

PHARMACOKINETICS OF BENZATHINE PENICILLIN IN
THE TREATMENT OF EARLY SYPHILIS IN
ZAMBIAN PATIENTS

BY

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DECLARATION

I hereby declare that this dissertation is my own work and that it has not been previously submitted for Degree purposes here or at any other University.


Sangeeta Wagle

A B S T R A C T

The study comprises 15 male patients between the ages of 20 and 29 years. Bacteriological examination established a definite diagnosis of early syphilis. Baseline studies like full blood count, ESR and random blood sugar, blood urea and electrolytes, serum creatinine and liver function tests were carried out on each patient. The patients were given a single injection of benzathine penicillin G, 2.4MIU intramuscularly. Blood was collected at intervals of 8, 24, 48, 72 hours, 7th, 14th and 28th day after injection of penicillin. Serum was separated and penicillin assay carried out on each sample. Peak serum levels of an average of 0.135IU/ml were established between 24-48 hours. The levels gradually dropped in the following weeks to 0.049 - 0.082 IU/ml on the 28th day. Thus showing that treponemicidal concentrations were maintained for sufficiently long periods.

Pharmacokinetic analysis was carried out with the assumption that penicillin behaves in the body in a first-order fashion (one compartment model). The K_a which is the absorption rate constant was 4.063 min^{-1} and the half-life was 39.7 hours. The results have been discussed in detail.

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LIST OF ABBREVIATIONS

CDC	:	Centre for Disease Control
DGI	:	Dark-ground Investigation
ELISA	:	Enzyme-linked immunosorbent assay
ESR	:	Erythrocyte sedimentation rate
FTA-ABS	:	Fluorescent treponemal antibody absorption test
HIV	:	Human immunodeficiency virus
HTLV-III	:	Human T-cell Lymphotropic virus type III
MIU	:	Mega International Units
RPR	:	Rapid plasma reagin test
SGOT	:	Serum glutamate - oxalate transaminase test
SGPT	:	Serum glutamate - pyruvate transaminase test
TPHA	:	<u>Treponema pallidum</u> hemagglutination test
TPI	:	<u>Treponema pallidum</u> immobilisation test

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CHAPTER I

VENEREAL AND NON-VENEREAL TREPONEMATOSES

INTRODUCTION

The treponematoses are a group of infections that are caused by spirochaetes belonging to the genus Treponema which is a taxonomic group of the family 'Treponemataceae' (a more detailed classification is presented in chapter III). Of all the venereal and non-venereal treponematoses, venereal syphilis (commonly referred to as 'Syphilis') is the most important. The other treponemal infections - endemic syphilis, yaws and pinta are transmitted (generally) by non-venereal modes and are usually endemic. They commonly occur in tropical regions.

VENEREAL AND NON-VENEREAL SYPHILIS

Syphilis was defined by Stokes as an infectious disease; due to T. pallidum; of great chronicity, systemic from the outset, capable of involving practically every structure of the body in its course, distinguished by florid manifestations on the one hand and years of complete asymptomatic latency on the other; able to simulate many diseases that are known in medicine and surgery. Transmissible to offspring in man, transmissible to certain laboratory animals, and treatable to the point of presumptive cure.

The above definition more-or-less summarises the concept of syphilis. The transmission of this disease may be venereal or non-venereal.

The non-venereal form of syphilis known as 'Syphilis Insontium', is also caused by T. pallidum. This form is not as common as the venereal infection and is usually endemic or sporadic. It is believed that in the early historical times, this non-venereal form was highly prevalent among the primitive tribes and was a contagious infection that presented in children. It was relatively rare amongst adults. With massive population movements this disease spread to the more civilised societies and environmental and socio-economic factors were responsible for the infection that gradually became an adult venereal infection - i.e. venereal syphilis. Non-venereal syphilis is still prevalent in many poor and developing countries, where it is known by various local names. It is found in various regions of Africa - Sudan, Mauritania, Senegal, Ghana, Nigeria, Botswana (dichuchwa), Zimbabwe (njovera) and in South Africa. In the Middle-East it is known as bejel and is found in many countries such as Iraq, Iran, Transjordan and Saudi Arabia (balash). It is also noted in Turkey, Macedonia, Lower Mongolia and Tibet.

In the past, it was quite common in Bosnia of present-day Yugoslavia, but mass eradication campaigns organised by the Yugoslavian Government and the World Health Organisation have virtually eliminated the disease from this state.

In the seventeenth century, endemic syphilis was found in Scotland (sibbens), Ireland (button scurvy), Norway (radesyge), Jutland (dithmarsch evil) and Greece (spirocolon). It was also observed in various parts of North America.

The symptoms of non-venereal syphilis are usually similar to those found in the secondary stage of syphilis. Mucous patches of the lips, mouth, pharynx, larynx, nose are often found. Condylomata lata of the axillae, under the breasts, anogenital region and occasionally on the abdomen, face and buccal mucosa, are also found. Generalised rashes - macular, papular or papilloform may be present, severe in some cases and absent in others.

Primary chancres are seen in endemic syphilis only when a large inoculum of T. pallidum is injected into a susceptible patient. Late lesions are generally gummatous and occur rarely. Osteitis, arthritis, alopecia, cardiovascular and neurological involvement are all rare in endemic syphilis.

Chances of transmission of the disease to the infant in utero are more common in venereal syphilis than in endemic syphilis.

The similarity in clinical manifestations, serological reactivity and darkground observations makes it difficult to diagnose sexually-transmitted syphilis in areas of endemic treponematoses (Wray, 1966).

It is believed that eradication of endemic syphilis from areas of high prevalence has resulted in the inhabitants of these areas becoming more susceptible to venereal syphilis e.g. rural areas of New Guinea (Rhodes and Anderson, 1970). and in rural areas of Western Samoa and Thailand (Guthe et al 1972). In some areas endemic treponematoses and sexually transmitted syphilis coexist. In such areas endemic infection occurs more commonly in rural areas and venereal syphilis in urban areas (Kooy, 1970, Du Toit, 1969).

The other treponematoses i.e. yaws and pinta are caused by T. pertenue and T. carateum respectively. The organisms are morphologically indistinguishable from T. pallidum. They are also found to give true positive serological tests for syphilis. Neither can be cultured on artificial media.

Y A W S

This is an endemic non-venereal infection commonly seen in children in humid tropical countries. The causative organism is T. pertenue. Lesions are usually seen on the arms and legs. Lesions form scars and sometimes the lesions may extend deep into the bone, causing bone destruction. (Fluker & Bouton-Hewitt 1970). Yaws is generally not transferred in a vertical manner i.e. from an infected mother to her foetus. Neurological and visceral involvement in late yaws is present but rare. Smith et al. (1971) carried out a detailed study showing various cases of yaws with neuro-ophthalmological involvement. This makes clinical distinction between late yaws and late syphilis even more difficult. The antibodies elicited by T. pertenue and T. pallidum in an infected person are similar. Thus sera from patients with yaws react in a similar manner to sera from syphilitic patients in conventional tests for syphilis and the TPI test. Garner et al. performed the FTA-ABS & TPI test in yaws patients and found that the tests showed 91.9% agreement (Garner et al., 1970). Cross immunity has been seen between yaws and syphilis. Lesions of yaws may be mistaken for extragenital syphilitic lesions, however, the age group (under 15) in which these lesions are seen may rule out syphilis. Diagnostic procedures and therapy are similar to those for syphilis. Penicillins are highly effective in the treatment of yaws.

P I N T A

This is a non-endemic infection found in Central and South America. This disease can occur at any age but is more common in people less than twenty years of age. Transmission is by casual contact or by insect vectors of the genus Hippelates. This disease commonly occurs among negroids and native Red Indians.

Pintides or the primary lesions of pinta are non-ulcerating papules which occur on the exposed areas of the body. Some months after the appearance of these, flat hyperpigmented lesions appear on the skin; depigmentation and hyperkeratosis takes place. Years later, cardiovascular and neurological involvement is thought to occur. Bone pains and aortic lesions with aneurysms have been described so have splenomegal eosinophilia and lymphocytosis. Sufferers of pinta give positive serological tests for syphilis and T. carateum has been demonstrated in early lesions. Penicillins are used in treating this infection.

A table comparing the characteristics of the various treponematoses is given below (Baron, 1982)

CHARACTERISTICS OF THE TREPONEMATOSES

CHARACTERISTICS	SYPHILIS	YAWS	PINTA	ENDEMIC SYPHILIS
EPIDEMIOLOGY				
Agent	<u>T. pallidum</u>	<u>T. pertenue</u>	<u>T. carateum</u>	<u>T. pallidum</u>
Other names	Venereal Syphilis	Framboesia, Pian	Carate, Cute	Bejel, Dichuchwa
Prevalence	Worldwide	Hot, humid areas	Hot, humid areas	Hot areas
Locations	Worldwide	Tropics	Central & South America	Deserts

(..... table continued)

CHARATERISTICS	SYPHILIS	YAWS	PINTA	ENDEMIC SYPHILIS
Age group	Adults	Children	Children adolescents	Children, adults
Spread	Venereal	Skin	Skin	Mucous membranes
Congenital Infection	Yes	Yes	Yes	Rarely
DISEASE CHARACTERISTICS				
Incubation	10-90 days	14-28 days	2-6 months	—
Invasiveness	High	Intermediate	Low	Intermediate
Perivascular cuffing	Yes	No	Yes	No
Tissues involved	All	Soft bones, soft tissues	Skin	Mucous membranes, muscles, bones
Predominant cellular infiltrate	Plasma cells lymphocytes	Mostly plasma cells	Mostly lymphocytes	Plasma cells lymphocytes
Destructive lesions	Yes	Yes	No	Yes
Granulomas	Yes	Yes	No	Yes
Gummas	Yes	Yes	No	Yes
Condylomata lata	Yes	Yes	No	Yes

CONCLUSION

It is believed that the organisms responsible for the venereal and non-venereal treponematoses i.e. T. pallidum, T. pertenue, T. carateum and any other different local treponemes may have evolved from the a common ancestor.

It has also been suggested that the more specialised manifestations of pinta, arose from being for many centuries more or less isolated in the South American continent. The particular disease syndromes appear to be modified by racial and particularly climatic environmental conditions. Whether the causative organism has become differentiated at some time in the distant past in adaptation to these circumstances, or whether it is the same treponeme still adapting itself today, is perhaps an academic question. Either way certain environmental circumstances favour the spread of one disease syndrome or the other.

CHAPTER II

HISTORY AND EPIDEMIOLOGY OF SYPHILIS

HISTORY

No one knows the origin of syphilis. There are many views regarding its origin, however, two well-defined theories are widely held and accepted. One theory suggests that syphilis is an ancient disease which has been mentioned in the Bible. They have cited passages in the Bible where the bibliographical characters could be afflicted with syphilis. However a closer inspection of such references has shown that the ailments mentioned in the Bible could be plague, chancroid, leprosy, gonorrhoea but not syphilis. No mention of syphilis or any disease resembling it has been found in the writings of the Greek, Egyptian or Arabic treatises on medicine. This shows quite clearly that syphilis was not known in Europe before the end of the fifteenth century.

The other, more popular theory, regarding the origin of syphilis is the Columbian theory. Christopher Columbus in his search for India, set out on a voyage and discovered the New World. The members of his crew mixed freely with the local Red Indian inhabitants. It is believed that they picked up this infection from these locals.

Evidence of bone syphilis has been reported in the bones of American Indians who lived before this time. So syphilis was probably present in the American Indians but was not so virulent amongst them as it was an endemic infection and they had developed some resistance to it over the years. In the Europeans, on the other hand, immunity to this infection was nil as they had never been exposed to it before and so it appeared in a highly virulent form in these sailors.

This may be true, because Ruy Diaz de Isla, a physician who had set out for this voyage with Columbus has mentioned a strange new disease which afflicted the members of Columbus' crew on their journey home. The signs and symptoms shown by these afflicted people were carefully recorded by him and seem to resemble syphilis closely. After returning to Spain in 1493, these sailors joined the army of King Ferdinand of Aragon and were sent to help in the siege of Naples in 1494. These sailors rapidly spread the infection to the people of Spain and Naples and to the soldiers of the French army. At the time promiscuity was rampant and many wars were being fought. Soldiers and mercenaries moved all over Europe thus spreading the infection to Portugal from where it was carried to India and the Far East by Portuguese sailors. This theory seems highly credible because a mention of syphilis has appeared in European medical literature only after the fifteenth century. Also the wave of spread of the disease seems to follow the path taken by Columbian crew that is from Palos in Spain, to Naples, France and so on. The writings of Fransisco Lopez de Villalobos, Hieronymus Fracastorius and Juan de Vigo besides a host of other medical writers who lived in the fifteenth century are valuable in medical literature because these writers have clearly outlined the various signs and symptoms of the disease. In those early days, syphilis was highly virulent amongst Europeans and had a high rate of morbidity. Its venereal mode of transmission was recognised even then, however, since the cause of the infection was not known it was believed that it could also be acquired through bad air and bad water. Syphilis acquired its name in 1521 from a poem written by Hieronymus Fracastorius called 'Syphilis sive Morbus Gallicus'. The incubation period of syphilis is quite long and thus the full extent of its attack on the body was not recognised until the sixteenth century when the French surgeon, Ambroise Pare attributed aortic aneurysm to syphilis.

The history of congenital syphilis and treatment of syphilis have been dealt with in relevant chapters (vide Chapters IV.B and VIII).

Over the centuries syphilis seems to have become less virulent as the human race has gradually developed some resistance to it. The morbidity rate for syphilis too has dropped considerably.

EPIDEMIOLOGY

There has been a progressive decline in the incidence and morbidity rate of syphilis in Western countries since 1860. Epidemics of syphilis were usually observed during wars, political unrest and large population movements. The general decline may be due to improvement in the socio-economic conditions of people following the Industrial Revolution.

There has been a marked decrease in the number of cases reported from treatment centres in England and Wales from 1940 to 1977 (King & Nicol, 1980). The rate per 100,000 population in 1977 was 3.74. The rate in 1982 was 2/100,000 amongst females in 1978 and 10/100,000 amongst males. In 1984, this rate dropped to less than 2/100,000 in females and 8/100,000 in males (WHO, 1986). In the USA, the rate of reported cases of early syphilis was as high as 100/100,000 in 1950. Extensive penicillin therapy caused a sharp decline in rate from 100/100,000 in 1950 to 3.4/100,000 in 1956. This could be due to the use of penicillin used for other conditions rendering undiagnosed syphilitic cases non-infectious by chance (so-called 'happenstance' treatment) However, in recent years from 1970 onwards, there has been an increase in the incidence of reported cases of syphilis. In 1977, the rate was 9.5 per 100,000 population and it has gone up to 20 per 100,000 population.

Statistics on the incidence of syphilis in men as compared to women show an increase in the men:women or male:female syphilitics ratio. This could be due to the high incidence of homosexuality in various countries. In the U.K., 42.4% of cases of primary and secondary syphilis were acquired homosexually (British Co-operative Clinical Group, 1973). The ratio of male:female incidence of sexually transmitted diseases is higher in developing countries than in developed countries. This is because the incidence of premarital sexual experience in members of both sexes is equal in the developed countries, while in the developing countries men have premarital and extramarital contacts with prostitutes and acquire infections (WHO, 1986).

In Zambia, the total number of cases reported for various sexually transmitted diseases in 1982 was 820,756 cases*. In 1986, reported cases of syphilis were approximately 67,000.

Congenital syphilis still accounts for about one-third of the stillborn infants in African countries (Larson and Larson, 1970; Hira et al, 1982; Ratnam, 1982).

Studies on pregnant women attending prenatal clinics using VDRL/RPR and MHA-TP/FTA-ABS as the screening tests show the following:-

<u>COUNTRY</u>	<u>VDRL/RPR</u>	<u>FTA/ABS/MHA-TP</u>
Zambia	14.3%	12.5%
Swaziland	10.0%	13.3%
Ethiopia	12.7%	10.9%

This shows that if prenatal screening and antenatal care of pregnant women is not carried out carefully, there is still considerable risk of congenital syphilis.

China has claimed the total eradication of congenital syphilis and many countries in both Eastern and Western Europe (e.g. Poland and the U.K.) as well as Japan, have been able to reduce its incidence to very low levels (WHO, 1982).

Spirochaetes are grouped under the order Spirochaetales which consists of two families (i) Spirochaetaceae (ii) Treponemataceae. The family Spirochaetaceae consists of three genera of free-living, large spiral organisms. The other, Treponemataceae, includes three genera pathogenic for humans : (a) Treponema, in which there are the causal agents of syphilis, bejel, yaws and pinta. (b) Borrelia, which is the pathogenic for relapsing fever and (c) Leptospira, which are pathogens resulting in systemic infections with fever, jaundice and meningitis.

T. pallidum was first observed by Schaudinn and Hoffmann in 1905, when they were studying exudates from syphilitic lesions in an attempt to establish the aetiology of syphilis.

MORPHOLOGY

T. pallidum is a flexible, helically shaped treponeme with tapering ends. It is usually 6-15µm (mean 7µm) long and has a uniform cylindrical thickness of approximately 0.25µm. The number of spirals vary from 0-14 in number and each spiral is 0.8-1.0µm in length. The width of the spiral which is rigid and regular is approximately 1.0µm.

The structure of T. pallidum has been elucidated by studying T. pallidum and associated treponemes by electron microscopy. T. pallidum has been found to have a complex internal structure. The basic structure consists of an outer cellular envelope beneath which is the peptidoglycan - cytoplasmic membrane complex.

CHAPTER III

CHARACTERISTICS OF TREPONEMA PALLIDUM

Treponema pallidum (T. pallidum) belongs to a large group of spiral, flexible organisms known as Spirochaetes. Spirochaetes may be free-living, saprophytes or they may be pathogenic. Spirochaetes are grouped under the order Spirochaetales which consists of two families (i) Spirochaetaceae (ii) Treponemataceae. The family Spirochaetaceae consists of three genera of free-living, large spiral organisms. The other, Treponemataceae, includes three genera pathogenic for humans : (a) Treponema, in which there are the causal agents of syphilis, bejel, yaws and pinta. (b) Borrelia, which is the pathogenic for relapsing fever and (c) Leptospira, which are pathogens resulting in systemic infections with fever, jaundice and meningitis.

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It has been suggested (Swain 1955, Christiansen 1963, Hovind-Hougen 1974) that a protective slime layer may be found on pathogenic treponemes. This layer surrounds the outer layer of the cell and seems to explain the lack of serological reactivity in freshly isolated T. pallidum cells from rabbits. (Christiansen, 1963). The capsular slime layer is deposited from the infected host itself and therefore does not react with syphilitic antibodies in serum which are directed against unprotected parts of T. pallidum cells. Ziegler et al (1976) were able to demonstrate this slime layer with the dye ruthenium red. It also stains with toluidine blue and is therefore, believed to be an acidic mucopolysaccharide.

Whereas, Christiansen (1963) has suggested that this slime-layer may be host-derived, Fitzgerald et al (1978) postulate it to be the product of treponemes rather than host cells. Ageing or treatment with heat, lysozyme, trypsin or papain restores the serological reactivity of the spirochaetes.

Outer envelope:- The outer envelope (OE) of T. pallidum is the primary target of the antibody - complement system. The motility and viability of T. pallidum depends on an intact outer envelope. In a hypotonic environment, the OE of T. pallidum is distended and it converts to a spherical form. The OE is comprised of lipids, proteins and carbohydrates, which differ in ratios to one another in various strains of Treponema.

Peptidoglycan — Cytoplasmic membrane complex - The peptidoglycan layer is almost continuous with cytoplasmic membrane and it is difficult to distinguish between the two. But Hovind-Hougen (1974) in electron microscopic studies has shown that the peptidoglycan layer is slightly more electron dense than the cell-membrane.

It is a thin layer. The peptidoglycan layer contains muramic acid, glucosamine, alanine and glutamic acid as major components. The diamino acid present in the cell wall of T. pallidum is ornithine.

Cytoplasm and Cytoplasmic inclusions:- The cytoplasm of T. pallidum is found to consist of various cell organelles such as ribosomes, vacuoles, nucleus and mesosomes. Besides these, cytoplasmic tubules are found originating at each end of the cell just inside the innermost layer of the cell-membrane (Hovind-Hougen & Birsch-Andersen, 1971). These tubules are in two sets of 6-8 bundles (one at each end of the cell) and are about 7.5 nm in diameter. Tubules rising from opposite ends of the cell, overlap at the centre. Their function is unknown.

Flagella:- Spirochaetal flagella (or axial filaments) are similar to other ^{*}flagella. They consist of 3 basic structural components; the hook, the filament and the basal body. The filament is surrounded by a striated sheath. The flagella of Treponema have basal bodies which have a single pair of rings. They are similar to the flagella of Gram positive bacteria such as Bacillus subtilis (De Pamphilis and Alder, 1971). These flagella arise from basal granules located subterminally in the cytoplasm at both ends of the cell. They wrap around the cell in a helical fashion and overlap at the cell centre with those from the opposite ends. Flagella penetrate the protoplasmic cylinder at the site of origin. The number of flagella inserted at each end of the cell varies for the different species of Treponema - but the number is usually 3 for pathogenic treponemes and 1-8 for non-pathogenic or cultivable treponemes. T. pallidum thus has flagella arising from each end of the cell. Flagella are composed mainly of protein with a molecular weight of 33,000 - 36,000 with small amounts (3%) of hexose. The acid composition of the protein is very similar to that of bacterial flagellin (Bharrier and Allis, 1974).

Flagella are believed to be organs of motility and there is good proof to support this theory. It has been suggested that if the protoplasmic cylinder is rigid and the external sheath flexible, treponemes may move by the rotation of the axial filaments (flagella), their insertion plates acting as a kind of ball and socket joint. This would cause the protoplasmic cylinder to rotate in the* direction while the flexible outer sheath would rotate in the same sense as the filaments and roll about the protoplasm like the thread on a tank. (Berg, 1976 - cited in Harris, 1981). The above model for treponemal motility ascribes a primary role to the flagella.

Bharrier and Rittenberg (1971) on the other hand, whilst not completely excluding the role of flagella in treponemal motility, have shown that rabbit antiserum against whole cells of T. zeulzeriae immobilises these cells, whereas antiserum directed against the axial filaments only, does not do so.

BIOCHEMICAL, METABOLIC AND CULTURAL CHARACTERISTICS

Since it is not yet possible to cultivate T. pallidum in vitro researchers have used cultivable treponemes e.g. T. phagedenis to determine the metabolic and chemical properties of the genus Treponema. The OE of T. pallidum contains 4-5% lipids (Watcher and Johnson, 1976). These are mainly phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The major glycolipid is monoglycosyl diglyceride. The hexose component of this glycolipid varies in the different species of Treponema.

* addendum - opposite

T. pallidum has been found to degrade glucose and pyruvate (Nichols and Baseman, 1975). The end products of pyruvate degradation are CO₂ and acetate. Pyruvate is degraded by a combination of EMP and HMP pathways. Pyruvate is degraded from the carboxyl position. Thus the TCA cycle may occur in T. pallidum. Glucose is degraded by a combination of EMP and HMP pathways (Schiller and Cox, 1977).

Protein synthesis is optimal at 34°C and pH7.6 (Baseman and Hayes, 1974). The majority of amino acids are incorporated but serine and valine are used most commonly. Synthesis of proteins is inhibited by erythromycin. A spectrum of high molecular weight proteins are synthesised. Motility of the treponemes is found to diminish during protein synthesis.

Motility of T. pallidum remains unaffected during RNA synthesis which is optimal at 37-39°C in an atmosphere of 20% O₂. T. pallidum is capable of synthesising and processing RNA to 23s, 16s and 4-5s classes (Nichols and Baseman, 1978)

The guanine and cytosine content used normally in the classification of organisms are found to show differences between T. pallidum (83%) and the cultivable treponemes - T. phagedenis (38-39%) and T. refringens (41.5%).

There have been various conflicting theories as to whether T. pallidum is an anaerobe or an aerobe. Earlier T. pallidum was believed to be a strict anaerobe (Nelson and Mayer, 1949, Johnson, 1977) despite the fact that actively motile T. pallidum were found in serous fluid from surface lesions which are exposed to air.

In order to maintain T. pallidum in cell monolayers, oxygen is necessary. Fitzgerald et al. (1977) and Sandok et al. (1978) have found that incubating T. pallidum in an atmosphere of N₂, CO₂ and 3-6% O₂, instead of under total anaerobiosis, enhanced survival. Virulence was maintained from 5-21 days. Cox and Barber (1974) and Steiner et al. (1984) have suggested that the reported oxygen toxicity may be either due to substrate depletion or due to the formation of toxic intermediates. Baseman et al. (1976) found that optimal substrate degradation, protein synthesis and motility were found in T. pallidum at 10-20% concentrations of O₂. These were markedly reduced during anaerobiosis. Though these studies indicate that T. pallidum is capable of accepting O₂ and may possess an oxygen-dependant cytochrome oxidase electron acceptor, the presence of CO and CN which are metabolic poisons for the cytochrome oxidase of other aerobes did not affect T. pallidum.

Cultivation of non-pathogenic treponemes have shown these to require a medium which contains amino acid, purines, pyrimidines, carbohydrates, inorganic ions and crystallised serum albumin (Steinman et al., 1952). The addition of foetal calf serum (FCS) and bovine serum albumin to the medium enhances survival of the pathogen (Chalmer and Taylor-Robinson, 1979). Since an atmosphere of more than 10-20% oxygen is detrimental to the organism, reducing agents such as cysteine, glutathione etc, are incorporated to maintain optimal redox potentials. Though studies on the cultivation of T. pallidum in tissue cultures have revealed that the organism can survive and even multiply for a short time, sustained multiplication has not been possible, though Jones et al. (1976) claimed to have accomplished a growing aerobic culture of T. pallidum in baby hamster kidney cells (BHK cells)

These studies were not verified by Foster et al (1977). T. pallidum can survive on culture cell types of a variety of tissues: testis, skin, cervix, spleen, kidney, lung, urethra, nerve, lymph nodes, embryonic liver, prepuce, foreskin, ear, cornea, nose and footpad.

PATHOGENESIS OF T. PALLIDUM

T. pallidum has a capsular slime layer which is believed to be made of an acidic mucopolysaccharide (Zeigler et al, 1976). Being an acidic mucopolysaccharide, the treponemal capsule is highly water-soluble and constantly dissipates from the surface of the organisms. The capsule is extremely important to the survival of T. pallidum as it can block the entry of large molecules into the T. pallidum thus preventing too many antibodies from getting near the cell. It is also antiphagocytic and also masks the antigen markers on the OE thus limiting the host's defence system. The capsule can interfere with immobilising antibodies (Fitzgerald et al, 1985). Mucopolysaccharides (or glycosaminoglycans, as they are now called) are loosely bound on the surface of the T. pallidum cells and can be detected by the addition of bovine serum albumin which forms a precipitate with them. Both chondroitin sulphate and hyaluronic acid have been shown to be a part of the treponemal capsule.

Since the treponemal capsule is important to the survival of T. pallidum they need to resynthesise the capsule. The acidic mucopolysaccharides of T. pallidum are composed of N-acetyl-D-glucosamine subunits. Though T. pallidum cannot synthesise this compound it can assemble the sub-units to form the capsule. Therefore, T. pallidum has to rely on an exogenous source for its N-acetyl-D-glucosamine sub-units. This compound is the main component of hyaluronic acid which is present in the tissue ground substance present on the surface of host cells.

In order to obtain this N-acetyl-D-glucosamine, T. pallidum cells require close contact with host cells. This attachment process is mediated by a treponemal mucopolysaccharidase enzyme. This enzyme is not present in non-pathogenic treponemes. Mucopolysaccharide ground substance is found in relatively large amounts surrounding capillary vessels. It provides structural integrity to these vessels. There is also a smaller amount of mucopolysaccharide material on the inside walls of the capillaries. The capillary walls are joined together by mucopolysaccharide material and are composed of endothelial cells. T. pallidum has a high affinity for tissues which have a higher concentration of mucopolysaccharide ground substance such as the dermis, testis, aorta, eye, placenta and umbilical cord. These sites are thus the commonest targets of infection. T. pallidum^{*} enters via breaches in the intact skin or via the mucous membranes. Initially, large numbers of T. pallidum are present on the host cell without any overt histopathological damage. The binding of T. pallidum to host cells is believed to be receptor-mediated. These receptors are present at the tip of the T. pallidum cells and are found to be 3 major treponemal proteins - proteins 1 - 3. (Baseman and Hayes, 1980). These receptor binding proteins are found to be associated with various host plasma proteins such as albumin, and β - 2 macroglobulin, fibronectin, transferrin etc. (Alderette and Baseman, 1979), however, fibronectin is the most important in the pathogenesis of T. pallidum. (Peterson et al, 1983; Fitzgerald and Repesh, 1984). On entering the host cell, selective T. pallidum cells bind to the fibronectin, collagen 1 and hyaluronic acid within extracellular matrix at the site of entry, while others make their way through the matrix, reach the vascular basement membrane and rapidly disseminate. (Fitzgerald et al, 1984).

At the site of entry the T. pallidum preferentially multiplies and induces the primary lesion which is a histological manifestation of the infiltration of lymphocytes and plasma cells to the site of entry and which also occurs due to the breakdown of hyaluronic acid and chondroitin sulphate at the site of entry causing breakdown of capillaries and necrosis. Mucopolysaccharide material accumulates at the site of the primary lesion. This may explain the hardening of the chancre (Fitzgerald et al, 1982). Wrzolkowa and Kozakiewicz (1980) suggest that the painless course of the primary indurated chancre could be due to the degenerative changes in the axon terminals at this site.

T. pallidum rapidly disseminates to other tissues through the blood and lymphatics, possibly attaching at the inner surface of capillaries. The treponemal mucopolysaccharidase may degrade the mucopolysaccharides that join the capillary endothelial cells. By splitting these cells apart, the cells may gain access to the perivascular area. Further degradation by the treponemal enzymes would produce the characteristic degradation of the mucopolysaccharide layer that provides support for vessels. The collapse of these vessels would produce the characteristic obliterative endarteritis, inhibited blood supply, necrosis and ulceration. This causes secondary lesions of syphilis and similarly tertiary lesions except the gumma which may originate due to a delayed hypersensitivity phenomenon.

The host immune mechanisms which may limit the primary stage and prevent the successive stages from occurring are outlined later (Vide Chapter V).

CHAPTER IV . A

EARLY ACQUIRED SYPHILIS

Syphilis is an infectious, chronic, systemic disease, the casual agent of which is Treponema pallidum. The pathogen is transmitted mainly through sexual contact. Other modes of transmission include transplacental transmission from the infected mother to the foetus, through transfusion of contaminated blood, through contaminated needle-stick injuries, through the use of improperly sterilised medical appliances such as catheters, needles (syringes) and other contaminated fomites. Nevertheless, these treponemes are, however, very delicate organisms which are unable to survive for long away from the human body and soon undergo dessication. This, therefore, limits the possibility of their spread through fomites.

Doctors and other medical and paramedical professionals may acquire the disease on their part due to the lack of proper precautions (such as the use of gloves) when examining patients. Syphilis has been known as the great imitator and it can simulate various other diseases which infect different parts of the body. A diagnosis of the disease on clinical judgement alone, may sometimes prove difficult. This disease is characterised by periods of florid manifestations interrupted by periods of asymptomatic latency.

The incubation period of syphilis in humans has been found to vary between nine and ninety days, however, it is usually between seventeen to twenty-eight days before the first visible signs appear at the site of entry of the treponemes into the body. The medium incubation period may vary and is usually inversely proportional to the size of the inoculum. The primary stage is manifested by the appearance of a primary lesion and swelling of the inguinal lymph nodes. This stage usually lasts for 3-8 weeks after which a spontaneous healing of the primary lesion occurs, is followed by the secondary stage. This stage generally occurs 6-8 weeks after the appearance of the primary lesion. Sometimes, however, the symptoms may appear after a longer interval which may last for even a year or more. The secondary stage is systemic and occurs due to a dissemination and multiplication of the treponemes in various parts of the body. The primary stage, in contrast, is locally manifested and occurs due to a replication of the treponemes at the site of entry. The secondary stage is generally characterised by generalised lymphadenopathy and a mucocutaneous rash which may appear on any part of the body as a result of tissue reaction to the presence of the treponemes. Involvement of various organs of the body such as the liver, spleen, kidneys, heart, skeleton, joints, larynx, eyes, meninges and brain has been observed. The skin, lymph nodes and genital tissue are commonly involved. Treponemes have been observed in blood, the milk of lactating females, in tears, saliva and CSF in various cases in the secondary stage. This stage usually lasts for 3-9 months and is followed by an early latent phase which may last into the second year after initial infection. At this stage, the treponemes still are present within the body. However, this condition is asymptomatic.

This period may be followed by a late latent stage which may last for many years. 30-50% of untreated patients in this late latent stage may go on to the tertiary stage which is characterised by progressive destructive mucocutaneous, mucoskeletal or parenchymal lesions, aortitis and CNS disease.

EARLY ACQUIRED SYPHILIS

Primary syphilis, secondary syphilis and early latent syphilis are together classified as early acquired syphilis. Another state which may occur as a result of inadequate treatment of secondary syphilis is that of early acquired relapsing or recurrent syphilis in which lesions of the secondary stage may appear repeatedly alternating with period of latency. However, this stage does not occur after the second year of infection.

PRIMARY SYPHILIS

This stage is characterised by the appearance of a primary lesion or chancre, at the site of infection of the treponemes. The primary lesion can occur on any part of the body. The most common sites (90% of chancre sites) in males and females are on the genitalia and associated organs - in males these sites are the coronal sulcus of the penis, inner surface of the prepuce, the glans and the shaft of the penis (Fig.1) and in females they are the vulva, the labia majora or minora, the fourchette, the clitoris or near the urethral orifice (Fig.2)

They may also be
vaginal wall. Ex
fingers, breasts,
also been reported



Fig.1: Primary syphilitic chancre on the genitalia in a male patient.



Fig.3: Co
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The chancre is bel
at the site of trep

lesion is single. Multiple lesions may occur. The treponemes enter the body through abrasions in the skin or mucous membranes. The organisms quickly spread to the draining lymphatics and different tissues.

Fig.2: Primary syphilitic chancre on the genitalia in a female patient. A pustular rash is also seen in the anogenital region.

At this point, treponemes present in the rest of the body are apprehended by the body's immune system and it is only the treponemes at the site of entry which multiply. In response to the presence of these multiplying treponemes, an influx of lymphocytes and plasma cells to the site of infection occurs.

They may also be found on the cervix and sometimes on the vaginal wall. Extragenital primary chancres of the mouth, fingers, breasts, eyelid, ear, between toes (Fig.3) have also been reported.



Fig.3: Condylomata lata
between the toes

The chancre is believed to appear as a result of local trauma at the site of treponemal entry. In most cases the primary lesion is single. Multiple lesions also have been found to occur. The treponemes enter the body via any damage or abrasions in the skin or mucous membranes. The organisms quickly spread to the draining lymphatics to different tissues. At this point, treponemes present in the rest of the body are apprehended by the body's immune system and it is only the treponemes at the site of entry which multiply. In response to the presence of these multiplying treponemes, an influx of lymphocytes and plasma cells to the site of infection occurs.

These lymphocytes and plasma cells accumulate around the blood vessels and lymph vessels in the infected area. As a result of this lymphocyte-plasma cell-treponeme infiltration, the integrity of these vessels is breached, resulting in haemorrhage, inhibited blood supply, erosion and eventual necrosis. This results in the typical, primary ulcerative lesion of syphilis i.e. the chancre. The chancre is circular, about 1cm in diameter with a clean granular base. It may sometimes have a crust of dried-up secretion covering it. When pressed, the chancre is hard, indolent and ahaemorrhagic. It exudes a serous fluid which is loaded with treponemes. In fact, it is this serous exudate which is used for examining under the darkground microscope to determine the aetiology of the chancre (see chapter VI: Diagnosis of syphilis). This ulcer has a hard edge and base and is therefore, firm and indurated. Approximately a week after the appearance of the chancre, the inguinal lymph nodes become enlarged.

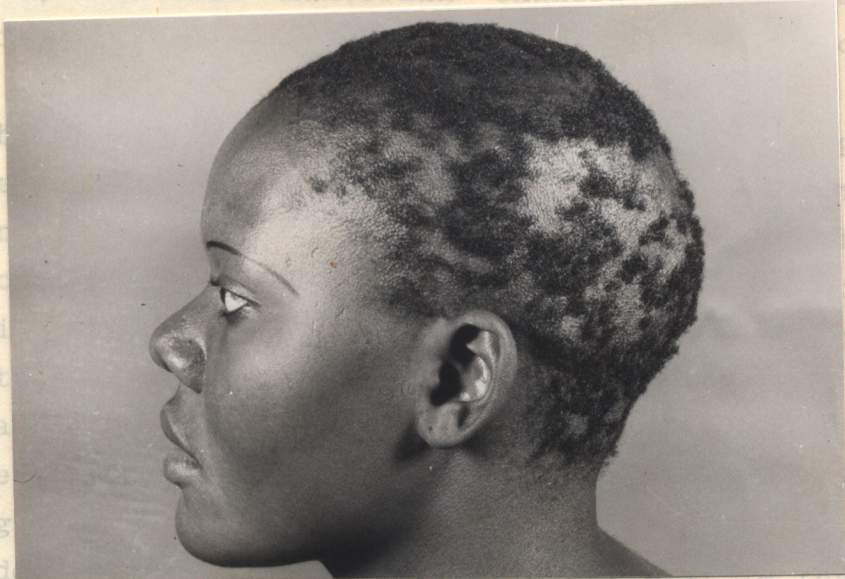


Fig.4: Syphilitic alopecia (loss of hair)
in a female patient.

They are discrete and painless but of a firm, rubbery consistency. With syphilitic chancres on the genitalia, the enlargement is often bilateral but with extragenital lesions it may be unilateral. The syphilitic chancre is usually painless and the status of the inguinal lymphadenopathy is non-tender. (The only discomfort caused is when the patient walks). However, if the lesion is infected, secondarily by pyogenic bacteria, the chancre and the lymph nodes may become very painful.

DIFFERENTIAL DIAGNOSIS OF GENITAL CHANCRES

A combination of clinical evaluation, darkground examination and serological testing is more than sufficient to establish a diagnosis of syphilis. But if one were to come across a genital lesion which did not appear to be like the typical painless, indurated 'Hunterian' chancre of syphilis, the following differential diagnosis would have to be considered:-

(i) Chancroid: The incidence of chancroid in temperate climates is low, however, it is one of the most commonly occurring sexually transmitted diseases in the tropics and sub-tropics. The sore of chancroid can be differentiated from a syphilitic sore by the following characteristics:-
(a) it is soft (b) it bleeds (c) it is painful (d) more importantly, the darkground investigations of the chancre exudate present negative results (e) the patient presents negative serological tests for syphilis. Besides the sore, the inguinal lymphadenopathy is distinctly different - it is tender and inflammatory, whereas it is normally non-tender and indolent in syphilis.

(ii) Genital herpetic lesions: Usually appear as small vesicles which may sometimes fuse to form a large chancre-like eruption. DGI and lack of induration in lesions help differentiation. The culture of Herpes simplex virus (HSV) from these lesions is useful.

(iii) Scabies with secondary infection: Presence of other burrows on different parts of the body, a history of nocturnal itching and demonstration of Sarcoptes Scabiei (or its ova) in the lesions, helps in differentiating these from syphilitic lesions.

(iv) Lymphogranuloma venereum (LGV): The primary lesion is a vesicle which heals quickly. Inguinal adenitis is usually unilateral. It is possible to cultivate Chlamydia from the pus which exudes from an eroded bubo.

(v) Granuloma inguinale: Occurs in the tropics. The initial lesion is a painless ulcer which spreads to form a red granulating mass. Donovan bodies are found in biopsy specimens.

(vi) Epithelioma: This usually occurs in older men. The lesion is usually typically raised, rolled and has an everted edge. If the infection is allowed to continue, the inguinal lymph nodes become very indurated.

(vii) Other syphilitic lesions. Lesions of secondary syphilis though DGI positive and also positive for serological tests can be differentiated due to their multiplicity and also due to generalised symptoms (see below) appearing on different parts of the body. Granulomatous lesions can be differentiated by their negative DGI test, though they (patients) are seropositive.

(viii) Tuberculous lesions, erosive balanitis, Behcet's disease may sometimes confuse the clinician.

Sometimes, primary syphilitic chancres may assume a pseudoneoplasmic appearance.

Cases of primary chancre being initially misdiagnosed as a cancer of the cervix have been reported. Dogliotti (1971) has described a number of cases among the Bantu who presented with atypical primary chancres and a suppurative inguinal lymphadenopathy. Lejman & Bogdas-Zewska (1969) have reported a case of a primary chancre secondarily infected with fusospirochaetal organisms leading to a perforation of the anterior wall of the preputial sac.

DIFFERENTIAL DIAGNOSIS OF EXTRAGENITAL CHANCRES

Extragenital chancres may appear on any part of the body. They can be diagnosed by darkground microscopy of the chancre exudates or aspirate obtained from puncture of a regional lymph nodes, serological tests and immunofluorescent staining. A history of sexual contacts may prove useful. The common sites of extragenital lesions are:-

(i) Lip or tongue: A primary chancre on these parts must be differentiated from a mucous patch of secondary syphilis, from a gummatous lesion, from herpes simplex virus with secondary pyogenic infection, from aphthous ulcers and from the oral ulceration of Behcets syndrome.

(ii) Tonsil: Primary syphilis of the tonsil might resemble Vincent's angina, diphtheria or lymphosarcoma.

(iii) Nipple: Syphilitic chancre of the nipple must be differentiated from Paget's disease.

(iv) Finger: Primary chancre of the finger must be differentiated from a simple paronychia

(v) Eyelid: Syphilitic chancre of the eyelid must be distinguished from a sty.

(vi) Anal margin: A chancre at this site may simulate a fissure, a thrombosed external pile, or Bowen's disease.

Sometimes a biopsy section may need to be examined before completing an initial diagnosis.

SECONDARY SYPHILIS

This stage may occur approximately 6-8 weeks after the appearance of the primary chancre. Sometimes the symptoms may take longer to appear. The most common manifestations of the secondary stage are mucocutaneous lesions and a generalised lymphadenopathy. Lesions of the meninges, bones or joints and conditions such as hepatitis, gastritis, uveitis, nephritis and alopecia are manifestations which occur rarely.

Clinical Features of Secondary Syphilis

Skin lesions	75-80%
Mucous membrane lesions	30%
Generalised lymphadenopathy	50-60%
Arthritis, arthralgia, periostitis]	
Hepatitis]	
Glomerulonephritis & nephrotic]	
syndrome]	
Iridocyclitis & choroidoretinitis]	10%
Neurological disease (meningitis,]	
cranial palsies)]	
Alopecia (Fig. 4)]	

The lesions of the skin and mucous membrane may be associated with non-specific constitutional symptoms such as malaise, fever, anorexia, nausea or vomiting, nocturnal headaches, constipation and pain in the long bones. These symptoms are more common in female patients (50%) than in males (30%).

If lesions are present on the throat the patient may suffer from sore throat and if on the larynx, the patient may suffer from hoarseness when vocal.

Lesions of secondary syphilis: Secondary syphilitic mucocutaneous lesions vary greatly. They are divided into four main groups (i) macular or roseolar (ii) papular (iii) papulosquamous (iv) pustular. These lesions are generally widespread and symmetrically disposed. They are non-irritating. Rashes vary from a pink or dusky rose to a coppery red in colour. Pigmentation of the rash is not clearly visible in patients of negroid origin. The rash is commonly seen on the flexor surfaces of the body. The lesions are usually indolent, asymptomatic and indurated. However, most papules and condylomata lata may cause severe itching. These lesions may appear in the different stages and types on the same patient simultaneously. This condition is called pleomorphic or polymorphic. The lesions are usually rounded.

(i) Macular or roseolar rash - The first eruption to appear. The lesions are rounded, discrete, flat and between 0.5 - 1.0cm in diameter. They have a light pink colour which is believed to be due to the presence of T. pallidum in the skin and engorgement of the blood vessels in the surrounding area. These eruptions are usually very faint in appearance and can hardly be seen in artificial light. (The rash is quite transient and may sometimes go unnoticed). It usually appears on the shoulders, chest, back, abdomen and on the flexor surfaces of the upper arms.

Sometimes the rash may spontaneously disappear and at other times it may persist and become papular giving rise to a maculopapular rash (Fig. 5).

Fig.5: An extensive papulosquamous syphilide on the back of a syphilitic patient.



Fig.6: Palmar

(ii) Papular rash - The most common of the secondary syphilitic lesions. These lesions are present on the trunk, arms and legs and (unlike in the case of the macular lesions), these are also found on the face, genitals, palms and soles. (Fig.6, Fig.7) The lesions are slightly raised, dull pink or red in colour and indurated. They are approximately 25mm - 1cm in diameter. Sometimes a line of papules is seen on the forehead, just below the hairline called the corona veneris.