the original pressor potency in the assay and the whole process can be repeated several times.

(d) Changes in conformation of the polypeptide molecule induced by high pH: It is already known that substances suddenly injected into the bloodstream do not readily mix and equalization is attained only after several recirculations. If such is also true for alkaline angiotensin it is possible that a substantial proportion of the injected angiotensin molecules could reach the receptors at a high pH, as *in vitro*, and thus act in a different, and more active, conformation.

It is very difficult to devise a direct biological experiment to test this hypothesis but an independent piece of evidence in favour of a change in conformation of the angiotensin molecule with rising pH of the solvent has been obtained by differential ultraviolet spectroscopy against an equimolar mixture of its constituent amino acids, at similar pH's. It has been observed that, when the pH is increased, a pronounced peak of absorption appears at 250 mμ when the pH is 10 or higher. Similar changes have been usually ascribed to alterations in the conformation of proteins and polypeptides.

In summary, a potentiating effect of high pH on the pressor activity of angiotensin has been found. Different possible explanations have been discussed and a change of the angiotensin molecule conformation seems to fit best the facts described. Differential ultraviolet spectroscopic studies appear to support this conclusion.

The authors are indebted to Drs. O. A. SCORNIK and M. R. MALINOW for helpful discussions. The assistance of Miss M. POUCHAN with the spectrophotometric measurements is gratefully acknowledged. This work has been supported in part by the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina and the National Institutes of Health, U.S. Public Health Service (Grant No. HE 05153-03).

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Received January 18th, 1963
Revised manuscript received April 18th, 1963

Biochim. Biophys. Acta, 74 (1963) 168–170

PN 1271

A role of the cell wall as a "primary reservoir" of vitamin B_{12} in a B_{12}-requiring Lactobacillus

The cells of *Lactobacillus delbrueckii*, an organism which requires vitamin B_{12} as an essential growth factor, elongate abnormally when the organism is grown in a B_{12}-deficient medium[1]. In a B_{12}-rich medium, however, they accumulate this vitamin

far in excess of the normal growth requirement and can use the accumulated vitamin for normal cell growth when the cells are transferred into a B_{12}-deficient medium. It is interesting that this B_{12}-accumulating property has been found to be characteristic only for B_{12}-requiring lactobacilli[2].

The location of B_{12} accumulated by the cell has been studied using *L. delbrueckii* No. 1. It has been found that the principal site of accumulation is in the cell-wall fraction. According to the conventional idea on the mechanism of incorporation of nutrients into bacterial cells, only the cytoplasmic membrane is involved, while the cell wall exists merely for the maintenance of cell rigidity. The authors would like to propose that there are cases in which the cell wall plays an active role in the uptake of certain nutrients in bacteria, taking as an example the mode of incorporation of vitamin B_{12} by the B_{12}-requiring Lactobacillus. The argument is based on the following observations:

(a). The cells of *L. delbrueckii* (400 mg dry wt.) grown at 45° in glucose–yeast extract–peptone–sodium acetate medium were harvested by centrifugation and suspended in a dilute B_{12} solution (55 ml containing 80 μg B_{12}). After gentle shaking for 30 min at room temperature, the cells were collected again by centrifugation, washed twice with distilled water and then sonicated (a full description of the method will be published elsewhere). The cellular components were separated by differential centrifugation and the B_{12} content analyzed by bioassay with *L. leichmannii* ATCC 4797. Most of the accumulated B_{12} was found in the cell-wall fraction (Fig. 1).

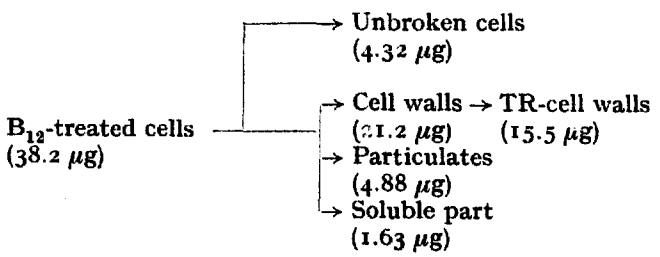

Fig. 1. Distribution of vitamin B_{12} in B_{12}-treated cells. The centrifugal conditions for the fractionation of cell components were as follows: unbroken cells, 400 × *g* for 30 min; cell walls, 15000 × *g* for 20 min; particulates, 100000 × *g* for 120 min. The numerical values in parentheses represent the B_{12} content of each fraction. TR-cell walls, cell walls that were treated with trypsin and RNAase.

(b). The B_{12} bound to the cell-wall fraction was not released by repeated washings with distilled water and/or phosphate buffer. Successive enzymatic treatment of the cell walls with trypsin (EC 3.4.4.4) and RNAase (EC 2.7.7.16) using a slight modification of the method of CUMMINS AND HARRIS[3] did not cause a significant decrease in the B_{12} content of this fraction (Fig. 1). Digestion with pepsin (EC 3.4.4.1) did not release B_{12} bound to the cell walls.

(c). When "protoplasts" were prepared from the cells which had accumulated B_{12}, using treatment with lysozyme (EC 3.2.1.17) in a hypertonic solution, most of the B_{12} was detected in the surrounding medium (Table I).

(d). Cell walls isolated from the cells which had not accumulated B_{12}, also had the ability to accumulate B_{12} even after successive treatment with trypsin, RNAase and lipase (EC 3.1.1.3). No contamination of the cell-wall preparation by cytoplasmic material could be detected by means of electron microscopy.

(e). Cellular components were obtained from the cells which had not accumulated

TABLE I

VITAMIN B_{12} CONTENT IN THE "PROTOPLASTS"

For preparation of the "protoplasts", a 4.4 mg/ml suspension of cells which had accumulated B_{12} was incubated with lysozyme (200 μg/ml) for 70 min at 37° in a solution containing 0.15 M lactose, 0.01 M glucose and 0.05 M sodium acetate. Control cells were incubated under the same conditions in the absence of lysozyme. The B_{12} contents of the cells and the "protoplasts" were determined on material which was sedimented by 15000 × *g* for 15 min.

	B_{12} content assayed in		*Total recovery (μg)*
	precipitate (μg)	*supernatant (μg)*	
Cells 130 mg	23.4	0.036	23.4
Equivalent "protoplasts"	9.5	18.0	27.5

B_{12}. Cell walls were treated with the enzymes and then [^{60}Co]B_{12} was added. The [^{60}Co]B_{12} bound to the cell walls was never released by washings. A part of radioactivity was, however, transferred (mobilized) to the particulates when the cell walls were incubated for 60 min at 37° in 0.01 M Tris buffer (pH 7.4) together with the same amount of particulates. This experiment was carried out with two levels of [^{60}Co]B_{12} (400 and 1.3 mμg/mg cell walls) with similar results.

(f). A B_{12}–peptide complex (mol. wt. approx. 15000) was obtained by treatment with 0.2 N HCl (not 0.02 N). The complex was used for the growth of *L. delbrueckii* suggesting that B_{12}-moiety was incorporated (mobilized) into the cytoplasm. (Data concerning Expts. t–f will be published elsewhere.)

These results clearly indicate that the cell wall plays an important role in the uptake of B_{12} in this organism.

KASHKET and coworkers who studied the accumulation of B_{12} by *L. leichmannii* ATCC 7830 (more correctly identified as *L. delbrueckii*[4,5]) recently stated[6,7] that the vitamin is bound almost exclusively in the ribosomes. This does not agree with the results of the present communication.

Since vitamin B_{12} is a large and complex molecule, containing a nucleotide-like moiety, it appears unlikely that it could readily pass through the cell surface to be incorporated into the cytoplasm. In view of the present findings it appears more reasonable to consider that B_{12} is first bound to the cell wall and then, when needed, only gradually exuded into the cytoplasm.

Full details of these experiments will be published elsewhere.

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Received February 15th, 1963
Revised manuscript received May 10th, 1963