

EFFECT OF COLCHICINE AND VINBLASTINE ON THE TOPOGRAPHICAL SEPARATION OF MEMBRANE FUNCTIONS*

By THOMAS E. UKENA† AND RICHARD D. BERLIN§

(From the Department of Physiology, Harvard Medical School,
Boston, Massachusetts 02115)

(Received for publication 27 March 1972)

Some of the proteins associated with the surface membrane of mammalian cells are distributed in a nonhomogeneous manner. Concanavalin A (Con A)¹ binds only to the posterior region of the plasma membrane of motile lymphocytes (1) and Con A binding sites are distributed in patches on the surface of some transformed cells (2) and polymorphonuclear leukocytes (3).

Furthermore, many proteins seem to have a degree of mobility within the plasma membrane (4). Thus when some normal cells are transformed by viruses, the concanavalin binding sites change from a homogeneous to a clustered arrangement (2). Membrane antigens, localized initially on one of a pair of cells fused by Sendai virus, are later distributed over the entire surface of the fused membrane (5).

The mechanisms which control the geographic distribution of membrane proteins are unknown, and little is understood about the relationship between components of the cytoplasm and the cell surface.

Phagocytosis of inert particles by alveolar macrophages and polymorphonuclear leukocytes (PMN) has recently been studied by Tsan and Berlin (7). It was shown that internalization of large portions of the plasma membrane during phagocytosis was not accompanied by a decrease in membrane transport by any of five specific carrier-mediated systems. The kinetic parameters of the transport systems were identical before and after phagocytosis. It was also shown that new carriers (presumably specific proteins) were not introduced into the membrane during this process. These results indicate that transport carriers are not internalized during phagocytosis. Thus the membrane of these cells behaves as if it were a mosaic, with topographically separate transport and phagocytic sites.

The experiments described here were designed to investigate the role of the microtubule system in determining the distribution of transport carriers in the membrane of the PMN. Colchicine and vinblastine are plant alkaloids that bind specifically at low concentrations to the microtubular proteins of mam-

* This work was supported by US Public Health Service and National Science Foundation grants.

† Supported by a US Public Health Service Training Grant.

§ Holds a US Public Health Service Career Development Award.

¹ *Abbreviations used in this paper:* Con A, concanavalin A; PMN, polymorphonuclear leukocytes.

malian cells, disrupting microtubular function. When PMN are pretreated with low doses of these alkaloids there is little effect on transport. However, in contrast to untreated cells, when such PMN are allowed to phagocytize inert particles in the presence of the same drug, membrane transport in two unrelated systems is markedly depressed.

Our observations are consistent with the conclusions that membrane carriers are internalized during phagocytosis when the microtubule system has been disrupted, but not when the microtubules are intact. The apparent topographic separation of transport and phagocytic sites previously described is eliminated by drugs which dissolve the microtubules.

Materials and Methods

Rabbit PMN were obtained from sterile peritoneal exudates by the method of Kaiser and Wood (6). PMN were harvested in a modified Hanks' solution (135 mM sodium chloride and 10 mM potassium monohydrogen phosphate adjusted to pH 8.0 with KOH) and cell monolayers were formed on round glass cover slips. After a 30 min preincubation in modified Hanks' solution with or without colchicine or vinblastine, the monolayers were drained and allowed to phagocytize polyvinyl toluene latex particles (2.2 μ diameter) in media containing 2 mM Mg for 30 min as previously described (7) in the continued presence or absence of alkaloids. After incubation with the particles, the cover slips were drained, thoroughly rinsed, and transport of adenine or lysine was measured using the rapid sampling technique previously developed in this laboratory (8). Test concentrations employed were adenine 10^{-5} M and lysine 10^{-4} M. Control monolayers also underwent two 30-min incubations, but the second incubation medium contained Mg^{++} and either Hanks' solution or Hanks' plus alkaloid, but no particles.

The protein content of monolayers was determined by the method of Lowry et al. (9) after overnight digestion in 2% Na_2CO_3 in 0.1 N NaOH (Lowry's solution A). Phagocytosis was quantitated by spectroscopy of dioxane extracts as described (10) and modified (7).

RESULTS

The effects of phagocytosis on alkaloid pretreated cells were tested on two transport systems: adenine which had been examined (7, 8) and a previously uncharacterized system based on the amino acid lysine. Lysine transport by this system obeys saturation kinetics with a V_{max} of 0.25×10^{-4} μ mole/min per 10^6 cells and an apparent K_m of 0.25 mM. As reported for the adenine system, after phagocytosis there was also no depression of lysine transport (Table I). Two consecutive 30-min incubations with 10^{-6} M colchicine alone (no particles) also had little effect. In contrast, preincubation of monolayers in 10^{-6} M colchicine followed by phagocytosis in the presence of the same concentration of the drug resulted in a dramatic decrease in lysine transport (Table I). The possibility that the effects on lysine transport were due to its modification by exchange diffusion (whereby the uptake of label is affected by the intracellular concentration of amino acids) was ruled out by preloading the PMN with 10 mM nonradioactive lysine during the second incubation period before testing transport (7).

In the adenine transport system, treatment with colchicine caused variable

(5–25%) decreases in transport, of the control monolayers, but phagocytosis with colchicine again resulted in large decreases in transport relative to the monolayers treated with colchicine alone (Table I). In control studies (as shown previously [7]) transport of adenine was unaffected after phagocytosis in Hanks' alone. However, after phagocytosis in the presence of colchicine, transport decreased significantly relative to monolayers treated only with colchicine.

The mean depressions of adenine and lysine transport shown in Table I agree remarkably well even though the data for the two systems were obtained in separate experiments. In three experiments it was possible to determine the effects of phagocytosis on transport of both lysine and adenine in colchicine-treated cells using the same cell preparation. Adenine transport was depressed by 63, 41, and 37% and lysine by 69, 34, and 34% in the respective experiments

TABLE I
Effect of Phagocytosis on Membrane Transport of Nonelectrolytes with and without Colchicine Pretreatment

Transport system	Per cent depression relative to nonphagocytizing monolayers*	
	No colchicine	Colchicine
Adenine	2.5 ± 2.3 (14) <i>P</i> † > 0.9	42.8 ± 5.0 (14) <i>P</i> < 0.001
Lysine	6.3 ± 3.9 (5) <i>P</i> > 0.8	43.5 ± 6.3 (5) <i>P</i> < 0.001

* Values given as per cent depression ± standard error of the mean. Number of observations is given in parentheses.

† *P* values refer to significance of depression from control (after phagocytosis).

relative to controls. These results indicate that two distinct and unrelated systems are affected in the same quantitative fashion by phagocytosis in the presence of colchicine.

Substitution of 10^{-6} M vinblastine sulfate for colchicine in the adenine and lysine systems gave identical results (three experiments).

The dose of colchicine employed in these experiments is within the range of concentrations causing specific effects in other animal cell systems (11) and somewhat less than described for some other effects of the drug in PMN attributed to microtubule dysfunction (12). The effects of colchicine on the shape of erythrocytes occur at 2000-fold higher concentrations (13), as does the non-specific precipitation of proteins by vinblastine (14). Fig. 1 shows a dose-effect curve for the depression of adenine transport after phagocytosis, in the presence of variable concentrations of colchicine. 10^{-6} M colchicine is the lowest dose that consistently produces the full transport effect. At higher concentrations, transport is depressed in nonphagocytizing monolayers due in part to loss of cells from the cover slip. However, the relative decreases in transport show little

change. Thus the decrease in transport associated with phagocytosis is not proportional to the concentration of colchicine employed, tending to eliminate a mechanism in which the alkaloid serves as a simple reactant. On the other hand, the essential relationship of phagocytosis to the effect of colchicine on transport is emphasized in experiments in which the depression of transport was determined as a function of the duration (and thus amount) of phagocytosis.

Fig. 2 shows the relationship of the transport effect to the uptake of polyvinyl toluene particles in a single experiment. The depression of trans-

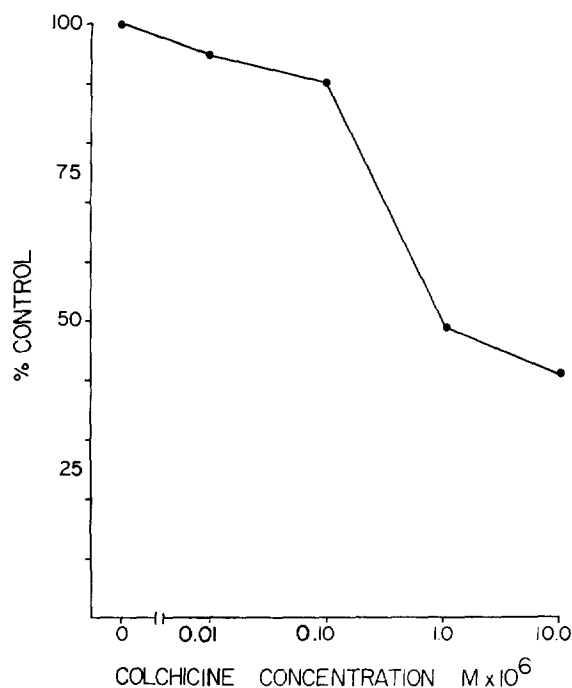


FIG. 1. Adenine transport after phagocytosis expressed as a per cent of the nonphagocytizing control *versus* colchicine concentration, $M \times 10^6$.

port closely parallels the uptake of particles, and thus the amount of membrane internalized. In control experiments it was shown that, beyond 10 min, the effect of the alkaloids was independent of the duration of exposure to them. The depression of transport due to phagocytosis in the presence of colchicine is not due to an increase in phagocytosis since under the conditions of our experiments colchicine depresses particle uptake by approximately 30%. Thus the amount of membrane internalized in the presence of colchicine is presumably actually less than in control phagocytosis experiments which show no change in transport.

Phagocytosis in the presence of colchicine does not alter the initial linear

period of adenine uptake, and the K_m for the system is unchanged. However the V_{max} of transport is decreased. These kinetics are most likely correlated with a decrease in the number of carrier proteins at the cell surface as previously discussed (7).

In order to test the possibility that the depression of transport was due to losses of cells from the cover slips during phagocytosis, we determined the protein content of cover slip monolayers by the method of Lowry et al. (9). There was no difference ($P > 0.9$) in the quantity of adherent cells between phagocytizing and control monolayers (with or without colchicine).

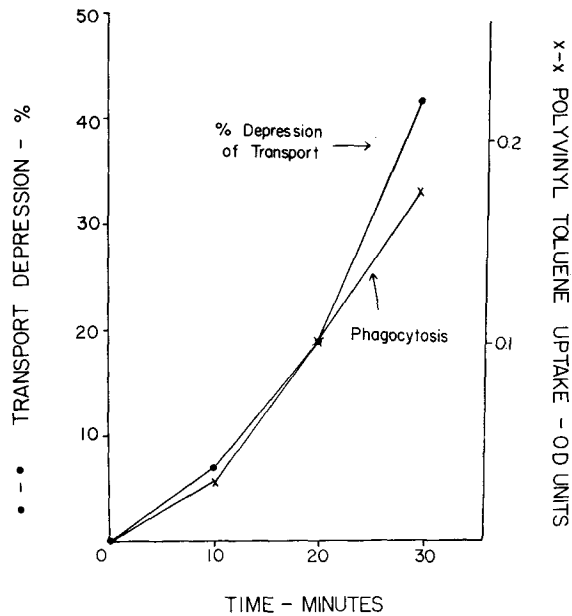


FIG. 2. Depression of transport (left ordinate) tested following variable periods of phagocytosis (abscissa), and the uptake of particles corresponding to the same time period (right ordinate).

CONCLUSION

During phagocytosis, PMN internalize a significant portion of their surface membrane. However this does not normally alter membrane transport, indicating that transport carrier proteins are not being internalized (7). The foregoing experiments demonstrate that when cells are treated with colchicine or vinblastine, phagocytosis results in large decreases in transport, suggesting that after alkaloid treatment, the internalization of large parts of the membrane during phagocytosis now removes transport carriers from the cell surface. Thus, the apparent geographical separation of phagocytic and membrane transport areas is abrogated by treatment with low doses of these drugs which bind specifically to microtubular proteins.

Two mechanisms are suggested: (a) Colchicine and vinblastine may interact directly with the membrane in a hitherto undescribed fashion to disrupt the long-range organization of membrane protein. We consider this unlikely, since at low concentrations these compounds do not appear to bind to membrane components (15). On the other hand, although they differ considerably in structure, they are known to bind specifically at very low concentrations to microtubules (16, 17). (b) Microtubules are involved in determining the topographical distribution of membrane proteins. Bridges between microtubules and the cell membrane have been identified in electron micrographs of plant cells (18) and lower animals (19). Vasiliev et al. found that colcemid relieved contact inhibition in cultured cells (20). We have recently shown that colchicine and vinblastine inhibit the agglutination of polymorphonuclear leukocytes by concanavalin A (a specific carbohydrate-binding plant protein) (21). Agglutination by Con A appears to be related to the organization of specific binding sites on the cell surface into patches (2), and such a distribution has been shown for PMN (3). The inhibitory effect of these drugs on Con A-induced agglutination of PMN suggests that the binding sites assume a more homogeneous distribution when the microtubules are not present. These experiments and the study described here indicate a new and important role for the microtubular system, and point to a possible link between cytoplasmic events and the control of surface function.

SUMMARY

The topographical separation of membrane functions into phagocytic and transport areas, inferred from physiological studies, is not demonstrable in cells treated with colchicine and vinblastine, alkaloids which bind to microtubular proteins.

REFERENCES

1. Smith, C. W., and J. E. Hollers. 1970. The pattern of binding of fluorescein-labeled concanavalin A to the motile lymphocyte. *Res. J. Reticuloendothel. Soc.* **8**:458.
2. Nicholson, G. L. 1971. Difference in topology of normal and tumor cell membranes shown by different surface distributions of ferritin-conjugated Concanavalin A. *Nature (London)*. **233**:244.
3. Smith, S., and J. P. Revel. 1972. Mapping of concanavalin binding sites on the surface of several cell types. *Develop. Biol.* **27**:434.
4. Singer, S. J., and G. L. Nicholson. 1972. The fluid mosaic model of the structure of cell membranes. *Science (Washington)*. **175**:720.
5. Frye, L. D., and M. Edidin. 1970. The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons. *J. Cell Sci.* **7**:319.
6. Kaiser, H. J., and W. B. Wood. 1962. Studies on the pathogenesis of fever. IX. The production of endogenous pyrogen by polymorphonuclear leukocytes. *J. Exp. Med.* **155**:127.

7. Tsan, M. F., and R. D. Berlin. 1971. Effect of phagocytosis on membrane transport of nonelectrolytes. *J. Exp. Med.* **134**:1016.
8. Hawkins, R. A., and R. D. Berlin. 1969. Purine transport in polymorphonuclear leukocytes. *Biochim. Biophys. Acta.* **173**:324.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.
10. Roberts, J., and J. H. Quastel. 1963. Particle uptake by polymorphonuclear leukocytes and Ehrlich ascites carcinoma cells. *Biochem. J.* **89**:150.
11. Williams, J. A., and J. Wolff. 1970. Possible role of microtubules in thyroid secretion. *Proc. Nat. Acad. Sci. U.S.A.* **67**:1901.
12. Malawista, S. E., and P. T. Bodel. 1967. The dissociation by colchicine of phagocytosis from increased oxygen consumption in human leukocytes. *J. Clin. Invest.* **46**:786.
13. Jacob, H., T. Amsden, and J. White. 1972. Membrane microfilaments of erythrocytes: alteration in intact cells reproduces the hereditary spherocytosis syndrome. *Proc. Nat. Acad. Sci. U.S.A.* **69**:471.
14. Wilson, L., J. Bryan, A. Ruby, and D. Mazia. 1970. Precipitation of proteins by vinblastine and calcium ions. *Proc. Nat. Acad. Sci. U.S.A.* **66**:807.
15. Borisy, G. G., and E. W. Taylor. 1967. The mechanisms of action of colchicine: binding of colchicine-³H to cellular protein. *J. Cell Biol.* **34**:525.
16. Weisenberg, R. C., G. G. Borisy, and E. W. Taylor. 1968. The colchicine-binding protein of mammalian brain and its relation to microtubules. *Biochemistry.* **7**:4466.
17. Marantz, R., M. Ventilla, and M. Shelanski. 1968. Vinblastine-induced precipitation of microtubule protein. *Science (Washington).* **165**:498.
18. Kiermayer, O. 1968. The distribution of microtubules in differentiating cells of *Microsterias denticulata* breb. *Planta.* **83**:223.
19. Silveira, M., and K. R. Porter. 1964. The spermatozooids of flatworms and their microtubular systems. *Protoplasma.* **59**:240.
20. Vasiliev, Ju. M., I. M. Gelfand, L. V. Domnina, O. Y. Ivanova, S. G. Komm, and L. V. Olshevskaia. 1970. Effect of colcemid on the locomotory behavior of fibroblasts. *J. Embryol. Exp. Morphol.* **24**:625.
21. Berlin, R. D., and T. E. Ukena. 1972. Effect of colchicine and vinblastine on the agglutination of polymorphonuclear leucocytes by concanavalin A. *Nature (London)*. In press.