In 1492 Christopher Columbus sailed west and found an undiscovered New-World. On his return he and his companions brought back many strange tales and treasures and, it is widely believed, syphilis – the GREAT POX.

Over the next few decades this infection spread like wildfire throughout the known world and was, for four and a half centuries, the HIV/AIDS of its day. It was feared, took no account of the race, creed or social standing of its victims and destroyed the lives, well being and reputations of millions of people. Only the use of penicillin for the treatment of this infection from the mid 1940’s allowed medical science the opportunity to treat, cure and bring this scourge under control.

This presentation aims to give an insight into the history of the disease, the role of Medical Laboratory Science and Scientists in elucidation of the cause and its laboratory diagnosis.

Not all Historians agree that syphilis was a new disease many believe that it was already present in Old World. The arguments are complex and only summarised below:

- The first documented outbreak of syphilis, or “the great pox,” followed the siege of Naples by the French in 1494. Giving rise to the legend that Columbus’ men had brought the disease back from the New World.
- It is said that there is no evidence of treponematoses on mainland Europe prior to 1492.
- Whatever the bones of a medieval woman found in a churchyard in Rivenhall, near Chelmsford Essex, who died between 1236 and 1445, show signs of what is believed to be syphilitic, already present in England before Columbus discovered the New World.
- But skeletons have also been found in the United States, which are believed to show that the disease was present before 1492.
- There is disagreement whether it is possible to reliably differentiate syphilis and syphilitic in ancient skeletons. Unfortunately as yet there is no DNA evidence to prove or disprove which of these trepanematoses is involved.
- There are allusions to “Venereal Leprosy” and “Congenital Leprosy” before 1492 suggesting that some medieval “lepers” might have syphilis.
- But Italian physicians had been attributing death to Leprosy since at least 1452 and did not attribute any of the symptoms associated with Leprosy to syphilis.
- Some hold the view that this “outbreak” was the result of large scale population movements across Europe during times of war leading to new strains being moved around. There were large armies assembling in several areas and large-scale troop movements. Whenever there are armies there is likely to be illicit sexual activities that spread sexually transmitted diseases; these, coupled with troop movements, hastened the spread of syphilis.
- Descriptions recorded by first hand observers at the time tell of patients with many pustules, rather than the single pustule usually seen today, and with the symptoms of rash and painful swellings that are now associated with secondary syphilis occurring much more rapidly than they do today. After about 50 years, this particularly virulent form of syphilis seems to have subsided and was replaced by the more slowly progressing form that we see today.

- What everyone does agree on is that the form of syphilis which spread at the time was much more dangerous and deadly than it had been in the past or was to become in the future. Spread by sexual contact, it was highly contagious and caused pustules, pain, and itching of the skin, often spreading all over the body. These symptoms were followed by internal pains and a deterioration of the bones. This stage of the disease often ended in death. A possible explanation for these symptoms is that they were caused by a new disease, but by a more virulent or deadly form of a long-occurring organism. This is not an uncommon phenomenon among bacterial infections; with a modern-day example being that of toxic shock strains Staphylococcus aureus, arising in the 1980s.

- Another view is that it did arise in the New World but, it was brought back by the Vikings, and it was through them that it spread and became established in Europe.

Christopher Columbus

What’s in a Name?

It has been called “The Scourge of the Renaissance”. It was a disease greatly feared by the Tutors who called it “The Frankish Pox” differentiated it from the Small Pox.

- In 1530 an Italian poet named Francesco Domenico Arquato called it Syphillis De Morbus Gallicus (On Syphilis, or the French Disease) which described the plight of a mythical shepherd but named Syphilis afflicted with the disease as a punishment for cursing the gods. The poem recognised the venereal nature of the infection and was a compendium of knowledge of the time regarding the disease.
- Paraenesis (1437-1514) called the new venereal disease “French Gonorhosa” and suggested that it arose through sexual intercourse between a leper and a prostitute with gonorrhoea.
- It has been called the “great miasm” because its symptoms are similar to those of many other diseases.
- In fact, before the introduction of specific bacteriological and immunological tests, many physicians believed that “whenever known all of syphilis knows all of medicine”.

The Fleet of Columbus

The Great Pox

A History of Syphilis and its Laboratory Diagnosis

New World – New disease

The Great Pox is a pox that spread from the New World. It is a disease that threatened the health and livelihood of people all over the world. It is known as the Great Pox and is a serious threat to public health.

In 1492, Christopher Columbus sailed west and discovered a new world. On his return, he and his companions brought back many strange tales and treasures. One of the most intriguing tales was about a disease that had been discovered in the New World. This disease was called syphilis, or the GREAT POX.

The Great Pox spread like wildfire throughout the known world and was, for four and a half centuries, the HIV/AIDS of its day. It was feared, took no account of the race, creed or social standing of its victims and destroyed the lives, well being and reputations of millions of people. Only the use of penicillin for the treatment of this infection from the mid 1940’s allowed medical science the opportunity to treat, cure and bring this scourge under control.

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Christopher Columbus

The Great Pox

A History of Syphilis and its Laboratory Diagnosis

New World – New disease

The Great Pox

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The Great Pox

A History of Syphilis and its Laboratory Diagnosis

New World – New disease
Syphilis and Blood Transfusion

The early practice of blood transfusion usually involved connecting the donor directly to the patient creating a serious problem through the potential transmission of syphilis from donor to recipient. The introduction of effective anticoagulation and storage of blood during the 1914-18 War, resulted in the transmission of syphilis by transfusion being virtually eliminated.

In two reports, published in 1941, Bitch and Turner & Cleek were among those who reported that 30% of a strength of 101,695 to be infected with venereal disease (including syphilis, soft chancre and gonorrhoea). Of these, 41 were constantly sick and 6% invalids. The number of candidates for recruitment that were refused on account of syphilis, per 10,000 offering for enlistment, ranged from 160 in 1870 down to 16 by 1910.

Testing was the original method of Wassermann, Neisser and Bruck (1906) for the WR, with modifications for patients under treatment "to recognise the last traces of Wassermann substance". The final report concluded with a design for a treatment block and a schedule for existing hospitals. "The measures to be adopted in each case must be carefully considered and adapted to existing conditions, with due regard to efficiency and economy." (Nothing new here!)

A Royal Commission on Venereal Disease was established in 1916 to consider syphilis, gonorrhoea and soft chancre. The report contained a memorandum on the results of treatment of venereal diseases with Salvarsan and Neo-Salvarsan in the Royal Navy. 4,203 cases were treated and 9,912 injections were given. The course of treatment was usually by the injection of mercurial salts, by mouth and mercurial baths.

In a report by Leval, Colonel Gilbert (1908) noted the chief cause of a decrease of syphilis in the Army as:

1. Improved methods of treatment
2. Lectures and individual talks
3. Increased temperature
4. Increased attractions in barracks
5. In India the provision of the Cantonment Act

In further reference to temperance, "Many of the admissions are from 298 in 1900 to 117 in 1906 based on the admission ratio per 1000 strength. Army records appear to first record the incidence of syphilis in men in 1861. Records from 1898 show that 30% of a strength of 101,695 to be infected with venereal disease (including syphilis, soft chancre and gonorrhoea). Of these, 41 were constantly sick and 6% invalids. The number of candidates for recruitment that were refused on account of syphilis, per 10,000 offering for enlistment, ranged from 160 in 1870 down to 16 by 1910. In 1905 saw the most serious epidemic of syphilis ever recorded in the British Army. This was due to the use of infected blood from the Influenza epidemic. In the early stages of syphilis, the disease is not easily detectable. The disease grows to epidemic proportions among knights and foot soldiers alike, reaching Italy and Spain by 1444 and arriving in England by 1449. It had catalysed the disease from beyond the ocean into an epidemic that would last more than 200 years.

In 1903 the Advisory Board for Army Medical Services requested an inquiry into "The Treatment of Venereal Disease in the Army and its Prevention in the Treatment of the Itch (Scabies)". The plan of investigation was (a) To ascertain the exact references and records at headquarters dealing with the subject, and (b) To classify this information. This involved methods of prevention and measures adopted for prophylaxis.

In a report by Stansfield (1906) for the WR, with modifications for patients under treatment "to recognise the last traces of Wassermann substance". The final report concluded with a design for a treatment block and a schedule for existing hospitals. "The measures to be adopted in each case must be carefully considered and adapted to existing conditions, with due regard to efficiency and economy." (Nothing new here!)

The manual VDRL test did not transfer to automation and screening blood donations. This test remained popular until fully automated screening of blood was introduced. The manual VDRL test did not transfer to automation and the Treponema pallidum Haemagglutination test (TPHA) became the method of choice. Testing was by the original method of Wassermann, Neisser and Bruck (1906) for the WR, with modifications for patients under treatment "to recognise the last traces of Wassermann substance". The final report concluded with a design for a treatment block and a schedule for existing hospitals. "The measures to be adopted in each case must be carefully considered and adapted to existing conditions, with due regard to efficiency and economy." (Nothing new here!)

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• Edwin Klebs in 1879 was apparently the first to see spirochetal bodies in syphilitic material and to transmit the disease to monkeys.

• Haarboe in 1881 was the first to convey the disease to lower animals; by inoculation of the eye he produced local lesions in the rabbits.

• S. Lautzgarten 1884 & 1885 used staining methods to demonstrate “Spirochaæta pallida” in syphilitic serum of the patients and other sites. But it was shown that similar if not identical bacilli were present in normal secretions.

• The studies by Metchnikoff and Rosse published between 1903 & 1905 showed that syphilis was transmissible to apes.

• Many workers tried to grow the organism in-vitro. Finger and Landsteiner in 1904 demonstrated that the cause of syphilis was particulate.

• Causative organism was discovered by F. Schaudin and E. Hoffmann in 1905. They used a modified Germa stain to demonstrate the presence of a spirogan organism, of characteristic appearance, in the chancres and inguinal glands of syphilitic patients.

• They first called the organism Spirochaeta pallida. Later they accepted the suggestion of William G. that it should be called Spiroplasma pallidum. However as Schaudin was of the view that the organism was a prokaryon and that the name Spiroplasma had already been used for another prokaryon in 1905 he introduced the name Treponema pallidum. Although in the first edition of Koch’s Manual of Determinative Bacteriology 1901 the name Treponema pallidum, the name Spiroplasma pallidum was still used by Kahn in 1905 and in the chapter on syphilis in volume 11 of the Medical Research Council’s Series ‘A System of Bacteriology in Relation to Medicine’ published in 1931.

• Landsteiner and Mucha proposed using dark-ground illumination to detect the organism.

• In 1903 Laevisetti invented the light impregnation method for demonstrating the presence of syphilis.

• Many workers test the organism in vitro. Schwartschezky in 1893 and Bongrino in 1911 & 1912 reported success. Nocht used serum water, utilizing steel ratel kidney or tissue, covered the surface of the bull with liquid paraffin and incubated aerobically at 37°C. Subculture studies have shown that starker “T. pallidum” has not been grown in artificial culture.

From the 1900s until the end of the 1920s in most routine Bacteriology Laboratories there was one day a week known as “WR Day”. This was the day on which the WRs – the Wassermann Reaction a complement fixation test for syphilis and VDRL a flocculation test for syphilis – were done; and until it was known that they had tested it was often a tense day. This was particularly so when a Junior Technician was allowed to do the tests on their own and it was known that they had worked it was often a tense day. This was particularly so when a Junior Technician was allowed to do the tests on their own and it was known that they had worked it was often a tense day.

COMPENDIUM FIXATION

1901 Bordet and Negguo describe the complement fixation reaction

1905 Wassermann, Neisser and Bruck described a complement reaction using aseptic extract of testicular fluid in syphilis as antigen – Wassermann Test. Wassermann Reactions (WR). Rokitansky, Wassermann Test

1907 Marks and Leduc used aqueous extracts of normal liver and other organs as antigen. They found that an alcoholic extract was even better.

1913 A. Neisser and Schaudin showed that the reacting substance was the definite reaction of syphilis, soluble in alcohol but not acetone.

1929 Browning & MacKenzie improved the sensitivity of the test by adding an alcoholic solution of cholesterol.

FLOCCULATION TESTS

1916 Sache and Georg published a flocculation test

1922 R.L. Kahn published a simple quantitative precipitation reaction for syphilis. The Kahn test which became the standard test for many years.

1946 Hama et al described the Venereal Diseases Research Laboratories test (VDRL).

1948 The Price Precipitation Reaction (PPR) was published

1950 The Unheated Serum Reagin (USR) which uses VDRL antigen plus China Chloride to block mixing substances in plasma

1953 Purkhy described Rapid Plasma Reagin test (RPR)

1958 Automated Reagin Test (ART) used on the Technicon Auto Analyzer uses VDRL carbon antigen.

Antis-Treponema Tests

AGGLUTINATION

1935 Laevisetti observed agglutination of T. pallidum in specimens made from the contents of bullae in syphilitic pemphigus

1936 Tomizawa and Kasamatsu published the Treponema pallidum haemagglutination test (THT) in which T. pallidum antigens are absorbed onto formalised tanned sheep red cells. Later Chicken or Turkey cells were used as these are nucleated and settle faster.

COMPLEMENT FIXATION

Extracts of Reiter’s treponeme are used as the antigen in the Reiter Protein Complement Fixation Test (RPCFT).

IMMUNOFLUORESCENT TEST

1936 Kaufman and Brule reported that CSF from secondary syphilis, especially from those patients with general paralysis, immobilised the organism.

SKIN TEST

1912 Nocht prepared extracts from “culture” of Treponema pallidum and used them in a skin test in a similar way to the laboratories test. A positive reaction was often obtained in cases of latent syphilis in which the WR was negative.

1940 Nelson and Mayer TPI Treponema Pallidum Immobilisation Test

FLUORESCENT ANTIBODY

1957 Deacon Falcon & Harris published the Fluorescent Treponemal Antibody test (FTA) – originally the serum diluted 1 in 5, but this produced too many false positives a dilution of 1 in 200 (FTA 200) was found to be more specific.

1958 Wilkins and Reyner described the FTA absorbing test (FTA-ABS) in which antibodies to Treponemal group antigens were absorbed with ultrasonically disintegrated Reiter treponemes. Indirect, Direct, Fluorescence test can be used for the detection of spirochates in sera.

Other Tests

1972 Lange and Snegoryev published the Coulter Gold Sol Test for use with CSF in cases of suspected the Neurosyphilis.

Reagin Tests

1901 Bordett and Gingep described the complement fixation reaction

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For the WR

Materials & Equipment

• 96% Water bath, 37°C water bath

• Racks for 3 x 7 inch glass tubes

• 37°C incubator

• Donald’s Dripper – a separating funnel filled with a short glass tube 7mm o.d. diameter (all 30 drops per minute this delivers 0.1ml)

• Pasteur pipette with an end diameter equal to 56 of a Stenholm-Morse gauge (Edwerts 0.150mm) (i.e. 1/5th volume)

• 100 ml glass measuring cylinders, graduate 10ml pipettes

Reagents

• 0.5% Sodium Chloride (proc free)

• Complement (C) – Guinea pig serum preserved by Richardson’s method

• Wasserman antigen

• Control sera

• 2.5% suspension of Formalised Sheep cells sensitised with 5 MLD – ammoniac

• 2 x 7 inch glass tubes or VDRL Porex trays

• Minimum Haemolytic Dose

Method

9.00am Inivate test and control sera in 5FC water bath to for 30 min.

• Wash sheep cells (RBC) in x 3. Check PCV and make a 5% aqueous solution

Run out dilant serum for tests and for Complement reaction (CT) with Donald’s Dripper

6% RBC antigen leave to ripen for 20 min

3.90am Remove sera for 5FC and add control sera to CT Add antigen to CT

Prepare 10 ml volumes of dilution from 1:100 to 1:10 and add to CT

Inactivate CT at 37°C for 30 min

Add 1/5th volume dilution of from 1:100 to 1:10 to CT

Inactivate C’ at 37°C for on hour

10.00am Go to coffee

10.30am Remove C’ from incubation add (volume sensitised RBCs, then reincubate at 37°C for 15 min, shake and incubate for a further 15 min.

Add one volume of antigen to test wells and one volume diluent to control wells

11.00am Read C’ formation, calculate MHD C’

Pepared 1.25 MHD and 1 MHD dilution

Add 1 volume of 1.25 MHD C’ to test wells

Add 1 volume MHC C’ to control wells

Inublate and add RBCs as in CT

Set up, perform and read (thai tests)

Do Kahn confirmation tests

Read WR Results

Record and report WR & Kahn results

Tidy up

Read and report WR & Kahn results

Final and if successful become a qualified Technician – with a pay rise!

August von Wassermann
1866–1925
Sexually transmitted

Primary Disease
- A local infection involving mucocutaneous sites and their draining lymph nodes
- Incubation: 10 days to 10 weeks
- Painless ulcer with a border and base of induration
- Eventually cleans up

Secondary Disease
- Six to eight weeks after primary lesion
- Spirochaetes with fever, rash, and generalized lymphadenopathy
- Rash is generalised maculopapular involving the palms and soles
- ‘Snail track’ ulcers in the mouth
- May also be meningitis, arthritis, arthralgia and iritis or retinitis

Latent Disease
- Asymptomatic phase which may persist for years
- Slow tissue damage occurs, some show signs of CNS involvement

Tertiary Disease
- May occur after one year or may take 10 years to develop
- Affect many systems of the body
- Patients develop gumma, which is an indolent granulomatous lesion which may undergo central mucoid degeneration
- Skeletal damage may give rise to Charcot joints
- Paresis of the nerves may occur giving rise to syphilitic psychosis
- Tabes dorsal disease in the spinal cord resulting in a typical shuffling gait
- Cardiovascular disease can include aortitis, aneurism aortic regurgitation

Pregnancy
- In pregnancy syphilis infection can result in miscarriage, premature birth, still birth or infant death

Congenital
- Infants born with syphilis can suffer from
  - Deafness
  - Hutchinson’s teeth and facial disfigurement
  - Interstitial Keratitis
  - Hepato-splenomegaly
  - Rashes
  - Sabre shins
  - Saddle nose
  - etc etc

Historical Treatment of Syphilis

The earliest documented treatment of the pox (syphilis) involved the use of mercury from the early part of the XVI century onwards. There are accounts of ointments and balms, almost certainly of a mercurial base, being used by Arab physicians, to treat ‘yaws’ at the time of the Crusades in the XI century. Treatment of leprosy with mercury preparations was known at this time and possibly syphilis also?

There are numerous descriptions of topical and oral preparations being administered, some of the effects of the treatment being as hideous as the disease itself.

The other treatment described alongside mercury in the XVI century is gaiac. Gaiac is powdered wood from the guaiacum, a tropical tree found in the Caribbean and Central American region. The powdered wood is made into a potion by boiling it down to a decoction, which is administered in large doses after the patient has previously been given purgatives and dieted to a meagre ration over several weeks.

This treatment took place in a heated room with the patient wrapped in blankets to induce sweating until ‘the sickness had been rooted out’

There was strong opinion for and against both treatments and many scholars reported that both treatments were often administered together, rather than as a single remedy.

However, mercury treatments held sway for around 300 years, even though many researchers and scholars reported that both treatments were often administered together, rather than as a single remedy.

In Victorian times the advent of commercial potions to treat almost anything was life and many preparations were offered to treat syphilis including potassium iodide, which was described in the Lancet in 1835.

In 1820, Paul Ehrlich and Sahachiro Hata created ‘Compound 606’ an arsenical, which was the constituent of the drug they called Salvarsan.

The use of bismuth, as an effective treatment was described by Sazarac and Lavaditi in 1921, who used tolerable doses to treat their patients, although Balzer had first described its use in 1899.

Undoubtedly, the turning point in the treatment of syphilis was the use of the antibiotic, penicillin, first described by Alexander Fleming in 1928. Following the work of Chain and Florey to purify the substance for safe human use, commercial production was possible.

Mahoney, Arnold and Harris, successfully treated four cases of syphilis in 1943 in U.S.A, with the newly produced drug. This treatment found rapid acceptance especially by military doctors throughout the forces in the Second World War.

The effectiveness of penicillin was demonstrated against all stages of the infection and in a timescale of a few weeks, compared with the years of treatment with previous remedies.