CLIV. THE ULTRAVIOLET ABSORPTION AND POTENTIOMETRIC TITRATION CURVES OF HUMAN SERUM PROTEINS AND SOME OTHERS

By ENSOR ROSLYN HOLIDAY[1] and ALEXANDER GEORGE OGSTON[2]

From the Research Laboratories, The London Hospital

(*Received 25 April 1938*)

This work is a continuation of that of Holiday [1936] and of Ogston [1936]. The work of Tiselius [1937], on the separation of serum proteins by electrophoresis which has appeared since this work was begun, has explained some of the earlier results and in some degree reduced the value of ours. It is now clear from his investigations and from those of McFarlane [1935] that homogeneous preparations of serum proteins are not obtained by precipitation with ammonium sulphate.

In our earlier work some degree of variation was found in the quantitative properties of different specimens of normal horse serum proteins. Our first object was to establish whether these variations are due to causes other than detailed differences in the method of separation; measurements were therefore made on the pseudoglobulins of the serum of a number of normal persons, separated by a standardized technique. As the opportunity arose, measurements were made also on some preparations of normal serum albumin, on serum and urinary proteins of two nephrotic patients, on some purified antibodies to types II and III pneumococcus and on a solution of seroglycoid prepared by Dr L. F. Hewitt.

Ultraviolet absorption spectra were measured as described by Holiday [1936]. Potentiometric titration curves were obtained by the method of Ogston [1936]: at least two concordant runs were made over each limb of the curve. Discordant results were very rare. The number of primary amino groups was obtained by potentiometric titration in presence of 4 % formaldehyde.

Materials

(1) *Normal pseudoglobulin* in serum obtained from 30 ml. of human blood was thrice precipitated from 20-fold dilution by 40 % saturated ammonium sulphate, allowing minimum time for floculation: the time of contact with strong ammonium sulphate never exceeded 8 hr. The separated globulin was dialysed in rotating cellophane sacs against running distilled water until negative to the Nessler test for ammonia, performed on the protein solution. The precipitated euglobulin was removed by filtration.

(2) *Normal albumin.* A specimen of normal human albumin was prepared from human blood by two successive precipitations by 80 % saturated ammonium sulphate after removal of the globulin, and dialysis against distilled water. Of three specimens of crystalline horse serum albumin, one was prepared by Dr Wolff in these laboratories; one was kindly sent to us by Dr L. F. Hewitt; the third was prepared by the method of Hewitt [1936] and had a carbohydrate content of less than 0·1 %.

[1] Owen Williams Scholar. [2] Freedom Research Fellow.

(3) *Nephrotic proteins* were prepared from the serum and urine of two patients. From serum, the pseudoglobulin was obtained by the method given above: two fractions of albumin were obtained by 66% and 80% saturated ammonium sulphate respectively. From urine, after thorough dialysis against tap water, two pseudoglobulin fractions were obtained by 40 and 50% saturated ammonium sulphate, and albumin by 60% saturated ammonium sulphate.

(4) Concentrates of protein antibodies to types II and III pneumococcus were prepared by Prof. J. R. Marrack from serum of immunized horses; the antibodies were obtained from their sera by elution of the specific precipitates.

Results

(1) *Tyrosine and tryptophan.* Values of the tyrosin and tryptophan contents of the proteins derived from their ultraviolet absorption curves [Holiday, 1936] are given in Table II.

Corrected values for the extinction coefficients of tyrosine and tryptophan were used in the calculation, based on a rematching of spectrograph plates with a photoelectric microphotometer: these corrected values are given in Table I. The revised equations for the analysis of mixtures are:

Table I. *Molecular extinction coefficients of tyrosine and tryptophan*

In $N/10$ NaOH $\epsilon \times 10^{-3}$												
Wave-length mμ ...	260	265	270	275	280	285	290	295	300	305	310	315
Tyrosine	1·75	1·14	1·08	1·24	1·54	1·96	2·24	2·26	1·89	1·14	0·471	0·182
Tryptophan	3·10	3·80	4·45	5·15	5·43	4·65	4·35	2·15	1·10	0·45	0·140	0·051
In $N/10$ HCl $\epsilon \times 10^{-3}$												
Wave-length mμ ...	260	265	270	275	280	285	290	295	300	305	310	315
Tyrosine	0·55	0·86	1·15	1·24	1·08	0·55	0·07	—	—	—	—	—
Tryptophan	3·90	4·65	5·23	5·37	5·46	4·45	3·72	1·35	0·57	0·10	—	—

$$M_{\text{tyrosine}} = (0{\cdot}99\, E_{305} - 0{\cdot}032\, E_{280}) \times 10^{-3}.$$
$$M_{\text{tryptophan}} = (0{\cdot}207\, E_{280} - 0{\cdot}280\, E_{305}) \times 10^{-3}.$$

The following is a more reliable method of correcting for haze or pigment than that given formerly:

(1) $M_{\text{tryptophan}}$ is obtained from the nomogram.

(2) From this E_{305} (tryptophan) is calculated $= M_{\text{tryptophan}} \times 0{\cdot}45 \times 10^3$.

Table II. *Amino, tyrosine, tryptophan: milliequivalents per g. protein*

Protein	Amino	Tyrosine	Tryptophan
Mean of 13 normals	0·449	0·380	0·150
Standard deviation	0·030	0·040	0·013
A 13	0·600	—	—
Horse crystalbumin (Hewitt)	0·680	0·278	0·016
Horse crystalbumin	0·670	0·290	0·005
Crystalbumin, horse albumin	0·650	—	—
Horse seroglycoid (Hewitt)	0·415	0·320	0·114
H 40 (serum)	0·415	0·365	0·164
H 50 (urine)	0·530	—	—
I 40 (urine)	0·500	0·304	0·168
I 50 (urine)	0·395	0·315	0·163
H 66 (serum)	0·640	0·304	0·080
H 80 (serum)	0·680	0·392	0·033
H 60 (urine)	0·570	0·355	0·020
Anti type II	0·500	0·410	0·103
Anti type II	0·590	0·470	0·160
Anti type III	0·410	0·380	0·160

Table III. *Titre from* p*H 7: milliequivalents per g. protein*

*p*H ...	3	3·5	4	4·5	5	5·5	6	6·5	7	7·5	8	8·5	9	9·5	10	10·5	11
Normal pseudoglobulins																	
Mean of 13	0·684	0·507	0·347	0·231	0·151	0·102	0·062	0·028	0	0·026	0·048	0·069	0·094	0·135	0·206	0·307	0·436
Standard deviation	0·029	0·020	0·020	0·014	0·013	0·008	0·005	0·003	0	0·003	0·003	0·006	0·006	0·009	0·011	0·014	0·025
Normal albumins																	
A 13 (human)	1·214	0·959	0·667	0·431	0·272	0·175	0·105	0·049	0	0·050	0·090	0·130	0·172	0·231	0·327	0·472	0·679
Horse seroglycoid (Hewitt)	0·863	0·678	0·503	0·373	0·268	0·186	0·118	0·056	0	0·040	0·077	0·111	0·137	0·182	0·261	0·387	0·574
Mean of three horse crystalbumins	1·268	1·003	0·668	0·406	0·244	0·151	0·092	0·044	0	0·051	0·105	0·163	0·208	0·260	0·340	0·463	0·689
Nephrotic proteins																	
Pseudoglobulins:																	
H 40 (serum)	0·742	0·565	0·400	0·264	0·167	0·112	0·064	0·026	0	0·028	0·055	0·076	0·098	0·140	0·207	0·323	0·452
H 50 (urine)	0·715	0·549	0·380	0·247	0·165	0·102	0·057	0·027	0	0·023	0·051	0·074	0·108	0·158	0·245	0·385	0·563
I 40 (urine)	0·753	0·580	0·405	0·278	0·182	0·100	0·056	0·028	0	0·027	0·056	0·092	0·130	0·187	0·261	0·358	0·495
I 50 (urine)	0·732	0·560	0·386	0·251	0·162	0·102	0·061	0·029	0	0·028	0·052	0·077	0·108	0·151	0·213	0·313	0·446
Albumins:																	
H 66 (serum)	1·036	0·805	0·530	0·325	0·215	0·146	0·090	0·043	0	0·046	0·087	0·127	0·171	0·231	0·318	0·459	0·680
H 80 (serum)	1·160	0·880	0·583	0·368	0·241	0·157	0·105	0·051	0	0·043	0·086	0·129	0·175	0·240	0·331	0·468	0·635
H 60 (urine)	1·238	0·996	0·708	0·468	0·296	0·182	0·105	0·050	0	0·058	0·112	0·153	0·200	0·275	0·387	0·537	0·762
Antibody proteins																	
Anti type II	1·050	0·860	0·643	0·435	0·260	0·140	0·058	0·018	0	0·022	0·043	0·069	0·098	0·150	0·248	0·395	0·615
Anti type II	0·855	0·705	0·525	0·340	0·195	0·113	0·062	0·024	0	0·021	0·040	0·072	0·114	0·190	0·320	0·515	0·825
Anti type III	0·778	0·638	0·468	0·323	0·198	0·121	0·066	0·027	0	0·022	0·040	0·057	0·080	0·122	0·207	0·325	0·472

(3) Since tyrosine in neutral solution has no absorption at 305 mμ the difference between E_{305} (tryptophan) and the extinction at 305 mμ of a neutral or acid solution of protein represents absorption due to haze or pigment (E_{pig}).

(4) Assuming that E_{pig} is the same at 305 mμ and at 280 mμ and the same in acid and alkaline solutions, correction is made by subtracting E_{pig} from the extinctions of the protein in alkaline solution at these wave-lengths: from the corrected extinctions $M_{tyrosine}$ and $M_{tryptophan}$ are calculated.

(2) *Titration curves.* The titration curves are expressed in Table III as titres from *p*H 7 in milliequivalents per g. protein. In Table II are given the free amino groups in milliequivalents per g. protein.

Discussion

Tiselius [1937] has shown that globulins separated by ammonium sulphate precipitation are mixtures, with the α-globulin predominating in "pseudo-globulin": he states (p. 1476, line 27) that his fractions differ in light absorption. Since their mobilities differ, their titration curves may differ. McFarlane [1935] found that pseudoglobulin preparations obtained by precipitation are somewhat heterogeneous in the ultracentrifuge. It is therefore not surprising that variations are found in the quantities which we have measured. Nevertheless we believe that our mean results represent fairly well the properties of normal pseudoglobulins prepared by our methods, and that, in lack of general availability of the electrophoretic method of separation, comparisons with these of values obtained with other proteins may be of some value.

There is no correlation with age or blood group of the variations found; comparison of these with our previous results for horse serum pseudoglobulin does not suggest any significant differences between them. The pseudoglobulins from the serum and urine of nephrosis give absorptions and titration values which vary somewhat from the normals, but it is doubtful whether these differences are sufficient to show with certainty that they are constitutionally different.

The values obtained for the antibodies should strictly be compared with those of horse serum euglobulin whose solubility properties they imitate, but reliable results for comparison are not available [Ogston, 1936]. However, it seems clear that the antibodies to types II and III pneumococcus are different from each other and that both are considerably different from serum globulin. Type II antibody shows a greater amino-titre than normal. Type III antibody resembles pseudoglobulin except in showing higher titres below *p*H 5, indicating a greater number of carboxyl groups. Neither antibody solution was completely precipitable by specific polysaccharide.

The two preparations of crystalbumin show closely concordant properties and there is no reason to doubt that our results represent the behaviour of this protein. The cruder preparation of human serum albumin differs from these to some extent, but since it was not crystallized, it probably contains seroglycoid: its titration curve accords with this.

Comparison with normals of the serum and urinary albumin of nephrosis, shows that the latter differ from normal and from each other: we regard these differences as significant.

The results obtained with seroglycoid are interesting since both the content of tyrosine, tryptophan and amino groups and the form of its titration curve suggest the properties of a globulin rather than of albumin. Comparison of the titration curves of seroglycoid and of crystalbumin suggest that, if their iso-electric points do not differ much, electrophoretic separation will be easy only at a *p*H below 4·5 or above 9.

SUMMARY

1. The ultraviolet absorption and potentiometric titration curves of a number of proteins have been measured. From them the contents of the proteins in tyrosine, tryptophan and amino groups have been estimated.

2. Satisfactorily concordant results have been obtained with normal human serum pseudoglobulin: serum and urinary pseudoglobulin of nephrosis do not differ significantly from normal.

3. Antibodies to types II and III pneumococcus are considered to differ in their properties from normal globulins.

4. Measurements have been made on purified serum albumins: serum and urinary albumin of nephrosis are significantly different from normal.

5. Seroglycoid resembles globulin rather than albumin in its properties.

The authors thank members of the laboratory staff for gifts of blood; Dr L. F. Hewitt for a gift of crystalbumin and of seroglycoid prepared by him; Prof. J. R. Marrack for preparation of the antibody solutions; and Dr Wolff for a specimen of crystallized albumin.

REFERENCES

Hewitt (1936). *Biochem. J.* **30**, 2229.
Holiday (1936). *Biochem. J.* **30**, 1795.
McFarlane (1935). *Biochem. J.* **29**, 407.
Ogston (1936). *Biochem. J.* **30**, 1845.
Tiselius (1937). *Biochem. J.* **31**, 1464.