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STREPTOCOCCUS ANTIGEN

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It is the object of my invention to produce an antigen that is specific in the treatment of (so-called) trichomonas vaginalis vaginitis.

The trichomonas vaginalis has been known for about one hundred years. (Donné, 1837.) Since 1856 (Scanzoni) it has been rather generally accepted that it is the causative agent of the particular type of vaginitis that is known as trichomonas vaginalis vaginitis.

But there have been some in recent years who have doubted that the trichomonas vaginalis was the true causative agent of this disease, and have even doubted that it was pathogenic at all; although no one prior to my work, so far as I have been able to discover, has established what the real causative agent is. Thus in 1926 Dobell conducted certain experiments on monkeys, which tended to establish, and I think did establish, that the trichomonas vaginalis was not the causative agent of this type of vaginitis; for he repeatedly infected the vaginal tracts of monkeys with the trichomonas vaginalis, and reported that none of the symptoms of the so-called trichomonas vaginalis vaginitis resulted.

In a paper by me, published in the American Journal of Obstetrics and Gynecology for April, 1933, page 465 et seq., I confirmed Dobell's observations, but in human cases. As reported in that paper, I observed many human cases where the trichomonas vaginalis was present in the vagina, but there were none of the symptoms of trichomonas vaginalis vaginitis. In addition, I deliberately implanted several patients with trichomonas vaginalis, and obtained a fairly active growth of it in the vagina; but although that growth persisted for weeks, there were absolutely no symptoms of trichomonas vaginalis vaginitis.

These observations are supported by the recent publication of Craig and Faust (1937), who say: "Although the trichomonas vaginalis is usually found in an abnormal vagina, and while gynecologists almost universally regard a form of vaginitis as being caused by the organism, there is no evidence of scientific value demonstrating that it is a pathogenic parasite. On the other hand there is much experimental evidence proving that it is harmless and incapable of causing any pathological lesions."

In my aforesaid 1933 paper, which indicated that the so-called trichomonas vaginalis vaginitis in human beings was almost certainly caused by some agent other than the trichomonas vaginalis, I suggested that some streptococcus probably played a very important part in that causation, and that that streptococcus was non-hemolytic. The discussion that followed the presentation of that paper (as that discussion appears on pages 471 to 474 of the April, 1933, issue of the American Journal of Obstetrics and Gynecology), made it evident that other gynecologists were not then in accord with my views, and felt that my work as reported at that time did not establish either that the trichomonas vaginalis was not the cause of that vaginitis or that a streptococcus was such cause.

As a result of continuous work since that reported in my 1933 paper, I have now been able to isolate a specific streptococcus which will cause the so-called trichomonas vaginalis vaginitis. It is the streptococcus subacidus. This is reported in a paper which I read before the Central Association of Obstetrics and Gynecology at its meeting in Dallas, Texas, in October of 1937, and which has been published in the American Journal of Obstetrics and Gynecology for August, 1938, volume 36, No. 2, pages 219–229.

While that streptococcus subacidus had been identified earlier as a species of streptococcus (in Holman's classification of streptococci), so far as I know it had never been associated with the so-called trichomonas vaginalis vaginitis; and neither it nor anything derived from it had been used either to produce or to treat the so-called trichomonas vaginalis vaginitis. Holman's classification of streptococci is published in the Journal of Medical Research, volume 34, 1916, pages 377–443; and the position of streptococcus subacidus in the streptococci group is shown in the chart appearing on page 388 of that paper. Table 9 of that paper, on pages 408 and 409, and the discussion of it which begins on page 409, are specifically directed to the streptococcus subacidus and its identifying characteristics. The identifying characteristics of streptococcus subacidus are also given on page 57 of Bergey's Manual of Determinative Bacteriology, fourth edition, published in 1934.

I have now isolated streptococcus subacidus from the vaginal discharge in human cases of the so-called trichomonas vaginalis vaginitis. I have made cultures of that isolated streptococcus subacidus. I have implanted that culture into the vaginal tracts of normal human beings—those with no trace of any vaginitis—and have produced the characteristic pathology of so-called trichomonas vaginalis vaginitis. After the production of that characteristic pathology, and its persistence for several weeks, I have recovered the streptococcus subacidus from those same indi-

viduals, and have isolated and identified it. Thus I have complied with Koch's postulates.

In addition, from that recovered, isolated, and identified streptococcus subacidus, I have prepared a specific vaccine and a specific antigenic filtrate, and have used both of them to treat these artificially produced cases (as well as other cases), and have succeeded in rendering them symptom-free; and they remained symptom-free for periods of months.

Further, I have injected rabbits intravenously with streptococcus subacidus, using repeated and progressively increasing doses, and after several weeks have obtained the blood serum from the rabbits so treated. When that blood serum was tested against streptococcus subacidus, agglutination or clumping occurred; thus demonstrating that the streptococcus subacidus is antigenic.

Also, I have taken blood serum from patients who have had the so-called trichomonas vaginalis vaginitis, and who have been rendered symptom-free by treatment with my specific filtrate and vaccine obtained from the streptococcus subacidus. I have tested that human blood-serum against the streptococcus subacidus, and have found that agglutination or clumping occurs; thus demonstrating that the streptococcus subacidus is antigenic in human beings, and that specific anti-bodies produced through its use are associated with patients who have been made symtom-free.

In isolating and identifying the streptococcus subacidus from human cases of so-called trichmonas vaginalis vaginitis, I obtained a sample of the vaginal discharge on a sterile swab, and implanted sterile broth with that sample. This broth was allowed to incubate for approximately twenty-four hours, during which a growth of organisms occurred. Then a drop from the culture so produced was streaked on a sterile blood-agar plate, and that plate was allowed to incubate for approximately twenty-four hours. Various colonies—growths from single organisms—appeared. Among these were some that were hemolytic, as indicated by a definite circular area of hemolysis about the colony; and a stained smear showed these to be streptococci. Contrary to what might have been supposed from what was reported in my aforesaid 1933 paper, namely that non-hemolytic streptococci played a very important part in trichomonas vaginalis vaginitis, I found that it was these hemolytic streptococci which are the causative agent of the so-called trichomonas vaginalis vaginitis. These hemolytic streptococci were identified as the streptococcus subacidus.

This identification showed the following characteristics of the so-isolated hemolytic streptococcus. It appears as a gram-positive slightly elongated small diplococcus, non-capsulated, and develops short chains both on blood-agar and in broth. In sugar media it ferments dextrose, saccrose, maltose, levulose, and trehalose; while in thirteen other sugars it produced no fermentation. The zone of hemolysis on blood-agar plates is small but definite, although perhaps not so definite as some other strains of hemolytic streptococci; and no green coloration was noted. It is killed in ten minutes at a temperature of 65° F.

It was this hemolytic streptococcus, thus isolated and identified which was used in the implantations previously referred to and which caused the symptoms of trichomonas vaginalis vaginitis.

The implantation in the human patients was as follows:

First, a sterile cotton swab which had been saturated with a living broth culture of the isolated and identified streptococcus was applied to the vaginal mucosa. Second, a sterile cotton tampon which had been saturated with the same culture was placed in the vagina, and allowed to remain there for twenty-four hours. Some of the subjects were normal women of child-bearing age, while others had shortly before undergone supra-pubic hysterectomy. Both groups developed the characteristic symptoms of so-called trichomonas vaginalis vaginitis; the symptoms produced in the former group were more severe than those produced in the latter group.

From this isolated and identified streptococcus, thus shown to be the causative agent of trichomonas vaginalis vaginitis, I have prepared antigens which are useful in the treatment of that disease. I have prepared two forms of such antigens, which will be referred to as the filtrate and the vaccine. These may be prepared as follows:

The isolated and identified streptococcus subacidus is transferred to a sterile culture medium, conveniently agar or broth, and incubated under usual incubating conditions for about one day. Propagation of the organisms is carried out in the usual manner.

To make the filtrate:

The organisms are transferred to a sterile growth-promoting liquid medium, most conveniently broth, and incubated for about two days, under usual incubating conditions at about normal blood temperature, and then held at room temperature for ten or twelve days.

After this procedure, the culture is passed through a sterilizing filter, and the resultant filtrate is the filtrate which I use.

To make the vaccine:

The organisms are transferred to a sterile growth-promoting medium (liquid or solid, such as agar or broth), and incubated for about one day. If agar is the medium used, the organisms are harvested in normal saline solution; and if broth is the medium used, the culture is centrifuged to get the organisms closely compacted, and the supernatant liquid discarded and the compacted organisms are taken up in nomal saline solution. In either event, the organisms in the suspension are then killed in any suitable way, as by heat. The suspension is then standardized to a definite count, conveniently about 250 million per cc. This is the vaccine which I use.

Out of abundant caution, it is desirable to add a preservative to both the filtrate and the vaccine, such as 1 part in 10,000 of "Merthiolate" (sodium ethyl mercuri thiosalicylate, Lilly); although no preservative is essential, and none was used in the experimental work for establishing the facts hereinbefore set forth.

Both antigens, the filtrate and the vaccine, are desirably used in treating so-called trichomonas vaginalis vaginitis. The treatment, whether of those already suffering from the vaginitis or those in whom it had been artificially produced, is as follows:

1. The vagina and external genitalia are wiped free of discharge, with dry sterile cotton pledgets.

2. A sterile tampon, preferably cotton, saturated with the filtrate, is inserted in the vagina, and removed by the patient after about twenty-four hours. Cotton is better for the tampon than is lamb's wool, because lamb's wool is not sufficiently absorbent; and I believe that it is better than any other absorbent, although of

course other absorbent materials, if sufficiently absorbent, may be used.

3. The filtrate is applied to the external genitalia, as by swabbing, and allowed to dry in place.

4. At the same time, an injection of the vaccine is given, preferably an intradermal injection and conveniently on the flexor surface of the forearm.

5. This double treatment, with both filtrate and vaccine, is repeated at intervals, conveniently of about a week, taking care to avoid the periods of menstruation.

6. For best results I deem it desirable that no other topical treatment be made than with the filtrate—as by douches, powders, vaginal suppositories, or any other antiseptic or germicide.

In administering the vaccine intradermally, it is desirable to start with a fairly small dose, of the order of about one-twentieth of a cc. Successive doses may be increased or not, depending upon the severity of the skin reaction; for I deem it desirable that care be taken that the skin reaction should not be more severe than is sufficient to produce an area of redness of about two inches in diameter. Ordinarily the maximum dose should not be above about one-half of a cc.

In the majority of cases, not more than seven treatments are required to cause the symptoms to disappear. A number of patients so treated have now been symptom-free for periods ranging from six to nine months. In some instances there has been recurrence, especially following menstruation; and in that event it is desirable to repeat the treatment through at least another menstrual cycle. While not all cases have responded to the treatment, all the artificially produced cases did so respond, and at least two-thirds of those reporting to the clinic with already existing cases of so-called trichomonas vaginalis vaginitis have been rendered symptom-free.

A few instances have been noted of transmission of the disease to the male. In those instances, symptoms somewhat similar to those in the female are observed. Those symptoms can be made to disappear, certainly in the majority of cases, by use of the vaccine intradermally in the male as above described for the female, and by anterior urethral injections of the filtrate.

I claim as my invention:

1. An antigen that is specific in the treatment of so-called trichomonas vaginalis vaginitis, comprising a vaccine consisting substantially of heat-killed streptococci subacidus.

2. An antigen that is specific in the treatment of so-called trichomonas vaginalis vaginitis, comprising a vaccine consisting substantially of killed streptococci subacidus.

3. An antigen that is specific in the treatment of so-called trichomonas vaginalis vaginitis, comprising a sterile filtrate of a broth culture of streptococcus subacidus.

4. An antigen that is specific in the treatment of so-called trichomonas vaginalis vaginitis, comprising a sterile filtrate of a culture of streptococcus subacidus.

5. A sterile antigen from streptococcus subacidus and specific in the treatment of so-called trichomonas vaginalis vaginitis.

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