IMMUNOLOGICAL RESPONSE OF CASES OF RECURRENT TROPICAL LYMPHANGITIS TO HAEMOLYTIC STREPTOCOCCI AND THEIR PRODUCTS *

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For many years filarial disease has been associated directly or indirectly with infection with other bacteria.

Unna [1] believed that the true sporadic cases of elephantiasis developed from incompletely healed erysipelas and that the streptococci may remain latent in the tissues and excite proliferative tissue changes which subsequently lead to elephantiasis.

Leber and Prowazek [2] found streptococci in filarial swellings and in the blood of a patient who suffered from lymphangitis in Samoa. Abadie [3] describes finding a streptococcus without filaria in a case of elephantiasis, and Wise and Minett [4] examined 30 cases of abdominal filariasis and observed streptococci in pus smears in 28 cases and were able to isolate the organism in pure culture in 20 instances.

Anderson [5] reports:

"Infestation by *Filaria bancrofti per se* produces no symptoms; all the pathological manifestations associated with filariasis are due to secondary infection by pyogenic organisms".

Suárez [6] has reported the isolation of haemolytic streptococci from cases of lymphangitis. Grace and Grace [7] studying the bacterial complications of filariasis comment:

"We believe on the clinical and bacteriological grounds already stated that the lymphangitis of both diseases is the same and also that the streptococcus recovered from the blood or abscess during the lymphangitis attack is the exciting cause of the attack."

The problem of the production of chronic lymphedema in animals has been solved by Homans, Drinker and Field [8]. These authors produced characteristic lymphedema in dogs

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by injecting a solution of quinine and urea and a suspension of silica into lymph vessels which produced thrombosis of the vessels in several areas. The protein content of the lymph increased steadily, and the deep layers of the skin and subcutaneous tissue became the sites of increasing fibrosis which was evidence of active proliferation of connective tissue. Attacks of acute lymphangitis which produced fever and prostration occurred spontaneously. Haemolytic streptococci could be recovered from the edematous tissue at the beginning of the attack, but never at any other time.

The authors [9] have associated the attacks of recurrent tropical lymphangitis with infection with haemolytic streptococci and in this article will attempt to describe the immunological response of these cases to haemolytic streptococci and their products.

Type of Cases Studied

The cases studied are those that have recurrent attacks of lymphangitis. The inflammation is sometimes not only confined to the lymphatics, but at times involves the subcutaneous and deep tissues, giving the appearance of a cellulitis.

The acute attack manifests itself usually with pain in some part of the lymphatic tract. The patient may be feeling well, when suddenly he takes a rigor and complains of severe pain localized over some area of the limb; he may become nauseated, with vomiting and rising temperature. After the first few hours, the lymphatics of the affected part are marked as a red streak, the skin becomes firm, tender and thickened, and the reddened area spreads until a considerable portion of the extremity is swollen, red, hot and tender. The proximal lymph nodes are tender, swollen, and the high fever persists for a period ranging from a few hours to two or three days, and is accompanied by general malaise which may persist after the fever is over. The abnormal condition of the extremity recedes slowly over a period of from one to three weeks. In some cases after all clinical signs of acute inflammation have disappeared, swelling is present in a greater degree than before the attack. Repeated attacks produce increasing lymphedema, and in some cases of long duration marked chronic lymphedema results. (See fig. 1.)

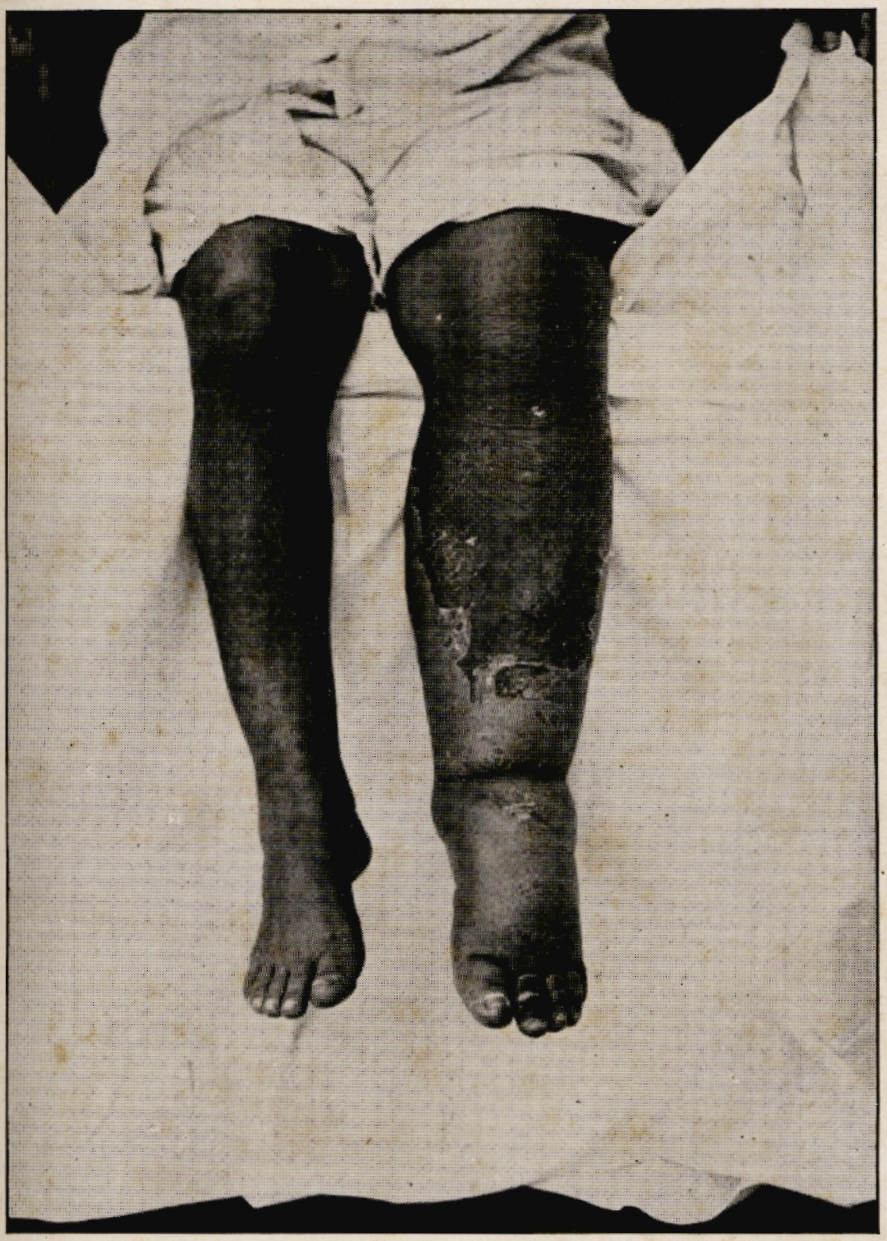

FIG. 1. Repeated attacks. Acute attack subsiding. Note marked lymphedema and desquamation. Virulent, toxigenic, group "A" beta-hemolytic streptococci isolated from this case.

Caso No. 1694. Pierna después de muchos ataques linfangíticos. Nótese la gran intensidad del linfoedema y la descamación. En este caso se aisló un estreptococo betahemolítico virulento, toxígeno, perteneciente al grupo "A".

Agglutinins:—In an attempt to demonstrate the formation of agglutinins against the streptococci in cases of recurrent tropical lymphangitis, we have tested a group of sera from persons suffering from recurrent tropical lymphangitis and a group of sera from persons suffering from other diseases. The sera from nine apparently normal subjects were also tested.

The test antigen * was prepared as follows: Stock cultures that had been kept on blood agar slants in the ice box for one to two months were transplanted to tryptic digest broth and incubated for 20 hours at 37° C. A flask containing 150 cc. of sterile tryptic digest broth and a few sterile potato strips were inoculated with 1 cc. of this culture and incubated at 37° C., its contents being shaken every 15 or 20 minutes to insure rapid growth. The flask was incubated for two hours, at the end of which time its contents were transferred to centrifuge tubes and centrifuged at low speed to throw down the coarse particles. To the bacterial suspension enough formalin was added to give a final concentration of 0.1 per cent.

Dilutions of the sera were made from 1 in 20 to 1 in 5120. To each 0.5 cc. of the serum dilutions an equal volume of the bacterial suspension was added, mixed thoroughly and incubated in a water bath at 37° C. for 20 hours, at the end of which, readings were made. The results of the agglutination test are shown in tables I, II, and III.

The results obtained with sera from cases of lymphangitis show that practically every case has streptococcus agglutinins in the blood. It is apparent that they are found in higher concentration during an acute attack. In a few cases in which they were present during the attack they could not be detected from one to four months after the attack.

* This procedure is similar to that recommended by Spicer (J. Immunol., 19: 445: 1930) with minor modifications.

TABLE I

AGGLUTINATION TESTS IN SERA FROM CASES OF RECURRENT TROPICAL LYMPHANGITIS

Name	Date sample was taken	Final dilutions									
		20	40	80	160	320	640	1280	2560	5120	Control
G. O.	9/22/34 (during attack)	±	3	4	4	2	2	2	2	2	0
G. O.	9/27/34 (8 days after attack)	±	1	2	4	4	3	±	0	0	0
G. O.	10/13/34 (during attack)	4	4	4	4	4	3	0	0	0	0
Z. C.	3/8/34 (6 days after attack)	1	2	2	2	2	2	1	0	0	0
Z. C.	6/27/34 (3 months 19 days after attack)	0	0	0	0	0	0	0	0	0	0
J. L.	6/28/34 (during attack)	1	4	4	3	3	3	1	±	0	0
J. L.	2/16/34 (46 days after first attack)	0	0	0	0	0	0	0	0	0	0
C. P.	1/31/34 (1 day after attack)	±	4	4	3	3	1	0	0	0	0
C. P.	2/13/34 (14 days after attack)	0	±	±	±	±	0	0	0	0	0
C. M.	11/16/34 (during attack)	3	4	2	2	2	2	±	0	0	0
C. M.	12/6/34 (20 days after attack)	±	±	±	3	3	2	0	0	0	0
C. D.	10/2/34 (during attack)	0	1	1	1	±	0	0	0	0	0
C. D.	10/11/34 (9 days after attack)	1	1	2	3	2	±	±	±	0	0
T. M.	11/1/34 (during attack)	3	3	3	4	4	3	0	0	0	0
T. M.	11/15/34 (14 days after attack)	±	3	3	3	3	3	1	0	0	0
N. C.	5/2/34 (during attack)	2	4	4	4	4	1	1	1	0	0
N. C.	5/12/34 (10 days after attack)	—	±	1	1	1	1	0	0	0	0
C. M.	3/29/34 (during attack)	2	3	3	4	3	3	1	0	0	0
C. M.	1/26/34 (3 days after attack)	±	1	2	2	2	1	0	0	0	0
J. C.	8/11/34 (2 years after attack)	±	1	3	3	3	1	0	0	0	0
J. C.	10/16/34 (2 months after attack)	3	3	4	4	3	±	0	0	0	0
J. R.	10/29/34 (during attack)	±	1	2	3	3	2	0	0	0	0
J. G.	9/22/34 (during attack)	0	3	4	3	3	±	0	0	0	0
G. R.	4/28/34 (during attack)	4	4	4	4	4	3	±	0	0	0
A. I.	2/21/34 (during attack)	4	4	4	3	2	±	0	0	0	0
M. L.	2/16/34 (during attack)	3	3	3	3	2	2	±	±	0	0
A. E.	11/16/34 (during attack)	±	2	1	±	0	0	0	0	0	0
Dr. J. P.	11/13/34 (during attack)	1	3	4	4	3	±	±	0	0	0
S.	2/3/34 (during attack)	±	±	±	±	±	±	±	±	±	0
F. C.	3/13/34 (during attack)	2	2	2	2	2	2	±	0	0	0
P. M.	5/11/34 (during attack)	4	4	3	3	2	±	0	0	0	0
P. L.	7/24/34 (1 day after attack)	1	2	4	4	4	4	1	±	0	0
I. Q.	10/25/34 (3 days after attack)	2	2	3	3	3	3	3	±	0	0
M. F.	11/10/33 (7 days after attack)	1	1	1	1	2	3	3	2	±	0
E. O.	8/17/33 (8 days after attack)	0	0	0	0	0	0	0	0	0	0
F. J.	8/14/34 (8 days after attack)	±	±	±	±	0	0	0	0	0	0
P. J.	1/12/34 (10 days after attack)	±	1	2	2	1	±	0	0	0	0
C. N.	8/11/33 (12 days after attack)	±	±	±	±	±	0	0	0	0	0
P. L.	1/12/34 (13 days after attack)	0	±	±	1	±	±	±	0	0	0
C. F.	9/16/33 (14 days after attack)	±	1	±	±	±	0	0	0	0	0
M. R.	7/24/34 (18 days after attack)	0	±	1	2	2	2	1	0	0	0
M. T.	8/11/33 (20 days after attack)	0	±	1	1	2	2	2	1	±	0
R. M.	8/11/33 (21 days after attack)	0	0	0	0	0	0	0	0	0	0
L. C.	2/15/34 (22 days after attack)	0	0	±	±	±	±	0	0	0	0
M. I.	1/5/34 (30 days after attack	1	1	2	2	2	2	1	±	±	0
R. R.	1/12/34 (30 days after attack)	±	0	0	0	0	0	0	0	0	0
F. S.	8/11/33 (30 days after attack)	±	±	1	3	3	3	±	0	0	0
J. T.	2/9/34 (30 days after attack)	±	1	2	2	2	1	0	0	0	0
N. C.	6/27/34 (30 days after attack)	±	1	1	1	1	0	0	0	0	0
C. R.	4/1/34 (30 days after attack)	0	0	0	0	0	0	0	0	0	0
N. R.	8/11/33 (30 days after attack)	2	3	3	3	3	2	±	0	0	0
D. A.	1/5/34 (30 days after attack)	1	2	3	3	3	2	1	0	0	0
A. A.	1/5/34 (35 days after attack)	2	3	4	4	3	3	1	0	0	0
A. L.	8/11/33 (35 days after attack)	0	0	0	0	0	0	0	0	0	0
C. J.	8/11/33 (35 days after attack)	0	0	0	0	0	0	0	0	0	0
E. H.	8/11/33 (40 days after attack)	0	0	0	0	0	0	0	0	0	0
M. F.	8/11/33 (40 days after attack)	±	±	1	1	1	1	0	0	0	0
J. C.	2/2/34 (40 days after attack)	±	1	2	3	4	4	3	±	0	0
N. R.	2/16/34 (46 days after attack)	±	±	±	±	0	0	0	0	0	0
D. M.	3/2/34 (52 days after attack)	2	2	2	2	1	1	0	0	0	0
M. C.	1/19/34 (60 days after attack)	2	1	±	±	±	±	±	0	0	0
E. A.	8/17/33 (60 days after attack)	0	0	0	0	0	0	0	0	0	0
C. D.	3/8/34 (67 days after attack)	±	±	±	±	±	±	±	0	0	0
D. R.	1/26/34 (90 days after attack)	±	1	2	±	0	0	0	0	0	0
D. C.	4/1/34 (90 days after attack)	0	0	0	0	0	0	0	0	0	0
D. S.	2/8/34 (5 months after attack)	0	0	0	0	0	0	0	0	0	0
M. F.	8/17/33 (6 months after attack)	0	0	±	0	0	0	0	0	0	0
J. C.	2/16/34 (14 months after attack)	±	±	±	±	±	0	0	0	0	0

TABLE II

AGGLUTINATION TITERS OF PATIENTS SUFFERING FROM VARIOUS DISEASES

Name	Diagnosis	Final dilutions									
		20	40	80	160	320	640	1280	2560	5120	Control
R. P.	Tubo-ovarian abscess. Peritonitis	4	4	4	4	4	4	1	0	0	0
J. R. R.	Otis media	1	1	0	0	0	0	0	0	0	0
R. C.	Renal calculus	2	3	3	0	0	0	0	0	0	0
A. L. V.	Burn	3	1	0	0	0	0	0	0	0	0
S. M.	Headaches. Insomnia	3	2	1	±	0	0	0	0	0	0
B. J.	Lupus erythematosus	4	3	3	2	1	1	±	0	0	0
F. G.	Chronic perihepatitis	4	3	3	3	2	2	2	0	0	0
L. M.	Chronic mastitis	3	3	3	3	3	2	2	2	0	0
G. G.	Thrombo-angitis obliterans	3	3	3	4	3	0	0	0	0	0
M. C.	Syphilis	3	3	3	3	1	0	0	0	0	0
C. L.	Carcinoma of the tongue	2	2	2	3	3	3	2	1	0	0
B. R.	Carcinoma of the vocal cords	4	3	3	2	2	2	0	0	0	0
G. F.	Simple goitre	3	3	2	1	±	0	0	0	0	0
L. C.	Paralysis agitans	0	1	1	2	2	0	0	0	0	0
G. P.	Chronic appendicitis	2	3	2	1	0	0	0	0	0	0
I. B.	Esophageal stricture	4	4	4	4	4	3	2	1	0	0
V. V.	Angina pectoris	1	1	1	1	±	±	0	0	0	0
I. M.	Schistosomiasis	2	2	1	1	±	±	0	0	0	0
A. R.	Prolapse of the uterus	1	1	2	1	1	±	0	0	0	0
A. M.	Vitiligo	3	3	3	3	2	0	0	0	0	0
I. R.	Hypertrophied tonsils	2	2	3	4	3	2	2	±	0	0

Table II shows that streptococcus agglutinins were also demonstrable in the sera of patients suffering from other diseases. In these cases the test was done only once in each instance. The results show no appreciable difference from those obtained in the cases of lymphangitis.

TABLE III

AGGLUTINATION TESTS IN NORMAL SUBJECTS

Name	Final dilutions									
	20	40	80	160	320	640	1280	2560	5120	Control
J. H.	0	0	0	0	0	0	0	0	0	0
R. S.	3	±	±	±	0	0	0	0	0	0
M. J.	2	2	2	2	1	1	±	0	0	0
F. P.	1	1	1	±	±	±	±	0	0	0
A. P.	2	2	2	2	2	2	0	0	0	0
A. L.	1	±	±	±	±	0	0	0	0	0
R. R.	±	1	1	1	1	±	0	0	0	0
E. F.	1	1	±	±	±	±	±	0	0	0
E. S.	±	0	0	0	0	0	0	0	0	0

Table III shows that streptococcus agglutinins are also demonstrable in the sera obtained from apparently normal individuals although in lower concentration.

The presence of streptococcus agglutinins in the sera of patients suffering from recurrent tropical lymphangitis can not be taken as evidence leading to diagnosis of an active streptococcus infection.

Allergic Reactions:—Our studies have dealt also with the response of cases of recurrent tropical lymphangitis to the filtrate of haemolytic streptococci. This reaction has been observed during the attack and in periods of quiescence.

The antigen used in our observations was streptococcus filtrate, strain L_7. The medium used for growing the streptococci was streptococcus toxin broth.* Two hundred and fifty cc. of broth were inoculated from a 24-hour blood agar slant. The flasks were incubated at 37° C. for 48 hours. They were then stored in the ice box for 48 hours longer. The culture was filtered through a "W" Berkefeld filter, and the filtrate thus obtained was distributed in 25 cc. amounts into 1 ounce vaccine bottles.

Dilutions of this filtrate were made with normal saline (1:25, 1:50, 1:100) and the dilution 1:25 was found more suitable for our observations.

One-twentieth of the 1:25 dilution was injected intracutaneously on the flexor aspects of both arms and both thighs. Uninoculated streptococcus toxin broth was used as a control. Reactions were read after 30 minutes and after 24 hours.

A group of twenty cases was tested *after the attack had subsided.* All twenty cases had their attacks in the lower limbs. Two of them had both lower limbs involved. All cases reacted positively in upper limbs. All but four cases reacted positively in the lower limbs. Of these four cases, two reacted positively in the normal limbs and negatively in the affected limb. The other two had both legs affected and reacted negatively in both.

Eighteen cases were inoculated during the attack of acute lymphangitis. In all cases the affected extremity was one of the lower extremities; in one case both lower extremities were affected. In five of the eighteen cases none of the extremities reacted, the test being negative in both lower and upper limbs. In the remaining thirteen, the upper extremities (non-affected) reacted positively. In four of these thirteen cases, both of the lower extremities reacted negatively; only one of these cases had both extremities affected. The remaining nine cases reacted negatively in the affected limb and positively in the non-affected limb.

* Wadsworth: Standard Methods", p. 88, 1927.

From these results it is apparent that patients suffering from recurrent attacks of lymphangitis react to haemolytic streptococcus filtrates during the period of quiescence.

A.L.P., female, has had attacks of recurrent tropical lymphangitis for the last five years. Attacks recurred frequently. The last two months, attacks have occurred at monthly intervals. Both legs are affected. Present attack began September 26, 1933, and was tested with Streptococcus filtrate the same day. Both upper and lower extremities are negative to the test. On October the 3rd, 1933, the patient has completely recovered from the attack and the test is again made. This time the upper extremities react positively, giving an area of erythema of about 30 mm., but the lower extremities react negatively.

M.R., white female, 25 years old, is admitted to the hospital suffering from an acute attack of lymphangitis of her left leg, October 11, 1933. On day of admission the patient is tested with Streptococcus filtrate and the reactions are negative in all the extremities. On October 24, 1933, 13 days after the onset of the attack, the patient has recovered completely and new tests are made. This time the patient reacts in the upper extremities with an area of erythema of 25×30 mm. Both lower extremities react very faintly with areas of erythema of 5×5 mm.

A.R., male, white, 35 years old, comes to the hospital with an attack of acute lymphangitis of the left leg. Patient is admitted to the hospital on October 19, 1933, and tested the same day. Patient reacts negatively in both lower limbs, but gives a positive reaction 12×12 mm. in both upper extremities. On October 25, 1933, the patient feels well and the acute attack has subsided. The patient is tested again, reactions being negative in the left leg (affected limb) and slightly positive in the right leg, right arm and left arm. On November 3, 1933, the patient is again admitted to the hospital with another acute attack. The tests are made during the acute attack with the following results: The left leg (affected limb) negative, the other three extremities reacting slightly positive (8×8 mm.).

There exists the possibility that the fever may be the cause of the change in reactivity. We decided to test a few cases suffering from other conditions with fever, some normal persons, and an individual suffering from attacks of recurrent sore throat.

Eight cases with high fever, suffering from various conditions (gastric ulcer, abdominal abscess, appendicitis, lymphocytic leukemia, typhoid fever, etc.) were tested in a similar way as the cases of lymphangitis. All gave completely negative reactions. From the throat of one of these cases (lymphatic leukemia) beta haemolytic streptococci have been cultured repeatedly in large numbers and in practically pure culture. Six apparently normal persons were tested with the same amount of diluted filtrate. Four reacted slightly and two gave an area of slight erythema about 8 mm. in diam-

eter with slight swelling. A case of recurrent sore throat, due to beta haemolytic streptococci, when tested during the afebrile period gave a four plus reaction with a painful area of intense erythema twenty millimeters in diameter and tendency to sloughing at the site of inoculation.

Apparently, high fever inhibits the skin reactions to haemolytic streptococcus filtrate in cases of recurrent tropical lymphangitis reacting positively previous to the febrile disease.

Development of Antistreptolysins:—Todd [10] and Coburn [11] have demonstrated that these antibodies are found in abnormally high concentrations in sera of patients suffering from rheumatic fever and in individuals recovering from haemolytic streptococcus infections. Furthermore, Coburn [11] has observed that antistreptolysins are not abnormally increased in the sera of subjects with bacterial infection other than haemolytic streptococcus, and he concludes that abnormally high antistreptolysin titer is strong evidence of recent infection by haemolytic streptococci, and that this relationship is apparently specific. While studying the probable relationship of *Streptococcus haemolyticus* to recurrent tropical lymphangitis, we have made antistreptolysin determinations in a group of person suffering from this condition, and in a group of normal individuals.

Material and Methods:—The culture medium and the method employed by us in the production of the reduced haemolytic streptococcus hematoxin was that described by Hodge and Swift [12] (1933). N.Y.5 scarlet fever strain of *Streptococcus haemolyticus* was used.

For the antistreptolysin determinations we proceeded as follows: Amounts of the reduced filtrate equal to 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50 cc. were pipetted into a series of Wassermann test tubes. The volume was made up to 1.5 cc., in each case with normal saline and 0.5 cc. of a 5 per cent suspension of washed fresh rabbit red blood cells in physiological saline added. The contents were mixed and the tubes placed in a water bath at 37° C. for one hour. The smallest amount of filtrate producing complete haemolysis was taken as the minimal haemolytic dose, which, in this case, was 0.2 cc.

Testing Sera for Antistreptolysin Content:—A preliminary test using serum dilutions of 1:25, 1:100 and 1:500 was

made in each case. One cc. of each dilution was pipetted into three Wassermann test tubes. Two tenths of the filtrate were added and the volume made up to 1.5 cc. with normal saline. The contents were mixed and incubated in a water bath at 37° C. for 15 minutes. Five tenths of a cc. of 5 per cent suspension of washed fresh rabbit red blood cells were added, mixed thoroughly, and incubation continued for 45 minutes longer, the tubes being shaken occasionally. At the end of this period the tubes were centrifuged and the highest dilution showing no haemolysis recorded. Assuming this to be the 1 to 100 dilution, then a new titration was made of the above dilution ranging from 0.1 cc. to 1 cc. in 0.1 cc. doses, using 0.2 cc. of the filtrate. The smallest amount of serum dilution completely inhibiting haemolysis was then recorded, in this case, 0.2 cc. Another titration was carried, ranging from 0.11 to 0.2 in .01 cc. increments. The smallest amount of serum dilution that completely inhibited haemolysis was recorded—in this case, 0.15 cc. This meant that 0.15 cc. of the 1 : 100 dilution of the serum tested neutralizes 0.2 cc. of the lytic filtrate; in other words, 0.0015 cc. of the undiluted serum. In reporting results the reciprocal of the fraction, as recommended by Todd and others, is used. We used a given dose of lytic filtrate and our units were not comparable with those of other investigators. In order to obviate this difficulty we determined the antistreptolysin values of eight of our sera and sent the same samples to Dr. David Seegal of New York who kindly tested them for us. He reported his results in unit values equivalent to those of Todd, Coburn and others. By calculation, we were able to change our figures accordingly. In all the tests a serum of known antistreptolysin content was included as control.

When a series of tests was done at varying intervals in sera from the same patient, each time a new determination was made the previous ones were repeated, to check results and obviate the possibility of error. Each new batch of streptolysin was standardized with a serum of known antistreptolysin content.

The antistreptolysin content of the sera of patients suffering from attacks of recurrent tropical lymphangitis was determined during the attack, a few days after the attack, and, in some instances, several weeks after the acute attack. The findings are presented in table IV.

TABLE IV

ANTISTREPTOLYSIN CONTENT OF THE SERA OF CASES OF RECURRENT TROPICAL LYMPHANGITIS

Case	Condition	Date determination was made	Days after acute attack	Units of antistreptolysin
Z. C.	repeated attacks	3/8/34	6 days	606
		4/10/34	38 days	538
		5/7/34	90 days	333
		9/23/34	during	760
N. C.	repeated attacks	5/2/34	during	1111
		5/12/34	10 days	1111
J. L.	repeated attacks	6/28/34	during	151
		7/23/34	during	151
		8/4/34	11 days	222
P. G. S.	repeated attacks	2/3/34	1 day	259
		2/16/34	13 days	231
F. M.	repeated attacks	9/30/33	during	76
		12/7/33	during	83
F. S.	repeated attacks	9/15/33	during	333
		8/11/33	30 days	230
*G. O.	repeated attacks	9/19/34	during	81
		9/27/34	8 days	81
		10/13/34	during	81
M. L.	first attack	2/16/34	during	83
		2/19/34	3 days	64
		2/27/34	11 days	64
		3/16/34	20 days	64
		3/21/34	35 days	47
L. C.	repeated attacks	2/6/34	7 days	151
		2/15/34	16 days	166
C. M.	repeated attacks	1/26/34	3 days	185
		3/29/34	during	208
C. de M.	repeated attacks	7/3/34	during	79
		7/21/34	18 days	53
T. M.	first attack	11/1/34	during	81
		11/15/34	14 days	81
C. D.	repeated attacks	10/2/34	during	98
		10/11/34	9 days	128
P. L.	repeated attacks	7/24/34	1 day	165
		9/4/34	41 days	256
M. R.	repeated attacks	7/7/34	during	277
		7/28/34	18 days	238
		9/4/34	59 days	238
*F. C.	repeated attacks	7/9/34	6 days	25
		7/31/31	20 days	64
		9/4/34	55 days	66
F. J.	repeated attacks	8/14/31	8 days	333
		10/15/31	59 days	185
J. G.	repeated attacks	9/23/34	during	208
		9/27/34	4 days	238
J. R.	repeated attacks	10/29/34	during	151
*G. R.	repeated attacks	4/28/34	during	98
*A. I.	repeated attacks	2/21/34	during	76
F. C.	repeated attacks	3/13/34	during	117
P. M.	repeated attacks	5/11/34	during	437
M. R.	repeated attacks	7/31/34	during	476
F. O.	repeated attacks	8/11/34	during	151
*I. Q.	repeated attacks	10/25/34	3 days	76
J. P.	repeated attacks	9/8/34	4 days	138
M. F.	repeated attacks	11/10/33	7 days	219
F. M.	repeated attacks	9/4/34	9 days	104
E. O.	repeated attacks	8/17/33	8 days	104
C. V.	repeated attacks	10/6/33	1 day	138
A. L. P.	repeated attacks	9/25/33	during	83
M. E. R.	repeated attacks	1/25/34	3 days	170
P. J.	repeated attacks	1/12/34	10 days	256
C. N.	repeated attacks	8/11/33	12 days	83
P. L.	repeated attacks	1/12/34	13 days	277
C. F.	repeated attacks	9/16/33	14 days	1041
M. T.	repeated attacks	8/11/33	20 days	277
R. M.	repeated a tacks	8/11/33	21 days	151
M. L.	repeated attacks	1/5/34	30 days	138
D. A.	repeated attacks	1/5/34	30 days	333
A. A.	repeated attacks	1/5/34	35 days	231
A. H.	repeated attacks	8/11/33	35 days	128
E. H.	repeated attacks	8/11/33	40 days	538
M. F.	repeated attacks	8/11/33	40 days	222
J. C.	repeated attacks	2/2/34	40 days	252
N. R.	repeated attacks	2/16/34	46 days	138

TABLE IV—Continued

ANTISTREPTOLYSIN CONTENT OF THE SERA OF CASES OF RECURRENT TROPICAL LYMPHANGITIS

Case	Condition	Date determination was made	Days after acute attack	Units of antistreptolysin
R. R.	only one attack	1/12/34	30 days	22
J. T.	only one attack	2/9/34	30 days	69
C. R.	only one attack	4/1/34	30 days	98
C. J.	repeated attacks	8/11/33	35 days	92
D. M.	only one attack	3/2/34	52 days	79
M. C.	only one attack	1/19/34	60 days	53
E. A.	only one attack	8/17/33	60 days	83
C. D.	only one attack	3/8/34	67 days	92
D. R.	repeated attacks	1/26/34	90 days	208
D. C.	repeated attacks	4/1/34	90 days	138
M. P.	repeated attacks	8/17/33	6 days	208
J. C.	repeated attacks	2/16/34	14 days	117
F. A.	repeated attacks	2/9/34	55 days	444
E. C.	only one attack	1/12/34	75 days	98
J. N.	only one attack	2/16/34	10 days	41
J. L.	only one attack	8/11/33	2 years	62
I. M.	only one attack	8/11/33	6 days	92
J. N. C.	repeated attacks	2/26/34	during	406
		3/9/34	during	406
M. R.	repeated attacks	2/7/34	during	133
B. A.	repeated attacks	11/18/33	30 days	151
C. vda. V.	repeated attacks	8/11/33	21 days	238
C. H.	repeated attacks	8/11/33	29 days	520

*In these cases virulent haemolytic streptococci were isolated from a local lesion or abscess. Average=198 units.

From table IV it is seen that the antistreptolysin content varies in different cases and in the same case at different periods. It also varies in the cases that have had only one attack and in those that have had several repeated attacks, as a rule being higher in the latter. In most cases, the antistreptolysin values of the patient's serum are low at the onset of the attack. A sharp rise may occur at any time from a few hours to seven or eight days, but it usually occurs during the first twenty-four hours. Then the titer is more or less sustained for a few weeks, decreasing gradually. From the second month on the decrease is more rapid, until a second acute attack ensues and the antistreptolysin values rise again. There are several cases on the table that show normal values; most of them are cases suffering from the first attack, while others were investigated from thirty to sixty days after the attack subsided.

According to most authors, the antistreptolysin contents of the blood of normal persons vary from 20 to 100 units. It is really very hard to determine what is a normal case. We studied a group of patients that were normal at the time that they were studied, but some of them had a previous

history of streptococcous infection, especially of recurrent sore throat. This group gave the following results:

TABLE V

ANTISTREPTOLYSIN CONTENT OF THE SERA OF NORMAL SUBJECTS

Case	Units of antistreptolysin
P. M. O.	138
C. L.	46
N. R.	151
J. F.	22
A. P.	25
J. A. P.	52
E. R.	66
M. O.	64
A. L.	18
A. C.	46
. . F.	62
L. H.	64
A. R.	52
J. M.	28
R. M.	21
C. F.	62
A. G.	62
L. G.	111
E. S.	46
E. C.	151

Fluctuation of Antistreptolysin Content

The fluctuation of antistreptolysin titer in a case of recurrent tropical lymphangitis was studied during the attack, forty-five days and ninety days after the attack, and when the next attack ensued.

Chart 1 shows the fluctuation of antistreptolysin titer in a case of recurrent tropical lymphangitis. During the acute attack the antistreptolysin titer was 600 units; forty-five days after the attack the titer was 510 units; ninety days after the attack, 300 units. The patient left the hospital well but returned with another attack six months after being discharged. His antistreptolysin titer then was 750 units.

We have made a composite curve with a hundred determinations on different patients at varying intervals. The curve shows that at the onset of symptoms the streptolysin values are low (below 100 units); there is a sharp rise usually within the first forty-eight hours, this value being more or less sustained for several days, and then gradually dropping within sixty days. This is seen in chart 2.

There is a definite increase in the antistreptolysin content of the blood of most cases suffering from recurrent tropical lymphangitis if compared with a normal subject. As a rule, there is also a fluctuation in the antistreptolysin content of the blood of these cases, it being lower before the attack and increasing from one to seven days after the onset. The values gradually decrease within sixty days.

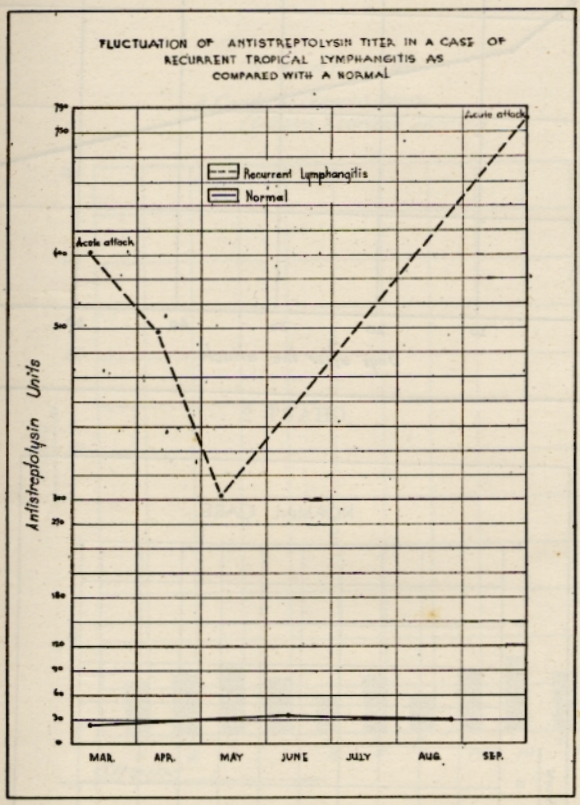

CHART 1

Antifibrinolytic Determinations in Cases of Recurrent Tropical Lymphangitis

Tillett and Garner [13] (1933) have shown that broth cultures of haemolytic streptococci of human origin rapidly dissolve human fibrin clot. Tillett, Edwards and Garner [14] (1934) demonstrated the development of resistance to dissolution in the plasma clot obtained from individuals follow-

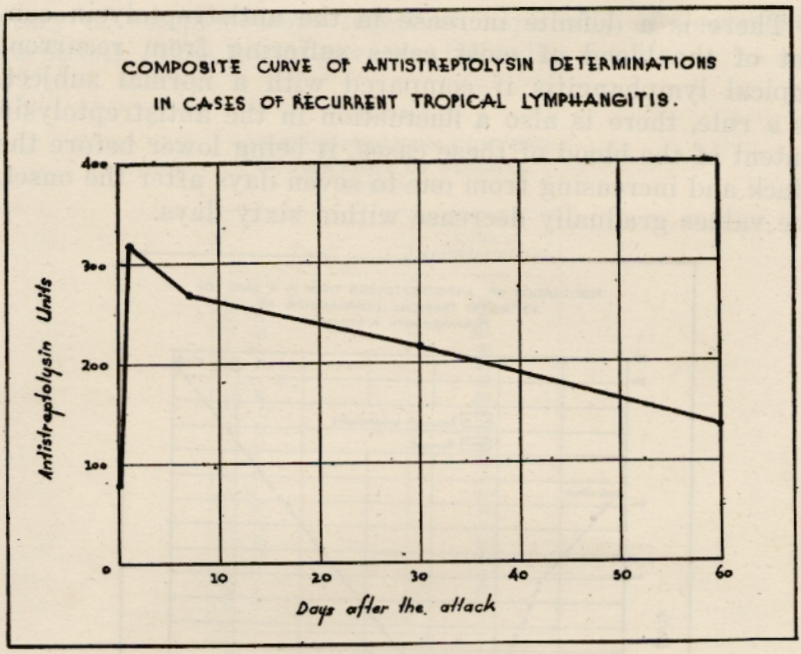

CHART 2

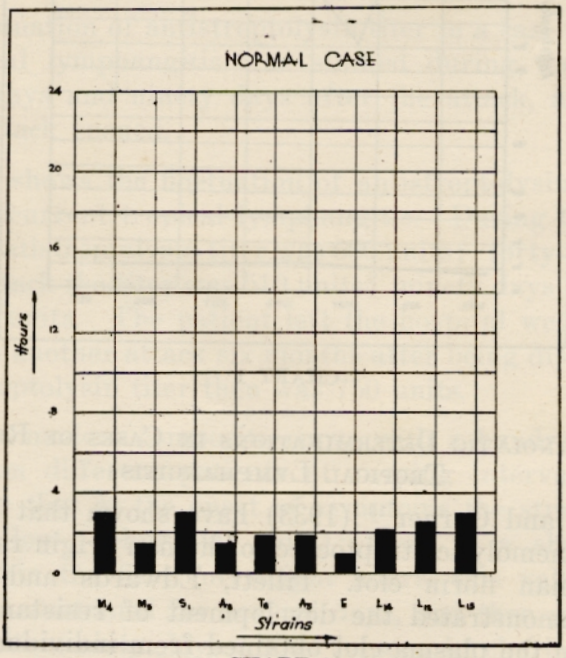

CHART 3

ing acute haemolytic streptococcous infections. They also showed that this antifibrinolytic property is absent in the fibrin clot derived from a group of patients convalescing from other infections. The blood from the great majority of healthy adults and from persons suffering from other diseases was found to be susceptible to fibrinolysis. The authors believe that this insusceptibility to dissolution is specifically

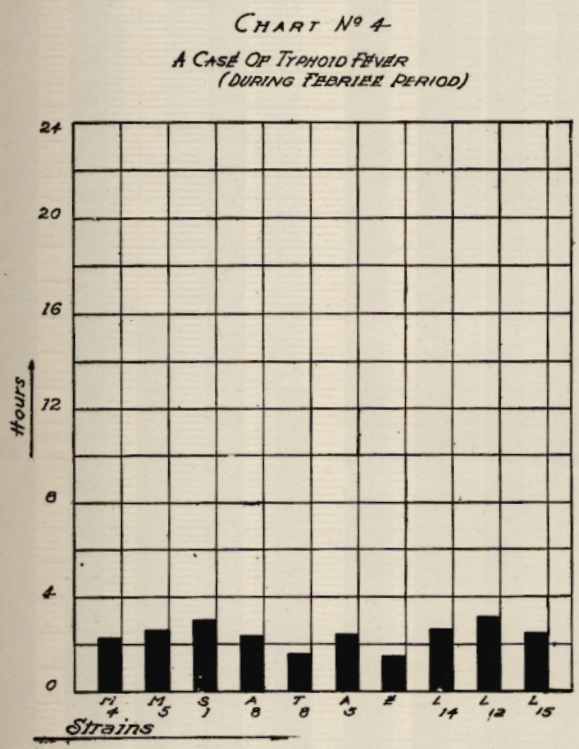

CHART 4

induced and that "the fibrinolysin of haemolytic streptococci in the body makes a definite response directed against the lytic action of the bacteria." The resistance to fibrinolysis of the plasma clot derived from a group of patients suffering from recurrent tropical lymphangitis was studied. In the first determinations several strains (M_4, M_5, S_1, A_8, T_8, A_5, E, L_{14}, L_{12}, and L_{15}) were used.

Chart 3 shows the susceptibility to lysis exhibited by the plasma clot of a normal subject. It should be noticed that there is a wide variation in the lytic power of the different

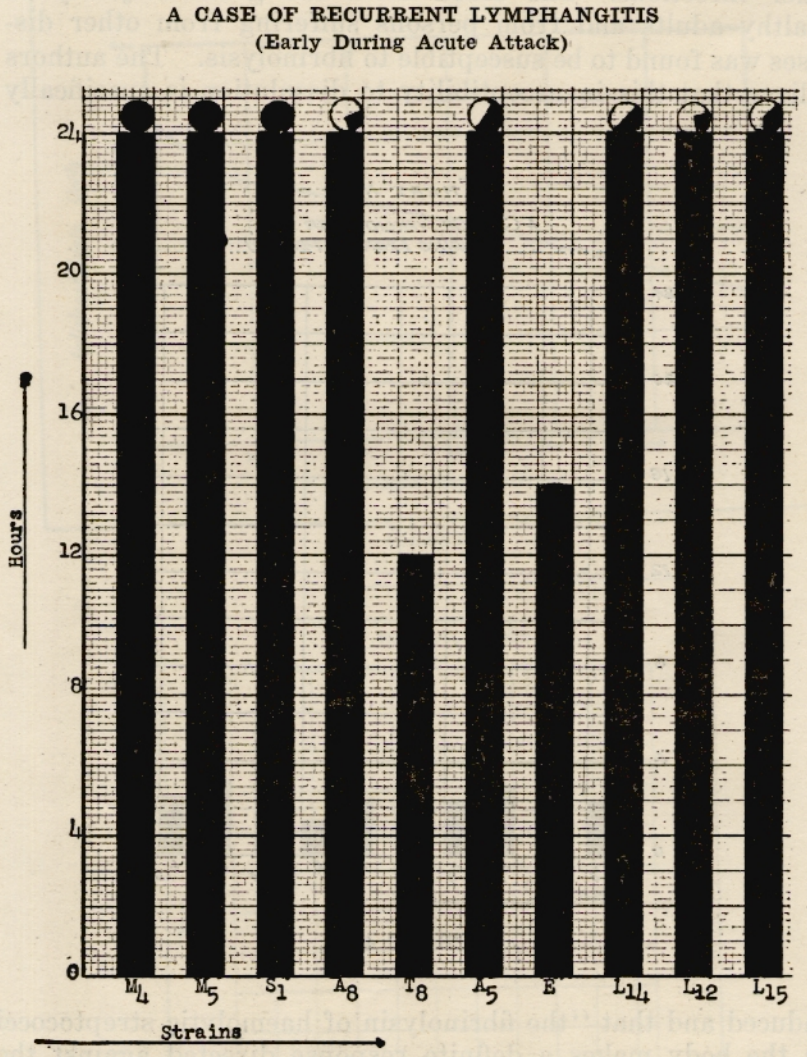

CHART 5

strains, some strains dissolving the normal human plasma clot in one hour, while it took others three hours to dissolve the same clot.

A CASE OF LYMPHANGITIS
(During the Acute Attack)

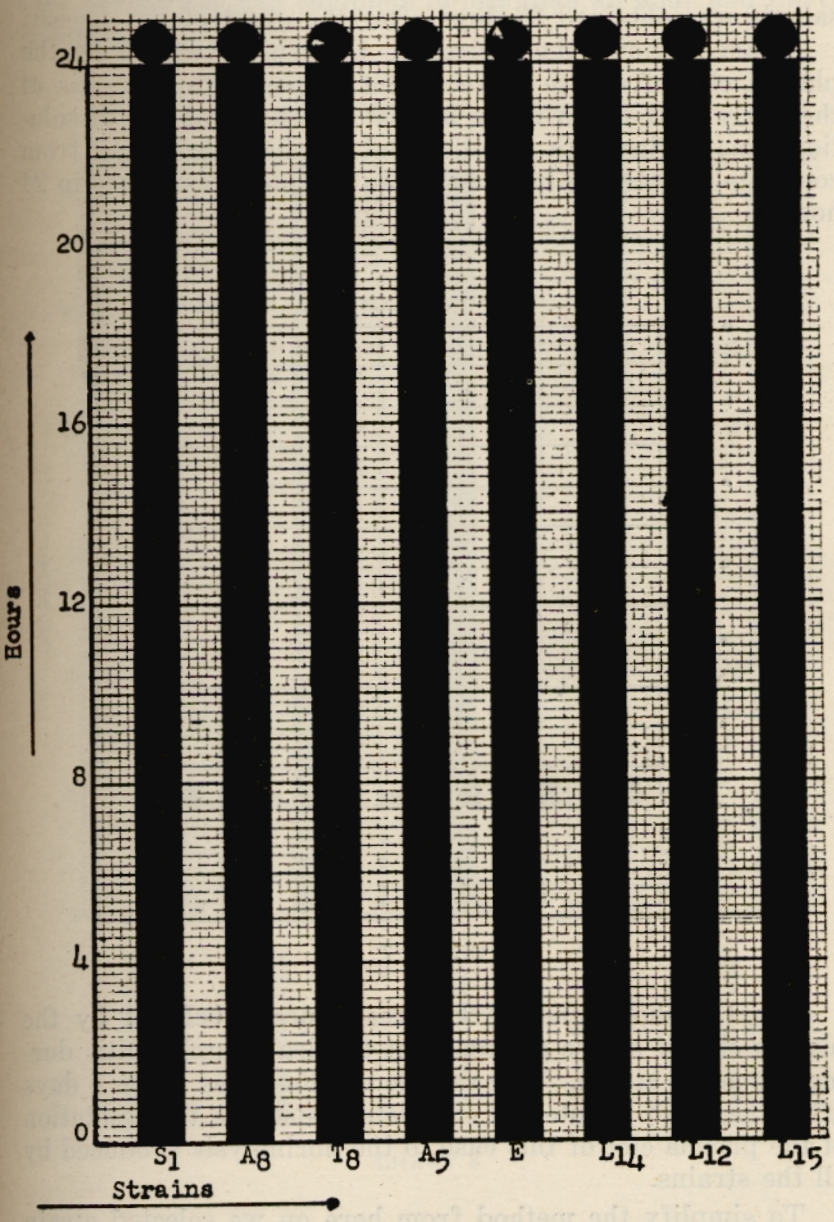

CHART 6

Chart 4 shows the dissolution time of the plasma clot obtained from a case of typhoid fever used as a control. The sample was obtained 38 days after the onset of the disease.

Chart 5 shows the resistance to lysis developed by the plasma clot of a case of recurrent tropical lymphangitis at the early part of an acute attack. Notice that the dissolution time varies with the different strains, fluctuating from complete dissolution in 12 hours to complete resistance in 24 hours.

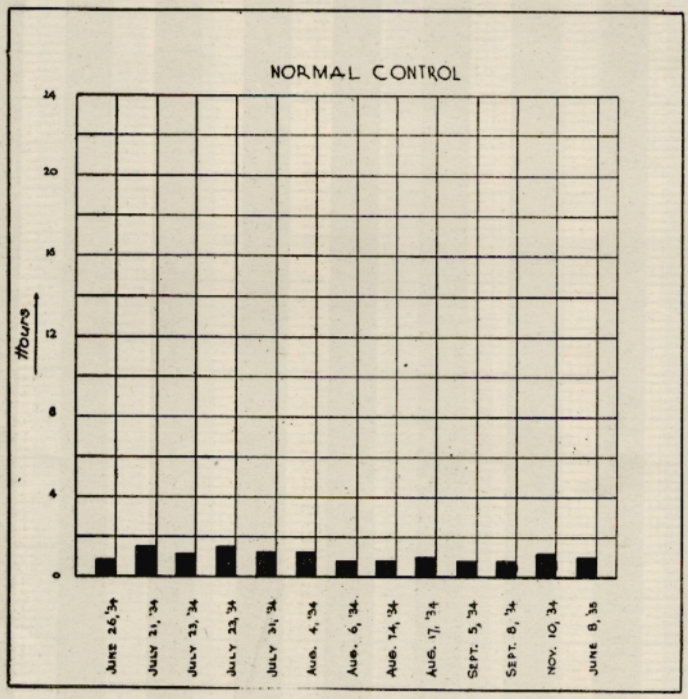

CHART 7

Chart 6 shows the marked resistance developed by the plasma clot of a case of recurrent tropical lymphangitis during the acute attack. The sample of blood was taken 4 days after the onset. Note the complete resistance to dissolution of the plasma clot of this case to the fibrinolysis produced by all the strains.

To simplify the method from here on we selected strain T_8 for carrying out the antifibrinolytic determinations. Repeated determinations were made on a normal control from

June, 1934, to June, 1935. In all determinations made in this case fibrinolysis was complete in less than an hour and thirty minutes. (Chart 7).

A CASE OF RECURRENT LYMPHANGITIS

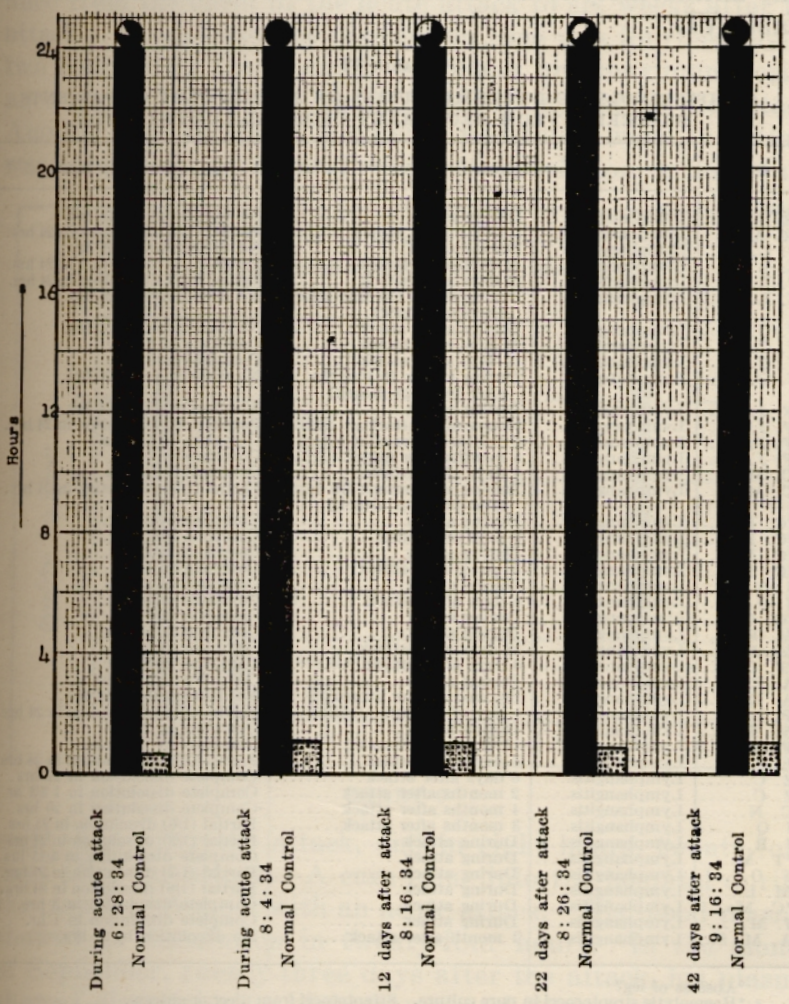

CHART 8

Repeated determinations were similarly made in a case of recurrent lymphangitis from June to September, 1934. The results are shown in Chart 8.

There was complete resistance to fibrinolysis during the period the determinations were made.

The resistance to fibrinolysis of the plasma clot derived from a group of patients suffering from recurrent tropical lymphangitis was studied. Strain T_8 was used in making the antifibrinolytic determination. The results are shown in table VI.

TABLE VI

ANTIFIBRINOLYTIC DETERMINATIONS ON PLASMA CLOT OF PATIENTS SUFFERING FROM RECURRENT TROPICAL LYMPHANGITIS

Case	Disease		Dissolution time
R. A.	Lymphangitis	During attack	No dissolution in 24 hrs.
R. A.	Lymphangitis	1 month after attack	Partial (1/8) dissolution in 24 hrs.
J. L.	Lymphangitis	During attack	No dissolution in 24 hrs.
J. L.	Lymphangitis	12 days after attack	Partial (1/8) dissolution in 24 hrs.
J. L.	Lymphangitis	22 days after attack	Partial (1/2) dissolution in 24 hrs.
J. L.	Lymphangitis	During attack (30 days after previous attack)	No dissolution in 24 hrs.
J. C.	Lymphangitis	During acute attack	No dissolution in 24 hrs.
J. C.	Lymphangitis	23 days after attack	Complete in 8 hrs.
J. C.	Lymphangitis	59 days after attack	Complete in 3 hrs.
J. C.	Lymphangitis	During attack (6 months after previous attack)	No dissolution in 24 hrs.
F. J.	Lymphangitis	During attack	No dissolution in 24 hrs.
F. J.	Lymphangitis	8 days after attack	Partial (1/6) dissolution in 24 hrs.
F. J.	Lymphangitis	2 months 8 days after attack	Complete in 3½ hrs.
F. J.	Lymphangitis	5 months after attack	Complete in 3 hrs.
C. M.	Lymphangitis	4 days after attack	Complete in 20 hrs.
C. M.	Lymphangitis	During acute attack (26 days after previous attack)	Partial (1/4) dissolution in 24 hrs.
°C. D.	Lymphangitis	During attack	Complete in 2/3 hr.
°C. D.	Lymphangitis	9 days after onset	Complete in 1/3 hr.
*C. Ma.	Lymphangitis	During attack	Complete in 2/3 hr.
*C. Ma.	Lymphangitis	18 days after attack	Complete in 1 hr.
J. G.	Lymphangitis	During attack	Complete in 22 hrs.
J. G.	Lymphangitis	4 days after attack	Complete in 14 hrs.
*G. O.	Lymphangitis	During attack	Complete in 7 hrs.
M. R.	Lymphangitis	During attack	Complete in 14 hrs.
M. R.	Lymphangitis	18 days after attack	Complete in 6 hrs.
*F. C.	Lymphangitis	6 days after attack	Complete in 1/2 hr.
A. E.	Lymphangitis	During attack	Complete in 1 1/6 hr.
A. E.	Lymphangitis	1½ month after attack	No dissolution in 24 hrs.
P. L.	Lymphangitis	1 day after acute attack	Partial (1/10) dissolution in 24 hrs.
M. R.	Lymphangitis	During attack	Complete in 22 hrs.
F. M.	Lymphangitis	12 days after attack	Complete in 7-17 hrs.
J. P.	Lymphangitis	4 days after attack	Partial (1/10) dissolution in 24 hrs.
Z. C.	Lymphangitis	6 days after attack	Complete dissolution in 22 hrs.
Z. C.	Lymphangitis	2 months after attack	Complete dissolution in 1 2/3 hr.
C. N.	Lymphangitis	4 months after attack	Complete dissolution in 20 hrs.
I. Q.	Lymphangitis	3 months after attack	Partial (1/6) dissolution in 24 hrs.
J. R.	Lymphangitis	During attack	Partial (1/3) dissolution in 24 hrs.
*T. M.	Lymphangitis	During attack	Complete dissolution in 3/15 hrs.
F. O.	Lymphangitis	During attack	Partial (1/2) dissolution in 24 hrs.
M. L.	Lymphangitis	During attack	Partial (1/8) dissolution in 24 hrs.
*C. Mo.	Lymphangitis	During attack	Complete dissolution in 3 hrs.
J. M.	Lymphangitis	During attack	Complete dissolution in 1 hr.
A. M. S.	Lymphangitis	1 month after attack	No dissolution in 24 hrs.

°Abscess of leg?
*Haemolytic streptococci in pure culture. Streptococci from ulcer or abscess.

It is seen in table VI that the plasma clot derived from cases of recurrent tropical lymphangitis develops a definite resistance to the fibrinolytic activity of haemolytic streptococci. However, in two cases of lymphangitis in which viru-

lent haemolytic streptococci were isolated from a local lesion in the affected limb during the acute attack, fibrinolysis was complete in thirty minutes during the attack, and in two hours, eight days after the attack.

In four cases the plasma clot exhibited maximum resistance from the onset of the acute attack to six weeks after the attack. In one case, the dissolution time was fourteen hours, two hours after the onset of symptoms, and maximum resistance on the eighteenth and fifty-ninth days after the attack. Another case exhibited maximum resistance when the attack was subsiding and showed complete dissolution in ten hours,

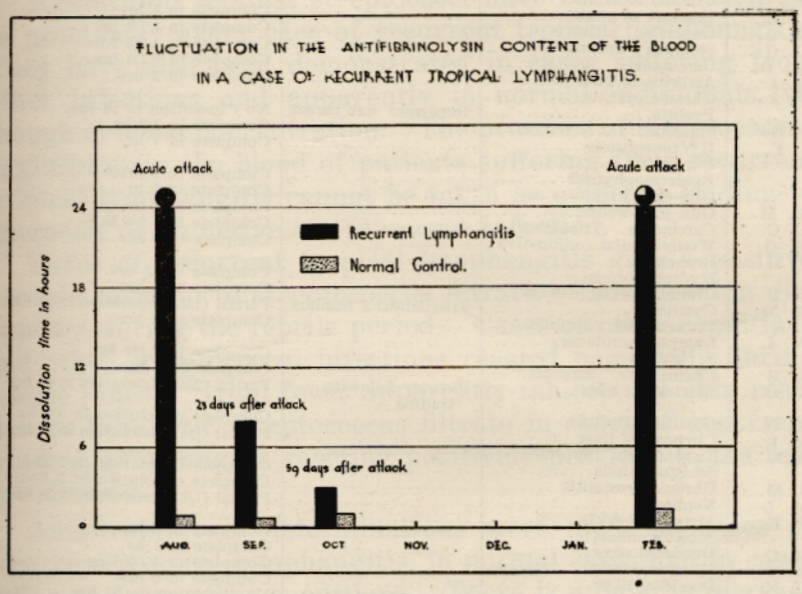

CHART 9

eight days after the attack, and in eight hours, twenty-four days after the attack. A case came into the hospital during the month of August with an acute attack. His blood plasma showed no dissolution in twenty-four hours. In the month of September, twenty-three days after the attack, his plasma showed dissolution in eight hours and during the month of October, fifty-nine days after the attack, his plasma clot dissolved in three hours. The patient was discharged from the hospital and came back with another acute attack in the month of February and his plasma clot exhibited complete resistance to dissolution in twenty-four hours. This is shown in chart 9.

Antifibrinolysin determinations were made on the plasma clot of patients suffering from various diseases, as well as of normal persons. The results are shown in table VII.

TABLE VII

ANTIFIBRINOLYTIC DETERMINATIONS ON PLASMA CLOT OF PATIENTS SUFFERING FROM DIFFERENT DISEASES AND OF NORMAL SUBJECTS

Case	Disease		Dissolution time
G. V.	Typhoid	Convalescence	Complete dissolution in 1 2/3 hr.
C. A.	Typhoid	3rd week	Complete dissolution in 1 2/3 hr.
A. S.	Typhoid	3rd week	Complete dissolution in 1 1/2 hr.
A. C.	Typhoid	1st week	Complete dissolution in 7 hrs.
L. M.	Typhoid	3rd week	Complete dissolution in 1/8 hr.
N. C.	Thromboflebitis		Complete dissolution in 1 1/4 hr.
Ma. R.	Chronic tonsilitis		No dissolution in 24 hrs.
J. La.	Fracture of ankle		Complete in 1 hr.
R. Q.	Gonorrheal arthritis		Complete in 1 1/4 hr.
C. G.	Secondary anemia		Complete in 1 2/3 hr.
M. G.	Carcinoma		Complete in 2 1/4 hrs.
R. J.	Arthritis of knee joint		Complete in 2 hrs.
P. S.	Chronic cystitis		
	Pyelitis	Recurrent sore throat	No dissolution in 24 hrs.
J. Go.	Sinusitis		Complete in 1/2 hr.
J. F.	Hydronephrosis		Complete in 1 hr.
S.	Dermatitis		
	Epidermophytosis		Complete in 3 hrs.
J. N.	Gun-shot wound		Complete in 1 hr.
R. M.	Gun-shot wound		Complete in 1 hr.
L. C.	Carcinoma. Trichiniasis		Complete in 1 1/4 hr.
H. G.	Wound ocular conjunctiva		Complete in 6 hrs
F. G.	Bronchitis		
	Hydronephrosis		Complete in 15 hrs.
C. P.	Chronic cardiac disease		Complete in 2 hrs.
R. M.	Cystitis	Friedlander's bacillus	Partial (1/3) dissolution in 24 hrs.
J. A.	Tuberculosis. Syphilis		Complete in 2 hrs.
W. L.	Lymphogranuloma		
	Syphilis		Complete in 2 1/2 hrs.
A. R.	Uncinariasis. Sarcoma		Complete in 5 hrs.
A. C.	Fracture of ulna	Pleurisy following trauma	Partial (2/3) dissolution in 24 hrs
K. G.	Cystitis. Pyelitis		Complete dissolution in 1,2 hr.
N. L.	Cirrhosis of liver		Partial (1/8) dissolution in 24 hrs.
O. V.	Tuberculosis. Syphilis		
	Schistosomiasis		Complete dissolution in 1 hr.
S. M.	Chronic prostatitis		Complete dissolution in 5 hours.
M. L.	Nephrosis		Partial (1/3) dissolution in 24 hrs.
F. R.	Acute bronchitis		
	Schistosomiasis		Complete in 3 hrs.
R. C.	Duodenal ulcer		Complete in 1 hr.
R. O.	Schistosomiasis		Complete in 4 1/2 hrs.
C. Ni.	Duodenal ulcer		Complete in 2 hrs.
E. B.	Duodenal ulcer	Recurrent sore throat	No dissolution in 24 hrs.
D. M.	Duodenal ulcer		Complete dissolution in 2 hrs.
	Rheumatoid arthritis		No dissolution in 24 hrs.
C. R.	Carcinoma		Complete in 1 hr.
C. D.	Pernicious anemia		Complete in 3 1/4 hrs.
A. P.	Normal		Complete in 2/3 hr.
E. M.	Normal		Complete in 2/3 hr.
M. G.	Normal		Complete in 2/3 hr.
E. R.	Normal		Complete in 2/3 hr.
J. M.	Normal		Complete in 2 hrs.
M. N.	Normal		Complete in 1/3 hr.
M. P.	Normal		Complete in 1 1/2 hr.
A. Po.	Normal		Complete in 1 1/2 hr.
P. V.	Normal		Complete in 1 hr.
R. A.	Normal		Complete in 2 hrs.
E.	Normal		Complete in 1 hr.
Jo. G.	Normal		Complete in 2 1/2 hrs.

In normal controls where repeated determinations were made at short intervals during several months, the dissolution

time varied slightly from thirty minutes, the lowest, to two hours and thirty minutes, the highest. This confirms the findings of Tillett, Edwards and Garner [14].

In the cases of other conditions there were four cases that showed complete resistance to dissolution in twenty-four hours and it was found that they had a history of previous streptococcus infection. All other cases showed dissolution of the plasma clot at varying intervals, the lowest being twenty minutes, and the highest fifteen hours.

Summary and Conclusions

Agglutinins against streptococci have been demonstrated in practically every case of recurrent tropical lymphangitis. They have also been demonstrated in cases suffering from other infections and apparently in normal individuals, although in lower concentration. The presence of streptococcus agglutinins in the blood of patients suffering from recurrent tropical lymphangitis cannot be taken as evidence leading to diagnosis of an active streptococcus infection.

Cases of recurrent tropical lymphangitis give a positive allergic reaction to streptococcus filtrate. This reaction disappears during the febrile period. Cases of other conditions and other streptococcal infections reacted negatively during febrile illness. High fever apparently inhibits the skin reaction to hemolytic streptococcus filtrate in cases of recurrent tropical lymphangitis reacting positively previous to the febrile disease.

Antistreptolysin determinations were made in cases of recurrent tropical lymphangitis, in normal cases and in cases suffering from other conditions. There is a definite increase in the antistreptolysin content of the blood of most cases suffering from recurrent tropical lymphangitis if compared with normal subjects. As a rule there is also a fluctuation in the antistreptolysin content of the blood of these cases, it being lower before the attack and increasing from one to seven days after the onset. The values gradually decrease within sixty days.

Antifibrinolysin determinations have been made on plasma clot of patients suffering from recurrent tropical lymphangitis, of patients suffering from various diseases as well as of normal individuals. Plasma clot derived from cases of recur-

rent tropical lymphangitis develops a definite resistance to the fibrinolytic activity of haemolytic streptococci, while plasma clot derived from non-streptococcal conditions and from normal individuals shows dissolution of the plasma clot at varying intervals, the lowest being twenty minutes and the highest, fifteen hours.

The decided increase in the antistreptolysin and antifibrinolysin content in the blood from cases of acute recurrent tropical lymphangitis and the fluctuation of the antifibrinolysin and antistreptolysin content of the blood in these cases is evidence that the acute attacks of recurrent tropical lymphangitis are preceded by haemolytic streptococcus infection.

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