

STUDIES ON SERUM PROTEINS IN LEPROSY
THE ALPHA, BETA AND GAMMA GLOBULIN FRACTIONS

SISTER HILARY ROSS
AND FRANK GEMAR

*From the Laboratories of the U. S. Public Health Service Hospital
Carville, Louisiana*

In an earlier report (9) it was shown that the serum proteins in leprosy patients show characteristic and significant departures from normal values. An increase in the globulin component was found, with a decrease in the albumin fraction which maintained the total protein concentration within the normal range. An increase in the euglobulin fraction has also been noted (5). In recent years increasing clinical significance is being attached to the various serum protein components, principally the albumin and the alpha, beta and gamma globulin fractions.

On the basis of electrophoretic analysis of the blood serum of patients with a variety of diseases, certain generalizations have been made. In febrile conditions the alpha globulins are high. In rheumatic fever, amyloidosis, relapsing malaria and sarcoidosis there are marked increases in the gamma fraction. In certain diseases affecting the liver, there is often an elevation of all three globulin fractions. Changes in the relative distribution of these proteins during the course of many diseases bear no relationship to total protein levels, although the total protein level is not necessarily normal (1, 3).

The increasing clinical significance which is being attached to the various fractions of the serum proteins has necessitated a simple and rapid procedure for their estimation. Wolfson and associates (8) have devised a simple chemical fractionation method, utilizing the biuret reaction of Weichselbaum, which approximates the results obtained by electrophoresis. Since we were not aware of any reports of determinations of the alpha, beta and gamma globulin fractions in leprosy, it seemed desirable to undertake such a study.

There have been studies on the relationship of specific protein fraction alterations and the results of some of the liver function tests in leprosy, in which references were made to some of the mechanisms connected with the thymol turbidity and cephalin-cholesterol flocculation tests (6). In the report referred to it was pointed out that Cohen and Thompson (2)

have indicated that the protein involved in the complex of the thymol turbidity reaction was beta globulin, while Hanger and associates (4) showed by electrophoresis that the cephalin-cholesterol flocculation test depended upon the presence of gamma globulin. Our observations include an attempt to determine possible reciprocity between the alpha, beta and gamma globulin fractions and the results of the thymol turbidity reaction and the cephalin-cholesterol flocculation test in leprosy.

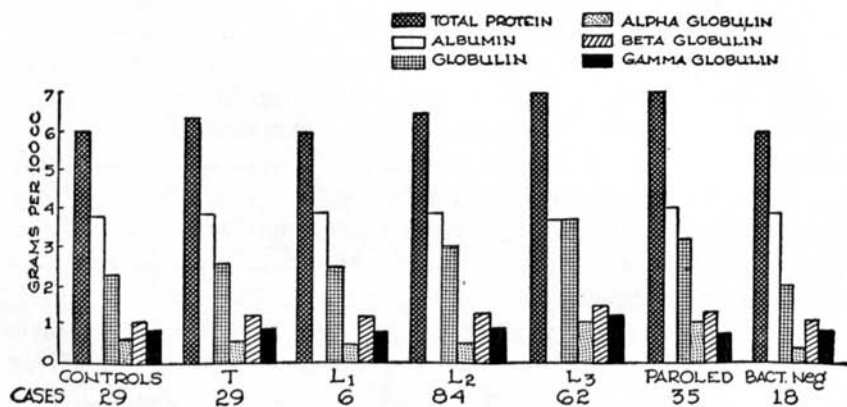
The work reported here involved 234 cases, varied as regards the type and duration of the disease and its stages of progression. Since the pattern of the alpha and gamma globulins changes with the progress of tuberculosis (7), we have excluded leprosy patients with pulmonary tuberculosis, whether active or arrested. Normal control specimens obtained from 29 employees of this institution were examined.

ANALYTICAL METHODS

Approximately 15 cc. of fasting blood was drawn by vein puncture and centrifuged after clotting, the serum being collected and analyzed on the same day. The total serum proteins, albumin, total globulin, and alpha, beta and gamma globulins were determined by the method of Wolfson and co-workers. The various protein fractions were standardized by the micro-Kjeldahl method. The Klett-Summerson photoelectric colorimeter was used for the readings. The thymol turbidity test and the cephalin-cholesterol flocculation test are described in a previous report (6).

RESULTS

The average findings of the total protein, albumin, total globulin, and the alpha, beta and gamma globulin fractions in



TEXT-FIG. 1. Serum protein fractions in 234 cases of leprosy of various types and stages of progression. T = tuberculoid; L1 = early lepromatous; L2 = moderately advanced lepromatous; L3 = far advanced lepromatous.

the various types and stages of leprosy are shown in Text-fig. 1. The figures for globulin fractions differ from those of Wolfson in that, in our work, the alpha and gamma fractions are lower and the beta fraction higher. Our figures were determined in 29 normal individuals, while the averages of those of Wolfson were obtained from four samples of pooled serum, each pool containing serum from between 30 and 150 persons.

In the 29 tuberculoid cases, the average value for the beta globulin was slightly higher than normal. There was an increase in the alpha and gamma fractions in 3 of the cases, and in 2 of them increase in the beta fraction.

In the 6 early lepromatous cases, the average for the alpha globulin was slightly lower than normal, while the averages for the beta and gamma fractions were slightly above normal. There was an increase in the beta fraction in 2 cases, and in 1 the gamma fraction was increased.

In the 84 moderately advanced lepromatous cases, the averages for the alpha, beta and gamma globulin fractions were higher than those of our controls. Of these cases, 7 showed an increase in the alpha globulin, 9 in the beta globulin and 25 in the gamma globulin fraction.

In the 62 far advanced lepromatous cases, the averages for the three globulin fractions were not within the normal limits. Of these cases, the alpha globulin was increased in 14, the beta fraction in 14, and the gamma fraction in 30.

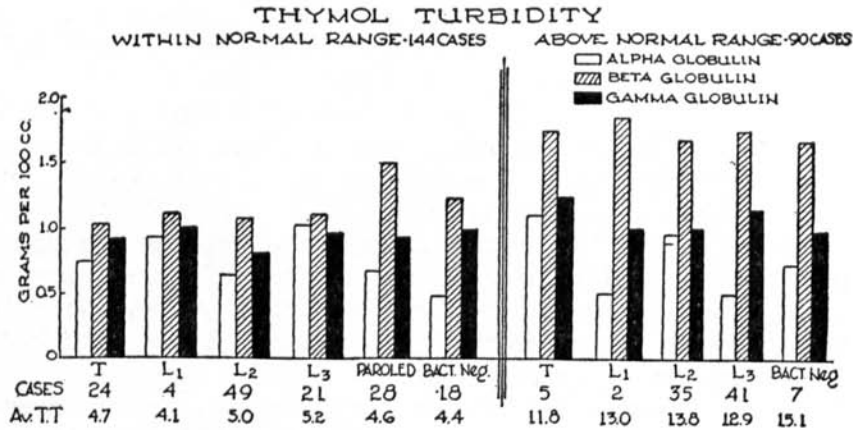
In the 35 paroled cases, the averages for the globulin fractions were also higher than those of the controls. There was an increase in the alpha globulin in 3 of the cases, an increase in the beta fraction in 1, while in 3 the gamma fraction showed an increase.

In the 18 cases which had been bacteriologically negative for a period of six months, the averages for the globulin fractions were within the normal range, although 1 case showed a slight increase in the gamma fraction.

Elevation of serum globulin fractions seemed on the one hand to be more marked in the moderately and far advanced lepromatous cases, and on the other hand they were less abnormal in the early lepromatous and the tuberculoid cases. Although the results varied greatly in different patients, there was more regularly an increase in the gamma globulin than in either the alpha or beta fractions.

Correlation of the results of the thymol turbidity test with the globulin fractions is shown in Text-fig. 2. Distinction is

made there between the 144 cases in which the readings of the turbidity test were within the normal range of 8 units, and the 90 cases in which the values were above that limit.



TEXT-FIG. 2. Correlation of the serum globulin fractions with the results of the thymol turbidity test. Symbols as in Text-fig. 1.

Of the 144 cases with normal thymol turbidity, 143 showed beta globulin values within the normal range; 1 tuberculoid case, only, showed an increase. The alpha globulin fraction was found to be normal in 137 of these cases; increases were noted in 3 tuberculoid and in 4 far advanced lepromatous cases. The gamma globulin fraction was within the normal range in 134 cases; increases were noted in 2 tuberculoid, 1 early lepromatous, 3 moderately advanced lepromatous, 2 far advanced lepromatous, 1 paroled and 1 bacteriologically negative case.

Of the 90 cases in which the results of the thymol turbidity test were above the normal range, 12 showed an increase in the alpha globulin, 27 in the beta fraction, and 51 in the gamma globulin fraction. The greater numbers of abnormal findings were noted in the moderately and far advanced lepromatous cases. The findings are shown in Table 1.

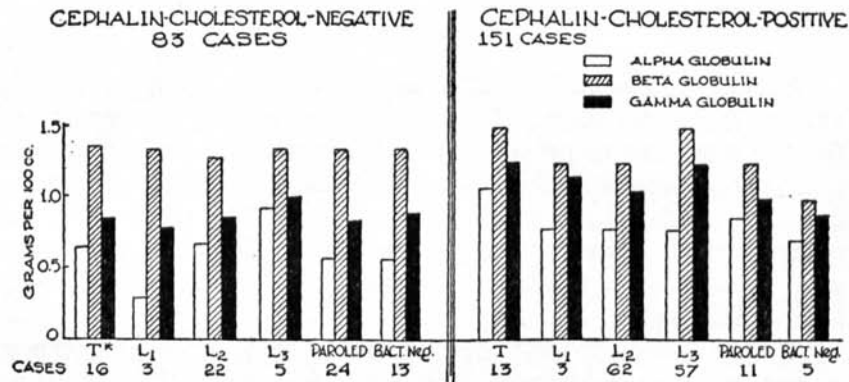
The significant observation seems to be an increase in the gamma globulin fraction rather than in the beta fraction, although there is no definite correlation between the gamma globulin increase and the thymol turbidity readings. Although the gamma globulin fraction is more consistently elevated, it appears that increases in both the beta and gamma globulins are part of the thymol turbidity reaction in leprosy.

Correlation of the results of the cephalin-cholesterol flocculation test with the globulin fractions is shown in Text-fig. 3.

TABLE 1.—Abnormal globulin findings in 90 cases with thymol turbidity readings above normal; cases with high globulin fractions.

Case group	Globulin		
	Alpha	Beta	Gamma
Tuberculoid	2	2	1
Early lepromatous	0	2	0
Moderate lepromatous	7	9	19
Advanced lepromatous	0	13	28
Paroled	3	1	3
Bacteriologically negative	0	0	0
TOTAL	12	27	51

Distinction is made between the 83 cases in which the flocculation reaction was negative and the 151 in which it was positive.



TEXT-FIG. 3. Correlation of the serum globulin fractions with the results of the cephalin-cholesterol flocculation test. Symbols as in Text-fig. 1.

Of the 83 flocculation-negative cases, the alpha globulins were increased in 4, the beta in 3, while the gamma globulins were negative in all.

Of the 151 flocculation-positive cases 23 showed an increase in the alpha fraction, 25 in the beta fraction, and 63 in the gamma fraction. The greater numbers of abnormal findings were noted in the moderately and far advanced lepromatous cases, as is to be seen in Table 2.

Comparison of the values for the beta and gamma globulin fractions of patients who had positive cephalin-cholesterol flocculation

TABLE 2.—*Abnormal globulin findings in 151 cases with positive cephalin-cholesterol flocculation reactions; cases with high globulin fractions.*

Case group	Globulin		
	Alpha	Beta	Gamma
Tuberculoid	3	1	3
Early lepromatous	0	2	1
Moderate lepromatous	5	7	25
Advanced lepromatous	14	14	30
Paroled	1	1	3
Bacteriologically negative	0	0	1
TOTAL	23	25	63

culatation reactions leads to a conclusion similar to that of the analysis of those cases in which the thymol turbidity reaction was found to be high.

SUMMARY

Serum specimens from 234 cases of leprosy, representing the various types and stages of activity of the disease, were examined for the total serum protein, albumin, total globulin, alpha, beta and gamma globulin fractions. Specimens from 29 employees used as controls were similarly examined. The findings in the patients with respect to the globulin fractions are correlated with the results of the thymol turbidity and cephalin-cholesterol flocculation tests.

Of the 234 cases, 118, or 50.4 per cent, showed an increase in one or more of the serum globulin fractions; 27 showed an increase in the alpha globulin, 28 in the beta globulin, 63 in the gamma globulin fraction.

Thymol turbidity units were increased in 90, or 38.5 per cent, of the 234 cases. Of these 90 cases, 12 showed an increase in the alpha globulin, 27 in the beta globulin, and 51 in the gamma globulin fraction. Of the 144 cases with normal thymol turbidity units, 7 showed an increase in the alpha globulin, one in the beta globulin, and 10 in the gamma globulin fraction.

Positive cephalin-cholesterol flocculation tests were noted in 151, or 64.5 per cent, of the 234 cases. Of these 151 cases, 23 showed an increase in the alpha globulin, 25 in the beta globulin, and 63 in the gamma globulin fraction. Of the 83 cases with negative cephalin-cholesterol flocculation tests, 4 showed an in-

crease in the alpha and 3 in the beta globulin fractions; no increases were noted in the gamma fraction.

Elevation of the serum globulin fractions was found to be more frequent in the moderately and far advanced lepromatous cases. Although the results varied greatly in different patients, there was more regularly an increase in gamma globulin than in either the alpha or beta fractions.

RESÚMEN

Se determinó, en 234 casos de lepra representativos de varios tipos clínicos y diferentes estados en el desarrollo de la enfermedad, la proteína total del suero sanguíneo, la albúmina, la globulina total y las fracciones alfa, beta y gama. Muestras de 29 sujetos no-leprosos sirvieron de control y fueron examinadas en forma idéntica. Los hallazgos con respecto a las fracciones de globulina fueron correlacionados con los resultados de las pruebas de turbidez al timol y la de la flocculación de la cefalina colesterol.

De los 234 casos, 118 (50.4%) demostraron un aumento en una o más de las fracciones de la globulina; 27 demostraron aumento en la fracción alfa, 28 en la beta y 63 en la gama.

La prueba de turbidez al timol se observó aumentada en 90 (38.5%) de los 234 casos. De éstos 90, 12 demostraron aumento en globulina alfa, 27 en globulina beta y 51 en globulina gama. De los 144 casos con valores normales a la prueba del timol, 7 tuvieron aumento en globulina alfa, 1 en globulina beta, y 10 en globulina gama.

La prueba de flocculación a la cefalina colesterol fué positiva en 151 (64.5%) de los 234 casos. De estos 151, 23 tuvieron la globulina alfa aumentada, 25 la beta, y 63 la gama. De los 83 casos negativos a la cefalina colesterol, 4 tuvieron la globulina alfa aumentada y 3 la beta, no hubo aumento en la fracción gama.

Se observó que el aumento en las fracciones de la globulina del suero fué más frecuente en casos del tipo lepromatoso moderadamente o muy avanzados. Aunque los resultados variaron considerablemente en distintos pacientes, se notó que la fracción gama estuvo más regularmente elevada que las alfa o beta.

REFERENCES

1. BAILER, M. M., ESKER, E. and SPIES, T. Serum proteins in hypoproteinemia due to nutritional deficiency. *J. Lab. & Clin. Med.* **32** (1947) 130-138.

2. COHEN, P. P. and THOMPSON, F. L. Mechanism of the thymol turbidity test. *J. Lab. & Clin. Med.* **32** (1947) 475-480.
3. [EDITORIAL] Electrophoresis and protein fractionation. *J. American Med. Assoc.* **136** (1948) 832.
4. RECANI, L., CHARGAFF, E. and HANGER, F. Comparison of the cephalin-cholesterol flocculation with thymol turbidity test. *Proc. Soc. Exper. Biol. & Med.* **60** (1945) 245-247.
5. ROSS, SISTER HILARY. Euglobulin in leprosy. *Internat. J. Leprosy* **11** (1943) 23-26.
6. ROSS, SISTER HILARY. The thymol turbidity and cephalin-cholesterol tests, euglobulin, albumin-globulin ratio and serology in leprosy. *Internat. J. Leprosy* **17** (1949) 237-245.
7. SEIBERT, F., NELSON, W. J. and SEIBERT, M. Correlation of extent of tuberculosis with amount of polysaccharide in the serum. *Proc. Soc. Exper. Biol. & Med.* **52** (1943) 219-222.
8. WOLFSON, W. Q., COHEN, C., CALVARY, E. and ICHIBA, F. Studies in serum proteins; a rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin in 1.0 ml. of serum. *American J. Clin. Path.* **18** (1948), Technical Section, 723-730.
9. WOOLEY, J. G. and ROSS, SISTER HILARY. Calcium, phosphorous and protein metabolism in leprosy. *Publ. Hlth. Rep.* **47** (1932) 380-389.