Estimation of intrinsic factor and detection of intrinsic factor antibodies using non-radioactive cyanocobalamin and microbiological assay

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SYNOPSIS A method employing non-radioactive vitamin B₁₂ and microbiological assay is described for estimating intrinsic factor in gastric juice and for detecting antibody to intrinsic factor in serum. Satisfactory agreement was obtained between the results by this method and by a modification of the method of Ardeman and Chanarin (1963).

During the first hour after gastric stimulation 11 patients with pernicious anaemia secreted between 0 and 240 units of intrinsic factor compared with between 1,600 and 39,000 units in 21 patients with other conditions. The results in three out of four patients with gastric atrophy were higher than those in pernicious anaemia but lower than in other conditions.

Estimation of intrinsic factor and detection of antibody to intrinsic factor in serum have become important in the study of megaloblastic anaemia. Several *in vitro* methods, all requiring radioactive vitamin B₁₂, have been described for these purposes. Such methods may not be practicable in all laboratories. The method of Ardeman and Chanarin (1963) has been modified by using non-radioactive cyanocobalamin and estimating bound cyanocobalamin by microbiological assay.

METHODS

ESTIMATION OF INTRINSIC FACTOR Filtered, neutralized gastric juice (0.1 ml.) and 0.2 ml. normal serum or 0.2 ml. serum containing antibody to intrinsic factor are mixed and left for 20 minutes, then 0.8 ml. cyanocobalamin solution (50 μ g./ml.) prepared from Cytamen (Glaxo Laboratories Ltd.), by dilution with water is added. The solutions are again left for 20 minutes, water (8.9 ml.) is added, followed by about 15 mg. charcoal (Norit OL, Hopkin & Williams Ltd.). The suspensions are mixed three times during 15 minutes and then centrifuged. Aliquots (0.5 ml.) of the clear solutions are mixed with 0.4 ml. 0.1% w/v NaCN solution and 1 ml. 0.4N acetate buffer pH 4.5. The volumes are made up to 20 ml. with water, the solutions are autoclaved at 5 lb. pressure for 20 minutes, cooled and the vitamin B₁₂ activity in 0.5 ml. and 1 ml. portions is estimated by microbiological assay with *Lactobacillus leichmannii* as described by Spray (1955). Serum blanks were not included because the

samples of normal serum and antibody-containing serum used bound equal amounts of cyanocobalamin.

The difference between the vitamin B₁₂ content of the solution containing normal serum and that containing antibody serum is used to calculate the result, which is expressed as millimicrograms vitamin B₁₂ bound by the intrinsic factor in 1 ml. gastric juice.

DETECTION OF ANTIBODY TO INTRINSIC FACTOR The amount of vitamin B₁₂ bound by a mixture of 0.2 ml. normal serum and 0.1 ml. gastric juice known to contain intrinsic factor is compared with that bound by mixtures of 0.1 ml. of the same gastric juice and 0.2 ml. of the unknown sera. A reduction of more than 20% in bound vitamin B₁₂ is taken to indicate the presence of antibody in the unknown serum.

RESULTS

RECOVERY OF ADDED CYANOCOBALAMIN FROM MIXTURES OF GASTRIC JUICE AND SERUM To ensure that bound cyanocobalamin was released quantitatively during autoclaving, known amounts of cyanocobalamin were added to mixtures of 0.1 ml. gastric juice from various subjects with 0.2 ml. normal serum or antibody-containing serum. The mixtures were treated as described, except that charcoal was omitted, to measure the recovery of the cyanocobalamin.

In a preliminary experiment, when 40 μ g. cyanocobalamin was added, the recoveries in eight de-

terminations were between 133 and 167%. Experience showed that after treating mixtures of gastric juice, serum, and cyanocobalamin with charcoal, the amount of vitamin B₁₂ remaining seldom exceeded the equivalent of 20 μ g. in the original mixture. In later experiments, therefore, 20 μ g. vitamin B₁₂ was added, and in 14 determinations in nine experiments the recoveries were 76-143% (mean 111%). The results are close to those for the recovery of cyanocobalamin from serum (Spray, 1955) and suggest that, within the errors of microbiological assays, all cyanocobalamin is released during autoclaving. Three of the highest recoveries—132%, 136%, and 143%—were all in the same experiment. Thus occasionally there may be uncontrollable factors causing high recoveries, which could explain the high values in the preliminary experiment.

REPRODUCIBILITY OF RESULTS The intrinsic factor content of three samples of gastric juice was estimated in different experiments. Table I shows that the maximum variation between individual values was about 37%.

TABLE I
INTRINSIC FACTOR CONTENT OF GASTRIC JUICE
MEASURED IN DIFFERENT EXPERIMENTS

Experiment No.	Intrinsic Factor Content of Gastric Juice (units ¹ /ml.)		
	Sample 1	Sample 2	Sample 3
1	106	82	138
2	82	57	116
3	73	64	106
4	90	65	102
5	—	—	109
6	98	73	118

1 unit = 1 μ g. vitamin B₁₂ activity uptake inhibited by serum containing antibody to intrinsic factor

COMPARISON OF RESULTS BY THE PRESENT METHOD WITH THOSE BY A MODIFICATION OF THE METHOD OF ARDEMAN AND CHANARIN (1963) Intrinsic factor was estimated in 45 samples of gastric juice from 29 subjects by the present method and by the method used by Wangel and Callender (1965), modified in the present work by using 100 μ g. ⁵⁸Co-cyanocobalamin in the system instead of 40 μ g. The results showed reasonable agreement in most instances (Fig. 1) with a highly significant correlation ($r = 0.856$, $P < 0.001$).

RESULTS IN PATIENTS WITH VARIOUS DISEASES Estimations were carried out on gastric juice from 37 patients who were given histamine or gastrin to stimulate gastric secretion. During the first hour after stimulation, 11 patients with pernicious anaemia secreted between 0 and 240 units of intrinsic factor, compared with between 1,600 and 39,000 units in subjects with other conditions (Table II); two patients with latent

FIG. 1. Comparison of the intrinsic factor content of 45 samples of gastric juice, expressed in units per ml., measured by the present method and by a modification of the method of Ardeman and Chanarin (1963) also expressed in units per ml.

The line is the theoretical line of complete correlation.

pernicious anaemia secreted 0 and 580 units respectively; a patient suspected of having subacute combined degeneration secreted 760 units. A man who was thought to have pernicious anaemia but absorbed 55% of a test dose of ⁵⁸Co-vitamin B₁₂, gave a result of 730 units. The values from the basal juices in pernicious anaemia were not so clearly separated from those in other conditions.

DETECTION OF ANTIBODY TO INTRINSIC FACTOR IN SERUM Thirty-two sera which had been tested for antibody to intrinsic factor by the isotope method were studied. Twelve samples clearly contained antibody by the present method and antibody was absent in 18. Identical results were obtained by the isotope method. Two samples depressed the uptake of cyanocobalamin by 19% and 14% respectively, values which were just too low to indicate the presence of antibody, whereas the results using ⁵⁸Co-cyanocobalamin suggested the presence of small amounts of antibody.

DISCUSSION

Comparison of results by the microbiological method and by a modification of the method of Ardeman and Chanarin (1963), both for intrinsic factor and for detecting antibody to intrinsic factor, suggests that results by the present method are valid. As expected, the method gives lower values in pernicious anaemia than in other conditions. In pernicious anaemia the results resemble those of Ardeman and Chanarin

TABLE II
SECRETION OF INTRINSIC FACTOR BY PATIENTS WITH VARIOUS DISEASES

Diagnosis	Stimulant ¹	Basal Juice (30 min.)				After Stimulation (60 min.)			
		Vol. (ml.)	pH	Intrinsic Factor Content		Vol. (ml.)	pH	Intrinsic Factor Content	
				Units per ml.	Total Units			Units per ml.	Total Units
Pernicious anaemia	H	8	N ²	0	0	25	N	0	0
Pernicious anaemia	H	—	—	—	—	4.5	N	2	9
Pernicious anaemia	H	—	—	—	—	45	N	0	0
Pernicious anaemia	H	—	—	—	—	18	N	5	90
Pernicious anaemia	H	—	—	—	—	9	N	27	240
Pernicious anaemia	H	—	—	—	—	11	N	0	0
Pernicious anaemia	H	4	N	51	200	8	N	0	0
Pernicious anaemia	G	12	N	4	48	20	N	9	180
Pernicious anaemia	G	30	N	4	120	68	N	0	0
Pernicious anaemia	H	5	N	2	10	18	N	0	0
Pernicious anaemia	H	11	N	0	0	34	N	0	0
Latent pernicious anaemia	H	—	—	—	—	35	N	0	0
Latent pernicious anaemia	H	7	N	14	98	12	N	48	580
? pernicious anaemia	H	31	N	19	590	22	N	33	730
? subacute combined degeneration	H	—	—	—	—	69	N	11	760
Control subject	H	55	1.6	43	2,400	225	1.5	102	23,000
Control subject	H	45	2.1	21	940	142	1.5	29	4,100
Control subject	H	115	1.8	18	2,100	354	1.6	69	24,000
Duodenal ulcer	H	28	1.8	54	1,500	212	1.4	138	28,000
Duodenal ulcer	H	—	—	—	—	300	1.6	17	5,100
Duodenal ulcer	H	—	—	—	—	178	1.6	45	8,000
Duodenal ulcer	G	10	N	35	350	129	1.4	119	15,000
Duodenal ulcer	H	55	2.4	13	720	220	1.6	92	20,000
Duodenal ulcer	H	25	1.7	8	200	324	1.6	96	31,000
Gastric ulcer	H	40	N	2	80	86	1.7	97	8,300
Iron deficiency	H	—	—	—	—	19	N	82	1,600
Iron deficiency	H	12	N	87	1,000	24	N	182	4,400
Iron deficiency	H	16	N	89	1,400	—	—	—	—
Iron deficiency	H	17	2.5	104	1,800	139	1.4	78	11,000
Iron deficiency	H	17	1.8	51	870	164	1.5	36	5,900
Cholecystitis	H	35	N	100	3,500	290	1.6	100	29,000
Cholecystitis	H	40	1.5	52	2,100	88	1.5	68	6,000
Post-cholecystectomy	G	13	1.7	37	480	155	1.4	92	14,000
Hyperthyroidism	H	—	—	—	—	66	1.7	106	7,000
Polycythaemia vera	H	—	—	—	—	67	1.6	106	7,100
Disseminated sclerosis	H	—	—	—	—	190	1.7	207	39,000
Myeloid sclerosis	G	40	1.8	36	1,400	98	1.6	59	5,800

¹H = histamine, G = gastrin

²N = neutral to universal indicator paper

(1963), whose values were also between 0 and 240 units during the hour after stimulation. Using modifications of the charcoal assay, Irvine, Davies, Haynes, and Scarth (1965) and Rødbro, Christiansen, and Schwartz (1965) obtained values below 100 units in all patients with pernicious anaemia. Ardeman and Chanarin (1963) reported somewhat higher results in patients having gastric atrophy without pernicious anaemia than in pernicious anaemia. The three patients in this study with results of 580, 730, and 760 units (Table I) all had gastric atrophy.

The results in the hour after stimulation in subjects with conditions unrelated to pernicious anaemia are higher than those of Ardeman and Chanarin (1963) and of Irvine *et al.* (1965). This could be partly a reflection of the occasional high recoveries of vitamin B₁₂. However, several values of over 30,000 units and one over 40,000 units were obtained in patients with

duodenal ulcer by Rødbro *et al.* (1965), whose results generally resemble the present ones.

Results for the detection of antibody to intrinsic factor in serum by both methods were identical except for a slight disagreement in two out of 32 samples of serum. Serum blanks were not included in the present study, and allowance for the uptake of cyanocobalamin by serum could affect the interpretation of the results.

The effects of varying the proportions of gastric juice, serum, and vitamin B₁₂ were not studied, but the results suggest that the method is a practicable means of measuring intrinsic factor and detecting antibodies to intrinsic factor without using isotopically-labelled cyanocobalamin. No facilities are needed other than those for the widely-used microbiological assay of serum vitamin B₁₂ with *Lactobacillus leichmannii*. The method is considerably less accurate than isotope

methods, which can give results reproducible to ± 3 units / ml. gastric juice (Irvine, 1966). These methods are also preferable on grounds of speed, but they require expensive special facilities. The results also confirm the method of Ardeman and Chanarin (1963), using a different principle to measure bound cyanocobalamin.

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