CLEARANCE OF VITAMIN B-12 (Co-60) INJECTED INTO THE DELTOID REGION

ELIMINAÇÃO DE VITAMINA B-12 (Co-60) INJETADA NA REGIÃO DELTOIDIANA

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I - Introduction

1 - Kety[7,8], in 1948 was the first one to study the clearance of radioactive material injected sub-cutaneously or intramuscularly, in order to obtain informations concerning to local circulatory conditions. Former investigators had already used natural[1,2,12] and artificial[8,18,20] radioisotopes in the investigation of blood circulation, employing, however, the intravenous route.

2 - Mc Girr[13], in 1952, reviewing previous papers, concluded that the injection of radioactive substances followed by the observation of the rate of disappearance of local activity, furnished indeed valuable information on circulatory conditions in the region were the substances were introduced. Shortly afterwards, several researchers tried to establish a correlation between the data obtained through other techniques[15,17] and those provided by Kety's method. Then the first discrepancies[17,19] appeared; some groups found a fair correlation and others a bad one.

2.1 - After the development of human serum albumin tagged with I-131 and its use by intravenous injection[11,21], Kety's method began to be gradually discarded. Nevertheless, at the Conference on the Use of Radioisotopes in Scientific Research[2] (Unesco-Paris, 1957), Gemmel and co-workers[7] made a critical analysis of the various data and methods, concluding that they could be used in many cases, as for instance, in the evaluation of the fitness of skin grafts in plastic surgery.

3 - The clearance of the material introduced by parenteral injection presents however a feature which has not been dealt with. As it is known certain drugs (medicines, vitamins, etc.) have been developed which are thought to be "slowly absorbed" when injected intramusculary. It should be of real practical interest to measure the rate at which such drugs are really absorbed. If such substances may be tagged with any gamma emitter radioisotopes or a positron emitter (and in certain cases even with beta emitters) the problem can be studied in a simple way. This is, for instance, what happens with the vitamin B-12 which having cobalt in its molecule may be obtained with a fair activity of radioactive cobalt (Co-60, Co-58, Co-56).

4 - It is known that the vitamin B-12, besides its curative action in cases of

pernicious anaemia (where relatively low doses are sufficient), seems to play an important role in other diseases (where it is used in high and maintained doses). Thus, it would be desirable to find a kind of administration of the vitamin which could avoid the need for repeated injections. This led us to compare the clearance of vitamin B-12 (Co-60) administered through an aqueous vehicle (the usual one) with that of the same substance in a gelatinous medium (supposedly of "slow absorption"). At the same time, we studied the aspect of the clearance curve, in order to compare it with those referred in the literature on the clearance of radiosodium chloride (Na-24).

II - Material and method

1 - The vitamin B-12 (Co-60) was injected in the deltoid region of patients without any circulatory disorder and fifteen clearance curves were traced. In seven cases it was employed a solution in an aqueous vehicle and, in eight, in a gelatinous one. In some cases, the test was repeated after different intervals, in the same patient.

2 - The solutions for parenteral use were obtained starting from Radio-Cyanocobalamine (Co-60) from the Abbott Laboratories. The amount of injected material, in each case, averaged 0,5 microcuries, in a volume of ca. 2 ml.

3 - In all the cases the injection was done deeply into the deltoid muscle, with the patient in a lying position.

4 - The rate of disappearance of local activity was observed by means of a scintillation head connected to a high tension power supply, a ratemeter, and a scaler. The indications of the ratemeter were continously registered by a Speedomax recorder (L.& N. - mod. G); the response of the system was previously adjusted by placing the syringe with the substance to be injected near the collimator of the scintillation head and in its axis.

4.1 - The position of the collimator was adjusted before, the active substance being injected with the needle in the direction where the axis of the collimator would be placed. Immediately after the injection, which was done rapidly, the

collimator was put in the chosen position and the recorder turned on. In no case more than five seconds elapsed between the injection and beginning of the record. The recording was always performed at least during 45 minutes; in most of the cases it was kept until ca.60 minutes after the beginning of the test.

4.2 - In six cases, besides the graphical record, a series of one minute countings was performed, with thirty seconds of waiting time between the countings and starting from the instant the injection was done.

5 - The injection of the active product was preceded by another, of non radioactive vitamin B-12 (1 mg) into the deltoid region of the other arm. This application was made with two purposes: first to avoid retention of radioactive material in the body and, second, to study the urinary recovery under conditions similar to those of the Schilling test[16]. Another injection of untagged vitamin B-12 was done after 24 hours.

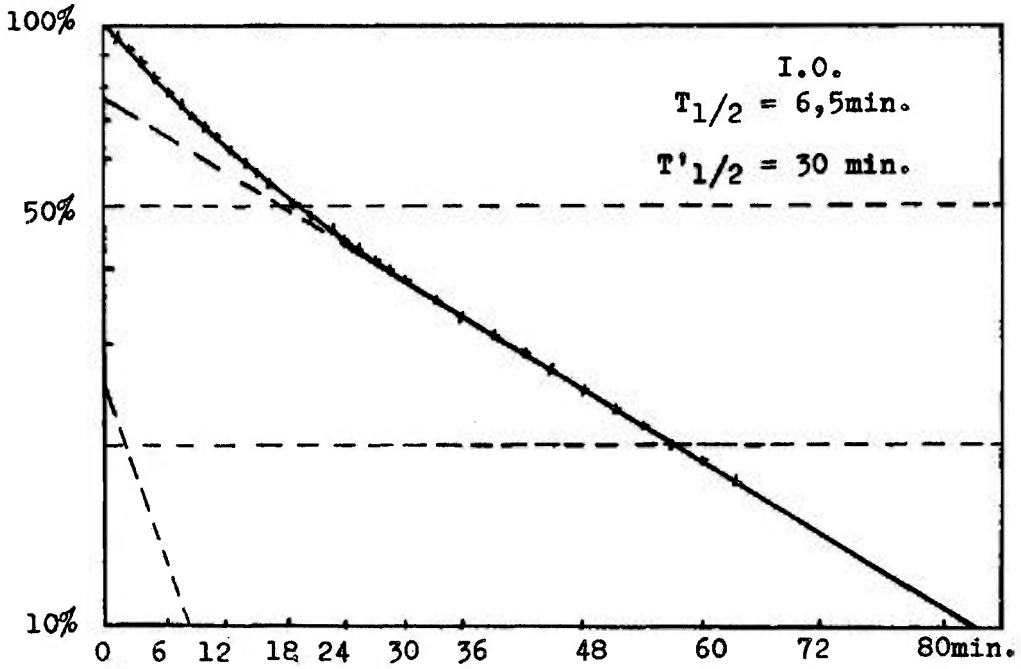
6 - Obviously, during the performance of the tests, the arm which received the radioactive material was fixed in relation to the scintillation head.

III - Results

1 - Starting from the linear graphic recording, and from the countings data when these were done simultaneously in every case a percentual semi-logarithmic graph was plotted. In some cases, the countings were represented as a function of the time logarithm[6]; in other cases, a log-log graph was built.

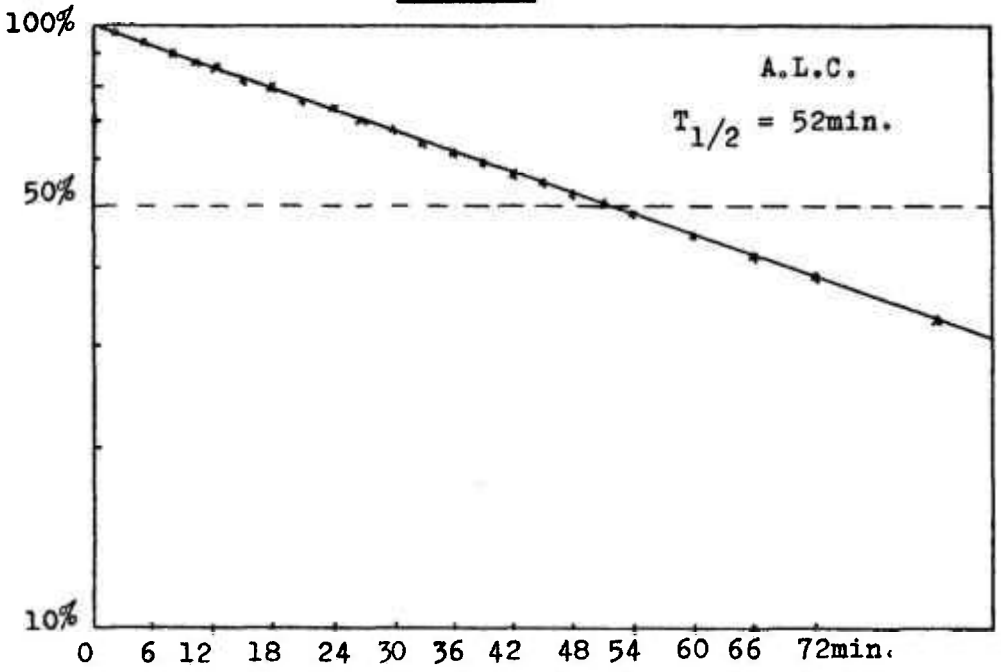
1.1 - All graphswere plotted using the net counts, i.e. the background was subtracted. This one grows during the experiment, due to the distribution of the radioactive material through the body of the patient. To examine such variation the background counting was determined at the beginning and end of each experiment putting a second scintillation head on the deltoid of the arm opposite to that where the radioactive vitamin was injected. In two cases the continuous recording of this residual counting was done. The observed increase did not exceed 10% in any case.

Figure 1



In the cases where the vitamin was administered in an aqueous medium the semi-logarithmic curve could be decomposed into two straight lines (Fig. 1). In those where a gelatinous vehicle was used a single straight line was obtained (Fig. 2).

Figure 2



3 - In table I are shown the results obtained for the "clearance constant" k, after Kety, and for the "half clearance" times, referring to the cases where an aqueous vehicle was employed. It seems that in such cases two phases could be distinguished in the phenomenon: a "fast" and a "slow" one. It should be noticed that the half-clearance times found for the fast component fall within the range of the results found by several authors for the clearance of sodium 24- injected through the intramuscular route.

3.1 - For the discussion of the results it is interesting to remark that in nearly all the cases where the clearance curve can be decomposed into two straight lines, the percentage corresponding to the "slow" component represents - at the beginning - ca.70% of the injected activity.

TABLE I

Patient	k	$T_{1/2}$ (min)	$T'_{1/2}$ (min)	Mass of the patient (kg)
M.M.S.	0'0722	5'3	26	65
A.L.C.	0'0364	8'5	33	48
D.G.S.	0'0577	7'2	34	68
I.O.	0'0398	6'5	30	38
J.B.	0'0411	5'5	33	65
H.F.	0'0413	6	29	49
C.T.L.	0'0554	4'5	29'5	43
Average	0'0491	6'2	30'8	

4 - Table II shows the results obtained for k and $T_{1/2}$ when a gelatinous vehicle was used. The half-clearance times are in the average higher than those registered for the slow component of the curves of the other group. The difference between the averages is statistically significant, the same occuring for the average values of k.

TABLE II

Patient	k	$T_{1/2}$ (min)	Mass of the patient (kg)
A.L.C.	0'0133	52	48
D.D.A.	0'0173	40	46
A.F.	0'0182	38	65
H.S.	0'0158	44	50
C.P.P.	0'0182	38	54
H.S.	0'0165	42	50
C.A.L.	0'0175	39'5	53
A.F.	0'0178	39	65
Average	0'0168	41'6	

4.1 - Patient A.L.C. was submitted to two assays: one with the vitamin in an aqueous medium and another using a gelatinous one. The half-clearance time changed-from 33 minutes (slow component) to 52 minutes. In two patients (H.S. and A.F.)where the assay with the gelatinous vehicle was repeated, the difference between the results was not significant.

4.2 - In one case (A.F.) two scintillation heads were adjusted with their axis at 90º one from another, in order to verify the possible influence on the results of the presence of a little amount of active material along the course of the needle. The resulting curves were perfectly similar.

5. - Figure 3 shows the graph that was obtained when the countings were plotted in function of the time logarithms; figure 4 is the log-log graph corresponding to case A.L.C. (aqueous medium), which is presented as an illustration.

6. - Figure 5 shows the straight lines corresponding to the different cases from table II and the "slow" component of the cases from table I.

IV - Summary and conclusions

1. - According to Kety the clearance curves plotted on semi-logarithmic paper - would be straight lines, since the phenomenon would be a simple removal of material

Figure 3

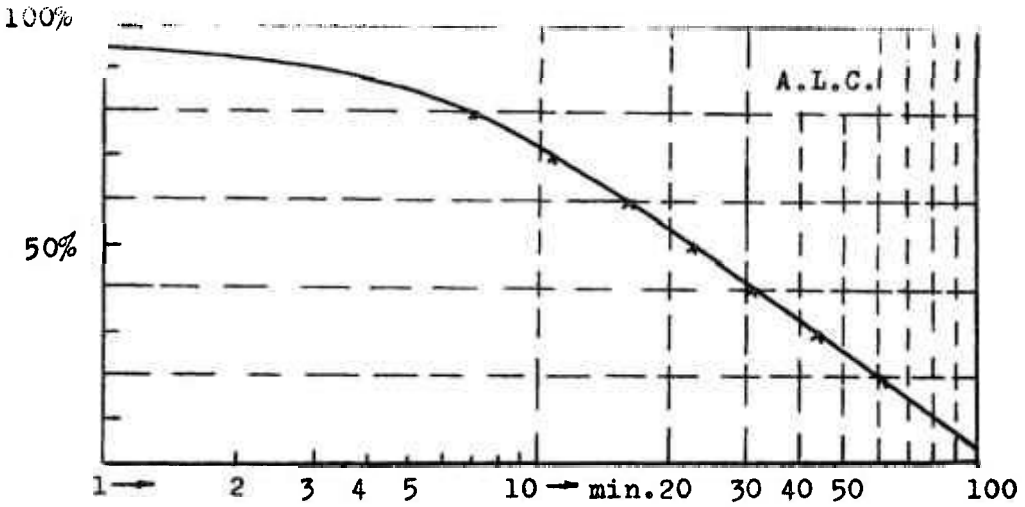


Figure 4

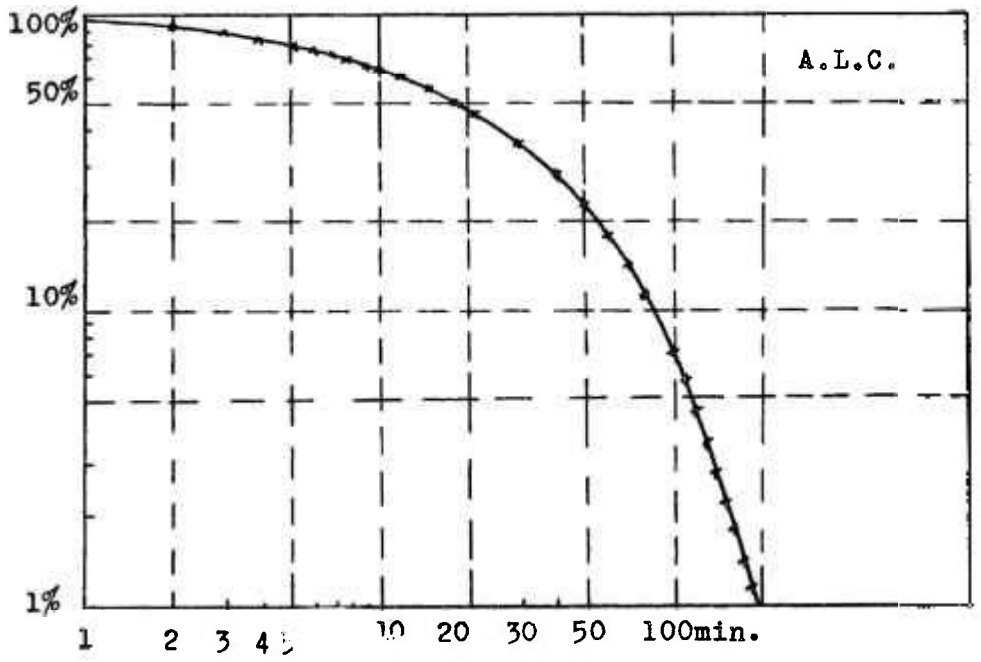
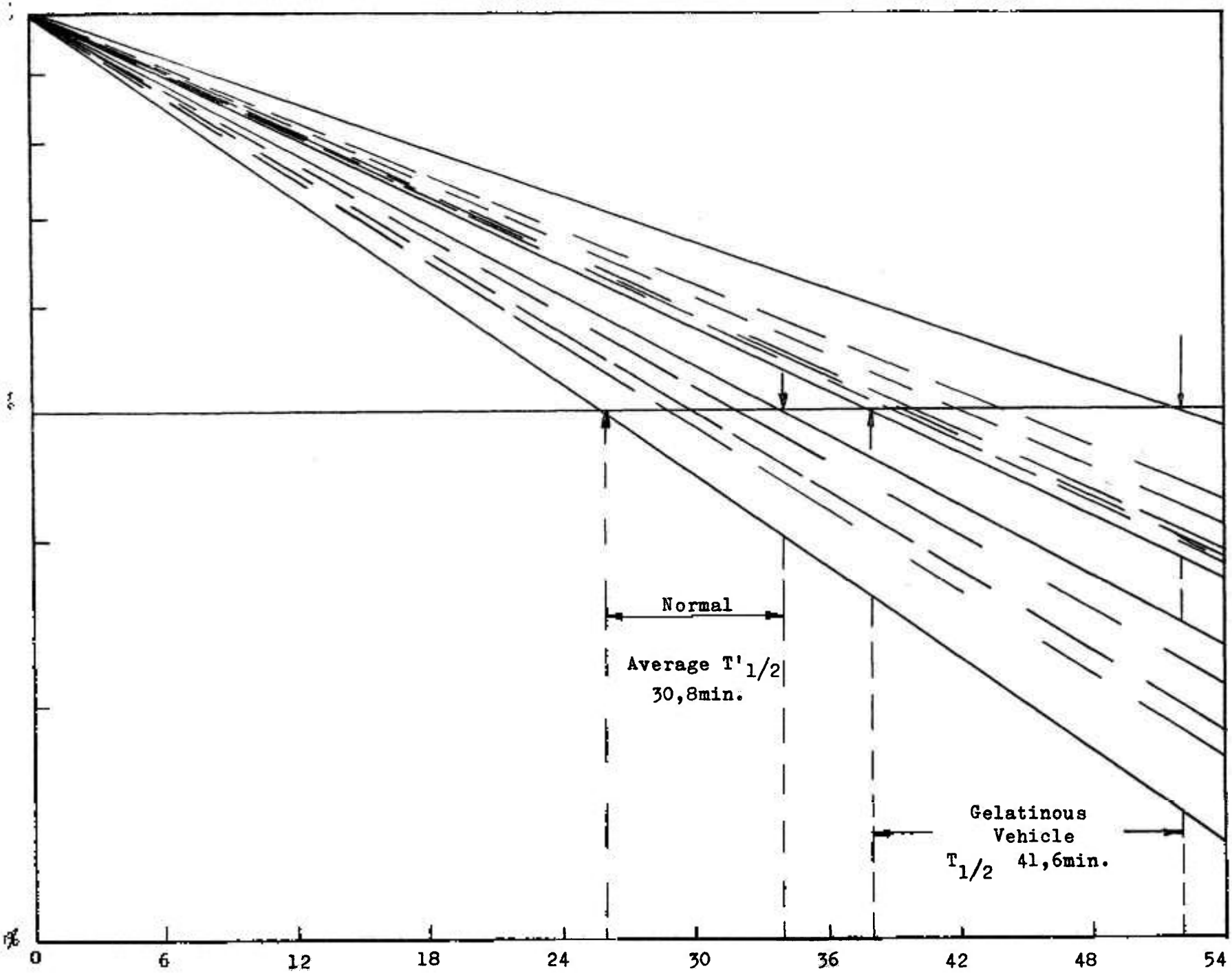


Figure 5


Normal
Average T'1/2
30,8min.
Gelatinous
Vehicle
T1/2 41,6min.
0
6
12
18
24
30
36
42
48
54 mi

2. - A similar remark is found in the paper of Elkin and co-workers[3] and in - others, where the observations were conducted up to 10 or 20 minutes after the injection of the material.

3. - In 1950, Frank and co-workers[4] conducting the observations up to 161 min - utes got a semi-logarithmic curve which is not a straight line. They call the attention to this fact, only to emphasize that the index introduced by Kety (clearance constant), estimated with the data obtained until after 10 minutes, does not have the meaning that was tried to be lent to it at the beginning. Wisham and co-workers [22,23] and Freund and co-workers[5] shortly afterwards, got clearance curves of sodium chloride (Na-24) into two straight lines. They explain this fact by the storage of sodium in the subcutaneous tissue: they deduct from the data the straight line obtained with the last points of the curve, and presume that the "difference" represents - actually the clearance of the sodium which remained in the muscle. In this their results for the half-clearance time between 6 and 11 minutes are not very different - (though a little lower) from those described by previous workers.

4. - It seems that this supposition may be doubted, once it is very unlikely - that ca. 50 to 70% of the injected substance leaves the muscle and enters the neighboring tissues: the study of the clearance curves presented by them allows the conclusion that the fraction which "disappears" according to the "slow" component is of about that value.

5. - The fact of having met half-times comparable to those described by other authors using sodium chloride, for the fast component, when dealing with aqueous solutions, suggests that this straight line is related to the spreading of the active material in the interior of the own muscular mass and eventually in the neighboring-tissues. It would thus be removed from the axis of the collimator, causing a decrease of the countings. The "slow" component would actually represent the clear - ance. Employing a gelatinous medium, of slow diffusion, the fast component was not observed: the semi-logarithmic plot is only one straight line.

6. - Accepting the slow component of the clearance curve as representing the absorption of the vitamin B-12 (Co-60) in an aqueous medium, the study of our results allows the conclusion that the gelatinous medium does not increase the absorption - time so that the product cannot be considered as of "slow absorption".

7. - This interpretation as to the meaning of the components of the semi-logarithmic clearance curves is but a hypothesis which must be tested in order to be proved. - However, even though the muscular clearance is represented by the straight line of - greater inclination, the above conclusion about considering vitamin B-12, as of "slow-absorption" in a gelatinous medium remains valid.

8. - Finally, according to the pattern of figure 4 graph, the phenomenon cannot be subjected to an analysis similar to that suggested by Friedell and co-workers[6] for the study of peripheral circulation by means of sodium radiophosphate (P-32). Besides, figure 5 clearly shows that a law from the type $C = C_o.t^n$, could not be applied to the process (in such a case the plot would be a straight line).

SUMMARY

The clearance of vitamin B-12 (Co-60) injected into the deltoid muscle was studied by means of external counting and graphic recording. When an aqueous medium - was used as a diluent of the vitamin, the obtained results could be decomposed in two straight lines when plotted in semi-log paper. When the adopted medium was a gelatinous one, only one straight line was obtained. A hypothesis was raised to explain this observation. Besides, the gelatinous medium does not delay the clearance of the injected material in such a way as to be called of "slow absorption". Friedell and co-workers[6] type of analysis is not applicable to the described phenomenon.

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