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CLINICAL OBSERVATIONS ON A SERIES OF CASES TREATED BY VACCINES.

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Thesis submitted for the M.D. Edinburgh

by

David Macnair,

M.B. Ch.B. Edinburgh, 1908.

M.D. 1912.

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Introduction.

The rapid progress in medical science which has taken place within recent years, has produced many new methods in the diagnosis and treatment of disease. These advances are chiefly due to our improved knowledge of bacteriology.

The initial work of Lister and Pasteur is still within living memory, yet the rapid evolution of bacteriology, the practical application of which has achieved such marvellous results is such that now, as an auxillary science, it is of the first importance in every branch of medicine. The application of bacteriology as an aid to accurate diagnosis and rational treatment is incontestable.

The most recent advance in this science is the introduction, for therapeutic purposes, of inoculations with bacterial vaccines. This, as will be shown, is the outcome of experimental research into the problems of Immunity.

It is not, however, strictly accurate to describe Vaccine Therapy as a new treatment. The name vaccine is derived from analogy with vaccination, originally performed by Jenner, the pioneer of inoculation, who used the virus of cow-pox, a modified small-pox. Then followed Pasteur who introduced the attenuated virus for Hydrophobia, and Koch succeeded the latter with his tuberculin.

Although this method has only been scientifically used during the last few years, it has, chiefly owing to the investigations of Wright and his school, already undergone

remarkable and extensive developments.

It is my intention to give a brief history of the modern researches which led to the introduction of Vaccine Therapy. I also propose to give an account of personal clinical trials with vaccines on a series of cases which were under my care when House Physician at the South Devon & East Cornwall Hospital, together with a description of the technique employed. These vaccines were prepared by myself in the Hospital laboratory from the patients' infecting organism.

I must acknowledge my indebtedness for the valuable aid I received, to Dr. Pethybridge the Hon. Pathologist, and to the Hon. Medical and Surgical Staff of the South Devon & East Cornwall Hospital.

General Principles of Vaccine Therapy.

A consideration of Nature's method of recovery from a disease of bacterial origin, reveals clinically, first of all the onset, when the patient may be seriously ill. Then, after a varying interval, recovery begins, the symptoms subside and the patient regains health. The scientific explanation of this phenomenon is that active pathogenic bacteria gain access to the tissues. There they multiply, produce toxins, and cause the symptoms of the disease. Meanwhile the tissues of the host react and produce antibodies. These prevent the spread of the infection, and the process going still further, effectually sterilises the tissues to which the organisms have gained access, and the patient is cured. The recovery of the patient depends on the sufficient production of these antibodies.

Numerous experiments have been performed to demonstrate the theory of the formation of antibodies. E.g., Wright has shown that if ricin is added to the blood of an animal, the red blood corpuscles are destroyed. If however, ricin in injected into the animal in small but increasing doses, an antibody develops in the blood so that subsequently when it is treated with ricin, destruction of the red blood corpuscles no longer occurs.

Again, if the serum and red blood corpuscles of two closely allied animals of the same species, such as a sheep and goat, are mixed together, no change occurs. But if the red blood corpuscles of the goat are injected

into a sheep in a series of gradually increasing doses, a substance develops in the sheep's blood, so that when the serum is added to the goat's red blood corpuscles, they are dissolved and a solution of haemoglobin is produced.

Experiments on animals with pathogenic micro-organisms, which will be referred to later, demonstrate the formation of anti-bacterial substances.

Thus the body elaborates its own anti-bodies to invading bacteria.

A considerable amount of research has been done with regard to the nature of these antibodies and various theories have been devised to explain their mode of action. Metchnikoff first propounded the theory that immunity depends on the digestive action of certain cells of the body alone, termed phagocytes.

Wright and Douglas believe phagocytosis is entirely dependent on the presence of certain substances in the serum which prepares the micro-organisms for injection. These substances are termed opsonins.

Neither of these theories are wholly accepted. It is not, however, within the scope of this thesis to enter into a full and critical discussion on all the theories and research in immunity. Our knowledge of events which actually take place in the tissues during the process of recovery from an infection, is still very imperfect. The mechanisms and substances concerned have been thoroughly investigated.

Various antibodies have been described such as agglutinins, bactericides, bacteriolysins, opsonins, alexins, antitoxins.

The nature of the antibody apparently varies according to the variety of the infecting bacteria. These have been termed by Wright "bacteriotropic" substances.

The sequence of events following the inoculation of a bacterial poison, was first demonstrated by Ehrlich[1].

A milch goat, previously immunised was inoculated with tetanus toxin, and the antitoxin in the milk was estimated at different intervals subsequently.

The results set forth graphically as a curve of immunity demonstrate first of all a depression, succeeded by a rise considerably above the previous level. After an interval, the curve descends to a higher base line. The fall in the curve of immunity is termed the 'Negative Phase,' after which succeeds the 'Positive Phase.' The higher base line represents the gain in immunity. The same succession of negative and positive phases was reproduced after a second and a third inoculation. Salomonsen and Madsen[2] were the next to demonstrate the reaction of immunity, (1887) in connection with inoculation of Diphtheria Toxin on a previously immunised milch mare. The antitoxin content in the milk and blood respectively showed negative and positive phases similar to the curves obtained by Ehrlich. Subsequent experiments with other immunising processes produced

similar curves. E.g. by Bulloch[3] in connection with the inoculation of ox-blood into rabbits.

Then followed the discovery of agglutination first described by Widal. Wright[4] and his fellow workers ingeniously applied this as the method of serum diagnosis in the discrimination of certain continued fevers, viz. typhoid and Malta fevers. This specific agglutinating power was observed to develop in the blood during the progress of the fever, and to persist for an indefinite period afterwards, in some cases for years, during which time the patient was immune to further attacks. The relation between the production of agglutinins and immunity was fully demonstrated. When bacteria were subjected to the influence of their corresponding agglutinin, they became immobile and distorted and their growth on a culture medium was inhibited. Inoculation experiments with typhoid bacilli (in monkeys) caused the production of agglutinins.

The evidence and deductions of these experiments resulted in the successful preventive inoculation against typhoid in man. This was first introduced by Wright,[5] who, with his collaborators, has made innumerable and exhaustive experiments demonstrating the presence of other bacteriotropic substances, notably bactericidins and opsonins.

With regard to the reaction of immunity (as set forth in a curve), Wright[6] in a series of typhoid inocu-

lations in men observed negative and positive phases in the bactericidal power of the blood. He demonstrated the maintenance of a higher base line, two years after inoculation.

The curve of immunity is not unvarying. Wright found, particularly with typhoid vaccine, that differences in the size of the dose, produced differences in the intensity and duration of the negative phase. A small dose, in one case, induced a positive phase in 24 hours; while large doses may be followed by a negative phase of many months duration.

Idiosyncrasy on the part of the subject was also found to play an important part. Again, it has been demonstrated that the effect of a series of inoculations is a cumulative one. Excessive doses of vaccine and too frequently repeated inoculations, will produce a cumulative negative phase, and thus poison the organism. While a cumulative positive phase and greater immunity results from properly adjusted doses inoculated only when the curve has reached a higher level.

The application of the production of immunity by vaccines, for therapeutic purposes was first introduced by Sir A. E. Wright.

The existence of a localised bacterial infection associated with inflammation at the site of inoculation suggested a parallel to the cases of the partially immunised animals employed in the initial experiments of Ehrlich and Salomensen. These cases have a

considerable balance of resisting power. The whole object of vaccine therapy is to develop these unexercised immunising properties in order to combat the infection.

A consideration of the conditions which obtain at the seat of a localised bacterial infection, e.g. staphylococcus, resulting in abscess formation, reveals clinically, a fluctuating mass surrounded by a definite zone of congestion. Here the flow of lymph is considerably retarded owing to coagulation of fibrin in the capillaries. Wright and Douglas[7] have shown that the antibacterial elements present in fluid obtained from pus are considerably reduced and may be nil, compared with those present in the blood serum. Consequently the organisms multiply and produce toxins in a region of lowered bacteriotropic pressure.

In order to cure such a condition, it is necessary to cause a free flow of lymph through the affected region, and secondly to raise the antibacterial power of the lymph.

The first is done by ordinary surgical measures - incision, rubefacients, etc. The second is done by appropriate doses of vaccine.

The reaction of immunity caused by a vaccine, is not a mere replica of the previous infection. There the reaction is hampered and often frustrated by the virulence of the organisms and toxins causing processes of necrosis and cell degeneration. The vaccine being

a devitalized culture, the organisms cannot multiply in the tissues. They stimulate the formation of antibacterial bodies so that the defensive forces of the body are considerably increased. Thus septicaemic invasion is counteracted and the occurrence of secondary foci prevented. In addition, there is circulating in the blood a considerable reserve of antibacterial fluid, available for flushing any bacterial nidus.

The initial tentative cases treated by inoculation with vaccines were those with purely localised infections, chiefly caused by varieties of staphylococci, and these cases have, according to literature, yielded the best results. Since then, however, vaccines have been successfully used in all kinds of infections due to practically every known species of micro-organisms.

The cases I shall presently detail were not specially selected. They occurred amongst the in-patients of the South Devon & East Cornwall Hospital. Vaccine therapy was only resorted to because the cases were intractable to ordinary medical or surgical treatment.

It is more rational to use vaccines prepared from the individual patient, than to employ stock vaccines. Wright strongly maintains this, claiming that the best results are obtained by the use of autogenous vaccines, and that they should be resorted to whenever possible in dangerous and obstinate infections. Indeed, there are frequently so many different strains of

genus of organism, that to obtain a completely successful result, it would seem imperative to employ autogenous vaccines.

Before describing the cases, I have devoted a section to the technique I used in preparing the vaccines.

Remarks on the 'Opsonic' Index.

The theory that both forms of immunity, natural and artificial, are due to a specific substance or special property of the blood serum, which prepares micro-organisms for ingestion by leucocytes, is not yet fully proved and is not generally accepted.

The theory is founded on an experiment by Wright and Douglas[8] who found that if a suspension of bacteria is mixed with washed leucocytes in a neutral medium, little or no phagocytosis occurs. If then, some blood serum be added, it is found that the bacteria accumulate in or upon the leucocytes. Hence the idea that something exists in the serum which acts as previously mentioned and is termed an 'Opsonin.'

The 'opsonic index' of a patient's blood is obtained by comparing the phagocytic count of the patient's blood with the phagocytic count of normal blood.

This index is regarded by Wright as a measure of the immunity of a patient to any particular micro-organism. With regard to the original experiment however, there are no control experiments which disprove that the phenomenon is not purely a physical condition. That the smaller particles adhere to the larger owing to the glutinous character of the medium. Heating the serum to 60°C. before adding it to the mixture, when no opsonic effect becomes manifest is also open to fallacy

on account of the chemical changes occurring in serum at this temperature. Again, no allowance is made for the bactericidal power of the serum, which, acting apart from the leucocytes, can be shown to exist.

Regarding the theory from a practical point of view, it is claimed that the patient's immunising power can be accurately measured and that invariably with the rise and fall of the 'opsonic' index there is a corresponding progress and regress in the clinical condition of the patient.

This latter claim has been shown to be fallacious by several observers, who have found the index continue to rise while the patient is progressively sinking, and die with the index abnormally high. And per contra, the 'opsonic' control has bade them desist while they have continued the vaccine inoculations with good results. It may be conceivable that the continued existence of a lesion in spite of a high opsonic index, is due to the failure of the serum or leucocytes to gain access to the lesion, owing to the density of the surrounding zone of inflammation. But it is otherwise when there is definite clinical improvement with a low index; and it is impossible to explain the formation of new lesions when the index is high.

With regard to the accuracy of the determination of the index, there are a great many sources of error in the technique. The difficulty in obtaining a

uniform bacillary emulsion and the treatment of the blood and leucocytes acting under abnormal conditions. Wright[9] says, "no matter how closely we think we may have reproduced 'in vitro' the conditions which obtain 'in vivo' we can never be sure that we have reproduced all the essential conditions, and it will never be legitimate to treat the measurements obtained 'in vitro' as if they held true, without qualification, in the living body.

Then on the slide, difficulties are encountered owing to the clumping of bacteria due to agglutinins, the dissolution of bacteria under the influence of bacteriolysins, the intracellular digestion of the microbes, the clumping of the corpuscles, the variation in the bacterial content of the leucocytes and the disparity of results obtained from different parts of the slide. There is moreover, the personal equation of the operator. Horder and Andrews[10] made some experiments in the estimation of indices, using the same slides, and found their results widely different.

Thus the margin of error in 'opsonic' counts is sufficient to negative all value claimed for them.

Again, in diseased conditions, difficulties are encountered owing to the variations caused by auto-inoculations. Indeed the wide variations which obtain in the 'opsonic' content of the blood of healthy individuals, are sufficient to show that it is difficult to

say which is normal or abnormal.

Consequently no confidence can be placed in estimations of the 'opsonic' index to give definite evidence as to the presence of a negative or positive phase and they are unreliable as a guide to the progress of immunity.

Preparation of Vaccine.

A vaccine consists of an emulsion of micro-organisms obtained free from extra cellular toxins, killed and suspended in a neutral medium, for the purpose of subcutaneous injection.

To prepare a vaccine it is first of all necessary to have a pure culture of the special organism, of 24 hours' growth on a dry medium. Sloped agar tubes are a suitable medium for the majority of organisms, but for certain varieties, e.g. streptococci, pneumococci, etc., blood agar is better.

The isolation and cultivation of the causal organism of the disease varies according to the nature of the infection. I have described the different methods employed separately in connection with each case.

When the growth of the organism is of considerable size, e.g. staphylococci, B. coli, etc., it is heaped up on the surface of the medium by means of a sterilised loop of platinum wire. The collection is then transferred to 5 c.c. of sterilised salt solution (0·8%) in a test tube. First of all, in order to ensure a uniform emulsion, about 1 c.c. of the salt solution is placed in a small sterilised agate mortar and two or three loopfuls of the culture are added to it. The whole is then well ground up and mixed with

the pestle and then added to the remaining 4 c.c. of saline in the test tube.

When the colonies are small and delicate, e.g. streptococci, the condensation fluid in the culture tube is first decanted off and 2 c.c. of the salt solution poured over the culture. By means of the platinum wire, the culture is then scraped off the surface of the medium, ground up in the mortar, and added to the remaining 3 c.c. of saline in the test tube. In some cases it may be necessary to use two or more culture tubes in order to obtain a sufficiently strong emulsion.

The emulsion is then sterilised by plugging the tube with sterile cotton wool and placing it in a water bath at 65°C. for half an hour.

The strength of the emeulsion is then estimated that is, the number of organisms per c.c. The method I adopt is that originally described by T. J. Horder. It consists in making a direct count of the organisms by means of a Thoma Zeiss Haemocytometer. The leucocyte pipette is used and the emulsion is diluted with a special staining solution composed of the following formula :

Giemsa staining solution.	10 pts.
Formalin.	2 pts.
Salt solution (0·1%).	100 pts.

The emulsion is drawn up to the 0·5 mark and diluted to the 11 mark. The pipette is well rotated

and a drop placed on the counting slide as in making a blood count. This is allowed to settle for half an hour; then the number of organisms in 40 squares is counted and from this the number per c.c. can be calculated.

Thus if 200 organisms are counted in squares, the number per c.c.:

$$= \frac{200 \times 4000 \times 20}{40} \times 1000 = 400 \text{ millions.}$$

At the same time, it can be readily seen if the emulsion is uniform, that no clumps exist and chains of streptococci are disintegrated.

Having now obtained a uniform emulsion and knowing the number of organisms per c.c., it can be diluted with sterilised salt solution to any desired strength per c.c.

One c.c. is then transferred to each of several sterilised glass ampoules prepared for the purpose. This is done by means of a special pipette which can readily be made from a piece of glass tubing about 12 inches long and $\frac{3}{8}$ inch bore. It is bent at right angles about three inches from one end, the short end being fitted with a rubber teat. The longer limb is drawn out in the flame to a fine point so that it can enter an ampoule. It is then graduated to measure 1 c.c. exactly.

Having transferred 1 c.c. of the emulsion to each ampoule, they are placed in a water bath at 65°C.

for 10 minutes to ensure sterilisation; then each is hermetically sealed at the neck in the flame of a blow-pipe.

The contents of one ampoule are run over a culture medium which is incubated for 24 hours. If no growth results, the vaccine is ready for use.

The following is a short account of the technique I employed in preparing the blood culture in case 9.

The blood was withdrawn from a vein in the forearm. The venous return was prevented by a bandage round the upper arm, and the vein rendered still more prominent by causing the patient to grasp a roll of bandage firmly. The skin over the selected vein was washed with soap, then ether applied. The blood was then withdrawn in a special 5 c.c. glass syringe fitted with a glass piston which slides easily. The syringe and needle having been boiled and allowed to cool, are fitted together and rinsedwith sterile water. The needle and neck of the syringe are then filled with a warm sterile solution of sodium citrate (0·5%). This prevents rapid clotting of the blood. The patient's arm, having been prepared is held out from the side of the bed at right angles to the patient and depressed. The operator, with his back to the patient, plunges the needle into the vein in the direction opposite to the venous circulation. This is done by puncturing the skin and passing the needle along the vein parallel to

the skin. If quickly and dexterously performed, little or no pain is caused. When the needle is in the vein, the blood flows rapidly into the syringe displacing the piston with its own force. When the syringe is full, the needle is rapidly withdrawn, the arm raised and the constricting bandage removed. The haemorrhage stops and a dressing is seldom required.

The blood is then injected into several culture tubes. No special culture media are, as a rule, necessary. I use two sloped agar tubes and two 10 c.c. broth tubes. The blood is distributed between them as follows. Into one agar tube a few drops are injected and allowed to spread well over the surface; into the other, a larger quantity is put and allowed to remain at the bottom. The broth tubes are treated similarly, a small quantity being injected into one, and from 2 to 3 c.c. in the other. The object of the larger quantity of blood is to dilute any antibodies which might be present and inhibit the growth of the organisms; and if the organisms are scanty, there will be more chance of cultivating them.

The broth tubes are rolled between the hands, and the whole are placed in the incubator for 24 hours or more.

If organisms are present, they usually appear within the first 24 hours. In the broth tubes, the blood pigment settles as a deposit at the bottom of the tube, and immediately above there is a translucent clot suspended in the broth. It is in this clot that the colonies develop.

Cases.

Case I. Mabel C., aged 25. Domestic servant admitted July 6/10 under Mr. Woolcombe.

This was a gynaecological case. There was a history of dysmenorrhoea and leucorrhoea. The latter was more or less constant. Patient was unmarried. There was no pain on micturition. Bimanual examination revealed two tumours in each broad ligament. The smaller, on the left, was about the size of uterus. An operation was performed and a double pyosalpinx was found. Bilateral salpingo-oophorectomy was performed, and the right side was drained per vaginam. Patient began to improve, but three weeks later her temperature again rose and another collection of pus could be felt. Under an anaesthetic, the abdominal wound was reopened and a drainage tube inserted. This continued to discharge copiously for a fortnight. A culture was made from the pus and staphylococci only were found. A vaccine was made containing 300,000,000 in 1 c.c. This was injected every seven days. At first there was a slight reaction T. 101° but the discharge rapidly became less and a week after the fifth injection, only a small sinus was left. This healed up in the course of another 10 days.

Patient was discharged September 20th, 1910.

Case II. Chas. D., aged 8. December 4th, 1909 under Mr. Webber.

Patient was admitted to Hospital looking very ill and emaciated. Both legs were drawn up on the abdomen in full flexion. The history was rather indefinite; the child had been unable to walk for some months and the deformity gradually developed. There was some fullness over each hip-joint, more marked on the right. The case was obviously one of advanced tubercular disease of the hip-joints. Double extension was applied with a light weight, and the deformity was gradually remedied. A tubercular abscess developed over the right hip. This was aspirated and filled with iodoform emulsion. This was followed by an abscess on the left side which was similarly treated. The condition did not improve however, and it was necessary to incise and scrape out the abscess cavity on both sides. The infection spread and various counter openings had to be made, resulting in the formation of sinuses. Eventually there were nine of these discharging sinuses; three over each hip and three on the back. The child became seriously ill, emaciation was extremely marked, and the temperature was typically hectic. Meanwhile Koch's T.R. had been injected every fourteen days in doses of 0·004 mg. After four injections, the general condition remained stationary. The pus from the sinuses had a greenish tinge. There was obviously a mixed infection. A culture made from the pus yielded

a mixed growth of staphylococci and B pyocyaneus. A pure growth of each was obtained by subculture on agar. B pyocyaneus was easily recognised. A gelatin medium was liquefied and became of a greenish yellow colour. On agar, there was a moist growth and the medium was stained a greenish colour. It was gram negative.

Vaccines were then made from each of these organisms and injected alternately each week. 200 million B pyocyaneus were injected first followed by 100 million staphylococci seven days later. These were continued for four infections of each, together with Koch's T.R. At the end of which time, no B pyocyaneus could be isolated from the pus. The local condition had rapidly improved, only three sinuses remained open, and the child had gained in weight and was considerably better. The injections of T.R. were still continued and one month later the sinuses were completely healed. There were altogether 10 injections of T.R. The child was sent home June, 1910 in a Bryant's splint.

Case III. Mrs. L., aged 52 years, under Mr. Lucy.

Patient was admitted in March 1910. She walked with a limp and complained of 'sciatica' in theleft leg. The pain commenced sixteen months previously. It had been almost constantly present as a dull ache down the back of the left leg. Latterly it had become much more acute and prevented her from sleeping.

Two and a quarter years previously, she had an

attack of Malta fever and cystitis, and one and a half years ago enteric fever. Shortly after recovery from the latter, the 'sciatica' commenced. Patient was pale and thin and looked ill.

On examination there was a fluctuating swelling immediately behind the great trochanter on the left hip. This was separated and a few ounces of pus withdrawn. A culture on agar and gelatin was negative. Aspiration did not relieve the symptoms, so a few days later an incision was made and two and a half pints of pus drained away. A large probe passed in apparently through the sacro-sciatic notch for a distance of eight inches; that is to the opposite side of the pelvis. Bare bone was encountered round the whole of the posterior wall of the pelvis. The pus evacuated was also sterile. A drainage tube was inserted but it did not drain well and caused discomfort, so two weeks later an incision was made down the left side of the sacrum, the whole cavity explored and drained direct through the great sacro-sciatic notch.

Meanwhile, the general condition of the patient gradually became worse, despite the improvement in her temperature, which at first rose to 103° or 104° each evening, and after the second operation became practically normal. In June, 1910, the temperature again rose, her condition became very serious, the emaciation was extreme, and her life was despaired of.

A culture from the pus yielded a pure growth of

staphylococcus aureus on agar. A vaccine was made from this and was at first injected in small doses every week, 25 million first, followed by 40 million. After the second injection a swelling was observed in the left grain. This was incised and half a pint of gaseous pus with a faecal odour was evacuated. It was then discovered that the drainage tube, passing across the pelvis, had, by pressure on the rectum, caused a slight faecal fistula. B. coli were readily grown from the pus and a vaccine was made from the culture. This was given every other week, alternately with the staphylococcal vaccine, first in doses of 40 millions, then after three injections it was increased to 50 millions, and the staphylococcal vaccine was coincidently increased to 250 millions. The patient began to manifest immediate improvement. The temperature became normal and remained so. The pain soon disappeared. The change in the general and physical condition of the patient was very striking. She put on weight rapidly; the hopeless cadaveric appearance gradually changed to that of robust health. Indeed, four months later the patient was stouter than ever she had been previously. The improvement in nervous energy was no less remarkable. From a condition of severe neurasthenia, she became bright and optimistic.

In January 1911, pus culture yielded a growth of staphylococci only, so that B. coli vaccine was stopped; 14 inoculations having been administered

altogether.

At the end of April 1911, only the posterior sinus remained open, the probe passed in four inches, but only soft granulations could be felt. There was only a slight discharge. The patient was able to get up and walk quite comfortably. She went home to the country under the care of her own medical attendant. There were given in all, 21 inoculations of staphylococcal vaccine.

Case IV. Elizabeth K., aged 47 years, single. Admitted May 2nd, 1910 and placed under Mr. Webber.

Patient complained of a swelling in the lower part of the back. She had first felt a small 'lump' there six weeks previously. On examination a soft fluctuating swelling was felt over the left lumbar region posteriorly. There were no local signs of inflammation, but the evening temperature rose to 99°.

A 'cold' abscess was diagnosed. Under an anaesthetic an incision was made, the pus evacuated and the cavity curetted and swabbed with a solution of zinc chloride. The skin was sutured and a few days later, the patient got up and felt perfectly well. Three weeks later however, the wound became painful and finally broke down, discharging some malodourous pus. The temperature rose to 100°. A culture from the pus yielded a pure growth of B. coli. A vaccine was made from this culture and eight million were injected.

There was no general reaction but the discharge became less. Seven days later 25 million were injected and this dose was repeated a week after. At the end of a month from the commencement of the vaccine treatment, the sinus which had previously been four inches deep, would only admit a probe for the distance of half an inch. There was only some sterile serous discharge. The patient was discharged on July 8th, 1910.

Case V. Ida E., aged 22, domestic servant.

Patient was admitted with the diagnosis of Meningitis(?) October, 1910. There was a history of 10 days illness commencing with pain in the lumbar region and fever. Three days prior to admittance to hospital she was seized with intense headache. On admission, patient was very drowsy, and vomited incessantly. Temperature varied between 100° and 104°. Then the abdomen became somewhat distended and there were one or two suggestive spots in the hypogastric region. Widal reaction proved positive. The cerebral symptoms gradually cleared up and the case became typical of enteric fever. About the beginning of the third week, patient began to complain of pain on micturition. Meanwhile the temperature subsided in the fourth week but the urinary symptoms became more marked. The pain on micturition was intense, catheter necessary; there was also some vaginitis and inflammation of the perineum. Urotropine produced no improvement. In the fifth week

the temperature again rose. There was a leucocytosis of 26,000 and enormous numbers of B. coli in her urine which was acid. These were cultivated and isolated on surface plates of Conradi Drigalski agar. The urine was diluted in 1 in 10 and a few drops run over the surface. Confirmatory tests were made with stab cultures in gelatin, and litmas milk. Pure cultures of the B. coli were then made on agar and from these a vaccine was prepared (50,000,000 in 100). The patient was inoculated with 1 c.c. of this vaccine. This was followed by a slight reaction on the third and fourth days (temp. 100°, pulse 106, and slight increase of pus present in the urine). The symptoms rapidly subsided, and 10 days later another 50,000,000 bacilli were injected hypodermically. This was also followed by some constitutional disturbance, but less marked. A third inoculation (1 c.c.) after a further interval of 10 days produced a very transitory reaction. Meanwhile the urinary symptoms rapidly improved. The inoculations (1 c.c.) were continued every 10 days. After the fifth inoculation the temperature remained normal. The pain and frequency of micturition had quite disappeared and no B. coli could be found in the urine.

The patient's general health had meanwhile markedly improved, and she was sent to the Convalescent Home, January 30th, 1911.

Case VI. Florence O, aged 13 years. Admitted February 1911 under Dr. Sotteau.

History. Patient had been feeling poorly for some weeks prior to admission. She was confined to bed for three weeks by her Dr.'s orders just before admission. She complained of no definite symptoms, except that for some months she noticed her urine had a bad odour and was dark in colour. There was also an increased desire to micturate at times. Apart from these symptoms patient felt quite comfortable except for a feeling of general weakness. She looked pale and emaciated. Weight 4 stone 12 lbs. Temperature and pulse normal. The urine was examined daily. At first it was dark amber and turbid, alkaline in reaction. Sp. gr. 1020. Albumen 1·2%. Blood and pus present. Microscopic examination of the deposit showed blood and pus cells, triple phosphates, and various putrefactive organisms. T.B. were absent.

Patient was confined to bed; urotropine was administered, bladder washed out daily and ·003 c.c. Koch's Old Tuberculin injected subcutaneously for diagnostic purposes. There was no reaction to T.B. At the end of a week, the urine had cleared.considerably, it was still dark in colour with a tenacious deposit of pus; blood was also present. The reaction then became acid. A culture of the deposit on agar yielded a copious growth of B. coli in pure culture. This was

confirmed by a stab culture in gelatin. A vaccine was made from these bacilli and 30,000,000 were injected. This produced no constitutional symptoms but the urine became clearer. Ten days later the patient was inoculated with 50,000,000. This resulted in still greater improvement. The albumen fell to 0·3% with only a trace of blood and a little pus in the urine. Three more inoculations were given of 50,000,000 at intervals of 10 days. During this time the urine remained practically unaltered. The albumen varied between 0·2% and 0·3%, the blood finally disappeared, but a trace of pus was occasionally present. The patient however, made considerable improvement in her general health and gained weight rapidly. She expressed herself as feeling better and left the Hospital in April 1911, although B. coli were still present in the urine.

Case VII. Mrs. S., aged 38 years, admitted March 2nd, 1910 under Dr. Fox.

Patient complained of frequency and pain on micturition. The history dated from her second pregnancy, two years previously. There had apparently been some retained products; the uterus was curetted 14 days after delivery. Thrombosis of the left femoral vein supervened. Since then the patient had been in delicate health and never felt quite well. About six months before admission, she began to experience pain on micturition and there was increased desire to do so. She also noticed that her urine was often thick, and

high coloured. For the last three months prior to admission to Hospital these symptoms had become more aggravating and latterly the patient had been unable to retain her urine for more than one hour.

When first examined, the urine was alkaline. Sp. gr. 30. It was opaque and there was a deposit of phosphates and pus. A culture from the pus yielded a copious growth of organisms which liquified gelatin. Most of the bacilli were not entirely decolorised by Gram's method of staining. They were chiefly B. Proteins and other organisms of putrefaction. The bladder was washed out daily and urinary antiseptics administered. A week later the urine was acid in reaction and the deposit less dense. Inoculation of gelatin and agar tubes yielded a pure culture of B. coli. This was confirmed as previously described, and a vaccine was made. 5 millions were injected every 10 days. The urine rapidly cleared and the symptoms disappeared. After the second injection, the patient felt perfectly well and left the Hospital three weeks after admission. B. coli were still present in the urine, but the growth on cultivation was very small.

Miss B., aged 30 years. Case VIII.

The patient was admitted to Hospital in March, 1910. Her illness commenced about 12 months previously. She complained of pain in the loins, chiefly the left, and noticed her urine was thick. The pain was more or less continuous. Latterly, however, there had been

severe exacerbations of the pain at night with temperature of 101° and 102°. These attacks were becoming more frequent and occasionally it had been necessary to inject morphia. The attacks were followed by the passage of thick urine. The patient looked pale and thin, and stated that she had been losing flesh.

April 1st. Urine was acid with a deposit of pus. Microscopic examination revealed enormous numbers of motile bacilli which, on culture, proved to be B. coli. No T.B. were found although the urine was repeatedly examined for that purpose. (It is interesting to note however, that there was a general and local reaction following an injection of Old Tuberculin.) A B. coli vaccine was prepared.

April 6th. 8 millions were injected. There was no reaction.

April 16th. 10 million were injected. There was some slight inflammation at the site of injection and temperature rose to 101°.

April 26th. 8 million injected.

Meanwhile the urine became much clearer, there was very little pain in the loins and the general health was much improved. Patient left the Hospital but returned 10 days later for another injection of the vaccine (8 million), and again on May 20th for a similar dose. On this last occasion the urine was found to be slightly alkaline, and no B. coli were present. A tonic was prescribed and when last seen on June 1st the patient felt quite well, the urine was normal, the pain had ceased and she had regained weight.

Case IX. Mrs. L., aged 25 years. Admitted under Dr. Fox, May 8th, 1910.

When the patient first came under observation, she complained of no definite symptoms, except of dull headache and a feeling of general malaise. The illness had come on gradually without any apparent cause a month previously. She had been confined to bed most of the time. Occasionally there had been attacks of vomiting.

Patient had had two attacks of rheumatic fever when 17 and 18 years of age. Since thenshe had been quite healthy.

On examination the patient looked sallow and delicate. There was a slight icteric tinge in the sclerotics. The temperature was slightly raised, usually between 99^{o}-100^{o}, occasionally rising to 101^{o}. There were no definite rigors, but sometimes, with the raised temperature, patient complained of feeling ill and vomited. The heart was found to be somewhat dilated, the dulness being increased transversely about ½". There was a blowing systolic bruit in the mitral area. The pulse rate averaged 100 per minute. No other physical signs were manifest except two attacks of cutaneous emboli. The first crop appeared round the left elbow a fortnight after admission, the second, over the front of the left thigh a week later. The condition did not yield to salicylates and cardiac tonics so a blood culture was made. The technique employed

is described in the special section, pages 18 and 19. There was a vigorous growth of streptococci in all the tubes. The cultures were quite atypical. In the agar tubes they were white and raised similar to that of staphylococcus albus. In the broth tubes, there were several larger opaque colonies in the translucent clot. Only on subculture were the typical cultural appearances of streptococci manifest. From this a vaccine was prepared.

The patient was first inoculated with 5 million. This resulted in a slight improvement in her general condition, but the pulse and temperature remained unaltered. After an interval of 7 days, 10 millions were given. This induced a very transitory phase of well being, but a similar dose 10 days later produced no effect. 15 millions were then given 7 days after but there was no response; indeed the clinical condition became gradually worse; the temperature oscillated higher, rigors were frequent, emaciation became more marked and the heart more dilated.

The dose of vaccine was increased to 20 millions after an interval of 10 days. Three days after this inoculation, there was intense pain and swelling in the left leg due to an embolus in the femoral artery and 48 hours later the patient became suddenly blind in the left eye owing to embolism of the retinal artery. No further inoculations were given. The patient sank rapidly and died 4 days later from cardiac failure.

The cases described in the foregoing pages may be considered in three groups.

The first four cases although suffering more or less from severe constitutional symptoms in the early stages, fall into the category of localised infections.

Case I. There was very dificient power of resistance to the staphylococci. The abdominal wound discharged copiously for a fortnight without any sign of healing. The vaccine caused a very satisfactory immunising response.

Case 2. This case, originally one of chronic tubercular infection of the hip-joints, became very acute, and the occurrence of the mixed infection rendered the case very desperate. The patient reacted successfully to all three vaccines, especially those of the B. pyocyaneus and staphylococcus. He did not experience, contrary to my expectation, any difficulty in responding to each vaccine. The immunising responses evoked by the vaccines of the secondary infections appeared to have a beneficial effect in clearing up the original tubercular infection.

Case 3. The original cause of the suppuration in this case is an open question. Whether the Malta or Enteric fever were the existing or predisposing causes, or whether the lesion was entirely tubercular in origin, was neversettled. No micro-organisms, unfortunately could be grown from the original pus. The

case ran a chronic course, and it is impossible to state definitely how much improvement was due to the vaccines beyond curing the secondary infections. At all events, clinical progress dated from the commencement of the inoculations. The difficulty in establishing efficient drainage and of encouraging a free flow of bacteriotropic lymph prevented rapid healing. That the patient was capable of immunising responses, was shown by the more rapid improvement with increasing doses of the vaccines.

The staphylococcal vaccine was increased from 25 millions to 250 millions and the B. coli from 40 millions to 50 millions.

In addition to the cure of the pathological conditions, it is interesting to note the co-incident psychic improvement which was induced. This feature, although present in the other cases, was strikingly manifest in this case.

Case 4. Here the B. coli was a secondary infection, and it cleared up rapidly with vaccine treatment.

The second group of cases (5-8) are those of B. coli infections of the urinary tract.

Case 5. This was doubtless a case of secondary B. coli infection following the B. typhosus. The symptoms were very acute at first but were very amenable to vaccine treatment.

Case 6. This case was chronic from the outset. It was sent into Hospital as Tubercular Kidney. The secondary infection of putrefactive organisms, at first obscured the original B. coli infection. This was particularly resistent to the vaccine inoculations, which however, resulted in the disappearance of clinical symptoms and considerable improvement in health.

Case 7. This was an acute primary infection following retained products of conception. A small dose of vaccine caused an excellent response, as shown by the rapid disappearance of symptoms. It is difficult to say whether or not a cure would have been established in this case if the inoculations had been continued.

Case 8. This was also a primary infection. The patient reacted to a small dose of vaccine and was very sensitive to any increase. In this case a cure was effected.

I have been unable to ascertain if a relapse occurred in this last case, or in any of these cases.

Experience has shown that B. coli vaccines are more potent in producing constitutional disturbances than most other vaccines. Certainly in the acute primary infections, a small dose (5 and 8 millions) produced a marked reaction and in these cases it would appear that the prognosis is more favourable than in the more chronic cases. Even though, in some cases, it would seem impossible to effect an absolute cure,

nevertheless, vaccine inoculations will cause alleviation of the subjective symptoms.

Case 9. This is the only case of generalised infection in which I had the opportunity of observing the effects of vaccine treatment. It was an obvious case of septicaemia, the bacteria being cultivated from the circulating blood.

The employment of vaccine in such a case would, at first, seem an unreasonable proceeding. However, from a consideration of experimental evidence, it appears that bacteriotropic substances are produced in the tissues at the seat of inoculation, the connective tissues yielding the maximum amount.

It has been shown in the production of diphtheria antitoxin in the horse, that little, if any, antitoxin is formed when the toxin is injected into the blood stream, whereas it is developed in considerable quantity when injected subcutaneously.

Again, in connection with anti-typhoid inoculations, Wright observed that a more effective immunising reaction is induced in patients who show considerable local reaction at the seat of inoculation, rather than in those who suffer severe constitutional symptoms with slight local reaction.

Consequently, it seems feasible to suppose that, in the case of a general infection where the patient fails to make satisfactory immunising responses, if the

antigen can be introduced into the connective tissues in concentrated form, rather than diluted with the whole volume of blood, a much greater immunising response will be evoked, and a cure effected. In actual practice this has been successfully performed in septicaemia cases.

In the case described however, despite the transitory improvement following the first two inoculations, the patient seemed incapable of any immunising response. Variations in the intervals between the inoculations and the size of the dose produced no result. It may be that the vigorous growth obtained from the blood in the culture tubes indicated a virulent strain of streptococci.

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