

# Identity of hepatic membrane transport systems for bile salts, phalloidin, and antamanide by photoaffinity labeling

(hepatocytes/bile salt uptake/phallotoxins/organotropism)

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**ABSTRACT** Phalloidin, a bicyclic heptapeptide, and antamanide, a monocyclic decapeptide from the poisonous mushroom *Amanita phalloides*, interact with bile-salt-binding polypeptides of the hepatocyte membrane, as demonstrated by photoaffinity labeling using the photolabile bile salt derivative 7,7-azo-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -cholan-24-oic acid, either unconjugated or taurine conjugated. With the photolabile derivatives of phalloidin,  $N^{\delta}$ -{4-[(1-azi-2,2,2-trifluoroethyl)benzoyl]- $\beta$ -alanyl}- $\delta$ -aminophalloin, and of antamanide,  $\{N^{\epsilon}$ -[4-(1-azi-2,2,2-trifluoroethyl)benzoyl]lys<sup>6</sup>}-antamanide, the same membrane polypeptides with apparent  $M_r$ s of 54,000 and 48,000 were labeled as with the photolabile derivatives of unconjugated and conjugated bile salts. The presence of bile salts decreased markedly the extent of labeling of these phalloidin- and antamanide-binding polypeptides. These results indicate that hepatic uptake systems for bile salts, phallotoxins, and the cycloamanide antamanide are identical, thus explaining the organotropism of phallotoxins.

Phalloidin, a bicyclic toxic heptapeptide (1, 2) from the poisonous mushroom *Amanita phalloides*, is readily and selectively taken up by hepatocytes and exerts intracellularly its fatal effects on the stability of plasma membranes (3, 4). Antamanide, a monocyclic decapeptide (5) from the same toadstool, inhibits the uptake of phalloidin into hepatocytes and, in appropriate concentrations, prevents the impairment of cells (6).

A series of observations suggests that the uptake of phalloidin and other phallotoxins occurs by the same transport mechanism that is physiologically responsible for the membrane transport of bile salts into hepatocytes (7-10), thus leading to an explanation for organotropism.

Since the identification of bile-salt-binding polypeptides in sinusoidal membranes of hepatocytes has been achieved by photoaffinity labeling using different photolabile bile salt derivatives (11-14), differential labeling in the presence of phalloidin or antamanide should give indirect proof for the involvement of the same polypeptides in hepatic membrane transport. Direct proof is established if photoaffinity labeling of plasma membranes and of isolated hepatocytes, using photolabile derivatives of phalloidin and antamanide, results in the labeling of identical polypeptides. This labeling must be decreased in the presence of bile salts. Photoaffinity labeling studies with 7,7-azo-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -cholan-24-oic acid and its taurine conjugate, with  $N^{\delta}$ -{4-[(1-azi-2,2,2-trifluoroethyl)benzoyl]- $\beta$ -alanyl}- $\delta$ -aminophalloin and with  $\{N^{\epsilon}$ -[4-(1-azi-2,2,2-trifluoroethyl)benzoyl]lys<sup>6</sup>}-antamanide demonstrated that identical polypeptides are involved in the uptake of bile salts, phalloidin, and antamanide into hepatocytes.

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## MATERIALS AND METHODS

**Materials.** Phalloidin was a sample from the laboratory of Theodor Wieland. The water-soluble *O*-carboxymethyl-tyr<sup>6</sup>-antamanide was prepared as described (15, 16) and its sodium salt was used.  $N^{\delta}$ -{4-[(1-azi-2,2,2-trifluoroethyl)benzoyl]- $\beta$ -[2,3-<sup>3</sup>H]alanyl}- $\delta$ -aminophalloin with a specific radioactivity of 25 Ci/mmol (1 Ci = 37 GBq) and 7,7-azo-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -[3 $\beta$ ,12 $\beta$ -<sup>3</sup>H]cholan-24-oic acid<sup>§</sup> and its taurine conjugate with a specific radioactivity of 20 Ci/mmol were synthesized as described (17-19).  $\{N^{\epsilon}$ -[4-(1-azi-2,2,2-trifluoroethyl)benzoyl]-[4,5-<sup>3</sup>H]lys<sup>6</sup>}-antamanide with a specific radioactivity of 15 Ci/mmol was prepared by catalytic hydrogenation of the unsaturated antamanide precursor by using <sup>3</sup>H<sub>2</sub> gas in the laboratory of Pierre Morgat (Saclay, France) and subsequent coupling with 4-(1-azi-2,2,2-trifluoroethyl)benzoic acid (18, 20).

**Animals.** Male Wistar rats (Tierzuchtanstalt Ivanovas, Kisslegg, F.R.G.) weighing 150-200 g and maintained on a standard rat diet (Altromin 300 R, Altromin GmbH, Lage, F.R.G.) and tap water ad lib were used.

**Isolation of Hepatocytes and Membranes.** Preparation of plasma membrane subfractions from liver, isolation of intact hepatocytes, and preparation of plasma membranes from isolated cells were carried out as described (14, 21, 22).

**Photoaffinity Labeling.** Photoaffinity labelings of membrane subfractions from rat liver (14) and of intact hepatocytes (22) were performed at 350 nm and 30°C after 5 min of preincubation with the respective photolabile derivative; other preincubation times are indicated separately.

**Polyacrylamide Gel Electrophoresis.** Membrane subfractions isolated either from rat liver or from isolated intact hepatocytes were subjected to discontinuous NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis on vertical slabs (180 × 140 × 1.6 or 200 × 140 × 2.8 mm, respectively) following the conditions described (23). Isolated hepatocytes were analyzed by electrophoresis after being washed at least three times and resuspended in 62.5 mM Tris-HCl buffer (pH 6.8) containing 2% NaDodSO<sub>4</sub>, 10% glycerol, 5% 2-mercaptoethanol, and 0.001% bromophenol blue. After agitation for 15 min the samples were heated to 95°C for 5 min. Aliquots of 5-25  $\mu$ l corresponding to 200-800  $\mu$ g of protein were applied onto the gels.

**Detection of Radioactivity.** Radioactivity was detected either by liquid scintillation counting following the procedure described (23) or fluorographically (24, 25).

## RESULTS

To prove that the polypeptides involved in the uptake of bile salts by the sinusoidal membrane of hepatocytes are identical with those responsible for the uptake of phallotoxins and

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<sup>§</sup>Alternative nomenclature: 7-azi- instead of 7,7-azo-.

antamanide, photoaffinity labeling studies have been performed by using membrane subfractions enriched with sinusoidal surfaces and isolated hepatocytes.

**Photoaffinity Labeling of Sinusoidal Membranes.** Fig. 1 demonstrates a clear concentration-dependent decrease in the extent of labeling of bile-salt-binding polypeptides in a subfraction of plasma membranes enriched with sinusoidal surfaces by the taurine-conjugated 7,7-azo-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -cholan-24-oic acid in the presence of 100 and 250  $\mu$ M phalloidin. Antamanide and its derivatives inhibit the uptake of phalloidin into hepatocytes (5, 6, 10, 26). The presence of the soluble *O*-carboxymethyl-tyr<sup>6</sup>-antamanide (15, 16) results clearly in a reduced labeling of the bile-salt-binding polypeptides of sinusoidal membrane fractions by the photolabile bile salt derivative, as shown in Fig. 2 with *O*-carboxymethyl-tyr<sup>6</sup>-antamanide concentrations of 50 and 100  $\mu$ M. As with phalloidin, the protecting effect of the soluble antamanide derivative was most distinct for the polypeptides with apparent  $M_r$ s of 54,000 and 48,000. Both polypeptides, the  $M_r$ s of which have been estimated previously as

52,000 and 48,000 (11–14), are assumed to have a function in membrane transport of bile salts.

Photoaffinity labeling of membrane subfractions with photolabile bile salt derivatives results in the labeling of two further polypeptides, the one with the  $M_r$  of 67,000, albumin (27), and the other with the  $M_r$  of 43,000, actin (13, 14, 23). Both polypeptides are included in membrane subfractions to a varying extent. The decrease in labeling by the photolabile bile salt derivatives in the presence of phalloidin or the soluble antamanide derivative shows that both cyclopeptides also interact with the proteins.

Direct proof of interaction of the bile-salt-binding polypeptides of hepatocyte membranes with phalloidin and antamanide was obtained from photoaffinity labeling experiments using photolabile derivatives of these cyclic oligopeptides. In Fig. 3 the labeling patterns of membrane polypeptides are compared after photoaffinity labeling of the same membrane fraction with the photolabile derivatives of the conjugated bile salt (Fig. 3A), of phalloidin (Fig. 3B), and of antamanide (Fig. 3C). Although the labeling patterns exhibit quantita-

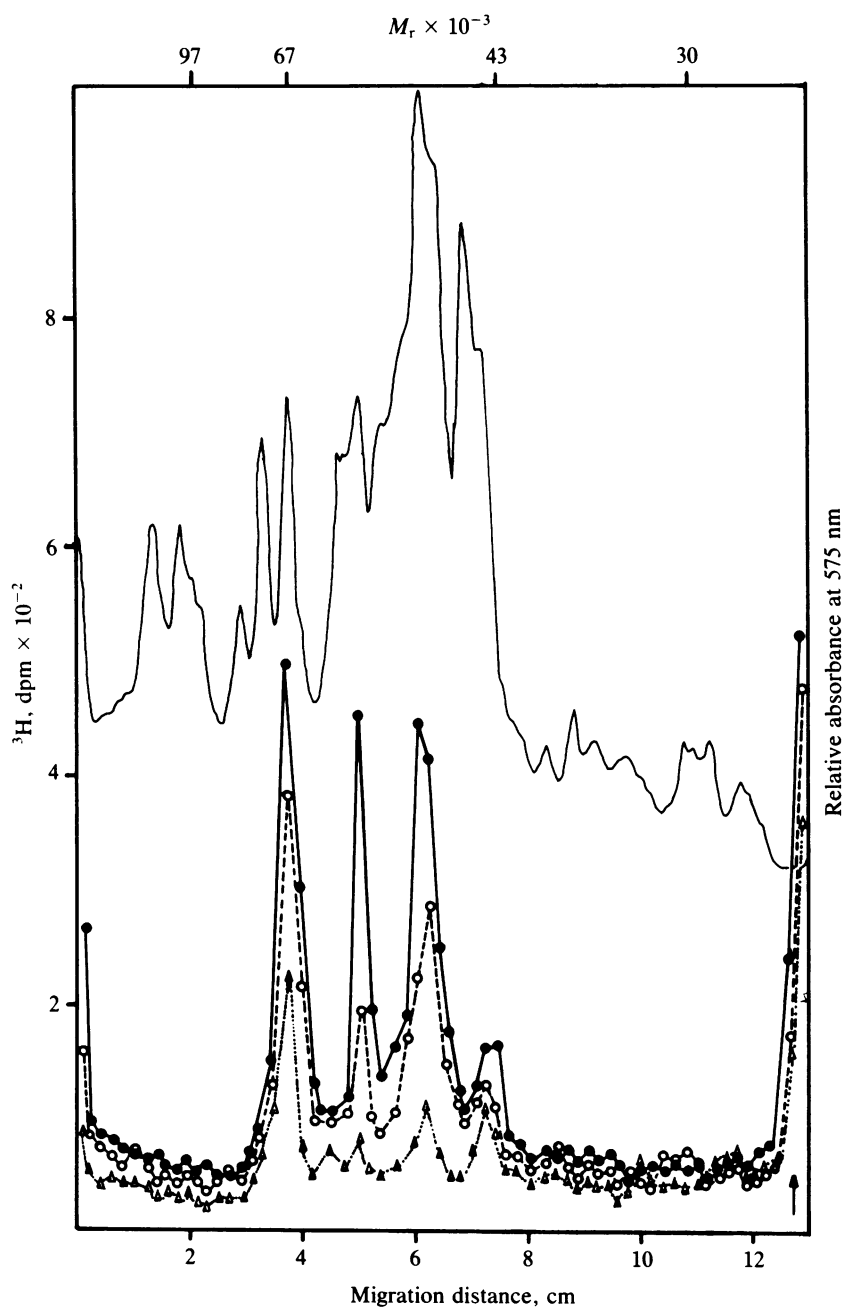


FIG. 1. Incorporation of radioactivity into polypeptides of plasma membrane subfraction of liver by photoaffinity labeling with the sodium salt of (7,7-azo-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -[3 $\beta$ ,12 $\beta$ -<sup>3</sup>H]cholan-24-oyl)-2-aminoethanesulfonic acid in the absence and presence of phalloidin. One-half milligram of sinusoidal membranes was photolabeled with 1.02  $\mu$ M (9.3  $\mu$ Ci) taurine-conjugated 7,7-azo derivative in the presence of 0 ( $\bullet$ ), 100 ( $\circ$ ), or 250 ( $\Delta$ )  $\mu$ M phalloidin. NaDod-SO<sub>4</sub>/polyacrylamide gel electrophoresis was performed at a total acrylamide concentration of 9% at a ratio of acrylamide/bisacrylamide of 97.2:2.8. The arrow indicates the position of bromophenol blue. The thin solid line shows the densitometer scanning of the polypeptides at 575 nm after staining with Coomassie blue.

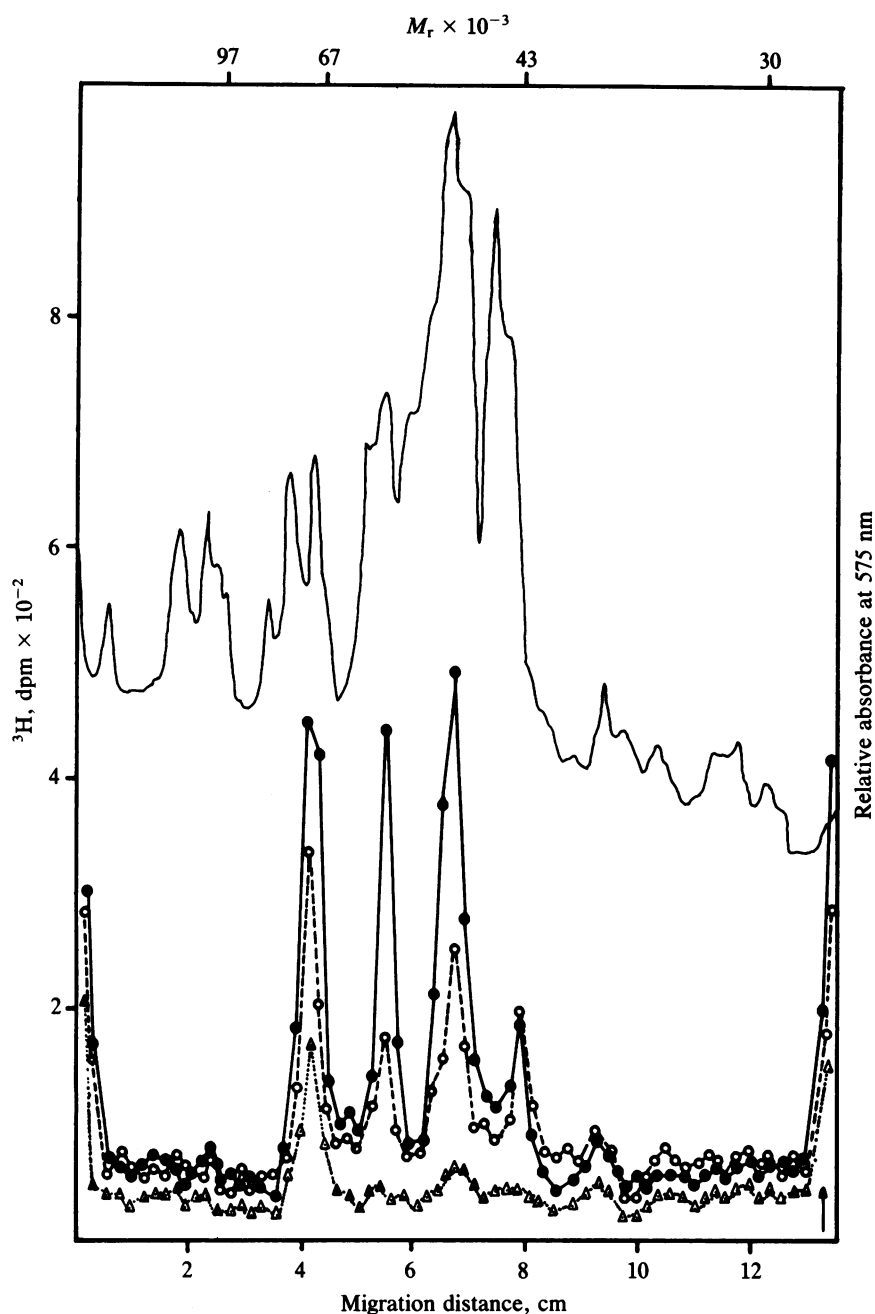


FIG. 2. Incorporation of radioactivity into polypeptides of plasma membrane subfraction from liver by photoaffinity labeling with the sodium salt of (7,7-azo-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -[3 $\beta$ ,12 $\beta$ - $^3\text{H}$ ]cholan-24-oyl)-2-aminoethanesulfonic acid in the absence and presence of antamanide. One-half milligram of sinusoidal membranes was photolabeled with 1.02  $\mu\text{M}$  (9.3  $\mu\text{Ci}$ ) taurine-conjugated 7,7-azo derivative in the presence of 0 (●), 50 (○), or 100 ( $\Delta$ )  $\mu\text{M}$  water-soluble antamanide derivative. For all other conditions, see the legend to Fig. 1.

tively large differences, the qualitative correspondence is obvious. Photoaffinity labeling with the bile salt derivative led to the strongest labeling of albumin (Fig. 3A). At the low concentrations used, photoaffinity labeling of albumin with the photolabile derivative of phalloidin was relatively low as compared to the other labeled polypeptides (Fig. 3B) and practically without significance with the derivative of antamanide (Fig. 3C). However, the interaction of both phalloidin and antamanide with albumin became apparent at high concentrations, as demonstrated by the differential photoaffinity labeling experiments (Figs. 1 and 2).

As to be expected with the photolabile derivative of phalloidin, the most outstanding labeling was seen in actin, the polypeptide with the  $M_r$  of 43,000 (Fig. 3B). This polypeptide was only slightly labeled by the photolabile derivative of antamanide (Fig. 3C).

With the photolabile derivative of phalloidin, as well as of antamanide, a polypeptide with the apparent  $M_r$  of 98,000 was labeled (Fig. 3B and C). This polypeptide is practically not labeled in membrane subfractions and isolated hepatocytes (11–14) by photolabile bile salt derivatives and there-

fore is assumed not to be involved in the uptake of bile salts by hepatocytes.

**Photoaffinity Labeling of Isolated Hepatocytes.** The results obtained with the membrane subfraction were confirmed through photoaffinity labeling studies performed on isolated intact hepatocytes. The comparison of the labeling patterns obtained after photoaffinity labeling of hepatocytes with the different photolabile derivatives and subsequent isolation of the plasma membrane fraction is shown in Fig. 4. With the photolabile derivatives of all three compounds the two membrane polypeptides with the apparent  $M_r$ s of 54,000 and 48,000 were clearly labeled (Fig. 4A–C). The labeling of a further polypeptide with an apparent  $M_r$  of 33,000 was caused by contamination of the membrane fraction by mitochondrial material (22) and is of no significance for the uptake of bile salts. The labeling of the polypeptide with the apparent  $M_r$  of 98,000 and of actin by the photolabile phalloidin derivative shows that phalloidin interacts with these polypeptides not only in isolated membranes but also in intact hepatocytes. The interaction of phalloidin and antamanide with the two bile-salt-binding polypeptides of hepato-

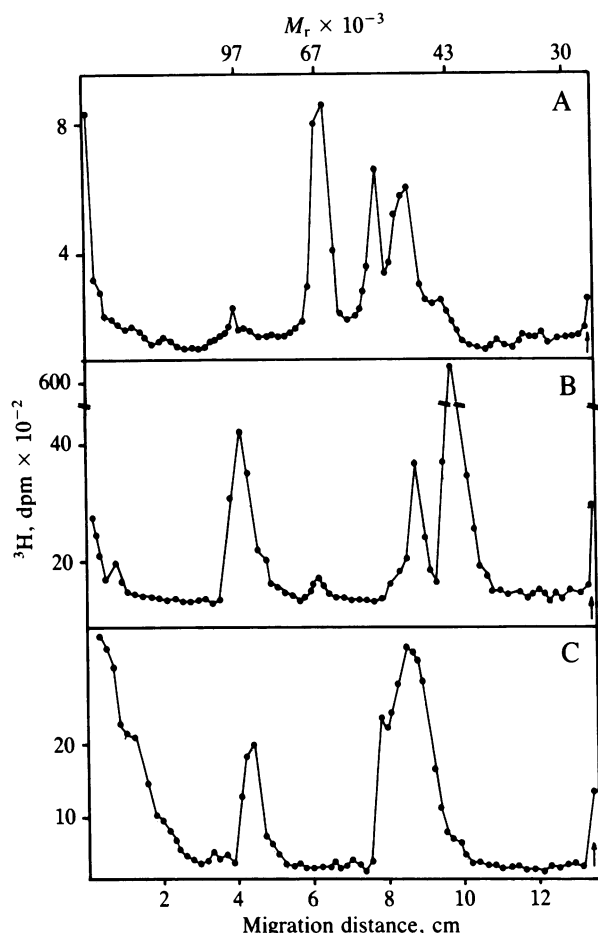


FIG. 3. Distribution of radioactivity after NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis of plasma membrane subfraction from liver. One-half milligram of sinusoidal membranes was photolabeled with 3.25  $\mu$ M (13  $\mu$ Ci) sodium salt of (7,7-azo-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -[3 $\beta$ ,12 $\beta$ -<sup>3</sup>H]cholan-24-oyl)-2-aminoethanesulfonic acid (A), 1.6  $\mu$ M (8  $\mu$ Ci) *N*<sup>6</sup>-[4-[(1-azi-2,2,2-trifluoroethyl)benzoyl]- $\beta$ -[2,3-<sup>3</sup>H]alanyl]- $\delta$ -aminophalloin (B), or 1.8  $\mu$ M (9  $\mu$ Ci) *N*<sup>6</sup>-[4-(1-azi-2,2,2-trifluoroethyl)benzoyl]-[4,5-<sup>3</sup>H]lys<sup>6</sup>]-antamanide (C). For all other conditions, see the legend to Fig. 1. Gel polyacrylamide concentration was 7.5%.

cyte membranes was revealed even more by differential photoaffinity labeling of intact hepatocytes and subsequent analysis of the whole cells by NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis.

Fig. 5 illustrates that among the polypeptides labeled by the photolabile phalloidin derivative, the labeling of the polypeptides of *M<sub>r</sub>*s 54,000 and 48,000 was clearly depressed in the presence of the water-soluble derivative of antamanide as well as by taurocholate and cholate. A decrease in the extent of labeling in the presence of competing ligands has also been observed in photoaffinity labeling experiments with the photolabile derivative of antamanide. Phalloidin as well as bile salts reduced the incorporation of the antamanide derivative into the two membrane polypeptides (not shown).

Fig. 6 demonstrates that incubation of the hepatocytes with the photolabile derivative of phalloidin prior to photoaffinity labeling resulted in decreased labeling of the membrane polypeptides with *M<sub>r</sub>*s of 54,000 and 48,000 with increasing incubation time. After 30 min of incubation, the photolabile derivative of phalloidin was nearly completely taken up into hepatocytes and therefore the membrane polypeptides involved in the uptake of the toxin were only slightly labeled, whereas F-actin inside the cells was heavily labeled.

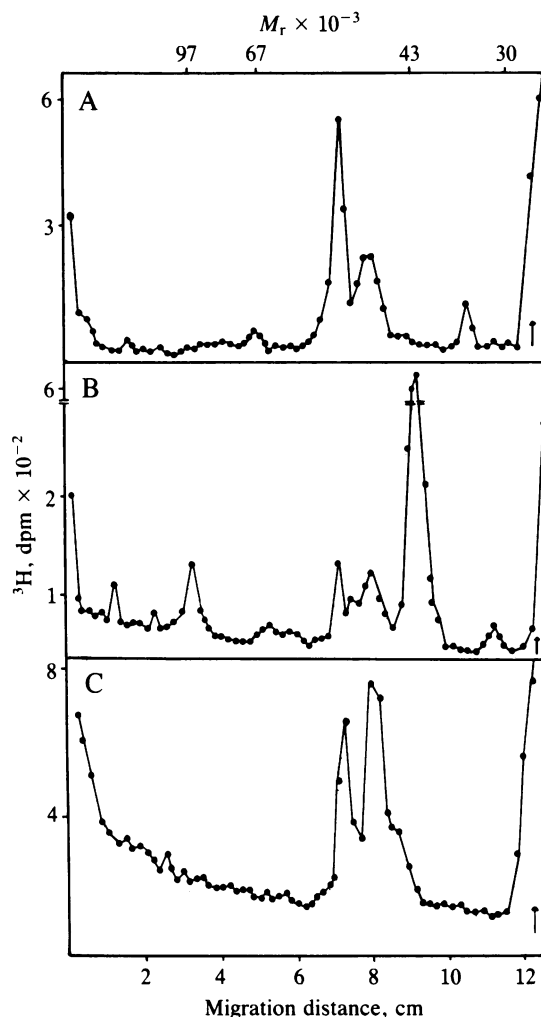


FIG. 4. Distribution of radioactivity after NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis of the membrane fraction obtained after photoaffinity labeling of isolated intact hepatocytes. A 5-ml cell suspension ( $50 \times 10^6$  hepatocytes) was preincubated for 10 min with 0.44  $\mu$ M (44  $\mu$ Ci) sodium salt of (7,7-azo-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -[3 $\beta$ ,12 $\beta$ -<sup>3</sup>H]cholan-24-oyl)-2-aminoethanesulfonic acid (A), 0.29  $\mu$ M (37  $\mu$ Ci) *N*<sup>6</sup>-[4-[(1-azi-2,2,2-trifluoroethyl)benzoyl]- $\beta$ -[2,3-<sup>3</sup>H]alanyl]- $\delta$ -aminophalloin (B), or 0.32  $\mu$ M (40  $\mu$ Ci) *N*<sup>6</sup>-[4-(1-azi-2,2,2-trifluoroethyl)benzoyl]-[4,5-<sup>3</sup>H]lys<sup>6</sup>]-antamanide (C). Subsequent to photoaffinity labeling the plasma membranes were isolated. For all other conditions, see the legend to Fig. 1. Gel polyacrylamide concentration was 7.5%.

## DISCUSSION

Photoaffinity labeling using appropriate photolabile derivatives of structurally different substances and mutual differential labeling can be employed to determine whether the same polypeptides are involved in membrane transport of these substances. Because the bile-salt-binding polypeptides of hepatocyte membranes have been identified by photoaffinity labeling (11–14) and because the toxic effect of phalloidin in hepatocytes is prevented by bile salts (7, 9) and by antamanide (6), the hypothesis that the transport systems for these substances are identical was examined with photolabile derivatives of these compounds. All photolabile derivatives used behave analogously to the naturally occurring compounds (14, 17, 20, 28) and their photolysis at 350 nm does not impair the viability of hepatocytes (22).

In sinusoidal membranes photoaffinity labeling with photolabile derivatives of bile salts, phalloidin, and antamanide resulted in the labeling of the same membrane polypeptides. The labeling of these polypeptides by any of the photolabile derivatives was clearly depressed by each of the other two

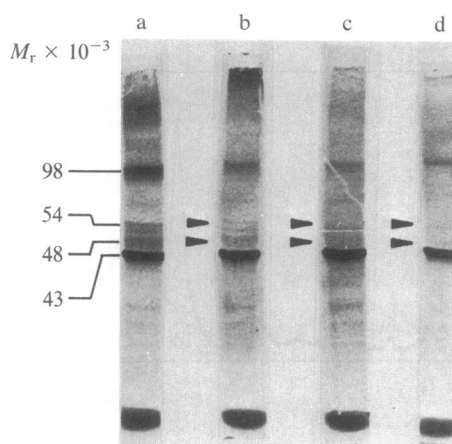


FIG. 5. Effect of water-soluble antamanide derivative and bile salts on photoaffinity labeling of hepatocyte proteins by the photolabile phalloidin derivative. A 5-ml cell suspension ( $2-4 \times 10^6$  cells) was submitted to photoaffinity labeling in the presence of no addition (lane a),  $100 \mu\text{M}$  *O*-carboxymethyl-tyr<sup>6</sup>-antamanide (lane b),  $500 \mu\text{M}$  taurocholate (lane c), or  $250 \mu\text{M}$  cholate (lane d). After photoaffinity labeling the cells were washed and aliquots corresponding to 200–400  $\mu\text{g}$  of protein were applied to NaDodSO<sub>4</sub>/polyacrylamide gels. Subsequently the gels were exposed to fluorography. For all other conditions, see the legend to Fig. 1.

substances present as a nonphotolabile derivative. The physiological significance of the membrane polypeptides with the apparent  $M_r$ s of 54,000 and 48,000 for the uptake of bile salts, phalloidin, and antamanide is shown by photoaffinity labeling of isolated intact hepatocytes. The absence of these two polypeptides in enterocytes of terminal ileum (23), which have the capability for active bile salt transport but are not able to take up phalloidin and antamanide (29, 30), is a further indication that a common transport exists in hepatocytes for bile salts, phalloidin, and antamanide. The involvement of different polypeptides in bile salt uptake by he-

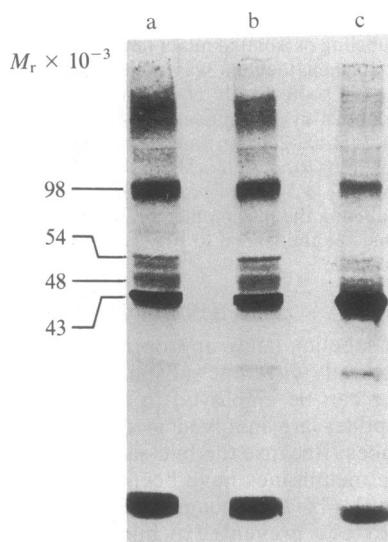


FIG. 6. Time-dependent uptake of photolabile phalloidin by rat hepatocytes. A 5-ml cell suspension ( $2-4 \times 10^6$  cells) was subjected to photoaffinity labeling after 0 min (lane a), 5 min (lane b), or 30 min (lane c) of incubation in the dark with  $0.17 \mu\text{M}$  of the photolabile phalloidin derivative. For all other conditions, see the legend to Fig. 5.

patocytes and by enterocytes explains the organotropism for phalloidin.

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1. Wieland, T. & Schön, W. (1955) *Liebigs Ann. Chem.* **593**, 157–178.
2. Wieland, T. (1983) *Int. J. Pept. Protein Res.* **22**, 257–276.
3. Reh binder, D., Löffler, G., Wieland, O. & Wieland, T. (1963) *Hoppe-Seyler's Z. Physiol. Chem.* **331**, 132–142.
4. Wieland, T. (1977) *Adv. Enzyme Regul.* **15**, 285–300.
5. Wieland, T., Lüben, G., Ottenheim, H., Faesel, J., de Vries, J. X., Konz, W., Prox, A. & Schmid, J. (1968) *Angew. Chem. Int. Ed. Engl.* **7**, 204–208.
6. Faulstich, H., Wieland, T., Walli, A. & Birkmann, K. (1974) *Hoppe-Seyler's Z. Physiol. Chem.* **355**, 1162–1163.
7. Frimmer, M., Petzinger, E., Rufeger, U. & Veil, L. B. (1977) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **301**, 145–147.
8. Frimmer, M., Petzinger, E. & Ziegler, K. (1980) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **313**, 85–89.
9. Petzinger, E. & Frimmer, M. (1980) *Arch. Toxicol.* **44**, 127–135.
10. Petzinger, E., Joppen, C. & Frimmer, M. (1983) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **322**, 174–179.
11. Kramer, W., Bickel, U., Buscher, H.-P., Gerok, W. & Kurz, G. (1980) *Hoppe-Seyler's Z. Physiol. Chem.* **361**, 1307.
12. Abberger, H., Bickel, U., Buscher, H.-P., Fuchte, K., Gerok, W., Kramer, W. & Kurz, G. (1981) in *Bile Acids and Lipids*, eds. Paumgartner, G., Stiehl, A. & Gerok, W. (MTP Press, Lancaster, England), pp. 233–246.
13. Fricker, G., Kramer, W., Buscher, H.-P., Gerok, W. & Kurz, G. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 897.
14. Kramer, W., Bickel, U., Buscher, H.-P., Gerok, W. & Kurz, G. (1982) *Eur. J. Biochem.* **129**, 13–24.
15. Wieland, T. & Rietzel, C. (1971) *Liebigs Ann. Chem.* **754**, 107–112.
16. Wieland, T., Rietzel, C. & Seeliger, A. (1972) *Liebigs Ann. Chem.* **759**, 52–70.
17. Wieland, T., Hollosi, M. & Nassal, M. (1983) *Liebigs Ann. Chem.*, 1533–1540.
18. Nassal, M. (1983) *Liebigs Ann. Chem.*, 1510–1523.
19. Kramer, W. & Kurz, G. (1983) *J. Lipid Res.* **24**, 910–923.
20. Nassal, M., Buc, P. & Wieland, T. (1983) *Liebigs Ann. Chem.*, 1524–1532.
21. Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520.
22. Abberger, H., Buscher, H.-P., Fuchte, K., Gerok, W., Giese, U., Kramer, W., Kurz, G. & Zanger, U. (1983) in *Bile Acids and Cholesterol in Health and Disease*, eds. Paumgartner, G., Stiehl, A. & Gerok, W. (MTP Press, Lancaster, England), pp. 77–87.
23. Kramer, W., Burckhardt, G., Wilson, F. A. & Kurz, G. (1983) *J. Biol. Chem.* **258**, 3623–3627.
24. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
25. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341.
26. Petzinger, E., Ziegler, K. & Frimmer, M. (1979) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **307**, 275–281.
27. Kramer, W., Buscher, H.-P., Gerok, W. & Kurz, G. (1979) *Eur. J. Biochem.* **102**, 1–9.
28. Burckhardt, G., Kramer, W., Kurz, G. & Wilson, F. A. (1983) *J. Biol. Chem.* **258**, 3618–3622.
29. Wieland, T., Faulstich, H., Jahn, W., Govindan, M. U., Puchinger, H., Kopitar, Z., Schmaus, H. & Schmitz, A. (1972) *Hoppe-Seyler's Z. Physiol. Chem.* **353**, 1337–1345.
30. Petzinger, E., Burckhardt, G., Schwenk, M. & Faulstich, H. (1982) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **320**, 196–200.