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Statistical analysis of paper electrophoretic patterns in 89 cases of albumin and globulin ratio reversals

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STATISTICAL ANALYSIS OF PAPER ELECTROPHORETIC PATTERNS IN 89 CASES OF ALBUMIN AND GLOBULIN RATIO REVERSALS

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INTRODUCTION

History and Definitions

Human blood serum contains an extremely complex mixture of proteins - "simple" proteins, lipoproteins, glucoproteins - and probably hundreds of distinct protein components (52). Considerable time and effort have been expended to produce a rapid and reliable method usable for general clinical studies for the fractionation of serum proteins. Those procedures or their combinations have resolved this very large and exceedingly complex system of components into a relatively few arbitrary fractions, satisfying only certain criteria inherent in the method employed (36). By general agreement serum proteins are subdivided into four main categories as separated by electrophoretic fractionation. Those four electrophoretic fractions are not pure. All contain lipid and carbohydrate materials which probably are combined as prosthetic groups (5, 18). Albumin fractions also apparently contain bilirubin and it has been estimated that at least fifty percent of the lipid and carbohydrate content of serum is bound to the albumin and gamma globulin fractions. In addition substances like calcium, phosphorous and sulfonamide drugs are also bound to the albumin fractions (14). It has been observed that the carbohydrate content of the various protein fractions differs not only from fraction to fraction but also from disease to disease (63). By refinement of methods, several subfractions have been identified

in addition to the main fractions albumin, alpha globulin, beta globulin, gamma globulins. (For instance the alpha globulins have been separated on paper electrophoresis into two groups.) So far no general agreement has been reached in the designation of the minor component whose mobility is intermediate between the normal beta and gamma globulins. Some authors refer to this fraction as $beta_2$ globulins, but equal justification exists to name this minor fraction $gamma_1$ globulin and the normally occuring large gamma globulin fraction, $gamma_2$ globulin (27). A new subdivision is discussed by Aly (2) who mentions "tryptophanreiches praealbumin", the nature of which is not definitely identified. One also considers that some of the known beta globulin anomalies, are due to the $beta_1$ lipoproteins of Oncley (14). From this we can deduce that the whole concept of purity of protein is relative and not absolute, it has no meaning other than as a reference to the method employed and to the assumptions necessary in studying the substance under description. The nomenclature of serum proteins is determined by convention, and depends on the method employed to break down the serum proteins into various fractions. Those fractions are summarized in Table I.

The principal chemophysical methods for characterization of proteins are (10, 18, 19, 36, 37, 71, 73),

1). Neutral salts (Na_2SO_4 - Howe, Na_2SO_4 - Wolfson, Cohn, K_2SO_4 - Butler)

TABLE I.

TERMINOLOGY OF SERUM PROTEINS OF VARIOUS METHODS OF FRACTIONATION

Boundary Electrophoresis	Paper Electrophoresis	Cohn's Fractions		Ultracentrifugation		"Salting out" c $(NH_4)_2SO_4$	
		Major	Minor	Major	Minor	Major	Minor
Albumin	Albumin	V	IV-4, VI	S_{4-5}		Albumin	
Alpha-Globulin	$Alpha_1$-Globulin	IV-1	I III III-0	S_{4-5}		pseudo-globulin	euglobulin
	$Alpha_2$-Globulin	IV-1 IV-4	V VI	S_{4-5}			
Beta Globulin	Beta Globulin	III-0		four fractions		euglobulin	
	$Gamma_1$ (β_2) Globulin	III-1	II+III	S_{19}	S_7	euglobulin	pseudo-globulin
Gamma Globulin	$Gamma_2$ Globulin	II	I+III III-0	S_7	S_{8-11}	pseudo-globulin	euglobulin

Based on 5, 10, 19, 21, 27, 36.

2). Water miscible organic precipitation (Ethanol - Cohn, Methanol - Pillemer)

3). Electrophoresis

4). Ultracentrifugation

5). Immunochemical techniques

6). Immunoelectrophoretic techniques

Electrophoresis can be subdivided into boundary electrophoresis and zone electrophoresis. In boundary electrophoresis the movement and separation of boundaries are observed by optical or analytical methods (68). This was first described by Tiselius in 1937 (67). Zone electrophoresis is that method in which the fractions migrate as separate zones which are stabilized by a supporting medium against convection (68). Filter paper electrophoresis uses filter paper as a suporting medium.

Paper electrophoresis has proved to be the method of choice in the clinical separation of serum proteins. It has several advantages over the Classical Tiselius Technique (23).

1). The inexpensive, robust apparatus occupies little space.

2). Only microquantities of serum are required.

3). No preparation of serum is necessary.

Much progress has been made since the introduction of this method by Koenig in 1937. Paper electrophoresis was first used for the separation of a protein mixture by Klobusitsky and Koenig in 1939, in attempting to extract a yellow pigment from snake ven-

om (7). This work attracted relatively little attention until about ten years later several laboratories independently developed various methods of paper electrophoresis (6, 12, 16, 30, 43, 48, 69, 70). Those methods were enthusiastically received and various pathological variations of serum analyzed before the methodical basis of the system was worked out satisfactorily. Grassmann was the first who made paper electrophoresis quantitative by direct photo-electric scanning of dyed paper electrophoresis patterns of serum (31, 32). This, as well as the elution technique of quantification were described in 1951. Paper electrophoresis in its clinical aspects has expanded at a remarkable rate and the use of the technique has grown so phenomenally that this procedure has moved into the front rank of important tools in biochemistry and clinical chemical research (49). Much of the published data are not subject to quantitative comparison however; the reason for this will be discussed later.

At the present time three distinct types of filter paper apparatuses are utilized in different laboratories.

1). Inverted V types - Durrum 1950, Flynn 1951 - (16, 23)

2). Horizontal type - Wieland 1948, Cremer & Tiselius 1950 - (12, 70)

3). Vertical type - Grassmann 1950 - (30)

Those paper electrophoretic analyses separate the various protein fraction only by their mobility in an electric field (23, 34, 36). Many variables influence this property.

Variables of Method

The physical chemistry of paper electrophoresis is complex. Many different phenomena influence the fractionation and quantification. It is important to understand the variables in order to get a satisfactory grasp of the problem involved. Some of the factors which play an important part in the resolution and distribution of the migrating ion and the quantification of the various protein fractions on paper electrophoresis will be mentioned. This should give insight into the problem of why it is absolutely necessary to follow a well established method for quantitative evaluation of any electrophoretic pattern.

Characteristics of the ion. The ions in solutions have different net electric charges. At a pH of 8.6 the serum proteins behave as anions and move at a differential velocity toward the anode (23, 27, 36, 68). The various names applied to normal serum components refer, as mentioned, to the relative position of the protein fractions after electrophoresis. The apparently homogenous peaks may consist of molecules of various sizes, shapes, and chemical composition (36). Their biological significance has inherently no influence on the naming of those fractions. In addition it must be realized that the function of mobility is not only dependent on the properties, concentration and environment of a component, but is an integral function of the mobilities and concentrations of all fractions of the system (7, 36). Even the absolute

concentration of protein in a specimen may effect the final differentiation pattern and may alter the mobilities and therefore the apparent percentages (21). Pathological serum fractions may migrate to positions intermediate to the expected normal homogenous peaks, or the abnormal peaks may be superimposed on normal fractions. It is also important to realize that proteins have a tendency for associations, dissociations and amphoteric behavior.

Characteristics of the apparatus and the three main phases of electrophoresis. Pezold (55), after detailed comparative studies, came to the conclusion that Durrum's inverted V method has several advantages in comparison to the horizontal method of other authors. He mentions that hydrostatic forces help in the fractionation. This is especially noticeable in the gamma globulin fraction which has a tendency to move toward the cathode because of the electroendosmotic flow (23, 55). Also the sagging of the filter paper is prevented. The size of the chamber is important in that the buffer solution must reach the proper equilibrium between evaporation and condensation. This brings us to the three physiochemical phases involved in this system (66).

1). The porous hydrophylic water insoluable filter paper.

2). The electrolytic wetting solution.

3). The gas-vapor phase adjacent to the filter paper strip.

The filter paper matrix must be evenly matched, be of high wet strength, chemically inert to the substance to be measured, and

must keep the electroendosmotic forces low (42, 47, 68). If the paper has a tendency to absorb proteins in its fibers, a tailing factor will result. This has been found to be three to five per cent of the albumin concentration (38, 55, 60, 68). Various correction factors have been suggested but specific pre-preparation of the paper itself appears most satisfactory (7, 42). The type of buffer and its ionic strength is of major importance and was one of the earliest variables controlled in paper electrophoresis. The mobility is increased with decreasing ionic strength (36, 68). Buffer at a pH of less than 8.5 will result in poorer separation of albumin from alpha$_1$ globulins (36, 44), than buffer above a pH of 8.5. At a pH 4.0, albumin will separate into two fractions (21). If calcium is added to the solution in a specific concentration the resolution of beta globulins from gamma globulins is improved, but the separation of gamma$_1$ globulins from gamma$_2$ globulins is decreased (44). This brings us to the third physiochemical phase, that between vapor, gas and paper. Increased temperature will produce increased vapor pressure.

Characteristics of the applied field. Increased voltage produces increased temperature which will increase the evaporation and therefore influence the phase interaction mentioned above (23). Applying smaller amount of voltage will prolong the amount of time required for proper resolution of the various protein fractions (7, 44, 47, 61).

Characteristics of quantitation. Distortion of protein patterns during the drying stage by excessive amount of liquid on both dependent ends has been reported (72). Several steps have been instituted to avoid this pitfall. Those steps include the application of a wick and rapid drying procedures in an oven above 110° C. (7). The selection of the proper dye is of maximal importance. Much detailed and excellent work has been done in this area (7, 38, 42, 45, 47, 53, 55). The quantitative aspects of the interaction between denatured proteins and the dyes and the measurement of dye uptake has given repeated difficulties in interpretation, since no stoichiometric relationship exists. The rate of dye absorption varies for the different protein fractions and also depends on the duration of heat denaturation of the protein, the length of staining, and the amount of rinse. The simplified staining procedure of Connerty (10) changes the oven drying step without testing its effect on pathological sera. The most commonly used dye today is bromphenol blue, introduced by Durrum in 1949. This is the only dye found to be homogenous on paper chromatography, which can be completely eluded from protein free filter paper, and still has excellent drying oven properties (7, 53, 55). Turba introduced Azocarmine B in 1950, and Grassmann Amidoschwarz 10 B in 1951. None of the latter two colors has the three excellent characteristics mentioned for bromphenol blue.

Correction factors have been proposed not only for the var-

ious dyes but also for the various methods. None of those are acceptable, because those correction factors are only applicable for a definite period of staining, a definite concentration of dyes and for the biochemically pure protein fraction. However, electrophoretically homogenous peaks of resolved serum proteins are chemically or biochemically not necessarily the same, and correction factors applicable to normal sera do not necessarily hold true for pathological samples (7, 42, 47, 55). It has been observed that albumin binds more dye than globulin (38). Also at a higher concentration of albumin, densitometers fail to follow Beer's law and no linear logrithmic relation is achieved unless a specific absorption filter is utilized (7, 13, 38, 76). In case of bromphenol blue the optimal wave length is 590 lambda. Various densitometers have been utilized and the results differ very significantly. Some use monochromatic light, some have integrators built in, others require the planimetric measurements of the pattern by hand. In most it is necessary to make the filter paper transparent but not in all. According to Pieper (56) the available automatic machines show more unreliability in repeated measures and their integrator is less precise in the calculation of the per cents of each component. Another variable is the area under the peak. According to McDonald (50) the perpendicular method may differ as much as 7.5% relative to the extended curve method in the determination of the area under the peak.

From this discussion it can be deduced that it is of utmost importance to use a strict technique and to establish agreement on the method used, in order to obtain consistent results in the various laboratories. Much of the published data of normal values and other numerical relationships which do not follow the same multiple step procedure may not be subject to quantitative comparison, see Table II (7, 8, 17, 25, 29, 37, 39, 45, 55, 64, 68). Therefore, only after agreement as to the detailed procedure has been achieved can the great practical diagnostic and prognostic value of paper electrophoresis on human serum be utilized to its maximum.

Quantitative Comparison of Normal Serum Proteins

TABLE II

Method Employed	No. of Sera	Albumin %	Alpha$_1$ Globulin %	Alpha$_2$ Globulin %	Beta Globulin %	Gamma Globulin %	References
Grassmann + Hannig	25	61.3	4.1	8.1	11.0	15.5	Grassmann
Grassmann + Hannig	200	54.3 ± 4.7	6.2 ± 1.5	7.5 ± 1.4	11.3 ± 1.6	20.6 ± 3.5	Fuchs
Modified Grassmann + Hannig	26	51.1 ± 4.3	5.8 ± 1.4	9.4 ± 1.7	12.7 ± 1.8	21.4 ± 2.5	Sundermann
Aminco-Gordon	37	56.3 ± 2.6	5.7 ± 0.7	9.3 ± 1.3	14.7 ± 1.6	14.1 ± 2.6	Gordon
Turba + Enankel	200	61.7 ± 3.1	5.5 ± 0.7	6.8 ± 0.9	9.2 ± 1.0	16.7 ± 2.4	Fuchs
Durrum	185	68.9 ± 4.2	2.9 ± 0.9	7.3 ± 1.5	9.0 ± 1.9	12.0 ± 2.5	Jencks
Durrum	11	62.5 ± 5.2	4.5 ± 0.6	9.2 ± 2.2	9.5 ± 2.4	14.3 ± 2.1	Link

Statement of Purpose

As shown in the foregoing discussion quantitative comparison of paper electrophoresis requires conformity as to the details of the multiple step procedure. Since in the past conformity was rare, quantification of the data could not be utilized. The only exception was the ratio of the concentration of albumin relative to the concentration of globulin in the blood serum. A reversal of this ratio expressed as less than 1.0 is a relatively well established index (1, 52) and is used as a measure of the health of the patient or the progression of his disease. However, this albumin/globulin ratio is simply a summation of a complex pattern of changes of serum proteins, without the need for identification of the specific physiological or pathological reasons responsible. Abdel (1) makes the suggestion that several such ratios may be worked out and found more successful and efficient in following certain diseases. Such another ratio had been suggested by Gilliland and Stanton (26) in 1954, at which time they reported that a albumin/alpha$_2$ globulin ratio below 3.0 is suggestive of amyloid changes in the kidney and rather diagnostic for diabetes. An increase above 3.0 was felt by those investigators to be indicative of rheumatic fever.

Established indexes of this nature could be of value in the direct evaluation of quantitative paper electrophoresis without reference to total proteins and their chemical determination.

This paper is a review of 466 electrophoretic patterns. Eighty-nine were selected in which paper electrophoresis has demonstrated a reversal of the concentration of the albumin relative to the concentration of globulin. In addition to the two ratios mentioned above, it will consider eleven other ratios and evaluate their significance by statistical means and point out their values, if any.

MATERIALS AND METHODS

The electrophoretic separation of serum proteins and their staining were made using the operating instructions of the Spinco Division of the Beckman Instrument Company (62). A Durrum type electrophoretic cell, regulated power supply and a calibrated recording densitometer and automatic integrator were employed. For a summary of pertinent details see below:

A). FRACTIONATION

Cabinet: Durrum type electrophoretic cell (Model-R, Series C)

Serum: 6 lambda; Schleicher and Schuell filter paper strips (2043 A-mgl)

Buffer: pH 8.6; ionic strength 0.075; 16 hours; 85 volts; 2.5 milliampere.

B). PROCESSING

Dye: gm Bromphenol blue/ l liter methanol

Drying: electric oven at 250° F. (121° C.)

Rinse: 5% glacial acetic acid

C). MEASUREMENTS

Densitometer:
- Analytrol calibrated recorder (Model RA)

Integrator:

The Durrum type chamber was used for the electrophoretic migration. The chamber contains two wicks of Schleicher and Schuell 300-029 (2 3/32 x 12½ inches) filter paper and 8 filter strips, (Schleicher and Schuell 2043 mg-l, 3.0 x 3.6 cm). This system was

saturated and equilibrated with 2.76 gm. diethyl barbituric acid and 15.40 gm. sodium diethyl barbiturate. The buffer was tested with a Beckman pH Meter model GS, and whenever necessary, adjusted with concentrated sodium hydroxide to a pH of 8.6 and an ionic strength of 0.075. The 6 lambda of serum were applied to the filter paper strips with minimal break of equilibration. The actual separation was performed in a cupboard of an air-conditioned controlled room at a temperature of 24.5° C, away from any possible drafts or air current. A direct current of 85 volts and 2.5 milliamperes was applied for 16 hours. After removal of the paper strips from the Durrum cell, they were placed in a preheated oven for 30 minutes, at a temperature of 250° F. (121° C.). This was followed by a prerinsing for 6 minutes in methanol. The staining was performed with bromphenol blue (0.1% by weight) in methanol. The filter strips were then rinsed in three different solutions of 5% by volume glacial acetic acid for 6 minutes each. The filter strips were reintroduced into the drying oven for 15 minutes at above temperature. The colors were intensified by a 15-minute exposure to ammonium vapors. The "Analytrol", which was warmed for at least 15 minutes, recorded and integrated the stained, nontranslucent filter strips. The perpendicular lines were dropped from the troughs between the peaks and the base line.

The complete procedure of fractionation, processing, measuring and recording was performed by the same two technicians, during all of the two-year period.

RESULTS

During a two-year period 22,097 patients entered the hospital; of those patients, 466 (2.1%) had their serum protein distribution analyzed by paper electrophoretic distribution. The cases in which the protein distribution revealed less than 50% albumin were selected. Those 89 cases (18.5% of all patients tested) were analyzed using statistical methods. It may be seen from Table III and IV that nearly one out of two patients who had an albumin/globulin ratio reversal had either a neoplastic condition or an infection. One out of five patients had multiple myeloma, leukemia or lymphoma. This differs from the connective tissue diseases ("collagen diseases") in which only one out of twelve patients with reversal of the albumin/globulin ratio were identified as such. It is important to note that in one out of twelve patients with this reversal, no significant disorder was identified; even so the patient was hospitalized and careful clinical and laboratory studies were performed.

The analysis of the protein electrophoretic patterns expresses its results in terms of percent of total proteins. Percents are the units given directly from the electrophoretic strip with the help of a densitometer and integrator. In this way possible errors resulting from the attempt of seeking for a universal constant or equating electrophoretic fractions with fractions achieved by other methods, have been avoided.

TABLE III.

DISTRIBUTION OF 89 CASES WITH ALBUMIN - GLOBULIN RATIO REVERSALS

A). Neoplastic Diseases - 22

1. Multiple Myeloma -- 8
2. Leukemia 6
3. Hodgkin's Disease - Lymphosarcoma -- 4
4. CA of Pancreas -- 1
5. CA of Stomach -- 1
6. Angiosarcoma -- 1
7. Fibroid Uterus -- 1

B). Infections, Wound Healing - 20

1. Wound Healing, Local Infection, Gangrene -- 10
2. Pneumonia -- 6
3. Infectious Mononucleosis -- 2
4. Boeck's Sarcoidosis -- 1
5. Infectious Polyneuritis -- 1

C). Hepatic, Biliary Diseases - 10

1. Cirrhosis -- 4
2. Hepatitis -- 4
3. Cholelithiasis -- 2
4. Cholelithiasis with Hepatitis -- 1

D). Renal Diseases - 8

1. Nephrotic Syndrome -- 4
2. Glomerulonephritis -- 4

E). Collagen Diseases - 7

1. Rheumatoid Arthritis -- 3
2. Periarteritis Nodosa -- 2
3. Lupus Erythematosus -- 1
4. Acute Rheumatic Fever - 1

F). Endocrine Diseases - 6

1. Diabetes Mellitus -- 3
2. Addison's Disease -- 2
3. Diabetes Insipidus -- 1

G). Cardiovascular Diseases - 5

1. Arteriosclerosis -- 5

H). Miscellaneous - 4

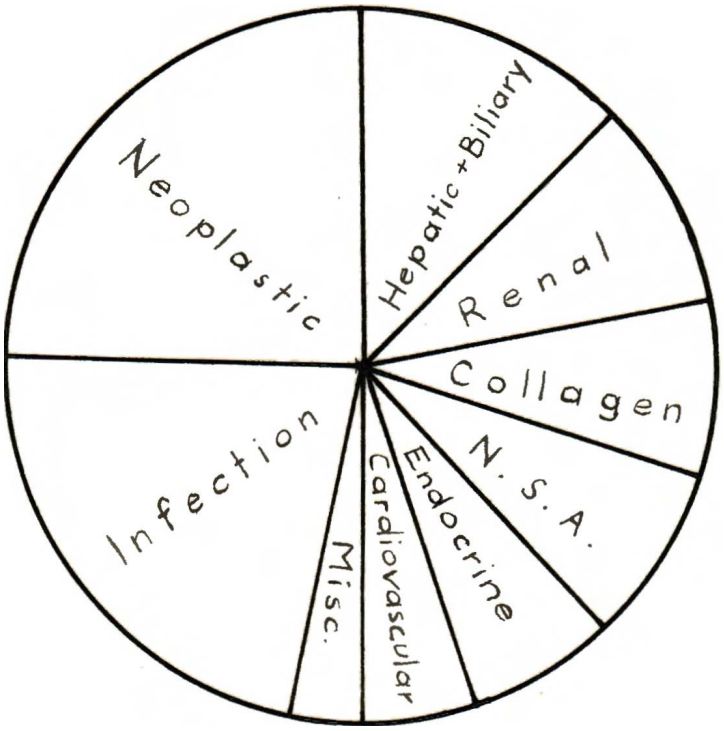
1. Urethral Stricture -- 1
2. Epilepsy -- 1
3. Ganglion (Cyst) -- 1
4. Pernicious Anemia -- 1

I). No Significant Abnormalities - 7

TABLE IV.

CONDITIONS WITH ALBUMIN - GLOBULIN RATIO REVERSALS

(N - 89)



1.	Neoplastic	24.7%	6.	No Significant Abnormalities	7.9%
2.	Infection	22.5%	7.	Endocrine	6.7%
3.	Hepatic, Biliary	11.2%	8.	Cardiovascular	5.6%
4.	Renal	9.0%	9.	Miscellaneous	4.5%
5.	Collagen	7.9%			

It has been pointed out previously that correction factors are not universally applicable to all types of serum, and that the attempt of comparing various methods may introduce gross errors. Nevertheless one must realize that results expressed in terms of percentage have one peculiar property. A change in one fraction will lead to a complimentary change in the percentage distribution of all other fractions. If the percentage change varies with different diseases in the different fractions and if this change is of statistical significance this could be picked up by various ratios.

The data was statistically analyzed with the following assumption. The distribution of various normal protein components follows the normal Gaussian curve. The arithmatic mean of each ratio was calculated and the standard deviation was expressed, using small sample techniques. To determine the significance between the differences of the means of each ratio, the standard error of the difference between two means was considered. The significance of this comparison was measured by the t-test and the probability of chance occurance of equal or greater value was expressed by p-scores.

Of the thirteen ratios analyzed (Table V), six can be considered to be of no value in terms of diagnostic separation of any of the diseasesunder consideration. The albumin/alpha$_2$ and gamma globulin ratio appears in its diagnostic significance very similar to the albumin/globulin ratio, except that the standard deviation

Mean and Standard Deviation of thirteen Electrophoretic Indices

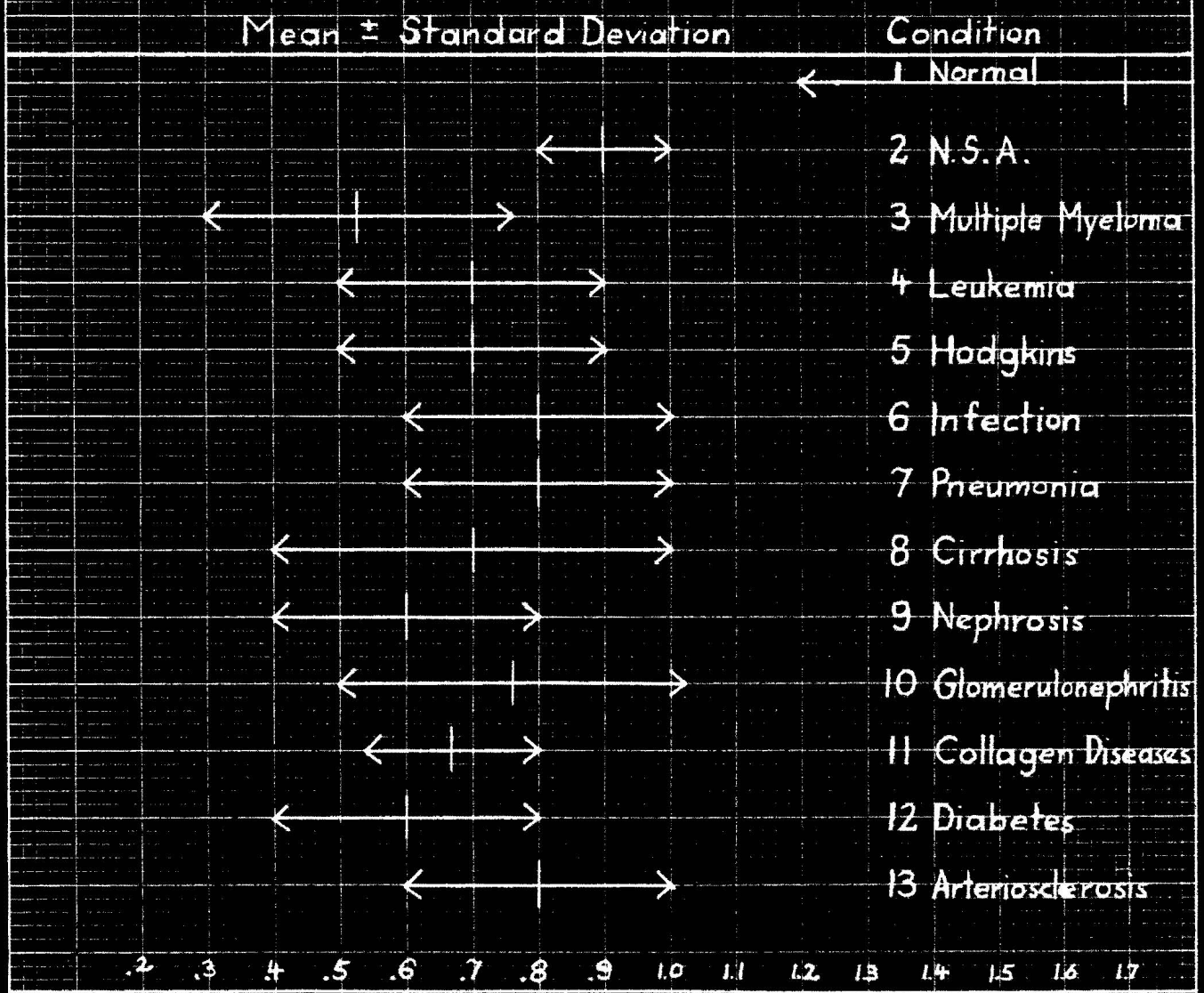
TABLE V

Ratio	Normal	N.S.A.	Multiple Myeloma	Leukemia	Hodgkins	Infection	Pneumonia	Cirrhosis	Nephrosis	Glomerulo-nephritis	Collagen Diseases	Diabetes	Arterio-sclerosis
Albumin/Globulin	1.7 ± .5	0.9 ± .1	0.5 ± .2	0.7 ± .2	0.7 ± .2	0.8 ± .2	0.8 ± .2	0.7 ± .3	0.6 ± .2	0.8 ± .3	0.7 ± .1	0.6 ± .2	0.8 ± .2
Albumin/Beta+Gamma Gl.	2.7 ± .7	1.5 ± .1	0.8 ± .6	1.3 ± .6	1.1 ± .4	1.3 ± .3	1.2 ± .3	1.0 ± .5	1.6 ± .2	1.2 ± .4	1.3 ± .4	0.9 ± .3	1.3 ± .2
Albumin/Alpha$_2$ Globulin	7.4 ± 3.0	3.6 ± .4	3.2 ± 1.4	3.1 ± 1.0	2.6 ± 1.1	2.9 ± .9	3.4 ± 1.1	5.6 ± 1.3	1.4 ± .9	3.2 ± 1.4	2.8 ± .9	1.9 ± .4	3.4 ± .8
Albumin/Gamma Globulin	4.5 ± 1.0	2.4 ± .2	1.3 ± .8	2.0 ± 1.1	1.7 ± .6	2.4 ± .9	1.7 ± .7	1.4 ± .8	2.8 ± .5	1.8 ± .7	1.5 ± .4	1.7 ± .6	2.3 ± .7
Albumin/Alpha$_2$+Beta Gl.	3.6 ± 1.4	1.9 ± .3	1.8 ± .8	1.6 ± .5	1.4 ± .5	1.6 ± .5	1.8 ± .4	2.0 ± .4	1.0 ± .5	1.8 ± .7	1.5 ± .4	0.9 ± .3	1.6 ± .3
Albumin/Alpha$_1$+Alpha$_2$ Gl.	4.8 ± 1.5	2.3 ± .3	2.1 ± .9	1.9 ± .8	1.8 ± .8	2.1 ± .5	2.4 ± .8	3.6 ± 1.3	1.2 ± .7	2.1 ± 1.0	2.0 ± .7	1.5 ± .3	2.3 ± .4
Albumin+Alpha$_{1+2}$/Gamma	5.5 ± 1.1	3.5 ± .4	2.1 ± 2.0	3.2 ± 1.9	2.8 ± 1.0	3.6 ± 1.2	2.5 ± 1.0	1.8 ± 1.1	6.0 ± 2.1	2.7 ± .9	2.3 ± .8	2.9 ± .7	3.2 ± .8
Albumin/Alpha$_2$+Gamma Gl.	2.7 ± .7	1.5 ± .1	0.8 ± .4	1.1 ± .3	0.9 ± .3	1.3 ± .4	1.2 ± .3	1.0 ± .5	0.9 ± .4	1.1 ± .3	1.2 ± .5	0.9 ± .2	1.4 ± .4
Alpha$_2$+Beta/Gamma Gl.	1.3 ± .3	1.3 ± .3	0.9 ± .9	1.3 ± .7	1.3 ± .6	1.6 ± .7	1.0 ± .4	0.6 ± .4	3.5 ± 1.7	1.1 ± .4	1.0 ± .4	1.9 ± .4	1.4 ± .3
Beta Gl./Gamma Gl.	0.7 ± .2	0.6 ± .2	0.4 ± .3	0.5 ± .2	0.5 ± .6	0.7 ± .4	0.4 ± .2	0.4 ± .2	0.8 ± .1	0.5 ± .2	0.4 ± .1	1.0 ± .2	0.7 ± .3
Alpha$_{1+2}$+Beta/Gamma Gl.	1.6 ± .3	1.7 ± .4	1.2 ± 1.1	1.7 ± 1.0	1.7 ± .9	2.0 ± .8	1.2 ± .4	0.8 ± .4	4.0 ± 2.0	1.4 ± .5	1.2 ± .5	2.1 ± .4	1.7 ± .5
Gamma Gl./Alpha$_2$ Gl.	1.6 ± .5	1.5 ± .2	5.1 ± 5.2	2.0 ± 1.3	1.8 ± 1.5	1.4 ± .8	2.2 ± .8	2.9 ± 1.2	0.5 ± .3	1.9 ± .8	2.0 ± .7	1.1 ± .3	1.5 ± .2
Beta+Gamma/Alpha$_{1+2}$	1.8 ± .4	1.6 ± .2	3.8 ± 3.1	1.8 ± .9	1.8 ± 1.2	1.6 ± .6	2.2 ± .6	6.9 ± .9	0.8 ± .4	1.8 ± .6	1.6 ± .4	1.7 ± .3	1.8 ± .2

Table VI

Distribution of $\frac{\text{Albumin}}{\text{Globulin}}$ Ratio

in 89 cases with albumin < 50% of total serum proteins

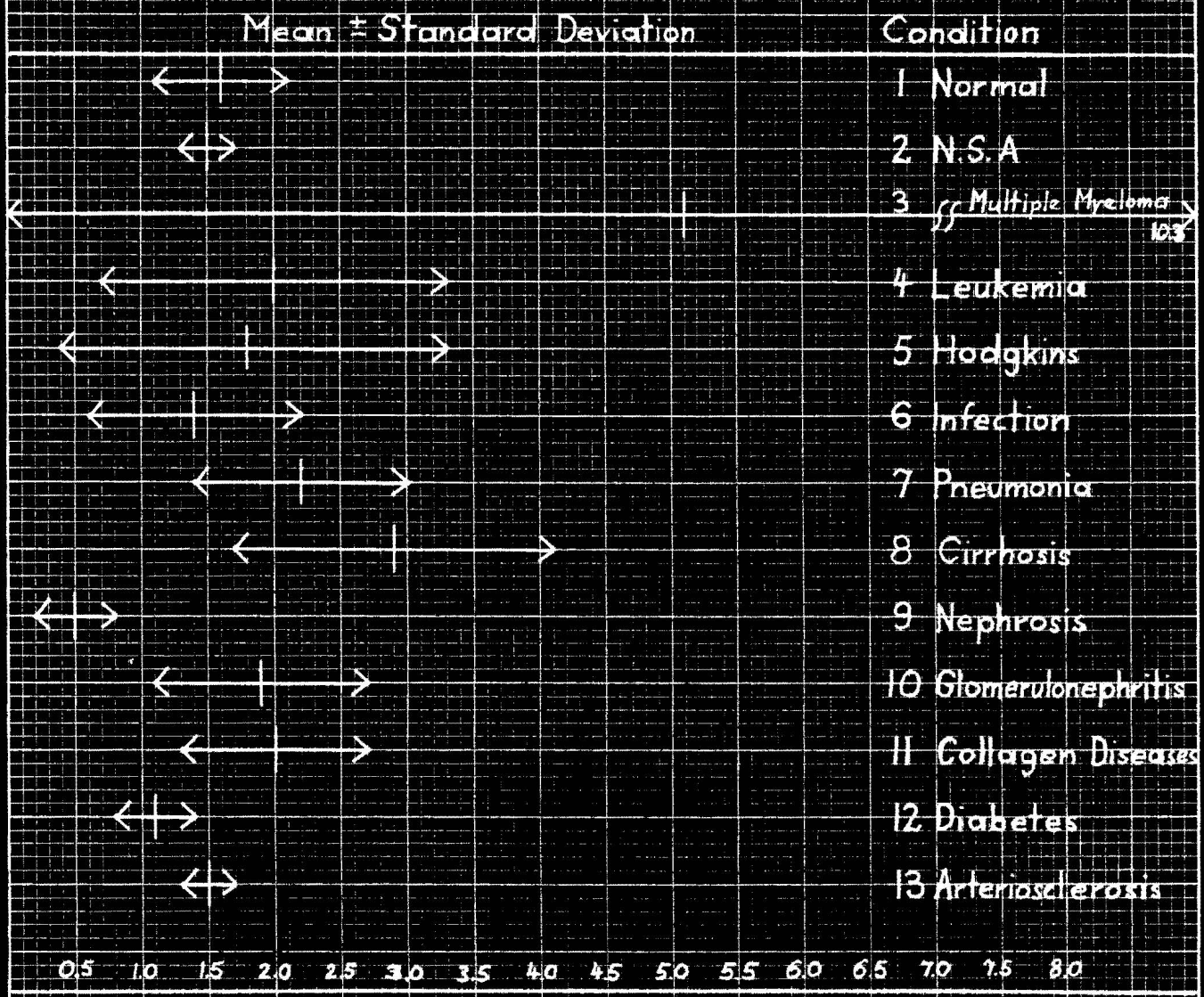


Probability p	Standard error of the difference between two means
<.001	Normal - Cirrhosis

Table VII

Distribution of $\frac{\text{Gamma Globulin}}{\text{Alpha}_2 \text{ Globulin}}$ Ratio

in 89 cases with albumin < 50% of total serum proteins

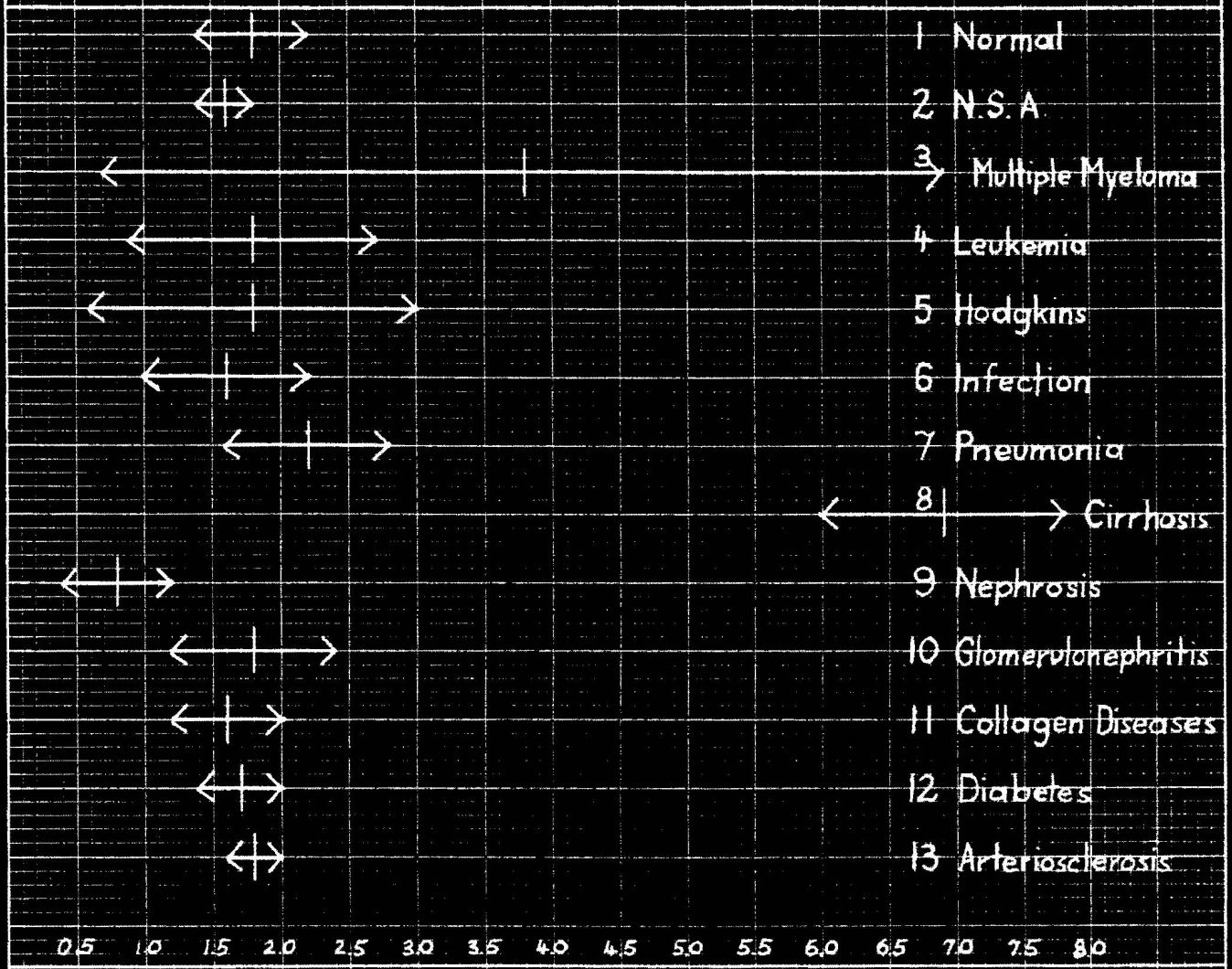


Probability p	Standard error of the difference between two means
< .001	N.S.A. – Nephrosis
< .01	Infection – Nephrosis
.02	Multiple Myeloma – Nephrosis

Table VIII

Distribution of $\frac{\text{Beta + Gamma Globulin}}{\text{Alpha}_1\text{ + Alpha}_2\text{ Globulin}}$ Ratio

in 89 cases with albumin < 50% of total serum proteins

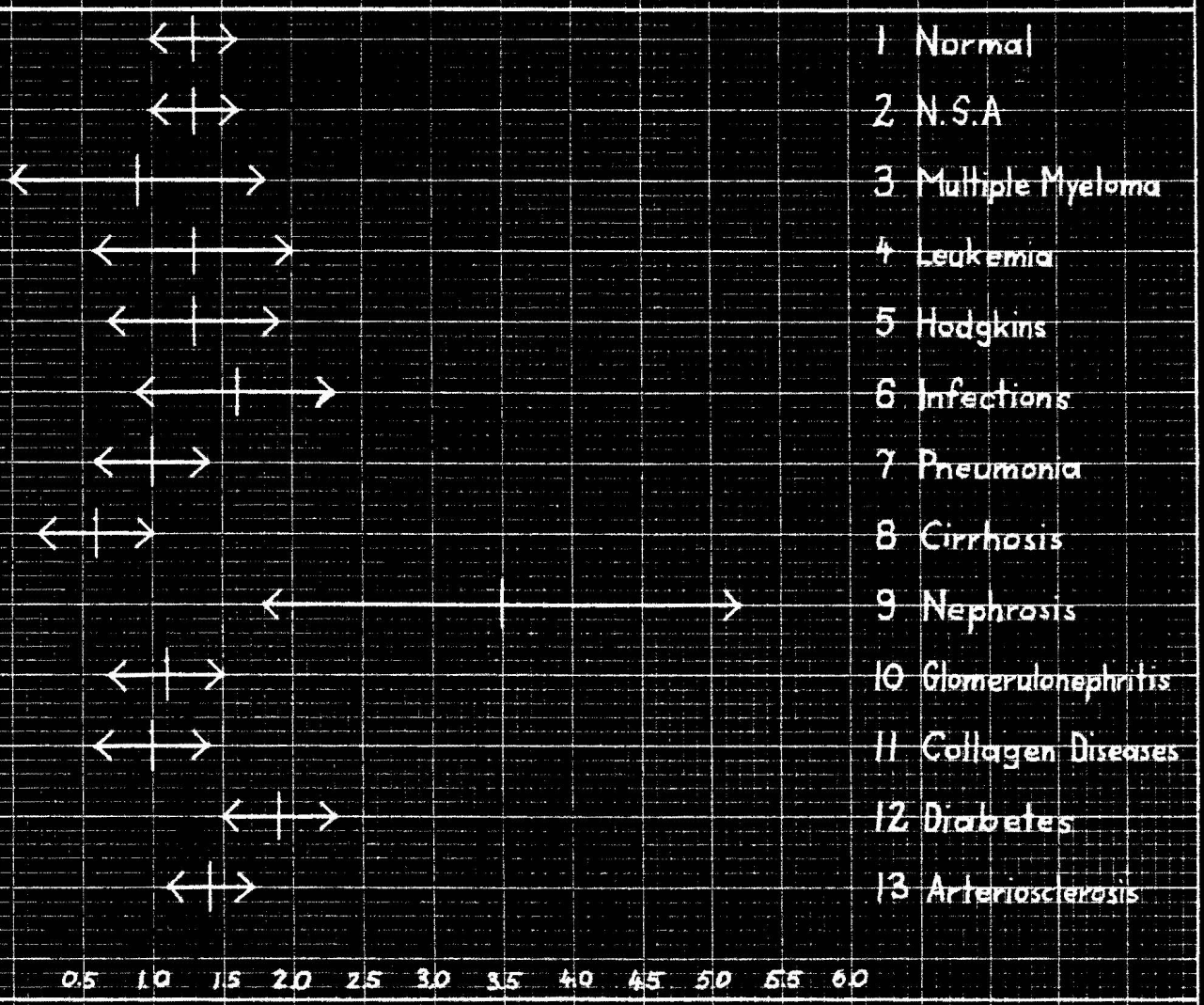


Probability p	Standard error of the difference between two means
< .01	N.S.A. – Nephrosis
< .001	N.S.A. – Cirrhosis
.02	Cirrhosis – Multiple Myeloma
< .001	Cirrhosis – Pneumonia

Table IX

Distribution of $\frac{\text{Alpha} + \text{Beta Globulin}}{\text{Gamma Globulin}}$ Ratio

in 89 cases with albumin < 50% of total serum proteins

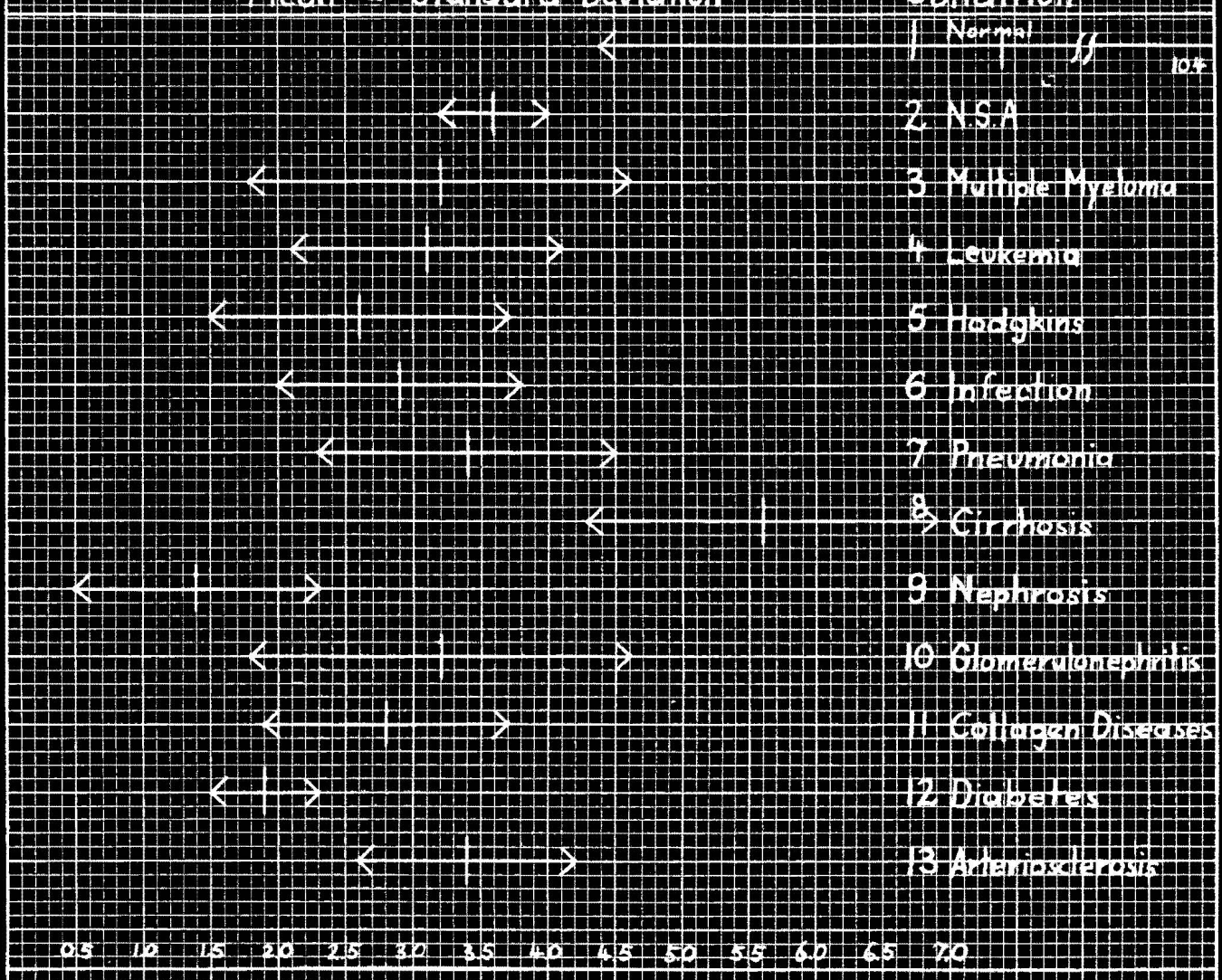


Probability p	Standard error of the difference between two means
.01	N.S.A. – Cirrhosis
.02	N.S.A. – Nephrosis

Table X

Distribution of $\frac{\text{Albumin}}{\text{Alpha}_2 \text{ Globulin}}$ Ratio

in 89 cases with albumin < 50% of total serum proteins

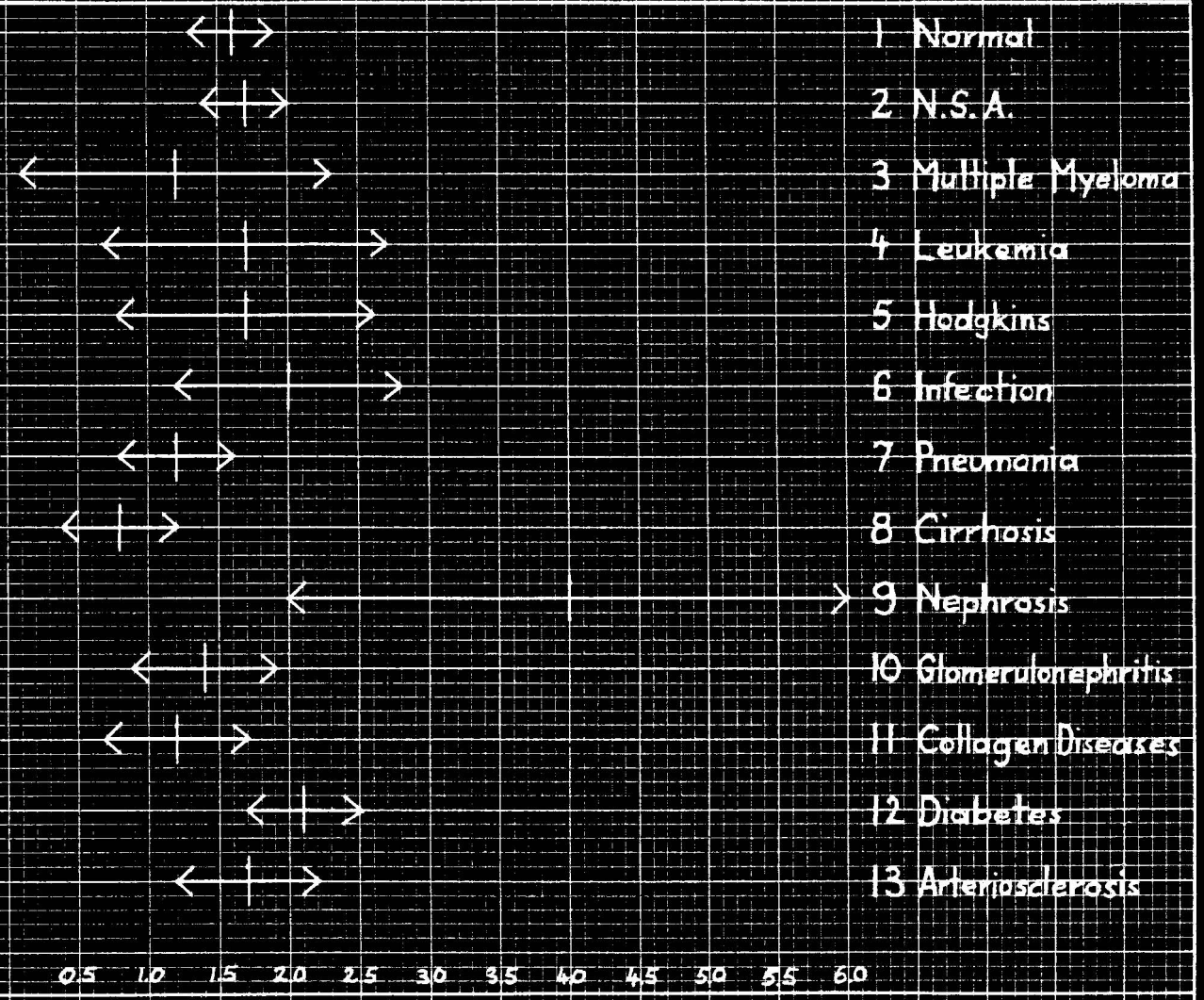


Probability P	Standard error of the difference between two means
< .001	N.S.A. – Diabetes
.02	Infection – Diabetes
< .01	Cirrhosis – Infection
< .02	Cirrhosis – Pneumonia

Table XI

Distribution of $\frac{\text{Alpha}_{1\&2}+\text{Beta Globulin}}{\text{Gamma Globulin}}$ Ratio

in 89 cases with albumin < 50% of total serum proteins



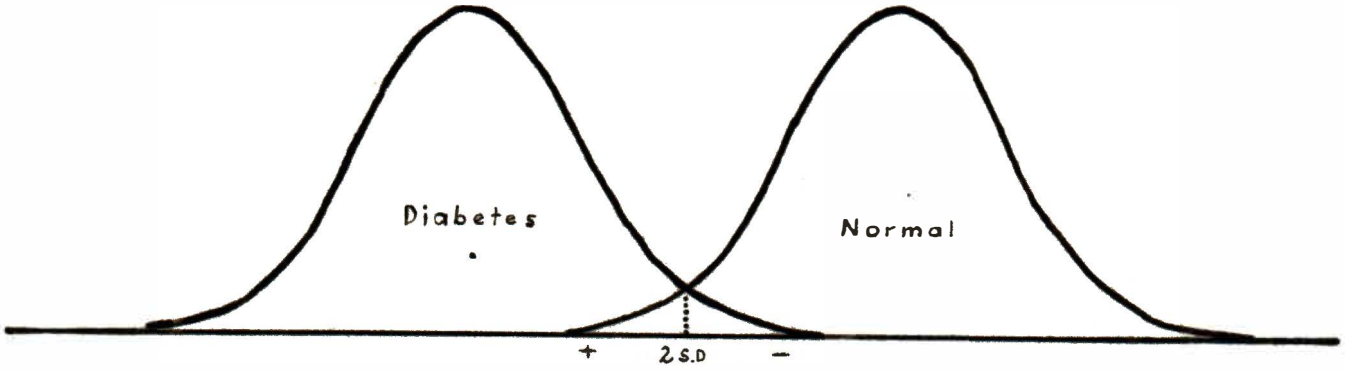
Probability p	Standard error of the difference between two means
.001	N.S.A. – Cirrhosis
> .02	N.S.A. – Nephrosis
> .10	Cirrhosis – Pneumonia

of the various conditions appears significantly larger and therefore gives less reliability than the well established index. It is interesting to notice that an albumin concentration of less than 50% only appears to influence those ratios in which the albumin concentration is used as one of the factors. In five ratios in which the albumin concentration has been excluded, no significant difference can be found between the normal group and the so-called "norm group." In the so-called normal group the albumin percent range in 95% of the cases is between 52.1 - 72.9%. The "norm group" are those cases in which albumin was below 50% even so no significant abnormality could be identified. This so-called "norm group" will be utilized for comparison with the other cases in which an albumin/globulin ratio reversal has occurred. Having excluded seven of the indices due to obvious insufficient separation of the various diseased conditions, let us concentrate on the six remaining ratios. (See Table VI to Table XI.) It can be seen from those tables that the expression of a statistical significant difference between the disease and the "norm group" is of importance, but not necessarily diagnostic. This concept can be expressed more adequately by the calculation of sensitivity and specificity of the various ratios. By sensitivity we have a measure of the percent of cases positively identified with a specific disease. In contrast, specificity expresses the percent of cases which, not having the disease under question, will be excluded by this ratio. As an example, let us

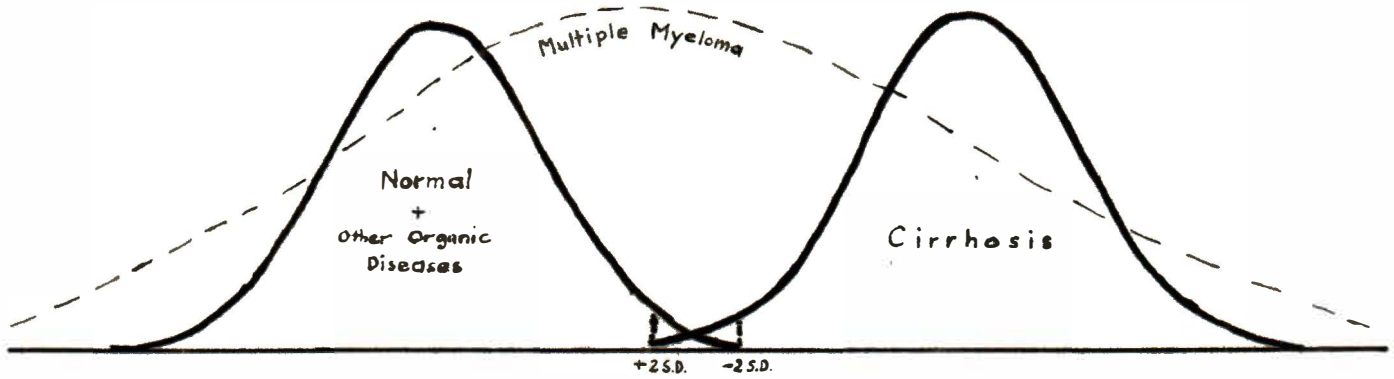
consider the albumin/alpha$_2$ globulin ratio in which the difference between the "norm" and the diabetic group is statistically highly significant at a p-value of .001. Nevertheless, this ratio does not separate diabetes from nephrosis, collagen diseases, glomerulonephritis, Hodgkin's disease, infections and leukemia. This can be expressed by a 10% sensitivity for organic diseases and a 95% specificity for excluding normal cases. This type of sensitivity appears to be of little clinical value. On the other hand, we are able to find a statistically significant ratio between normal and cirrhotic on one hand and the other cases with albumin/globulin ratio reversal. At a dividing point of 4.5, 80% of the normal and cirrhotic patients will be above, while 80% of the organic disease with albumin and globulin ratio reversals were below this point. The different tables refer to the various relationships of the remaining six ratios, and Table XIII summarizes the data.

The change in concentration of the various serum protein fractions in the 89 cases is shown in Table XIII. Again the "norm group" has been used as a reference point, and only those changes are recorded in which the mean of the disease group falls outside of two standard deviations of the "norm group". It can be seen that in the differential diagnosis of glomerulonephritis and nephrosis the electrophoretic pattern may be helpful. The marked increase of alpha$_2$ globulins and the decrease in gamma globulins is distinctive. Similarly in cirrhosis the marked decrease in alpha$_2$

TABLE XII.



Distribution Curve of Cases with < 50% of Albumin on Utilization of the Albumin/Alpha$_2$ Ratio.



Distribution Curve of Cases with < 50% of Albumin on Utilization of the Beta + Gamma Globulin/Alpha$_{1\&2}$ Globulin Ratio.
(This ratio does not appear to change in cases with Albumin >50%.)

TABLE XIII.

DIAGNOSTIC DIVIDING POINTS OF VARIOUS ELECTROPHORETIC INDICES

in 89 cases with albumin less than 50% of total serum proteins.

	Ratio	Dividing Point	Condition	Sensitivity	Specificity
1.	Albumin / Globulin	 1.0	not diagnostic organic disease	20%	90%
2.	Gamma Globulin / Alpha$_2$ Globulin	 5.0 0.5	multiple myeloma not diagnostic organic disease	50% 10%	95% 95%
3.	Beta & Gamma Gl. / Alpha$_1$ & $_2$ Gl.	 4.5 1.0	cirrhosis multiple myeloma not diagnostic organic disease	95% 40% 10%	95% 95% 95%
4.	Alpha$_2$ & Beta Gl. / Gamma Globulin	 3.2	nephrosis not diagnostic	55%	95%
5.	Albumin / Alpha$_2$ Globulin	 4.5 1.5	normal cirrhosis not diagnostic nephrosis	80% 50%	80% 80%
6.	Alpha$_1$ & 2 Beta Gl. / Gamma Globulin	 3.8	nephrosis not diagnostic	55%	95%

Key: not diagnostic 

organic disease 

diagnostic 

TABLE XIV.

CHANGES IN PROTEIN FRACTIONS IN VARIOUS DISEASES

in 89 cases of albumin concentration less than 50%.

Condition	Albumin	Alpha$_1$-Gl.	Alpha$_2$-Gl.	Beta Gl.	Gamma Gl.
N.S.A. ("Norm group") (Mean ± 2 S. D.)	44.0-51.6	4.4-10.0	11.3-15.3	6.0-17.6	16.2-23.4
Glomerulonephritis	↓	—	—	—	↑
Nephrosis	↓	—	↑↑	—	↓
Arteriosclerosis	—	—	—	—	—
Infection	↓	—	↑	—	—
Pneumonia	↓	—	—	—	↑
Cirrhosis	↓	—	↓↓	—	↑
Multiple Myeloma	↓↓	—	—	— ↑↑	—
Diabetes	↓↓	—	↑↑	↑	—
Leukemia	↓	—	—	—	↑
Hodgkin's	↓	—	↑	—	↑
Collagen Disease	↓	—	—	—	↑

Key:

1). Single arrow - Mean of disease group outside of two standard deviations of "norm" group. (50% of disease group outside the range of 98% of "norm group")

2). Double arrow - Mean ± S.D. of disease group outside of two standard deviations of "norm" group. (84% of disease group outside the range of 98% of "norm group")

globulins and the increase in gamma globulins appear to be specific for this condition. The changes in multiple myeloma and certain types of diabetes also appear to be highly significant in relation to the "norm group". The other alterations appear to be significant but not specific for the disease under question.

DISCUSSION

In various disease conditions changes in the serum protein pattern, as analyzed by paper electrophoresis, may manifest an increase or a decrease in the concentration of the normal components; this is known as disproteinemia. It may also reveal serum proteins which are not seen under normal conditions; this is known as paraproteinemia (21). It is generally recognized that the most significant information gained from paper electrophoretic analysis of serum proteins reveals the severity or the prognosis of a specific disease (39, 57). Its use as a diagnostic instrument is somewhat more limited.

Albumin concentration is rarely increased except in cases of acute dehydration or shock. The most common disproteinemia is a decrease in the albumin concentration of the serum protein. This was the factor used in selection of the cases under discussion. This decrease may have three main physiological reasons (26, 52, 57, 77).

1). Deficient intake of proteins.

An absolute deficiency of albumin and other proteins exists in cases of malabsorption syndromes, such as sprue, cystic fibrosis, celiac disease, and in cases of starvation.

A relative deficiency may occur in cases in which the need is greater than normal, such as may be seen in pregnancy, lactation, growth, hyperthyroidism and diabetes mellitus.

2). Deficient synthesis of albumin.

This may occur in liver diseases.

3). Excessive loss of albumin.

This may be found in the nephrotic syndrome, severe hemorrhage, and thermal burns (52, 57).

From this we can see that a reversal of the albumin/globulin ratio may be due to a decreased albumin concentration, while the globulin concentration may be slightly decreased, normal or increased. It also may occur that the albumin concentration is normal, while the globulin concentration is increased to such an extent as to reverse the albumin/globulin ratio. This is seen in multiple myeloma.

The $alpha_1$ globulin is relatively constant in most cases (77). This was found in the cases analyzed in this paper. Even so, a few reports mentioned an increase of $alpha_1$ globulins in osteomyelitis, Hodgkin's disease, and rheumatoid arthritis; they also refer to a decrease in $alpha_1$ globulins in lymphatic leukemia and chronic hepatitis. Quantitative examination and statistical evaluation have shown that those conditions appear to be nonspecific.

$Alpha_2$ globulins may increase in many conditions, but a significant decrease was only found in cirrhosis (39, 64, 77). This was found to be most significantly the case in the 89 cases analyzed. The increase in $alpha_2$ globulins is often associated with inflammation and tissue destruction and has been found to correlate with the concentration of glycoproteins in the serum and the sedi-

mentation rate (24, 39).

The beta globulins are also frequently increased, especially in nephrosis. In this condition the concentration of lipoproteins appears also increased (39). In cirrhosis with jaundice the increase in beta globulins appears to parallel thymol turbidity, if there is an increase in phospholipids and cholesterol (24). In the cases discussed, an increase in beta globulins was found in diabetes.

An increase in gamma globulins occurs frequently in cases with chronic infection, connective tissue diseases as well as liver diseases (24, 39). A decrease in this component was only found in the nephrotic syndrome and in cases of hypogammaglobulinemia. (39, 64). Those changes have been essentially the same in the cases mentioned in this paper.

The protein patterns which have been observed in most diseases have been found to be nonspecific changes in the various serum protein fractions. Usually the changes appear to be proportional to the severity and the progression of the disease, as mentioned previously. Only in five conditions were the serum protein changes found to be pathognomic.

1). Multiple myeloma:

In this condition there appears to be a paraproteinemia in which the component has a mobility close to beta and gamma globulins in 70-80% of the cases, but may advance as far as the albumin fraction (11, 15, 52, 57). Not in all cases of multiple myeloma can a

qualitative and quantitative change in the serum protein pattern by paper electrophoresis be detected (9, 21). In this disease the remarkable variability of the protein pattern appears to have little or no relation to the severity of the clinical symptoms (57). The variant mobility mentioned above, produces a wide spread of the standard deviation of many of the indices considered in this paper.

2). Nephrosis:

This condition reveals a highly atypical pattern which has been generally substantiated (21, 24, 39, 57, 64, 77). There is a marked decrease in albumin, a marked increase in alpha globulins. Occasionally electrophoretic fractionation is unable to completely resolve the $alpha_2$ and beta globulins because of the high concentration of lipoproteins (21, 39, 57). It also has been found that a decrease in gamma globulins is part of the pattern usually found (15, 39, 64, 77). The marked increase in $alpha_2$ globulins and the decrease in albumin and gamma globulin has been found in this analysis.

3). Hypogammaglobulinemia:

This disease was first described in 1952 (46). Today, close to 100 cases have been reported (28). This syndrome is characterized by extreme suggestibility to infection and a deficiency of serum gamma globulins (28, 46, 78). The condition is subdivided into congenital, acquired, and transient, with each having a distinct age level and sex incidence (28). The half-life of adminis-

tered gamma globulins in this condition appears to average 36 days (46, 57).

4). Cirrhosis:

We find a decrease in albumin, a marked decrease in alpha$_2$ globulins and an increase in gamma globulins. These findings are similar to those in the modern literature (8, 15, 64), but are at variance with the older reports relying on boundary electrophoresis (52).

5). Hypoalbuminemia:

This extremely rare abnormality has been referred to by Grassmann (33) and Gitlin (28). This condition appears to be familial, and is characterized by edema of unknown origin, increased sedimentation rate in an otherwise healthy individual. No case of this condition has been found in this survey.

Nonspecific changes in serum proteins were found in most other conditions (21). The most characteristic change in connective tissue diseases is a decrease in albumin and an increase in gamma globulin (52, 64). The specific protein for L. E. cell phenomena, was associated with the gamma globulin fraction (23). In addition a minor increase of alpha$_2$ globulins has been reported (24, 57). This increase was not found to be significant in our series. In rheumatoid arthritis there appears to be general agreement that the albumin is decreased and the alpha$_2$ and gamma globulin is increased (39, 52, 59). Endocrine disorders also reveal changes

in the protein patterns mostly nonspecific. Only in some cases of diabetes is the protein pattern similar to nephrosis except for the nonspecific changes in gamma globulin. In the average case well under control the protein patterns should be near normal (57). In other conditions the administration of ACTH or steroids should not only produce a leukopenia but an increase of beta and gamma globulins. Leukemias demonstrate a nonspecific reaction and the changes appear proportional to the severity and progression of the disease (3, 21, 57, 58). A few case reports of chronic lymphocytic leukemia with agammaglobulinemia have been reported (40). In Hodgkin's and lymphosarcoma the various reports (3, 15, 21, 39, 57, 77), appear to vary from each other, thus pointing to the nonspecific response of the systemic reaction produced by those diseases. In addition, clinical and morphological transition forms occur between lymphatic tumors and plasmacytomas (4, 15). In infectious and inflammatory diseases the decrease in albumin and increase in globulins have been demonstrated indirectly by the sedimentation rate (21). It appears to be debatable from the literature whether the concept of increased $alpha_2$ globulins in acute conditions and increased gamma globulins in chronic conditions has scientific basis. It appears more likely that those changes depend more on the nature of the disease (15, 24, 52, 64). In the 89 cases discussed, the $alpha_2$ globulins were increased in general tissue infections while the gamma globulins were increased in pneumonia. Glomerulonephritis appears to reveal non-

specific changes, but those nonspecific changes are in distinct opposition to the findings in nephrosis. Arteriosclerosis, malignancies and malnutrition (8, 21, 39, 51, 52), show variable features and the changes are not diagnostic.

Serum electrophoretic pattern analysis with the somewhat more elaborate filter paper system capable of quantitation of protein components by scanning with a photodensiometer and automatically plotting the peaks with an integrator, is rapidly gaining favor and replacing chemical fractionation (15, 59). In addition, the determination of lipoproteins and glucoproteins is gaining in importance (33). Those methods must be considered as important laboratory tools available to the physician. They are not only of value in the diagnosis of various diseases, but give an indication of the severity and prognosis of the patient's condition. This test measures the changes of the protein fractions in a more direct manner than the sedimentation rate, thymol-turbidity, and cephalin flocculation.

SUMMARY

After a short discussion on the history and methods of serum protein fractionation, it was pointed out that it is necessary to have agreement on the multiple step procedure on paper electrophoretic analysis of serum proteins, before quantitative measure from one laboratory to the next can be compared. The pattern of human serum proteins has been determined by paper electrophoresis in 466 cases representing various pathological conditions. Eighty-nine cases in which the concentration of albumin was less than 50% were selected and statistically analyzed. Nearly 50% of the patients with albumin/globulin ratio reversal had either a neoplastic condition or an infection. Thirteen different ratios were analyzed and their significance determined. In addition, the percentage changes of the various protein fractions were reported if they were found to be of significance. It was discussed that paper electrophoresis was diagnostic for nephrosis in which a decrease in albumin and gamma globulin and a marked increase in $alpha_2$ globulins occured. This was most reliably expressed in the ratio of $alpha_2$ and beta globulins/gamma globulins ratio. A quantitative dividing point of 3.2 revealed a sensitivity of 55% and a specificity of 95%. In cirrhosis a decrease in albumin and a marked decrease in $alpha_2$ globulins with an increase in gamma globulins were noted. The ratio found to be most reliable was beta and gamma globulins/$alpha_1$ and $alpha_2$ globulins. The quantitative dividing point was found to be 4.5 At

this point excluding multiple myeloma, the sensitivity for cirrhosis was 95%, the specificity also was 95%. Multiple myeloma was characterized by its variable globulin peak, in addition to a decreased albumin concentration. The ratio most diagnostic was gamma globulin/alpha$_2$ globulin. At a dividing point of 5.0 the sensitivity was 50%, specificity 95%. A short discussion over the various nonspecific changes in the serum protein fractions was given.

CONCLUSION

1). Eighty-nine cases in which the concentration of albumin was less than 50% were selected from 466 paper electrophoretic patterns. Thirteen different ratios as well as variations in each fraction are analyzed and their significance is evaluated.

2). The changes in the serum protein fractions of multiple myeloma, cirrhosis and nephrosis are specific.

3). Multiple myeloma is characterized by its variable globulin peak and a decreased albumin concentration. The index of gamma globulin/alpha$_2$ globulin at a quantitative dividing point of 5.0 shows a sensitivity of 50% and a specificity of 95% for multiple myeloma.

4). Cirrhosis is characterized by a decrease in albumin concentration, a marked decrease in alpha$_2$ globulin, and an increase in gamma globulin. The index, beta and gamma globulins/alpha$_1$ and alpha$_2$ globulins at a quantitative dividing point of 4.5 shows a sensitivity of 95% and a specificity of 95% for this disease if multiple myeloma is excluded.

5). Nephrosis is characterized by a decrease in albumin and gamma globulin concentration in addition to a marked increase in alpha$_2$ globulins. The index, alpha$_2$ and beta globulins/gamma globulins at a quantitative dividing point of 3.2 shows a sensitivity of 55% and a specificity of 95% for this disease.

APPENDICES

APPENDIX I

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH NO SIGNIFICANT ABNORMALITIES

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
63334	40	F	49.4	4.4	11.8	13.2	21.2
63403	17	F	46.3	6.9	14.1	11.1	21.5
63994	28	F	49.7	7.2	14.1	9.8	19.2
64028	55	F	48.3	8.4	12.6	10.7	19.9
64387	17	F	46.2	8.6	14.1	11.1	20.0
64615	53	F	45.1	7.1	13.9	17.7	16.2
65050	33	M	49.6	7.8	12.5	9.3	20.8
Mean			47.8	7.2	13.3	11.8	19.8
Standard Deviation±			1.9	1.4	1.0	2.9	1.8
Mean± 2 S. D.			44.0-51.6	4.4-10.0	11.3-15.3	6.0-17.6	16.2-23.4

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH GLOMERULONEPHRITIS

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
46164	45	M	42.7	6.8	14.5	10.3	25.6
47143	11	M	49.0	4.9	9.4	8.9	27.9
51894	53	M	29.6	10.5	16.3	15.0	28.6
64674	31	M	47.5	6.9	16.6	11.8	17.3
Mean			42.2	7.3	14.2	11.5	24.9
Standard Deviation±			8.8	2.4	3.3	2.7	5.2
Mean ± 2 S. D.			24.6-59.8	2.5-12.1	7.6-20.8	6.1-16.9	14.5-35.3

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH NEPHROTIC SYNDROME

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
47860	46	M	41.6	3.7	24.2	13.3	17.3
53757	5	F	27.0	7.8	46.0	7.9	11.3
59700	65	F	33.9	7.9	38.5	10.2	9.6
60221	2½	F	47.7	5.3	18.8	11.7	16.4
Mean			37.6	6.2	31.9	10.8	13.7
Standard Deviation±			9.0	2.0	12.6	2.3	3.8
Mean ± 2 S. D.			19.6-55.6	2.2-10.2	6.7-57.1	6.2-15.4	6.1-21.3

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH ARTERIOSCLEROSIS

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
51249	49	F	36.5	6.4	16.6	15.2	25.4
54985	50	M	48.5	4.8	12.9	16.6	17.2
61977	52	F	48.4	4.8	14.9	9.9	22.0
63248	39	M	47.5	8.2	11.2	17.4	15.6
66240	79	F	45.1	4.6	13.3	13.0	24.0
Mean			45.2	5.8	13.7	14.4	20.8
Standard Deviation±			5.1	2.0	2.1	2.7	3.8
Mean ± 2 S. D.			35.0-55.4	1.8-9.8	9.5-17.9	9.0-19.8	13.2-28.4

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH WOUND HEALING, LOCAL INFECTIONS, OR GANGRENE

Hospital Number	Age	Sex	Albumin (%)	$Alpha_1$ Globulin (%)	$Alpha_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
46080	44	M	36.8	6.2	17.1	13.5	26.5
50784	69	M	49.0	5.9	15.7	17.6	11.8
53033	33	F	47.8	5.7	17.0	13.1	16.4
53334	45	M	33.9	7.1	22.9	19.5	16.6
57977	35	M	48.6	6.1	13.5	14.2	17.6
60843	16	M	39.9	9.4	19.5	14.4	16.7
62055	20	F	41.7	11.1	16.4	11.4	19.3
62927	28	F	37.0	4.8	10.2	11.0	37.0
65050	33	M	49.6	7.8	12.5	9.3	20.8
65061	37	F	49.2	6.6	12.6	9.9	21.8
Mean			43.4	7.1	15.7	13.4	20.5
Standard Deviation‡			6.1	1.9	3.7	3.3	7.0
Mean ± 2 S. D.			41.2-55.6	3.3-10.9	8.3-23.1	6.8-20.0	6.5-34.5

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH PNEUMONIA

Hospital Number	Age	Sex	Albumin (%)	$Alpha_1$ Globulin (%)	$Alpha_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
52174	18	F	32.6	4.4	14.3	8.7	40.0
52607	63	F	38.4	4.7	14.2	8.9	33.8
52691	44	F	47.4	3.5	8.9	11.2	29.0
56892	42	F	43.0	5.0	16.2	11.6	24.2
64428	32	M	48.0	5.0	12.5	10.8	23.6
64499	37	F	48.2	7.9	13.2	13.8	16.9
Mean			42.9	5.1	13.2	10.8	27.9
Standard Deviation±			6.3	1.5	2.5	1.9	8.2
Mean ± 2 S. D.			30.3-55.5	2.1-8.1	8.2-18.2	7.0-14.6	11.5-44.3

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH CIRRHOSIS

Hospital Number	Age	Sex	Albumin (%)	$Alpha_1$ Globulin (%)	$Alpha_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
51883	48	M	49.7	4.6	11.3	12.0	22.4
53709	53	M	46.5	4.8	9.7	12.2	26.8
54925	50	M	21.0	0.9	2.9	10.8	64.4
63636	44	F	40.9	8.1	6.9	11.8	32.4
Mean			39.5	4.6	7.7	11.7	36.5
Standard Deviation±			12.9	3.0	3.7	0.6	19.0
Mean ± 2 S. D.			13.7-65.3	0.0-10.6	0.5-14.9	10.5-12.9	0.0-74.5

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH MULTIPLE MYELOMA

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
51742	81	F	27.7	8.4	21.8	17.3	24.8
54316	67	M	24.9	3.8	13.4	8.1	49.8
57332	44	F	32.6	5.4	11.7	50.3	
59333	46	M	47.2	10.0	20.9	9.1	12.7
60794	58	F	24.0	2.4	4.9	3.7	65.1
60917	62	F	26.8	3.1	5.3	4.0	60.8
62577	67	M	37.0	4.8	10.2	11.0	37.0
45858	71	F	46.3	5.2	13.1	13.8	21.7
Mean			33.3	5.4	12.7	48.9	
Standard Deviation ±			9.3	2.6	6.3	15.0	
Mean ± 2 S. D.			14.7–51.9	0.2–10.6	0.1–25.3	18.9 – 78.9	

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH DIABETES

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
49093	77	M	28.2	7.1	19.6	24.0	21.2
50090	23	M	41.5	4.5	19.0	17.5	17.5
54634	65	M	36.0	4.1	17.8	17.5	24.6
Mean			35.2	5.2	18.8	19.7	21.1
Standard Deviation ±			6.7	1.6	0.9	3.8	3.5
Mean ± 2 S. D.			21.8–48.6	2.0–8.4	17.0–20.6	12.1–27.3	14.1–28.1

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH LEUKEMIA

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
41935	65	M	43.5	5.2	8.1	9.3	34.0
50393	73	M	37.5	5.4	13.6	12.6	31.0
59333	46	M	47.2	10.0	20.9	9.1	12.7
62165	76	F	45.3	10.2	17.2	12.3	14.9
63139	5	M	45.1	8.6	12.6	10.9	22.8
65034	53	F	28.1	11.9	15.6	15.2	29.3
Mean			41.1	8.6	14.7	11.6	24.1
Standard Deviation ±			7.2	2.7	4.4	2.3	8.8
Mean ± 2 S. D.			26.7-55.5	3.2-14.0	5.9-23.5	7.0-16.2	6.5-41.7

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH HODGKINS AND LYMPHOSARCOMA

Hospital Number	Age	Sex	Albumin, (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
56808	70	F	37.3	3.4	9.3	11.3	38.7
59365	43	M	43.0	7.3	18.5	10.4	20.8
64877	21	F	26.1	15.3	23.6	15.1	19.9
65170	62	M	45.1	6.1	15.9	12.2	20.7
Mean			37.9	8.0	16.8	12.3	25.0
Standard Deviation ±			7.4	4.4	5.2	1.8	7.9
Mean ± 2 S. D.			23.1-52.7	0.0-16.8	6.4-27.2	8.7-15.9	9.2-40.8

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH COLLAGEN DISEASES

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
46767	47	F	41.5	2.7	9.7	13.9	32.2
46601	53	F	37.8	6.2	13.5	13.5	29.0
62588	61	F	44.8	6.3	13.2	6.8	28.8
46733	73	F	34.0	5.1	17.4	12.7	30.9
64208	18	M	39.8	9.5	21.4	11.1	18.2
51774	13	F	45.5	7.9	14.9	8.9	22.8
45608	22	M	34.1	5.5	16.1	11.8	32.4
Mean			39.6	6.2	15.2	11.2	27.8
Standard Deviation ±			4.7	2.2	3.0	2.6	5.3
Mean ± 2 S. D.			30.2-49.0	1.8-10.6	9.2-21.2	6.0-16.4	17.2-38.4

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH MISCELLANEOUS DISEASES

Hospital Number	Age	Sex	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
52911	48	M	40.4	4.7	14.0	8.6	32.4
53200	54	F	49.1	4.9	11.5	16.1	18.4
62076	20	M	45.8	8.6	15.2	12.1	18.2
63054	44	M	40.0	8.1	15.1	12.0	24.8
50616	58	F	40.5	8.0	10.6	13.8	27.1
45527	77	M	41.7	4.7	13.9	9.3	30.5
59292	59	M	36.9	7.0	15.1	13.0	28.0
65181	91	M	46.9	7.0	17.5	11.8	16.7

APPENDIX II

STATISTICAL METHODS*

Arithmetic Mean $$\bar{X} = \frac{\Sigma (x)}{N}$$

Standard Deviation for Small Samples $$s = \sqrt{\frac{\Sigma (x^2)}{N-1}}$$

Standard Error of Mean $$S_{\bar{x}} = \frac{s}{\sqrt{N}}$$

Standard Error of Difference between two Means $$S_{\bar{x}_1 - \bar{x}_2} = \sqrt{S_{\bar{x}_1}^2 + S_{\bar{x}_2}^2}$$

Probability of Occurance of deviations not greater than p $$t = \frac{\bar{X}_1 - \bar{X}_2}{S_{\bar{x}_1 - \bar{x}_2}}$$

(t was changed to p with help of t-table.)

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APPENDIX III

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