Etiology of pernicious anemia

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THE ETIOLOGY OF PERNICIOUS ANEMIA

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I
INTRODUCTION

"Much has been done; much remains to do; a way has been opened."
Sir William Osler "Aequanimitas and other Addresses".

In 1824 an Edinburgh physician, Dr. J.S. Combe, published in the Transactions of the Edinburgh Medico-Chirurgical Society the first accurate report of the symptoms, signs, and post-mortem findings of a case of pernicious anemia. More than a century has since elapsed, but the fundamental cause of pernicious anemia remains undetermined, although the missing links of a pathogenetic chain are slowly being forged and the future holds promise of solutions to the basic questions of pernicious anemia. The complexity of the symptomatology, the wide involvement of tissues, the occurrence of remarkable remissions and relapses, and the formerly almost invariably fatal issue have attracted great interest from the medical profession. Controversy has raged around the etiology of the disease.
and has resulted in a vast literature concerning the etiological and other aspects of pernicious anemia. Our knowledge of the disease has undergone frequent and fundamental revisions as a result of laboratory and clinical research and advances. Discoveries have not come by chance but represent the fitting-together of accumulated discoveries and individual advances into a growing logical pattern.

In the realm of therapeutics the treatment of pernicious anemia was formerly empirical and rather unsatisfactory. Every conceivable method of treatment had its ardent exponents and false hopes, based on insufficient realization of the occurrence of fairly complete remissions with or without treatment, were continually being raised only to be discarded as failures. Previous to the work of Minot and Murphy innumerable methods of treatment existed, each claimed by its exponents to have some particular beneficial action. The very large number of advocated treatments bespoke their lack of efficiency and specificity. The introduction in 1926 of a specific active therapeutic agent radically altered the therapeutic rationale in pernicious anemia and in addition to materially reversing the inevitably unfavorable prognosis of the disease and restoring health, it has served to inspire much research that has proven of great worth.
The past decade has witnessed the development of a new and useful knowledge in spite of the fact that many questions of etiology and pathogenesis are not wholly answered.

In addition to therapeutic and other advances great gains have been made during the past decade in the knowledge of blood formation, blood destruction, and pigment metabolism which have greatly helped in the solution of the riddle of pernicious anemia. Refined methods in hematology, bacteriology and bio-chemistry have added their quota of aid in solving the problems of blood diseases.

I shall attempt in this paper to discuss some of the etiological conceptions of pernicious anemia. Stress will be laid on the modern views in etiology and pathogenesis that have been brought forth in the past decade. An introduction to the modern concepts will be furnished by a brief review of the more rational concepts that have preceded our modern views. Fundamental problems such as erythropoiesis, erythrocyte destruction and pigment metabolism will be discussed and will serve to emphasize the fundamental difficulties encountered in establishing an etiological basis for the disease termed pernicious anemia that is slowly being conquered by modern medical science.

The history of medicine is replete with instances of controversy over various medical problems but the contro-
versy that has raged over the etiology and pathogenesis of pernicious anemia has been long and varied. Many theories have been proposed but none has completely swept aside the veils hiding the ultimate factors concerned in the production of the pernicious anemia as a disease entity. A certain number of definite facts have been established but their implications have led to a much larger number of hypothetical ideas. Positive findings have been emphasized even when they have failed to explain other manifestations of pernicious anemia. The work of Minot and Murphy has led many to believe that the etiological question has at last been settled but this belief is far removed from the truth. The position is somewhat analogous to that of diabetes mellitus. In both diseases a valuable therapeutic agent is at hand but the fundamental mechanisms whereby the diseases are produced are still unknown.
Many treatises on anemia have appeared from the earliest times but the descriptions contained therein have not been sufficiently clear to definitely establish the case-reports as those of pernicious anemia. The historical aspect of pernicious anemia is found to divide itself into four periods. These are:

1. The earliest recognition of the disease as a clinical entity (1822-1855).

2. The independent recognition of the disease by Biermer and the union of several forms of the condition under the name of progressive pernicious anemia (1871).

3. The era of Hunter and Ehrlich and their controversy as to the relative importance of blood destruction and blood formation in the pathogenesis of the disease.

4. The modern period marked by increased knowledge of blood formation and destruction, pigment metabolism, the epochal advance in therapy, and the newer knowledge of
physiological mechanisms.

In 1822 a Scottish physician, Dr. J.S. Combe, (1), published in the Transactions of the Medico-Chirurgical Society of Edinburgh a description of a case of profound anemia in such detail and accuracy of description that there is little doubt that the description was that of a description was that of a patient suffering from pernicious anemia. Combe's description was as follows:

"The case now recorded appears to me entitled to still further attention as exhibiting a well-marked instance of a very peculiar disease, which has excited little attention among medical men, and which has been altogether overlooked by any English author with whose writings I am acquainted. Unfortunately, however, such is the allowable diversity of opinion on most medical subjects that it is very possible the following case may be viewed in different lights, and receive different appellations; and while some may be disposed to regard the peculiar characteristic from which it derives its denomination of anemia as constituting a morbid state sui generis, others may consider the defect of the red circulating mass as an accidental and occasional circumstance, denoting some peculiar change in the assimilative powers, the primary stages of which we have been unable to detect. Doubtful myself which of these opinions may be the most correct, I shall do little more than state correctly the phenomena of the case and minutely the appearances presented on dissection. One remark only I may at present offer, that if any train of symptoms may be allowed to constitute anemia a generic disease the following may be considered an example of it in its most idiopathic form.

The case was a man, aged forty-seven, who had been born and had spent the greater part of his life in the country, where his duties were neither laborious nor unhealthy; who had led a regular and temperate life, and had enjoyed perfect health since childhood, and had never lost any blood.

I was much struck by his peculiar appearance. He exactly resembled a person just recovering from an attack of syncope; his face, lips and whole surface were of a deadly pale colour; the whites of the eyes bluish; his motions and speech languid; he complained much of weakness; his respiration, free when at rest, became hurried on the
slightest exertion; pulse 80 and feeble; inner part of the lips and fauces nearly as colourless as the surface; bowels very irregular, generally lax, his stools very dark and foetid; urine reported to be copious and very pale; appetite unimpaired, of late his stomach has reject-ed almost every kind of food; constant thirst; he has no pain referable to any part, and a minute examination could not detect any structural derangement of any organ.

It was only about two months ago since he began to complain, but not until his friends had observed his altered complexion; he then lost strength and said his head troubled him. Of his last symptom he has no distinct recollection. His feet became oedematous and his appetite failed him.

My attention was drawn to the skin, which was of wax-en colour, soft and delicate, the cellular tissue about the eyes and breast slightly distended with watery effusion. The pulse was feeble and easily excited by any emotion. A very minute examination of the case, and a careful consider-ation of its history, scarcely solved the nature of the affection; and its long continuance and inveteracy rendered our prognosis much more doubtful. ***

POST-MORTEM.--The subcutaneous fat was scanty, of a pale yellow colour, and semi-fluid. Not a drop of blood ex-caped on dividing the scalp. The heart when cut into was a pale colour, and did not tinge linen when rubbed on it; it appeared like flesh macerated many days in water. The right ventricle contained a pale coagulum. The left side was wholly empty. There was a considerable moisture be-dewing the viscera of the abdomen. The liver was of its proper size and structure, but of a light-brown colour. The spleen was the only viscus of its usual colour; it was very soft, and its contents, on pressure being applied, turned out like a sac. The kidneys were nearly bloodless; pancreas of a pale reddish hue. The stomach and intestines were perfectly sound, thin, showing no vessels, and trans-parent. The muscular substance throughout the body was, like the heart, very pale, and exuded no blood, but only a pale serum, when cut into. The arteries were empty, and so were the jugular, femoral and humoral veins.

The only morbid appearances detected may be considered the effusion into the thorax and abdomen, the ossification of the dura mater, and the nearly bloodless state of every viscus and structure in the body, with the exception of the spleen. A state like that of our patient's in which every organ was nearly deprived of its red blood, is one, I believe, of very rare occurrence, and of which we have very few cases on record."

His statement "that if any train of symptoms may be
allowed to constitute anemia a generic disease, the fol-
lowing may be considered an example of it in its most id-
iopathic form" is of prime importance, since this was
the first time any author had claimed that any special
type of anemia existed. Another important point he brought
out was that he was able to find nothing in the clinical
or post-mortem findings to explain the condition. He sug-
gested, however, that "it is probably owing to some dis-
order of the digestive and assimilative organs that its
characteristic symptoms have their origin," thus antici-
pating Hunter and the exponents of the intestinal theory
by nearly three-quarters of a century.

Dr. Thomas Addison (3) of Guy's Hospital, first in
1849 and later in 1855, described in detail, as a preface
to his work "On the Constitutional and Local Effects of
Disease of the Suprarenal Capsules", the type of severe
anemia which still bears his name. As he brought out in
his paper, he accidentally "stumbled" on the suprarenal
syndrome while trying to solve the etiology of "idiopathic
anemia" which is now occasionally called Addison's anem-
ia in tribute to his clear-cut description which definitely
established the syndrome as a clinical entity. His de-
tailed and remarkably complete description of "a remarkable
form of anemia" is fitting of quotation:

"As a preface to my subject, it may not be altogether
without interest or unprofitable to give a brief narrative
of the circumstances and observations by which I have been
led to my present convictions."
For a long period I had from time to time met with a very remarkable form of general anemia, occurring without any discoverable cause whatever--cases in which there had been no previous loss of blood, no exhausting diarrhoea, no chlorosis, no purpura, no renal, splenic, miasmatic glandular, strumous, or malignant disease.

Accordingly, in speaking of this form in clinical lecture, I perhaps with little propriety applied to it the term "idiopathic," to distinguish it from cases in which there existed more or less evidence of some of the usual causes or concomitants of the anaemic state.

The disease presented in every instance the same general character, pursued a similar course, and with scarcely a single exception, was followed, after a variable period, by the same fatal result.

It occurs in both sexes generally, but not exclusively, beyond the middle period of life, and so far as I at present know, chiefly in persons of a somewhat large and bulky frame, and with a strongly-marked tendency to the formation of fat.

It makes its approach in so slow and insidious a manner that the patient can hardly fix a date to his earliest feeling of languor which is shortly to become so extreme. The countenance gets pale, the whites of the eyes become pearly, the general frame flabby rather than wasted; the pulse, perhaps, large, but remarkably soft and compressible, and occasionally with a slight jerk, especially under the slightest excitement; there is an increasing indisposition to exertion, with an uncomfortable feeling of faintness or breathlessness on attempting it; the heart is readily made to palpitate; the whole surface of the body presents a blanched, smooth, and waxy appearance; the lips, gums, and tongue seem bloodless; the flabbiness of the solids increases; the appetite fails; extreme languor and faintness supervene, breathlessness and palpitations being produced by the most trifling exertion or emotion; some slight edema is probably perceived about the ankles; the debility becomes extreme. The patient can no longer rise from his bed, the mind occasionally wanders, he falls into a prostrate and half-torpid state, and at length expires. Nevertheless, to the very last, and after a sickness of, perhaps, several months' duration, the bulkiness of the general frame and obesity often present a most striking contrast to the failure and exhaustion observable in every other respect.

With perhaps a single exception the disease, in my own experience, resisted all remedial efforts, and sooner or later terminated fatally.

On examining the bodies of such patients after death I have failed to discover any organic lesion that could properly or reasonably be assigned as an adequate cause
of such serious consequences; nevertheless, from the disease having uniformly occurred in fat people, I was naturally led to entertain a suspicion that some form of fatty degeneration might have a share, at least, in its production; and I may observe that, in the case last examined, the heart had undergone such a change, and that a portion of the semilunar ganglion and solar plexus, on being subjected to microscopic examination, was pronounced by Mr. Quekett to have passed into a corresponding condition.

Whether any or all of these morbid changes are essentially concerned—as I believe they are—in giving rise to this very remarkable disease, future observation will probably decide.

The cases having occurred prior to the publication of Dr. Bennett's interesting essay on "Leucocythaemia," it was not determined by microscopic examination whether there did or did not exist an excess of white corpuscles in the blood of such patients."

We see that Addison emphasized the fact that there were none of the usual concomitants of the anemic state to account for its cause and he accordingly termed the anemia "idiopathic". Addison omitted a description of the remarkable phases of remission and relapse, and the symptoms referable to the degenerative changes in the central nervous system. His description placed idiopathic anemia among the diseases which exist as clinical entities.

In the Guy's Hospital Reports for 1857, Dr. Wilks (5) was able to report the absence of "leucemia" in that class of cases which has specially gained the attention of Dr. Addison, and which he has designated "idiopathic" anemia. This seems to be the first microscopic examination of blood in pernicious anemia.

In 1867 and again in 1872, Prof. Biermer (7) of Zurich published papers describing a very severe anemia, which he named Progressive Pernicious Anemia. He apparently was unaware of Addison's previous publication and he
independently came to the conclusion that there existed
the severe type of progressive anemia which constituted
a separate generic disease. His descriptions of the
clinical and post-mortem findings were almost identical
with those of Addison except that he drew attention to
the tendency to small capillary hemorrhages. Biermer
viewed the disease as a symptom complex associated with
puerperal sepsis, unsuitable feeding, unhealthy surround­
ings, and chronic diarrhea. Whereas Addison had emphasized
the idiopathic nature of the disease.

Whereas Addison's description passed almost unremarked
Biermer's account of the disease aroused widespread inter­
est, and his name has been associated with this type of
anemia on the Continent up to the present time. Biermer,
like Addison, laid stress on the fatty degeneration of
the cardio-vascular system which he found post-mortem,
but differed from him on the following fundamental point.
While Addison held that the essence of the disease was its
idiopathic nature, namely that no cause during life or
death could be found to account satisfactorily for its pro­
duction, Biermer stated that the absence of clear etiologi­
cal factors was the exception, and that the disease ap­
peared particularly in the poverty-stricken classes, and
was associated with various causes, such as puerperal sep­
sis, unsuitable feeding, unhealthy surroundings, and
particularly with chronic diarrhea. Moreover, the disease was found in certain cases of cancer and Bothriocephalus infestation. In short, Biermer, under the title of Progressive Pernicious Anemia, included a group of conditions of which one was the idiopathic variety described by Addison. The view that pernicious anemia was a symptom complex was hailed by Biermer's continental contemporaries and by the majority of later investigators, such as Ehrlich, as the correct one, and in consequence has influenced the continental investigators up to the present time"(2).

"A careful study of Biermer's work shows conclusively that he added nothing new to our knowledge of the disease described by Addison twenty-one years earlier beyond pointing out the frequency of retinal haemorrhage. On the other hand, he added much confusion by including under a single name both Addison's "idiopathic anaemia" and a great variety of other conditions. The designation Biermer's anaemia," which is employed in Germany, Scandinavia and Switzerland, is therefore quite unjustified."(9)

In 1875, Dr. Pye-Smith (8), a pupil of Thomas Addison, drew the attention of the medical world to Addison's claim to be the first to describe idiopathic anemia as a clinical entity. At that time he described a case of "idiopathic anemia of Addison" and reviewed 103 collected cases. "The following case of a remarkable and obscure
disease seems to find an appropriate place in these re-
ports (Guy's Hospital Reports), since it was within the
walls of Guy's Hospital that the malady in question was
first recognized and its characters defined (by Addison).
He strongly upheld Addison's view of the idiopathic nat-
ure of the disease and stressed its progressive course.

In the period beginning about 1870 the work of
William Hunter (10, 11, 12) emphasized certain aspects
of the disease. His work directed investigation along
lines other than purely hematological studies. Hunter
emphasized the clinical signs and symptoms of the digest-
ive system. He demonstrated that the liver in pernicious
anemia contains more iron than the liver in other anemias,
and believed that the hepatic sederosis was a specific
finding. Then Hunter by a process of synthetic reasoning
alleged that the deposits of iron-bearing pigment in the
liver were the result of abnormal blood destruction in
the portal area. He believed that a toxin, produced a-
llegedly by a specific organism in the intestine, was ab-
sorbed into the portal blood and there caused an intense
destruction of blood corpuscles, the freed pigment of
which was then deposited in the liver lobules. His view-
point on the relation of infection to pernicious anemia is
best stated in his own words "Septic infection plays an
important part in the development of this disease, although,
however severe it may be, it is unable of itself to pro-
duce it. It is, however, a potent antecedent condition creating an unhealthy condition of the stomach and intestine which favours the contraction of the specific hemolytic infection responsible for the disease, and which favours its continuance after its contraction. The typical mode of development of the disease is an antecedent history of oral, gastric, or intestinal trouble associated with sepsis for some years previous to onset of the disease.

Hunter (12) ardently upheld his theory of the hemolytic nature of pernicious anemia. In his own words "There exists a form of anemic disease, possessing the clinical features and the mysterious origin described by Addison. This form is always marked by an extensive hemolysis as a constant pathological feature. This hemolysis is not a terminal feature. On the contrary, it is one of the initial features and marks its progress from first to last. In pernicious anemia we have to do with an organism whose blood-corpuscles are being destroyed with unusual rapidity. The conclusion seems unavoidable that excessive destruction of blood corpuscles is a far more important factor in the anemia than has hitherto been considered probable or possible."

In 1881 Ehrlich's discovery of the value of aniline dyes in the staining of blood films revolutionized the science of hematology. Rapid advances were then made in
the study of the formed elements of the blood in circulation and blood formation in the bone marrow.

Pepper (13) had described the megaloblastic appearance of the bone-marrow and believed that this change denoted a reversion to an embryonic type of blood formation.

In 1898 Ehrlich and Lazarus (14) published a monograph on the disease almost simultaneously with that of Hunter. The Ehrlich treatise dealt with megaloblastic blood formation, and the role of infection and hemolysis was briefly dismissed. Hunter (12) dismissed blood formation in a few paragraphs and devoted nearly the whole work to the advancement of the thesis of gastro-intestinal sepsis and portal hemolysis. Muir (15) described his extensive bone marrow investigations and came to the conclusion that these could be interpreted as compensatory to the portal blood destruction. Thus Hunter cited Muir's views as evidence of the secondary importance of the blood formation in relation to portal hemolysis, and stated that these changes were most marked when siderosis was present in the highest degree, while Ehrlich's adherents as firmly stated that the siderosis was most prominent when the megaloblastic degeneration was at its height. Ehrlich stated that the megaloblastic bone marrow changes were degenerative and were the essential pathological and pathognomonic features of the disease. Ehrlich believed that the only diagnostic criterion of the disease was the megaloblast. And main-
tained that all anemias showing megaloblasts were pernicious.

Thus were established two schools of thought. The one led by Ehrlich taught that the anemia was due to a primary alteration in the marrow and if the alteration was in the nature of an embryonic reversion or as a megaloblastic degeneration. Ehrlich's supporters viewed the disease from an almost purely hematological angle. The other school of thought, led by Hunter, taught that abnormal blood destruction was the essential fundamental process, and that the marrow changes were to be regarded as compensatory regenerative activity in response to a physiological need. This school approached the subject from a physiological and clinical, as well as a hematological angle.
III

A DEFINITION OF PERNICIOUS ANEMIA

It would be well at this point to venture a definition of the disease with which this paper is concerned in the hope a composite viewpoint may serve to accentuate the difficulties met in endeavoring to establish its cause. A complete definition, based upon known facts concerning the disease, should also lead to an unbiased approach to the possibilities of etiological significance.

Pernicious Anemia (Primary Anemia, Addisonian Anemia, Biermer's Anemia) (16) is a chronic disease of unknown causation characterized by achlorhydria, certain gastrointestinal and neurological disturbances, a progressive course interrupted by periods of remission and relapse, a characteristic response to certain forms of therapy and an anemia of which the chief features are perverted hemogenesis, macrocytosis and hemolysis.

Though any anemia that progresses to a fatal issue merits the designation "pernicious"; for the purposes of
in accord with modern usage, the term of pernicious anemia will refer only to the well known and definitely characterized Addison-Biermer type of anemia.

The disease under consideration, though somewhat variable in its course, duration and response to therapy, presents when fully developed, if modern therapy has not been instituted, one of the most sharply defined clinical pictures of disease with characteristic clinical symptoms, course and blood findings. The severe anemia of insidious onset, the lemon tint to the skin, the asthenic, the slight fever, the disturbances of the gastro-intestinal tract, the urobilinuria, the parasthesias and other nervous system symptoms, together with the blood picture indicative, on the one hand, of increased blood destruction (anemia, leucopenia, reduction of the platelets and bilirubinemia) and, on the other, of impaired blood formation, but with reversion to the embryonal type (relative hyperchromemia, as shown by the lacolor index, megalocytosis, microcytosis, poikilocytosis, polychromasia, erythroblastosis and et.) and a course that, in the absence of modern therapy and though marked characteristically by remissions, is inevitably fatal. These criteria are so clean-cut and so distinctive that they render the diagnosis relatively easy, at least when the disease is full-blown, and they also point to a disease entity, for
for which the principal etiologic factor or factors at least must be rather constant, rather than to a mere clinical syndrome that could be due in different instances to principal pathogenic factors that are different and distinct from one another.
IV
SOME FUNDAMENTAL PROBLEMS

The study of pernicious anemia must necessarily involve an appreciation of the problems of blood formation, blood destruction, and pigment metabolism. A proper understanding of the pathological changes occurring in pernicious anemia imply an understanding of normal erythrogenesis, erythrocyte destruction, and pigment metabolism. A detailed discussion of the physiological processes thus concerned is necessarily precluded by the scope of this paper and in consequence the most widely accepted views only will be briefly presented.

In different species of animals and in different ages of their development, blood formation occurs in different parts of the body. In man the appearance of the blood islands of the yolk sac is followed in the third week by a similar development within the mesoderm of the embryo. For the first half of embryonic life
active formation of both white and red cells is concentrated in the liver. In the second half of embryonic life, the spleen is the principal organ of blood formation. Erythrogenesis is found to occur in the medullary canals of the bones in the second or third months of foetal life. The progressive development of the red marrow is accompanied by a concomitant decrease in the hematopoietic activity of the liver and spleen. These organs at the time of birth completely cease their activity in normal blood formation. In certain severe pathological conditions, however, the liver and spleen may revert to their embryonic activity in blood formation.

The origin and development of the erythrocyte is highly controversial and no benefit can be gained here by recapitulating the arguments between the theories of single or multiple origin of blood cells. The recent work of Sabin, Doan, and Cunningham (17) (18) has found wide acceptance, and the descriptions below are summarized from their work.

Sabin and her associates adopted the plan of simplifying the red marrow by making it hypoplastic by means of starvation. In such hypoplastic marrow we can recognize two sets of vessels: (1) Sinusoids, containing actively circulating blood (2) Intersinusoidal capillaries, which have only a potential cavity, the endothelial walls being in apposition, so that the capillaries are shut off
from the general circulation. It is in this second set of vessels that the red cells are formed. Resumption of feeding again stimulates blood formation and by studies at suitable intervals the process of blood formation has been accurately observed. A megaloblast is formed by mitotic division of one of the endothelial cells, and this new cell, itself dividing, gives rise to numerous erythroblasts thereby spreading the capillary walls. The megaloblast is a large round cell with a deeply basophilic cytoplasm and a large vesicular nucleus containing finely divided chromatin. Hemoglobin gradually appears in the cytoplasm of the megaloblasts during their active proliferation in the intersinusoidal capillaries. Successive generations attain maturity by reduction in size, with more but smaller mitochondria, more haemoglobin in the cytoplasm, and with a denser chromatin network in the nucleus which may have a cartwheel appearance. The last stage of the development of the nucleated red blood cell is that of the normoblast, a cell which has a pycnotic nucleus made up of a solid mass of chromatin and a cytoplasm closely resembling that of a mature erythrocyte.

The fate of the erythroblast nucleus is a moot question and is said to be either dissolved intracellularly or extruded as a whole. Cooke (19) has recently thoroughly studied this problem and offers convincing evidence
that the nucleus undergoes intro-cellular disintegration. The proliferation incidental to maturation causes a distention of the intersinusoidal capillaries and a subsequent discharge of the matured elements into the circulating blood. When the red cell leaves the marrow and enters the circulation it retains some traces of immaturity which are seen as the reticulation phenomenon of vitally stained films. The presence of these reticulated cells is of fundamental diagnostic and prognostic importance in pernicious anemia.

The normal physiological process of blood formation results in constant production of formed elements in the circulation. The number of cells, their size and shape, and hemoglobin content remains constant from day to day within narrow limits. This fact implies the presence in the body of some inhibitory mechanism, under normal conditions, which regulates the entrance of the formed elements into the active circulation. Further, it implies the presence of some stimulating mechanism which in response to body needs replaces the elements of the blood lost as a result of hemorrhage and etc. In pernicious anaemia, the essential pathological condition is the inability of the bone-marrow to undergo normoblastic hyperplasia, in spite of body demands.

The average life of the red blood corpuscle is considered to be from ten to forty days under normal condi-
tions (20). Under pathologic conditions the life of the corpuscle may be much shorter. Ashby's (21) observation on the persistence of cells in the circulation of transfused erythrocytes suggests that such cells remain in the circulation thirty days or longer.

It has been demonstrated (22) that a definite increase in the percentage of reticulocytes after exercise occurs in animals which had previously led a sedentary life. Concomitant changes in hemoglobin percentage, plasma to cell ratio, and red blood cells are evidence of increased blood destruction during exercise. Exercise is an important factor in the maintenance of an efficient hematopoietic tissue.

The manner in which the destruction takes place is not fully understood. Three possible methods of blood destruction have been suggested, namely, hemolysis, phagocytosis, and fragmentation.

Rous and Robertson (20)(23) insist that the normal fate of the erythrocyte is fragmentation, one by one, while still circulating, to a fine hemoglobin-containing dust and that the fragmentation products are removed from the circulation by the spleen.

Until quite recently the prevalent view credited the liver as the sole site of bilirubin formation but later work (25) has shown conclusively that the manufacture of bilirubin occurs in the cells of the reticulo-endothelial
system to a large extent. Whipple and Hooper (24) experimentally determined that the liver is not essential to the rapid conversion of haemoglobin into bile pigment. They found that bile pigment formation goes on in a dog whose liver, spleen, and intestines have been isolated from the circulatory system. They indicate that the vascular endothelium may be the agent which brings about the rapid change of hemoglobin to bile pigment. Mann and others (25) studied the bilirubin content of the large organs of the body as measured by the spectrophotometer. Studies of both venous and arterial systems of most vascular areas showed an equal bilirubin content in the venous and arterial bloods but the bilirubin content of the venous blood of the spleen and bone marrow was greater than their arterial blood. They believe that the spleen and bone-marrow are sites of bilirubin formation.

The fate of hemoglobin (26) is represented in the accompanying diagram which represents a working hypothesis based on available evidence. The hemoglobin is probably first converted into globin and an iron-containing pigment. The ultimate fate of the globin is unknown but since it is a protein it is probably converted to amino acids. The iron containing pigment, which in the reduced form is known as hemochromogen and when oxidized is known as hematin, is freed of iron and converted into bilirubin. This change may occur in any of the components
FATE OF HEMOGLOBIN

Hemoglobin
(in reticula-endothelial system)

Hemochromogen plus Oxygen

Globin?

Amino Acids?

Hematin
(in reticulo-endothelial system)

Bilirubin
(in blood stream)

Urobilin (in urine)
excreted

Bilirubin (in bile)

Urobilinogen (in intestine)

Plus oxygen

Iron (hemosiderin)
(in liver and spleen)

? Urobilin excreted (in feces)

Hemoglobin
of the reticulo-endothelial system cells. The liberated iron is stored and utilized in the formation of new hemoglobin. The bilirubin travels in the plasma to the liver where it is extracted by the liver cells and excreted into the bile capillaries as a constituent of bile. It may be mentioned here that the investigations of Van den Bergh (27) suggest that there are two types of bilirubin: (1) bilirubin which is poured into the blood stream by the reticulo-endothelial system, and (2) bilirubin which has passed through the liver cells into the bile. These two types of bilirubin give different reactions to the Van den Bergh test which is of diagnostic importance in differentiating types of jaundice.

In the light of the physiological processes discussed in previous paragraphs we can now turn our attention to the controversy involving the relative importance of abnormal blood formation versus excessive blood destruction.

The two views which have held the field up to the present day may be summarized as follows (2):

(1) Pepper and Cohnheim, and later Ehrlich, insisted that the primary pathological site was the bone-marrow which had reverted to an embryonic type, thus producing abnormal cells. Hemolysis was of secondary importance, since it was the result of phagocytosis of these abnormal circulating elements by the cells of the reticu-
lo-endothelial system. No satisfactory explanation was given as to why this megaloblastic degeneration of the bone marrow occurred, unless the view was accepted that the developmental factor was of prime importance. The bone-marrow changes were explained by some as being the result of the action of a toxin.

(2) The other view is associated with the name of Hunter, and has been supported by the majority of English workers. The primary site of the disease was held to be the gastro-entestinal tract: the absorption of a hemolytic toxin was believed to produce great blood destruction in the portal area, and the marrow changes were interpreted as a secondary compensatory process.

Hurst (9) states Quincke, in 1876, discovered that the viscera contained an excess of iron in fatal cases of pernicious anemia. Hunter's work (12) in later years demonstrated the marked hemosiderosis of the liver and exerted great influence on subsequent thought. He demonstrated the increased iron content of the liver by gravimetric analysis and attributed the hepatic siderosis to an intense portal hemolysis.

The microscopic evidence of exaggerated phagocytosis of red blood corpuscles by the cells of the reticulo-endothelial system, the hyperbilirubinemia, and the siderosis of organs has been the basis of the view that excessive blood destruction leads to the anemic state.
in pernicious anemia. It has been generally agreed that the anemia which is quite characteristic, is due to excessive hemolysis or destruction of the red cells at a more rapid rate and in larger numbers than occurs in the normal individual (16).

Whipple (26), in 1922, suggested that pernicious anemia represents a condition of faulty blood construction rather than increased blood destruction. He advanced the hypothesis that the importance of blood destruction is not an essential feature of pernicious anemia. He offers evidence of an increased formation of a substance which he calls "pigment complex" and a decreased formation of stroma. Pigment complex may be formed from food or body protein, including hemoglobin, and may give rise to hemoglobin, bile-pigments and possibly to urochrome and urobilin. Reformation of hemoglobin from bile-pigments is denied by him, as is also the absorption of sterocobilin in the intestine.

"The increased stercobilin content of the feces in cases of pernicious anemia is generally held to be definite evidence of the importance of the role of hemolysis in the disease. That a different view is compatible with these facts is clearly stated in the following paragraphs by Whipple (26).

"We are told that the stercobilin in a case of pernicious anemia is an index of blood destruction. Let us
examine some of these figures and, further, let us assume the normal red cell count as 5,000,000, and the pernicious anemia count as 1,000,000, for the sake of simplicity of comparison. If the anemia red cells disintegrate at the same rate of speed as the normal control, and if these products result in bilirubin and then stercobilin, we must say the stercobilin figures should be one-fifth of normal. But the stercobilin figures during periods of remission in pernicious anemia often exceed twice or three times normal stercobilin excretin, making no allowance for similar pigments in the urine. This can only mean that the pernicious anemia patient with one-fifth the number of red cells, and two or three times the amount of stercobilin output, must regenerate its total red cell mass every three days instead of the assumed normal every thirty days. The normal replacement factor for red cells and haemoglobin is believed to be 3 per cent. per day. We must postulate from 30 to 40 per cent. replacement of red cells per day in a pernicious anemia case if we persist in explaining the stercobilin content as being due to blood destruction. Those who wish to accept this explanation are welcome to do so, but it would be a fleeting and troublous life period endured by the red cell in pernicious anemia.  

Ashby (28) studying a series of pernicious anemia patients during transfusion therapy found that in perni-
cious anemia the red cells exist in the circulation as long as in the normal human case. She has expressed doubt that blood destruction is an important factor in producing the anemia of pernicious anemia.

Minot (29) has also expressed his disbelief that the anemia of pernicious anemia is fundamentally hemolytic. He believes that the proliferating megalocytes of the bone-marrow fail to differentiate toward erythrocytes, so that the anemia is explained by a functional ineffectiveness of the bone-marrow rather than by a process that is fundamentally hemolytic. His view is that dysfunction of the hemoglobin pigment metabolism is a feature of the disease and leads to an increase of pigments in the blood plasma tissues and excreta but this is a phenomenon secondary to a defect in the manufacture of red blood cells.

Peabody and Brown (30) have found in certain cases of pernicious anemia a remarkable degree of phagocytosis of red blood cells in the bone marrow and they have suggested that the phagocytosis may be a factor in the production of the disease. Their studies of control materials indicate that phagocytosis of erythrocytes occurs to a very limited extent in normal marrow but in the active stages of pernicious anemia it occurs in a marked degree and tends to decline with the advent of remission.

Peabody has shown by his examinations of the bone-marrow obtained by biopsy in cases of pernicious anemia
that the myeloid hyperplasia is most marked during a relapse and that the structure of the marrow tends to return to normal after suitable therapy. During a relapse there is rapid and extensive proliferation of megaloblasts and a diminished tendency towards differentiation of mature cells of the erythrocyte series. He states that although the bone-marrow is hyperplastic it is functionally inefficient. During remissions there are few megaloblasts and a great relative increase of normoblasts and mature red blood cells in the marrow. Peabody explains the anemia of relapse as due to the functional inefficacy of the bone-marrow. The blood picture of the remission is due to resumption of a normal type of cell development with an increase in normocytes and erthrocytes. (31).

Recent work (32) includes some important observations of the bone-marrow of pernicious anemia patients obtained by biopsy. In relapse, the marrow is characteristic. It is hyperplastic, without fat cells, and presents large numbers of large embryonic nucleated red blood cells which are often present in islands with numerous mitotic figures. Numerous cells resembling small lymphocytes are seen, and histiocytes are in increased numbers. The percentage of normal nucleated red blood cells is diminished. In severe relapse only a few erythroblasts and normoblasts are seen. After liver therapy, the marrow resumes its normal appearance to a great extent; the normoblasts pre-
dominate and magaloblasts are rare with a decrease in
erthrogones and histiocytes.

Hurst (9) has ascribed the changes in the marrow to
the action of a specific poison which acts upon the bone
marrow and may be identical with the hemolysin. Another
factor is an attempt on the part of the marrow to compen­
sate for the prolonged and excessive blood destruction.
The characteristic features of the blood in Addison's a­
nemia are due to the reaction of the bone-marrow to the
toxemia, and the stage when it appears and its degrees
depend upon the varying power of reaction of the marrow
in different individuals.

Gulland and Davidson(2) have succintly summarized the
evidence against the theory of portal hemolysis as the
cause of pernicious anemia. Their views may be outlined
as follows: (1) No evidence exists that intravascular
hemolysis occurs in pernicious anemia. (2) The quanti­
tative increase of the iron content of liver, kidneys,
and spleen does not imply portal hemolysis inasmuch as
hemolysis at other sites may produce similar results.
(3) No specific hemolytic agent has been demonstrated
by anyone studying this problem. (4) There is no proof
that the hemolytic substance extractable from dead body
of Bothriocephalus latus can be absorbed. The majority
of persons harboring the parasite do not suffer from a
megaloblastic anemia. (5) The experimental anemias
produced by the injection parenterally of numerous toxic
agents are not identical with pernicious anemia. (6)
Excessive amounts of iron in the liver may be equally well accounted for by either increased hemolysis or increased storage due to decreased demands by the bone-marrow for material from which to manufacture hemoglobin, or a combination of both.

Farquharson and his associates (32) have made quantitative studies of the excretion of urobilinogen in pernicious anemia in the feces and urine, before and following liver therapy, with complemental studies of the blood pictures. They have shown that in pernicious anemia, in relapse, the excretion of urobilinogen is greatly increased. In severe cases the excretion of urobilinogen is equivalent to 8-13 grams of hemoglobin. Following liver therapy and beginning at the peak of the reticulocyte crises there is a sharp decrease in the excretion of urobilinogen. In cases with little anemia, with general macrocytosis but little microcytosis or polikilocytosis, the urobilinogen is slightly increased. In severe cases of anemia the excretion of urobilin and urobilinogen are greatly increased when the blood cells display marked deviations from normal morphology. They have concluded that the irregular forms are less resistant to destruction, and that longer lived red cells approach more closely the normal morphology. Following liver therapy, the bone-marrow produces red-blood cells which are
closer to normal with increased resistance to destructive influences and there is a concomitant decline in urobilinogen and urobilin excretion. Farquharson and his co-workers believe that the primary factor in excessive pigment metabolism is an abnormality of red-blood cells which renders them susceptible to destructive influences.

Minot and Lee (33) have pointed out that it is possible to recognize five somewhat distinct types of the disease depending upon the balance maintained between blood destruction and blood regeneration. There are two main types. These are: (1) A type characterized by a rapid unremitting course with oligocythemia, a marked degree of abnormal blood formation, and of bilirubinemia, and (2) A type characterized by a long monotonous course with much evidence of abnormal blood formation and a lesser degree of bilirubinemia—their are the so-called myelotoxic cases. Between these two extremes lie three types of remitting cases in which the evidences of abnormal formation, hyper-bilirubinemia, and immature forms tend to assume a balance. Minot remarks that the middle type bears a strong resemblance to acquired hemolytic jaundice.

Boyd (34) has observed "It is true that in prenicious anemia large amounts of iron are found in the reticuloendothelial cells of liver, spleen, and bone marrow, but it appears more probable that the increase is due to a disturbance of iron metabolism rather than to increased
blood destruction. No doubt there is some increased destruction of the immature red cells, thus accounting for the increase of bilirubin in the blood serum, but it is extremely unlikely that mere hemolysis is at the bottom of the disease. The hemolysis in Addison's anemia is a consequence rather than a cause of the disease."
THE CONSTITUTIONAL FACTOR AND FAMILIAL OCCURRENCE.

This problem involves a description of certain alleged constitutional factors common to pernicious anemia patients and some reference to familial pernicious anemia.

Addison (3), in his original description, noted that the disease occurred mainly in large persons with large and bulky frames. "It occurs, so far as I at present know, chiefly in persons of a somewhat large and bulky frame, and with a strongly-marked tendency to the formation of fat". Maitland Jones, according to Cornell (35), and Levine and Ladd (36) have noted the great frequency of grey or white hair. Sheard (37), among 15 cases, found but one exception to this rule. In the fourteen cases showing the feature he determined that the average age at which greyness began was 29.4 years, which was 15 years prior to the onset of the disease. He further noted the frequency of "silky" or very fine hair.
Achlorhydria, one of the striking features of pernicious anemia, is usually interpreted as a constitutional one. Bassler and Gutman (38) in a study of achlorhydric individuals have placed them in the "adrenotrope" groups, females of masculine build and males of apoplectic diathesis with tendency to pigmentation, very firm and stained teeth, and great mental susceptibility to external influence.

Draper (39) has made painstaking anthropological measurements on forty-five cases of pernicious anemia. He found that people with pernicious anemia have short, broad faces; large mandibular angles; very short noses; short but deep and wide chests; especially wide subcostal angles; and very long thin ears. "The male of the pernicious anemia race, therefore is a medium to tall individual with short chest, high placed umbilicus, long abdomen, and a tendency to enuchoidal habitus as is shown by his relatively long lower extremities. The males of this group show definite feministic tendencies in the domain of secondary sex characteristics." Stockard (40) in commenting on Draper's work stated "The pernicious anemia group tended in their measurements very decidedly to approach the acromegalics. The anemic persons may be possibility be a peculiar deviation from the bone growth state of the acromegalics. Both conditions may have some connection with calcium metabolism, and
blood disturbance and bone disturbance in this was may be somehow correlated."

Friedlander (41), in an analysis of 500 cases of typical pernicious anemia concludes that "there is a definite constitutional predisposition embodied in individuals endowed with a diathesis characterized by fair complexion, light hair and light colored eyes."

The factor of heredity in the production of pernicious has received much attention in the studies of the disease.

Klein, according to Meulengracht (42), was the first to notice the familial incidence of pernicious anemia when he observed the disease in 3 brothers and sisters. Bramwell (43) later described a family in which 7 individuals in two generations had suffered from pernicious anemia.

Gulland (44) in 1907 stated "I have come across three cases of people in middle life whose fathers had died of pernicious anemia." "It seems probable that in people who become affected with pernicious anemia that there may be an inherent weakness of the resisting power and of the blood-forming organs." Fetics (45), in 1911, cited a family wherein five members were pernicious anemia patients. Two brothers, one sister, a paternal cousin, and a paternal uncle presented the typical clinical picture of Addison's anemia and the blood picture
substantiated the diagnosis.

Bartlett (46), in 1913, reported a family wherein, over a period of thirty years, there were four deaths. Two of the deaths were definitely proven to be due to pernicious anemia and the other two judged by autopsy findings and symptomatology were probably due to pernicious anemia.

Cabot (47), in his review of 1200 cases of pernicious anemia, stated, "The writer twice found the disease in the same family, two sisters being attacked in one instance and a brother and a sister in another."

Gilford (48), in 1923, reported the case of a woman of 65 who had an advanced case of pernicious anemia and whose two first cousins had the same disease. In another instance, in a family of ten brothers and sisters there were three deaths definitely due to pernicious anemia. In addition an uncle had died at the age of 63 because of a severe case of pernicious anemia.

Dorst (49) reported an unusual family group wherein a mother and four children died of Addison's anemia. Gastric analysis of six other members of the same family showed that only two had a normal gastric secretion and two others had a very marked hypochlorhydria. Levine and Ladd (36) in their comprehensive review of a series of pernicious patients had reported that past histories in nine cases were definitely suggestive of a familial
incidence.

Hurst reported a positive family history in four out of twenty-four cases of the disease (50).

Mac Lachlan and Kline (51) observed a series of cases occurring in a family in Pennsylvania and accumulated data covering four generations. In all there were 17 cases of pernicious anemia of whom 13 were dead and 4 living. Of the 13 dead the family physician was certain that 7 had had pernicious anemia. All four of the living members of the family had pernicious anemia. In the first and second generations there were respectively one and three deaths from anemia, but the nature of the anemia could not be ascertained. In the third generation there were 7 deaths from anemia and three cases of pernicious anemia. In the fourth generation there were six cases of secondary anemia, one of pernicious anemia and two deaths from anemia.

Meulengracht (42) has emphasized the etiological significance of the hereditary factor in pernicious anemia. He cited one family in which a thorough investigation disclosed a high percentage of pernicious anemia incidence. Two brothers in the family had succumbed to pernicious anemia. The son of one of the brothers dying was immediately examined and showed a color index of "one plus", a megalocytic blood picture, gastric achylia and other characteristic evidences of pernicious
anemia. A further investigation showed that six other members of the family showed gastric achylia and of these six, four had a typical glossitis and one later developed a typical case of pernicious anemia.

Carey (52) in an analysis of ninety cases of Addisonian anemia was able to elicit positive histories of familial anemia in ten cases.

Barker (16), in considering this factor, has stated that all patients, like healthy people, are in the biologic sense to be looked upon as human "phenotypes"; that is, each human phenotype (healthy or diseased) consists of two parts: (1) a part acquired through inheritance—the so-called genotype and (2) a part acquired from the environment—the so-called paratype. The 'pathologic phenotype' known as a 'patient suffering from pernicious anemia' owes its pathologic state to (1) an abnormality of the genotype (2) injurious conditions (external) acting a phenotype of normal genotypic content, (3) abnormality of genotype plus the action of injurious external conditions. "A study of family trees in which pernicious anemia has occurred in more than one case has given rise to the hypothesis that the predisposition to the disease may depend on a Mendelian dominant, perhaps on the hereditary transmission of a single pathologic id or gen. Those who support the view of such hereditary disposition explain the small number of cases observed in affected families (1)
by the fact that, since pernicious anemia is a disease predominantly of later life, many members of affected families die before the disease becomes manifest; (2) by the probability that many mild and atypical cases are overlooked, and (3) by the possibility that, in addition to the inherited disposition, certain releasing factors resident in external circumstances are also necessary in order that the disease shall become manifest, the phenotype remaining apparently healthy (despite its carrying the pathologic genotype constituent), because of the lack of this releasing factor or factors."

"Even if the occurrence of pernicious anemia should be proved in the main to be due to the inheritance of a genotypic disposition thereto, the actual pathogenic skein would still have to be slowly unraveled. It would be necessary to determine, for example, whether this genotypic disposition led to a primary inferiority of the bone-marrow, or to a primary hyperactivity of reticulo-endothelial blood destruction."

Cornell (35) concludes that "The genotypic(heredity) conception of pernicious anemia is at present of little more than academic interest and whether it eventually proves convincing or otherwise, ordinary pathological studies must be pursued to determine the sequence of events in the pathogenesis."
VI

AGE INCIDENCE

Barker has viewed pernicious anemia as "A disease of later life, not occurring in childhood or during adolescence, and rarely between 20 and 30, but then increasing steadily in incidence during each quinquennium (in ratio of the number of cases to the number of persons of the same age alive up to the age of 70") (16).

Cabot's series of cases (47) revealed that 822 cases of 1200 occurred after the age of thirty-five. Panton and others (52) found that the average age on admission was 46 years with extremes of 20 years and 68 years.

Eason (54) compiled statistics concerning pernicious anemia on the basis of admissions to Edinburgh hospitals over a period of twenty-five years. He found the greatest incidence between the ages of 45 and 59. Gulland and Davidson (2) studying 300 cases admitted to the Royal Infirmary in Edinburgh found that 80 per cent of the cases were persons of between 40 and seventy years
of age. They found two cases under the age of twenty, and twelve cases between the ages of 70 and 75.

In Sturgis' and Isaacs' series of 1850 cases (54) there occurred an age incidence between 22 years and 80 years. Fifty percent of the cases occurred between the ages of fifty and sixty while eighty-six percent occurred between forty and seventy years of age.

Lichty's (55) group of cases show an average age of 50 years while the average age of females was 41 years. The youngest of the series was a female of 2 years and the oldest was a male of 79 years. Smithburn and Zerfas (56) report the highest incidence between 51 and 60 years. In Heath's group the ages of patients varied from 20 years to 79 years with the highest incidence between 50 and 59 years. (57).
VII

SEX INCIDENCE

Cabot's analysis of 1200 cases of pernicious anemia compiled from his and other records found that 723 males were afflicted while 434 females made up the rest of the series (47). Panton, and Maitland-Jones, series comprised 68 males and 49 females (52). Cornell (35) collected figures from various countries and found that of a total of 1,1726 cases, 819 occurred in males and 907 in females. Reznikoff (58) states that in a compilation of 371 cases 209 were males and 162 females.

Gulland and Davidson (2) investigated the sex incidence in 300 consecutive cases. One hundred and forty-one cases occurred in males and 159 in females. Sturgis and Isaacs (54) in an analysis of 150 patients with pernicious anemia reported the disease in 87 males and 63 females. Lichty's group of cases showed a distribution of 75 males and 68 females. Smithburn's and Zerfas' group was composed of 64 males and 51 females (56).
Gullani Davidson have expressed the view that "Both sexes are liable to this disease, and in our experience the liability is about equal. About the age of fifty, males are affected more frequently than females, but below the age of fifty the females equally or even more frequently exhibit the condition. In America, opinion is in favor of a preponderance of cases occurring in males, while Continental figures show the reverse."
VIII

GEOGRAPHICAL DISTRIBUTION

Gulland and Davidson(2) have allocated the disease to the regions of North America and Northern Europe. It appears that races inhabiting the tropical countries are seldom afflicted.

Cornell (35) writes that the disease is commoner in Northern Europe, the British Isles and North America, than elsewhere, and that it is uncommon in Brazil and China, and never occurs in a full-blooded negro. He has collected statistics from many countries which indicate that in those countries with available figures that the death rate per 100,000 population ran usually from 3 to 6. In the provinces of Canada, New Brunswick was high with 9.0 and British Columbia had a rate from 6 to 10 per 100,000 population. Ontario showed a yearly incidence of from 14 to 15 per 100,000 of population and the deaths from pernicious anemia constituted 1.28 to 1.31 per cent of total deaths. Cornell has remarked about the high incidence of pernicious anemia in Ontario, and has noted the co-existence of simple goitre
in 3 patients.

Montgomery (59) has made an interesting survey of the incidence of pernicious anemia in Western Canada. He observed in these cases that the individuals acquiring the disease were the Anglo-Saxon, life-long inhabitants, and never the more recently settled inhabitants. The survey also disclosed the curious fact that whereas certain localities reported only occasional cases of pernicious anemia, other localities of equal population furnished a large percentage of cases. These patients were the long-time inhabitants of Anglo-Saxon race. A feature of the "high-incidence" districts was the fact that they are sections wherein the drinking water contains a high concentration of alkaline earths. This finding suggests the possibility of an achlorhydria induced by the continual drinking of alkaline water. In one village of 400 people which had reported four cases of pernicious anemia within a period of five years, and where the drinking water was very alkaline, a very high incidence of hypochlorhydria and achlorhydria was found. On the other hand in districts where the water was not as alkaline, hyperacidity, constipation, and hemorrhoids were more common.

Levine and Ladd's (36) figures from John Hopkin's Hospital, which are reproduced here, seem to indicate that the races inhabiting northern climes are more susceptible to pernicious anemia, and support Barker's (16) view that "pernicious anemia occurs in all countries of temperate climate,
<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Born in U.S.</th>
<th>Foreigners</th>
<th>Russia</th>
<th>Canada</th>
<th>Ireland</th>
<th>England</th>
<th>Italy</th>
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<tbody>
<tr>
<td>Total admissions to Hospital</td>
<td>23323</td>
<td>13696</td>
<td>8627</td>
<td>2193</td>
<td>2070</td>
<td>1813</td>
<td>79</td>
<td>795</td>
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<tr>
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<td>41.3</td>
<td>9.4</td>
<td>8.9</td>
<td>7.8</td>
<td>3.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Total cases of Pernicious Anemia</td>
<td>143</td>
<td>86</td>
<td>57</td>
<td>3</td>
<td>23</td>
<td>11</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Percentage of Pernicious Anemia</td>
<td>100</td>
<td>60.1</td>
<td>39.9</td>
<td>2.1</td>
<td>16.1</td>
<td>7.7</td>
<td>5.6</td>
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</tr>
<tr>
<td>Percentage of all admissions(born in various countries) having Pernicious Anemia</td>
<td>0.61</td>
<td>0.63</td>
<td>0.59</td>
<td>0.14</td>
<td>1.11</td>
<td>0.61</td>
<td>1.01</td>
<td>0.18</td>
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</table>

Analysis of 143 cases of Pernicious Anemia showing nationality distribution of patients Levine and Ladd (36)
### Analysis of 143 cases of Pernicious Anemia showing nationality distribution of patients Levine and Ladd (36)

<table>
<thead>
<tr>
<th></th>
<th>Sweden</th>
<th>Denmark</th>
<th>Germany</th>
<th>Greece</th>
<th>Miscellaneous</th>
<th>All foreign countries except Ireland, England, Sweden and Denmark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total admissions to Hospital</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>278 37 358 323 1092 4634</td>
</tr>
<tr>
<td>Percent admissions to Hospital</td>
<td>1.2</td>
<td>0.2</td>
<td>1.5</td>
<td>1.4</td>
<td>4.7</td>
<td>19.9</td>
</tr>
<tr>
<td>Total cases of Pernicious Anemia</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Percentage of Pernicious Anemia cases</td>
<td>4.2</td>
<td>1.4</td>
<td>6.7</td>
<td>0</td>
<td>1.4</td>
<td>4.9</td>
</tr>
<tr>
<td>Percentage of all admissions (born in various countries) having Pernicious Anemia</td>
<td>2.16</td>
<td>5.4</td>
<td>0.28</td>
<td>0</td>
<td>0.18</td>
<td>0.15</td>
</tr>
</tbody>
</table>
but seems to be rare in the tropics."

Reznikoff states "Little is known concerning the geographical distribution of pernicious anemia except that it is wide-spread in the white race, particularly among people of Nordic origin. It has been observed in mulatto negroes".(58)
IX

ACHLORHYDRIA IN PERNICIOUS ANEMIA.

It would perhaps be well to define at the outset just what is implied by the term 'achlorhydria'. A certain looseness in terminology occurs in the literature with respect to the terms 'achlorhydria' and 'achylia'. Some writers have assumed that the terms are interchangeable and can be used indiscriminately. In both conditions no free hydrochloric acid is present in the fasting gastric juice, or in any sample of a fractional test meal. In achylia gastrica there is a complete absence of any secretion of either acid or pepsin, and hence combined acid is also absent. In achlorhydria free acid is absent, but combined acid may be present (2).

- "Since 1886, when Cahn and Von Mehring first drew attention to the absence of free hydrochloric acid from the gastric contents in a case of Addison's anemia, it has gradually become recognized that the association is quite invariable.

Ewald found achlorhydria in every one of 75 cases,
Zadek obtained similar results in his fifty cases, and, according to Weinberg, it was present in every one of 105 cases of Addison's anemia in Martins' clinic at Rostock between 1900 and 1907." (60)

"Faber and Bloch, in 1900, collected 33 cases of pernicious anemia in which gastric analyses had been done and all of them showed an achlorhydria. Cabot collected 79 cases and all but one showed the absence of free hydrochloric acid." (35)

Panton and Maitland-Jones analyzed 117 cases of pernicious anemia in London Hospital from 1911 to 1920 and in 33 of 35 patients studied with Ewald test-meals achlorhydria was demonstrable in 33. (52)

Levine and Ladd (36), using an Ewald test-meal and obtaining samples of the gastric contents while fasting and every forty-five minutes thereafter, found free hydrochloric acid only three times in a series of 108 cases. In one of the exceptions the blood and symptomatology were typical of Addison's anemia and the diagnosis was confirmed post-mortem, but in the other two post-mortem examination did not establish a definite diagnosis.

Faber and Gram (61), in 1924 reported that 'achydia' (?) was demonstrated in 47 of 54 pernicious anemia cases and only four showed free hydro-chloric acid (three patients were not tested). They also found a simple anemia in 41 per cent of ninety cases of 'achylia'. Knott's series of
of 37 cases from Guy's Hospital all showed an achlorhydria (62).

Hurst (60) found an achlorhydria in all of a series of 36 cases examined by means of fractional test-meals. He also recorded the results of Ewald, Zadek, Weinberg, Levine and Ladd, Faber, and Striech in which an achlorhydria was present in all of 579 cases.

Sturgis and Isaacs (54) have recently carefully studied 150 cases of pernicious anemia in whom the diagnosis had been established by every known clinical procedure of value including therapeutic responses to liver extract or desiccated stomach. An achlorhydria was present in all of the 150 cases. They conclude that "a patient has never been observed who had free hydrochloric acid in the gastric contents and later developed pernicious anemia. A patient with pernicious anemia always has an achlorhydria. It is possible that the few cases in which an achlorhydria has not been observed may be accounted for by technical errors or erroneous diagnoses".

Wintrobe (63) in a summary of 1141 cases states that all but 27 cases showed no free hydrochloric acid. The diagnosis of pernicious anemia in the 27 cases with free hydrochloric acid was confirmed at autopsy in only 5 instances. Heath's series of cases has shown achlorhydria present in all of 19 cases (57). Lichty's analysis of 143 cases shows the presence of achlorhydria in all cases. (55) Chas. Hunter (64) has
reported 25 cases all of which were achlorhydric. Wilkinson's study of 200 cases demonstrated achlorhydria in all and total acidity was greatly lowered (65).

Moschowitz (66) has well expressed the present day view of the association between achlorhydria and pernicious anemia. He states "Achlorhydria is such a constant sign in pernicious anemia that probably no case is valid unless achlorhydria is present. In my experience I do not recall an instance in which achlorhydria was absent. Cases in which hydrochloric acid was present eventually proved to be some other disease. Therefore achlorhydria may be regarded as the most decisive differential sign in establishing a diagnosis of pernicious anemia."

Cornell (35) has written "If one fact has received ample confirmation in connection with the entire subject of pernicious anemia it is this,—the stomach contents do not contain free hydrochloric acid."

Conner (67) has recently made an extensive survey of 154 blood relatives of 109 patients with pernicious anemia from the view point of gastric acidity. Sixty marital partners were also studied to ascertain the effects, if any, of common living conditions. He found 30 per cent incidence of achlorhydria among blood relatives of pernicious anemia patients as compared with an incidence 19.15 per cent among a control group of patients who had received test meals, because of various gastro-intestinal complaints. The aver-
age age of the "relative" group was six years less than the control group age. Conner feels that achlorhydria has a distinct tendency to occur familialy because of a constitutional predisposition to its development.

Various observers have demonstrated that the achlorhydria in many instances has preceded the anemia. Hurst (50) has collected 14 cases from the literature in which the achlorhydria preceded the anemia by periods of time varying from 3 months to 13 years. Sturtevant (68) has noted a case in which the achlorhydria preceded the anemia by 14 years. Riley (69) states that he has observed achlorhydria precede the development of anemia by intervals of from 8 to 25 years. In Lichty's series of cases he has noted that several patients with achlorhydria later developed the blood picture and symptoms of pernicious anemia (55). Hunter (64) reports one case wherein achlorhydria preceded blood changes by two years.

Achlorhydria antedated the onset of blood changes by a period of 13 years in a case reported by Bie (70). In Carey's collection of 60 cases of pernicious anemia seven of the cases in which histories were detailed and complete gave histories suggesting the existence of a gastric achlorhydria before the onset of a pernicious anemia symptomatology. (71) Sturgis and Isaac's group included one patient who had been achlorhydric for 26 years and another for 10 years preceding the onset of the anemia. (54) Davis and Vanderhof (72) have observed a hospitalized patient in whom a routine examination
demonstrated an achlorhydria but was symptom-free insofar as pernicious anemia symptomatology was concerned. Five years later the patient was again observed and presented a typical picture of pernicious anemia with characteristic response to liver therapy.

The persistence of achlorhydria after liver and other suitable therapy has been reported by the vast majority of observers. In Sturgis' and Isaacs' series (54) achlorhydria was found to persist in all cases. Heath's 19 cases did not evidence any return of free acid in the gastric juices after therapy. (57) Johansen's studies (73) in this respect have been quite thorough. He has observed 19 pernicious anemia patients before and after treatment with liver and liver preparations for periods of time varying from three to seventeen months. Histamine stimulation was employed in all cases. His results indicate a persistence of the achlorhydria in all cases in spite of objective and subjective clinical improvement. Gulland and Davidson (2) have expressed the opinion that a lack of free hydrochloric acid is a persistent finding.

Connery and Jeliffe (74) have described one case wherein free acid was found in the gastric juice after liver therapy. Achlorhydria had been present previous to treatment despite histamine stimulation. A year after the institution of liver therapy free hydrochloric acid equivalent to 36 c.c. of tenth-normal sodium hydroxide was demonstrated.
A possible relationship between achlorhydria of achylia and pernicious anemia is suggested by the clinical evidence offered by the occurrence of pernicious anemia following removal of the stomach. Moynihan (75), in 1911, reported a case wherein complete gastrectomy had been performed because of a pathological condition. The patient died three years and eight months later and death had been preceded by a profound anemia. Hartman (75) has made thorough blood studies of a patient gastrectomized because of a carcinomatous ulcer. Pre-operative examinations revealed a red blood count of 5,520,000, a leucocyte count of 8,200, and a 80% hemoglobin. Achlorhydria was also present. One year and a half later a blood examination showed a 53% hemoglobin, a red count of 2,000,000 and a white count of 2,200. Clinically, the patient showed signs and symptoms of pernicious anemia which underwent typical relapses and remissions with a gradual decline and death. Poole and Foster (77) have described the occurrence of pernicious anemia in a woman who underwent total gastrectomy because of a syphilitic gastritis. Five years after the operative procedure the patient displayed a typical blood picture and symptomatology of pernicious anemia. Achlorhydria had been present previous to the operation. Liver and stomach therapy invoked a response similar to that characteristic of primary pernicious anemia. Lang (78) has observed an unusual case wherein a posterior gastro-jejunostomy was performed in the treatment of pre- and post-pyloric
<table>
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<tr>
<th>Authors</th>
<th>Date</th>
<th>Age at onset</th>
<th>Operation</th>
<th>R.B.C.</th>
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<td>.6</td>
<td>1.24</td>
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<td>1.08</td>
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<td>55</td>
<td>Gastrectomy</td>
<td>1.5</td>
<td>1.7</td>
<td>---</td>
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<td>56</td>
<td>Gastrectomy</td>
<td>3.4</td>
<td>0.7</td>
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<td>Gastrectomy</td>
<td>1.5</td>
<td>1.7</td>
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<td>1930</td>
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<td>0.8</td>
<td>1.1</td>
<td>---</td>
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<td>---</td>
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<td>Yes</td>
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ulcers with the development two years and a half later of an achlorhydria and the clinical and blood picture of pernicious anemia responsive to liver and stomach preparations.

Ivy (79) has noted the development of a severe anemia of secondary type in dogs experimentally gastrectomized. Mann and Graham (80) in an experimental study of gastrectomy in dogs have reported that they did not observe any blood pictures similar to pernicious anemia following gastrectomy. Two of the animals did develop a secondary anemia. However, they failed to describe the diet of the animals which may have prevented the development of an anemia.

Rowland and Simpson (81) have reported the development of an Addisonian anemia following gastrectomy for ulcer and malignancy respectively in two cases. The first case developed the anemia six years after the operation and exhibited a marked response to liver therapy characteristic of pernicious anemia. In the other case, a partial gastrectomy was performed on a male aged 41 for carcinoma of the stomach. The patient remained well for 15 years following operation and then developed signs of pernicious anemia and subacute combined cord degeneration without recurrence of the carcinoma.

Little and Zerfas (82) have described a case of severe anemia developing in a man, aged 28, in whom two entero-enterostomies had been performed, in such a manner that the absorptive area of the small bowel was out of function. With the exception of free HCl in the gastric contents the laboratory
The Gastric Secretion in Pernicious Anemia  
(Averages of 200 Cases)

<table>
<thead>
<tr>
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<th>Pernicious Anemia Patients</th>
<th>Normal Controls</th>
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<tbody>
<tr>
<td>Emptying Time</td>
<td>1-6 hours</td>
<td>2 hours</td>
</tr>
<tr>
<td>Fasting Contents</td>
<td>15.3 c.c.</td>
<td>52-54 c.c.</td>
</tr>
<tr>
<td></td>
<td>(min.-0:max-86 c.c.)</td>
<td></td>
</tr>
<tr>
<td>Mucus</td>
<td>2 plus</td>
<td>trace</td>
</tr>
<tr>
<td>Blood</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Bile</td>
<td>15%</td>
<td>4%</td>
</tr>
<tr>
<td>Pus</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>27</td>
<td>nil</td>
</tr>
<tr>
<td>Free HCl Acid</td>
<td>nil</td>
<td>0-60</td>
</tr>
<tr>
<td></td>
<td>(absent in all cases)</td>
<td></td>
</tr>
<tr>
<td>Total Acidity</td>
<td>2-28 units</td>
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</tr>
<tr>
<td>Total Chlorides</td>
<td>22-88 units</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(fasting)</td>
<td></td>
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<tr>
<td>Inorganic Chlorides</td>
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<tr>
<td>Peptic Activity</td>
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<td>12-18 minutes</td>
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and clinical observations were typical of pernicious anemia. The blood response and clinical improvement following therapy were similar to those of pernicious anemia.

Helmer, Fouts and Zerfas (83) in a recent thorough study of the gastric juice in forty-seven patients found more or less dysfunction of the gastric glands as indicated by

1. Small volumes of juice containing large amounts of mucous.
2. Absence of free HCl with an alkaline reaction.
3. Practical absence of the enzymes, pepsin and rennin.
4. Low chloride concentration with high N and P values.
5. No relation between total acid, pH, Chlorides, N and P as found in normal controls.

Goldhamer (84) has studied the relationship between the amount of gastric juice secreted by pernicious anemia patients and the number of red blood cells of adult type. 17 patients were studied and a total of 117 observations were made. The amount of gastric juice secreted by this group of patients varied from 5 c.c. to 19 c.c. per hour (normal output averages 150 c.c. per hour). Goldhamer determined that the red blood count bears a direct relationship to the amount of gastric secretion, e.g., the greater the amount of gastric juice the higher the red blood count level. He further observed that the constant drainage of gastric juice caused a decrease in the number of red blood cells in the peripheral blood. Wilkinson (85) in a study of 200 cases found the average volume
output of gastric juice in pernicious anemia to be 15.3 c.c.
The maximum output was 86 c.c. while several patients failed to secrete any gastric juice.

The exact relationship of achlorhydria and pernicious anemia, whether one be the cause and the other the effect is a highly debatable question. That it has some significance is attested to by their invariable co-existence. Little can be gained here by a detailed discussion of the questions of the exact significance of achlorhydria in pernicious anemia. But as Cornell (35) has stated "It would seem advisable to adopt the following tentative conclusion: Achlorhydria, a functional abnormality of constitutional origin, is almost invariably associated with pernicious anaemia, and forms, in these cases, a necessary link in an etiological chain, to which by the addition of further unknown links, pernicious anaemia is made to appear."

"Changes in the gastric secretion, though at times seemingly minor in character, may have a profound influence in leading not only to disturbances of digestion and nutrition, but also in producing changes of the gravest character in the hemopoietic system". (87).
Every organism that inhabits the lumen of the gut in pernicious anemia has been suspected of causing the disease. (35).

In 1900 Adami (89) suggested that pernicious anemia represented a subinfection...a chronic inflammatory condition of the intestinal tract which was associated with excessive passing into the portal blood of colon and allied bacilli which subsequently destroy red blood corpuscles".

Hunter (10) (11) (12) conceived an etiological point of view composed of the factors of septic and specific infection. The septic infection, by staphylococci or streptococci, consisted of septic lesions in the mouth ("oral sepsis"), septic gastritis, septic enteritis, and septic colitis. In Hunter's mind the septic infection was a potent antecedent condition creating an unhealthy condition in the stomach and intestine which favored the contraction of the specific infection causing pernicious anemia. The specific infection
was designated as a hemolytic process by Hunter and was the immediate cause of pernicious anemia. In 1909 Hunter made microphotographs of the glossitic lesions showing a long streptococcus in the subepithelial layer of tongue at the eroded areas where the mucosa was gone. From these lesions he claims to have obtained streptococci in a highly virulent state in pure culture. To this organism he attributed largely the septic manifestations which he maintained must precede or antedate the true specific infection directly responsible for the anemia. He made no attempts to incriminate any specific organism as the specific cause of the specific infection.

Hunter carefully studied the organs of defunct pernicious anemia patients and made careful gravimetric studies of the liver and spleen. He was able to demonstrate that the liver in pernicious anemia contains more iron than the liver in other anemias. For him, the marked hepatic siderosis became a specific finding and its presence enabled him to evolve a full blown theory of etiology. Thus, he predicated that a specific infection by virtue of its toxin formation in the lumen of the intestine set up a typical train of events. The toxin was absorbed into the portal circulation and there caused an intense hemolysis, the freed pigment of which was then lodged in the liver lobules. The intense anemia thus created led to the symptomatology of pernicious anemia.

Hester (90), in 1906, first suggested the possible relat-
ionship between B. welchii and pernicious anemia. At that time he commented upon; (1) The abundance of the organism in the feces of pernicious anemia patients as compared with their moderate occurrence in the feces of normal individuals. (2) The gradual reduction in the number of these organisms as the clinical conditions underwent improvement and (3) The ability of the B. Welchii organism to make a hemolytic substance. Hester evidently believed that the organism was capable of producing some toxic product which when absorbed into the portal circulation led to hemolysis.

Klotz and Holman (91), in 1911 called attention to the marked anemia and evidences of hemolysis that occurred in patients with gas gangrene due to B. welchii. Leroy (92) had previously reported a case of B. welchii infection characterized by an anemia.

Simonds, in 1915, isolated four strains of B. welchii from the stools of pernicious anemia patients. Two of the isolated strains were experimentally found to have the property of elaborating a hemolytic toxin, which caused marked hemolysis in experimental animals (93). Cornell (94) later demonstrated that the intrasplenic or subcutaneous injection of B. welchii caused a chronic infection characterized by an anemia of varying intensity marked by anisocytosis but not partaking of the character of pernicious anemia.

Vogel, in 1916, viewed pernicious anemia as a syndrome based essentially on a condition of increased hemolysis plus
a toxic action on the bone-marrow of which the origins are obscure and involved and depend on a variety of partial causes which are differently associated in different cases. One of these factors is hypersplenism while another is the presence of some toxic agent of endogenous nature. The toxin is derived from the alimentary tract and is possibly a lipoid which produces a new lipoid in the liver which is actively hemolytic. In addition there is a general disturbance of the lipoid metabolism of the body. He further stressed the existence of some constitutional anomaly which renders the body susceptible and permits the other agencies to act. (95).

Nye has recently endeavored to experimentally substantiate the hypothetical etiological role of B. welchii in pernicious anemia. Nye believed that B. welchii is not a normal inhabitant of the upper small intestine but migrates to it from the colon in the presence of an achlorhydria. He determined that:

(1) In stools from cases of pernicious anemia there is a great increase in the number of B. welchii spores as compared with normal stools and stools from the majority of cases of miscellaneous diseases.

(2) An increase in B. welchii spores is also present in stools from cases of gastric achylia with pernicious anemia.

(3) Counts of vegetative forms of B. welchii seemed to indicate that in any of the above conditions the number of active or vegetative forms of B. welchii are practically the same.
Nye concluded that the gastric achylia accounts for the increase in spore forms because of the tendency of B. welchii to spore formation in an alkaline medium, and that the chronic intestinal infection with B. welchii is the causative factor in pernicious anemia. (96).

Reid, Orr, and Burleigh experimentally inoculated rabbits with B. welchii cultures and were able to produce acute and chronic infections and concomitant severe hyperchromic anemias, with many of the features of pernicious anemia (97). Kahn and Torrey (98) were able to produce blood pictures simulating those of pernicious anemia by the injection of B. welchii toxin into monkeys but the color indices were higher and the leucocytic depression more marked. Moench, Kahn, and Torrey (99) later made bacterial analyses of seventy-two stool specimens from thirty-three cases of pernicious anemia which they found unusually large numbers of viable organisms among which were B. coli, B. strep., and B. welchii. B. welchii and B. coli showed uniformly high counts and these investigations inclined to the view that B. welchii is of etiological significance in pernicious anemia due to its increased numbers and migration from the colon to the ileum.

Draper and Barach (100) (101) (102) have thoroughly studied the etiological significance of B. welchii in pernicious anemia. They found that the injection of B. welchii hemotoxin into rabbits produced acute and chronic anemias which in many cases tended to disappear spontaneously. The blood pictures
produced were not similar to those of pernicious anemia and the pathological tissue changes were those due to the active hemotoxin, but did not resemble those typical of pernicious anemia. The administration of saline extracts from pernicious stools caused an anemia of secondary type in the rabbits and pathological study of the bone-marrow, liver, and spinal cord revealed no changes characteristic of pernicious anemia. Saline extracts of normal stools produced changes similar to those produced by pernicious anemia stools. The anemias showed a tendency to remission in spite of continual injection of toxins. They also ascertained that the injection of B. welchii toxin caused the production of an anti-hemotoxin. The sera of normal rabbits, normal humans, patients with secondary anemia, and patients with pernicious anemia did not possess any anti-hemolytic activity against the B. welchii toxin.

Cornell (35), has answered the proponents of the B. welchii theory in the following manner. "There is no convincing evidence to support this hypothesis. The toxin has neurotoxic properties, but the chronic infections in rabbits showed no combined system disease, no alterations in the gastric secretion, and no remissions. The toxin has never been shown capable of absorption from the intestine. In fact it is questionable if the organism living in the gut could produce a toxin comparable to that produced under very special culture conditions. It probably cannot be regularly found in the intern-
al organs after death and its presence might always be due to agonal invasion. In some animals this organism may be cultured from the liver during life and health. No agglutination or complement-fixation by the serum on the organism or its toxin has been found, although both have been looked for. The blood picture produced by the chronic infection or by the continued injection of toxin, while definitely suggestive, is really not the blood picture of pernicious anemia and may best be classified as a toxic, macrocytic, hemolytic anemia. Finally B. welchii antitoxin has been repeatedly administered to patients without visible benefit."

Seyderhelm (quoted in Moench (et al) (99)) performed some interesting experiments in order to study the enteric nature of pernicious anemia. He established ileostomies in ten pernicious anemia patients at points from 10 to 20 cm. above the ileocecal valve. He observed that a brown fluid was evacuated which could not be distinguished from stains in appearance, odor, or bacterial content. Following the ileostomy a process of automatic sterilization seems to have occurred and the fecal flora greatly decreased with concomitant clinical improvement. These experiments seemed to implicate the colon as the source of an intoxicant following a migration of the toxic agents from the colon to the ileum where absorptive conditions are optimal. Seyderhelm regarded the colon bacillus as the pathogenetic agent.

Further experimental work by Seyderhelm (quoted in Win-
trobe (63) seemed to support his views. He produced strictures in ten dogs in the ileum at points 10 to 20 cm. above the ileocecal valve, and two of the dogs subsequently developed hyperchromic anemia with megalocytosis, anisocytosis and urobilinogenuria. Autopsy examination demonstrated an abundant colonic type of flora above the stricture and hemosiderosis of the liver and spleen with a megaloblastic reaction in the bone-marrow. In one dog which did not develop anemia, the abundant colonic type of flora was absent above the stricture.

Hurst (9) (50) (60) is a contemporary supporter of Hunter's etiological views, and has firmly upheld the streptococcus as an etiological agent in pernicious anemia. He was able to culture strep. longus from duodenal contents in sixteen of twenty-cases of Addison's anemia and in all of nine cases of subacute combined degeneration of the cord. The streptococci cultured were found to be of the hemolytic variety. In his own words "That the streptococcus itself is the organism which produces the toxin responsible for the nervous disease as well as the anemia, appears very probable from the fact that in two of my patients with subacute combined cord degeneration, each injection of an autogenous vaccine, made from a strep. longus isolated from the socket of an infected tooth and from the duodenum respectively, gave rise after an interval of some hours to a temporary aggravation of the sensation of pins and needles in the hands and feet, which was followed
eventually by the almost complete disappearance of the symptom." Hurst views achlorhydria as an essential predisposing cause which allows bacteria to reach the stomach from the mouth when oral sepsis is present. Oral sepsis due to a special hemolytic streptococcus in the presence of achlorhydria leads to pernicious anemia. "It is probable that one poison is especially toxic for the blood and leads to a hemolytic form of anemia, and that another is specially toxic for the nervous tissues and leads to combined degeneration of the cord."

Knott's (103) investigations of the intestinal flora in pernicious anemia revealed an abundant flora in the small intestine. He viewed the finding as a result of intestinal invasion by pyogenic and catarrhal organisms from the mouth and pharynx, and fecal and putrefactive bacteria from the large intestine. Cultures revealed a 90% incidence of hemolytic strains of bacteria from both groups. Knott has expressed the opinion that any of these hemolytic organisms may lead to pernicious anemia. His later investigations led him to report that effective liver therapy does not influence the nature or intensity of the intestinal infections.

Krumbhaar (104) has expressed some interesting views on the active processes in pernicious anemia. He has advanced the belief that pernicious anemia might well be due to an intestinal noxious agent somewhat associated with achlorhydria and absorbed by the portal circulation. Its appearance in
the liver upsets that organ's hypothetical relationship to hemapoiesis by hindering it from furnishing a necessary ingredient with the result that inefficient erythrocytes are prematurely sent into the blood stream. In spite of the bone-marrow hyperplasia an insufficient number of inefficient erythrocytes are turned out to be destroyed in excessive numbers by the spleen which is stimulated to overactivity by the passage of the noxious agent through that organ. Krumbhaar, by removal of dog's spleens, or by diverting the splenic blood from the liver, produced transient anemias which led him to believe that the normal spleen secreted something into the blood which, after its passage through the liver, exerted a stimulating effect on the erythropoietic function of the bone-marrow. He pictures the removal of a spleen that is diseased to have the opposite effect on the blood picture. The spleen may have some influence in preventing the liver from exerting its maturating effect on the erythroblasts which defect the administration of liver corrects.

Allport (10) suggested that pernicious anemia is due to an unknown substance present in normal blood, but diminished in pernicious anemia the function of which is to regulate normal phagocytosis of blood elements. He assumed this protective factor to reside in the blood or bone-marrow and tested its therapeutic value by making an alcoholic extract of ox-blood with some measure of success in one case. He called the protective factor "Sanguinin".
Macht (106) has recently analyzed the blood serum of pernicious anemia patients to determine the presence in it of a toxic substance or substances. His method consisted of observing the effect of such sera on the growth of seedlings of Lupinus albus and he was able to observe a decided inhibition of maturation and growth. He was led to believe that such inhibition implied the presence of some toxin in the blood serum of pernicious anemia patients and has ascribed etiological significance to the toxin. He states that this phytotoxic property is not shown by sera from patients with various other blood diseases and from normal controls. Liver therapy eventually led to a decrease in the phytotoxic index of the blood serum. Repetition of these experiments by Upjohn and his co-workers failed to confirm the work of Macht and they could find no appreciable difference in growth rates of seedlings in the presence of pernicious anemia blood serum and the serum of normal individuals. (107)

Dock and Mermod (108) (109) have found that there is increased reticulocyte resistance to hemolysis after successful treatment of pernicious anemia. Previous to therapy they found that plasma from pernicious anemia patients was strongly hemolytic but became less so after therapy. They have suggested the presence of a hemolysin to which reticulocytes are particularly vulnerable. They later found that intravenous injections of repeated doses of Congo Red in 6% glucose was found to invoke a marked reticulocyte response
and a decrease in the serum bilirubin. Congo Red injected daily for five days intraperitoneally into anemic guinea pigs produced reticulocyte response maximal within 5 to 7 days. They have postulated that the Congo Red neutralizes a toxin enterogenous in origin which is hemolytic and have stated that "these observations make imperative a further exploration of the old theory that pernicious anemia is due to excessive absorption or deficient detoxication of noxious substances derived from gastro-intestinal tract."

Wakerlin and Brunner (110) (111) claimed to have found an anti-anemic principle in normal urine which significantly increases the reticular material in the red blood cells of pigeons in a manner analogous to the liver principle. They have found that sterile urine specimens from untreated pernicious anemia patients, when unheated, caused a decrease in reticulocytes below 5%. Similar specimens, when heated, did not cause such a decrease. Fifteen of twenty-one pigeons receiving unheated urine showed no ill-effects. However, following the primary decrease in reticulocytes most of the surviving pigeons showed a subsequent reticulocytosis, which Wakerlin and Brunner have assumed indicates the presence of anti-pernicious anemia urinary substance. They have further postulated a thermolabile, toxic, reticulocyte decreasing factor and a partially thermostable relatively non-toxic reticulocyte-stimulating factor, in such urines. Normal human urine contains the former but not the latter.
Cornell (35) has outlined the following requirements for any hypothetical toxin and states that no definite proof of its existence and of its etiological importance has been demonstrated. (1) Any toxin must be shown to be specifically active in the body. (2) Any toxin to be seriously considered would have to produce: (a) a megaloblastic marrow reaction, (b) a characteristic blood picture, (c) nerve tissue degeneration without neuroglial increase, (d) remissions either by intermitting doses or on constant doses: (3) It has not been shown that the truly hemolytic products are absorbable from the human intestine. (4) If a toxin is hypothecated then a unity of toxin must be predicated by the strikingly similar clinical and pathological pictures in all cases. Plurality of toxins finds its best argument in the occurrence of subacute degeneration of the cord temporarily without anemia, and in the occurrence of splenomegaly in certain cases.

Gulland and Davidson (2) have examined bacteriologically the gastro-intestinal content of 41 cases of pernicious anemia qualitatively and quantitatively. In addition, the intestinal flora of other pathological conditions and of healthy subjects were investigated for the purposes of comparison. They have declared that:

1. No evidence was found to indicate that any individual type of organism was specifically related to pernicious anemia.
2. No evidence was found that bacterial haemolysins are of aetiological importance in the disease.
3. A great numerical increase of organisms, normal inhabitants of the bowel, e.g., B. coli, streptococci and especially B. Welchii was found to occur in the gastro-intestinal contents of cases of pernicious anaemia.

4. No evidence was found that these organisms differed qualitatively from those found in healthy persons.

5. The great quantitative increase of bacteria, especially at levels of the small intestine which in normal persons are relatively bacteria-free, may be a factor of great aetiological importance.

On the other hand they have pointed out that it can be definitely said that in pernicious anemia "We know that gastritis and marked "sepsis" of the gastro-intestinal tract exist. In spite of the want of scientific evidence, we are forced to admit that clinical information is strongly in favour of the possibility of intestinal intoxication being the cause of many chronic ailments, and the view of clinicians, based on careful observation over long periods, cannot be disregarded".
LIVER THERAPY, ITS SIGNIFICANCE, AND THE ROLE OF THE STOMACH IN PERNICIOUS ANEMIA.

The observation of Minot and Murphy (112) in 1926 that adequate liver feeding induces and maintains remissions has led not only to far reaching results in the domains of therapy, but it has served to stimulate investigations that are slowly unraveling the perplexing problems intrinsic to the disease and has resulted in a marked revision of ideas concerning gastric physiology and deficiency disease.

Though the idea of dietary therapy in pernicious anemia was not new, the earlier reports that appeared were not sufficiently encouraging and convincing enough to rule out the possibility of spontaneous remissions and the prognosis was not materially changed.

Diet as a factor in the treatment of pernicious anemia has been referred to by most writers on the subject. Thus, in 1863, Habershon (113) wrote "many patients at an early stage completely recover under the influence of bracing air and a nutrient and stimulating diet." He apparently recognized
the importance of the gastro-intestinal tract as a factor in the disease. Pepper (13) drew attention to the liver degeneration and atrophy of the mucosa of the stomach with consequent impaired digestive functions and stressed the need for attention to nutrition and the administration of food in an easily assimilable form. Osler (114) speaks of cases "that appear to have got well with change of air and a better diet, after resisting all ordinary measures."

Hunter, (10) (11) (12) in accord with his views on the pathogenesis of pernicious anemia attributed great importance to dieto-therapy, and advocated a farinaceous diet. Frazer, (115) in 1894, described a pernicious anemia patient whom the administration of raw bone marrow proved of great benefit. Barker and Sprunt (116) advised "an abundant roborant diet, rich in protein". Fitch (117) advocated a diet including bone marrow, liver, and spleen because of their abundance of iron. Gibson and Howard (118) suggested an iron-rich and vitamin adequate diet, incidentally containing an abundance of liver, and found that the diet established a positive nitrogen and iron balance and there was an apparent increase in remissions. Mosenthal (119) also demonstrated the restoration of the nitrogen balance in pernicious anemia by the forced feeding of a diet rich in meats.

The work of Whipple and Robscheit, (120) in 1920 and subsequently, may be cited as the focal point from which the observations of Minot and Murphy emanated. Whipple and Robscheit demonstrated the efficacy of a liver diet in secondary anemia
of dogs, induced by bleeding, in hemoglobin regeneration. Jene\textsuperscript{121}, in 1922, also reported that hemoglobin regeneration was accelerated by protein and a vitamin-rich diet. In 1925, Whipple and Robscheit-Robbins found beef liver feeding was associated with maximal blood regeneration in constantly maintained severe secondary anemias.

Minot and Murphy, (112) in August, 1926, reported on a series of cases treated by a "special diet". The series consisted of 45 patients in various stages of pernicious anemia and had been under observation for periods of time varying from six weeks to two years. The diet used consisted essentially of the following components daily:

1. From 120 to 240 grams of cooked calf's or beef liver.
2. 120 grams or more of beef or mutton muscle meat.
3. Not less than 300 grams of vegetables containing from 1\% to 10\% of carbohydrates.
4. 250-500 grams of fruit.
5. 40 grams of fat.
6. An egg and 240 grams of milk.
7. Breads, potatoes, and cereals to bring the caloric intake to 2000-3000 calories.

Thus the diet was made up of foods rich in complete proteins and iron, contained an abundance of fruits and fresh vegetables, and had a low fat content. Proportionately it contained, 340 grams of carbohydrate, 135 grams of protein and 70 grams of fat. In addition, 15 c.c. of dilute hydro-
chloric acid was administered daily and the general regimen included abundant rest.

All patients showed a prompt, rapid and distinct remission of the anemia with marked symptomatic improvement. Preceding the institution of the diet the patients showed an average red blood cell count of 1,470,000; one month thereafter the average red blood cell count was 3,400,000 and four to six months later 27 cases had an average red count of 4,500,000. All cases showed a good reticulocyte response and icteria indices became normal. Three of the patients discontinued the dietetic regime and showed a typical relapse of pernicious anemia, and one of these rapidly improved after a reinstitution of the diet.

The work of Minot and Murphy in this series of pernicious anemia patients served to place the dietetic treatment of pernicious anemia on a firm basis. It also seemed to indicate that something contained in the foods was active in the blood of pernicious anemia. Minot and Murphy took cognizance of the phenomenon of spontaneous and natural remissions in pernicious anemia, but the regularity with which the diet induced remission ruled out the possibility of natural remissions. Also, the diet-induced remissions were more complete than natural remissions which tend to decrease in level as the disease progresses. Thus, the work of Minot and Murphy brought the problem of nutrition in pernicious anemia to a sharp focus of interest and proved the active role of dietary factors as
therapeutic agents, and threw a new light on etiological problems.

Koessler, et al. (121) in 1926 postulated an etiological theory that combined several factors. They indicated their belief that primary pernicious anemia represents an intoxication through bacterial poisons formed by either streptococci B. Coli, or B. welchii. These poisons are hemolytic, neurotoxic, and myelotoxic and are found in the upper small intestine of persons with achylia. Vitamin deficiency and underfeeding, leads to a change in intestinal structure and function which allows absorption of the bacterial poisons. The blood changes and the changes in the gastro-intestinal tract are due to vitamen A deficiency, the neurologic manifestations are engendered by vitamin B. deficiency, and the hemorrhagic tendency is the result of a deficiency of vitamin C. They studied the effects of chronic vitamin A. deficiency in rats and were able to observe the development of a chronic anemia and found that vitamin A must be present in the diet of the anemic rats in order to foster blood regeneration. Koessler and his associates later reported (122) the results of treating 45 pernicious anemia patients by a vitamin rich and high caloric diet for from six months to two years and found that remissions were immediate and blood pictures became normal in from 8 to 14 weeks, with clinical improvement. Liver and kidney constituted a large part of their diets, but in the light of their previous work, they postulated that the therapeutic
value or liver and kidney was intrinsic to their high vitamin content, and criticized Minot and Murphy's low-fat diet because it led to a decrease in vitamin intake.

In a later paper Murphy and others reported the complete blood studies in ten cases of pernicious anemia treated by the "liver diet". Rapid and distinct remissions were noted in all of the ten cases and there occurred the rapid delivery of new, young red blood cells from the bone-marrow into the general circulation as evidenced first by a prompt increase of the reticulocytes in the circulating blood. Icteric indices indicated a sharp decrease to normal limits of bile pigment concentration in the serum with a coincident rise in red blood cell count and hemoglobin percentages. The morphologic appearance of the red blood cells became normal with simultaneous reduction of erythrocytic size to normal. Heath (57) at the same time published confirmatory results as did Middleton (124) and Means Richardson (125). Ordway and Graham (126) reported markedly beneficial results in 25 cases treated by liver and liver extract. In addition they made a critical survey of all reported cases in an attempt to gather evidence bearing on the successful application of the liver diet in pernicious anemia. 578 cases were collected and exhibited uniformly successful and remarkable results. Starting with an average red blood count of 1,500,000, all of the cases reached a level of 4,000,000 or more red blood cells with very few exceptions. Clinical changes were con-
stant and led to clinical recovery. Other striking blood changes were (a) the increase in reticulocytes (b) increase of hemoglobin percentages, (c) the decrease to normal of the icteria indices (d) the gradual return to normal of red cell morphology and (e) the increase of the white blood cells further indicating bone-marrow activity.

The isolation of the "active" principle of liver was first attempted by Cohn, Minot and their associates (127) (128). The early stages of fractionation showed that the active principle was not precipitated with the proteins removed by heat or acidification to pH5 nor by subsequent precipitation of the filtrate in alcoholic solution of 70 per cent. by volume. An active fraction "G" was, however, precipitated by raising the concentration of alcohol to 95 per cent. by volume, and was the first extract extensively used in the treatment of pernicious anemia. It was determined that all active material could be precipitated from the filtrate by phosphotungstic acid or by Reineke's salt, and it could be regenerated from these precipitates (129). Salts of gold, silver, platinum, or mercury destroy clinical activity, as does exposure to normal sodium hydroxide at room temperature, or boiling with mineral acids. Heating to 80 degrees C. or even to 100 degrees C. at pH 5 does not destroy clinical activity. The active material is readily soluble in water, and when partly purified it is also soluble in large volumes of slightly acid 95% alcohol. It is insoluble in ether.
The present conception of the nature of the "anti-ane-
mic principle" in liver can be summarized as follows. The
active principle appears to be non-protein in nature and pro-
ably is a relatively small nitrogenous base which can be
precipitated by picric and tannic acids. Cohn, Mc Meekin,
and Minot (130) have concentrated the activity in a fraction
precipitable by picric acid which was low in alpha-amino ni-
trogen. Dakin and West (129) have concentrated the prin-
ciple in a fraction precipitable by picric and flavianic acids.
The concentrated fractions isolated by each of these groups
of workers appear to be comparable. Maximal effects upon
blood production in pernicious anemia have resulted from the
injection of from 80 to 100 mgm. of the most active fractions.
Fiske, Subbarow, and Jacobson (130) have recently separated
from liver three different fractions of which two have been
chrystallized. Each of the three different fractions ex-
hibits a quantitatively different degree of activity when
tested on guinea-pig, but when administered to a pernicious
anemia patient proved therapeutically inert. On the other
hand, a mixture of the fractions was highly active thera-
peutically.

In 1928 and subsequently the experiments of Castle (131)
(132) (133) (134) (135) did much to clarify the role of the
stomach in pernicious anemia and his results and conclusions
have led to much work that has slowly resulted in a modern
conception of the nature of the fundamental errors in the hu-
man body giving rise to pernicious anemia. The almost universal relationship of achlorhydria to Addison's disease has been discussed elsewhere as has the fact that the achlorhydria precedes clinical symptoms and persists after otherwise successful liver therapy. The work of Castle has done much to clarify the role of the liver and stomach in hematopoiesis, and to elucidate the nature of the antianemic factor.

The action of liver in benefiting cases of pernicious anemia suggested to Castle the possibility of a deficiency basis for the disease. "The deficiency would, however, not seem to be of the ordinary type, for liver is ordinarily absent from the diet of unaffected normal humans. The incidence of a marked reduction of hydrochloric acid and pepsin in the stomach which is not affected in the general improvement of the patient on a liver diet suggests that the achylia may possibly play an intermediary role in causing the deficiency. An obvious possibility, especially in view of the probable polypeptide nature of the effective principle in liver extract, is a deficiency of the gastric digestion of protein."

Castle in his experiments determined that when beefsteak which had previously been digested by a normal person's gastric juice was given to a person suffering from pernicious anemia, a typical reticulocyte response could be elicited. Artificially pepsin-digested beefsteak failed to evoke any reticulocyte response as a measure of hematopoietic stimula-
tion.

From these experiments Castle concluded "It is possible that the achylia gastrica of the pernicious anemia is operative in the production of a deficiency disease through a failure of the patient's stomach to produce the substance apparently formed during digestion in the normal stomach."

Later, to each of 3 cases of pernicious anemia was given daily for a period of ten days between 200 and 300 grams of beef muscle without demonstrable effect on blood formation for 14 days. Immediately thereafter to each of these patients and to 7 others were given daily the incubated contents of a normal human stomach recovered after the ingestion of similar quantities of beef muscle. In each of the three patients first-mentioned and in five of the seven others there appeared before the tenth day an increase of the immature red blood cells followed by a progressive improvement in the anemia similar to that obtained with liver therapy.

Castle subsequently tested the hematopoietic effect produced in pernicious anemia patients by the administration of gastric juice alone and of beef muscle alone. Each of these substances alone proved inactive. However, to the patients previously tested there was administered the material resulting from the incubation of 300 C.C. of gastric juice, from normal individuals, with 200 gm. of ground beef at 37 degrees C. and at a pH of 2.5 to 3.5 and later neutralized to pH5 prior to administration. In all but two of these cases there oc-
curred a significant reticulocytosis and distinct clinical improvement.

As a result of these experiments Castle concluded that the interaction of beef and gastric juice produces some substance capable of causing a remission in certain cases of pernicious anemia. On the basis of these experiments Castle elaborated a theory of the existence of an "intrinsic" factor in stomach or gastric juice and of an "extrinsic" factor in beef which by their interaction lead to the formation of a substance capable of stimulating bone-marrow which he termed an anti-anemic principle. Thus, all patient's with Addison's anemia are unable to carry out this essential reaction.

In 1930, Castle et al reported further experiments conducted to substantiate his previous results and to attempt to ascertain the nature of the intrinsic and extrinsic factors. Incubation of beef muscle first with human saliva and subsequently with duodenal contents free of gastric secretion failed to elicit hematopoietic response when administered to pernicious anemia patients in relapse. Incubation of beef with pepsin and hydrochloric acid yielded no response. Heating of the material resulting from incubation of beef with normal gastric juice to a temperature of 70 degrees C. leg to negative results when the material was administered to pernicious anemia patients in relapse.

From these experiments Castle drew the following conclusions:
1. The active constituent (intrinsic factor) of the normal human fasting gastric contents is secreted by the stomach.

2. The substance is organic thermolabile, possibly an enzyme, capable of niteration with protein (extrinsic factor) in neutral solution, resulting in the production of a material having a marked hematopoietic effect in pernicious anemia patients.

3. The lack of the intrinsic factor is probably the essential defect leading to the development of pernicious anemia.

Castle later showed that there may be a dissociation of gastric acidity and the intrinsic factor. Cases of tropical sprue with normal stomach acid values may present an anemia which responds to liver therapy, while elderly individuals often have an achlorhydia but no anemia. Acid gastric juice from an anemic patient with sprue, when mixed with meat and fed to a patient with pernicious anemia failed to induce a reticulocyte response while achlorhydic gastric juice from an elderly non-anemic individual was found to contain the intrinsic factor by the same test.

Thus, Castle believed that the intrinsic factor was absent from the otherwise normal gastric contents of pernicious anemia patients and that it was present in the otherwise achylic gastric contents of patients without anemia and of three patients with hypochromic anemia. Some of the patients with pernicious anemia with apparently normal gastric
juice in which the intrinsic factor was shown to have been absent in relapse was later shown to be present after remissions induced by liver therapy.

Castle and Strauss later turned their attention to the extrinsic factor. Various substances were substituted for the beef of their original experiments and digested by normal gastric juice. By this method the extrinsic factor was found to be absent from casein, gluten, nucleoprotein from hen's blood and nucleic acid of yeast and of animal origin. Washed beef muscle protein, splenic pulp and autolyzed yeast contained the extrinsic factor. (136)

Wills and Naish (137) used the white of hen's egg for a vitamin B2 preparation and administered 40 to 80 rat doses of the preparation alone or incubated with normal gastric juice and found it unable to influence the regeneration of red blood cells in typical pernicious anemia. However, the patient was not studied later with a combination of gastric juice and an extrinsic factor of known quantity so their results are not conclusive.

In 1932, Wills, (138) studying tropical macrocytic anemia, a condition wherein the intrinsic factor is assumed to be present, presented some observations on the nature of the hemopoietic factor in "Marmite" (a preparation said to be similar to "Vegex"). In these experiments the B2 content of the preparations were checked and dosage governed accordingly. Dried distiller's yeast, aqueous extracts of brewer's
yeast, the acid clay vitamin preparation of Janse, and egg white were found to be inactive. However, maximal or marked reticulocyte responses were obtained with preparations of Marmite (crude acid, alkaline, and aqueous extracts, alcoholic extracts and autoclaved Marmite). Wells concluded that the extrinsic factor is not identical with vitamin B2 and suggests the possibility of some unknown protein breakdown product as the extrinsic factor.

Lassen and Lassen (139) have also recently studied the possible role of Vitamin B2 as the extrinsic factor in pernicious anemia. Eight patients with typical pernicious anemia were treated with various yeast preparations given partly without additions of any kind, partly after incubation with normal gastric juice. They found that yeast was completely without any antianemic effect. Gastric juice did not, with any degree of certainty, increase the content of active antianemic principle.

Castle has summarized the results of his work and interpreted them as follows. (140).

"It is believed that a physiological mechanism exists in the normal individual which is usually absent in the patient with Addisonian pernicious anemia in relapse. Both the food and the stomach are involved in this process, which will obviously be initiated in the normal individual whenever extrinsic factor is taken in the food. It is logical to conclude that the integrity of this mechanism prevents the dev-
velopment of pernicious anemia in the normal individual; and that its absence leads to a deficiency of the active principle of liver and so to pernicious anemia. It is, however, evident that a deficiency of the active principle finally effective in the body may be brought about by at least three different types of derangement of this mechanism in the gastrointestinal tract. A lack of intrinsic factor in the food or a failure of the absorption or the destruction of these substances or their end products in the intestinal tract would have the same result. Furthermore, an interruption or inhibition within the body of any link in the chain of chemical events leading to the production of the end product active in the bone marrow would also have a similar effect."

"Deficiency of the gastric factor is apparently the dominant mechanism in Addisonian pernicious anemia in relapse."

The work of Castle was ample evidence of the essential role of the stomach in the causation of pernicious anemia. In 1929 Sturgis and Isaacs (141) and Sharp (142) administered to pernicious anemia patients the powdered stomachs of hogs: defatted and dessicated at low temperatures. The daily administration of from 20 to 30 gm. of the dessicated material derived from 150 to 200 gm. of the fresh organ was promptly followed by a reticulocyte response greater than that obtained by feeding the extract from 300-600 gm. of liver. These experiments indicated that the stomach tissue contains a red blood cell maturing substance, and indicates either the
presence of an active hemotopoietic principle in the stomach wall or the presence of an enzyme which may act on the protein present in the stomach, during the interval following removal of the stomach, to form an active hemopoietic substance. In 1930 Sturgis and Isaacs (143) ascertained that the response was negligible when either the gastric mucosa or muscularis was fed separately. However, Wilkinson, (144) reported that either muscularis or mucosa was active in stimulating the hemopoietic system. Conner (144) treated six patients with raw swine stomach, and was able to stimulate a hemopoietic response with marked clinical improvement. Renshaw (145) found dessicated hog's stomach highly effective in a patient in whom liver therapy had been unsatisfactory.

Middleton and Strahm (146) administered pooled normal gastric juice to two pernicious anemia patients, but failed to evoke a remission. They offer their results as support of Castle's view that the gastric juice alone does not contain the active factor essential to the stimulation of erythropoiesis.

Morris et al. tested the efficiency of a normal gastric juice as a hemopoietic agent in pernicious anemia. They found that gastric juice concentrated by vacuum distillation evoked maximal reticulocyte responses when injected intramuscularly (147). They infer that the gastric juice contains a specific hemopoietic hormone which they have called "Addisin". Zerfas
and Fouts endeavored to isolate the Addisin principle of Morris, from the known gastric enzymes by ultrafiltration methods. They ascertained that concentration by vaccum distillation and refrigeration for 2 months were necessary before the hemopoietic principle could be demonstrated. From this work they conclude that some change must occur in the gastric juice before a hemopoietically active gastric juice can be demonstrated and that the gastric juice contains both the intrinsic and extrinsic factors of Castle but require some mode of activation before it is liberated. (148).

The intrinsic factor of Castle has not been identified with any known constituent of the normal gastric secretion. It has been shown not to be hydrochloric acid, pepsin, rennin, or lipase. (133) (134). It is present in the gastric secretions of certain persons with gastric achlorhydria, who either have no anemia, or have other types of anemia.

It may be absent from the gastric secretion in certain unusual cases of pernicious anemia in the presence of normal amounts of hydrochloric acid and enzymes. (134). Castle's work has shown that it is not present in normal heman saliva. Castle (134) has also shown that it was not present in normal duodenal contents from which gastric juice has been excluded. It is readily destroyed by heat:boiling for five minutes, heating to 70 degrees to 80 degrees C. for half an hour, or to 40 degrees C. for 3 days.

The extrinsic factor of Castle also has not been identi-
fied. It is present in beef muscle (132), eggs, and rice-polishings, and wheat germ (149). Fouts, Helmer and Zerfas have found the liver fraction "G" of Cohn, Minot and their associates to be an efficient source of extrinsic factor (150). They have found that 10 c.c. of normal gastric juice will increase the potency of 4.5 grams of liver extract. They have assumed that the increase in potency represents a reaction similar to that which occurs when normal gastric juice acts on beef muscle rather than an increase in the active principle present in the liver.

Wilkinson and Klein (151) were able to obtain stomach extracts that were hematopoietically active in the treatment of pernicious anemia, by methods similar to those used to obtain the enzyme zymase from yeast. They were unable to produce an active extract by methods similar to those used for liver fractionation. They termed the active preparation 'hemapoietin' and view it as analogous to Castle's intrinsic factor.

Goldhamer (152) has recently predicated that the so-called intrinsic factor of Castle in pernicious anemia represents a deficiency in quantity rather than quality and that the rate of hemopoiesis is dependent on the quantity of intrinsic factor produced. He has raised the following objections to Castle's theory:

1. The characteristic spontaneous remissions which occur in pernicious anemia are not explained by Castle's views.
2. The fact that at least some maturation of red blood cells occurs in patients who apparently have no intrinsic factor in their gastric juice despite the postulation that the intrinsic factor is necessary to maturation.

Goldhammer studied the gastric juices of pernicious anemia patients in relapse to determine the quantitative presence of extrinsic factor. He found that such gastric juice incubated with meat was potent in producing reticulocyte responses. Concentrates of the juice proved active when injected intramuscularly. Red blood counts and hemoglobin percentages were restored to normal levels and the reticulocyte response reached calculated peaks. Thus, Goldhamer has inferred that the gastric juice in pernicious anemia patients is quantitatively deficient in the intrinsic factor, and that the red blood count level depends on the amount of intrinsic factor secreted.

Greenspan (153) has reported the results of a series of well conducted experiments that serve to clarify many confusing elements concerning the role of the stomach in hematopoiesis. He based his experiments on the following theory:

"An erythrocyte stimulating hormone resides in the gastric mucosa. In pernicious anemia, in which atrophy of the gastric mucosa takes place, a loss of this hormone occurs coinciding with the disappearance of acid from the gastric juice. Thus, in addition to the well known external secretion (digestive) the glands of the gastric mucosa produce an inter-
nal secretion (hematopoietic); this suggests an analogy of the pancreatic gland. It is possible that this hematopoietic hormone may control the level of erythrocyte production in normal individuals."

This conception of the role of the stomach in pernicious anemia differs from Castle's theory in that it recognizes no "extrinsic factor" but postulates deficiency of a single substance as causing subnormal hematopoiesis.

In order to test this theory certain animal experiments were carried out in 1924 and 1925. These showed that extracts of hog stomach mucosa when injected into normal rabbits and guinea-pigs produce (1) a temporary erythrocytosis with an absolute increase of from 1-4 million erythrocytes within two hours after injection. (2) a moderate reticulocytosis and (3) bone marrow hyperplasia after repeated injections. Unfortunately, the hog stomach mucosa was not used clinically in pernicious anemia cases.

Later experiments carried out by Greenspon to clarify the work of Castle and others has swept aside many difficulties and contradictory results. Dessicated stomach was intubated with pepsin and hydrochloric acid and subsequently administered to a patient with pernicious anemia without any reticulocyte response. Stomach tissue was then depepsinized using the method of Fenger and Andrew ( ) and its subsequent administration in a pernicious anemia induced a definite reticulocyte response and remission of symptoms. The depep-
sinized tissue was then incubated with pepsin and hydrochloric acid, and found to be inactive; but its incubation with hydrochloric acid alone resulted in an effective preparation producing a definite reticulocyte response.

The results of these experiments indicate that (1) the pepsin content of normal gastric hog mucosa could be removed without destroying the anti-pernicious anemia principle and (2) that hydrochloric acid alone does not destroy the active anti-pernicious principle and (3) that pepsin destroys the anti-pernicious anemia.

Greenspon farther administered calcium carbonate orally to two subjects followed by histamine stimulation of gastric juice production. The gastric juice was collected, iced and the reaction of the juice adjusted to neutrality and maintained in such a condition. To a pernicious anemia patient was then administered 250c.c. of the cold, neutralized gastric juice daily. It was given in the morning on an empty stomach and no food was allowed for four hours in order to avoid the introduction of any "extrinsic factor". A 14 percent reticulocyte response occurred on the seventh day. This experiment showed that the oral administration of normal gastric juice is effective in pernicious anemia if peptic activity is prevented; it also shows that the physical presence of pepsin does not destroy the anti-pernicious anemia principle if the pepsin is inactivated chemically. It also shows that the gastric juice is effective without an "extrinsic factor".
Meutengracht (154) investigated the antianemic principle in the stomach in an attempt to localize the particular portion of the stomach concerned in its production and to correlate its function with its histological structure. He ascribes to the pyloric glands the function of secreting an unknown substance essential to blood and the central nervous system. He reasons therefore that the pernicious anemia is caused by an atrophy of the pyloric glands. The similarity of certain glands in the duodenum to the pyloric glands explains why not all cases of gastric atrophy lead to pernicious anemia.

Jones et al. (155) have reported the results of a gastroscopic, roentgenologic and surgical study of five cases of pernicious anemia, before and after successful treatment. Observations of the stomach were made by gastroscopic and roentgenologic examination, by direct observation at laparotomy and by histologic examination of biopsy material. They present evidence indicating that gastric atrophy occurs particularly during a relapse and not as an invariable accompaniment of the disease. Following specific therapy of the pernicious anemia, evidences of atrophy and hypertrophy of the stomach tended to disappear. They believe that the change from an appearance of atrophy to that of a normal gastric mucosa represents an epithelial change associated with successful treatment of a specific deficiency state rather than the healing of a chronic inflammatory process. They believe that
the apparent return to normal from the hypertrophic condition of the mucous membrane represents the subsidence of a chronic gastritis.

Goldhamer, Isaacs, and Sturgis (156) have observed the effects of administration of various livers in pernicious anemia. They administered the following types of liver to various pernicious anemia patients—
1. Liver of human foetus.
2. Liver from an adequately treated case of pernicious anemia.
3. Liver from an inadequately treated pernicious anemia.
5. The liver of acute yellow atrophy.

The administration of 1, 2, 5 evoked reticulocyte responses, but 3, 4, proved ineffective. "It is suggested that a liver may be sufficiently damaged so that the active principle may be present but not presented to tissues for utilization." "A pernicious-like anemia may be present if liver is so damaged that it cannot store the active principle or cannot present it to body tissues."

Recent work has tended to prove that the liver is 'storage house' of the anti-pernicious anemia principle. Richter and Ivy (157) demonstrated that a liver extract made from the liver of an adequately treated pernicious anemia patient when administered to pernicious anemia patients is effective. Extracts made from the livers of untreated pernicious anemia...
patient's proved ineffective. They conclude that the liver does not fabricate the anti-anemic principle but rather acts as a storage depot.

Wintrobe and Schumacker (158) have described several cases of macrocytic anemia associated with hepatic disease. In one of the cases a reticulocyte response was secured by intramuscular injection of liver extract which suggested a storage of the hemapoietic factor in the liver, which is interfered with in hepatic disease.

Goldhamer's case of cirrhosis of the liver with a blood picture of pernicious anemia also responded to liver therapy. He has concluded that in addition to defective gastric secretion, defective intake of food and inadequate absorption a fourth factor operative in pernicious anemia is the ability to store the material necessary for the maturation of red blood cells. (159)

Wilkinson, and Klein have stated (160) that their stomach extract "Hemapoietin" acting on substances of diet produces an active principle that is stored in the liver. They have prepared various concentrates of hemapoietin which they believe differs from the active principle of liver in that it is extremely sensitive to heat and chemical treatment.

Goodman and others (161) have studied the anti-anemia potency of livers obtained from totally gastrectomized swine. Seven totally gastrectomized swine were observed
for from two to six months and fed a diet low in anti-anemic principle. They showed no great changes and post-mortem examinations of liver and spinal cord revealed nothing abnormal. However, extracts of liver made from the animals showed definitely that after total gastrectomy the anti-anemia potency of liver becomes depleted and that a significant loss could be detected after three months.
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