present inability to increase transport after 18 h of hyperglycemia, suggests that altered transport might be related to a modification of newly forming cells.

Lack of effect of inhibitors of protein synthesis on intestinal amino acid[8,9] and carbohydrate movement (although other organs may be affected)[10] does not mean that all intestinal transport systems are stable once formed, since a time factor might be involved. Ca^{2+} transport is not adversely affected by inhibition of protein synthesis, but the increase in its transport brought about by vitamin D can be blocked by actinomycin D[9,11]. We must likely begin thinking of intestinal transport systems with inducible components. The transport systems possess at least short-term stability.

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Actinomycin D inhibition of amino acid transport in *Streptococcus faecalis*

Actinomycin D has been reported recently to inhibit respiration and glycolysis in human leukemic leukocytes, suggesting that the inhibition of DNA-directed RNA synthesis may not be the only metabolic effect of this substance[1]. The reversal by glucose of actinomycin D inhibition of protein synthesis in ascites cells may reflect a related activity[2]. While investigating the amino acid transport systems of *Bacillus subtilis*, we found several years ago that the usefulness of actinomycin D as an inhibitor of amino acid incorporation into protein was limited in such experiments by its inhibition of amino acid transport. This effect has been examined further, and, in view of the current interest in additional metabolic effects of actinomycin D, we des-

cribe below the inhibition by this antibiotic of α-aminoisobutyric acid, cycloleucine, glutamic acid and leucine accumulation in *Streptococcus faecalis*.

Cells were grown in a synthetic medium[3]. Amino acid uptake was measured using previously described methods with minor modifications[4]. Cells exposed to ^{14}C-labeled amino acids (3 mM) in phosphate buffer (0.18 M) containing glucose were centrifuged, and the cell pellets were extracted with hot water to liberate the accumulated amino acid which was measured by assay of radioactivity. Chromatographic experiments have established that, with glutamate, most, and, with α-aminoisobutyric acid and cycloleucine, all of the isotope extracted from the cell occurs in the respective amino acid.

Fig. 1 shows the time course of α-aminoisobutyric acid and glutamic acid uptake in *S. faecalis*, and its inhibition by actinomycin D. Significant inhibition of transport was observed with 3 μg/ml, and maximal inhibitory effects were observed

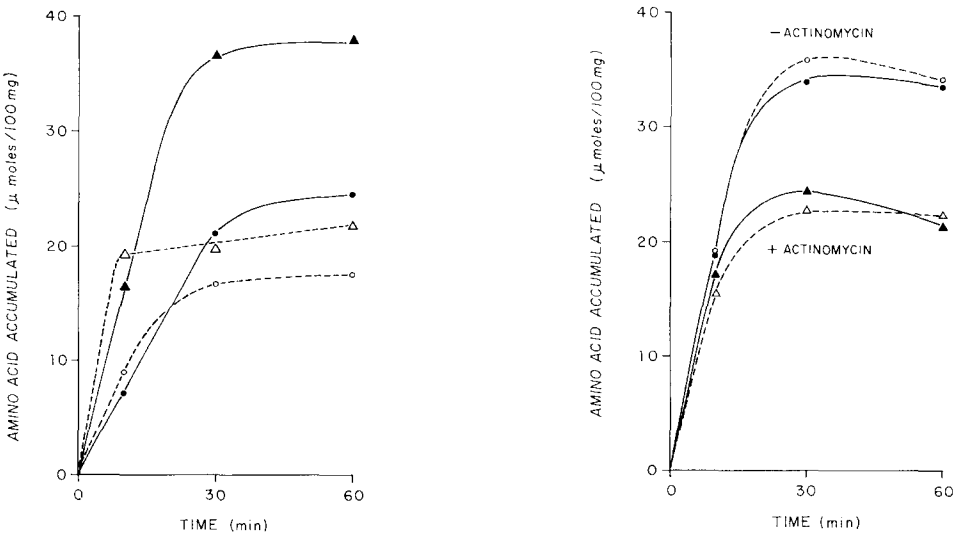

Fig. 1. Effect of actinomycin D on α-amino[^{14}C]isobutyric acid and L-[^{14}C]glutamic acid accumulation in *S. faecalis*. Cells at 1.6 mg/ml were incubated in buffer containing glucose, the ^{14}C-labeled amino acid (3 mM), and actinomycin D (20 μg/ml), as indicated below. ▲—▲, α-aminoisobutyric acid, control; △- - - - -△, α-aminoisobutyric acid, + actinomycin; ●—●, glutamic acid, control; ○- - - -○, glutamic acid, + actinomycin.

Fig. 2. Effect of preincubation with actinomycin D on inhibition of α-amino[^{14}C]isobutyric acid accumulation. Cells were added to buffer containing glucose and actinomycin D, as indicated. α-Amino[^{14}C]isobutyric acid was added immediately (no pretreatment), or after 20 min (pretreated), and incubation was continued for the times shown. ●—●, no pretreatment, no actinomycin; ○- - - -○, pretreated, no actinomycin; ▲—▲, no pretreatment, + actinomycin; △- - - -△, pretreated, + actinomycin.

using 10–20 μg/ml of actinomycin D; higher levels did not produce greater inhibition. Increasing the extracellular osmotic pressure with sucrose did not prevent these effects, indicating that they do not originate in cell wall changes. This inhibition, which was not observed until after 10 min (α-aminoisobutyric acid) or 20 min (glutamic acid) of uptake, did not occur earlier if cells were pretreated with the antibiotic

for 20 min before the labeled amino acid was added (Fig. 2). Comparable results were obtained using cycloleucine to measure accumulation. It appears that inhibition depends on the simultaneous presence of the amino acid and the antibiotic. In some experiments with such pretreated cells, α-aminoisobutyric acid uptake stopped more abruptly after 10 min, resulting in the accumulation of smaller amounts of amino acid.

It is unlikely that this phenomenon depends solely on a block in protein synthesis. Levels of puromycin which inhibited glutamate and leucine incorporation into the cell residue, at least as well as actinomycin D, had no discernible effect on α-aminoisobutyric acid, cycloleucine, glutamate, or leucine transport (Table I). The glutamic acid incorporated into the cell residue, which was resistant to inhibition by both antibiotics, appears to be present in cell wall material. While these observations tend to exclude a block in the terminal steps of protein synthesis as being the causative event in this phenomenon, they do not exclude an effect on RNA synthesis from such a role. In *Lactobacillus plantarum*, preincubation of cells in buffer led to a considerable (25%) increase in glutamic acid accumulation capacity, which also could be inhibited by actinomycin D. Puromycin had only a slight effect on this

TABLE I

EFFECT OF ACTINOMYCIN D AND PUROMYCIN ON AMINO ACID TRANSPORT AND INCORPORATION INTO CELL RESIDUE

Cells (1.6 mg/ml) were incubated for 60 min with the [^{14}C]amino acids in the absence or presence of actinomycin D (20 μg/ml), or puromycin (300 μg/ml). The amino acid pool was extracted, and the cell residue was washed with acetone and ether and dried (glutamate), or digested with 3 M HCl and dried (leucine), prior to counting.

	Cell extract				*Cell residue*	
	α-Aminoisobutyric acid	*Cycloleucine*	*Glutamic acid*	*Leucine*	*Glutamic acid*	*Leucine*
	(μmoles/100 mg cells)				*(μmoles/100 mg cells)*	
Control	38.4	5.8	25.2	2.6	3.6	3.2
Actinomycin D	22.6	3.1	18.7	1.9	2.1	0.8
Puromycin	41.3	5.8	24.3	3.0	1.9	0.3

increase in capacity, despite a pronounced inhibition of amino acid incorporation into protein.

The inhibition of transport by actinomycin D also appears not to be caused by an inhibition of glycolysis. Titrimetric determinations of hydrogen ion production from glucose indicated, at most, a small (8%) inhibition by the antibiotic of the glycolytic rate. The removal of glucose from the buffer was completely unaffected. Although these results appear to exclude inhibition of reactions in the glycolytic pathway as a causative factor, it is not known whether the subsequent utilization of high-energy compounds in the transport process has been affected. The possibility also must be considered that a small portion of the total glycolytic activity is coupled to the transport process, and that this fraction is specifically inhibited by actinomycin D.

There have been several previous reports that actinomycin D and puromycin inhibit transport systems. The stimulation by aldosterone of sodium transport in the

toad urinary bladder was inhibited by actinomycin D and puromycin[5,6], and the stimulation by vitamin D of intestinal calcium transport was inhibited by actinomycin D[7,8]. Puromycin inhibited amino acid transport in embryonic chick bone, but only after a long period of essentially normal uptake[9]. In all these cases, it has been suggested that the synthesis of protein catalysts involved in the respective transport processes is inhibited by these antibiotics. BERLIN AND STADTMAN[10] recently observed actinomycin D inhibition of adenine uptake in *B. subtilis*. They suggested that the purine nucleotide pyrophosphorylase, which metabolizes adenine after its transport into the cell, is subject to feed-back inhibition by intracellular nucleotides present in higher concentrations when nucleic acid synthesis is blocked by actinomycin D. Since α-aminoisobutyric acid and cycloleucine are not significantly metabolized in *S. faecalis*, such an explanation is not applicable in terms of a metabolic pathway, but it might apply for the transport catalyst itself.

The absence of information regarding correlative effects on other cell components makes any suggestion regarding the possible basis of the inhibition described here a speculative undertaking. In view of the lack of inhibition by puromycin, the most likely possibilities are that actinomycin D prevents the synthesis or regeneration of nucleotide or polynucleotide-dependent catalysts required for the operation of the transport systems, that it causes an accumulation of inhibitory nucleotides, or that it interferes with the utilization of high-energy substances.

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