but it is certainly not trivial, and it is much more amenable to control. We still do not know which of the many particulate and gaseous pollutants we are exposed to in our towns and at our work are significant or how they act. But from the point of view of the prevention of bronchitis this is unimportant. We know that clean air cannot cause bronchitis, and we can have air as clean as we are willing and able to pay for. We cannot afford to get rid of all atmospheric pollution—the country's prosperity is built on the rate at which we burn up coal and oil—but it could be greatly reduced at not too great a cost to our economy.

So far as urban pollution is concerned, the Clean Air Act (1956) has pointed the way. Since that Act the level of smoke pollution in our towns has been falling, and it is probably no coincidence that the gradient of bronchitis mortality from large towns through medium and small towns to rural areas is also beginning to show signs of flattening. Progress in the control of atmospheric pollution at work is slower. The Factories Act provides legal machinery for the control of dusts, gases, and fumes at places of work, but the machinery is not being used as effectively as it might be. This is attributable in part to the uneven distribution of occupational health services, which because they are still largely non-statutory are provided only by the more enlightened private firms and by some of our nationalized industries, and in part to the serious inadequacy of laboratory facilities for measuring and monitoring the working environment. We are in urgent need of a national occupational health service supported by a full occupational hygiene laboratory service.

Finally I must refer to the misery and frustration caused by the present social security legislation, which permits the payment of special benefit to men with demonstrable *x*-ray evidence of dust in the lungs but withholds it from men who have been working in the same occupations and are equally or even more disabled by chronic bronchitis. This has led to an insistent demand that bronchitis should be recognized as a prescribed disease in certain occupations. However, except on the very rare occasions when the onset of the disease can be traced to a single massive exposure to an irritant gas such as chlorine or nitric oxide, it is never possible to estimate with any confidence how much, if any, of a man's bronchitis is attributable to his job, particularly if he is a heavy smoker.

The demand should be for prevention, not for compensation. We must insist on a much stricter observance of measures to protect men against excessive exposure to particulate and gaseous atmospheric pollutants at work. We must also, I believe, get rid of the National Insurance (Industrial Injuries) Act, and with it the nineteenth century concept of compensation for industrially induced disease. Legislation to relate social security benefits for long-term sickness or disability to family needs rather than to the cause of the wage-earner's incapacity is long overdue.

REFERENCES

Daly, C. (1954). *Brit. med. J.*, **2**, 687.
Elwood, P. C., Pemberton, J., Merrett, J. D., Carey, G. C. R., and McAulay I. R. (1965). *Brit. J. industr. Med.*, **22**, 27.
Fairbairn, A. S., and Reid, D. D. (1958). *Brit. J. prev. soc. Med.*, **12**, 94.
Gough, J. (1960). *Industr. Med. Surg.*, **29**, 283.
Higgins, I. T. T., and Cochrane, A. L. (1961). *Brit. J. industr. Med.*, **18**, 93.
Higgins, I. T. T., Oldham, P. D., Cochrane, A. L., and Gilson, J. C. (1956). *Brit. med. J.*, **2**, 904.
Lowe, C. R. (1968). *Proc. roy. Soc. Med.*, **61**, 98.
Lowe, C. R., Pelmear, P. L., Campbell, H., Hitchens, R. A. N., Khosla, T., and King, T. C. (1968). *Brit. J. prev. soc. Med.*, **22**, 1.
Medical Research Council (1966). *Brit. med. J.*, **1**, 101.
Pemberton, J., and Goldberg, C. (1954). *Brit. med. J.*, **2**, 567.
Roberts, F. (1952). *The Cost of Health.* London.
Schilling, R. S. F. (1956). *Lancet*, **2**, 261, 319.
United States Department of Health, Education and Welfare (1967). *The Health Consequences of Smoking*, P.H.S. Publication No. 1696. Washington.

Dissociation of Intrinsic Factor from its Antibody: Application to Study of Pernicious Anaemia Gastric Juice Specimens

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Summary: Intrinsic factor antibody may sometimes be concealed in the gastric secretions of pernicious anaemia subjects, being complexed with residual amounts of intrinsic factor.

A method is described for dissociating intrinsic factor from its antibody. Antibody to the vitamin-B_{12}-binding site of intrinsic factor was identified in 16 (57%) out of 28 samples of pernicious anaemia gastric juice after dissociation but in only 10 before dissociation. There was no clear relationship between the incidence of antibody in the serum and in the gastric juice of these patients.

Introduction

Antibody to Castle's intrinsic factor is detectable in the serum of 55% of patients suffering from Addisonian pernicious anaemia (Taylor, 1959 ; Ardeman and Chanarin, 1963). There is good reason to suppose that this antibody may interfere with intrinsic factor production as well as prevent its function in vitamin-B_{12} absorption, and these aspects have been reviewed by Chanarin (1968). Intrinsic factor antibodies are of at least two types. The commoner type reacts with that portion of the intrinsic factor molecule which links with vitamin B_{12}, thus preventing union with this vitamin (synonyms: vitamin-B_{12}-binding-site antibody ; blocking antibody ; type I antibody). The other type of antibody reacts with intrinsic factor evidently elsewhere than on the vitamin-B_{12}-binding site (synonyms: complex antibody ; precipitating antibody ; type II antibody). Two general types of in-vitro test have been devised to identify these two kinds of antibody. Antibody localized in the gastrointestinal tract is likely to be of greater importance in pernicious anaemia patients than the antibody in the serum, since the antibody can interfere with intrinsic factor function only at sites of confrontation. As intrinsic factor is probably not absorbed, this encounter must take place in the gastrointestinal lumen or at the mucosal surface.

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Fisher *et al.* (1966) found intrinsic factor antibody in the gastric juice of 5 out of 14 patients with pernicious anaemia, including one who did not have such an antibody in the serum. Others have investigated the gastric juice from patients in whom additional intrinsic factor failed significantly to improve the absorption of vitamin B_{12}. Gastric juice antibody was present in the case reported by Schade *et al.* (1966) and in all nine cases reported by Herbert *et al.* (1967).

These observations have been concerned with free antibody in the gastric juice. Intrinsic factor secretion is often not abolished in pernicious anaemia, and small amounts are present in about one-third of the patients (Ardeman and Chanarin, 1965). If intrinsic factor antibody is present in the gastric juice samples from such patients, it is likely to be concealed in a complex with intrinsic factor and hence it will not be demonstrable by direct examination. The purpose of this study was to devise a means whereby such antibody could be released from intrinsic factor and then assayed by a standard procedure. This method would then be used for the detection of antibody in gastric juice samples from pernicious anaemia patients.

Materials

Normal human gastric juice obtained from several donors was pooled, depepsinized, restored to neutral *p*H, and frozen in aliquots at −20° C. until used. The vitamin-B_{12}-binding capacity and intrinsic factor titre were measured by the method of Ardeman and Chanarin (1963).

Gastric juice from subjects with pernicious anaemia was aspirated by nasogastric tube for 30 minutes before and for one hour after stimulation with either histamine or pentagastrin (Peptavalon). These samples were centrifuged, filtered, and stored at −20° C. until tested.

Serum from five subjects with pernicious anaemia, containing an antibody to the vitamin-B_{12}-binding site of intrinsic factor, in titres ranging from 100 to 250 units/ml. were used in the pilot studies. The antibody titre was measured by the charcoal adsorption method of Ardeman and Chanarin (1963). These samples were stored at −20° C. in aliquots until used.

$^{57}CoB_{12}$ (Radiochemical Centre, Amersham) with a specific activity of 25 mμCi/ml. and diluted with non-radioactive cyanocobalamin to give a final concentration of 200 mμg./ml. Activated charcoal (British Drug Houses) was prepared by heating at 160° C. for two hours and then mixed with normal plasma at a concentration of 100 mg./ml., immediately before use. Measurements of γ-ray emission were made in a Packard, well-type scintillation counter. *p*H was measured on a direct reading glass electrode *p*H meter (Electronic Instruments Limited.)

Methods

(*a*) *Velocity of Combination of Intrinsic Factor with Vitamin B_{12} and with Antibody to Vitamin-B_{12}-binding Site.*—(1) Estimates were made of the speed of combination of normal gastric juice samples with vitamin B_{12}. A 10-ml. volume from a pool of five normal gastric juice samples (binding about 80 mμg. of vitamin B_{12}/ml.) was mixed with 5 ml. of $^{57}CoB_{12}$ (200 mμg./ml.). One-millilitre aliquots were expelled into saline containing serum-coated charcoal, which absorbed the unbound vitamin B_{12}, thus curtailing the combination. After centrifugation the supernatant radioactivity was expressed as a percentage of the radioactivity observed 10 minutes after mixing, by which time no further vitamin B_{12} was bound. (2) Ten millilitres of the gastric juice pool was mixed with 4 ml. of serum containing intrinsic factor antibodies (250 units of antibody per ml.). One-millilitre aliquots were removed at stated intervals and expelled into saline containing 100 mμg. of $^{57}CoB_{12}$. After 10 minutes serum-coated charcoal was added to extract the unbound vitamin B_{12}. After centrifugation at 3,000 r.p.m. for 10 minutes the activity of the supernatant was measured. Antibody combination was expressed as a percentage of the value observed after 10 minutes. These studies were carried out at *p*H 7·5, 3·8, and 3·2.

(*b*) *Effect of pH on Combination of Intrinsic Factor and Antibody Against Vitamin-B_{12}-binding State of Intrinsic Factor.*—This was studied in mixtures of serum containing antibody to intrinsic factor and with normal gastric juice. Suitable controls were also set up as indicated in Table I. The quantities chosen were such that no free antibody could be detected. After incubation at room temperature for 30 minutes the test mixture and controls were exposed to the sequence of steps outlined below. The *p*H was reduced to 3·2 with 0·2 M disodium-phosphate/citric-acid buffer. Cyanocobalamin was added in about tenfold excess of the binding capacity of the components. The mixture was agitated for 15 minutes. The unbound vitamin B_{12} was then removed by two additions of 100 mg. of serum-coated charcoal, added at an interval of three minutes. The mixture was again shaken, centrifuged at 3,000 r.p.m. for 10 minutes, and the supernatant was then tested for antibody by the method of Ardeman and Chanarin (1963). The charcoal control was set up to confirm complete removal of the unbound vitamin B_{12} excess.

TABLE I.—*Test and Control Preparations to Measure Dissociation of Antibody from Complex With Intrinsic Factor*

	Test	Controls		
Gastric juice	2·5 ml. (125 u. intrinsic factor)	2·5		
Saline			2·5	2·5
Pernicious anaemia serum	0·5 ml. (100 u. Ab)		0·5	
Normal serum ..		0·5		0·5

(*c*) *Dissociation of Antibody from Sites of Intrinsic Factor Other than those Reacting with Vitamin B_{12}.*—Mixtures containing normal gastric juice (100 units of intrinsic factor) and serum with intrinsic factor antibody in increasing quantities were lowered to *p*H 3·2 for 15 minutes with 0·2 M sodium-phosphate/citrate buffer. These were decanted into 1-ml. volumes of normal gastric juice complexed with $^{57}CoB_{12}$. The antibody was precipitated with 40% saturated NH_4SO_4 (Jacob and Schilling, 1966) and radioactivity in both supernatant and deposit were measured. The radioactivity in the deposit was expressed as a percentage of total radioactivity.

(*d*) *Pernicious Anaemia Gastric Juice.*—Manœuvres applied to these specimens were the same as the sequence outlined in section (*b*).

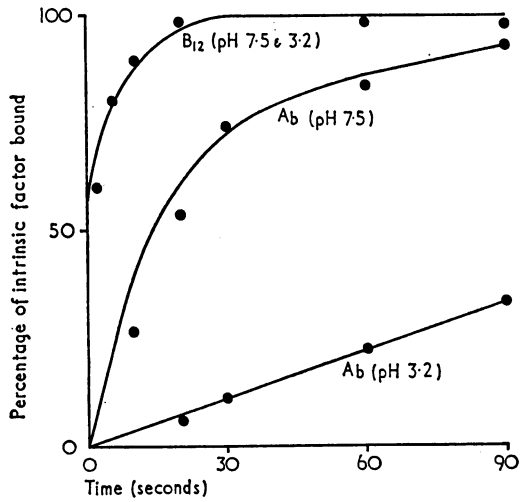
(*e*) *Suppression of Antibody with Anti-human Serum.*—An anti-human serum preparation was used (Netherlands Red Cross Transfusion Service PHO—13—P2); also a specific anti-human serum IgA (X80H kindly supplied by Dr. R. Drew, Department of Immunological Pathology, University of Birmingham). These were mixed with gastric juice specimens found to have an antibody to intrinsic factor. After incubation at 37° C. for two hours they were frozen at −20° C. for 24 hours and then thawed and tested for antibody as described.

Results

(*a*) *Reaction Velocities* (see Chart).—(1) *Intrinsic Factor and Vitamin B_{12}*: The combination was extremely rapid both at room temperatures and at 4° C. Vitamin B_{12} binders were 60% saturated in two seconds and 100% saturated within 20 seconds. There was no significant difference in the rate of binding at *p*H 7·5, *p*H 3·2, and *p*H 3. (2) *Intrinsic Factor and Antibody*: At *p*H 7·5 50% of the antibody had combined by 20 seconds and complete combination occurred only after 90 seconds. At *p*H 3·2 30% of the antibody united with intrinsic factor after 90 seconds.

(*b*) *Dissociation of "Vitamin-B_{12}-binding-site Antibody" from Intrinsic Factor.*—Five separate sera were mixed with

normal gastric juice and tested as described. Under optimum conditions between 75 and 100% of antibody added to normal gastric juice was recovered. The degree of dissociation was measured over the *p*H range from 7·5 to 3·2. A *p*H of 3·2 was chosen since this *p*H gave maximum dissociation without a significant degree of denaturation of released antibody. A fall in antibody activity occurred below *p*H 3·0 when the duration of exposure was greater than 15 minutes.



Speed of combination of intrinsic factor with vitamin B_{12} and of intrinsic factor with antibody to vitamin-B_{12}-binding site.

(*c*) Endeavours to dissociate "non-binding-site" antibody by lowering the *p*H to 3·2 and then permitting random reassociation at neutral *p*H with intrinsic factor-$^{57}CoB_{12}$ provided no convincing evidence that this antibody behaved similarly to the vitamin-B_{12}-binding-site antibody under these conditions. Correspondingly, application of this manœuvre has provided no evidence concerning the presence of this antibody in gastric juice from pernicious anaemia cases.

(*d*) *Pernicious Anaemia Gastric Juice.*—Twenty-eight gastric juice specimens from subjects with pernicious anaemia and 17 control specimens were treated and tested, as described, for the release and identification of antibody to the vitamin-B_{12}-binding site. Of the 28 pernicious anaemia gastric juices, nine were from patients with an antibody to intrinsic factor in the serum and 11 from subjects without such an antibody. Of the 28 gastric juice specimens, 16 gave positive results for the presence of antibody. Of these 16, 8 were from patients with a serum antibody and 8 from patients without (Table II). Among the 17 controls, including four specimens from patients with autoimmune thyroid disorders, who had serum antibody to intrinsic factor without evidence of impaired vitamin-B_{12} absorption, no positive results for gastric juice antibody were obtained. A general observation among the pernicious anaemia samples was that dissociation showed more antibody than was found on simple testing and that where some intrinsic factor was found in the sample, antibody was demonstrated only after dissociation.

TABLE II.—*Correlation of Incidence of Gastric Juice Antibody with Serum Antibodies to Intrinsic Factor*

			Gastric Juice Intrinsic Factor Antibodies	
			+	−
Serum intrinsic factor antibodies ..	*M*	+	8	3
	M	−	8	9
			16	12

(*e*) In three gastric juice specimens found to have an antibody to intrinsic factor mixture with an equal volume of anti-human serum caused a clear drop in antibody activity. This observation was likewise made on using specific anti IgA serum, thus identifying the antibody in these specimens as of class IgA.

Discussion

Free antibody in pernicious anaemia gastric juice was present in 10 out of 28 samples; in six more cases antibody could be demonstrated on dissociation from intrinsic factor. In those with free antibody the dissociation procedure produced some rise in titre of antibody. Antibody was present in the gastric juice of four patients in the absence of serum antibody; Fisher *et al.* (1966) described one similar case. This emphasizes that it is local gastrointestinal antibody which is of importance in impairing vitamin-B_{12} absorption, particularly since serum antibody may be present and in no way interferes with vitamin-B_{12} absorption.

In the present group of 28 patients antibody was found in the serum or gastric juice of 19 (68%). It cannot, however, be concluded that no antibody was present in the remaining 32% in whom we failed to find it.

Observations on the transplacental transmission of antibody to intrinsic factor suggest that this antibody may suppress intrinsic factor production in the infant without itself appearing in the gastric juice. Fisher and Taylor (1967), however, failed to observe any impairment of infantile intrinsic factor production as a consequence of transplacental passage of intrinsic factor antibody. This antibody may therefore be synthesized in the gastric submucosa and function locally in the mucosa (Bar Shany and Herbert, 1966; Goldberg *et al.*, 1967) and either not appear in the gastric secretion or be found only in small quantities. Endeavours to identify antibody in concentrates of pernicious anaemia gastric juice, where the undiluted sample evidently contained no antibody, have failed. Antibody may be directed against other sites of the intrinsic factor molecule than the vitamin-B_{12}-binding site. Such antibodies were not identified in gastric juice by the procedures described.

REFERENCES

Ardeman, S., and Chanarin, I. (1963). *Lancet*, **2**, 1350.
Ardeman, S., and Chanarin, I. (1965). *Gut*, **6**, 436.
Bar Shany, S., and Herbert, V. (1966). *Clin. Res.*, **14**, 291.
Chanarin, I. (1958). *Gut*, **9**, 373.
Fisher, J. M., Rees, C., and Taylor, K. B. (1966). *Lancet*, **2**, 88.
Fisher, J. M., and Taylor, K. B. (1967). *Lancet*, **1**, 695.
Goldberg, L. S., Barnett, E. V., and Desai, R. (1967). *Pediatrics*, **40**, 851.
Herbert, V., Carmel, R., and Li, J. G. (1967). *New Engl. J. Med.*, **276**, 61.
Jacob, E., and Schilling, R. F. (1966). *J. Lab. clin. Med.*, **67**, 510.
Schade, S. G., Feick, P., Muckerheide, M., and Schilling, R. F. (1966). *New Engl. J. Med.*, **275**, 528.
Taylor, K. B. (1959). *Lancet*, **2**, 106.