

COMPARATIVE OBSERVATIONS ON BIOLOGICAL CHARACTERS OF *SP. PALLIDA* AND *SP. PERTENUIS*.*

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The problem of the relation between syphilis and yaws has been approached from several directions and practically all modern observers have agreed that these diseases are distinct. Clinical differences have been clearly pointed out by Castellani.¹ Differences in immunity tests, in experimental lesions and in serum reactions have been recorded by Charlonis,² Castellani,³ Neisser, Baermann and Halberstadter,⁴ Ashburn and Craig,⁵ Nichols,⁶ Baermann and Wetter,⁷ and others. Since the discovery of *Sp. pallida* and *Sp. pertenuis*, comparative observations on the organisms themselves have naturally been attempted, and in this paper it is proposed to summarize the results of these observations. Our knowledge of the causative organisms is still rudimentary in most respects, and we cannot expect at this stage to correlate biological characters and pathogenic properties; but enough observations have already been made to warrant some consideration.

Before attempting to differentiate within this group of organisms, it is natural to inquire what relations the group as a whole bears to other organisms. Eventually, no doubt, such an inquiry can be answered with some certainty, and the answer will help in rounding out our conceptions of nature and of disease, but at present it must be confessed that little can be learned by looking in this direction. As Fantham⁸ says, "The literature on spirochaetae, at present, is in a state of the utmost confusion." Evidences of this confusion are not far to seek. On every hand we find directly contradictory statements concerning the presence or absence of an undulating membrane, on the presence or

absence and significance of flagella, on the occurrence of transverse or longitudinal division, on the nature of granules, and on the various stages of life history. To quote Fantham again, "The accounts of *Sp. plicatilis*, the type species of the genus, given by Ehrenberg, by Schaudinn and by Zuelzer, vary so much that they cannot be reconciled, and suggest that the authors may have dealt with different organisms." Some authors, such as Metchnikoff⁹ and Doflein,¹⁰ hold that our knowledge is not sufficient to mark the limits of the spirochaetae from those of the spirilla and vibrios; others, such as Holling,¹¹ find it easy to make clear distinctions. Some workers, such as Gross¹² and Zuelzer,¹³ hold that many heterogeneous organisms have been included among the spirochaetae, and that the group must be radically split up; others, as Fantham,¹⁴ hold that there are no sufficient reasons for forming new genera. It is evident that until the protozoologists or protistologists come to some agreement it is hopeless for the mere medical man to attempt to construct any etiological philosophy of the spirochaetoses.

In passing, however, two rather significant developments may be mentioned. In the first place, Doflein¹⁵ has rearranged his classification of Protozoa on the basis of the conception that the spirochaetae form a bridge between the bacterial spiral forms and the flagellated protozoa. This displacement of the rhizopods as the lowest protozoa, in favor of the flagellates, in order to conform to his conception of genetic relationship, by such an authority as Doflein, commands attention. In the second place, salvarsan has proved to be equally

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efficient against the mouth spirochaetes, those of relapsing fever, those of foul spirochaetosis, and those of syphilis and yaws. This fact seems to argue for some fairly close relationship among these different forms, as salvarsan undoubtedly has quite a strict selective action."

Morphology.

Coming now to the organisms in question, comparative studies of morphology have yielded some slight differential data. Schaudinn and Castellani¹² considered the two organisms practically identical in form. Prowazek¹³ and Russell,¹⁴ on the other hand, have described certain minute differences: *Sp. pertenuis* is thicker and less rigid; the coils are looser and not as regular; the ends tend to curl up to a greater extent; and forms showing longitudinal division are more numerous.

Such observations as I have been able to make on this subject have been made on one strain of *pertenuis* whose source has been described elsewhere, and which has been carried on in rabbits since February, 1910. On comparing fresh specimens of *pertenuis* and *pallida* from the rabbit, with the dark field apparatus, the two organisms are strikingly alike, and in some preparations, are indistinguishable. On the whole, however, one gets the impression that *pallida* has a more active corkscrew motion, that it is more rigid, and that its twists are closer and more regular. These observations, made with fresh material in which there is less apt to be artificial distortion, tend to confirm the conclusion of Prowazek and of Russell. In regard to thickness one must proceed with caution, as Nogouchi has cultivated a thick and a thin variety of *pallida*. Altogether, we may say that slight morphological differences, which, to be sure, seem insufficient for ordinary diagnosis, nevertheless do exist, and it may be that the greater rigidity of *pallida*, like the more glassy ectoplasm of *Entamoeba histolytica*, can be correlated with its greater pathogenic power. In regard to the vexed questions of the method of division, the significance of pointed

ends, of flagella, and of granules, no comparative statements can be made.

Cultural Characters.

Both *Sp. pallida* and *Sp. pertenuis* have, within the last year, been grown in pure culture by Noguchi, and through his kindness I have been able to observe one strain of *pertenuis* and several strains of *pallida* for nearly a year. Both organisms are obligate anaerobes and grow slowly and diffusely in the depths of tissue-serum-agar. The first evidence of growth comes only 4 or 5 days after a transfer, when the lower part of the tract of inoculation becomes more distinct, and the media appears granular. Then a faint haze appears leading off from the tract to the side of the tube, and this extends and becomes more dense until most of the tube is opaque, with a sharp and even line of demarcation about one-half cm. from the surface of the agar. It is suggestive that occasionally no sign of growth appears for two, three, or even four weeks. After inoculating one strain of *pertenuis* and one of *pallida* on the same day and on the same media, I have noticed repeatedly that growth appeared in the *pertenuis* tubes one to three days earlier than in the *pallida* tubes, and that the growth became more dense. No other differences have been noted, and even here due allowance must be made for the fact that some varieties of *pallida* grow more rapidly and more profusely than others.

These cultures rapidly lose their virulence. Nogouchi, who first grew *pallida* in pure culture and reproduced lesions with cultures, had three takes out of four inoculations in a strain which had been grown for a month, and only two out of eight in a strain which had been grown four months; while on transfer from rabbit to rabbit the percentage of takes is practically 100. After a number of transplants, running from two to eight months, I have failed in six attempts to produce lesions with cultures of *pertenuis*.

Antibodies.

It was hoped that when cultures of the

spirochaetes were obtained these could be used for various diagnostic and differential serum tests, such as agglutination, precipitation and lysis, but no definite progress has been made in this direction, partly on account of the technical difficulties of handling anaerobes and of examination by the dark field microscope, and partly because the cultures, along with loss of virulence, may possibly lose their antigenic powers. The anaerobic nature of the growth has made the separation of the organisms from the media difficult, and these organisms are so sensitive that even the addition of ordinary salt solution will immobilize them. Schereschewsky," working with mixed cultures of pallida and their filtrates, was unable to demonstrate any agglutinins or precipitins in syphilitic sera. Of course such antibodies may not exist, but the presumption is that they do exist.

Complement Fixation.

Both pallida and pertenuis give rise to the Wassermann reaction in man and in experi-

were made as follows: two tubes with a rich growth of one and two months were selected; the oil was poured off and the tube filled and broken open; the agar column was put in a sterile Petri dish, the upper part was cut away and the tissue picked out. The media was then shaken up with ten times its weight of absolute alcohol and allowed to extract for ten days, with frequent shaking. The fluid was then filtered, evaporated to one-third its volume and used in a dilution of one to ten; titrated and 0.1 c.c. used as antigen. The sera were inactivated and used in amounts of 0.1 and 0.2 c. c.

Rabbits infected with pallida and pertenuis gave a double plus reaction with the stock antigen of syphilitic foetal liver, but no reaction with specific culture antigens either at the height of the lesion or after recovery. This is one more fact which tends to prove that the usual Wassermann reaction is not a strict antibody reaction, but is more in the nature of an indifferent chemical or physical reaction.

| Sp. Pallida | Stock Antigen | Pal. | Per. | Dent. | Control | |
|---------------|---------------|------|------|-------|---------|------------------------------------|
| Rabbit 1-77 | — | — | — | — | — | Before lesion |
| Rabbit 1-79 | ++ | — | — | — | — | Active lesion |
| Rabbit 1-75 | ++ | — | — | — | — | Active lesion |
| | — | — | — | — | — | 12 days later, lesion disappearing |
| Sp. Pertenuis | | | | | | |
| 84 | — | — | — | — | — | Before lesion |
| 86 | — | — | — | — | — | Before lesion |
| 85 | ++ | — | — | — | — | Active lesion |
| | — | — | — | — | — | 13 days later |
| | — | — | — | — | — | 30 days later, lesion gone |
| Normal rabbit | — | — | — | — | — | |
| Normal rabbit | — | — | — | — | — | |

mental infections in animals, especially in the rabbit. In man the reaction with pallida is stronger and much more persistent. With specific antigens, however, as Nogouchi²³ has pointed out, no reaction occurs in rabbits. Craig and I have had the same experience, as is shown in Table I.

TABLE I.

Complement fixation tests with sera of rabbits infected with sp. pallida and sp. pertenuis and specific culture antigens.

The stock antigen was made from the liver of a syphilitic foetus. The other antigens

Nogouchi has obtained some results with specific culture antigens in the later stages of syphilis, but there is some question as to the nature of these reactions, as Craig and I have seen the same results with an alcoholic antigen of Sp. dentia. The possible significance of these findings will be discussed in a paper which will appear shortly. One significant feature is the fact that no positive reactions have ever been obtained with an uninoculated culture medium used as an antigen. Schereschewsky, on the other hand, using horse serum media obtained positive results with

his uninoculated control. It may be recalled that a year ago I reported to the Society some results of efforts to use extracts of infected testicles as antigens.²¹ Nothing conclusive could be learned in this way, however, as extracts of normal testicles frequently give positive reactions. In the case of our culture antigens the uninoculated control behaves satisfactorily, but non-specific or possibly group reactions occur with cultures of other spirochaetae. Specific fixation in human yaws has been reported by Castellani²² and Bowman,²³ using extracts of yaws as antigen, but these results could not be duplicated by Baermann and Wetter²⁴ in man, nor by ourselves in animals.

Cutaneous Reaction.

Nogouchi²⁵ was the first to succeed in producing definite skin reactions in man with his culture material—luetin. This subject is still in the developmental stage, however, as some strains seem more suitable than others, and the reactions so far occur chiefly in the tertiary and hereditary forms of the disease. As far as I know no comparative experiments have been made with different spirochaetae.

Comparative observations on strictly biological characters of the two organisms come to an end here, and it must be admitted that they are sufficiently meagre. Possibilities, however, have been opened up in several directions, and we may expect further information in the near future. Already we have additional and positive knowledge of an indirect sort in recent experiments with rabbits and in the action of salvarsan.

Experimental Lesions in the Rabbit.

As is stated above, distinctly different lesions have been produced in monkeys by inoculating the two spirochaetes. These have been noted by Halberstadter and many others. The lesion with *pertenuis* is more like the human yaw in being fungating and rich in serum; local autoinoculation is also distinctive. In the rabbit there is no marked difference in testicular or scrotal lesions except that the incubation period with *pertenuis* is considerably

shorter in the case of scrotal lesions. Castellani, however, working in Ehrlich's laboratory with a strain of *pertenuis* which I supplied, has recently succeeded in producing a generalized infection with *pertenuis*. According to Castellani²⁶ the lesions produced by *pertenuis*, which occur chiefly about the head, are distinctive. In a personal communication he says, "According to my opinion those appearances on the head are characteristic for frambesia in their macro and microscopic signs. I have never had corresponding results in rabbits injected with syphilis." These differences in lesions in the last analysis must, of course, be referred to biological, or, in other words, chemical or physical differences in the two organisms.

Reactions to Drugs.

In regard to mercury, Baermann and Wetter have observed that the Wassermann reaction may disappear in *pertenuis* infections after one or two injections of the salicylate—which is certainly not the experience with *pallida* infections. The most marked difference in reaction, however, is seen with salvarsan. In experimental infections in the rabbit I found that *pertenuis* infections could be permanently cured with doses which allowed relapses with *pallida* infections,²⁷ and Castelli says, "As to the chemotherapeutic effects, I can confirm your results that frambesia heals with a smaller quantity of salvarsan than syphilis does." Striking confirmation of these laboratory findings has been given by clinical experience. For example, Rost²⁸ has reported that out of 500 cases of yaws 81.8% were cured with one intramuscular injection, 15% with two, and the remainder with three injections, a total of 100% of cures. It is common knowledge that in syphilis the results have been worse than the reverse of these figures with one injection; it is only too true that less than 20% of miscellaneous cases can be cured with one injection. What is the cause for such radically different results with organisms which apparently resemble each other so closely? Evidently underneath apparent re-

semblances there lies a very real difference in resistance—but in what this consists we do not yet know. Is it a mechanical property which allows one organism to penetrate the tissues more deeply, or a resistant stage or some other defensive power?

Conclusion.

In conclusion, in spite of the well-known fallacies of genealogical theories, one is tempted to speculate on Doflein's grouping of the spirochaetae. Doflein places first the saprophytic spirochaetes which are found about the genitals and mouth, then the parasitic spirochaetes which may cause or at least grow in local lesions, then the blood, and finally the tissue parasites. In this connection one fact about yaws, which is rarely mentioned, should be emphasized, and that is that yaws frequently is a purely local skin disease like pemphigus, and if it spreads, spreads by autoinoculation. Of course, generalized eruptions are probably the rule, but I have seen a number of cases in which one or two yaws would occur at the point of contact, as on the neck of a parent who carried a child with yaws on the lips, and no other lesions would occur in months of observation. Then again, when the spirochaetae do circulate they find their way directly back to the skin, for which they seem to have an especial affinity. With such considerations as a starting point one may imagine that pallida and pertenuis were originally represented by one form or two forms which lived on man as a saprophyte, then as a local parasite, and that certain pre-pallida strains developed into blood and lymph parasites, while the pertenuis strain kept to the skin directly or after circulation.

A review of our present knowledge of the biological characters of *Sp. pallida* and *Sp. pertenuis* throws but little light on their distinctive pathogenic properties and shows the need for further investigation along some of the lines indicated.

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