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THE TREATMENT

OF

SOME SKIN DISEASES

BY

BACTERIAL VACCINES.

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The subject of my thesis is the treatment of some Skin Diseases by Bacterial Vaccines. I have chosen this subject for the reasons that I have been engaged for some time in the treatment of Skin Diseases by Vaccines, and that Dermatologists are even at the present day by no means agreed as to the value of Bacterial Vaccines in certain Skin Diseases, notably Acne Vulgaris.

I shall begin by giving a short account of immunity, followed by a detailed description of the method of preparing a Vaccine, and the technique of the opsonic index. I shall then describe in detail some Skin affections, and more especially Acne Vulgaris, treated by bacterial vaccines, and the conclusions arrived at by such treatment. Immunity may be defined as the non-susceptibility of the body to a disease, or to the invasion of an organism. It may be natural or acquired. We speak of natural immunity when the body is naturally insusceptible to disease, and of acquired immunity when it results from an attack of the disease, or is produced artificially by inoculation. Acquired immunity may be active or passive, according as it is produced by injections of non-lethal doses of an organism or its toxins, or by injections of the serum of an immunised animal.

In order to understand how immunity is acquired by the introduction of a bacterial vaccine it will be necessary to refer to certain terms which are constantly

used in humoral pathology, such as Antigens, Antibodies, Receptors, Amboceptors and Complement. All substances of an organic character which when introduced into the body produce harmful effects are called Antigens. It is a generic name and includes bacteria, animal cells, such as the cells of various organs, red corpuscles or animal fluids, as the serum of the blood, or animal proteids, animal secretions, such as snake venom, and certain vegetable products such as Ricin, Crotin and Abrin. When pathogenic bacteria gain access to the body they may produce their harmful effects by their intra-cellular or extra-cellular products. As the result of the introduction of these Antigens, various antibodies or immune substances are produced, such as Antitoxins, Agglutinins, Precipitins, Lysins, Opsonins and Antiaggressins, which are supposed to be defensive reactions. In the case of toxins, Ehrlich assumes that they possess two atom groups, a toxophorous group acting harmfully, but unstable, and a haptophorous group retaining more persistently its affinity for antitoxin. When toxin produces its harmful effect it is because the haptophorous atom-group chances to have an affinity for a particular receptor or side chain and, becoming attached to the cell, conditions the living cell adversely.

By way of compensation other receptors of the same kind are produced, until there is over-production, when

the superfluous receptors are thrown off and occur free in the blood. These free receptors are the antitoxin, and possess the original affinity for the haptophorous atom-groups. If toxin meets it combination occurs and the affinity of the toxin-molecules being satisfied, they cannot become fixed in the tissues and are consequently harmless. In the case of Bacteria, Ehrlich explains the origin of immune body on lines similar to the production of antitoxin. It is assumed that there exist receptors for molecules which are more complex than those comparable with toxin, and that such receptors have a second affinity satisfied by a substance which is present in all animals apart from their being infected. This substance is known as complement, cytase or alexin, and those receptors which have the power of combining with the antigen which gave rise to them as well as with complement are called amboceptors or immune bodies.

Complement is thermolabile, i.e., it is readily destroyed by heating for half an hour at a temperature of between 50° and 60° C. It possesses another peculiarity in that it can combine with many different amboceptors. The Complement which at one time combines with the amboceptor produced by the introduction of bacteria into an animal, can when not so engaged combine with the amboceptor produced by the

introduction into the animal of the red corpuscles of some different species. Amboceptor is thermostable, and is not altered by the heating above mentioned. In addition to complement it is possible that all animals possess a certain amount of natural amboceptor, and when pathogenic bacteria infect the body more amboceptors are formed which are however specific for the bacteria giving rise to them. When complement and amboceptor are combined they form a combination which is spoken of as lysin, and if they can operate against bacteria in this conjunction they constitute bacteriolysins. It is the complement which when so fixed brings about the lysin or solution of the antigen. Pfeiffer observed this take place when cholera vibrios were injected into the peritoneal cavity of a guinea-pig. The same effect was observed in vitro with the peritoneal fluid when quite fresh. Bordet's explanation of Pfeiffer's phenomenon is that immune body merely sensitises the related bacteria so that complement becomes effective. He found that a similar action occurs if red blood cells of one species of animal are injected into another. Metchnikoff again holds the view that bacteriolysis is the result of phagocytosis, and that immune body and cytase (comparable with complement) originate in the leucocytes and thus bacteria must become englobed before they are destroyed. When virulent organisms gain access to an immunised

animal the leucocytes at once migrate to the site of infection, surround the organisms, ingest and so destroy them. This property of the leucocytes was at one time ascribed by Metchnikoff to "Education" of the leucocytes, but since the same thing happens when the serum of the immunised animal is injected into a non-immunised one he ascribed the action to substances named stimulins which increase the activity of the leucocytes, but as the existence of stimulins has not been proved he subsequently conceived the serum as acting, not on the leucocytes but on the organisms, causing them to become positively chemotactic and to attract the phagocytes.

Kanthack and Hardy[1] have observed the discharge of granules from eosinophile cells in the neighbourhood of bacteria, and considered that this matter produced a change in the organisms upon which phagocytosis followed. Lastly, Wright has demonstrated the presence in the blood of substances which he calls opsonins. These act upon the bacteria and prepare them as it were for their destruction by the phagocytes. He found that washed leucocytes without serum are non-phagocytic, but become so on the addition of normal serum. If, however, the serum is first heated to 60°-65° C. before being added to the mixture of leucocytes and microbes, phagocytosis does not take place, but if the unheated serum is mixed with the bacteria, the

micture kept at 37° C. for 15 minutes and then heated to 60° C. for 15 minutes, phagocytosis can still take place, thus demonstrating that the serum acts in some way on the bacteria.

The exact nature and constitution of opsonins are still unknown. Yorke[2] in an article entitled "Observations on the behaviour of opsonins and serum proteids during pressure filtration" showed that they were probably of the nature of colloid, and Noguchi[3] in an article entitled "On the influence of the reaction and of desiccation upon opsonins" has shown that they are not destroyed by drying the serum at 23° C. and that a temperature of 150° C. even does not destroy them entirely.

Again Yorke and Smith[4] have shown that there is a difference in the opsonins of normal and immune serum, that there is evidence of the existence of a preopsonin in normal serum which can be converted into active opsonin by the addition of any micro-organism, and that there is lowering and ultimate suppression of opsonic power of normal serum to all organisms, by the addition of one. In fact much the greater proportion of opsonin in normal serum is non-specific.

That there is a difference in the behaviour of the opsonins of normal and immune serum has been demonstrated by Bulloch and Western[5]. They tested the serum against staphylococci and tubercle bacilli, and

found that injections of tuberculin produced a rise in the tuberculo-opsonin but did not affect the staphylococcic opsonin. Injections of killed staphylococci had the reverse effect. As regards the structure of opsonins there are many views. Muir and Martin (6) believe that in immune serum a specific immune thermostable opsonin is present, and also a normal thermolabile opsonin.

Cowie and Chapman (7) hold the same view. Metchnikoff, Dean and others suggest that they are identical with the substance sensibilisatrice while Wright, Douglas, Bulloch and others consider that they are distinct from amboceptor and complement and form a class by themselves. From the observations of Allen (8) it would appear that opsonins are formed in the muscle or subcutaneous tissues and are then conveyed into the blood (8). If this is the case it will be evident why artificial immunisation should be beneficial in general blood infections.

The foregoing being a brief resumé of the principal views held with regard to immunity, I shall next describe the preparation of a bacterial vaccine. A bacterial vaccine is a sterile standardised emulsion of micro-organisms, and as the success of treatment of bacterial infections depends on the quality of the vaccine and its dosage, it will be obvious that its preparation is of the first importance. So far as the

preparation of a vaccine is concerned infections may be described as general or local. In general or systemic infections the organisms are cultivated from the blood, and in many cases from urine. In localised infections they are cultivated from the secretions from the site of the disease.

A blood culture is made as follows. The skin over the bend of the elbow is thoroughly cleansed with soap and warm water, next with some antiseptic such as 2% Lysol or Carbolic Acid, and finally rubbed with Ether. A tourniquet is then applied above the bend of the elbow to cause the veins to stand out prominently. A 10 c.c. sterile all glass syringe into which about 2 c.c. of sterile sodium citrate solution has been drawn, is used. The needle is inserted into the median basilic vein or median cephalic vein preferably against the stream, and the syringe slowly filled with blood, easing the piston to assist the flow. The tourniquet is then removed and the needle withdrawn. Into 6 tubes each containing about 15 c.c. of sterile broth, equal quantities of the blood are introduced. The tubes are then well shaken and incubated, three aerobically and three anaerobically at 37° C. They are examined after 18 or 24 hours, by making films and staining by simple dyes and after Gram's method. In the event of no growth they should be examined again after 48 and 72 hours. If a growth is found a subculture is made

on Agar or blood Agar - the great majority of organisms growing on these media. Care must be taken that the medium is sterile to avoid contamination with extraneous organisms, as the bacillus subtilis, a common source of contamination.

In the case of local infections it is necessary to examine the pus or other secretion microscopically before making a culture, as by this means one gets an idea of the nature of the organism, its morphological characters, and whether the infection is mixed or single, and the most suitable medium for its growth. Having ascertained this information a culture is next made by taking a tube of sloped Agar or blood Agar and with a sterile platinum loop smearing a small quantity of the secretion gently over the surface, care being taken to flame the mouth of the tube and plug of cotton wool before and after inoculation. The tube is then incubated at 37° C. for 18 to 24 hours. Next a film is prepared, stained by Gram's method and examined microscopically for identification. If the culture is pure, the vaccine can be made straightway. If it is a mixed culture it is necessary to get a pure culture and this is done by plating out as follows:- To the culture add about 10 c.c. of sterile 0.75% solution of sodium chloride and with a sterile platinum loop or glass rod gently separate the culture from the medium and emulsify as well as possible, then pour it into another sterile tube, and shake for

10 or 15 minutes. Next boil up 3 Agar tubes and allow them to cool down to 45° C. From the emulsion take one platinum loopful and add to No.1 tube and mix well by rolling the tube between the hands, from No. 1 tube take 2 loopfuls and add to No. 2, mix well and from No. 2 take 4 loopfuls and add to No. 3 tube, again mix well. Next, pour the contents of the tubes into sterile Petri dishes, first having flamed the tubes, and incubate for 18 or 24 hours at 37° C. It is thus a process of dilution, and in No. 3 Petri's dish pure colonies will be found which can be picked out and sub-cultured for 18 or 24 hours on sloped medium.

Having by this method obtained a pure culture the vaccine is made as follows:-

To the culture add from 3 to 6 C.C. of sterile 0.75% sodium chloride solution, according to the luxuriance of the growth, and with a sterile platinum loop or glass rod gently separate the growth from the medium, emulsifying as well as possible. Next pour into another sterile tube containing a few glass beads, which help to break up clumps, or it may be poured into an Ehrlenmeyer's flask, also containing a few sterile glass beads. Next shake for 15 or 20 minutes to thoroughly emulsify and break up clumps.

The emulsion is next centrifugalised for a few minutes to throw down clumps and any of the medium which may be in it. It is absolutely necessary to remove clumps in order to standardise the emulsion accurately.

Next the emulsion is pipetted off from the sediment into a second tube or flask, and is ready for standardisation which is carried out as follows:-

An opsonising pipette to which a rubber teat is fitted is marked about half an inch from the end by a grease pencil - this mark is the unit volume. By gently compressing the teat and then slightly relaxing it, two volumes of a sterile 2% sodium citrate solution, one volume of normal blood, and one volume of the emulsion - each of the volumes being separated by a bubble of air, are drawn into the pipette. The whole is then expelled on to a clean glass slide, and thoroughly mixed by alternately sucking it up and expelling it.

Thorough mixing is essential in order to have the red cells and bacteria as equally distributed as possible. A drop of the mixture is then placed near one end of a clean glass slide, and a thin film made by drawing the edge of another slide across it. The film is allowed to dry in the air, and is then stained by Leishman's, Jenner's, or Giemsa's Stain. In the case of tubercle bacillus, the film is stained by Ziehl-Neelsen's Stain. In using Leishman's or Jenner's Stain it is important that the stain be not allowed to evaporate, as there will be a deposit which may spoil the film for counting. The film being stained is now ready for counting. A 1/12th inch oil-immersion lens is necessary, and a counter such as Ehrlich's, or division of

the field into squares by marking the eyepiece with a grease pencil greatly facilitates the count. A mechanical stage is also an advantage although not absolutely necessary. In order to get an accurate estimate twenty successive fields should be counted. The number of red blood corpuscles counted in twenty fields are added up in one column, and the number of bacteria in the same fields are added up in another column. Suppose in the twenty fields 300 red cells and 250 bacteria have been counted, and as there are 5 millions of red cells in a cubic millimetre or 5000 millions in a cubic centimetre, then the number of organisms in a cubic centimetre will be obtained as follows:- The number of red corpuscles counted, is to the number of bacteria counted, as the number of red corpuscles per cubic centimetre in normal blood, is to the number of bacteria per cubic centimetre in the emulsion, thus:-

$$300 : 250 :: 5000,000,000 - \frac{250 \times 5000,000,000}{300} = 4166,666,666 \text{ organisms per C.C.}$$

This again can be diluted to any strength required by dividing the original emulsion by the strength required, e.g. a dosage of 300,000,000 per C.C. is required, then 4166,666,666 is divided by 300,000,000 = 13.8 or the number of times one C.C. of the orignal emulsion has to be diluted to give a strength of 300 millions per C.C.

Another method of standardising a vaccine is that

described by Dr. Leith Murray in the Lancet Vol. I, 1908, p.790. A Thoma-Zeiss haemocytometer is used and it is practically the method of enumerating the red blood corpuscles. Some of the bacterial emulsion is sucked into the pipette up to the mark .5, and then the diluting fluid is sucked into it till the mixture of emulsion and diluting fluid reaches the mark 101. The diluting fluid is composed as follows:-

Sodium chloride	0.1 part
Formalin	4 parts
Giemsa's Stain	5 "
Sterilised distilled water to	100 "

The pipette is then taken between the second finger and thumb and thoroughly shaken to mix the emulsion and diluting fluid. The diluting fluid in the capillary part of the pipette which is not mixed with the emulsion is blown out, and then a drop of the mixture is transferred to the centre of the platform of the slide, and a very thin cover glass put over it. The drop should be sufficiently large almost to cover the platform after the cover slip has been applied. After waiting for about half an hour to allow the organisms to fall to the bottom, counting is commenced with a 1/12th inch oil-immersion lens.

The number of organisms in two or three sets of sixteen squares is counted, and the average number in one square calculated. This number x 200 x 4000 x 1000 gives the number of organisms in one cubic centimetre, and can be diluted to any strength by dividing

by the strength required. Having now standardised the emulsion, the next step is its sterilisation, and this is usually done by placing the tube or flask containing the emulsion in a water bath at 56° or 60° C. for one hour, taking care that the immersion is complete. It is then removed from the bath and allowed to cool. The method of sterilising a vaccine is important, thus:-

Semple and Matson[9] have shown in the case of typhoid vaccine that if heat be employed the vaccine will not possess immunising properties of value after 6 months, whereas a similar vaccine sterilised by the addition of 0.5% of carbolic acid instead of heat remained efficient three years after preparation. In the typhoid and coli group autolysis occurs with the liberation of Endotoxin, and this is increased by heating the vaccine. Probably in most cases a vaccine can be rendered sterile in 2 or 3 days by the addition of 0.5% carbolic acid alone. This I have found to be the case with staphylococcus vaccines.

The vaccine having been sterilised can now be diluted to any required strength by the addition of sterile 0.75% sodium chloride solution, or sterilised distilled water may be used. To the diluted vaccine 0.5% pure carbolic acid or 0.3 tricresol is added with the view of inhibiting the growth of any air-borne organisms that may contaminate the vaccine during manipulation. The vaccine is now put into sterile ampoules in the doses required, using a sterilised glass syringe graduated

with ½ c.c. and 1 c.c. graduations or a graduated burette may be used. The ampoules are then sealed off in a Bunsen flame or blow pipe, and the doses marked on each. As the bulk of the dose is a factor in the amount of the local reaction produced by the injection of a vaccine, it is advisable to put the dose in as small a bulk as possible, from ½ c.c. to 1 c.c. The vaccine is then put aside for two days or so when it is tested for sterility, before use, by inoculating a tube of medium, and incubating at 37° C. for 48 hours aerobically or anaerobically according as the organism is an aerobe or anaerobe. If it should be found not to be sterile it is necessary to make a fresh test after a few days, or each dose may be sterilised for half an hour at 55° C. before use.

Having prepared the vaccine the next point to be considered is its method of administration. A vaccine is generally administered subcutaneously, although good results have been recorded by their oral administration. Latham (10) states that if given with 10 c.c. normal saline solution or horse serum they are absorbed and produced exactly similar results as when given subcutaneously. Their use has also been advocated per rectum by Calmette & Breton (11) In injecting a vaccine subcutaneously it is advisable to select a site free from pressure or friction, and the usual sites recommended are the flank, high up in the buttock, between the scapulae

or two or three inches below the centre of the clavicle, but when one is inoculating a good many patients daily the upper arm is convenient and saves time. The site of injection having been selected the skin should be thoroughly cleansed by washing with soap and warm water, then with an antiseptic as lysol or acid carbolic 2% and finally rubbed with ether. For the injection an all glass syringe is best as it can be disinfected by boiling in water. The needle can also be boiled or sterilised in absolute alcohol or 5% carbolic acid. Platinum iridium needles are best, and can be kept in absolute alcohol or in 5% carbolic acid. The effects produced by an injection may be constitutional and local. It may be stated as a rule that in chronic infections the constitutional effects are nil. In my experience it is the rare exception to have the patient complain of any constitutional symptoms whatever. The local effects vary not only afterthe first but after subsequent injections.

It is usual to have some redness and aching at the site of injection for one or two days, but rare to have anything else. In a few cases - probably in not more than one in two or three hundred, a nodular lump forms which may be painful for a week or ten days, and may take some weeks to disappear. Cases of severe cellulitis have been reported but in a series of several thousand injections given during the past 18 months I have not met a case. With regard to the local reaction, I

have found that it is kept at its minimum by giving the injection in as small a bulk as possible, i.e. the amount of local reaction depends not on the dosage of the vaccine but on its bulk. Further, the amount of local reaction is no guide to immunisation as one frequently sees in the same patient, with the same dose and the same vaccine, a difference in the local reaction, notwithstanding that the patient is responding well to the vaccine.

Having described the method of preparing a vaccine I shall next describe the technique of the opsonic index. The opsonic index may be defined as the ratio of the patient's serum to the normal person's serum which is taken as unity. For its demonstration the following are required:-

1. An emulsion of the infecting organism.
2. A drop or two of the blood serum of the patient.
3. A drop or two of the blood serum of the normal person.
4. A quantity of washed blood corpuscles.
5. Capillary pipettes about 6 or 7 inches long, and marked about half an inch from the end and fitted with a rubber teat.

The method is as follows:-

(a) With a pipette take up in the following order, one volume of washed blood corpuscles, one volume of emulsion, and one volume of the patient's serum each being separated by a bubble of air.

(b) Expel on to a glass slide and mix thoroughly by repeatedly sucking up and expelling, after

sucking up for the last time seal the end of the pipette in the flame, taking care to draw the mixture some distance away from the extremity.

(c) The pipette is marked for identification.

(d) With another pipette repeat the process, using normal serum instead of the patient's. Mark for identification.

(e) The pipettes are now incubated for 15 minutes at 37° C. (in the case of tubercle bacillus for 25 minutes).

(f) Break off the sealed ends and thoroughly mix on a slide as before.

(g) Place a small drop near one end of a clean glass slide, and make a film, allow to dry in the air.

(h) Fix and stain, in the case of tubercle bacillus with methylic alcohol for 15 minutes, or equal parts of alcohol and ether for half an hour to an hour and stain by Ziehl-Neelsen's method, counterstaining with Thionin blue or Loeffler's methylene blue, for other organisms stain by Leishman's, Jenner's or Giemsa's stain.

(i) With a 1/12th inch oil-immersion lens, and a mechanical stage (not absolutely necessary) count the number of organisms ingested by 50 or 100 polymorphonuclear leucocytes. The number of bacteria counted in the patient's slide divided by the number of bacteria counted in the normal slide, gives the patient's opsonic index - e.g. In the patient's slide 100 polymorphonuclear leucocytes contain 300 organisms, and in the normal slide 400 organisms $\therefore$ $300 \div 400 = 0.75$ - the patient's opsonic index.

The above is the actual technique and with practice proficiency may be obtained by the majority, but the pitfalls are numerous and if not carefully guarded against an accurate result is impossible, and at times, even with the greatest care an unreliable result will

be obtained.

Of the opsonic index it may be said that familiarity does not breed contempt. I shall now point out some of the pitfalls and how they may be avoided. Beginning with the emulsion which is of the first importance, it should be made from a culture of the patient's own organisms when possible, and should be a recent culture, from 12-18 hours growth, young organisms staining better than old ones, a matter of importance when it comes to the count.

Fleming[12] has pointed out that some organisms when freshly isolated are very little susceptible to phagocytosis, and in many cases the serum of the uninfected person exerts much less opsonic action than that of the infected person with the result that the index is very high. When, however, the organisms have been carried through many generations on artificial media it becomes more easily opsonised by the healthy serum with the result that the index steadily comes down, although the patient is doing well. This is especially seen in the bacillus coli group. In the case of the tubercle bacillus it would appear, according to Wright[13] that the mode of culture and preparation does not make any difference in the opsonic index. In the case of the tubercle bacillus, gonococcus, and some other organisms, spontaneous phagocytosis has been observed by Wright and Reid[14] and that it disappeared when 1.5% salt solution was used in making the

emulsion. Another source of fallacy is the agglutination of some organisms such as the tubercle bacillus, meningococcus, typhoid and coli groups, by the serum of infected persons, while others are agglutinised by all sera, such an one being the micrococcus neoformans. In the case of the tubercle bacillus this can be avoided by heating the bacillus to 100° C. in the typhoid group by using a strain which has lost its susceptibility to the agglutination of the serum, and in the case of the meningococcus this clumping can be greatly obviated, according to Allen(15) by employing a growth 6 to 10 hours old and incubating the opsonic mixture for only 10 minutes. These are all important when working with the organisms in question, but for the ordinary organisms the chief thing is to make a careful emulsion, and then to get rid of clumps by centrifugalising. As a rule two or three minutes will suffice. Finally the density of the emulsion is most important, if too strong the cells will be crammed with organisms, and an accurate count rendered impossible. Experience will help one to gauge the strength of an emulsion by its opacity, but it can only be accurately determined by opsonising with a normal serum. A strength which will give from 3 to 5 organisms per leucocyte is a good working strength. The chief pitfalls to be avoided with the ordinary organisms are clumps, which render an accurate count impossible, and too strong

an emulsion, both of which can generally be avoided by centrifugalising and standardising by opsonising with a normal serum.

2. The preparation of the washed blood corpuscles.

The source of the blood corpuscles is important. Wright and Douglas[16] and Bulloch and Atkin[17] have shown that the phagocytic power of corpuscles is the same, whether derived from healthy or infected persons, but it has been found that the red cells of some individuals are agglutinated by their own serum or the serum of others, and Fleming[18] has shown that auto-agglutination, although extremely uncommon in healthy people, occurs in about 90% of Hospital patients, all of whom are affected with some bacterial disease, and as agglutination of the red cells in an opsonic mixture gives an unduly high phagocytic count varying from 24% to 220%, it will be obvious that the blood from those patients should be avoided. Apart from agglutination the practical point is that the source of the blood is immaterial.

The blood is collected as follows:-

The finger or thumb having been selected, it is first rendered aseptic, next a piece of bandage is wound round it so as to cause congestion at the tip, and with an aseptic surgical needle it is pricked near the root of the nail, and the blood allowed to

run into a blood capsule or centrifugalising tube containing a 2% sodium citrate solution, blood may be added to the citrate solution in the proportion of 1 to 5. The blood and citrate solution are then mixed and washed by closing the mouth of the tube with the thumb and alternately inverting it, not by shaking. It is then centrifugalised until the blood cells are thrown down, when the clear supernatant sodium citrate solution is carefully pipetted off, and then some 0.75% sodium chloride solution, equal in quantity to the sodium citrate solution used, is added well mixed with the layer of blood. corpuscles and again centrifugalised. This process is repeated, the object being to thoroughly free the corpuscles from blood plasma. Finally as much as possible of the salt solution is pipetted off, and the red and white cells thoroughly mixed with what is left are now ready for use.

Some of the pitfalls to be avoided are as follows:

(1) The solutions of citrate of sodium and chloride sodium should be made with sterile distilled water, and should be centrifugalised before used to free from hairs and filaments for these entangle the leucocytes and cause clumps which spoil the films for counting.

(2) In centrifugalising the blood the time required will depend on the speed of the centrifuge and the quantity of the blood mixture. With a centrifuge of 10,000 revolutions per minute, and about 10 c.c. of

the blood and sodium citrate solution 5 or 6 minutes will usually be sufficient to clear the fluid and show a distinct zone of white cells lying over the reds. With the chloride of sodium solution a shorter time is required owing to the lower specific gravity of the sodium chloride solution. The time of centrifugalising is very important, if too prolonged the white cells are clumped, and are apt to be disintegrated, and if not long enough there is a dearth of leucocytes, which may render a satisfactory count difficult or impossible.

(3) Great care is required in pipetting off the supernatant fluid not to disturb the white layer of cells, otherwise when it comes to the count one may be surprised to find that there are few or none to count.

3. The next item is the Serum.

This is obtained by pricking the finger in the manner described for obtaining blood for the washed corpuscles. Break off the points of a Wright's blood pipette, and touch the blood with the curved end when it will flow into the tube spontaneously. Next seal the end of the pipette away from the blood, taking the precaution to hold the pipette at a point between the blood and the end to be sealed, so that the blood may not be overheated. When the sealed end is cooled shake down the blood and seal the other end. In collecting the blood it should flow spontaneously, the finger should not be forcibly squeezed. The blood is then put aside

to coagulate or it may be put in the incubator for 15 minutes. If the serum is not clearly separated it is centrifugalised, as a rule 4 or 5 minutes suffice. The principal precautions necessary for collecting blood for the serum are to see that it is not overheated, for opsonins are readily destroyed by heating at 60° C, and that it is perfectly clear, for if mixed with red corpuscles there is a diminution in the count, and this in proportion to the amount of red corpuscles it contains, as has been shown by Fleming[19]. From some experiments of Fleming[20] it would appear that normal serum retains its full opsonic power for about a week at room temperature, but in the case of pathological serum the time it retains its full opsonic power is more variable. In serum kept in an incubator at 37° C. for 36 hours there was a very appreciable drop in the opsonic index, and if serum is left open for six hours the same happened.

In the actual method itself there are several precautions which must be taken to obtain accurate results.

(1) The cells, emulsion and serum should be taken in the order mentioned, in this way contamination of the cells by the emulsion, and of the emulsion by the serum is avoided.

(2) In order to get a regular count the mixing must be thorough, and air bubbles carefully avoided when drawing up the mixture for the last time. Air bubbles are best avoided by mixing gently and using

thin pipettes with square cut ends.

(3) In making the film, which is usually done on a glass slide, it is well to use a special spreader, somewhat narrower than the slide. Such a spreader can be made by nicking the edges of another slide with a file, and breaking it across, when it will be found to have a sharp edge and a slightly concave end, the corners are next broken off to make it narrower than the slide. By using such a spreader most of the leucocytes will be found along the edges and at the end of the film. It is important that the drop should not be large enough to extend to the end of the slide, as the leucocytes will be too crowded to count properly, or they may be swept off the slide altogether. In making the film it need not be so thin as the film for a differential count.

(4) It is important that the film be properly stained, otherwise an accurate count is impossible. For all organisms except tubercle bacillus, Leishman's stain is probably the best, but Giemsa's stain is also very good, In using Leichman's stain it is important to prevent evaporation of the stain, as otherwise there is apt to be a deposit which may spoil the film for counting. It is used as follows:-

The unfixed film is covered with the pure stain for 2 minutes, taking care to prevent evaporation, then double the quantity of distilled water is dropped on, and the two mixed by carefully moving the slide from

side to side. It is then allowed to stand for 10 minutes, and thoroughly washed off with distilled water, and allowed to dry in the air.

(5) Finally, in the count itself the following precautions are necessary. Only typical polymorphonuclear cells should be counted. Cells showing clumps of bacteria should be avoided, as also cells which are clumped, and the same portion of the film should be used both for the patient's count and the control, as there is apt to be a difference in the count between cells found along the edges and those at the end. In order to obtain a reliable count, it is usual to count 50 or a 100 cells, but more depends upon the regularity of the count than the number of cells counted. In one case 50 cells may be sufficient to give a reliable count, in another, 100 cells may be too few.

The essentials of a good film are:-

(a) It should not be sufficiently large to cover the whole slide.

(b) The staining should be efficient to show up the bacteria and polymorphonuclear leucocytes.

(c) The cells should not be cramped or crowded together.

(d) It should not contain many clumps of bacteria or leucocytes.

(e) The number of bacteria in each cell should, approximate each other.

The accuracy of the method of estimating the opsonic index has been disputed by many. Among others

by Jeans, and Sellards & Moss[21], Walker[22] and Strangeways[23] but the results obtained by Wright, Douglas, Fleming, Bulloch, Frazer,[24] Urwick,[25] and others, must appeal to any unbiassed reader. Fleming in his article in the Practitioner, May 1908, p. 629, showed that out of 76 slides counted by two and three persons the difference seldom exceeded 10%, and in duplicate estimations of 26 sera from tuberculous patients, the great majority showed variations of less than 10%, Bulloch (26) in determining the indices towards the tubercle bacillus in forty four students and forty nurses, all healthy, against his own serum found an average index of 0.96. Seventy-five had indices between 0.90 and 1.10, and of the remaining nine, one had an index of 1.20, and five had an index as low as 0.80, but none were outside these limits. Wells & Freeman[27] estimating the indices in a large number of infants under one year old, to different organisms, came to the following conclusions:-

(1) A low opsonic index is not diagnostic in children under one year old.

(2) In infants a low opsonic index is not inconsistent with health, and the child may be thriving well with a declining index.

(3) When the opsonic index is low this will rise in response to the stimulus of an inoculation with a bacterial vaccine.

(4) That the healthy breast fed infant possesses

no advantages over the healthy artificially fed child.

(5) The anti-bacterial defence in children cannot depend upon opsonic content of the serum.

Again, Greenwood [28] has pointed out in connection with the indices of tuberculous subjects that there is an error probably inherent, and apart from the experimental error, and that it may exceed 10%. The practical suggestions that he makes are, that high indices should be more closely scrutinised than low values, and that it is better to work with tolerably thick emulsions, giving an average for the normal serum of not less than three bacilli per cell.

From the foregoing remarks it will be evident that the estimation of the opsonic index is sufficiently accurate, and that also an index at or near unity is the rule in health. The fact that a low index is not diagnostic in infants may be ignored, as infants do not frequently come under treatment by vaccines, and the inherent error pointed out by Greenwood is not sufficiently great to be of much practical importance. In infected persons on the other hand the opsonic index varies. In chronic and apyrexial conditions it is generally below unity.

Lawson and Stewart [29] in between 2000 and 3000 observations upon apyrexial Phthisis found the index to vary between 0.5 and 1.0. Bulloch [30] again in 150 cases of lupus in all stages, found in 75% of cases an index below 0.8, while the average for the 150 cases

was 0.75. The same holds good for chronic cases due to other organisms. In acute cases it may be above or below unity according as immunising responses are satisfactory or not, or fluctuating where auto-inoculations are occurring. The effect of graduated exercise in afebrile pulmonary tuberculosis is to produce auto-inoculations which act in the same manner as injections of tuberculin, (Meakin & Wheeler[31] and Paterson).[32] And in febrile cases rest steadies the index by diminishing auto-inoculations.

When a dose of bacterial vaccine is injected into an infected person it produces the following effects on the opsonic index:- There is first a fall in the index, named by Wright the negative phase, this is followed after a variable time by a rise which is called the positive phase, when the full rise has been attained, the index remains steady for a variable time, and this is called the stage of equilibrium, it then begins to fall again gradually or rapidly. By repeating the injections the same phenomena will be observed. The initial fall is explained as being due to the using up of some of the opsonin by the vaccine at the site of injection, and the subsequent rise to the formation of fresh opsonin. Within certain limits the negative and positive phases depend on the dose of vaccine injected, and thus by a study of the opsonic index we have a means of gauging the strength and frequency of our doses: e.g. in a case of staphylococcus infection, we have ascertained the opsonic index, and have then given an initial

dose, say of 50 millions of staphylococcus vaccine, if next day we find a slight rise in the index, and 5 or 6 days later it is just about the same, we may conclude our dose has beentoo small, or if we have given a dose of 1000 millions and find a fall next day, followed by a further fall in 6 or 7 days, we may conclude our dose has been too large, or again, if we have given a dose of 200 or 300 millions, and next day there is a slight rise or fall, followed by a more pronounced rise in 6 or 7 days, we conclude our dose has been correct. It has been found that a second injection given during a negative phase produces a further fall in the index, and in tuberculous infections it is said that cumulation of positive phases cannot be produced, and that therefore the full effect of one dose should be waited before another be given, in other infections the dose should be repeated while the index is still high, and thus one positive phase is superimposed upon another. Since the opsonic index has been found to be consistently at or near unity in health it follows that a marked deviation from the normal may be taken as an aid to diagnosis in many cases, e.g. the discrimination between a case of tuberculosis, syphilis and malignant disease, or between a rheumatic and a gonorrhoeic joint, etc., and again in forming a prognosis it may come to our aid, although here the difficulties are greater, for it has been shown by Bulloch [33] and Lawson and Stewart [34] in cases of phthisis in the early

stage, that many had indices ranging between 0.4 and 0.8. Nevertheless it would seem reasonable to suppose that cases responding to vaccine treatment, as evidenced by an index consistently above unity, are more likely to do well than those with an index persistently at a low ebb.

The value of the opsonic index as a guide in vaccine therapy has been assailed in many quarters, but it would appear to me that the crux of the whole matter lies, not in the method itself but in the manner of its performance. The technique itself is not difficult but the pitfalls are numerous and until these have been surmounted - and they can only be surmounted by attention to detail and practice - reliable results are impossible. As Wright aptly remarks[35] "Although the opsonic readings cannot be remotely compared with the methods by which the modern navigator can steer from point to point with accuracy, they are comparable with the soundings and compass bearings which enable the navigator to know whether he is shaping a proper course, and whether he is keeping on that course." Again Wright has pointed out that the increase of opsonins in the blood is not the only factor required in the production of artificial immunisation. It is necessary that the blood rich in bacteriotropic substances be conveyed to the seat of infection. It can be shown that the pus from an abscess cavity may contain no opsonin from the absence of phagocytosis, and in fluid exudations the

opsonin may be greatly diminished, although the blood may contain its full complement of opsonin. Here the ordinary rules of Surgery apply. Efficient circulation through the infected area and efficient drainage are necessary.

In dependent parts the circulation can be improved by position and bandaging as in the case of a varicose ulcer, in other cases hot fomentations will serve the same purpose, as will X rays and Bier's method of producing congestion. Abscesses will be opened and drained. Sinuses and tuberculous glands will be scraped, and in the case of brawny infiltrations, as in Carbuncles, Wright has shown that the hypercoagulability of the blood can be diminished by the administration of a decalcifying agent such as citric acid in dram doses t.d.s. and that a flow of lymph can be determined to the surface, after evacuation by excision, puncture or the removal of scabs, etc., by the local application of a citrated hypertonic salt solution - 2-4% sodium chloride and .5% sodium citrate.

Having given a short resumé of some of the principal views held with regard to immunity, and having described the preparation of a vaccine, and the technique of the opsonic index with comments thereon, I shall now proceed to the subject matter of my thesis, viz:- The treatment of some skin diseases by bacterial vaccines. The diseases treated include, Acne Vulgaris in its various stages, Seborrhoeic Alopecia, Alopecia Areata,

Rosacea (pustular stage), Coccogenic Sycosis, Boils, Carbuncles, Chronic Septic Eczema, and Chronic Ulcers of the lower extremities.

During the past 18 months I have treated over a hundred cases of Acne Vulgaris by vaccines, and as it constitutes the largest number of my cases, and is the most important clinically, with perhaps the exception of Coccogenic Sycosis, I shall begin with it.

The etiology of Acne Vulgaris is still a matter of dispute. It is generally conceded that the bacillus first described by Unna[36] and named by him the acne bacillus is the cause of the comedo, but the cause of the pustulation is still a matter of dispure. Sabouraud agrees with Unna that the acne bacillus is the cause of the comedo, but considers that the pustulation is due to a secondary staphylococcic infection[37], while Gilchrist[38] maintains that the acne bacillus, or as he calls it the bacillus acnes, is the cause of acne in all its stages, for he found it in all his smears, and grew it in 30% of his cases. Whitfield[39] again agrees with Sabouraud, viz: that the acne bacillus is the cause of the comedo, but that the pustulation is due to a secondary staphylococcic infection, and Fleming[40] holds that the acne bacillus is the cause of the pustulation in a large number of cases. In 44% of pus films he examined, he found the acne bacillus alone, and accompanied with staphylococci in 53% of

cases. My own observations accord with Fleming's. In the course of my vaccine work I have examined hundreds of smears from pustules and have almost invariably found the acne bacillus present, very frequently the only organism, in other cases in conjunction with the staphylococcus, and when both are present the acne bacillus generally predominates. In some cases the staphylococci exceed the acne bacilli. In these small pin point evanescent pustules the acne bacillus is invariably present and generally the only organism found, and in the deep indurated pustules the acne bacillus always predominates and is frequently the only organism present. It is in the ordinary superficial pustule that the staphylococci may equal or exceed the acne bacilli.

In smears from pustules it is not uncommon to find phagocytosis of the bacillus and in cases which are responding to treatment it is frequently very marked. A very good method of demonstrating phagocytosis is to put a drop of pus on a slide, and to apply another slide over it, and gently press the two together, and separate by sliding. The films are allowed to dry in the air and are then stained by Leishman's method, or they may be stained by Gram's method, using a weak solution of eosin or carbol fuchsin as a counterstain. Morphologically, Sabouraud[41] divides the acne bacillus into two forms, the young and the old. The young form is punctiform, oval or barrel-shaped and less than 1μ in breadth. Superficially they resemble cocci. The old

form is longer, sigmoid shaped, single or united in chains or arranged in bundles. As a rule the acne bacillus is from 1μ to 3μ in length and about ½μ in breadth. In the comedo they are in general short and stout, while in the pustule they are thinner and longer. In young cultures they are often short, but in older cultures they are longer and very much resemble those found in pus. Their grouping is irregular, in pus they may be single, in pairs, V shaped, crescent shaped, or S shaped, or they may be arranged in bundles or clusters. They stain readily with most of the basic anilin dyes, but Gram's is the best stain. They are more easily decolourised than cocci, and for this reason methylated spirit or 50% alcohol should be used. It is a non-mobile organism, hanging-drop preparations show only active Brownian movements. Culturally it is a difficult organism to grow, and for this reason many media have been tried. For my part I always use the medium recommended by Sabouraud. His medium has the following composition:-

Agar-agar	15 grams
Peptone, garnulated	20 "
Glycerine, neutralised	20 "
Glacial, acetic acid	5 drops
Distilled water	1 litre

It is put in sloped tubes and incubated at 37° C. As a rule I have not much difficulty in getting a growth, and I sometimes find it easier to get a growth from pus than from a comedo. Roughly speaking I am able to get a growth from pus in over 30% of cases,

and generally sufficient to make an emulsion of 4 or 5 c.c., with a strength of from 4000 to 6000 million organisms per c.c.. The appearance of a growth from a comedo is usually as described by Sabouraud, viz:- Colonies of microbacilli begin to develop on the 4th day and after 24 hours they take the form of a cone or button, 1 to 3 millimetres above the surface and generally extending a similar distance into the medium. They are brownish or brick-red in colour and continue to grow for 5 weeks. Frequently in association with these colonies of micro-bacilli, colonies of a coccus with a grey culture (Unna's micrococcus) develop. These appear about the second day. The brick-red culture of microbacilli may develop in the centre of the grey colony of cocci. In 28 days the grey culture dies and the culture of microbacilli remains pure. Although the above description is substantially correct, there are not a few exceptions. It is not very rare to find no sign of growth until 7 or 10 days after incubation. Almost invariably in cultures from comedones one observes in 24 or 48 hours a growth of a greyish colour, surrounding the comedo, and frequently with crinkled edges, which gradually enlarges for a few days. Microscopical examination at this time reveals no bacilli, and the culture may be looked upon as a failure, whereas if one examines the culture a few days later one or two small raised points may be observed which on examination microscopically prove to be the acne bacillus.

Sometimes the Colonies will continue to grow for 6 or 8 weeks or longer, and occasionally they begin to shrivel up in 2 or 3 weeks, and the cocci may survive for more than 4 weeks, which can be proved by subculture. A culture from pus has the same characteristics, but the surrounding growth of cocci is more frequently absent. In inoculating the medium with the comedo it is necessary to use Sabouraud's method, and it should be imbedded in the medium, In inoculating with pus it is necessary to plant a loopful on one spot, a thin smear will not suffice for a growth. The acne bacillus is said to grow in broth producing cloudiness in two days with a flocculent deposit on the sides and bottom of the tube, the broth is not rendered acid but I have failed to grow it aeobically.

It does not grow on ordinary Agar.

On Acid Agar it grows, the Colonies appearing about the 3rd day, and resembling those of staphylococcus albus.

Fleming has found oleic acid glycerine agar the most suitable medium for its growth, and that sub-cultures in this medium show an appreciable growth in 24 hours, and in 2 days a mass of white opaque colonies which resemble those of staphylococcus albus in size and appearance.

Molesworth, in an article in the British Medical Journal, May 21st 1910, disputes the identity of Fleming's bacillus with the microbacillus of Sabouraud,

for the reasons that Fleming's organism was found to be strictly aerobic growing only on the uppermost centimetre of a shake or Stab culture, and producing no growth on slopes incubated anaerobically, and that it grew quite readily on ordinay Agar, and in ordinary broth in 48 hours. He holds the view that the microbacillus is an anaerobe, and mentions that Hallé and Civatte[42] in making anaerobic cultures from comedones, found constantly colonies of an organism which corresponded very closely in microscopic characters with the organism seen in the comedo, and that by inoculating one of Sabouraud's cultures in a deep glucose agar tube by the stroke method the organism only grew anaerobically. He recommends a 2% glucose agar in deep shake tubes or stabs or slopes, incubated anaerobically. In about 4 days colonies appear in myriads confined to the aeaerobic areas, these slowly increase in size, and when discrete assume a lenticular form, greyish white in colour and morphologically identical with Sabouraud's microbacillus. The agglutination test is unreliable. I have found the patient's serum agglutinate up to 50 & in. one case it agglutinated better at 40 than at 20, and with regard to the <u>normal</u> serum the results were very variable, some agglutinating up to 30 and 40. With regard to its pathogenicity Sabouraud found inoculation experiments invariably negative, while Gilchrist found that it killed mice and guinea-pigs. Fleming by rubbing a broth culture into the

skin of the forearm of a person suffering from acne produced a pustular eruption. I have not had the opportunity to try these experiments.

With regard to the part played by the staphylococcus albus in acne - the staphylococcus aureus is so exceptionally found that it may be ignored - in only one of my 100 cases did I get a growth of staphylococcus aureus and that was from a superficial pustule on the shoulder of a woman aged 26, I may state that I gave a vaccine of staphylococcus albus a prolonged trial in some of my earlier cases, but failed to cure one of them, certainly there was some improvement in the pustulation, in some cases it disappeared for a few weeks only to return again. Whitfield (43) mentions two cases of severe indurated acne, in one, a young man, he had no difficulty in demonstrating staphylococci in the pus from his lesions, or in procuring a culture from which he made a vaccine, yet, although afterinoculation, his index was successfully raised, and maintained at a high level, very little if any benefit was observed. In the other, a lady a little over 30, who had been the subject of extreme nodose acne for more than ten years, the staphylococcus was demonstrated in small numbers in the pus, but he could never get a free growth. On two occasions he succeeded in getting a feeble growth of a gram, staining staphylococcus, which he could not distinguish from staphylococcus pyogenes albus but on both occasions it rapidly died out, so that he was unable to prepare a

vaccine of her own strain of organism. The use of a stock vaccine raised her index to the organism, from which the vaccine was prepared, but no corresponding clinical improvement was observed, and the treatment was therefore given up. This is just what one would expect, for if the microbacillus is the cause of the pustulation in acne, it is pre-eminently so in the deep nodose type. It is in this type that one almost invariably finds the acne bacillus in large numbers, frequently the only organism present, and it is in this type that one very frequently fails to get a sufficient growth to make a vaccine, no matter how thickly one makes the smear, and it is also in this type that one gets a free growth of the acne bacillus on Sabouraud's medium. The same applies even more forcibly to those small evanescent pin-head pustules which come dribbling out, so to speak, day by day. Their clinical significance is that they are suppurating comedones which are on the point of being extruded. It is only in the ordinary superficial pustule that one usually gets a good growth of the staphylococcus albus. As the result of examination, microscopical and cultural, and, above all, the result of treatment, I hold the view that the <u>acne bacillus is the causal organism of acne vulgaris, in all its stages</u>, and that the staphylococcus albus is simply a surface contamination. So much am I convinced of this that for the past nine months I have practically ceased to use a staphylococcus vaccine in acne. Another organism which is constantly found in the comedones and

pustules is the bottle bacillus, indeed it is found in all scurfy conditions of the skin. As the name suggests, it is frequently bottle or flask shaped, but it may be ovoid, barrel-shaped or round. It varies much in size but is always much larger than a coccus. It closely resembles the yeasts. It grows on Sabouraud's medium best at a temperature of 30° C, but will also grow at 37° C. Usually after 36 hours a growth of small whitish round colonies occur, which develop into a profuse raised growth, creamy in colour. It attains its maximum growth in 4 or 5 days. Frequently, especially on plates it takes the shape of a bottle. It is an aerobe and facultative anaerobe. It stains well with the ordinary basic stains, and also with Gram's stain. In acne it is probably only a Saprophyte. At the present time I am treating two cases of pityriasis of the scalp with a vaccine prepared from it, but it is too early to speak of the result.

TREATMENT:

In the treatment of localised diseases by vaccines it must be remembered that it is not sufficient to increase the opsonic power of the blood, but means must be taken also to enable it to permeate the site of the disease. In no infection does this apply more forcibly than in acne. It is a localised disease which encysts itself and is thus cut off more or less effectively from the general circulation, and, therefore from the oposinins and although the blood may be rendered rich

in opsonins by the introduction of vaccines, but little benefit may accrue from their use unless measures be taken to secure an adequate supply to the site of the disease. The means by which this may be accomplished have been outlined under the technique of the opsonic index.

Before commencing treatment with a vaccine it is essential to examine the various lesions microscopically and culturally, as emphasised under the preparation of a vaccine. The opsonic index is then taken and the patient receives his initial injection of the appropriate vaccine. The index is again taken one or two days after the injection, and again in 5 to 7 days, when the patient receives another injection according to the finding of the index, and so the treatment is continued. This has been my method of procedure in about one third of my cases, but owing to the increase in the number of patients and the difficulty of getting them to attend at the hospital for the index alone, I have had to be satisfied with the symptoms as the guide to dosage in about two thirds of my cases. From my observations of the opsonic index in acne cases, it reacts much the same as in other bacterial infections, but the range in infected persons (before treatment) is much greater than in staphylococcic infections, especially those above normal. It is not uncommon to find an index anywhere between 0.6 and 1.4 or even higher. I was rather surprised at this in my earlier cases, but as

duplicates gave much the same results, I was inclined to the view that the marked variations were due to auto-inoculation, especially as they occurred most frequently in the deep pustular or indurated type.

After a given dose of an acne vaccine the index will respond according to the dose. If the dose has been too small there will be no negative phase, but an immediate rise which falls again in two or three days. If the dose has been excessive there may be a fall of 4 or 5 points, and it may be some days before the index reaches the point at which it was before the injection, to be followed by a higher level which at the end of from 4 to 7 days begins to fall again, generally rapidly. If the dose is moderate it will be followed by a drop of a point or two, which will recover itself in one or two days, and will reach a higher level by several points, and with slight fluctuations will begin to fall again in four or five days. As treatment progresses it will be found that larger and larger doses have to be given to maintain the index above unity. To keep on with small doses is to keep on indefinitely. I usually begin with an initial dose of 5 millions of acbe vaccine, followed by weekly doses of 10, 15, 20, 25, and 30 millions unless a pronounced reaction follows a given dose, when I wait for a few days and repeat the same dose. It is so seldom that a pronounced reaction follows these weekly graduated doses that the above is my usual method. With the above doses the index fluctuates between a point or two below and three to five

points above unity, and in some of the milder cases other three or four injections of the same strength suffice for a cure. On the other hand after a time a large number of cases fail to respond to these doses- many of the comedo or punctata type, and nearly all the indurated type - In these cases it is necessary to go on pushing the dose by increments of from 5 to 10 millions at intervals of from 10 to 12 days. Even with largest dose - I frequently go up to 100 millions, and have given as much as 200 millions for a dose - the index begins to fall in 10 or 12 days. Apart from the opsonic index, it may be stated that in the pustular forms of acne, the pustulation diminishes when the index is high, and increases when the index is low, and thus a guide is given as to dosage and its frequency. Suppose with a given dose there is an improvement in the appearance of the lesions in 24 or 48 hours, followed in the course of a few days by fresh lesions, one may conclude that there has been no negative phase, and that the dose has been too small, or again, when, for the same period there is an aggravation of the symptoms, with perhaps fresh lesions followed by a period of improvement lasting for perhaps a week or so, one may conclude that there has been a negative phase, followed by a period of increased resistance, and that the dose has been correct. On the other hand when fresh spots continue to come out daily for perhaps a week or longer, the inference is that

the dose has been excessive. It may therefore be taken that when no symptoms follow a given dose the indication is to increase the dose. In those cases where the dose appears correct, the re-appearance of fresh lesions is the indication for repeating the dose - usually 7 to 12 days. As treatment progresses it will be found that gradually increasing doses have to be given to produce any effect on the lesions. Even with the above guidance it is at times most difficult to interpret the clinical signs, and to be sure that one is giving a correct dose.

The doses which I have found necessary to effect a cure have ranged from 30 to 100 millions as a rule. In the mildest forms it may not be necessary to exceed 30 millions, in cases of moderate severity with comedones and superficial pustules 30 to 40 millions may suffice. In the severe comedo types doses of from 50 to 60 millions or more will probably have to be reached and in the indurata type it may be necessary to exceed 100 millions.

PROGNOSIS:

Probably in no disease is it more difficult to give a definite prognosis than in acne. Taking into consideration its development at puberty and its natural tendency to die out beforethe age of 30, there is evidently something more than a bacillus at the back of it. Toxic intestinal absorption, evidently plays an important part, as frequently evidencedby a

recrudescence of the disease in gastric disturbances and intestinal stasis. Still it may be said that the great majority of cases can be greatly benefited, and a fair proportion cured, but to give anything like an accurate percentage is impossible for how often does it happen that a cured case returns for further treatment in a few weeks or months.

For the vaccine treatment it may be said, however, that it is a striking advance on the older methods of treatment, for one sees, almost daily, cases which have resisted every other form of treatment, begin to improve almost from the first. From my experience of the cases treated I would say that the mild comedo and superficial pustular types are most amenable to treatment, next the pure comedo type, and last the deep indurated type. I have seen a good many of the first and second types clear up in from 3 to 5 months, but the last type is more obdurate and may take from 9 to 12 months to cure. With regard to the vaccine, about 20 of my cases were treated with an autogenous vaccine, the remainder with a stock vaccine, sometimes from one source at other times polyvalent, and they were always made from cultures from comedones or pustules - about equal in number - and not more than five weeks old, and the same vaccine, whether autogenous, from one source or polyvalent, was used in the treatment of a case from beginning to end. In mature cultures the appearance of the bacillus is the same whether grown from a comedo

or pustule. In young cultures from the comedo the bacillus is shorter and thicker, thus it would appear that any morphological difference in the bacillus is due to the age of the culture.

While admitting that an autogenous vaccine is the ideal in vaccine therapy, I cannot say that I have seen better results from the use of an autogenous than from a stock vaccine. It would thus appear that the acne bacillus is an organism which varies very little in its virulence. There can be little doubt that the virulence of a vaccine depends within certain limits on the temperature at which it is sterilised. The thermal death point of the acne bacillus is given by Sabouraud as 75° C. I never allow the temperature to exceed 55° C. and the vaccine after the addition of 0.5% Acid Carbolic is kept for a week before use. From a practical point of view this is a decided gain, as the patient is not kept waiting several weeks for his vaccine, to say nothing of the greater cost of an autogenous vaccine.

From the foregoing remarks on acne vulgaris I would deduce the following conclusions, viz: that,

1. The acne bacillus is the cause of acne in all its stages.

2. The staphylococcus is a surface contamination, and at the most can only aggravate existing pustulation.

3. Doses of 5-10 millions do not suffice to cure unless one is prepared to go on indefinitely. The doses required will range from 30 to a 100 millions according to the type of the disease.

4. The duration of treatment will extend from three to five months in the milder cases, to six to twelve months in the severe forms.

5. As it is found that there is a close relation between the opsonic index and the amount of pustualtion, the latter may be taken as the guide to dosage and frequency of administration.

6. While it is impossible to give anything like a definite prognosis, it may be said that marked improvement can be brought about in the great majority of cases, and a fair proportion cured, and that it is a striking improvement on the older methods of treatment. The mild comedo and superficial pustular type being most amenable to treatment, next the pure comedo type, and last the deep indurated type.

7. I have not found an autogenous vaccine give better results than a stock vaccine.

Another group of cases which I have treated with an acne vaccine is seborrhoeic alopecia, alopecia areata and rosacea (pustular stage).

According to Sabouraud the acne bacillus is the cause of seborrhoea oleasa, alopecia areata and rosacea. If one examines a smear from a sebaceous filament one invariably finds the microbacillus in large numbers, just as one does in the comedo of acne. Examination of the hairs also shows the bacillus, frequently in large numbers. In cases of alopecia areata one almost invariably finds the bacillus in the stumps or point of exclamation hairs. Again in cases of rosacea one

invariably finds the acne bacillus in the pustules, just as one does in cases of acne. Since the bacillus is constantly found in these conditions I accept Sabouraud's views, and I have tried a vaccine in four cases of seborrhoeic alopecia, in four cases of alopecia areata, and in six cases of rosacea.

I shall take the cases of seborrhoeic alopecia first,

CASE I. A man aged 40, married, an insurance agent by occupation, came to the Hospital in March 1911 for treatment for a small pustular eruption of the scalp, brow, chin and sides of nose (rosacea). The vertex was completely bald. His history was that he had been completely bald from the age of 18, and that the eruption (follicular) first came out on the scalp 4 months ago, and rapidly extended to the brow, chin and sides of nose. He hasthree brothers who became bald at about the age of 20, and his father also lost his hair at an early age, but a sister about his own age has not lost her hair. On examination of the scalp the skin was freely movable and not atrophied, and the follicles were prominent and plugged with sebum. The scalp and face were greasy. Microscopical examination of the pustules showed the acne bacillus in profusion, also a few staphylococci, and bottle bacilli. From the pustules I was able to cultivate the bacillus on Sabouraud's medium. As he was sent to me for treatment for the pustular eruption in accordance with

Sabouraud's dictum, notwithstanding the microscopical examination revealed the acne bacillus out of all proportion to the staphylococcus, I put him on a staphylococcus vaccine. From the middle of March to the end of April he had weekly injections of the staphylococcus albus in doses from 100 to 750 millions without any improvement. I then added 5-10 millions acne bacillus to the staphylococcus and this was given during May with very little result. In June I left off the staphylococcus vaccine, and gave him an initial dose of 25 millions acne bacillus, which was increased weekly until he was having 200 millions for a dose in September, when treatment terminated. By the end of June the pustulation had greatly diminished, and had entirely disappeared by the end of July. About this time a perceptible growth could be seen of hair/over the occipital and parietal regions, and he noticed that the scalp did not "perspire" so much. In September when the treatment was discontinued the whole of the occipital and parietal regions were covered with hair sufficiently thick and long to cover the baldness. Over the temples and forehead there was a certain amount of growth, but it was feeble. Throughout he had practically no local treatment. I asked him to come and see me in a month or two, and he came in January of this year. The hair was still growing and with no tendency to fall out, and there had been no return of the pustules.

CASE II. A man, aged 42, married, a clerk by occupation, came for treatment in August 1911. He had total alopecia of the scalp and eyebrows, and thinning of the moustache and beard, but the rest of the body was not affected. Two years ago his hair began to fall out and in a few weeks he was completely bald except for a few hairs here and there round the circumference of the scalp. The eyebrows fell out about the same time. The scalp and face were oily and the follicles were prominent and plugged with sebum. His general health was good, and he had not had any illness or mental strain before the hair fell out. Examination of smears from the follicles showed the microbacillus in large numbers, there were also some cocci and bottle bacilli. He had injections of a vaccine of acne bacilli from the middle of August to the end of December 1911 for the first two months weekly, then at intervals of from 10 to 12 days. After some weeks treatment it was noticed that there was less oiliness of the scalp and face, and about two months from the beginning of treatment, a visible growth of hair could be seen over the occipital and parietal regions, and extending forward, the eyebrows and moustache were also growing. Towards the end of December the occipital and parietal regions and part of the frontal regions were covered by a fine downy growth of hair, and the eyebrows and moustache were practically normal again. Previous to the injections he had been under treatment for 18 months. The

doses given were from 25 to 300 millions. During the course of injections the only local treatment he had was a 5% Lotion of Lysol.

CASE III. A man aged 23, married, a Hebrew minister, came to the Hospital in July 1911. For three or four months his hair had been falling out rapidly, and when I saw him there was a general thinning of the hair with diffuse patches of baldness all over the scalp. The eyebrows and hair of the face were not affected. The complexion was greasy. His general health was good and he had had no illness or mental strain. No history of baldness in his family. Smears from the follicles showed the microbacillus in large numbers, also some cocci and bottle bacilli. The hairs also showed the microbacillus. He had injections of an acne vaccine from July 19th to the second week in October at weekly intervals. The doses ranged from 25 to 175 millions. After four injections the hair had practically ceased to fall out, the skin was less greasy, and his complexion clearer, and by the second week in October his hair had completely grown and looked quite normal. He came to see me about the end of January of this year, and he had had no further trouble with his hair. He used no local application while under vaccine treatment.

CASE IV. A woman aged 51, who had been under treatment for 15 months without any improvement, came

under my care for vaccine treatment in August 1911. She is married and has had 6 children, no miscarriages. One sister aged 60 has been completely bald for 5 years. Her father had some loss of hair, but never became completely bald. As to her mother, she cannot speak. At the age of 17 she had a bald patch on the head but the hair soon grew again. At the age of 40 the hair began to fall in patches but it grew again completely. Two years ago she suffered from overstrain and worry and the hair began to fall out, and in a month or two she was completely bald. A few months later the eyebrows and eyelashes fell out, and next the hair of the pubic region and rest of the body. At the present time she is completely bald. The scalp is free and movable and the follicles are not atrophied. Tiny droplets can be seen oozing from the follicles. She says her scalp is always wet. Smears from the follicles show the microbacilli in large numbers with some cocci and bottle bacilli. On August 16th she had an initial injection of 50 millions of an acne vaccine, and the injections were repeated at intervals of -7 - 10 - 12 days and the doses gradually increased until by the end of December she was having 400 millions for a dose. After 3 or 4 injections there was a diminution in the oiliness of the scalp, and by the end of September hair could be seen with the aid of a lens springing from the follicles round the circumference of the scalp posteriorly. The growth was slow but by the end of December the hair

could be felt and also seen by the naked eye. Over the occipital and parietal regions, and the eyebrows were quite visible. The patient is still under treatment and I cannot therefore speak as to the ultimate result.

From these few cases it would be unwise to draw a general conclusion, but from the result of treatment the presumption is that the acne bacillus is at least an important factor in the causation of seborrhoeic alopecia. Cases one, two and three clearly point to this, and although a neurotic origin cannot be excluded in Case IV, vaccines should have a recognised place in its treatment. It will be observed that the doses given were very much larger than those usually given in cases of acne. With the largest dose a marked negative phase was not produced, and the index fluctuated between a point or two below normal and three or four points above, occasionally as much as six or seven points above. The explanation may be that the bacillus is more securely cut off from the general circulation and that the inflammatory process is of a lower and more chronic type. The largest doses produced no constitutional symptoms, and there was no connection between the local reaction and the dose given.

The next group of cases is alopecia areata, and I shall give details of four cases treated.

CASE I. A girl, aged 14, came under treatment in July 1911 suffering from alopecia areata and severe acne of the face (comedones and deep indurated pustules). Her hair began to fall out two years ago in the form of patches, on some of the patches a fine downy growth of hair had grown only to fall out again in a few weeks. When I saw her she had a large patch about 2 inches in diameter to the right of the occipital region, another patch about the same size just above the left ear, a third patch about 1½ inches in diameter over the vertex, and a patch about 2 inches in diameter over the centre of the forehead, and several smaller patches scattered here and there. She is a strong healthy looking girl, her sight is normal and her teeth are good. Examination of the stumps showed the acne bacillus in large numbers, also some cocci and bottle bacillus. A smear from a pustule on the face showed myriads of acne bacilli with some cocci. The bacillus was grown on Sabouraud's medium. She had previously had 12 injections of a staphylococcus vaccine for her acne without any benefit. From the middle of July till the middle of October she had weekly injections of an acne vaccine beginning with 25 millions until by the middle of Octobershe was having 200 millions for a dose. From the end of October until the end of December the injections were given fortnightly and the dose increased to 400 millions. No sign of improvement was observed

until she had been a month under treatment, when the pustulation of the face began to improve and by the end of October her face was practically well. About two months after beginning treatment the hair was beginning to grow freely over the bald patches, and with very little tendency to fall out. By the end of December all the patches were covered with a good growth of hair and it was assuming the normal colour. She had her last injection about the end of January 1912, and was discharged as cured.

CASE II. A man, aged 38, married, a furniture porter by occupation, came under treatment in July 1911. Two years ago he noticed a bald patch over the chin, this was quickly followed by patches of baldness over the temporal and occipital regions, vertex and nape of neck. The moustache and eyebrows were also affected. The scalp and face were very greasy. Examination of the hairs showed the acne bacillus in abundance with some cocci. From July 12th to September 27th he received 12 weekly injections of acne bacillus, beginning with 25 millions and increasing to 200 millions for a dose. After he had been under treatment for a month, the hair began to grow over the patches of the scalp, and in two more weeks over the chin, eyebrows and moustache. By the end of September all the patches were completely covered, as well as the eyebrows. He came to see me two months later and the growth had been maintained, and the hair was regaining

its natural colour. I saw him again in February of this year when the scalp and other regions were practically normal.

CASE III. A man, aged 30, a grocer by occupation, came under treatment in August 1911. He had a bald patch extending across the nape of the neck from ear to ear. It commenced a year ago as a small patch in the centre of the nape of the neck, and gradually extended upward for 1½ inches, and outwards until it reached from ear to ear. He has also two bald patches on the chin the size of a shilling which he first noticed six months ago. He has had no illness or worry and his present health is good. His sight is normal and his teeth are good. Examination of the hairs showed numerous bacilli in the shaft and extending round the sheath of the hair, also a few cocci and bottle bacilli. He had weekly injections of the acne vaccine from August to the end of October, in doses from 25 to 200 millions, and from October to the middle of January 1912 the injections were given fortnightly, and the dose gradually increased to 350 millions. By this time he had a good growth of hair over the bald patch with the exception of a spot the size of a halfpenny, and hair can be seen coming on this spot also. The hair had also re-grown on the chin. Improvement commenced about 5 weeks after beginning the treatment, and steadily progressed.

CASE IV. A man, aged 27, single, a commercial traveller, came under treatment in September 1911. About 4 months ago he noticed a small patch of baldness over the nape of the neck, this had gradually extended and when I saw him he had two patches about 1½ inches by 1 inch, separated by a narrow band of hair. His general health is good, has had no worry, his sight is normal, and he has a good set of teeth. Examination of the hair showed the acne bacillus in profusion. He had injections of an acne vaccine from September 15th to the end of January 1912 in doses ranging from 50 to 300 millions. About one month after the commencement of treatment, improvement was visible, and by the end of January 1912 the patches were almost completely covered with hair from ½ to 1 inch in length. Here, again, as in the case of Seborrhoeic Alopecia the result of treatment would point to the conclusion that the acne bacillus is an important factor in the causation of the disease, and that a vaccine should be used in stubborn cases, by no means an inconsiderable number. In cases I and II the disease had lasted for two years, in Case III for one year, and they had all been under treatment with little apparent effect. In Case IV the disease had only lasted four months, and had not been previously treated. During the course of vaccines there was practically no other treatment.

The last group of cases which I have treated with

CASE IV. A man, aged 27, single, a commercial traveller, came under treatment in September 1911. About 4 months ago he noticed a small patch of baldness over the nape of the neck, this had gradually extended and when I saw him he had two patches about 1½ inches by 1 inch, separated by a narrow band of hair. His general health is good, has had no worry, his sight is normal, and he has a good set of teeth. Examination of the hair showed the acne bacillus in profusion. He had injections of an acne vaccine from September 15th to the end of January 1912 in doses ranging from 50 to 300 millions. About one month after the commencement of treatment, improvement was visible, and by the end of January 1912 the patches were almost completely covered with hair from ½ to 1 inch in length. Here, again, as in the case of Seborrhoeic Alopecia the result of treatment would point to the conclusion that the acne bacillus is an important factor in the causation of the disease, and that a vaccine should be used in stubborn cases, by no means an inconsiderable number. In cases I and II the disease had lasted for two years, in Case III for one year, and they had all been under treatment with little apparent effect. In Case IV the disease had only lasted four months, and had not been previously treated. During the course of vaccines there was practically no other treatment.

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a vaccine of acne is Rosacea in the pustular stage, and as these cases reacted to a vaccine much the same as acne does and with similar doses, I shall only give details of one case, although I have treated six.

A woman aged 47, came under treatment in September 1911. She is married and has had nine children, all of whom are alive with the exception of one which died at the age of 21 months from diphtheria. For the past 9 years she has suffered almost continuously from deep pustular nodules which are almost exclusively confined to the chin and nose. There is thickening of the skin of the face and marked dilatation of the capillaries of the cheeks and nose. The scalp is scurfy and there is thinning of the hair. Her general health is good upon the whole but she suffers at times from constipation. Her habits are regular and she does not drink. Smears from the pustules showed the acne bacillus in large numbers with a few cocci, and I was able to obtain a growth of the bacillus from a pustule. She had injections of a stock vaccine of acne from the 21st September to the end of January 1912. The initial dose was 10 millions and was gradually increased to 70 millions. The doses were given weekly to begin with, and with the higher doses every 10 to 14 days. After the first 2 or 3 injections there was an improvement, and the pustules which had been constantly coming out, and lasting for 2 or 3 weeks showed a diminution in number and size until by the end of December her face was

practically clear, and for the last month she has only had one small pustule. She had been under treatment off and on for years. The only local treatment used during the vaccine course was a calamine lotion for the face and Hebra's soap spirit for washing the head. In Rosacea, as in Acne, I believe the pustulation to be due to the acne bacillus, and look upon the staphylococcus as simply a surface contamination.

The next group of cases which I treated with a vaccine are those due to staphylococcic infection, and includes Coccogenic Sycosis, Boils, Carbuncles, Chronic Septic Eczemas and Chronic Ulcers of the lower extremities.

I shall begin with Sycosis having treated over 50 cases. With regard to the etiology of sycosis, I shall only mention that microscopical examination of hairs, and smears from pustules invariably shows the staphylococcus, sometimes in enormous numbers especially in the hairs. Bottle bacilli are also generally to be seen. In over 40 of my cases culture showed the organism to be the staphylococcus pyogenes aureus. The remainder were due to the staphylococcus albus. In three cases cultures from the hairs showed the staphylococcus aureus, and from the pustules the staphylococcus albus. Thus it will be seen that by far the greater number of cases are due to the staphylococcus aureus. The citreus is so rare that it may be excluded. I have seldom seen it in a culture a few days old, young growths

looking like the citreus becoming golden in a few days. Probably in no disease of the skin is it more important to make a careful bacteriological examination than in sycosis. In this connection I may perhaps allude to 3 cases.

CASE I. A man, aged 51, a coachman who has been under treatment at the Hospital for three months, was referred to me for vaccine treatment in January 1911. Four months ago one or two small pustules appeared on the cheeks, next he had a pustule on the back of the left hand, followed by one or two on the right hand, these were followed by more pustules over the cheeks, chin and upper lip. A month ago a painful lump formed on the upper lip and another at the angle of the mouth, and another on the chin. The lump over the chin was as large as a small walnut, hard to the touch, and showed numerous suppurating points over the surface. The lumps over the upper lip and angle of the mouth were about the size of a hazel nut and also showed numerous suppurating points. The lump over the chin looked remarkably like a kerion, and he gave a history of having handled calves suffering from ringworm. Careful examination of the hairs failed to detect the fungus, and cultures on French proof Agar also gave a negative result, but they were teeming with cocci, and culture on Agar Agar gave a growth of staphylococcus pyogenes aureus. He had 15 injections of an autogenous vaccine from 11th January to 3rd May 1911. The doses ranged from 150 to 750 millions. He was discharged cured in May and he has remained well.

CASE II. A man, aged 40, a barber. He had suffered from a pustular eruption of the face for many years, and had been treated for sycosis. On examination he had numerous comedones and superficial pustules over the brow, cheeks and chin, also over the shoulders and upper arms. No cocci were found in the hairs which were normal. Smears from the pustules showed the acne bacillus in large numbers with some cocci. He had injections of an acne vaccine for 8 months and left the Hospital practically cured.

CASE III. A man aged 43, a commercial traveller, had suffered from sycosis for nearly 20 years. At one time he says it almost got well, but it has been much worse again for the past two years. This case was interesting in that I isolated a bacillus from the hairs and pustules which morphologically and culturally was undistinguishable from the bacillus diphtheria, and answered most of the tests for that organism. I suggested the use of antitoxin, but before trying it, another examination of the hairs and pustules was made, a fortnight after the first examination and to my surprise no bacilli were found, only the staphylococcus aureus. I may mention that during the interval between the two examinations he had been rubbing in a strong ammoniated mercury ointment. One year ago he had 12 injections of a stock staphylococcus vaccine without any benefit. I also gave him 6 injections of a stock vaccine with very little benefit. In January of this

year I started with an autogenous vaccine. He has had 5 injections in doses of from 200 to 750 millions, and he says his face has not been so well for years. He is still under treatment, but I am dubious as to the result, for in addition to the pustulation, the skin is much altered, being thickened, red and scaly.

In sycosis, the great depth to which the organisms descend, and the fact that they are securely cut off from the general circulation by their position in the follicles, render them difficult of access. Nevertheless in the acute and sub-acute stages, I have had excellent results, so much so that I incline to the view that nearly all the acute cases and many of the sub-acute, can be cured by a vaccine. With regard to chronic cases, those which have lasted perhaps for years, it is different. The tissues have become altered, and their resistance so diminished, that in many cases all that one can hope for is some improvement in the condition. There is a certain type of case which is not benefited at all by a vaccine. I refer to those chronic cases in which there is practically never any pustulation, but a chronic dermatitis. The skin is markedly thickened, frequently dry scaly and reddened, at other times it is cracked and there is exudation of serum. In these cases, to my mind, local treatment by hyperaemia, such as cupping by Bier's method, X Rays, counter-irritation by a strong solution of iodine, or even a blister, are the indications for treatment. Those cases

also where there is a nasal discharge, or where the eyelids are affected, resist treatment so long as these complications continue. In some cases the patient's occupation and surroundings have to be taken into consideration before a cure can be brought about. With regard to the vaccine, I am convinced that an autogenous is superior to a stock vaccine. I have frequently seen cases improve up to a certain point with a stock vaccine, and then refuse to make further progress until an autogenous was used. If a stock vaccine be used it should be polyvalent, for there can be but little doubt that the staphylococcus group varies in virulence in different persons, and in different diseases. In this connection it has appeared to me that cases due to the staphylococcus albus are more amenable to treatment than those due to the staphylococcus aureus. The doses which I have found it necessary to give have ranged from 100 to 200 millions for an initial dose, to 750-1000 millions for a maximum dose. Very rarely have I been obliged to exceed 1500 millions for a dose. The intervals between the injections were seven days for the smaller doses, and from ten to twelve for the larger doses. The average number of injections required were 15. In a few cases as many as 25 to 30 have been given.

From the foregoing remarks I would draw the following conclusions.

1. That the staphylococcus pyogenes aureus is the casual organism in by far the greater proportion of cases.

2. That the great majority of acute cases and many of the sub-acute can be cured by a vaccine.

3. That in the chronic cases - those which have lasted perhaps for years - complete cure is rare.

4. That in a certain type of case, already mentioned, a vaccine is of no use.

5. That an autogenous vaccine is superior to ,a stock vaccine, and that a case should never be looked upon as a failure until an autogenous vaccine has been tried.

The next group of cases which I have treated with a vaccine are Boils and Carbuncles. I have treated over 30 cases of the former and 6 of the latter, and in the case of boils, I refer not to isolated boils due to a local source, but to those which continue to come out in crops here and there all over the body for months or longer.

One of my cases had suffered from a continuous succession of boils for about 12 months. The staphylococcus pyogenes aureus was the organism found in every case, I exclude the furuncular lesions which one frequently sees in severe cases of acne which are really due to the acne bacillus. In practically every case a cure resulted, and the average number of injections required was six. In a few cases as many as 12 or 15 injections had to be given. The injections were usually given at intervals of seven days, and the doses usually ranged from 100 to 750 millions. Some of the most troublesome cases were those complicating Seborrhoeic Dermatitis, and in stout people at the sites of contact,

or where there was friction or discharge, as in the neighbourhood of the external genitals. Several cases relapsed but again got well after two or three injections. It was found in these cases as in coccogenic sycosis, that an autogenous vaccine was superior to a stock vaccine. Improvement was quicker, and the doses required were smaller. About half the number of cases were treated with an autogenous vaccine. For the vaccine treatment of boils it may be said that it excels all other forms of treatment, cases which have resisted every other form of treatment, frequently clearing up in three or four weeks.

In the case of carbuncles the results were much the same. Sometimes after the first injection there was marked alleviation of the symptoms, and in those cases which were already discharging, relief from pain, and healing was more rapid. The number of injections given were from 4 to 6, and the doses from 150 to 750 millions staphylococcus aureus vaccine.

I have also treated a fair number of cases of eczema with secondary pyogenic infection with good results. I have seen cases which had resisted treatment for months, begin to clear up after one or two injections, and get well after a few more injections of a staphylococcus aureus vaccine.

The last group of cases which I shall mention are those of chronic ulcers of the lower extremities. I

have tried a vaccine in six outpatients but without any improvement, and in my opinion a vaccine is worthless in these cases, unless the patient can rest and have other appropriate treatment.

This brings me to the end of my cases, and it only remains to give a brief summary of my paper. As I have already given the conclusions arrived at under each group of cases, little more than a recapitulation will be necessary.

I have given a short resumé of the various theories held with regard to immunity, and how it can be produced artificially by a bacterial vaccine, and I have described the preparation of a vaccine, and the technique of the opsonic index with comments thereon. With regard to acne I have arrived at the following conclusions;-

1. The acne bacillus is the cause of acne in all its stages.

2. The staphylococcus is a surface contamination, and at the most can only aggravate existing pustulation.

3. Doses of 5-10 millions do not suffice to cure unless one is prepared to go on indefinitely. The doses required will range from 30 to 100 millions according to the types of the disease.

4. The duration of treatment will extend from 3 to 5 months in the milder cases, to six to twelve months in the severe forms.

5. As it is found that there is a close relation between the opsonic index and the amount of pustulation, the latter may be taken as the guide to dosage and frequency of administration.

6. While it is impossible to give anything like a definite prognosis, it may be said that marked improvement may be brought about in the great majority of cases, and a fair proportion cured, and that it is a striking improvement on the older methods of treatment.

7. I have not found that an autogenous vaccine gives better results than a stock vaccine.

In the case of seborrhoeic alopecia, and alopecia areata, although the number of cases treated are not sufficient to enable one to draw a general conclusion, I think the result of treatment points to the acne bacillus as being the casual organism, as advocated by Sabouraud, and that a vaccine is indicated in cases which resist ordinary treatment.

With regard to rosacea, I have no hesitation in saying that the pustulation is due to the acne bacillus, and that it can only be cured by an acne vaccine.

In the case of coccogenic sycosis the conclusions I arrived at are as follows:-

1. That the staphylococcus pyogenes aureus is the casual organism in by far the greater proportion of cases.

2. That the great majority of acute cases and many of the sub-acute cases can be cured by a vaccine.

3. That in chronic cases - those which have lasted perhaps for many years - complete cure is rare.

4. That in a certain type of case, already mentioned, a vaccine is of no use.

5. That an autogenic vaccine is superior to a stock vaccine, and that a case should never be looked upon as a failure until an autogenous vaccine has been tried.

With regard to boils it may be said that the vaccine treatment excels all other forms of treatment, cases which had resisted every other form of treatment frequently clearing up in three or four weeks. In the case of carbuncles the results were also satisfactory. Sometimes after a single injection there was marked alleviation of the symptoms, and the healing process was quicker.

Cases of eczema with secondary pyogenic infection, gave very good results. I have seen cases which had resisted ordinary treatment for months clear up after a few injections of a staphylococcus aureus vaccine.

With regard to chronic ulcers of the legs, in my cases there was no improvement. A vaccine is worthless unless the patient can rest and have other appropriate treatment.

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