VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

Edited by

ROBERT S. HARRIS
Massachusetts Institute of Technology
Cambridge, Massachusetts

IRA G. WOOL
The University of Chicago
Chicago, Illinois

JOHN A. LORAINE
Clinical Endocrinology Research Unit
Medical Research Unit
The University
Edinburgh, Scotland

Consulting Editors

G. F. MARRIAN
The Imperial Cancer Research Fund Laboratories
London, England

KENNETH V. THIMANN
Harvard University
Cambridge, Massachusetts

ACADEMIC PRESS, New York and London

ACADEMIC PRESS INC.
111 Fifth Avenue, New York 3, New York

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 43-10535

PRINTED IN THE UNITED STATES OF AMERICA

Contributors to Volume 21

Numbers in parentheses refer to the page on which the author's contribution begins.

ANN M. BUDY, *Department of Physiology, University of Chicago, Chicago, Illinois (51).*

G. P. CREAN, *Clinical Endocrinology Research Unit, University of Edinburgh, Edinburgh, Scotland (215).*

LEON ELLENBOGEN, *Lederle Laboratories, Division of American Cyanamid Company, Pearl River, New York (1).*

DEREK R. HIGHLEY, *Lederle Laboratories, Division of American Cyanamid Company, Pearl River, New York (1).*

JENG M. HSU, *Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland (113).*

CHIKATARO KAWASAKI, *Laboratory of Chemical Hygiene and Nutrition, School of Pharmacy, Osaka University, Toyonaka, Japan (69).*

FRANKLIN C. MCLEAN, *Department of Physiology, University of Chicago, Chicago, Illinois (51).*

CHARLES A. NICHOL, *Department of Experimental Therapeutics, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York (135).*

FRED ROSEN, *Department of Experimental Therapeutics, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York (135).*

Preface

The Editors are pleased to present the twenty-first volume of *Vitamins and Hormones*.

The authors have been encouraged to prepare selective interpretive reviews that are critical in character and that record their own outlook. The papers are not intended as exhaustive digests of the literature. The value of the volume (and of the serial publication) is conditioned, of course, by how well the authors achieve that objective. In the present instance we feel they have done very well indeed and are greatly in debt to them for their scholarly contributions and for the time and effort they have so unselfishly devoted to the task.

November 1963

R. S. Harris
J. A. Loraine
I. G. Wool

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Intrinsic Factor

LEON ELLENBOGEN AND DEREK R. HIGHLEY

Biochemistry Research Department, Biochemical Research Section, Lederle Laboratories, American Cyanamid Company, Pearl River, New York

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I. Introduction

In discussing the development of knowledge concerning intrinsic factor and its relationship to pernicious anemia, Castle (1953) said: "Thus, this

disease would not develop if the patient could effect daily the transfer of a millionth of a gram of vitamin B_{12} the distance of a small fraction of a millimeter across the intestinal mucosa and into the blood stream. This he cannot do, principally as a result of failure of his stomach to secrete into its lumen some essential but still unknown substance." The existence of this essential but unknown substance called intrinsic factor was postulated by Castle and his associates (Castle and Townsend, 1929; Castle *et al.*, 1930) more than thirty years ago.

Castle *et al.* believed that beef muscle (extrinsic factor) reacted with normal human gastric juice to yield an "anti-pernicious anemia principle" which induced remission when fed to pernicious anemia patients. When vitamin B_{12} was isolated (Rickes *et al.*, 1948; Smith, 1948), it was quickly identified as extrinsic factor. Physiological doses of vitamin B_{12} were inactive orally, but were effective in treating pernicious anemia when given parenterally. Castle's theory therefore, had to be modified because it is now believed that intrinsic factor functions by transporting vitamin B_{12} across the intestinal mucosa (Berk *et al.*, 1948). Pernicious anemia therefore results from a vitamin B_{12} deficiency due to absence or diminution of intrinsic factor secretion by the patient.

The role of intrinsic factor in the absorption of physiological amounts of vitamin B_{12} has attracted more interest than the absorption of any other vitamin. It is of interest that so special an absorption mechanism is needed for a vitamin for which the daily requirement is only about 1 μg.

Excellent reviews dealing with pernicious anemia and/or vitamin B_{12} have appeared in the last few years (Gräsbeck, 1960; Herbert, 1959a; Witts, 1961; Castle, 1953; Ungley, 1955). The present article will be concerned with a critical review and discussion of intrinsic factor and closely related subjects. Discussion of vitamin B_{12} will be limited to its direct relationship to intrinsic factor.

II. Site of Secretion and Sources of Intrinsic Factor

A. Human

Shortly after the demonstration of the effectiveness of beef muscle and gastric juice in pernicious anemia, Castle *et al.* (1930) showed that normal human duodenal juice did not contain intrinsic factor activity. This was confirmed by Landboe-Christensen and Bohn (1947). Further studies by Landboe-Christensen *et al.* (1952) showed that desiccated preparations of normal human duodenum, but not jejunum, contained a small amount of intrinsic factor activity. This was undoubtedly due to contamination of the tissue by gastric juice, as patients who have undergone total gastric

resection cannot absorb vitamin B_{12} and intestinal juices of such patients are devoid of intrinsic factor activity (MacDonald *et al.*, 1947; Paulson *et al.*, 1950; Swendseid *et al.*, 1953).

Wilkinson *et al.* (1938), showed that the normal human stomach possessed intrinsic factor activity. More specifically, in man, the pyloric region of the stomach is inactive whereas the fundic (corpus) and cardiac regions are active (Fox and Castle, 1942; Landboe-Christensen and Plum, 1948; Meulengracht, 1952). These results are in agreement with histological findings that atrophy of the stomach characteristically seen in pernicious anemia occurs in the fundic area, but not in the pyloric region (Magnus and Ungley, 1938; Meulengracht, 1939; Motteram, 1951).

Cellular fractionation studies of human gastric mucosal extracts indicate that intrinsic factor activity is present in both the mitochondria and particale-free supernatant fluid (W. H. Taylor *et al.*, 1961).

B. Hog

Sturgis and Isaacs (1929) were the first to show that hog stomach was an effective source of intrinsic factor for oral treatment of pernicious anemia. These investigators used fresh, whole, hog stomach which was desiccated before use. Sharp (1929), Wilkinson (1930), and Snapper and du Preez (1931) confirmed these findings. Surprisingly, in the hog, in contrast to man, intrinsic factor is found not in the fundic area of the stomach, but mainly in the pyloric region, probably with a trace in the cardiac portion (Fox and Castle, 1942; Meulengracht, 1934, 1935; Landboe-Christensen and Plum, 1948). As pointed out by Meulengracht (1952) the contrast between the hog and human is rare in the study of comparative physiology. While it is true that the assay of intrinsic factor in the early years was not as quantitative as in the last decade, nothing has appeared in the recent literature which would cause one to doubt the significance of these data. Meulengracht (1934, 1952, 1953) also showed that the mucosal layer of the pyloric section of the hog is the richest source of intrinsic factor. The trace of activity found in the muscularis was probably due to contamination from the mucosa (Meulengracht, 1953).

Intrinsic factor activity is also present in hog gastric juice and duodenal juice and in the duodenum itself (Braun, 1934; Landboe-Christensen and Bohn, 1947; Bethel *et al.*, 1949; Hall *et al.*, 1950). It is not certain whether the intrinsic factor activity found in the duodenum was due to contamination from the secretions of the stomach passing into the small intestine. Dexter *et al.* (1939) presented evidence that intrinsic factor activity, which Uotila (1936, 1938) had reported to be present in the hog ileum, was due to adsorption on the ileal wall of intrinsic factor which had been secreted

higher in the tract. Heinrich (1957) reported that crude lyophilized duodenum from the pylorectomized hog did not exhibit intrinsic factor activity in the pernicious anemia patient.

C. Rat

Gastric juice, whole stomach homogenates, and extracts of the glandular portion of the stomach of the rat possess intrinsic factor activity in the rat (Watson and Florey, 1955; Nieweg *et al.*, 1956). Rat gastric juice appears to be as effective as human gastric juice in patients with pernicious anemia (Abels *et al.*, 1957). The anatomical distribution of intrinsic factor in the rat is similar to that observed in man; the fundic portion of the rat stomach contains most, if not all, of the intrinsic factor activity (Keuning *et al.*, 1959). This was determined by measuring the effect of different areas of the rat stomach on vitamin B_{12} absorption in the gastrectomized rat. These investigators also showed by autoradiographic studies that the chief (pepsinogen) cells of the fundus appeared to secrete intrinsic factor.

D. Other Species

Very little work has been published on the intrinsic factor of other species. Schwartz *et al.* (1958) and Abels (1959) reported that intrinsic factor preparations from the wild boar and monkey were active in man.

Intrinsic factor preparations from the guinea pig, hamster, rabbit, rat, hog, and human stimulated vitamin B_{12} uptake in guinea pig ileum (Wilson and Strauss, 1959). Cooper *et al.* (1962) confirmed the observation that vitamin B_{12} bound to human intrinsic factor is absorbed by the intact guinea pig. Stimulation of vitamin B_{12} uptake by liver slices, caused by dog gastric juice, has been described (Johnson and Driscoll, 1958). However, it is not certain whether stimulation of liver-slice uptake is related to intrinsic factor activity.

III. Assay of Intrinsic Factor

A. Use of Gastrectomized Animals

The lack of a rapid, readily available, and unequivocal assay method is one of the most important factors that has hampered progress toward isolation, characterization, and study of the mechanism of action of intrinsic factor. There is no animal counterpart to human Addisonian pernicious anemia. In man, total gastrectomy causes complete loss of ability to absorb orally administered vitamin B_{12}. Eventually, megaloblastic anemia indistinguishable from pernicious anemia, will result, due to vitamin B_{12} deficiency, if treatment with vitamin B_{12} is not instituted (Paulson *et al.*, 1950; Paulson and Harvey, 1954; Swendseid *et al.*, 1953; Halsted *et al.*,

1954; MacDonald *et al.*, 1947; MacLean and Sundberg, 1956; Callender *et al.*, 1954a; Badenoch *et al.*, 1955). As in pernicious anemia, this absorption defect can be corrected by feeding normal human gastric juice or hog intrinsic factor together with vitamin B_{12}.

Because the anatomical distribution of intrinsic factor in the stomach of the rat is similar to that of man, it was originally hoped that the use of the gastrectomized rat might serve as a means of measuring hog intrinsic factor activity. (Most of the studies on the isolation of intrinsic factor have been performed with hog gastrointestinal tissue because it is more readily available than human gastric juice and is active in pernicious anemia.) The gastrectomized rat fails to absorb orally administered vitamin B_{12} (Watson and Florey, 1955; Chow *et al.*, 1955; Clayton *et al.*, 1955; Nieweg *et al.*, 1956), and rat gastric juice or rat stomach preparations restore vitamin B_{12} absorption to normal. This absorption defect cannot, however, be corrected with hog intrinsic factor. In fact, in many instances vitamin B_{12} absorption is depressed in the normal rat by hog intrinsic factor. Using a different technique, Holdsworth and Coates (1956) came to the same conclusion. A ligature was placed at the distal end of the duodenum of the normal rat to eliminate the influence of gastric juice. A second ligature was made at the ileocecal junction. Radioactive vitamin B_{12} was introduced alone or in combination with hog intrinsic factor or rat-stomach extract. Radioactivity measurements showed that absorption took place with vitamin B_{12} alone and was decreased when hog intrinsic factor was added; and rat-stomach extract increased vitamin B_{12} absorption.

The inability of hog intrinsic factor to effect vitamin B_{12} absorption in the gastrectomized rat was a great disappointment in the search for a simple, readily available assay for hog intrinsic factor. There is promise that the gastrectomized rat may be useful as an assay animal for human intrinsic factor. However, further work is needed to amplify the finding by K. B. Taylor *et al.* (1958) that low concentrations of purified human intrinsic factor promoted absorption of vitamin B_{12} in the gastrectomized rat. The results of Wilson and Strauss (1959) and Cooper *et al.* (1962) on stimulation of vitamin B_{12} uptake by human intrinsic factor in the guinea pig also show promise as a useful assay for human intrinsic factor. Limited studies attempting to use the hog or gastrectomized hog to assay hog intrinsic factor have proved unsuccessful (Heinrich, 1957; Holdsworth and Coates, 1960, 1961). Totally gastrectomized hogs absorb vitamin B_{12} very well. This does not necessarily conflict with the findings of Heinrich (1957) that only the pylorus of the hog is a source of intrinsic factor, as the mechanism of action of intrinsic factor in hogs is different from that in humans or rats (see Section V).

B. Binding of Vitamin B_{12}

The observation by Ternberg and Eakin (1949) that intrinsic factor concentrates bind vitamin B_{12}, has caused more confusion and misunderstanding for many years than probably any other aspect of the intrinsic factor field. Confusion regarding the quantitative relationship between vitamin B_{12} binding and intrinsic factor activity has been compounded by the numerous methods used to measure vitamin B_{12} binding. It has been measured by microbial growth inhibition (Ternberg and Eakin, 1949), electrophoresis (Barlow and Frederick, 1959; Heinrich *et al.*, 1956), adsorption onto charcoal (O. N. Miller, 1957), gel filtration with Sephadex (Daisley, 1961), ultrafiltration (Gregory and Holdsworth, 1955a, b), and dialysis binding (Rosenblum *et al.*, 1954). Measurement of microbial growth inhibition has the disadvantage that some organisms have enzymes which liberate the bound vitamin B_{12}. However, microbial inhibition permits measurement of vitamin B_{12} binding by small molecules. This is not possible with either dialysis, ultrafiltration, or gel filtration. The dialysis, ultrafiltration, or gel filtration methods are quite amenable to the use of radioactive vitamin B_{12}, and since it is believed that intrinsic factor is nondialyzable, these techniques have been the most widely used of the various binding techniques in recent years.

Much of the confusion about whether intrinsic factor binds vitamin B_{12} or not appears to have been dissipated with the preparation of more highly purified intrinsic factor. All purified preparations bind vitamin B_{12}, and binding is considerably greater than that observed with crude preparations (Holdsworth, 1961; Ellenbogen and Williams, 1960; Bromer and Davisson, 1961; Andresen, 1954; Berlin *et al.*, 1959). Many substances other than intrinsic factor bind vitamin B_{12}, e.g., lysozyme (C. E. Meyer *et al.*, 1950; Bird and Hoevet, 1951), saliva (Beerstecher and Edmonds, 1951a, b, 1952; Beerstecher and Altgelt, 1951), serum (A. Miller and Sullivan, 1958; Ross, 1950, 1952; Pitney *et al.*, 1954; Rosenthal and Sarett, 1952), milk (Gregory and Holdsworth, 1955a), cerebrospinal fluid (L. M. Meyer *et al.*, 1959). More extensive reviews on vitamin B_{12} binding by substances other than intrinsic factor were presented by Gräsbeck (1960) and Wijmenga (1957).

Binding of vitamin B_{12} by materials not derived from gastrointestinal tissue does not in itself invalidate the usefulness of binding in the preparation of purified intrinsic factor concentrates. Binding by intrinsic factor is considerably more specific than that observed with materials other than intrinsic factor. Intrinsic factor preferentially binds vitamin B_{12} in a mixture of vitamin B_{12} and an excess of various vitamin B_{12} analogs (Bunge and Schilling, 1957). Furthermore, the equilibrium between unbound vitamin B_{12} and vitamin B_{12} bound to intrinsic factor is strongly in favor of the

bound form (Highley and Ellenbogen, 1962), even in the presence of an excess of the unbound vitamin. This is not true for other biological materials which bind vitamin B_{12}. The vitamin B_{12} binding capacity of lysozyme has been reported to differ from that of intrinsic factor in that the vitamin B_{12} is readily dialyzable (Bird and Hoevet, 1951).

Vitamin B_{12} bound to intrinsic factor is absorbed by the intestine in preference to unbound vitamin B_{12} (Bishop *et al.*, 1955), an extremely important observation (see under Section V). When vitamin B_{12} binding was decreased by treatment of intrinsic factor with various specific group inhibitors, a corresponding decrease in intrinsic factor activity was observed (Gräsbeck, 1958, 1959a, b).

Intrinsic factor preparations have been purified with the aid of vitamin B_{12} binding-capacity measurements. Berlin *et al.* (1959) and Barlow and Frederick (1959) obtained good correlation between binding capacity and intrinsic factor activity. Berlin and associates found that correlation was better as sample purity increased. Barlow and Frederick utilized paper electrophoresis as a means of separating the various vitamin B_{12}-binding components in intrinsic factor concentrates. The vitamin B_{12} binding capacity of one of the components was found to correlate with the activity of the original concentrate. This technique has neither been generally accepted nor confirmed. It would appear that the successful application of this technique to the assay of intrinsic factor would require separation of pure intrinsic factor from inactive material.

In the studies of Glass *et al.* (1959), it is of interest that the vitamin B_{12} binding capacity of hog-stomach preparations increased proportionately to the increase in ammonium sulfate used to precipitate the various preparations. The correlation between activity and binding capacity, however, was not as good as that observed by other investigators.

The disagreement regarding the possible correlation between intrinsic factor activity and binding can most readily be explained by the recent reports by Holdsworth (1961) and Highley and Ellenbogen (1962). These investigators were able to purify two fractions from hog pylorus. Both fractions combined with vitamin B_{12}, but only one possessed high intrinsic factor activity. It is uncertain whether the inactive vitamin B_{12}-binding component resulted from inactivation during fractionation or was present originally. Gräsbeck (1958, 1959a, b) showed that intrinsic factor activity can be lost without loss of vitamin B_{12} binding.

In addition to these findings, it is important to remember that vitamin B_{12} binding by intrinsic factor is only one of its properties. There may be several groups which are necessary for activity. Glass *et al.* (1957) and Gräsbeck (1959a) speculated that intrinsic factor may be thought of as containing a vitamin B_{12}-binding group and an "absorption group." Both groups

would be necessary for activity. If the "absorption group" were absent or destroyed, the material would be inactive but would retain its ability to bind vitamin B_{12}. A somewhat similar view has been expressed by Bunge and Schilling (1957). These hypotheses help explain why different investigators have obtained materials differing in vitamin B_{12} binding capacity but with similar activity; or conversely, why materials of similar binding capacity may vary greatly in potency. Inactivation of the "absorption group" could also explain the inhibition of vitamin B_{12} absorption sometimes associated with increasing doses of intrinsic factor (Glass *et al.*, 1955; Toporek *et al.*, 1955).

It thus becomes apparent that the use of vitamin B_{12} binding as a criterion of intrinsic factor activity is not unequivocal. However, with careful interpretation, this property of intrinsic factor can be helpful even though it is not completely quantitative or free of false positive results.

C. *In Vitro* Techniques

1. Enhancement of Vitamin B_{12} Uptake by Tissue

Numerous investigators have explored the possibility of an assay of intrinsic factor based on its *in vitro* enhancement of vitamin B_{12} uptake by tissues. O. N. Miller (1957) reported that intrinsic factor stimulated the uptake of vitamin B_{12} by serum proteins and suggested that this might be useful as an assay method. This technique proved to be nonspecific and appeared to be an indirect measure of vitamin B_{12} binding; thus, it is not being used at present. The same year, O. N. Miller and Hunter (1957) showed that hog intrinsic factor stimulated uptake of labeled vitamin B_{12} by rat-liver slices. This observation was rapidly confirmed by Herbert (1958a, b), Latner and Raine (1957, 1959), and Johnson and Driscoll (1958). Following this, it was also noted that intrinsic factor stimulated uptake of vitamin B_{12} by liver homogenates (Minard and Wagner, 1958) and enhanced vitamin B_{12} uptake by everted small intestine of the hamster, guinea pig, rabbit, and monkey (Herbert and Spaet, 1958; Herbert, 1959b; Wilson and Strauss, 1959; Strauss and Wilson, 1960; Johnson *et al.*, 1958; Wolff, 1962). *In vivo* studies showed that intrinsic factor also stimulated hepatic uptake of vitamin B_{12} in rats (Herbert and Spaet, 1958; Okuda *et al.*, 1959). This is surprising in view of the fact that hog intrinsic factor does not promote vitamin B_{12} absorption in the rat. Many of the above investigations have shed light on the mechanism of vitamin B_{12} absorption and transport. These will be discussed under Section V.

Although it is uncertain whether these techniques actually measure intrinsic factor activity, there is some preliminary evidence which indicates there is a fair correlation between intrinsic factor activity in the *in vitro*

system and clinical activity. Rhodes *et al.* (1959) used the *in vitro* system of Miller and Hunter (1958) to prepare hog intrinsic factor concentrate active at 0.6 mg, and Bromer and Davisson (1962) utilized the technique of Minard and Wagner (1958) in conjunction with clinical assays to obtain potent intrinsic factor-vitamin B_{12} complex. Even so, these techniques have not found general acceptance. More thorough studies are needed to determine their quantitative accuracy, particularly with purified preparations and inactive vitamin B_{12} binders obtained during fractionation. As with vitamin B_{12} binding methods, these techniques appear to be useful but have not proved to be completely quantitative or free of false positive results.

2. Inhibition of Cobamide Coenzyme Activity

In a collaborative study by Barker and Ellenbogen (Ellenbogen *et al.*, 1960a), the inhibition of cobamide coenzyme activity by hog intrinsic factor was determined. Good correlation was observed between the clinical activity of intrinsic factor preparations and their specific activity as inhibitors of the coenzyme-dependent, glutamate isomerase reaction (Barker *et al.*, 1960a, b). A comparison is given in Table I of eight intrinsic factor preparations with respect to clinical activity as measured by the Ellenbogen and Williams (1958) modification of the Schilling urinary excretion test (Schilling, 1953) and inhibition of the glutamate isomerase reaction. Correlation is good except for preparation number 16, a very crude sample with barely detectable vitamin B_{12} binding. It is of interest that heat-inactivated intrinsic factor showed a reduction in clinical activity proportional to its loss of specific activity in glutamate isomerase inhibition, e.g., preparations 2 and 3 (Table I), obtained by partial heat inactivation of preparation no. 1. These results are similar to those obtained when vitamin B_{12} binding by intrinsic factor was compared with intrinsic factor activity (Ellenbogen and Williams, 1960). This suggests that inhibition is due to binding of the coenzyme by intrinsic factor; further evidence is provided by the observation that inhibition by intrinsic factor can be completely overcome by increasing the coenzyme concentration. Heparin, lysozyme, and other proteins that bind vitamin B_{12} caused some inhibition of coenzyme activity, but their specific activities were quite low.

The measurement of cobamide coenzyme activity in the presence of intrinsic factor may serve as a useful *in vitro* assay method for purified intrinsic factor preparations. It is a much more rapid technique than dialysis binding. Studies of clinically inactive fractions, with high binding capacity, recently obtained during intrinsic factor fractionation (Highley and Ellenbogen, 1962), are needed to ascertain whether this technique might be more specific than measurement of vitamin B_{12} binding capacity. The use

of intrinsic factor in the study of the role of cobamide coenzymes in intermediary metabolism is discussed in Section VI, A.

D. *In Vivo* Techniques in Humans

The most widely used and accepted methods for measuring hog and human intrinsic factor activity are still based on the identical techniques that are used to measure vitamin B_{12} absorption in pernicious anemia patients; namely, fecal excretion, urinary excretion, hepatic uptake, blood plasma radioactivity, and total body radioactivity. These methods are used with pernicious anemia patients (treated or in remission) or in patients with total gastric resection. In addition, in pernicious anemia patients in relapse, intrinsic factor activity is measured by noting the clinical and hematological response obtained. These methods continue to be most unequivocal for the assay of intrinsic factor.

TABLE I

Comparison of Inhibitor and Intrinsic Factor (IF) Activities of Intrinsic Factor Preparations[a]

IF preparation no.	Protein conc. at 50% inhibition (μg/ml)	Specific activity as inhibitor (100 μg/ml) (a)	Intrinsic factor		Activity ratio (a)/(b)
			Minimum effective dose (mg)	Specific activity (1/mg) (b)	
1	61	1.64	1.0	1.0	1.6
2	139	0.72	2.5	0.4	1.8
3	136	0.74	2.5	0.4	1.9
4	60	1.67	1.0	1.0	1.7
5	105	0.95	1.5	0.7	1.4
6	106	0.94	2.5	0.4	2.3
9	316	0.32	8.0	0.12	2.6
16	390	0.25	100.0	0.01	25.0

[a] Ellenbogen *et al.* (1960a).

Until the last decade, intrinsic factor activity could be measured only in untreated pernicious anemia patients. This technique involved about 1 month of observation of hematological response based upon an increase in red blood cell count and increase in the number of reticulocytes. Today, untreated pernicious anemia patients are not available in sufficient numbers for testing. In addition, the hematological assay involves a risk while the patient is receiving a preparation of unknown potency. However, until 1960, this was the only official method for assaying commercial intrinsic factor preparations. Its details have been described by the U.S.P. Anti-Anemia Preparations Advisory Board (Pharmacopeia of the United States, 1955).

The availability of cobalt-labeled vitamin B_{12} has made it possible to

investigate quantitatively the absorption of vitamin B_{12} following oral administration. Various radioactive isotopes of cobalt have been used (Co^{56}, Co^{57}, Co^{58}, and Co^{60}) (Rosenblum, 1962a). Fortunately, all the cobalt isotopes have strong gamma emission permitting sensitive counting of the radiation. Excellent reviews of the radiological use and stability of the different isotopes of cobalt-labeled vitamin B_{12} have been presented by Rosenblum (1962a,b).

The enthusiasm with which the five radio-vitamin absorption techniques have been received is indicated by the fact that more than one hundred papers have been published on modifications, usefulness, and quantitative comparison of the different methods. The five methods will be reviewed briefly because of their importance in the clinical evaluation of intrinsic factor potency. The reader should consult the original references for detailed information. An excellent summary of the various methods used for clinical purposes is given by Mollin (1959).

1. Fecal Excretion

This technique, described by Heinle *et al.* (1952), was the first isotopic method used for the study of vitamin B_{12} absorption. An oral dose of 0.5–1.0 μg of vitamin B_{12} is usually given. The difference between the dose and the amount excreted in the feces represents the quantity of vitamin B_{12} absorbed. Normal subjects absorb from 50 to 60% of a 1.0-μg oral dose, whereas pernicious anemia subjects absorb less than 10%. When the same dose is given to pernicious anemia patients, together with fully active intrinsic factor preparations, absorption is increased to normal levels. Representative reports on the use of the fecal excretion test are given by Callender and Evans (1955a), Baker and Mollin (1955), Mollin *et al.* (1957), Swendseid *et al.* (1954), Callender *et al.* (1954a,b), and Halsted *et al.* (1956). A comparison of this method with the hepatic uptake and urinary excretion tests has been reported by Pollycove and Apt (1956).

The fecal excretion technique is in theory the most accurate and quantitative means of determining absorption since it yields an absolute result. Its disadvantages are that (1) 7–10 days are required to collect all unabsorbed fecal radioactivity; (2) it is the least pleasant of the methods; (3) because only a small percentage of a large dose is absorbed, it cannot be used to accurately measure absorption of doses much above 5–10 μg; and (4) fecal radioactivity does not represent exclusively unabsorbed vitamin B_{12} since it has been shown that some absorbed vitamin is excreted into the feces by the bile (Okuda *et al.*, 1958).

2. Urinary Excretion

The urinary excretion test was introduced by Schilling and is frequently called the "Schilling test." It is without doubt the most widely used method