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As a complement to Dr. Grin's study on the campaign against endemic syphilis in Bosnia, Dr. T. B. Turner and Dr. D. H. Hollander of the International Treponematosis Laboratory Center were invited to submit this contribution on two strains of Treponema pallidum *isolated from two Bosnian patients.*

STUDIES ON TREPONEMES FROM CASES OF ENDEMIC SYPHILIS

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Introduction

In 1950 the World Health Organization made a grant to Johns Hopkins University for the establishment of an International Treponematosis Laboratory Center. The purpose of the centre was to study, in collaboration with field teams and other laboratories, certain problems which appeared to be basic to a long-range worldwide attack on the treponematoses. One of these problems was conceived to be an elucidation of the biological relationship between species and strains of treponemes isolated from different clinical syndromes as they existed in various parts of the world.[4]

Among the first of such syndromes selected by this laboratory for investigation was endemic syphilis, which was then under extensive field study in Yugoslavia by Dr. E. I. Grin [c] and his associates, with the support of WHO. In collaboration with Dr. Grin, two strains of treponemes from patients in Bosnia were successfully established in laboratory animals and subjected to further study in this laboratory.

Isolation of Strains

For the attempted isolation of strains, rabbits and hamsters were shipped by air from Baltimore to Sarajevo. The transfer of human material to animals was made by Dr. Grin, and the animals were returned promptly by air to Baltimore, where they were subsequently observed by the writers.

Transfers were made from three patients on 5 September 1950, with successful isolations in two instances. These two strains of treponemes have been designated as Bosnia A and Bosnia B.

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Bosnia A strain

The patient from whom this strain was obtained was a 35-year-old man, K. A. S., History No. 86/50. According to Dr. Grin's notes, the patient had mucous patches under the tongue and on the tonsils, papular secondary lesions on the face, trunk, and extremities, with some becoming pustular, and moist condylomata on the genitalia. Kahn and Meinicke serological reactions were positive.

The material for inoculation of animals was collected, in a mixture of serum and saline, from an ulcer on the shaft of the penis; dark-field examination of this material showed many treponemes. One rabbit was inoculated as follows : 0.5 ml was injected into the body of each testis, and 0.1 ml intradermally at each of four sites on the clipped back. One hamster was given intradermal inoculations in each side of the scrotum and at four sites on the back, the amount injected at each site being approximately 0.2 ml.

The animals arrived in Baltimore in good condition. On the 70th day after inoculation, slight changes were noted in each epididymis of the rabbit and, upon excision of the testis, typical motile treponemes were demonstrated. This material was transferred to the testes of two normal rabbits, which developed lesions in about 30 days. This strain has now been through 19 successive animal passages. No evidence of infection developed in the hamster inoculated directly from the patient, although the lymph nodes of the hamster were not examined.

Bosnia B strain

The patient from whom this strain was obtained was a 38-year-old man, N. G. G., History No. 19/50. Four other members of his family—his wife and three children—were also infected. Examination of the patient revealed mucous patches in the mouth and moist condylomata lata on the skin of the scrotum. Material collected from the latter lesions in serum-saline showed treponemes on dark-field examination. The Kahn and Meinicke tests were positive.

One rabbit was inoculated as follows : 0.4 ml into the body of each testis and 0.1 ml intradermally at four sites on the clipped back. The animal arrived back in Baltimore in good condition. On the 76th day after inoculation, lesions were noted in the testes, and on excision each showed enlargement of the head of the epididymis and pinpoint lesions on the surface of the testis. Transfers were made to two other rabbits, which developed testicular and cutaneous lesions in 21 days. This strain also has now been through 19 successive animal passages.

Bosnia C strain

Material was transferred to two hamsters only. Since the strain was not recovered from these animals, no further details will be given.

Character of the Lesions Induced in Rabbits

The Bosnia A and Bosnia B strains behaved so much alike in laboratory animals that the statements made here will apply to both unless otherwise indicated. Comparisons will be made with nine recently isolated strains of treponemes, as well as with the Nichols strain of *Treponema pallidum* which has been so widely used in laboratory studies. Among the recently isolated strains, three were isolated from patients with venereally acquired syphilis : Chicago, Baghdad A, and Baghdad B ; three from patients with bejel : Syria A, Syria B, and Iraq B ; and three from patients with yaws : Haiti A, Haiti B, and Indonesia B.

The Bosnia A strain has now been through 19 animal passages in rabbits. Of 38 animals inoculated intratesticularly, lesions in which *T. pallidum* has been demonstrated developed in 37. The Bosnia B strain has also been through 19 passages in rabbits at the time of writing with positively identified lesions in 40 of 42 animals inoculated intratesticularly. Twelve rabbits have been inoculated intradermally with the Bosnia A strain : in four it was the sole method of inoculation ; in the other eight, it was combined with testicular inoculation. Twelve rabbits have also been inoculated intradermally with the Bosnia B strain ; in four of these animals this was the sole method of inoculation.

Detailed comparative data on these strains will be published elsewhere. Suffice it to say here that the main difference among all these strains is in their capacity to produce indurated lesions in rabbits. This, in turn, appears to be related to the production, presumably by the treponemes, of a mucoid material which has been identified as hyaluronic acid. A study of histopathological material from rabbit lesions shows no significant qualitative difference between the Bosnia strains and other syphilis strains. Quantitatively, however, less metachromatic staining mucoid material is, in general, observed than with the other syphilis strains, but much more than with the yaws strains. The hypothesis has been advanced [7] that the principal difference in the reaction of the rabbit to the treponemal group of organisms resides in the production of hyaluronic acid. This difference may lie either in the amount of mucopolysaccharide produced, or in an actual chemical difference in the hyaluronic acids produced by different strains and species and a difference in the susceptibility of these products to the enzymes of the rabbit which are concerned with the disposal of hyaluronic acid.

Using as a criterion the extent to which mucoid material is present and indurated lesions are produced, both the Bosnia strains conform more to the pattern of that exhibited by rabbits inoculated with strains of venereally acquired syphilis—the Nichols, Chicago, Baghdad A, and Baghdad B strains—than they do to that induced by the strains of yaws treponemes under study. Indurated lesions of a type commonly encountered in syphilis

were observed in the first two or three passages ; and, while not all the inoculated animals have shown these typical lesions in subsequent passages, the Bosnia strains unquestionably have the capacity to induce syphilis-like lesions in the rabbit.

Character and Frequency of Lesions in Hamsters

Some years ago Bessemans and de Moor [1] noted that, when the golden or Syrian hamster (*Mesocricetus auratus*) was inoculated with *T. pallidum*, lesions ordinarily did not develop ; but the animal acquired a symptomless infection as manifested by the presence of readily demonstrable treponemes in the lymph nodes. More recently Geiman [2] has reported that lesions can be produced in hamsters by yaws treponemes but not usually by syphilis strains.

In our hands each of the 12 newly isolated strains has at one time or another produced specific, local, ulcerated lesions in hamsters after intradermal inoculation in the groin or scrotum or into the tissues of the lip. These lesions do not differ significantly in character according to the species of treponemes inoculated ; but, from our experience as a whole, it may be said that the yaws and bejel treponemes have produced lesions in a higher proportion of the hamsters inoculated than have the new syphilis strains. Moreover, most of the hamsters which have developed very extensive lesions have been inoculated with either yaws or bejel treponemes. Since a number of factors, such as the temperature of the environment and the size of the inoculum, have not been adequately controlled, it cannot be said at this time that there is a real difference in the behaviour of this animal species to different strains of treponemes. Almost without exception, however, motile treponemes can be demonstrated in the lymph nodes of hamsters inoculated with syphilis as well as with yaws and bejel treponemes.

It is of interest, however, that, unlike the lesions induced in rabbits by *T. pallidum*, the lesions in hamsters exhibit little, if any, induration, although they are customarily swarming with treponemes. What may be the biological basis of this variation in the response of these two animal species to the same strain of *T. pallidum* is not known ; again, it is possible that the hyaluronic-acid/hyaluronidase system may be involved.

Of eight hamsters inoculated with the Bosnia A strain, five developed external dark-field-positive lesions, and treponemes were demonstrated by dark-field examination of the inguinal nodes of the other three. The Bosnia B strain produced lesions in five of seven hamsters inoculated, and each of the other two animals showed motile treponemes upon dark-field examination of the inguinal lymph-nodes. These results are cited as a matter of interest only, since no conclusions of a comparative nature can be drawn from them.

Immunological Relationship

The immunological relationships between the newly isolated strains and the Nichols strain which was isolated in North America in 1911 were examined by a series of challenge inoculations. Rabbits were inoculated intratesticularly with the Nichols strain of *T. pallidum* and the animals allowed to progress through the usual evolution of the disease. Between three and four months after the original inoculation, at a time when the animals were known to be immune to the homologous strain, groups of four of these rabbits, together with four normal rabbits as controls, were inoculated intradermally on the back with the challenge strain. Failure of lesions to develop in the previously infected rabbits, when characteristic lesions developed in the controls, was regarded as evidence of a substantial degree of cross-immunity between the two strains.

Employing the foregoing experimental method, the following results were obtained : of four animals previously infected with the Nichols strain, none developed lesions upon challenge with 5,000 treponemes of the Bosnia A strain injected into each of four sites, whereas four control animals similarly inoculated developed a characteristic pattern of lesions. The same results were obtained when a group of four rabbits immune to the Nichols strain was similarly inoculated with the Bosnia B strain, together with four normal controls.

It is concluded on the basis of the foregoing evidence that both of the Bosnia strains are closely related immunologically to the Nichols strain. Since numerous other syphilis strains have also been shown to bear a similar relationship to the Nichols strain [5] it is concluded that the Bosnia strains behave, in this respect, like most other syphilis strains isolated in various parts of the world, but more particularly in North America.

Penicillin Sensitivity of Bosnia Strains

Laboratory studies on the sensitivity of a particular strain of treponemes cannot be interpreted directly in terms of treatment of the clinical disease in patients. Nevertheless, laboratory data of a comparative nature can be very useful when viewed in the light of clinical experience with one or another of the treponematoses. The Bosnia strains have been subjected to laboratory study with this point in mind.

The penicillin sensitivity of these strains was tested by in vivo and in vitro methods. The former is a relatively crude method devised in this laboratory [6] and has been used for screening purposes only. Using the Nichols strain as a standard of reference, it has been determined that the Bosnia strains when tested in either rabbits or hamsters respond in essentially the same manner as the reference strain and the other newly isolated syphilis strains.

A more sensitive index of penicillin effects is provided by the in vitro methods developed and applied by our associate Miss Ellen Nell. These methods are essentially those employed in testing other organisms by the dilution method, with special adaptations to the more difficult problem of assay against the treponemal group of organisms, as described elsewhere.[3]

Again employing the Nichols strain for reference, it has been found that the amount of penicillin which will render 50% of the treponemes non motile in 18 hours, using control tubes for purposes of comparison, is of the order of 0.0019 μ per ml of test material. The corresponding figure for Bosnia A is 0.0013 and for Bosnia B, 0.0012, both of which are well within the limits of variation of the method. It is of interest, too, that all other strains tested fell within these general limits.

It seems fair to conclude, therefore, that the Bosnia strains of endemic syphilis do not vary significantly in their penicillin sensitivity from other strains of treponemes isolated in other parts of the world and from various clinical syndromes. These results suggest that types of penicillin treatment which are found to be effective in other treponemal diseases will be equally effective in the endemic syphilis of Bosnia. Should significant clinical differences be noted between the reaction of the host in endemic syphilis and that commonly observed in venereally acquired syphilis, it appears that they may best be explained by epidemiological or sociological factors rather than by important biological differences in the infecting treponemes.

SUMMARY

Two strains of treponemes, designated as Bosnia A and Bosnia B, were established in laboratory animals which were flown from Sarajevo, Yugoslavia, to Baltimore, Md., USA, where their biological relationship to strains isolated from other clinical syndromes appearing in various parts of the world was studied at the International Treponematosis Laboratory Center. Both strains went through 19 successive animal passages in rabbits, and it was found that they resembled each other in all the characteristics noted in the laboratory. Moreover, the disease picture which the strains produced in rabbits and hamsters had many characteristics in common with that produced by other strains of *Treponema pallidum* in North America and elsewhere.

A close immunological relationship was also demonstrated between the Bosnia

RÉSUMÉ

Deux souches de tréponèmes, dénommées « Bosnie A » et « Bosnie B », ont été inoculées à des animaux de laboratoire qui ont été envoyés par avion de Sarajevo (Yougoslavie) à Baltimore, Md. (Etats-Unis d'Amérique). Les auteurs ont étudié, au Laboratoire central international des Tréponématoses, la relation biologique existant entre ces souches et celles qui avaient été isolées d'autres syndromes dans différentes parties du monde. Après 19 passages successifs sur le lapin, toutes les caractéristiques de ces souches observées au laboratoire se sont avérées identiques. En outre, les manifestations pathologiques déterminées par ces deux souches chez le lapin et le hamster présentent de nombreux traits communs avec celles qui ont été produites par d'autres souches de *Treponema pallidum* en Amérique du Nord et ailleurs.

Une relation immunologique étroite a pu être démontrée entre les souches de

strains and a reference strain of *T. pallidum* (the Nichols strain) to which numerous other strains of the same treponeme have been shown to be similarly related. Strains of treponemes isolated from some patients suffering from bejel or yaws have also shown this same close immunological relationship.

The Bosnia strains demonstrated the same order of sensitivity to penicillin as the Nichols strain and other recently isolated strains of syphilis, bejel, or yaws treponemes.

The authors conclude that the Bosnia A and Bosnia B strains belong to the general group of strains commonly designated as *T. pallidum* and that the response to therapy of patients with endemic syphilis should follow much the same pattern as that observed in patients with venereally acquired syphilis.

Bosnie et une souche de référence de *T. pallidum* (souche Nichols), dont la parenté avec de nombreuses autres souches du même tréponème a été également prouvée. Des souches de tréponèmes isolées de quelques malades atteints de béjel ou de pian ont montré la même étroite relation.

Les souches de Bosnie présentent une sensibilité à la pénicilline analogue à celle de la souche Nichols et d'autres souches récemment isolées de tréponèmes de la syphilis, du béjel ou du pian.

Les auteurs concluent que les souches Bosnie A et Bosnie B appartiennent au groupe général communément désigné comme *T. pallidum* et que l'effet du traitement devrait être à peu près le même chez les malades atteints de syphilis endémique que chez les malades souffrant de syphilis vénérienne.

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