) Normal livers, $t^{1/2}$ 37 and 32 (min); (▲ . . . ▲, △——△) colchicine-treated livers, $t^{1/2}$ 37 and 30 (min).

of albumin (and other serum proteins) began to rise slowly (fig.2).

The release of vesicles from the pericanalicular space into the bile was examined by measuring the rate of the fall in biliary IgA levels with time after removal of the liver from the rat. Initially the vesicles in the pericanalicular space will be loaded with IgA. When the liver is removed from the rat the IgA supply is cut off and vesicles reaching the pericanalicular cytoplasm thereafter will have little or no IgA. Fig.3 shows that the fall in biliary IgA levels is apparently unaffected by the presence of colchicine.

## 4. Discussion

Colchicine clearly inhibits the transfer of $^{125}$I-labelled IgA from blood to bile, it would therefore appear that microtubules are important in at least some part of this process. As described in section 1, colchicine might interfere with the process at several points. The release of IgA from the pericanalicular cytoplasm into the bile, the transfer of endocytic shuttle vesicles carrying secretory IgA and free secretory component and the supply of secretory component to the sinusoidal plasma membrane might each or all be affected by colchicine. An effect on endocytic vesicle formation is unlikely [8].

From our results it appears that colchicine has no effect on the final part of the process, i.e., the release from endocytic vesicles of secretory IgA into bile. Fig.3 indicates that in both normal and colchicine-treated liver the release of IgA was a first order reaction with random release of the contents of endocytic shuttle vesicles waiting near the bile canalicular plasma membrane [1].

Colchicine would, however, appear to inhibit the movement of endocytic vesicles from the sinusoidal plasma membrane to the bile canalicular plasma membrane. As pointed out in section 1, if only the transport of secretory component to the sinusoidal plasma membrane were inhibited, the secretory component already on the plasma membrane should move to the bile in the normal way taking ~30 min. to do so. However, measurements of the amount of free secretory component in the bile (fig.2) showed that the steady rise seen in normal livers (due to only trace amounts of IgA with which to combine) was immediately stopped by the addition of colchicine and levels of free secretory component thereafter begin to fall. We conclude that the rapid movement of endocytic shuttle vesicles depends on intact microtubules. A similar conclusion was reported from an immunoelectron microscopic study of the movement of IgA-containing vesicles across cultured human neoplastic colon cells [13].

The movement of shuttle vesicles from sinusoidal to bile canalicular plasma membranes was not completely inhibited in colchicine-treated livers since some labelled IgA appears in bile even when colchicine is added beforehand (fig.1b). Such partial inhibition by colchicine (~60%) is in accordance with results on both secretion [7] and endocytosis [9,14] in hepatocytes.

The time course of the appearance of labelled IgA in the bile from a colchicine-treated liver suggests that the supply of secretory component to the sinusoidal plasma membrane was inhibited in addition to the inhibition of endocytic vesicle movement. Initially, when the sinusoidal plasma membrane was loaded with secretory component, amounts of IgA appearing in bile whilst clearly subnormal, did increase (fig.1b). However, ~90 min after colchicine addition, amounts of labelled IgA reaching the bile began to fall and a second addition of labelled IgA produced little response. It appears likely, then, that colchicine inhibits the supply of free secretory component to the sinusoidal plasma membrane, the amounts of labelled IgA reaching bile falling off as soon as the secretory component initially present on the plasma membrane has been used up. Such an inhibition resembles that seen for plasma protein secretion [7].

Microtubules would thus appear to be required both for the rapid and specific transfer of free secretory component from the Golgi region to the sinusoidal plasma membrane and for the movement of endocytic vesicles from the region of the sinusoidal surface to the region of the bile canalicular surface of the cell. Such involvement of microtubules in the rapid movement of membraneous vesicles both to and from a surface could be similar to that observed in nerve cells [15].

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