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THE EVOLUTION OF THE TREATMENT OF ADDISONIAN ANAEMIA*

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The rational treatment of any disease is governed entirely by the depth of understanding of its fundamental cause and of the factors which may contribute. The treatment of Addisonian anaemia has therefore evolved with the accumulation of knowledge through the century since 1855, when Addison, in the preamble to his classical monograph, *On the Constitutional and Local Effects of Disease of the Suprarenal Capsules*, wrote his few brief paragraphs on an "idiopathic anaemia."

It would not be within the terms of my title to enter into a long discussion on whether the first recognizable clinical descriptions of Addisonian anaemia should be ascribed to James Scarth Combe, of Edinburgh, to Thomas Addison, of Guy's, or to Professor Anton Biermer, of Zurich. But it would be appropriate to mention that Addison recognized that the malady was fatal, the onset was insidious, the usual symptoms of anaemia were present, that the body fat was often well preserved, and that treatment was ineffective. He stated that the condition arose from "no discoverable cause whatever," but speculated that "from the disease having uniformly occurred in fat people" he was "naturally led to entertain a suspicion that some form of fatty degeneration might have a share at least in its production."

Addison's most clear description—though submerged in his classical monograph on suprarenal disease, and consequently open to the criticism that the author was confusing two diseases—is nevertheless far superior to that given by Combe (1824), whose patient had, indeed, a severe anaemia, but with no convincing features suggestive of Addisonian anaemia.

There is no doubt, however, that the account of 15 cases of fatal anaemia which he had met with in the course of five years, given by Biermer in 1872, came nearer than any other that had previously appeared to depicting Addisonian anaemia as it is recognized to-day.

Addison's position was, however, firmly established some years after his death by two staunch advocates: first, his devoted pupil—Samuel Wilks—who in 1874, in a letter to the *British Medical Journal*, asserted that Thomas Addison had lectured about the disease as far back as 1843; and, secondly, William Pepper, professor of clinical medicine in the University of Pennsylvania, himself greatly interested in the disease, who gave, in 1875, some of the first reports of the bone marrow—

albeit not very convincing, since they suggest leukaemia rather than pernicious anaemia—and who considered that "the older and equally clear description by Addison of idiopathic anaemia was one and the same affection as the progressive pernicious anaemia reported by Biermer."

Addison as a Diagnostician

Addison was outstandingly famous as a diagnostician; his obituary notice in the *Medical Times and Gazette* (1860, 2, 20) says: "Diagnosis was his forte, and those who knew him best are aware that he stood unrivalled in his power of searching into the complex framework of the body and dragging the hidden malady to light. His very meagre and simple prescriptions—if he remembered to prescribe at all—showed that he made no study of therapeutics."

Nevertheless, in what is always regarded as his first public pronouncement on Addisonian anaemia, his communication to the South London Medical Society in 1849, there are some scant descriptions of treatment, but it is difficult to know whether Addison was then speaking of suprarenal disease or his "idiopathic anaemia." The probability is the former, but there is clearly confusion of both. Whatever may have been the nature of these cases, the somewhat non-specific treatment can be regarded as appropriate to all of them.

In referring to two patients who recovered, he says of one, "A man below the middle period of life, who was looked upon as past all hope, and suspected to be suffering from some latent malignant disease, slowly, but steadily, recovered under the free use of brandy, but with the singular result that the hair on one side of his head turned permanently grey, whilst the other side retained its original brown colour."

Treatment of anaemia at that time had not progressed since the time when Sydenham wrote, "We give Mars in the pale colours, the pallor disappears, and once again the face is rosy and ruddy." Then, as now, the iron-deficiency anaemias would have responded to iron in the form of the oxide, the sulphate, and the carbonate, provided sufficient was given, and this irrespective of mercury and other metals, the astringents, diet according to the powers of the stomach, as well as formidable purgings, which were put into operation at the same time.

Pepper (1875), however, appreciated that Addisonian anaemia was in a class by itself, and doubtless he had found, like others, that iron was ineffective. Unhindered by our modern knowledge of such trivialities

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as blood groups, he made at least a rational if somewhat perilous approach to symptomatic treatment by advocating and practising blood transfusion, with reactions and results which strike terror into the heart of the modern reader.

Approach to Rational Treatment

At this time—the 'seventies—the current views on aetiology can hardly be regarded as pointing the way to rational treatment, but even then the possibility of a psychosomatic influence was being seriously considered.

Samuel Wilks (1874), in the letter to which I have already alluded, and in which he succinctly established the priority of Addison over Biermer, said: "I express an opinion that nervous shock may be the starting point of the morbid process, and I know of a case where a young lady poisoned her father, and was so overwhelmed with grief that she took to her bed, became anaemic and died. Two of my cases gave a distinct history of moral shock, and a young lad, who died in one of Sir W. Gull's beds, with extreme anaemia, had never been well since he had been attacked by a sheep in a field." He then delivers the knock-out blow to the Continental claim: "The German and the Swiss doctors do not allude to this probable cause."

The last quarter of the nineteenth century provided a steady accumulation of clinical detail about Addisonian anaemia, but relatively little in the way of fundamental understanding, with the one exception of the finding of changes in the stomach, which, however, were not properly comprehended at that time. Consequently, though the clinical entity itself became clearer, the approach to rational and specific treatment was wanting. Glossitis, a number of characteristic changes in the peripheral blood, the hereditary factor, retinal haemorrhages, and neurological changes all came to be built into the picture of the disease.

It was Samuel Fenwick (1877), physician to the London Hospital, who supplied the first appreciation of the importance of stomach function. In a lecture on "Atrophy of the Stomach" he made a clear distinction between Addison's anaemia and Addison's disease, and he described four cases of the former which, after death, had a well-marked lesion of the glandular structure of the stomach. He says: "The stomach showed no signs of post-mortem solution; the whole of the glandular structure of the organ was in a state of atrophy." He actually tested an infusion of the stomach with half a drachm of hydrochloric acid for its power to digest hard-boiled egg. This observation—the failure of stomach function—was, as we now know, the key not only to the proper understanding of the disease but also to its specific treatment as practised to-day.

However, the correct interpretation of these findings was side-tracked—in fact, obscured—for almost half a century after Samuel Fenwick's lecture, largely as the result of the work of William Hunter (1901), a physician at Charing Cross Hospital, who in an incisive and convincing monograph postulated a septic origin for the disease.

A Septic Origin?

Hunter's thesis was based on the belief that Addisonian anaemia was essentially haemolytic by reason of infection of the alimentary canal with haemolytic organisms. The preface to his monograph contains a table in the form of a genealogical tree emanating from the question "What is Pernicious Anaemia?" and evolving to be answered in the later "generations" by confident statements that the disease must originate in the gastro-intestinal area from a "special poison" in the liver, from "infection" in the intestine and stomach, the latter with *Streptococcus longus*, and a "special infection" with associated dental cario-necrosis and glossitis.

Hunter's monograph, convincingly written, greatly influenced the treatment of Addisonian anaemia. First, he

advocated the prevention and removal of the infective cause of the disease by paying attention to the hygiene of the teeth and of the mouth. Secondly, he said it was essential to change such conditions in the gastro-intestinal tract as favoured the operation of the infective cause. This was done by washing out the stomach, by administering what was regarded as a gastric antiseptic—salicylic acid or bismuth salicylate—as well as intestinal antiseptics, such as salol, naphthol, calomel, or mercuric chloride, and, not least, enemata containing salicylic acid. Thirdly, there were certain measures designed to combat the chief symptoms, of which the most important and most widely used was arsenic in the form of Fowler's solution. This had been introduced by Byrom Bramwell (1877), with high hopes of effecting permanent cure, though experience had shown that it had only a temporary beneficial influence.

It is perhaps interesting to note that Minot and Castle (1935) took the trouble to investigate the action of arsenic in pernicious anaemia when they were carrying out their studies on the significance and interpretation of the reticulocyte count. They found that arsenic would produce an irregular and often sustained reticulocyte response in pernicious anaemia, sometimes associated with showers of nucleated cells, but that the reaction was not physiological, since any subsequent rise in the red-cell count, if such occurred at all, was irregular, disorderly, and incalculable. It is now known that arsenic has no real blood-building value and that it acts as a bone-marrow depressant rather than as a stimulant.

Hunter also practised dietetic treatment, stating as his view that "the most important factor regulating the amount of blood destruction in health is the nature of the diet, a nitrogenous diet causing a much greater destruction than a farinaceous one." He also made a very limited trial of antistreptococcal serum, but even though most of the cases which he records seem to have had a remission he clearly rated this remedy quite low.

Hunter's views dominated the treatment of pernicious anaemia for a quarter of a century, and were supported by Arthur Hurst (1923), who showed that, as the result of oral sepsis, the *Str. longus* became implanted in, and could be recovered from, both stomach and duodenum. Hurst is best known for his emphasis upon the loss of stomach function, particularly the complete achlorhydria so characteristic of Addisonian anaemia, which permitted the implantation of the toxin-producing streptococcus in the upper part of the alimentary tract. Hurst realized the primary importance of the achlorhydria, since it preceded the anaemia and was not, therefore, a secondary phenomenon, whilst it persisted even in remission. He held the view that the anaemia and nervous system changes were both toxic in origin.

When I entered the wards of the Middlesex Hospital in the early 'twenties, the main treatment of Addisonian anaemia followed the views of William Hunter, as expanded by Hurst. Wholesale dental extractions, the administration of arsenic, dilute hydrochloric acid three times a day before meals, for life, together with acidophilus milk. Splenectomy had also a considerable vogue, especially following the reasonably favourable report of Krumbhaar (1916).

Transfusion, with or without splenectomy, was also widely practised, especially in America, with, however, no more than a temporary effect, and this method of treatment must have given George Minot (Minot and Lee, 1917) some of his first experience with pernicious anaemia.

The Work of Whipple, Minot, and Murphy

Meanwhile, in the year following the first world war the way was being prepared for one of the most far-reaching discoveries in medicine. As early as 1918, George Whipple, first in California, and later in Rochester, began a long-term fundamental study upon the power of different foodstuffs—meat, liver, and other substances—to effect the regeneration of haemoglobin in dogs, otherwise maintained on a

standard basic diet, and exsanguinated to a fixed degree. This original and ingenious programme was both simple and effective.

During the period from 1918 to 1925, in association with his colleagues C. W. Hooper and Mrs. Robscheit-Robbins, Whipple published a series of papers, unravelling the basic physiology of haemopoiesis in a systematic and orderly manner, and with a thoroughness and pertinacity which can scarcely have been equalled before or since. The most significant papers in relation to my theme are entitled: "Blood Regeneration following Simple Anaemia: Influence of Meat, Liver and Various Extracts Alone or Combined with Standard Diets" (Hooper, Robscheit, and Whipple, 1920); "Favourable Influence of Liver, Heart, and Skeletal Muscle in Diet on Blood Regeneration in Anaemia" (Whipple and Robscheit-Robbins, 1925). It should also be noted that Whipple (1922) had suggested that, "In pernicious anaemia, there may be a scarcity of the material from which the stroma of the red-blood cells are formed or that a disease of the stroma-forming cells of the marrow exists."

Critics have sometimes said that though Whipple and his colleagues clearly demonstrated the virtues of liver and other substances in blood regeneration, their standard of reference, a dog rendered anaemic by bleeding, bore no resemblance whatever to pernicious anaemia. But no one can read the first fully documented paper of Minot and Murphy (1926) without feeling Whipple's influence, and, indeed, the paper contains this significant paragraph: "Following the work of Whipple and Robscheit-Robbins, we made a few observations on patients, concerning the influence of a diet containing an abundance of liver and muscle-meat, on blood regeneration. The effect appeared to be quite similar to that which they obtained in dogs. These observations, together with the information given above, led us to investigate the value of a diet with an abundance of food rich in complete proteins and iron—particularly liver—and relatively low in fat, as a means of treatment for pernicious anaemia."

The award of the Nobel Prize in 1934 to Whipple, Minot, and Murphy expressed the judgment of an impartial world towards these great men.

Looking back over the intervening period and re-reading Minot and Murphy's simple conclusion that "the dietetic treatment of pernicious anaemia is of more importance than hitherto recognized," one must greatly admire their modesty and the care with which they attempted to control their remarkable results. Their most commendable caution in interpreting therapeutic successes in a disease well known to be subject to one or more spontaneous remissions ought to be blazoned as an example to all who undertake therapeutic trials. After a most critical discussion of results with other remedies, they say, in relation to the obvious constancy and endurance of their own: "There is no data to indicate whether the remission in these 45 cases will last longer than those of others." This simple paper, patently honest and diffident in the extreme, resembles the gentle opening bars of a great unfinished symphony, of which some movements have even now not yet been composed, much less played.

A Developmental Sequence

The developments have been remarkable: In therapeutics, the sequence from liver itself to oral and parenteral extracts, crude and refined, to folic acid, folinic acid, and vitamin B₁₂. In clinical medicine, the comprehension of much about the megaloblastic anaemias—not only pernicious anaemia, but also the nutritional anaemias of pregnancy, infancy, and alimentary disturbances such as sprue. In physiology and pathology, a steady advance in knowledge of the fundamental processes of blood formation, including the cytochemistry and biochemistry of erythropoietic metabolism, all of which has illuminated the field of protein chemistry far beyond the haemopoietic system.

With the discovery of the efficacy of liver the true pathology and aetiology of Addisonian anaemia was shown to be not a haemolytic phenomenon but a maturation defect causing dyshaemopoiesis—a shift from a destructive process to a deficiency disease. Nevertheless, there is ample evidence that excessive red-cell destruction contributes to the anaemic state, even though this haemolytic aspect is of quite secondary importance compared with dyshaemopoiesis (Singer *et al.*, 1948).

The concept of Addisonian anaemia has, however, been greatly extended, so that it is now regarded as a generalized cellular disease, and not simply as a dyshaemopoietic anaemia with neurotrophic complications. Cellular degenerations or changes are found not only in the erythroblasts of the bone marrow and the tissue of the central nervous system, but also in the granular leucocytes of the marrow giving rise to macropolycytes, in the megakaryocytes, in the epithelial cells of the tongue, oesophagus, stomach, and even the respiratory tract (Graham and Rheault, 1954). These multiple changes are all rectified with liver or vitamin B₁₂.

Many times during the past hundred years has diet been considered important in the treatment of Addisonian anaemia. Many times also has the fashion changed, as is so frequently the case with dietetic measures for any pathological condition even to this day. Fenwick (1880), for example, recommended protein foods rather than farinaceous, whereas Hunter advocated the reverse. It should also be appreciated that others (Gibson and Howard, 1923) before Minot and Murphy—some also basing their work on that of Whipple and his colleagues—had prescribed liver for patients with Addisonian anaemia, and had reported improvement, but none had recognized that liver possessed a complete curative effect.

What is remarkable is not that Minot and Murphy thought of trying the effect of liver, since there were a number of pointers, from the well-known beneficial effect of liver-soup in sprue to the experimental work of Whipple, but that they had the foresight or instinct or courage to exhibit it in sufficient amount—half a pound or more of raw or lightly cooked liver a day for life—and with sufficient persistence to obtain and maintain the required effect.

There should be a plaque somewhere to record the fortitude and faith of the first 45 patients with Addisonian anaemia whom Minot and Murphy treated, who endured this nauseating treatment and so gave this great boom to mankind.

When I was in Boston in 1946 there was a dramatic clinical demonstration of two cases of Addisonian anaemia at the Peter Bent Brigham Hospital. Minot and Murphy were both present. The first case was one of the original series of 45 reported in 1926; the patient, a woman, had reached the age of 80, and was dying of carcinoma of the stomach; she had had more than twenty years of happy life, and had graduated through all the advances in liver therapy to that time. The second case, a newly identified one, presented as a contrast, had, with one single parenteral injection of a concentrated extract, shown a maximum reticulocyte response and all the other expected features of a complete remission.

Minot's technique for estimating an early response to treatment by means of a reticulocyte count was a neat and most effective clinical method for control. It was probably crucial in the discovery of liver, since the occurrence of a reticulocyte crisis encouraged persistence and indicated dosage. It has since found numerous applications in clinical haematology, as well as in the elucidation of the pathology of blood disease and the evaluation of methods of treatment.

Castle's Hypothesis

The remarkable investigations of William B. Castle and his colleagues, which began in 1929 and continue even to this day, have clearly shown the role of the stomach in the production of Addisonian anaemia. The experiments,

brilliantly planned and faultlessly executed, showed the relationship between normal haemopoiesis, the diet, gastric secretion, and liver function.

Castle's work can be accounted among the greatest advances in the study of disease processes. It called attention to the vital point in Addisonian anaemia—that certain stomach secretions were essential for fundamental nutrition. The weak antiseptic action of the normal stomach juices in preventing the implantation of a haemolytic organism in the intestine, the basis of twenty-five years of treatment for Addisonian anaemia, ceased to be important almost overnight.

Castle's experimental work led him to propose (Castle *et al.*, 1930) his hypothesis that normal erythropoiesis depended upon a substance of unknown composition generally referred to as the haemopoietic or anti-pernicious anaemia principle. This principle, he suggested, was formed in the normal stomach or in the small intestine by the interaction of two factors—an extrinsic factor present in certain protein constituents of food, and an intrinsic factor secreted by the epithelial cells of the fundus of the stomach.

The haemopoietic principle, absorbed from the alimentary tract, was stored in the liver, whence it ultimately reached the bone marrow by way of the blood stream. Castle's hypothesis explained Addisonian anaemia by an absence of intrinsic factor; it had also the merit of supplying a rational explanation for most megaloblastic anaemias either by other deficiencies or by failure in the processes of absorption, transport, or storage of the haemopoietic principle.

The treatment of Addisonian anaemia quickly evolved from Minot and Murphy's great announcement, largely as the result of the work of Edwin J. Cohn and his associates, who in 1928 introduced a refined and highly potent liver extract for parenteral injection which was subsequently improved through the following decade. Somewhat later, Sharp (1929), Sturgis and Isaacs (1929), and Wilkinson (1930) showed that hog's stomach contained a substance active in the treatment of Addisonian anaemia. This factor, at first thought to be the same as that present in liver, was found to have different properties; it can now, in the terminology of Castle's hypothesis, be regarded as intrinsic factor, and can, in fact, be used to effect absorption of vitamin B₁₂ from the stomach.

With increasing knowledge, however, it became clear that Castle's hypothesis was not a complete explanation, since it did not always fit new facts as they were discovered. It was, for example, found that a number of megaloblastic anaemias would respond to crude liver extracts, but not to refined and concentrated products, and this suggested that liver contained more than one substance, each or all of which might be necessary for supplying the deficiency in different types of megaloblastic anaemia.

Folic Acid and Vitamin B₁₂

This belief was eventually confirmed by the synthesis of folic acid in 1945 by Angier and his team, and the isolation of vitamin B₁₂, almost simultaneously, in 1948, by Lester Smith in England and Ricketts and his colleagues in America. Studies with these substances have shown that the anti-anaemic properties of liver itself, as well as the crude extracts or products, can be attributed to both folic acid and vitamin B₁₂, whereas the refined liver extracts owe their activity to vitamin B₁₂ alone. Addisonian anaemia, including the neurological complications, is now known to be due essentially to a deficiency of vitamin B₁₂, whereas most if not all of the other megaloblastic anaemias of similar haematological type are due to a deficiency of both folic acid and vitamin B₁₂, or, in some cases, of folic acid alone.

So far as Addisonian anaemia is concerned, the limitations of folic acid are now fully appreciated. Notwithstanding haemopoietic activity, folic acid fails to prevent or cure the nervous lesions associated with the disease. Indeed, exces-

sive administration would seem to precipitate neurological complications in advanced and untreated cases of the disease by rapidly immobilizing any traces of vitamin B₁₂ in the tissues, thereby creating an acute and sudden shortage of essential neurotrophic material.

Vitamin B₁₂, or cyanocobalamin, the unique cobalt-containing material which was isolated by modern methods of chemical separation, was originally prepared by an expensive process from ox-liver. It is now obtained from the metabolism fluids of a number of bacteria.

In Addisonian anaemia it is active in a daily dose as low as 1 µg., whilst a satisfactory haematological response can be obtained with as little as 20 µg. a day, and a maintenance dose of some 45 µg. every six weeks. It is also protective against the neurotrophic complications in the same somewhat uncertain and unpredictable manner as occurs with liver extracts. Experience has shown that a dosage higher than the minimum should always be used, and especially in arteriosclerotic subjects or those in whom an infection supervenes. Something of the order of 100 µg. a day is advisable until remission is complete, with a maintenance dose of 50 µg. every two weeks. Maintenance by means of massive doses at long intervals is unsatisfactory.

It is now also appreciated that most cases of Addisonian anaemia require to take iron in order to complete recovery, whilst a number may be thyroid-deficient.

Some Difficulties in Treatment

The evolution of liver treatment for Addisonian anaemia from 1926 to the present day has emphasized certain therapeutic principles, has revealed a number of complications or difficulties, and, above all, has brought to light something of the nature and mode of interaction of various related substances in the processes of haemopoiesis, especially with regard to the synthesis of substances which are fundamental constituents of a biological cell—namely, nucleic acid and its derivatives.

None would now question that Addisonian anaemia, a deficiency disease due to failure of stomach function, is incurable, and that adequate treatment must be maintained for life if haematological relapse and, above all, neurotrophic complications are to be avoided. A critical analysis (Wilkinson, 1932) of such cases as have been reported to have re-established the essential gastric function has shown quite clearly that most, if not all, are likely to have been sprue or some other alimentary disease, rather than Addisonian anaemia. Fully restored cases usually relapse in two to ten months if treatment is omitted, but some have remained well for nearly two and a half years.

Proper maintenance, with haematological control, is, however, a prime requirement, since it is really no kindness to cure a patient of Addisonian anaemia if he is afterwards to develop, and die from, preventable spinal paralysis.

Clinical experience has shown that the dosage of vitamin B₁₂, whether as such or as liver extract, should be increased in the aged, or the arteriosclerotic, and in such as develop an infective process; while septic foci should be removed. Other deficiencies may also complicate the picture, so that some with Addisonian anaemia may be found to need regular courses of iron or thyroid extract, and sometimes vitamin C, while those with neurotrophic disturbances are the better for large doses of the complete vitamin B complex, which, with calcium pantothenate, may be very effective in relieving other deficiency symptoms, such as glossitis.

In brief, the treatment of Addisonian anaemia has evolved to the point where specific curative treatment is available, with which the physician can ensure that no patient who is co-operative need die of the disease *per se*. But this is not to say that the treatment has reached a mechanical stage where neither skill nor art is required. Optimum dosage differs from patient to patient. Constant haematological and clinical supervision are necessary. Minor difficulties, such as sensitivity to liver extract, may be troublesome, and

the possibility of other incidental deficiencies should always be borne in mind, since their treatment can greatly increase the comfort and well-being of the patient.

Pathology and Biochemistry of Erythropoiesis

So much for the evolution of treatment. This survey would not, however, be complete without some short and simple account of the revelations arising from this evolution in respect of the fundamental pathology and biochemistry of erythropoiesis.

Knowledge has come from many sources, but above all from the clinical field (which would have pleased Addison), since no satisfactory technique for assay and evaluation in an animal has ever been devised. Castle, for example, had to carry out the whole of his classical work on the human subject, and likewise with the primary testing of various extracts of liver, as well as folic acid and vitamin B₁₂.

Throughout the years the hypothesis of Castle has provided an invaluable working basis for the clinician in all cases of megaloblastic anaemia. It has to be admitted, however, that the hypothesis is no longer acceptable in its original form, even though in the clinical field it still has most valuable applications, and has also provided a focusing point for new discoveries which might otherwise have become inextricably confused.

But vitamin B₁₂, which, at the outset, was thought to be the haemopoietic principle, has the natural distribution and all the properties of extrinsic factor. It cannot be absorbed in active form without gastric intrinsic factor, but, once absorbed, it behaves like the haemopoietic principle. Evidence has now accumulated to show that vitamin B₁₂ itself is microbiologically active, but not haemopoietically so unless it becomes bound to intrinsic factor, possibly a mucoprotein, in the gastric juice or its counterpart in the tissues. The bond is heat-labile, and when dissociated by heat the vitamin B₁₂ is no longer haemopoietically active. In Addisonian anaemia vitamin B₁₂ is highly active by parenteral injection by reason of a tissue-binding substance, since it appears in the serum in bound form, but it is only active by mouth when given in massive dose (3 mg.) or in association with normal gastric juice (Ungley, 1949).

Knowledge concerning erythropoiesis has also come from studies in bacterial metabolism and animal nutrition, and, indeed, the fact that intestinal bacteria may immobilize such substances as vitamin B₁₂ has an influence on therapeutics, which may be profoundly altered by a change of flora brought about by an antibiotic. Thus, the binding of vitamin B₁₂ so that it is no longer available to bacteria may, in fact, be one of the essential functions of Castle's intrinsic factor, irrespective of conversion of vitamin B₁₂ to a haemopoietically active form.

Functional Relationship between Folic Acid and Vitamin B₁₂

A problem of fundamental importance is a determination of the functional relationship between folic acid and vitamin B₁₂, particularly whether they are independent haemopoietic agents or whether their actions are inter-related so that the presence of one is necessary for the functioning of the other. Folic acid was at one time thought to correspond to Castle's extrinsic factor, but this obviously is not the case; and, indeed, it is difficult to fit folic acid into Castle's hypothesis at all.

The activity of folic acid seems to be dependent upon its conversion to folinic acid, otherwise known as the citrovorum factor, and so called because it is an essential growth factor for *Leuconostoc citrovorum*. Folinic acid has been shown to be therapeutically active in certain megaloblastic anaemias (Davidson and Girdwood, 1951), but, unfortunately, attempts to throw light on the relationship of folic acid, folinic acid, and vitamin B₁₂ by *in vivo* and *in vitro* studies of bone marrow have produced confusing and inexplicable results. When vitamin B₁₂ is injected into the bone marrow of a case of Addisonian anaemia in relapse

the material is utilized by the cells, but this is not the case with either folic acid or folinic acid. On the other hand, when the materials are tested *in vitro* with megaloblastic marrow cultures, folic acid can bring about normal maturation, whereas vitamin B₁₂ will not do so unless associated with normal gastric juice.

Studies of the metabolic requirements of bacteria have also shown that the pyrimidine base, thymine, can replace folic acid, and that the desoxyriboside of thymine, thymidine, can replace vitamin B₁₂ in satisfying certain essential growth requirements of organisms. Both of these substances, as well as a number of others (Welch and Heinle, 1951), possess some haemopoietic activity in megaloblastic anaemias.

This rapidly accumulating tangle of facts has not as yet been unravelled, but from the angle of basic biochemistry the views of Vilter and his colleagues (1950) offer a logical and rational approach to the problem, particularly in the co-ordination of bacteriological metabolic experiments with therapeutic results in the megaloblastic anaemias.

In brief, it is suggested that all the substances which I have considered are concerned in the formation of nucleic acid, which is essential for haemopoiesis. The synthesis of nucleic acid is thought to be brought about by a "chain reaction" in which, in the first stage, folic acid, probably first converted to folinic acid, exercises a coenzyme function in the formation, from uracil and other simple substances, of purines and pyrimidines (such as thymine). In the second stage, vitamin B₁₂ is thought to act as the coenzyme for the conversion of pyrimidines to nucleosides (such as thymidine) which are available for the formation of nucleotides, and so of nucleic acid. On this theory a deficiency in either folic acid or vitamin B₁₂ may cause a breakdown in nucleic acid metabolism.

These views point to there being some interplay between folic acid and vitamin B₁₂ in certain metabolic processes, and it may well be that the riddle of the interchangeable haemopoietic roles of these substances in Addisonian anaemia will be solved only through detailed studies of biochemical reactions.

We know that we have not yet solved all the therapeutic problems of Addisonian anaemia, much less drawn all the dividends from the discoveries which have been made since Addison first lighted a beacon a century ago. At that time he could hardly have been aware of the magnitude of the vista which he was about to illuminate, even as we do not know what will happen in the next century.

Conclusion

In a brief hour I have tried to look over the past, including the near-past in which the tempo of advance has so rapidly increased. In so doing, one remembers with gratitude the heritage of this great hospital through its many sons whom Addison inspired, most of whose contributions in the field of my subject can be found in succeeding volumes of the *Guy's Hospital Reports*.

I think of Wilks, Pye-Smith, Taylor, French, Campbell, Hurst, John Conybeare, Izod Bennett, whose pupil I once was because he came to us at Middlesex, and John Ryle, who was my immediate predecessor at Cambridge.

"Let us now praise famous men."

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FIG. 1.—Orlon replacing portion of abdominal aorta of dog. Carrel suture used (see text).

ARTERIAL REPLACEMENT BY "ORLON" CLOTH*

A PRELIMINARY REPORT OF ITS EXPERIMENTAL AND CLINICAL USE

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During the years since the second world war it has gradually become clear that the grafts used as substitutes for arteries served chiefly as scaffolding on which the tissues of the host developed a new vessel. The survival of cells from the donor has not proved essential for success. Indeed, good results may be obtained where no effort is made to maintain the viability of the cells of the graft. K. Nakayama (1953, personal communication) has used human homografts preserved in alcohol to replace the aortic bifurcation. In our own hands the results with heterografts sterilized and preserved in neutral formalin and used in animals have been most satisfactory over considerable periods of time. It therefore seemed a logical consequence that artificial materials might be used as a scaffold, and Voorhees and his colleagues (1952) reported the successful use of an inert plastic cloth, "vinyon-N."

Experimental Studies

Vinyon-N was difficult to obtain in England, and so we chose for trial "orlon," a plastic developed by duPont and available in Great Britain from Zurrer Silks (Darwen) Ltd., Anchor Mills, Darwen, Lancs. It was one of a variety of woven plastic materials which were easily available and likely to be chemically inert in the body. The choice was made chiefly on the naked-eye appearance of the weave and its power to hold sutures. Its ability to prevent excessive leakage of water or blood through its pores was also tested on the laboratory bench before it was used surgically. It was found to be more suitable in these ways than other materials that were tried.

Orlon is an acrylic resin, a polymer of acrylonitrile, and possesses the qualities of strong resistance to acids, alkalis,

salts, and greases. The fabric used was 175 on the weft and 260 on the warp. It was inelastic, soft, and pliable, and sewed easily without tearing.

Mongrel or greyhound dogs were anaesthetized with intravenous pentobarbitone sodium, and under aseptic conditions the peritoneum was opened through a long midline incision. The posterior peritoneum was incised, exposing the distal abdominal aorta, the lumbar arteries were ligated, and 2 to 3 cm. of aorta was resected after Blalock clamps had been applied

FIG. 2.—Aortogram two months after insertion of orlon. Carrel suturing not used. The upper anastomosis (arrow) is barely discernible, the lower is located by the small notch.

to it above and below. The tube of orlon fabric was then sutured end-to-end to the aorta with over-and-over continuous stitches of 5/0 silk moistened with liquid paraffin, and the clamps were temporarily removed to allow blood to fill the tube and test for leaks at the anastomoses. Blood oozed through the orlon fabric for about a minute while clotting was occurring in the interstices of the fabric.

Animal Studies with Orlon

Dog No.	Type of Anastomosis	Result	Time (Weeks) and Method of Check	Total Time Followed (Weeks)
G.2	End-to-end	Patent	12 Necropsy	12
G.3	"	Ruptured anastomosis	16 "	16
G.4	"	Patent	21 Aortogram	21*
G.6	"	"	3 " Clinical	15*
G.7	"	"	8 Aortogram	16*
G.9	End-to-side	"	16 Clinical	8
G.10	End-to-end	"	4 Aortogram	8
G.11	Side-to-side†	Thrombosis	8 Necropsy	—
G.12	"	Patent	10 hrs. " Aortogram	5
			3 Necropsy	4*
			4 Clinical	

* Indicates animal still surviving for further study.

† All prostheses were inserted in the aorta, except in G.11 (aorta to external iliac).

*Based on a communication to the Surgical Research Society, London, November 26, 1954.