SERUM PROTEIN PATTERNS IN LEPROSY, I
CARVILLE SURVEY[1]

RUDOLPH J. MUELLING, JR., M.D.
Louisiana State University School of Medicine
New Orleans, La.

CATHERINE GOETZ, M.D.[2]
Charity Hospital of Louisiana
New Orleans, La.

AND SISTER HILARY ROSS
U. S. Public Health Service Hospital
Carville, La.

One hundred and sixty samples of serum from leprosy patients at the Public Health Service Hospital (National Leprosarium) at Carville, Louisiana, have been subjected to chemical and paper-electrophoretic analysis of the proteins. These form the basis of a survey. No historical review of protein analysis by these methods will be given, as there are many in the literature. Of the 160 patients, 143 were lepromatous and 17 tuberculoid. Clinically the patients were grouped as follows:[3]

Lepromatous		
Active	98	
Inactive	30	
With amyloidosis	15	143
Tuberculoid		
Active	6	
Inactive	11	17
Total		160

TECHNIQUES

Whole blood was collected and allowed to clot. The serum was separated at 37°C in order not to miss a cryoglobulin which might be present, as this would cause a lower total protein value.

CHEMICAL FRACTIONATION

The total protein determination was done by the biuret method, using Ellerbrook's modification of the Weichselbaum ([12]) biuret reagent.[4] The fractionations were accomplished by means of the Wolfson-Cohn ([13]) technique, also modified by Ellerbrook.[4]

[1] Presented to the International Academy of Pathology, Houston, Texas, April 1955. This work was supported in part by Grant E-1120 from the Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

[2] Present address: Natchez General Hospital, Natchez, Mississippi.

[3] The authors use the Carville designations, "active" meaning bacteriologically positive and "inactive" meaning bacteriologically negative, regardless of the status of the case in other respects.—EDITOR.

Because of the high values encountered it was necessary to modify some of the volume measurements. The fractions have been checked electrophoretically and run around 96 per cent pure. The exact procedure ("water" always meaning distilled water) is as follows:

Reagents.—1. Rochelle salt solution: Dissolve 180 gm. NaOH in 500-700 cc. water. Dissolve 112 gm. potassium sodium tartrate in 500-700 cc. water. Combine these two solutions by pouring into a 2-liter volumetric flask. Make to volume with water.

2. Copper sulfate solution: Dissolve 15 gm. $CuSO_4 5H_2O$ in water and dilute in 100 cc.

3. Potassium iodide solution: Dissolve 5 gm. in water and dilute to 100 cc. Store in a brown bottle in the dark.

4. Biuret reagent: To 8 volumes of the Rochelle salt solution add 1 volume of the $CuSO_4$ solution. This will make a white, curd-like material on first mixing; shake until the solution is clear. The absolute amounts of these solutions in the reagent is not critical, so we use a glass-stoppered 100 cc. graduated cylinder to mix this solution. We make only enough for a two-day period; if the reagent is to be stored longer, use a paraffined bottle. In either case the reagent should be kept in the refrigerator.

5. Standard albumin solution: Dilute 25% purified human albumin (type used for intravenous therapy) with normal saline to give approximately a 4 gm./% solution. Determine the exact protein concentration by the Kjeldahl method, running at least 6 to 8 determinations. Store this standard in small rubber-stoppered vials in the deep freezer, and take out one vial at a time for use. The vial being used should be kept in the refrigerator. Bacterial contamination may reduce the protein content, and this should be avoided.

6. Sodium sulfate, 23%: Dissolve 230 gm. reagent-grade Na_2SO_4 in water at 37°C. Dilute to one liter and store in 37°C incubator or water bath.

7. Sodium sulfite, 28%: Dissolve 280 gm. reagent-grade Na_2SO_3 in water at 28°C. Dilute to one liter. Store at room temperature.

8. Saline ammonium sulfate: In a one-liter volumetric flask dissolve 193 gm. reagent-grade $(NH_4)_2SO_4$ in about 500 cc. water. Add 40 gm. NaCl, dissolve, dilute to one liter. Store at room temperature.

9. Ether: USP.

10. Span-Ether reagent: Mix one cc. of Span 20 (Atlas Powder Co., Wilmington, Del.) with 99 cc. ether. Filter through a moderately fast filter paper into a 100-cc. graduated cylinder. Make up to 100 cc. with ether. Store in a tightly corked bottle.

Procedure for total protein.—1. Place 0.2 cc. serum in a cuvet.

2. Add exactly 4.8 cc. water.

3. Add 5 cc. biuret reagent and mix.

4. Blank: 5 cc. biuret and 5 cc. water.

5. Standard: Prepare using 0.2 cc. standard albumin solution, adding water and biuret as in the unknown.

6. After 30 minutes read in the spectrophotometer at 550 mu after setting blank to 0. If turbidity develops in the unknown, add about 2 cc. of ether to the blank, unknown, and standard, and shake vigorously for 30 seconds; centrifuge and read.

The optical density is essentially proportional to the protein concentration over the usually-encountered range.

$$\text{Gm./\% protein} = \frac{\text{OD unknown}}{\text{OD standard}} \times \text{concentration of standard in gm./\%}$$

Serum albumin plus alpha globulin (Howe albumin).—1. To 4.60 cc. 23% Na_2SO_4 in a centrifuge tube add 0.4 cc. serum; mix.

2. Add 2-3 cc. ether and shake vigorously for 30 seconds. We use rubber stoppers for this step. Be careful to release the ether pressure every now and then when shaking, without letting the ether blow the contents from the tube.

3. Centrifuge at 1500-2000 rpm for 10-15 minutes. The globulin layer will be on top of the sodium sulfate solution.

---

[4] The modifications of technique referred to, by L. D. Ellerbrook, are to be found in a laboratory syllabus used in the postgraduate course in chemical pathology, University of Washington School of Medicine, Seattle, Washington.

4. Carefully tilt the tube to dislodge the globulin from the wall of the tube. Cautiously insert pipette through the other layer along the side of the tube. Place finger tightly on top of pipette to prevent ether from getting into pipette. Withdraw about 3 cc. of the sodium sulfate solution and transfer to a clean test tube. Transfer 2.5 cc. to a cuvet.

5. Add 2.5 cc. water and 5 cc. biuret reagent; mix.

6. Blank: 2.5 cc. sodium sulfate and 2.5 cc. water; mix. Add 5 cc. biuret reagent; mix.

7. Standard: 0.2 cc. standard albumin solution plus 2.5 cc. water; mix. Add 2.3 cc. sodium sulfate; mix. Add 5 cc. reagent; mix.

8. After 30 minutes read as in total protein.

Serum albumin.—1. Place 9.6 cc. of 28% sodium sulfite in a centrifuge tube.

2. Pipet 0.4 cc. serum into the sulfite, and mix well by inversion using a clean, dry, rubber stopper.

3. Add about 2-3 cc. span-ether, stopper, and invert gently 10 times.

4. Centrifuge 10-15 minutes at 1500-2000 rpm.

5. Carefully remove 5 cc. of the clear solution and place in a cuvet.

6. Add 5 cc. biuret; mix well.

7. Blank: 4.8 cc. sodium sulfite and 0.2 cc. water; mix. Add 5 cc. biuret reagent; mix.

8. Standard: 4.8 cc. sodium sulfite and 0.2 cc. standard albumin; mix and add 5 cc. biuret.

9. After 30 minutes read as above.

Serum alpha globulin.—Serum alpha globulin = serum albumin plus alpha globulin minus serum albumin.

Serum beta plus gamma globulin.—Beta plus gamma = total protein minus albumin plus alpha.

Serum globulin.—Total protein minus albumin.

Serum gamma globulin.—1. Pipet 9.6 cc. saline ammonium sulfate into 15 cc. centrifuge tube. Layer 0.4 cc. serum carefully on top. Stopper with a clean, dry, rubber stopper and mix carefully by slow repeated inversions until, within a minute or so, the visible turbidity has reached an apparent maximum—about 30 inversions.

2. Remove 1 cc. of the mixture and discard.

3. Stopper the tube and centrifuge at 2250-2750 rpm for thirty minutes. If the supernate is still cloudy, cool the tube in cold water for a few minutes and centrifuge again. The supernate must be clear.

4. Taking care not to disturb the precipitate, gently decant most of the supernate and centrifuge the unstoppered tube again at the same speed for about five minutes.

5. Slowly invert the tube on paper toweling and let stand for a few minutes.

6. Add 5 cc. biuret reagent. Mix and get all of the precipitant into solution. The best way to do this is to hit the end of the tube on the hand while shaking laterally. If all does not go into solution, add one drop of concentrated NaOH and add a drop to the standard and one to the blank.

7. Add 5 cc. water. Let stand 15 minutes, centrifuge down any slight turbidity, and decant the supernate into a cuvet.

8. Blank and standard: The same as for total protein.

9. Let stand thirty minutes and read.

10. Divide the calculated protein concentration by 1.8 to obtain the gamma globulin.

Serum beta globulin.—Serum-beta-plus-gamma minus gamma.

PAPER ELECTROPHORESIS

Paper electrophoresis was performed on Whatman #1 chromatography paper, 1 inch wide and 12 inches long. Our electrophoresis cabinet was made in our department and accommodates 20 one-inch strips. A barbital buffer, pH 8.6, is used in a concentration of 0.05 ionic strength. The strips are wetted with buffer, blotted with a paper towel and placed in the cabinet. The current is allowed to run for 30 minutes to equilibrate. Voltage is adjusted to give a milliamperage reading between 0.7-1 mu. per strip. The strips are then loaded with 10 lambda of serum in a linear fashion with a Kirk micro pipette. Electrophoresis proceeds for a period of four hours. The strips are removed and dried in a hot air oven at 60°C. Strips for the study of total protein are stained with amidoschwarz, lipid-bound protein with oil red 0 ([3]), and carbohydrate-bound protein

with the periodic acid Schiff reaction (PAS) ([6]). After drying, the strips are oiled and are read in a gamma chromatophore densitometer. A graph is obtained by plotting the optical density against the millimeters of migration from the loading point. The procedures are as follows:

Buffer.—Stock barbitol buffer, pH 8.6, ionic strength 0.1, 0.1 M: 20.62 gm. sodium barbituric acid; 2.49 gm. barbituric acid. Dilute to one liter with water. The solution may have to stand for a day or so before all of the acid barbiturate goes into solution. This buffer is diluted one-half, giving an ionic strength of 0.05.

Amidoschwarz stain for total protein.—1. Stain the dried strip 10 minutes in saturated solution of amidoschwarz 10B in absolute methyl alcohol containing 10% acetic acid.

2. Use successive washes of methyl alcohol containing 10% acetic acid until the background of the strip is pale blue. Leave the strips in the washes about 30-45 minutes. Gentle agitation during destaining hastens the process and saves wash solution.

3. Air-dry flat on paper toweling or glass plate.

4. Oil with white mineral oil (USP) and read without a filter.

Oil red O stain for lipid detection.—1. Stain the dried strip 16 hours in 60% ethanol saturated with oil red O.

2. Rinse away excess stain in tap water, blot with a paper towel and dry.

3. Oil with glycerin (Merck Reagent) and read with a green filter. (Mineral oil would take up some of the dye and so is unsatisfactory.)

PAS stain for mucopolysaccharide components.—1. Fix the dried strip in absolute ethyl alcohol.

2. Periodic acid solution, 5 minutes.
3. Rinse in 70% alcohol.
4. Reducing solution, 5 minutes.
5. Rinse in 70% alcohol.
6. Fuchsin-sulfite solution, 25 minutes.
7. Wash three times in sulfite rinse.
8. Wash 10 minutes in running tap water.
9. Dehydrate in ethanol and dry in air on a glass plate.
10. Oil with mineral oil and read with a green filter. The PAS strips must be read the same day they are stained, as the color deepens with time and the background color is usually quite intense on the following day.

(N. B.: Each new lot of mineral oil and glycerin should be tested on a standard strip, as some lots of these reagents may take up stain and prove unsatisfactory.)

Periodic acid solution: Dissolve 1.2 gm. periodic acid in 30 cc. water. Add 15 cc. of 1/5 M sodium acetate and 100 cc. ethyl alcohol. This solution will keep for several days in the dark.

Reducing solution: Dissolve 5 gm. potassium iodide and 5 gm. sodium thiosulfate in 100 cc. water. Add with stirring 150 cc. ethyl alcohol and then 2.5 cc. 2N/HCl. The solution is ready for use immediately.

Fuchsin sulfite solution: Dissolve 2 gm. basic fuchsin in 400 cc. boiling water. Cool to 50°C and filter. To the filtrate add 10 cc. 2N/HCl and 4 gm. potassium metabisulfite. Stopper and let stand in a cool, dark place overnight. Add 1 gm. decolorizing charcoal, mix and filter immediately. Add 10 cc. or more 2N/HCl in small amounts until, after the last addition, the mixture does not become violet-red when drying spontaneously on a glass slide. The slide for testing the fuchsin sulfite should be placed on white paper, as the change is delicate. Keep well stoppered, in the dark, in the refrigerator.

Sulfite rinse water: To 100 cc. water add 1 cc. concentrated HCl and 0.4 gm. potassium metabisulfite. Store in the dark.

Because of the different filters used in obtaining the density readings, the total protein, lipoprotein and mucopolysaccharide values are not directly comparable with each other. In some cases one of the latter may exceed the total protein values. However, each of the protein moities may be compared on different patients.

RESULTS

CHEMICAL FRACTIONATION

The results of the chemical fractionation of the total group of 160 sera studied are summarized in Table 1.

TABLE 1.—*Chemical protein fractionation values in the sera of 160 leprosy cases, 143 lepromatous and 17 tuberculoid, in gram/per cent.*

	Patients		Normal	
	Range	Mean	Range[a]	Mean[b]
Total protein	4.1-11.8	8.84	5.85-8.09	6.97
Albumin	1.2- 5.5	3.39	3.28-4.88	4.08
Total globulin	2.3- 9.3	5.33	1.58-4.18	2.88
Alpha globulin	0.3- 3.1	1.15	0.12-1.42	0.77
Beta globulin	0.5- 4.5	1.90	0.39-1.83	1.11
Gamma globulin	0.6- 5.0	2.28	0.34-1.68	1.01

[a]Mean, ±3 S.D.[4]
[b]From Ellerbrook ([4]).

RESULTS

ELECTROPHORETIC FRACTIONATION

Lepromatous, active (*98 cases*).—The albumin may be normal to slightly decreased and the globulin is usually increased (Fig. 1b, c, d, and Fig. 2a, b). The globulin increase may be a total increase of all of the globulin moities, or may be of a single fraction, or of any combination of fractions. The most common occurrence is an elevation in the gamma region (Fig. 1b and Fig. 2a, b). The next most common is a beta-gamma increase, and then a total elevation with predominant gamma (Fig. 1c). Other combinations occur in smaller numbers; predominant beta (Fig. 1d).

The lipoprotein is usually not remarkable. However, the curves Fig. 1c and Fig. 2a represent increase of this fraction.

Of this group of patients, 45 gave a normal mucopolysaccharide pattern (Fig. 1a, c, and Fig. 2b), while 53 had mucopolysaccharide elevation and abnormal patterns. This elevation is usually not the generalized increase seen in chronic infections, but presents one or several peaks. These may be quite high, traveling within abnormal protein components or with the normal components. In Fig. 1b there is a sharp peak traveling with the slower gamma globulin. A moderate peak in beta is seen in Fig. 1d, concomitant with the abnormal beta globulin rise. Two well-defined peaks are present in Fig. 2a, one moving with the most rapid components of the albumin and the other in the alpha$_2$ globulin.

Lepromatous, inactive (*30 cases*).—This group proved to be a very interesting one, as only 4 cases out of 30 had normal curves. The aberration is within the total protein pattern. The chemical value also was increased. The same general features of the patterns of the first group persisted (Fig. 2d and Fig. 3a). The lipoprotein was not remarkable, but the mucopolysaccharide was peaked in two instances. One of these is shown in Fig. 2d. This entire curve is very abnormal.

Lepromatous with amyloidosis (*15 cases*).—The total protein may be elevated or depressed, depending on the level of albumin (Fig. 3b, c, d). The level of albumin would appear to be related to the amount

lost in the urine as the amyloidosis progresses. The globulin is usually elevated. There are more single-fraction elevations than double-fractions, and if there is a general increase it is overwhelming (Fig. 3b). In several cases there was identified a protein fraction more rapid than

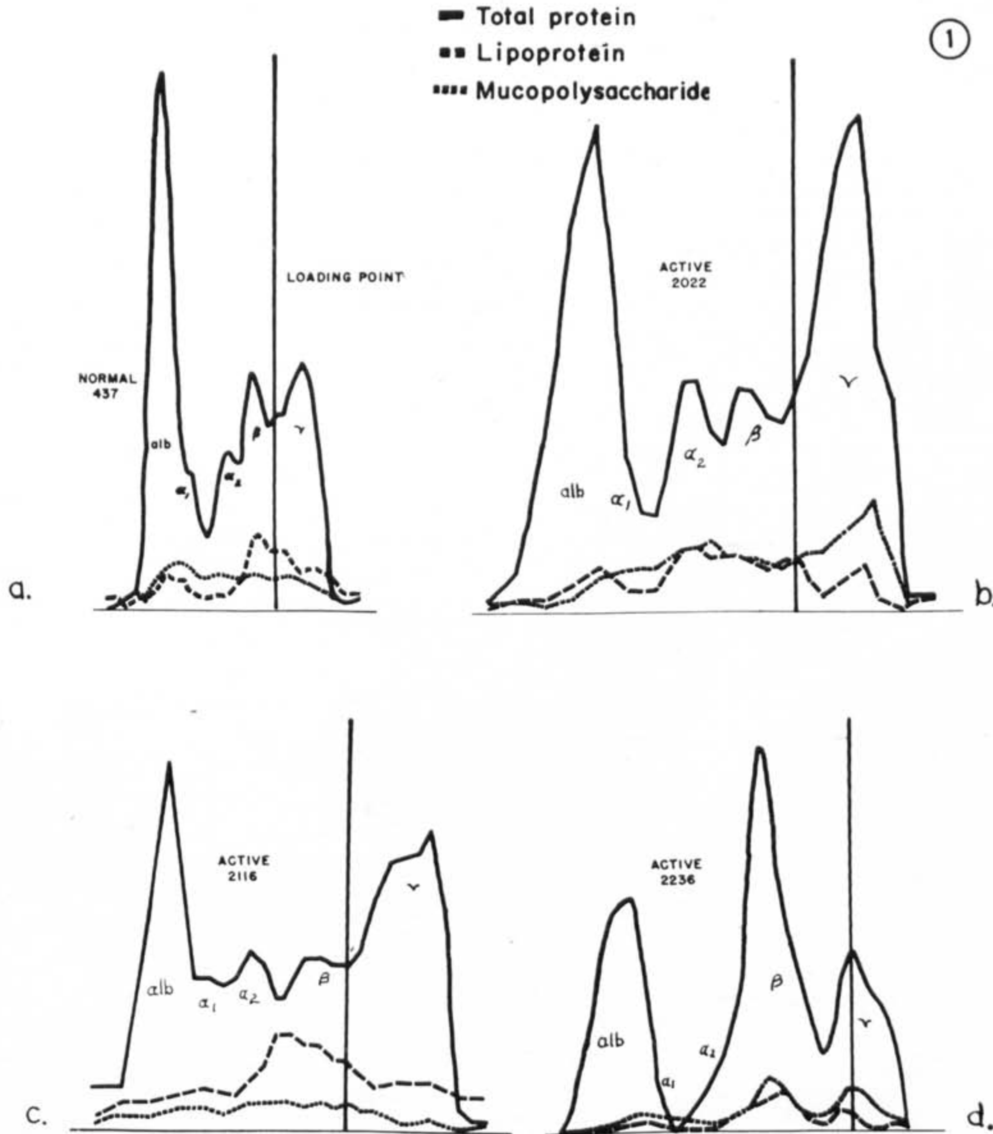

FIG. 1. (*a*) Normal. This whole curve appears somewhat compressed in comparison with the other curves, a discrepancy due to a difference in the time of the electrophoresis run. Notice the normal mucopolysaccharide and lipoprotein levels. (*b*) Active lepromatous case. Massive increase of gamma globulin with a single-spike increase in the gamma mucopolysaccharide. (*c*) Active lepromatous case. Sharp albumin peak with generalized globulin elevation. The gamma fraction is most greatly affected. A lipoprotein peak is seen between $alpha_2$ and beta. (*d*) Active lepromatous case. Very distorted curve. Protein increase is mainly beta. Increase of beta mucopolysaccharide.

albumin and named "rho" for rapid. As the albumin disappears in the urine, curve reversal occurs (Fig. 3b, c) as the serum albumin decreases and the already-increased globulin becomes relatively more important. The lipoprotein is usually increased in the beta region. This is apparently due to the nephrosis, as the same thing occurs in the nephrotic stage of glomerulonephritis and lipoid nephrosis ([9]). There is an aberration in the mucopolysaccharide pattern. In many cases this aberration is peaked (Fig. 3b, c). This peak may occur anywhere, as in the pattern of the active (bacteriologically-positive) patients. There is no constant anomaly. Upon occasion, a generalized type of elevation is seen (Fig. 3d).

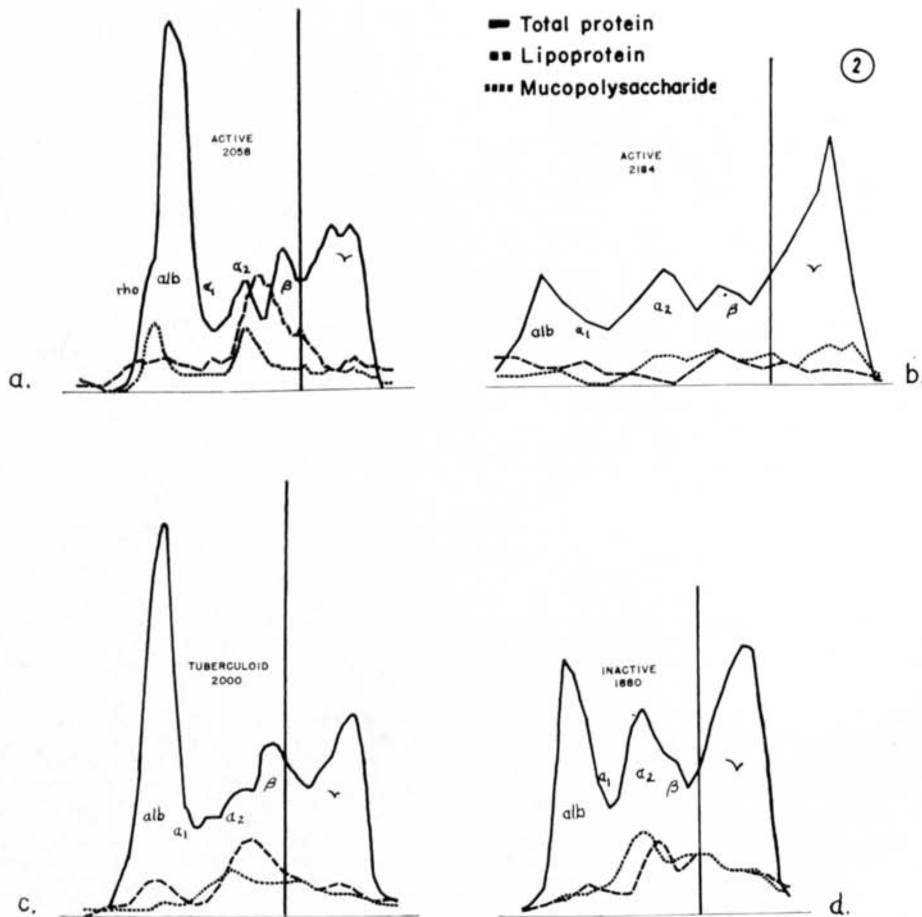

FIG. 2. (*a*) Active lepromatous case. Pronounced "rho" (prealbuminoid) fraction. Slight gamma increase with marked lipoprotein increase between alpha$_2$ and beta. Mucopolysaccharide peaks are present in rho and alpha$_2$. (*b*) Active lepromatous case. This shows complete curve reversal because of decrease in albumin and increase in gamma. (*c*) Tuberculoid case. Increase in beta and slight increase of alpha$_2$. The lipoprotein is an exaggeration of the normal curve. (*d*) Inactive lepromatous case. Markedly abnormal curve. Gamma increase with inseparability of alpha$_2$ and beta. Spiking increase of mucopolysaccharide in this latter area.

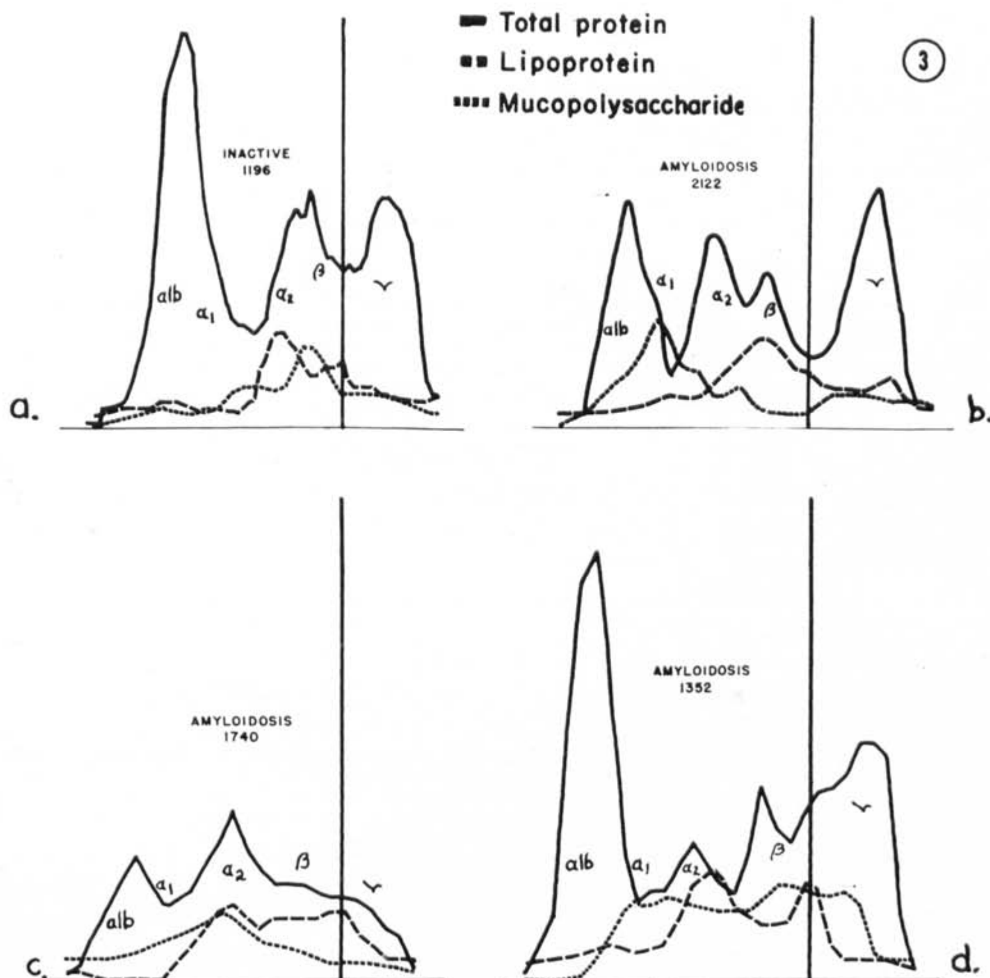

FIG. 3. (*a*) Inactive lepromatous case. $Alpha_2$ and beta inseparability, with $alpha_2$ mucopolysaccharide and beta lipoprotein elevations. (*b*) Lepromatous case with amyloidosis. Decrease in albumin with generalized globulin increase. Elevation of $alpha_1$ mucopolysaccharide and beta lipoprotein. (*c*) Lepromatous case with amyloidosis. Very abnormal curve. Decrease in albumin, beta and gamma globulin with elevation of $alpha_2$ globulin. Increase of $alpha_2$ mucopolysaccharide with generalized $alpha_2$ and beta lipoprotein. (*d*) Lepromatous case with amyloidosis. Relatively normal protein curve with generalized mucopolysaccharide increase and $alpha_2$ lipoprotein increase.

Tuberculoid, active and inactive (*17 cases*).—The albumin was normal in these cases. The total protein varied from 7.3-10.4 gm/per cent. The beta globulin is usually elevated over the gamma (Fig. 2c); an alpha elevation is rare. The mucopolysaccharide is usually not elevated; only 4 cases showed a mild elevation. The type of elevation was generalized, without sharp peaks. The lipoprotein was usually elevated, probably because of the elevation of the beta globulin.

DISCUSSION

The results of the chemical studies on total protein and globulin parallel the findings of one of us [Sr. H. R. ([10])] in 1943, in 150 Carville cases. There is some difference in the albumin findings, as the Howe albumin includes most of the alpha globulin.[4] Other reports available in the literature are difficult to compare because of the use of different techniques and methods of reporting results. Chakravarti's ([2]) total protein values for 47 patients are somewhat below ours, and he presents no very low albumin values. Ishihara's ([5]) study of 85 patients with the Wolfson-Cohn fractionation and biuret determination of the protein gives no low albumin figures. The beta-globulin figures are decidedly low in comparison with ours, the gamma-globulins also appear to be low.

There are not many reports of electrophoresis studies on leprosy. Seibert and Nelson ([11]) presented 3 cases of advanced leprosy studied with the Tiselius apparatus. They concluded that the total proteins were high normal, the albumin was markedly decreased, and the alpha and gamma globulin fractions were markedly increased. This is not always the case, as the albumin may be normal to decreased. Exceptionally low values for albumin are usually related to amyloid disease of the kidneys. The alpha globulin may or may not be increased. Ishihara ([5]) studied 21 cases with the Tiselius apparatus and concluded that the alpha and beta fractions do not vary from normal in any form of this disease, and that the gamma globulin increase is alone responsible for the increase in total globulin. Electrophoretically we are able to show other increases, and on occasion these are at the expense of the gamma globulin. Arcuri and Inzerillo ([1]) ran 15 patients' sera on the Tiselius apparatus and decided that the beta globulins showed some increase and that the gamma was constantly increased.

Paper electrophoresis has been applied to the study of 45 leprosy serum by Miguel *et al.* ([8]), and on 100 sera by Mauze and Arnaud ([7]). The former authors concluded that when the gamma globulin was increased markedly, the alpha globulin was decreased. We agree with the latter authors that "electrophoresis is the best and most precise method of investigating sera." However, neither of these groups used differential staining of the electrophoresis strips to study the various components of the serum.

No absolutely diagnostic curve or pattern is seen in leprosy. We have not found the determination of lipoprotein to be particularly helpful. On the other hand, the mucopolysaccharide element may give an interesting insight into this disease. The mucopolysaccharide appears to be related to activity (bacteriologic positivity). This is a hypothesis which can only be checked by study of multiple specimens from the same patients. If this hypothesis should prove to be true, then the leprologist would have an additional aid in determining clinical

activity in cases in which the degree of activity is questionable. This determination might be utilized in the testing of antileprosy agents.

Elevations of the mucopolysaccharide must have some relationship to the metabolism of the leprosy bacillus. Whether this compound is produced by the body in an attempt to regulate the infection has yet to be determined. Hanks ([4]) has shown that lipoprotein and mucopolysaccharide are inhibitory to the metabolic activity of mycobacteria. Since mucopolysaccharides can occur in various fractions of the sera, it would be interesting to know if all the fractions of mucopolysaccharide are inhibitory, or if only certain fractions behave in this manner. The deposition of the mucopolysaccharide amyloid must be related to abnormal protein metabolism. This complication is a great problem at Carville and some other places, although in some countries (e.g., Central America) it is seen rarely in lepromatous cases. Studies are now under way in an attempt to elucidate this difference in behavior.

SUMMARY

1. The chemical and paper electrophoretic findings in the sera from 160 cases of leprosy at Carville are presented.

2. The paper electrophoresis strips were studied by differential means. The findings regarding lipid-bound protein were not remarkable. The mucopolysaccharide appears to be related to activity.

3. This pilot study is being followed by other studies to elucidate the problems that have been raised.

RESUMEN

1. Preséntanse los hallazgos electroforéticos químicos y en papel obtenidos en los sueros de 160 casos de lepra de la leprosería de Carville.

2. Se estudiaron con métodos diferenciales las tiras electroforéticas de papel. Los hallazgos relativos a la proteína lípidofija no fueron notables. El mucopolisacárido parece guardar relación con la actividad.

3. Este estudio explorador irá seguido de otros estudios para dilucidar los problemas planteados.

REFERENCES

1. Arcuri, F. and Inzerillo, R. La disprotidemia nella lebbra. Studio elettroforetico del siero nelle diverse forme cliniche di lebbra prima e dopo terapia con tiosemicarbazone. Acta Med. italica **7** (1952) 29-34.
2. Chakravarti, H. Studies on plasma protein. IV. Leprosy. Indian Med. Gaz. **86** (1951) 196-199.
3. Durrum, E. L., Paul, M. H. and Smith, E. R. B. Lipid detection in paper electrophoresis. Science **116** (1952) 428-430.
4. Hanks, J. H. and Gray, C. T. The application of metabolic studies to leprosy research. Internat. J. Leprosy **22** (1954) 147-161.
5. Ishihara, S. A study of the serum proteins in leprosy. Internat. J. Leprosy **21** (1953) 187-199.
6. Köiw, E. and Gronwall, A. Staining protein-bound carbohydrates after electrophoresis of serum on filter paper. Scandinavian J. Clin. & Lab. Invest. **4** (1952) 244-246.
7. Mauze, J. and Arnaud, G. L'electrophorese du serum de lépreux. Internat. J. Leprosy **22** (1954) 55-60.

8. MIGUEL, S., ROLDAN, A., GUILLEN, J., TERENCIO, J. and PONCIANI, J. Proteínas plasmáticas en la lepra. Internat. J. Leprosy **22** (1954) 47-54.
9. MUELLING, R. J., JR. Unpublished data.
10. ROSS, SR. HILARY. Euglobulin in leprosy. Internat. J. Leprosy **11** (1943) 23-26.
11. SEIBERT, F. B. and NELSON, J. W. Electrophoresis of serum; serum proteins in tuberculosis and other chronic diseases. American Rev. Tuberc. **47** (1943) 66-77.
12. WEICHSELBAUM, T. E. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. American J. Clin. Pathol., Tech. Sect. **10** (1946) 40-49.
13. WOLFSON, W. Q., COHN, C., CALVARY, E. and ICHIBA, F. Studies in serum proteins; rapid procedure for estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin, and gamma globulin in 1.0 ml. of serum. American J. Clin. Pathol. **18** (1948) 723-730.