

A STUDY OF THE SERUM PROTEINS IN LEPROSY

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INTRODUCTION

In cases of lepromatous leprosy, in which the viscera are involved, the sera frequently react strongly to the formol-gel, Takata and other tests, and the erythrocyte sedimentation rate is often high. Many reports have been published on the findings with such tests, but there have been fewer studies of the serum proteins and fractions. Here are reported the results of various examinations of that kind, made in the different forms of leprosy and in cases of erythema nodosum leprosum. Part of the work was done with patients in the Aisei-en National Leprosarium, at Nagashima, and part with patients in the Suruga National Leprosarium, at Shizuoka.

SPECIFIC GRAVITY AND ALBUMIN-GLOBULIN RATIO

On the basis of the specific gravity of the sera and correlation with their viscosity, the serum proteins were investigated in 50 cases, 32 of lepromatous leprosy and 18 of the neural type, at Nagashima.²

Method.—The specific gravity of the serum was first determined by the van Slyke copper sulfate method, and from the value so obtained the total protein was calculated by this formula: total protein = $389.6 \times (\text{specific gravity of serum} - 1.0079)$. The albumin-globulin ratio was then estimated by the method devised by Yoshikawa, which correlates the specific gravity with viscosity. This is a modification of the method devised by Rohrer *et al.*, in which the albumin-globulin ratio is estimated by correlating the refractive index and viscosity.

The results are shown in Table 1. No statistically significant difference was found in the concentration of the serum proteins in the lepromatous and neural forms, when estimated by the specific gravity method. There was, however, a conspicuous difference in the specific viscosity determinations, that

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² In his manuscript the author followed the Japanese custom of distinguishing "neural" and "macular" (or "tuberculoid macular") cases. To avoid confusion the usual term "tuberculoid" is applied to the latter kind. The general term "maculoneural," which frequently appears, is understood to include both of these forms, and it is therefore used in the plural sense.

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of the lepromatous cases being the higher. The albumin-globulin ratio, therefore, was over 1.0 (average 1.62) in the neural cases, but less than 1.0 (average 0.75) in the lepromatous cases, in which there was increase of globulin.

TABLE 1.—*Specific gravity, protein concentrations and albumin-globulin ratios of sera of 50 leprosy cases (Nagashima).*

Type of cases	No. of cases	Specific gravity	Total protein gm./dl.	Specific viscosity	Albumin p. c.	Globulin p. c.	Albumin-globulin ratio
Neural	18	1.027	7.44	1.77	62	38	1.62
Lepromatous	32	1.028	7.83	1.99	43	57	0.75

Note: Total serum protein = $389.6 \times (\text{specific gravity} - 1.0079)$.

CONCENTRATION AND FRACTIONS OF SERUM PROTEINS

In this work done at Shizuoka, the concentration of the total serum proteins was measured by refractometry. The protein fractions were determined by two methods: (a) colorimetry in connection with the Biuret reaction, and (b) Tiselius' electrophoretic method.

A. FRACTIONS BY THE BIURET METHOD

Method.—The Biuret method devised by Cohn and Wolfson was used, salting out by the Yoshikawa-Saito method with 28 per cent sodium sulfite.

(a) Three flasks, Nos. 1, 2 and 3, are set up containing 12 cc., 9 cc. and 7 cc., respectively, of 28 per cent sodium sulfite solution. Water is added to flasks 2 and 3 to bring the total volume to 12 cc. Serum, 0.5 cc., is then added to each flask. After vigorous shaking they are set aside for 30 minutes.

(b) Five flasks, marked A to E, are then set up. To A, 2 cc. of the suspension in No. 1 is transferred and 2 cc. of water is added. To C, 4 cc. of the suspension in No. 2 is transferred. The suspensions in the three original flasks are now filtered, and 4 cc. of the clear filtrate from No. 1 is transferred to B, and the same amount from Nos. 2 and 3 to D and E. To each of these five flasks is added 4 cc. of Biuret reagent,³ and they are set aside at 37°C. for 20 minutes.

(c) Colorimetric readings are made, and the protein fractions are calculated as follows:

$$\frac{\text{reading of A}}{\text{reading of B}} \times 50 = \% \text{ albumin} \dots\dots\dots (1)$$

³ The Biuret reagent used is as follows: sodium potassium tartrate, 9.0 gm.; cupric sulfate, 3.0 gm.; potassium iodide, 5.0 gm. These are dissolved in 0.2 N sodium hydroxide and the total volume is adjusted to exactly 1,000 cc.

$$\frac{\text{reading of C}}{\text{reading of D}} \times 100 = \% \text{ albumin} + \alpha \text{ globulin} \dots\dots\dots(2)$$

$$\frac{\text{reading of C}}{\text{reading of E}} \times 100 = \% \text{ albumin} + \alpha + \beta \text{ globulins} \dots\dots\dots(3)$$

$$(2) - (1) = \% \alpha \text{ globulin}$$

$$(3) - (2) = \% \beta \text{ globulin}$$

$$100 - (3) = \% \gamma \text{ globulin}$$

The percentages thus obtained were used to calculate the concentration of each fraction, on the basis of the estimations of total proteins obtained with the refractometer.

These determinations were made on the sera of 50 leprosy cases of various forms and degrees of advancement, and also on 25 lepromatous cases with the reactional condition called erythema nodosum leprosum. For controls, determinations were made on the sera of 10 healthy members of the staff of the leprosarium. The data are shown in Table 2, and graphically in Text-fig. 1, but those on the erythema nodosum leprosum cases will be considered later.

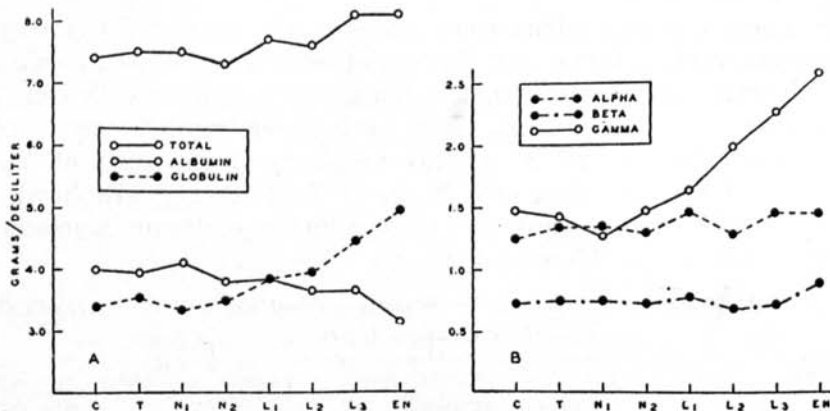
TABLE 2.—Total serum proteins and protein fractions (Biuret method) in 50 leprosy cases (Suruga).

Type and degree advancement	No. of cases	Total protein gm./dl.	Albumin p. c. and gm./dl.	Globulin			Albumin-globulin ratio
				Alpha p. c. and gm./dl.	Beta p. c. and gm./dl.	Gamma p. c. and gm./dl.	
Normal controls	10	7.4	54 (51-57) 4.00	17 (15-19) 1.25	10 (7-13) 0.74	19 (15-23) 1.41	1.17
Tuberculoid	3	7.5	53 (52-57) 3.98	18 (17-20) 1.35	10 (10-11) 0.75	19 (14-21) 1.42	1.12
Neural, N1	4	7.5	55 (52-57) 4.13	18 (15-22) 1.35	10 (7-13) 0.75	17 (13-22) 1.27	1.22
Neural, N2	8	7.3	52 (50-58) 3.80	18 (15-20) 1.31	10 (5-12) 0.73	20 (13-23) 1.46	1.08
Lepromatous, L1	6	7.7	50 (48-51) 3.85	19 (15-20) 1.46	10 (7-13) 0.77	21 (16-25) 1.62	1.00
Lepromatous, L2	14	7.6	48 (38-54) 3.65	17 (13-22) 1.29	9 (6-12) 0.68	26 (20-37) 1.97	0.92
Lepromatous, L3	15	8.1	45 (38-51) 3.65	18 (15-22) 1.45	9 (5-14) 0.72	28 (20-37) 2.27	0.81
E. N. L.	25	8.1	39 (22-54)† 3.15	18 (13-25) 1.45	11 (5-14) 0.89	31 (20-43) 2.59	0.64

Total proteins.—No essential differences with respect to the total proteins were found among the different patient groups, or departure from the control value, except for the advanced lepromatous cases. In them there was an increase.

Albumin.—Normal figures for the albumin fraction were obtained in the tuberculoid and neural groups, the average per-

centages (of the total protein) ⁴ varying from 52 to 55 (the individuals ranging from 50 to 58), as compared with 54 for the controls (individual range 51-57). In the nodular cases, however, the values decreased with advancement of the disease, from 50 per cent in the minimal cases, to 48 per cent in the moderately advanced ones and 45 per cent in the far advanced ones. There was some lessening of the actual concentration of albumin (grams/deciliter of serum) in the two more advanced lepromatous groups.



TEXT-FIG. 1. Concentrations of (A) total serum proteins, albumin and globulin, and of (B) the globulin fractions, in the different groups of leprosy cases, including erythema nodosum reaction cases (EN) and controls (C).

Total globulins.—The total globulin percentages, not given in Table 2 but readily apparent by difference from those for albumin (see also Text-fig. 1A), were normal in the less serious forms of the disease. In the lepromatous cases they differed from normal inversely as the albumin, being most increased in the advanced cases—55 per cent in the L₃ group as compared with 46 per cent in the controls.

Albumin-globulin ratio.—This ratio was in the normal range (over 1.10) in the tuberculoid and less severe neural cases, slightly reduced (1.08) in the more advanced neural cases, and progressively decreasing in the lepromatous groups with advancement of the disease, from 1.00 to 0.92 and 0.81. These

⁴ The terms "per cent" and "percentage" are used only to signify the proportions of given fractions in the total proteins. The expression "grams/deciliter" ("gm./dl.") is used for the concentration in the whole serum or plasma.

results are consistent with those obtained by the copper sulfate method, and with those of other workers.

Globulin fractions.—In the determinations of the globulin fractions by colorimetry, as described, the values for alpha and beta globulins did not differ from the normals in any form of the disease, with respect to either the absolute concentrations or the percentages. The gamma globulin values on the other hand, differed from normal in the lepromatous cases, increasing with the degree of advancement and being alone responsible for the increase of the total globulins. The average percentages for the three groups of these cases are 21 for the L_1 cases, 26 for the L_2 cases, and 28 for the L_3 cases. The increases of absolute concentration, which reached 2.27 gm./dl. in the advanced lepromatous group, are best seen in Text-fig. 1B.

B. FRACTIONS BY ELECTROPHORESIS

To compare the findings obtained by the colorimetric method with serum and those obtained with the electrophoresis apparatus of Tiselius with plasma, both procedures were applied to specimens from 15 cases of leprosy and 6 healthy persons.⁵ The leprosy cases were: 1 tuberculoid, 5 neural and 9 lepromatous. Both serum and plasma were taken at the same time except from 3 patients, from whom two blood specimens were taken one day apart. The results are shown in Table 3.

TABLE 3.—*Protein fractions in 15 cases of leprosy and 6 healthy controls, obtained by electrophoresis of plasmas and by colorimetry of sera of the same individuals in percentages.*

Case group	No. of cases	Electrophoresis					Colorimetry			
		Albu- min	Alpha globu- lin	Beta globu- lin	Gamma globu- lin	Fibri- no- gen	Albu- min	Alpha globu- lin	Beta globu- lin	Gamma globu- lin
Normal controls	6	56	7	11	18	8	54	17	10	19
Neuromacular/ ^a	6	56	8	10	19	7	56	18	9	20
Lepromatous	9	46	8	10	26	10	45	18	11	26

^a Neural cases, 5, and tuberculoid, 1.

By electrophoresis, the albumin fraction was more than 50 per cent in all but one of the 6 cases of tuberculoid and neural leprosy, and less than 50 per cent in all but one of the 9 lepromatous cases. No essential differences were observed for the alpha and beta globulin fractions and fibrinogen between the

⁵ Dr. H. Hirai, of the Biochemistry Department of the Tokyo University Medical School, helped me with the electrophoresis. For this study he used the plasma of the same individual patients whose sera I used for colorimetry.

leprosy cases and the controls. The gamma globulin fraction, however, which averaged 19 per cent in the maculoneural cases and 18 per cent in the controls, was 26 per cent in the lepromatous group. These figures are closely consistent with those obtained by colorimetry in the specimens from the same individuals, as shown in the second part of Table 3. Here again the only departure from normal is the much higher percentage of the gamma globulin fraction in the lepromatous form.

SERUM PROTEIN FRACTIONS IN ERYTHEMA NODOSUM LEPROSUM

Erythema nodosum leprosum is an interesting condition, about the nature of which there has been much discussion. Many regard it as an antigen-antibody reaction phenomenon. I have studied the serum protein fractions of 25 cases; the data are included in Table 2. In 8 cases determinations were made during and after the reactions. These reaction cases were of the L₂ and L₃ grades.

The average figure of 8.1 gm./dl. for the total proteins in the 25 cases (Table 2) is precisely the same as for the L₃ group. However, the albumin percentage (39) was the lowest of all the

TABLE 4.—Total serum proteins and fractions in erythema nodosum leprosum, determinations in 8 cases during and after the reactions.

Case No.	Reaction	Total protein gm./dl.	Albumin p. c.	Globulin, per cent			Albumin-globulin ratio
				Alpha	Beta	Gamma	
1	During	8.0	40	16	9	35	0.66
	After	7.8	45	14	10	31	0.82
2	During	8.0	42	24	8	26	0.72
	After	8.2	46	22	10	22	0.85
3	During	7.8	37	22	9	32	0.58
	After	8.6	41	19	10	30	0.69
4	During	7.9	43	19	9	29	0.75
	After	7.4	46	18	14	22	0.85
5	During	7.9	47	22	10	21	0.88
	After	7.9	46	20	14	20	0.85
6	During	7.4	40	24	10	26	0.66
	After	7.7	54	17	8	21	1.10
7	During	8.8	22	18	11	49	0.28
	After	7.6	38	19	10	33	0.61
8	During	9.0	41	16	8	34	0.69
	After	8.6	44	18	9	30	0.78
Average	During	8.1	39	20	9	31	0.64
	After	8.0	45	18	11	26	0.82

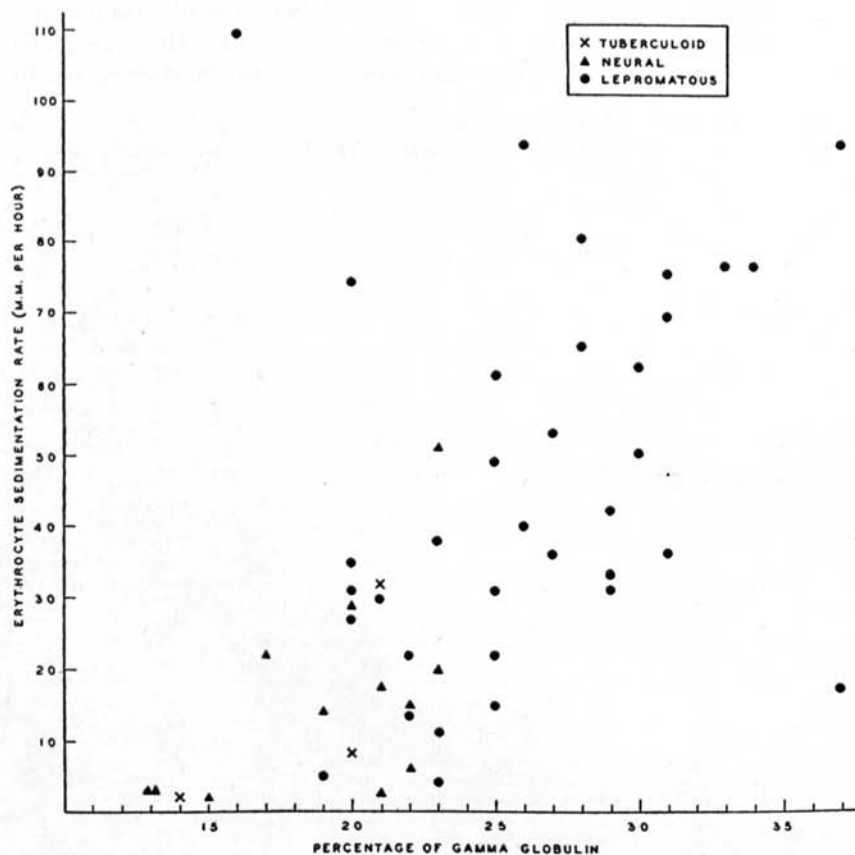
groups, and with the globulin percentage of 61 the ratio of those elements was the lowest of all, 0.64.

Examination of the data of the 8 cases examined twice, shown in Table 4, shows that the averages for the total proteins did not change significantly, being 8.1 gm./dl. during the reactions and practically 8.0 gm./dl. afterward. However, the proportion of albumin increased and of globulin decreased in

TABLE 5.—Gamma globulin percentages and erythrocyte sedimentation rates in 50 cases of leprosy and the results of the Takata test in 28 cases.

Case No.	Gamma globulin p. c.	Erythrocyte sedimentation mm./hr.	Takata reaction	Case No.	Gamma globulin p. c.	Erythrocyte sedimentation mm./hr.	Takata reaction
<i>Tubercloid cases (3)</i>				<i>Lepromatous cases (cont.)</i>			
1	21	32	1+	25	21	30	1+
2	20	8	—	26	20	27
3	14	2	—	27	29	42
<i>Neural cases (12)</i>				28	27	36	2+
4	13	3	—	29	26	40	2+
5	15	2	—	30	25	47
6	22	6	—	31	28	80	2+
7	17	22	—	32	30	50	1+
8	22	15	33	31	36
9	21	17	34	37	93
10	13	3	—	35	25	31
11	19	14	36	26	93
12	23	51	37	23	38
13	20	29	38	31	75
14	21	2	—	39	20	31	2+
15	23	20	40	30	62	4+
<i>Lepromatous cases (35)</i>				41	27	53
16	22	14	42	34	76	4+
17	25	22	1+	43	20	35	2+
18	19	5	±	44	23	11
19	22	22	2+	45	31	69	4+
20	25	15	2+	46	25	61	2+
21	16	109	47	33	76	4+
22	20	74	1+	48	29	31	3+
23	28	65	49	37	17
24	23	4	1+	50	29	33

all but one case, twice in considerable amount, so that the averages changed from the low of 39 to 45 per cent, the latter figure being the same as the average for the nonreactional L_3 cases. Thus the albumin-globulin ratio increased from 0.64 to 0.82.



TEXT-FIG. 2. Correlation between the gamma globulin fractions of serum and the erythrocyte sedimentation rates.

ERYTHROCYTE SEDIMENTATION RATE AND SERUM PROTEINS

The erythrocyte sedimentation rate is commonly accelerated in leprosy, more often in the lepromatous form than in the maculoneural ones. Acceleration of sedimentation is said to be due to instability of the blood colloids, stability depending on the amount of fibrinogen and globulin which are ready to disperse. When they are increased, erythrocytes coagulate more rapidly and their sedimentation is accelerated, an effect which is increased if their number is decreased.

The gamma globulin fraction of the serum proteins being

the one which varies in different forms and stages of the disease, the percentages of that fraction and the blood sedimentation rates observed in 50 cases of different types are shown in Table 5 and Text-fig. 2.

It will be seen that there is relatively little increase of globulin, and that the sedimentation rates are low in the tuberculoid and neural forms of the disease, whereas there is increase of gamma globulin and acceleration of sedimentation rate in the lepromatous form. It may be concluded that, in general, there is a correlation between the percentages of gamma globulin and the blood sedimentation rates.

THE TAKATA REACTION AND SERUM PROTEINS

The Takata reaction, used as a test of liver function, is generally believed to depend on an unstable condition of the serum colloids. The stability is affected by increased dispersion of the colloids, to which increase of globulin and decrease of albumin contribute primarily. Jetzler, Shinde and Barth, Schrender, and others have reported that the occurrence of the positive Takata reaction is due to a decreased albumin-globulin ratio.

Many studies have been reported of the Takata reaction in leprosy. More positive cases have been observed in lepromatous leprosy than in the neuromacular forms. My own results with this test in 62 cases are shown in Table 6.

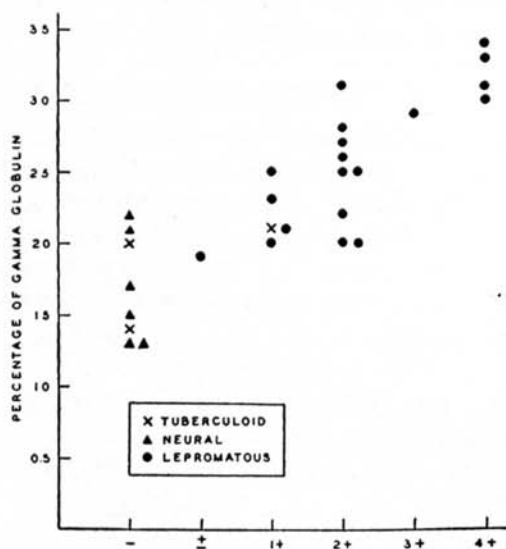
TABLE 6.—Results of the Takata test in 62 cases of leprosy.

Type and degree	No. of cases	Results of the test					
		Neg.	±	1+	2+	3+	4+
T	4	3	0	1	0	0	0
N ₁	5	5	0	0	0	0	0
N ₂	3	2	0	0	1	0	0
L ₁	12	3	2	2	4	1	0
L ₂	23	0	5	7	4	6	1
L ₃	15	0	0	0	5	1	9
Total	62	13	7	10	14	8	10

Almost all cases of the maculoneural forms turned out negative, while contrarily most of the lepromatous cases were

positive, and the grade of the reaction increased with the evolution of the disease.

A marked positive correlation was observed between the percentage of gamma globulin and the Takata reaction, as is clearly shown in Text-fig. 3. My observations also agree with the cited views in connection with the albumin-globulin ratio, which was less than 1.0 in almost all the Takata-positive sera, while all sera with a ratio of more than 1.0 were Takata negative.



TEXT-FIG. 3. Correlation of the gamma globulin percentages and the results of the Takata test in 2 cases of leprosy (cf Table 5).

DISCUSSION

When the concentration of serum proteins was measured by the specific gravity method, no significant difference was found between the lepromatous and neural forms of leprosy. Regarding the specific viscosity of serum, the lepromatous form showed 1.99 and the neural one 1.77. The albumin-globulin ratio, computed from specific gravity and viscosity, was 1.62 for the neural form and 0.75 for the lepromatous one.

When the concentration of serum proteins was measured by the refractometric method, the following figures (gm./dl.) were obtained: tuberculoid cases, 7.5; neural cases, 7.3-7.5; slight and moderately advanced lepromatous cases, 7.6; advanced lepromatous cases, 8.1. Except for the last one there were no

significant differences between the groups, nor any departure from the figure for normal persons, 7.4. In the advanced lepromatous form, however, there was a distinct increase.

Regarding the serum protein fractions, the albumin content was almost the same in the neuromacular forms of leprosy as in the healthy controls, but in the lepromatous form it decreased with advancement of the disease. Total globulins showed the reverse change, being almost within the normal range in the neuromacular forms but increasing with severity in the lepromatous form. Consequently, the albumin-globulin ratio is over 1.0 in neuromacular forms and below 1.0 in the lepromatous one.

The alpha and beta globulin fractions remain at almost the same level in all forms and stages of leprosy. On the average, alpha globulin was 17-19 per cent and beta globulin 9-10 per cent, in comparison with 17 per cent and 10 per cent respectively, in healthy people. Gamma globulin, also, gave normal values except in lepromatous leprosy: tuberculoid, 19 per cent (range 14-21%), minimal neural, 17 per cent (range 13-22%); and advanced neural, 20 per cent (13-23%), in comparison with 19 per cent (12-22%) for healthy people. In the lepromatous cases, however, the average for the least advanced ones was 21 per cent (range 16-25%), for the moderately advanced ones 26 per cent (20-37%), and for the far advanced ones 28 per cent (20-37%).

In lepromatous leprosy there is a marked increase of gamma globulin, compared with the maculoneural forms, the increase becoming greater as the disease advances, and its concentration may become as high as 2.27 grams/deciliter. The gross increase of globulin is due to the increase of this fraction.

Measurements of the fractions of blood plasma by electrophoresis yielded results closely consistent with the findings in the serum by colorimetry. As before, the measurements in cases of the maculoneural forms were essentially the same as in healthy persons, whereas in the lepromatous form the albumin was decreased and the gamma globulin increased, while the alpha and beta globulins and the fibrinogen were in normal amounts.

The serum protein fractions in several lepromatous cases with the erythema nodosum type of lepra reaction proved to be much the same as in far advanced lepromatous cases without reaction, with the lowest albumin-globulin ratio of all groups. The same cases tested later, in the stage of absorption of the

reactions, showed decrease of the gamma globulin and increase of the albumin and corresponding elevation of the albumin-globulin ratio.

There is a positive correlation between the amount of gamma globulin and the erythrocyte sedimentation rate, increase of gamma globulin causing acceleration of the sedimentation rate.

Many cases of lepromatous leprosy are Takata positive, with a correlation between the strength of the reaction and the advancement of the disease. Globulin increase was found when this reaction was positive, such reactions being common among the cases with less than 1.0 albumin-globulin ratio. There is direct correlation between the amount of gamma globulin and the positive rate of the Takata reaction.

CONCLUSIONS

1. The concentration of serum proteins in leprosy patients evidently increases in far advanced lepromatous leprosy, but not in other forms or stages of the disease.

2. On fractioning the serum or plasma proteins of leprosy patients by either colorimetry or electrophoresis, cases of the maculoneural forms yield essentially the same results as do normal, healthy persons, whereas in cases of the lepromatous form there is a decrease of albumin and increase of globulin.

3. The fractions in cases of erythema nodosum leprosum resemble those of far advanced lepromatous leprosy, with decrease of albumin and increase of gamma globulin. After the subsidence of the reaction the gamma globulin decreases and the albumin increases.

4. Gamma globulin plays an important role in the acceleration of blood sedimentation in cases of leprosy.

5. The Takata reaction, which is supposed to be caused chiefly by increased gamma globulin, becomes positive in lepromatous leprosy.

CONCLUSIONES

1. La concentración de las proteínas del suero en pacientes leprosos, está aumentada en lepra lepromatosa avanzada, pero no en otras formas de la enfermedad.

2. Fraccionamiento de las proteínas del suero por electroforesis o colorimetría, demuestra valores normales en casos maculo-neurales, pero en los casos lepromatosos hay una disminución en la albúmina y un aumento en la globulina.

3. Las fracciones en "erythema nodosum leprosum" son similares a las de lepra lepromatosa avanzada, aumento en globulina y disminución en albúmina. Después de la reacción, se observa una disminución en la globulina y un aumento en la albúmina.

4. La globulina gamma es importante en la aceleración de la sedimentación de hematíes en casos de lepra.

5. La reacción de Takata, que se supone dependa del aumento en la globulina gamma, es positiva en lepra lepromatosa.