A Protein-tyrosine Kinase-regulated, pH-dependent, Carrier-mediated Uptake System for Folate in Human Normal Colonic Epithelial Cell Line NCM460*

Chandira K. Kumar‡, Mary Pat Moyer§, Pradeep K. Dudeja¶, and Hamid M. Said**

From the (Veterans Administration Medical Center, Long Beach, California 90822, University of California School of Medicine, Irvine, California 92717, Center for Human Cell Biotechnology, The University of Texas Health Science Center, San Antonio, Texas 78284, and the *University of Illinois and Westside Veterans Administration Medical Center, Chicago, Illinois 60612

A significant proportion of the bacterially synthesized folate in the large intestine exists in the form of folate monoglutamate. Recent studies in our laboratory using human colonic apical membrane vesicles have shown the existence of an efficient carrier-mediated system for folate uptake. Nothing, however, is known about the cellular regulation of the colonic uptake process. In this study, we used a recently established human normal colonic epithelial cell line NCM460 to address this issue. Uptake of folic acid by NCM460 cells was: 1) linear with time for 4 min of incubation and occurred with minimal metabolic alterations, 2) temperature- and pH- (but not Na+) dependent, 3) saturable as a function of concentration (apparent Km of 1.4 μM), 4) inhibited by structural analogs and anion transport inhibitors, and 5) energy-dependent. These characteristics of folic acid uptake by NCM460 cells are similar to those seen with apical membrane vesicles derived from human native colonic tissue. Using these cells, we found that protein kinase C-and Ca2+/calmodulin-mediated pathways have no role in regulating folic acid uptake. On the other hand, cAMP (through a mechanism independent of protein kinase A) and protein-tyrosine kinase-mediated pathways were found to play a role in the regulation of folic acid uptake by these cells. These results establish the suitability of NCM460 cells as an in vitro model system for investigating the details of the mechanism of colonic folate uptake and its regulation. Folic acid uptake by these cells appears to involve a carrier-mediated system, which is temperature-, pH-, and energy-dependent and appears to be under the regulation of cAMP and protein tyrosine kinase.

Folate is an essential micronutrient, which acts as a coenzyme in the synthesis of DNA and RNA and the interconversion and degradation of several amino acids (1–4). An adequate supply of folate is therefore necessary for normal cellular function, growth, and development. Folate deficiency has been suggested as one of the most common vitamin deficiencies in the Western Hemisphere (5, 6). Humans and other mammals cannot synthesize folate and rely on exogenous sources to meet their metabolic requirements. Folates are presented to the host from the diet and are also synthesized in the large intestine by normal microflora. The mechanism of absorption of dietary folate has been intensively examined over the past two decades at the tissue, cellular, subcellular, and more recently, molecular levels (6–15). Absorption of dietary folate has been shown to occur mainly in the proximal small intestine and involves a specialized, carrier-mediated system (6–12).

As to the bacterially synthesized folate in the large intestine, a significant amount of that folate exists in the monoglutamate, i.e., the absorbable form. Using [3H]p-aminobenzoic acid to label the newly synthesized folate by the intestinal flora, Rong et al. (16) have shown that a portion of this folate is indeed absorbed by the rat and is incorporated into its various tissues. Very limited studies, however, are available describing the mechanism and regulation of folate uptake by the colocytes. Addressing this issue is of physiological importance because the colon has a unique structure, luminal environment, absorption mechanisms, and energy metabolism when compared with the small intestine. Folate uptake may also be of nutritional importance especially under conditions of massive disease or extensive resection of the small intestine (17–20). Furthermore, studies of folate metabolism at the cellular level may help clarify the causes of the localized folate deficiency believed to be associated with premalignant changes in colonic epithelia (21, 22).

Prompted by the above and the recent observation in our laboratory that a mRNA species from a mouse colon hybridizes with cDNA of a recently cloned folate carrier from mouse small intestine (the intestinal folate carrier-1) (14), we performed a study to directly test for a folate transporter in the colon. Using purified apical membrane vesicles prepared from human colonic tissue, we demonstrated the existence of an efficient carrier-mediated system for folate uptake that is pH-dependent and DIDS1-sensitive (23). Nothing, however, is known about the intracellular regulation of the folate uptake process in the colon. Studies with colonic apical membrane vesicles cannot provide such information because these structures lack the intracellular components. A colonic cell line that possesses a folate uptake mechanism that is similar to that of the native colocytes would be an ideal model system to address this issue. The NCM460 cells (a normal, non-transformed epithelial cell line derived from the human transverse colonic mucosa) (24) were chosen as a model system because they possess char-

*This study was supported in part by grants from the Department of Veterans Affairs (to H. M. S.) and National Institutes of Health Grants DK47203 (to H. M. S.) and HL 48497 (to M. P. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed: UCI and Long Beach VA Medical Program, VA Medical Center-151, Long Beach, CA 90822. Tel.: 310-494-5811; Fax: 310-494-5675.

1 The abbreviations used are: DIDS, H,H- diaiosiethiocyanostilbene-2,2‘-disulfonic acid; PTK, protein-tyrosine kinase; PBC, protein kinase C; PKA, protein kinase A; Bt2cAMP, dibutyryl cAMP; IBMX, isobutylmethylxanthine; SITS, 4-acetamido-4‘-isothiocyanostilbene-2,2‘-disulfonic acid; MES, 4-morpholinoethanesulfonic acid.
Folate Uptake in Normal Human Colonic Cells

MATERIALS AND METHODS

[3H]Folic acid (specific activity, 30 Ci/mmol; radiochemical purity, \(>97\%\)) was purchased from American Radiolabeled Chemicals (St. Louis, MO). [3H]Biotin (specific activity, 46.8 Ci/mmol) was obtained from Du Pont NEN. Trypsin and other cell culture ingredients were from Sigma. All other chemicals were of analytical grade and were purchased from commercial sources.

The human normal colonic epithelial cell line NCM460 was propagated to maintain its colonocyte features (24) in the culture medium M3:10TM (INCELL Corp., San Antonio, TX). The M3:10TM medium is M3TM base medium supplemented with 10% (v/v) fetal bovine serum and antibiotics and contains many growth factors and nutrients, some of which have been described elsewhere (26-28). NCM460 cells were used between passage 36 and 48 for this study. The cells were grown in 75-cm² plastic flasks (Costar) at 37°C in a 5% CO₂ air atmosphere with media changes every 4 days. NCM460 cells were subcultured by trypsinization with 0.05% trypsin and 0.9 mM EDTA in Ca²⁺- and Mg²⁺-free phosphate-buffered saline solution and plated onto 12-well plates at a concentration of 5 \(\times 10^5\) cells/well. Uptake of folate acid was studied 3-6 days following confluence. Preliminary experiments showed no difference in folate acid uptake by NCM460 cells between day 3 and 12 postconfluence (data not shown). Cell growth was observed by periodic monitoring with an inverted microscope. Cell viability was tested by the trypan blue dye exclusion method and found to be \(>95\%\).

Uptake experiments were performed at 37°C, unless otherwise mentioned. The incubation buffer was Krebs-Ringer phosphate buffer containing (in mM): 123 NaCl, 4.9 KCl, 1.2 MgSO₄, 0.85 CaCl₂, 5 glucose, 5 glutamine, 10 HEPES, and 10 MES, pH 5.0 (unless otherwise stated). [3H]Folic acid was added to the incubation buffer at the beginning of the experiment, and uptake was terminated after 3 min of incubation (unless otherwise specified) by the addition of 1 ml of ice-cold buffer followed by immediate removal by aspiration. The monolayers were rinsed twice with ice-cold buffer and digested with 1 ml of 1 N NaOH, neutralized by HCl, and then counted for radioactivity in a liquid scintillation counter. Protein contents of cell digests were estimated on a spectrophotometer. Protein contents of cell digests were estimated on a scintillation counter. Protein contents of cell digests were estimated on a spectrophotometer. Protein contents of cell digests were estimated on a scintillation counter. Protein contents of cell digests were estimated on a spectrophotometer. Protein contents of cell digests were estimated on a scintillation counter. Protein contents of cell digests were estimated on a spectrophotometer. Protein contents of cell digests were estimated on a scintillation counter. Protein contents of cell digests were estimated on a spectrophotometer.

RESULTS

Mechanism of Folic Acid Uptake by NCM460 Cells

Uptake with Time and Effect of Incubation Temperature—Fig. 1 shows the time-dependent uptake of low (5.4 nM) and high (3 \(\mu\)M) concentrations of folic acid by NCM460 cells. In both cases the uptake was found to be linear with time for up to 4 min of incubation and occurred at a rate of 0.021 and 0.53 pmol/mg of protein/min for low and high concentrations, respectively. Based on these results, 3 min of incubation was chosen as the standard incubation time for all subsequent experiments.

In a separate study, we examined the effect of incubation temperature on the uptake of folic acid (5.4 nM). Uptake was found to be significantly (\(p < 0.01\)) higher at 37°C compared with uptake at 4°C (61 ± 2 (n = 6) and 29 ± 1 (n = 6) fmol/mg of protein/3 min, respectively).

We also examined the metabolic form of the radioactivity taken up by NCM460 cells following 3 and 15 min of incubation with [3H]folic acid (21.6 nM). In this experiment cells were quickly washed at the end of incubation with ice-cold buffer, suspended in 50% water/methanol solution as described previously (10), homogenized, and then centrifuged. The supernatant was then applied to cellulose-precipitated thin layer chromatography plates. The plates were run using a solvent system of 0.1 M anhydrous NaHPO₄ solution (pH 7.0). The results showed that 97.6 and 89.4% of the [3H]radioactivity taken up by the monolayers to be in the form of intact [3H]folic acid after 3 and 15 min of incubation, respectively.

Role of Na⁺ and the Effect of Incubation Buffer pH—The role of Na⁺ in folic acid uptake by NCM460 cells was investigated in this study. We determined by examining the effect of iso-osmotically replacing Na⁺ (123 mM) in the incubation buffer with chloride salts of other monovalent cations such as K⁺ and choline (123 mM) or with the non-ionic mannitol (246 mM) on the uptake of folic acid (5.4 nM). The results showed no significant change in folic acid uptake under all conditions tested (56 ± 1 (n = 5), 57 ± 2 (n = 5), 52 ± 1 (n = 5), and 56 ± 6 (n = 5) fmol/mg of protein/3 min for control (Na⁺), K⁺, choline, and mannitol, respectively).

In a separate experiment we examined the effect of varying the incubation buffer pH over the range of 3.5-8.5 on the uptake of folic acid (5.4 nM). The results showed an increase in folic acid uptake with decreasing incubation buffer pH with a maximum uptake around pH 5 (Fig. 2). Thus, we chose the buffer of pH 5 for all other studies.

Uptake of Folic Acid as a Function of Concentration—Uptake of folic acid uptake by monolayers of NCM460 cells was examined as a function of increasing the substrate concentration in the incubation medium. Uptake was found to be a saturable component at low concentrations and to be linear at high concentrations. Uptake by the saturable component was determined by subtracting uptake by diffusion from the total uptake at each concentration (Fig. 3) (uptake by diffusion was determined from the slope of the linear uptake at high folic acid concentrations). Kinetic parameters of the saturable uptake process were then determined as described under "Materials and Methods" and found to be 1.4 ± 0.2 \(\mu\)M for the apparent \(K_m\) and 9.7 ± 0.6 pmol/mg of protein/3 min for the \(V_{max}\).

Effect of Unlabeled Folic Acid and Folate Structural Analogs on the Uptake of [3H]Folic Acid—The effect of unlabeled folic acid and the related structural analogs (5-formyltetrahydrofolate, 5-methyltetrahydrofolate, and methotrexate) on the uptake of 5.4 nM [3H]folic acid by NCM460 cells was examined in this study. Unlabeled folic acid and its related compounds (at 1 \(\mu\)M) caused significant inhibition (\(p < 0.01\) for all) in the uptake of 5.4 nM [3H]folic acid (79 ± 2 (n = 6), 22 ± 1 (n = 6), 30 ± 3

---

Fig. 1. Uptake of folic acid by NCM460 cells as a function of time. NCM460 cells were incubated at 37°C in Krebs-Ringer phosphate buffer containing 10 mM of MES and 10 mM of HEPES, pH 5.0, in the presence of low (A, 5.4 nM) or high (B, 3 \(\mu\)M) concentrations of folic acid. Each data point represents mean ± S.E. of four to six separate uptake experiments. For A, \(Y = 0.021X + 0.007, r = 0.997\); for B, \(Y = 0.528X + 0.207, r = 0.987\).
anion transport inhibitors DIDS, 4-acetamido-4’-isothiocyanato-stilbene-2,2’-disulfonic acid (SITS), and probenecid on the uptake of folic acid (5.4 nM) by NCM460 cells. The results showed significant inhibition ($p < 0.01$) in folic acid uptake by all compounds tested ($81 \pm 2$ ($n = 6$), $16 \pm 2$ ($n = 6$), $25 \pm 1$ ($n = 6$), and $43 \pm 3$ ($n = 6$) fmol/mg of protein/3 min for control, DIDS, SITS, and probenecid, respectively).

In another study we examined the effect of the short chain fatty acids, acetate, propionate, and butyrate (10 mM, sodium salt), in the incubation buffer on the uptake folic acid (5.4 nM) by NCM460 cells. The results showed that no significant change in the uptake of folic acid by NCM460 cells was observed in the presence of these anions compared with control ($63 \pm 2$ ($n = 4$), $62 \pm 2$ ($n = 4$), $64 \pm 3$ ($n = 4$), and $61 \pm 3$ ($n = 4$) fmol/mg of protein/3 min for control, acetate, propionate, and butyrate, respectively).

**Regulation of Folic Acid Uptake in NCM460 Cells and the Role of Protein Kinase-mediated Pathways**

Following the determination of existence of a carrier-mediated system for folate uptake by NCM460 and the characterization of its nature, we examined possible regulation of the function of this carrier by specific protein kinase-mediated pathways. We focused on pathways that involve protein kinases for which consensus sequences have been shown to exist in recently cloned folate transporters (namely protein kinase C (PKC) and A (PKA)) (13–15, 31) and on pathways that have been shown to play a role in the regulation of uptake of other nutrients by intestinal and other epithelia (namely, PTK and Ca$^{2+}$/calmodulin) (32–40).

The possible role of PKC in the regulation of folic acid uptake by NCM460 cells was tested by examining the effect of pretreating NCM460 cells for 1 h with either the PKC activator phorbol 12-myristate 13-acetate or with the PKC inhibitors bisindolylmaleimide or chelerythrine on the uptake of 5.4 nM folic acid. The results showed that none of these pretreatments significantly affected folic acid uptake ($74 \pm 9$ ($n = 11$), $68 \pm 8$ ($n = 11$), $69 \pm 9$ ($n = 11$), and $66 \pm 9$ ($n = 11$) fmol/mg of protein/3 min for control and $1$, $10$, and $100 \mu M$ phorbol 12-myristate 13-acetate-treated cells, respectively; $69 \pm 2$ ($n = 6$), $79 \pm 7$ ($n = 6$), $72 \pm 9$ ($n = 6$), and $68 \pm 3$ ($n = 6$) fmol/mg of protein/3 min for control and $1$, $10$, and $100 \mu M$ bisindolylmaleimide-treated cells, respectively; $70 \pm 4$ ($n = 6$), $64 \pm 2$ ($n = 6$), and $61 \pm 9$ ($n = 6$) fmol/mg of protein/3 min for control, 2.5, and $25 \mu M$ chelerythrine-treated cells, respectively).

Involvement of PTK-mediated pathway in the regulation of folic acid uptake was also tested. This was done by examining the effect of pretreating NCM460 cells for 1 h with compounds that are known to increase intracellular CAMP levels (isobutylmethylxanthine (IBMX) and dibutyryl cAMP (Bt2cAMP)) and thus activate PKA and that of the specific PKA inhibitor H-89 on the uptake of 5.4 nM folic acid. The results showed that IBMX and Bt2cAMP cause a significant ($p < 0.01$) decrease in the uptake of folic acid. On the other hand H-89 did not cause any appreciable effect (Table I). We also examined the effect of pretreating cells with Bt2cAMP (1 mM) on folic acid (5.4 nM) uptake in the presence of H-89 (100 \mu M). No reversal in the inhibitory effect of Bt2cAMP by H-89 was observed ($70 \pm 4$ ($n = 4$), $45 \pm 2$ ($n = 4$), $45 \pm 1$ ($n = 4$) fmol/mg of protein/3 min for the control, Bt2cAMP-treated, and both Bt2cAMP- and H-89-treated, respectively).

In another study we tested for the involvement of PTK in the regulation of folic acid uptake by NCM460 cells. This was done by examining the effect of pretreating the NCM460 cells for 1 h with the PTK inhibitors genistein and tyrphostin A 25 on the uptake of 5.4 nM folic acid. Genistin and tyrphostin A 1, respec-
NCM460 monolayers were preincubated for 1 h at 37 °C with the compound under investigation. [3H]Folic acid (5.4 nM) was then added and incubation continued at 37 °C in Krebs-Ringer buffer, pH 5.0. Data are mean ± S.E. Number of separate uptake determinations is in parentheses. NS, not significant.

| Compound Uptake | p value* |
|-----------------|----------|
| Exp. A          |          |
| Control         | 79 ± 17 (6) |  <0.01 |
| IBMX            | 63 ± 3 (6)  |  >0.05 |
| 1 mM            | 46 ± 3 (6)  |  >0.01 |
| 2.5 mM          | 37 ± 1 (6)  |  <0.01 |
| 5 mM            | 45 ± 2 (6)  |  >0.01 |
| Dibutyryl cAMP, 1 mM | 69 ± 2 (6)  |  NS    |
| Exp. B          |          |
| Control         | 70 ± 4 (6)  |        |
| H-89            | 69 ± 2 (6)  |  NS    |
| 50 μM           | 70 ± 2 (6)  |  NS    |

* p values were calculated using the Student’s t test; comparison was made relative to the simultaneously performed controls.

NCM460 monolayers were preincubated for 1 h at 37 °C with the compound under investigation. [3H]Folic acid (5.4 nM) was then added and incubation continued for 3 min at 37 °C in Krebs-Ringer buffer, pH 5.0. Data are mean ± S.E. Number of separate uptake determinations is in parentheses. NS, not significant.

| Compound Concentration Uptake | p value* |
|-------------------------------|----------|
| Exp. A                        |          |
| Control                       | 52 ± 3 (6) | <0.01 |
| Genistein                     | 25 ± 3 (6) | <0.01 |
| Genistin                      | 25 ± 3 (6) |  NS   |
| Genistein                     | 100 ± 3 (6) |  NS |
| Exp. B                        |          |
| Control                       | 70 ± 3 (6) |        |
| Tyrphostin A-25               | 45 ± 2 (6) |  <0.01 |
| Tyrphostin A-1                | 68 ± 3 (6) |  NS    |

* p values were calculated using the Student’s t test; comparison was made relative to the simultaneously performed controls.

**DISCUSSION**

The major aim of the present study was to establish the suitability of NCM460 cells as a model to study the details of colonic folate uptake mechanism and its cellular regulation. We chose this normal non-transformed human colonic epithelial cell line because these cells possess many of the characteristics of the native colonocytes (24, 25). Our results showed that uptake of folic acid was temperature-dependent and occurred with minimal metabolic alterations to the transported substrate. Na+ in the incubation medium appeared to play no role in folic acid uptake as indicated by the lack of effect of Na+ removal on uptake. On the other hand, incubation buffer pH (i.e. H+ concentration) appeared to play an important role in driving folic acid uptake. Increasing the H+ concentration in the incubation medium by lowering the incubation buffer pH led to a marked increase in folic acid uptake. The effect of pH on folic acid uptake by NCM460 cells may represent the existence of a folate /OH− exchange mechanism (or a folate /H+ cotransport) and/or represent a direct effect of pH on the folic acid uptake carrier, as suggested before (9, 11).

The uptake process of folic acid by NCM460 was saturable as a function of increasing the substrate concentration with an apparent \( K_m \) and \( V_{max} \) of 1.4 ± 0.2 μM and 9.7 ± 0.6 pmol/mg of protein/3 min, respectively. This finding indicates the involvement of a carrier-mediated system in the uptake process. This conclusion was further supported by the finding of a significant inhibition in [3H]folic acid uptake by unlabeled folic acid and by its structural analogs. The inhibition constants (\( K_i \)) for the folate structural analogs 5-formytyethylhydrofolate, 5-methyltetrahydrofolate, and methotrexate were 1.1, 1.9, and 1.8 μM, respectively. The finding that the \( K_i \) values of these analogs are similar to the apparent \( K_m \) of the substrate (folic acid) transport suggests that these analogs share the same uptake mechanism with folic acid in NCM460 cells. The process of folic acid uptake by NCM460 cells was also energy-dependent as indicated by the significant inhibition in the uptake process by different metabolic inhibitors.

The folic acid uptake process was sensitive to the effect of the anion transport inhibitors DIDS, SITS, and probenecid, further suggesting the possible involvement of a folate /OH− exchange
mechanism. Because (i) the process is sensitive to these anion transport inhibitors and (ii) colonic lumen contains high concentrations of the anions acetate, propionate, and butyrate (31), we also tested the effect of these short chain fatty acids on the carrier-mediated uptake of folic acid by NCM460 cells. Our results, however, showed that these anions have no effect on the uptake process.

All of the above described characteristics of folic acid uptake mechanism by the NCM460 cell line are similar to those recently observed with purified apical membrane vesicles prepared from native human colonic tissue (23). This clearly establishes the suitability of this cell line as a model with which to study the cellular regulation of folate uptake by the human colon. Using these cells we then tested the possible involvement of specific protein kinase- and Ca\(^{2+}\)/calmodulin-mediated pathways in the regulation of folic acid uptake by colonocytes. We focused on pathways that involve protein kinases (PKC and PKA) for which consensus sequences have been shown to exist in the recently cloned folate carriers (PTK- and Ca\(^{2+}\)/calmodulin-mediated pathways) (33–41). When specific modulators of these pathways were used, we found that PKC-mediated pathways had no role in regulating folate uptake by NCM460 cells. In contrast, compounds that increased the intracellular cAMP level, namely IBMX and Bt\(_2\)cAMP, caused a significant decrease in folate acid uptake. However, the specific inhibitor of PKA H-89 had no significant effect on folate acid uptake, and when cells were pretreated with Bt\(_2\)cAMP in the presence of H-89, the inhibitory effect caused by Bt\(_2\)cAMP was not reversed. These findings suggested that intracellular cAMP affects folate acid uptake through a PKA-independent mechanism. Similar observations and conclusions were reported by Muller et al. (42) in their findings on the inhibition of the H\(^+\)/peptide cotransporter by intracellular cAMP level in the human intestinal epithelial cell line Caco-2. Assuming that the expressed folate carrier in NCM460 cells is similar to the recently cloned folate carriers in that it has consensus sequences for PKC and PKA, our findings may suggest that either phosphorylation of these sites has no effect on the function of the folate acid uptake carrier or that these consensus sites are not accessible to these protein kinases.

Although the regulation of folate acid uptake by NCM460 cells was apparently not mediated by the PKC- and PKA-mediated pathways, a role for the PTK-mediated pathway was suggested by the observations that inhibitors of PTK activity, namely genistein and tyrphostin A25, caused a significant decrease in folate acid uptake. The effect of these compounds appeared to be specific because their negative controls (genistin and tyrphostin A1, respectively) did not affect folate acid uptake. Furthermore, the uptake of the unrelated biotin was up-regulated by genistein. The inhibitory effect of genistein appeared to be mediated through a decrease in the V\(_{\text{max}}\) of the folate acid uptake process and an increase in the apparent K\(_{\text{m}}\). These findings suggest that the inhibitory effect caused by genistein is mediated through a decrease in the affinity, activity, and/or number of the folate acid uptake carriers. The ability of the PTK phosphatase inhibitor orthovanadate to cause a significant increase in folate acid uptake further supports the suggested involvement of PTK in the regulation of folate acid uptake by NCM460 and raised the possibility that phosphorylation may be involved. It should be mentioned here, however, that the sequences of the folate carriers cloned so far contained no consensus sites for PTK phosphorylation. Our findings, therefore, may suggest that a “cryptic” rather than a consensus (“canonical”) site(s) for PTK phosphorylation may be involved in the regulation of folate transport. Alternatively the possibility that PTK is acting on an auxiliary protein, which then exerts its effect on the folate acid uptake carrier, cannot be ignored. Further studies are needed to address these issues. Similar findings have been reported in the case of the rat renal Na/P\(_i\) cotransporter clone where no consensus sites for PKA phosphorylation were found in the cloned carrier, yet transport activity is regulated by a PKA-mediated pathway (43). Further, PKC-mediated inhibition of the rat renal Na/P\(_i\) cotransporter was not prevented by the removal of the protein kinase C consensus sequences (44). Similarly, a cryptic site for PKC has been identified at the amino terminus of the Na\(^+\)/K\(^+\)-ATPase (45).

In summary, our results demonstrate the suitability of the NCM460 cell line as an in vitro model system to investigate the detailed mechanism and regulation of folate acid uptake by colonocytes. Uptake appears to be via a carrier-mediated system which is temperature-, pH- and energy-dependent and appears to be under the regulation of PTK and cAMP. Regulation of folate uptake by PTK and cAMP may suggest that the process is under the influence of growth factors and other biologically active agents. Further studies are needed to address this issue.

Acknowledgments—We thank Lawrence A. Manzano for technical assistance and Drs. David Dyer and Taoi Nguyen for helpful discussions.

REFERENCES

1. Blakley, R. L. (1969) The Biochemistry of Folic Acid and Related Pteridines, North-Holland, Amsterdam.
2. Blakley, R. L., and Benkovic, S. J. (1984) Folates and Pterins: Chemistry and Biochemistry of Folates, Vol. 1, John Wiley & Sons, Inc., New York.
3. Blakley, R. L., and Benkovic, S. J. (1985) Folates and Pterins: Chemistry and Biochemistry of Pterins, Vol. 2, John Wiley & Sons, Inc., New York.
4. Blakley, R. L., and Whitaker, V. A. (1986) Folates and Pterins: Pharmacological, Pharmacological, and Physiological Aspects, Vol. 3, John Wiley & Sons, Inc., New York.
5. Elsborg, L., Lynghue, J., and Ryttig, K. (1981) Folic Acid and Folic Acid Deficiency, AJR Research, Copenhagen.
6. Zimmerman, J. (1990) Gastroenterology 99, 964–972.
7. Said, H. M., and Strum, W. B. (1983) J. Pharmacol. Exp. Ther. 226, 95–99.
8. Said, H. M., Ghishan, F. K., and Murrell, J. E. (1985) Am. J. Physiol. 249, G567–G571.
9. Schron, C. M., Washington, C., and Blitzer, B. (1985) J. Clin. Invest. 76, 2039–2043.
10. Said, H. M., and Redha, R. (1987) Biochem. J. 247, 141–146.
11. Said, H. M., Ghishan, F. K., and Redha, R. (1987) Am. J. Physiol. 252, 2229–2236.
12. Mason, J. B., and Selhub, J. (1991) in Biochim. Biophys. Acta 108, 705–710.
13. Prasad, P. D., Ramamoorthy, S., Leibach, F. H., and Ganapathy, V. (1995) J. Pharmacol. Exp. Ther. 274, 141–146.
14. Nguyen, T. T., Dyer, D. L., Dunning, D., Rubin, S. A., and Said, H. M. (1997) Gastroenterology, in press.
15. Elshorbagy, A., Syed, R., and Tyagi, S. (1994) Am. J. Clin. Nutr. 59, 1955–1959.
16. Hylander, E., Ladhofeged, K., and Jarnum, S. (1988) Scand. J. Gastroenterol. 15, 55–60.
17. Ginststead, W. C., Pak, C. Y. C., and Kre, J. S. (1984) Am. J. Physiol. 247, G189–G192.
18. Hylander, E., Ladhofeged, K., and Jarnum, S. (1990) Scand. J. Gastroenterol. 25, 705–710.
19. Elshorbagy, A., Syed, R., and Tyagi, S. (1994) Gastroenterology 106, A230 (abstr.)
20. Lashner, B. A., Heidenreich, P. A., and Su, G. L. (1989) Gastroenterology 97, 1450–1454.
21. Cravo, M. L., Mason, J. B., and Selhub, J. (1991) Am. J. Clin. Nutr. 53, 1450–1454.
22. Manzano, G., in press.
23. Torania, S. A., Naom, H. F., Said, H. M., and Dudeja, P. K. (1995) Gastroenterology 108, A277 (abstr.)
24. Moyer, M. P., Stauffer, J. S., Manzano, L. A., Tanzer, L. L., and Merriman, R. L. (1997) In Vitro Cell. Dev. Biol. Anim., in press.
25. Sahi, J., Stauffer, J. S., Ladven, T. J., Moyer, M. P., and Rao, M. C. (1995) Gastroenterology 108, A321 (abstr.)
26. Stauffer, J. S., Manzano, L. A., Balch, G. C., Merriman, R. L., Tanzer, L. R., and Moyer, M. P. (1999) Am. J. Surg. 179, 190–196.
27. Moyer, M. P., Dixon, P. S., Culpepper, A. L., and Aust, J. B. (1990) in Colon Cancer (Moyer, M. P., and Poste, G., eds) pp. 85–136, Academic Press, San Diego.
28. Moyer, M. P. (1991) J. Tissue Cult. Methods 13, 107–116.
30. Wilkinson, G. N. (1961) Biochem. J. 60, 324–332
31. Wrong, O. M., Edmonds, C. J., and Chadwick, V. S. (1980) The Large Intestine: Its Role in Mammalian Nutrition and Homeostasis, pp. 113–119, John Wiley & Sons, Inc., New York
32. Dixon, K. H., Lanpher, B. C., Chiu, J., Kelley, K., and Cowan, K. H. (1994) J. Biol. Chem. 269, 17–20
33. Donowitz, M., and Welsh, M. J. (1987) in Physiology of the Gastrointestinal Tract (Johnson, L. R., ed) pp. 1351–1388, Raven Press, Ltd., New York
34. Rood, R. P., Emmer, E., and Wesoleck, J. (1988) J. Clin. Invest. 82, 1091–1097
35. Cohen, M. E., Reimihl, L., Watson, A. J. M., Gorlick, F., Sikora, K. R., Tse, M., Rood, R. P., Czernik, A. J., Sharp, G. W., and Donowitz, M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8990–8994
36. de Jonge, H. R., and Rao, M. C. (1990) in Textbook of Secretory Diarrhea (Lebenthal, E., and Duffey, M., eds) pp. 191–207, Raven Press, Ltd., New York
37. Fliegel, L., Walsh, M. P., and Singh, D. (1992) Biochem. J. 282, 139–145
38. Brandes, M., Miyamoto, Y., Ganapathy, V., and Leibach, F. H. (1993) Am. J. Physiol. 264, G936–G946
39. Piper, R. C., James, D. E., Slot, J. W., Puri, C., and Lawrence, J. C., Jr. (1993) J. Biol. Chem. 268, 16557–16563
40. Donowitz, M., Montgomery, J. L. M., Walker, M. S., and Cohen, M. E. (1994) Am. J. Physiol. 266, G647–G656
41. Said, H. M., and Ma, T. Y. (1994) Am. J. Physiol. 266, G15–G21
42. Muller, U., Brandes, M., Prasad, P. D., Fei, Y. J, Ganapathy, V., and Leibach, F. H. (1996) Biochem. Biophys. Res. Commun. 218, 461–465
43. Magagnin, S., Werner, A., Markovich, D., Sorribas, V., Stange, G., Biber, J., and Murer, H. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5979–5983
44. Hayes, G., Busch, A. E., Lang, F., Biber, J., and Murer, H. (1995) Pfleugers Arch. Eur. J. Physiol. 430, 819–824
45. Beguin, P., Beggah, A. T., Chibalin, A. V., Burgener-Kairuz, P., Jaisser, F., Mathews, P. M., Rossier, B. C., Cotecechia, S., and Geering, K. (1994) J. Biol. Chem. 269, 24437–24445