THE AMERICAN JOURNAL OF Clinical Nutrition

MARCH-APRIL 1960 VOLUME 8, NUMBER 2

Symposium on Absorption Mechanisms and the Malabsorption Syndrome

Transport of Amino Acids Into and Across Cells

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THIS symposium is concerned with the massive disappearance of nutrients from the alimentary canal and their reappearance in various body channels; more particularly with the intervening events and their disorders. What is the nature of these events? We are dealing with the problem of transport, usually of structurally-specific mediated transport, and often active transport.

An axiom of biochemical advance is that a phenomenon should be studied in simple form, if possible in isolated form, in order to understand it. Do the chemical reactions of transport across the intestinal mucosa exist in simpler form? We will try to show that the simpler, probably universal phenomenon of active transport *into* cells is fully capable of producing active transport *across* cells.

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Presented at the Symposium on Absorption Mechanisms and the Malabsorption Syndrome, under the sponsorship of The National Vitamin Foundation, Inc., March 3, 1959, New York, New York.

This study was supported in part by a grant (C-2645-C2) from the National Cancer Institute, National Institutes of Health of the U. S. Public Health Service.

Figure 1 shows how this may occur: if a cell is able to concentrate a solute from the cellular environment, it should be able to release the solute at an elevated concentration into another separated segment of its environment. All that is required is that concentration be interrupted or inhibited with regard to the restricted segment (side B); or instead concentration might be stimulated or facilitated from the other part of the environment (side A). Undoubtedly cells of the intestinal mucosal do pump the amino acids into their interior as other cells do; indeed Hird[1] has shown that this is the case. The problem then is to show whether they have supplemented facilities for concentrating amino acids from their mucosal side, or handicapped concentrating ability on their serosal aspect; or whether instead they have an entirely unrelated apparatus for transcellular transfer, which seems unlikely to us. If their ordinary transport apparatus is asymmetric, we should like to know whether the polarization is humoral or anatomic in origin.

TRANSPORT ACROSS CELLS ARRANGED TO FORM A BARRIER

We recently demonstrated experimentally that concentrative uptake into cells can indeed

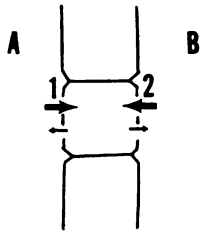

FIG. 1. Concentration into a cell can produce concentration across the cell. Inhibition of process 2 or stimulation of process 1 can produce accumulation in compartment B.

produce concentration across cells.[2] We have been able to form Ehrlich ascites tumor cells into an exceedingly thin membrane by catching them on a very fine cellulosic Millipore* filter. The filter very quickly becomes plugged to further filtration, a finding that had originally plagued us before we realized the usefulness of the resulting membrane. Cells that are not firmly attached are washed off with a saline solution. Figure 2 illustrates the microscopic appearance of these membranes in fixed sections prepared for us by Dr. Burton L. Baker (Department of Anatomy). Gravimetric determinations are in approximate agreement as to the illustrated number of cells forming the barrier.

When we placed a suitable saline medium (Krebs-Ringer bicarbonate solution) above and below this membrane, with 10 mM glycine in each phase, the only change in distribution was the uptake of glycine by the 5 or 6 mg. of cells present, causing an indetectible change in the glycine level of the two phases. We then tried to stimulate concentration at one face of the cells by placing 0.8 mM pyridoxal in the solution on that side. The membranes are sufficiently impermeable so that the pyridoxal gradient is not dissipated for several hours. After a twenty-minute lag the glycine level of the opposite phase began to rise, reaching a maximum level in about one hour averaging 1.17 times the concentration of the pyridoxal-containing phase (Fig. 3). The two opposed gradients then gradually disappeared together.

The phase volumes for this experiment were about 35 ml. for that containing pyridoxal,

* One inch type HA filters. Millipore Filter Corporation, Bedford, Massachusetts.

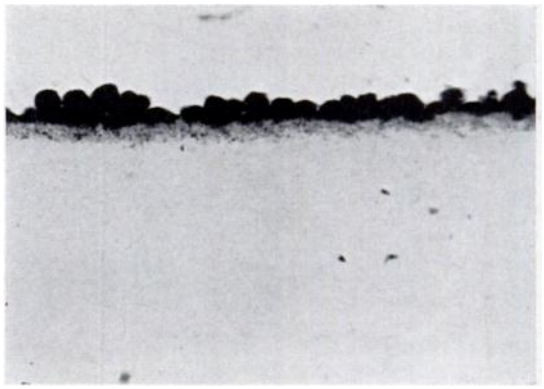

FIG. 2. Photomicrograph of cells in place on the Millipore filter, fixed with Bouin's fixative and stained with Regaud's iron hematoxylin and Masson's stain.

0.5 ml. for the other, the latter being kept small so that we would not have to wait too long for the concentrating ability to become apparent. Note how unlikely it is that these cells can release enough of a binding agent to bind 2 mM glycine in a 0.5 ml. volume, roughly 100 times the volume of the cells, or, considering both compartments, about 6,000 times that of the cells. Thus we have glycine being concentrated into a second extracellular compartment, where the glycine activity is very accessible to study, rather than into a mysterious cell interior where doubts can always be raised as to the free state of the amino acid.

This concentrating effect is independent of the relative position of the filter and the cells; in fact both surfaces of the cells may be covered with the filter material without significantly changing the result.

Pyridoxal itself is a binding agent for amino acids, but the stability constant for the resulting Schiff base is too low to make this a significant factor in the extracellular phases. In fact, if this factor operates at all, it would only intensify the problem of explaining the asymmetry of distribution.

Similar results are obtained with other α-amino acids including α-aminoisobutyric acid but not with N-dimethylglycine or α-methylglutamic acid, in precise agreement with the specificity of the intracellular concentration process. The process is similarly temperature-sensitive and dependent upon the presence of extracellular potassium (the latter acting to maintain an adequate level of cellular K^+).[3]

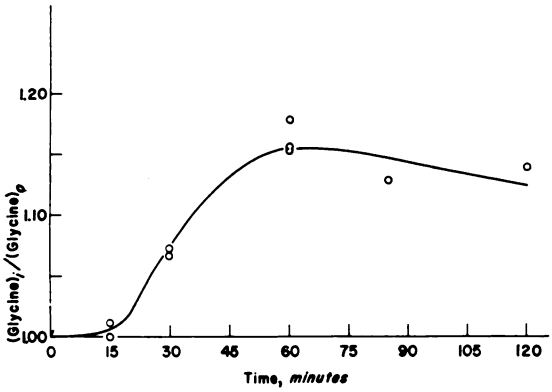

FIG. 3. Change of glycine distribution ratio with time after the addition of 8.0×10^{-4} M pyridoxal to phase o.

So far we have not been able to discover a single factor to which only one of the two processes is sensitive. Therefore we are convinced that intracellular concentration is serving to produce transcellular concentration. At the same time we believe this experiment has finally made unequivocal the evidence that transport into cell of these solutes is really active or concentrative. This conclusion, however, should not be freely extended to other solutes that are accumulated by cells.

Before we consider why pyridoxal produces this strange effect we should like to mention other means that we have used to produce asymmetry of amino acid distribution. If we set up a potassium gradient of 4 or 5 mM by initially placing 15 mM K^+ on the one (less voluminous) phase and omitting K^+ from the other phase (adjusting the Na^+ level in each case to maintain isoosmotic conditions) glycine moves for twenty-five minutes toward the solution (that is, losing K^+), to produce a distribution ratio of 1.1 (in one case 1.5) (Table I). This again recalls the behavior of these cells in giving up part of their potassium as they gain amino acid, and in being able to gain amino acid only in proportion to the K^+ they have available to lose.[3] Potassium ion penetrates the membrane more rapidly than pyridoxal, presumably mainly through spaces between the cells, since sucrose also gradually penetrates. This fairly rapid permeation of K^+ shortens the period during which this phenomenon may be observed and presumably limits its maximal intensity. Conversely, in analogy to concentrative uptake into the free cells, a potassium gradient arises across the membrane in the opposite sense to the glycine gradient when 50 mM glycine is placed on one side of the membrane (Table II). Accordingly, the idea gains strength that potassium migration may drive amino acid transport, and that the energy for both may be applied primarily to the maintenance of the potassium gradient,[3] the potassium gradient then serving to drive amino acid transport. This substantial evidence for common ground in the uptake of such highly dissimilar substances as amino acids and K^+ is interesting when we come to consider the specificity of absorption defects in diseases of intestinal absorption or of tubular transport.

As for the action of pyridoxal we note that

TABLE I

Effect of a Potassium Gradient on Glycine Distribution

Experiment	Final [Glycine] c.p.m./ml.		Ratio $[Glycine]_1/[Glycine]_2$
	Side 1	Side 2	
1	4,650	2,990	1.55
2	7,250	6,680	1.09
3	6,760	6,200	1.09
4	4,780	4,550	1.05

NOTE: Side 1 was made 15 millimolar in K^+ at zero time, whereas side 2 contained no potassium. At the time of observation (30 min.) the (K^+) was 4.5 mM on side 1, and about 0.2 mM on the voluminous side 2. The cells had been brought to equilibrium with 10 mM labeled glycine before preparing the membrane. The labeled glycine level was 10 mM initially on both sides of the membrane.

TABLE II

Effect of a Glycine Gradient on Potassium Distribution

Time (min.)	Final $[K^+]$mEq./L.		Ratio $[K^+]_1/[K^+]_2$
	Side 1	Side 2	
15	6.05	5.90	1.03
25	6.45	5.90	1.09
25	6.43	6.07	1.06
40	6.50	5.90	1.10
60	6.35	6.00	1.06

some moves onto the cells, staining them yellow, but this migration is almost instantaneous and not in step with the production of an amino acid gradient. The interior of the cell is apparently not generally accessible to the aldehyde. Prahbat Pal[4] has further shown in our laboratory that at least down to 0.5 mM pyridoxal levels the addition of neutral amino acids to the cell suspensions only decreases pyridoxal fixation by the cells despite the relative enrichment of the cell interior with a pyridoxal binding agent. Obviously the role for which we are considering pyridoxal here is as a possible carrier for amino acid transport. Although pyridoxal is chemically suitable for the types of carrier combination that must be considered,[5] the evidence, as recently summarized elsewhere,[6] is not entirely unequivocal for this role. At the levels through which vitamin B_6 varies in moderate depletion states, it is certainly a significant factor in the distribution of a model amino acid in the intact rat.[7] Dr. Masani Suda of the Institute for Protein Research, Osaka University, Japan, recently told us that depletion of vitamin B_6 by administration of penicillamine has permitted him to show a stimulating effect of pyridoxal on amino acid absorption from the intestine of the intact rat. Disturbances in placental transport of amino acids in toxemias of pregnancy[8] may be related to the apparently disturbed vitamin B_6 economy of pregnancy.

SOME ATTRIBUTES OF AMINO ACID TRANSPORT

We trust the foregoing results show that whatever properties may be found for the process of concentration of solutes into isolated cells are also significant for transmucosal transport. We may summarize some of the attributes that have been observed during a decade of preoccupation with this problem:[6,9,11]

(1) The amino acids fall into transport families. These divisions may depend on whether the amino acid contains a single carboxyl group or two, and whether it contains a single amino group or two. Within each of these families competitions characteristically occur, but not between members of different families. Some of the charged amino acids actually stimulate the transport of some of those of the neutral family. As Wiseman[10] has pointed out, the existence of such competitions may cause a partial stratification as to the levels in the upper intestine where various amino acids principally cross. The gradual release of amino acids by proteolysis may protect us to a considerable degree from excessive competitions for absorption. We also have to think about the competition among the amino acids for transport beyond the mucosa, into various cells, especially when amino acids are injected for nutritional purposes.

(2) For vigorous transport the amino group should be α or β to the carboxyl group, and if a second amino group is two or three but not more carbon atoms from the first, striking intensification of uptake occurs. These properties would make lower homologs of lysine and ornithine biologically dangerous, and may explain their limited occurrence. Effects on pyridoxal distribution completely different from those of other amino acids also result, suggesting pyridoxal binding within the cells by such amino acids.[4]

(3) Intensified transport may be signalled either by a decreased level at the site from which the amino acid derives or an increased level in the compartment into which it is pumped. Plasma amino acids, in relationship to tissue levels, frequently show the operation of this principle. Decreased plasma levels may signal that concentrative uptake has been stimulated (e.g., endocrinologically[11–13]) by a major tissue, or that an additional concentrative organ, for example the placenta in pregnancy, has been interposed.[13] The restraint of concentrative uptake may be essential for the transport of solutes from cell to cell, and may be a means for the control of growth.[11–13]

(4) Because active transport is subject to saturation, the concentration gradients at high loads may come to be in the reverse direction, and the anatomic barrier to diffusion which is necessary for effective concentration may become conspicuous.

(5) The energy sources for amino acid transport may be either oxidative or glycolytic and

do not appear distinctive, except for the possible channelling through the potassium pump to amino acid transport.

(6) The minimal reaction for transport (and or course we are interested in discovering the minimal reaction and not all the unessential fates that may follow) remains unidentified but involves primarily the amino group, and is sensitive to pyridoxal, a substance that reacts appropriately with the amino group.

SPECULATION

For this process we visualize the existence of a chemical reactant essential to transport. This reactant may become accessible to the transported solute despite the barrier to diffusion either by extruding a portion of itself to form an active site at the cell periphery or by receiving the solute secondarily from a superficial active site. The reactant could be visualized as able to receive the free solute from one aspect of a macromolecule forming part of the outer barrier of the cell, but able then to pass the solute on to the carrier only on the opposite aspect of the molecule[14] inside the osmotic barrier. In the latter case either reaction may serve to increase the energy level of the solute, so that it may be released into the interior of the cell at a higher concentration. If this process is less effective on one aspect of the cell, transcellular concentration will result. An osmotic pump that concentrates a solute outward (e.g., Na^+) may be similarly organized to produce transcellular concentration.

We might expect to isolate the transport reaction even further if we could shear off with an accurate microtome the one face of the cell barrier, to leave us with a single plasma membrane to study rather than two. We can perhaps achieve this purpose with a chemical "microtome" which can destroy the transport activity of the exposed surface. If we then place a suitable potassium-rich intracellular medium as one phase and an extracellular medium for the other, perhaps we can still obtain the characteristic transport activity. Note, however, that we lose the usual indicator of transport if we simply isolate cell membranes in a randomly orientated preparation. Such a preparation will be useful only if we discover a way to mark the transport reaction so we can recognize it in the absence of separate "extracellular" and "cellular" phases.

SUMMARY

The question of whether or not transport across the intestinal mucosa or similar barriers can be studied in simpler forms has been considered. We have shown that cells can be arranged to form a membrane able to concentrate amino acids from one and into the other of two phases separated by the membrane. This was achieved by adding pyridoxal or pyridoxal phosphate to the first phase, or by adding an excess of potassium ion to the second phase. The characteristics for the transport across the cells were exactly similar to those for accumulation into the cells.

These results show that the concentrative process for cells produces elevated levels of free amino acids, and is able to simulate the behavior of a secretory tissue. They further show that the attributes found for the transport into or out of cells are significant for transmucosal transport. Accordingly, these attributes have been summarized, and possible ways in which active transport may take place have been considered.

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DISCUSSION

Question: Do enzyme inhibitors modify the ratio?

Dr. Christensen: The question was whether or not chemical inhibitors are able to modify the development of gradients across the artificial membrane of cells. This has been tried, and the answer is yes. Several inhibitors produce results in the membrane system rather similar to those produced in suspensions of the Ehrlich ascites tumor cells. However, the chemical agents used are often reactive with the pyridoxal with which the gradient is produced, for example cyanide or mercury or a number of other substances have been tried; so that the results are preliminary and not unequivocal.

We have felt that we ought to be able to produce an actual gradient with such agents inhibiting the transport on one of the exposed surfaces. But we need here to limit ourselves to inhibitors that influence carrier transport *per se* across the membrane, rather than those which interfere with the delivery of energy to the transport apparatus.

For example, we have thought of establishing anoxia on one surface of the cells by simply not having oxygen present in the corresponding atmosphere. But whether or not we could maintain an oxygen gradient across the barrier for an adequate length of time is rather dubious, in view of the diffusability of oxygen. Furthermore, oxygen deficiency may be expected to influence the availability of energy for transport at all surfaces of the cell, and not merely at the one exposed to anoxia.