Original Articles.

GONOCOCCUS VACCINES AND GLYCERINE EXTRACTS OF THE GONOCOCCUS IN THE DIAGNOSIS OF GONORRHEAL INFECTIONS.*

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INTRODUCTION.

THE experiments described below were undertaken with the hope of finding a reliable method of diagnosis for gonorrhea by means of a specific reaction. The theoretical grounds for believing this to be possible have been discussed by earlier writers referred to below, and need not be detailed here. At this point it suffices merely to mention that changes have been shown to exist in the blood serum of patients suffering from various infectious diseases; that supposed anaphylactic reactions, like that to tuberculin, are believed to depend on changes in the serum; and that several investigators have found in the serum of patients suffering from gonorrheal infection, evidence of changes which they considered specific for gonorrhea (¹¹ and ¹²).

As in tuberculosis, so in gonorrhea, three types of reaction have been observed after subcutaneous injection of vaccine: (a) the *local reaction* at the point of injection; (b) the *focal reaction* at the seat of the lesion; and (c) the *general reaction*, fever, malaise, etc.

Whereas previous attempts at diagnosis by means of subcutaneous injection of gonococcus vaccine have not yielded very satisfactory results, we thought it worth while to try the effects of a vaccine far more concentrated than any used heretofore, and to administer it intradermically instead of subcutaneously.

In another series of cases inoculations of vaccine were made by the method devised by v. Pirquet for the skin tuberculin test; and, in a third group, glycerine extracts were employed in like manner.

PART I: SERIES 1 AND 2.

METHOD, DOSAGE, MATERIAL.

The vaccine was prepared from nine strains of gonococci, which had been under artificial cultivation for varying lengths of time. The organisms were suspended in normal salt solution, standardized by count to 500 million per 0.1 c.c., and mixed with $\frac{1}{2}$ per cent. of lysol.

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The first injections were given several days after the preparation of the vaccine when it had been shown by culture to be sterile. Heat was not used to kill the bacteria as they die quickly under the above conditions. Subsequent cultures showed that the vaccines remained sterile while in use.

Normal salt solution containing $\frac{1}{2}$ per cent. of lysol was used as a control solution.

Before administering the vaccine or control, the skin of the back of each arm was carefully cleaned. The vaccine was introduced intradermically; i.e., beneath the superficial layer of the skin in the same way in which cocaine is used for local anesthesia. The control solution was given similarly in the other arm.

The dose in the first, and in nearly all subsequent cases, was 500 million gonococci, or 0.1 c.c. of the vaccine. One patient, having fever, was given 300 million to begin with, but received 500 million when convalescent. Another received 150 million, and a third 100 million. There were no other exceptions.

The clinical material came partly from the out-patient department of the Massachusetts General Hospital, but chiefly from the hospital wards. Thirty-two patients were studied, of whom fourteen showed evidence of active gonorrheal infection in some form; thirteen had unrelated diseases; and five were healthy. "Table No. I" shows more definitely the distribution of material.

FIRST SERIES: RESULTS.

The local reactions generally began in about six hours, were fully developed after twenty-four hours and disappeared gradually in the course of two or three days. They consisted of redness, swelling and tenderness over a zone several centimeters in diameter, surrounding a central area which was more deeply injected and sometimes elevated. Severe reactions showed larger zones and were somewhat painful.

The injections of the control solution left behind only the mark of the needle puncture. Table I shows that local reactions occurred in all the gonorrheal cases and in all but three of the non-gonorrheal or control cases; that most of the reactions were marked; and that the proportion of marked reactions was nearly as great in the control as in the gonorrheal cases.

Closer analysis shows that the first test was made on a case of chronic prostatitis, and that a severe local reaction developed. The three following tests were made on control cases, and they did not react at all. Their respective diagnoses were abscess following typhoid, fistula in ano, and carcinoma of the breast. The fifth case had acute anterior urethritis, and gave a marked reaction. These five tests were made within the first week after the preparation of the vaccine, and their outcome was distinctively encouraging.

All subsequent injections of vaccine, however, gave positive local reactions; and although tests were made over a period of 109 days after the first use of this vaccine, no other striking change in its effect was seen.

These results led us to believe that the vaccine, which had seemed to act specifically during the first week, had subsequently developed irritating properties of a nonspecific nature. Acting on this hypothesis, we made a second series of tests with a fresh vaccine. The results are shown in Table II.

TABLE I.

LOCAL REACTIONS: FIRST SERIES.

Gonorrheal Cases: 14.

Diagnosis.	No re- action.	Slight Reac- tion.	Marked Reac- tion.	Severe Reac- tion.	Num- ber of Cases
Acute urethritis	—	—	3	—	3
Chronic urethritis or prostatitis	—	—	3	1	4
Salpingitis	—	1	1	1	3
Arthritis of gonorrheal type	—	—	3	1	4

Control Cases: 18.

Normal persons	—	1	3	1	5
Various diseases	3	5	4	1	13
Totals of reactions	3	7	17	5	32

TABLE II.

LOCAL REACTIONS: SECOND SERIES.

Gonorrheal Cases: 3.

Diagnosis.	Slight Reaction.	Marked Reaction.	Number of Cases.
Acute urethritis with arthritis	1	—	1
Chronic gonorrheal infection	—	2	2

Control Cases: 11.

Normal persons	—	2	2
Various diseases	4	5	9

SECOND SERIES.

All cases inoculated with the second vaccine showed reactions, although the tests were made within a week of its preparation.

The two vaccines used were prepared in the same way by the same person; both were standardized by count to 500 million per 0.1 c.c. and both were administered in the same way and in the same dose. Nevertheless, the vaccines were not identical. The first was made from several strains which had been kept for some time in the laboratory and to these were added several freshly isolated cultures. The second vaccine was made from a new set of fresh cultures. Such differences of origin might, perhaps, cause

a difference of toxicity or of irritating properties in two vaccines. Irons³ points out that the degree of reaction depends not only on the number of gonococci inoculated, but also on the source and age of the cultures from which the vaccine is prepared. He found that cultures isolated within three months furnished a vaccine ten times as strong as a culture which had been grown in the laboratory for two years.

We are inclined to believe that our vaccines possessed irritating properties of a non-specific nature, and that if there was any specific reaction, it was masked by the response to the irritant.

If the hypothesis be advanced that better results might have been obtained with smaller dosage, it may be replied that our dosage was not larger than that frequently used by Irons for diagnostic tests; and that general and focal reactions in our series were neither many nor well-marked; also, this hypothesis does not explain the three negative tests. There seems little reason to suppose that the outcome of the local reactions would have been essentially different had injections been given subcutaneously instead of intradermally.

A focal reaction was observed in only one case of the entire number, 46 in all. The reaction consisted of increase of pain in the affected joints of a patient supposed to have gonorrheal arthritis. Three other cases of arthritis, probably gonorrheal, and four salpingitis cases gave no such reaction.

Symptoms attributable to a general reaction were carefully looked for, and four hourly charts were kept for 32 ward-patients. Eight of these, including two control patients, showed slight elevations of temperature appearing some hours after the injection. One of the controls had had variations of temperature before the test, and after it his temperature rose from 97 degrees to 100.3 degrees, the greatest increase noted.

In the gonorrheal cases the temperature rise ranged from $\frac{1}{2}$ degree to 1.2 degrees. The patients who had been running slightly elevated temperatures before the test showed increase of fever. Elevations of temperature occurred in three of the four cases of arthritis, and in two of the four salpingitis cases. None of those who responded with fever complained of malaise, but one of them showed the focal reaction mentioned above.

Malaise was noted, however, in two other gonorrheal and in two control cases.

Stated briefly, the results from injection of vaccine in 46 cases show that local reactions appeared in all gonorrheal and in nearly all non-gonorrheal cases; that there were signs suggesting a general reaction in eight gonorrheal cases and in two non-gonorrheal cases; and that a focal reaction was observed in one case only, the diagnosis in this case being gonorrheal arthritis.

LITERATURE.

The local reaction is generally conceded to be unreliable for diagnosis. Although the most common of the three reactions, local manifestations have been observed repeatedly when it was believed that active and preexisting gonorrhea could be excluded. Such reactions may closely resemble those of gonorrheal cases; and, according to Guggisberg⁸, indifferent subcutaneous injections may cause painful efflorescence in sensitive persons. The local reaction in gonorrhea is generally more marked and of longer duration than that which may appear in normal individuals, but these reactions have not yet been sharply differentiated.

General reactions consisting of fever, rapid pulse, headache, lassitude and leucocytosis, or of any of these symptoms, have been observed in a comparatively small number of cases. According to Guggisberg the general reaction occurs most often in fresh infections when acute inflammation has existed recently. Irons¹ says that the fever may be so slight as easily to escape notice, but Fromme and Collmann⁷ think that a rise of temperature following the injection of the vaccine may perhaps help in differentiating extrauterine pregnancy from salpingitis. This hypothesis lacks confirmation. Apparently, no general reaction has been recorded in a non-gonorrheal case.

The focal reaction merits closer analysis. Pain and swelling were observed by Irons¹ in a considerable number of cases of gonorrheal arthritis, and he attributed to these signs, plus fever, a certain value for distinguishing gonorrheal from other forms of arthritis. He says that a positive reaction can generally be obtained at will in gonorrheal arthritis by means of graduated doses. In a case of post-operative periurethral abscess without secondary infection or retention of pus he saw a "marked increase of redness and tenderness about the wound." He says, further, that ocular reactions are sometimes observed in cases in which iritis has previously been present, and that increased inflammation or effusions into the eye are not infrequent after therapeutic inoculations of vaccine in active gonorrheal iritis.

In women having gonorrheal salpingitis or allied diseases, increase of pain and of localized tenderness, increase of discharge, and hemorrhages or premature menstruation have been observed repeatedly. Some of these symptoms have been noted in a considerable proportion of cases of this kind by Reiter⁶, Köhler⁶, Fromme and Collmann⁷ and by Guggisberg⁸. The latter has twice found gonococci in the discharge after injection, having failed to find them previously.

Fromme and Collmann, on the other hand, saw marked focal reactions in three cases thought not to be infected with gonorrhea and emphasized the occurrence of a focal reaction in

a virgin who had a myoma. Guggisberg recorded a marked positive reaction in one case of septic infection.

Of fundamental importance to those interested in vaccine diagnosis, and in treatment as well, is the work of E. C. Rosenow⁹ on autolysis of bacteria. He showed that the gonococcus and some other organisms undergo rapid autolysis in normal salt solution; that at a certain period of autolysis the mixture becomes extremely toxic; and that the time the acutely toxic substance appears in solution is directly proportional to the activity of the autolytic ferment and the rate of disintegration of the organisms. Suspensions of the gonococcus in salt solution when incubated at 37° C. became toxic to guinea-pigs after 10 minutes, more so after one hour, and were non-toxic again after 20 hours. With the staphylococcus aureus, little or no proteolysis occurred under similar conditions.

DISCUSSION.

Although irritating properties manifested by injection into human beings and toxicity in guinea-pigs are not the same, there seems to be an analogy between the development of toxic products by autolysis in Rosenow's experiments and the appearance of irritating properties in our vaccines; moreover, it is the experience of Dr. Albert E. Steele, Assistant in Clinical Bacteriology at the Massachusetts General Hospital, that gonococcus vaccine autolyzes within a few days, even when prepared with lysol and kept in an ice-chest. If autolysis of bacterial suspensions be delayed by lysol and by cold, it follows that the lysol must be added promptly to suspensions of gonococci, and the suspensions must be placed as soon as practicable in an ice-chest if the formation in them of toxic substances is to be delayed.

If the same line of reasoning be applied hypothetically to the supposed formation in gonococcus vaccine of substances irritating to human beings it becomes apparent that two vaccines similarly prepared might be different in action provided that, in the first instance, the lysol were added to the suspension immediately and the mixture placed at once in an ice-chest and, in the second instance, the lysol were added some hours later, the suspension having stood meanwhile at room temperature. Another important discovery of Rosenow is the fact that recently isolated cultures of the gonococcus autolyse more rapidly than do older cultures.¹⁰

Whether or not any such factors may have caused the difference in action of our two vaccines, one of which appeared to have been non-irritating during the first week, and the other irritating from the start, is a question which suggests itself to the writers, but which they are unable to answer from information at hand.

Returning to the facts that suspensions of gonococci become quickly toxic to guinea-pigs

and later again non-toxic, and that this change corresponds to the rate of autolysis, it may be asked what influence this autolysis has on the immunizing properties of a vaccine, and whether or not these properties may be destroyed by autolysis.

Some light may perhaps be thrown on the question by comparing the clinical value of gonococcus vaccine with that of staphylococcus vaccine, which Rosenow showed did not autolyse to any extent in normal salt solution. Staphylococcus vaccine has to a high degree the power of influencing favorably localized foci of staphylococcus infection; whereas, gonococcus vaccine is generally conceded to be one of the less efficacious of the vaccines similarly used.

When a vaccine has undergone autolysis to an unknown degree, when changes in it may still be taking place, and when the peculiar proteins of the original organism may perhaps have been destroyed or modified, the results of diagnostic tests and of treatment with such a vaccine may well prove unreliable; and, did a specific reaction occur, it might be masked by the effects of irritating or non-specific toxic products of disintegration of the bacteria.

These considerations indicate that more knowledge is needed of the changes which take place in gonococcus vaccine, and raise the hope that more satisfactory results may follow improved methods of preparing vaccine.

PART II: INOCULATION OF VACCINE BY THE VON PIRQUET METHOD.

While the second series of injections was being conducted in the wards, a group of out-patients was tested with the same vaccine, inoculated by the method devised by v. Pirquet for the tuberculin test. Inoculations of the vaccine were always made in two places with a control scarification between them, and, in every instance, a control solution was used in like manner.

The cases, 15 in number, ranged from acute anterior urethritis to chronic posterior urethritis and prostatitis. One of the patients was seen again on the day following the inoculation, 10 were seen on the second day, and 4 not until the third day.

There resulted, as a rule, only a minute scab at every scarification point, but occasionally there was found a slight redness a few millimeters in diameter. No swelling and no papule was observed in any of these cases on the days subsequent to the inoculation; but sometimes a small wheal formed quickly at all scarifications. In consideration of the dirtiness of many of the patients and the fact that the redness was seen nearly as often at control-scarifications as at the points of inoculation of the vaccine, slight redness was attributed to superficial infection. It is conceded that very slight or evanescent specific reactions may have been overlooked be-

cause close observation of results was impossible under the circumstances.

DISCUSSION AND SUMMARY.

The von Pirquet technic of inoculation was first used by Köhler⁶, who tested, in this way, several gonococcus vaccines. He says that during the first hour after inoculation a slight swelling appeared, and that it continued to increase for four or five hours until it reached about the size of a lentil. After from 18 to 24 hours it began to fade, and on the second day it was scarcely recognizable. The swelling was often surrounded by a red areola. Köhler observed this phenomenon in many gonorrheal cases, and likened it to the von Pirquet tuberculin reaction, but he says that it was never so definite as is a positive reaction to tuberculin. Control inoculations showed a similar slight swelling after one hour. It decreased after 4 or 5 hours, and, as a rule, it was entirely gone in from 8 to 12 hours.

Although their vaccine was, probably, much more concentrated than any used by Köhler, the writers obtained no results comparable with his. They failed to observe anything resembling a positive tuberculin test in 15 cases of gonorrheal infection, which included all stages of the disease from the acute to the most chronic types of genitourinary infection in the male.

PART III: GLYCERINE EXTRACTS.

PREPARATION AND TECHNIC.

Three extracts were prepared. For each of them the gonococci in 12 culture tubes of human blood agar were washed off with a small amount of distilled water, 0.5 per cent. of carbolic acid added, and the mixture placed in the incubator 37° C. "Extract I" was incubated for 24 hours, and then heated at 60° C. for 2 hours. "Extract No. II" was incubated for 48 hours, and heated at 60 degrees for 2 hours, and "Extract III" was not incubated but was heated immediately at 60 degrees for 2 hours. To each of these extracts 2 c.c. of glycerine were added after the heating, and, finally, all was evaporated in an air blast to a small volume. The resulting extracts were viscid and cloudy.

The control solution was made similarly, but from sterile culture tubes.

The extracts were kept in bottles covered with rubber nipples and they remained in an ice-chest except when taken out to withdraw material for the tests. This was done aseptically by means of a hypodermic syringe and enough of the vaccine was taken out each time to suffice for the work of the day.

Inoculations were made by the von Pirquet method; a control scarification without any inoculation was made first, and then the vaccine was inoculated on either side of it. The control

solution was inoculated in the same way at two other points with a scarification between.

Cultures on blood serum and on plain agar showed the extracts to be sterile before they were used, and again after completion of the tests.

WORK AND RESULTS WITH "EXTRACT I."

Twelve of the seventeen patients in this series had gonorrhea, in three other cases its presence was suspected, and two were believed to be free from it. Seven of the patients, including four gonorrheal cases and one in which gonorrhea was suspected, were seen at 24 and 48 hours after the inoculation. The remaining ten, being out-patients, were not seen until the third day. There were among them eight cases of acute or chronic gonorrhea and two of arthritis due, presumably, to gonorrhea.

Very slight redness was noted once at a control mark and once where the gonococcus extract had been inoculated.

Nothing resembling a specific reaction was observed in this series.

WORK AND RESULTS WITH "EXTRACT II."

In the series of 15 patients 12 had gonorrhea, and in the remaining three its presence was suspected. Acute and chronic urethritis, acute exacerbation of chronic urethritis, epididymitis and salpingitis were included in the series. Seven ward-patients were revisited after 24 hours, 48 hours, and 4 days. Five out-patients were seen on the second day, and three on the third day.

Seven cases, two of them in the wards, showed no reaction at all.

Five cases, three of which were in the wards, showed slight redness at both points of inoculation of the vaccine. These reactions were considered of questionable significance.

Three cases, two of them in the wards, showed a small area of redness with central swelling at both points of vaccine inoculation. These reactions were classed as positive.

Slight redness without swelling occurred at one control mark in two patients, and at two control marks in one patient. It was attributed to infection.

The result with "Extract II" was no reaction in seven cases, a questionable reaction in five cases, and a positive reaction in three cases.

WORK AND RESULTS WITH "EXTRACT III."

There were 15 patients in the series of whom 11 had chronic urethritis or prostatitis, 2 sub-acute urethritis, and 2 arthritis, supposedly of gonorrheal origin. Six ward patients were seen daily after the inoculation as long as any reaction was visible, and of the 9 out-patients 5 were

seen on the second, 3 on the third day, and one which reacted returned on the second and third days.

Six out-patients and 3 ward-patients showed no change, 2 ward cases and one out-patient case gave questionable reactions, and 3 gave positive reactions. The control marks in two cases of doubtful reaction showed slight redness. The outcome of reactions was judged by the same criteria as for "Extract II."

SUMMARY OF WORK WITH EXTRACTS I, II AND III.

The total number of tests with these extracts was 48, and the number made with each extract was about the same.

Extract I gave nothing resembling a specific reaction.

Of 30 tests with Extracts II and III 16 were negative, 8 were doubtful, and 6 gave reactions resembling those seen in positive skin-tests made with tuberculin by the v. Pirquet technic. The papules, however, were decidedly smaller than those of a well-marked tuberculin test. It is worthy of note that all positive tests occurred in patients having a definitely active gonorrhea, and that the outcome of the tests in the more chronic and the latent cases was uniformly negative.

DISCUSSION: EXTRACTS.

The technic followed by Dr. Steele in preparing our extracts was outlined by Dr. E. E. Irons of Chicago in a letter to Steele, and the latter followed the directions with the greatest care. Owing to Dr. Steele's experience in the preparation of vaccines, we believe that errors of technic can be excluded.

The extracts and also the control solution were cloudy and contained a brownish sediment due apparently to blood elements washed from the media with the bacteria. The sediments were amorphous as seen under an oil-immersion lens. They showed no signs of recognizable bacterial elements.

Iron does not state in figures the proportion of gonorrheal cases in which he observed well-marked reactions with gonococci, but his paper leaves with us the impression that the reactions occurred in a considerable proportion of the gonorrheal cases. Fairly marked reactions were seen by him in adults occasionally, and in children more frequently when previous gonococcal infection could be ruled out; and reactions were obtained with gonococci in epidemic meningitis and with meningococcus extracts in gonorrhea. Believing that infections with the micrococcus catarrhalis and other allied organisms might give a like response to gonococci, Irons points out that such infections must be excluded before a positive reaction can be attributed to gonorrhea. He suggests also that the reaction may be characteristic of a group of infections rather than of a single disease, and says that

a positive reaction should be regarded merely as confirmatory evidence of gonorrhea.

The comparatively small number of tests made by us does not admit of detailed deductions. We infer from them, however, that our proportion of definitely positive reactions was probably less than that of Irons and that doubtful results were relatively numerous. We are inclined to believe that the papules seen in the "positive" cases indicate a specific or a group reaction; and that redness without papule formation should be regarded as due, probably to infection or to other accidental causes.

In a letter received recently from Dr. Irons, he attributes the cloudiness of our extracts to blood particles washed from the media, and advises the use of ascites agar or of blood agar in which the blood has been mixed with the agar before it hardens. He says, further, that some extracts are not nearly so satisfactory as others, and suggests that this may be due to differences in the degree of autolysis of the bacterial protein.

CONCLUSION.

A. FROM PUBLISHED WORK OF OTHERS REFERRED TO ABOVE:

1. Reactions thus far observed after injections of gonococcus vaccine have not been clearly specific for gonorrheal infection.
2. The reactions seem to show a selective action in some types of gonorrheal disease.
3. Local reactions after injections are common in non-gonorrheal cases. They are not valuable for the diagnosis of gonorrhea or for demonstration of its cure.
4. Focal reactions are seen frequently in active gonorrheal arthritis and salpingitis; but, whereas they have been observed in cases showing no other evidence of gonorrhea, their diagnostic value is small.
5. A general reaction is seldom noticeable, and it may be easily simulated by slight symptoms due to other causes. It has, *per se*, no diagnostic value.
6. Gonococcus vaccine autolyses rapidly, and thus undergoes changes little known.

B. FROM WRITERS' WORK:

1. The concentrated vaccine used by the writers showed no superiority for diagnostic tests.
2. It produced in gonorrheal and in control cases a local lesion like that of a chemical irritant. We think that autolysis may have been a factor in producing the supposedly irritating properties of our vaccine; that the changes in the vaccine may have prevented it from producing a specific reaction; and that the unsatisfactory therapeutic effects of gonococcus vaccine may, perhaps, be traceable to autolysis.
4. Glycerine extracts of the gonococcus in-

oculated by the method of von Pirquet caused, in a few cases, the formation of peculiar papules which may have represented a specific reaction. Most of the cases showed no definite reaction.

5. Although our tests were negative in the main, and although the work of others using similar methods has yielded unsatisfactory results for diagnosis we desire to emphasize our belief that failures serve as guideposts to progress, and that present knowledge justifies the hope that a valuable diagnostic test for gonorrheal infection may yet be devised through improved methods of preparing vaccines or extracts of the gonococcus.

It is with great pleasure that we express our thanks and obligation to Dr. Albert E. Steele for preparing the vaccines and the extracts which we used, and to Dr. Hugh Cabot, as well as to many other members of the Staff of the Massachusetts General Hospital, for the opportunity to study their patients.

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THE PATHOLOGICAL LESION OF WHOOPING COUGH.*

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THE whooping cough bacillus was discovered by Bordet and Gengou in 1900, but was not obtained by them in pure culture until 1906. They found it in large numbers in the sputum of all early cases of the disease examined. The organism is a minute ovoid, Gram-negative coccobacillus which stains lightly by the ordinary methods. It is about the size of the influenza bacillus. They were able to start cultures only on a potato-blood-agar medium which they devised. They were also able to obtain a complement fixation test by using an emulsion of the organism and serum from a patient's blood. This test showed fairly conclusively that the patient produced an antibody for the organism, and that reaction has been the basis of the proof since that date that the organism causes the disease. Still, in spite of the fact that the bacillus has been generally accepted by bacteriologists as the cause of whooping cough, it has not been accepted to any extent by the medical profession because the organism has never been dem-

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