The Wassermann Test in Leishmaniosis

by

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Leishmaniosis americana is taken to be one of the diseases in which the WASSERMANN test is frequently positive. This would appear, however, to be the result of erroneous observation, probably on cases having suffered from syphilis and in whom the disease was either not identified or not looked for. The same might perhaps be said of many other diseases, malaria, measles, scarlet fever etc. in which positive results have also been reported. Of these we will treat later but we are already entitled to assert, even though our statistics are still small, that we only obtained positive results on patients of scarlet fever measles or malaria that also suffered from syphilis. We will later submit proofs and arguments.

Syphilis is sometimes difficult to diagnose, inasmuch as the patients deny suffering from it. But after a positive test carried out with care, syphilis should be looked for by every other means possible.

The WASSERMANN test, although not based on a specific reaction, is positive in an enormous number of cases in syphilis. The reaction reveals the modifications introduced in the contaminated organism by the disease: a well done specific treatment makes them disappear sooner or later. For these reasons, and although we do not know the fundamental cause, the WASSERMANN test preserves a certain specificity in syphilis.

In how many cases in which it was impossible to diagnose syphilis clinically has not the WASSERMANN test aided in establishing a diagnostic which followed by specific treatment resulted in considerable improvements in the patient’s health? As it is a very complex reaction, which depends on numerous elements, it is necessary that they should all be perfectly titrated: only like that can the results be relied on.

The differences in results of different authors are certainly due to the different technic used by each one. If the test is carried out under the same conditions and with the same technic it will give the same results even if done by different workers.

It is extremely necessary to use
more than one, and at least three antigens in the reaction, all of them titrated and studied as to their fixing power: the WASSERMANN test should never be practised with one antigen, because this might bring about grave errors. Cholesterinised antigens should be used with care and cholesterinising should be made by a previous titration with known sera and comparatively with antigens already tried: thus the optimum quantity of cholesterin to add to a given volume of antigen is reached. This titration is lengthy and the antigen must only be made use of after it has been well verified with numerous known sera and compared with antigens already tried.

We tested the serum of 60 patients of Leishmaniosis americana in the 19th Ward of the Santa Casa de Misericordia of Rio de Janeiro, thanks to the courtesy of the chief, Prof. Dr. FERNANDO TERRA to whom we are very much indebted for his kindness.

In all patients we searched for syphilis most painstakingly, so as to be able to come to exact conclusions.

The clinical diagnostic of leishmaniosis, especially in the mucous form of the disease, lends itself to confusion with syphilis in its similar lesions: it is a capital fact that syphilis attacks the bony tissue whereas leishmaniosis may destroy the tissues and the cartilage but always leaves the bone intact.

The clinical precedents of each of the cases of great importance in revealing or excluding concomitant syphilis.

The serologic examination renders, we think, invaluable services, for out of the 60 cases we observed, not a single one of pure leishmaniosis, free from syphilis deviated the complement when mixed with the antigens used in the WASSERMANN test.

Technic employed in carrying out these reactions.

Antigens:—The antigens used were six in number: leukic liver (alcoholic extract of liver of foetus with hereditary syphilis); human heart (cholesterinised alcoholic extract); ox-heart (cholesterinised alcoholic extract); antigens of acetone-insoluble lipoids, according to NOGUHI’s technic; antigen of BORDET and RUELENS (used in the Pasteur Institute of Brussels, a modification of NOGUHI’s process of extracting acetone-insoluble lipoids); antigen of LESSER (etheric extract of ox-heart). Acetone-insoluble lipoids, both for NOGUHI’s and for BORDET and RUELENS’ process were extracted from ox-heart.

All these antigens were perfectly known having been tried on numerous known syphilitic and known normal sera. Nevertheless, to make sure, they were all titrated again on the day of the reaction as to their inhibitory power and controlled with known positive and known negative sera. For verifying the inhibitory power we used two or three times the dose employed in reactions together with a fixed dose of complement and sheep-corpuscles sensitised with two or three hemolyzing units.

Complement:—We employed fresh guinea-pig serum; to obtain a more homogeneous complement we employed from eight to ten guinea-pigs which were bled on the same day the reaction was carried out. Complement titration was done by mixing with a fixed dose of hemolysins and the dose of antigen to be used in the reaction.

Hemolytic system:—Rabbit anti-red corpuscles of sheep, the potency of the serum for the corpuscles being always controlled on the day it is made use of.

We also verified all the other elements, complement, hemolysins, antigens and saline solution, taken one by one and mixed with sheep corpuscles to see
if by themselves they would produce hemolysis.

All these indispensable complementary tests having been performed so as to make sure of the working of all the elements entering into the reaction, we next carried out the test with the serum of the patients of leishmaniosis. These sera were collected, separated from coagulum and inactivated in a water-bath at 56°C, during half an hour, and were then kept on ice until next day when the test was carried out.

0.2 of serum were placed in each of seven tubes: each of them received 0.2 of a different antigen except the last one which remained as control of serum. We next added the quantity of complement determined by the previous titrations and filled all the tubes up to 2 cc. with saline solution at 0.85%.

The tubes were then placed in a water-bath at 37°C to undergo the first incubation for the space of an hour. An hour after, we added the sheep corpuscles (5% emulsion in saeine solution) sensitised with 2 to 3 hemolytic units. Reading was done at the end of an hour when controls were completely hemolysed.

With this technic it never happened for serum of patients of leishmaniosis alone, on whom a careful clinical observation had excluded syphilis, to give positive WASSERMANN tests.

Of all the cases we observed, all the ones in which positive WASSERMANN tests were obtained were found to be suffering also from syphilis and specific treatment would support our views. In these cases the ministering of tartar emetic by GASPAR VIANNA's process was not enough to completely cure the patient and specific antisyphilitic treatment would hasten and complete the treatment. This was thus therapeutic proof to confirm the clinical one and the result of the WASSERMANN test. Tartar emetic although taken to be an active cicatrissant has no curative action on syphilitic lesions nor does specific antisyphilitic medication, mercury and arsenicals, cure leishmaniosis. In medical literature there is no lack of observations of cases of leishmaniosis cured or improved by 914 and even in treatises on pathology 914 is currently prescribed in the treatment of this disease. In leishmaniosis americana we never observed a case to be cured or improved by this treatment: the only patients that profit and profit considerably by this treatment are the ones suffering at the same time from syphilis.

Out of the patients we observed, one of them, the patient of observation 27 died: as this observation is of some interest since it was a case of mixed cutaneous leishmaniosis and tertiary syphilis with WASSERMANN test strongly positive with the six antigens used and absence of total hemolysis with all of them, we describe the clinical case and the results of the autopsy carried out in the Department for Pathological Anatomy of the Instituto Oswaldo Cruz by the assistant Dr. CEZAR GUERREIRO to whom we are very much indebted for the information given.

Obs. XXVII.—C. F.—Subject of black colour, with a big ulceration on the nose extending from both sides and destroying the external nostrils: nasal septum destroyed and bony parts of nose affected. From nose ulceration extended to upper lip, which besides oedema, had a circular ulceration 4 centimetres in diameter rounded and with projecting borders. Within mouth could be noted ulceration of tonsils; epiglottis partly destroyed; vocal chords totally ulcerated and destroyed; the patient was almost aphonie, it being almost impossible to perceive his words. Pharynx covered by a big ulceration, of irregular borders, rough and purulent surface. Oesophagus affected in the neighbourhood of the pharynx. On the limbs ulcer-
rations of different sizes from three to ten centimetres in diameter. Ulcerations generally rounded and covered with a dark yellow and adhering crust. Below this crust surface was rough and purulent. Ulcerations bled with ease, as soon as the crust was removed and the patients complained of acute pains. Inguino-crural and epitrochlear ganglia swollen in size.

On glass a quite noticeable white scar, from a chancre in the patient's previous history. Smears made with pieces of ulcers and stained by GIEKMSA process, revealed numerous leishmanias, either isolated in a microscopic field or else in great numbers parasiting big mononuclear leucocytes and heaped up in their protoplasm, leaving on sight the intensely stained nucleus and the outlines of the protoplasm.

In symbiosis with the leishmanias could be seen in the microscopic field spirilla and fusiform bacilli. We have already seen this fuso-spirillar symbiosis in other lesions besides angina and oriental sore, such as ulcerated epithelioma, blastomycosis, syphilitic or simple chancres etc.

It is probably a factor of contamination, which the ulceration, giving it immediately a phagedenic character and the treatment is very much helped by the local ministration of antiseptics.

We made a biopsy of the ulceration of the nose and the small piece removed was fixed in SCHAUDINN sublimate-alcohol and embedded according to the ordinary methods. The sections stained with GIEKMSA, hematoxylin-eosin and VAN GIESON showed numerous leishmanias, sometimes isolated and between the tissue cells as those emigrated from the blood or, more commonly, parasiting the great mononuclear leucocytes.

The patient died when still under treatment and right in the beginning of it from a process of acute miliary tu-

berculosis as will be seen in the protocol. Let it be remarked in passing, that leishmaniosis in its mucous form, the most serious and resistant to treatment, with its frequent recidives destroying the cartilages of the nose, hindering thereby normal breathing, making breathing through the mouth necessary and attacking all the organs found at the back of the mouth (tonsils etc.) which are the means of defense of the body places the human body in a state of inferiority rendering the invasion by other diseases whose saprophytic germs are found under normal conditions in the mouth (tuberculosis, diphtheria etc.) easy.

Leishmaniosis is not a disease producing by itself death; generally the cases become chronic and when death ensues it is very often due to tuberculosis which finding a favourable ground has developed meanwhile.


whole lip: slight depth with granular ground. Nose also with losses in cartilaginous septum. Left nostril with vegetative formations the size of an almond. Right nostril with an ulceration covering it entirely. Both fossae very much narrowed. Cornea transparent pupils equally dilated. Section shows a somewhat reduced fatty stratum, 2 mm. on the abdominal wall, muscular layer 7 mm. Muscles brown, rather dry. In the deeper parts of the abdomen no liquid. Liver reaching beyond xyphoid process 6 cm, covered by costal border at the height of the papilla. Location of diaphragm 6th rib on either side. Pericardium on view in an extension of 9 cm. In the pericardial sac 30 cc. of a serous yellow liquid. Mucous tissue at the base of the tongue reddened with the glandules in evidence. Pharynx with an ulceration covering it completely. Epiglottis with border partly destroyed. Aryteno-epiglottic fold narrowed with granular thickened mucous membrane. Complete destruction of vocal chords in their place an ulceration of granular ground. Tonsils also ulcerated with almost complete loss of tissue-substance. Oesophagus with the mucous layer of its upper part completely destroyed. Upper third of trachea with turgid vessels, the upper part swollen and the mucous membrane destroyed. Left lung adhering to the costal pleura in its whole surface by weak fibrous strands. Right lung free, the cavity almost empty. Left lung enlarged, with increased consistency. On section shows a diminute volume of air: distributed all over the tissue whitish nodules, bigger than pin-heads: interstitial part of the nodules of a reddish hue and jelly-like consistency. Pleura covered by a fibrous thickening of jelly-like aspect. Peri-bronchial lymphatic glands enlarged, of dark-coloured tissue. Lower margins of lungs containing a little air and giving a diminished crepitation. Right lung: pleura smooth; under it on lung-tissue several whitened, unequally-sized nodules. The three lobules connected by fibrous strands. At section all the lung-tissue shows nodules similar to the ones of left lung, but sparser: relatively more air is kept in parenchyma. Interstitial tissue also with jelly-like appearance and consistency. Heart not enlarged in relation to stature. Pleural leaf of epicardium smooth. Sub-epicardial fat rather reduced. Right ventricle: endocardium smooth; thickness of ventricle 3 mm.: muscle brownish. Right auricle: endocardium smooth; mitral valves not thickened. Spleen adhering strongly to diaphragm and to neighbouring organs by fibrous strands: dimensions at convexity $14 + 10 + 4$. On section splenic pulp of a dark colour; follicular apparatus not perceptible. Supporting apparatus fairly evident: dispersed on pulp, whitish nodules larger than a pin-head and clearly limited in the tissue are to be seen. Liver with strong fibrous connections with diaphragm several vertical indentations. On section lobular outlines faint; a volume of blood kept in the organ; in isolated points, several nodules more or less of the size of a pin-head, clearly limited in tissue. Gall bladder with thin walls containing a few cc. of greenish and fluid bile.

Medulla of both adrenals darkish showing ill-defined whitish spots. Both kidneys somewhat reduced in volume; fibrous capsules easily detachable. On section cortex not much reduced limits between the two layers indistinct. Outline of pyramids slightly injected. Pelvis of kidney narrow; ureter permeable. Bladder contracted containing a few centimeters of cloudy urine. Prostate small whitish. Vesicula seminalis with thin walls freeing, when pressed, a cloudy mucous fluid. Both testes not enlarged; tissue brownish. Tubules not easily isolated. Abdominal aorta with a smooth and shining inner coat. Mesenteric and
mesocolic ganglia enlarged some with the tissue in advanced stage of caseification. Mucous membrane of the intestine of a diffuse reddish colour all over: some ankylostoma are to be seen. At the height of jejenum a loss of tissue of 1—4 centimeters with projecting margins and rough ground. In ileum two similar losses close to each other, about 1 centimetre in diameter with projecting borders and a rough ground. Gastric mucous membrane diffusely reddish: no loss of tissue. Skull asymetric with somewhat thickened walls. Dura mater equally tense on both sides. Meninges of a milkish appearance both at base and in convex part: no granules. Frontal and longitudinal sections show nothing abnormal in brain, cerebellum and medullary bulb. Microscopic sections of organs and ulcerations showed tubercular and syphilitic lesions.

Protocol


Obs. II.—C. G. S.—Brazilian, white, 42 years, single, resident at S. João Marcos, State of Rio de Janeiro. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.

Obs. III.—J. P. S.—Brazilian, white, farm-labourer, 44 years, married, resident at Itaperuna, State of Minas Geraes Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.

Obs. IV.—J. S. J.—Brazilian, mulatto, 52 years, single resident at Itaocara, State of Rio de Janeiro. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. V.—C. P.—Greek, white, workman, 43 years, married, no residence. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. VI.—O. A. S.—Brazilian, white, 34 years, single, resident at Estação do Livramento, State of Minas Geraes. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.

Obs. VII.—A. M.—Portuguese, white, 60 years, married, mason, resident at Campo Grande. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. VIII.—A. M.—Brazilian, black, single, 26 years, mason, resident at Rua Conselheiro Octaviano, Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. IX.—O. A. S.—Brazilian, white, 34 years, single, workman, resident at Estação do Livramento, State of Minas Geraes. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. X.—M. B. O.—Brazilian, white, 60 years workman, resident at Lençóes do Rio Verde, State of Minas Geraes. Leishmaniosis tegumentaria cutanea; syphilis tertiaris. WASSERMANN test: Positive.

Obs. XI.—M. A. L.—Brazilian, white, 32 years, married, workman, resident at Rua Paysandú 18, Federal District. Leishmaniosis tegumentaria mixta; syphilis tertiaris. WASSERMANN test: Positive.

Obs. XII.—J. F. A.—Brazilian, mulatto, 35 years, married, workman, resident at Santa Isabel. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XIII.—A. M.—Turk, white, 35 years, single, grocer, resident at Rua Buenos Ayres 348, Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XIV.—A. F.—Brazilian, white, 19 years, married, shoemaker, resident at Rua Tavares 276, Federal District. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.


Obs. XVII.—Brazilian, black, 45 years, single, workman, resident at Trinagem, Federal District. Leishmaniosis tegumentaria mixta: syphilis. WASSERMANN test: Negative.

Obs. XVIII.—A. M. G.—Portuguese, white, 63 years, single, workman, resident at Rua Theophilo Ottoni, Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XIX.—A. R. L.—Brazilian, mulatto, 22 years, single, workman, resident at Estação Lagão, State of Minas Gerais. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XXI.—O. S. A.—Brazilian, white, 35 years, married, resident, State of Rio de Janeiro. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XXII.—M. S.—Brazilian, black, 35 years, single, resident at Itaborahy. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XXVI.—A. M. G.—Portuguese, white, 60 years, single, workman, resident at Bauru, State of São Paulo. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.


Obs. XXVIII.—J. C.—Brazilian, black, 28 years, single, farm-hand, resident in the State of Minas Gerais. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XXX.—J. S.—Turk, white, 33 years, married, resident at Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XXXII.—C. J.—Brazilian, white, 27 years, single, resident in State of Minas Gerais. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.

Obs. XXXIII.—M. P.—Brazilian, white, 32 years, married, resident in Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XXXIV.—M. L.—Brazilian, black, 24 years, single, resident in State of Rio de Janeiro. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XXXV.—F. I.—Brazilian, white, 35 years, single, resident in State of Minas Gerais. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XXXVI.—J. C.—Brazilian, white,

Obs. XVII.—Brasilian, black, 45 years, single, workman, resident at Triagem, Federal District. Leishmaniosis tegumentaria mixta: syphilis. WASSERMANN test: Negative.

Obs. XVIII.—A. M. G.—Portuguese, white, 63 years, single, workman, resident at Rua Theophilo Ottoni, Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XIX.—A. R. L.—Brazilian, mulatto, 22 years, single, workman, resident at Estação Lagão, State of Minas Geraes. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XXI.—O. S. A.—Brazilian, white, 35 years, married, resident, State of Rio de Janeiro. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XXII.—M. S.—Brazilian, black, 35 years, single, resident at Itaborahy Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XXVI.—A. M. G.—Portuguese, white, 60 years, single, workman, resident at Bauru, State of São Paulo. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.


Obs. XXVIII.—J. C.—Brazilian, black, 28 years, single, farm-hand, resident in the State of Minas Geraes. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XXX.—J. S.—Turk, white, 33 years, married, resident at Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XXXII.—C. J.—Brazilian, white, 27 years, single, resident in State of Minas Geraes. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.

Obs. XXXIII.—M. P.—Brazilian, white, 32 years, married, resident in Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XXXIV.—M. L.—Brazilian, black, 24 years, single, resident in State of Rio de Janeiro. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XXXV.—F. I.—Brazilian, white, 35 years, single, resident in State of Minas Geraes. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XXXVI.—J. C.—Brazilian, whi-

Obs. XXXVII.—A. C. M.—Portuguese, white, 45 years, married, resident at Federal District. Leishmaniosis tegumentaria mixta: syphilis tertiaria. WASSERMANN test: Positive.


Obs. XXXIX.—A. N. O.—Portuguese, white, 32 years, single, resident in Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XLIII.—J. M.—Brazilian, black, 28 years, single, resident in Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. XLV.—A. C.—Portuguese, white, 50 years, single, resident in Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. XLVI.—A. J. C.—Portuguese, white, 42 years, single, resident in Federal District. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.

Obs. XLVII.—M. G. G.—Brazilian, black, 29 years, single, resident in State of Minas Gerais. Leishmaniosis tegumentaria mixta: syphilis tertiaria. WASSERMANN test: Positive.

Obs. XLVIII.—S. P.—Brazilian, white, 36 years, single, resident in State of Rio de Janeiro. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.


Obs. L.—P. A.—Portuguese, white, 26 years, single, resident in Federal District. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.

Obs. LI.—A. G.—Brazilian, white, 34 years, single, resident in Federal District. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.


Obs. LV.—J. G.—Brazilian, black, 36 years, single, resident in State of Espírito Santo. Leishmaniosis tegumentaria mixta. WASSERMANN test: Negative.

Obs. LVI.—M. N.—Brazilian, black, 24 years, single, resident in State of Paraná. Leishmaniosis tegumentaria cutanea. WASSERMANN test: Negative.

Obs. LVII.—A. W. M.—American,
white, 38 years, single, no residence. *Leishmaniosis tegumentaria cutanea.* WASSERMANN test: Negative.


Obs. LX.—M. E. F.—Brazilian, black, 31 years, married, resident in state of Minas Geraes. *Leishmaniosis tegumentaria cutanea.* WASSERMANN test: Negative.

**Conclusions.**

The serum of patients of *Leishmaniosis americana,* in any of its clinical forms, cutaneous, mucous or mixed, does not deviate the complement when mixed with the antigens usually employed in WASSERMANN test.

In the cases in which positive results are obtained with WASSERMANN tests on the sera of patients of this disease, syphilis exists simultaneously.