CLINICAL APPLICATION OF A SIMPLE METHOD FOR ESTIMATING "GAMMA GLOBULIN" [1]

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The electrophoretic technique offers the most accurate method for serum fractionation. This permits a quantitative separation of serum into albumin and into three major globulin fractions which are designated as alpha, beta and gamma components. In recent years electrophoretic analyses of serum proteins have been performed by many investigators in a variety of diseases. The accumulated data from these studies have now attained sufficient size and agreement that they may be of value as an aid in the differential diagnosis of certain diseases or for following the course of specific infections, particularly with reference to persistence or cessation of activity. While any one or all of the serum fractions may be altered in various diseases, it would appear that, for certain types of pathologic disorders, a knowledge of the quantitative amount of serum gamma globulin and of serum albumin would offer considerable assistance to the clinician.

Because of the cost of the electrophoretic apparatus, the skill required in its operation and the time consumed in making these determinations, it is unlikely that this technique will become a routine laboratory procedure. Many chemical methods are available for the estimation of serum albumin. However, available chemical methods for quantitative gamma globulin measurement have not been employed extensively in clinical laboratories. Recently we developed a simple salting-out method for estimating gamma globulin. This method which will be described in detail elsewhere (1) will be considered briefly here.

To 1 ml. of fresh serum in a centrifuge tube is added slowly and with thorough mixing 0.5 ml. of saturated ammonium sulfate. After the tube has stood in the ice box over night, it is centrifuged at 3,000 R.P.M. for 30 minutes. The supernatant is discarded. The packed precipitate is finely emulsified in 3.0 ml. of 33.3 per cent saturated ammonium sulfate. The tube is recentrifuged for 30 minutes and the supernatant fluid is discarded. The precipitate is dissolved in 10 ml. of saline and an aliquot is employed to determine its protein content, using a biuret method such as that of Weichselbaum (2).

Triplicate determinations may be made with an accuracy of $\pm$ 2 per cent. Electrophoretic studies indicate that 73 to 83 per cent of this protein fraction consists of gamma globulin, the remainder consisting of alpha and beta globulins. From 70 to 82 per cent of the total gamma globulin present in the serum is recovered in this fraction. Numerical comparisons were made between this chemical fraction (G. G. 33.3) and gamma globulin as determined by electrophoretic estimation in 33 human sera of which 7 came from normal controls, the remainder from subjects with a variety of diseases. The standard deviation of the chemical values from the electrophoretic values was 2.01 per cent (expressed as gamma globulin/total serum protein) with a coefficient of variation of 10 per cent. In general the chemical values for "gamma globulin" were somewhat higher than the corresponding electrophoretic values in instances in which the gamma globulin content is not increased. When the gamma globulin content is greatly increased, the chemical values are somewhat low. A number of solubility studies on this fraction reveal reasons why the precipitation from undiluted serum has advantages over the usual procedures in which serum is diluted greatly prior to precipitation.

Together with the determination of serum albumin by the method of Howe (3), we have carried out measurements of "gamma globulin" by our simple procedure in sera of normal persons and of patients with a number of diseases. The fluctuations in this constituent from time to time in normal persons have been ascertained by repeated determinations in a few healthy individuals over a prolonged period. The clinical usefulness of this method is indicated by serial determina-

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TABLE I

Mean values for serum protein constituents in 30 normal human subjects contrasted with electrophoretic values for 20 samples of pooled plasma (6)

*Chemical values

	T.S.P.	"G.G."	Alb.	Glob.	A/G	A/T.S.P.	"G.G."/A	"G.G."/G	"G.G."/T.S.P.
Mean value	6.96	0.860	4.96	2.00	2.5	71.3	17.4	44.3	12.3
Standard deviation	0.37	0.144	0.26	0.38	0.6	4.4	3.0	7.2	1.6
Coefficient of variation in per cent	5.3	16.7	5.3	19.0	23.1	6.2	17.2	16.3	13.0
† Electrophoretic values (Armstrong *et al.* [6])									
Mean	(7.30)	0.803	4.03	2.79	1.4	55.2			11.0
Coefficient of variation						2			6

* Values expressed in grams per 100 ml. serum or as per cent.

† The values of Armstrong *et al.* (6) are expressed only in per cent, to indicate the proportion which each fraction comprised of the whole plasma protein. The value for total plasma protein of 7.30, used for the conversion of his percentages to grams per cent, was derived by adding a value of 0.304 gram for fibrinogen to our mean value for total serum protein.

tions of the "gamma globulin" during the course of a few specific illnesses.

Values for total serum protein, serum albumin and "gamma globulin" in normal subjects

Simultaneous determinations have been made in 30 normal subjects of the total serum protein (T.S.P.) serum albumin (A), total serum globulin (G) and of the globulin fraction precipitated with 33.3 per cent saturated ammonium sulfate which is referred to as *G.G. 33.3.* The serum albumin and total serum globulin were measured by the method of Howe (3) using 21.5 per cent sodium sulfate. The initial one-third of the albumin filtrate was discarded as recommended by Gutman and coworkers (4). The G.G. 33.3 was determined by our method, as already described (1). All proteins were measured colorimetrically by the dilute biuret method of Weichselbaum (2). This method was calibrated and checked frequently with determinations of protein nitrogen, using a semimicro-Kjeldahl technique (5). The factor 6.4 was used for conversion of nitrogen to total protein.

In the control subjects there were 18 males and 12 females ranging in age from 18 to 35 years. All blood specimens were obtained in the morning approximately 2 to 3 hours after breakfast. Venous stasis was minimized by applying a tourniquet only immediately before withdrawal of the blood. All patients were sitting when samples were withdrawn. The results are presented in Table I which also, for comparison, indicates the electrophoretic values obtained by Armstrong and coworkers (6) from 20 samples of pooled plasma.

It is difficult to compare our normal values for protein fractions with those obtained electrophoretically for normal plasma. The value for serum albumin is higher and the value for serum globulin is lower with the Howe technique than the corresponding electrophoretic values (7 to 9). It has been demonstrated that the precipitation of serum with 21.5 per cent sodium sulfate does not remove all of the alpha and beta globulin (4, 7). Likewise, as we have indicated, our "gamma globulin" is not entirely pure and does not contain all the gamma globulin present in serum.

Weekly fluctuation in chemically determined protein fractions in normal control subjects

If one wishes to apply the chemical fractionation of proteins to clinical disorders, it is necessary to know the magnitude of fluctuation of these components from time to time in a given individual. In 5 healthy control subjects we have determined the total serum protein, serum albumin, total serum globulin and the G.G. 33.3 fraction many times at intervals of 1 to 3 weeks over a period ranging from 6 to 12 months. The observed fluctuations are indicated in Table II. It appears that the total globulin of serum exhibits the greatest range of variation while the total serum protein and the serum albumin seem to have the greatest stability.

Values for G.G. 33.3, serum protein and serum albumin in pathologic sera

In a variety of pathologic states, we have determined the protein fractions, using the methods previously described. In Table III only single determinations from a given individual were em-

TABLE II

Weekly fluctuations of chemically determined protein constituents in 5 normal subjects

Name	No. deter.	Total protein			"Gamma globulin"			Albumin			Globulin			Albumin/globulin		
		Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.
H. A.	21	6.66	0.31	4.7	0.903	0.089	9.9	4.62	0.24	5.2	2.07	0.38	18.4	2.3	0.6	26.1
V. J.	30	6.92	0.34	4.9	0.748	0.079	10.6	5.00	0.28	5.6	1.94	0.38	19.6	2.7	0.5	18.5
G. G.	23	6.81	0.30	4.4	0.813	0.095	11.7	4.66	0.26	5.6	2.14	0.32	15.0	2.2	0.4	18.2
V. D.	18	6.74	0.35	5.2	0.692	0.098	14.2	4.68	0.31	6.6	2.01	0.34	16.9	2.4	0.5	20.8
R. B.	13	6.68	0.34	5.1	0.680	0.068	10.0	4.82	0.19	3.9	1.87	0.34	18.2	2.7	0.7	25.9

Name	No. deter.	Albumin/T.S.P.			"G.G."/albumin			"G.G."/globulin			"G.G."/T.S.P.		
		Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.
H. A.	21	68.7	4.3	6.2	19.2	2.3	12.0	44.8	8.5	19.0	13.4	1.4	10.4
V. J.	30	72.2	4.5	6.3	15.2	1.5	9.9	40.9	9.7	23.7	10.8	1.2	11.1
G. G.	23	68.4	4.1	6.0	17.7	2.2	12.4	40.5	6.4	15.8	11.9	1.5	12.6
V. D.	18	70.1	4.0	5.7	15.4	2.0	13.0	36.4	5.2	14.3	10.3	1.2	11.7
R. B.	13	72.1	4.3	5.9	14.1	1.8	11.7	37.9	9.8	26.9	10.2	1.0	10.0

ployed to obtain the mean values and the total ranges for different disorders.

It will be noted that, in this group of disorders, there often was a reduction in serum albumin and a considerable increase in the G.G. 33.3 fraction as contrasted with normal values. In instances where the G.G. 33.3 fraction was increased greatly, there usually was an increase in total globulin. However, the increase in total globulin was sometimes small and yet a substantial increase in the G.G. 33.3 fraction occurred, as in the case of the patients with beta hemolytic streptococcal pharyngitis.

Because of the variations in plasma volume which may occur during infections, it seems preferable when following the course of a specific dis-

TABLE III

Disease	Approximate duration	Number patients	T.P.	Alb.	Glob.	"G.G."	A/G	A/T.P.	"G.G."/A	"G.G."/G	"G.G."/T.P.
Normal controls		30	6.96	4.96	2.00	0.860	2.5	71.3	17.4	44.3	12.3
Streptococcal pharyngitis	7–14 days	13	7.27	4.73	2.55	1.280	2.1	65.3	27.2	55.0	17.8
Malaria inoculata	21–28 days	4	6.67	2.87	3.81	1.427	0.7	47.8	50.2	37.6	21.4
Chronic staph. osteomyelitis	months	1	7.10	3.81	3.29	1.850	1.2	53.7	48.7	56.5	26.2
Acute rheumatic fever	10–21 days	17	7.11	4.07	3.11	1.570	1.4	57.7	40.9	51.0	22.0
Gonococcal arthritis	10–30 days	3	7.00	3.88	3.12	1.212	1.6	55.2	31.5	38.8	17.3
Subacute bacterial endocarditis	months	2	A. 6.91 B. 6.39	3.40 4.02	3.51 2.37	1.365 1.526	1.0 1.7	49.1 63.0	40.0 38.0	39.0 64.4	19.8 23.9
Chronic rheumatoid arthritis	months-years	29	7.16	4.27	2.90	1.458	1.6	60.1	34.8	50.6	20.0
Erythema multiforme	14–21 days	3	7.40	4.07	3.37	1.617	1.2	58.7	39.4	47.7	21.5
Disseminated lupus erythematosus	unknown	3	5.68	2.45	3.56	1.901	0.7	48.3	62.3	59.2	28.5
Dermatomyositis	unknown	4	7.23	3.56	3.72	1.793	1.1	56.1	51.7	48.9	25.1
Cirrhosis of liver	unknown	9	6.57	3.92	3.38	1.591	1.0	48.6	51.2	47.8	21.9
Infectious hepatitis	6 weeks	1	8.17	3.86	4.31	1.625	0.9	47.2	42.0	37.6	20.0
Lymphogranuloma inguinale	unknown	3	8.52	4.23	4.28	2.547	1.1	50.3	61.5	58.5	29.1
Nephrotic syndrome	unknown	6	4.89	2.45	2.47	0.443	1.2	49.2	19.1	17.9	8.8

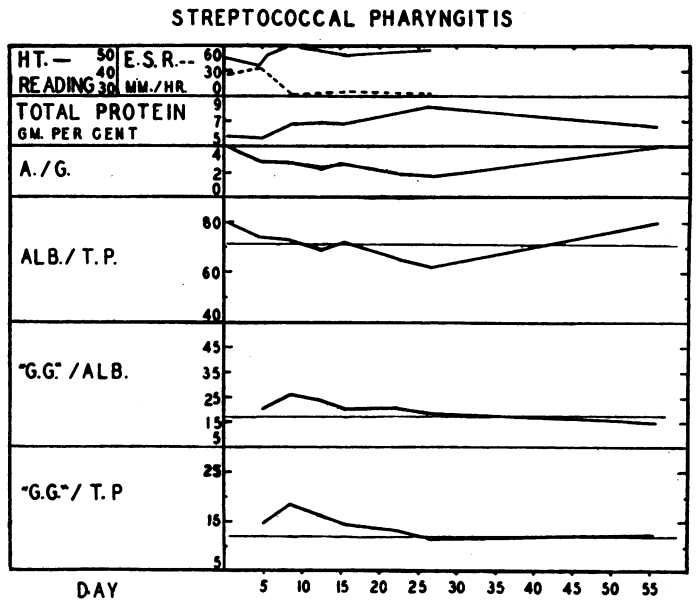

FIG. 1. NOTE THAT IN THIS UNCOMPLICATED CASE OF STREPTOCOCCAL PHARYNGITIS, PROTEIN ABNORMALITIES PERSISTED AFTER THE ERYTHROCYTE SEDIMENTATION RATE BECAME NORMAL

The mean values for the ratios of these protein constituents in normal subjects are indicated by fine horizontal lines.

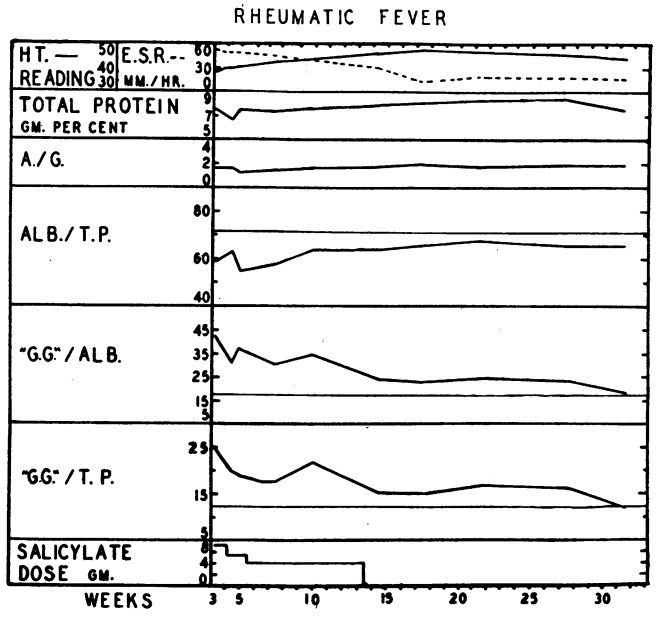

FIG. 2. IN THIS YOUNG ADULT WITH ACUTE RHEUMATIC FEVER, MODERATE ABNORMALITIES IN PROTEIN CONSTITUENTS PERSISTED FOR A PROLONGED PERIOD AFTER CLINICAL EVIDENCE OF ACTIVITY HAD SUBSIDED

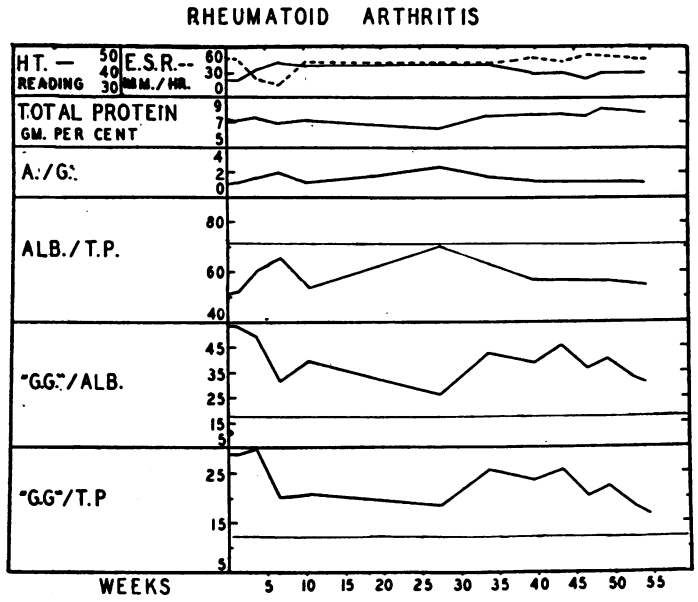

FIG. 3. IN THIS PATIENT WITH CHRONIC ARTHRITIS, ABNORMALITIES IN PROTEIN CONSTITUENTS INCREASED DURING PERIODS OF INTENSE ACTIVITY AND DIMINISHED DURING PERIODS OF RELATIVE QUIESCENCE OF THE PROCESS

ease to give attention to the ratios of various protein fractions to each other rather than the absolute values. For detecting qualitative alterations in serum protein during acute and chronic infections, the ratio of G.G. 33.3 to albumin usually gives the most striking abnormality since each component tends to change in a different direction. In certain pathologic sera as in normal sera, the albumin value, obtained with the sodium sulfate technique, may be excessively high as compared to electrophoretic measurements (4, 10, 11).

The increases in G.G. 33.3 which we have observed in such diseases as malaria, beta hemolytic streptococcal pharyngitis, acute rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus, cirrhosis of the liver, infectious hepatitis and lymphogranuloma inguinale are qualitatively similar to the increases that have been observed electrophoretically in these disorders by other investigators (4, 12 to 19). Likewise the decrease in this constituent in the nephrotic syndrome is in agreement with electrophoretic findings (10, 20).

Serial determinations of protein fractions by chemical means during specific illnesses

In a number of diseases, the electrophoretic pattern of the plasma or serum has been determined repeatedly during the course of the illness in individual cases. In Figures 1 to 4 are presented changes in protein constituents as determined by chemical measurements in a few selected cases of specific diseases. It is not pertinent at this time to discuss the clinical significance of these changes but rather to indicate that they correlate at least qualitatively with the changes in similar cases in which repeated electrophoretic measurements have been obtained.

In Figure 1 the protein changes in an uncomplicated case of streptococcal pharyngitis are shown. In this particular case the protein abnormalities persisted for a considerable period after clinical recovery had ensued and the erythrocyte sedimentation rate had become normal. This pattern is somewhat similar to that obtained by Dole and coworkers (13) in electrophoretic studies made during the course of scarlet fever.

Figure 2 illustrates the changes in protein con-

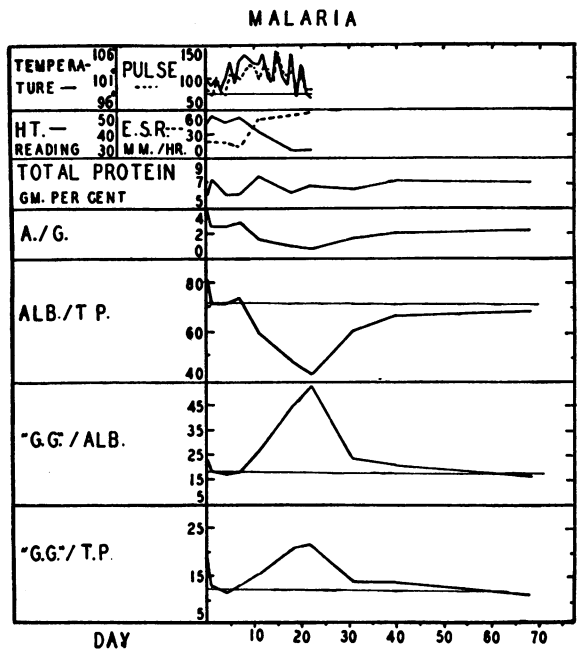

FIG. 4. THIS PATIENT WITH MALARIA INOCULATA (PLASMODIUM VIVAX) DEVELOPED MARKED ABNORMALITIES IN THE PLASMA PROTEINS DURING THE FEBRILE ILLNESS

These changes slowly subsided after the fever was terminated on the 22nd day by administration of quinine.

stituents as determined chemically in a somewhat protracted case of acute rheumatic fever. In this instance, the "gamma globulin" remained elevated for a number of weeks after evidence of clinical activity had subsided and after the erythrocyte sedimentation rate had become normal. Somewhat similar observations were made in serial electrophoretic determinations of protein constituents in this disease by Dole and coworkers (13) and by Rutstein and colleagues (14).

The alterations in protein constituents in a protracted case of rheumatoid arthritis are presented in Figure 3. In this patient who was followed for one year, there were great fluctuations in the intensity of the disease as evidenced by clinical observations. Throughout most of the period of observation, the erythrocyte sedimentation rate remained greatly elevated. A rough correlation existed between clinical activity of the arthritis and the degree of abnormality in protein constituents. The changes observed in this patient are somewhat similar to the electrophoretic changes observed in an arthritic patient by Dole and Rothbard (15).

Finally, Figure 4 illustrates the changes in the protein pattern of a patient with central nervous system syphilis who was subjected to malaria inoculata (plasmodium vivax). Fever was terminated on the 22nd day by quinine administration. In this patient a marked hypoalbuminemia developed during the febrile illness and was accompanied by an increase in the "gamma globulin." With subsidence of the infection, the protein constituents gradually returned toward normal values. Electrophoretic studies of protein components in similar patients have demonstrated similar findings (21).

Possible clinical usefulness of gamma globulin determinations as indicated from reported electrophoretic studies

Many of the electrophoretic findings in sera of diseased subjects have been summarized in the recent paper by Stern and Reiner (22). It has been demonstrated that many antibodies to human infections are concentrated in the gamma globulin fraction (23, 24). Significant increases in gamma globulin occur during acute and chronic infections as well as in cirrhosis and congestive heart failure. Because of the numerous disorders in which this fraction is increased, its diagnostic value, as evidenced from a single determination, appears to have limited value. Even single determinations, however, in selected instances, when taken in conjunction with other clinical studies, may offer useful information. For example, in cirrhosis of the liver, the gamma globulin is greatly increased whereas in livers involved with metastatic carcinoma, this fraction is not increased (18). The finding of an elevated rather than a reduced gamma globulin content in the nephrotic syndrome suggests an acute exacerbation of the renal disease (10). The occurrence of a greatly elevated gamma globulin in a patient, suspected of psychogenic rheumatism, would cause one to consider more seriously the possibility of rheumatoid arthritis or a related disorder. Likewise, the finding of a normal gamma globulin content in the sera of patients, suspected of having certain diseases in which this fraction usually is increased, would have some value in exclusion of such processes.

The real value of serial gamma globulin deter-

minations during the course of specific illnesses remains to be learned. From serial electrophoretic studies, it would appear that the rise and fall of gamma globulin in such diseases as beta hemolytic streptococcal pharyngitis, rheumatic fever, rheumatoid arthritis, pulmonary tuberculosis and sarcoid might be a useful aid in evaluating the activity of these diseases (13 to 15, 25, 26). In tuberculous infections at least, the gamma globulin fraction appears useful in determining the prognosis (25).

SUMMARY

From an examination of 30 normal sera, the mean values were determined for total serum protein, serum albumin, serum globulin and "gamma globulin" as determined by chemical procedures. The standard deviation and coefficient of variation for these constituents are indicated.

Similar studies were made repeatedly in 5 healthy subjects in order to determine the variation in these constituents in a given individual from time to time.

Serum albumin, serum globulin and serum "gamma globulin" were measured in a number of patients with various diseases. It is demonstrated that these values show at least qualitative similarity to electrophoretic findings in the same diseases.

The possible usefulness of serial determinations of these protein constituents during the course of specific illnesses is illustrated by a few examples.

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