SERIAL CEPHALIN FLOCCULATION TEST IN LEPROSY
A PRELIMINARY REPORT

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In 1943 Bruger (1) proposed a fractional cephalin-cholesterol flocculation test to be used as an index of increasing or decreasing hepatic dysfunction. This modified test consisted in the use of increasing dilutions of serum with saline, noting the flocculation according to the procedure originally described by Hanger. The data presented in support of the modified test do show that, when it is repeated at various intervals, changes occur which can be interpreted as indicative of increasing or decreasing function.

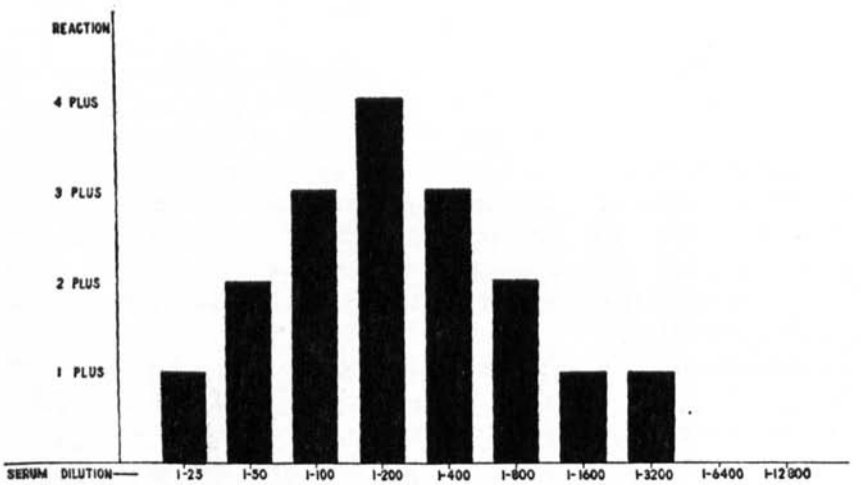
Because of differences in the dilutions used by Bruger (1:10 serum dilution in the first tube), Makari (2) found that false positive reactions occurred which resulted in minor alterations of the curves. He devised a serial dilution technique in which the amount of serum added to the first tube was identical to that used in the original one-tube Hanger method. In a recent study in this laboratory of liver function tests in leprosy (5), it was found that the cephalin-cholesterol test of Hanger was positive in 68.4 per cent of the 152 cases studied. It was thought that the serial dilutions of sera with saline in conjunction with the cephalin-cholesterol method might be of interest.

EXPERIMENTAL

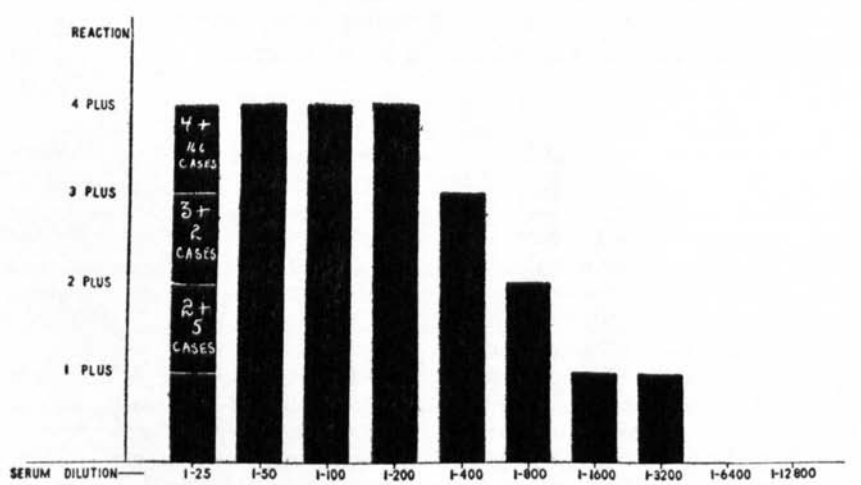
For the work reported here, 425 cases were used which were varied as regards type and duration of the disease and its state of progression. All but a very few, including recently admitted patients, were under sulfone therapy. Blood specimens were obtained from 133 of the 425 cases at weekly intervals for a period of one month to observe whether variations in flocculation would occur over a short period of time. Control specimens were collected from 30 employees of this institution and analyzed coincidentally with the patient's blood.

Approximately 10 cc. of fasting blood was drawn by vein puncture and centrifuged after clotting, the serum being collected and analyzed the same day. The cephalin-cholesterol emulsion was prepared according to the method of Hanger, employing Difco antigen. The test consisted of setting up 10 chemically cleaned test-tubes each containing 4 cc. of 0.85 per cent saline. Serial dilutions of the serum were made beginning with a 1:25 dilution, then 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, and 1:12800; and then to each tube 1 cc. of the cephalin-cholesterol

emulsion was added, making the total amount of fluid in each tube 5 cc. Serial flocculation charts were obtained by plotting the degree of flocculation, read in accordance with Hanger's method, as ordinates against serial dilutions as abscissae. Zero reactions were not charted since five of the controls gave a 1-plus flocculation in a 1:25 serum dilution, which is considered normal.



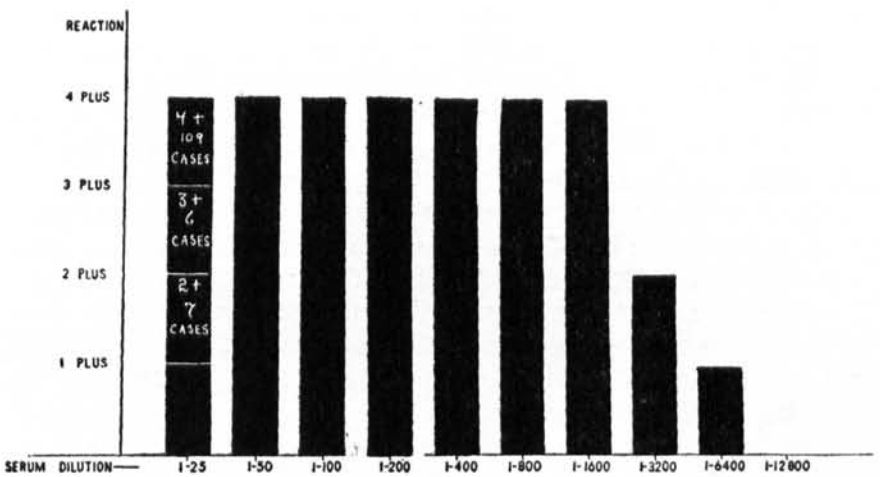
TEXT-FIG. 1. Curve of serial cephalin-cholesterol flocculation reactions of sera of apparently normal, nonleprous controls. Also Pattern 1 of leprosy patients, observed in 30 lepromatous cases, all bacteriologically negative.



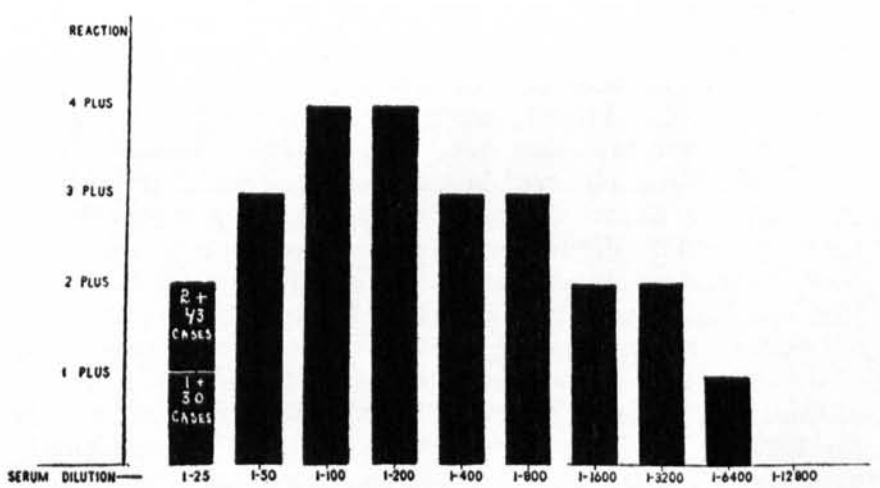
TEXT-FIG. 2. Pattern 2 of the serial cephalin-cholesterol flocculation reactions in lepromatous leprosy, observed in 173 cases. Variations of positivity occurred in the 1:25 serum dilution, but not in the others.

RESULTS

The results of this serial test obtained in the 30 control sera from employees are shown in Text-fig. 1. In the first tube, containing the 1:25 serum dilution, the flocculations were either zero or 1-plus. A 2-plus flocculation occurred with dilutions of 1:50, and 3-plus with dilutions of 1:100, while complete flocculation occurred with the 1:200 dilution. The flocculations then showed a decrease, with 3-plus in the 1:400 dilution, 2-plus in



TEXT-FIG. 3. Pattern 3 of the reactions, observed in 122 lepromatous cases; variations in the 1:25 dilution only.



TEXT-FIG. 4. Pattern 4 of the reactions, observed in 73 lepromatous cases; variations in the 1:25 dilutions only.

the 1:800 dilution, and 1-plus in the 1:1600 and 1:3200 dilutions. No flocculation occurred in the 1:6400 and 1:12800 dilutions.

The results obtained in 398 lepromatous cases are grouped into four patterns. Pattern 1, pertaining to 30 such cases, is identical with that of the control group as shown in Text-fig. 1, and the chart is therefore not reproduced here. Pattern 2 (Text-fig. 2) is characterized by either 2-plus, 3-plus or complete flocculation in the 1:25 dilution, with complete flocculation in the 1:50, 1:100, and 1:200 dilutions. The flocculation then showed a decrease to 3-plus in the dilution of 1:400, 2-plus in the dilution of 1:800, and 1-plus in dilutions of 1:1600 and 1:3200; the higher dilutions showed no flocculation. Pattern 3 (Text-fig. 3) is characterized by 2-plus, 3-plus or 4-plus flocculation in the 1:25 dilution, with complete flocculation in the dilutions from 1:50 to 1:1600, 2-plus in 1:3200, and 1-plus in the 1:6400 dilution, with no flocculation in the highest dilution. Pattern 4 (Text-fig. 4), shows a 1-plus flocculation in the 1:25 dilution, a 3-plus flocculation in the 1:50 dilution, complete flocculation in the dilutions 1:100 and 1:200, with decreasing effects thereafter—3-plus in 1:400 and 1:800, 2-plus in 1:1600 and 1:3200, and 1-plus in 1:6400, the 1:12800 dilution again showing no flocculation.

The 30 cases showing the normal curve (Pattern 1) were all bacteriologically negative for *Mycobacterium leprae.* Though inactive, 7 were classified as early cases, 19 as moderately advanced, and 4 as far advanced.

Of the 173 cases which fell within the second pattern, 28 were early, 55 moderately advanced, and 90 far advanced. Five of the 28 early cases showed 2-plus flocculation in the 1:25 serum dilution, and these cases were also bacteriologically negative. The other 168 cases were bacteriologically positive.

Pattern 3 was observed in 122 cases, 2 of which were early, 29 moderately advanced, and 91 far advanced. A 2-plus flocculation in the 1:25 dilution was noted in 2 of the early cases and 5 of the moderately advanced ones, and these cases were also bacteriologically negative. The other 115 cases were positive.

Pattern 4 was observed in 73 cases, of which 8 were early, 27 were moderately advanced, and 38 were far advanced. Thirty of these cases showed a flocculation within the normal range in the 1:25 serum dilution, and of them 20 were bacteriologically negative. Forty-three showed a 2-plus flocculation in the 1:25 dilution, and of them 10 were bacteriologically negative.

In addition to the 398 lepromatous cases, the serial dilution

test was applied in 27 tuberculoid (neural) cases. Of these, 25 had serial flocculations comparable to those of our controls (first pattern), and all of them were bacteriologically negative. Two cases fell within the fourth pattern.

In the entire series of 425 cases, 57 (13.4%) showed normal values throughout. Of the 398 lepromatous cases, 173 (43.3%) exhibited the second flocculation pattern; 122 (30.6%) fell within the third pattern; and 73 cases (18%) fell in the range of the fourth pattern. Since the first tube (dilution 1:25) is comparable to the one-tube Hanger method, results of Patterns 2 and 3 represent 69.4 per cent of the 425 cases. In a recent article (4) we reported a positive cephalin-cholesterol test in 68.4 per cent of 152 cases using that one-tube method. Our present findings are in close agreement with the previous results. The results in Pattern 4 closely approach those of our controls, though they are not within the normal range.

Serial repetitions of the test at weekly intervals for a period of one month on 133 of the 425 cases, did not reveal variations.

In previous work, we observed (6) that the progress of leprosy is associated with changes in serum proteins consisting of an increase in total globulins as well as euglobulins (5), with a lowering of the albumin-globulin ratio. Recant and his associates (3) state in substance that, in disease, positive cephalin-cholesterol flocculation may be obtained with serum due to any of the following alterations: (1) increase of gamma globulin in such quantity that there is an insufficiency of the normal components of the serum albumin fraction to inhibit the reaction; (2) diminution of the serum albumin fraction below the initial levels necessary to inhibit the reaction; and (3) diminution in the flocculation inhibiting properties of the albumin fraction. From this it is conceivable that many conditions other than liver disease might occasion a change in the pattern of the serum proteins. It seems that the results of the serial dilution cephalin-cholesterol test in our series of patients could be looked upon as a measure of abnormal serum protein pattern, or of liver dysfunction, or both.

SUMMARY

Blood specimens from 425 cases of leprosy (398 of the lepromatous type and 27 tuberculoid) were examined for the cephalin-cholesterol flocculation reaction using a serial serum dilution method. Blood specimens from 30 employees were similarly examined as controls. Serial repetitions of this test were

performed at weekly intervals over a period of one month on 133 of the 425 cases.

Four patterns of flocculation are reported. Pattern 1 is presented by apparently normal individuals and was observed in the 30 controls, and also in 57 of the 425 leprosy cases examined. Of these 57 cases, 50 were bacteriologically negative for *M. leprae.* Pattern 2 was noted in 173 cases, and Pattern 3 in 122, the greatest number occurring in the moderately and far advanced lepromatous cases. These patterns are suggestive of liver dysfunction, since in both patterns the first tube is positive according to the one-tube Hanger method. Pattern 4, which closely approaches the normal pattern, was observed in 73 cases.

The serial cephalin flocculation patterns are not found to vary in leprosy when examinations are made at weekly intervals for a period of one month.

It is pointed out that many conditions other than liver disease might occasion a change in the pattern of serum proteins, and that this in turn may be reflected in the serum serial dilution cephalin-cholesterol flocculation.

The repeated use of this test several times a year may be useful as a guide to therapy and prognosis in the treatment of leprosy, or of concomitant hepatic dysfunction.

RESÚMEN

Muestras de sangre de 425 casos de lepra (398 del tipo lepromatoso y 27 del tipo tuberculoide) fueron sometidas a la prueba de floculación de la cefalina-colesterol usando un método de dilucion en serie del suero sanguíneo. Muestras de sangre de 30 empleados fueron similarmente examinadas y sirvieron de control. La prueba fué repetida serialmente a intérvalos de una semana por un período de un mes en 133 de los 425 casos.

Se notaron cuatro tipos distintos a la reacción de floculación, a saber: El tipo I se presentó en individuos aparentemente normales, y se observó en los 30 casos control, y también en 57 de los 425 casos de lepra. De éstos 57 casos, 50 eran bacteriológicamente negativos para *M. leprae.* El tipo II se notó en 173 casos, y el tipo III en 122 casos, la mayoría de los cuales eran casos moderadamente avanzados o muy avanzados de lepra lepromatosa. Estos tipos (II y III) son sugestivos de disfunción hepática, puesto que en ámbos el primer tubo es positivo de acuerdo con el método de un solo tubo de Hanger. El tipo IV, que se asemeja al normal, fué observado en 73 casos.

La prueba en serie de floculación de la cefalina no varió en

pacientes leprosos cuando los exámenes fueron hechos a intérvalos de 1 semana por un período de 1 mes.

Se hace notar que muchos otros factores además de enfermedad hepática pueden ocasionar un cambio en el mosáico de las proteinas del suero, y que éste a su vez se puede reflejar en la prueba de dilución seriada de la floculación de la cefalina-colesterol.

El uso repetido de esta prueba varias veces al año puede ser útil en guiar la terapia y el pronóstico en el tratamiento de la lepra, o de disfunción hepática asociada.

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