la that confer tolerance; these cells could give rise to components of the skin itself, in addition to blood cells. In the older literature, the simultaneous origin of vascular endothelium and blood from the same mesenchymal elements in the embryo is emphasized, suggesting that, even in the adult, blood-forming tissues may on occasion give rise to epithelial or connective tissue cells (*11*). Dunn observed the development of a stratified, squamous epithelium on the surface of skin wounds is rats when the wounds were covered by thymus tissue, and suggested that this epithelium was derived from reticulum cells of the thymus (*12*). Furthermore, Andrew (*13*) believes that lymphocytes can transform into epithelial cells in the intestine and the skin. According to the stem-cell hypothesis, the greater immunizing ability of retransplanted skin isografts as opposed to first-passage isografts would follow from their greater proportion of allogeneic components due to the extensive revascularization and regenerative hyperplasia that invariably accompanies the union of a skin graft with its host. In both cases, the proportion of allogeneic components would have to be large enough to immunize, yet small enough so that its destruction would not result in substantial damage to the graft as a whole.

As yet, there is no decisive evidence for any of these alternatives. If the leukocyte containment hypothesis is correct, it raises the question of the extent to which the immunizing ability of skin grafts in general is dependent on contained leukocytes.

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Actinomycin D Effect on Amino Acid Absorption from Rat Jejunal Loops

Abstract. *The absorption of amino acids from jejunal loops was suppressed in anesthetized rats treated previously with 1.0 or 1.5 micrograms of actinomycin D per gram of body weight. The absorption of the acidic, neutral, and basic amino acids showed selective differences in response that were relative to the degree of inhibition and to the time interval required to demonstrate actinomycin sensitivity.*

Inhibitors of RNA formation and protein synthesis affect the membrane transport of biological materials (*1*). Piperno and Oxender (*2*) reported results suggesting the necessity of a specific binding protein in the uptake of branched-chain amino acids by *Escherichia coli*. We have studied the effect of actinomycin D on the absorption of amino acids from the intestine.

Jejunal loops were prepared in 200-g male rats (Sprague-Dawley) according to the procedure of Delhumeau *et al.* (*3*). The rats were randomly allotted to the various groups for treatment and were injected intraperitoneally with actinomycin D (1.0 or 1.5 μg per gram of body weight) 2, 4, or 8 hours before preparation of the jejunal loop. Some animals were not treated with actinomycin and served as controls. Experiments were also performed on animals injected with saline. All animals were fasted for 30 hours and then anesthetized with sodium pentobarbital.

Two loops were made in the upper part of the jejunum of each animal; 1 ml of a solution of amino acids (total of 90 μmole) simulating the composition of casein with 0.05 percent glucose added was placed in one loop, and 1 ml of 0.05 percent glucose solution was placed in the other as a blank. After 15 minutes, the loops were removed and washed several times with citrate buffer (*p*H 2.2). The combined washings for each loop were analyzed for amino acid content by ion exchange chromatography (*4*). The blank loops all contained very minute quantities of the amino acids (from 0.01 to 0.29 μmole), and these amounts were disregarded in the calculations.

In some experiments a dose of 1.5 μg per gram of body weight was required for actinomycin to reduce amino acid absorption; in others, a dose of 1.0 μg/g was sufficient (Table 1). In the experiment reported in Table 1, inhibition of absorption was obtained with 1.0 μg of actinomycin per gram of body weight. Compared to controls, rats that received actinomycin 2 hours before the loop operation had no significant decreases in the absorption of amino acids, except proline. In animals 4 hours after injection of actinomycin, the percentages of absorption for all acidic and neutral amino acids were significantly less than those of the controls. For the same time interval, the absorption of the basic amino acids appeared to be slightly decreased, but not significantly. In animals injected with actinomycin 8 hours before the loop operation, the absorption of the basic amino acids further decreased, and the absorption of some, notably lysine, arginine, and tryptophan, was significantly reduced relative to that in control rats. In experiments carried out on animals injected with saline instead of actinomycin, the amounts of amino acids that were absorbed were similar to those of control rats in all instances, and the data were not included in the table.

In addition to differences in the length of time necessary for actinomycin to influence the absorption of the various amino acid groups, treatment with the antibiotic appeared to elicit differences in the degree of inhibition. Absorption of the acidic amino acids was inhibited to the greatest extent by actinomycin administration. Among the neutral amino acids, the antibiotic treatment inhibited the absorption of some more than it did of others. The amino acids in these two subgroups paralleled the amino acids that Oxender and Christensen (*5*) postulated were absorbed at the

so-called leucine-preferring and alanine-preferring transport sites. Leucine, valine, isoleucine, tyrosine, and phenylalanine, designated as being absorbed predominantly at the leucine-preferring site, were inhibited to a lesser extent by actinomycin than alanine, glycine, threonine, and serine were. Four hours after injection of actinomycin, the mean inhibition for the leucine-preferring subgroup was 46.8 percent, and that for the alanine-preferring subgroup was 72.8 percent (calculated from values in Table 1). The alanine-preferring site which Oxender and Christensen (*6*) found was more sensitive than the leucine-preferring site to respiratory inhibitors and variations in sodium and potassium ions was also affected to a greater degree by actinomycin. In these experiments with actinomycin, the absorption of methionine and of proline was inhibited to the same degree as that of leucine.

The specific effect of actinomycin on the absorption of the various groups of amino acids is some indication that the antibiotic does not act by producing a generalized toxic condition in the intestinal tissue, which interferes with all absorption processes. We also measured the absorption of D-glucose from jejunal loops in rats injected with the antibiotic 4 hours before the test. D-Glucose solution (1 ml, 100 μmole) was placed in the jejunal loops, and the amount remaining after the incubation period was analyzed enzymatically by the Glucostat method (Worthington Biochemical Corp., Freehold, New Jersey). The average absorption was 57.7 $\pm$ 3.2 percent in four control rats and 74.1 $\pm$ 10.6 percent in four rats injected with actinomycin. The absorption of glucose was apparently not inhibited by actinomycin treatment and may have been slightly enhanced.

These effects of actinomycin on amino acid absorption occurred while the concentrations of amino acids in the plasma were maintained. Other experiments (*7*) have shown that for at least 8 hours after the injection of actinomycin the concentrations of amino acids in plasma from treated animals were similar in all instances to those of control rats. When amino acids were determined in plasma of cardiac blood obtained immediately after an absorption test, there were no differences between treated and control rats for any of the amino acids (results not tabulated). Apparently, the effect of actinomycin on intestinal amino acid absorption antedates any alteration in plasma amino acid concentrations.

In some biological systems actinomycin D blocks DNA-directed RNA synthesis and subsequent protein synthesis (*8*). On this basis, one explanation of our results may be that absorption of amino acids from the intestine is dependent upon protein carriers specific for the acidic, basic, and neutral amino acids. Some integral parts of the mechanism for template or protein synthesis may have short and characteristic half-lives and thus express varying degrees of sensitivity to actinomycin. It should be emphasized that these effects on amino acid absorption were observed after administration of high doses of actinomycin and may not be the result of a direct action of the antibiotic on DNA-directed RNA synthesis. More recent evidence has shown that smaller doses of actinomycin D (0.25 μg per gram of body weight) enhance amino acid absorption from intestinal loops (*7*). This effect may result from a stimulation of corticosterone secretion, since hydrocortisone also increases amino acid absorption from rat intestinal loops (*7*), and the studies of Leppe and Szego (*9*) have demonstrated elevated corticosterone levels in animals treated with actinomycin.

Table 1. Administration of actinomycin D and the absorption of amino acids from rat jejunal loops. The amino acid solution placed in the jejunal loop contained the following amino acids in micromoles: leucine (Leu), 8.00; trytophan (Try), 0.70; lysine (Lys-HCl), 5.70; arginine (Arg-HCl), 2.44; histidine (His-HCl), 2.04; phenylalanine (Phe), 3.42; isoleucine (Ileu), 5.24; valine (Val), 6.60; methionine (Met), 2.18; threonine (Thr), 3.78; serine (Ser), 6.68; glutamic acid (Glu), 16.32; tyrosine (Tyr), 3.34; cysteine (Cys, included with basic amino acids), 0.64; proline (Pro), 10.66; asparagine (Asp), 5.80; alanine (Ala), 3.96; glycine (Gly), 2.76. The absorption in percentage equals the amount added to loop minus the amount found in loop after 15 minutes, divided by the amount added to loop, times 100. All values are means $\pm$S.D. The percentage of inhibition equals the percentage of absorption in control rats minus the percentage of absorption in rats treated with actinomycin at 2, 4, or 8 hours, divided by the percentage of absorption in control rats, times 100. All values are means. Six control rats, four rats treated with actinomycin D 2 hours before testing, nine rats treated 4 hours before testing, and five rats treated 8 hours before testing were studied.

Amino acids	Absorption by control rats	Time between actinomycin D treatment and testing: 2 hours Absorption (%)	2 hours Inhibition (%)	4 hours Absorption (%)	4 hours Inhibition (%)	8 hours Absorption (%)	8 hours Inhibition (%)
				Acidic			
Glu	13.2 $\pm$ 6.4	11.3 $\pm$ 3.0	14.4	2.0 $\pm$ 4.5*	84.8	3.7 $\pm$ 4.0†	72.0
Asp	32.9 $\pm$ 11.6	22.9 $\pm$ 9.9	30.4	6.1 $\pm$ 9.0‡	81.5	8.7 $\pm$ 7.9*	73.6
				Neutral			
Ala	48.7 $\pm$ 8.7	39.7 $\pm$ 10.6	18.5	13.9 $\pm$ 15.6‡	71.5	17.2 $\pm$ 12.4‡	64.7
Gly	49.3 $\pm$ 10.0	34.4 $\pm$ 9.8	30.2	10.3 $\pm$ 10.8‡	79.1	15.4 $\pm$ 10.1‡	68.8
Thr	32.7 $\pm$ 7.9	30.4 $\pm$ 11.7	7.0	9.8 $\pm$ 11.7*	70.0	14.5 $\pm$ 9.3*	55.7
Ser	41.5 $\pm$ 8.9	37.7 $\pm$ 12.5	9.2	12.2 $\pm$ 13.6*	70.6	20.1 $\pm$ 5.0*	51.6
Leu	72.0 $\pm$ 5.7	67.3 $\pm$ 12.6	6.5	41.3 $\pm$ 10.7‡	42.6	43.5 $\pm$ 12.2‡	39.6
Val	73.1 $\pm$ 5.8	66.8 $\pm$ 13.1	8.6	42.9 $\pm$ 10.7‡	41.3	43.6 $\pm$ 12.1‡	40.4
Ileu	77.7 $\pm$ 4.6	71.4 $\pm$ 12.1	8.1	46.7 $\pm$ 9.9‡	39.9	45.9 $\pm$ 13.4‡	40.9
Tyr	56.3 $\pm$ 8.3	52.7 $\pm$ 12.6	6.4	22.8 $\pm$ 15.5‡	59.5	29.6 $\pm$ 13.6*	47.4
Phe	58.9 $\pm$ 8.2	56.8 $\pm$ 12.0	3.6	28.9 $\pm$ 13.1‡	50.9	33.8 $\pm$ 13.3*	42.6
Met	75.5 $\pm$ 3.6	70.2 $\pm$ 12.0	7.0	44.3 $\pm$ 10.8‡	41.3	41.3 $\pm$ 11.2‡	45.3
Pro	77.7 $\pm$ 6.4	61.5 $\pm$ 8.4*	20.8	32.0 $\pm$ 16.2‡	58.8	32.9 $\pm$ 19.6‡	57.7
				Basic			
Lys	68.6 $\pm$ 8.9	56.7 $\pm$ 11.1	17.3	46.4 $\pm$ 18.6	32.4	34.3 $\pm$ 9.3*	50.0
Arg	82.0 $\pm$ 5.0	71.6 $\pm$ 12.3	12.7	60.1 $\pm$ 19.8	26.7	45.9 $\pm$ 10.7*	44.0
Try	69.5 $\pm$ 8.7	77.1 $\pm$ 17.1	0	61.9 $\pm$ 14.3	10.9	39.0 $\pm$ 8.8†	43.9
His	42.5 $\pm$ 11.2	41.2 $\pm$ 8.5	3.1	37.9 $\pm$ 17.8	10.8	26.8 $\pm$ 4.9	36.9
Cys	84.4 $\pm$ 17.5	87.5 $\pm$ 3.1	0	77.4 $\pm$ 10.0	8.3	69.4 $\pm$ 19.2	17.8

*, †, ‡: Significant difference from value of control rats at the 1.0 percent, 2.5 percent, and 0.1 percent level, respectively, by *t*-test.

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