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ALMROTH WRIGHT—PIONEER IN IMMUNOLOGY*

BY

LEONARD COLEBROOK, F.R.C.S., F.R.C.O.G., F.R.S.

Almroth Wright was not much given to boasting, but, like Bernard Shaw, he sometimes took pleasure in shocking people by a remark that smacked of arrogance. On one such occasion, when a visitor to his laboratory, who had been rather talking down to him, asked whether he had read a certain paper, Wright replied somewhat tersely, "No, we don't read bacteriology—we make it."

The time has come, I think, when we may profitably consider how far he did "make bacteriology," and perhaps ask ourselves whether some of the unconventional ideas he stood for may not be worthy of re-examination.

When he graduated in 1883 (from Trinity College, Dublin) medical bacteriology was beginning to make quite a stir in the world. The great mystery of tuberculous disease (which was killing 1,000 people a week in England and Wales) looked a little nearer a solution with Koch's discovery of the tubercle bacillus in 1882. A year before that he had shown that many microbes could be grown, and pure cultures obtained, on solid nutrient media. A year later he isolated the cholera vibrio. In France, too, great things were happening. The patient work of Davaine and Pasteur and others had at last established the relationship of *Bacillus anthracis* to anthrax; and by his classical experiment with 50 sheep Pasteur had shown that it was possible to make animals immune to that deadly disease by the inoculation of an attenuated culture of the bacillus.

Very soon the concept of "antibodies" was born—from the work of Ehrlich and von Behring, of Roux, and of Pfeiffer. Antitoxins were detected in the blood of animals inoculated with the poisonous principles contained in cultures of diphtheria and tetanus bacilli. A little later a bactericidal antibody was discovered in the peritoneal fluid of guinea-pigs which had been inoculated with a killed culture of the cholera vibrio. And after that yet another antibody (agglutinin) was found which caused the clumping of typhoid bacilli by the serum of animals inoculated with that microbe or of man suffering from typhoid fever.

Diagnosis and Prevention of Typhoid Fever

It was at this point that Wright started his bacteriological work—in 1893. He had recently been appointed Professor of Pathology in the Army Medical Services, with his headquarters at the Royal Victoria Hospital at Netley on Southampton Water. There he frequently saw men suffering from typhoid fever, and others

invalided home with Malta fever, and some with fevers to which it was not easy to attach a diagnostic label. There was no such thing as laboratory diagnosis in those days—nor, indeed, in most hospitals was there any laboratory. But the clumping of typhoid bacilli by serum quickly suggested to Wright, as well as to others on the Continent, that it might be possible to develop a laboratory test which would serve for the differential diagnosis of typhoid from "Malta" and, perhaps, other fevers.

In devising a technique for carrying out such a test Wright made his first contribution to bacteriological methods—the use of a wide-bore, graduated capillary pipette instead of the more conventional test-tube. As an indirect result of this work Wright was soon able to demonstrate that Malta fever was not by any means confined to that island.

Then he turned his attention to the control of typhoid fever, which was a serious public health problem in those days. In England it was killing 5,000 people a year, and in the U.S.A. 15,000. The British Army serving abroad lost many men from it even in peacetime, and had far heavier losses in every campaign. Ten years had gone by since Pasteur's great achievement with immunization against anthrax, and no one had attempted a similar preventive therapy in man by the inoculation of a bacterial vaccine. But things were moving in that direction. Haffkine, a Russian bacteriologist who had been working at the Pasteur Institute, was making plans to carry out anti-cholera inoculations in India. On his way there he visited Wright at Netley, and in course of conversation suggested that it might be worth trying a similar procedure against typhoid.

Wright promptly took up the idea. It had been shown in Germany that the destruction of cholera vibrios in the peritoneum of a guinea-pig was brought about by the agency of a bactericidal antibody in the animal's circulating blood. Wright saw that, if it was possible by an inoculation of vaccine to elicit the formation of a similar bactericidal antibody to the typhoid bacillus in the circulating blood of a man, it would probably constitute a barrier against the invasion of his blood stream by those bacilli from the intestine. It might even prevent the intestinal infection itself.

He went to work on that concept—but there were difficulties. Pasteur had used an attenuated but still living culture for the inoculation of his animals; and had demonstrated their immunity by "challenging" them with a dose of virulent culture which would certainly

*Being the first of the 1953 series of Almroth Wright Lectures delivered at St. Mary's Hospital on June 5.

kill an uninoculated animal. Wright "saw with his mind" that it should be possible to employ a non-living vaccine (this had indeed been done with the cholera guinea-pigs in Germany), and that it should also be possible to get evidence of a man's response to the inoculation by testing his blood *in vitro* for bactericidal antibody. But no test of that kind was then known. So Wright devised one, using, as he always did, very simple tools—a little glass tubing, a blow-pipe, some rubber teats, and a glass-writing pencil. The procedure he achieved remains to this day a model of ingenuity, and has not been improved upon for the object Wright had in view. It enabled him to determine approximately how many typhoid bacilli would be killed by a given volume of serum.

Then he began his inoculations—on himself and some of the "surgeons on probation" working in his class at Netley. Very soon his tests of the blood showed that, following the inoculations, there was usually a big increase in its power to kill typhoid bacilli—often a hundredfold or even more—and this enhanced power was maintained for weeks or months.

To Wright that meant that it would probably be possible to protect soldiers against typhoid fever. Preliminary trials on volunteers in the Indian Army gave encouraging results, but when Wright urged the War Office to mount a large-scale trial on troops embarking for the South African War there was immediate opposition. Some of those in authority were not at all impressed by what happened in Wright's capillary pipettes; they wanted clear-cut statistical proof that the men would in fact be protected by the inoculations. Such proof could, of course, not be obtained until a large-scale trial had been made.

So it happened that less than 4% (about 12,000 men) of the troops were inoculated, and during the three years of that war the Army had nearly 58,000 cases of typhoid, with about 9,000 deaths. Although an attempt was made to collect evidence of the degree of protection afforded to the inoculated men the conditions of active service made it very difficult to get reliable data. Dr. John Freeman has told how, when he visited a hospital where there were some cases of typhoid, he asked the sergeant in charge whether they had been inoculated. The reply was, "Oh, yes, Sir; I always scores 'em up as inoculated, because it stands to reason that they wouldn't have got typhoid if they hadn't been."

Statistical Data

After the war Wright might well have let the matter rest. He had published his laboratory data about the increased bactericidal power, and he could have left it at that. Most men would have done so. But his experience with the War Office had made him realize that the proposal to inject microbes, whether dead or alive, into healthy human beings was bound to arouse antagon-

ism—it was too novel. And Wright was not half an Irishman for nothing. So after the war he continued to press for proper statistical trials to be carried out by the Army, with the result that ultimately, in 1909, satisfactory data were produced by his successor at Netley, Colonel W. B. Leishman. Table I shows that the records, obtained with special precautions against error, were strongly in favour of the use of vaccine.

A little later a similar trial, with similar results, was reported by Colonel Russell, who compared the data from two camps in the U.S., one of which had all the men inoculated and the other very few. The records are shown in Table II.

TABLE II.—Typhoid Fever in Two Comparable Camps of the United States Army (Both with a Good Water Supply)

	Uninoculated	Inoculated	Cases of Typhoid	Deaths
Camp A (1909)	82,100	1,900	282	22
" B (1913)	0	91,000	3	0

Figures taken from Russell, *War Dept. Bull. Wash.*, No. 2, 1914.

Had it not been for the striking success of these trials between the wars we might well have entered upon the first world war with our fighting Forces uninoculated. As it was, an active policy of immunization was adopted from the beginning in all Services, and by the end of 1915 almost 100% of our Expeditionary Force had been inoculated. As the war went on cultures of paratyphoid A and B were added to the vaccine.

The sickness and deaths due to enteric fevers in that war are shown in Table III and compared with the figures for the South African War.

TABLE III

	Annual Incidence per 1,000 Strength	Annual Death Rate per 1,000 Strength	Total Cases	Total Deaths	Mean Strength
South African War, 1899–1902	105.00	14.6	57,684	9,022	208,226
1914–18 war	2.35	0.139	20,139	1,191	2,000,000 (approx.)

Table taken from the *Medical and Surgical History of the Great War* (Section of Pathology, p. 211). 1923.

It has been estimated that if our losses from typhoid and paratyphoid fevers during the first world war had been on the same scale as in South Africa there would have been nearly 1,000,000 cases and about 125,000 deaths. They might easily have been on a greater scale, since the land fighting in the 1914–18 war was chiefly trench warfare and fought along the Franco-German frontier, where typhoid was always endemic. Not all the reduction of incidence and mortality from enteric fevers in the first world war can be ascribed to the policy of inoculation, because in the years between the wars there had been progress also in military hygiene—particularly with regard to water supplies—but in view of the figures shown in Tables I and II it seems very probable that a large part of the improvement was the result of immunization.

That great achievement—the control of typhoid fever—will always stand out as one of the biggest landmarks of medicine in the twentieth century. History will surely award the credit for it, in the first place to Pasteur for his pioneer work on anthrax in animals, and after him to Wright. Wright had certainly "made" bacteriology—by his masterly elaboration of a method of measuring the antibody response in the inoculated man, so affording a guide to that procedure; by his method of enumerating the bacterial bodies in his vaccine, and so making dosage more precise; and by his dogged fight to get the vaccine used in the British Army against considerable opposition.

TABLE I.—Antityphoid Inoculation in Sixteen Units of the British Army Serving Abroad During 1905–9

	No.	No. of Cases of Typhoid Fever	No. of Deaths from Typhoid Fever
Inoculated	5,473	21	2 (i.e., 0.38 per 1,000 inoculated men)
Uninoculated	6,610	187	26 (i.e., 3.93 per 1,000 uninoculated men)

Table taken from Leishman, *J. roy. Army med. Cps*, 1909, 12, 163.

Vaccine Therapy

When that work was done Wright left the Army Medical Services (1902) and came to St. Mary's as Professor of Pathology and Bacteriology. Speaking of that appointment in later years, he used to say that every large hospital needs two staffs—one to teach all that is known, the other to teach that we don't know anything, and to get on with it. "Getting on with it" meant for Wright embarking upon a fresh chapter in the story of immunization.

In this undertaking Pasteur was again his leader, for Pasteur had shown that it was possible to do preventive inoculation successfully in the case of rabies even when the patient had been bitten by a mad dog *several days before* the inoculations were given. Reflecting upon this, Wright said to himself: "The microbes introduced by the bite have only won a bridgehead—the rest of the man's tissues are uninfected and should be able to respond to an inoculation just as the tissues of a healthy man against typhoid." And he went on to apply the same conception to other localized infections. At Netley he had seen a number of men suffering from boils. "Were they not," he asked himself, "in much the same case as the man bitten by a mad dog?"

As he pondered upon this another question arose in his mind. "How does the man with a boil recover from his infection?" Tests of the blood of men with boils had shown that there was no bactericidal antibody to the staphylococcus. There must then be some other mechanism. Metchnikoff's recent work suggested that it might be phagocytosis, and Wright decided to go to work on that hypothesis. But very little was known about phagocytosis—and there were no methods for studying it. Gradually a plan took shape in Wright's mind. He must first devise methods for finding out whether the blood of a sick man had less capacity for phagocytosis than that of a healthy man; and, if it was so, whether it was possible to increase his phagocytic activity by appropriate doses of vaccine—in other words, "to build up his resistance."

That was an entirely new approach to infectious disease—and it called for a lot of preliminary work in the laboratory. Very soon this brought an unexpected discovery. When he collected leucocytes from his own blood and washed them free of serum (by the centrifuge), and then mixed them up with microbes, there was almost no phagocytosis, but when serum was present in the mixture it was abundant. Apparently there was something in the serum which made the microbes acceptable to the leucocytes. Wright christened it an "opsonic factor" from the Greek word *opsono*=I prepare victuals for.

That discovery immediately suggested to Wright that he might *measure* this opsonic factor in the serum of different individuals, and in the serum of the same individual before and after an inoculation of vaccine. To do that he had first to prepare the way by a series of technical tricks. He had to devise a convenient method of collecting small samples of blood (for the syringe in those days was a rather primitive affair). For this he again resorted to glass tubing—and "Wright's capsule" gradually evolved. Next he had to find a method of spreading blood films in such a way that the leucocytes were all assembled at one end, more or less in a straight line, like soldiers on parade. This he achieved by means of the "spreader"—a microscope slide broken across in such a way that the line of fracture presented a very slightly concave arch. When a drop of blood was drawn along under this arch the leucocytes, being larger than the red cells, were carried to the end of the film. Thirdly, he had to find out how to prepare a suspension of leucocytes from blood in such a way that they would retain their capacity for phagocytosis for some hours.

"Therapeutic Immunization"

When these preliminaries were completed Wright was ready to embark upon his plan of "therapeutic immunization." Very soon the laboratory off the museum became a sort of unofficial out-patient department where, at almost

any hour of the day, you might have found patients waiting to have a sample of blood taken or to get an inoculation. And at almost any hour of the night you might have found Wright and his assistants—Douglas, John Freeman, Alexander Fleming, and others—patiently working out the "opsonic indices" of those specimens of blood; or discussing, over that midnight cup of tea, the progress of this or that patient. And frequently there were visitors from other countries helping with the work, for this new concept of Wright's had kindled the imagination of doctors all over the world. St. Mary's was, for the time being, the centre of the medical stage.

It would not be very profitable to-day to discuss in any detail that campaign of vaccine therapy. We tried it for all kinds of infections: for boils and carbuncles, for erysipelas, for tuberculous glands and joints and lupus, for phthisis, for urinary and respiratory tract infections. No attempt was made to assess the results by statistical methods, partly because the clinical material was much too heterogeneous to give data of any value, and partly because Wright himself held strong views about the fallibility and the pitfalls of statistical procedures applied to medical problems.

There were some striking successes, and quite a few failures (especially in septicaemic conditions and phthisis), and in many other cases it was difficult to be sure how much the patient's recovery had been assisted by our treatment.

Looking back, it seems to me that the road Wright took was well worth while at that time, when neither the sulphonamides nor the antibiotics were on the horizon. We certainly learned much about infections and the response to inoculations of vaccine; and we helped many people. I think we might have won more substantial scientific gains if we had concentrated our attack upon a smaller target, treating fewer patients; and perhaps studying the effect of vaccines in some animal infections so that we could have checked our clinical impressions by experimental investigations and by statistical comparisons. To-day, after nearly 50 years, we still have little precise knowledge about the train of events set going by an infection or an inoculation of vaccine. Wright's working hypothesis that phagocytosis, activated by an opsonic antibody, was the dominant factor may well prove to have been too simple. He himself, in his later years, abandoned the view that opsonic power of the serum *per se* could serve as an indication of a patient's resistance; but he held to the belief that the key to that resistance must be sought for in the circulating blood. And he spent 20 years in that search. It may be that, with fuller understanding of the factors which make for recovery from a microbial infection, future years may see a return to Wright's programme of evoking the body's potential response to an immunizing stimulus.

Summarizing, I think one may fairly say that in this phase of his life Wright did again "make bacteriology" by his discovery of the opsonic power of serum, which "married" the concept of salvation by phagocytosis with that of humoral antibodies; by his many technical devices for the study of phagocytosis; by working out some of the principles of therapeutic immunization; and, perhaps not least, by bringing the whole medical profession to think of infectious disease in terms of microbes and the patient's resistance to those microbes. Together these constituted a notable advance in the application of science to medicine, and in that advance Wright was unquestionably the leader.

Infections of War Wounds

During the first world war Wright did perhaps his best scientific work. That war quickly brought many new problems. High explosives were being used on a scale never known before and were causing terrible injuries which gave rise to appalling sepsis. Gas-gangrene became a frequent instead of a rare event. For the first time in the history of war medical research was called upon to play its part. Wright, with Fleming, was commissioned to set up a laboratory in France for the study of these wound infections.

The surgeons of that day had just witnessed the astonishing disappearance of sepsis in their civilian work, following the application of Lister's principles and asepsis in the operating theatres. From that experience there had grown up a largely uncritical faith in the ability of all sorts of antiseptic substances to cope with the bacterial infections of wounds, and it was assumed that these same agents would suffice in war emergency. But that hope was quickly disappointed—the antiseptics appeared to have little or no effect. Great numbers of men were dying or losing their limbs, sometimes even when their injuries were comparatively slight.

Wright, almost alone, saw what was happening. Although the antiseptics killed microbes so well in test-tubes they were not killing them—or even restraining their growth—in the wounds. Reflecting upon this, he recalled the earlier work of Koch and von Behring, which had shown that, whereas perchloride of mercury in watery solution would kill anthrax bacilli at a dilution of 1 in 1,000,000, the solution required to be 100 times as strong if the antiseptic was dissolved in serum. And Wright had found the same thing

FIG. 1.—Antiseptics can favour the growth of microbes in blood (Fleming).

with some other antiseptics. Apparently they forfeited much of their antiseptic potency by combination with serum proteins. As Wright put it, they were “quenched.” The situation was even worse than that, for on investigation it transpired that many antiseptics, when added to serum in concentrations which failed to kill microbes, actually favoured their growth. This effect was well shown by Fleming in the experiment illustrated in Fig. 1. Here several volumes of serum, equally inoculated with a gas-forming

anaerobic bacillus, received increasing amounts of carbolic acid (from right to left), and were then covered with sterile melted petroleum jelly. (The volume of gas formed under the plug of petroleum jelly served as a rough indicator of the amount of growth in the serum.) It is seen that, whereas there was little gas-formation in the control serum, it increased very considerably with each addition of carbolic from a concentration of 0.03 to 0.06%, and was suppressed only when the concentration reached 0.5%—a strength at which blood cells and most fixed tissue cells would be severely damaged or destroyed. There can be little doubt that the application of dilute solutions of antiseptics to wounds in the past must often have resulted in an increased growth of microbes, as shown in this experiment, rather than in a suppression of growth.

There was a second reason why the antiseptics so often failed to check microbial growth. They did not reach the microbes, which were hidden in a thousand nooks and crannies among torn muscle fibres, impacted bony fragments, etc. Another experiment of Fleming's illustrated this difficulty in a striking fashion. The lower end of a test-tube was pulled out into spikes as shown in Fig. 2 to simulate a ragged wound cavity. It was then half-filled with serum broth and heavily inoculated with a great variety of microbes from an infected wound, and incubated. Next day, when there was a profuse growth, it was emptied out and the tube refilled with a strong antiseptic solution, which was left for some hours. This in turn was thrown out and the tube again filled with sterile broth and incubated—to see whether any microbes had remained alive. The result was always the same. Whatever antiseptic had been used the broth yielded a copious growth. Evidently the antiseptic had failed to reach the microbes in the tips of the spikes.

Having satisfied himself that there was little or nothing to be hoped for from the antiseptic policy, Wright decided

to go back to the beginning and try to find out what was happening in the wounds. First he must know what were the conditions which allowed so great a swarming of microbes in them. Would the fresh lymph as it exuded into a wound support such a copious growth of many species? or did this occur only in exudate after it had lain in the wound for a time and become changed? To answer that Wright collected small samples of fresh lymph in what he called a “lymph leech”—a simple contrivance for exerting gentle suction on the wound wall. When he incubated lymph collected in such a “leech” he obtained only a sparse growth of microbes, and usually only one or two species—streptococci or staphylococci; whereas fluid which had been incubated by the patient himself in his wound cavity showed some 20 to 30 species growing in great profusion. It appeared, therefore, that certain microbes were able to multiply to some extent in fresh lymph while others would grow freely only in stale exudate.

FIG. 2.—Fleming's experiment on the failure of antiseptics to sterilize wounds.

Wright next asked himself: Were there nutrient substances available in the stale lymph which were not present in the fresh? Reflecting upon this, he recalled two recent discoveries which might well throw a light on these findings. Opie, in New York, had found that the pus of tuberculous abscesses contained proteolytic ferments which he believed were set free from the disintegrating leucocytes. And other American workers, Jobling and Peterson, had demonstrated that normal human serum exerted an antiproteolytic effect. In the wounds there were plenty of disintegrating leucocytes, and if proteolytic ferments were being produced from these they would probably break down the proteins of the exuding lymph to provide simpler foodstuffs for microbial growth. These proteolytic ferments would also presumably neutralize any antiproteolytic influence of the fresh lymph.

Devising appropriate techniques, Wright soon found that the stale exudates of all the heavily infected wounds were in fact actively proteolytic; and that the fresh lymph from his “leeches” had an antiproteolytic effect, although usually somewhat less than that of serum.

It remained to show that, while normal serum (or lymph) with its full antiproteolytic power was a poor medium for the growth of microbes (because most of these could not utilize its unaltered proteins), it became a very good medium when sufficient proteolytic ferment was added to it to neutralize the antiproteolytic factor—or, better still, to make the serum actively proteolytic. By a simple experiment Wright and Fleming soon obtained clear evidence that this was the case. Taking a fresh sample of serum, they first estimated its proteolytic power, inoculated it with a gas-forming anaerobic bacillus, and then distributed it in equal volumes in miniature test-tubes. To each of these volumes (except one) they added increasing quantities of a proteolytic ferment (trypsin); and finally covered them all with melted petroleum jelly. The progressive increase of growth as the point was reached at which the antiproteolytic power was abolished and the fluid became actively proteolytic is well shown in Fig. 3.

FIG. 3.—Wright's experiment on the antiproteolytic power of normal serum.

From this and similar experiments with other microbes it became clear that: (a) In normal serum, or lymph, with its full antiproteolytic power, very few of the microbic species found in war wounds (notably certain of the streptococci and staphylococci) can initiate growth and multiply fairly freely. Wright called these "serophytes." (b) Most of the species found in the war wounds cannot grow in normal serum or lymph until its antiproteolytic power has been blunted off or completely neutralized. Wright called these "serosaprophytes." (c) Some microbic species are intermediate between the above two classes.

Three Propositions

These findings gradually built themselves up in Wright's mind into a generalization which seemed to him, as it seems to me, of fundamental importance to our understanding of infectious diseases. It can perhaps best be formulated in the form of three distinct propositions. I cannot recall that Wright ever put it exactly in that way, but I think I do no violence to his ideas in so expressing it. The three propositions are these: (1) A microbe's capacity to initiate human disease is determined in part—an important part—by its capacity to multiply freely in human body fluids. (2) The capacity to multiply freely in human body fluids varies widely among the many microbic species which man encounters. (3) The capacity to multiply freely in body fluids is largely dependent upon reduction of the antiproteolytic power of the blood.

The first of these propositions may seem self-evident, yet, strangely enough, I have found no reference to it, or to the other two, in any textbook (in the English language) of pathology or of bacteriology or of medicine. But I suggest that there is much to support Wright's contention.

So far as our knowledge goes (there is room for further study of this point) there are only two microbic species which are able to multiply freely in human blood serum—namely, the haemolytic streptococcus and the staphylococcus. Is it an accidental coincidence that these two species have also caused such varied manifestations of human disease, and afflicted so many people in every part of the world? Consider first the haemolytic streptococcus—it has brought us scarlet fever, a formidable killer not long ago, and tonsillitis, and otitis media, with its heavy toll of deafness; it has brought us puerperal fever and rheumatic fever; countless septicaemias and disabilities resulting from wounds of war and of civil life; and it has proved a deadly complication of other disease, notably of influenza, as in the great pandemic of 1918, and of measles. The staphylococcus in its turn has brought us osteomyelitis, boils and carbuncles; much wound sepsis and many septicaemias; it has in the past added greatly to the heavy toll of smallpox and the misery of skin diseases; it has been responsible for many fatal respiratory infections of infants; for food-poisoning; and to-day it is still perhaps the most serious cause of cross-infection in hospitals.

May it not be that the ability of these two microbes to do so much damage to man is closely linked with their capacity to multiply freely in his body fluids? If that view is correct, surely Wright's generalization is one which pathology and medicine can ill afford to ignore. Even if his interpretation of his experimental facts was mistaken (which should certainly not be taken for granted) the facts themselves are of sufficient interest to warrant further investigation and discussion. And yet for nearly 40 years they have been completely ignored. I do not propose to consider here why that has happened. Suffice it to say that it is no new thing in science. It will be recalled that Mendel's classical work on inheritance attracted no attention for 35 years after his death; and that the same happened to Oliver Wendell Holmes's clear warning that puerperal fever was being transmitted by doctors and nurses. In our own time Fleming's suggestion that penicillin might be of value in medicine went almost unnoticed for more than 10 years, and, but for Florey's intelligent hunch that it was worthy of further investigation, we might still be without penicillin.

Experiments on the Properties of Leucocytes

To come back to the war wounds. When Wright had satisfied himself about the conditions which favoured proliferation of microbes in the wounds he turned his attention to the other side of the medal—the factors which check that proliferation and the invasion of tissues to which it so often gave rise. Naturally his thought focused on leucocytes. Surgeons had long speculated upon the part played by these cells in connexion with wound sepsis. Their possibly beneficial role was suggested by the term "laudable pus," but in sober fact there was still very little precise knowledge about the leucocytes and their functions. Wright set to work to study them as he might have done some new species of animal. Entirely new methods had to be devised for collecting leucocytes from blood; for observing their responses to microbes, to chemical agents, and to physical conditions. It amounted almost to a new chapter in physiology.

I will describe only two of his experimental procedures—which gave results so illuminating that they ought to be shown to every student. First what Wright called the "lath

FIG. 4.—The "lath experiment."

experiment"—which demonstrated so clearly the remarkable capacity of leucocytes to restrain the growth of microbes such as staphylococci or streptococci. For this purpose two narrow strips of glass were placed back to back in a tube of quite fresh blood which was immediately centrifuged and then incubated for an hour (Fig. 4, left). During this incubation very large numbers of leucocytes left the blood clot and stuck on to the middle section of the two "laths," as could readily be seen by removing one of them from the blood, and washing and staining it (Fig. 4, centre). The other lath was then removed from the blood and placed, leucocyte-coated surface down, on an agar surface previously inoculated with staphylococci or streptococci. A third lath (from another tube of blood similarly treated) was removed from the blood and dried for half an hour in the incubator before being placed on the agar surface. In Fig. 4, right, it is evident that there has been a complete suppression of growth under the part of the undried lath where the leucocytes were most thickly massed. (In other experiments, conducted rather differently, it was shown that the microbes were completely killed by the cells.)

The second experiment was done with pus collected from a healing wound and was also intended to demonstrate the remarkable antimicrobial power of leucocytes (or their products). A drop of the pus was placed on a sterile agar surface and immediately covered with a portion of a microscope slide which, by its weight, pressed out the pus into a very thin layer. After incubation it was observed that no colonies had grown except at the extreme periphery of the drop of pus, where a few organisms had been squeezed out in the fluid, out of reach of the cellular elements (Fig. 5, A). Other samples of the same pus in which the leucocytes had

been put out of action—for example, by drying as at B, or by heating to 45° C. as at C—gave an abundant growth of colonies all through the specimen. Finally, in order to find out whether the pus could deal with a greater number of microbes than it originally contained, other drops were placed on a heavily infected agar surface (see lower half of Fig. 5). Under the drop of fresh untreated pus (at D) there was complete suppression of growth; while under the dried pus (at E) there was no suppression. We were unable by this method to arrive at the limit of the antimicrobial power of the leucocytes. Wright gave the name "bio-pyo-culture" to this type of experiment.

By these and many other ingenious experiments Wright contributed more, in my judgment, to our understanding of the factors at work in an infected wound than anybody else in the last 100 years. Again he had "made bacteriology" and also immunology.

As his work developed he was able to formulate the lines along which "treatment by physiological agencies" should proceed. In the early stages, he told the surgeons, the emphasis should be on the removal of foreign bodies and

FIG. 5.—Experiment demonstrating the antimicrobial power of leucocytes.

necrotic tissues (since these would create "dead spaces") and on the promotion of an abundant flow of fresh lymph, rich in anti-proteolytic factor, to restrain the proliferation of microbes. In the later stages the emphasis should be on securing the best conditions for the functioning of leucocytes and on healing. "The leucocyte is the best antiseptic," he would say, and closure of the wound—by suture or skin-grafting—will usually give it the best conditions. In its essence, the present-day treatment of wounds finds its scientific basis in Wright's laboratory work during the first world war, although the textbooks of surgery tell the student nothing of that work.

I have spoken of only three points at which Wright broke new ground in medical science. There were of course many more. He was not greatly distressed that so much of his work failed to win general acceptance during his lifetime. He knew too well that the unconventional view is seldom popular, and he was confident that, in so far as he had found truth, his work would endure. "After all," he would say, "the Almighty is in no hurry; why should I be?"

Figs. 1, 2, and 3 are taken from Professor Fleming's paper in the *British Journal of Surgery*, 1919, 25, 99; Fig. 5 from the paper by Almroth Wright in the *Lancet*, 1919, 1, 489.

SERUM VITAMIN B₁₂ CONCENTRATIONS OF PATIENTS WITH MEGALOBlastic ANAEMIA AFTER TREATMENT WITH VITAMIN B₁₂, FOLIC ACID, OR FOLINIC ACID

BY

D. L. MOLLIN, M.B., Ch.B.

Assistant Lecturer in Pathology (Haematology)

AND

G. I. M. ROSS, M.B., Ch.B., D.C.P.*

Assistant Lecturer in Pathology (Bacteriology)

(From the Department of Pathology, Postgraduate Medical School of London, W.12)

The vitamin B₁₂ concentrations in the serum and urine of normal subjects and of patients with untreated megaloblastic anaemia have already been reported (Mollin and Ross, 1952). Measurements of the vitamin were made by microbiological assay using *Euglena gracilis* var. *bacillaris* as test organism. The mean vitamin B₁₂ concentration in the serum of normal subjects was found to be about 320 µg. per ml.; the lower limit of the range in normal subjects was about 100 µg. per ml. The concentrations in the serum of patients with untreated pernicious anaemia were below the normal range; the mean concentration was less than 40 µg. per ml.

The changes in vitamin B₁₂ concentrations in serum and urine during the first 72 hours after intramuscular injections of vitamin B₁₂ have also been described. After the injections the concentrations of the vitamin in the serum of patients with pernicious anaemia rose from low pretreatment levels to within or above the normal range (Mollin and Ross, 1953).

In this paper we report the length of time that haemopoiesis was normoblastic and the serum vitamin B₁₂ concentration was normal in patients with pernicious anaemia given single intramuscular injections of vitamin B₁₂—observations which have a direct bearing on the treatment of pernicious anaemia. In addition, the effect of daily injections of vitamin B₁₂ and of treatment with folic acid or folinic acid is reported.

Materials and Methods

Observations are reported on 33 patients with pernicious anaemia in relapse and 13 patients suffering from other megaloblastic anaemias. While in hospital these patients received a diet from which meat was excluded and in which fish, butter, cheese, and eggs were restricted. Patients usually received the vitamin-B₁₂-deficient diet for five to seven days before treatment.

Method of Assay.—The method using *Euglena gracilis* var. *bacillaris* as test organism is based on that introduced by Hutner *et al.* (1949); details of the procedure followed have been described previously (Ross, 1952). Serum for assay purposes was usually taken daily; serial samples taken over a period of one to two weeks were generally assayed in one batch. All the samples were stored frozen and assayed at least twice. The concentrations recorded are total concentrations of vitamin B₁₂ equivalent, but, as no significant amount of uncombined vitamin was present in the specimens

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