xylose which can inhibit growth completely under photosynthetic conditions.

According to the scheme for the photosynthetic carbon cycle proposed by Calvin⁶ largely as a result of work on *C. pyrenoidosa*, xylulose 5-phosphate is a product of two transketolase reactions. During the course of its assimilation, a carbon dioxide molecule must pass through one or other of these transketolase pathways. This offers a possible explanation of the specific action of xylose. If xylose or a phosphorylated derivative competed with xylulose 5-phosphate for an enzymic surface, a blocking of the photosynthetic carbon cycle could be anticipated. On the other hand, the process of photolysis, with the possibility of growth promotion in light through the formation of energy-rich materials, could proceed unhindered in the presence of xylose.

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Biosynthesis of Gramicidin-S

A FUNDAMENTAL problem of biochemistry is the mechanism by which the sequence of amino-acids in peptides and proteins is determined. We are studying the formation by *Bacillus brevis* of gramicidin-S—a cyclic decapeptide in which the sequence valyl-ornithyl-leucyl-D-phenylalanyl-prolyl occurs twice¹.

When a culture of *Bacillus brevis* (A.T.C.C. No. 9999) was aerated at 37° C. in a medium supplemented with the five amino-acids of gramicidin-S, a normal bacterial growth-curve was obtained. Biological assay with *Streptococcus* sp. (A.T.C.C. No. 9854) of alcoholic extracts of the bacteria showed that antibiotic activity appeared rapidly towards the end of growth and was finally equivalent to 50–100 mgm. of gramicidin-S per litre of culture. These alcoholic extracts, when subjected to paper chromatography with butanol-acetic acid, gave a spot with the same *R_F* value as an authentic sample of gramicidin-S, and which on hydrolysis gave the expected five amino-acids in equimolar amounts.

For studying the formation of gramicidin-S the cells were always harvested when the culture had reached one of three optical densities, and these cells can be called early, middle and late. The cells were washed and resuspended in solutions the compositions of which are given in Table 1; on incubation overnight at 37° C. these cells did not divide, but formed gramicidin-S in amounts similar to those of the experiment shown in Table 1. It is seen that in the complete medium all the cells formed gramicidin-S; when the medium lacked D-phenylalanine, or all five amino-acids, the formation of gramicidin-S by early cells was prevented, but that by late cells was unaffected. Paper chromatography showed that extracts of late cells did not contain a significant pool of free amino-acids. If the medium lacked any one of the amino-acids other than D-phenylalanine, gramicidin-S formation by early cells was reduced to

Table 1. GRAMICIDIN-S FORMED (MG./100 ML. OF SUSPENSION) BY RESTING SUSPENSIONS OF *Bacillus brevis*

Medium	Bacteria		
	Early	Middle	Late
Complete	18	14	8.4
.. less D-phenylalanine	1.7	7.1	8.5
.. less all amino-acids	1.8	3.9	8.2

The cells were harvested at three stages of growth and each batch was washed and incubated for 16 hr. in a complete medium or one lacking certain amino-acids. The complete medium contained glucose (1 gm.), *tris* (0.66 gm.), L-valine, L-ornithine, L-leucine, D-phenylalanine and L-proline (66 mgm. each), minerals and growth factors in 100 ml. of water at pH 7.25.

about one-half. If suspensions of early or middle cells were disintegrated for 2½ min. in a sonic disintegrator they formed no gramicidin-S on incubation, but suspensions of late cells formed about half as much as usual.

These results suggest that when the five amino-acids are incorporated into gramicidin-S they pass through a number of intermediate compounds, and that the final production of gramicidin-S is a simple reaction which does not require free amino-acids, and which readily occurs in cell-free suspension. That intermediate compounds are stored in the cells is also suggested by experiments in which carbon-14-DL-phenylalanine and carbon-14-L-valine were added to the solution in which the washed cells were resuspended. As shown in Table 2, the specific activities of the phenylalanine and valine in the newly formed gramicidin-S were less than one-third of those in the solution. Paper chromatography proved that this was not due to the bacteria containing a pool of unlabelled phenylalanine and valine.

Table 2. SPECIFIC ACTIVITIES OF AMINO-ACIDS OF NEWLY FORMED GRAMICIDIN-S

Amino-acid	Specific activity (counts/min./mgm.)	
	Medium	Gramicidin-S
Valine	23,700	7,130
Phenylalanine	15,300	3,200

Cells at the middle stage of growth were harvested and incubated in the complete medium of Table 1 but with the D-phenylalanine replaced by carbon-14-DL-phenylalanine and the L-valine by carbon-14-L-valine.

Paper chromatography of extracts of the bacteria has so far revealed no intermediate compounds with certainty, but three peptides have been found. One peptide, on hydrolysis, gives all the amino-acids of gramicidin-S except ornithine, together with an unidentified one, in roughly equimolar amounts. The other two give valine, leucine, proline and another unidentified amino-acid.

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