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## ORIGINAL COMMUNICATIONS.

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### VACCINE THERAPY IN SOME SUPPURATIONS OF THE NOSE AND EAR; ALSO TECHNIC FOR DETERMINING OPSONIC INDICES.\*

BY JOSEPH C. BECK, M.D., CHICAGO.

#### THE TECHNIC OF DETERMINING OPSONIC INDICES.

The facts and theories upon which opsonic treatment is based are as follows:

1. Bacteria infecting the body are attacked by leucocytes which ingest them.
2. The number of bacteria which can be ingested is of varying quantity.
3. The number of bacteria which can be ingested depends upon their preparation by substances present in the plasma of the blood known as opsonins.

The exact nature of opsonins is not known, but it is known that they are not identical with the agglutinins, antitoxins, etc., which are also found in the plasma. Their action is not on the leucocytes, but on the bacteria which, as stated, they prepare for ingestion. Opsonins are present in normal blood as well as in the blood of infected individuals. The opsonic strength of normal blood is practically constant, but varies slightly with the individual, and general health, nutrition, etc.

The opsonic strength of an infected individual is lower than that of a normal individual, consequently his blood can prepare fewer bacteria and fewer will be ingested by the leucocytes. The relation between the number of bacteria ingested by the leucocytes of the infected person to the number of bacteria ingested by the leucocytes of a healthy individual, gives us a value which we call "the opsonic index." For instance, if ten bacteria are ingested on the average

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by the leucocytes of a healthy person, and five of the same bacteria by the leucocytes from an infected person, the opsonic index of the latter would be .5.

The opsonic index of an infected person may be increased by injecting into him killed cultures of his infecting organisms. For instance, if an infection is due to the *staphylococcus albus*, some of these particular germs are taken, grown on suitable culture media, and when sufficient quantity has been obtained, the culture is washed off with .85 per cent sodium chloride solution, to which a little carbolic acid has been added. The mixture, well shaken, is standardized to contain 300,000,000 cocci to the cubic cm., which represents one hypodermic dose. It is important to inject cultures made from the particular infecting organisms, because it is well known that even such well known organisms as the *staphylococcus albus* are subject to greater variation in virulence. Furthermore, this prevents errors in the diagnosis of the infecting germs.

#### I. METHOD OF DETERMINING OPSONIC INDICES.

In a small test-tube, place about two cubic cm. of a solution containing 1½ per cent of sodium citrate, and .85 per cent sodium chloride. Clean the finger with soap and water, followed by alcohol; prick the finger, and allow fifteen drops of blood to flow into the solution in the test-tube, mixing well. The sodium citrate prevents coagulation of the blood. The test-tube is now taken to the centrifuge and centrifuged until all the solid particles of the blood are thrown down. The red blood corpuscles being the heavier, will form the lower layer, the leucocytes appearing as delicate, grayish film (known as "cream") on the surface of the reds. The supernatant liquid has dissolved in it the plasma of the blood and is withdrawn by means of a delicate pipette, care being taken not to disturb the cream. The test-tube is then filled with .85 per cent sodium chloride solution, the corpuscles well shaken up, and the mixture again centrifuged. The corpuscles will then arrange themselves as before, and after withdrawing the supernatant liquid, we will have freed them from the original plasma and the sodium citrate that was previously added. By means of special tubes, known as capsules, a quantity of blood is collected from the patient (capsule half-filled). Capsules of blood are similarly collected from several healthy individuals (two or three). The capsules are placed in the incubator at 37° C. for about five minutes, when the blood will be found to have coagulated. The capsules are then centrifuged, the

clot being thrown down, leaving the clear serum above. Meanwhile a suspension of the particular bacteria has been made by taking a loopful from the culture and mixing thoroughly with a quantity of .85 per cent salt solution, the amount of the solution being such that the mixture will be turbid, the turbidity to be judged only by experience.

We now have prepared: a. The cream consisting of washed leucocytes; b. a suspension of the infecting bacteria; c. serum from patients; d. serum from several normal individuals.

We now take a pipette with a long capillary stem. At a distance of about 5 millimeters from its tip, we make a mark with a blue pencil. We now draw from the cream sufficient to fill the stem up to the blue pencil mark. Then we remove the pipette from the cream, drawing the volume a little ways up to the stem so as to leave an air space. Then dip the same pipette into the suspension of bacteria of which we draw up an equal quantity. We leave another air space and draw up an equal quantity of serum from the patients' capsule. We now blow the contents of the pipette upon a glass plate and mix thoroughly by drawing up and blowing out several times. After mixing, the mixture is drawn well up into the stem of the pipette, the tip of the pipette sealed in a flame, the pipette marked on its bulb for identification and laid aside. We now take equal quantities of the sera from the capsules of blood collected from the normal individuals and mix them together in a little test-tube. This mixed serum is called "the pool." The object of mixing several sera rather than relying on one serum is to overcome fluctuations which are present in normal sera (fluctuating opsonic strength.) Another long-stemmed pipette is now taken, a distance marked off from its point as before, and equal quantities of cream, bacterial suspension, and pool serum taken and mixed, drawn up into the pipette, the end of the pipette sealed, its bulb marked for identification, and laid aside.

It will be noticed that these two pipettes vary only in that one contains patient's serum in the mixture, while the other is made up with pool serum. The two pipettes are now taken, placed in the incubator at 37° C. for fifteen minutes. They are then removed from the incubator, the contents of each is separately blown on a glass plate, drawn back and forth several times to insure mixture (this second mixing is required because the corpuscles tend to settle during incubation), and now a drop of the mixture is blown onto the

end of a perfectly clean glass microscopical slide, and the drop spreads evenly over the slide. At least three slides (two to be held in reserve for accidents) should be made from each pipette. The slides are now stained with Nocht's stain and examined with the microscope. The number of bacteria in each of fifty leucocytes is counted on each side. As before stated, the relation between the number of bacteria ingested by the leucocytes on the patient's slides compared with the number ingested by leucocytes obtained from the control slides, gives us the opsonic index. As will be noticed, the serum was the only variable factor in the preparation of these slides, therefore the variation in the number of bacteria ingested must be due to this difference of sera, and the substance in the sera which is responsible for this variation is "opsonin."

## II. PREPARATION OF VACCINE.

A culture of the specific germs is washed off with salt solution and carbolic acid as before stated, standardized, placed in homeopathic phial (number for staphylococci being 300,000,000, other organisms requiring a strength of from 50,000,000 up) subjected to a temperature of 60° C. for a half to one hour in order to kill the organisms, a higher temperature to be avoided so as not to interfere with the chemical action of the bacterial ferments.

A control culture is made from the vaccine, and in certain cases also guinea pig inoculations to insure sterility of the vaccine, especially the absence of tetanus must be insured.

## REPORT OF CASES.

The total number of cases that I have treated by this method are eleven, which are as follows:

2 Unilateral sinusitis of the staphylococcus variety of infection, of a chronic type.

1 Polysinusitis, chronic, of a staphylococcus aurius, pseudo-diphtheria bacillus.

1 Chronic aptral infection, unilateral of dental origin, staphylococcus infection.

2 Subacute unilateral sinusitis (most probably confined to the frontal sinus.) Staphylococcus pyogenes aureus.

2 Chronic purulent otitis media of a double infection; viz., staphylococcus and pneumococcus (bilateral).

1 Chronic purulent otitis, radical mastoid, Friedlander bacillus infection.

1 Chronic purulent otitis, unilateral, ossiculectomy, diphtheria infection.

I Acute otitis media purulenta bilateralis diphtheria-like bacillus infection, following a violent grip attack.

All these cases had, previously to the vaccination treatment (auto-genous), the usual accepted method of treatment without the desired results. All of them were subjected to the opsonic index which was lower than the normal; one as low as 0.3 and the nearest to the normal was 0.76. In all but one case, and that was the chronic bilateral sinus infection in which more than one index was taken, I depended almost exclusively on the clinical manifestations as an index and used the average time of ten days between the times of vaccination. Complete records were kept on the observations such as reaction and other symptoms and will be published in detail in a subsequent paper. Suffice it to say at this time that without exception there is a distinct improvement and some of the cases are cured, although not long enough time has elapsed in the chronic cases to be absolutely certain. My purpose in bringing this subject before you is not to come with any positive conclusions because in the first place it requires a great number of cases of various pathological conditions and a much longer time to observe before one can make positive statements, but, to encourage if I can, some of you to take up this line of work if you are not already doing it, and in a symposium, report our results. So far as complications or accidents are concerned in this treatment, I must say that not in one case was there a single bad result and only one that gave me any thought and anxiety, and that was the following:

In the case of subacute unilateral sinus disease of staphylococcus pyogenus aureus infection, at the second injection into his left arm, there followed what clinically one would diagnose as an erysipelas with marked infiltration from the point of inoculation above the elbow, down to the finger tips, but absolutely no general symptoms. The patient felt as well as he ever did, and there was no infection of the axillary glands. Most of the cases showed slight general disturbances for from an hour to four hours after the vaccination, such as nausea, malaise and slight headache, and occasionally a slight rise of temperature.

The specific technic of vaccination is as follows: Scrub up thoroughly the part where the injection is made, either the arm or interscapular region, and under strictly aseptic precautions, draw up into a hypodermic syringe the dose prescribed, usually one cubic centimeter of the vaccine, and inject subcutaneously, seal up the puncture with collodion. As said above, about ten days are allowed to

elapse, when a second vaccination is made and so often repeated in those intervals until the patient is cured. If one should observe that a patient reacts badly and becomes markedly depressed after the injection, it is necessary to prolong the interval.

In conclusion, I wish to say that at the present time I have the following cases under treatment, but not sufficient length of time has elapsed for me to even report any partial results. Three cases of chronic nasopharyngitis, all staphylococcus infection (mild).

Two cases chronic tubercular laryngitis (ulcerated).

Four chronic purulent otitis media bilateral, in tubercular individuals, in which a positive ophthalmic reaction was obtained.

Two cases of atrophic rhinitis in tubercular subjects, one in non-tubercular subject.

All these tubercular patients are treated by tuberculin injections instead of the autovaccine.

The laboratory work of determining opsonic indices and making the vaccines are at present done for me by the *Columbus Medical Laboratory* and the *Chicago Laboratory*.

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