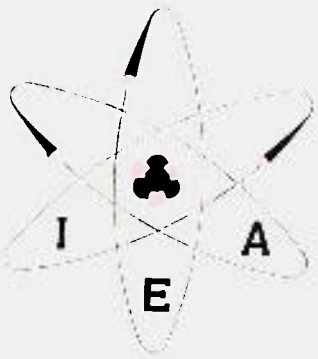

URINARY RECOVERY OF VITAMIN B-12 (Co-60) ADMINISTERED BY PARENTERAL ROUTE

RECUPERAÇÃO URINÁRIA DA VITAMINA B-12 (Co-60) ADMINISTRADA POR VIA PARENTERAL

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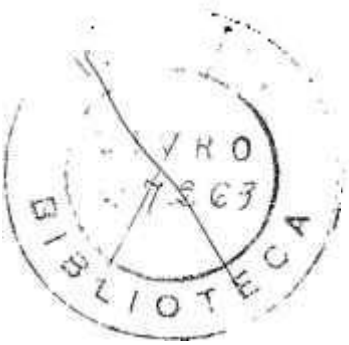

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Urinary Recovery of Vitamin B-12 (Co-60) Administered by Parenteral Route.

I - Introduction

1 - The amount of vitamin B-12 excreted through the urine and evaluated by microbiological methods is measured in millimicrograms[1]. The quantity which is introduced into the body with a common diet is thought to be of some micrograms. Thus, there is a disproportion between the amount introduced into the body and that excreted through urine.

2 - Okuda and co-workers[2], trying to clear the problem, formulated and discussed three hypotheses, by which the vitamin B-12 could be: a) changed to biologically inactive forms; b) stored in certain tissues or organs; c) excreted through another route in a significant way. From the beginning, however, they dismissed the first hypothesis, due to the lack of sufficient evidence. The second one was also discarded, for the continuous retention of vitamin B-12 would lead, after some decades, to the storage of some milligrams in the body, and it is known that this is not the case. Remaining only the third possibility, an extrarenal path of elimination was sought.

3 - Considering that the liver is also an excretory organ and that a great number of drugs are selectively eliminated through it, and in view of the quantity of bile excreted (500ml to 1300ml daily), they concluded that the biliary route is the searched one.

4 - They employed tagged vitamin B-12 (Co-60) on rats, administered by parenteral injection, in order to remove any doubt concerning the amount really introduced into the organism of the animals. They concluded that the amount eliminated through the bile, in the same period (approximately 30 days) was twice as great as that excreted through the urine. This result was obtained with and without a "flushing dose".

5 - The results presented by these authors came to our knowledge in a period when some of us[2] were studying the determination of radiocyanocobalamine in the urine and the clearance of vitamin B-12 administered by intramuscular injection.

II - Material and method

1 - We employed vitamin B-12 (Co-60) prepared for parenteral use, starting

from the radiocyanocobalamine supplied by Abbott Laboratories. Fourteen assays were performed, six using an aqueous vehicle and eight using a gelatinous one. The adopted doses of vitamin B-12 showed an activity of ca. 0,5 μc of Co-60. Shortly before and 24 hours after the administration of the radioactive material, the patients were given one milligram of non radioactive vitamin B-12, as a "flushing dose"; no assay was performed without such doses, in order to minimize the irradiation of the patients (normal ones).

2 - The injected amount was determined by weighing, establishing the difference between the mass of the serynge with the active material and prepared for injection and its mass after the injection. A standard was prepared from the solution employed for administration, by diluting approximately one gram in 500 ml.

3 - Urine was collected in periods of 6, 12, 24 and 48 hours. Aliquots were counted in a well-type crystal scintillation counter. The activity retained by active carbon and that extracted by resins were studied and the results compared with those obtained by measuring the urine. We had already verified[2] that active carbon removes practically all vitamin B-12 (Co-60) after one or two successive extractions; this does not occur with resins. However, when there is a degradation of the vitamin, a R-H resin is able to retain the active material, while the efficiency of active carbon shows a significant decrease. In this way, the comparison of the activity of urine and that retained by carbon and by resin permits to detect the presence of degradation products of the vitamin.

III - Results

1 - In the following table we summarize the results obtained for urinary recovery. The administered quantity was evaluated in "counts per minute", comparing the injected mass with that of the standard and the counting rate of this one.

2 - The percentage of active material excreted in 48 hours through the urine varied from 58,0% to 85,6% of the administered dose with the average value of 74,86%.

3 - At the same time, we were able to notice that in five cases the urinary

activity was little retained by the active carbon: such urines when percolated through a R-H resin, left a significant part of its activity bound to it. It was further[2] observed that, while the elution of the activity retained by those resins when percolated by a solution of vitamin B-12 (Co-60) was difficult, in these five cases it was easy; this showed clearly that one had to deal with ionized cobalt originated from the eventual breakdown of the structure of the vitamin B-12 (Co-60) molecule.

3.1 - Furthermore, while the employed resins retained a little fraction of the activity of the vitamin B-12 (Co-60) solution, it extracted almost completely the activity of those urines. In three of those five cases the assayed urines had been collected recently; the other two were examined after one week. The urines which had no degradation whatever of vitamin B-12 showed the same initial features, when kept during four weeks and reassayed.

4 - The data reveals also that in those cases where vitamin B-12 (Co-60) was administered in a gelatinous vehicle the urinary excretion in 48 hours averaged 79,29% while in the cases where an aqueous vehicle was used, the average elimination was 68,97%. The difference between these results is slightly significant.

Patient	"counts/min" administered	Periods	"counts/min. " eliminated	% of the admin. doses	Recovery in 48 h. %
A.L.C.	331000 (S.G.)	0-12 h	265800	80,3	
		12-24 h	11021	3,3	
		24-48 h	3700	1,1	84,7
D.D.A.	365424 (S.G.)	0-6 h	229040	62,7	
		6-12 h	48282	13,2	
		12-24 h	7215	2,0	77,9
A.F.	379326 (S.G.)	0-6 h	238620	62,9	
		6-12 h	32320	8,5	
		12-24 h	10488	2,8	
		24-48 h	9120	2,4	76,6
H.S.	365424 (S.G.)	0-6 h	289803	79,3	
		6-12 h	14480	4,0	
		12-24 h	1066	0,3	
		24-48 h	7344	2,0	85,6

Patient	"counts/min" administered	Periods	"counts/min" eliminated	% of the admin. doses	Recovery in 48 h. %
C.P.P.	367410 (S.G.)	0-6 h	283716	77,29	
		6-12 h	22380	6,1	
		12-24 h	3111	0,9	
		24-48 h	1872	0,5	84,79
M.S.	375354 (S.G.)	6 h	270336	72,0	
		6-12 h	31995	8,5	
		12-24 h	3204	0,8	
		24-48 h	6300	1,7	83,0
C.A.L.	371382 (S.G.)	0-6 h	237600	64,0	
		6-12 h	48714	13,1	
		12-24 h	5500	0,2	77,3
A.F.	365424 (S.G.)	0-6 h	157140	43,0	
		6-12 h	58370	15,9	
		12-24 h	8313	2,3	
		24-48 h	11655	3,2	64,4
M.M.S.	433600 (S.A.)	0-12 h	365540	83,0	
		12-24 h	7470	1,7	
		24-48 h	n.d.		84,7
A.L.C.	424900 (S.A.)	0-12 h	311300	73,0	
		12-24 h	2875	0,6	
		24-48 h	n.d.		73,6
D.G.C.	524365 (S.A.)	0-6 h	296200	56,5	
		6-12 h	52900	10,1	
		12-24 h	15700	3,0	
		24-48 h	5300	1,0	70,6
I.O.	478570 (S.A.)	0-6 h	293400	61,2	
		6-12 h	17550	3,7	
		12-24 h	2970	0,6	
		24-48 h	3915	0,8	66,3
J.B.	537540 (S.A.)	0-6 h	266000	49,5	
		6-12 h	46280	8,6	
		12-24 h	9450	1,8	
		24-48 h	3525	0,7	60,6
H.F.	353090 (S.A.)	0-6 h	152028	43,1	
		6-12 h	37987	10,8	
		12-24 h	7480	2,1	
		24-48 h	7062	2,0	58,0

IV - Conclusions

1 - Our results, which were obtained in human beings, are not consistent with those found in rats by Okuda and co-workers. In experiments with human beings, the renal route is undoubtedly more important than the biliary one, at least when "flushing doses" are used. As a matter of fact, in no case the urinary recovery in 48 hours was less than 58%; in the average it reached 74,86%. Since the activity in feces was not examined, it is not possible to formulate any indication concerning the percentage which would have been eliminated through the bile (and that retained by the body) in the same period.

2 - At the same time, the fact of finding five cases where the urinary activity in Co-60 could not be wholly attributed to vitamin B-12, explains perhaps why the microbiological experiments show so low values in the average. We have no elements, in our cases, to infer the "situs" of degradation of vitamin B-12.

3 - Kulback and co-workers[4], studying recently the excretion of vitamin B-12 administered by parenteral route in cases of renal insufficiency detected for the "control-group" (ten subjects), an urinary recovery of 72,15% in 48 hours. The excellent agreement between their results and those obtained by us seems very significant and suggests the convenience of conducting further studies concerning the problem of the elimination of vitamin B-12.

V - Summary

The urinary recovery of vitamin B-12 (Co-60) in human beings administered by parenteral route was studied by the authors, with the purpose of comparison with the results found in rats by Okuda and co-workers[1]. In each one of the fourteen cases studied, the urinary recovery in 48 hours was more than 58% of the injected dose, with an average of recovery of 74,86%. These results agree with those of Kulback et alls[4], in a group of ten "normals".

Five of the fourteen cases presented an activity of Co-60 which could not be wholly attributed to the tagged vitamin. It seems that a degradation of this vitamin occurs, at least partially. The renal route of excretion seems to be the major one (when flushing doses are used) with some evidence that the low levels seen in microbiological determinations are due to a partial degradation of the vitamin.

VI - References

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