

**Part I**

**BIOLOGY OF TREPONEMAL INFECTIONS**



## SOURCES OF STRAINS STUDIED

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### Clinical and Epidemiological Entities

Syphilis and yaws as distinct disease syndromes have been recognized for over 400 years. Speculation concerning their relationship considerably antedates the discovery of the etiologic agents, *Treponema pallidum* and *T. pertenue*, in 1905, the former by Schaudinn & Hoffman<sup>16-18</sup> and the latter by Castellani.<sup>4</sup> While this speculation still continues, few informed persons deny that clinical and epidemiological differences do, in fact, exist between the naturally occurring diseases; and most will agree that distinguishing names for the two syndromes are both useful and biologically justifiable.

Since these two diseases obviously have many features in common, the real question is: What is the basis for the observed differences, and how stable are the distinguishing characteristics? Ultimately the question of whether two disease syndromes are the same or different becomes a philosophical one and perhaps even a semantic argument; it is probable that arm-chair dialectics have contributed about all that may be expected to the syphilis-yaws problem, and there remains the need for fundamental comparative data under more or less controlled conditions.

Another disease syndrome that comes clearly within the general category of the treponematoses is pinta, or mal del pinto. Since the clinical and epidemiological pattern of this disease does not closely resemble syphilis or yaws, its biologic relationship to these syndromes was not suspected until about twenty-five years ago when an unusually high incidence of positive Wassermann reactions in pinta patients was commented upon by Menk,<sup>12</sup> and by González-Herrejón & Pallarés.<sup>7</sup> In 1938 Sáenz, Grau Triana & Armenteros<sup>15</sup> found treponemes in a patient with pinta, and León-Blanco<sup>10</sup> and others subsequently showed that treponemes resembling *T. pallidum* were regularly present in certain types of pinta lesions.

The three foregoing syndromes represent perhaps the most clear-cut entities within the treponematoses group. Yet there are many others that also clearly belong within the group, but which for lack of extensive study or because of marginal differential criteria have not achieved standing as

distinct clinical entities. Among this group may be mentioned bejel, which occurs characteristically among nomadic tribes of the hot dry areas of Asia Minor and the Mediterranean area; endemic syphilis, in sections of the Balkans; dichuchwa, in Bechuanaland and adjacent areas; njovera, in Southern Rhodesia; and siti, in British West Africa. All these are basically endemic syphilitic infections acquired in infancy.

In addition to these syndromes observed in man, there is a naturally occurring disease of domestic rabbits, known in the medical literature as venereal spirochetosis of rabbits,<sup>1, 14</sup> which is biologically related to the treponematoses of man.

In this monograph comparative data on the behavior of treponemes of most of these syndromes will be presented, and an attempt made to determine wherein they resemble one another or differ.

### The Experimental Approach

It is only possible to carry out adequately controlled experiments on disease in man under exceptional and almost always difficult circumstances. And so it has been with the treponematoses. Many observations of great value have been made on the treponemal diseases of man; but in general these have been of a descriptive nature, or else—as in the therapeutic use of penicillin—so striking that there is no question of the validity of the results.

There remain, however, many questions of the fundamental biology of the disease which can scarcely be studied in man under controlled conditions, and it is necessary, therefore, to turn to the laboratory for clues as to the answer to these questions. The limitations of such an approach are readily conceded, yet it is the only one which at the moment gives promise of worth-while results.

In the laboratory, it is practicable to study the behavior of different strains and species of treponemes in the same host species maintained under virtually identical conditions; or by working with the same strain of treponeme to observe its behavior in a host species subjected to various modifying procedures. Even here, however, the relatively leisurely pace of the evolution of the treponemal disease process poses difficulties, and at the least makes it necessary to carry out experimentation on a time-scale much too extended to be fully consonant with the time-scale for the development, maturation and senescence of the individual human investigator.

Then, too, while one is not handicapped in experimental treponemal research by some of the enormous difficulties under which the investigator of cancer or leprosy, for example, must work, inability to cultivate *in vitro* the etiological agents of the treponemal diseases imposes serious limitations, particularly in attempts to study the metabolism of the treponeme or its antigenic structure. Such limitations, however, properly serve as a challenge to the investigator and indeed at times become ends in themselves.

Emphasis will be placed on what has been accomplished, without undue preoccupation with investigations in which failure has been the result.

### *Definitions*

In every laboratory a scientific jargon—a kind of shorthand or abbreviated language—develops which is useful in conveying ideas with the least number of words. The reader will be spared most of these, but a few expressions, of necessity, recur so frequently that it seems permissible to define them here and continue to use them throughout this monograph. The principal examples are the following:

*Syphilis treponeme* (or yaws treponeme, bejel treponeme etc.)—One or another strain of *T. pallidum*, *T. pertenue* or *T. carateum* originally derived from a typical case of syphilis, yaws, bejel or endemic syphilis or pinta. Since much of the experimental work in this laboratory was carried out with the Nichols strain of *T. pallidum*, this strain will usually be the one used when no other identification is given. The word treponeme as used here denotes a pathogenic spirochete belonging to the genus *Treponema*.

*Cuniculi infection, cuniculi treponeme*—The disease “spontaneous venereal spirochetosis of rabbits” originally described by Ross<sup>14</sup> and Bayon,<sup>1</sup> and the treponeme, *T. cuniculi*, which is the etiological agent of the disease.

*Wassermann antibody*—The substance in the serum of human beings or experimental animals infected with treponemes that is measured by the standard serological tests using lipoidal antigens. The term “reagin” is synonymous with Wassermann antibody.

*Standard serological tests (STS)*—Those tests which detect Wassermann antibody by flocculation or complement-fixation reactions with lipoidal antigens, including Wassermann, Kahn, Eagle, Mazzini, Kline, Hinton, and other similar tests. These tests are grouped together in contradistinction to tests which measure other antibody, for example immobilizing or agglutinating antibody. (For further discussion of this subject see Chapter 5.)

Cardiolipin antigen and the VDRL test are included, for the purposes of this monograph, in the category of standard serological tests.

*TPI test*—Treponemal immobilization test.

*TPA test*—Treponemal agglutination test.

*Darkfield examination: darkfield positive, darkfield negative*—The microscopic examination of material using darkground illumination. The material is said to be darkfield positive, or darkfield negative, depending on whether or not treponemes are demonstrated by this method.

*Infectivity test*—Unless otherwise specified, this term refers to the inoculation of material into an animal in order to determine whether the inoculated material contains virulent treponemes. Such tests are commonly made by inoculating the material into one or both testes of two normal

rabbits, which are then observed for 90 days. The development of characteristic lesions, in which treponemes can be demonstrated by darkfield examination, constitutes a positive test. While a positive infectivity test is proof that the inoculated material contained rabbit-virulent treponemes, a negative test does not necessarily exclude their presence, although it constitutes valuable evidence on that point particularly with a well-adapted laboratory strain of syphilis treponemes.

*Normal or negative animal*—These terms are colloquial but useful. Strictly speaking there is probably no such thing as a “normal” animal. As used here it means simply that the animal has not previously been infected with the particular agent under discussion.

The term “negative” is used to indicate that the animal shows no signs or symptoms suggestive of the specific disease process in question.

#### *Classification of spirochetal organisms*

It is of the essence of the thoughtful biologist that he is at once busily engaged in cataloguing the individuality of living forms, while at the same time he is seeking for common denominators that will reveal relationships and similarities hitherto hidden.

In the microscopic world of the bacteria three principal morphologic categories have long been distinguished. These three, or modifications thereof, are the spherical forms, the rod-like or perhaps more strictly speaking the cylindrical forms, and the helical, or those that have a spiral shape. Because perhaps the spiral micro-organisms have not been as well studied as many representatives of the other two groups, they are less familiar and a little more mysterious to most medical biologists. There is a tendency, therefore, stemming perhaps from this ignorance, to regard all these spiral micro-organisms as being in some way related biologically. And indeed they are, in that they have a common form; but it is unwise to go much further than this, except where modern methods provide a solid basis for conjecture. Unfortunately, for the course of easy assumptions, studies have frequently revealed differences rather than similarities when varieties of spirochetal organisms have been compared.

In the last edition of Bergey's *Manual of determinative bacteriology*,<sup>2</sup> which represents the consensus of informed American opinion, essentially the following classification of the spiral organisms (not including the vibrios which are only slightly spiral) is given:

#### ORDER V *Spirochaetales* Buchanan

##### FAMILY I *Spirochaetaceae* Swellengrebel

Spirals 30-500  $\mu$ , with definite protoplasmic structures

Genus I *Spirochaeta* Ehrenberg—No periplast or cross-striations

Genus II *Saprospira* Gross—Free living, periplast and cross-striations

Genus III *Cristispira* Gross—Parasitic, periplast and cross-striations

FAMILY II *Treponemataceae* SchaudinnSpirals 4-16  $\mu$ , without obvious protoplasmic structureGenus I *Borrelia* Swellengrebel—Stain easilyGenus II *Treponema* Schaudinn—Anaerobes, stain with difficultyGenus III *Leptospira* Noguchi—Aerobes, stain with difficulty

There is no conclusive evidence indicating a close biological relationship between the *Treponema*, and either the free-living *Spirochaeta*, *Saprosyria* and *Cristispira* on the one hand, or the more dependent genera such as the *Borrelia* and *Leptospira* on the other. Indeed, there is much bacteriological, immunological and pathological evidence which suggests that the *Treponema* are no more closely related to these other genera of spiral organisms than to many micro-organisms that are spherical or rod-shaped.

It would be attractive to speculate about such relationships in the light of modern genetics, and to conceive the spiral form as a thread linking all these groups, but there is no evidence to support such a linkage and one can scarcely afford the luxury of this idle speculation if one is not prepared to apply some well-established technical methods to the study of the problem.

So let us, therefore, dismiss from consideration for the time being other groups of spiral organisms not morphologically identical with the *Treponema*, on the basis that there is little or no evidence indicating a useful degree of biological relationship between these other groups and the organisms with which this monograph is primarily concerned.

*Classification within the Treponema group.* No entirely satisfactory classification of organisms belonging to the *Treponema* group is now available. Unfortunately, despite much application to this problem, we have no new or improved scheme to propose, although the subject will be discussed again in Chapter 10. As a working classification we have adopted the following, which clearly rests on clinical and epidemiological considerations:

1. *Human pathogens primarily:* Includes the causative agents of
  - (a) syphilis (*T. pallidum*)
  - (b) yaws (*T. pertenue*)
  - (c) pinta (*T. carateum*, sometimes called *T. herrejoni*, *T. pictor*, *T. americana*, *T. discromoderma*, or *T. pintae*)
  - (d) bejel and other non-venereal syndromes which clinically and epidemiologically appear to be closely related (*T. pallidum*, or bejel treponemes etc.)
2. *Animal pathogens primarily:* Includes the causative agent of non-venereal spirochetosis of rabbits (*T. cuniculi*).
3. *Human saprophytes primarily:* Includes the mouth spirochetes (*T. macrodentium* and *T. microdentium*) and related spirochetes often present about the anus and in fecal material. Included here are also

the culture spirochetes of which the best known are the Reiter; Kroo; Noguchi; Kazan; and Nichols strains.

*Strains of treponemes studied*

Over the years some 76 different strains of *Treponema* have been isolated, and most of them propagated for a time at least in animals, at the International Treponematoses Laboratory Center—the senior author's laboratory. We shall use the term "strain" here in its commonly accepted sense as being a collection of individual *Treponema* which were obtained originally from the same source, commonly a human being or an animal with a naturally occurring infection, and propagated in straight-line succession.

Some of these strains were isolated primarily for the purpose of making comparative studies of one sort or another; others were isolated during the study of some other aspect of the biology of the treponematoses. In most instances, however, care was taken to assure that a particular strain was obtained from a source which could be regarded as typical of the clinical disease to which medical custom has attached a distinguishing name. Thus, with rare exceptions the strains of syphilis spirochetes and yaws spirochetes were secured from patients selected precisely because they presented classical evidence of the respective disease syndromes according to the clinical and epidemiological criteria of informed observers. The same statement can be made for the other treponemal syndromes studied, although often in these instances clinical and epidemiological criteria may have been less clear-cut.

In describing below the strains studied in this laboratory, therefore, no apologies will be made for designating these as syphilis or yaws strains (*T. pallidum* and *T. pertenue*, respectively) as the case may be. We have, however, in the recent studies in collaboration with the World Health Organization, designated these strains by the locality of origin in an effort to divest ourselves of any prejudicial notion, in so far as this is possible, concerning the biological inter-relationships existing among these strains.

Of the 76 strains studied, 39 were from patients with a clinical diagnosis of syphilis; 20 were from patients with a clinical diagnosis of yaws; 3 from patients with bejel; 8 from endemic syphilis or one of the treponemal syndromes which is recognized by a local name only; and 6 strains were isolated from a naturally occurring disease in rabbits: venereal spirochetosis of rabbits. In addition, treponemes from patients with pinta were recovered three times in the initial passages in hamsters, but the strains were lost on subsequent passage.

These 76 strains were isolated at four different periods. The first group in point of time comprised 21 strains from syphilis and yaws patients isolated during the years 1932-35 by the senior author and his associates—at that time on the Jamaica Yaws Commission. All strains in this group were

TABLE IA. STRAINS OF TREPONEMAL ORGANISMS ISOLATED AND PROPAGATED IN LABORATORY ANIMALS: STRAINS ISOLATED FROM PATIENTS LIVING IN JAMAICA, B.W.I.

Strain designation	Date of transfer to laboratory animals	Patients' initials	Clinical diagnosis	Incubation <sup>a</sup> period in 1st animal-passage (days)	Total animal passages observed	Still available in this laboratory <sup>b</sup>
YA	30. 3.32	S. C.	Yaws—generalized	28	47+	Yes
Undesignated <sup>c</sup>	2. 5.32	M. S.	„	35	3	No
YB	30.11.32	J. D.	„	—	10	„
Undesignated	6. 3.33	L. P.	„	50	1	„
„	23. 3.34	A. W.	„	54	1	„
„	27. 4.34	E. S.	„	67	1	„
„	2. 5.34	M. C.	„	52	3	„
YC	21. 6.34	A. W.	„	40	9	„
YD <sup>d</sup>	6. 7.34	C. T.	„	43	25+	Yes
YE	27. 7.34	M. S.	„	60	6	No
YF	24. 8.34	R. W.	„	—	5	„
YH	7. 2.35	L. W.	„ <sup>e</sup>	19	15	„
YK	12. 2.35	A. G.	„ <sup>e</sup>	26	6	„
S1	19. 8.32	H. S.	Primary syphilis	53	10	No
S2	10. 2.33	J. R.	„	60	4	„
S3	24.11.33	A. R.	Secondary syphilis <sup>f</sup>	30	8	„
S4	1.12.33	K. P.	Primary syphilis	35	8	„
S5	16. 2.34	L. D.	„	30	8	„
S6	18. 1.34	L. H.	Secondary syphilis <sup>f</sup>	40	22	„
S8	2. 2.34	S. M.	„ <sup>f</sup>	48	8	„
S10	10. 7.34	A. F.	„ <sup>f</sup>	23	8	„

<sup>a</sup> All isolations were made by intratesticular inoculation of rabbits, except where noted.

<sup>b</sup> As of 1 September 1955

<sup>c</sup> "Undesignated" refers to strains not propagated in serial passage.

<sup>d</sup> See Chapter 7 for history of strain YD and designations. YD-pre-1949 and YD-post-1949.

<sup>e</sup> Isolations made on granulating wound of rabbit by: *Hippelates fly* transmission (Kumm & Turner<sup>3</sup>)

<sup>f</sup> Transfer material from lymph node

isolated from patients who were permanent residents of Jamaica, B.W.I.; 8 strains were from adults with characteristic histories, including strong presumption that the disease was acquired by sexual exposure, and physical signs of early syphilis; and 13 strains were from persons, mostly children, in whom the epidemiological and physical findings were characteristic of yaws.

For identification, these strains, pertinent details concerning which are given in Table IA, were designated S1, S2, etc., if they were isolated from a yaws patient. All transfers from patients to rabbits were made by inoculation of material into the body of the rabbit's testis, except in the case of two strains, YH and YK, which were transmitted from man to rabbit through *Hippelates* flies. The isolation of these two strains has been described in more detail by Kumm & Turner.<sup>3</sup>

TABLE Ib. STRAINS OF TREPONEMAL ORGANISMS ISOLATED AND PROPAGATED IN LABORATORY ANIMALS: STRAINS ISOLATED FROM PERSONS LIVING IN THE USA, AND FROM NATURAL INFECTIONS OF RABBITS \*

Strain designation	Date of transfer to laboratory animals	Clinical diagnosis	Incubation period in 1st animal-passage (days)	Total animal passages observed	Available in this laboratory
K.C. 37	1. 3.37	Secondary syphilis	43	3	No
S.M. 37	14. 9.37	Primary syphilis	36	5	"
D.T. 37	25.10.37	Secondary syphilis	82	3	"
S.R. 37	2.11.37	Primary syphilis	50	3	"
F.S. 37	16.11.37	Secondary syphilis	34	3	"
P.A. 37	3.12.37	"	—	3	"
N.P. 37	14.12.37	" (blood)	37	4	"
B.T. 38	3. 1.38	Primary syphilis	—	3	"
E.C. 38	10. 1.38	Secondary syphilis	40	2	"
V.M. 38	18. 1.38	Primary syphilis	63	3	"
L.W. 38	24. 1.38	"	36	3	"
C.J. 39	12. 9.39	Secondary syphilis	30	6	"
A.G. 39	18. 9.39	"	39	3	"
M.S.I 39	13.10.39	"	40	18	Yes
M.S.II 39	18.10.39	"	60	4	No
L.W. 39	1.11.39	"	47	5	"
M.S.S. 39	30.11.39	"	32	4	"
M.J. 41	21.11.41	" (blood)	42	4	"
Cuniculi A	28.12.39	Rabbit non-venereal spirochetosis	26	37	Yes
Cuniculi B	3. 2.40	"	24	14	No
Cuniculi C	28.10.40	"	30	2	"
Cuniculi D	22. 1.41	"	20	1	"
Cuniculi E	22. 1.41	"	20	1	"
Cuniculi F	24.10.41	"	32	1	"

\* All strains were isolated from patients living in Maryland, unless otherwise noted ; all isolations were made by intratesticular inoculation of rabbits.

A second group of isolations was made during the years 1937-41 from two sources: 18 strains from patients with typical signs of early syphilis, all of whom lived in Maryland; and 6 strains from as many domestic rabbits which were observed by chance in this laboratory and which had characteristic evidence of cuniculi infection. All isolations were made in this laboratory by the senior author and his associates. Pertinent data on this group of strains are given in Table Ib. Strains from human beings were designated by the initials of the patient from whom they were obtained, together with the year of isolation, as for example K. C. 37; strains from rabbits with cuniculi infections were designated cuniculi A, cuniculi B, and so on.

A third group of 9 strains was isolated during 1947 and 1948. All were from patients with syphilis; 6 patients lived in Maryland, 2 in the Los Angeles area of California, and one in St Louis, Missouri. As in the previous groups, all isolations were made by intratesticular inoculations of rabbits. Strains were designated in a manner similar to that adopted in the case of the preceding group. Pertinent data on these strains are shown in Table Ic. It will be noted that several of these strains were obtained from material secured by biopsy, rather than from cutaneous lesions.

A fourth group of 22 strains was isolated during 1950-55 by the authors and their associates from patients living in various parts of the world.

TABLE IC. STRAINS OF TREPONEMAL ORGANISMS ISOLATED AND PROPAGATED IN LABORATORY ANIMALS: STRAINS OF SYPHILIS ISOLATED IN THE UNITED STATES OF AMERICA

Strain designation	Date of transfer to laboratory animals	Clinical diagnosis	Incubation period in 1st animal—passage (days)	Total animal passages observed	Available in this laboratory
A.H. 47	12.11.47	Secondary syphilis	31	3	No
F.R. 47	17.11.47	Secondary syphilis	49	3	"
St Louis 47	27.11.47	Early syphilis (node) <sup>a</sup>	14	17	"
L.J.W. 47	5.12.47	Secondary syphilis	25	2	"
H.W. 48	21. 1.48	Secondary syphilis (liver biopsy) <sup>b</sup>	55	2	"
W.M. 48	2.11.48	Late syphilis (liver biopsy) <sup>b</sup>	65	4	"
C.C. 49	17. 1.49	Late syphilis (node) <sup>c</sup>	39	2	"
Cal I	19.10.48	Primary syphilis <sup>d</sup>	—	2	"
Cal II	21.12.48	Primary syphilis <sup>d</sup>	—	6	"

<sup>a</sup> Received from Dr Virgil Scott (Washington University, St Louis, Mo.); transfer from axillary node of patient with early syphilis

<sup>b</sup> Transfers made from liver biopsy material—patient H.W. had hepatitis (see Calkins et al.<sup>3</sup>)

<sup>c</sup> Abdominal lymph node—late lesion of stomach (see Calkins et al.<sup>3</sup>)

<sup>d</sup> Received from Dr Ruth Boak, University of California, Los Angeles, California, 2 February 1949

TABLE 1b. STRAINS OF TREPONEMAL ORGANISMS ISOLATED AND PROPAGATED IN LABORATORY ANIMALS:  
STRAINS ISOLATED FROM PERSONS LIVING IN VARIOUS PARTS OF THE WORLD

Strain designation	Date of transfer to laboratory animals	Source of strain			Incubation period in 1st animal-passage (days)		Total animal passages observed	Available in this laboratory <sup>a</sup>
		Patients' initials and age	Transfers (See reference numbers in Appendix 1)	Clinical diagnosis	Rabbit	Hamster		
Syria A	6. 5.50	A.M. 6 years	1	Bejel	53 testis; 27 back	43, 30, 32	31	No
Syria B	6. 5.50	W.D. (child)	2	Bejel	67	—	35	Yes
Bosnia A	5. 9.50	K.A.S. 35 years	3	Endemic syphilis	60	—	33	"
Bosnia B	5. 9.50	N.G.G. 38 years	4	"	77	—	33	"
Baghdad A	30.12.50	S.H. 20 years	5	Veneral syphilis	35 back	—	41	"
Baghdad B	30.12.50	J.A. 40 years	6	"	30 back	—	35	"
Samoa A	12. 1.51	J. 1½ years	7	Yaws	21 back	32	3	No
Chicago	9. 2.51	W. McD. 25 years	8	Syphilis	11 back	—	55	Yes
Indonesia B	3. 3.51	S. 11 years	9	Generalized yaws	5 back	—	24	"
Haiti A	7. 3.51	M.E. 9 years	10	Generalized yaws	75	—	27	"
Haiti B	7. 3.51	J.L.S. 11 years	11	"	70	—	38	"
Iraq A	29. 4.52	S. 7 years	12	Bejel	69	30	37	"
Mexico A	13. 1.53	G. A. 18 years	13	Primary syphilis	—	22, 28, 35 <sup>b</sup>	9	"
Samoa D	24. 1.53	I. 1 year	14	Generalized yaws	—	13, 12, 10	15	"
Samoa E	24. 1.53	M. 3 years	15	"	—	15, 10, 10	13	"
Samoa F	24. 1.53	M. 4 years	16	"	—	47, 24, 30	14	"
Bechuanaland C	3. 4.54	G. G. 22 years	17	Non-veneral treponematosi	—	52	8	"
Bechuanaland D	4. 4.54	M.M. 6 years	18	"	—	23	7	"
Gambia A	9. 7.55	B.K. 4 years	19	"	—	40	2	"
Gambia B	9. 7.55	S.S. 11 years	20	"	—	37	2	"
Gambia C	9. 7.55	T.S. 4 years	21	"	—	46	2	"
Gambia D	9. 7.55	O.G. 4 years	22	"	—	43	2	"

<sup>a</sup> As of 1 September 1955

<sup>b</sup> No lesions—lymph nodes darkfield positive on indicated day

Isolations were made under the auspices of the World Health Organization with the collaboration of scientists who made the initial transfers from patients to animals in the respective areas. Additional details of these isolations are given in Appendix 1 (page 269).

Altogether during this last period 4 strains were isolated from patients with typical syphilis; 7 strains from typical yaws; 3 strains from patients with bejel; and 8 strains from individuals with one or another of the treponematoses which locally are known by other names. Pertinent data on these strains are shown in Table ID, but reference will be made to them again in Chapters 7, 8 and 9.

Throughout this period, the well-known Nichols strain of *T. pallidum* was used in the nature of a "standard" laboratory strain, and many of the special studies were made with this strain. The Nichols strain was isolated in 1912 by Major H. J. Nichols of the United States Army from the spinal fluid of a patient with recurrent neurosyphilis,<sup>13</sup> and has been maintained more or less continuously in laboratory animals since that time. About every decade, one or more accidental laboratory infections of human beings have been observed with this strain, thus attesting to its pathogenicity for man despite long propagation in laboratory animals. (See Chapter 7.)

### Methods of Isolation of Treponemal Strains

#### *Types of lesions used*

In most instances skin lesions, showing numerous treponemes, of patients with characteristic signs of the disease under study were selected as sources of transfer material. In yaws patients this was ordinarily a frambesiform lesion, and in syphilis patients a penile chancre or a skin papule of the secondary stage.

The lesion was grasped about the base with a hemostat to fix the lesion and to effect a degree of hemostasis. The surface of the lesion was washed with sterile water or saline and was gently abraded. Usually the serum, which oozed freely, was rich in actively motile treponemes. Serum from the lesion was collected with a capillary pipette or small syringe with or without slight dilution in normal saline solution. The addition of 10% inactivated normal serum to the saline is desirable since treponemes appear to survive poorly in saline. As soon as 0.2-1.0 ml of material was collected it was inoculated into one or more laboratory animals.

#### *Inoculation of rabbits*

All isolations prior to 1950 were made by direct transfer of infective material to rabbits, usually by intratesticular inoculation. It had previously

been shown, contrary to what might be supposed, that the monkey (*Macacus rhesus*) is a less favorable animal for initial isolation of yaws strains than the rabbit, and subsequent experience in this laboratory is in agreement with that observation. (See Chapter 2.)

Inoculations were commonly made into the body of one or both testes of the rabbit with amounts up to 1.0 ml, preferably less. A small amount (0.1 ml) was often injected intradermally into one or both scrota. Intracutaneous inoculation on the clipped back of the animal was occasionally employed, and yielded positive results in some cases. Inoculation on to the surface of a granulating wound, as described by Chesney, Turner & Halley,<sup>5</sup> was used in 63 instances with positive results in only 3.

During the course of experiments on the possible transmission of yaws by *Hippelates* flies,<sup>8</sup> rabbits were infected by this means. In one type of experiment the animal was infected by flies feeding first on an infectious yaws lesion of man and then upon a granulating wound or a freshly scarified area on the scrotum; of 68 rabbits on which supposedly infected flies were permitted to feed, specific yaws lesions developed in the wound in 7. In another type of experiment, the esophageal diverticula of *Hippelates* flies which had fed on infectious yaws lesions were dissected out at various intervals after feeding and inoculated into wounds of the back or scrotum. Of 28 rabbits so exposed, 8 showed definite evidences of infection. Incubation periods in the rabbits ranged from 16 to 87 days, with all but 3 being between 26 and 45 days. These experiments have been previously reported by Kumm, Turner & Peat.<sup>9</sup>

The evolution of disease phenomena in the inoculated rabbits resembled that seen after the inoculation of adapted strains, except that on the whole the lesions in the first animal passage tended to be smaller and more evanescent. This was particularly true of the lesions produced initially by the yaws strains. Often the only lesion detected was slight enlargement of the head of the epididymis on the inoculated side; the time for the development of this type of lesion, frequently over 50 days, suggests that it is a metastatic lesion rather than the initial focus.

As a group, syphilis strains tended to have shorter incubation periods than yaws strains. While the difference in incubation periods appears to be a significant one, it must be borne in mind that the syphilis patients as a group were in a substantially earlier phase in the evolution of their disease than were most of the yaws patients. To what extent this factor influenced the incubation period in the initial transfer, it is difficult to say. Moreover, yaws lesions in rabbits are as a rule much smaller and more difficult to detect, so that this factor may have delayed recognition of the changes in the rabbit's testis. But whatever the explanation for the differences observed in incubation period, these same differences do not appear to be restricted to the initial transfers, and were commonly noted also in many subsequent passages.

Comparative data on the incubation period of the disease in the first-passage animals are also of limited value because of the differing circumstances under which the isolations were made: some in the relatively high environmental temperature of the tropics, others in the rather cool temperature of the temperate zone, and still others partly under both conditions. Moreover, inocula from different sources probably varied considerably in the number of treponemes injected. For what they are worth, however, the data on initial incubation periods following intratesticular inoculation of rabbits are given.

Of 31 syphilis strains the shortest incubation period was 14 days, the longest 82, the median being 40 days. Of 11 yaws strains the shortest incubation period was 28 days, the longest 75, the median being 52 days. Three strains of bejel treponemes were isolated by intratesticular inoculation of rabbits with incubation periods of 53, 67, and 69 days, respectively. The two strains of so-called endemic syphilis isolated in rabbits had incubation periods of 60 and 70 days, respectively. Most of the strains isolated in the past three years were initially inoculated into hamsters rather than rabbits, and data for these are not included in the foregoing analysis.

#### *Inoculation of hamsters*

With the demonstration in 1949 by Geiman & McKee<sup>6</sup> that the hamster was susceptible to yaws as well as to syphilis treponemes, this laboratory animal was utilized either alone or in conjunction with the rabbit for subsequent isolations. However, not until 1952 was it appreciated that hamsters infected with one of the treponemal group of organisms customarily contain numerous treponemes in their lymph nodes even when no clinically discernible lesion is present, and undoubtedly some of the initial transfers into hamsters were erroneously regarded as unsuccessful prior to that date. Eight strains have been isolated since 1952 solely in hamsters (Table Ic).

#### *Unsuccessful isolations*

During the course of these studies 76 different strains of treponemal organisms have been successfully isolated in laboratory animals. In the same period transfers of material, known to contain treponemal organisms from 10 patients with syphilis and 17 patients with yaws, for one reason or another failed to result in successful isolations. Taken at their face value, however, these figures may be misleading, for in many instances failure was associated with secondary bacterial contamination in a situation in which transfer had been made to one animal only, a practice not uncommon where patients with yaws and syphilis were numerous and rabbits rather scarce. Following inoculation of human material into the testis of rabbits there is often initially a slight non-specific reaction manifested as a palpable

infiltration, or even an actual nodule, in the body of the testis, but in the presence of substantial numbers of contaminating bacteria, an abscess may form, under which circumstances there will be much less chance of a successful isolation.

Of course, any of the other factors such as temperature, antibiotics, or prior cuniculi infection, which are known to influence treponeme infections (see Chapter 3), may adversely affect the chances of a successful isolation, and it is perhaps surprising that more failures have not been encountered, particularly in animals shipped long distances after inoculation.

#### *Attempted isolation of pinta treponemes*

Although the same methods were employed in attempting to isolate pinta strains, we have been unable to establish a strain in animals. Three of 30 hamsters, however, when examined 10 weeks after the inoculation of pinta material, contained actively motile treponemes in their inguinal lymph nodes as follows:

(1) H-146, a male hamster was inoculated with material from A. S., a 15-year-old male Indian, by Drs D. H. Hollander, J. Olarte and G. Varela, 17 January 1952. When the animal was sacrificed after 73 days, no symptoms having been present in the meantime, the apparently normal inguinal lymph node contained 2 typical treponemes identified in the emulsion with 0.1 ml of serum saline by 3 observers.

(2) H-154, a male hamster was inoculated with material from M. R., a 42-year-old male Indian in Mexcala, Guerrero, Mexico, 19 January 1952. A scaly area noted in one groin after 4 weeks was repeatedly darkfield negative. When the animal was sacrificed 67 days after inoculation one inguinal node appeared definitely enlarged. An emulsion in 0.1 ml of serum saline contained 3 typical treponemes identified by 3 observers. This and the first hamster were part of a group of 8 hamsters and 2 rabbits shipped from Mexico City to Baltimore at the same time; 3 hamsters did not survive, all animals remained asymptomatic, and the 3 other hamsters had darkfield-negative lymph nodes.

(3) H-439, a male hamster was inoculated with material from J. H., an 18-year-old male Indian in Iguala, Guerrero, Mexico by Dr J. Olarte, 15 January 1953. When the hamster was sacrificed after 72 days without lesions the inguinal nodes were enlarged. Emulsion of one node in 0.1 ml of saline contained 2 treponemes identified by 4 observers. Two other hamsters inoculated from the same patient and 6 from another pinta patient included in the same shipment were negative, while 3 hamsters inoculated with Mexico A, a strain of syphilis which was also in this shipment, were each positive. In two other shipments 15 hamsters were inoculated from pinta patients without further success.

Altogether 8 hamsters were inoculated from the 3 positive hamsters, and 4 blind passages were made into another generation with no evidence of disease in these 12 animals.

The known variation among species of syphilis, yaws, and cuniculi treponemes in their susceptibility for humans, rabbits, and hamsters, respectively, may be an argument that strains or species of treponemes may exist to which laboratory animals are not susceptible. On the other hand, the demonstration of treponemes in 3 animals inoculated from 3 different pinta patients suggests that hamsters, at least, are not wholly resistant.

Another explanation for the failures may lie in the fact that human infections of relatively long standing were used for transfer. The presence of specific antibody in this material may greatly decrease the infectivity, particularly when the inoculation is made into a new animal species. It is perhaps pertinent that León-Blanco,<sup>11</sup> who reported a single successful transmission from a case of Cuban pinta into a rabbit, first inoculated the material into a human volunteer, and subsequently inoculated the rabbit from the early primary lesion of the human volunteer.

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\* \*

With the reservation that our experience in the isolation of strains is far from a controlled experiment, and that it is necessary to rely on impressions only, it appears that both the rabbit and the hamster are satisfactory animals for the isolation of most new strains of treponemes, and indeed there are indications that of these two the hamster is the more satisfactory, particularly for isolation of the yaws type of organism.

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