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Primary pernicious anemia versus idiopathic sprue : diagnostic differentiation by astric and small bowel biopsy

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PRIMARY PERNICIOUS ANEMIA VERSUS IDIOPATHIC SPRUE: DIAGNOSTIC DIFFERENTIATION BY GASTRIC AND SMALL BOWEL BIOPSY

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Introduction

The pathologic states that underly the development of megaloblastic anemia have been classified into three groups: 1) insufficient availability of vitamin B 12, 2) failure of absorption of vitamin B 12, and 3) failure of utilization of vitamin B 12. Considerable attention has recently been focused on the components of the second group, particularly in the differentiation of primary pernicious anemia and occult nontropical and tropical sprue. (14)

The sprue conditions are characterized by many, often subtle, manifestations. In the absence of overt symptoms and signs, idiopathic sprue may masquerade as primary pernicious anemia. (14)

Since the development of an instrument and technique for the transoral biopsy of small intestine mucosa by Shiner in 1956 and others (1,2,3,6,7,11, 15, 16, 17, 18), histologic changes of the mucosa in sprue states have been recognized which, while not specific, are highly suggestive of the condition. Previously the autolysis of gastrointestinal mucosa in surgical and autopsy material had prevented recognition of these changes.

It has recently been brought to light that

within each clinic group of primary pernicious anemia patients a few "occult" nontropical and tropical sprue conditions may exist. (14) The transoral small intestine biopsy procedure has provided a method for obtaining small intestine biopsies in which characteristic histologic changes can be recognized in cases of megaloblastic anemias which are secondary to sprue conditions A pilot study was therefore carried out on a small group of pernicious anemia patients to ascertain if a significant percentage of sprue like conditions existed.

Method

The patients studied were those being treated regularly for pernicious anemia in the hematologic clinic of the University of Nebraska College of Medicine Dispensary. Past records including history and physical, hematological results, general laboratory, and roentgenological studies were carefully reviewed.

Biopsies of gastric and jejunal mucosa were obtained with the Crosby small intestine biopsy tube. (5) The instrument is comprised of a cylindrical capsule 11 mm. in diameter and 20 mm. in length with rounded ends divided in circular cross section by a rubber diaphragm to form two chambers. In the

proximal chamber, which has a 5 mm. port, is located a knife on a cylindrical block which sweeps the wall of the capsule through an arc of 90 degrees and moves on its long axis in a linear direction. The knife block is activated by a coiled spring attached to an off center pin in the proximal end of the capsule and to the block itself. A small plastic tube extends from the proximal chamber for any desired length.

The instrument is operated through the effect of negative air pressure. As suction is applied through the plastic tube, intestinal or gastric mucosa is "sucked" into the port of the capsule occluding it. As the suction is continued, the rubber diaphragm dividing the chambers bulges into the proximal section of the capsule pushing the knife block proximally before it thereby releasing it from the key on which it had previously been cocked. The tightly coiled spring then whirls the knife edge across the port amputating the mucosa into the capsule.

The patient were instructed not to eat or drink twelve hours prior to the biopsy procedure. The capsule and tube were filled with saline to prevent foreign material from entering the port and then

the capsule was swallowed into the stomach. No premedication or oropharyngeal topical anesthesia was required. Under fluoroscopic control the capsule was maneuvered into the proximal jejunum. The saline was forced from the system by the injection of air through the tubing. Negative pressure was gently applied to the system by means of a syringe which "sucked" mucosa into the aperature. When resistence was encountered, a sudden sharp negative pressure was applied releasing the knife. The capsule and tube were then withdrawn. The specimen was extracted from the capsule with a forceps and placed in ten percent formalin solution for fixing. The capsule was passed a second time for a biopsy of the mid body of the stomach along the lesser curvature. Slides were made from sections cut twenty-four hours later and stained with hemotoxylin-eosin and Periodic Acid Schiff dyes.

Histology

Stomach In the normal stomach there are three histologic regions: 1) cardia, a 5 to 30 mm. ring shaped area at the cardio-espphageal junction containing coiled glands, 2) the fundus and proximate two-thirds or body, containing simple tubular glands

and 3) the lower one-third or pylorus containing glands similar to those found in the cardia region. (9)

Throughout the stomach the surface epithelium is composed of tal , regular columnar cells with basal nuclei and clear, transparent, only faintly granular cytoplasm. (9,12)

The glands in the cardia are terminally branched tubules containing columnar or cuboidal cells with a pale granular cytoplasm which often give the mucin reaction. They open directly into gastric pits and show enlargements in many places. The clear glandular cells are found either above or rarely alternating with parietal cells. (9,12)

The gastric glands in the body portion open into comparatively shallow gastric pits. The blind ends of the tubules are club-shaped and often divide into two or three branches. These glands contain the neck and body chief cells, parietal cells and argentaffine cells. (9,12)

The neck chief cells are identical with the cells lining the cardiac and pyloric glands. They are mucin producing columnar cells which line the upper, narrower part of the tubules. The body chief cells produce pepsin which is present in the cells in the

form of a granular precursor. The parietal cells produce acid and are scattered singly, internal to the body chief cells. They are most numerous at the necks of the tubules and are spherical or triangular in shape. The cytoplasm stains readily with acid dyes.

Argentaffine cells may be present at the base of the tubules.

In the pyloric mucosa the glands open into gastric pits which are much deeper than body gastric pits. These glands are lined by mucin producing cells but no parietal cells are present. An occasional argentaffine cell may be found. (9,12)

Throughout the stomach the glands are separated by scanty connective tissue which consists of a deli-cate network of collagenous and reticulum fibers in which sparse cells consisting of fibroblasts, reticuloendothelial cells, lymphocytes, plasma cells and eosinophils are found. (9,12)

The average thickness of normal gastric mucosa is 520 microns. (9)

In pernicious anemia the gastric mucosa shows varying degrees of inflammation and atrophy. (3,10,11) In lesser degrees of involvement there is a cellular infiltration composed of plasma cells and polymorphs

in the superficial portions of the lamina propria but accompanied by only a minor reduction in the size of the tubules. In more severe involvement the cell infiltration is marked and lymphocytes are found. The surface epithelium is flattened and goblet cells are numerous on the surface and in the pits. The tubules are great y reduced in number and changed in configuration. Cells staining for acid and pepsin (i.e. parietal and chief cells) are rare. The surface sometimes appears villous. In the most severe involvement the epithelium is composed almost entirely of goble cells. The pits end blindly or in small tubules in which only a very occasional cell stains for acid or pepsin and the surface appears very villous. (9)

The average thickness of the gastric mucosa in pernicious anemia range from 420 to 470 microns. (9)

These findings are referred to as intestinal-ization of the gastric mucosa but are not necessarily specific for pernicious anemia in as much as they also are found in idiopathic sprue and chronic atrophic gastritis.

Jejunum In normal jejunum the villi are long and finger-like ranging from 250 to 1000 microns in length and 75 to 150 microns in width. (7) They are

covered with a regu ar columnar epithelium which contains elongated nuclei situated toward the base. Homogenous material which stains pink with hemotox-ylin-eosin stain fills the rest of the cell. (7,15,17)

Mitosis is common in the cells lining the glands of Lieberkuhn but is not seen in the villous epithelium. The latter rests upon a basement membrane and a striated or brush border is located on its free surface. Goblet cells are scattered throughout the covering epithelium. (7)

The lining cells of the glands of Lieberkuhn are columnar cells but are much shorter than the villous columnar cells. They contain ovoid nuclei. Goblet cells are more numerous in the glands. Paneth cells containing granules are seen at the bases. Argen-taffine cells are also seen in the glands in hemotoxylin-eosin preparations. (7,17)

The interstitial connective tissue contains histiocytes, plasma cells, lymphocytes, eosinophils, mast cells and neutrophil polymorphs. (7,15,17)

In idiopathic sprue, jejunal changes are marked. The main abnormality is blunting, clubbing and reduction in the size of the villi which are 250 to 300 microns long. (7) The cells of the villi epithelium are lo columnar cells or cuboidal with

small irregular nuclei and vacuolated cytoplasm. The basement membrane is absent or interrupted. Goblet cells are usually increased and choked with mucous. The villous stroma is edematous and there is an increase in the cellular infiltration consist-ing of lymphocytes, plasma cells, eosinophils and occasionally polymorph neutrophils. (2,7,8,17)

The glands of Lieberkuhn appear as gaping crypts. The epithelium is flattened and the nuclei are irregular in size. Mitosis is reduced.

Results

Four females, 55 to 81, and two males, 67 and 77 years of age were studied.

Symptoms and Signs The signs and symptoms of primary pernicious anemia and idiopathic sprue and the incidence in the patients studied are recorded in Table I. (page 11)

Premature gray hair was present in two patients. Three patients had lemon colored skin at the time of diagnosis and two of these had yellow sclerae.

The loss of vibratory sense in the lower extremities was found in five patients. Two of these had incoordination of the lower extremities and one of these had loss of coordination of the fingers. Half of the patients had a positive Rhomberg.

In three patients reflexes were decreased. Two had sphincter disturbances, two vertigo and two tinnitus.

Three patients with anemia had symptoms referable to the heart, predominantly of the coronary insufficiency type.

Three patients had had smooth tongue margins and two had had a sore beefy red tongue at the time of diagnosis. One patient had both symptoms.

Two patients suffered from bloating and distention. Three patients admitted to stools that floated upon occasion. Two admitted to occasional mushy stools. No one reported greasy or pungent stools. Weight loss was reported by all six patients.

Laboratory ertinent laboratory studies associated with pernicious anemia and idiopathic sprue and the results in the patients studied are recorded in Table II. (page 14).

The calcium, inorganic phosphorus, serum protein and cholesterol levels plus prothrombin time were not significantly altered from the normal ranges of the laboratory doing the procedures.

Hemoglobin alues ranged from 6.2 to 13.8 Gm.%. Red blood cell counts ranged from 1.64 to 4.3 million. All original blood smears were reported as domon-strating macrocytosis, however the indicies were

TABLE I

PATIENT SYMPTOMS AND SIGNS RELATIVE TO PERNICIOUS ANEMIA AND IDIOPATHIC SPRUE

Patient	A	B	C	D	E	F	Total
Age	67	77	55	73	77	81	
Sex	M	M	F	F	F	F	
Race	Cau	Cau	Cau	Cau	Cau	Cau	
Premature gray hair	*				*		2
Blue eyes	*			*			2
Long ear lobes	*						1
Large frame							0
Eyes apart	*						1
Short deep chest							0
Lemon colored skin	*	*	*				3
Yellow sclerae	*	*					2
Nervous System							
Loss of vibration sense of lower extremities	*	*	*	*	*		5
Incoordination of lower extremities			*	*			2
Loss of coordination of fingers			*				1
Rhomberg	≠	-	≠	≠	-		
Disturbed position sense		*			*		2
Babinski							0
Spasticity		*					1
Drawing or cramping of tetany							0
Reflexes-Increased or Decreased	nor	dec	dec	dec	nor	nor	

TABLE I Cont'd

	A nor	B nor	C stag	D stag	E nor	F nor	Total
Gait							
Sphincter disturbances	*			*			2
Vertigo			*	*			2
Tinnitis	*					*	2
Cardio-vascular							
Palpitation			*	*			2
Extra beats			*				1
Precordial pain		*		*			2
Dyspnea			*	*			2
Gastro-intestinal							
Beefy red tongue, sore				*	*		2
Tongue margins smooth	*				*	*	3
Belching		*					1
Flatus	*	*					2
Bloating			*	*			2
Distention			*	*			2
Diarrhea							0
Constipation		*	*				2
Anorexia	*	*		*			3
Weight loss (pounds)	47	?	?	12	60	?	
Indigestion							
Burning							
Aching							
Gnawing							
Cramping							
Stabbing							
Nausea							
Vomiting							

TABLE I Cont'd

	A	B	C	D	E	F	Total
Stools							
Volume (bulky)							
Color (brown, clay, yellow)	bro	bro	bro	cla	bro	bro	
Foamy							0
Formed	*	*	*	*	*	*	6
Semisolid							0
Mushy	*			*			2
Liquid				*			1
Greasy							0
Odor (sick sweet, sour, pungent)							0
Float	*	*	*				3
Sink				*	*		2
Signs of Vitamin Deficiency							
Splenomegaly		*		*			2
Hepatomegaly		*	*	*			3

* Indicates the presence of the sign or symptom

TABLE II

LABORATORY STUDIES

	Normal	A	B	C	D	E	F
Calcium	9-11 mg%	10.0	9.3	9.8	9.3	9.7	10.0
Phosphorus	2-5 mg%	4.1	3.6	2.5	2.6	3.1	3.4
Albumin	3.5-5 Gm%	4.3	4.2	4.4	4.5	4.2	4.3
Globulin	2.5-4 Gm%	2.3	2.4	3.2	2.2	3.3	2.7
Cholesterol	150-300 mg%	408	325	442	345	317	208
Hgb	12-16 Gm%	6.5	6.2	12.2	10.5	13.8	9.5
RBC	4.3-6.0 M	2.22	1.64	4.30	3.10	4.10	2.88
Hematocrit	36-54%	20	22	?	34	?	?
MCH	27-33 micro-micrograms	29	40		34		
MCHC	33-38%	32.5	42		31		
MCV	80-95 cu. microns	90	126		100		
Gastric Analysis							
Free		0	0	0	0	0	0
Total		9	11	16	46		
Gastric Analysis after stimulation with histamine							
Free		0	0	0	0	0	0
Total		4	7	10	8		9
Prothrombin time Patient-control		14/14	14/13	14/14	14/14	14/14	13/14

determined in only three, or 50 percent, of the cases. The indicies were compatable with a macrocytic anemia in patients B and D but were normal in patient A.

Achlorhydria to histamine stimulation was found in all six patients.

Biopsies-Stomach The gastric specimens measuring 3 to 10 mm. in length and 2 to 3 mm. in width included mucosa, muscularis mucosa and varying amounts of submucosa. In all five cases the following pattern was identified.

The thicknes of the mucosa ranged from 414 to 478 microns.

TABLE III

THICKNESS OF GASTRIC MUCOSA IN MICRONS

Patient	Normal		C	D	E	F
Thickness	520	457	426	478	414	432

In the hemotoxylin-eosin stained slides the mucosal surface appeared somewhat villous in all specimens probably due to widening of the gastric pits. The epithelial cells were of the mucin producing type and showed basal, dark, elongated to ovoid shaped nuclei. The tubules were very sparse and gaping. The lining cells were vacuolated and had dark staining, small, basal nuclei. No parietal

cells or chief cel s were identified. A marked diffuse infiltration of lymphocytes and plasma cells was present in all specimens in varying degrees. In all cases, focal collections of lymphocytes were noted somewhat similar in appearance to lymph follicles in the small intestine.

A definite muscularis mucosa could not always be identified.

Periodic Acid Schiff stain showed the entire surface and gastric pit epithelium to be stained purple to red in granular pattern. A well circumscribed area of staining in each individual cell was noted. The basal end containing the nucleus was free of the red-purple color. The lining cells of the tubules also demonstrated a similar staining patterns There was some variation from tubule to tubule with some cells showing marked staining while others were comparatively free of any stain.

Jejunum The jejunal specimens measuring 3 to 10 mm. in length and 2 to 3 mm. in width consisted of well preserved mucosa, muscularis mucosa and varying amounts of submucosa. There was considerable variation in the length of the long finger like villi with less variation in the width. Mucosal thickness including villi varied from 740 to 840 microns. Villi length

ranged from 350 to 658 microns and the width from 98 to 196 microns.

TABLE IV

JEJUNAL MUCOSA THICKNESS AND VILLI LENGTH AND WIDTH IN MICRONS.

Patient	Normal	A	B	C	D	E	F
Mucosa thickness	400-850	830	840	756	740	840	840
Villi length	250-1000	560	350	560	350	658	630
Villi width	75-150	140	98	140	130	196	168

The covering epithelium was columnar with basal elongated nuclei. This rested upon a prominent basement membrane. A definite striated border covered the epithelium.

The villi stroma appeared to have the same components as the stroma between the glands of Lieberkuhn which comsisted of plasma cells, lymphocytes, histiocytes, eosinophils, mast cells and neutrophil polymorphs plus reticular tissue and elastic fibrils. Smooth muscle fibers we e noted in some villi.

Paneth cells with granules were observed at the bases of the glands of Lieberkuhn. Argentaffine cells were identified with more difficulty in the glands.

The lining cells of the glands of Lieberkuhn displayed ovoid nuclei. Mitosis was common as opposed to none bserved in the epithelium cells of the villi.

A well defined muscularis mucosa was present

in all specimens.

Periodic Acid Schiff atained the goblet cells in the villi with a granular pattern in a definite circumscribed area. The striated border was also stained. In some of the glands of Lieberkuhn a diffuse granular attern was noted at the lumen end of the cells. Other cells in the glands stained as the goblet cells in the villi which are probably mucopolysaccharide secretion cells.

Discussion

Signs and Symptoms The history and physical findings were compatable with primary pernicious anemia in all six patients, however in four patients there were symptoms that are frequently seen in idiopathic sprue.

The only common finding was weight loss. The loss of vibratory sense in the lower extremities in five patients was the second most common sign. No other single finding was present in a majority of the patients.

Cardio-vascular symptoms and anemia were associated in three cases.

A sore red tongue with smooth margins can be found in either primary pernicious anemia or

idiopathic sprue. (6,18) In only one case were both signs present.

Belching, bloating, flatus, distention, and indigestion are common signs of both pernicious anemia and sprue. There was no consistent correla-tion of these signs with stool symptoms indicative of sprue. (6)

Laboratory In idiopathic sprue, because of malabsorption, decreased calcium levels, increased inorganic phosphorus levels, decreased serum protein levels, decreased cholesterol levels, and increased prothrombin time might be expected. (6) The normal findings in this study would not substantiate mal-absorption phenomena.

The hemoblobin, red cell count, hematocrit and gastric analysis determinations were done at the time of diagnosis. All other laboratory results are current or were determined after the diagnosis of primary pernicious anemia.

All hematologic and gastric analysis determinations were compatable with the diagnosis of pernicious anemia. (18)

Biopsies The gastric mucosa biopsies showed marked variations from the normal which were compatable with the inflammatory and atrophic changes found in

primary pernicious anemia.

The jejunal mucosa was normal in all six biopsies with no blunting or clubbing of the villi.

While no instances of sprue were suggested by the tissue specimens studied, this by no means rules out the possibility that such cases might be discovered in the future through continued use of the small intestine biopsy tube.

Of considerable significance is the fact that in these pernicious anemia patients supposedly in a state of remission, marked histologic changes from the normal were evident. This would suggest that other factors are involved in this disease state, presumably of a deficiency nature, which, due to their absence resulted in the persistence of the abnormal gastric mucosa. Magnus has suggested that this procedure might be used as a method confirming a diagnosis of pernicious anemia in patients receiving vitamin B 12 (10), however the gastric lesion is not patho-gnomonic of this type of megaloblastic anemia in that it can be confused with lesions caused by chronic atrophic gastritis and nontropical and tropical sprue.

Value of the Periodic Acid Schiff dye cannot be determined at this time because of lack of sufficient control biopsies in normal states stained with this

material.

All biopsies were obtained on an outpatient basis. The specimens were well preserved and quite satisfactory for discriminating histological study. There was virtually no danger to the patient. All patients, even though elderly, found it comparatively easy to swallow and tolerate the tube and capsule. Healing is believed to take place by contraction of muscularis mucosa which approximates the epithelial margins around the defect. Epithelial cells migrate over the lesion to complete the healing process which probably takes place within two to three days. (19)

Infection, hemorrhage, and perforation are possible complications but were not observed.

Failure to obtain a biopsy specimen occurred in a patient with distal esophageal stenosis through which the capsule could not pass. An unforeseen complication was encountered in the first two patients. The distal section of the capsule separated from the proximal portion but was recovered in the stool in both cases without incident.

An increased resistence to withdrawal of the capsule occurred in one patient. Incomplete transection of the mucosa was the most likely cause. The tube was easily withdrawn after waiting ten minutes.

Summary

The pathologic states found to underly the development of megaloblastic anemias have been classified either as insufficient availability, failure of absorption or failure of utilization of vitamin B 12.

Primary pernicious anemia and idiopathic sprue can both cause failure of absorption of vitamin B 12. Recent studies have shown a change in jejunal mucosa suggestive of idiopathic sprue and therefore a means of distinguishing between these two conditions was sought by use of the Crosby small intestine biopsy tube and technique.

Six patients being treated for primary pernicious anemia at the hematologic clinic of the University of Nebraska College of Medicine Dispensary were studied.

Pertinent signs and symptoms, and laboratory determinations were recorded. All hematologic and gastric analysis laboratory determinations were compatable with the diagnosis of pernicious anemia and there was no significant alteration from the normal of other laboratory tests.

The gastric mucosal changes in five patients showing marked chronic inflammatory and atrophic changes were compatable with pernicious anemia. The jejunal mucosa in all six patients was found to be normal.

Conclusions

1. Gastric and small bowel biopsying through use of the Crosby small intestine tube is a simple and safe procedure that can be accomplished on an outpatient basis.

2. The tube can be utilized extensively in both research and in diagnosis of diseases manifesting themselves in the gastrointestinal tract.

3. The gastric mucosal lesion in five patients biopsied revealed inflammatory and atrophic changes compatable with primary pernicious anemia.

4. No histologic abnormalities indicative of idiopathic sprue were found in the jejunal biopsies of six patients with supposed primary pernicious anemia.

5. Further studies are required to prove or disprove the value of small intestine biopsying in the differential diagnosis of primary pernicious anemia and idiopathic sprue.

6. The gastric lesion of primary pernicious anemia remains markedly altered from the normal even though the patient is in a state of remission indicating other factors, presumably of a deficiency nature are involved in this disease state.

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