

## **APPENDICES**



## APPENDIX 1

## THE SOURCES AND ISOLATION OF STRAINS

In the isolation of treponemal strains from different parts of the world the International Treponematoses Laboratory Center has had most willing and helpful collaboration from physicians and scientists in many countries. The following notes which refer to numbers listed in Table I<sub>D</sub> can give only feeble credit to those who assisted in this undertaking; doubtless the names of some individuals who contributed time and effort have been unintentionally omitted. Without the assistance of the Venereal Diseases and Treponematoses Section of WHO, under the direction of Dr T. Guthe, in the coordination of research, many of these investigations would not have been possible.

Details concerning unsuccessful isolations are not included.

(1) *Syria A strain*—Transfers made by Dr John C. Hume and Dr Emil Rizk from lip lesion of patient A. M., aged 6 years, who had typical bejel lesions of about 3-4 months' duration. The animals were inoculated on 6 May 1950, and arrived in this laboratory on 17 May 1950.

(2) *Syria B strain*—Transfers made by Dr Hume and Dr Rizk from vulva lesion and lower lip lesion of patient W. D., a child of unstated age. Lesions were regarded as typical bejel. The animals were inoculated on 6 May 1950, and arrived in this laboratory on 17 May 1950.

(3) *Bosnia A strain*—Transfers made by Dr E. I. Grin, who wrote as follows: "I selected three typical cases with early secondary syphilis lesions. All three are peasants, residents of the north-east part of Bosnia where syphilis is endemic." All patients were from remote villages. This strain was isolated from a 35-year-old male patient K. A. S. (No. 86/50), who had mucous patches under the tongue, on the tonsils and papular secondary lesions on the face, less on the trunk and extremities, some of which, however, were pustular, and moist condyloma on the genitalia. Serological tests—Kahn and MKR II—were positive. Material for inoculation was taken from an ulcer on the shaft of the penis. Darkfield showed many treponemes. Inoculations were made on 5 September 1950, and the animals arrived in this laboratory on 22 September 1950.

(4) *Bosnia B strain*—Transfers made by Dr E. I. Grin. The comments made on Bosnia A strain apply here, too. This strain was isolated from patient N.G.G., a 38-year-old male, whose wife and three children also had early syphilis. Patient had secondary syphilis lesions in mouth and on skin of scrotum. Darkfield-positive material was collected from scrotal lesions. Kahn and MKR II tests were strongly positive. The animals were inoculated on 5 September 1950, and arrived in this laboratory on 22 September 1950.

(5) *Baghdad A strain*—Transfers made by Dr M. Tuomioja and Dr E. H. Hudson from patient S. H., a 20-year-old auctioneer, single, living in Baghdad. There was a history of sexual exposure about two months previously and physical examination showed a primary syphilitic lesion on the penis with typical regional adenitis. Material from the lesion was darkfield positive and blood serological tests (Laughlen, Wassermann, Kahn and Rein-Bossak) were strongly positive. The animals were inoculated on 30 December 1950, and received in this laboratory on 6 January 1951.

(6) *Baghdad B strain*—Transfers made by Dr M. Tuomioja and Dr E. H. Hudson from patient J. A., an unmarried male fisherman of about 40 years of age. There was a history of repeated sexual exposure and a penile lesion of about 15 days' duration. Examination showed a typical primary syphilitic lesion on the glans penis with regional lymphadenopathy. Material from the lesion was darkfield positive and blood serological tests (Laughlen, Kahn, Wassermann, and Rein-Bossak) were strongly positive. Transfers were made on 30 December 1950, and the animals were received in this laboratory on 6 January 1951.

(7) *Samoa A strain*—Transfers made by Dr M. J. Marples from patient No. 11—J., an 18-month-old male resident of Apia, West Samoa. The patient had frambesiform lesions scattered in large numbers over the body. Material for transfer was taken from a large frambesioma on the right thigh and from two smaller lesions on the left ankle. After dilution with saline, the material was darkfield positive, showing about three treponemes per field. The animals were inoculated on 12 January 1951, and received in this laboratory on 25 January 1951.

(8) *Chicago strain*—Transfers made by Dr T. B. Turner and Dr J. Rodriguez from patient W. McD. (No. 3163), a Negro male about 25 years of age. There was a large, greatly indurated annular primary syphilitic lesion on the prepuce of about 3 weeks' duration. Material collected from the lesion showed approximately 12 000 000 treponemes per ml on darkfield examination. Transfer was made on 9 February 1951, and the animals were received in this laboratory on 17 February 1951.

(9) *Indonesia B strain*—Transfer made by Dr Huang-Ying Li from patient S. a female of about 11 years of age, who had had frambesiform yaws lesions for approximately one month. Residence, Kemajoran Bendungan, Djakarta. A brother and a sister also had early yaws at the time. Transfers were made from typical frambesioma on cheek; darkfield on inoculated material showed 8-10 treponemes per field. The animals were inoculated on 3 March 1951, and received in this laboratory on 8 March 1951.

(10) *Haiti A strain*—Transfer made by Dr S. Levitan from patient M. E. (No. 480), a male aged 9 years, resident in Commune de Bainet. The patient had typical generalized frambesiform yaws, with history of initial lesions 6 months previously. Material transferred was darkfield positive. The animals were inoculated on 7 March 1951, and received in this laboratory on 10 March 1951.

(11) *Haiti B strain*—Transfer made by Dr S. Levitan from patient J. L. S. (No. 482), an 11-year-old male resident of Commune de Côtes de Fer. Patient had typical generalized frambesiform yaws with history of initial lesion about 5 weeks previously. Lesions on the lower abdomen were used for transfer. The animals were inoculated on 7 March 1951, and arrived in this laboratory on 10 March 1951.

(12) *Iraq B strain*—Transfer made by Dr E. H. Hudson, Dr M. Tuomioja and Dr G. Csonka from patient S. (No. 3920), a female aged 7 years, who had oral mucous patches and anal condyloma regarded as typical of bejel. Inoculated material was received in this laboratory on 8 May 1951.

(13) *Mexico A strain*—Transfers made by Dr J. Olarte from patient G. A., an 18-year-old male who had a typical primary syphilitic lesion of 10 days' duration. The transferred material was darkfield positive. The animals were received in this laboratory on 17 January 1953.

(14) *Samoa D strain*—Transfers made by Dr M. J. Marples from patient I., a 7-month-old male resident of Apia, Western Samoa, with typical generalized lesions of yaws. Transfers were made from frambesiomias, which were darkfield positive. The animals were inoculated on 24 January 1953, and arrived in this laboratory on 30 January 1953.

(15) *Samoa E strain*—Same note as for (14), except transfers were made from patient M., a 3-year-old male with typical generalized yaws. Material was obtained from fram-besiform lesions.

(16) *Samoa F strain*—Same note as for (14), except transfers were made from patient M., a 4-year-old male with typical generalized yaws lesions.

(17) *Bechuanaland C strain*—Transfers made by Dr J. F. Murray from patient G. G. (No. R.N. 8465), a Bantu female, aged 22 years, resident in the Suping district of Bechuanaland. This patient had vulva and anal condylomata and mucous patches on the soft palate and fauces of about 4 months' duration. These lesions were darkfield positive and were regarded as typical of the non-venereal treponematosi s syndrome designated "dichuchwa", although since the patient was an adult, the presence of venereally acquired disease could not be ruled out. Inoculations were made on 3 April 1954, and the animals were received in this laboratory on 8 April 1954.

(18) *Bechuanaland D strain*—Transferred by Dr J. F. Murray from patient M. M. (R.N. 8458), a Bantu female 6 years of age residing in the Molepolole district of Bechuanaland. This patient showed mucous patches on fauces and buccal mucosa of about 2 months' duration. Darkfield examination of the lesions was positive and the case was regarded as typical of non-venereal treponematosi s or "dichuchwa". The animals were inoculated on 3 April 1954, and arrived in this laboratory on 8 April 1954.

(19) *Gambia A strain*—Transfers made by Dr A. H. Davies from B. K. (Case No. 7) a 4-year-old female of the Losola (Fula) tribe in Gambia, West Africa. The patient had an ulcer on the buccal mucosa and inner surface of the lip which had been present one year. The lesion was darkfield positive and was regarded as typical of the non-venereal treponematosi s syndrome known as "siti". Transfers were made on 9 July 1955 to two hamsters, and the animals were received in this laboratory on 23 July 1955.

(20) *Gambia B strain*—Transfers made by Dr A. H. Davies from patient S. S. (Case No. 8) an 11-year-old member of the Tukulor (Fula) Tribe, residing in Diganteh Central Division, Gambia. The patient had had a small rounded ulcer on the buccal surface of the upper lip for about one year. The lesion was darkfield positive and was regarded as typical of the non-venereal treponematosi s syndrome known as "siti". Transfers were made on 9 July 1955, and the animals were received in this laboratory on 23 July 1955.

(21) *Gambia C strain*—Transfers were made by Dr A. H. Davies from T. S., a 4-year-old male of the Tukulor (Fula) Tribe who had had darkfield positive ulcers on the upper lip for 10 months. The inoculations were made on 9 July 1955, and the animals were received in this laboratory on 23 July 1955.

(22) *Gambia D strain*—Transfers made by Dr A. H. Davies from O. G. (Case No. 10), a 4-year-old male who had had sore areas on the face, upper lip, right shoulder and scrotum for about one year. Transfers were made from the lip lesion, which was darkfield positive. This was regarded as a typical case of "siti". Inoculations were made on 9 July 1955, and the animals were received in this laboratory on 23 July 1955.

We wish to thank Dr James A. McFadzean, who made the initial arrangements for the transfers of the Gambia strains.

## APPENDIX 2

## PREVENTION AND TREATMENT OF LABORATORY ACCIDENTS

It appears that pathogenic treponemes do not lose their virulence for man on repeated animal passage (see Chapter 7). There is, therefore, an inherent risk involved in experimentation with this group of organisms, although the risk can be minimized by attention to details which at first glance might seem trivial. In our laboratory most accidents involving the risk of treponemal infection to laboratory personnel have arisen from the following circumstances: (*a*) inadvertent use of unplugged pipettes; (*b*) use of syringes to which the needle is not firmly attached and which becomes separated under pressure, and (*c*) piercing of the operator's hand with an infected needle when an animal suddenly struggles.

In regard to the last eventuality, even with ordinary care an animal may struggle or jump suddenly during an injection. For intratesticular inoculation rabbits should be held by an assistant, the ears and the scruff of the neck being firmly grasped with one hand, and the hind legs with the other. In all inoculations and bleedings, the syringe should be held in such a manner that the needle is never pointed at the hands of the operator or his assistant. Difficult inoculations are more safely performed under nembutal anesthesia in the case of rabbits or ether in that of hamsters.

The availability of penicillin as a relatively non-toxic treponemicidal agent has materially altered the approach to the clinical management of laboratory accidents with these organisms. It now seems wise to give treatment in cases which would not have warranted the risk of arsenical therapy. Nevertheless, the use of penicillin is not without some attendant risk, and there remains the need for care in handling treponemes in order to avoid accidents.

Laboratory accidents with treponemes should be dealt with by the general principle that if any parenteral treatment is warranted, sufficient should be given to destroy all the treponemes which may have entered the body. The precise manner of treatment should take into consideration the material and the route of inoculation, on the one hand, and the best contemporary clinical judgement of therapy, including the risks of hypersensitivity, on the other. The clinical decision is arrived at after consciously or unconsciously applying the laws of chance and weighing the risks of infection against the risks of treatment complications.

Accidents involving contact of the treponeme with the unbroken skin involve relatively little risk, since treponemes are readily destroyed by soap and water. At the other extreme, accidents involving penetration of known infectious material, in effect a direct inoculation, clearly warrant treatment. Intermediate situations may require careful evaluation of the possibility that treponemes have entered the body, in order to arrive at a decision on the advisability of treatment.

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