2 Thomson, N.C., Patel, K.R., and Kerr, J.W., Thorax *33* (1978) 694.
3 Cerrina, J., Denjean, A., Alexandre, G., and Lockhart, A., Am. Rev. resp. Dis. *123* (1981) 156.
4 Patel, K.R., Br. med. J. *282* (1981) 932.
5 Rolla, G., Bucca, C., Polizzi, S., Maina, A., Giachina, O., and Salvini, P., Lancet *1* (1982) 1305.
6 Foreman, J.C., and Garland, L.G., Br. med. J. *1* (1976) 820.
7 Douglas, W.W., in: The pharmacological basis of therapeutics, p.609. Eds A. Goodman Gilman, L.S. Goodman and A. Gilman. Macmillan, New York 1980.
8 Tanazaki, Y., and Townley, R.G., Int. Archs Allergy appl. Immun. (1983) in press.
9 Fanta, C.H., Venugopalan, C.S., Lacouture, P.G., and Drazen, J.M., Am. Rev. resp. Dis. *125* (1982) 61.
10 Cheng, J.B., and Townley, R.G., Archs int. Pharmacodyn. Ther. *263* (1983) 228.
11 Farley, J.M., and Miles, P.R., J. Pharmac. exp. Ther. *207* (1978) 341.
12 Bellemann, P., Ferry, D., Lubbecke, F., and Glossmann, H., Drug Res. *31* (1981) 2067.
13 Glossmann, H., Ferry, D.R., Lubbecke, F., Mewes, R., and Hofmann, F., Trends pharmac. Sci. *3* (1982) 431.
14 Williams, L.T., and Tremble, P., J. clin. Invest. *70* (1982) 209.
15 Ehlert, F.J., Itoga, E., Roeske, W.R., and Yamamura, H.I., Biochem. biophys. Res. Commun. *104* (1982) 937.
16 Bolger, C.T., Gengo, R.J., Luchowski, E.M., Siegel, H., Trigle, D.J., and Janis, R.A., Biochem. biophys. Res. Commun. *104* (1982) 1604.
17 Murphy, K.M.M., and Snyder, S.H., Eur. J. Pharmac. *77* (1982) 201.
18 Cheng, J.B., and Townley, R.G., Life Sci. *30* (1982) 2079.

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Participation of microtubules and microfilaments in the transcellular biliary secretion of immunoglobulin A in primary cultures of rat hepatocytes[1]

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Summary. The biliary secretion of immunoglobulin A (IgA) in primary hepatocyte cultures was investigated by means of immunofluorescence. The characteristic accumulation of IgA in visible bile canaliculi was found to be strongly inhibited by vinblastine and colchicine or by cytochalasin B, but its surface binding was not.

Microtubules in conjunction with contractile microfilaments are known to play a role in phenomena of cellular and intracellular mobility, such as cellular organelle movements and endo- and exocytosis[2,3]. For instance, microtubular dysfunction in the liver caused by colchicine is associated with decreased secretion into the blood of albumin[4,5], fibrinogen[6], and and lipoproteins[7]. It is also established now that antimicrotubular and antimicrofilamentous drugs interfere with certain aspects of biliary secretion like transport of lipids[8], bile acids[9,10] or output of proteins into bile[11,12]. However, the mechanisms of this interference are only poorly understood and deserve further investigation in different experimental systems.

As pointed out in recent publications from this laboratory, primary monolayer cultures of adult rat hepatocytes form de novo a structural and functional biliary polarity[13,14] and provide a valuable model for examining the mechanisms of the transcellular and biliary transport of organic anions[15] and of immunoglobulin A[16] performed by the liver. The present report describes the influence of different drugs interacting with cytoskeletal elements on the biliary secretion of IgA in cultured hepatocytes.

Materials and methods. Male Sprague-Dawley rats (220–290 g) kept on a standardized diet of Alma[36] were used for isolating liver parenchymal cells as described[16]. Hepatocytes were maintained on collagen-coated cover slips in W/AB 77 medium[17] for up to 4 days. Details of cultivation are described[15,16]. Uptake and transcellular transport of IgA by the cultured cells was followed by direct immunofluorescence as described in a previous study[16]: on the 3rd day of cultivation cultures were exposed to human IgA (Biotest-Serum Institut, Frankfurt) at a concentration of 0.5 mg/ml for 2–5 h. Subsequently, cultures were fixed on ice with glutaraldehyde (2.5% for 3 min), methanol (5 min) and acetone (5 min) followed by a mixture of acetone and phosphate-buffered saline (PBS) pH 7.4 (1/1;v/v) (10 min) and were then rinsed extensively with PBS. The procedure for detection of IgA by direct immunofluorescence using fluoresceinated rabbit antihuman IgA (Behring Institute, Marburg) and the specificity of the immunologic reaction were described previously[16]. To define the influence of several drugs on uptake and secretion of IgA, hepatocyte cultures were incubated in the presence of these drugs either simultaneously with or prior to IgA as described in the legends to the figures. Individual experiments were repeated at least twice in order to evaluate the significance of the observations. Vinblastine sulfate and colchicine (Sigma, München) were used in a final concentration of 10 µg/ml and 50 µg/ml, respectively. Cytochalasin B (Sigma) dissolved in dimethylsulfoxide (10 mg/ml) was used in a final concentration of 100 µg/ml. Dimethylsulfoxide alone was without effect on the cells. Viability of the drug-exposed cells was routinely evaluated by several common viability tests[17] and on the basis of the ability of the cells to perform urea synthesis[18] and other metabolic functions[17].

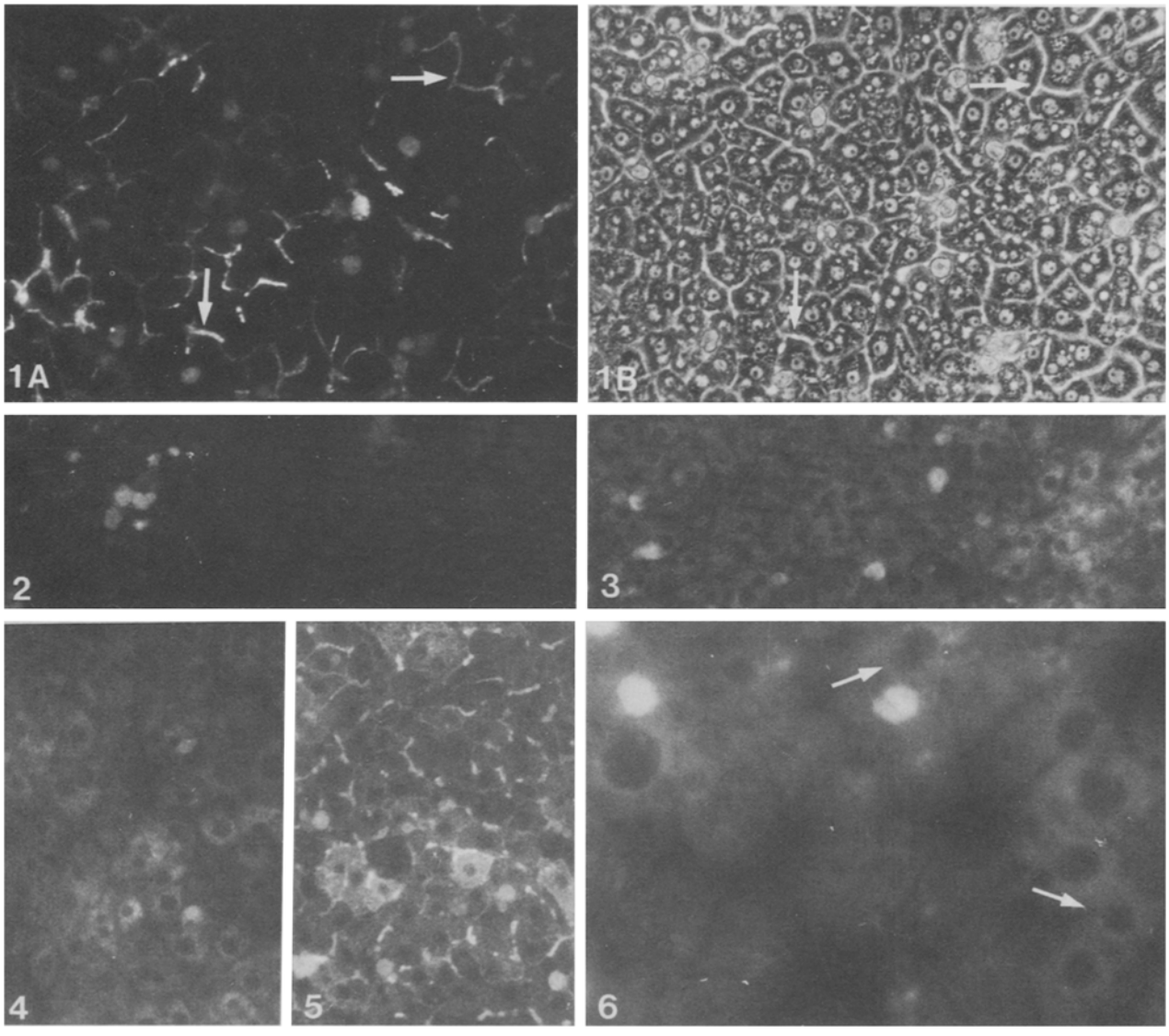
Results and discussion. Cultured hepatocytes exposed to IgA have been shown to take up the immunoglobulin in a process mediated by its recepter, the so-called secretory component[16,19,20]. In a previous publication we were able to show that the internalized IgA is subject to a transcellular biliary secretion[16]. As illustrated in figure 1 (A and B) the final event of this transport visualized by immunofluorescence is the accumulation of the IgA within bile canaliculi[16] which are discernible by phase contrast microscopy as lucid enlargements of the intercellular space[14,15,18].

The accumulation of IgA could be considerably influenced by various agents known to interfere with cytoskeleton elements. The antimicrotubular drug vinblastine produced a complete inhibition of the transport (fig.3) including the intermediate 'vesicular' stage[16], whereas surface binding of

IgA was not affected as indicated by the presence of an intensive fluorescence covering the cytoplasm (compare figs 2 and 3). Colchicine had a similar effect only when added prior to (fig. 4) but not simultaneously with IgA (fig. 5). These cytochemical findings are in agreement with the biochemical results of Mullock et al.[11] obtained with isolated perfused rat liver and with those of Godfrey et al.[12] obtained in vivo. The different response to vinblastine and colchicine reported here may be explained by their different mode of action, namely disruption of preexisting microtubules and inhibition of microtubule polymerization, respectively[21].

Exposure of hepatocyte cultures to cytochalasin B caused a pronounced dilatation of the bile canaliculi within less than 1 h (fig. 6) similar to that observed in vivo[22]. The drug also impaired the transcellular transport of IgA, apparently by paralyzing uptake and translocation, since a diffuse fluorescence covering the cytoplasm (indicating binding to a secretory component[16]) could be detected, but no particulate fluorescence nor fluorescence accumulated at the biliary pole. These findings indicate that microfilaments participate in this kind of transport. It is not possible, however, to tell whether microfilaments are involved only in the uptake of IgA into the hepatocytes or also in the subsequent events of translocation to and excretion at the biliary pole.

In the cases of both the agents vinblastine and cytochalasin B, there is no difference in the distribution of fluorescence between cultures exposed for 2 or 5 h, whereas in control cultures a pronounced increase in the intensity of



Figures 1–6. Hepatocyte cultures incubated for 5 h in media containing human IgA (except fig. 2); 3rd day of cultivation.
Figure 1. *A* Fluorescence micrograph of cell monolayer showing many branching bile canaliculi between adjacent hepatocytes which are intensely stained for IgA (arrows). *B* Phase contrast micrograph of same culture. Bile canaliculi (arrows) appear as lucid enlargements of the intercellular space, though they are often poorly preserved due to the fixation procedure. ×210. Figure 2. Control culture incubated without IgA. Unspecific staining is present only on dead round cells on top of the monolayer. × 180. Figure 3. Treatment with vinblastine added simultaneously with IgA. A diffuse fluorescence covering the cytoplasm can be seen. Canaliculi are not stained and no particulate fluorescence is detectable. ×180. Figure 4. Treatment with colchicine added 2 h prior to IgA. No accumulation of fluorescence is detectable. × 180. Figure 5. Treatment with colchicine added simultaneously with IgA. A marked accumulation of fluorescence within bile canaliculi can be seen. ×180. Figure 6. Treatment with cytochalasin B added simultaneously with IgA. Fluorescence covering the cytoplasm appears diffuse. No accumulation of fluorescence can be seen at bile canaliculi many of which appear dilated (arrows). ×400.

fluorescence covering the bile canaliculi is observed, resulting from an ongoing secretion during this period[16]. These observations are in accordance with the view that excretion of IgA follows a transcellular and not a paracellular pathway.

Studying the secretion of horseradish peroxidase Kacich et al.[23] have obtained different results, suggesting that only microtubules but not microfilaments are involved. Whether this difference is due to possible alternative routes for the secretion of IgA and horseradish peroxidase or could be explained in another way is currently being investigated.

Each of the observed effects of these drugs on IgA transport seemed to be specifically mediated by their influence on elements of the cytoskeleton, as has also been found for the effect of vinblastine on canalicular morphology[18]. A general toxic deterioration of cell metabolism caused by these agents could be ruled out by measuring metabolic functions such as urea synthesis[18] and by performing viability tests (e.g. staining with trypan blue or leakage of lactate dehydrogenase) which all revealed a normal behavior of the drug-exposed hepatocytes.

The findings reported here suggest that both microtubules and microfilaments are involved in the transcellular vesicular transport of IgA and that primary cultures of hepatocytes may provide a suitable model for further defining the particular role of these cytoskeletal elements in this kind of secretion.

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2 Allison, A.C., in: Locomotion of Tissue Cells. CIBA Foundation Symposium vol. 14, p. 109. Ed. M. Abercrombie. Associated Scientific Publishers, New York 1973.
3 Bardele, C.F., Cytobiologie *7* (1973) 442.
4 LeMarchand, Y., Patzelt, C., Assimacopoulos-Jeannet, F., Loten, E.G., and Jeanrenaud, B., J. clin. Invest. *53* (1974) 1512.
5 Redman, C.M., Banerjee, D., Howell, K., and Palade, G.E., J. Cell Biol. *66* (1975) 42.
6 Feldmann, G., Maurice, M., Sapin, C., and Benhamou, J.P., J. Cell Biol. *67* (1975) 237.
7 Stein, O., Sanger, L., and Stein, Y., J. Cell Biol. *62* (1974) 90.
8 Gregory, D.H., Vlahcevic, Z.R., Prugh, M.F., and Swell, L., Gastroenterology *74* (1978) 93.
9 Dubin, M., Maurice, M., Feldmann, G., and Erlinger, S., Gastroenterology *79* (1980) 646.
10 Reichen, J., Berman, M.D., and Berk, P.D., Biochim. biophys. Acta *643* (1981) 126.
11 Mullock, B.M., Jones, R.S., Peppard, J., and Hinton, R.H., FEBS Lett. *120* (1980) 278.
12 Godfrey, P.P., Lembra, L., and Coleman, R., Biochem. J. *208* (1982) 153.
13 Jung, W., Gebhardt, R., and Mecke, D., Eur. J. Cell Biol. *27* (1982) 230.
14 Gebhardt, R., Jung, W., and Robenek, H., Eur. J. Cell Biol. *29* (1982) 68.
15 Gebhardt, R., and Jung, W., J. Cell Sci. *56* (1982) 233.
16 Gebhardt, R., Gastroenterology *84* (1983) 1462.
17 Gebhardt, R., and Mecke, D., Exp. Cell Res. *124* (1979) 349.
18 Gebhardt, R., Exp. Cell Res. *144* (1983) 218.
19 Orlans, E., Peppard, J., Fry, J.F., Hinton, R.H., and Mullock, B.M., J. exp. Med. *50* (1979) 1577.
20 Socken, D.J., Jeejeebhoy, K.N., Bazin, H., and Underdown, B.J., J. exp. Med. *50* (1979) 1538.
21 Wilson, L., Bamburg, J.R., Mizel, S.D., Grisham, L.M., and Creswell, K.M., Fedn Proc. *33* (1974) 158.
22 Phillips, M.J., Oda, M., Mak, E., Fisher, M.M., and Jeejeebhoy, K.N., Gastroenterology *69* (1975) 48.
23 Kacich, R.L., Renston, R.H., and Jones, A.L., Gastroenterology *80* (1981) 1360.

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Rabbit immunization to xenogeneic red blood cells following anterior eye chamber inoculation

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Summary. Inoculation of human red blood cells (HRBC) into the anterior chamber of the eye (AC) of rabbits effectively stimulated systemic antibody production. Intraocular (i.o.) inoculation was observed to be more effective a route of immunization than i.v. or i.m. inoculation of antigen. These results contradict the accepted belief that the AC is an immunologically privileged site in the body.

It is well established that allografts transplanted to the AC survive longer than grafts transplanted orthotopically or heterotopically to other body sites[2-5]. Unfortunately, the concept arose that the AC is an immunologically privileged site in an absolute sense, i.e. that foreign grafts transplanted there stimulate no immunity and are not rejected. Although the AC lacks lymphatic drainage[6,7], it does not lack vascular drainage, and grafts to the AC are not permanent. The following experiments were performed in an effort to clarify our understanding of the immunologically privileged status of the AC. The results of these experiments demonstrate that inoculation of HRBC into the AC of the rabbits eye vigorously stimulates the hosts immune system and results in the production of high titers of systemic antibody.

Materials and methods. Human blood (type - 0) in Alsever's solution was washed 5 times in Earle's balanced salt solution (EBSS) to remove serum components and buffy coat lymphocytes. HRBC were collected and diluted 1:3 in EBSS and used to immunize New Zealand White rabbits (2.0-2.5 kg each). All immunizations consisted of 0.25 ml of diluted HRBC. The immunization protocol is shown in table 1. I.m. inoculations were to the right flank, i.v. inoculations to the right marginal ear vein, and i.o. (intraocular) inoculations to the right AC. Rabbits immunized by the i.o. route were heavily sedated with sodium pentobarbital (Nembutal, Abbot Laboratories) and the periorbital nerves blocked by s.c. administration of lidocaine HCl (Xylocaine, Astra Laboratories). After sedation and anesthesia the eye was immobilized and a 27-ga needle was inserted through the sclera at the lateral limbus and directly into the AC. AC fluid (0.25 ml) was removed and replaced with an equal volume of HRBC. Loss of AC fluid and trauma to the eye was minimal.