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A COMPARISON OF THE GRADIENT TUBE
AND THE MICRO-KJELDAHL
NITROGEN METHODS FOR THE
DETERMINATION OF SERUM PROTEIN
CONCENTRATION

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE

Janet Hope Traver
1949

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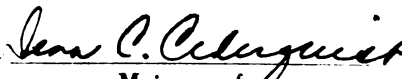
"A Comparison of the Gradient Tube and the
Micro-Kjeldahl Nitrogen Methods for
the Determination of Serum Protein
Concentration"

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A COMPARISON OF THE
GRADIENT TUBE
AND THE
MICRO-KJELDAHL NITROGEN
METHODS FOR THE
DETERMINATION OF SERUM PROTEIN
CONCENTRATION

By
Janet Hope Traver

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INTRODUCTION

INTRODUCTION

Plasma and serum protein determinations have been used with other clinical tests to assess nutritional status (Youmans et al., 1943). Normal human plasma and serum values reportedly range between 6.0 and 8.0 grams protein percent (Epstein, 1912; Rowe, 1916; Linder, Lundsgaard, and Van Slyke, 1924; Bruckman, D'Esopo, and Peters, 1930; Peters and Eisenman, 1933; Cameron and White, 1942; Kagan, 1942; Bieler, Ecker, and Spies, 1947). The significance of values near the extremes of the normal range is a matter of speculation.

The concept of a state of dynamic equilibrium between plasma proteins and food and body proteins was formulated by Madden and Whipple (1940). Experimental data to support this concept were published by Schoenheimer and Rittenberg (1940). These workers fed amino acids containing isotopic nitrogen to animals and found that the greatest concentration of the isotope was present in the serum proteins.

Since proteins are closely associated with many vital functions of the body, it might be expected that a constant concentration of blood protein would exist. Experimenters have demonstrated, however, that blood proteins may vary in concentration.

Some experimental work has shown that plasma or serum protein concentrations have decreased when

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animals were fasted or maintained on diets low in protein (Frisch, Mendel, and Peters, 1929; Metcalf, Favour, and Stare, 1945; Field and Dam, 1946). On the other hand, Leatham (1945 and 1947) demonstrated that animals on high protein diets (78 percent casein or lactalbumin) did not show significant increases in plasma protein concentration.

The quality as well as quantity of the dietary protein also may be a factor in altering blood protein concentration. Some proteins appear to be more efficient than others for the regeneration of plasma proteins (Kerr, Hurwitz, and Whipple, 1918; Madden and Whipple, 1940). This is probably due to varying amino acid contents of these proteins, for Madden and his co-workers (1943) have shown that the ten amino acids essential for the growth of the rat are needed for the production of serum proteins in the dog.

The relation between blood and dietary proteins also has been demonstrated in humans. Peters, Wakeman, and Eisenman (1927) observed that the plasma protein concentrations of 19 cases of severe malnutrition ranged from 3.44 to 7.17 grams percent. A decrease of 0.73 grams protein percent plasma was found by Keys et al. (1946) when they examined blood from 34 men who had been existing for six months on 49 grams of protein per day. Most of this protein came from vegetable sources. The

recommended daily allowance for an adult man is 70 grams of protein (Food and Nutrition Board, 1943). Walters, Rossiter, and Lehmann (1947) examined blood from 12 Indian war prisoners who had been eating diets low in protein. The control group was nine normal Indian subjects. Mean concentrations of 5.24 and 6.89 grams protein percent serum respectively for the prisoners and the controls were noted. A relationship was observed between protein intake and serum protein concentration by Arnell, Goldman, and Bertucci (1945) in a study of 11 pregnant women with nutritional edema.

Other studies have revealed normal protein concentrations in populations existing on low protein diets. Stuart (1945) determined serum albumin concentrations on French children who, during the war, had received approximately 63 grams of protein a day but only 19 grams from animal sources. He observed no edema or hypoproteinemia among the children studied. Youmans et al. (1943) studied a middle Tennessee rural population of 776 white and colored people. A dietary survey indicated mean intakes of 64 grams of protein per day for the whites and 48 grams of protein per day for the colored. The mean serum protein concentrations were 6.95 and 6.96 grams protein percent respectively. There appeared to be no correlation between the dietary intakes of calories or protein and the serum protein concentration.

The protein intakes in these studies although less than the recommended dietary allowance for this nutrient, evidently were not low enough to produce changes in the blood protein concentrations.

Arnell, Goldman, and Bertucci (1945) stated that "close correlation between the protein content of the diet and alterations in the composition of the blood cannot be expected"; however, a population study of protein intakes and blood protein concentrations might reveal some relationship between dietary and blood protein values. In other words, would a minimal protein intake be correlated with a blood protein value in the lower portion of the "normal" range? Similarly, would a very high protein intake be correlated with a blood protein value in the upper portion of the "normal" range? If such a relationship existed, a plasma or serum protein determination would be of value in assessing nutritional status.

The need for a simple but accurate method for the determination of serum or plasma protein concentration for survey studies is obvious. Although several methods have been suggested, their accuracy for the determination of serum or plasma protein may be questioned.

In the study to be reported, the use of one of the short methods, the Linderstrom-Lang gradient tube, as described by Lowry and Hunter (1945) has been investigated. The accuracy of serum protein concentration

calculated from specific gravity, as determined by this method, was found by comparison with values obtained from micro-Kjeldahl determinations. The relative effects of salt and sugar on the specific gravity of an albumin solution were also determined.

REVIEW OF THE LITERATURE

Methods for Determining Protein Concentration

The methods commonly used to determine protein concentration may be divided into two groups, physical and chemical. Firstly, it is assumed that non-protein substances have a negligible effect on the physical property being tested, and, secondly, that there is no appreciable uncorrected variation resulting from changes in the non-protein fraction. Kirk (1947) stated that "it is important to note that the physical property itself can generally be measured quite accurately and reproducibly, but the relation of that property to protein concentration may be uncertain due to the failure of one of the basic assumptions".

One of the physical properties which has been related to protein concentration is specific gravity. This property may be measured by the use of a gradient tube (Ponder, 1942; Lowry and Hunter, 1945; Hoch and Marrack, 1945) which consists of a cylinder containing kerosene and bromobenzene or chlorobenzene so mixed that the density of the mixture varies linearly throughout a portion of the column. Thus, a substance of unknown specific gravity, e.g. serum, dropped into the column will come to equilibrium at a point where the mixture and the serum have the same specific gravity. The specific gravity of the serum, however, may be altered by solution of the bromobenzene or chlorobenzene

in the serum. This alteration in specific gravity necessitates the standardization of the time between delivery of the serum into the mixture and reading of the cylinder. Solutions of copper or potassium sulfate of known density are utilized to relate cylinder readings to specific gravity.

Barbour and Hamilton (1926) calculated specific gravity from the time required for a 10 cubic millimeter drop of a substance to fall 30 centimeters through a mixture of xylene and bromobenzene of a density slightly lower than that of the substance being tested. This method has two disadvantages not present in the gradient tube method; the size of the drop and the timing must be very accurate.

Specific gravity also may be determined by allowing drops of a substance to fall into a graded series of copper sulfate solutions of known densities and noting the rise or fall of the drops (Phillips et al., 1944; Adams and Ballou, 1946; Meyer et al., 1947). Simeone and Sarris (1941), using a similar method, noted the movement of glass beads, calibrated for density, in the solution being tested. Mortensen (1942) devised a method using glass beads, one heavier and one lighter than the sample. The beads were placed in a pipette with the sample and the pipette inverted. The position at which the beads collided

was used as an indication of specific gravity.

The refractive index of a protein solution is another physical property which has been used to determine concentration. This method has been used by Neuhausen and Rioch (1923), Siebenmann (1937), and Sunderman (1944), but according to Kirk (1947) this method for determining protein concentration is not as sensitive as the specific gravity methods.

Bateman (1947) utilized another physical property of proteins, that of surface tension and the related spreading on surfaces, to determine concentration. He spread the protein over the surface of a concentrated salt solution and measured the area of the film when it was compressed to a standard pressure. The Kjeldahl determination of nitrogen was used to relate area to protein concentration.

Other methods based on physical properties also have been used. Infrared absorption spectroscopy was used by Buswell and Gore (1942) and electrophoresis by Cooper (1945). At present these methods are used primarily in research (Kirk, 1947).

Protein concentration may be determined by the weight of precipitated proteins after washing and drying. Gravimetric determinations have been done by Barnett, Jones, and Cohn (1932) and by Boyd (1939). The difficulty in procuring quantitative separations of protein is a major problem with this method of

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analysis (Kirk, 1947).

Looney and Walsh (1939) precipitated total protein with sulfosalicylic acid in the presence of gum ghatti and determined the concentration turbidimetrically. The volume of precipitated protein after centrifugation has been used to determine protein concentration by Rytand (1939) and by Lewis (1946). Kirk (1947), however, reported that it is difficult to standardize the conditions of precipitation and centrifugation sufficiently to make this procedure quantitative.

Among the chemical methods used to determine protein concentration is the Kjeldahl with its several variations. After a sample is digested with acid in the presence of a catalyst, the ammonia formed upon the addition of alkali may be distilled into boric acid, and the ammonium salt formed titrated with standard acid (Ma and Zuazaga, 1942). The ammonia also may be distilled into standard acid and the excess acid titrated with standard base (Robinson, Price, and Hogden, 1937). Van Slyke (1927), using a gasometric method, measured the amount of nitrogen produced by the addition of hypobromite after digestion. Marcali and Reiman (1946) eliminated distillation by titrating the ammonium salts formed upon digestion with sodium hydroxide in the presence of formaldehyde. However, calcium, barium, copper, iron,

and phosphates interfere, so the latter method of analysis would probably not be applicable to blood. Levey (1948) suggested the use of the Conway diffusion cell for serum protein determinations. In this method the ammonia from the digested sample is allowed to diffuse into boric acid and the ammonium salt formed titrated with standard acid. Direct Nesslerization of the digested sample and colorimetric determination of the compound formed is another variation of the Kjeldahl method commonly used (Hubbard, 1931; Schales, Ebert, and Stead, 1942).

It is possible to estimate protein concentration by determining the amounts of certain amino acids which are present. The Folin phenol reaction for tyrosine (Greenberg, 1929) and the Sakaguchi reaction for free guanidine and, therefore, arginine (Albanese, Irby, and Saur, 1946) are but two examples.

The biuret reaction, a test for the presence of peptide linkages, has been used to determine protein concentration (Robinson and Hogden, 1940). Stiff (1949) devised a new modification of this method by determining the amount of copper held in solution with protein.

Another method for estimating protein concentration was used by Fishberg and Dolin (1930). The proteins were titrated directly with acid; the amount of acid required to take the protein solution from one

pH to another was found to be a function of the protein concentration.

Of the many methods available for the determination of protein concentration, Kirk (1947) stated that the Kjeldahl nitrogen method, the biuret reaction, and specific gravity have shown themselves to be particularly useful despite the many limitations inherent in each method.

Accuracy of Specific Gravity Methods for Determining Protein Concentration

Kagan (1938) stated that " since the protein content of serum is between 75 and 80 percent of the dry weight, a close correlation with specific gravity is to be expected". Formulae have been calculated to relate specific gravity to protein concentration by experimentally determining both specific gravity and protein concentration on serum and plasma samples. Nevertheless, some workers disagree as to the validity of this procedure.

Some investigators have compared the protein concentration values secured from the gradient tube and the Kjeldahl methods. Lowry and Hunter (1945) analyzed serum samples from 240 patients with a variety of diseases but with no liver damage. They reported a mean deviation between the values from the two methods of 0.24 gram percent for a rather wide range of protein values, 3.5 to 13.5 grams protein percent. Twenty-five normal serum samples were analyzed by Hoch and Marrack (1945) who reported good correlation; however, they recorded differences up to 1.9 grams protein percent. Ponder (1942) stated that the gradient tube method gives values correct to ± 0.1 gram protein percent and concluded that this method may be used for clinical purposes.

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The results obtained from the copper sulfate and the Kjeldahl methods also have been compared. A mean difference of +0.15 gram protein percent (copper sulfate value minus Kjeldahl value) was reported by Atchley and co-workers (1945). Meyer et al. (1947) using 47 samples of dog plasma found a correlation of 0.9061 between the results obtained by using these methods. These workers also reported that 68 percent of the samples agreed within 0.2 grams protein percent. On the other hand, Adams and Ballou (1946) discovered that only 35 percent of the 128 human serum samples they analyzed agreed within 0.2 gram protein percent and reported a correlation of 0.74. Cook, Keay, and Mc Intosh (1945) found good correlation between the results of the two methods but large individual differences and, therefore, concluded that specific gravity gives only an approximation of plasma protein concentration.

Values for protein concentration obtained from the falling drop method of determining specific gravity have been compared with values obtained from the Kjeldahl method. A highly significant correlation of 0.9522 for dog plasma was reported by Meyer and co-workers (1947). They found that 77 percent of the samples gave results that agreed within 0.2 gram protein percent and concluded that the falling drop method may be

used clinically. Kagan (1938) testing the falling drop method with both serum and plasma reported mean deviations from the macro-Kjeldahl of ± 0.16 gram protein percent for serum and ± 0.23 gram protein percent for plasma. On the other hand, a very low correlation of 0.18 was revealed by Looney (1942) when he compared values obtained from the falling drop method and the turbidimetric method. Although Looney believed the latter method to be an accurate standard, the use of this method for a standard is not universally accepted (Kirk, 1947).

Protein concentration calculated from specific gravity measured directly by the weight of a measured volume of plasma was found to agree fairly closely with the Kjeldahl gasometric values by Moore and Van Slyke (1930); the maximum deviation was 0.6 gram protein percent. Weech, Snelling, and Goettsch (1933) using canine plasma verified the close relationship between specific gravity and protein concentration and the formula calculated by Moore and Van Slyke (1930) to relate specific gravity and protein concentration. Weech, Snelling, and Goettsch (1933) also demonstrated the applicability of this formula at specific gravities as low as 1.0121 which represented 1.84 grams protein percent as determined by the Kjeldahl method. Extremely high correlations, 0.993734 for dog serum and 0.991532 for dog plasma,

were reported by Weech, Reeves, and Goettsch (1936). They used a pycnometer to determine specific gravity and the Kjeldahl method to determine protein content. On the other hand, Zozaya (1935) found a correlation of 0.28 between the values obtained from these methods. He thought this poor agreement was due to the use of serum, but this assumption was shown to be false by other workers (Weech, Reeves, and Goettsch, 1936)

The glass bead methods also have been tested for their accuracy in determining protein concentration. Simeone and Sarris (1941) analyzed 118 samples of human serum finding an average deviation of 0.25 gram protein percent between the values from their method and the micro-Kjeldahl method. Mortensen (1942) compared his method with the Kjeldahl and found fair correlation.

The disagreement between workers as to the validity of the specific gravity methods to determine serum and plasma protein concentration may be due to variations in serum and plasma constituents other than protein which also affect specific gravity. These constituents are discussed in the next section.

In view of the conflicting evidence in the above reports, the use of specific gravity methods to determine protein concentration cannot be assumed to give satisfactory results. For this reason, this

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for ensuring the integrity of the financial system and for providing a clear audit trail. The document also highlights the need for transparency and accountability in all financial dealings.

In the second part, the document outlines the various methods used to collect and analyze data. It describes the process of gathering information from different sources and how this data is then used to identify trends and patterns. The document also discusses the importance of using reliable and valid data sources to ensure the accuracy of the findings.

The third part of the document focuses on the results of the analysis. It presents the findings of the study and discusses the implications of these results. The document also provides a detailed explanation of the factors that may have influenced the outcomes and offers suggestions for further research.

Finally, the document concludes with a summary of the key points and a statement of the author's conclusions. It reiterates the importance of maintaining accurate records and the need for transparency and accountability in financial matters. The document also expresses the author's hope that the findings of the study will be useful to others in the field.

study was designed to contribute more information concerning the correlation between the results obtained from the gradient tube and the micro-Kjeldahl methods.

Factors Which Alter Specific Gravity

Blood constituents other than proteins which may vary in concentration have been shown to effect the specific gravity of serum and plasma. Simeone and Sarris (1941) demonstrated that glucose produces a linear error, but the glucose concentrations used were as high as 1050 milligrams percent serum. A glucose concentration at this level would represent an abnormal condition, for normal post-absorptive concentrations range from 90 to 120 milligrams percent blood (Meyers, 1924). The work of Simeone and Sarris (1941) suggests that normal concentrations of glucose do not produce a large error in protein concentration as calculated from density. They found a difference of only 0.07 gram protein percent when calculated from specific gravity at a glucose level of 200 milligrams percent when contrasted to the calculated protein concentration at a glucose level of 100 milligrams percent. A blood glucose level of 200 milligrams percent might be possible in the normal individual after the ingestion of carbohydrate.

It has been recognized that salts have an effect on the specific gravity of serum and plasma (Barbour and Hamilton, 1924; Looney, 1942). However, Kagan (1938) found no relation between the level of salt and the deviation between the protein values obtained from the falling drop and the Kjeldahl methods.

Lowry and Hunter (1945) stated that " in order to influence the apparent serum protein concentration [calculated from specific gravity] by as much as 0.1 gram percent, it would be necessary to double the normal concentrations of either serum lipids, the blood glucose, or the non-protein nitrogen ".

Zozaya (1935) believed that the specific gravity of serum depends on the relation between the bound and the free water. He stated that bound water is dependent on the kind and percent of the protein fraction, euglobulin having a greater density per unit concentration than albumin. Nugent and Towle (1934), however, stated that " beef serum albumin and serum globulin exert effects upon the specific gravities of their synthetic solutions which are identical within the limits of experimental error of the methods usually employed for the accurate determination of specific gravity values ".

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Use of Serum and Plasma for Protein Determinations

Both serum and plasma have been used for the determination of protein; however, it has been shown that the addition of oxalate or heparin to blood alters the protein concentration. A rather conclusive study was done by Peters, Eisenman, and Bulger (1925). They determined serum protein concentrations on defibrinated blood before and after the addition of an anticoagulant. When 0.2 gram neutral potassium oxalate percent was added to the defibrinated blood, a decrease of 0.3 to 0.4 gram protein percent was noted, although plasma could be expected to contain about 0.3 gram protein percent more than serum (Kagan, 1942).

Serum, oxalated plasma, and heparinized plasma were analyzed by Kagan (1942). He observed that there was no consistent difference between the protein concentration of oxalated plasma and serum and that heparinized plasma gave higher results than oxalated plasma. This effect of oxalate also was noted by Gettler and Baker (1916), by Lehman and Scott (1935), and by Cameron and White (1942).

As was previously noted, Kagan (1938) found a lower mean deviation between the protein values obtained from the falling drop and the micro-Kjeldahl methods for serum than for plasma. He (Kagan, 1942) stated that "the values obtained for oxalated plasma are not as reliable as those for serum, probably because the amount

of water withdrawn from the red blood cells varies with the amount of oxalate used as well as with the varying effect that oxalate has on the cells of different persons".

The actual protein concentration of the serum of living organisms is probably better represented by experimentally determined serum protein values than by experimentally determined plasma protein values. For this reason, serum was used throughout this study.

EXPERIMENTAL PROCEDURES

Collection of Samples

The blood samples used for testing the gradient tube method for the determination of serum protein concentration were obtained from albino rats (250 to 500 grams) which had been used for other experiments. The animals at the time of sacrificing had been maintained for at least one month on the following rations:

	Percent
Casein	13.7
Yeast	8.4
Salt mixture	4.2
Cod liver oil	1.1
Corn oil	4.2
Cerelose and/or corn starch	68.4

The metabolic activity of the rats was kept as uniform as possible by removing both the food and water the evening before the animals were killed. The period of fasting ranged from 16 to 18 hours. Halatol was injected intraperitoneally, the heart exposed, and a blood sample taken with a 10 milliliter syringe and a number 25 needle. Four to nine milliliters of blood could be secured in this manner. Hemolysis was minimized by removing the needle before the blood was transferred to a 15 milliliter centrifuge tube.

The sample was allowed to clot and the serum transferred to a corked centrifuge tube. The serum was centrifuged for 15 minutes at 3300 revolutions per minute (diameter of head, 20 inches), decanted, and recentrifuged. The serum samples were tightly corked

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is essential for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It includes a detailed description of the data collection process, which involves gathering information from multiple sources and using statistical techniques to interpret the results.

3. The third part of the document provides a comprehensive overview of the data analysis process. It describes how the collected data is processed and analyzed to identify trends, patterns, and insights that can inform decision-making.

4. The fourth part of the document discusses the importance of data security and privacy. It outlines the measures taken to protect sensitive information and ensure that data is handled in a secure and compliant manner.

5. The fifth part of the document provides a summary of the findings and conclusions of the study. It highlights the key insights gained from the data analysis and discusses the implications for the organization's future operations.

6. The sixth part of the document includes a list of references and a bibliography. It cites the various sources of information used in the study, including academic journals, books, and other relevant documents.

7. The seventh part of the document provides a detailed description of the data collection process. It includes a list of the various methods and tools used, as well as a description of the data sources and the way in which the data was collected.

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whenever possible throughout the analyses to prevent evaporation and absorption of ammonia. Aliquots were taken for the gradient tube, the Kjeldahl total nitrogen, and the non-protein nitrogen determinations within one hour after centrifugation.

Specific Gravity Method

A gradient tube was established as described by Lowry and Hunter (1945). Mixtures of kerosene and bromobenzene (see Appendix) were used; the lighter mixture was layered over the heavier in a 500 milliliter graduated cylinder. The graduated cylinder was placed in a larger cylinder containing the jacket solution and paraffin used for a seal (see diagram in Appendix). A spiralled copper wire was used for mixing. About five minutes of mixing were required to form the gradient.

Potassium sulfate solutions of known specific gravities were prepared. The specific gravities of the solutions covered the range of the specific gravities usually found in serum. These solutions were used to relate the cylinder readings of the gradient tube to specific gravities.

Both serum and standards were allowed to reach room temperature before use. Pipettes calibrated to 0.01 milliliter were employed for delivery into the gradient. The pipette was rinsed two times with each standard before use; a clean pipette was used for the serum sample. The cylinder readings of the center of the drops were recorded four $\pm$ one-half minutes after delivery. Fine sea sand was used to settle the drops, and the gradient was covered while not in use to prevent evaporation.

A graph was constructed (Figure I.) by plotting the cylinder readings of the standard solutions against their specific gravities. The specific gravity of the serum was determined from such a graph.

In order to determine the relative effects of salt and sugar on the specific gravity of serum, an albumin solution of a concentration approximating human serum protein concentration was prepared. Glucose and sodium chloride were added to portions of this albumin solution.

Normal serum sodium values range from 300 to 330 milligrams percent (Hawk, Oser, and Summerson, 1947, p. 596) and serum chloride from 347 to 376 milligrams percent as calculated from 98 to 106 milliequivalents per liter (Hawk, Oser, and Summerson, 1947, p. 574). Sodium chloride in amounts representing 650, 670, 690, and 710 milligrams percent were added to portions of the albumin solution.

Normal post-absorptive blood glucose values range from 70 to 120 milligrams percent depending on the method of analysis used (Hawk, Oser, and Summerson, 1947, pp. 520 to 529). Glucose in amounts representing 85, 120, and 250 milligrams percent were added to portions of the albumin solution. The specific gravities of all the solutions were determined by the gradient tube and the nitrogen concentration by the micro-Kjeldahl method.

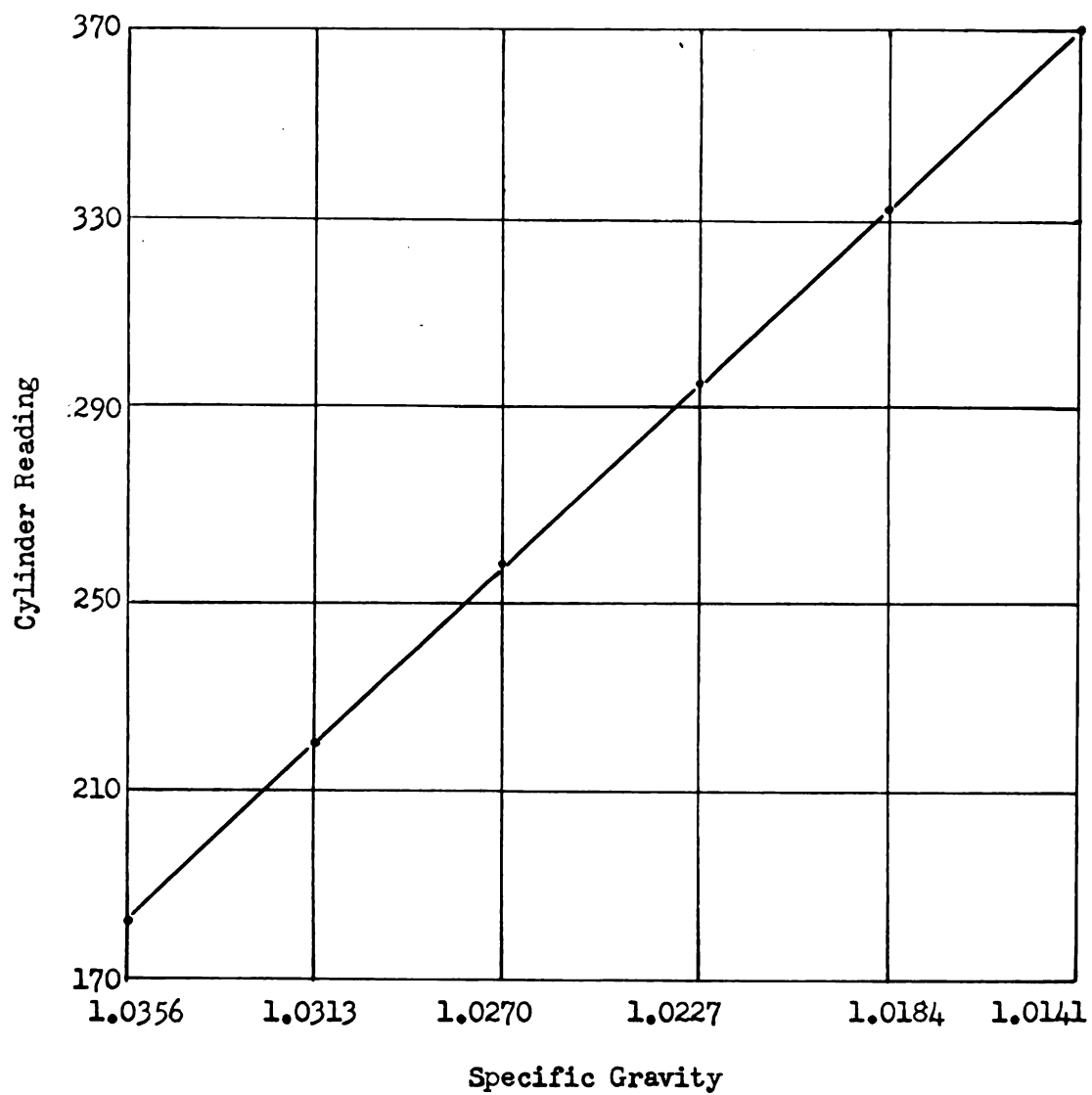


Figure I. Gradient Tube Cylinder Readings
for Solutions of Known Specific Gravities.

Total Nitrogen Method

Special Van Slyke-Ostwald blood pipettes were used to transfer at least two 0.5 milliliter samples of serum to 10 milliliter volumetric flasks. The samples were diluted to volume with 0.9 percent sodium chloride (Robinson, Price, and Hogden, 1937). At least two two milliliter aliquots of each dilution were transferred to 30 milliliter Kjeldahl flasks. Three milliliters of selenium digestion mixture and three glass beads were added to each (Robinson, Price, and Hogden, 1937). The aliquots were digested until colorless (one to one and one-half hours), the necks of the flasks washed with ammonia-free distilled water, and the heating continued for one-half hour. A diagram of the digestion rack may be found in the Appendix.

After the flasks had cooled, 10 milliliters of ammonia-free distilled water and a piece of red litmus paper were added. Before the cooled flask was connected to the distillation apparatus (see diagram in Appendix), the apparatus was steamed out for at least five minutes. Five milliliters of two percent boric acid and three drops (approximately 0.005 milliliter) of the indicator (Ma and Zuazaga, 1942) were placed in a 50 milliliter Erlenmeyer flask which in turn was floated in a beaker of water. The tip of the delivery tube was placed just above the surface of the liquid before eight

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milliliters of concentrated sodium hydroxide were added to the Kjeldahl flask from the reservoir. Immediately the delivery tip was lowered into the boric acid-indicator mixture, and the Kjeldahl flask was heated with a micro burner. After the sample had been distilled for 10 minutes (Robinson, Price, and Hogden, 1937), the collecting vessel was lowered until the delivery tip was again just above the liquid. The delivery tube was washed in the following manner: the micro burner was removed, the clamp on the trap opened, and ammonia-free distilled water was allowed to flow from the reservoir into the Kjeldahl flask; the sample was reheated for one minute and water from the reservoir was allowed to flow from the reservoir into the collecting flask. The tip of the delivery tube was rinsed and the Erlenmeyer flask removed for titration. The total volume of the distillate was kept at approximately 15 milliliters (Ma and Zuazaga, 1942). The distillate was titrated with 0.01 N hydrochloric acid to the purple end point of the indicator (pH 4.52); the amount of acid required was read to 0.1 milliliter and estimated to the nearest 0.01 milliliter. A blank using two milliliters of 0.9 percent sodium chloride was analyzed for each five digestions.

The protein concentration was calculated from the protein nitrogen concentration; protein = factor (total nitrogen - non-protein nitrogen).

1937年10月，日本帝国主义侵华战争爆发，上海沦陷，许多文化界人士流亡到香港。在这一特殊时期，许多文化界人士在香港创办了《南华》杂志，这是当时香港唯一的一份综合性文化刊物。《南华》杂志的创办，不仅为流亡文化界人士提供了一个发表作品的平台，也为香港的文化事业注入了新的活力。

《南华》杂志的创办，是当时文化界人士在特殊时期的一种无奈之举，也是他们对文化事业的执着追求。在战火纷飞的环境中，他们依然坚持创办刊物，传播文化，这体现了他们高尚的爱国情怀和坚定的文化信念。

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Although 6.25 is the factor commonly used to relate the concentrations of protein and protein nitrogen, Cook (1946) found that 6.6 is more applicable to blood proteins. Both factors were used to calculate protein concentration in this study.

Five milliliters of the ammonium sulfate standard solution containing 0.125 milligram of nitrogen per milliliter were used to determine the time required for distillation and also were added to serum aliquots to determine recovery of nitrogen. Digestion times for one-half, one, two, and four hours after the solutions were colorless were tested to determine the digestion time required for maximum recovery of nitrogen.

1. The first part of the document is a letter from the President of the United States to the Congress, dated January 3, 1862. It is a very long letter, and it contains a great deal of information about the state of the country at that time. The President talks about the war, the economy, and the future of the nation. He also talks about the role of the government and the people. The letter is written in a very formal and dignified style, and it is one of the most important documents in American history.

2. The second part of the document is a report from the Secretary of the Treasury, dated January 10, 1862. It is a very long report, and it contains a great deal of information about the state of the Treasury at that time. The Secretary talks about the revenue, the debt, and the future of the Treasury. He also talks about the role of the Treasury and the people. The report is written in a very formal and dignified style, and it is one of the most important documents in American history.

3. The third part of the document is a report from the Secretary of the Interior, dated January 17, 1862. It is a very long report, and it contains a great deal of information about the state of the Interior at that time. The Secretary talks about the land, the minerals, and the future of the Interior. He also talks about the role of the Interior and the people. The report is written in a very formal and dignified style, and it is one of the most important documents in American history.

4. The fourth part of the document is a report from the Secretary of the War, dated January 24, 1862. It is a very long report, and it contains a great deal of information about the state of the War at that time. The Secretary talks about the army, the navy, and the future of the War. He also talks about the role of the War and the people. The report is written in a very formal and dignified style, and it is one of the most important documents in American history.

5. The fifth part of the document is a report from the Secretary of the Navy, dated January 31, 1862. It is a very long report, and it contains a great deal of information about the state of the Navy at that time. The Secretary talks about the ships, the sailors, and the future of the Navy. He also talks about the role of the Navy and the people. The report is written in a very formal and dignified style, and it is one of the most important documents in American history.

Non-protein Nitrogen Method

At least two 0.5 milliliter samples of serum were pipetted into 15 milliliter centrifuge tubes and 4.5 milliliters of 10 percent trichloroacetic acid added (Robinson, Price, and Hogden, 1937). The corked tubes were allowed to stand with occasional shaking for at least two hours. The samples then were centrifuged for 15 minutes at 2000 revolutions per minute (diameter of head, 20 inches) and the supernatant decanted. Two one milliliter aliquots of the supernatant were transferred to 18 x 25 centimeter pyrex test tubes which had been calibrated to seven milliliters. One milliliter of diluted copper sulfate digestion mixture (Hawk, Oser, and Summerson, 1947, p. 495) was added, and the aliquots were heated over a micro burner until a few drops remained. Two drops of 30 percent hydrogen peroxide were added and the tubes reheated. The process was repeated using one drop of hydrogen peroxide. The digests were diluted to seven milliliters with ammonia-free distilled water. Blanks using one milliliter of 10 percent trichloroacetic acid were also digested.

A series of standards was set up as follows:

Ammonium sulfate standard (0.02 mg. N/ml.)	Digestion mixture	Ammonia-free distilled water
ml.	ml.	ml.
0	1	6.0
1.0	1	5.0
1.5	1	4.5
2.0	1	4.0
2.5	1	3.5

After all the tubes had been thoroughly chilled with running water, three milliliters of Nessler's reagent were added to each, and in five minutes the percent transmissions were determined with a Cenco-Sheard-Sanford photolometer using a green filter (wave length, 525 millimicrons). The undigested blank was set at 100 before the percent transmissions of the standard solutions were determined. Similarly, the digested blank was set at 100 before the percent transmissions of the samples were determined.

A curve was drawn on semi-logarithmic paper (Figure II.) by plotting the percent transmissions of the standard solutions against their nitrogen content. One milliliter of the ammonium sulfate standard solution containing 0.02 milligram nitrogen per milliliter was added to supernatant aliquots to test the recovery of nitrogen.

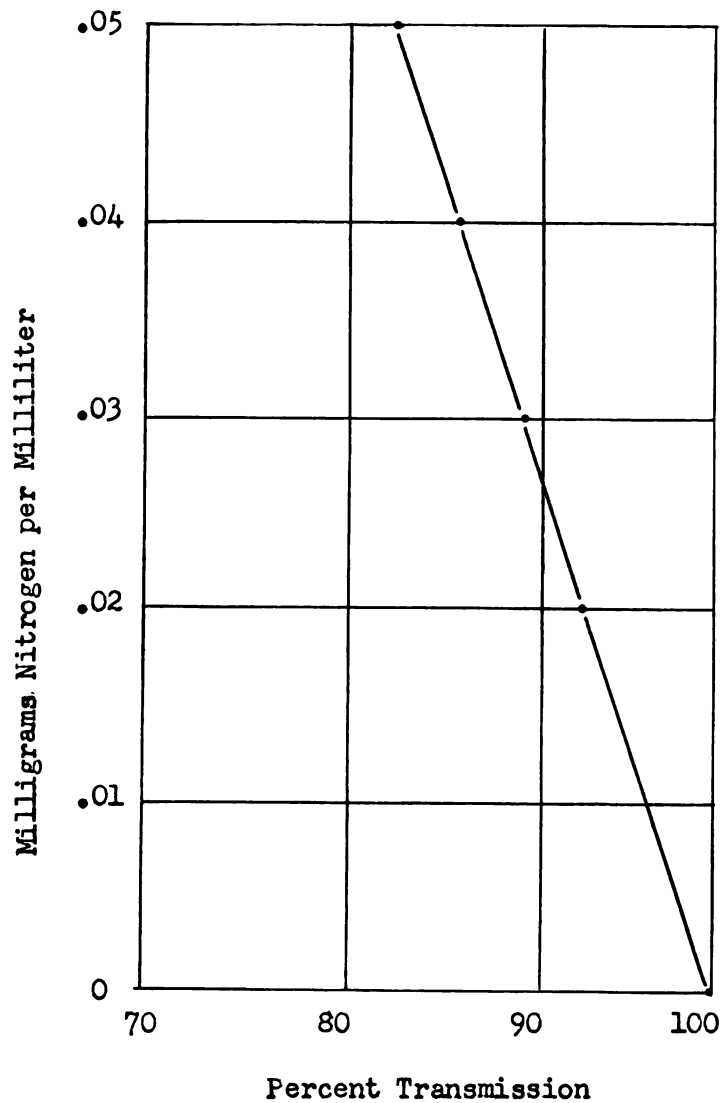


Figure II. Percent Transmissions for
Solutions of Known Nitrogen Concentrations.

RESULTS

Accuracy of the Methods for Total and Non-protein Nitrogen

Recoveries for the total nitrogen were determined on three serum samples. Table I summarizes the results. Osborn and Krasnitz (1934) also noted a maximum recovery and then a decrease with longer digestion times when selenium was used as a catalyst. Although Chibnall, Rees, and Williams (1943) suggested an eight hour heating period after clearing for macro-Kjeldahl complete digestion, Cook, Keay, and McIntosh (1945) found no differences among two, four, eight, and sixteen hours total digestion for the micro-Kjeldahl. These workers secured recoveries between 98 and 102 percent.

The differences in the titration values for aliquots from the same diluted serum sample and from different dilutions of the same serum sample from the 20 rats are summarized in Table II. The differences also are expressed in terms of protein concentration (grams nitrogen times 6.6) and in terms of percent of the mean protein concentration.

The titration values used to calculate protein concentration by Milam (1946) were within 0.2 milliliter of 0.2 N acid for 0.2 milliliter of plasma. The quantity being titrated in this study represented 0.1 milliliter serum. All titration values were averaged to calculate protein concentration.

Table I
Total Nitrogen Recoveries for Three Serum Samples
with Varying Digestion Times

Time heated after colorless	Serum sample	Sample plus 0.625 mg. N	Recovery	Mean recovery
hours	mg. N %	mg. N %	%	%
$\frac{1}{2}$	0.792	1.407	98.5	
	0.796	1.415	99.1	
	0.801	1.416	98.3	
	0.800	1.409	97.7	
	0.786	1.430	100.0	
	0.793	1.416	99.6	
				99.4
1	0.834	1.451	98.8	
	0.824	1.422	95.7	
	0.804	1.421	98.7	
	0.815	1.418	96.4	
				97.4
2	0.644	1.258	98.2	
	0.668	1.269	96.1	
	0.675	1.264	94.2	
				96.2
4	0.644	1.251	97.0	
	0.658	1.255	95.4	
	0.663	1.258	95.2	
				95.9

Table II
Effect of Sampling and Dilution on the Determination of Total Nitrogen

	Differences in titration values			Differences in protein concentration			Percent of mean protein concentration		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Same dilution	ml. 0.01 N acid			grams %			0.3	2.7	1.4
	0.02	0.18	0.10	0.02	0.17	0.09			
	0.06	0.32	0.21	0.05	0.30	0.19			
Different dilution							0.8	4.8	2.9

The blank determinations averaged 0.4 milliliter (range 0.20 to 0.55 milliliter) 0.01 N acid or 5.8 percent of the mean titration which is somewhat higher than the three percent reported by Cook, Keay, and Mc Intosh (1945). Perhaps this was due to the fact that the distillations and titrations were done in a room in which other determinations were being done.

The recoveries for non-protein nitrogen ranged from 95 to 105 percent which was considered sufficiently accurate due to the relatively low concentration of non-protein nitrogen when compared with the total nitrogen.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for transparency and accountability, particularly in financial matters. The text suggests that organizations should implement robust systems to track and document every aspect of their operations.

2. The second part of the document addresses the challenges associated with data management and security. It highlights the need for organizations to protect their sensitive information from unauthorized access and breaches. The text recommends the use of secure storage solutions and the implementation of strict access controls to ensure the integrity and confidentiality of the data.

3. The third part of the document focuses on the importance of regular audits and reviews. It states that periodic assessments are necessary to identify potential weaknesses and areas for improvement. The text encourages organizations to conduct thorough audits of their financial records, internal controls, and overall operational efficiency.

4. The fourth part of the document discusses the role of technology in enhancing organizational performance. It mentions that leveraging modern tools and software can significantly streamline processes and improve productivity. The text suggests that organizations should invest in reliable technology solutions that can support their growth and expansion.

5. The fifth part of the document concludes by emphasizing the importance of continuous learning and improvement. It states that organizations should foster a culture of innovation and encourage their employees to stay updated with the latest industry trends and best practices. The text suggests that regular training and development programs can help organizations maintain a competitive edge in the market.

Correlation Between Specific Gravity and Protein Nitrogen Concentration

The results of the analyses of the 20 samples of rat serum may be found in Table III. The means and the standard deviations of the values have been reported. Sex differences could not be compared, because there were nearly twice as many male as female rats. A correlation of 0.9938 was found between the specific gravities and the protein nitrogen concentrations.

A scatter diagram representing the relationship between the specific gravities and the protein nitrogen concentrations in grams percent serum is shown in Figure III. The formulae for the lines which best fit the data were calculated and rearranged* so that protein nitrogen and protein concentration could be calculated from specific gravity. The following formulae were calculated:

(1) Protein nitrogen (P) in grams percent serum:

$$P = 52.1 (\text{specific gravity} - 1.0075).$$

(2) Protein (P) in grams percent serum (protein N $\times$ 6.25):

$$P = 325.7 (\text{specific gravity} - 1.0075).$$

*Protein = A + B (specific gravity) (or)

$$\text{Protein} = B (\text{specific gravity} + A \div B).$$

B = the sum of (the deviations of the specific gravities from their mean $\times$ the deviations of the concentrations of protein from their mean) $\div$ the sum of the squares of the deviations of the specific gravities from their mean.

A = the mean of the specific gravities - (B $\times$ the mean of the concentrations of protein).

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author to the editor of the journal.

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Table III

Specific Gravity, Total and Non-protein Nitrogen, and Protein Concentrations for Twenty Samples of Rat Sera

Specific gravity	Total nitrogen minus non-protein nitrogen	Non-protein nitrogen	Protein (protein nitrogen $\times 6.25$)	Protein (protein nitrogen $\times 6.6$)
	grams %	grams %	grams %	grams %
1.0197	0.636	0.022	3.98	4.20
1.0218	0.752	0.041	4.70	4.97
1.0233	0.791	0.029	4.94	5.22
1.0236	0.834	0.030	5.21	5.50
1.0241	0.832	0.048	5.20	5.49
1.0248	0.880	0.016	5.50	5.81
1.0248	0.914	0.041	5.72	6.03
1.0253	0.922	0.041	5.76	6.08
1.0254	0.925	0.045	5.78	6.11
1.0255	0.935	0.039	5.84	6.17
1.0256	0.953	0.039	5.96	6.29
1.0259	0.958	0.046	5.98	6.32
1.0260	0.988	0.044	6.18	6.52
1.0266	1.013	0.042	6.33	6.68
1.0270	1.022	0.038	6.39	6.74
1.0274	1.024	0.029	6.40	6.76
1.0283	1.081	0.024	6.76	7.14
1.0283	1.107	0.029	6.92	7.30
1.0287	1.111	0.035	6.95	7.33
1.0306	1.190	0.046	7.44	7.85
Mean 1.0256	0.943	0.036	5.90	6.23
S. D. 0.0024	0.013	0.009	0.81	0.86

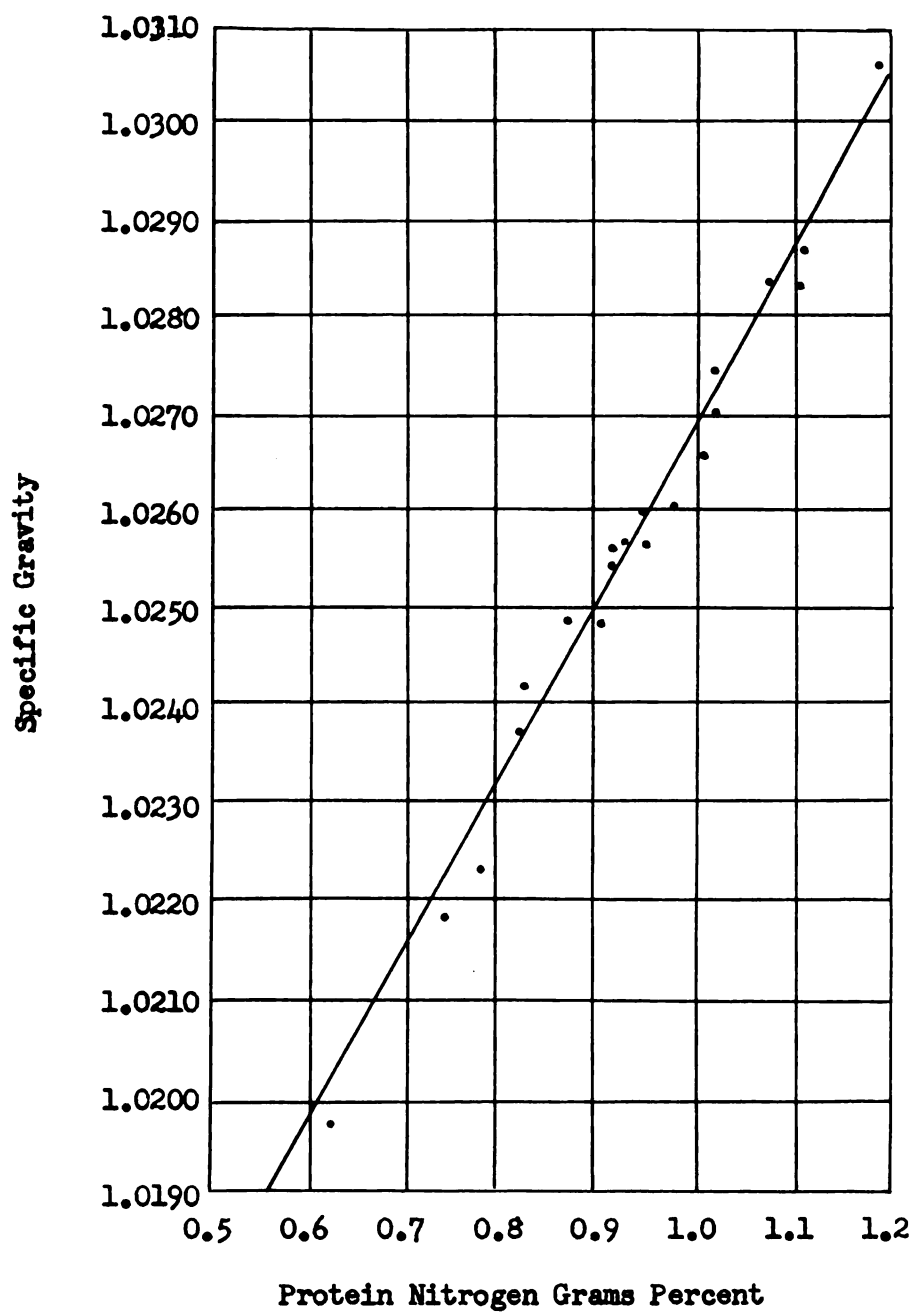


Figure III. Relation Between Specific Gravity
and Protein Nitrogen Concentration.*

*Line calculated from formula (1).

(3) Protein (P) in grams percent serum (protein $\times 6.6$):

$P = 343 (\text{specific gravity} - 1.0075)$.

The theoretical specific gravity for serum containing no protein was calculated to be 1.0075. The other numbers in the above formulae are factors calculated to convert specific gravity to the desired expression.

The standard error of estimate for formula (1) was found to be 0.0145 gram nitrogen percent; for formulae (2) and (3) was 0.09 gram protein percent.

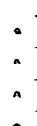
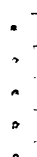
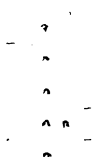
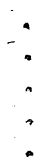
Effect of Salt and Sugar on the Specific Gravity of an Albumin Solution

Table IV summarizes the effects of the addition of various amounts of sodium chloride and glucose on the specific gravity of an albumin solution. Within the range used, each increase of 20 milligrams sodium chloride percent resulted in the specific gravity being increased by 0.0004 and the calculated protein concentration by 0.15 gram percent.

No differences could be detected between the specific gravities of the albumin solutions containing 85 and 120 milligrams glucose percent, but a value of 250 milligrams percent increased the calculated protein concentration by 0.14 gram percent when compared with the protein values calculated at glucose concentrations characteristic of normal fasting blood.

Table IV
Effects of Sodium Chloride and Glucose on the
Specific Gravity of an Albumin Solution

	Specific gravity	Protein P-343 (Sp. Gr.-1.0075)	Protein Kjeldahl (protein nitrogen x6.6)
milligrams % Sodium chloride added		grams %	grams %
0	1.0292	7.44	5.13
650	1.0358	9.71	5.14
670	1.0362	9.84	5.13
690	1.0367	10.02	5.13
710	1.0371	10.51	5.11
Glucose added			
0	1.0292	7.44	5.13
85	1.0294	7.51	5.10
120	1.0294	7.51	5.14
250	1.0298	7.65	5.17



DISCUSSION OF RESULTS

Use of the Gradient Tube to Determine Protein Concentration

Although a high correlation of 0.9938 was found to exist between specific gravity and protein concentration under the experimental conditions described, several points should be mentioned concerning the use of the gradient tube for the determination of serum protein concentration.

The effect of variations of glucose within the normal post-absorptive range on the specific gravity of an albumin solution was not detectable. However, a higher blood glucose value of 250 milligrams percent, which would be possible after the ingestion of carbohydrates, would probably affect the accuracy of specific gravity methods for the determination of serum protein concentration. Although in many nutrition surveys carbohydrate ingestion is not controlled, an abstinence period of several hours probably would be desirable if the specific gravity of serum is to be determined.

The addition of salt, varied within the normal range, had a marked effect on the specific gravity of an albumin solution. In this study salt concentration may not have been an important factor in the accuracy of the gradient tube to determine serum protein concentration, for it is possible that experimental animals under controlled conditions such as diet and period of fasting and water abstinence would have a more uniform

1. The first part of the paper is a review of the literature on the topic of the paper.

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3. The third part of the paper is a presentation of the results of the study.

4. The fourth part of the paper is a discussion of the results of the study.

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serum salt concentration than a group of humans. It is interesting to note that the high correlations between specific gravity and protein concentration reported by Weech, Reeves, and Goettsch (1936), 0.993734 and 0.991532, and by Meyer et al. (1947), 0.9522, were observed with canine plasma and serum while the specific gravity and protein concentration of human plasma and serum usually showed a lower correlation (Adams and Ballou, 1946). It is possible that these differences were due to a larger variation in the salt concentrations of the human samples.

Several investigators have reported the effects of large doses of salt and of salt abstinence on blood sodium chloride content. Two humans were given 50 grams of sodium chloride a day for two days by Torbert and Cheney (1936) who noted increases in plasma chloride (expressed as sodium chloride) from 555 to 582 milligrams percent (94.9 to 99.6 milliequivalents chloride per liter) and from 554 to 602 milligrams percent (94.8 to 103.0 milliequivalents chloride per liter). Bourdillon (1937) fed a human 210 milliequivalents (12.3 grams) of sodium chloride and observed an increase in serum sodium from 142.7 to 147.1 milliequivalents per liter and in serum chloride from 102.8 to 108.4 milliequivalents per liter. Based on the data obtained in the present study, there would be a difference of approximately 0.22 gram protein percent between the

The first part of the report discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the company's financial health and for providing reliable information to stakeholders. The report then outlines the current state of the company's record-keeping system, highlighting several areas where improvements are needed. These include the need for more standardized procedures, the implementation of new software, and the training of staff in proper record-keeping practices. The report also discusses the importance of regular audits to ensure the accuracy and integrity of the records. Finally, the report provides recommendations for how the company can improve its record-keeping system in the future.

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protein values calculated from specific gravities at the lower and the higher sodium and chloride concentrations reported by Bourdillon (1937). An error of this magnitude, however, would probably be clinically important only at borderline values.

Cutler, Power, and Wilder (1943) analyzed the blood from 28 patients (with no Addison's disease) who had been on a salt free diet for two days. Plasma sodium values between 300 and 322 milligrams percent (130.5 to 140.0 milliequivalents per liter) and plasma chloride values between 333 and 371 milligrams percent (93.9 to 104.6 milliequivalents per liter) were observed.

Although these experiments demonstrated changes in blood sodium chloride concentrations, the values reported were within or near the normal ranges of serum sodium values, 129.4 to 154.0 milliequivalents per liter (a composite range from the literature), reported by Snyder and Katzenelbogen (1942) and the serum chloride values, 95.2 to 113.8 milliequivalents per liter, reported by Eisenman (1929). If blood salt and glucose concentrations were determined in a nutrition survey, the effects of these substances in extreme concentrations on the determination of protein values from specific gravity could be considered.

A comparison of the formulae used to calculate protein concentration from specific gravity determined

Table V

Formulae Used to Relate Specific Gravity and Protein Concentration *

Specific gravity method	Substance analyzed	K**	A**	Protein grams % for specific gravity of 1.0227	Reference
Gradient tube	rat serum	325.7	1.0075	4.95	Present study Lowry and Hunter (1945) Hoch and Marrack (1945)
	human serum	348	1.0069	5.50	
	human serum	359.2	1.0069	5.68	
Falling drop	human serum	345	1.0076	5.20	Kagan (1938) ibid. Meyer et al. (1947)
	human plasma	340	1.0099	4.35	
	dog plasma	315	1.0050	5.58	
Copper sulfate	human serum	343	1.007	5.38	Adams and Ballou (1946) Meyer et al. (1947)
	dog plasma	325	1.0059	5.46	
Direct	human plasma	343	1.007	5.38	Moore and Van Slyke (1930) Weech, Snelling, and Goettsch (1933) Schousboe (1939) Weech, Reeves, and Goettsch (1936) ibid. Hoch and Marrack (1945)
	dog plasma	343	1.007	5.38	
	human serum	343	1.007	5.38	
	dog serum	347.9	1.00726	5.37	
	dog plasma	340.1	1.00687	5.38	
	human serum	365	1.007	5.73	

*Protein concentration determined by Kjeldahl method.

**Protein grams percent K (Specific gravity - A).

by various methods may be found in Table V. It was assumed that when no factor was mentioned, 6.25 was used to convert grams protein nitrogen to grams protein. The formula calculated from the data in this study using the conversion factor, 6.25, was included for comparison. It should be noted that formulae differ not only with the method used to determine specific gravity and the investigator but also with the substance being analyzed. The protein value calculated from the formula from this study is with one exception the lowest of those listed. The lower value might be due to a species difference in blood salt concentration, for Anderson et al. (1930) found rat blood chloride concentration slightly higher than human. It is obvious that the formulae calculated in this study could not be applied to human studies without further investigation.

If the conditions for studies with human subjects could be sufficiently controlled, the gradient tube might prove to be an accurate as well as convenient method for the determination of serum protein concentration in nutrition surveys.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The correlation between specific gravity and protein nitrogen (total nitrogen minus non-protein nitrogen) was determined on 20 samples of rat serum. Specific gravity was determined by the Linderstrom-Lang gradient tube method, total nitrogen by the micro-Kjeldahl nitrogen method, and non-protein nitrogen by Nesslerization. The effects of physiological concentrations of blood glucose and serum sodium chloride on the specific gravity of an albumin solution also were determined.

A correlation of 0.9938 was found to exist between specific gravity and protein nitrogen concentration under the experimental conditions described. Formulae based on the data were calculated to convert specific gravity to protein and protein nitrogen concentrations. The standard error of estimate was 0.09 gram protein percent.

No differences could be detected between the specific gravities of the albumin solutions containing concentrations of glucose characteristic of normal post-absorptive blood. However, a glucose concentration of 250 milligrams percent increased the calculated protein concentration by 0.14 gram percent when compared with the protein value at normal post-absorptive glucose concentrations.

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Similarly, within the range used, each increase of 20 milligrams sodium chloride percent increased the calculated protein concentration by 0.15 gram percent. Such an error probably would not be important clinically except at borderline values. In this study salt concentration may not have been an important factor in the accuracy of the gradient tube method for the determination of serum protein concentration, for it is possible that the blood of experimental animals under controlled conditions would have a more uniform salt concentration than a group of humans.

It was concluded that if the conditions for studies with human subjects could be sufficiently controlled, the gradient tube might prove to be an accurate as well as convenient method for the determination of serum protein concentration in nutrition surveys.

LITERATURE CITED

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- Adams, M. A. and A. N. Ballou 1946 A Comparison Between the Values for Plasma or Serum Protein as Obtained by the Specific Gravity and Micro-Kjeldahl Methods. J. Lab. Clin. Med., 31:507-513.
- Albanese, A. A., V. Irby and B. Saur 1946 The Colorimetric Estimation of Proteins in Various Body Fluids. J. Biol. Chem., 166:231-237.
- Anderson, A. K., H. E. Honeywell, A. C. Santy and S. Pedersen 1930 The Composition of Normal Rat Blood. J. Biol. Chem., 86:157-165.
- Arnell, R. E., D. W. Goldman and F. J. Bertucci 1945 Protein Deficiencies in Pregnancy. J. Am. Med. Assoc., 127:1101-1107.
- Atchley, J., R. Bacon, G. Curran and K. David 1945 A Clinical Evaluation of the Copper Sulfate Method for Measuring Specific Gravity of Whole Blood and Plasma. J. Lab. Clin. Med., 30:830-838.
- Barbour, H. G. and W. F. Hamilton 1924 Blood Specific Gravity: Its Significance and a New Method for Its Determination. Am. J. Physiol., 69:654-661.
- Barbour, H. G. and W. F. Hamilton 1926 The Falling Drop Method for Determining Specific Gravity. J. Biol. Chem., 69:625-640.

1. The first part of the paper discusses the importance of the study of the history of the United States. It is argued that the study of history is essential for a full understanding of the present and for the development of a sense of national identity. The author also discusses the role of the federal government in the development of the country and the importance of the Constitution.

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- Barnett, C. W., R. B. Jones and R. B. Cohn 1932 The Maintenance of a Normal Plasma Protein Concentration in Spite of Repeated Protein Losses by Bleeding. J. Exp. Med., 55:683-693.
- Bateman, J. B. 1947 Determination of Protein in Serum and Cerebrospinal Fluids by Formation of Unimolecular Films. J. Cell. Comp. Physiol., 29:85-89.
- Bieler, M. N., E. E. Ecker and T. D. Spies 1947 Serum Proteins in Hypoproteinemia Due to Nutritional Deficiencies. J. Lab. Clin. Med., 32:130-138.
- Bourdillon, J. 1937 Distribution in Body Fluids and Excretions of Ingested Ammonium Chloride, Potassium Chloride, and Sodium Chloride. Am. J. Physiol., 120: 411-419.
- Boyd, E. M. 1939 Separations of Lipids in Gravimetric Acetone Method for Plasma Total Protein. Proc. Soc. Exp. Biol. Med., 42:263-264.
- Bruckman, F. S., L. M. D'Esopo and J. P. Peters 1930 The Plasma Proteins in Relation to Blood Hydration. IV. Malnutrition and the Serum Proteins. J. Clin. Invest., 8:577-590.
- Buswell, A. M. and R. C. Gore 1942 Quantitative Spectroscopic Analysis of Proteins. J. Phys. Chem., 46:575-581.
- Cameron, A. T. and F. D. White 1942 The Diagnostic Value of Plasma Protein. Canadian Med. J., 46:255-261.

The first part of the document is a list of names and their corresponding addresses. The names are listed in a column on the left, and the addresses are listed in a column on the right. The names are: John Doe, Jane Smith, Robert Brown, Mary White, and Thomas Green. The addresses are: 123 Main St, 456 Elm St, 789 Oak St, 101 Pine St, and 202 Cedar St.

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- Chibnall, A. C., M. W. Rees and E. F. Williams 1943
The Total Nitrogen Content of Egg Albumin and Other
Proteins. Biochem. J., 37:354-359.
- Cook, R. P. 1946 The Determination of Nitrogen and of
Protein in Pooled Samples of Human Plasma. Biochem.
J., 40:41-45.
- Cook, R. P., D. M. Keay and D. G. Mc Intosh 1945 Methods
for Determining Plasma Protein. Brit. Med. J., 2:
456-457.
- Cooper, G. R. 1945 Electrophoretic Analysis of Mixtures
of Protein. J. Biol. Chem., 158:727-728.
- Cutler, H. H., M. H. Power and R. M. Wilder 1943 Concen-
trations of Chloride, Sodium, and Potassium in Urine
and Blood; Their Diagnostic Significance in Adrenal
Insufficiency. J. Am. Med. Assoc., 123:28-30.
- Eisenman, A. J. 1929 A Note on the Van Slyke Method for
the Determination of Chloride in Blood and Tissue.
J. Biol. Chem., 82:411-414.
- Epstein, A. A. 1912 A Contribution to the Study of the
Chemistry of Blood Serum. J. Exp. Med., 16:719-731.
- Field, J. B. and H. Dam 1946 Influence of Diet on Plasma
Fibrinogen in the Chick. J. Nutrition, 31:509-523.
- Fishberg, E. H. and B. T. Dolin 1930 Determination of
Serum Proteins. Proc. Soc. Exp. Biol. Med., 28:
205-206.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the transparency and accountability of the organization. This section also outlines the various methods used to collect and analyze data, ensuring that the information is reliable and up-to-date.

2. The second part of the document focuses on the implementation of the proposed changes. It details the steps involved in the process, from the initial planning stage to the final execution. This section also addresses the potential challenges that may arise during the implementation phase and provides strategies to overcome them.

3. The third part of the document discusses the impact of the proposed changes on the organization. It analyzes the potential benefits and risks, as well as the expected outcomes. This section also includes a comparison of the current state of the organization with the proposed changes, highlighting the areas of improvement.

4. The fourth part of the document provides a summary of the key findings and conclusions. It reiterates the importance of the proposed changes and the need for continued monitoring and evaluation. This section also includes a list of recommendations for future actions and a timeline for implementation.

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- Food and Nutrition Board 1948 Recommended Dietary Allowances, Revised. National Research Council. Reprint and Circ. Series No. 129.
- Frisch, R. A., L. B. Mendel and J. P. Peters 1929 The Production of Edema and Serum Protein Deficiency in White Rats by Low Protein Diets. J. Biol. Chem., 84:167-177.
- Gettler, H. O. and W. Baker 1916 Chemical and Physical Analysis of Blood in Thirty Normal Cases. J. Biol. Chem., 25:211-222.
- Greenberg, D. M. 1929 The Colorimetric Determination of the Serum Proteins. J. Biol. Chem., 82:545-550.
- Hawk, P. B., B. L. Oser and W. H. Summerson 1947 Practical Physiological Chemistry. The Blakiston Company. Philadelphia, Pa., 12th ed.
- Hill, R. M. and V. Trevorrow 1941 Plasma Albumin, Globulin, and Fibrinogen in Healthy Individuals from Birth to Adult Life. J. Lab. Clin. Med., 26:1838-1849.
- Hoch, H. and J. Marrack 1945 Estimation of Serum Protein by the Linderstrom-Lang Gradient. Brit. Med. J., 2: 876-878.
- Hubbard, R. S. 1931 The Determination of Blood Proteins by a Direct Micro-Kjeldahl Method. J. Lab. Clin. Med., 16:500-503.

- Kagan, B. M. 1938 A Simple Method for the Estimation of Total Protein Content of Plasma and Serum. J. Clin. Invest., 17:373-376.
- Kagan, B. M. 1942 Studies on the Clinical Significance of Serum Protein. I. The Protein Content of Normal Human Venous and Capillary Serum and Factors Affecting the Accuracy of Its Determination. J. Lab. Clin. Med., 27:1457-1463.
- Kerr, W. J., S. H. Hurwitz and G. H. Whipple 1918 Regeneration of Blood Serum Proteins. II. Influence of Diet Upon Curve of Protein Regeneration Following Plasma Depletion. Am. J. Physiol., 47:370-378.
- Keys, A., H. L. Taylor, O. Mickelsen and A. Henschel 1946 Famine Edema and the Mechanism of Its Formation. Science, 103:669-670.
- Kirk, P. L. 1947 The Chemical Determination of Proteins. In Anson, M. L. and J. T. Edsall Advances in Protein Chemistry. Academic Press Inc., Publ., New York, N. Y., Vol. III.
- Leatham, J. H. 1945 The Plasma Protein Concentrations and Organ Weights of Rats on a High Protein Diet. Endocrinology, 37:157-164.
- Leatham, J. H. 1947 Plasma Protein Concentrations and Organ Weights of Rats as Related to a High Protein Diet. Proc. Soc. Exp. Biol. Med., 64:90-92.

Figure 1

- Lehman, W. and F. H. Scott 1935 Note on the Total Protein Content of Plasma and Serum. J. Biol. Chem., 111:43-44.
- Levey, S. 1948 A Simple Method of Determining Non-protein Nitrogen, Total Protein, and Albumin in Blood Serum Samples by Using Conway Cells. Am. J. Clin. Path., 18:435-438.
- Lewis, J. H. 1946 A Centrifuge Method of Determining Blood Proteins. Arch. Path., 42:350-354.
- Linder, G. C., C. Lundsgaard and D. D. Van Slyke 1924 The Concentration of Plasma Proteins in Nephritis. J. Exp. Med., 39:887-920.
- Looney, J. M. 1942 The Relation Between Specific Gravity of Blood Serum and Its Protein Concentration. J. Lab. Clin. Med., 27:1463-1469.
- Looney, J. M. and A. I. Walsh 1939 The Determination of Globulin and Albumin in Blood Serum by the Photo-electric Colorimeter. J. Biol. Chem., 130:635-639.
- Lowry, C. H. and T. H. Hunter 1945 The Determination of Serum Protein Concentration with the Gradient Tube. J. Biol. Chem., 159:465-474.
- Ma, T. S. and G. Zuazaga 1942 Micro-Kjeldahl Determination of Nitrogen. A New Indicator and a Improved Rapid Method. Ind. Eng. Chem., Anal. Ed., 14:280-282.
- Madden, S. C., J. R. Carter, A. A. Kattus, L. L. Miller, and G. H. Whipple 1943 Ten Amino Acids Essential for

- Plasma Protein Production Affective Orally or Intravenously. *J. Exp. Med.*, 77:277-295.
- Madden, S. C. and G. H. Whipple 1940 Plasma Proteins: Their Source, Production, and Utilization. *Physiol. Rev.*, 20:194-217.
- Marcali, K. and W. Reiman 1946 Kjeldahl Determination of Nitrogen. Elimination of Distillation. *Ind. Eng. Chem., Anal. Ed.*, 18:709-710.
- Markham, R. 1942 A Steam Distillation Apparatus Suitable for Micro-Kjeldahl Analysis. *Biochem. J.*, 36:790-791.
- Metcoff, J., C. B. Favour and F. J. Stare 1945 Plasma Protein and Hemoglobin in the Protein Deficient Rat. A Three Dimensional Study. *J. Clin. Invest.*, 24:82-91.
- Meyer, F. L., W. E. Abbott, M. Allison and C. Mc Kay 1947 A Comparison of Plasma Protein Concentration, Hemoglobin, and Hematocrit Values Determined by Chemical Methods and Calculated from Specific Gravity. *Arch. Biochem.*, 12:359-366.
- Meyers, V. C. 1924 Chemical Changes in the Blood and Their Clinical Significance. *Physiol. Rev.*, 4:274-328.
- Milam, D. F. 1946 Plasma Protein Levels in Normal Individuals. *J. Lab. Clin. Med.*, 31:285-290.
- Moore, N. S. and D. D. Van Slyke 1930 The Relationships Between Plasma Specific Gravity, Plasma Protein Content, and Edema in Nephritis. *J. Clin. Invest.*, 8:337-355.

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- Mortensen, R. A. 1942 A Rapid Method for the Determination of Serum Protein. J. Lab. Clin. Med., 27: 693-700.
- Neuhausen, B. S. and D. M. Rioch 1923 The Refractometric Determination of Serum Protein. J. Biol. Chem., 55:353-356.
- Nugent, R. L. and L. W. Towle 1934 The Specific Gravity of Synthetic Solutions of Serum Albumin and Serum Globulin. J. Biol. Chem., 104:395-398.
- Osborn, R. A. and A. Krasnitz 1934 A Study of the Kjeldahl Method. III. Further Comparisons of Selenium with Mercury and with Copper Catalysts. J. Assoc. Official Agri. Chem., 17:339-342.
- Peters, J. P. and A. J. Eisenman 1933 The Serum Proteins in Diseases Not Primarily Affecting the Cardiovascular System or Kidneys. Am. J. Med. Sc., 186:808-833.
- Peters, J. P., A. J. Eisenman and H. A. Bulger 1925 The Plasma Proteins in Relation to Blood Hydration. I. In Normal Individuals and in Miscellaneous Conditions. J. Clin. Invest., 1:435-450.
- Peters, J. P. and D. D. Van Slyke 1932 Quantitative Clinical Chemistry. Vol. II. Methods. The Williams and Wilkins Co., Baltimore, Md.
- Peters, J. P., A. M. Wakeman and A. J. Eisenman 1927 The Plasma Proteins in Relation to Blood Hydration. III. The Plasma Proteins in Malnutrition. J. Clin. Invest., 3:491-495.

- Phillips, R. A., D. D. Van Slyke, V. P. Dole, K. Emerson, Jr., P. B. Hamilton and R. M. Archibald 1944 Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma with Line Charts for Calculating Plasma Protein, Hemoglobin, and Hematocrit from Plasma and Whole Blood Gravities. Bull. Hygiene, 19:140.
- Ponder, E. 1942 A Simple Method for Determining Plasma Density and Plasma Protein Concentration. J. Lab. Clin. Med., 28:232-234.
- Robinson, H. W. and C. G. Hogden 1940 The Biuret Reaction in the Determination of Serum Proteins. J. Biol. Chem., 135:707-725.
- Robinson, H. W., J. W. Price and C. G. Hogden 1937 The Estimation of Albumin and Globulin in Blood Serum. I. A Study of the Errors Involved in the Filtration Procedure. J. Biol. Chem., 120:481-498.
- Rowe, A. H. 1916 The Albumin and Globulin Content of Human Blood Serum in Health, Syphilis, Pneumonia, and Certain Other Infections, with the Bearing of Globulin on the Wassermann Reaction. Arch. Int. Med., 18:455-473.
- Ryland, D. A. 1939 A Simple Rapid Method for the Determination of Total Protein and Albumin Concentrations in Blood Plasma, Serum, or Other Body Fluids. J. Lab. Clin. Med., 24:439-443.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the transparency and accountability of the organization. This section also outlines the various methods used to collect and analyze data, ensuring that the information is reliable and up-to-date.

2. The second part of the document focuses on the financial aspects of the organization. It provides a detailed overview of the budget, including the projected income and expenses for the upcoming year. This section also discusses the various financial risks and how they can be mitigated, ensuring that the organization remains financially stable and secure.

3. The third part of the document addresses the human resources of the organization. It discusses the current state of the workforce, including the number of employees, their skills, and their experience. This section also outlines the various strategies used to attract and retain talent, ensuring that the organization has a strong and capable workforce.

4. The fourth part of the document discusses the marketing and sales efforts of the organization. It provides a detailed overview of the various marketing campaigns and sales strategies used to promote the organization's products and services. This section also discusses the various challenges faced in the marketing and sales process and how they can be overcome.

5. The fifth part of the document discusses the overall performance of the organization. It provides a detailed overview of the various key performance indicators (KPIs) used to measure the organization's success. This section also discusses the various factors that have contributed to the organization's success and how they can be leveraged to achieve even greater success in the future.

6. The sixth part of the document discusses the future of the organization. It provides a detailed overview of the various strategic initiatives and goals for the upcoming year. This section also discusses the various challenges and opportunities that the organization will face in the future and how they can be addressed.

7. The seventh part of the document discusses the various stakeholders of the organization. It provides a detailed overview of the various groups and individuals who have an interest in the organization's success. This section also discusses the various ways in which the organization can engage with its stakeholders and ensure that their needs and interests are met.

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9. The ninth part of the document discusses the various legal and regulatory requirements that the organization must comply with. It provides a detailed overview of the various laws and regulations that apply to the organization's operations. This section also discusses the various ways in which the organization can ensure that it is in full compliance with all applicable laws and regulations.

10. The tenth part of the document discusses the various ethical considerations that the organization must take into account. It provides a detailed overview of the various ethical principles and standards that guide the organization's operations. This section also discusses the various ways in which the organization can ensure that it is acting in an ethical and responsible manner.

- Schales, O., R. V. Ebert and E. A. Stead, Jr. 1942
Capillary Tube Kjeldahl Method for Determining Protein
Content of Five to Twenty Milligrams of Tissue Fluid.
Proc. Soc. Exp. Biol. Med., 49:1-4.
- Schoenheimer, R. and D. Rittenberg 1940 The Study of
Intermediary Metabolism of Animals with the Aid of
Isotopes. **Physiol. Rev.**, 20:218-248.
- Schousboe, J. 1939 Use of Krogh's Precision Syringe
for Rapid Specific Gravity Measurement of Serum as
an Indicator of Protein Content. **J. Biol. Chem.**,
129:371-375.
- Siebenmann, C. 1937 Refractometric Methods for Deter-
mining Total Protein. **Biochem. J.**, 31:205-211.
- Simeone, F. A. and S. P. Sarris 1941 A Simple Method
for the Determination of Serum Protein. **J. Lab. Clin.
Med.**, 26:1046-1052.
- Sobel, A. E., A. M. Mayer and S. P. Gottfried 1944 A
Convenient Titrimetric Ultramicromethod for the
Estimation of Urea and Kjeldahl Nitrogen. **J. Biol.
Chem.**, 156:355-363.
- Snyder, R. and S. Katzenelbogen 1942 The Distribution
of Sodium, Potassium, Calcium, Magnesium, Inorganic
Phosphorous and Chloride Between the Blood Serum and
Cells of Normal Individuals. **J. Biol. Chem.**, 143:
223-226.

- Stiff, H. A. 1949 A New Colorimetric Method for the Determination of Serum Protein. J. Biol. Chem., 177:179-185.
- Stuart, H. C. 1945 Review of the Evidence as to the Nutritional State of Children in France. Am. J. Pub. Health, 35:299-307.
- Sunderman, F. W. 1944 A Rapid Method for Estimating Serum Proteins. Formula for Calculating Serum Protein Concentration from the Refractive Index of Serum. J. Biol. Chem., 153:139-142.
- Torbert, H. C. and G. Cheney 1936 Reduction in Colloidal Osmotic Pressure of Blood Serum After Salt Ingestion. J. Am. Med. Assoc., 106:683-684.
- Van Slyke, D. D. 1927 Gasometric Micro-Kjeldahl Determination of Nitrogen. J. Biol. Chem., 71:235-248.
- Walters, J. H., R. J. Rossiter and H. Lehmann 1947 Blood Volume Changes in Protein Deficiency. Lancet, 1:244-249.
- Weech, A. A., E. B. Reeves and E. Goettsch 1936 The Relationship Between Specific Gravity and Protein Content in Plasma, Serum, and Transudate from Dogs. J. Biol. Chem., 113:167-174.
- Weech, A. A., C. E. Snelling and E. Goettsch 1933 The Relation Between Plasma Protein Content, Plasma Specific Gravity, and Edema in Dogs Maintained on a Protein Inadequate Diet and in Dogs Rendered Edematous by

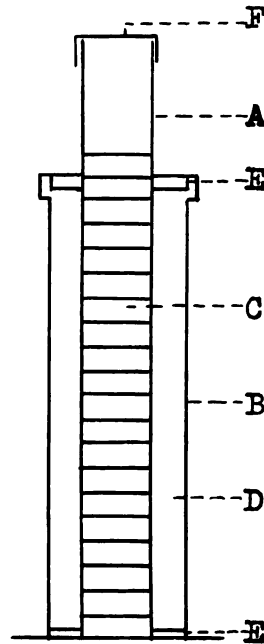
Plasmaphoresis. J. Clin. Invest., 12:193-216.

Youmans, J. B., E. W. Patton, W. R. Sutton, R. Kern
and R. Steinkamp 1943 Surveys of the Nutrition of
Populations. II. The Protein Nutrition of a Rural
Population in Middle Tennessee. Am. J. Pub. Health,
33:955-964.

Zozaga, J. 1935 A Physicochemical Study of Blood Sera.
J. Biol. Chem., 110:599-617.

APPENDIX

Gradient Tube*



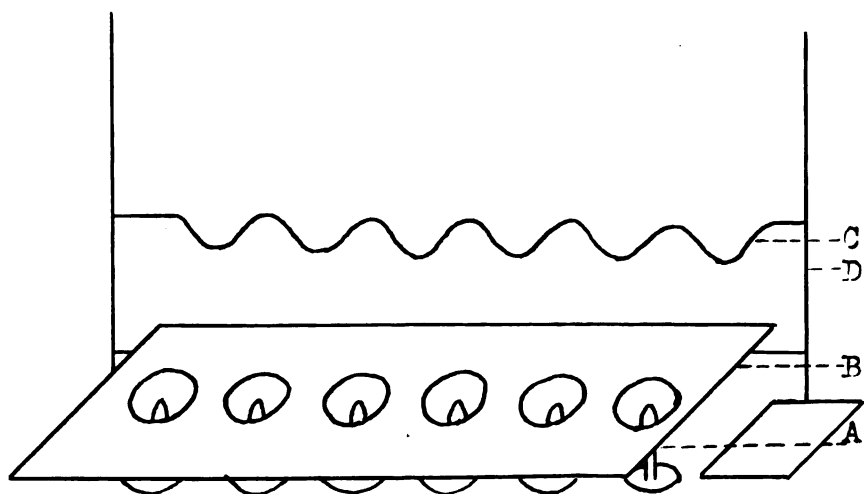
Scale
one inch equals
approximately
five inches.

Key

- A- 500 milliliter graduated cylinder.
- B- Ungraduated cylinder.
- C- Gradient.
- D- Jacket solution.
- E- Paraffin seal.
- F- Cover.

*Lowry and Hunter (1945).

Digestion Rack*



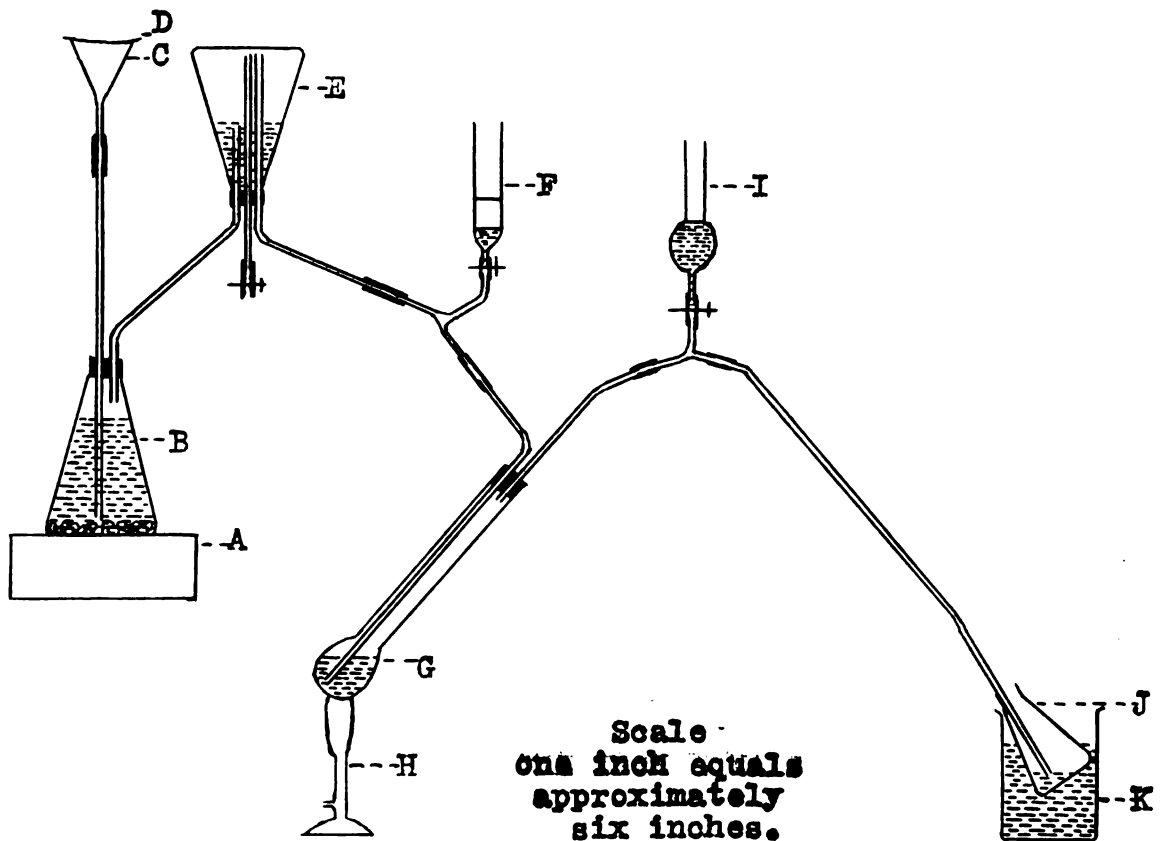
Scale
one inch equals
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Key

- A- Micro burner (flame one inch high).
- B- Asbestos board.
- C- Heavy wire.
- D- Ring stand. and clamp.

*Ma and Zuazaga (1942).

Distillation Apparatus*



Key

- A- Hot plate.
- B- 300 milliliter Erlenmeyer flask containing ammonia-free distilled water and boiling chips.
- C- Funnel.
- D- Watch glass.
- E- Trap and equalizer of steam pressure.
- F- Reservoir containing concentrated sodium hydroxide.
- G- 30 milliliter Kjeldahl flask containing digested sample.
- H- Micro burner.
- I- Reservoir containing ammonia-free distilled water which has been adjusted to the purple color of the indicator.
- J- 50 milliliter Erlenmeyer flask containing boric acid and indicator.
- K- 250 milliliter beaker containing water.

Rubber tubing was boiled in dilute ammonium hydroxide, washed with distilled water, and boiled in two percent boric acid (Sobel, Mayer, and Goettfried, 1944).

*Peters and Van Slyke (1932); Hill and Trevorrow (1941); Markham (1942).

Solutions

Albumin solution (C.P. blood albumin containing 15% salt):

20 milliliters of albumin solution (approximately 6.5 grams per liter) were diluted to 25 milliliters.

162.5 milligrams of sodium chloride were dissolved in approximately four milliliters of water, 20 milliliters of the albumin solution added, and the mixture diluted to 25 milliliters.

This was repeated with 167.5, 172.5, and 177.5 milligrams of sodium chloride and with 21.2, 30.0, and 62.5 milligrams of glucose.

Ammonia-free distilled water:

10 milliliters of concentrated sulfuric acid and a few crystals of potassium permanganate were added to 1500 milliliters of distilled water. The mixture was redistilled and the distillate collected after the first 50 to 100 milliliters were discarded. The distillate used was tested with Nessler's reagent for the presence of ammonia.

The pH of a portion of the water was adjusted to the purple color of the indicator.

***Ammonium sulfate standards (C.P.-neutrality A.C.S. tested):**

The ammonium sulfate was dried to constant weight at 105 degrees centigrade (Sobel, Mayer, and

***Solutions were made with ammonia-free distilled water.**

Gottfried, 1944). 0.5896 gram was diluted to one liter (0.125 milligram nitrogen per milliliter). 80 milliliters of this solution were diluted to 500 milliliters once a week (0.02 milligram nitrogen per milliliter).

***Boric acid - 2 percent:**

10 grams of boric acid were diluted to 500 milliliters with boiled water.

***Digestion mixtures:**

One milliliter of selenium oxychloride was diluted to 250 milliliters with concentrated sulfuric acid and added to 250 milliliters of saturated potassium sulfate.

300 milliliters of 85 percent phosphoric acid, 50 milliliters of five percent copper sulfate solution, and 100 milliliters of concentrated sulfuric acid were combined. The solution was diluted one to ten for use.

Gradient jacket solution:

Two grams of copper sulfate and 0.6 milliliter of concentrated sulfuric acid were diluted to one liter.

Gradient mixtures:

150 milliliters of kerosene** were combined with 100 milliliters of bromobenzene*** and adjusted to

*Solutions were made with ammonia-free distilled water.

**Eimer and Amend, odorless.

***Eimer and Amend, special for falling drop method.

a specific gravity of 1.07.

180 milliliters of kerosene were combined with 70 milliliters of bromobenzene and adjusted to a specific gravity of 0.99.

Hydrogen peroxide - 30 percent.

Hydrochloric acid standard solutions:

0.1 N acid was made by carefully adjusting the acidity of an acid solution which was approximately 0.1 N. The acid was titrated with sodium hydroxide which had been standardized against potassium acid phthalate (A.C.S. standard).

0.01 N acid was made by diluting the 0.1 N acid. This was done once a week.

Indicator:

0.1 gram of brom-cresol green was diluted to 100 milliliters with 95 percent ethanol. 0.1 gram methyl red was diluted to 100 milliliters with 95 percent ethanol. Five parts of the brom-cresol green solution were combined with one part of the methyl red solution for use.

Nessler's reagent:

100 grams of mercuric iodide and 70 grams of potassium iodide were added to 400 milliliters of distilled water. 100 grams of sodium hydroxide were dissolved in 500 milliliters

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of distilled water and added to the above solution. The combined solutions were made up to one liter and diluted one to one for use (Hawk, Oser, and Summerson, 1947, p. 1230 - method of Bock and Benedict).

Potassium sulfate standards:

Potassium sulfate (A.C.S. standard) was dried to constant weight at 110 degrees centigrade and the following amounts were each diluted to 500 milliliters:

Potassium sulfate	Specific gravity of solutions
grams	
8.8200	1.0141
11.5400	1.0184
14.2650	1.0227
17.0200	1.0270
19.7900	1.0313
22.5500	1.0356

The stock solutions were kept in a refrigerator in paraffined sealed bottles. Smaller bottles of the solutions were refrigerated and maintained for use with the gradient tube. They were renewed every two weeks.

*Sodium chloride - 0.9 percent.

*Sodium hydroxide (C.P. pellets):

100 grams of sodium hydroxide were added to 100 milliliters of distilled water and allowed

*Solutions were made with ammonia-free distilled water.

to stand a week. The solution was filtered
and stored in paraffined bottles.

*Trichloroacetic acid - 10 percent.

*Solutions were made with ammonia-free distilled water.

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