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Mean Molecular Weights of Synthetic Mixtures of Bovine Plasma Albumin and Globulin.

HENRY L. TAYLOR AND ANCEL KEYS

From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.

Albumin and globulin, though generally considered to be separate entities, show sedimentation anomalies when they are studied in mixtures with the ultracentrifuge (Pederson[1]). The data of McFarlane[2] suggest that globulin may dissociate in such mixtures. We have studied purified bovine plasma albumin and globulin, alone and in synthetic mixtures, by osmometric methods.

Sterile bovine plasma was carefully fractionated with ammonium sulfate at 0°C by a technic similar to that used by McMeekin.[3] The albumin and globulin fractions were reprecipitated, freed of ammonia by dialysis and then dialyzed to equilibrium with phosphate buffer of pH 7.4, $\mu = 0.16$. Osmotic pressures were measured at 0°C by the method to be described by Keys. Protein concentrations were determined with the Pulfrich refractometer in every sample and checked by the micro-Kjeldahl method of Keys[4] in about every third sample. Mean molecular weights were determined by the method of Adair and Robinson[5] in which the pressure-concentration ratio is extrapolated at zero concentration. At least 4 acceptable P/C values at below 3% protein concentration were obtained for each sample.

The molecular weights of the albumin and globulin used here were 69,900 and 170,000, respectively, and these values were used to compute the theoretical mean mol. wts. of the mixtures from Dalton's law of partial pressures. The observed mol. wts. of the mixtures deviated widely and systematically from the simple predictions. As shown in Fig. 1, the mean mol. wts. of the mixtures are lower, and the osmotic pressures are higher than predicted. The deviation approaches a linear function of the mol. wt. in the range of A/G from 0.1 to 2.1. Similar results were obtained with other preparations.

1 Pederson, K. O., *Compt. Rend. Lab. Carlsberg, Copenhagen*, 1938, **22**, 426.

2 McFarlane, A. S., *Biochem. J.*, 1935, **29**, 407.

3 McMeekin, T. L., *J. Am. Chem. Soc.*, 1939, **61**, 2884.

4 Keys, A., *J. Biol. Chem.*, 1940, **132**, 181.

5 Adair, G. S., and Robinson, M. E., *Biochem. J.*, 1930, **24**, 1864.

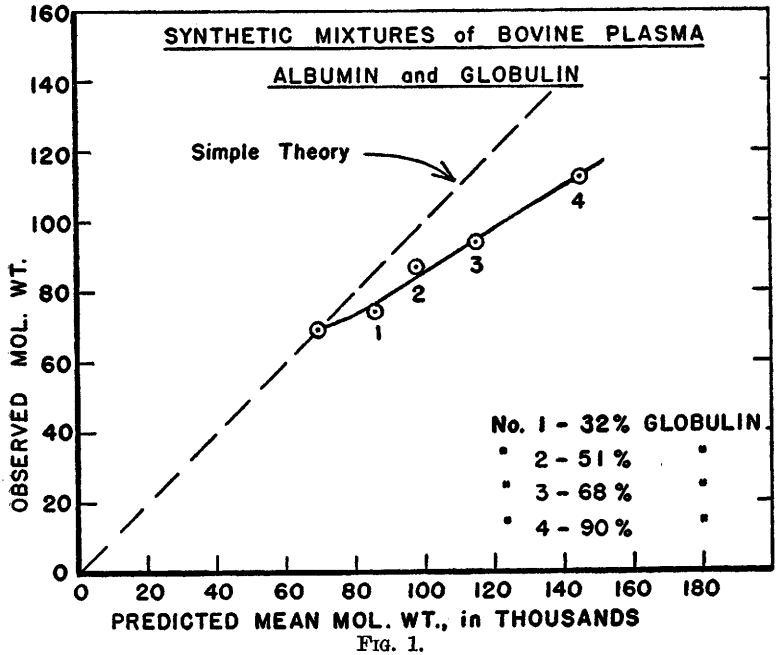

FIG. 1.

Sedimentation diagrams of such mixtures clearly show 2 fractions corresponding in molecular size with albumin and globulin but McFarlane's (op. cit.) data on horse plasma proteins indicate that the proportion of the smaller molecules is higher than the composition of his mixtures would warrant. This strongly reinforces our conclusion that globulin dissociates in the presence of albumin. Assuming that the dissociation product is a particle like albumin in size, our results would indicate that about 35% of the globulin undergoes dissociation; in other words, in an albumin-globulin mixture, for every globulin 170,000 molecule, there would be a globulin 70,000 molecule. Alternatively, globulin 170,000 may split into equal halves (mol. wt. 85,000, cf. McFarlane, op. cit.).

These results explain the divergent values reported for the mol. wt. of globulin—145,000 to 192,000. The presence of minute amounts of albumin depresses the mean mol. wt. to a remarkable degree. It is not easy to separate globulin cleanly from albumin; on the other hand, we have had no difficulty in preparing pure albumin. It appears that the formation of dissociated globulin is reversible, that the dissociated globulin salts out with the other globulin and it becomes associated when it enters an albumin-free phase. These phenomena are most striking at very low A/G ratios and are seen in purest form at infinite dilution, but they are de-

monstrable at an A/G ratio of 2.1 and protein concentrations of over 3%. In human plasma (A/G = 2, tot. prot. = 7%) the effect is sufficiently obscured so that the simple relation of Keys[6] holds approximately.

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Skin Sensitization to a Simple Compound by Injections of Conjugates.

KARL LANDSTEINER AND MERRILL W. CHASE.

From the Laboratories of the Rockefeller Institute for Medical Research.

In view of a recent paper[1] touching upon the subject, we wish to make a preliminary communication of a study under way for some time on the possibility of producing in animals skin sensitivity to drugs by immunizing with conjugates. We have in fact been able to render guinea pigs sensitive to superficial application of picryl chloride[2, 3] by intraperitoneal injections of a conjugate resulting from the treatment of guinea pig erythrocyte stromata with picryl chloride in alkaline solution, killed tubercle bacilli as in previous work[4] having been injected beforehand.

Since even minute quantities of the simple substance can sensitize under certain conditions and must be avoided, the chief concern in these experiments was to guard against the inclusion of unchanged picryl chloride in the injection material. This was carried out by adding an excess of glycine which removed any possible remainder of the substance through chemical combination, and by washing with aqueous alcohol.

The large majority of animals treated in this way have shown upon subsequent testing with the simple substance typical reactions of the contact dermatitis type.

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6 Keys, A., *J. Phys. Chem.*, 1938, **42**, 11.

1 Haxthausen, H., *Acta Derm.-Vener.*, 1940, **21**, 158.

2 Landsteiner, K., and Jacobs, J., *J. Exp. Med.*, 1935, **61**, 643.

3 Landsteiner, K., and Chase, M. W., *J. Exp. Med.*, 1937, **66**, 337.

4 Landsteiner, K., and Chase, M. W., *J. Exp. Med.*, 1940, **71**, 237.