PN 1238

Assay of intrinsic factor with anti-intrinsic factor serum *in vitro*

One of the characteristics of Castle's intrinsic factor is its ability to bind vitamin B_{12}. Since, however, this property is shared by many other substances, the determination of vitamin B_{12}-binding power by dialysis, ultrafiltration or microbiological methods has up till now lacked sufficient specificity to be useful as an assay for intrinsic factor[1] *in vitro*.

It has been shown that serum of some patients with pernicious anaemia inhibits the physiological action of intrinsic factor on the intestinal absorption of vitamin B_{12} in this condition[2,3]. Sera with anti-intrinsic factor activity were found to decrease the vitamin B_{12}-binding power of normal human gastric juice[4]. In these experiments 1 ml serum was mixed with 1 ml pooled neutralized normal human gastric juice and kept at room temperature for 15–30 min before the addition of 0.050 μg ^{58}Co-labelled vitamin B_{12}. When this procedure was applied to 55 normal sera and 12 pernicious-anaemia sera without anti-intrinsic factor activity the residual radioactivity after 24-h dialysis in Visking tube against running tap water was on the average 90 % of the initial radioactivity (range 77–100 %). After exposure to 11 pernicious-anaemia sera inhibiting intrinsic factor *in vivo*, an average of 55 % of the radioactivity was recovered (range 49–60 %).

Subsequently we have investigated the effect of sera with anti-intrinsic factor activity on the affinity for vitamin B_{12} of vitamin B_{12} binders other than intrinsic factor. The substance to be tested was also exposed to anti-intrinsic factor serum for 15–30 min at room temperature before [^{58}Co]cyanocobalamin was added. Control tests were performed by substituting saline or inactive serum for the anti-intrinsic factor serum. Separation of bound and free vitamin B_{12} was obtained by dialysis (performed in duplicate to exclude faulty results due to leakage) or by the use of Sephadex G-25 or G-75 (medium size) gel-filtration columns. In Table I data are presented showing the lack of effect of anti-intrinsic factor serum on the binding power of saliva, which does not contain intrinsic factor, though it has a high binding capacity.

TABLE I

VITAMIN B_{12} BINDING OF SALIVA ON SEPHADEX G-75 COLUMNS AFTER INCUBATION WITH ANTI-INTRINSIC FACTOR OR NORMAL SERUM

Saliva (ml)	*Anti-intrinsic factor serum (ml)*	*Normal serum (ml)*	*[^{58}Co]Vitamin B_{12} added (μg)*	*[^{58}Co]Vitamin B_{12} bound (% of added)*
2	1	–	0.05	95
2	–	1	0.05	95
1	1	–	0.20	72
1	–	1	0.20	67

Results obtained in tests of hog fundus and pylorus-mucosal extracts using dialysis are shown in Table II. The vitamin B_{12}-binding capacity of the fundus extract was not depressed by the anti-intrinsic factor serum. In the test on pylorus extract this serum decreased the retention in the dialysis bag, when the amount of vitamin B_{12} added nearly saturated the vitamin B_{12}-binding power. In tests on

pernicious anaemia patients only the pyloric preparation was found to have intrinsic factor activity as could be expected on the basis of the known facts about the distribution of intrinsic factor in the hog[5].

We have also studied samples of neutralized gastric juice from different patients. The gastric juice was obtained by aspiration for 1 h after histamine stimulation while the patient was encouraged to avoid contamination with saliva. The results are summarized in Table III. The amount of vitamin B_{12} bound by the samples of gastric

TABLE II

DIALYSIS OF HOG-FUNDUS AND HOG-PYLORIC-MUCOSAL EXTRACTS

The extracts are incubated with 1 ml saline or 1 ml anti-intrinsic factor serum: 0.5 μg ^{58}Co-labelled vitamin B_{12} added.

		[^{58}Co]Vitamin B_{12} retained (%)	
		+ Saline	+ Anti-intrinsic factor serum
Fundus (ml):	1.2	87	89
	1.0	83	87
	0.8	75	79
	0.5	55	64
	0.25	36	47
	0.10	32	38
Pylorus (ml):	1.0	93	95
	0.5	93	96
	0.3	90	76
	0.25	83	65
	0.20	69	64
	0.10	45	45

TABLE III

DIALYSIS OF HUMAN GASTRIC JUICE

Vitamin B_{12} binding after dialysis of 1 ml gastric juice samples incubated with 1 ml saline (a) or 1 ml anti-intrinsic factor serum (b)

Diagnose	Gastric juice			% radioactivity after dialysis***					
	Volume in 1 h (ml)	Free HCl	Intrinsic factor in vivo	0.05 μg B_{12}		0.10 μg B_{12}		0.20 μg B_{12}	
				a	b	a	b	a	b
Pernicious anaemia	25	negative	negative	84	94	51	54		
Multiple myeloma	50	positive	positive	94	45				
Nutritional megablastic anaemia	25	positive	positive	84	88	89	56		
Non-tropical sprue	15	negative	positive	89	90	77	33		
Haemolytic anaemia	20*	negative	positive			86	65	45	39
	30*	positive	positive			74	42	41	29
Non-tropical sprue	10	negative	?			93	78		
Pernicious anaemia	10	negative	negative	92	92	66	70		
Idiopathic thrombocytopenic purpura + iron deficiency anaemia	7**	negative	positive			90	91	76	67
	25**	negative				79	69	43	43

*, ** Different samples from the same patients.

*** In 100 duplicate dialysis experiments the mean difference between the two values was 1.64% and the standard deviation 1.8%. On the basis of these data we can assume $P = 0.05$ for differences higher than 5.24%.

juice was decreased by active serum with the exception of the samples obtained from pernicious-anaemia patients.

In these experiments exposure to anti-intrinsic factor serum was found to decrease the vitamin B_{12}-binding capacity only of materials having intrinsic factor activity *in vivo*. Apparently anti-intrinsic factor serum can be used for the differentiation of non-specific and specific intrinsic factor binding.

It should be pointed out that one has to add different amounts of vitamin B_{12}, when testing samples with unknown vitamin B_{12}-binding capacity because it has been found that the percentage depression of vitamin B_{12} power in normal human gastric juice is more marked when its binding capacity is nearly saturated.

The vitamin B_{12}-binding capacity of serum itself may influence the results to a limited degree. This can be avoided by the use of a 34% ammonium sulphate precipitate of serum, which contains all anti-intrinsic factor activity in the precipitated γ-globulins and does not bind the vitamin B_{12} (ref. 4).

The estimation of vitamin B_{12}-binding power susceptible to inhibition by anti-intrinsic factor serum provides a more specific method for detecting intrinsic factor *in vitro*, which can be used for screening fractions obtained in purification procedures. The assay of human gastric juice by this method shows promise as a convenient diagnostic technique in pernicious anaemia, because it has the advantage of avoiding the administration of radioactive material to the patient, which is required for the performance of the presently available diagnostic tests based on the absorption of labelled vitamin B_{12}.

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Gene-controlled facilitated diffusion and active transport of α-thioethylglucopyranoside in *Saccharomyces cerevisiae*

HAWTHORNE[1] has established that the complementary gene pairs MG_1MG_2, MG_2MG_3, or MG_4MA_1 control α-methyl glucoside fermentation in *Saccharomyces cerevisiae*. MG_1 and MG_3 are regulatory genes for isomaltase synthesis[2,3] and MA_1 a structural gene for α-glucosidase (EC 3.2.1.20).

Abbreviation: TEG, α-thioethylglucopyranoside.