Molecular weights of rat, rabbit, and guinea-pig serum albumins

Although the serum albumins of the rat, rabbit, and guinea-pig have been used in many biochemical and biological studies, and although occasionally sedimentation coefficients have been mentioned by various workers, there do not appear to be any published figures for the molecular weights of these proteins. The ARCHIBALD[1] ultracentrifugal procedure has now been used to obtain this information.

In order to establish the validity of the measurements, it was necessary to make comparisons of albumin samples isolated by entirely independent methods, and examined when possible by at least two different analytical techniques. Preparative methods which were used included free-boundary electrophoresis, separation at low pH in organic-solvent systems, and chromatography on packed columns followed by gradient elution. The quality of the products was in all cases checked by high-velocity ultracentrifugation, whilst some samples were also subjected to electrophoresis, either in the Tiselius apparatus or on paper*.

Molecular weight determinations were done at 0.6–0.8 % albumin concentration in 0.15 M KCl containing sufficient phosphate buffer (pH 6.4) to give a total ionic strength of 0.2. The Spinco ultracentrifuge was run at low speed (12,590 or 8,210 rev./min), and measurements and calculations were made as previously described[2]. It was assumed that the partial specific volume in all cases was the same as that of human serum albumin near its iso-electric point ($\bar{v} = 0.736$)[3].

Since differences of less than 5 % may be attributed to experimental error in the molecular weight determinations, the typical results in Table I demonstrate good agreement between the values for corresponding albumins prepared by alternative methods. The molecular weights of the rat and rabbit albumins did not differ significantly, although the former might have been slightly high because of the presence of small traces of heavier material which were detectable in the ultracentrifuge at high speed (59,780 rev./min). Guinea-pig albumin differed from the other albumins by giving a molecular weight nearly 10 % lower. None of the proteins referred to in Table I showed any time dependence of molecular weight during the low-speed experiments, further evidence for the absence of contaminants which might seriously affect the results. Of course, the conclusions drawn here assume that the sedimentation and diffusion coefficients of the albumins do not have an unusually high

TABLE I

MOLECULAR WEIGHTS OF SERUM ALBUMINS

Source	*Isolation*	*Examination*	*Molecular weight*
Rat	Column chromatography	Paper electrophoresis	69,900
Rat	Ethanol–trichloracetic acid		68,900
Rabbit	Column chromatography	Paper electrophoresis	68,900
Rabbit	Ethanol–trichloracetic acid		66,300
Guinea-pig	Free electrophoresis	Free electrophoresis	62,300
Guinea-pig	Ethanol–trichloracetic acid		62,300

* Samples prepared by column chromatography and examined by paper electrophoresis were kindly provided by Dr. S. COHEN.

concentration dependence, and that the partial specific volumes do not differ greatly from the value used in the calculations. Although there is no information available about the partial specific volumes, experimental evidence indicates that there are probably no unexpected concentration effects in the sedimentation behaviour.

The provisional conclusion that guinea-pig albumin differs from the other two albumins, which are closely similar, is supported by the finding that its sedimentation coefficient in the neutral range (pH 6–8) was slightly lower than the 4.4 S found for both rat and rabbit albumins. An additional distinction lay in the sedimentation velocity behaviour in more acid solution (pH 5–2). Here, rat and rabbit albumins followed the trend already established for human, bovine, and horse serum albumins[4], the sedimentation coefficient falling throughout the range pH 5 to pH 2, whereas a more complicated dependence on pH was found for guinea-pig albumin. These observations indicate, therefore, that guinea-pig albumin differs from the others not only in the size of its molecule, but also in some structural feature which remains to be elucidated. Full details of this work will be published later.

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Received December 10th, 1958

Adenosine polyphosphate requirement of baker's yeast phosphopyruvate carboxylase

The enzyme responsible of carbon dioxide fixation by baker's yeast (*Saccharomyces cerevisiae*)[1,2] is a phosphopyruvate carboxylase. It can be extracted in a soluble form from acetone-dried yeast[3] and purified 500 times by a procedure involving treatment with $Ca_3(PO_4)_2$ gel, three precipitations with $(NH_4)_2SO_4$ between 0.42–0.55 satn., dialysis against 0.025 *M* borate buffer pH 8.0, treatment with protamine sulphate, two fractionations with ethanol and a final chromatography on a hydroxylapatite column[4]. If $^{14}CO_2$ is used for the carboxylation of phosphopyruvate, radioactive oxaloacetic acid can be isolated as the 2,4-dinitrophenylhydrazone and the latter identified by paper chromatography and radioautography; further, if the carboxylation takes place in the presence of glutamate-aspartate transaminase and glutamate, or of malic dehydrogenase and reduced diphosphopyridine dinucleotide, the oxaloacetic acid reacts to form respectively aspartic or malic acid, which can be isolated and identified by the chromatographic and radioautographic methods.

The purified enzyme preparations decarboxylate oxaloacetate, catalyse the exchange of $^{14}CO_2$ into the β-carboxyl group of oxaloacetate, and carboxylate phosphopyruvate. The exchange reaction requires ATP and Mn^{++}, but for oxaloacetate decarboxylation, only ATP is necessary. The carboxylation of phosphopyruvate takes