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Bruce A. Voyles  
Grinnell College

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## Characterization of Aminoisobutyric Acid Transport Systems in MCF-8, a Line of Malignant Mouse Mammary Epithelial Cells

BRUCE A. VOYLES

Department of Biology, Grinnell College, Grinnell, Iowa 50112

Uptake of  $\alpha$ -aminoisobutyric acid (AIB) by MCF-8, a line of malignant mouse mammary epithelial cells derived from a D2 hyperplastic nodule outgrowth, was found to be mediated by two different transport systems. In actively dividing cells about 60% of total AIB uptake is mediated by an A-like system: transport was  $\text{Na}^+$ -dependent, was inhibited by N-methyl-AIB, was maximal at pH 7.5 and ceased at pH 6. Transport by this system was reduced by nearly 65% when cell division ceased in confluent cultures, but the addition of insulin and/or calf serum to the medium of quiescent cells restored activity. Transport by the A system had a  $K_m$  of 1.0 mM AIB and  $V_{max}$  of 5.5  $\mu\text{M}$  AIB transported per  $\mu\text{g}$  cellular protein per minute. About 20% of total AIB uptake was mediated by an L-like transport system which was  $\text{Na}^+$ -independent, was inhibited by  $\alpha$ -2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid, and was active at both pH 7.5 and pH 6. The activity of the L system was not influenced by cell growth rate or by insulin or serum. The remaining 20% of total AIB uptake occurs via a non-saturable route. No ASC-like system was detected.

INDEX DESCRIPTORS: Mammary cell line, AIB, transport, mammary tumor

The transport of neutral amino acids was originally studied in the Ehrlich ascites carcinoma cell line in which three transport systems called A (preferring alanine), ASC (preferring alanine, serine and cysteine), and L (preferring leucine) were described (Oxender and Christensen, 1963). These systems are distinguished not only by the nature of the amino acids transported but also by their sodium dependence or independence (Christensen, 1969). The A system requires the presence of sodium and transports most strongly amino acids with short linear side chains, including the non-metabolizable amino acid analogue  $\alpha$ -aminoisobutyric acid. The ASC system is also sodium dependent but excludes N-methylated amino acid analogues such as N-methyl-aminoisobutyric acid. The L system is sodium-independent and transports most strongly amino acids with branched or aromatic side chains. Transport systems with these characteristics have been found to occur in both normal and transformed fibroblast cell lines (Foster and Pardee, 1969; Oxender, et al., 1977; Dubrow, et al., 1978; Weber, et al., 1979; Boghetti, et al., 1980).

More recently studies have been undertaken to investigate the transport of amino acids in epithelial cells taken from a variety of tissues. Transport systems with the characteristics of the A, ASC and L systems have been described in kidney epithelial cells from the pig (Rabito and Karish, 1982; Amsler, et al., 1983; Sepulveda and Pearson, 1982) and dog (Boerner and Saier, 1982), and in Chinese hamster ovary cells (Shorwell, et al., 1981). Malignant mammary epithelial cells have been studied as ascites tumor cells grown in suspension (Christensen, 1979; Neville, et al., 1980), as single cells suspensions tested immediately following dissociation of solid tumors by enzymatic digestion (Hissin and Hilf, 1978a, 1978b, 1978c, 1979) and as primary monolayer cultures prepared from solid tumors (Gay and Hilf, 1980). Normal mammary epithelial cells have also been studied, but only as explants of mammary glands taken from late pregnant and lactating mice (Neville, et al., 1980).

This paper describes studies to define the characteristics of amino acid transport in MCF-8 cells, a line of malignant mouse mammary epithelial cells. The MCF-8 cell line was derived from an adenocarcinoma which arose within a transplanted D2 hyperplastic nodule outgrowth in BALB/c mouse (Soule, et al., 1973). These cells form scirrhous mammary carcinomas (Russo and McGrath, 1974) and do not release mouse mammary tumor virus (Arnold et al., 1976). In culture MCF-8 cells have retained some "normal" characteristics such as epithelioid morphology and density-dependent growth regulation,

but exhibit the strong reactivity in concanavalin A-mediated hemadsorption assays characteristic of malignant mammary epithelial cells (Voyles and McGrath, 1976, 1978). In this paper I describe the transport systems for aminoisobutyric acid in MCF-8 cells and the hormone and density-dependent regulation of their activities.

### MATERIALS AND METHODS

#### Cell Culture

The mouse mammary epithelial cell line MCF-8 (clone 5<sup>3</sup>) was obtained from Dr. Herbert Soule, Laboratory of Tumor Biology, the Michigan Cancer Foundation, Detroit, Michigan. All experiments were carried out on cells between passages 49 and 60, with new cells being retrieved from ultra-low temperature storage as needed.

The cells were grown in Dulbecco's modification of Eagles's minimum essential medium with 4500 mg/1 glucose (Grand Island Biological) supplemented with 100  $\mu\text{g}/\text{ml}$  sodium pyruvate, 100 U/ml penicillin, 100  $\mu\text{g}/\text{ml}$  streptomycin, 10  $\mu\text{g}/\text{ml}$  bovine insulin and 15% v/v of newborn calf serum (Grand Island Biological). Cultures were maintained in a humidified atmosphere with 7.5%  $\text{CO}_2$  in air.

#### Transport Measurements

Transport measurements were performed on cells grown in 35mm tissue culture dishes. The cells were dispersed by brief treatment with 0.1% trypsin and 0.025% EDTA, plated at  $7.5 \times 10^4$  cells per dish, and then incubated for 48 hours without a change of medium, by which time the cultures achieved about 30-40% confluency. Most cells have only very limited cell-cell contacts at this level of confluency.

To determine transport, the growth medium was removed by aspiration and the dishes washed twice at 37°C with 2.5ml of phosphate buffered saline (PBS). The cells were incubated at 37°C in 0.6ml of PBS containing  $\alpha$ -aminoisobutyric acid (AIB) and  $^3\text{H}$ -AIB. Unless otherwise noted, the incubation medium contained 1mM AIB and 1 $\mu\text{Ci}/\text{ml}$   $^3\text{H}$ -AIB and the incubation period was 5 minutes. Transport was terminated by aspirating the labeling medium and washing the dishes three times with 2.5ml of ice-cold PBS containing 48mM glycyl-glycine to improve the removal of unincorporated  $^3\text{H}$ -AIB from the plastic surface of the dishes (Ronquist, et al., 1976). The cells were then lysed by the addition of 1ml of 0.1M NaOH and duplicate 0.2ml samples taken for determination of radioactivity by liquid scintillation spectrometry and of protein by the Lowry method using bovine serum albumin fraction V as the standard. The number of cells per dish for each experiment was determined using the nuclear count method of Sanford (Sanford et al., 1951) on three unlabelled dishes. Since the average protein content of a dish was directly

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proportional to the number of cells per dish over the range  $0.4-15 \times 10^5$  cells per dish (linear regression  $r^2 = .89$ ), the transport rate was calculated from the specific activity of the substrate in the labelling medium and was expressed as pM AIB taken up per  $\mu\text{g}$  protein per minute. Assays were performed in duplicate or triplicate.

#### Composition of Solutions

Phosphate buffered saline (pH 7.2) was made from Dulbecco's formulation (Dulbecco and Vogt, 1954; 137mM NaCl, 2.7 mM KCl, 8.1mM  $\text{Na}_2\text{HPO}_4$ , 1.5mM  $\text{KH}_2\text{PO}_4$ , 0.9mM  $\text{CaCl}_2$ ) supplemented with 0.5mM  $\text{MgCl}_2$  and 5.6mM glucose. Sodium-free phosphate buffered saline was prepared by replacing NaCl with choline chloride and  $\text{Na}_2\text{HPO}_4$  with choline phosphate.

#### Materials

$\alpha$ -aminoisobutyric acid ( $\alpha$ -methyl- $^3\text{H}$ ) was obtained from New England Nuclear. 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid was purchased from Calbiochem. All other materials were obtained from Sigma Chemical Co.

## RESULTS

### Discrimination of Transport Systems for AIB

**The A System:** Figure 1 presents the transport of 1mM AIB by actively growing MCF-8 cells under three different conditions. Transport of 1mM AIB in  $\text{Na}^+$ -containing medium was linear from one to 15 minutes and proceeds at a rate of 1.82 pM AIB taken up per microgram cellular protein per minute over that interval. Christensen (1969) defined the A system as N-methyl-AIB inhibitable AIB uptake in the presence of  $\text{Na}^+$ . When N-methyl-AIB was added to  $\text{Na}^+$ -containing incubation medium at twenty-five times the concentration of AIB used, the transport of 1mM AIB remained linear from one to 15 minutes, but the rate of transport was reduced to only 0.72 pM per microgram cellular protein per minute. The A system thus appears to be a major mechanism of uptake of AIB in subconfluent, actively-growing MCF-8 cells, being responsible for about 60% of total AIB uptake at 1 mM AIB.

**The ASC System:**  $\text{Na}^+$ -dependent transport of AIB which is not inhibited by N-methyl-AIB is considered to be mediated by the ASC system (Christensen, 1969). When  $\text{Na}^+$  was replaced with choline, AIB transport was still linear from one to 15 minutes, but the rate of uptake was decreased to 0.68 pM AIB per microgram cellular protein per minute (Fig. 1). Since the two sets of points for uptake in the presence of N-methyl-AIB and in  $\text{Na}^+$ -free medium lie within each other's standard error limits, a single line was drawn for both sets. In a separate series of experiments, uptake of 1mM AIB over a five minute incubation period was determined in  $\text{Na}^+$ -free medium or in  $\text{Na}^+$ -containing medium plus a twenty-five fold excess of N-methyl-AIB averaged. In eight determinations the difference was not statistically significant by the T-test. It thus appears that the ASC system is of very minor importance for the transport of AIB by MCF-8 cells. Based on these observations all sodium-dependent transport of AIB by MCF-8 cells can be considered to be mediated by the A system alone.

**L System:** Transport by the L system is defined as transport in  $\text{Na}^+$ -free medium that is inhibited by  $\alpha$ -2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) (Tager and Christensen, 1971). Thirty to forty percent of the total transport of 1mM AIB by MCF-8 cells was  $\text{Na}^+$ -independent (Fig. 1). In a series of four experiments using five minute incubations, BCH inhibited between 42% and 53% of  $\text{Na}^+$ -independent uptake of 1mM AIB. Thus about 20-25% of total AIB transport appeared to be mediated by the L system. The remaining portion of total AIB uptake (15-20%), which is both  $\text{Na}^+$  independent and not inhibited by BCH, was considered to be due to a nonsaturable (NS) component.

**Cross-inhibition studies:** Cross inhibition studies using naturally occurring amino acids were used to examine the contribution of

multiple transport systems to AIB transport in MCF-8 cells. Figure 2 shows the effect of addition of increasing concentrations of various amino acids on the transport of 1mM AIB in  $\text{Na}^+$ -containing medium.

Addition of the amino acid proline, which is transported exclusively by the A system in R3230AC rat mammary carcinoma cells (Hissin and Hilf, 1978b), produced levels of inhibition of AIB transport which were similar to that produced by the addition of N-methyl-AIB. In another series of experiments testing combinations of inhibitors, 25mM proline and 25mM N-methyl-AIB added together caused a  $65.2 \pm 1.8\%$  inhibition of transport compared to  $64.2 \pm 3.2\%$  for N-methyl-AIB alone or  $61.7 \pm 7.1\%$  for proline alone, suggesting that they share a common transport system. Two other amino acids transported primarily by the A system, glycine and alanine, also did not significantly increase the level of inhibition produced by N-methyl-AIB alone.

The ability of the amino acid phenylalanine to inhibit AIB transport is also shown in Figure 2. At concentrations between 1 and 10mM when phenylalanine is transported primarily by the L system, phenylalanine caused less inhibition than either N-methyl-AIB or proline. At a concentration of 25mM, however, when phenylalanine is more likely to be taken up by both the A and L systems (Hissin and Hilf, 1978a), the degree of inhibition of AIB transport was increased. In another experiment, addition of both phenylalanine and N-methyl-AIB resulted in a  $85.3 \pm 1\%$  inhibition of AIB transport in  $\text{Na}^+$ -containing medium. This level compared to that of  $82.6 \pm 2.2\%$  found in  $\text{Na}^+$ -free medium containing 25mM BCH. Neither phenylalanine nor N-methyl-AIB can be transported by the ASC system and yet together reduced transport to the level produced by non-saturable uptake alone, suggesting that the ASC system is not

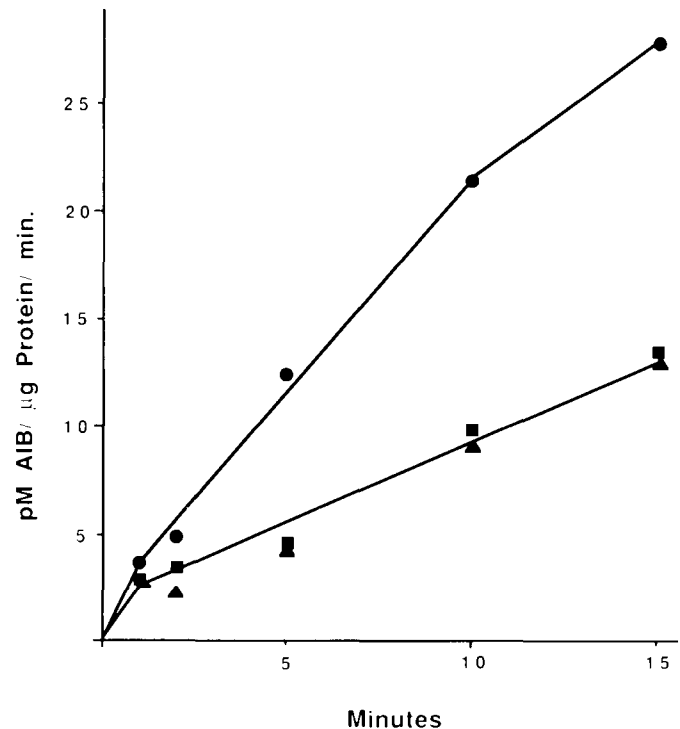


Fig. 1: Aminoisobutyric acid transport by actively growing MCF-8 cells in  $\text{Na}^+$ -containing medium (●), in  $\text{Na}^+$ -free medium (▲) and in  $\text{Na}^+$ -containing medium plus 25 mM N-methyl-AIB (■). Uptake of 1mM AIB was determined for cells at a density of  $1.3 \times 10^5$  cell/dish forty-eight hours after plating.

significant for transport AIB in MCF-8 cells.

Serine, which is transported both by the A and L systems in rat mammary tumor cells (Hissin and Hilf, 1978a; Gay and Hilf, 1980) was more effective than N-methyl-AIB in inhibiting AIB uptake in MCF-8 cells (Fig. 2). Serine in combination with N-methyl-AIB produced  $85.8 \pm 1.3\%$  inhibition, similar to phenylalanine plus N-methyl-AIB.

**pH Dependency of Transport:** Another means of distinguishing the A and L transport systems is by the influence of pH on transport rate. The A system functions only at neutral or slightly basic pH's, while the L system functions at acidic pH's as well. Figure 3 illustrates the rates of  $\text{Na}^+$ -dependent and  $\text{Na}^+$ -independent transport of AIB at different pH's.  $\text{Na}^+$ -dependent uptake was clearly pH-dependent as well; maximal uptake was obtained at pH 7.5 and uptake ceased at pH 6.  $\text{Na}^+$ -independent transport rates did not vary significantly over the pH range of six to eight.

#### Properties of the A System in MCF-8

**Kinetics of the A System:** Figure 4 presents the transport of AIB by actively growing MCF-8 cells over a concentration range of 0.1 to 10mM AIB. The  $\text{Na}^+$ -independent and  $\text{Na}^+$ -dependent components of that transport are also shown. Total uptake in the presence of  $\text{Na}^+$

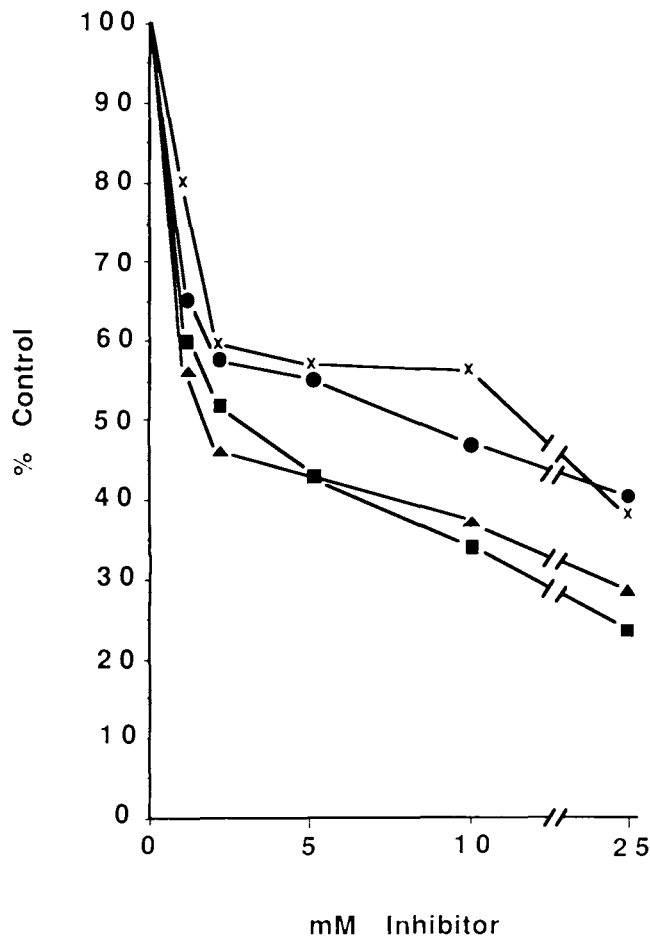


Fig. 2: Inhibition of aminoisobutyric acid transport by various amino acids. The amount of transport of 1mM AIB during a five minute incubation was determined in  $\text{Na}^+$ -containing medium in the presence of increasing concentrations of the following amino acids (●): N-methyl-AIB, (■): proline, (x): phenylalanine, (▲): serine.

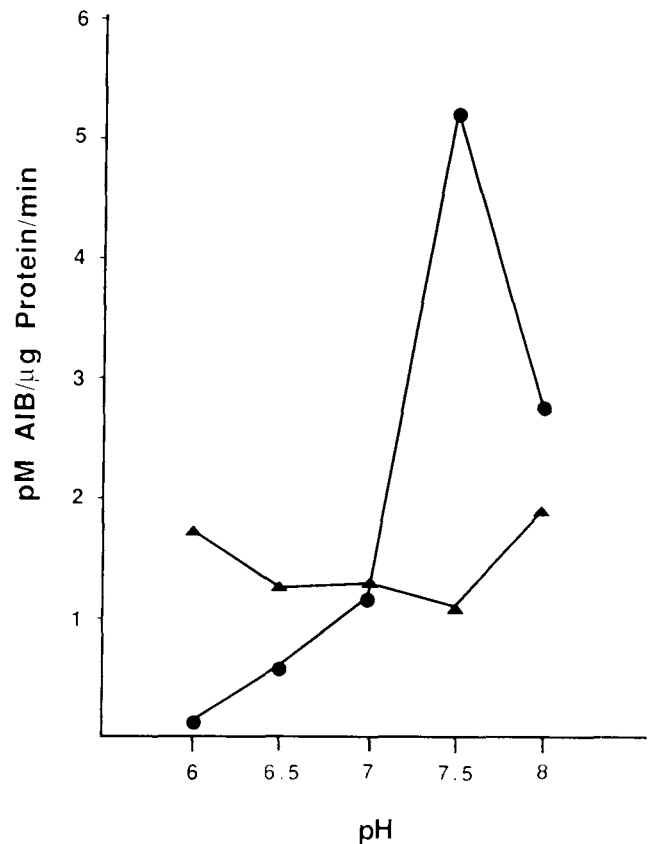


Fig. 3: Aminoisobutyric acid transport as a function of pH of the incubation medium. Sodium-containing and  $\text{Na}^+$ -free media containing 1mM AIB were adjusted to the desired pH's with KOH or HCl before transport during a five-minute incubation period by actively growing MCF-8 cells at a density of  $1.4 \times 10^5$  was measured. (●):  $\text{Na}^+$ -dependent transport determined by subtraction of  $\text{Na}^+$ -independent transport from total transport in  $\text{Na}^+$ -containing medium; (▲):  $\text{Na}^+$ -independent transport.

did not appear to saturate over the range of concentrations shown. An Eadie-Hofstee plot of the total uptake of AIB in MCF-8 cells (Fig. 5) was clearly biphasic, indicating that more than one component was responsible for transport of AIB.

The  $\text{Na}^+$ -dependent component of total AIB uptake (Fig. 4) appeared to be responsible for the majority of transport at low concentrations of AIB and to saturate at a concentration of about 4mM AIB. From the Eadie-Hofstee plot (Fig. 5), the A system of MCF-8 cells has a  $V_{\text{max}}$  of 4.6 pM AIB per  $\mu\text{g}$  protein per minute and a  $K_m$  of 0.79 mM AIB. The  $\text{Na}^+$ -independent component, which includes both the L system and non-saturable uptake, did not saturate over the range of AIB concentrations tested. An Eadie-Hofstee plot of  $\text{Na}^+$ -independent uptake was also biphasic (not shown). The two arms of this plot could be resolved into a component with a  $V_{\text{max}}$  of 3.6 mM AIB per  $\mu\text{g}$  protein per minute and a  $K_m$  of 3.2 mM AIB describing the L system, and a second component representing the non-saturable uptake.

**AIB Transport as a Function of Cell Density:** Figure 6 shows the relationship of cell density and AIB transport in MCF-8 cells. At low cell densities when the cultures were less than 50% confluent so that cell-cell contacts are limited and the cells were actively dividing, transport in  $\text{Na}^+$ -containing medium was divided about equally between the A and L/NS transport systems as measured by N-methyl-AIB inhibatability. As cell-cell contacts increased and the cultures

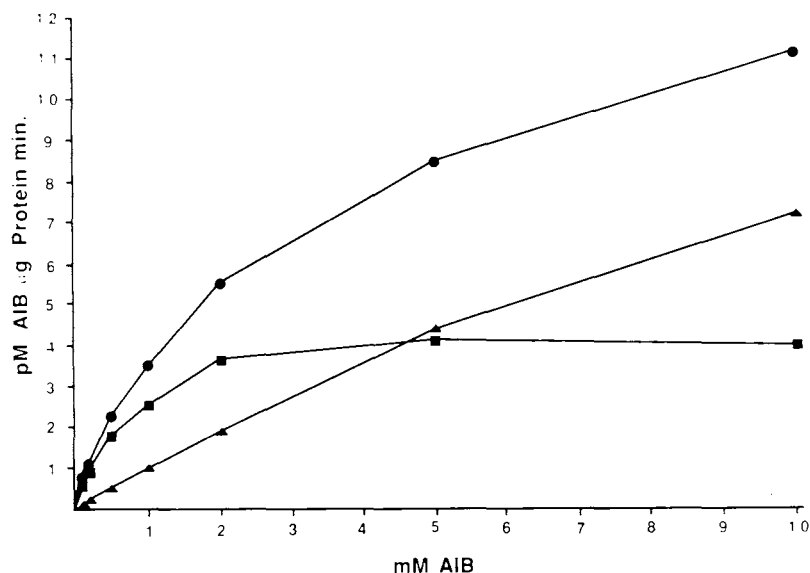


Fig. 4: Aminoisobutyric acid transport as a function of concentration of AIB. Uptake of AIB during the five minute incubation period was determined over the concentration range 0.1-10mM in actively growing MCF-8 cells at a density of  $3.5 \times 10^5$  cells/dish. (●): total transport in  $\text{Na}^+$ -independent transport; (■): sodium-dependent transport determined by subtraction of  $\text{Na}^+$ -independent transport (▲) from total transport.

became confluent and "contact-inhibited," total transport decreased gradually to about half of its previous extent. This decrease appeared to be due to a preferential reduction of transport by the N-methyl-AIB inhibitable A system, since the amount of AIB transported by the L/NS systems decreased only about 15% while A system transport decreased nearly 65%. In quiescent cultures which had been confluent for 48 hours only about 18% of the total AIB uptake was mediated by the A system, compared to more than 50% for growing cells.

*Serum and Hormone Responsiveness of A System Transport:* The role of serum and insulin in the maintenance of proliferation of mammary epithelial cells is well established (Bern and Nandi, 1961; Topper, 1970). Insulin has also been shown to be a mitogen in stationary phase primary cultures of malignant mouse mammary epithelial cells (Voyles and McGrath, 1979). Studies were therefore conducted to determine the ability of serum and insulin, as well as several steroid hormones, to stimulate A system transport in stationary phase cultures of MCF-8 cells. Confluent cultures which had ceased cell division were transferred to medium containing 1% calf serum and no insulin for 24 hours. Insulin, newborn calf serum or steroid hormones were added without a medium change and four hours later the uptake of 1mM AIB in  $\text{Na}^+$ -containing and  $\text{Na}^+$ -free medium was determined to measure the contributions of the A and L/NS systems to total transport of AIB. The results are shown in Figure 7.

In control cultures which received no additions, the levels of transport in  $\text{Na}^+$ -containing and  $\text{Na}^+$ -free media were the same, indicating that A system transport had ceased in the confluent cultures. Added individually insulin (10  $\mu\text{g}/\text{ml}$ ) and calf serum (10% v/v) stimulated the activity of the  $\text{Na}^+$ -dependent A system, increasing transport about 45-60% over the level found in untreated cells using only the  $\text{Na}^+$ -independent L/NS system. When insulin and serum were added at the same time, the amount of stimulation was about 55% above that of untreated cells. In neither circumstance, however, was the level of A system activity restored to that of actively growing cells (equivalent to 256% stimulation). L/NS activity, as measured by transport in  $\text{Na}^+$ -free medium, was unaffected by either

serum or insulin addition. The addition of  $10^{-6}\text{M}$  dexamethesone or hydrocortisone to stationary cultures produced small reductions in transport of 1mM AIB in both  $\text{Na}^+$ -containing and  $\text{Na}^+$ -free media. Addition of  $10^{-6}\text{M}$  estradiol had no effect on the level of AIB transport in quiescent cultures.

## DISCUSSION

Although many studies to determine the ability of mammalian cells to transport neutral amino acids have been conducted, only limited information is available concerning the properties of transport systems in mammary epithelial cells. As part of a series of experiments to compare normal and malignant mouse mammary epithelial cells, I have investigated the uptake of the amino acid analogue  $\alpha$ -aminoisobutyric acid by MCF-8, a cell line of mouse mammary epithelial cells derived from a tumor which arose in the transplanted D2 hyperplastic alveolar nodule line. Since these cells do not express any mouse mammary tumor virus information, their metabolism is not perturbed by virus-specific changes and thus can be studied and compared to normal mammary epithelial cells, to tumor cells in primary cultures which do synthesize and release copious amounts of mouse mammary tumor virus, and to epithelial cells of other tissues of origin as well.

My results indicate that MCF-8 mammary tumor cells have two transport systems for AIB. The primary transport system, which accounts for about 60% of the total uptake of AIB in growing cells, is  $\text{Na}^+$ -dependent, has a pH optimum of 7.5 and is inactive at pH 6, and is inhibited by N-methyl-AIB. These are characteristics of the A system defined by Christensen (1969). The second transport system is  $\text{Na}^+$ -independent, insensitive to the pH of the medium and is inhibited by 2-aminobicyclo [2,2,1] heptane-2-carboxylate, all characteristics of the L system. The L system accounts for about 20% of total uptake. The ASC system, which is  $\text{Na}^+$ -dependent but is not inhibited by N-methyl-AIB, if present at all, transports less than 5% of the total AIB taken up in growing MCF-8 cells.

Hissin and Hilf (1978a) reported finding the A system contribut-

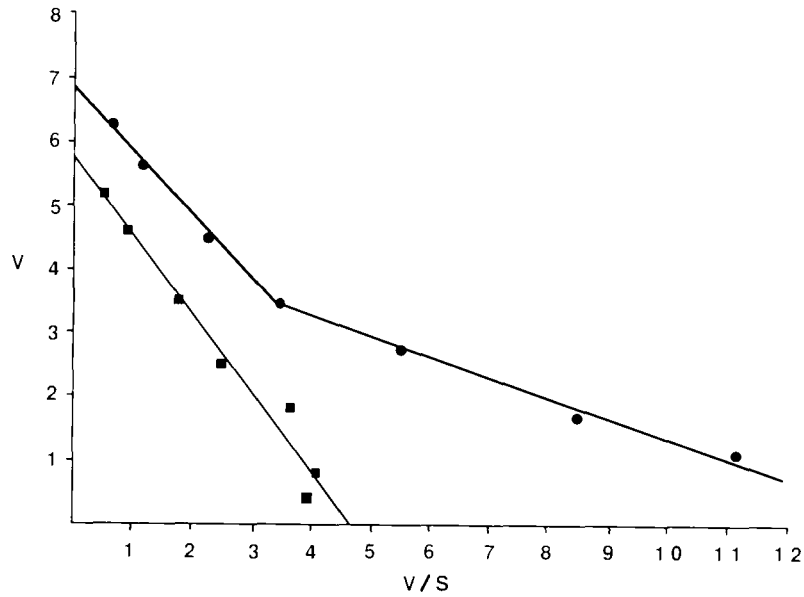


Fig. 5: Aminoisobutyric acid transport as a function of concentration of AIB plotted according to Eadie-Hofstee.  $V = \text{pM AIB}/\mu\text{g protein}/\text{min}$ ;  $V/S = V/\text{concentration of AIB}$ . (●): total AIB transport in  $\text{Na}^+$ -containing medium, with two lines fit by linear regression to represent the high affinity component (1-1mM,  $r = .997$ ) and the low affinity component (1-10 mM,  $r = .994$ ). (■):  $\text{Na}^+$ -dependent AIB transport determined by subtraction of  $\text{Na}^+$ -independent transport from total AIB transport ( $r = .991$ ).

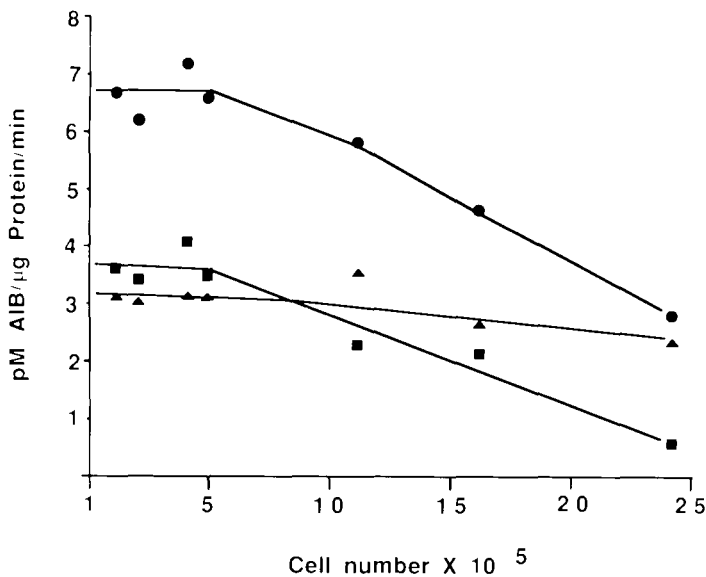


Fig. 6: Aminoisobutyric acid transport as a function of cell density. MCF-8 cells were plated a various densities and the transport of 1mM AIB during a five minute incubation period was determined 48 hours later. Confluency of the cell monolayers was achieved at about  $11 \times 10^5$  cells per dish. Cells at the density of  $16 \times 10^5$  achieved confluency 24 hours before assay; those at a density of  $24 \times 10^5$  achieved confluency 48 hours before assay. (●): total transport in  $\text{Na}^+$ -containing medium; (▲):  $\text{Na}^+$ -independent transport; (■):  $\text{Na}^+$ -dependent transport determined by subtraction of  $\text{Na}^+$ -independent transport from total transport.

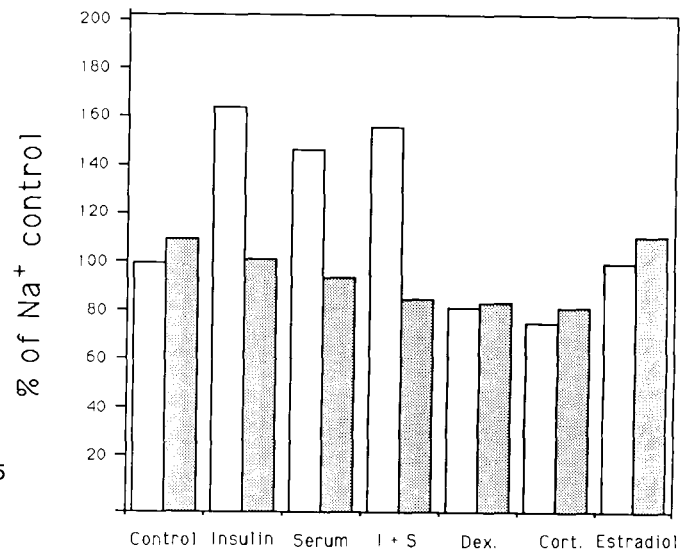


Fig. 7: Effect of hormones or serum on aminoisobutyric acid transport. Additions were made to stationary phase cultures. Uptake of 1mM AIB in  $\text{Na}^+$ -containing (open bar) and  $\text{Na}^+$ -free (stippled bar) media was determined 4 hours after the additions.

ing about 75% and the L system about 25% to the saturable uptake of AIB in the R3230AC rat mammary adenocarcinoma line which is maintained by transplantation. No ASC activity was detected. Neville, et al. (1980) found that explants of normal mouse mammary gland transported AIB by the A and L systems in about equal proportions and that ASC activity was absent. They also found that Ehrlich ascites cells (derived from a mouse mammary tumor) had six times more A system activity than L system activity for the transport of AIB. Although the ASC system was defined by Christensen in the Ehrlich ascites cell line (Christensen, 1969), no transport of AIB was found by that system in Neville's studies. In a note added in proof, they indicated that explants of the D1 solid mouse mammary tumor had A and L system activities in about equal amounts. It thus appears that rodent mammary epithelial cells taken from solid tissues, whether normal or malignant, transport AIB primarily only by A and L systems and lack the ASC transport system found in epithelial cells from other tissues such as the kidney (Sepulveda and Pearson, 1982; Rabito and Karish, 1982) and ovary (Shorwell, et al., 1981).

The kinetic properties of the A transport system in MCF-8 cells ( $K_m = 0.79$  mM and  $V_{max} = 4.6$  pM AIB/ $\mu$ g protein/minute) appear to be similar to those of other mammary cells, although direct comparisons are difficult because of differences in experimental procedures (Neville, et al., 1980; Hissin and Hilf, 1978a; Gay and Hilf, 1980).

The A system of MCF-8 mammary epithelial cells appears to be subject to several forms of regulation. One of these is related either to the degree of confluency or to the rate of cell division in the culture. As the population density of a culture increases and the growth rate slows, transport of AIB by the A system decreases until almost all AIB uptake occurs by the L system alone in cultures which have been 100% confluent for 48 hours. Both cessation of growth and entry into a  $G_0$  state (Dubrow, et al., 1978) and cell-cell contacts (Lieberman, et al., 1979) have been shown to cause a reduction in the rate of AIB transport by the A system. Other workers have found that insulin simulates AIB transport of fibroblasts tested in suspension cultures (Guidotti, et al., 1976) and in liver parenchymal cells growing in monolayer cultures (Kletzien, et al., 1976). Both insulin and serum appear able to stimulate A system transport of AIB in quiescent MCF-8 cells, and to work synergistically when given together. I have previously found that insulin is a mitogen in confluent primary cultures of malignant but not normal BALB/c mammary epithelial cells (Voyles and McGrath, 1979), so it is possible that the stimulatory effect of insulin is related to its mitogenic activity. It is interesting to note that Hissin and Hilf (1978c) found that administration of insulin to diabetic rats decreased the rate of development of R3230AC mammary tumor, but increased the rate of AIB transport by the A system when given in tissue culture. They suggest that this paradoxical effect of insulin may be due to the presence of other hormones in vivo which modulate the effects of insulin.

Although Russell, et al. (1982) found that the steroid hormone hydrocortisone stimulated AIB uptake by dermal fibroblasts, both hydrocortisone and dexamethasone produced modest decreases in AIB transport in quiescent MCF-8 cells. Since these cells express no mouse mammary tumor virus information, this observation suggests that it may be possible to study virus-specific changes in transport independently of density-dependent changes in primary cultures of malignant mammary epithelial cells in which virus production is regulated by those compounds. The hormone estradiol had no effect on AIB transport in quiescent MCF-8 cells, although Hissin and Hilf (1979) have reported that A system uptake is somewhat inhibited by the compound in cultures of R3230AC mammary tumor cells.

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