ISOLATION OF AN INTRINSIC FACTOR-VITAMIN B_{12} COMPLEX

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The purification of intrinsic factor has been the object of intensive work since Castle proposed the existence of the substance about three decades ago. Recently Ellenbogen and Williams (1960) described a preparation of porcine intrinsic factor (PIF) that was effective in the urinary excretion test (Schilling, 1953) at 0.3 mg per daily oral dose. Other investigators (Andreson and Skouby, 1956; Gregory et al., 1957; and Wijmenga et al., 1954) have isolated highly purified PIF-Vitamin B_{12} complexes which either were clinically inactive or were less active than 0.3 mg per daily dose. This report describes the isolation and partial characterization of a PIF-B_{12} complex that was effective in the Schilling test and in a relapsed pernicious anemia patient at less than 50 µg.

Preparation. - A 2% (dry w/v) solution of ground porcine pyloric mucosa was incubated with 0.025% pancreatin in aqueous solution (pH 7.6-8.0, 37°) for 2.5 hours. The solution was filtered and the filtrate was dialyzed and lyophilized. 5-10 mg of this preparation gave a response in the Schilling test comparable to 1 N.F. unit of PIF. The salt-free preparation was dissolved in 0.05 M sodium acetate, pH 5.4, and was subjected to chromatography at 4° on a 13 cm (diam.) X 15 cm column of Amberlite XE-64 resin. Fractions of the effluent were collected automatically and were analyzed by measurement of ultraviolet absorption at 276 mµ. The initial protein peak was discarded and a fraction (E-3) assaying 1 N.F. unit per mg in the Schilling test was eluted with 0.58 M, pH 5.4 sodium acetate buffer. E-3 was chromatographed

at 4° on a 3.5 X 40 cm column of calcium phosphate gel in 0.001 M, pH 6.8 sodium phosphate buffer. After the first peak was eluted, the molarity of the buffer was increased to 0.005 to remove fraction E-4 which was highly effective in the clinical test (Table I) and in the Schilling test (Table II) at about 0.1-0.2 mg.

Table I

Potency of PIF Preparations as Measured* in Relapsed Pernicious Anemia Patients

Patient	Prep.	PIF Dose per Day	B_{12} Dose per Day	r.b.c. count		Hemoglobin		Peak Reticulocyte Rise
				Initial	After 28 da.	Initial	After 28 da.	
		mg	µg	millions/mm³		g/100 ml		%
C.B.	E-4	0.1	10	1.90	3.40	6.9	10.2	17.2
G.L.	EB-6	0.039	1	1.94	3.60	6.9	10.5	4.8**
A.W.	N.F. Std.	50	10	2.14	3.48	6.75	10.5	19.6

* The National Formulary, Supplement "Evaluation of Oral Liver and Stomach Preparations," 11th Edition, 1960, in press.

** The initial percentage of reticulocytes was 1.6; after 8 days the value rose to 4.5% and stayed between 4 and 5% for the duration of the test.

When E-4 was subjected to ultracentrifugal analysis in 0.043 M pH 7.2 potassium phosphate buffer, the material appeared to be homogeneous (S_{20} = 3.1 X 10^{-13}). However, when an excess of B_{12} was added to E-4 and the centrifugation was repeated, about 15% of the starting material combined with the vitamin and sedimented with an S_{20} of 4.4 X 10^{-13}. The B_{12}-binding component (EB-5) was purified by ultracentrifugal fractionations until nearly homogeneous by this criterion (cf. Figure 1).

The sedimentation constant was increased to 5.4 X 10^{-13} by the removal of the slower sedimenting component. Fraction EB-5 was also prepared using $Co^{60}B_{12}$ (0.045 µc per µg B_{12}) and, as indicated in Table II, elicited a strong response in the Schilling

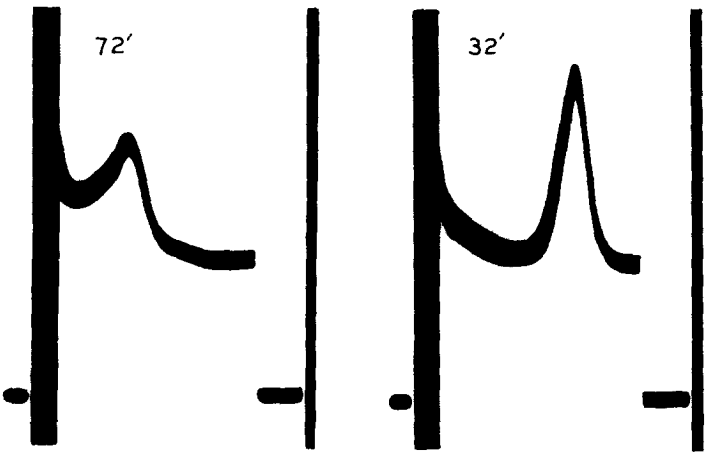

Fig. 1. Tracings of two frames of sedimentation pattern of intrinsic factor-vitamin B_{12} complex in pH 7.2, $\Gamma/2$ 0.1 potassium phosphate buffer at 56,100 r.p.m. Sedimentation from right to left.

test at less than 50 μg. Free electrophoresis of EB-5 in the same buffer demonstrated a minor impurity (Figure 2). A sample of the major component (EB-6) was removed from the ascending arm of the electrophoresis cell for clinical testing and partial

Table II

Potency of PIF Preparations as Measured by the Schilling Test

Fraction Number	Pernicious Anemia Patient*	Test Sample		Standard PIF	
		Dosage	B_{12} Recovery	Dosage	B_{12} Recovery
		μg	%	N.F. units	%
E-4	T.D.	250	13.0	0.75	9.5
E-4	J.W.	220	18.8	0.75	13.1
E-4	E.A.	150	12.2	0.75	11.9
E-4	C.T.	150	12.5	0.75	13.1
EB-5**	A.N.	28	29.7	1	21.6
EB-5**	A.P.	28	16.4	1	11.4

* Patients excreted in urine less than 0.5% of the administered dose of $Co^{60}B_{12}$ without PIF, with the exception of C.T. (1.6%)

**Total dose of $Co^{60}B_{12}$ was 0.44-0.46 μg for both test sample and standard. 2.2 μg $Co^{60}B_{12}$ (0.1 μc) was employed in other tests.

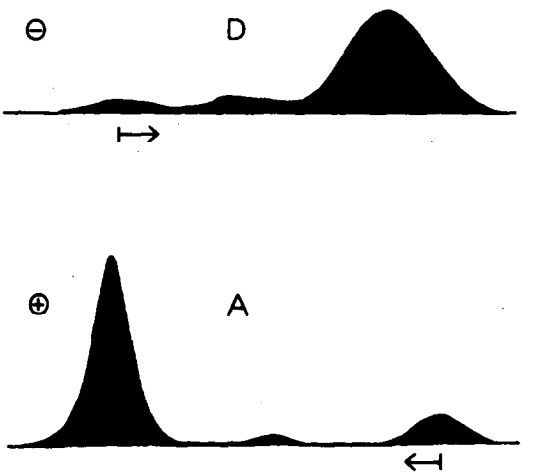

Fig. 2. Tracing of the 180 minute electrophoretic pattern of fraction EB-5 in pH 7.2, $\Gamma/2$ 0.1 potassium phosphate buffer. $\mu = 4 \times 10^{-5}$ cm²/volt sec. The main component (EB-6) represents 92% of mobile material.

characterization. The data in Table I indicate that the oral administration to G.L. of 40 µg of EB-6 (1 µg B_{12}) per day caused an increase in hemoglobin and in red cell count equivalent to that obtained with one N.F. unit of PIF and 10 µg of B_{12} per day. The reason for the low, prolonged reticulocyte rise is not known.

Properties. - EB-6 contained about 25 ug B_{12} per mg, based on spectrophotometric measurements. Preliminary analyses show that the complex contains 6.8% total reducing sugars and a wide variety of amino acid residues. If a stoichiometric binding of intrinsic factor and vitamin B_{12} occurs, PIF would have a molecular weight of about 53,000. However, the sedimentation constant of 5.4 is not consistent with this molecular weight, suggesting that either association occurs or that the complex is unusually dense. Investigations of these and other chemical, physical, and biological aspects continue, and will be the subject of a later report.

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