STUDIES ON TREPONEMA PALLIDUM AND SYPHILIS.

III. THE INDIVIDUAL FLUCTUATIONS IN VIRULENCE AND COMPARATIVE VIRULENCE OF TREPONEMA PALLIDUM STRAINS PASSED THROUGH RABBITS.

BY HANS ZINSSER, M.D., J. G. HOPKINS, M.D., AND MALCOLM McBURNEY, M.D.

(From the Department of Bacteriology of the College of Physicians and Surgeons, Columbia University, New York.)

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In the course of the experimental work carried out at this laboratory in connection with antibody formation against Treponema pallidum, it has been necessary to keep a number of strains of this microorganism alive, in a virulent condition, by passage through rabbits. Incidental to this work we have felt it desirable to study fluctuations in virulence in individual strains and to determine, if possible, whether there is a variation in virulence between races of treponema obtained from different human sources.

A great deal of work has been done on rabbit syphilis, the most extensive study probably being that of Uhlenhuth and Mulzer. These writers observed a distinct increase of virulence in the course of rabbit passage. They found that after the fourth passage they often had 69 per cent to 70 per cent of takes, which rose to a frequent 100 per cent after the 11th or 12th generation, with a coincident progressive tendency for generalization.

Noguchi, in doing his earlier work on rabbit syphilis, noticed a distinct difference in the morphology of various strains of treponemata in that some strains were conspicuously thinner than others, and that this morphological difference has some direct correlation to ease of cultivation, agility of motion, and infectiousness. He describes three forms: a thin one with an average width of 0.2 of a micromillimeter, a thick form with an average thickness of 0.3 of a micromillimeter, and an intermediate one measuring 0.25 of a micromillimeter. He states that the thicker types do not produce any changes for 5 to 6 weeks and then

result in hard, indurated nodules which are sharply demarcated, while the thinner
ones usually have a shorter incubation time, producing a swelling of the testicle
in 10 to 14 days, which by gradual swelling is transformed into a large diffuse
lesion. Nichols\(^3\) has studied a strain derived from the central nervous system;
that is, from spinal fluid in a case of nervous relapse following salvarsan. It
corresponded to the thick form of Noguchi, and Nichols confirms the observation
that this type produces hard cartilaginous nodules. He states that the lesions
developed by his strain were easily differentiated from the soft, edematous proc-
eses produced by the strains obtained from chancres and mucous patches, and
calls attention to the fact that patients with nervous relapse often show papular
rather than macular secondary eruptions. He states that his strain had a char-
acteristic localization on the scrotal side of the tunica vaginalis and was char-
acterized by a great tendency to generalization, in that it produced chancres
on the opposite scrotum, nodules in the opposite testis, keratitis, and nodules
on the eyelids, which developed after an unusually short incubation time. It was
Nichols' impression that *Treponema pallidum* strains may vary extensively in
their invasive powers, and that a certain amount of prognostic deduction can
be drawn from a study of the rabbit pathogenicity of a strain isolated from any
given case.

These observations are, of course, of great importance for the
knowledge of the biology of these microorganisms and might have
serious bearing upon the clinical and immunological comprehension
of syphilis. We have therefore included a comparative study of the
strains that we have used incidental to our work in other fields of
observation in the hope of throwing further light upon these relations.

We have inoculated rabbits altogether from fourteen human cases,
one of them nervous system cases, about half of them from chancres,
and the rest from mucous patches and condylomata. We were
successful in starting the strains and carrying them to the second
generation in six of these cases. One of these, Strain O, was lost
after the third generation; another, Strain K, obtained from the
New York Department of Health, was lost after ten generations.
The other four have been carried along up to the present day and are
now in generations varying from the twelfth to the twenty-first.
Strain T, which we have run in addition to these, is Dr. Nichols'
strain, which he kindly sent to us in his own seventeenth generation,
and which has now gone through an additional nine generations in
our hands.

Text-figs. 1 to 5 represent fluctuations in percentage takes of Strains A, T, F, L, and S. The dots on the lines, marking angles, represent not generations, but groups of rabbits inoculated at one time, since often two or three lots of animals were inoculated from various lesions in a single generation. The total number of generations of each strain is given in Arabic numerals in the heading for each chart. The figures at the bottom of the charts represent the number of rabbits injected in each lot in the uppermost row, and the number of takes in the lowest row. The middle row represents animals which died within 2 months without showing lesions. Since in the earlier part of our work we were unable to prove out by puncture every lesion, we have omitted from our statistical percentages all doubtful lesions which might have been abscesses and were not proved microscopically, and we have also disregarded in the statistics all rabbits that died within 2 months without showing lesions. It is not improb-
able that *Treponema pallidum* can remain latent for long periods in the testicle without either growing or dying out, and for some reasons, not entirely clear, can later enter into a sudden phase of growth by which a lesion is produced. The work was rendered difficult also (and by this we account for the great irregularities in the charts in certain places) by the fact that in the course of these experiments,

![Chart showing positive reaction in successive generations of Strain F.](image)

which have covered a period ranging from October, 1913, up to the present time, there occurred occasional epidemics of bacterial infection which killed a large number of rabbits. Often also rabbits of unsuitable size with very small, undeveloped testicles were the only ones that could be procured. Furthermore, on a number of occasions, the material for injection was not the best, and this too tended to make great differences in the number of takes obtained.
When one studies these charts first, without qualifying considerations, in regard to the percentages of takes, the following facts become apparent.

**Text-FIG. 3.** Chart showing positive reaction in successive generations of Strain L.

**Strain A.**—One of the rabbits inoculated developed a lesion in the first generation, which, of course, though technically 50 per cent, means nothing considering the small number of rabbits, but in the second generation 90 per cent of the rabbits became syphilitic. In the next generation only 20 per cent, then in quick succession two lots with 100 per cent lesions were found, and after a number of gener-
ations in which in one lot we obtained no takes whatever, again 100 per cent of the rabbits in four successive inoculations were positive.

It is not necessary to specify individually the other charts since throughout all of them it is apparent that 100 per cent of takes often occur early in the passage of the strain, which returns again later with fluctuations between, and it is our impression in studying in detail the long series of rabbit inoculations that we have observed, that there is neither a rise nor fall in the virulence of the strains studied by us, but that an originally high virulence possessed by treponema derived from the human body for rabbits, remains high and fluctuates

Text-Fig. 4. Chart showing positive reaction in successive generations of Strain S.

Strain S, 12 Generations.
only according to other factors which we shall discuss later. We do not believe that there is a gradual increase of virulence, as stated by Uhlenhuth and Mulzer, but that these strains remain about the same throughout succeeding generations. Indeed, we believe that with

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\text{Text-Fig. 5. Chart showing positive reaction in successive generations of Strain T.}
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the most favorable conditions for inoculation after the first inoculation from man, when it can be obtained pure and can be injected into suitable rabbits, with favorable technique and in sufficient quantity, 100 per cent of takes, or very nearly that, would be the rule, at least in all the strains studied by us.
The factors upon which we believe that the percentage of takes depends are: (1) The quality of the material. When we have had large diffuse lesions (which are spoken of by most writers as edematous, but in which the soggy, soft mass is filled with a gelatinous, sticky exudate), in which large numbers of treponemata can be obtained in the press juice, which can be injected without much tissue detritus, the chances of obtaining a high percentage of takes are excellent. (2) The type of rabbit injected. We do not believe that the race or color of the rabbit has much to do with its susceptibility to syphilis, since we have seen no difference in this regard. We do believe that intratesticular inoculation is more successful when we have been able to get well grown rabbits with relatively large testicles in which it has been easy to inject directly into the testicular substance and diffuse the material throughout the testicle while slowly pulling out the needle. When small rabbits with undeveloped testicles were used and a good deal of the material has gone into the sack and the other surrounding tissues, the number of takes has been smaller. (3) The technique of injection. We find that the technique of injection has been of great importance. When the material was prepared as described above, the syphilitic testicle macerated thoroughly in a masticator, and often squeezed through cheese-cloth after that to remove tissue particles, and then this relatively clean material carefully injected into the center of the testicle itself, the percentage of takes has been much larger than in cases where the original material consisted of chancres or small indurations, where a reasonable amount of material could be obtained only with tissue detritus.

It seems to us also that some of the irregularity, especially in the early part of our work, may have been due to the fact that we have learned in the course of our studies that great frequency of examination is necessary in order that none of the lesions may be missed. Within the last month we have seen large, diffuse lesions which within 3 or 4 days have diminished in size, changing into small nodules, and in the course of another week have seemed to undergo complete retrogression. Rabbit 2 is an example of this. In this animal a large, diffuse lesion has completely disappeared within the course of a single week. Such rapid retrogression, of course, is noticed only in the cases of large, soft, myxomatous lesions, where there is no hard,
compact infiltration. The nodular and ulcerated lesions do not, of course, manifest the same speed of healing.

Other factors which must be considered in all such statistical studies are the frequent secondary infection or rapid necrosis of lesions and the almost invariable secondary infection of ulcerated lesions, in which even when the original focus was syphilitic, puncture reveals pus and bacteria, and only repeated puncture, and this with the chances against the investigator, may show treponemata under the dark-field.

Statements as to the difference in incubation time possessed by various strains have also engaged our attention, and we have been able to determine no regularity in this regard. In the case of the strains studied by us the incubation times have varied from 14 to over 190 days, and have averaged from 21 to 42 days. In the same strain large fluctuations have appeared, and we believe that this is subject to the modifying factors introduced by the nature of the material and manner of injection on the one hand, and the size and individual resistance of the rabbit injected, on the other. It does not appear from any of our observations that the length of incubation time has any relation at all to special characteristics of an individual strain. The incubation times observed by us were as follows:

Strain A ................................................. 14 to 48 days.
" F ................................................. 14 " 71 "
" L ................................................. 14 " 192 "
" S ................................................. 8 " 49 "
" T ................................................. 14 " 74 "

In explanation of the table it should be said that two of the 14 day early lesions were proved by puncture, and that as regards the longest periods tabulated it may well have been that a more frequent examination of the rabbits might have shortened this possibly by as much as 5 days or a week. However, even subtracting such periods, the great length of incubation time in some cases is apparent.

An observation that has been emphasized, especially by Nichols, is the apparent similarity of lesions caused by the same strain, on the basis of which he believes that the human disease may be to a certain extent governed by the type of treponema with which the individual is infected. Although in our earlier studies we did not follow this
point carefully, during the later months of our work we have paid attention to it, and it does not appear that any of our strains, even the one sent to us by Dr. Nichols, has manifested such regularity in pathogenic powers. As illustrative examples we may cite a few only which we think are alone sufficient to show that, in our hands at least, the difference in lesion has been governed by other fortuitous factors rather than by any consistent and inherent properties of the particular treponema used. At the time of writing we have on hand the following lesions:

Strain A.

Rabbit 3. Small nodular lesions in both testicles.

" 4. A hard nodule in one testicle which has followed a large, diffuse lesion in the same testicle.

" 5. A chancre with beginning abscess formation.

" 6. A diffuse, gummy lesion in the left testicle.

Strain F.

" 7. A very small, superficial skin induration and a small nodule in the right testicle. Has had a small nodular lesion in the left.

" 8. A very small central nodule in one testicle.

" 9. A large, diffuse lesion in the right testicle, just beginning to become indurated in the center.

Strain S.

" 10. Two nodules, hard and indurated, one in each testicle at the lower pole. 2 weeks ago this rabbit had had diffuse lesions of the edematous or myxomatous variety.

" 11. Large, diffuse lesions 2 weeks ago which now show as ulcerated nodules or chancres.

" 12. A beginning small, hard nodule in one testicle.

" 13. Soft, general enlargement of the testicle which looks like an early diffuse lesion.

In Strain T, the strain sent us by Dr. Nichols, we have not been able to observe a uniformity of lesions, this strain apparently having changed either because of our technique or by reason of its prolonged rabbit passage. In this strain we have had, it is true, a preponderance of indurated plaques and nodules in the testicle, but in a number of animals, large, diffuse lesions and chancrous lesions have appeared. For instance, in our third and fourth generation of T, we have had the following lesions:
Rabbit 14. Developed a chancre.

15. Had an indurated plaque on the left testicle.

16. Had a small gelatinous nodule at the globo-testicular junction with an edematous or myxomatous appearance of the whole testicle, which was loaded with treponemata. This testicle on palpation appeared to be normal, though generally enlarged.

17. Showed large, diffuse lesions of the myxomatous, soft type.

18. Another rabbit of the same generation of this strain as Rabbit 17 showed a nodule in the left testicle.

Neither in percentage of takes nor in speed of incubation time has this strain been markedly different from any of our others. The incubation time has varied from 14 to 74 days, which is well within the ranges shown by the others.

Another important point made by previous writers has been studied by us; namely, the relation of difference in morphology to pathogenicity. A distinct difference in thickness, as stated above, has been noted by so authoritative a writer as Noguchi, and we have looked for this in the rabbit pallida, often feeling sure that some of the strains did appear more slender and active than others. We have, however, taken lesions of four different strains and examined them with this particular point in view, having three observers check each other on the observation of the slides, without being able definitely to determine consistent difference in size. It is unquestionable that occasionally a strain looks thicker than other strains when taken from the testicle, but we have also seen thick and thin forms together in the same preparation. We have not measured, and do not wish to speak finally of this point, except in so far as we cannot at present definitely confirm consistent differences in morphology between the rabbit strains in our possession. We do not believe that such difference in the morphology, if it exists (and even in Noguchi’s hands the difference between the narrowest and the thickest is estimated as 0.1 of a micromillimeter), has a constant relation to difference in invasive property.
CONCLUSIONS.

1. As regards these strains, at least, there is no consistent change, either increase or decrease, of rabbit pathogenicity during progressive rabbit passage.

2. There is no difference in pathogenicity between these different strains, although some of them were isolated from condylomata, some from chancres, one from a mucous patch, and one by Nichols from the nervous system.

3. We do not believe that there is any consistency of difference in speed of incubation between these various strains.

4. We think that the nature of the lesion produced by any individual strain, and the incubation time as well, are dependent upon the fortuitous factors incident to the nature of the material used, the technique of injection, and the size of rabbit in which the injection is made.

As to the tendency to generalize possessed by one strain or another, we are not prepared to make a statement. We have not inspected as carefully as we might all our rabbits in regard to the more obscure syphilitic lesions such as small nodules on the eyebrows, nodules on the nasal bones, and internal lesions of the eye. As a matter of fact, we have noticed three cases of keratitis, probably syphilitic, and a number of preputial metastases, we have seen occasional nodules about the anus, and in one case a peritonitis in the fluid of which living treponemata were found on examination. There has not been in our series an extensive number of generalizations. We attribute this largely to the fact that all the studies here recorded were made on rabbits intratesticularly inoculated. It appears from the writings of other investigators that intravenous and intracardial injection of rabbits leads to a more extensive metastatic or general distribution. Two of the few rabbits inoculated intravenously showed testicular lesions. A series of rabbits intracerebrally inoculated through trephine openings showed nothing definite on autopsy, though one of them showed interference with reflexes and rigidity of the limbs for a time which seemed significant of pathological change. This rabbit, however, recovered entirely.