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THE LABORATORY DIAGNOSIS OF WEIL'S DISEASE.

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(From the Wellcome Bureau of Scientific Research, London.)

WEIL's disease or spirochætal jaundice is characterized by fever, hæmorrhages from the nose and kidneys, abdominal pain, vomiting, conjunctival congestion, intense muscular pains, and jaundice on or about the fifth day after onset. Jaundice is not always present and occasionally the disease may be so mild that it is only known to have occurred when agglutinins are subsequently demonstrated in the blood of the patient. The disease is due to infection with *Leptospira icterohæmorrhagiæ* ; this organism occurs in the kidneys of up to 40 per cent of British brown rats and to a smaller extent in the black rat. The incubation period is generally in the neighbourhood of ten days but may vary between four and nineteen days (Schüffner, 1934). The mortality in 142 consecutive cases with jaundice was 15 per cent (Alston and Brown, 1937). It is essentially an occupational disease affecting persons who have come into intimate contact with the excreta of infected rats. Sewer workers, miners, fish workers, and canal workers are especially liable to contract the disease. Cases due to bathing in infected rivers are frequently met with.

A disease which is constantly being confused with Weil's disease is infectious or catarrhal jaundice. This occurs both sporadically and in widespread epidemics, chiefly affecting children. All degrees of severity up to a fatal termination may be met with. A severe case of this disease is hard, if not impossible, to diagnose clinically from Weil's disease.

Ocular congestion has been regarded by some as being of diagnostic importance; it is, however, frequently met with in both diseases.

The differential blood-count is of the utmost importance in differentiating the two diseases.

In Weil's disease there is a high polymorphonuclear count associated with a marked leucocytosis, and in infectious catarrhal jaundice, provided there is no septic focus, a leucopenia, and a high mononuclear count.

A typical count in Weil's disease would be :—

Polymorphonuclears	..	..	..	..	..	85
Small lymphocytes	..	..	..	..	..	11
Large mononuclears	..	..	..	..	..	3
Eosinophiles	..	..	..	..	..	1
						——
						100

whereas in catarrhal jaundice we should expect to find a count such as :—

Polymorphonuclears	..	..	..	..	..	55
Small lymphocytes	..	..	..	..	..	42
Large mononuclears	..	..	..	..	..	1
Eosinophiles	..	..	..	..	..	2
						——
						100

The cause of epidemic catarrhal jaundice or infective hepatitis is most probably a filtrable virus, *cf.* Findlay, MacCallum and Murgatroyd (1939); it has nothing whatever to do with leptospira.

Numerous observers, more especially Swan and McKeon (1938) have drawn attention to the high blood urea figure in Weil's disease and conclude that a grave prognosis must be attached to a blood urea figure of over 200 mg. per 100 c.c.

The Isolation of Leptospira from the Patient.

(1) *From the Blood.*—(a) By direct centrifugalization (Schüffner's technique) suitable in the first week of the disease. To 2 or 3 c.c. of blood add 0·25 c.c. of 20 per cent citrate in saline, centrifuge at 1,500 revolutions for five minutes, remove the supernatant fluid and spin this down at 1,500 revolutions for ten minutes. To the supernatant fluid add saponin to a total concentration of 1 : 1,000, then spin at 3,000 revolutions for half an hour. The deposit is then examined microscopically using dark-ground illumination. Using this procedure, Schüffner was able to give serum in the case of a laboratory infection within a few hours of the onset of the disease with striking result.

(b) By blood culture. During the first ten days of the disease a few drops of the patient's blood are inoculated into a modified Fletcher's medium consisting of 3 c.c. distilled water, 0·5 Lemco broth pH 7·4 ; to this, after autoclaving, is added 0·25 c.c. of inactivated rabbit's serum passed through

a Seitz filter. The cultures are incubated at 30° C. Growth occurs in three or four days. The serum of different rabbits appears to vary very much in the suitability for making this medium.

In making subcultures massive inoculation is necessary, the carrying over of one platinum loopful is not satisfactory.

(c) By animal inoculation during the first ten days of the disease.

At least two guinea-pigs are scarified and intraperitoneally inoculated with the patient's blood. In some cases leptospira can be found in the peritoneal fluid as early as the third day after inoculation. If the guinea-pigs become infected, the temperature rises rapidly on about the ninth day and then falls. If the animal is now killed, the following post-mortem appearances will be noted. Slight to intense jaundice of conjunctiva, pads and skin, petechial hæmorrhages in the groin, butterfly hæmorrhages in the lungs and scattered hæmorrhages in the intestines.

Cultures of the blood from the seared heart will, on incubation, show growth of leptospira and, on examination of a saline suspension of triturated liver by dark-field illumination, the organism will generally be seen.

In certain cases after the animal has been inoculated with the patient's blood, no rise of temperature or jaundice is seen, but if some of the liver of this animal is injected into a second guinea-pig, this animal may develop typical symptoms with death and typical post-mortem appearances on the tenth day after inoculation.

(2) *From the Urine.*—The best chance of finding leptospira in the urine is between the fifteenth and twenty-third days of disease.

It is essential to give the patient an alkali by the mouth before the examination in order to make the urine slightly alkaline. Leptospira rapidly die in acid urine, and it is quite useless either to search with dark-ground illumination or to inoculate animals with the urine of the patient if it has been passed some time previously. The urine must be absolutely freshly passed and the reaction slightly alkaline.

The centrifuged deposit is searched, using dark-field illumination, and is also scarified into two young guinea-pigs.

It is advisable to warn the animal attendants that there is danger of infection when attending to these animals unless gloves are worn, especially if there are any abrasions or cuts on the hands.

SEROLOGICAL TESTS.

Agglutination.—Agglutinins appear in the blood of the patient about the sixth day and generally reach their maximum titre about the twentieth day of disease or a little later. A titre of 1 : 10,000 or 1 : 30,000 is frequently met with.

Agglutinins may persist for many years, even a titre of 1 : 300 several years after the onset of the disease is not uncommon (Fairley, 1934 ; Brown, 1935), and Postmus (1933) demonstrated their presence 6,066 days after the onset of the disease. This survival of agglutinins in the blood of a

patient must be taken into account when diagnosing the disease, for in a case recorded by McKeon and Brown (1936) the patient suffered from jaundice, epistaxis and dark-coloured urine, and his serum agglutinated the leptospira to a titre of 1 : 100.   The serum in this case was examined on three separate occasions at approximately weekly intervals but no rise in titre of the agglutinins occurred.   The differential blood-count in this case was typical of epidemic catarrhal jaundice and so, in spite of the fact that there were agglutinins in the blood to *Leptospira icterohæmorrhagiæ*, this case was diagnosed as being epidemic catarrhal jaundice, the presence of agglutinins being due to a previous attack of Weil's disease.

The agglutination test (Schüffner's technique) is performed with a culture of *L. icterohæmorrhagiæ* formolized to 0·2 per cent, using A.R. formalin.

The dilutions of the serum to be tested are most conveniently made with peptone water as the diluent in the depressions of a painter's palette (Winsor and Newton).   A range of dilutions from a 1 : 5 to 1 : 15,000 is convenient ; to these dilutions are added an equal volume of the formolized culture, the contents of each depression are then mixed and interaction is allowed to take place at room temperature overnight.

Before the antigen is used it is centrifuged to throw down any clumps of leptospira, the supernatant fluid being used for the test.

As well as having a negative control, with peptone water and culture only, a known positive serum is also put up, diluted in the same way as the patient's serum.   The necessity for this is that formolized cultures, although sometimes they will last for six months or longer, may for some reason or other suddenly become inagglutinable.

The result is read by dark-field illumination using a 2/3-inch objective and a 10x ocular, droplets from the varying dilutions being placed on a slide and examined without a cover-glass.   A definitely positive reaction at 1 : 10 is diagnostic in the presence of satisfactory controls.

Reference must be made to the occasional occurrence of a false agglutination.   In these cases the aggregates which are seen under dark-field illumination are composed of small particles in which the leptospira are entangled.   Sometimes this state of affairs may cause the greatest difficulty in reading the results, one thing that casts doubt upon this being a true agglutination is that the background is not cleared of leptospira.

If there is any doubt about whether it is a true agglutination, a portion of the mixture should be critically examined under a cover-slip with a 1/12-inch objective.

It is interesting to note that in certain cases in which this false agglutination has occurred there has also been a false Wassermann reaction lasting for a few days only.

In using the above agglutination technique it is usual to find that the reaction is more marked at a 1 : 30 dilution than at a 1 : 10, and in certain cases both the 1 : 10 and 1 : 30 are negative but the higher dilutions are positive.

THE MACROSCOPIC TEST.

Pot (1936) describes a macroscopic agglutination test for Weil's disease and advocates the use of an antigen grown in Korthop's medium. Brown and Broom (1939) have drawn attention to the fact that human strains of *L. icterohæmorrhagiæ* isolated in England showed considerable differences in their agglutinability when using Schüffner's medium.

Brown (1939) described a rapid presumptive test for Weil's disease by means of which a result can be obtained within fifteen minutes of receiving the serum. This test is a modification of the technique used by Garrow (1917) and it is claimed that the results so far obtained are sufficiently accurate to warrant the administration of therapeutic serum to the patient ; in fact, in the case of the last five positive human sera tested by this method the results compare extremely favourably with those got either by Schüffner's method or the macroscopic technique. One noticeable feature is that in
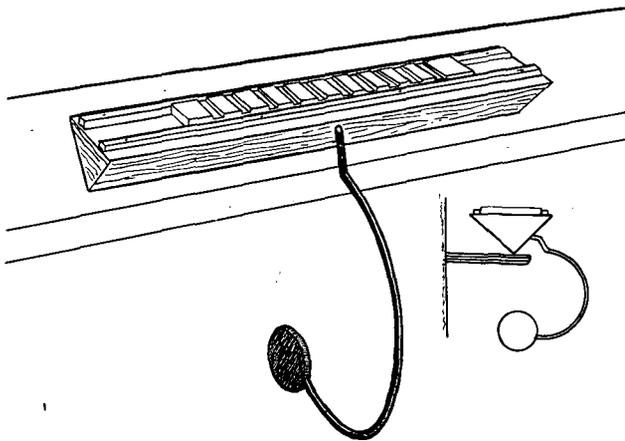
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none of these cases has there been a negative zone in the 1 : 10 and 1 : 30 dilutions which have been present when using the other two methods. The test essentially consists in rocking to and fro on a slide for ten minutes small quantities of varying dilutions of the patient's serum in presence of a heavy suspension of *L. icterohæmorrhagiæ* ; this saline suspension is formalized to a concentration of 0·2 per cent. Ordinary cultures of leptospira are not sufficiently concentrated for this method and therefore a well-grown culture is formolized to 0·2 per cent and then saponin is added to it to a total concentration of 1 : 1,000. This is then centrifuged at high speed for half an hour and the deposit is suspended in 0·2 per cent formolized saline ; sufficient of this diluting fluid is added to make the opacity of the suspension equal to that of a No. 1 Wellcome opacity tube used for the standardization of vaccines. In order to obtain this state of concentration, a

well-grown culture has to be reduced to approximately one-tenth of its original bulk.

The dilutions of the serum are made in the depressions of a painter's palette as in Schüffner's method. They are then mixed with an equal quantity of the concentrated antigen and placed on the slide shown in the diagram. This is made to rock for ten minutes and the result is read by means of a hand lens against a black background.

The Adhesion Test.—Brown and Davis (1927) described a rapid test for the detection of antibodies in the serum of a patient suffering from Weil's disease. The test is a modification of the Rieckenberg reaction. It consists in allowing the following to interact at 37° C. for thirty minutes.

One volume of a fivefold dilution of the patient's serum. One volume of a young actively motile leptospira culture. One volume of a saline

THE ADHESION TEST.

Positive reaction.   Negative reaction.

suspension of *B. coli*. One volume of a five-fold dilution of fresh guinea-pigs' serum.

A small drop, covered with a coverslip, is examined by dark-field illumination with a 1/12-inch objective ; in the case of a positive result the *B. coli* will be found to be firmly adherent to the leptospira. This adhesion test will be found to be positive to approximately the same titre as the agglutination test.

The appearance of a positive and negative adhesion test is shown in the above drawings.

The Protection Test.—Two pairs of guinea-pigs are inoculated intra-peritoneally with 0·5 c.c. and 1·5 c.c. of the patient's serum respectively ; one hour later the animals are inoculated with 0·2 c.c. of a young culture of virulent leptospira. If, with suitable controls with normal sera, the animals

receiving the patient's serum survive and those with normal sera die of the disease, the test is positive.

I wish to thank Dr. G. M. Findlay for his drawings of the adhesion test.

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