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Plasma Protein Distribution by the Modified Wu Colorimetric Method During Glucose Tolerance Tests.

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Wu¹ published a colorimetric method for the analysis of plasma proteins by use of a phospho-18-molybdictungstic acid (phenol) reagent. The method of fractionation and the standard used was modified by Gibson² and his method in essential details has been followed in this work. Six cc. of blood were removed by vena puncture, oxalated and centrifuged to obtain the clear plasma. One cc. of the plasma thus obtained was placed in a 15 cc. centrifuge tube and 2 cc. of distilled water added. To this was added 1 cc. of saturated ammonium sulphate thus bringing the solution to one-fourth saturation, precipitating out the fibrinogen. After standing 10 minutes the fibrinogen was lightly centrifuged and the fluid decanted into a dry tube. One cc. of the fluid was placed into a second 15 cc. centrifuge tube with $\frac{1}{2}$ cc. of water and 1 cc. of saturated ammonium sulphate. This brought the mixture to half saturation thus precipitating out the globulins. After standing 15-20 min. the tubes were centrifuged rapidly, and the supernatant fluid decanted off. One cc. of this fluid and 2 cc. of water were pipetted into a third centrifuge tube for the albumin determination.

After all the liquid had been drained from the fibrinogen and globulin, the precipitates were dissolved in 3 cc. of water. From this point the separated fractions were treated in the same manner. To each tube was added 1 cc. of trichloroacetic acid (20%) and the resulting precipitate centrifuged out. The fluid was removed, 2 cc. of distilled water blown from a pipette so as to cause a dispersion of protein and one drop of concentrated sodium hydroxide added to cause solution. The volume was brought to 9 cc.

Five cc. of standard solutions of fibrinogen, globulin and albumin (100 mg. per 100 cc.) were diluted to 9 cc. and placed in a water bath at 40°C. along with the unknowns. Clouding takes place if this is carried out at room temperature. Two cc. of phenol reagent and 3 cc. of 20% sodium carbonate were added and the tubes al-

¹ Wu, H., *J. Biol. Chem.*, 1922, **12**, 33.

² Gibson, R. B., private communication.

lowed to remain in the water bath for 10 min. They were then compared in a colorimeter with the respective standard.

Standards: The albumin and globulin used for the standards were obtained from human ascitic fluid by fractionation with ammonium sulphate and heat coagulation. The fibrinogen was obtained from beef blood in a similar manner. Sodium hydroxide was used to cause their solution. This type of standard is probably more accurate and is easier to compare than tyrosine.

Calculation of results are as follows:

Fibrinogen; $5/R = \% \text{ fibrinogen when standard is set at } 10.$
 Globulin; $50/R \times 0.4 = \% \text{ globulin}$ " " " "
 Albumin; $50/R = \% \text{ albumin}$ " " " "

Normal protein distribution was studied on 26 individuals most of whom were medical students. The following table summarizes the results obtained:

TABLE I.

	Fibrinogen	Globulin	Albumin	Total Protein
Maximum	0.59	2.74	5.21	7.70
Minimum	0.33	1.42	3.33	5.95
Average	0.46	1.93	4.35	6.71
Standard deviation	0.06	0.28	0.42	

Figures express grams protein per 100 cc. of plasma.

The protein distribution during sugar tolerance tests as determined for 9 normal individuals. As hemoglobin is always reduced when the blood glucose increases under these conditions it was supposed that the proteins would also be diluted. However this was not the case.

The sugar tolerance curve was obtained in the usual manner. Fifty grams of glucose were given by mouth. Blood sugar was determined at the fasting level and $\frac{1}{2}$, 1 and 2 hours after receiving the glucose. The protein distributions were studied at the fasting

TABLE II.

Protein	No change	Dilution	Concentration	Conc. 1 hr. Dilut. 2 hr.	Dilut. 1 hr. Conc. 2 hr.	No result
Fibrinogen	2	2	1	2	—	2
Globulin	2	2	3	1	1	
Albumin	1	5	3	—	—	
Total protein	1	4	3	1	—	

Numbers express the cases out of 9 falling into each class.

level and the 1 and 2 hour periods. A summary of the results obtained is given in Table II.

One individual showed no change in the proteins during this test. All of the others showed changes in one or all of the fractions but no correlation could be found between the protein variations and the blood sugar level, or the type of sugar curve. In 3 cases the albumin decreased and the globulin increased while in 1 case the reverse of this was true. In the other cases both proteins gave the same type of change.

Summary: The Wu method for protein distribution as modified by Gibson gives a rapid, accurate method for determination of plasma proteins. Protein is used as a standard. During glucose tolerance tests there is no relation between the protein distribution and blood sugar as is found with hemoglobin and the blood sugar.

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An Electrophysiological Study of the "Simple" Reflex Circuit.

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It is generally conceded that there are no well isolated reflex arcs. Nervous energy released in the reflex arc sense organs spreads apparently to immediately circumjacent and even to distant and perhaps to all parts of the central nervous system.* Positive evidence pertaining directly to this point has been furnished by recent experiments.¹ In the rat it has been demonstrated that the electrical energy released in the reflex arc sense organs was conducted to all parts of the cerebral cortex studied. These studies did not disclose the possible immediacy of spread of the electrical energy in the cord. The energy recorded in the cortex might have reached there by relatively sharply delimited circuits to spread subsequently to all cortical fields. It might have reached there by way of all of the afferent spino cerebral-paths. To shed further light upon this particular problem as well as upon the general problem of mass responsiveness in the central nervous system the present study was undertaken.

* The morphological concomitant of this physiological concept is presented by Michaels, J. J., *J. Nerv. and Mental Disease*, in press.

¹ Travis, L. E., and Herren, R. Y., *J. Comp. Psychology*, 1931, in press.