THE THYMOL TURBIDITY AND CEPHALIN-CHOLESTEROL TESTS, EUGLOBULIN, ALBUMIN-GLOBULIN RATIO AND SEROLOGY IN LEPROSY [1]

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During the course of experimental work on leprous patients in Hawaii, Dr. Eric Fennel observed a relationship between the thymol turbidity and cephalin-cholesterol tests, the albumin: globulin ratio and the degree of skin involvement in the cases he studied. He made observations on 25 cases and included some of his findings in a report on the thymol turbidity test (4), but he felt that they were insufficient to justify more extended publication. In a personal interview with him in 1947 it was agreed that I should repeat that work on a larger group of patients. While doing so, the opportunity was taken to compare these tests with the euglobulin of the serum as well as the serology.

Experience has shown that various serum turbidity and flocculation reactions used in the clinical laboratory respond differently with sera from patients with different diseases. It was hoped originally that one or the other of these tests would be specific for liver diseases, but it has since been shown that positive reactions are produced primarily when the concentration of some particular protein fraction of the serum is greater than normal.

The thymol turbidity test was first described by MacLagan (8) in 1944 as an index of liver function. He believed that the thymol turbidity is due to a globulin-thymol-lipid complex, and that the test gives essentially the same results as the colloidal gold procedure which has been used in the study of liver disease. The earlier studies of Hanger (5) likewise implicated a globulin —that is, gamma globulin—as the important factor in the cephalin-cholesterol flocculation. Subsequently, Hanger and coworkers (9) described evidence indicating that that there must be an alteration in the character of the serum albumin, together with an increased globulin, before flocculation will take place. It is evident that the underlying mechanism of the two tests is not identical. With other associates Hanger (10), using electro-

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phoretic methods, showed that gamma globulin was not involved in the mechanism of the thymol turbidity test, whereas the cephalin-cholesterol flocculation test depended upon the presence of that globulin. Cohen and Thompson (2) presented evidence that the protein in the complex of the thymol turbidity test was beta globulin.

For the work to be reported here, 152 cases were selected, all ambulatory but varied as regards type and duration of the disease and its state of progression. It did not seem feasible to classify the cases according to the stages of activity of the disease as in previous reports from this laboratory, since all but a very few of the patients are on sulfone therapy and all show improvement though they may be moderately and far advanced lepromatous cases. Blood specimens were obtained from some of the patients on two occasions at intervals of approximately three months. Control bloods were collected from ten employees of this institution and analyzed coincidentally with the patients' blood.

ANALYTICAL METHODS

Approximately 20 cc. of fasting blood was drawn by vein puncture and centrifuged after clotting, the serum being collected and analyzed on the same day. The proteins were determined by a micro-Kjeldahl method as in previous work on proteins in this laboratory (14). The euglobulin technique is also described in a previous report (11). The technique used for the thymol turbidity test was that of Shank and Hoaglund (12), who modified that of MacLagan.

In the thymol turbidity test 0.1 cc. of serum was added to 6.0 cc. of thymol barbiturate buffer in a calibrated test tube, which was stoppered, shaken, and allowed to stand for thirty minutes. At the end of that time the tubes were inverted once and read in a Klett-Summerson photoelectric colorimeter equipped with a red filter of light transmission limits of 640 millimicrons. The galvanometer was adjusted to 100 per cent transmission of light with a blank containing 6.0 cc. of the thymol buffer. The turbidity of a given reaction is expressed in units derived from a standard curve prepared by using barium sulphate suspensions as recommended by Shank and Hoaglund. The quantities of standards prepared were double those used by those authors, in order to adapt the procedure to the colorimeter used. It was found that the reagent developed a turbidity on standing after two weeks. However, reheating, cooling, and again seeding with thymol crystals resulted in a clear solution.

The cephalin-cholesterol flocculation test was performed according to the method of Hanger (5), employing Difco antigen. The tubes were kept at room temperature in the dark and read at the end of 24 and 48 hours.

For the serology, Kahn's standard precipitation test was run parallel with the Mazzini flocculation test (3).

In the thymol turbidity reaction the normal range is 0 to 8 units. In the cephalin-cholesterol reaction, 3+ to 4+ are considered positive. Albumin:globulin ratios of 1.5 to 2.5 are considered normal. Euglobulin values of 60 to 100 tyrosin indices are normal.

The data obtained are shown in Tables 1 and 2. In the heading of Table 1 the comparison of the thymol turbidity and cephalin-cholesterol tests is shown, and in the three sections of the body of that table are shown the relationships of those tests to the albumin:globulin ratio, the serum euglobulin, and the serology. Of the entire group of 152 cases, 59, or 38.8 per cent were positive in all of the tests including the serology; 5 or 3.3 per cent, were negative in all of the tests including the serology; while 88, or 57.2 per cent, were positive in one or more of the tests.

TABLE 1.—*Correlation of the thymol turbidity (T. T.) and cephalin-cholesterol (C. C.) tests, and comparison with* (a) *the albumin:globulin ratio,* (b) *the euglobulin-tyrosin indices, and* (c) *the serology (Kahn and Mazzini tests), in 152 cases.*

	T. T. positive C. C. positive (79 cases, 52%)	T. T. positive C. C. negative (14 cases, 9%)	T. T. negative C. C. positive (25 cases, 16%)	T. T. negative C. C. negative (34 cases, 22%)
Comparison with the albumin:globulin ratio [1]				
Above 1.5[1]	20 cases	1 case	15 cases	21 cases
Below 1.5	59 cases	13 cases	10 cases	13 cases
Comparison with the euglobulin-tyrosin index				
Normal	16 cases	6 cases	5 cases	21 cases
Increased	63 cases	8 cases	20 cases	13 cases
Comparison with the serology reactions				
Positive	61 cases	3 cases	22 cases	9 cases
Negative	16 cases	10 cases	3 cases	23 cases
Doubtful	2 cases	1 case	-------	2 cases

[1] A/G ratio 1.5 to 2.5 considered the lower limit of normal.

The cephalin-cholesterol tests were positive in 104 of the cases, or 68.4 per cent, and the thymol turbidity values were above normal in 93 of the cases or 61.2 per cent. Considering these two tests together, 79 cases, or 51.3 per cent of the total, gave positive results with both; while on the other hand 34 cases, or 22.3 per cent, were negative with both.

Of the 79 cases positive in both the thymol turbidity and the cephalin-cholesterol tests, 59 (75%) had albumin:globulin ratios below normal, with hyperglobulinemia, although the total proteins were within the normal range of 6.0 to 8.5 per cent in all but one of the cases. Of the 34 cases negative in both tests, 13 (38%) showed albumin:globulin ratios below normal, with increase of the globulin fraction. No less than 63 (80%) of the 79 positive cases showed an increase in the euglobulins, but that was seen in only 13 (38%) of the 34 negative cases. Again, of the 79-case group 61 (77%) gave positive results in the serological reactions, while of the 34-case group only 9 (26%) were positive.

Although there is experimental and clinical evidence that the thymol turbidity and cephalin-cholesterol flocculation tests depend upon different factors, it was of interest to compare the two in this series since both are generally regarded as liver function tests. The results of the thymol turbidity and cephalin cholesterol flocculation were in agreement in 51.3 per cent of the cases. Sixty-three of these cases had an increase in euglobulin, while 59 had a low albumin:globulin ratio. In my previous article on euglobulin in leprosy it was pointed out that the liver is the main source of much of the globulin, and that infections impair the manufacture of serum proteins in the liver despite the fact that protein intake may be maintained. As has been said earlier in the present article, cephalin-cholesterol flocculation is dependent upon the presence of gamma globulin and the thymol turbidity test upon beta globulin. Hanger and co-workers state in substance that the elaboration of the serum albumins is usually ascribed to the liver. When this organ becomes diseased, as in long-standing cirrhosis, there is commonly a distinct drop in the serum albumin fraction as well as an increase of serum globulin. 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.

Table 2 gives the minimal, maximal and average findings of the thymol turbidity test, the albumin:globulin ratio, and euglobulin, as well as the number of cases positive in the cephalin-cholesterol flocculation tests and the serology, in the various

TABLE 2. — *Thymol turbidity and cephalin-cholesterol tests, albumin:globulin ratio, euglobulins of serum, and serology in the various types of leprosy, 152 cases.*

	Thymol turbidity, units	Cephalin-cholesterol, cases positive	Albumin: globulin ratio	Euglobulin-tyrosin indices	Serology, cases positive
Controls (nonleprous), 10 cases					
Minimum	2.5		1.5	60	
Maximum	8.0	1 (10 %)	2.3	80	1 (10 %)
Average	5.2		1.7	69	
Tuberculoid leprosy, 6 cases					
Minimum	3.0		1.2	85	
Maximum	14.0	0 (—)	1.8	160	2 (33 %)
Average	6.9		1.6	117	
Neural leprosy, 6 cases					
Minimum	7.4		0.9	75	
Maximum	25.0	3 (50 %)	1.2	200	2 (33 %)
Average	7.5		1.0	92	
Early lepromatous (L1), 39 cases					
Minimum	2.5		0.7	70	
Maximum	24.0	26 (67 %)	3.0	260	19 (49 %)
Average	11.5		1.4	146	
Moderately advanced lepromatous (L2), 47 cases					
Minimum	2.5		0.6	62	
Maximum	28.0	33 (70 %)	1.9	280	33 (70 %)
Average	14.3		1.2	160	
Far advanced lepromatous (L3), 44 cases					
Minimum	5.0		0.5	70	
Maximum	28.4	37 (84 %)	1.6	298	28 (64 %)
Average	14.5		1.1	140	
Paroled patients, 10 cases					
Minimum	3.5		0.9	62	
Maximum	16.0	6 (60 %)	1.7	225	2 (20 %)
Average	9.0		1.2	135	

types of leprosy. The average figures for the albumin:globulin ratio and the euglobulin concentration in our controls agree with the findings in previous reports (11, 14). The figures for the thymol turbidity test are slightly higher than those of Shank and Hoaglund, who reported 4.7 units as the upper limit of normal. One of the controls gave a 3-plus cephalin-cholesterol reaction and was serologically positive.

Of the 6 tuberculoid cases, the average values for the thymol turbidity test and albumin:globulin ratio were within the normal limits, although one of the cases had an increased thymol turbidity as well as a low albumin:globulin ratio. The cephalin-cholesterol reaction was negative in all of these cases; 2 of them were positive serologically. The average euglobulin was slightly above normal, three of the cases showing an increase.

Of the 6 neural cases, the averages for thymol turbidity and the euglobulins were within the normal range, though one of the cases showed increases with respect to both determinations. Three cases were positive in the cephalin-cholesterol flocculation, and two were serologically positive. The albumin:globulin ratio was below normal in all of them. These neural cases were moderately and far advanced, with mutilations of the hands and feet.

In all of the lepromatous groups the averages for thymol turbidity, albumin:globulin ratio and euglobulin were not within the normal range. The greater number of positive cephalin-cholesterol and abnormal thymol turbidity tests, as well as the positive serological reactions, were found in the moderately and far advanced lepromatous cases. Of the 39 early cases, 14 had normal albumin:globulin ratios; while of the 47 moderately advanced ones 10, and of the 44 advanced ones 11, showed normal values. The euglobulin was normal in 13 of the early cases, in 9 of the moderately advanced ones, and in 7 of the far advanced ones. The thymol turbidity test was normal in 18 of the early cases, in 14 of the moderately advanced ones, and in 9 of the advanced ones.

Of the 10 paroled cases, 4 showed high thymol turbidity values, which made the average above normal; six of them were positive to the cephalin-cholesterol test, and two of the cases were serologically positive. The average albumin:globulin ratio was below normal. Six of the cases had hyperglobulinemia which lowered the ratio. These paroled cases like most of the neural ones were far advanced cases showing mutilations and much scar tissue.

The results show marked variations between the groups into which the cases have been divided on the basis of type and clinical findings. Thus, the most obvious differences occur between the moderately and far advanced lepromatous cases on the one hand and the early lepromatous and tuberculoid cases on the other hand. The total serum proteins were within the normal range in all of the cases but one, which showed 9.3 per cent. The majority of the patients at this institution are of the lepromatous type, though many show some degree of neural involvement. Hence the number of lepromatous cases is greater than that of the neural and tuberculoid forms. It has also been our experience that the greater number of our cases are moderately and far advanced when first admitted to this institution.

DISCUSSION

Disease of the liver has been found postmortem in almost all autopsies at this leprosarium (6), although symptoms of disease of the liver have not been frequently noted in life. In a study of the van den Burgh test in this laboratory (1), 138 out of 200 cases were found to be positive, indicating early hepatic lesions. It has also been observed (14) that the progress of leprosy is associated with changes in serum proteins which produce an increase in total globulins as well as euglobulins (11) with a lowering of the albumin:globulin ratio. It was suggested that hepatic dysfunction and liver damage might be an etiologic factor in the disturbed protein metabolism noted. Tilden (13) calls lepromatous leprosy a reticuloendothelial disease from the histologic point of view, because it involves those structures (skin, mucous membranes, lymph nodes, bone marrow, liver and spleen) which one would expect to be involved if the pathologic response were on the part of the reticuloendothelial system.

It is impossible to explain satisfactorily the interrelation between leprosy and our blood chemistry findings. The changes associated with leprosy appear to be the result of a widespread disorder of function of the body tissues involving the reticuloendothelial system (liver, spleen, lymph nodes, mucous membranes), probably other internal organs, and the skin. The determination of the functional efficiency of the liver by chemical methods offers unusual difficulties because of the multiplicity of duties performed by the liver, and because the activity of the liver is so intimately associated with other organs that, in investigating those functions, it is difficult to delineate the hepatic factor.

SUMMARY

Blood specimens from 152 cases of leprosy were examined for the thymol turbidity and cephalin-cholesterol flocculation tests, euglobulin, albumin:globulin ratio and serology (Kahn and Mazzini). Blood from 10 employees used as controls were similarly examined.

In the entire group of 152 cases, 59, or 38.8 per cent, were positive in all of the tests including the serological ones; 5, or 3.3 per cent, were negative in all tests; while 88, or 57.2 per cent of the cases, were positive in one or more of the tests.

The cephalin-cholesterol flocculation test was positive in 104, or 68.4 per cent, of the 152 cases, while the thymol turbidity value was above normal in 93, or 61.2 per cent, of them.

Of the 152 cases, 79, or 51.3 per cent, showed positive cephalin-cholesterol flocculation; and increased thymol turbidity was seen in 79 cases, or 51.3 per cent, while both tests were negative in 34 cases, or 22.3 per cent.

The greatest number of abnormal findings was in the group showing both positive cephalin test and increased thymol turbidity.

The albumin:globulin ratio was below normal in 95 of the cases, the greatest number (59) of such results being in those cases in which the cephalin test was positive and the thymol turbidity increased.

The euglobulins were increased in 104 cases, and again the greatest number (63) was in those cases giving abnormal cephalin and thymol turbidity results.

The serology was found to be positive in 86 cases, 63 having an abnormal cephalin as well as thymol turbidity reactions.

The results show marked variations between the groups into which the cases have been divided. The greater number of positive cephalin-cholesterol flocculation and abnormal thymol turbidity reactions as well as serological positivity was observed in the moderately and far advanced lepromatous cases. The average albumin:globulin ratio was below normal in all of the lepromatous cases, though the total protein was within the normal range of 6 to 8.5 per cent. Eighty of the lepromatous cases were positive serologically.

Reference is made to some of the mechanisms of the thymol turbidity test and the cephalin flocculation test. It appears that an increase of the beta and gamma globulins are a part of the reaction of both tests, although the underlying mechanism of the two tests is not identical.

The changes noted appear to be the result of a widespread disorder of function of body tissues involving the reticuloendothelial system, including the skin and probably certain internal organs.

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