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# A colchicine-sensitive uptake system in Morris hepatomas

(microtubules/cytochalasin B/vinblastine/α-aminoisobutyric acid/regenerating liver)

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ABSTRACT **The interference of microtubular disruptors with the uptake of amino acids and other low molecular weight substrates has been studied in Morris hepatomas, host liver, and regenerating liver. Colchicine inhibits amino acid transport (α-aminoisobutyric acid, L-methionine, and L-leucine) in hepatomas by 59–98% whereas transport in host and regenerating liver is not impeded but increased. In hepatomas, treatment with colchicine also reduces the uptake of L-fucose, cytidine, urea, and carbonate. Vinblastine, but not lumicolchicine or cytochalasin B, is an effective inhibitor. The inhibition of uptake is not linked to a decrease of cellular ATP and UTP. The data suggest that the transport of low molecular weight substrates in hepatomas is related to microtubules or other colchicine-binding structures, e.g., of the plasma membrane. This colchicine-sensitive uptake system in hepatomas may be due to the malignant transformation of hepatocytes.**

A variety of cellular functions primarily related to intracellular translocation of macromolecules or to cell membrane dynamics and architecture depend on an intact microtubular system. Evidence is largely based on the interference of colchicine and vinblastine with the assembly of microtubules (1, 2). Microtubular disruption inhibits secretion of salivary gland mucin (3), of hepatic very low density lipoproteins (4, 5), and of albumin and other plasma proteins (6). Microtubule-associated functions may also regulate lysosomal degranulation during phagocytosis (7, 8) and influence the conversion of prohormones into their active forms (9). The colchicine-induced inhibition of the mobility of membrane components (10) and changes in plasma membrane microviscosity associated with phagocytosis (11) may reflect the close relationship between the microtubular system and the cell membrane.

Little is known, however, about the role of microtubules in transmembrane transport. Colchicine has been found to prevent the increased capacity for amino acid transport of concanavalin A-activated lymphocytes during the prereplicative phase (12) and of proliferating hepatocytes after partial hepatectomy without affecting the basic uptake of amino acids (13). This report describes the influence of microtubule disruptors on the uptake of various low molecular weight substances by Morris hepatomas compared to normal liver and regenerating liver. Our results suggest a colchicine-sensitive uptake system as a characteristic feature of hepatocellular Morris carcinomas.

## MATERIALS AND METHODS

Female Buffalo and ACI rats (150–180 g) were bred in our laboratories and fed a commercial diet (Altromin R; Altromin GmbH, Lage-Lippe, West Germany; containing 18–20% protein) and water ad libitum. The animals were kept in windowless rooms at 24°C with constant humidity and with light from 7:30 a.m. to 7:30 p.m.

Morris hepatomas (originally obtained from H. P. Morris, Howard University, Washington, DC) were inoculated into both hind legs of ACI rats (9121) and Buffalo rats (7777).

Partial hepatectomy was performed between 8:00 and 9:00 a.m. (14); two-thirds of the liver was removed under slight ether anesthesia. Drugs (Sigma) were administered intraperitoneally between 8:00 and 9:00 a.m. at various time intervals prior to injection of the labeled substrates. Radioactively labeled compounds (Radiochemical Centre, Amersham, England) were injected into the tail vein. Tissue samples were obtained by freeze-clamping *in situ* under slight pentobarbital anesthesia and kept in liquid nitrogen. The frozen tissue was transferred to 10 vol of chilled 50 mM Tris·HCl, pH 7.5/25 mM KCl/5 mM $MgCl_2$ and was immediately homogenized. An acid-soluble fraction was obtained by precipitation of samples of the homogenate by addition of 3 vol of 10% (wt/vol) trichloroacetic acid. The precipitate was removed by centrifugation, and aliquots of the supernatant were assayed for radioactivity in a toluene/Triton X-100 scintillation fluid. Acid-insoluble radioactivity was determined as described (15). For quantitative analysis of intracellular amino acid concentrations, tissue samples were homogenized in 10 vol of 0.9 M $HClO_4$. After centrifugation, the supernatant was neutralized with $KHCO_3$. The acidified samples were subjected to automatic amino acid analysis. Nucleoside phosphate pools were measured enzymatically (16, 17). Protein content was measured by the biuret method (18). Lumicolchicine, prepared according to Wilson and Friedkin (19), was a gift from I. Stadler and W. W. Franke from the Krebsforschungszentrum Heidelberg, West Germany. Chemicals of analytical grade were obtained from Merck (Darmstadt, West Germany) and Roth (Karlsruhe, West Germany).

## RESULTS

**Effect of Colchicine on Uptake of Amino Acids and Other Substrates by Liver and Hepatomas.** The uptake of labeled amino acids into acid-soluble and acid-insoluble cellular material was significantly inhibited in hepatomas of rats treated with colchicine at intervals of 2–8 hr prior to injection of the isotope. Uptake into the acid-soluble material (Table 1) of Morris hepatoma 7777 was reduced by 77% for L-[$^{35}$S]methionine and by 59% for L-[$^{14}$C]leucine. Similarly, colchicine decreased the incorporation of methionine and leucine into the acid-insoluble material by 94% and 98%, respectively (Table 1). The same inhibitory effect of colchicine on methionine uptake was found in Morris hepatoma 9121.

In host liver, however, amino acid uptake and incorporation were not inhibited, but colchicine increased L-[$^{35}$S]methionine

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Abbreviation: AIB, α-aminoisobutyric acid.

Table 1. Effect of colchicine on uptake and incorporation of various substrates into host liver and hepatoma

| Substrate | Hepatoma | | Host liver | |
|---|---|---|---|---|
| | Colchicine-treated (% inhibition) | Control | Colchicine-treated (% increase) | Control |
| **Acid-soluble material** | | | | |
| 2-Amino[1-$^{14}$C]isobutyric acid* | 0.2 ± 0.08 (95) | 4.0 ± 1.4 | 7.6 ± 3.5 (76) | 4.3 ± 0.5 |
| L-[$^{35}$S]Methionine† | 130 ± 26 (77) | 541 ± 72 | 1878 ± 422 (204) | 616 ± 70 |
| L-[$^{35}$S]Methionine* | 34 ± 15 (81) | 174 ± 29 | 398 ± 13 (73) | 230 ± 31 |
| L-[1-$^{14}$C]Leucine† | 13 ± 3 (59) | 33 ± 1 | 64 ± 5 (52) | 42 ± 3 |
| L-[6-$^{3}$H]Fucose† | 95 ± 35 (81) | 485 ± 50 | 627 ± 262 | 695 ± 65 |
| [5-$^{3}$H]Cytidine† | 6.1 ± 1.4 (88) | 52 ± 8 | 62 ± 4 | 64 ± 8 |
| [$^{14}$C]Urea† | 2.1 ± 1.4 (62) | 5.4 ± 2.5 | 6.2 ± 0.5 | 5.8 ± 0.9 |
| Sodium [$^{14}$C]carbonate† | 17 ± 4 (84) | 104 ± 14 | 270 ± 62 (73) | 156 ± 20 |
| **Acid-insoluble material** | | | | |
| 2-Amino[1-$^{14}$C]isobutyric acid* | — | — | — | — |
| L-[$^{35}$S]Methionine† | 94 ± 27 (94) | 1594 ± 801 | 5815 ± 78 (44) | 4025 ± 643 |
| L-[$^{35}$S]Methionine* | 44 ± 29 (95) | 976 ± 252 | 1169 ± 13 (<1) | 1138 ± 192 |
| L-[1-$^{14}$C]Leucine† | 11 ± 6 (98) | 725 ± 119 | 908 ± 106 (<1) | 1005 ± 225 |
| L-[6-$^{3}$H]Fucose† | 111 ± 12 (69) | 349 ± 217 | 644 ± 311 (11) | 578 ± 116 |
| [5-$^{3}$H]Cytidine† | — | — | — | — |
| [$^{14}$C]Urea† | — | — | — | — |
| Sodium [$^{14}$C]carbonate† | 1.9 ± 1.2 (98) | 81 ± 24 | 404 ± 116 (46) | 276 ± 69 |

Buffalo rats bearing Morris hepatoma 7777 or ACI rats bearing Morris hepatoma 9121 were treated with 0.25 mg of colchicine (1 mg/ml of 0.9% NaCl) per 100 g body weight intraperitoneally (controls received saline). Six hours later the animals were pulse-labeled with one of the following substrates for 30 min (1 Ci = $3.7 \times 10^{10}$ becquerels): 160 μCi (Buffalo rats) or 40 μCi (ACI rats) of L-[$^{35}$S]methionine (945 Ci/mmol), 5 μCi of L-[1-$^{14}$C]leucine (59 Ci/mol), 0.5 mCi of $Na_2$[$^{14}$C]carbonate (56.5 Ci/mol), 5 μCi of α-amino[1-$^{14}$C]isobutyric acid (60 Ci/mol), 100 μCi of L-[6-$^{3}$H]fucose (16.6 Ci/mmol), 50 μCi of [5-$^{3}$H]cytidine (23.8 Ci/mmol), and 15 μCi of [$^{14}$C]urea (59 Ci/mol) per 100 g body weight each. Radioactivity was then determined. Percent inhibition or increase of uptake into drug-treated rats compared to controls (100%) is given in parentheses. Data represent mean uptake ± SEM in dpm per mg of tissue wet weight from three animals each.

* ACI rats bearing Morris hepatoma 9121.

† Buffalo rats bearing Morris hepatoma 7777.

radioactivity—e.g., 3-fold in the acid-soluble fraction and less so into liver proteins (Table 1). Regenerating liver 24 and 48 hr after partial hepatectomy behaved like host liver (Table 2). To distinguish between effects on transport and metabolism of amino acids, we determined the influence of colchicine on the uptake of the nonmetabolizable amino acid α-aminoisobutyric acid (AIB). In hepatoma 7777, the drug decreased the uptake of AIB by 95%; transport of AIB into host liver was not impaired, but increased by 76%. The initial rates of uptake immediately after the injection of the labeled amino acids were decreased by colchicine, indicating that the inhibitory effect in hepatomas is related to transport processes (Fig. 1).

The inhibition of uptake by colchicine in hepatoma tissue was not restricted to amino acids, but also involved the uptake of other small molecules such as L-fucose, cytidine, urea, and carbonate, as shown in Morris hepatoma 7777 (Table 1). In host liver, uptake and incorporation of these substrates (except for carbonate) were not changed by colchicine. The increase of acid-soluble and acid-insoluble radioactivity found in host liver after injection of [$^{14}$C]carbonate is possibly a result of its conversion to arginine.

Control hepatomas (7777 and 9121) had moderately less uptake and incorporation of substrates than did the control host livers (Table 1), confirming comparative studies on the uptake of AIB (20) and nucleic acid precursors (21) in Morris hepatomas 9618A, 7777, and 5123C. The diverse effects of colchicine upon the uptake of amino acids into hepatoma and host liver were dose dependent, as shown for L-leucine (Fig. 2). In Morris hepatoma 7777, colchicine inhibited the uptake of L-leucine into both the acid-soluble and acid-insoluble fractions at doses as low as 0.75 mg/kg body weight with a maximal effect at doses of 1.5–2.5 mg/kg body weight. In host liver, colchicine conversely increased the uptake of L-leucine into the acid-soluble material in a dose-dependent manner; after an initial decline, it moderately increased the uptake into the acid-insoluble material.

**Specificity of Inhibitory Effect of Microtubular Disruptors.** Vinblastine sulfate, which binds to tubulin and interferes with microtubule assembly, is also an effective inhibitor in Morris hepatomas, whereas lumicolchicine, a structural isomer of colchicine which does not interact with microtubule protein (22), was not effective (Table 3). Pretreatment with an equi-

Table 2. Effect of colchicine on uptake and incorporation of L-[$^{35}$S]methionine into regenerating liver

| Time after partial hepatectomy, hr | Acid-soluble | | Acid-insoluble | |
|---|---|---|---|---|
| | Colchicine-treated (% increase) | Control | Colchicine-treated (% increase) | Control |
| 24 | 414 ± 87 (36) | 304 ± 65 | 1653 ± 204 (<1) | 1790 ± 423 |
| 48 | 404 ± 8 (70) | 238 ± 4 | 1501 ± 76 ( 19) | 1259 ± 391 |

Twenty-four and 48 hr after partial hepatectomy, uptake and incorporation of L-[$^{35}$S]methionine (40 μCi/100 g body weight) into the regenerating liver of ACI rats were determined according to the procedures of Table 1. Data represent mean uptake ± SEM in dpm per mg of tissue wet weight from three animals each.

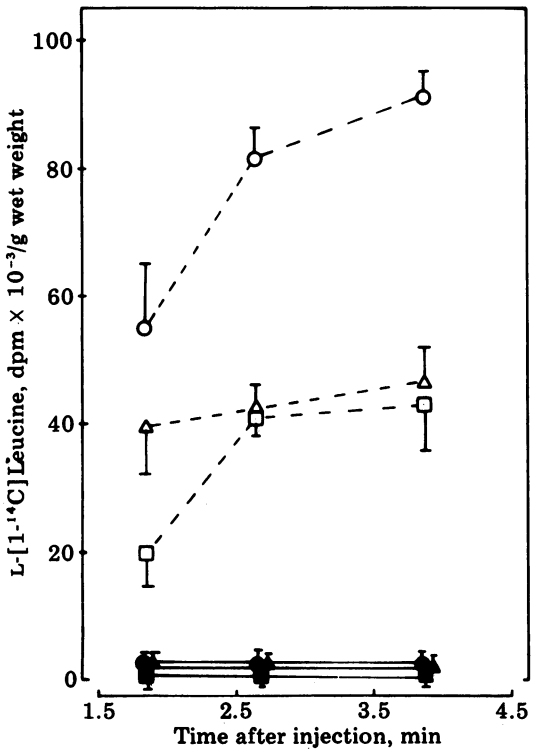


FIG. 1. Effect of colchicine on initial rates of uptake and incorporation of leucine into Morris hepatoma 7777. Buffalo rats bearing Morris hepatoma 7777 were injected with 5 μCi of L-[1-$^{14}$C]leucine (59 Ci/mol) per 100 g body weight intravenously 6 hr after intraperitoneal administration of 0.25 mg of colchicine per 100 g body weight. Organ samples were obtained at various times after injection of the label and radioactivity was determined. Each point represents the mean ± SEM for three tissues from control (---) and colchicine-treated (——) rats. ● and ○, Total radioactivity; ▲ and △, acid-soluble radioactivity; ■ and □, acid-insoluble radioactivity.

molar dose of cytochalasin B did not inhibit uptake of L-[$^{35}$S]-methionine. That the specific inhibition of uptake in hepatomas is caused by a preferential enrichment of the drug was ruled out by measuring the entry of [*ring-C-methoxyl*-$^{3}$H]colchicine into liver and hepatoma. Only 0.15 nmol was taken up per g wet weight into Morris hepatoma 7777 as compared to 0.76 nmol taken up per g wet weight into liver when 12.5 nmol of labeled colchicine per g body weight was intravenously injected into tumor-bearing rats.

Table 3. Specifity of inhibitory effect of microtubular disruptors on uptake and incorporation of methionine into host liver and hepatoma

| Drug | Hepatoma 7777 | | Host liver | |
|---|---|---|---|---|
| | Acid-soluble | Acid-insoluble | Acid-soluble | Acid-insoluble |
| Colchicine | 23 | 6 | 300 | 144 |
| Vinblastine | 40 | 3 | 98 | 84 |
| Lumicolchicine | 105 | 113 | 84 | 89 |
| Cytochalasin B | 177 | 150 | 111 | 117 |

Buffalo rats bearing Morris hepatoma 7777 were intraperitoneally injected with 0.25 mg of colchicine (1 mg/ml of 0.9% NaCl), 0.6 mg of vinblastine (1 mg/ml of 0.9% NaCl), 0.25 mg of lumicolchicine [0.25 mg/ml of 10% (vol/vol) ethanol], or 0.6 mg of cytochalasin B [1 mg/ml of 20% dimethyl sulfoxide/$H_2O$ (vol/vol)] per 100 g body weight each. (Controls received an equal amount of the respective solvent.) Six hours later, 40 μCi of L-[$^{35}$S]methionine per 100 g body weight was injected intravenously. Tissue samples were obtained 30 min later and assayed for radioactivity. The uptake and incorporation into livers and hepatomas were calculated as dpm/g tissue wet weight and expressed as percentage of uptake and incorporation into controls. Each value represents the results from three drug-treated rats as compared to three controls; the determinations did not diverge more than 20%.

**Influence of Colchicine on Nucleoside Phosphate Levels and Amino Acid Pools.** The effect of colchicine on nucleoside phosphate levels was investigated to exclude the possibility that drug action is due to interference with cellular energetics. There was no significant decrease in the concentration of adenine and uridine nucleotides, whereas the uptake of L-[$^{3}$H]fucose was significantly impeded (Table 4). In order to examine whether the effect of colchicine on amino acid uptake is related to changes in amino acid pool size, we determined intracellular amino acid concentrations in the acid-soluble fraction by quantitative analysis. In both host liver and hepatoma, the concentrations of free threonine, valine, isoleucine, leucine, tyrosine, and phenylalanine increased identically 2- to 3-fold by colchicine compared to untreated controls. The levels of other amino acids, including methionine, were not significantly changed in both tissues.

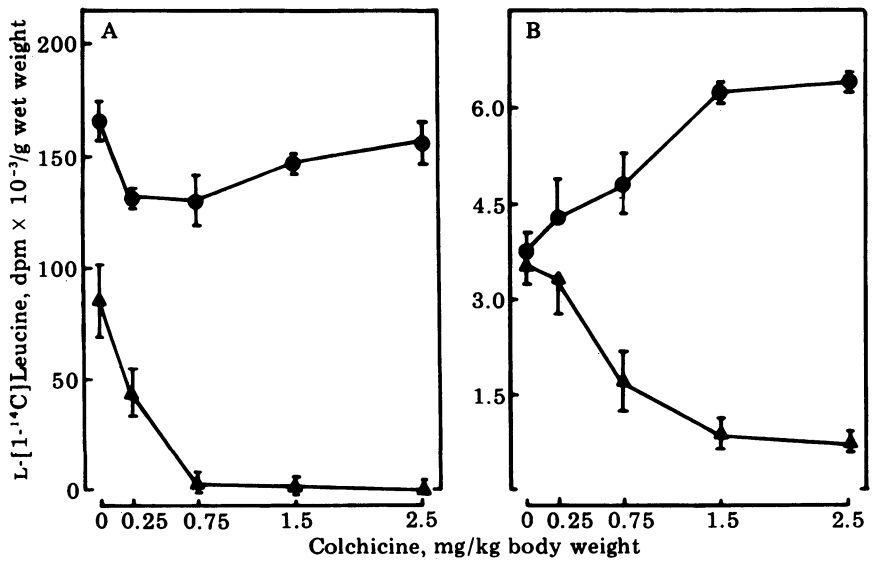


FIG. 2. Effect of colchicine dose on leucine uptake and incorporation in hepatoma and host liver. (*A*) Acid-insoluble radioactivity; (*B*) acid-soluble radioactivity. Buffalo rats bearing Morris hepatoma 7777 were intraperitoneally injected with the doses of colchicine indicated 6 hr prior to intravenous administration of 5 μCi of L-[1-$^{14}$C]leucine per 100 g body weight. Tissue samples were obtained 30 min after injection of the isotope and assayed for radioactivity. Each point is the mean ± SEM for hepatoma 7777 (▲) or host liver (●) from three animals.

Table 4. Effect of colchicine on concentrations of adenine and uridine nucleotides and on uptake of L-[$^3$H]fucose

| Tissue | Concentration, μmol/g of tissue wet weight | | | | | Uptake of L-[$^3$H]fucose, dpm/mg of tissue wet weight |
|---|---|---|---|---|---|---|
| | ATP | ADP | AMP | UTP + UDP | UMP | |
| Hepatoma 7777 | | | | | | |
| Colchicine | 0.53 ± 0.14 | 1.43 ± 0.10 | 1.06 ± 0.10 | 0.16 ± 0.05 | 0.16 ± 0.02 | 277 ± 90 |
| Controls | 0.46 ± 0.01 | 1.99 ± 0.25 | 1.17 ± 0.12 | 0.18 ± 0.02 | 0.20 ± 0.01 | 509 ± 132 |
| Host liver | | | | | | |
| Colchicine | 2.09 ± 0.14 | 1.90 ± 0.10 | 0.17 ± 0.08 | 0.29 ± 0.02 | 0.04 ± 0.01 | 738 ± 97 |
| Controls | 2.02 ± 0.06 | 1.64 ± 0.10 | 0.14 ± 0.01 | 0.28 ± 0.03 | 0.02 ± 0.01 | 826 ± 135 |

Adenine and uridine nucleotide concentrations were measured in Morris hepatoma 7777 and host liver of Buffalo rats 2.5 hr after intraperitoneal injection of 0.15 mg of colchicine per 100 g body weight. L-[6-$^3$H]Fucose (50 μCi/100 g body weight) was injected intravenously 10 min before the animals were killed. Data represent means ± SEM from three animals each.

## DISCUSSION

Colchicine blocks the transport of AIB and naturally occurring amino acids into Morris hepatomas but not into host liver. Inhibition is also not observed in regenerating liver, confirming the reported lack of effect on AIB transport by colchicine given 7 hr after partial hepatectomy (13). Therefore, these data suggest a colchicine-sensitive uptake system as a characteristic feature of hepatomas and, possibly, of other malignant cells. This assumption is supported by a decrease in the uphill transport of AIB by vinca alkaloids in Ehrlich ascites cells; however, the decrease was only 30% (23), whereas in Morris hepatomas we find a 95% inhibition of uptake. It is therefore advisable to investigate the influence of these drugs on transport systems in other malignant cells because changes in uptake appear to be crucial in malignant transformation (24).

The inhibition of uptake in hepatomas described here differs from the specific inhibition of nucleoside transport by colchicine and lumicolchicine in several cell lines (25). The blockage of uptake in Morris hepatomas is a general one, involving a variety of low molecular weight substrates and, unlike the study described in ref. 25, is restricted to drugs with a binding affinity for tubulin. Whereas vinblastine is also an effective inhibitor, neither lumicolchicine nor cytochalasin B interferes with amino acid uptake in hepatomas. Because the inhibition of uptake does not depend on a lack of cellular ATP, the effect of microtubule disruptors is not mediated by energy depletion. A preferential uptake of colchicine into hepatomas could be excluded. Furthermore, the opposite effects of colchicine on amino acid uptake in liver and hepatoma cannot be explained by changes in amino acid pool size; the increase by colchicine in the intracellular level of some amino acids, including L-leucine but not methionine, in host liver (26, 27) also occurs in hepatomas. The increased amino acid concentrations (e.g., of leucine) in colchicine-treated hepatomas despite the blockage of uptake indicate that the size of a given amino acid pool is presumably ruled not only by the rate of transport of the amino acid but also by its metabolism to other products and the overall rates of protein synthesis and degradation. Moreover, a concomitant inhibition of protein synthesis by colchicine must be considered in both tissues because, in drug-treated hepatomas, inhibition of uptake of naturally occurring amino acids into the acid-soluble material is less than into protein and is less marked than inhibition of AIB uptake. By contrast, the increase of acid-soluble radioactivity in host liver is followed by a smaller increase of protein-bound isotope.

Several molecular mechanisms could be invoked for the effect of colchicine and vinblastine on transport in hepatomas. One is direct interaction of both drugs with plasma membrane components. Tubulin or similar colchicine-binding proteins were detected in the plasma membrane of rat liver (28, 29). These colchicine-sensitive membrane components may be essential for the coordinated control of basic nutrient uptake. Such regulatory sites might be altered in transformed cells in view of changes in uptake in malignant cells (30, 31). Moreover, our observations might reflect a proximity among uptake systems newly appearing during malignant transformation of hepatocytes. During dedifferentiation and loss of specific cell functions such as protein secretion (32), the elaborate transport systems may also dedifferentiate, and uptake of nutrients would possibly be achieved by simple processes such as micropinocytosis, for example. These new uptake mechanisms could depend on variables such as plasma membrane microviscosity or the lateral mobility of membrane components, both of which are affected by colchicine (10, 11). The effect of colchicine and vinblastine could be due to changes in composition (33) and turnover (15, 34) of membrane proteins and glycoproteins in hepatomas. Finally, colchicine and vinblastine disaggregate the microtubular cytoskeleton in hepatomas. This system is involved in the flow of secretory vesicles from the endoplasmic reticulum to the cell surface (35). Conversely, the microtubular system may be essential for the formation and detachment of endocytotic vesicles from the plasma membrane or the movement of carrier proteins within the plasma membrane.

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1. Adelman, R. M., Borisy, G. G., Shelanski, M. L., Weisenberg, R. C. & Taylor, E. W. (1968) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **27**, 1186–1193.
2. Snyder, J. A. & McIntosh, J. R. (1976) *Annu. Rev. Biochem.* **45**, 699–720.
3. Rossignol, B., Herman, G. & Keryer, G. (1972) *FEBS Lett.* **21**, 189–194.
4. McMarchand, Y., Sincle, A., Assimacoupolus-Jeannet, F., Orci, L., Rouiller, C. & Jeanrenaud, B. (1973) *J. Biol. Chem.* **248**, 6862–6870.
5. Stein, O. & Stein, Y. (1973) *Biochim. Biophys. Acta* **306**, 142–147.
6. Redman, C. M., Banerjee, D., Howell, K. & Palade, G. E. (1975) *J. Cell Biol.* **66**, 42–59.
7. Malawista, S. E. & Bodel, P. T. (1967) *J. Clin. Invest.* **46**, 786–796.
8. Zurier, R. B., Hoffstein, S. & Weissmann, G. (1973) *J. Cell Biol.* **58**, 27–41.
9. Kemper, B., Habener, J. F., Rich, A. & Potts, J. T. (1975) *Endocrinology* **96**, 903–912.
10. Wunderlich, F., Müller, R. & Speth, V. (1973) *Science* **182**, 1136–1138.
11. Berlin, R. D. & Fera, J. P. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1072–1076.
12. Greene, W. C., Parker, C. M. & Parker, C. W. (1976) *J. Immunol.* **117**, 1015–1022.
13. Walker, P. R. & Whitfield, J. F. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1394–1398.

14. Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12,** 186–202.
15. Tauber, R. & Reutter, W. (1978) *Eur. J. Biochem.* **83,** 37–45.
16. Keppler, D., Gawehn, K. & Decker, K. (1974) in *Methods of Enzymatic Analysis,* ed. Bergmeyer, H. U. (Academic, New York), 2nd Ed., pp. 2172–2178.
17. Lamprecht, W. & Trautschold, I. (1974) in *Methods of Enzymatic Analysis,* ed. Bergmeyer, H. U. (Academic, New York), 2nd Ed., pp. 2101–2110.
18. Beisenherz, G., Boltze, H. J., Bücher, T., Czok, R., Garbade, K. H., Meyer-Arendt, E. & Pfleiderer, G. (1953) *Z. Naturforsch.* **8b,** 555–577.
19. Wilson, L. & Friedkin, M. (1966) *Biochemistry* **5,** 2463–2468.
20. Baril, E. F., Potter, V. R. & Morris, H. P. (1969) *Cancer Res.* **29,** 2101–2115.
21. Lea, M. A., Bullock, J., Khalil, F. L. & Morris, H. P. (1974) *Cancer Res.* **34,** 3414–3420.
22. Wilson, L. & Friedkin, M. (1967) *Biochemistry* **6,** 3126–3135.
23. Fyfe, M. J., Loftfield, S. & Goldman, I. D. (1975) *J. Cell. Physiol.* **86,** 201–212.
24. Holley, R. W. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 2840–2841.
25. Mizel, S. B. & Wilson, L. (1972) *Biochemistry* **11,** 2573–2578.
26. Redman, C. M., Banerjee, D., Manning, C., Huang, C. J. & Green, K. (1978) *J. Cell Biol.* **77,** 400–416.
27. Dorling, P. R., Quinn, P. S. & Judah, J. D. (1975) *Biochem. J.* **152,** 341–348.
28. Stadler, J. & Franke, W. W. (1974) *J. Cell Biol.* **60,** 297–303.
29. Bhattacharyya, B. & Wolff, J. (1975) *J. Biol. Chem.* **250,** 7639–7646.
30. Martin, G. S., Venuta, S., Weber, M. & Rubin, H. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 2739–2741.
31. Isselbacher, K. J. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 585–589.
32. Schreiber, G., Boutwell, R. K., Potter, V. R. & Morris, H. P. (1966) *Cancer Res.* **26,** 2357–2361.
33. Vischer, P. & Reutter, W. (1978) *Eur. J. Biochem.* **84,** 363–368.
34. Reutter, W. Tauber, R., Vischer, P., Harms, E., Grünholz, H. J. & Bauer, C. (1978) in *Protein Turnover and Lysosome Function,* eds. Segal, H. L. & Doyle, D. J. (Academic, New York), pp. 779–790.
35. Morré, D. J. (1977) in *Membrane Alterations as a Basis of Liver Injury,* eds. Popper, H., Bianchi, L. & Reutter, W. (MTP Press, Lancaster, England), pp. 15–28.