The Relation of Binding Power to Intrinsic Factor Activity

Effect of Pseudovitamin B_{12} on Absorption of Vitamin B_{12}

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THE QUESTION of whether or not "binding" was related to intrinsic factor activity has been under investigation for many years. In 1950, Prusoff and co-workers[1] concluded that binding had no relationship to intrinsic factor activity. Their statement was based on results that showed that intrinsic factor concentrates with the lowest "binding power" gave them the best vitamin B_{12} absorption under the conditions of their experiments. In 1954, Williams, Ellenbogen and Esposito[2] also stated that vitamin B_{12} binding was probably not a property of intrinsic factor. The validity of these statements and the experiments upon which they were based could appropriately be questioned on the basis of: (1) non-uniformity of the methods used to prepare the experimental intrinsic factor concentrates; (2) non-uniformity in activity of concentrates made exactly the same way; (3) lack of definitive knowledge concerning the composition and effects of the large fraction of impurities contaminating the intrinsic factor concentrates; (4) possible changes from the natural state of intrinsic factor as it finally appears in the concentrates; and (5) a lack of an adequate or uniform definition of binding power.

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In 1955 a number of publications appeared[3–5] from which it could be inferred that the pendulum was swinging in the direction of the possibility that binding was a necessary property of intrinsic factor activity. This report is not intended as a complete review of the literature but should serve to indicate some of the pertinent highlights. At approximately this time, it appeared from observations in this laboratory resulting from an extensive program of testing intrinsic factor preparations that binding was definitely essential for intrinsic factor activity. This paper presents the experimental procedure and results supporting the previously published preliminary reports[6,7] of the work carried out to prove this point. The urinary excretion test essentially as described by Schilling[8] was used as a measure of vitamin B_{12} absorption.

EXPERIMENTAL METHODS

Source of Intrinsic Factor: To circumvent most of the criticisms inherent to previous approaches to this problem of determining the relation of binding to intrinsic factor activity, pooled normal human gastric juice (NHGJ) was used as the source of intrinsic factor in the experiments to be described. After collection, the NHGJ was filtered through several layers of cheese cloth, adjusted to pH 7, and then frozen until used, thus giving a product only slightly removed from its native physiologic state.

Binding: When referred to with respect to the experiments described in this paper, binding means that property of the intrinsic

TABLE I

Net Percentage of Oral Dose of Co^{60}-Vitamin B_{12} Excreted by Totally Gastrectomized Patients in Twenty-Four–Hour Urine Collection

Patient	Mixture		
	Control	Cold	Hot
Daw	0.0	1.2	6.0
Daw	0.5	2.7	6.5
Bla	1.3	2.3	9.5
Noo	0.9	4.0	5.3
And	0.6	1.0	4.8
Mac	0.5	3.0	8.0
Kra	0.3	1.9	5.5
Gra*	1.1	1.0	4.8
Pol*	0.7	0.5	7.2

NOTE: See section on experimental methods for details regarding composition of mixtures and doses.
* Patient with pernicious anemia.

TABLE II

Net Percentage of Oral Dose of Co^{60}-Vitamin B_{12} Excreted by Patients with Pernicious Anemia in Twenty-Four–Hour Urine Collection

Patient	Mixture			
	Control Plus	(?)	Hot	Cold
Goe	B_{12} 2.7	14.9	12.4	5.7
Cla	P_s-B_{12} 2.7	13.7	17.6	0.2
Pet	B_{12} 0.6	11.6	13.1	5.4

NOTE: See section on experimental methods for details regarding composition of mixtures and doses.

factor component of NHGJ which made the added vitamin B_{12} unavailable to the test organism, L. leichmannii ATCC 7830. Each gastric juice-vitamin B_{12} preparation was assayed as is, to give a determination of "free" vitamin B_{12} in the presence of intrinsic factor, and also after autoclaving (destruction of intrinsic factor), to give "total" vitamin B_{12} in the preparation.

Preparation of Test Doses: The "cold mixture" was prepared by adding sufficient, non-radioactive, vitamin B_{12} to a sample of NHGJ to completely saturate its binding capacity and leave approximately 10 per cent free in the mixture. The mixture was allowed to stand at room temperature for thirty minutes before being used. In the first series of experiments (Table I), 2.2 μg. vitamin B_{12} (0.5 μc.) was added to 50 ml. NHGJ, approximately 2 μg. being bound by the intrinsic factor in the NHGJ and 0.2 μg. remaining free. In the second series of experiments (Table II), 0.46 μg. vitamin B_{12} (0.5 μc.) was added to 10 ml. NHGJ. The "hot mixtures" were prepared in the same way except that Co^{60}-labeled vitamin B_{12} was used instead of the non-radioactive vitamin B_{12}. The "(?) mixture" in the second series of experiments (Table II) was prepared by adding 0 46 μg. non-radioactive pseudovitamin B_{12} to 10 ml. NHGJ.

Schedule of Tests: All doses were orally administered. The NHGJ mixtures were given with any available fruit juice.

In the first series of experiments, the control mixture consisted of 2.2 μg. Co^{60}-vitamin B_{12}. The cold mixture consisted of 2.2 μg. Co^{60}-vitamin B_{12} followed immediately by a mixture of 2.2 μg. non-radioactive vitamin B_{12} in 50 ml. NHGJ. The hot mixture consisted of 2.2 μg. non-radioactive vitamin B_{12} followed immediately by a mixture of 2.2 μg. Co^{60}-vitamin B_{12} in 50 ml. NHGJ (Table I).

In the second series of experiments, the control mixture consisted of 0.46 μg. Co^{60}-vitamin B_{12} plus 0.46 μg. non-radioactive vitamin B_{12} or non-radioactive pseudovitamin B_{12}. The (?) mixture consisted of 0.46 μg. Co^{60}-vitamin B_{12} followed immediately by a mixture of 0.46 μg. non-radioactive pseudovitamin B_{12} in 10 ml. NHGJ. The hot mixture consisted of 0.46 μg. non-radioactive pseudovitamin B_{12} followed immediately by a mixture of 0.46 μg. Co^{60}-vitamin B_{12} in 10 ml. NHGJ. The cold mixture consisted of 0.46 μg. Co^{60}-vitamin B_{12} followed immediately by a mixture of 0.46 μg. non-radioactive vitamin B_{12} in 10 ml. NHGJ.

All tests were spaced one week apart to avoid any extraneous effects due to residual vitamin B_{12}.

Test Patients: The patients in series 1 had undergone total gastrectomy and were chosen to circumvent any controversy concerning binding power of their own gastric juice. Two patients with pernicious anemia were added

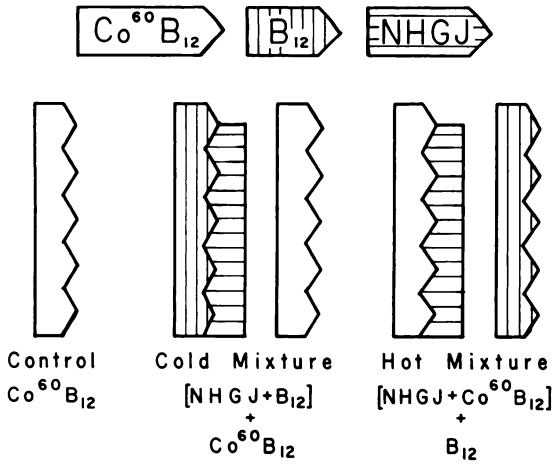

FIG. 1. Diagrammatic representation of oral test doses. *Control*, Co^{60}-labeled vitamin B_{12} only. *Cold Mixture*, normal human gastric juice with its binding capacity saturated with non-radioactive vitamin B_{12} as one component of the dose, and a second component of an equivalent amount of Co^{60}-labeled vitamin B_{12} to be taken orally, one immediately after the other. *Hot Mixture*, normal human gastric juice with its binding capacity saturated with Co^{60}-labeled vitamin B_{12} as one component of the dose, and a second component of an equivalent amount of non-radioactive vitamin B_{12} to be taken orally, one immediately after the other.

later for comparison. There was apparently no difference in response in the two types of patients. In series 2, all patients had pernicious anemia.

RESULTS

Figure 1 is a diagrammatic representation of the working hypothesis on which this work was based as well as a picture of the test doses used. The hypothesis held that if binding was a necessary property for intrinsic factor activity, that vitamin B_{12} which was bound to the intrinsic factor component of NHGJ by prior saturation of its "binding capacity," would be preferentially absorbed from the oral test mixtures presented to the patients. In other words, if the binding capacity of a sample of NHGJ was saturated by exposure to non-radioactive vitamin B_{12} and given to a patient with an equivalent amount of Co^{60}-vitamin B_{12} (cold mixture), very little Co^{60}-vitamin B_{12} would be absorbed. If, however, the binding capacity of NHGJ was saturated by exposure to Co^{60}-vitamin B_{12} and then given to a patient with an equivalent amount of non-radioactive vitamin B_{12} (hot mixture), a much greater quantity of Co^{60}-vitamin B_{12} would be absorbed. These expectations were adequately borne out (Table I). The hot mixture gave 2.5 to 14 times as much absorption as the cold mixture, with only one exception (patient Noo). These results can leave little doubt that binding, although still not adequately defined, is necessary for intrinsic factor activity. It must always be pointed out that binding by itself does not automatically indicate intrinsic factor activity, but intrinsic factor activity must carry with it the property of binding vitamin B_{12}. The possibility that the binding property may be linked with one factor and the absorption property with a second factor, intrinsic factor, does not appear to have any adequate support at the present time.

Implicit in the explanation for these results is the assumption that exchange between vitamin B_{12} bound by intrinsic factor and free vitamin B_{12} in the gastric juice mixtures would be slow or negligible. If the exchange were rapid, ratios of absorptions from hot mixture to cold mixture would be much closer to 1 rather than the values of 2.5 to 14 which were actually obtained.

Extending these experiments to the study of the effect that pseudovitamin B_{12} might have in competition for absorption with vitamin B_{12}, it was found that intrinsic factor preferentially binds vitamin B_{12} even though first exposed to pseudovitamin B_{12}. This can be seen by comparing the results obtained with the (?) mixture and the hot mixture (Table II). The absorptions measured with each mixture were almost identical. This is interpreted to mean that the binding of intrinsic factor with vitamin B_{12} is stronger than with pseudovitamin B_{12}. The ratios of absorption from hot mixture to cold mixture in this series were comparable to the ratios obtained in the first series discussed.

These results have been confirmed by the work of Bunge, Schloesser and Schilling.[9]

SUMMARY

Evidence is presented to show that binding is an essential property for intrinsic factor activity.

Exchange between bound and free vitamin B_{12} in normal human gastric juice is slow or negligible under conditions of these experiments.

Intrinsic factor preferentially binds vitamin B_{12} in the presence of pseudovitamin B_{12}.

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