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Absorption of Vitamin B_{12} in Uganda Africans

S. K. KAJUBI AND R. M. OKEL

Department of Physiology and Radioisotopes, Makerere Medical School, P.O. Box 7072, Kampala, Uganda

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Summary. The absorption of radioactive vitamin B_{12} has been found to be slow in Ugandan Africans, and the Ugandan serum has also been shown to have an increased capacity for binding vitamin B_{12}. These two findings are suggested to be the explanation for the finding that in order to obtain adequate excretions of vitamin in the standard Schilling Test the flushing dose has to be delayed from the standard 2 hr to 4 hr after the test dose, and increased from 1000 to 4000 μg intramuscularly. The underlying cause of the slow rate of absorption is not clear, but it could be the abnormal intestinal mucosa which exists in these people. The aetiology of the increased capacity of serum for binding vitamin remains unexplained.

Résumé. L'absorption du vitamin B_{12} radioactive ca été constaté d'être lente chez les Ougandais Africains et ca été bien demonstré en se servant du même étude que le serum de ces sujets possède une capacité plus élevée que d'habitude pour capter le vitamin B_{12}. Ces deux constatations semblent donner une explication au fait que pour obtenir une excretion adequate pour le vitamin B_{12} selon le test de Schilling autentique, le 'flushing dose' doit être retardée de 2 ou 4 heure après le 'test dose' et en même temps la dose est augmenté de 1000 ou 4000 μg intramusculaire. Le cause fundamentale à la base de l'absorption lente du vitamin B_{12} n'est pas encore elucidée mais peut-être la mucose intestinale qui est anomale qui peût exister chez ces sujets. L'etiologie la capacité accrue de capter le vitamin B_{12} par le serum reste encore inexpliquée.

The absorption of vitamin B_{12} is commonly investigated by means of the Schilling test (Schilling, Clatanoff & Korst, 1955). In the most frequently employed procedure a fasting subject receives 1 μCi of ^{57}Co B_{12} by mouth, after which 1000 μg of stable vitamin B_{12} is administered intramuscularly or subcutaneously 2 hr later as a 'flushing' dose. Normal food intake is allowed soon after the test dose, and the urine passed in the subsequent 24 hr period is saved and its radioactivity counted. Schilling *et al.* (1955) found excretions of 7–22% (average 14·2%) of the test dose in normal subjects.

Application of this procedure to the Ugandan has yielded very low excretion rates, 5%

or below. Normal results were not obtained unless (a) the flushing dose was delayed to 4 hr after the test dose, (b) the flushing dose was increased to 4000 μg intramuscularly.

This paper describes our investigations of this problem and discusses our findings.

MATERIALS AND METHODS

A total of twenty subjects have been subjected to the Schilling test. All were hospital patients who were afebrile and clinically free from intestinal and renal disease at the time of study.

After emptying his bladder each fasting subject was given orally 1 μCi capsules ^{57}Co vitamin B_{12} (Amersham, England) using small sips of tap water. Normal alimentation was resumed as soon as possible after the test dose. 4 hr later a flushing dose of stable vitamin B_{12} of 4000 μg was given intramuscularly. The urine passed in the subsequent 24 hr was saved and an aliquot of its used for counting its radioactivity.

Because it was necessary to study vitamin absorption by following plasma radioactivity, another thirty patients were also studied. In this case no flushing dose was given after the test dose, and urinary excretions were not studied. Venous blood was obtained before the oral dose and then 4, 6, 8, 10, 12, 14, and 24 hr later. The radioactivity of 3 ml of plasma or serum from each sample was then counted over a 5 min period in a Packard gamma counter.

Serum concentrations of vitamin B_{12} in Ugandans were also investigated in twenty of the above thirty patients before they underwent the study mentioned above, using the method of Frenkel, Keller & McCall (1966). This technique employs the principle of saturation analysis. The B_{12} in an unknown sample (first separated from the binding proteins by boiling) is allowed to compete with a measured quantity of radioactive vitamin for the binding sites on proteins in pooled serum. The required volume of pooled serum used is first determined in a separate experiment, and it is that volume of serum which binds 70–80% of 100 pg of ^{57}Co B_{12}. While Frenkel *et al.* (1966) found this to be 50–150 μl, the volume in Ugandans was found to be 40–50 μl of serum. This indicates increased binding capacity in the Ugandan serum as compared to that studied by Frenkel and his colleagues. The bound is separated from free vitamin by binding on cellulose and then centrifuging.

RESULTS

Though the subjects studied belonged to different tribes the results did not show any tribal differences.

Urinary excretion of ^{57}Co B_{12}

The ages of the twenty patients ranged from 18 to 57 years. There were five females and fifteen males. Their tribes were: Baganda, 11; Banyarwanda, 3; Baluo, 3; Basoga, 1; Bakiga, 1; Madi, 1. Their clinical diagnoses are shown in Table 1 and the urinary excretion of vitamin was 6·0–27·5%, mean 13·6%, which compares well with that reported by Schilling and his colleagues (1955).

Plasma radioactivity studies

The thirty patients were aged between 13 and 67 years. There were fourteen females and

TABLE 1. The clinical features recorded among the twenty patients who underwent the Schilling test

Diagnosis	No. of patients
Asthma	1
Fractured femur	1
Thrombocytopaenia	1
Hypertensive heart failure	1
Lobar pneumonia	1
Pulmonary tuberculosis	1
(R) hemiplagia? cause	2
Rheumatic heart failure	1
Rheumatoid arthritis	1
Chronic headache	2
Cirrhosis	2
(R) pleural effusion	2
Diabetes mellitus	1
Hypochronic anaemia	2
EMF—heart failure	1
Total	20

Haemoglobin: range 8·0–14·5 g/100 ml; mean 10·8 g/100 ml.

TABLE 2. The clinical features recorded among the thirty patients in whom plasma radioactivity following 1 μCi ^{57}Co B_{12} by mouth was studied

Diagnosis	No. of patients
Old polio (L) arm	1
Nephrotic syndrome	3
Cardiac failure	3
Diabetes mellitus	4
Hepato-splenomegaly? cause	2
Rhematoid arthritis	1
EMF+heart failure	2
haemolytic anaemia	3
Essential hypertension	1
Brochiectasis	1
Hepatoma	2
Chronic myeloid leukaemia	1
Simple goitre	2
Liver abscess	1
Chronic headache	3
Total	30

Haemoglobin: range 8·5–15·2 g/100 ml; mean 11·3 g/100 ml.

sixteen males. The tribes were: Baganda, 13; Batoro, 5; Banyarwanda, 5; Banyankole, 4; Basamya, 1; Lugbara, 1; Langi, 1. Their clinical diagnoses are shown in Table 2, and Fig. 1 shows the mean radioactivity of plasma in the thirty patients. The highest mean reading was obtained 10 hr after the oral dose.

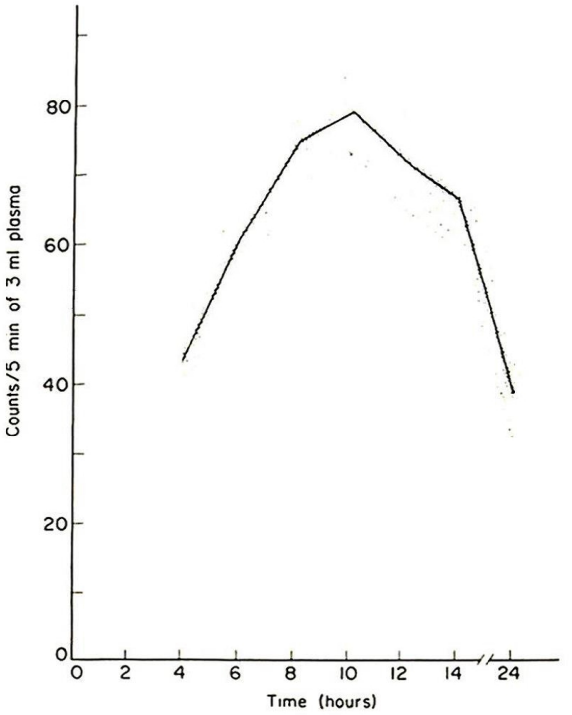

FIG. 1. Radioactivity in 3 ml of plasma after 1 μCi oral ^{57}Co vitamin B_{12}. Stippled area; range of counts ± SEM.

Concentrations of vitamin B_{12} in serum

The results of the serum vitamin B_{12} assay were 220–960 pg/ml, mean 550 pg/ml. They are in a similar range with those published elsewhere (Frenkel *et al.* 1966).

DISCUSSION

Two mechanisms are involved in the intestinal absorption of vitamin B_{12}; the first one is intrinsic factor mediated, and the second is intrinsic factor independent (Doscherholmen *et al.*, 1957). The latter is probably by simple diffusion and is only clearly demonstrable when high doses of the vitamin are given (Doscherholmen *et al.*, 1957). The peak plasma radioactivity due to this mechanism is recorded 4 hr after the oral dose or earlier, and it is a much more modest peak than that due to intrinsic factor activity.

The intrinsic factor dependent mechanism is the more important, and plasma radioactivity due to this rises more slowly and, in Caucasians, reaches a peak 8 hr after the test dose, though the activity at 10 and 12 hr is also fairly high. There is evidence that intestinal

absorption is responsible for this delayed rise. Firstly, in patients with pernicious anaemia, it only appears when small doses of (active) vitamin are given together with intrinsic factor. Large doses without intrinsic factor in these patients cause the early rise kind of response. Secondly, though it is conceivable for this later rise to be a consequence of early hepatic removal of some of the vitamin from the blood, this has been made unlikely by finding this response in a subject with porta-caval shunt (Doscherholmen *et al.*, 1957). Furthermore, in normal subjects radioactivity in the liver has not been demonstrated earlier than 3 hr after the test dose, making quick hepatic uptake of vitamin unlikely (Booth & Mollin, 1956). Thus it is clear that this response reflects intestinal–intrinsic factor interaction with the (active) vitamin during absorption.

The fact that the peak of this response was delayed in Ugandans suggests a delay in intestinal absorption in these people. The intestinal mucosa of the Ugandan has very few tall finger-like villi, most of them being shaped like leaves or spades, and the villus height is reduced (Cook, Kajubi & Lee, 1969). These minor structural differences have not been believed to impair absorption (Cook *et al.*, 1969). But about a quarter of the Ugandans studied by Banwell *et al.* (1964) showed impaired D-xylose absorption. It is possible that involvement of ileal mucosa by similar changes is responsible for these minor alteration in vitamin B_{12} absorption.

It is conceivable that a comparatively prolonged intestinal transit time in the Ugandan could account for this kind of delay, but studies reported from other African countries make this unlikely; a much shorter transit time has been recorded (Burkitt, 1972).

It is of interest that this 2 hr delay in the appearance of the plasma peak co-existed with the need to postpone the flushing dose from 2 to 4 hr after the test dose. A constant relation between these two events can be suggested from work referred to above, as follows:

Let X = the number of hours after the oral dose at which peak radioactivity in plasma is observed;
and Y = the number of hours after the test dose at which the flushing dose should be given.

Then it is obvious that y must be given $(x-y)$ hours before x. This would work out to be $8-2 = 6$ hr, so that y must be given 6 hr before x. Applying this to the Ugandan situation would give $x = 10$ hr, and since y must precede x by 6 hr, it should be given at 4 hr after the test dose. This was the time interval after which we obtained adequate excretions in our subjects. This work emphasizes once more that populations vary, so that in a previously 'unstudied population' investigation of the plasma peak would be recommended before assuming that the classical time arrangements of this test are applicable to it. No extra counting equipment is needed for doing this plasma study.

The explanation for the need for a bigger flushing dose in Ugandans must be related to the increased binding capacity of the serum in these people. A lot of stable vitamin is necessary to saturate this capacity before any can possibly remain free in the blood so as to be filtered across the glomerulus. The fact that plasma concentrations of vitamin B_{12} in Ugandans were in the same range as found elsewhere excludes the suggestion that low concentrations accounted for this anomaly. Moreover, the low concentrations encountered in patients with pernicious anaemia do not result in such increased requirements when the Schilling Test is performed with intrinsic factor in these patients. However, the cause for this increased binding of vitamin to serum is not clear. It is tempting to suggest that the

increased gamma globulins in the blood in this population (Shaper & Lewis, 1971) and the mechanisms involved in their generation may be related to this problem. It requires further study. Similar, though not identical, findings have been recorded in Nigerians (Fleming, 1968).

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