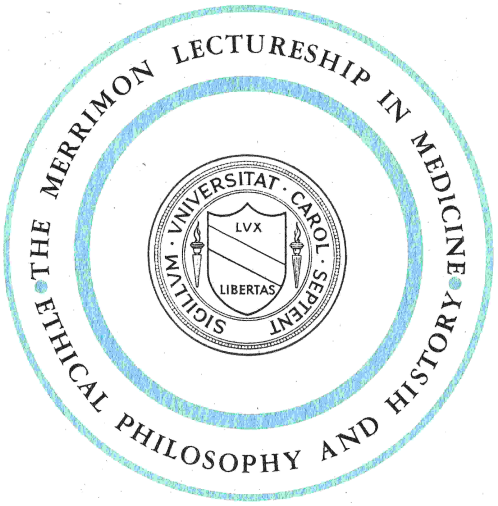
THE UNIVERSITY OF NORTH CAROLINA
SCHOOL OF MEDICINE

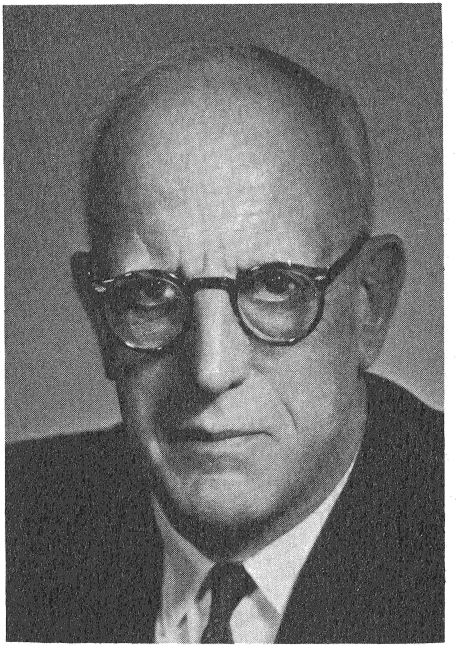


MERRIMON LECTURE

by

DR. WILLIAM BOSWORTH CASTLE

MARCH TWENTY-SECOND, NINETEEN HUNDRED AND SIXTY-SEVEN



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THE SECOND ANNUAL MERRIMON LECTURE

Pernicious Anemia: A Continued Study

by

DR. WILLIAM BOSWORTH CASTLE

Introduction

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MERRIMON LECTURE

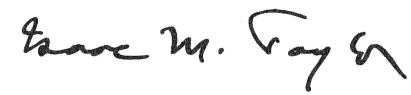
1967

Again, it is a pleasure to acknowledge with deep appreciation the bequest which, under the terms of the will of Dr. Louise Merrimon Perry, has made possible the Second Merrimon Lecture in Medicine. On Wednesday, March 22, 1967 Dr. William Bosworth Castle, Francis Weld Peabody Faculty Professor of Medicine, Harvard University, delivered the second annual lecture; "Pernicious Anemia: A Continued Study".

Like Dr. Nicholson J. Eastman, the first Merrimon Lecturer, Dr. Castle is an eminent medical statesman, and we are grateful that he was able to spend a week with us and, "to give to medical students and others interested an insight in the history, tradition, philosophy, and ethics of medicine".

The eminence of the first two Merrimon Lecturers augers well for this lectureship and promises to fulfill our hope of establishing the Merrimon Lectureship among the most honored in the Nation.

We look forward with anticipation and pleasure to future Merrimon Lectures.



ISAAC M. TAYLOR

Pernicious Anemia: A Continued Study

Introduction. It is a very great privilege to be invited by the Dean and Faculty of the University of North Carolina School of Medicine to present this second Merrimon Lecture, one of a continuing series concerning which the intent of the donor was "to give to medical students and others interested an insight in the history, traditions, philosophy and ethics of medicine." These high purposes could not have been better fulfilled than by the first lecture on "Induced Abortion and Contraception: A Consideration of Ethical Philosophy in Obstetrics," given last year by Dr. Nicholas J. Eastman of Johns Hopkins. It is indeed most fitting that this lecture series was initiated by a discussion of a subject of the first order of importance to society and medicine today. Recently, for the first time in history of Western medicine, physicians can no longer apply with unqualified benefit to society in general their traditional ethic of preserving life at every stage at all costs. Here in this university community this and related problems will be continuously presented for thoughtful consideration, because of the establishment of the Carolina Population Program. That Dr. Eastman so ably discussed subjects of such preeminent importance permits those Merrimon Lecturers who follow him to address themselves to matters of less urgency. Consequently, I hope that you will find some interest in listening for an hour to an account of some historical aspects of the development of knowledge concerning pernicious anemia, the fuller comprehension of which still occupies the active attention of medical students.

The chronological perspective of this disease first to be visualized is that until 1926 a diagnosis of pernicious anemia—unless mistaken—was a sentence of death within months or at most a few years as irrevocable as would still unhappily be today a diagnosis of wide-spread cancer (Fig. 1). Consequently, the conversion of a fatal human disease into perhaps the most easily and reliably treated of any is indeed a great medical achievement, certainly as viewed by a physician like myself within whose professional lifespan this miracle took place. Moreover, what at first seemed to be a unique human disorder has now been recognized as a process immediately due to a fundamental nutritional defect capable of affecting the nuclear metabolism of men, of mice and of microbes.

Therapeutic Triumph. Pernicious anemia has attracted the curiosity of the medical profession more or less continuously since 1855 when Addison described in the same monograph with his classic account of disease of the suprarenal capsules what his contemporaries sometimes found a recognizable disease entity. This is the more remarkable because his description included none of its clinically identifying features as we know them today: sore mouth, jaundice, and especially neural manifestations. However, Addison's

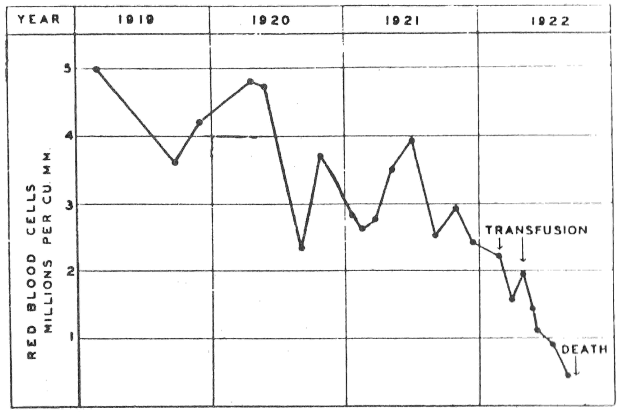


Fig. 1. Fatal course of a case of pernicious anemia before the advent of liver therapy. Note the progressively diminishing fluctuations in the number of red cells suggesting the dampening of a positive feed-back mechanism. Reversal of such a vicious cycle was possibly related to the tendency of temporarily improved nutrition to improve intrinsic secretion and thus vitamin B_{12} assimilation which in turn would increase red cell production and lessen hemolysis. (From a lantern slide in the collection of the late Dr. G. R. Minot)

inability to find a cause for the "idiopathic" anemia at autopsy served to segregate it from other varieties of less mysterious origin. Thus, he concluded only that "the disease having uniformly occurred in fat people . . . some form of fatty degeneration might have a share." Combe's earlier account of a fatal case in 1822, also said to be one of pernicious anemia, received little attention, although he was over a hundred years ahead of his time in correctly ascribing its proximate cause to "some disorder of the digestive and assimilative organs." However, in order to be an effective stimulus to the progress of such understanding, even repeated speculation of this sort had to survive the overwhelming professional concern with positive infective and toxic causes of disease introduced during the last half of the 19th century by the work of Pasteur, and to revive in a later era concerned with "the newer knowledge of nutrition."

More specifically, it was necessary for experimental work to develop a solid basis for the notion that a poor diet resulted in anemia. This, at last, was accomplished in the years following 1918 by George H. Whipple and his associates in Rochester, New York, through extensive experiments with chronically bled dogs. In these animals they established quantitative relations between enhanced hemoglobin production and diverse animal and vegetable supplements to a basal diet, such as liver, dog's muscle and spinach. Of these, liver was the most potent. Fortunately, it was not until more than a decade later that it was realized that the most significant influence upon hemoglobin production by these dogs was the amount and availability of the iron present in the various dietary items tested. Meanwhile, Whipple's experiments continued to pique the curiosity of himself and others concerning the effective properties of these foods. Indeed, his work, together with the subsequent dramatic application of liver feeding to pernicious anemia by George R. Minot and Willam P. Murphy of Boston, converted hematology from a largely descriptive to a dynamic subject characterized by productive clinical investigation that has lately contributed much even to molecular biology. As in other circumstances in medicine the success of an "empirical" treatment led the way to further understanding of the disease.

Whipple's observations were without doubt the immediate stimulus to several attempts in the early Twenties to treat pernicious anemia by dietary means. Indeed, remissions were actually produced by liver feeding in more than one clinic, but they were judged by experienced physicians to be "spontaneous." Minot had the impetus of a long-held clinical suspicion "that something in food might be of advantage to patients with pernicious anemia." It was, however, principally the use of the so-called "reticulocyte response" that enabled him to satisfy himself and shortly others that he and Dr. Murphy were on the right track. Their faith was founded on this Rock of Gibralter-like profile (Fig. 2). Reticulocytes, originally

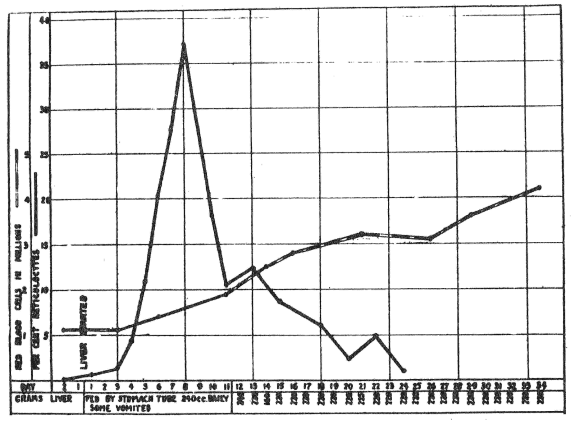


Fig 2. Response of a severely ill patient with pernicious anemia to raw liver feeding in the amounts indicated. Note the initial delay followed by the impressive rise in newly-formed red cells (reticulocytes) to a peak on the eighth day of therapy, followed by an increase in the number of red cells to over 4,000,000 in 34 days. (From a lantern slide in the collection of the late Dr. G. R. Minot)

thought by Ehrlich to be degenerating forms, were recognized as early as 1891 by Theobald Smith to be instead newcomers to the circulation from the bone marrow in response to the acute hemolytic anemia of cattle with Texas fever. He induced the appearance of these "granular forms" in the blood of a healthy cow after a delay of 4 or 5 days during which she was bled 2-4 liters daily—but never on Sunday! The significance of "curves plotted from frequent observations" of reticulocyte percentages as an index of red blood cell production was well-known to Minot as early an 1916 from clinical experience.

In 1926 Minot and Murphy using a diet containing "an abundance of food rich in complete proteins and iron—particularly liver—and relatively low in fat" reported consistent improvement in 45 patients. By 1928 collaboration with Prof. E. J. Cohn, later famous for his fractionation of plasma during World War II, had reduced the daily oral dose from half a pound of liver to 12.75 grams of so-called "Fraction G," a yellow powder commercially prepared through the collaboration of Eli Lilly and Company (Table I). In 1930 Gännslen in Germany introduced a nearly protein-free preparation for successful parenteral use, probably representing about 0.35 grams of dry material a day. Through the work of Dakin and West, who employed salting out procedures, by 1935 the daily parenteral dose of a commercial liver extract preparation had become as little as 15 milligrams parenterally. In 1945 came the false dawn of the synthesis of folic acid, soon to find its proper use in milligram dosage in a related type of anemia. Meanwhile, in 1948, vitamin B_{12} was isolated from liver extract by members of the pharmaceutical industry: Karl Folkers and his associates in the United States and almost immediately thereafter E. Lester Smith in England. Progress to this gratifying endpoint had been slow because of the necessity of clinical testing; and final success resulted from a combination of earlier fractionation methods with partition and adsorption chromatography, as well as from the long-overdue help provided by the discovery of the growth-stimulating

TABLE I

PROGRESS IN THE CONCENTRATION OF THE ANTI-PERNICIOUS ANEMIA PRINCIPLE

Date	*Substance**	*Dry Weight (gm)*
1926	Whole liver—half pound	60
1928	Liver "fraction G" (oral)	12.75
1931	Crude liver extract	0.35
1936	Refined liver extract	0.015
1945	Pteroylglutamic (folic) acid	0.001
1948	Vitamin B_{12}	0.000,001

*Parenteral administration unless otherwise indicated.

properties of highly purified liver extract fractions for a microbe, *Lactobacillus lactis* Dorner. Randolph West who had worked so long towards its isolation, was the first to demonstrate the clinical activity of crystalline vitamin B_{12} when injected in the form of cyanocobalamin in pernicious anemia. At 1 millionth of a gram a day, it is one of the most potent therapeutic agents now in use. Modern microbiological analysis indicates that the heroic pernicious anemia patients who ate a half a pound of raw beef liver a day were presented with about 60 micrograms of vitamin B_{12} and 600 micrograms of folic acid. Vitamin B_{12} is the largest water soluble vitamin (m.w. 1350) and is red because of the presence of a central cobalt atom in the corrin macro-ring, analogous to the position of iron in the porphyrin ring of hemoglobin (Fig. 3).

The unitarian concept concerning the various clinical forms of megaloblastic anemia, initially suggested by their response to liver feeding, was first shaken by Lucy Wills and her associates. Between 1930 and 1938, in a series of clinical observations on nutritional anemia in Bombay, especially in pregnant women, they showed that these macrocytic anemias responded to injections of crude, but not of the

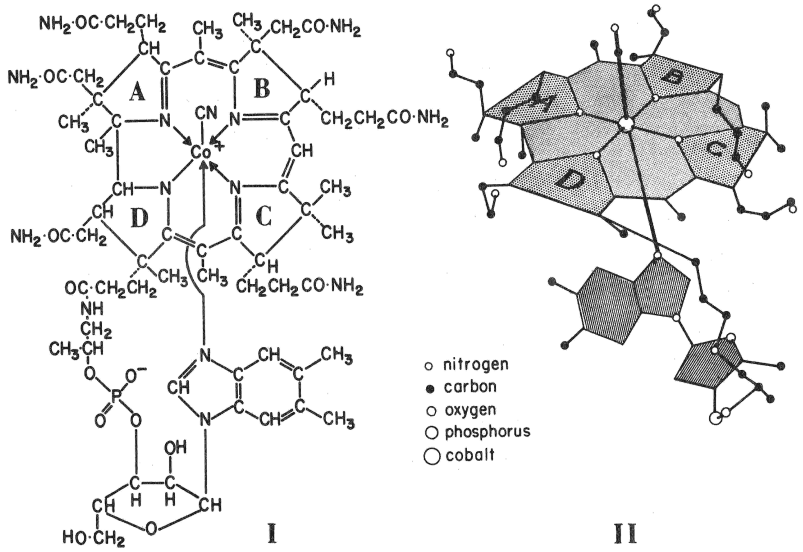


Fig. 3. Chemical formula of vitamin B_{12} (cyanocobalamin), I; and semi-diagrammatic, 3-dimensional representation of its structural formula, II, showing relations of the "planar" and "nucleotide" portions. In the physiologically active co-enzyme form in the body the CN group above the planar ring is replaced by an adenosine-like moiety (From Beck, W. S.: New England J. Med. 266: 708-714, 1962.)

then recently developed, purified liver extract, now known to be a fairly pure solution of vitamin B_{12}. These results were duplicated in monkeys on similarly defective diets. In 1940, Snell and Peterson described a microbial growth factor that was present in liver and yeast and could be adsorbed by and eluted from charcoal. The term folic acid was coined the next year by Mitchell for a similar substance in spinach and other green plants; and it remained only for Stokstad and for Pfiffner and his associates independently to isolate pteroylglutamic acid from liver in 1943 as a growth stimulant for *Lactobacillus casei.* Synthesis was soon accomplished; and in 1945 Spies and associates discovered that it was active in milligram daily doses in pernicious anemia. Subsequently, however, it was found that such patients when treated with only pteroylglutamic acid failed to maintain hematological remission and tended to develop neurological signs characteristic of pernicious anemia, sometimes very rapidly. Synthetic folic acid was soon discovered to be active in the tropical macrocytic anemia in India, in sprue with macrocytic enemia and in most cases of megaloblastic anemia of pregnancy of the temperate zone. We now recognize that anemias with morphologically indistinguishable blood and bone marrows are the result of nutritional deficiency, dietary or conditioned, of either vitamin B_{12} or folic acid or of both.

The Alimentary Tract. The limitations of time demand that from this point I pursue only the analysis of how the patient with pernicious anemia becomes a creature starving for vitamin B_{12} in the midst of plenty. For him, alas, it has become impossible to transport daily the essential millionth of a gram of the vitamin across the fraction of a millimeter depth of the small intestinal epithelial cell and into the blood stream. It will not be possible to discuss the fifty-year controversy between the opposing theories of decreased blood formation on the one hand and excessive blood destruction on the other. These are now happily reconciled as complementary causes of the anemia by our present understanding. Thus, vitamin B_{12} or folic acid deficiency plays a dual role. Not only is the synthesis of nuclear nucleo-protein (desoxyribosenucleic acid) in the blood-building cells of the bone marrow inhibited, but there is also an excessive destruction of red cells both within the bone marrow (ineffective erythropoiesis) and after their launching from the bone marrow into the circulation. These processes explain the characteristic megaloblastosis of the bone marrow and slight jaundice of the patient, respectively. Nor will time permit me to do more than point out that even today we have no understanding of how deficiency of vitamin B_{12}, but not of folic acid, brings about the grave neural disturbance characteristic of pernicious anemia.

The Tongue. Barclay first described sore mouth and tongue in 1851, but as his patient was a puerperal nursing female, she more

probably had folic acid than vitamin B_{12} deficiency as its cause. This was, of course, long before the recognition that glossitis is a frequent feature of vitamin deficiency diseases such as pellagra or sprue and is even observed in severe iron deficiency. The subjective discomfort and the "inflamed" or "smoothed-out" appearances of the tongue are now understood to be manifestations of the effect of such deficiencies upon the metabolism and morphology of its epithelial cells. Macrocytosis and nuclear abnormalities of such cells in oral and gastric washings as well as in intestinal biopsies have now been reported in vitamin B_{12} and folic acid deficiencies. However, in the closing years of the nineteenth century medical thought was still dominated by the fruitful application of the discoveries in bacteriology. This intellectual climate fostered the growth of a conviction on the part of William Hunter of London that the inflamed tongue, which he regarded as an essential feature of pernicious anemia, was due to an infective process. He obtained pure cultures of *Streptococcus longus* from the interior of the afflicted patients' tongues. He believed that the inflammatory process was due to such organisms and became by extension responsible for the esophagitis and gastritis and therefore eventually for the failure of hydrochloric secretion in the stomach in pernicious anemia. Although Hunter's bacteriological observations could not be confirmed and his therapeutic recommendations with respect to oral sepsis were without benefit to patients in London, removal of the broad tapeworm from anemic patients in Finland was curative of the associated anemia with some regularity. Here seemed to be an even more obvious source of a hypothetical toxin which, according to Faber, could produce nervous, digestive and hematological abnormalities resembling those of idiopathic pernicious anemia. Today, when interpreted in terms of vitamin B_{12} deficiency the etiologic significance of both types of intestinal parasites has been clarified.

The Stomach. Combe of Edinburgh, as has already been mentioned, deserves the credit for the correct surmise in 1822 that pernicious anemia was what we would call today a "conditioned deficiency disease." Although serious pursuit of this concept did not begin until over a hundred years later, cogent arguments in its support were presented in the interval. Thus, Austin Flint, Sr., he of the murmur and a remarkable American physician and teacher, narrowed the indictment by exclusion to the stomach. In two or three autopsied patients with pernicious anemia, Flint, like Addison, had found no gross abnormalities. However, he was aware that Handfield Jones had published in 1855 an account of the microscopic examination of 100 human stomachs in which he found considerable atrophy of the secretory glands in 14. Consequently, Flint in 1860 was led to remark that such an important organ might, like the kidney, "undergo degenerative disease not rendered distinctly apparent to the naked eye"; and he went on to say, "I suspect that in these cases there exists de-

generative disease of the glandular tubuli of the stomach . . . fatal anemia must follow an amount of degenerative disease reducing the amount of gastric juice so far that assimilation of food is rendered wholly inadequate for the wants of the body. I shall be ready to claim the merit of this idea when the difficult and laborious researches of someone have shown it to be correct." A decade later in 1870, Samuel Fenwick of the London Hospital examined post mortem the stomach of a patient considered to have Addison's "idiopathic anaemia." Under the microscope it appeared that the "glandular structure was in a state of atrophy" (Fig. 4). A suspension of scrapings from the withered mucous membrane acidified with hydrochloric acid failed to reduce the weight of a cube of hard boiled white of egg during incubation for 9 hours at blood heat. From these observations, he concluded that "the progressive atrophy of the stomach had prevented the digestion of the albuminous material of the food . . ." Nevertheless, he blunted the force of his argument by reporting in the same article that patients who had died of cancer of the breast had similar degenerative processes in the stomach.

This demonstration of a lack of peptic hydrolysis after death was not followed until 1886 by the first proof of a lack of hydrochloric acid in the stomach contents during life by Cahn and von Mering. However, by the turn of the century, the ability of the patient's stomach

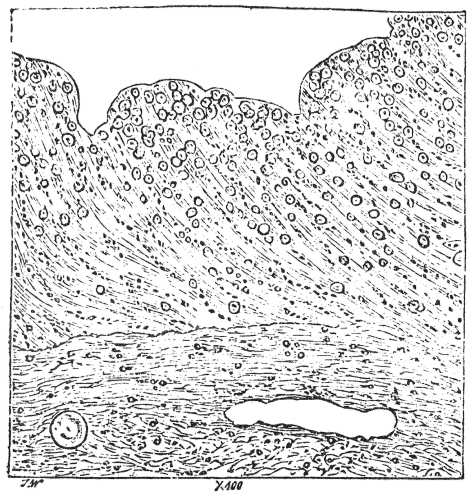


Fig. 4. Drawing of a microscopic section of the atrophic mucosal lining of the stomach of a case of pernicious anemia. A modern stained section of normal mucosa would show many glandular structures lined with "chief" cells capable of secreting pepsin and "parietal" cells capable of secreting hydrocloric acid and intrinsic factor (From Fenwick, S.: Lancet 2: 78-80, 1870.)

to secrete hydrochloric acid had become a convenient measure of gastric function and had been weighed and found wanting in many patients with pernicious anemia. The concept of the etiological role of achylia gastrica owes much to the work of Arthur Hurst of Guy's Hospital, who by the end of the first quarter of this century, recognized the constancy of a lack of hydrochloric acid in pernicious anemia and its persistence during spontaneous remissions. He also gathered instances of pernicious anemia developing after subtotal gastrectomy as well as in patients with cancer of the stomach. Moreover, achlorhydria was found to have preceded the appearance of pernicious anemia by many years and, like the disease itself, to be distinctly more common in the relatives of persons with established pernicious anemia.

Like Hunter, Hurst believed in the infective origin of the anemia, but as a failure of intestinal antisepsis resulting from a *primary* achlorhydria. However, at that time, twenty-five or more years after Hunter's work, an entirely different conception of certain disease states had gained firm recognition: that morbid processes may be due to defective nutrition. By the early twenties, beri-beri as well as pellagra with its sore mouth and neural changes and early sprue with its macrocytic anemia and diarrhea were being treated with considerable success as dietary deficiency diseases. In 1925, Elders, in Holland, reported increases in the blood values in a patient with pernicious anemia for whom he had prescribed the same dietary regimen that he had found, while previously working in Sumatra, to be "invariably successful" in cases of sprue. It included a kilogram or more of underdone meat daily. Only a year later came the work of Minot and Murphy.

The dramatic success of liver feeding immediately raised the question as to why normal persons did not need to eat a half-pound of liver a day in order to stave off pernicious anemia The sole irremediable difference between the normal individual and the patient, whether developed spontaneously or as a result of gastrectomy, seemed to be a lack of gastric secretion. How was it that the stomach of the normal person could derive something from ordinary food that was for him equivalent to eating liver? With these thoughts in mind, it was not difficult to suppose that "by substituting some digestive process of the normal stomach . . . it might be possible to affect the patient's disease favorably." The daily reticulocyte counts performed on Minot's patients seemed to provide a means of testing this idea promptly. Although unknown to us at the time, it was, of course, essentially that of Flint and of Fenwick.

On the basis of Elder's employment of underdone meat, conceivably successful because applied to an early stage of the disease, our first attempt in 1927 was to determine whether or not the daily administration by stomach tube of the liquified stomach contents of a healthy normal man removed one hour after the ingestion of 300

grams of lean beef muscle would cause new red cell production in untreated pernicious anemia. When this proved to be the case, it was soon shown in other patients that 159 milliliters of neutralized human gastric juice secreted by normal medical students under the stimulus of histamine were without effect, as were 200 grams of beef muscle. However, when such amounts of beef muscle and normal gastric juice were given together, a reticulocyte response appeared and the increased red cell production resembled that obtained with moderate amounts of liver given by mouth (Fig. 5). Consequently, it was assumed that some unknown but essential interaction between beef muscle as an *extrinsic factor* and normal human gastric juice as an *intrinsic factor* was required for the restoration of normal blood production in the patient with pernicious anemia.

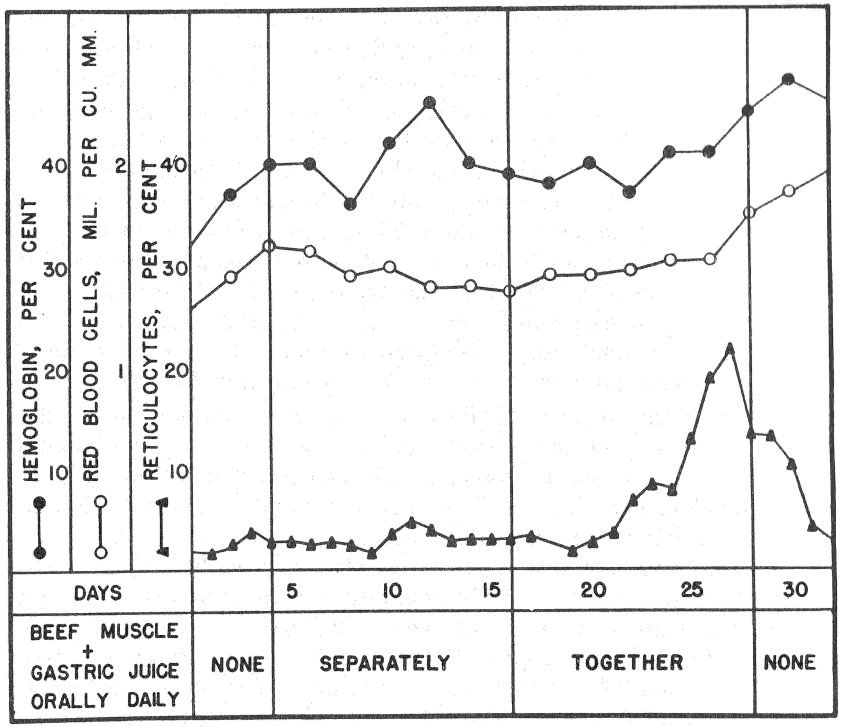


Fig. 5. Negative effect in a patient with pernicious anemia of separate administration of extrinsic and intrinsic factors followed by postive effect when these substances were given together. In the period of separate administration 200 gm. of beef muscle were given daily at 8:00 a.m. and 150 ml or normal gastric juice at pH 7 were given at 8:00 p.m. No reticulocyte response appeared until the seventh day of the period of combined administration, and then the reticulocyte peak was reached on the 12th day of this period. Only the beginning of the subsequent rises in red cell and hemoglobin values is shown. (From Castle, W. B: Chapter 14 in *Pathologic Physiology: Mechanisms of Disease,* edited by W. A. Sodeman, Philadelphia: W. B. Saunders Co., 1950.)

Over the next twenty years, the reticulocyte response method was employed in similar observations on patients with pernicious anemia with the object of determining the nature of both of the factors normally involved in blood formation and the conditions essential for their apparent interaction. As early as 1931, Reimann had demonstrated what appeared to be a 30-fold potentiation of liver by mixture with gastric juice; and later this test for the presence of extrinsic factor was shown by others to apply also to a crude liver extract. In 1948, a few months after the identification of vitamin B_{12} as the anti-pernicious anemia principle of liver, Lionel Berk, working in Boston found that a purified injectable liver extract when given simultaneously by mouth with gastric juice was rendered active in red cell formation. This at once led to a similar demonstration of the potentiation by gastric juice of vitamin B_{12} itself when given daily. Microbial analysis of 70 per cent alcoholic extracts of beef muscle then showed them to contain vitamin B_{12} in amounts sufficient to explain the potentiating effects of gastric juice upon the unknown extrinsic factor of beef muscle observed earlier. Indeed, I microgram of vitamin B_{12} was rendered erythropoietically active when given orally with as little as 10 milliliters of gastric juice, but not to the extent of the same amount of vitamin B_{12} given parenternally (Fig. 6). It was inferred that the essential physiological action of intrinsic factor was merely to enchance the assimilation of the extrinsic factor in the form of vitamin B_{12}. This surmise shortly became a fact when radioactive vitamin B_{12} became available to Heinle and Welch in 1952. Their pioneer work, followed by that of Schilling, Glass, Hagen and Doscherholmen and others provided convenient methods by which the enhanced absorption of radioactive vitamin B_{12} by intrinsic factor could be demonstrated, whether or not the patient was anemic. In retrospect the nature of intrinsic factor, might have become clear much earlier if, as pointed out in 1938 by William Dock, parenteral administration of beef muscle extract had been pursued more actively and so the probable identity of extrinsic factor and the anti-pernicious anemia principle of liver established.

Intrinsic factor was not detected in early observations on normal human saliva or on aspirated duodenal contents from which gastric juice had been excluded by a balloon inflated in the pylorus. Moreover, as is now known, its natural presence together with vitamin B_{12} made possible the successful therapeutic use of dessicated, defatted hog stomach in 1929 by Sturgis and Isaacs. Meulengracht later showed that the site of secretion in the hog stomach is, surprisingly, not the thick pepsin-secreting fundus area. Instead, the "pyloric

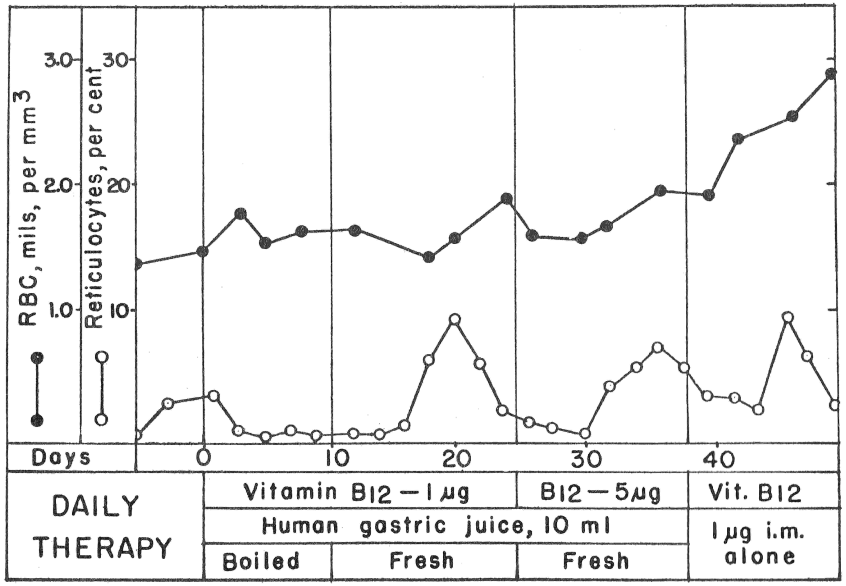


Fig. 6. Potentiation of the effect of small amounts of vitamin B_{12} by simultaneous daily administration of normal human gastric juice. Note the negative effect of 10 ml of boiled gastric juice with 1 µg of vitamin B_{12}, followed by a first reticulocyte response when 10 ml of fresh gastric juice was substituted. Evidently assimilation of small amounts of vitamin B_{12} will occur in pernicious anemia until the secretion of intrinsic factor has become reduced below this level. When the amount of vitamin B_{12} was increased to 5 µg daily second reticulocyte response developed. However, the parenteral administration of 1 µg. vitamin B_{12} daily produced a third reticulocyte response indicating its greater ability to cause increased red cell production. (From Castle, W. B.: Med. Clin. North America *50*: 1245-1256, 1966).

gland organ" and, to some extent, the cardia of the hog stomach are active sources, as is the duodenum. Clinical observations by Dr. Herbert Fox, now of Durham, with similar material of human origin demonstrated that intrinsic factor is secreted throughout most of the human stomach except the pyloric region, which is not involved in the atrophic gastritis characteristic of pernicious anemia. Recently Hoedemaeker et al. of Groningen have shown that the cytoplasm of the parietal, acid-secreting, cells of the human stomach is actively labeled by exposure to a solution of radioactive vitamin B_{12} in vitro. Thus, these cells are the probable souce of intrinsic factor in man.

It was early found that unlike the extrinsic factor, the intrinsic factor activity of normal human gastric juice and of hog stomach was destroyed at once by boiling or in 30 minutes by temperatures of from 70° to 80°C. as well as by 70 per cent alcohol. From this and by analogy with known functions of the stomach, it was assumed that some type of enzyme had been inactivated. However, commercial pepsin derived from the hog stomach was ineffective and no other type of enzyme tested was found to possess the essential property of intrinsic factor. In 1949, a quite different idea of intrinsic factors function was introduced through the discovery by Ternberg and Eakin that gastric juice inhibited the multiplication of bacteria requiring vitamin B_{12} for their growth. This property, referred to by them as "binding," like the clinical activity of intrinsic factor, was readily destroyed by boiling. Subsequently it has been learned that all native sources of intrinsic factor and its concentrates possess active binding capacity for vitamin B_{12} when studied by improved dialysis or absorption methods employing radioactive vitamin B_{12} and specific anti-intrinsic factor serum. That this binding property is of undoubted physiological significance was shown by Bishop et al. in observations demonstrating the preferential absorption in pernicious enemia of radioactive vitamin B_{12} after being in contact with gastric juice in vitro for some time before simultaneous administration of that "loaded" gastric juice together with an equal amount of "cold" vitamin B_{12} to the patient.

In 1952 Glass and his associates reported that the acid glandular mucoprotein of human gastric juice possessed the essential function of intrinsic factor with respect to vitamin B_{12}. Soon he and others using electrophoretic, chromatographic and other methods produced active preparations with molecular weights ranging from 5000 to 50,000 (Table II). All those tested have contained besides amino acids, hexose, hexoseamine, and sialic acid. As little as 100 micrograms have been shown to be active in the Schilling test. Recently Gräsbeck in Helsingfors and Ellenbogen in this country have produced homogeneous intrinsic factor–vitamin B_{12} complexes of simi-

TABLE II

INTRINSIC FACTOR

Stomach only (man)
Parietal (HCl-secreting) cells
Mucopolypeptide, m.w. 50,000±
Thermolabile 100° C.
Stable at pH 10
Slow anodic mobility at pH 8.6
Binds vitamin B_{12}
No blood group activity

lar molecular size from human gastric juice and hog stomach extract, respectively. Possibly in each instance two molecules of intrinsic factor are linked by one or more molecules of vitamin B_{12}.

The Intestine. In 1932 Birkeland pointed out that the decisively beneficial effects of expulsion of the broad tapeworm *Diphyllobothrium latum* practiced in Finland after about 1885 suggested that the parasite acted as a means of ushering in what sometimes appeared later to be classical pernicious anemia. In 1939 von Bonsdorf in Helsingfors began a series of observations that showed that such patients may not respond erythropoietically to mixtures of beef muscle and gastric juice until after the worm has been removed. Indeed, thereafter some patients exhibiting moderate ability to secrete hydrochloric acid and therefore probably intrinsic factor, responded to beef muscle alone. In 1951, vitamin B_{12} was substituted for beef muscle in his observations with similar results. The broad tapeworm is now known to contain about 2 micrograms of vitamin B_{12} per gram of dried weight and patients with Addisonian pernicious anemia have actually responded to mixtures of gastric juice and dried, pulverized tapeworm. Thus, probably with sufficient reduction of intrinsic factor secretion, the tapeworm may deprive the patient of most of the vitamin B_{12} in the food, especially if the parasite is large and is located in the proximal ileum well above the site of absorption of vitamin B_{12}.

Under certain conditions, intestinal bacteria are capable of similar competitive activity. As a consequence of various anatomical lesions of the small intestine, rapidly multiplying bacteria, normally not present in the small bowel, may either adsorb or utilize vitamin B_{12}. Thus packaged, the vitamin is carried out of the body of patients with small intestinal diverticula, intestinal anastomosis or a "blind loop" of intestine. In such patients temporary sterilization of the bowel by broad-spectrum antibiotics may greatly improve its ability to assimilate vitamin B_{12} (Fig. 7). Such recent observations explain Faber's pioneer finding in 1895 of pernicious anemia in a patient with an intestinal stricture. They clarify the experimental results of Seyderhelm, who in 1924 produced ileal strictures in 10 dogs in 2 of which a progressive hyperchronic anemia appeared, associated with a luxuriant growth of bacteria in the stagnant bowel above the partial obstruction. In 1929, Little and associates reported the first case of pernicious anemia with a "blind loop" following surgical anastomosis of the intestine as distinct from stricture. In many patients with regional disease (ileitis) or surgical resection of the distal part of the small bowel macrocytic anemia responsive to vitamin B_{12} has now been observed. In 1959 it was shown by Booth and Mollin at laparotomy of "normal" subjects 3 hours after the administration of radioactive vitamin B_{12} that the absorption of vitamin B_{12} from food normally does not take place in the proximal

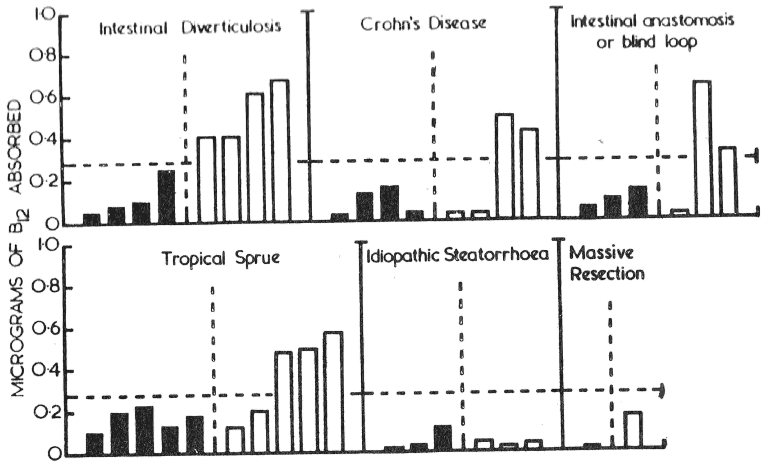


Fig. 7. Effect of antibiotics on the absorption of radioactive vitamin B_{12} by 20 patients with the malabsorption syndrome. Each patient received an oral dose of 1 μg. of radioactive vitamin B_{12} plus 50 mg. of hog intrinsic factor before and then after a course of antibiotics. The black columns denote the amount of vitamin B_{12} absorbed before the course of antibiotics. The open columns denote the amount of vitamin B_{12} absorbed after the course of antibiotics. In the patients with idiopathic steatorrhea and massive resection the negative effect is due to gross damage and to removal, respectively, of the special absorbing area of the distal ileum. (From Mlolin, D. L. Booth, C. C. and Baker, S. J. Brit. J. Haematol. 3: 412-428, 1957.)

ileum where folic acid is absorbed in man, but instead in the distal portion. Present knowledge of the clinical pathogenesis of vitamin B_{12} deficiency is summarized in Table III.

In 1955 came to an end the exclusive study of pernicious anemia in patients. In that "Year of the Rat" Watson and Florney demon-

TABLE III

PATHOGENESIS OF VITAMIN B_{12} DEFICIENCY*

1. Food defect: animal protein lack
2. Gastric defect: intrinsic factor lack
   a. P. A.—congenital, adult auto-immune (?)
   b. Gastrectomy, diffuse gastric cancer
3. Intestinal defect: malabsorption
   a. Parasites—*D. Iatum*, diverticuli "blind loop", I. F. antibiotics (?)
   b. Distal ileum—resection, inflammation, "receptor" lack
   c. Diffuse—steatorrhea. sprue, gastro-colic fistula

*Develops when less than 1 microgram is absorbed daily.

strated that removal of the secretory portion of the rat stomach rendered the animal unable to assimilate tracer does of radioactive vitamin B_{12} unless a source of rat intrinsic factor was simultaneously supplied. Human or hog stomach preparations were ineffective. Thereafter, the study of pernicious anemia or at least of the assimilation of radioactive vitamin B_{12} could be pursued in any laboratory, clinical or otherwise. Shortly, organic fragments of laboratory animals such as liver slices, everted loops of intestine and even mucosal scrapings were yielding new information at an accelerated pace, concerning the action of intrinsic factor. The guinea pig intestine was found to absorb increased amounts of radioactive vitamin B_{12} under the influence of hog or human intrinsic factor. In Table IV are shown the presumed sequence of events involved in the enhanced assimilation of vitamin B_{12} from the normal low concentrations in the food by virtue of intrinsic factor action.

Again, however, in 1957 new insights into the cause of the atrophy of the stomach in pernicious anemia arose from the study of patients by Schwartz of Copenhagen. He noted apparent refractoriness to orally administered combinations of partially purified hog stomach extract and vitamin B_{12}. When the serum of these patients was mixed with intrinsic factor, and given by mouth to ther patients with pernicious anemia, the assimilation of radioactive vitamin B_{12} was inhibited. Shortly thereafter Keith Taylor in Oxford showed that this was also true of the serum of pernicious anemia patients who had never even eaten a pork chop! Today, we recognize that about half of all patients with pernicious anemia develop antibodies against human intrinsic factor in their serums and that about 40 per cent have antibodies directed against a complex of vitamin B_{12} with intrinsic factor (Table V). Confined to the serum these antibodies do not impede the assimilation or transport by the blood of vitamin B_{12}; but there is now evidence that, especially the antibody directed against the vitamin B_{12}-intrinsic factor complex, may leak through

TABLE IV

PHASES OF INTRINSIC FACTOR (IF) ACTION

I. IF competitively binds food B_{12}, especially at acid pH value (B. Cooper)
II. IF–B_{12} is resistant to pepsin, chymotrypsin and parasites (R. Gräsbeck)
III. IF–B_{12} adheres to intestinal surface in presence of C++ and pH > 6.5 (V. Herbert)
IV. Species related pinocytosis* of IF–B_{12} complex; and release of B_{12} within intestinal cell (T. H. Wilson)

*Cell "drinking" or phagocytosis at a molecular level. This may be how the large molecular complex can enter.

TABLE V

ANTI-INTRINSIC FACTOR (IF) ANTIBODIES

Gastritis without P.A.	very rare
Congenital P. A. (free HCl)	none
Adult P. A. (no HCl)	usual
Against B_{12} binding site	(J. Abels)
In serum	50%
In gastric secretion	rare
Against B_{12}–IF complex	(R. Schilling)
In serum	40%
In gastric secretion	40%

or be secreted by the withered stomach mucosa. Here it may antagonize residual traces of intrinsic factor.

Another kind of serum antibody, this time directed against the cytoplasm of the parietal stomach cells that secrete intrinsic factor often appears in the serum before pernicious anemia develops and is present in about 90 per cent of such patients and in more than a third of patients with destructive disease of the thyroid gland, and in some of their relatives (Table VI). Moreover, there is a clinical association between pernicious anemia with gastric atrophy and myxedema with thyroid atrophy. Most of such thyroid patients have antibodies against the cytoplasm of thyroid cells, but about half of them also have a different antibody against parietal cell cytoplasm. At the moment, this is only guilt by association, but it seems possible that the underlying cause of the gastritis that destroys the stomach as a prelude to pernicious anemia may resemble that

TABLE VI

"AUTOANTIBODIES" IN PERNICIOUS ANEMIA AND THYROIDITIS

(Patients and Relatives)

Antibodies (Immuno-fluor.)	*Reactors Positive of Percentage*				
	Pernicious anemia		Hashimoto, myxedema		Hospital controls
	Pts.	Rels.	Pts.	Rels.	
Gastric parietal cytoplasm	89	36	32	20	2-16*
Thyroid acinar cytoplasm	55	50	87	46	0-15*

*Incidence increases with age; F >M

which destroys the thyroid and causes myxedema. It remains to discover whether the hereditary tendency to develop cell-specific antibodies in both diseases is due to a common susceptibility to a type of auto-immunity or is a response to as yet unknown antigens in the environment.

Conclusions. Whatever may be the future of clinical investigation, the study of pernicious anemia has already provided insight into matters previously unsuspected. Two new vitamins of fundamental importance to cellular metabolism were discovered, vitamin B_{12} and folic acid. Synthetic analogs of the latter help to control acute leukemia in children. The discovery of cobalt in the vitamin B_{12} molecule explained its importance as a trace mineral in animal nutrition. The intrinsic factor of gastric juice, a still obscure slime, is nevertheless the only indispensable secretion of the human stomach. Future study of the similarities between the gastritis involved in pernicious anemia and that involved in thyroiditis may lead to better understanding of a type of auto-immunity common to both. Clinical analysis of factors concerned in vitamin B_{12} absorbtion has stimulated renewed interest in the mechanisms of alimentary assimilation and has provided a scientific basis for the detrimental effects of certain intestinal parasites upon nutrition. The strikingly abnormal morphological and staining characteristics of the erythropoietic cells in pernicious anemia led to early histochemical studies concerning the biological functions of desoxyribose-and ribose-nucleic acids. Thus, using tools borrowed from physiology and biochemistry, physicians studying pernicious anemia have presented challenging new vistas in many fields of biology and medicine.

W. B. CASTLE

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