SEROLOGICAL DIAGNOSIS OF LYMPHOGRANULOMA VENEREUM*

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Lymphogranuloma venereum (L.G.V.) has been known for many years under such names as lymphogranuloma inguinale, lymphopathia venereum, climatic bubo, paradenitis, or Nicolas and Favre's disease. Frei (1925) introduced a skin test, for long regarded as specific, using a treated suspension of pus from a bubo as antigen. With this he obtained in patients with lymphogranuloma venereum a papular reaction $\frac{3}{4}$ –1 cm. in diameter, 36–48 hours after intradermal inoculation. Control patients with other diseases showed no reaction. Using the same antigen he attempted without success to demonstrate complement-fixing antibody in sera from positive patients.

During the next 15 years attempts were made, with equivocal results, to demonstrate complement-fixing antibody in sera from patients suffering from L.G.V., using antigens prepared in this manner. Coutts and others (1934) obtained positive fixation in sera from three out of five cases using a 1 per cent. aqueous phenolized pus suspension as antigen. Later, with a 1 in 30 suspension, complement fixation occurred in eighteen out of twenty sera from patients diagnosed clinically as suffering from L.G.V. Melczer and Sipos (1937), reviewing previous results, concluded that antigens varied in their potency according to the amount of virus present. Attempts to overcome this difficulty by preparing antigens from infected mouse brain, or guinea-pig or rabbit lymph nodes were not successful.

PREPARATION OF YOLK-SAC SUSPENSIONS OF VIRUS

Using the method of yolk-sac inoculation, introduced for rickettsia by Cox (1938), Rake and others (1940) were able to prepare concentrated suspensions of L.G.V. virus for use as antigens.

Methods of enhancing the potency of such antigens and increasing their specificity, by extraction with ether (Smadel and others, 1943), by suspension in 0.5 per cent. phenol saline (Nigg and Bowser, 1943), and by suspension in phenol saline followed by boiling (Nigg and others, 1946), were put forward. Bedson (1936) had introduced boiled psittacosis suspensions as antigens, and Bedson and others (1949) found this method equally suitable for L.G.V. virus suspensions. They confirmed the findings of Rake and others (1941) that cross reactions occurred in complement-fixation tests using L.G.V. and psittacosis antigens with their antisera. They showed also that, as well as the group heat-stable antigen, each virus possessed a specific heat-labile antigen. The demonstration of the specific heat-labile antigen required the use of living virus suspensions and serum from which the group antibody had been absorbed. It was not recommended as a routine method for differentiation.

With the introduction of yolk-sac antigens, extensive serological investigations were carried out. Grace and Rake (1943) regarded as positive any serum which fixed complement in a 1/4 or higher dilution, but Knott and others (1943) noted that sera from patients with no history or clinical manifestations of the disease often fixed complement in lower dilutions, and that cross reactions with sera from syphilitic patients occurred. Such effects were minimized by the demonstration of rising titres of antibody in successive sera.

Dulaney and Packer (1947) investigated the specificity of the complement-fixation test in the diagnosis of L.G.V. From 69 clinical cases they obtained 91 per cent. positive complement-fixation tests in contrast to 54 per cent. positive skin tests. In discussing non-specific reactions, they cited thirteen patients with upper respiratory tract

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infections whose sera reacted to a titre of 1/20 with L.G.V. antigen. After 3 months, ten had become negative, but 10 months later, three still reacted to a titre of 1/20.

In discussing titres which can be regarded as being diagnostic when only single specimens of serum are examined, Landau (1946), Dulaney and Packer (1947), and Bedson and others (1949) regarded a titre of 1/32 dilution of serum as beyond the range of non-specific effect, and, in conjunction with the clinical condition, as evidence of infection. *Without clinical manifestations* such a titre (1/32) would only indicate recent infection by a member of the psittacosis-L.G.V. group of viruses.

PRESENT INVESTIGATIONS

A serological investigation by the complement-fixation technique was carried out in Liverpool during the period 1948-49 to determine the incidence of L.G.V. among selected groups of the population.

Material and Methods

Sources of Sera

Group I.—Sera from seventy patients, the majority being seamen, under investigation for venereal disease. In many of these L.G.V. was suspected. As far as possible repeated samples of serum were examined, the first being obtained at the initial attendance for treatment soon after the onset of clinical manifestations.

Group II.—Sera from 192 blood donors.

Group III.—306 sera which had been submitted for a Wassermann test. These were subdivided into:

- (a) 150 negative
- (b) 156 positive.

Group IV.—51 sera from miscellaneous infections, comprising twenty from cases of active tuberculosis, eighteen from possible cases of paratyphoid fever, and thirteen from possible cases of toxoplasmosis.

Virus.—The J.H. strain of L.G.V. virus was used in the preparation of antigen. Seven-day-old fertile eggs were inoculated into the yolk-sac with a dilution of virus which would cause death of the embryo in 5 to 8 days. The yolk sacs were harvested within 24 hours of death of the embryo.

Antigens.—Lygranum C.F. antigen (Squibb) was used as a standard of comparison.

The preparation of L.G.V. antigen was based on the method of Bedson and others (1949).

Control normal yolk-sac antigen was prepared similarly from uninoculated yolk-sacs of 10 to 12-day-old eggs.

Complement-Fixation Tests.—The method used was that advocated in a monograph entitled "Lymphogranuloma Venereum" (1943).^{*} Increasing dilutions of serum were titrated against a single optimal dilution of

antigen in the presence of two units of complement for 75 minutes at 37°C. Two volumes of a final 1 per cent. suspension of sensitized sheep cells were then added, and the test re-incubated for 30 minutes at 37°C. A preliminary reading was made and the test allowed to stand overnight at 4°C. before the final reading. Sera were inactivated at 56°C. for 30 minutes before use. Complement consisted of pooled guinea-pig serum preserved by the method of Richardson (1941).

Interpretation of the Test.—An arbitrary scale from 4 (complete fixation) to 0 (complete haemolysis) was used. The titre of a serum was the highest dilution giving 4 or 3 fixation.

Titres of less than 1/20 were regarded as negative, a titre of 1/20 was doubtful except where there was a positive Frei test and histological evidence of infection, and titres of 1/40 or higher were accepted as positive where there was clinical evidence of infection. The titres stated refer to the initial dilutions of serum.

Results

Group I.—Of the seventy patients who were under investigation for V.D., 36 were negative, four doubtful, and thirty serologically positive. Only single samples of serum were examined from sixteen of the thirty patients with a positive reaction; of these sixteen, fifteen had a titre of 1/40 or higher and one a titre of 1/20 together with a positive Frei test and histological evidence of infection. From the other fourteen patients, two or more samples of serum were examined at different times with considerable variation in titres, although all at some stage showed titres higher than 1/40.

Details of the thirty serologically positive patients are given in Table I.

A Frei test was carried out on seventeen of the thirty serologically positive patients, using as antigen heated yolk-sac suspension of virus prepared by the method of Bedson and others (1949). As shown in Table II (overleaf), seven patients had positive reactions and eight had negative reactions. Doubtful reactions occurred in two patients (Nos 20 and 28) and these have not been included.

Two patients from the L.G.V. negative group, two from the doubtful group, and four from the L.G.V. positive group had strongly positive Wassermann reactions. One patient from the last group had a doubtfully positive Wassermann reaction.

In the four doubtful patients, all of whom had clinical signs suggestive of L.G.V., titres of 1/20 were recorded. Only single samples of serum were obtained from three of the patients, but from the fourth three samples of serum were examined without showing a rise in titre and he was ultimately diagnosed as having syphilis.

The incubation period in the positive cases varied from 5 to 10 days when primary lesions were

^{*} Published by E. R. Squibb and Sons, New York.

TABLE I
DETAILS OF THIRTY SEROLOGICALLY POSITIVE PATIENTS IN GROUP I

No.	Sex	Race	Place of Exposure	Primary Lesion Noted	Time of Appearance of Enlarged Inguinal Nodes after Exposure	Wassermann Reaction	Frei Test	Serological Results and Remarks
1	M.	White	Not stated	No	Left inguinal nodes enlarged and subsided. One month later right inguinal nodes enlarged, 8 days after exposure	Neg.	Not done	(1) 1/80 (2) 1 week later 1/160
2	M.	Coloured	Aden	Small ulcer 10 days after exposure	Shortly after primary lesion	Neg.	Not done	(1) 1/320 (2) 2 weeks later 1/640, probable recrudescence of chronic case
3	M.	White	Brazil	No	10 weeks	Neg.	Not done	(1) 1/40
4	M.	White	West Africa	No	10-14 days	Neg.	Not done	(1) 1/80
5	M.	White	Africa	No	Not stated	Neg.	Not done	(1) 1/80
6	M.	Coloured	Java	No	14 days	++	Not done	(1) 1/40. Syphilis in 1945
7	M.	White	Liverpool	Small ulcer 21 days after exposure	Shortly after initial lesion	Neg.	Not done	(1) 1/80 (2) 3 weeks later 1/40. Wassermann reaction then also negative
8	M.	White	Brazil	No	3 weeks	Neg.	Not done	(1) 1/40
*9	M.	White	West Africa	Primary syphilis	2 months	Neg.	Positive	(1) 1/20 (2) 3 weeks later 1/20 (3) 12 weeks later 1/160 (4) 20 weeks later 1/40 (5) 30 weeks later 1/20. Wassermann reaction negative five times. Frei test positive twice with 30 weeks interval
*10	F.	White	Not stated	No	Not stated	Neg.	Positive	(1) 1/20. Biopsy histological appearance suggested L.G.V.
*11	M.	Coloured	West Africa	No	11 weeks	Neg.	Neg.	(1) 1/20
*12	M.	White	South America	No	8 weeks	++	Positive	(1) 1/80 (2) 3 weeks later 1/80 (3) 7 weeks later 1/40. Also had syphilis
13	M.	White	West Indies	Small ulcer 11 days after exposure	Shortly after initial lesion	Neg.	Not done	(1) 1/40
*14	M.	White	British Guiana	No	3 months	Neg.	Positive	(1) 1/160
15	M.	Coloured	Batavia	No	1 month	Neg.	Not done	(1) 1/40
*16	M.	White	India	No	2 months	Neg.	Neg.	(1) 1/160 (2) 8 weeks later 1/160 (3) 10 weeks later 1/80 (1) 1/80
17	M.	White	West Indies	Small ulcer 8 weeks after exposure	Shortly after initial lesion	Neg.	Not done	(1) 1/80
*18	M.	Coloured	Many	Small ulcer	Shortly after initial lesion	Doubtful positive	Positive	(1) 1/80 Had syphilis 1947
19	M.	Coloured	Burma	No	3 weeks	Neg.	Not done	(1) 1/40
20	M.	White	Brazil	Gonorrhoea 5 days after exposure	Shortly after initial lesion; subsided but recurred a month later	Neg.	Doubtful positive	(1) 1/20 (2) 2 weeks later 1/40 (3) 5 weeks later 1/80 (4) 10 weeks later 1/20
*21	M.	White	Africa and Liverpool	No	1 month	Neg.	Strongly positive	(1) 1/160 (2) 4 weeks later 1/160 (3) 8 weeks later 1/160 (4) 11 weeks later 1/80 (1) 1/160
22	M.	White	Spain and Beirut	No	2 weeks	Neg.	Not done	(1) 1/160
*23	M.	White	Not stated	No	2 weeks	Neg.	Neg.	(1) 1/80 (2) 1 week later 1/80 (3) 4 weeks later 1/80 (1) 1/160
*24	M.	Coloured	West Indies	No	3-4 weeks; recurrences after 2 and 6 months	Neg.	Neg.	(1) 1/160
*25	F.	White	Liverpool	No	2 weeks after last exposure	++	Neg.	(1) 1/80 (2) 1 week later 1/80 (3) 5 weeks later 1/20 (4) 8 weeks later 1/20 (1) 1/160 (2) 4 weeks later 1/20 (1) 1/160 (2) 6 weeks later 1/80 (1) 1/160
*26	M.	White	Brazil and Liverpool	No	3 months after first exposure	Neg.	Neg.	(1) 1/160 (2) 4 weeks later 1/20 (1) 1/160 (2) 6 weeks later 1/80 (1) 1/160
*27	M.	White	India	No	3 weeks	Neg.	Positive	(1) 1/160 (2) 6 weeks later 1/80 (1) 1/160
28	M.	White	Spain	No	2 weeks	Neg.	Doubtful positive	(1) 1/160
*29	M.	Coloured	(Unable to speak English)	Small ulcer 1 month after first exposure	6 weeks	Neg.	Neg.	(1) 1/160 (1) 1/80 (2) 3 weeks later 1/20. Wassermann reaction negative initially but later positive
*30	M.	White	Brazil and Liverpool			++	Neg.	

* Cases detailed in Table II

TABLE II

RESULTS OF THE FREI TEST IN FIFTEEN PATIENTS OF GROUP I

Result	Case No.	I.P.*	Serological Titre
Positive	9	2 months	1/20-1/160
	10	not stated	1/20
	12	8 weeks	1/80
	14	3 months	1/160
	18	not stated	1/80
	21	1 month	1/160
	27	3 weeks	1/160
Negative	11	11 weeks	1/20-1/80
	16	2 months	1/160
	23	2 weeks	1/80
	24	3-4 weeks	1/160
	25	not stated	1/80
	26	3 months	1/160
	29	not stated	1/160
	30	6 weeks	1/80

* I.P.=Incubation Period (i.e. the time from exposure to the appearance of lesions).

noted, to 3 months when enlargement of lymph nodes occurred. An approximate time lapse from exposure to the appearance of the initial symptoms in 25 cases is given in Table III.

The diagnosis in the 36 negative cases was as follows:

chancroid	12	Hodgkin's	
syphilis	6	disease	1
gonorrhoea	3	rectal stricture ..	1
non-specific inguinal		malaria	1
adenitis	3	prostatic	
lymphangitis penis	2	inflammation ..	1
non-specific penile ulcer	1	tuberculosis ..	1
primary atypical pneumo-		carcinoma vulvae	1
nitis	1	not stated	2

Groups II, III, and IV.—The examination of sera from these groups was carried out to investigate the amount of L.G.V.-psittacosis group antibody in the selected populations. All sera were initially screened in a 1/5 dilution and those giving a positive reaction were further investigated. Only those having a titre of 1/40 or higher were regarded as specific.

The results of the whole investigation are summarized in Table IV (opposite).

Discussion

The value of the complement-fixation reaction as an aid in the diagnosis of L.G.V. has been sustained.

In Group I the diagnosis was confirmed in thirty cases and was doubtful in four. Among the negative cases were three diagnosed as non-specific inguinal adenitis which may have been L.G.V.

Although a titre of 1/40 has been regarded as beyond the range of non-specific effect, it is preferable to examine repeated samples of serum in making a diagnosis. The titres obtained cover a wide range and it is difficult to assess how far this is due to early and intensive therapy. Treatment does not prevent a rise in antibody level during the course of infection, but if virus is eliminated, as may occur, it hastens the fall in titre of antibody. Wall (1947), in experiments with mice infected with L.G.V. and treated with sulphonamide, showed that there was a relationship between the antibody level in the serum and the time and duration of therapy. When treatment was early and prolonged no demonstrable antibody was present. Meyer (1948) considered that the complement-fixation reaction might be a true indication of the presence of virus in the host tissues and might therefore be useful in the evaluation of clinical cure. In chronic cases, e.g. with rectal stricture, a high antibody level is often found, and Rodaniche (1941) showed that virus may persist in infected tissues for many years.

Although a titre of 1/20 may indicate specific infection in association with clinical manifestations or in the early stage of infection, it is often a non-specific effect unchanged in successive samples of serum, and has been noted in sera from cases of respiratory infection by Smadel (1943) and by Dulaney and Packer (1947).

Both Rake and others (1941) and Bedson and others (1949) showed that the Frei test was a group effect similar to the complement-fixation reaction. In this series it was less satisfactory than the latter test, and, although it was carried out shortly after the first sample of serum from each case was reported positive, there was no absolute correlation between the result of the skin test and the serological titre. The results, despite the limited number

TABLE III

NUMBER OF DAYS FROM EXPOSURE TO THE APPEARANCE OF SYMPTOMS

No. of Days	..	..	5-10	11-14	15-21	22-28	29-56	57-84	Total
No. of Cases	..	..	2	7	4	3	5	4	25

TABLE IV
RESULTS IN ALL FOUR GROUPS

Group	Dilutions of serum								No. of Positive Cases *	Per cent. Positive
	<1/20	1/20	1/40	1/80	1/160	1/320	1/640	Total		
I	36	5	6	11	11	—	1	70	30†	42.9
II	190	1	1	—	—	—	—	192	1	0.5
IIIa	133	6	9	—	2	—	—	150	11	7.3
IIIb	125	10	15	5	1	—	—	156	21	13.5
IV	50	1	—	—	—	—	—	51	0	0
Total	534	23	31	16	14	—	1	619	63	10.2

* Those having a titre of 1/40 or higher.

† including one case with a titre of 1/20, a positive Frei test, and histological confirmatory diagnosis.

of Frei tests, are in agreement with the findings of Dulaney and Packer (1947). The development in the serum of complement-fixing antibody occurs before the Frei test becomes positive, but the latter test, once positive, is considered to remain so for many years. It is essential that both tests be carried out on suspected cases of L.G.V., as the effects of early diagnosis and intensive therapy remain to be assessed.

Of the other sera examined, some of those from Group III, both Wassermann positive and negative, showed a greater incidence of reactions to a titre of 1/40 or higher than those in Groups II and IV. It is suggested that this indicates the presence of latent L.G.V. in this group. While this may be correct for a large seaport, it does not follow that it would apply in inland towns of an equivalent size, as the populations in the latter are less polyglot and infection is less easily introduced.

By itself a positive serological reaction is a group effect and may result from infection by any member of the L.G.V.-psittacosis group of viruses. Such reactions may occur in a small proportion of primary atypical pneumonia infections.

Summary

A serological investigation using the complement-fixation test has confirmed the diagnosis of lymphogranuloma venereum in thirty out of a group of seventy patients, mainly seamen, in Liverpool.

In the test, titres of less than 1/20 were regarded as negative, titres of 1/20 of doubtful significance in the absence of other confirmatory findings (e.g. a positive Frei test), and titres of 1/40 or higher as supporting evidence of infection.

Details of the patients showing positive reactions are presented in Table II. A Frei test was carried out on seventeen of these thirty cases; of these only seven had positive reactions.

Among other groups investigated, totalling 549 patients, titres of 1/40 or higher were obtained in 13.5 per cent. of Wassermann positive sera, in 7.3

per cent. of Wassermann negative sera, and in 0.5 per cent. of sera from blood donors.

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