Relationship of Glandular Mucoprotein from Human Gastric Juice to Castle's Intrinsic Antianemic Factor[1]

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CASTLE'S THEORY of the interaction of extrinsic food factor and intrinsic gastric factor in forming the hematopoietic principle (*1*) underwent some modification with the discovery of vitamin B_{12}. The extrinsic food factor is probably identical with vitamin B_{12} (*2*), whereas the chief role of the intrinsic factor appears to be that of promoting the intestinal assimilation of B_{12} (*2, 3*). Whether this is achieved by protecting B_{12} from destruction by intestinal bacteria is rather doubtful (*4*). Owing to the activity of the intrinsic factor, orally administered vitamin B_{12} becomes available for the bone marrow, where it exerts its maturation effect upon erythrocytes. Thus, the few micrograms of B_{12} ordinarily taken daily in food suffice to maintain the hematopoiesis at a normal level provided that the intrinsic factor is present in the stomach. If the latter is absent, as in pernicious anemia, the absorption of B_{12} from the food is deficient, and this may lead to the hematological and neurological changes observed in this disease. Under these circumstances the parenteral administration of B_{12} may allow it to reach the bone marrow.

Although the nature of the extrinsic food factor seems to be resolved, the other question, now over 20 years old, remains unanswered: What is the elusive substance present in normal gastric juice and absent from that of patients with pernicious anemia, which is responsible for the activity of the intrinsic factor? None of the gastric juice constituents suggested so far as intrinsic factor (*5*) has satisfied the requirements postulated for its acceptance as the intrinsic factor (*6*). This applies to secretory products of argentaffine cells and folic acid, as well as the enzymes, amino-polypeptidase and lysozyme.

In the course of studies on mucous substances of the human gastric juice (*7–12*) it occurred to us that one of the components of gastric "dissolved mucin" had a striking similarity to the intrinsic factor. The substance in question is the "glandular mucoprotein" (*10*), which we so named because it originates in the mucoid cells of the neck of the gastric glands (*8–12*). It was separated by fractionation (*8, 9*) from other mucous substances of the human stomach—i.e., visible surface epithelium mucus, derived from columnar cells of the surface epithelial lining, which forms the slimy protective coating on the gastric mucosa, and its degradation and dissolution product, dissolved gastric mucoproteose. After a simple colorimetric technique was developed (*8*) for its quantitation, we studied the chemical and physical characteristics of the glandular mucoprotein, as well as its occurrence in the human gastric juice under various physiological and pathological conditions (*7–12*). As more information was gathered on its properties, the similarities between them and the physical features (*13*) and the physiological characteristics of the intrinsic factor became more apparent.

Both substances are nondialyzable compounds containing a protein moiety, filtrable in native state through a Berkefeld filter, precipitated with 60–80% alcohol or acetone, but not with 10% trichloracetic acid at room temperature, and salted out with saturated ammonium sulfate. Both glandular mucoprotein and intrinsic factor are soluble in the native state in acid juice and are not hydrolyzed or destroyed by the action of dilute alkali at a slightly alkaline pH. Finally, both are rather resistant to the acid-peptic digestion, so that they are only partly digested by pepsin at 37° C at a low pH, and can be stored for an indefinite period of time in the acid gastric juice at a pH of about 1.5 and 5° C without undergoing marked disintegration (*3, 8, 9, 11, 13*).

Moreover, the glandular mucoprotein fits well into most of the physiological features of the intrinsic factor. Like the latter, it occurs in all normal human gastric juices, although its concentration varies according to the individual and the kind and intensity of the stimulus applied for its secretion (*7, 8, 11, 12*). Like the intrinsic factor (*1*), it is not necessarily absent from the gastric secretion whenever hydrochloric acid is lacking (*8, 11, 12*). Its concentration in the acid gastric juice of patients with duodenal ulcer is increased (*8*), a fact which is in line with recent observation on the increased concentration of intrinsic factor in such gastric juices (*3*). Glandular mucoprotein is absent from digestive secretions that are known to be free from intrinsic factor—i.e., human saliva, bile, and duodenal secretion (*1, 8, 9*). As shown by studies on pathological and operated stomachs

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(*8, 11, 12*), glandular mucoprotein is formed in the fundus and corpus of the stomach in humans, which is the site of formation of intrinsic factor in man (*14*). Finally, the fundal area, whence glandular mucoprotein is derived, undergoes most severe atrophic lesions in pernicious anemia (*15*).

Additional circumstantial evidence was obtained by the study of secretion of glandular mucoprotein in pernicious and other anemias. It was shown (*10, 16*) that glandular mucoprotein, like intrinsic factor, was entirely absent, or present only in traces, in gastric juice of patients with pernicious anemia. It was absent from the stomach of those patients, even when the strongest stimulus for its secretion was applied (*10*) in the form of intravenously administered insulin, which acts as a central vagal stimulus (*8*). Under these conditions the absence of glandular mucoprotein in patients with pernicious anemia contrasts with the regular occurrence of this substance in gastric juices of patients with other nonpernicious anemias (*10*), as well as with the occurrence of other mucous substances of surface epithelial origin in normal or even increased concentrations in stomachs of patients with pernicious anemia (*10, 16*).

To adduce direct evidence of the relationship between glandular mucoprotein and the intrinsic factor, advantage was taken of the recent investigations concerning the oral administration of B_{12} to patients with pernicious anemia. If B_{12} was given by mouth in doses even 50–100 times greater than those ordinarily used for parenteral administration, the hematopoietic response was irregular and, for the most part, unsatisfactory (*3, 4, 17*). It was necessary to administer it by mouth either in huge single doses of 3000–5000 μg so that some fraction of it could be assimilated and cause a hematopoietic effect (*4, 18*), or to give it with a material containing the intrinsic factor, such as extracts of animal gastric or duodenal mucosa (*19*), or normal human gastric juice (*2–4, 17*). Direct evidence for the intrinsic factor activity of glandular mucoprotein would be obtained if glandular mucoprotein were added to oral doses of B_{12} inefficient per se, and were found to potentiate its action in the same way as the total normal gastric juice from which it was derived and to be effective in similar doses to those contained in the volume of gastric juice sufficient to promote the hematopoietic response.

These studies were performed on nine patients with proved pernicious anemia untreated or in relapse.[2] All of them presented the classical signs and symptoms of this disease, including more or less advanced combined degeneration of the cord, a characteristic macrocytic and hyperchromic anemia, a megaloblastic bone marrow, complete absence of HCl from the stomach after histamine, and a negative x-ray of the gastrointestinal tract. There were two men and seven women, between 40 and 84 years of age.

Since our purpose was not only to evaluate the presence or absence of intrinsic factor activity in glandular mucoprotein, but also to determine the extent of this activity and to compare combined oral treatment with parenterally administered B_{12}, the experimental observation period in each case (except one) was long, ranging from 3 to 10 months. During that time daily reticulocyte counts, in addition to complete blood counts three times a week, were performed in each case. At frequent intervals hematocrit determinations were also made, as well as bone marrow studies with the use of the iliac crest technique (*20*).

The study in each case was divided into several observation periods. In most instances the initial baseline period was determined for a period of 3–10 days, during which no treatment was administered. Immediately thereafter in Cases 1, 2, 3, 7, 8, and 9, the second control period was started, during which vitamin B_{12} alone was given by mouth in a total daily dose of 7–30 μg, once a day 3–4 hours before breakfast, or divided in two doses given at intervals of 12 hours, (3–4 hours before breakfast and 3–4 hours after dinner). The control period, the usual duration of which was 10–18 days, was included at a later date in the observation of Cases 4 and 5. The next period consisted in administration of the same total dose of B_{12} for the same duration of time, together with glandular mucoprotein; both substances were given simultaneously once or twice a day as described above. After its conclusion an interval followed during which no treatment was given, and the carry-over effect upon hematopoiesis of the previous treatment period was carefully evaluated. The duration of this period, with the exception of Case 6, was between 20 and 77 days. Thereafter, B_{12} was administered parenterally during another period (in all except Case 9); this followed the preceding period immediately in Cases 1–3, 6–8, and another additional period of oral treatment in Cases 4 and 5. The parenteral doses of B_{12} ranged between 70 and 350 μg/week, and were given for a period of 3–10 weeks, in doses of 10 μg daily at the beginning, and in larger doses of 45–50 μg daily later on if the response to smaller doses was observed to be insufficient. Large parenteral doses of B_{12} were used in order to determine whether the response obtained with the oral treatment was optimal.

The results of this combined oral treatment of pernicious anemia were as follows:

In two cases (2 and 7) out of nine studied, the hematopoietic response obtained was not adequate. One of them (2) did not respond to a daily oral dose of 20 μg B_{12} given together with 50 mg mucoprotein, but her response to a subsequent parenteral administration of huge doses of B_{12} was also very sluggish.

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This patient was in a partial relapse, as shown by the absence of megaloblasts from the bone marrow. In the second of these cases (7) a daily dose of 10 μg B_{12} given for 2 weeks, together with a daily dose of 100 mg mucoprotein, resulted only in a stabilization of the blood status, which fell rapidly whenever the treatment was discontinued. No significant reticulocytosis developed during the oral treatment, but it appeared when parenteral treatment with large doses of B_{12} was started. In both cases the ratio of B_{12} to mucoprotein was neither proper nor optimal, as we know at present; this could account for the failure of this combined oral treatment.

In the remaining seven cases, listed in Table 1, a definite hematopoietic response was obtained by associating glandular mucoprotein to daily small oral doses of B_{12}. Five of these cases had a high caloric standardized diet, which did not contain liver, meat, or fish, and only 1 egg, 1 pint of milk, and 1 serving of cheese per day. In two other cases (6 and 8), a normal bland hospital diet, without liver, was given.

Vitamin B_{12} was administered orally in gelatinous capsules prepared and supplied for the purpose of this study by Eli Lilly and Company. Glandular mucoprotein was also administered as a dry powder carefully weighed and placed in gelatinous capsules. It was recovered from gastric juices rich in this substance—i.e., collected after intravenous administration of insulin (*8*) from young normal individuals or patients with duodenal ulcer or hyperacidity, or collected during the preoperative or postoperative gastric drainage in patients with abdominal (nongastric) surgery. The concentration of mucoprotein in insulin juices was up to 200 mg per cent; that in overnight secretions was much less (20–60 mg per cent usually), but larger volumes of juice were available. It was necessary to collect and process more than 50 l of gastric juice from more than 100 individuals to obtain about 20 g of glandular mucoprotein used in the treatment of the nine anemic patients.

The glandular mucoprotein was processed according to the previously described (*8, 9*) fractionation technique, with some slight modifications:

Acid gastric juices (pH 1.0–2.0) collected from above sources were pooled and kept under refrigeration. One-half vol 10% trichloracetic acid was added to centrifuged gastric juice and filtered through vacuum filters. After 20 min the mixture was centrifuged at high speed and the supernatant portion separated from the precipitate. One and one-half vol acetone was added to the supernatant liquid, and the mixture left for about 2 hr at room temperature until the precipitate of total mucin previously dissolved in the gastric juice settled. This was separated from the supernatant fluid by centrifugation and taken up with stirring into as much 0.1 *N* NaOH as was required to obtain a clear solution. To this was added 0.1 *N* HCl in an amount necessary to bring the pH just below 2.0. This usually required the addition of about 1½ vol HCl for each vol NaOH. The solution was left for 1–3 hr at room temperature until the flocculent and fluffy precipitate of glandular mucoprotein developed. This was again taken up in NaOH and reprecipitated with HCl just below pH 2.0. The precipitated glandular mucoprotein was separated from the supernatant by centrifugation, washed once with acetone water and twice with pure acetone, transferred into a watch glass, and dried in the open air at room temperature for 24–48 hr, until dry gray-green crystal-like particles formed. They were crushed into a powder, and measured amounts were put in capsules. In some of the experiments the material was dialyzed against distilled water for 48 hr at 6° C after the acid precipitation and lyophilized in the dry-freezer, instead of being dried at the open air. Exposure of the material to temperatures higher than 25° C was avoided, and the fractionation procedure was carried on as quickly as possible.

The content of B_{12} in glandular mucoprotein processed in the above manner was assayed by the Eli Lilly Research Laboratories with the *L. Leichmannii* test and found to be negligible (0.00025 μg before and 0.00006 μg after tryptic hydrolysis/1 mg of dry weight of material.

The potentiating effect of addition of glandular mucoprotein upon the hematopoietic activity of orally administered B_{12}, which has been reported by the authors of this paper in their preliminary publications (*21*), is manifest in all cases shown in Table 1.

Case 1. The total oral ose of 420 μg B_{12} given within a period of 2 weeks was without any hematopoietic effect. After mucoprotein was added to the same dose of B_{12} during the next period of 2 weeks (Period 3) the reticulocytes rose to 12.9%. This was followed by a sharp rise in RBC, Hb, and hematocrit values, as well as disappearance of megaloblasts from the bone marrow (Periods 3 and 4). The data are shown in Fig. 1.

Case 2. The total oral dose of 540 μg B_{12} was spread over 18 days. This resulted in the rise of reticulocytes up to 9.2% but was with little effect upon the RBC, hematocrit values, and the megaloblasts in the bone marrow (Period 2). The daily addition of small doses of mucoprotein to similar doses of B_{12} caused a quick rise in reticulocytes up to 19%, a sharp rise in RBC, Hb, and hematocrit values, as well as disappearance of megaloblasts from the bone marrow (Periods 3 and 4).

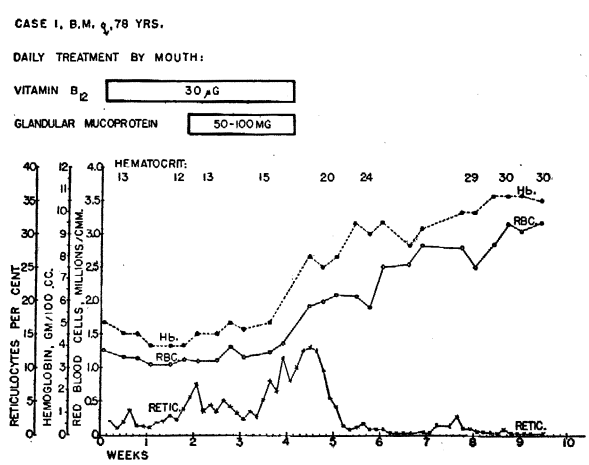

FIG. 1. Typical case of pernicious anemia (Case 1). Small oral doses of vitamin B_{12} are ineffective by themselves. Upon the addition of glandular mucoprotein a pronounced hematopoietic response is obtained.

TABLE 1

HEMATOPOIETIC RESPONSE IN PATIENTS WITH PERNICIOUS ANEMIA TO ORAL ADMINISTRATION OF

Case No.	Name, age, sex	Period		Treatment	Route of administration
		No.	Duration in days		
1	B. M.	1	3	None	—
	83	2	14	30 μg B_{12} daily	Orally
	years,	3	14	30 μg B_{12} + 50–100 mg mucoprotein daily	"
	f.	4	47	None (carry-over effect)	—
		5*	21	10 μg B_{12} daily for 2 weeks, and 45 μg B_{12} daily for the next 1 week	i. m.
3	T. M.	1	3	None	—
	40	2	18	30 μg B_{12} daily	Orally
	years,	3	18	30 μg B_{12} + 25–75 mg mucoprotein daily	"
	m.	4	42	None (carry-over effect)	—
		5*	70	70 μg B_{12} weekly for 10 weeks	i. m.
4	A. H.	1	10	None	—
	63	2	35	10 μg B_{12} + 100–200 mg mucoprotein daily	Orally
	years,	3	25	None (carry-over effect)	—
	f.	4	14	10 μg B_{12} daily for the first 10 days	Orally
		5	10	10 μg B_{12} + 200 mg mucoprotein daily	"
		6	10	None	—
		7*	90	10–45 μg B_{12} daily†	i. m.
5	L. C.	1	4	None	—
	85	2	9	30 μg B_{12} + 50 mg mucoprotein daily	Orally
	years,	3	20	None (carry-over effect)	—
	f.	4	15	30 μg B_{12} daily	Orally
		5	15	30 μg B_{12} + 50 mg mucoprotein daily	"
		6	77	None (carry-over effect)	—
		7*	42	10 μg B_{12} daily for 24 days, and 50 μg B_{12} daily for the next 18 days	i. m.
6	A. M.	1	8	None	—
	62	2	25	30 μg B_{12} + 50 mg mucoprotein daily for the first 20 days	Orally
	years,	3	16	50 μg B_{12} daily	i. m.
	f.	4	40	50 μg B_{12} + 25 mg testosterone daily	" "
8	M. V.	1	33	10–20 μg B_{12} t.i.w. for 12 single doses	Orally
	70	2	31	10–20 μg B_{12} + 100–200 mg mucoprotein t.i.w. for 12 doses	"
	years,	3	30	None	—
	f.	4*	30	35 μg B_{12} twice a week	i. m.
9	A. M.	1	23	10 μg B_{12} daily for the first 10 days	Orally
	60	2	23	200 mg mucoprotein daily for 7 days, followed by 10 μg B_{12} daily for the next 10 days	"
	years,				
	m.	3	24	10 μg B_{12} + 200 mg mucoprotein daily for the first 10 days	"
		4*	21	20 μg B_{12} daily for the first 10 days	"
		5	23	20 μg B_{12} + 200 mg mucoprotein daily for the first 9 days	"
		6	24	20 μg B_{12} + 200 mg mucoprotein daily for the first 11 days	"

* The period of administration of B_{12} did not follow immediately the preceding period; an interval of a few days or weeks intervened.

† 10 μg B_{12} i. m. daily for 10 days, 45 μg B_{12} i. m. daily for the next 10 days, 45 μg B_{12} i. m. twice a week for the next 3 weeks, 1 month—no treatment, and 30 μg B_{12} i. m. twice a week for the last 3 weeks.

TABLE 1

VITAMIN B_{12} JOINTLY WITH GLANDULAR MUCOPROTEIN PROCESSED FROM GASTRIC JUICE OF MAN

Total dose per period		Reticulocytes (%)				Red blood cells (millions/mm³)				Hematocrit (%)		Hemoglobin (g/100 ml)		Megaloblasts in bone marrow (%)	
				Maximal response**				Maximal response**							
B_{12} (μg)	Mucoprotein (mg)	Onset	End‡	Count	Day	Onset	End‡	Count	Day	Onset	End	Onset	End	Onset	End
—	—	—	1.9	2.6	(2)	1.25	1.14					5.0	4.5		
420	—	1.9	3.7	3.9	(10)	1.14	1.12			13	14	4.5	4.0		
420	1120	3.7	10.1	**12.9**	(14)	1.12	**1.92**			14	**20**	4.0	**8.0**	16	**0**
—	—	10.1	0.4	**12.6**	(1)	1.92	**3.09**	**3.17**	(43)	20	**30**	8.0	**10.0**	0	0
455	—	0.6	1.3	1.9		3.05	3.15	3.65	(14)	30	32	11.7	12.0		
—	—		0.9			1.90	1.33			18	14	6.0	4.5		
540	—	0.9	8.1	9.2	(9)	1.33	1.46			14	17	4.5	5.5	31	13
540	600	8.1	1.2	**19.0**	(5)	1.46	**2.41**			17	**30**	5.5	**9.5**	13	**4**
—	—	1.2	1.2	2.5		2.41	**3.20**			30	**35**	9.5	**12.5**	4	**0**
700	—	1.8	1.3	9.9	(7)	3.00	4.04	4.37	(30)			11.7	14.0		
—	—	1.0	0.4	1.8		1.32	1.00	1.47	(3)	14	12	5.0	3.5		
350	5200	0.4	1.1	**31.3**	(7)	1.00	**3.07**			12	**28**	3.5	**11.0**	25	**1**
—	—	.2	0.3	0.3		3.07	3.14	**3.21**	(23)	28	**30**	11.0	10.5	1	**0**
100	—	.3	.2	1.1		3.14	3.21	3.31	(10)	30	30	10.5	11.5		
100	1400	.2	.2	0.7		3.21	**3.75**			30	31	11.5	**12.5**		0
—	—	.2	.1	0.3		3.75	3.46			31	32	12.5	11.5		
1000	—	0.1	0.1	2.3		3.46	3.51	3.58	(44)	32	32	11.5	13.0		
—	—	1.9	3.6	4.5	(2)	0.96	1.03					4.5	4.5		
270	450	3.6	**28.4**	**30.8**	(8)	1.03	**1.63**					4.5	5.5	13	**1**
—	—	28.4	1.5			1.63	**2.40**					5.5	**8.0**	1	**0**
450	—	1.5	2.2	4.3	(12)	2.40	2.58	2.61	(13)	23	26	7.5	9.0		0
450	750	2.2	0.3			2.58	**3.26**			26	**31**	9.0	**10.2**		
—	—	0.3	0.7	1.7		3.26	**3.55**	**3.87**	(36)	31	**37**	10.0	**12.0**		0
1140	—	0.8	0.8	1.8		3.55	3.45	3.86	(30)	34	33	12.7	13.5		
—	—	0.2	0.8			2.18	2.24			25	25	9.2	8.2		
600	1000	0.8	1.5	**16.2**	(10)	2.24	**3.01**			25	**35**	8.2	**11.3**	30	**2**
800	—	1.5	1.2	5.2	(8)	3.01	2.65			35	34	11.2	10.5	2	0
2000	—	1.6	2.0			2.65	4.75			34	45	10.5	14.5		
180	—	1.1	1.0	1.4		3.00	2.15			36	32	10.5	10.5	12	
180	1400	1.0	1.6	**3.5**	(16)	2.15	3.20	**3.28**	(28)	32	**36**	10.5	**12.0**		**4**
—	—	1.6	1.1	**1.8**		3.15	2.70			36	30	12.0	10.9	4	6
315	—	1.2	2.1	11.0	(9)	2.26	**3.31**			26	35	8.3	10.7		0
100	—	1.0	1.1	9.2	(9)	1.80	1.83	2.10	(17)	20	20	8.5	7.5	21	16
100	1400	1.1	2.2	4.2	(20)	1.83	1.97	2.08	(10)	20	20	7.5	9.0	16	20
100	2000	2.2	0.6	3.5	(7)	1.97	2.75	**2.82**	(12)	20	**29**	9.0	**10.8**	20	**2**
200	—	0.9	0.2	1.4		2.53	2.87	2.99	(12)	32	34	11.0	10.5	2	3
180	1800	0.4	0.8	**4.5**	(12)	2.87	**3.78**	**3.81**	(21)	34	**39**	10.5	**13.5**	3	**0**
220	2200	0.8	1.2	**4.3**	(10)	3.78	**4.15**	**4.20**	(19)	38	**41**	13.5	**14.0**	0	**0**

‡ In most instances the count at the end of the preceding period has been used as the onset count of the subsequent period, if no interval between both intervened.

** The maximal response of reticulocytes and red blood cells is tabulated only if this value exceeds that observed at the onset or at the end of the particular period of observation. The days on which the maximal response was observed are listed only if significant for the evaluation of the response. Figures in bold-face type represent the most significant hematopoietic response to the mucoprotein administration.

Case 4. Because of the precarious status of the patient, the preliminary control period with B_{12} alone could not be included. The simultaneous administration of B_{12} in a daily dose of 10 μg together with glandular mucoprotein caused a sharp rise in reticulocytes up to 31.0% and a rapid rise in RBC from 1.0 to over 3.0 million, which was accompanied by a rise in Hb and hematocrit values, and the disappearance of megaloblasts from the bone marrow. The comparison of the hematopoietic effect of B_{12} alone, subsequently administered by mouth in a total dose of 100 μg within 10 days (during which no change in the blood status occurred [Period 4] with that of the same dose of B_{12} given together with mucoprotein [Period 5] during which a rise of ½ million RBC was observed), offers additional evidence of intrinsic-factorlike activity of mucoprotein in this case.

Case 5. This case is similar in many respects to the preceding. Because of the seriousness of patient's condition, B_{12} was given together with mucoprotein at once in a total dose of 270 μg during the period of 9 days (Period 2). A sharp rise in reticulocytes up to 30.8% was observed, which was followed by a rapid ascent of RBC and Hb (Periods 2 and 3) and disappearance of megaloblasts from the bone marrow. The effect of mucoprotein upon the hematopoiesis is evident also from the comparison of Periods 4 and 5 in this case: although 450 μg B_{12} given alone during a subsequent period of 2 weeks did not change the blood status significantly, the simultaneous administration of the same dose of B_{12} with mucoprotein during a similar period of time resulted in a distinct rise in RBC and hematocrit values.

Case 6. The administration of 30 μg B_{12} daily within a period of 20 days (in a total dose of 600 μg) together with mucoprotein caused a definite reticulocyte response (up to 16.2%), as well as a significant rise in RBC, Hb, and hematocrit values. No further control with oral doses of B_{12} alone was possible in this case.

Case 8. The oral administration of the total dose of 180 μg B_{12} within a period of 33 days resulted only in a steady deterioration of the blood status (Period 1). The addition of mucoprotein to the same dose of B_{12} during Period 2 of the observation, the duration of which was similar to the former, resulted in a significant improvement of the blood status and rise in RBC, Hb, and hematocrit values to the previous level. No significant response in reticulocytes was observed. It should be mentioned, however, that this was the only case in which the reticulocyte counts were done only twice a week, so that it could quite easily have happened that the reticulocyte peak was overlooked. The discontinuation of administration of B_{12} and mucoprotein during Period 3 of the observation resulted in a progressive decrease in RBC, Hb, and hematocrit values.

Case 9. The administration of 100 μg B_{12} within a period of 10 days resulted in a reticulocytosis (9.2%), which was, however, not followed by any rise in RBC or hematocrit values. A similar lack of response was observed during the second period, when the same dose of B_{12} spread over 10 days was administered subsequently, following a 7-day period during which mucoprotein alone was administered. A definite rise in RBC and hematocrit values, as well as almost complete disappearance of megaloblasts from the bone marrow was observed during Period 3 when the same dose of 100 μg B_{12} was administered within 10 days simultaneously with glandular mucoprotein. No further significant change in the blood status was observed during Period 4, when a daily dose of 20 μg B_{12} alone was given by mouth for 10 days. A definite rise was observed, however, in RBC, hematocrit, and hemoglobin values, which was preceded by a sustained rise in reticulocytes and complete disappearance of megaloblasts from the bone marrow, when similar daily doses of B_{12} were given by mouth, together with mucoprotein, during the next testing periods (5 and 6).

The hematopoietic response obtained was optimal in Cases 1, 4, and 5. This is shown by the inability to raise the RBC counts with the subsequent parenteral treatments with maximal doses of B_{12} (Period 5 in Case 1, and Period 7 in Cases 4 and 5) above values observed at the end of the oral treatment with B_{12} and mucoprotein. Also the complete clinical remission, not to mention the gradual disappearance of all neurological signs of disease obtained with combined oral treatment in these cases, points to the same conclusion. In Case 6[3] the parenteral administration of B_{12} during two subsequent weeks (Period 3) did not change the blood status. This indicates that the response to oral treatment at that period was probably optimal. Since the patient had a fracture of the spine with a negative nitrogen balance, daily administration of testosterone in injections, together with huge parenteral doses of B_{12}, was started and ultimately resulted in the improvement of the blood status of the patient (Period 4).

The hematopoietic response to oral treatment with B_{12} and mucoprotein in Cases 3 and 8 was suboptimal, although the general clinical response to this treatment was excellent. This was shown by the persistence of a few megaloblasts in the bone marrow after oral treatment and by the further improvement of the blood status with the parenteral administration of large doses of B_{12}. Finally, in Case 9 no definite evaluation of the extent of the hematopoietic response was possible, because the parenteral treatment has not yet been given. The poor reticulocyte response observed would indicate, however, that the response to oral treatment was suboptimal, in spite of the complete clinical remission observed in this patient.

The data reported indicate that the glandular mucoprotein separated from the human gastric juice by the Glass and Boyd fractionation technique (*8*) exhibits a definite intrinsic factor activity if administered simultaneously with small doses of B_{12} by mouth to patients with pernicious anemia. The daily dose of mucoprotein sufficient for promoting this hematopoietic response is far below the doses required when extracts of the gastric or duodenal hog mucosa were used for the same purpose (*19*). This indicates that glandular mucoprotein fraction of the human gastric mucin is apparently endowed with more powerful intrinsic factor activity than the total extracts of the hog mucosa just mentioned. Given to patients with pernicious anemia, mucoprotein does not exhibit by itself any hematopoietic activity (see Table 1: Case 9, first part of Period 2). Given together with B_{12} by

[3] Cases 2 and 6 were observed in the Medical Service of the Montefiore Hospital, New York City. The generous permission of L. Leiter, chief of the Medical Service, Montefiore Hospital, to include these observations in the present work is hereby gratefully acknowledged.

mouth in doses that correspond to those present in the usual volume of 75–100 ml of normal gastric juice collected under basal conditions or after histamine (*8*), which was found adequate for promoting the hematopoietic response of oral doses of B_{12} (*2–4*), it behaves in this respect like total gastric juice, from which it was recovered. In order to explore the practical possibility of oral treatment of pernicious anemia with B_{12} associated with glandular mucoprotein, it was necessary in the present studies to use doses of both substances larger than those that are sufficient for demonstrating the hematopoietic activity of these materials only. Although as little as 10 ml of gastric juice may exert a potentiating effect upon a daily dose of 5–10 μg B_{12}, no one has been able, so far as we know, to obtain an optimal hematopoietic response and a true clinical remission with these small doses of gastric juice and B_{12}, if given by mouth.

No answer can yet be given to the question of what is the mechanism by which glandular mucoprotein makes B_{12} administered by mouth assimilable by the gastrointestinal tract of patients with pernicious anemia. It appears that no stoichiometric binding of B_{12} by glandular mucoprotein takes place, a fact that is at variance with the results of microbiological *in vitro* tests on binding of vitamin B_{12} by gastric juice (*22*). This is evident from the observation that the greater the daily oral dose of B_{12} is, the less glandular mucoprotein is required to promote the hematopoietic effect, and vice versa. With a daily dose of 30 μg B_{12}, 50–75 mg glandular mucoprotein usually sufficed, if given daily for 10 days, to obtain an optimal hematopoietic response. But with the dose of 10–20 μg B_{12}, even 100 mg mucoprotein per day was usually insufficient, and the response was obtained only when this amount of B_{12} was administered with a daily dose of 150–200 mg of glandular mucoprotein. It is possible, however, that the actual technique used for separation of mucoprotein is not ideal for preservation of the intrinsic factor activity of this substance, because it causes a loss of solubility at the acid and neutral range of pH. Perhaps an improvement of the technique of fractionation, now in progress, would decrease the dose of mucoprotein required to promote the hematopoietic response of small oral doses of B_{12}.

Because of the significance which glandular mucoprotein seems to assume in the problem of the intrinsic factor, in addition to physiological and pathological data adduced above, some other available preliminary information on this substance should be cited. Human glandular mucoprotein contains 12.6 ± 0.4 g nitrogen and 7.5 ± 0.6 g tyrosine (and tryptophane) per cent as measured by Folin-Ciocalteu reaction (*8*). It gives a violet color with the biuret reagent. It contains hexuronic acid and hexosamine, as shown by positive Dische's carbazole and indole reactions, but no galactose and mannose, as determined by Dische's cysteine method (*23*). Its total reducing substance content as measured by indole reaction (*23*) is about 12.0 per cent. No blue color was obtained with Dische's diphenylamine reagent (*23*), which indicates the absence of desoxyribose in its molecule.[4] Also, no purple color was obtained on addition of Hess' "sensitized diphenylamine reagent," which indicates the absence of ketoses in the mucoprotein molecule (*24*). X-ray diffraction study of glandular mucoprotein, dried from acetone in open air, showed that most particles exhibit a weak birefringence.[5] The extinction was not complete at any single position, but moved as a band across the face of the particle. This appearance implied that the molecules involved were not symmetrical but were rather long in one direction. Under stress arising from drying they took a preferred orientation in one direction. The refractive index of glandular mucoprotein has been found to be 1.544.

The glandular mucoprotein moves fast in the electrophoretic field (*25*), and its mobility is only slightly less than that of crystalline pepsin, but much faster than that of other mucous components of gastric juice derived from surface epithelium. At pH 6.0 and 0.5 per cent concentration its mobility in the phosphate buffer at 0.015 amp and 200 v is about -8.5×10^{-5}, whereas that of pepsin under similar conditions is around -11.0×10^{-5}, and that of gastric mucoproteose is only -5.6×10^{-5}. The isoelectric point of glandular mucoprotein seems to be very low, in the neighborhood of pH 3.0, or is only slightly more alkaline than that of pepsin.

In contrast to other materials assayed for the intrinsic factor activity prepared from the gastric juice by precipitation with acetone, alcohol, Lloyd's reagent, casein, ammonium sulfate, etc. (*13*), which represented only indefinite fractions or precipitated protein complexes of indeterminate nature, glandular mucoprotein is a well-defined normal component of human (*8, 9*) and canine (*26*) gastric juice, and is a fairly homogenous substance (*8*). This is also evidenced on electrophoresis by the single, sharply outlined and, to great extent, symmetrical peak obtained over a large range of pH—from 4.6 to 8.0 (*25*). Since, however, the presence of contaminants in the glandular mucoprotein fraction in very small concentrations cannot be excluded by this method of study, further work on this problem with more sensitive techniques is required.

Because of this consideration it cannot be stated at present whether glandular mucoprotein is the pure intrinsic factor, or whether the intrinsic factor (enzyme?) is contained in or adsorbed to its molecule. However, at the present stage of information, taking into account normal occurrence of glandular mucoprotein in the human gastric juice, the rather homogeneous appearance of this substance on electrophoresis, the extent to which it fits into physiological and pathological data available on intrinsic factor, the

[4] These determinations were done in Dr. Dische's laboratory at the College of Physicians and Surgeons, Columbia University, New York, with the assistance of Mrs. M. Osnos, which is gratefully acknowledged. Further investigations on polysaccharides in glandular mucoprotein are in progress in this laboratory.

[5] These studies were done in the Eli Lilly Research Laboratories, Eli Lilly and Company, through the courtesy of E. D. Campbell, director, Biochemical Research Division.

close similarity of physical characteristics of mucoprotein and intrinsic factor, and the potent intrinsic factor activity of mucoprotein, it appears permissible to consider glandular mucoprotein fraction of the gastric "dissolved mucin" as the main carrier of the intrinsic factor activity of the human gastric juice.

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News and Notes

Symposium on the Evaluation of Optical Imagery

A Symposium on the Evaluation of Optical Imagery, held at the National Bureau of Standards Oct. 18–20, 1951, was attended by approximately 250 specialists in this field. Sponsored by NBS in cooperation with the Office of Naval Research, the meeting was the ninth of 12 symposia scheduled for the bureau's semicentennial year. The program, under the chairmanship of I. C. Gardner, of NBS, consisted of 21 technical papers organized to treat comprehensively a phase of lens design which is of great importance to the designer, but which has not yet received complete, systematic treatment in any one publication.

In applied optics, lens designs are usually evaluated on the basis of geometric optics, and the performance of optical systems is commonly measured in terms of their geometric aberrations. These practices are justified when aberrations are so large that diffraction plays but a small part in determining the quality of the imagery. Now, however, better optical systems are being produced; automatic computing machines make it possible to test an optical design completely by computation; the interferometer enables the wave front emergent from an optical system to be completely mapped; and integrating devices permit diffraction effects to be readily and fully determined. The purpose of the symposium was to re-examine and compare the present methods of image evaluation in the light of recent developments, with the purpose of placing these methods on a sound engineering basis and utilizing the principles of physical optics when justified. It is expected that the proceedings, including both papers and discussion, will be published as a single volume.

The six sessions of the symposium were under the chairmanship of I. C. Gardner, W. R. Brode (NBS), H. R. J. Grosch (International Business Machines), A. Maréchal (Institut d'Optique, Paris), S. S. Ballard (Tufts College), and Brian O'Brien (University of Rochester). The meeting began with a paper by F. Zernike (Natuurkundig Laboratorium, Groningen, The Netherlands) on the diffraction theory of aberrations. Tolerances for various aberrations were given; they are much larger than expected from geometric optics. Dr. Maréchal discussed the quality of optical images as determined by various quantities related to the diffraction pattern. Primary diffraction images produced