Technical Papers

Some Applications of the Rapid Uptake of Vitamin B_{12} by Resting *Lactobacillus leichmannii* Organisms

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In our previous paper (*1*), it was demonstrated that certain microorganisms such as *Lactobacillus leichmannii* (ATCC 4797) incorporated the radioactivity of exogenous vitamin B_{12} tagged with Co^{60} in the growing cells, whereas organisms like *L. arabinosus* did not. Experimental evidence indicated that the radioactivity was due to vitamin B_{12} per se and not to any simple Co^{60} compounds. This study has been extended to follow the uptake of vitamin B_{12} by "resting" bacterial cells. The results are reported here.

Quantitative uptake of vitamin B_{12} by "resting" cells. Two hundred ml of Skegg and Wright (*2*) medium, to which was added a total of approximately 10 mμg crystalline vitamin B_{12}, was inoculated with *L. leichmannii* and incubated at 37° for 43 hr. This concentration of vitamin B_{12} in media (i.e., 0.5 μg/10 cc) represented approximately one half the amount supporting the maximum growth of *L. leichmannii*, according to our data on dose-growth response curve. The organisms were harvested by centrifugation, washed once with 25 ml 0.85% saline solution, and resuspended in sufficient (approximately 40 ml) salt solution to give a turbidity reading of 400 in a Klett-Summerson photoelectric colorimeter with #42 filter. This suspension was used for subsequent studies on the quantitative absorption of vitamin B_{12} in test solutions.

One ml of the bacterial suspension was added to 20 ml 0.85% NaCl solutions containing measured amounts of radioactive vitamin B_{12}. The mixture was allowed to stand at room temperature for 30 min or less, with intermittent shaking, and then centrifuged at 1500 rpm for 15 min. The supernatant fluid was sucked off through a capillary tube, and the packed organisms were transferred quantitatively to a planchet. Radioactivity was measured with a Geiger-Müller thin mica window counter.

The results of a typical recovery experiment are given in Table 1. They demonstrate that the recovery was quantitative over an approximately 64-fold range between 0.5 to 32 μg or more. With larger quantities of the organisms, the amount of vitamin B_{12} recovered could be increased proportionately. Even smaller amounts of radioactive vitamin B_{12} could be measured with equal accuracy if a more sensitive counter were employed. No self-absorption corrections were made, since the solid content per sq cm of the planchet was found to be on the order of 3 mg/cm² or less.

TABLE 1

RECOVERY OF VITAMIN B_{12} FROM RESTING CELLS OF *L. leichmannii*

Radioactive vitamin B_{12} added (mμg)	Radioactivity in washed cells (measured as mμg radioactive vitamin)	Recovery in cells (%)
0.5	0.5	100
1.0	1.0	100
2.0	2.0	100
4.0	4.0	100
8.0	8.0	100
16.0	16.0	100
32.0	32.0	100
64.0	55.0	85
128.0	96.0	55
256.0	126.0	50

Effects of pH and salt concentrations. Attempts were made to ascertain the effect of pH and of salt concentrations on the recovery of radioactive vitamin B_{12}. To this end, 2.0-ml aliquots of radioactive vitamin B_{12} solution (10 mμg/ml) were added to 20-ml portions of Skeggs and Wright medium previously adjusted (with *N*/1 HCl or *N*/10 NaOH) to various pH's, ranging from 2 to 9, and to 20-ml portions of NaCl solutions of different concentrations ranging from 0.2 to 4.3%. In these adjustments of pH no effort was made to keep the ionic strength constant. One ml of a suspension of *L. leichmannii* was again added to each solution and allowed to stand at room temperature for 30 min. The radioactivity in different samples was measured according to the procedure described above. Recovery was essentially complete between pH 3 and pH 9. At higher pH's recovery was greatly reduced. For example, at pH 10 only 10% of the added radioactivity was found in the cells.

In the pH range of unbuffered NaCl solutions the recovery was markedly affected by salt concentra-

TABLE 2

EFFECT OF NaCl CONCENTRATION ON UPTAKE OF RADIOACTIVITY OF VITAMIN B_{12}

Salt concentration (%)	Recovery (%)
0.2–1.4	90–100
1.7	85
2.2	65
2.6	45
3.4	12
4.3	10

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tions, being practically quantitative at low salt concentrations, and progressively poorer at higher concentrations. For example, in 1.7% NaCl solution (i.e., twice isotonicity) recovery was only 85% (Table 2).

These results demonstrate that under appropriate conditions it is possible by these techniques to separate quantitatively the vitamin B_{12} from dilute solutions. If the vitamin B_{12} to be assayed contains Co^{60}, radioactivity in the cells may be taken as a measure of the vitamin content. This method is ideally suited to the estimation of radioactive vitamin B_{12} in the urine of rats given small subcutaneous injections. In this situation the samples could not be assayed satisfactorily by the usual microbiological method, both because of the low activity and because of the presence of inhibitors that prevented the use of larger aliquots. Approximate measurements could be made if the vitamin were separated by repeated extraction with normal butanol after the addition of solid ammonium sulfate to the specimens, and the radioactivity in the butanol determined. Results of determinations following both *L. leichmannii* uptake and the butanol extraction of radioactivity in urine collected 24 hr after subcutaneous injection of 1.0 μg of Co^{60} tagged vitamin B_{12} are compared in Table 3.

TABLE 3

DETERMINATION OF RADIOACTIVE VITAMIN B_{12} IN RAT URINE

Samples	Method employed*	
	Uptake by resting cells	Butanol extraction
Normal rat urine + 5 mμg vitamin B_{12}	4.2	3.5
Normal rat urine + 20 mμg vitamin B_{12}	18.5	14.0
Normal rat urine + 40 mμg vitamin B_{12}	37.0	26.0
Urine (*A*)†	9.0	6.0
Urine (*B*)†	12.0	7.8
Urine (*C*)†	13.6	10.2

* The radioactive vitamin B_{12} recovered by each method was expressed as mμg/sample (20 ml). One mμg was found to give 10 cpm.

† The samples of urine were obtained from adult rats 24 hr after injection of 1 μg radioactive vitamin B_{12}.

They demonstrate that the activity obtained by the method of absorption on microorganisms yielded consistently higher results than the extraction method. The higher values are probably more nearly correct, since control recovery experiments in which known amounts of radioactive vitamin B_{12} were added to normal urine gave more nearly quantitative results by the *L. leichmannii* uptake method.

The rapid and quantitative isolation from solution of vitamin B_{12} by microorganisms such as *L. leichmannii* also lends itself to assay of nonradioactive vitamin B_{12}. This has been accomplished either by the isotope dilution technique (i.e., by adding a known amount of radioactive vitamin B_{12} to the test solution followed by subsequent addition of a standardized suspension of organisms with uptake of a definite amount of vitamin B_{12}) or by microbiological determination of vitamin B_{12} in the cell mass. In the latter case, after centrifugation and washing, if the cells are suspended in normal saline solution and killed by heating at 60° for 1 hr, the vitamin B_{12} in the cell mass is available to the test organisms used in the assay method of Skeggs and Wright. The main advantage of our procedure lies in concentrating the small quantities of vitamin B_{12} by the use of bacteria. The details of these methods and further applications will be published elsewhere.

References

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On the Interaction of Mesons and Plural vs. Multiple Meson Production

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Recent experimental work by Bernardini *et al.* may throw some light on the nature of the interaction of π-mesons with nucleons as well as suggest a new interpretation of the problem of multiple vs. plural meson production. It is well known that the question of multiple vs. plural meson production may be in good part an outgrowth of our lack of knowledge concerning the nature of nuclear forces and the elementary interaction process (*1*).

Bernardini, Booth, and Lederman (*2*) have found that the very strong inelastic nuclear scattering of negative π-mesons in which the energy losses exceed 50%, as well as the negative π-meson nuclear absorption involving catastrophic processes, are strongly energy-dependent and occur only for energies greater than about 60 mev. The frequency of these anomalous scatterings, as well as the existence of cases showing energy losses close to 100%, casts doubt on the validity of the free nuclear model of the nucleus (*3*). The possibility of explaining the observations in terms of multiple elastic collisions inside the nucleus, instead of the single π-nucleon collisions, is also unlikely in view of the recent results of Chedester *et al.* (*4*). In a personal communication Bernardini has informed the writer that there is some direct evidence that this type of anomalous scattering could be interpreted as an interaction of a negative π-meson with a group of nucleons (containing 3 or 4 nucleons) acting as a *whole*. The scattering experiments cited above suggest the excitation of such nuclear groups or subunits of the nucleus, and may indicate the operation of a many-body type process. The experimental results also lend themselves to an interpretation which suggests that the anomalous scattering of π-mesons of energies greater than about 60 mev by complex nuclei