Inhibition of Amino Acid Transport in Rabbit Intestine by \( \rho \)-Chloromercuriphenyl Sulfonic Acid

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ABSTRACT Influx of phenylalanine across the brush border of rabbit intestine is markedly reduced by treatment with 5 mM \( \rho \)-chloromercuriphenyl sulfonate (PCMBS). The effect is rapidly and completely reversed by dithiothreitol. Phenylalanine influx into PCMBS-treated tissue can be competitively inhibited by other neutral amino acids and follows saturation kinetics. PCMBS causes an increase in the apparent Michaelis constant from the value observed in control tissue but does not alter the maximal influx significantly. Treatment of the tissue with PCMBS leads to a significant reduction in the Na-sensitivity of the transport, and a number of results indicate that the major effect of the reagent is to cause a marked reduction in the affinity of the transport system for Na. The transport system can be partially protected against reaction with PCMBS by phenylalanine and tryptophan but not by methionine or norleucine. The results suggest that PCMBS reacts with a sulphhydryl group in the region of the transport site and may alter conformational changes associated with the binding of substrates.

The transport of neutral amino acids by rabbit ileum has been investigated extensively by Schultz, Curran, and co-workers (1–4). Examination of the unidirectional fluxes of several amino acids from the mucosal solution into the cell across the brush border membrane has indicated that this influx follows Michaelis-Menten kinetics and is strongly dependent on the Na concentration in the mucosal bathing solution. Considerable evidence indicates that the transport system mediates a coupled entry of Na and amino acid into the cell (2, 5, 6). In addition, the process of amino acid influx is little affected by metabolic inhibitors under experimental conditions in which the cells are prevented from gaining Na (7). Thus, the system seemed to be a suitable one for an attempt to identify some of the functional chemical
groups in the mucosal membrane that are involved in coupled Na-amino acid transport. At least two studies had suggested that sulfhydryl groups might play a role. Burns and Faust (8) showed that p-hydroxymercuribenzoate, a sulfhydryl-reactive reagent, inhibited the binding of amino acids to isolated hamster intestine brush border membranes. Frizzell and Schultz (9) also suggested that a sulfhydryl group may be involved in the process of neutral amino acid transport in rabbit ileum. In addition, the modification of biological membranes by sulfhydryl-reactive reagents has been reported to alter transport of sulfate, K (10), choline (11), and glucose (12) in erythrocytes, glucose in fat cells (13), K and Cl in mitochondria (14), H and Cl in gastric mucosa (15), and sugars and amino acids in membrane vesicles from Escherichia coli (16). In order to investigate possible roles of sulfhydryl groups in amino acid transport in rabbit ileum, we examined the effects of p-chloromercuriphenyl sulfonic acid (PCMBS) on phenylalanine influx across the brush border. This organic mercurial reacts with protein sulfhydryl groups with relatively high specificity and appears to penetrate biological membranes rather slowly (17).

METHODS

New Zealand white rabbits were sacrificed by intravenous injection of sodium pentobarbital. A segment of small intestine beginning at the ileocecal valve was removed rapidly, opened along the mesenteric border, washed in normal Ringer solution, and mounted in the influx chambers described by Schultz et al. (1). Unidirectional influxes of phenylalanine, alanine, methionine and 3-O-methyl glucose were measured as described previously (1) by a 60 s exposure of the mucosal surface to a test solution containing [14C]amino acid or [14C]sugar and nonpenetrating [3H]inulin to correct for test solution that remains adhered to the surface of the intestine. Influx was evaluated from the amount of 14C taken up by the tissue after correction for the inulin "space." The normal Ringer solution contained 140 mM NaCl, 10 mM KHCO3, 0.2 mM KH2PO4, 1.2 mM K2HPO4, 1.2 mM MgCl2, and 1.2 mM CaCl2, and had a pH of 7.2 when equilibrated with 95% O2-5% CO2. Na-free solution was prepared by replacing all NaCl by choline Cl.

The mounted tissue was allowed to equilibrate at 37°C for 30 min in Na-free Ringer solution. All incubations before flux measurement were always carried out in Na-free medium in order to insure that poisoned tissues did not gain Na. Previous studies (7) with metabolic inhibitors and ouabain had shown that inhibition in the presence of Na led to some decrease in alanine influx, presumably due to cell swelling. The effect was minimized by preincubation in Na-free medium and this procedure was adopted in the present study in an effort to avoid possible similar effects of PCMBS. After the equilibration period, the mucosal surface of the tissue was exposed to PCMBS in Na-free solution for varying periods of time. At appropriate intervals, the PCMBS solution was removed from the chamber and the mucosal surface of the tissue was washed three times with fresh Na-free solution. The amino acid or sugar influx was then measured in PCMBS-modified tissue either from normal
Ringer solution containing 140 mM Na or from a Na-free solution. PCMBS was never present in the test solution used to determine influx. In each experiment, control measurements were made on tissue that had not been exposed to PCMBS but which was otherwise treated identically. In some experiments, the time-course of PCMBS inhibition was studied; in others, the tissue was modified by incubation for 30 min in Na-free Ringer containing 5 mM PCMBS and then studied in the manner described above. In a few experiments, intestine was mounted as a flat sheet in the apparatus described by Schultz and Zalusky (18) in order to measure short-circuit current and to evaluate effects of PCMBS, amino acids, and sugars. L-Phenylalanine, L-tryptophan, L-methionine, L-alanine, 3-O-methyl-D-glucose, and PCMBS were obtained from Sigma Chemical Co., St. Louis, Mo.; dl-α-amino-δ-hydroxyvaleric acid from Cyclo Chemical Corp., Los Angeles, Calif.; and D-phenylalanine from Calbiochem, Los Angeles, Calif. [14C]L-Phenylalanine, [14C]L-methionine, [14C]3-O-methyl-D-glucose, and [3H]inulin were purchased from New England Nuclear, Boston, Mass.

RESULTS

The effect of PCMBS on phenylalanine influx measured from an external concentration of 1 mM is shown in Fig. I. The ratio of influx into PCMBS treated tissue \( (J_\text{PCMBS}) \) to control influx \( (J_\text{C}) \) is given as a function of time of exposure to several concentrations of PCMBS. For each point, the control tissue was the area immediately adjacent to the treated tissue, and both pieces had been exposed to bathing solution for the same total time. Influx

![Figure 1](image-url)  
**Figure 1.** Effect of PCMBS on phenylalanine influx as a function of time. The points at 5 mM PCMBS are averages from six experiments; the other concentrations are single experiments.
was measured from solution containing 140 mM Na but until the time of influx measurement, the mucosal surfaces of all tissues were bathed in Na-free choline medium. Under these conditions, 5 mM PCMBS rapidly inhibits 80–90% of phenylalanine influx. Inhibition was observed at PCMBS concentrations as low as 10^{-5} M but the time-course of the effect was slowed considerably at concentrations below 1 mM. The average control influx observed in these experiments was 0.99 ± 0.08 μmol/l·cm^2, a value that agrees well with that expected from the results of Hajjar and Curran (4). Most subsequent experiments were carried out at 5 mM PCMBS in order to provide maximal inhibition in a relatively short period of time. Unless otherwise noted, phenylalanine influx was measured at a concentration of 1 mM.

The inhibitory effect of PCMBS on phenylalanine influx could not be reversed by prolonged washing of the tissue with Na-free solution as indicated by the experiment shown in Fig. 2. In this experiment, eight samples of tissue were exposed to 5 mM PCMBS at zero time; four tissues were used to determine the time-course of inhibition. The remaining four tissues were exposed to PCMBS for 30 min and then washed in Na-free solution for varying periods of time up to 60 min. There was no significant recovery of ability to transport phenylalanine; two additional experiments gave identical results. However, the effect of PCMBS could be rapidly and completely reversed by treatment with the reducing agent dithiothreitol (DTT) as also shown in Fig. 2. In this experiment, tissues were treated for 15 min with 5 mM PCMBS, washed three times rapidly, and then exposed for varying periods of time to

![Figure 2](image-url)
Na-free medium containing 50 mM DTT before influx measurement. At the earliest time studied, 5 min after addition of DTT, phenylalanine influx had returned approximately to control values. Additional experiments showed that 10 mM DTT was sufficient to reverse completely the effects of PCMBS.

In order to obtain a better estimate of the rate at which DTT acts, the following experiment was carried out. Tissues were treated with 5 mM PCMBS for 5 min and washed free of excess PCMBS. Reversal with DTT was then attempted by simply including DTT (50 mM) in the test solution used to measure phenylalanine influx so that the total time of exposure to DTT was 60 s. The results, summarized in Fig. 3, show that this procedure leads to complete recovery of phenylalanine influx. Thus the action of DTT must be complete in much less than 60 s. We also attempted a similar experiment in which the PCMBS-treated tissues were exposed to test solution (and DTT) for only 5–7 s. Although influx measurements are somewhat unsatisfactory under these conditions, the results indicated almost complete recovery (83%) of phenylalanine influx with this very short exposure to DTT.

Although DTT appears to reverse completely the effect of PCMBS, additional experiments were carried out to compare the normal transport system with the one treated with PCMBS and restored with DTT. To do this, we have examined the ability of methionine to inhibit phenylalanine influx under the two conditions. The experimental approach is similar to that used by Hajjar and Curran (4) in studies on phenylalanine transport. Influx of phenylalanine is measured at a concentration of 1 mM under control conditions and in the presence of three different concentrations of methionine. As previously discussed (4), the ratio of control influx \( \left( J_0 \right) \) to influx in the presence of methionine \( \left( J_i \right) \) is given by

\[
\frac{J_0}{J_i} = 1 + \frac{K_{\text{Phe}} [\text{Met}]}{K_{\text{Met}} (K_{\text{Phe}} + [\text{Phe}])},
\]

in which \( K_{\text{Phe}} \) is the "apparent Michaelis constant" of phenylalanine for the transport system and \( K_{\text{Met}} \) is the "inhibitory constant" for methionine; \( K_{\text{Met}} \) should be identical with the Michaelis constant for methionine if the inhibition is competitive. Test tissues were treated with 5 mM PCMBS for 30 min and then with 50 mM DTT for 15 min, always in Na-free medium. Control tissues were incubated for the same total length of time in Na-free medium, and fluxes were measured in all tissues from solution containing 140 mM Na. As shown in Fig. 4, a plot of \( J_0/J_i \) vs. methionine concentration yields straight lines with identical slopes in control tissues and in PCMBS-treated tissues restored with DTT. Assuming that \( K_{\text{Phe}} = 3 \text{ mM} \) (4), \( K_{\text{Met}} \) is calculated to be 1.7 mM for control tissues and 1.8 mM for those treated with PCMBS and DTT. Thus, the inhibited and restored system appears
to have the same characteristics as the control with respect to its handling of both phenylalanine and methionine. The value of $K_{Met}$ calculated from these results agrees reasonably well with the value of 1.4 mM determined by direct measurement of methionine influx (Preston, Schaeffer, and Curran, unpublished observations).

To determine whether PCMBS causes a relatively nonspecific inhibition of all transport processes in the brush border membrane, we examined its effects on the influx of 3-O-methyl glucose (3-MG), an actively transported sugar whose influx across the brush border is also strongly dependent on Na concentration in the mucosal solution (19). As shown in Fig. 5, 5 mM PCMBS causes relatively little inhibition of 3-MG influx, particularly at short times of exposure. At 20 min, amino acid influx was inhibited by 80–90% while influx of 3-MG was decreased by only 13%. The data shown in Fig. 5 also indicate that PCMBS alters the influxes of methionine and alanine with almost exactly the same time-course as that observed for inhibition of phenylalanine influx.

If the inhibition of amino acid transport by PCMBS occurs by reaction with a group (presumably a sulfhydryl) in the transport site, it should be possible to protect the system, to some extent at least, by adding an actively
transported amino acid to the solution containing PCMBS. Some experiments testing this possibility are shown in Fig. 6. When the tissue was exposed simultaneously to 5 mM PCMBS and 50 mM phenylalanine for varying periods, the rate at which PCMBS inhibited phenylalanine influx was reduced substantially. Virtually identical protection was obtained with tryptophan which also has a bulky aromatic side chain. However, the addition of methionine to the preincubation solution altered the time-course of PCMBS action only slightly. Norleucine, leucine, and 3-MG at concentrations of 50 mM gave results similar to those shown for methionine. Thus, only phenylalanine and tryptophan provided effective protection of the transport system under these particular experimental conditions.

There are, in principle, several possible interpretations of these observations. For example, PCMBS could react with the free amino acids present, thereby reducing the effective concentration of the inhibitor and slowing its reaction with the transport system. Since several amino acids fail to protect effectively, a general reaction of PCMBS, for instance with the α-amino group, seems unlikely. However, the protection observed in the presence of phenylalanine and tryptophan could be the results of a charge-transfer complex formed between the reagent and the aromatic nuclei of the amino acid which could facilitate the reaction of PCMBS with the free aromatic amino acid. To test this possibility, the effect of D-phenylalanine on the time-course
of PCMBS inhibition of phenylalanine influx was examined. Since the transport system has an affinity for d-phenylalanine that is at least on the order of magnitude lower than its affinity for L-phenylalanine (4), the d-isomer should provide much less effective protection. As shown in Fig. 6, d-phenylalanine does not protect as well as L-phenylalanine. Therefore, the reduced inhibitory effect of PCMBS in the presence of L-phenylalanine appears to be due to its protection of some site on the membrane from PCMBS attack. It seems unlikely that these protective effects can be ascribed to entry of amino acid into the mucosal cells during the initial incubation period, since previous studies (1) have shown that preloading the cells with alanine had no effect on alanine influx measured in the presence of 140 mM Na. However, to test this possibility, experiments were carried out in which tissues were first preincubated for 30 min in Na-free solution containing 50 mM phenylalanine in order to load the cells with amino acid. The time-course of the effect of PCMBS on phenylalanine influx was then compared in preloaded and control tissue. The resulting curves were identical, indicating that cellular amino acid does not protect against the action of PCMBS.

Experiments similar to those described above were also carried out with phenylalanine influx measured from Na-free choline medium rather than from solution containing 140 mM Na. Since influx is considerably reduced

![Figure 6](image-url)
under these conditions, phenylalanine concentration in the test solution was raised to 10 mM to provide influxes approximately the same as those obtained at 1 mM in normal Na-Ringer. The data on the right-hand side of Fig. 6 show that PCMBs also inhibits influx measured in the absence of Na and that addition of L-phenylalanine to the preincubation solution protects against this action. Comparison of the data for fluxes measured with and without Na present suggests that the inhibition caused by PCMBs is relatively greater for fluxes measured in the presence of Na than in its absence. This impression is confirmed by the data shown in Fig. 7 which indicate that three sulphydryl-reactive agents (PCMBs, N-ethylmaleimide [5 mM], and iodoacetate [5 mM]) all have a significantly greater relative effect on phenylalanine influx when it is measured in the presence of Na. These results suggest that a major effect of PCMBs may be to alter the nature of the Na-dependence of phenylalanine influx although this cannot be the sole effect because substantial inhibition is also observed under Na-free conditions.

A variety of experiments were carried out to obtain information on the properties of phenylalanine influx remaining after treatment with PCMBs. The abilities of tryptophan, $\alpha$-amino-$\delta$-hydroxyvaleric acid and methionine to inhibit this residual flux were examined with results summarized in Table I. Both in the presence of Na and in its absence, other neutral amino acids caused substantial inhibition of phenylalanine influx, suggesting that this residual flux still involved a mediated transfer mechanism. To examine this possibility and to obtain further insight into the nature of the PCMBs
effect, kinetic experiments were carried out at varying concentrations of Na and phenylalanine. In each experiment, a set of tissues was treated with PCMBS for 30 min. Influx was then measured at several concentrations of phenylalanine in solutions containing 0, 47, or 140 mM Na. Separate control experiments were carried out in the same manner on tissues not treated with PCMBS. Phenylalanine influx into PCMBS-treated tissue follows Michaelis-Menten kinetics both in the presence of Na and in its absence. Under both conditions, the major effect of PCMBS is on the apparent Michaelis constant, \( K_t \), with minimal changes in maximal flux, \( J_{max} \), as indicated by the double reciprocal plots shown in Fig. 8. In the presence of Na, \( K_t \) is increased from 4 mM to 13 mM by PCMBS. For fluxes measured

### Table I

**Competitive Inhibition of Phenylalanine Influx in PCMBS-Treated Tissue**

| Competitor | Comp | Phe | Na  | Phe influx |
|------------|------|-----|-----|------------|
|            | mM   | mM  | mM  | \( \mu \text{mol}\/\text{h cm}^2 \) |
| Tryptophan | 10   | 1   | 140 | 0.38 ± 0.06 |
| AHVA*      | 20   | 1   | 140 | 0.37 ± 0.03 |
| Methionine | 20   | 10  | 0   | 1.00 ± 0.08 |

*α-Amino-δ-hydroxyvaleric acid.

### Figure 8

Double reciprocal plots of phenylalanine influx in control tissues and tissues treated for 30 min with 5 mM PCMBS.
in Na-free medium, $K_i$ increases from 15 mM to 26 mM. Similar kinetic experiments were also carried out at a Na concentration of 47 mM in PCMBS-treated tissue and yielded a $K_i$ of 21 mM. The results shown in Fig. 8 for 140 mM Na were obtained at relatively low phenylalanine concentrations (0.5–5 mM). However, a similar set of experiments at higher concentrations (5–40 mM) yielded essentially identical results; PCMBS treatment caused a 3.6-fold increase in $K_i$ and no significant change in maximal flux.

A further test for a change in Na dependence as a result of PCMBS treatment was carried out by determining phenylalanine influx from a concentration of 2 mM in the presence and absence of Na in paired tissues from the same animal. In control tissues, the ratio of influx at 140 mM Na to that at zero Na was 5.69 ± 0.60 (12 observations) while in PCMBS treated tissues, the ratio was 1.84 ± 0.18 (12 observations), indicating a significant decrease in Na sensitivity of the process. The expected ratios can be calculated from the known values of $K_i$ in the presence and absence of Na. For control tissues, the data of Hajjar and Curran (4) yield an expected ratio of 5.2 and the data above give an expected ratio of 1.9 for PCMBS-treated tissues; both values agree well with those observed.

A final series of experiments was carried out to examine the effect of PCMBS on the ability of phenylalanine to stimulate short-circuit current ($I_{sc}$). This increase in $I_{sc}$ is due to stimulation of net Na transport across the tissue (20) and involves the coupled entry of Na and amino acid into the cell across the brush border membrane. Four pieces of tissue were mounted in chambers, bathed on both sides with normal Ringer (140 mM Na), and short-circuitcd. One piece served as control. Two pieces were treated for 5 min or 10 min with 5 mM PCMBS in the mucosal solution; the chamber was washed thoroughly and refilled with normal Ringer. The fourth piece was treated for 10 min with PCMBS followed by 10 min with 10 mM DTT. After these treatments were completed, 10 mM phenylalanine was added to the mucosal solution of each chamber and $I_{sc}$ followed for 10 min. The results of one such experiment are shown in Fig. 9 in terms of the ratio of $I_{sc}$ after addition of phenylalanine to the initial $I_{sc}$. Four additional experiments gave similar results. Treatment of the mucosal surface with PCMBS for 5 min reduced the response to phenylalanine by an average of 77% and 10 min exposure to PCMBS almost completely abolished the response (average inhibition, 94%). The effect of PCMBS could be reversed by DTT (average recovery, 80%). Fig. 9 also shows the results of a similar experiment carried out with an actively transported sugar, galactose. A 5 min exposure of the mucosal surface to PCMBS had virtually no effect on the increase in $I_{sc}$ caused by galactose while a 10 min exposure caused considerable inhibition. These observations are consistent with those shown in Fig. 5, indicating that PCMBS affects sugar influx more slowly than phenylalanine influx.
Figure 9. Effect of phenylalanine and galactose on short-circuit current across rabbit ileum bathed on both sides in normal Ringer solution. Results are expressed relative to current just before addition of 10 mM phenylalanine on galactose to the mucosal solution. (○) control; (×) 5 min exposure of mucosal surface to 5 mM PCMBS; (O) 10 min exposure to PCMBS; (△) 10 min exposure to PCMBS followed by 10 min exposure to 10 mM DTT.

Discussion

The effects of PCMBS on amino acid transport described above are presumably due to reaction of the reagent with sulphydryl groups in the brush border membrane since organic mercurials such as PCMBS are highly selective for such groups (21, 22). However, under conditions in which the reagent is present in excess, as it certainly is in these experiments, amino, carboxyl, and histidyl residues may also be modified. For example, PCMBS reacts with a histidyl residue in whale myoglobin (23). On the other hand, the ready reversibility by DTT of the effect of PCMBS on phenylalanine influx is consistent with the concept that the primary effect is on sulphydryl groups. The extreme rapidity with which DTT reverses the effects of PCMBS also suggests quite strongly that site of action is on the brush border membrane. It seems quite unlikely that the present results can be explained by metabolic inhibition caused by PCMBS that enters the mucosal cells, since previous studies (7) have shown that alanine influx is virtually unaffected by metabolic inhibitors, providing the incubation is carried out in Na-free solution (as it was in the present study). In addition, PCMBS appears to penetrate cell membranes slowly (17). Thus, on several grounds the hypothesis that PCMBS inhibits amino acid influx as a result of reaction with sulphydryl groups in the brush border membrane seems reasonable.
The effects of PCMBS could, however, be relatively nonspecific and result from general disruption of the membrane perhaps due to an increase in negative charge in certain regions as a result of reaction with the reagent. For example, Godin and Schrier (24) have reported that exposure of erythrocyte membranes to 4 × 10^{-5} M \( p \)-hydroxymercuriphenyl sulfonate, a compound closely related to PCMBS, for 15 min at 23°C solubilized 12% of the total membrane protein. Such nonspecific effects do not appear to be involved in the inhibition of phenylalanine influx for several reasons: (a) the effect of PCMBS is relatively specific since it inhibits phenylalanine influx to a much greater extent than 3-MG influx; (b) phenylalanine influx still involves a mediated process in PCMBS-treated tissue, and the maximal influx is essentially the same as that observed in control tissues; (c) the effect of PCMBS can be completely reversed by DTT, and the restored system appears to have characteristics identical to the normal transport system; (d) the transport site can be protected from PCMBS by certain amino acids.

In the presence of Na and in its absence, PCMBS modification causes an increase in the apparent Michaelis constant, \( K_t \), of the transport system with little change in the maximal influx. Thus, reaction with PCMBS does not render the transport system inoperative but rather alters its kinetic characteristics. Under these conditions, the model of the Na-dependent neutral amino acid transport system in the brush border of rabbit ileum described by Curran et al. (2) can be utilized to obtain further insight into the nature of the PCMBS effect. According to this model, the reciprocal of \( K_t \) should be a linear function of Na concentration in the mucosal solution, and Hajjar and Curran (4) have shown that such a relationship holds for phenylalanine influx. This relationship can be used to evaluate certain parameters of the model. Amino acid (A) is assumed to combine first with a transport site (X) to form a complex

\[
X + A \rightleftharpoons XA, \quad (2)
\]

and this binary complex can then combine with Na:

\[
XA + Na \rightleftharpoons XNa \quad (3)
\]

Both the binary and ternary complexes can be translocated across the membrane. Analysis of the steady-state kinetic behavior of this system using certain simplifying assumptions (2) indicates that

\[
1/K_t = 1/K_1 + [Na]/K_1K_2
\]

in which \( K_1 \) is the dissociation constant for reaction 2 and \( K_2 \) is the dissociation constant for reaction 3. The linear relation between \( 1/K_t \) and [Na] can be used to evaluate \( K_1 \) and \( K_2 \). As shown in Fig. 10, a similar relation-
ship is observed for tissue treated with PCMB. This relationship yields $K_1 = 25$ mM and $K_2 = 135$ mM compared to values of $K_1 = 17.8$ mM and $K_2 = 25$ mM obtained by Hajjar and Curran (4) for phenylalanine influx into normal tissue. Thus within the context of this model, which appears to provide an adequate working representation of the transport system, the major effect of PCMB is a substantial decrease in the affinity of the transport site for Na. There is also a relatively small decrease in the affinity for amino acid.

These relationships are supported by the observation that the sensitivity of phenylalanine to Na removal is decreased by a factor of nearly 3 by treatment with PCMB. The studies shown in Table I are also consistent with these conclusions. Given values of $K_i$ for phenylalanine, Eq. 1 can be used to calculate a $K_i$ for the other amino acids used as inhibitors (and this quantity should represent $K_i$ for those amino acids). In PCMB-treated tissue, $K_i$ for tryptophan measured at 140 mM Na is 10 mM compared to a value of 2.2 mM in normal tissue (4). Under the same conditions $K_i$ for $\alpha$-amino-$\delta$-hydroxyvaleric acid is 8.8 mM compared to a normal value of 1.8 mM (Preston, Schaeffer, and Curran, unpublished). For experiments in the absence of Na, $K_i$ of methionine is 21 mM in PCMB-treated tissue and 9.1 mM in normal tissue (Preston, Schaeffer, and Curran, unpublished). Since the effect of PCMB is considerably greater in the presence of Na than in its absence, these calculations also suggest a significant reduction in the affinity of the transport site for Na.

Finally the results shown in Fig. 9 on changes in $I_{sc}$ caused by phenylalanine also indicate a marked effect of PCMB on the interaction of Na with the transport site. A short (5 min) exposure of the mucosal surface to PCMB

**Figure 10.** Relationship between $I/K_i$ for phenylalanine and Na concentration in the mucosal solution. Control data from Hajjar and Curran (4).
almost completely inhibits the stimulation of $I_{se}$ caused by addition of phenylalanine to the mucosal solution. Since there is still a substantial entry of amino acid into the cells under the conditions of this experiment, the results suggest strongly that the ability of the transport system to combine with Na has been seriously impaired.

The present results do not provide information on the specific mechanism by which the transport system is altered by reaction with PCMBS. However some interesting speculations can be made on the basis of the model for the Na-amino acid interaction suggested by Schultz (25). He proposed that combination of the amino acid with its binding site results in a conformational change that “creates” the Na binding site. In his sketch of this model (Fig. 9 of reference 25) Schultz included a hypothetical “inhibitor” site adjacent to the amino acid site and suggested that reaction of an inhibitor with this site could prevent or impair the conformational change leading to formation of the Na site. The behavior of PCMBS seems consistent with this concept since its major effect is on the interaction of Na with the transport site. It would be necessary also to propose that reaction with PCMBS alters the amino acid site since the apparent affinity for phenylalanine is somewhat reduced. The observations that the transport system can be protected by amino acids with aromatic side chains but not by amino acids with less bulky side chains (Fig. 6) could also be explained in terms of Schultz’s model. For example, binding of the more bulky amino acids could lead to a conformational change that reduces the accessibility of the “inhibitor” site to PCMBS while the less bulky amino acids are unable to cause such a change. On the basis of these considerations, we would suggest that PCMBS alters amino acid transport in rabbit ileum by reacting with a site, presumably a sulfhydryl group, that is adjacent to the amino acid site and thereby influencing conformational changes in the transport system. Although these concepts seem consistent with our results, they must remain speculative until we have considerably more information about the molecular nature of the transport site.

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