

CLINICAL STUDY OF THE BLOOD GLUTATHIONE IN LEPROSY¹

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The literature dealing with the subject of the glutathione content of the blood in various pathological conditions is extensive, but the subject has not received sufficient attention in connection with leprosy. Contributions have been recorded by Takashima (1) and by Sakakibara (2), with which I have been able to acquaint myself only from abstracts.³ There are no records of similar studies in the Russian literature. As will be seen, studies of the variations in the amount of glutathione in the blood of leprosy patients permits a definite orientation with respect to the question of the state of protoplasmic dynamic factors.

In the organism affected by leprosy, impairment of the intermediary metabolism develops quite early. Even at the very onset of the disease, when clinical symptoms and subjective neural disturbances only begin to define themselves, there are indications of disturbances in the blood and lymph circulation, such as cutaneous erythema and cyanosis. These changes develop in parallel with or are accompanied by disorders of the vegetative nervous system. The frequent involvement of the upper respiratory tract by the leprous process also affects the gas metabolism. The chemical alterations in the organism of the patient become more pronounced as the leprous process becomes generalized.

According to recent studies, the oxidation process in the tissues may take place without the participation of atmospheric oxygen. Substances which require energetic oxidizing agents under artificial conditions are easily oxidized in the animal organism because of the presence there of a number of oxidizing ferments. Glutathione, first discovered in 1918 by Mayerhoff and studied in detail by J.

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² Chief Physician, A. A. Shelakowsky; Scientific Supervisor, Prof. I. N. Perevodchikov.

³ See also reference (3).

Hopkins, belongs to such ferments. It has been shown to be a tripeptide containing sulphur and composed of cystine, glycol, and glutamic acid.

The glutathione of the blood is contained in the erythrocytes and is of utmost importance in the processes of general and tissue metabolism. Although our knowledge of the physiology of this substance is still incomplete, the important part that it plays in the respiratory function of the erythrocyte, comparable with that of hemoglobin, is definitely established. It possesses the capacity of easily binding and splitting off its hydrogen and is therefore met in two forms, hydrated or reduced (sulphhydrated, SH) and dehydrated or oxidized (disulphide, SS). In the tissues the latter form quickly changes again into the other (reversible reaction). Thus glutathione fulfills the function of an oxidizing ferment.

In the present study the glutathione content of the blood was determined by the method of Woodward and Fry. The blood was taken from the antecubital vein, usually while the patient was fasting. Similar specimens from five healthy individuals served as the control. For the purpose of studying the possible variations in leprosy, determinations were made on groups of patients suffering from the various forms and stages of the disease.

Takashima and Sakakibara evidently studied only the reduced form of glutathione in their leprosy patients, as far as can be judged. In the present study the entire content of this substance, in both the reduced and oxidized forms, was determined. Moreover, blood taken for analysis of the hemoglobin served also for the calculation of the so-called Gabbe and Woodward indices. Since the glutathione is contained in the erythrocytes, the number of which varies, while none is present in the plasma, Gabbe suggested the following formula for calculation of the index:

$$K = \frac{\text{Reduced glutathione (mg. per 100 cc.)}}{\text{Erythrocytes (in millions)}}$$

Under normal conditions this index varies between 5.3 and 9.3 (average 5.8) and serves to express the amount of reduced glutathione per cell. According to the data of numerous authors, the Gabbe index is higher in anemias than normal. However, in some diseases not complicated by anemia, including leprosy, there is no parallelism or dependence between the amount of glutathione in the blood and the absolute number of erythrocytes.

Since in anemias an inverse relationship is observed between

the glutathione and hemoglobin values, Woodward suggested the following formula:

$$K = \frac{\text{Reduced glutathione (mg. per 100 cc.) X hemoglobin (per cent)}}{\text{Erythrocytes (in millions)}}$$

This formula expresses the degree of oxidation capacity of each erythrocyte. Under normal conditions the Woodward index varies between 5.3 and 7.8 (average 5.7).

In normal individuals the total glutathione in the blood varies between 34 and 41 mg. per 100 cc.; the reduced form varies from 26 to 33 mg. and the oxidized form from 5 to 10 mg. The normal controls examined in the present study gave the following average values: total, 37.5 mg. per 100 cc.; reduced, 30.0 mg.; oxidized, 7.5 mg. About the same values have been reported by other authors who have employed the Woodward and Fry method as well as the Gabbe index (Woodward and Fry, Goorevich, Guindes and Smirnov).

STUDY OF LEPROSY CASES

The leprosy patients investigated, a total of 82, were divided into four groups. (1) The first group was composed of 20 patients with the tuberculoid form of the disease or with only nervous symptoms (N1 and N2). In all of them the cutaneous manifestations and nervous symptoms corresponded to the maculo-neural type of the old classification. (2) The second group was made up of 18 patients with the lepromatous and mixed forms of leprosy (L1, L2, L3, L1-N2, L2-N2). The course of the disease in all of them at the time of the investigation was regressive. (3) The third group included 34 cases, mostly of active lepromatous leprosy, which may be classified as L3. The course of the disease may be characterized as slowly or rapidly progressive. (4) The fourth group, 10 patients, all suffered from the lepromatous type of the disease with exacerbations of the process (lepra reaction).

A total of 99 determinations were made on these 82 cases, seventeen duplicate ones having been made on thirteen of them. Erythrocyte counts and hemoglobin determinations (percentages) were also made, and the color indices were calculated. The details of the findings are submitted in Table 1.

TABLE 1.—Data for the 82 cases examined, and results of the determinations of blood glutathione and indices.

Case No.	Sex and Age (Years)	Leprosy		Blood		Glutathione (mg. per 100cc)			Glutathione Index		Treatment		
		Duration (Yrs.)	Form	Course	Erythrocytes (1000's)	Hemoglobin (Per Cent)	Color Index	Total	Reduced	Oxidized		Gabbe	Woodward
1	F ?	3	Tuberculoid	Regressive	4,560	75	0.83	41	37	4	8.1	6.0	Alepol
2	M 58	10	Tuberculoid	Regressive	4,910	80	0.81	41	38	3	7.8	6.2	Moogrol
3	F ?	2	Tuberculoid	Regressive	5,036	80	0.80	40	34	6	6.7	5.4	Moogrol
4	F 49	2	Tuberculoid	Stationary	5,270	80	0.77	33	29	4	5.5	4.4	None
5	M 52	3	Tuberculoid	Regressive	5,060	80	0.80	35	33	2	6.5	5.2	None
6	F 67	6	Tuberculoid	Regressive	3,900	85	1.0	40	37	3	9.2	8.0	Chaul. oil
7	F 53	2	Tuberculoid	Stationary	4,890	80	0.82	41	39	2	8.0	6.3	None
8	M 32	2	Tuberculoid	Regressive	4,850	68	0.70	37	37	0	7.8	5.2	None
9	M 50	2	Tuberculoid	Regressive	5,260	80	0.77	37	34	3	6.4	5.2	None
10	M 23	7	Tuberculoid	Trans. to lepromatous	5,180	80	0.76	37	36	1	6.9	5.6	None
11	M 43	5	Tuberculoid	Stationary	5,480	85	0.79	35	33	2	6.0	5.1	Moogrol
12	M 51	3	Tuberculoid	Trans. to lepromatous	5,010	80	0.80	38	33	5	6.6	4.8	None
13	M ?	6	Tuberculoid	Stationary	4,820	80	0.83	37	29	8	6.0	6.2
14	M 24	2	Maculoneural	Regressive	5,280	80	0.77	46	40	6	7.5	6.0	Alepol
15	F 27	12	Maculoneural	Stationary	4,860	75	0.78	32	26	6	5.0	4.0	Moogrol
16	F 54	9	Maculoneural	Regressive	4,830	80	0.83	35	33	2	7.0	5.5
17	F 33	8	Maculoneural	Regressive	4,810	75	0.78	34	30	4	6.2	4.7	None
18	F 16	3	Maculoneural	Stationary	5,260	90	0.86	35	29	6	5.6	5.0	None
19	F 66	2	Maculoneural	Stationary	4,810	75	0.79	36	29	7	6.0	4.1	None
20	M 47	3	Maculoneural	Regressive	4,380	75	0.91	30	27	3	6.1	4.6	None

Group I. Cases with tuberculoid or maculo-neural leprosy

Group II. Cases with lepromatous leprosy, not active

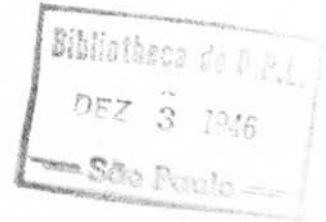
Case No.	Sex and Age (Years)	Leprosy			Blood			Glutathione (mg. per 100cc)			Glutathione Index		Treatment
		Duration (Yrs.)	Form	Course	Erythrocytes (1000's)	Hemo-globin (Per Cent)	Color Index	Total	Re-duced	Oxi-dized	Gabbe	Wood-ward	
1	F 19	2	Lepromatous	Regressive	5,480	80	0.74	43	40	3	7.3	5.9	None
2	M 19	1	Lepromatous	Regressive	4,630	80	0.87	40	32	8	6.9	5.5	Alepol
3	M 26	9	Lepromatous	Stationary	5,130	90	0.88	35	33	2	6.4	5.6	None
4	M 21	4	Lepromatous	Regressive	5,510	80	0.73	43	34	9	6.1	4.9	None
5	F ?	12	Lepromatous	Regressive	5,480	85	0.78	46	40	6	7.3	6.2	None
6	F 53	2	Lepromatous	Regressive	5,430	80	0.76	43	40	3	7.4	5.9	None
7	F 33	2	Lepromatous	Regressive	4,470	58	0.66	39	33	6	7.4	4.3	None
8	F 35	2	Lepromatous	Regressive	4,560	70	0.78	46	36	10	7.8	5.5
9	M 46	6	Lepromatous	Regressive	5,120	80	0.87	37	25	12	4.8	3.9	Chaul. oil
10	M 64	19	Lepromatous	Regressive	5,010	80	0.80	39	34	5	6.8	3.3	None
11	F 36	9	Lepromatous	Regressive	5,240	80	0.76	38	28	10	5.3	4.2	Alepol
12	M 35	10	Lepromatous	Regressive	4,820	75	0.78	39	35	4	7.2	5.4	None
13	M 39	9	Lepromatous	Regressive	5,480	90	0.83	34	28	6	5.1	4.6	Moogrol
14	F 30	3	Lepromatous	Regressive	5,230	80	0.77	40	37	3	7.0	5.6	None
15	M 28	3	Lepromatous	Regressive	4,970	85	0.87	37	30	7	6.0	5.1	Chaul. oil
16	M 38	9	Lepromatous	Regressive	4,520	80	0.88	35	31	4	6.8	5.4	Alepol
17	M 49	2	Lepromatous	Regressive	4,410	75	0.85	28	24	4	5.4	4.0	None
18	M 24	2	Lepromatous	Regressive	4,910	75	0.79	38	35	3	7.1	5.3	None

Group III. Cases mostly with lepromatous leprosy, more or less active

Case No.	Sex and Age (Years)	Leprosy			Blood		Glutathione ⁷ (mg. per 100cc)			Glutathione Index		Treatment	
		Duration (Yrs.)	Form	Course	Erythrocytes (1000's)	Hemoglobin (Per Cent)	Color Index	Total	Reduced	Oxidized	Gabbe ward		Woodward
1	M ?	5	Lepromatous	Progressive	4,970	88	0.89	26	26	0	5.2	4.6	None
2	M 48	2	Lepromatous	Progressive	4,280	70	0.82	28	27	1	6.3	4.2	None
3	F 28	5	Lepromatous	Progressive	5,510	80	0.73	28	27	1	4.9	4.1	Chaul. oil
4	M 29	11	Mixed	Torpid	4,810	75	0.78	29	28	1	5.8	4.4	None
5	M 24	12	Tuberculoïd	Torpid	5,020	80	0.80	32	31	1	6.1	4.9	None
6	M 27	3	Tuberculoïd	Torpid	5,020	75	0.75	29	26	3	5.1	3.7	Chaul. oil
7	M 53	10	Tuberculoïd	Torpid	5,250	80	0.76	29	28	1	5.3	4.3	Chaul. oil
8	M 26	11	Mixed	Torpid	4,370	75	0.87	32	29	3	6.6	4.9	None
9	M 30	11	Tuberculoïd	Stationary	5,060	80	0.80	28	25	3	4.9	3.9	None
10	M 57	5	Mixed	Torpid	4,540	70	0.78	30	20	10	4.4	3.1	None
11	M 53	9	Mixed	Torpid	4,820	80	0.83	33	27	6	5.6	4.5	None
12	M 42	13	Tuberculoïd	Torpid	5,030	80	0.80	30	26	4	5.1	4.1	None
13	M 56	18	Tuberculoïd	Torpid	5,210	80	0.76	28	24	4	4.6	3.7	None
14	F 31	11	Tuberculoïd	Progressive	5,360	80	0.75	33	27	6	5.0	4.0	None
15	M 29	8	Mixed	Torpid	4,380	70	0.75	28	21	7	4.9	3.3	Chaul. oil
16	M ?	11	Lepromatous	Torpid	5,600	60	0.82	31	25	6	4.4	3.3	Moogrol
17	M ?	1	Lepromatous	Progressive	4,720	75	0.54	29	24	5	5.1	3.6	None
18	F 56	11	Lepromatous	Torpid	4,380	70	0.81	31	27	4	4.6	3.7	Serum
19	M 38	5	Mixed	Progressive	5,010	80	0.80	27	25	2	5.0	4.0	None
20	F 29	12	Lepromatous	Progressive	5,430	80	0.75	27	21	6	3.9	3.1	None
21	F 46	5	Lepromatous	Progressive	6,040	70	0.59	29	28	1	4.6	3.2	Alepol
22	F ?	4	Lepromatous	Progressive	5,410	80	0.74	26	20	6	3.7	2.9	Alepol
23	M 24	2	Lepromatous	Progressive	4,030	75	0.93	29	25	4	6.2	4.6	None
24	M 33	2	Lepromatous	Progressive	4,830	90	0.93	24	23	1	4.7	4.3	None
25	M 53	2	Lepromatous	Progressive	4,830	85	0.80	29	24	5	5.0	4.2	None
26	F 56	9	Lepromatous	Progressive	5,230	80	0.76	28	26	2	4.9	4.0	Chaul. oil
27	F 24	8	Lepromatous	Progressive	5,320	80	0.75	30	26	4	4.9	3.9	None
28	M 34	10	Lepromatous	Progressive	4,480	75	0.85	33	32	1	7.1	5.3	None
29	M 27	10	Lepromatous	Progressive	5,340	80	0.75	30	29	1	5.4	4.3	None
30	M 34	5	Lepromatous	Progressive	5,480	75	0.83	28	24	4	5.3	3.9	None
31	F 35	3	Lepromatous	Progressive	4,660	75	0.81	27	19	8	4.0	3.0	None
32	F 19	10	Lepromatous	Progressive	5,260	80	0.76	30	28	2	5.3	4.2	Chaul. oil
33	F ?	12	Lepromatous	Progressive	4,820	75	0.78	30	23	7	4.9	3.6	None
34	M 38	6*	Lepromatous	Progressive	4,850	90	0.93	24	20	4	4.1	3.7	None

Group IV. Cases with lepromatous leprosy, in reaction

Case No.	Sex and Age (Years)	Leprosy		Blood		Glutathione (mg. per 100cc)			Glutathione Index		Treatment		
		Duration (Yrs.)	Form	Course	Erythrocytes (1000's)	Hemoglobin (Per Cent)	Color Index	Total	Reduced	Oxidized		Gabbe	Woodward
1	M ?	9	Lepromatous	Reaction	4,980	80	0.81	23	15	8	3.0	2.4	None
2	M 63	8	Lepromatous	Reaction	5,260	80	0.77	23	17	6	3.2	2.6	None
3	F 25	2	Lepromatous	Reaction	5,442	80	0.76	25	21	4	3.8	3.1	None
4	M 34	3	Lepromatous	Reaction	4,530	75	0.83	31	24	7	5.3	3.9	None
5	F 48	14	Lepromatous	Reaction	5,170	80	0.78	27	22	5	4.2	3.4	None
6	M 40	8	Lepromatous	Reaction	4,960	78	0.80	20	15	5	3.0	2.3	None
7	M 25	9	Lepromatous	Reaction	5,260	80	0.77	24	20	4	3.8	3.0	None
8	M 18	8	Lepromatous	Reaction	5,190	80	0.78	24	13	11	2.5	2.0	None
9	M 23	11	Lepromatous	Reaction	5,270	80	0.77	24	20	4	3.7	3.0	None
10	M 40	15	Lepromatous	Reaction	5,430	85	0.79	26	23	3	4.2	3.6	None



The average of the figures for total glutathione given in this table shows that, on the whole, there was a slight decrease as compared with the normal controls: total glutathione 32.6 mg. per 100 cc., the reduced form 28.2 mg., and the oxidized form 4.4 mg.; the Gabbe index averaged about 5.6 and the Woodward index 4.4. It will be seen, however, that the reduction is ascribable mainly to the last two groups. In the first two the variations, from 28 to 46 mg., differed little from normal, whereas in the third group the range was from 24 to 33 mg. and in the fourth group from 20 to 31 mg. The effects of these differences, as shown by average figures for the different groups, are shown in Table 2. Each group will be considered separately.

TABLE 2.—Average blood glutathione level, per 100 cc. by case groups, including controls.

Group	Glutathione			Index	
	Total	Reduced	Oxidized	Gabbe	Woodward
I	37.0	33.15	3.85	6.7	5.4
II	38.9	33.0	5.9	6.6	5.1
Average	37.9	33.1	4.8	6.7	5.6
III	28.9	25.3	3.6	5.1	3.9
IV	24.7	19.0	5.7	3.7	2.9
Average	28.0	23.9	4.1	4.8	3.7
Total Average	32.6	28.2	4.4	5.6	4.4
Controls	37.5	30.0	7.5	—	—

Group 1.—It will be observed that in this group, which comprised only cases with tuberculoid and ordinary maculo-neural leprosy (Table 1, I), the figures for total glutathione are of the order of normal values. Those for reduced glutathione, however, show a tendency to rise to the highest normal levels. Thus this form is actually somewhat increased, whereas there is, relatively, a considerable lowering in the oxidized glutathione as compared with the normal value. Thus the reduction processes in most of the patients of this group prevail over the processes of oxidation.

Group 2.—Very similar figures were obtained in the cases of the second group, in which the disease, for the most part of the lepromatous type, was of regressive character (Table 1, II). In them the values for total and reduced glutathione remain within normal levels, while on the average the content of oxidized glutathione is decreased, but to a lesser degree than in the first group. A study of the variations in the cases of this group reveals a normal corre-

lation between the different forms of the substance, reduced, oxidized, and entire.

Group 3.—In the third group, composed of cases with clinically active manifestations and mainly of the lepromatous type (Table 1, III), the figures for all three determinations were markedly decreased. Whereas in the first two groups together the values for glutathione varied from 28 to 46 mg. per 100 cc. (average 38) and of the reduced form from 24 to 37 mg. (average 33), in the third group the total figures ranged from 24 to 34 mg. per 100 cc. (average 28.9) and for the reduced form from 19 to 32 mg. (average 25.3).

Group 4.—Advantage was taken of the opportunity to examine the bloods of ten patients with lepromatous leprosy who were in a reactive condition generally known as "lepra reaction" (Table 1, IV). The data obtained show that during exacerbation of the disease process in such cases there is an especially marked lowering of the amount of reduced glutathione as compared with normal and materially greater reduction in the total amount than in the other groups, even in the third one composed of more or less active cases in the ordinary process of the disease. The range for total glutathione was from 20 to 31 mg. per 100 cc. (average 24.7), and of the reduced form from 13 to 24 mg. (average 19.0). The latter element was mainly responsible for the low total figures since the oxidized glutathione values remained relatively high, though still less than in the controls.

INDICES AND OTHER DATA

The Gabbe index, obtained by dividing the reduced glutathione by the number of erythrocytes (millions), which normally averages 5.8, was found (Table 2) to average somewhat higher than normal in the first two groups of cases (6.7 and 6.6), respectively. This fact confirms the indication of a prevalence of the reduced form of glutathione in the erythrocyte over the oxidized form. This predominance of reduced glutathione in the blood of cases of tubercloid and maculo-neural leprosy, and also in the regressive stage of cases of lepromatous leprosy, permits the conclusion that the aerobic oxidation is complemented by considerable anaerobic oxidation.

In the cases of the third group (i.e., those with the active form of the lepromatous process) there is demonstrated a reduction of the Gabbe index. It follows that no compensation by intracellular oxidation is taking place and that the amount of glutathione in each erythrocyte is lowered. Thus there is evidence in these cases of an impairment of the oxidation-reduction processes in the tissues.

The Woodward index, obtained by multiplying the reduced glutathione and hemoglobin values and dividing the result by the erythrocyte count (normal average 5.7), is seen to remain almost within the normal limits in the first and second groups. This corresponds with the concept that the oxidative capacity of the erythrocytes is not altered. On the other hand, the decrease of this index in patients of the third group (to an average of 3.9) seems to indicate a lowering of oxidative capacity.

With respect to the fourth group—lepromatous cases in reaction—the changes in the correlation of the two forms of glutathione are more demonstrable than the variations in the total glutathione values. The decrease in the reduced glutathione and the corresponding relative increase of its oxidized form indicate the presence of an altered oxidation process in the tissues. The Gabbe and Woodward indices are also decreased, a fact which seems to indicate a lowering of the oxidation-reduction capacity of each erythrocyte.

In this study, no correlation could be established between the glutathione values and the sex or age of the patients. Nor was any correlation found between the findings and the antileprosy treatment to which the patients had been subjected. On the other hand the length of the course of the disease has been found, in most instances, to have had some influence on the blood glutathione content. Thus in advanced cases its amount was usually lower than in earlier ones (Table 3).

TABLE 3.—Relation of duration of the disease to the glutathione values.

Group	Duration of the disease	Glutathione				
		Reduced	Oxidized	Total	Gabbe	Woodward
1 & 2	1-4 yrs.	33.7	4.7	38.4	6.8	5.2
	5-9 yrs.	31.0	5.0	36.0	6.3	5.3
	10 & over	34.6	4.8	39.4	6.8	5.4
3	1-4 yrs.	23.5	4.1	27.6	5.0	3.8
	5-9 yrs.	24.5	3.7	28.2	4.9	3.6
	10 & over	26.5	3.5	30.0	5.1	4.0
4	1-4 yrs.	22.5	5.5	28.0	4.6	3.5
	5-9 yrs.	16.0	6.8	22.8	3.1	2.5
	10 & over	21.7	4.0	25.7	4.0	3.3
Total	1-4 yrs.	30.4	4.6	35.0	6.2	4.7
	5-9 yrs.	25.7	4.7	30.4	5.1	4.2
	10 & over	27.7	3.8	31.5	5.4	4.2

Repeated determinations of the blood glutathione in the same patient, made for control purposes, indicate its stability. Only

very slight variations, not exceeding a few mg. per 100 cc. were observed.

DISCUSSION

On the basis of the data obtained in this study, it seems permissible to conclude that in the determination of the blood glutathione and its indices we have a new test applicable to leprosy, one which characterizes it as a disease in which the general metabolism is unstable and variable. In cases in which the symptomatology does not indicate any profound changes in the organism, or in which the process shows a tendency towards regression, the amount of glutathione in the blood does not differ from normal. In the slow course of progression of the disease, associated with further development of symptoms and of pathological functional disturbances, the diminishing amounts of the glutathione in the blood indicate a depression in the intracellular oxidative processes in the organism. This further confirms a lowering of the oxidation-reduction processes during this period of the disease. In the case of activation of the leprosy process (lepra reaction), the amount of glutathione in the blood is still further decreased, but a relative increase of the content of the oxidized form, as compared with the reduced form, indicates a compensatory tendency of the organism to combat the impaired equilibrium.

Stated summarily, it has been found that with improvement of the leprosy process (regression) the amount of glutathione in the blood rises, while exacerbation of the process is followed by a decrease. Thus a study of this element of the blood serves as a valuable index of the course of the pathological process in the leprosy patient and is of aid in the prognosis of the disease and in evaluating the treatment employed.

CONCLUSIONS

1. A study of the glutathione content of the blood in leprosy is of aid in determining the state of the oxidation-reduction processes in the patient.
2. In the tuberculoid and maculo-neural forms of the disease the total amount of glutathione in the blood does not differ from normal, but the amount of the oxidized form decreases with a corresponding increase of the reduced form.
3. The qualitative and quantitative values of blood glutathione in patients with lepromatous leprosy of slight and moderate degrees of advancement of the disease and in a regressive stage do not differ from normal.

4. In the active cases of the lepromatous type of leprosy in which the disease is progressing either rapidly or slowly all of the glutathione values are decreased.

5. In the reactive phase of leprosy there is a marked decrease of reduced glutathione and the total value though the amount of the oxidized form tends to remain within normal limits.

6. Determination of the blood glutathione values in leprosy is of value in ascertaining the extent of compensation of the leprosy process.

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