5231

Determination of Serum Proteins.

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There have been two methods for the estimation of the serum proteins, the refractometric and the chemical determination of the nitrogen through some modification of the Kjeldahl method. While titrating proteins electrometrically we found that the amount of acid required within certain ranges to take a protein from one pH to another was a direct function of the concentration of the protein actually present. This offered an ideal and simple method for the estimation of the serum proteins in the normal and pathological blood. For clinical purposes this can be done with indicators of which we have found methyl red the most satisfactory, and for more exact estimations, the hydrogen ion concentration can be determined electrometrically.

For the colorimetric estimation of the proteins in the serum the following method is used. One-half cc. of blood is diluted with an equal quantity of distilled water and one-quarter cc. of tenth normal HCl and 2 drops of methyl red are added. A color develops instantly.

Yellow—over 6% Protein.
Yellow orange—5% Protein.
Pink orange—4% Protein.
Pink—3% Protein.
Red—below 3% Protein.

This furnishes results sufficiently accurate for ordinary clinical purposes. If more accurate results are required a set of acetate buffers are made up between the ranges of pH = 4 and pH = 6. The serum proteins may then be matched as follows:

7.2%____pH 6.2	4.5%____pH 5.3
6.3%____pH 5.9	3.6%____pH 5.0
5.4%____pH 5.6	2.7%____pH 4.7

The application of protein titration given above is entirely designed for clinical purposes. The results when compared with buffer solutions give results that check within 0.4-0.5 gm. of protein per 100 cc. of serum with those obtained by the determination of the total protein nitrogen by digestion methods. This is sufficiently accurate for diagnostic purposes. One hundred sera were examined and parallel determinations of the serum protein nitrogen made and were

found to be within this limit. In 3 cases of nephrosis with edema a distinctly red color was obtained and the protein digestion method showed contents of 3.1, 2.9 and 3.4 gm. per 100 cc. respectively. The turbidity of the sera due to the high lipoid content did not interfere. One case of suspected nephrosis with slight edema showed an orange red color equivalent to a buffer of pH 5.2 and was shown by the standard methods to have 4.5% of protein. Certain of the sera which gave a yellow orange color (pH = 5.8) contained about 6% total protein and were to be classified as low normals. In cases of cachexia of cancer an orange pink color (pH = 5.3) was obtained and the sera showed a protein content of 4.6% and 4.8% respectively.

5232

Concentration of the Causative Agent in the Filtrate of the Rous Chicken Sarcoma.

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The studies of Lewis and Andervont,[1] and those of Michaelis,[2] also of Baker and MacIntosh,[3] Frankel[4] and Sugiura and Benedict,[5] within the past 2 or 3 years, have disclosed additional information concerning the physical and chemical properties of the causative agent of the Rous Chicken Sarcoma. Most of these observers investigated the range of H ion concentration in which the active agent retains its infectivity, and concluded that the nature of the buffer solution appears to be of no great significance, as long as the pH is between 3.8 and 10 to 11. Since the experiments of Lewis and Michaelis were carried out with tumor extracts, and those of Sugiura and Benedict with fragments of tumor tissue, we were interested in determining the activity of the causative agent in a cell-free filtrate under varying pH values, and to correlate this with the results obtained with the tumor extract, or tissue fragments.

[1] Lewis and Andervont, *Bull. Johns Hopkins Hosp.*, 1927, **41**, 185.

[2] Lewis and Michaelis, *Bull. Johns Hopkins Hosp.*, 1928, **43**, 92.

[3] Baker and MacIntosh, *Brit. J. Exp. Path.*, 1927, **8**, 257.

[4] Frankel, E., *Z. f. Krebsforschung*, 1929, **29**, 491.

[5] Sugiura and Benedict, *J. Can. Res.*, 1927, **11**, 164.