CXCI. NOTE ON THE PRESENCE OF A NEW SERUM PROTEIN IN THE BLOOD OF VARIOUS ANIMALS

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THE observation that horse serum albumin can be separated into fractions of widely different properties led to the preparation of two proteins, namely crystalbumin, which is carbohydrate-free, and seroglycoid, a new serum protein, to which is due the greater part of the polysaccharide content of horse serum albumin [Hewitt, 1934; 1936; 1937]. Some 10–20 % of horse serum albumin consists of seroglycoid which, when isolated, has very characteristic properties, not being heat-coagulable or precipitable by 2 % trichloroacetic acid. The present experiments were conducted with a view to determining whether horse serum is unique in this respect or whether proteins similar to seroglycoid occur in the blood serum of other animals.

FRACTIONATION METHODS

Globulins were removed from sera by addition of an equal volume of saturated ammonium sulphate solution either to the undiluted serum or after dilution with water. The filtrates containing the albumin were fractionated by salting out with ammonium sulphate and acetic acid, and further purification of the seroglycoid fractions was effected by heat coagulation or treatment with 2 % trichloroacetic acid. The course of the fractionations was followed by carbohydrate determinations as described in previous papers. Results were calculated as before, assuming that galactose-mannose-glucosamine is the carbohydrate present in serum proteins, and the figures quoted throughout the paper are in terms of this trisaccharide.

Ox serum

After removal of the globulins from ox serum by means of ammonium sulphate, addition of acetic acid to the filtrate containing the albumin fraction precipitated a protein which after seven reprecipitations contained a little less than 0.3 % of trisaccharide and was therefore largely composed of crystalbumin. Further fractionation was effected by dissolving in water, removing the first precipitate produced on adding ammonium sulphate and collecting and dialysing the fraction precipitated on further addition of ammonium sulphate. This fraction contained less than 0.15 % of trisaccharide.

From the mother liquor of the first precipitation of crystalbumin a fraction was recovered containing 4 % of trisaccharide. Refractionation with ammonium sulphate separated this into a sparingly soluble portion containing 1.6 % and a readily soluble fraction containing 6 % of trisaccharide. To a solution of this last fraction 2 % of trichloroacetic acid was added and the precipitate was found to contain 5.5 % and the unprecipitated seroglycoid 8.2 % of trisaccharide.

Fractionation by similar methods of a further small sample of ox serum yielded similar results. The first salting-out processes yielded a sparingly soluble crystalalbumin fraction containing 0.6% of trisaccharide and a readily soluble fraction containing 6% of trisaccharide. This latter fraction was heated in a boiling water-bath first at pH 6 and then at pH 4.7 as described in the case of horse seroglycoid [Hewitt, 1937] and yielded a coagulum containing 5% and uncoagulated seroglycoid containing 7.6% of trisaccharide.

Thus in each fractionation a seroglycoid fraction was obtained readily soluble in ammonium sulphate and containing about 8% of trisaccharide. As in the case of horse seroglycoid the protein was not coagulated when heated, or precipitated by 2% trichloroacetic acid, but it was relatively less readily precipitated by ammonium sulphate than horse seroglycoid. The fraction containing 6% of trisaccharide had an optical rotatory power, $[\alpha]_{5461}$, of -52.5° , which, as in the case of horse seroglycoid fractions, is considerably lower than that of crystalalbumin, which has a rotation of about -78° .

A sample of ox serum pseudoglobulin examined contained 3.3% of trisaccharide and the total albumin fraction about 2% of trisaccharide. This would correspond, after making allowance for the presence of other glycoproteins, to about 20% of the albumin fraction consisting of seroglycoid.

Rabbit serum

With rabbit serum the fractionation proceeded rather differently from those previously described, in that the fractions most readily precipitated by ammonium sulphate were richer in seroglycoid than the more soluble fractions. In a typical fractionation process, after removal of globulins by means of 50% saturated ammonium sulphate solution, the first precipitate obtained on addition of acetic acid was reprecipitated several times and then contained 4% of trisaccharide, whilst a mother liquor fraction contained only 0.3%. In another fractionation the total albumin filtrate was treated with ammonium sulphate and the protein first precipitated contained 6% of trisaccharide. To a solution of this protein trichloroacetic acid was added and the precipitate contained 4.3% of trisaccharide and the unprecipitated seroglycoid contained 7.8% of trisaccharide. From rabbit serum therefore it has been possible to separate a crystalalbumin fraction and a seroglycoid fraction containing a little less than 8% of trisaccharide. Unlike the seroglycoid obtained from ox or horse serum rabbit seroglycoid is relatively much more easily precipitated by ammonium sulphate than the crystalalbumin fraction. A rabbit serum globulin preparation contained 3.9% of trisaccharide.

Human serum

The relative solubilities of the fractions of human serum albumin resembled those of ox serum rather than those of the other animals examined. In the first fractionation the least soluble fraction contained 0.5% and the most soluble fraction 4.1% of trisaccharide. By further salting-out processes the low value was reduced to 0.3%. The seroglycoid fraction was purified by heating at the isoelectric point when the coagulum was found to contain 1.2% and the uncoagulated seroglycoid 8.6% of trisaccharide. In a second fractionation the original salting-out processes yielded a crystalalbumin containing 0.3% and a seroglycoid fraction containing 5.9% of trisaccharide. The optical rotatory powers, $[\alpha]_{5461}$, of the fractions containing 0.5, 4.1 and 5.9% of polysaccharide were respectively -72° , -59° and -56° . Like the other sera examined human

serum albumin, therefore, can be separated into a crystalalbumin and a seroglycoid fraction. A specimen of human serum globulin was found to contain 3.8 % of trisaccharide.

Chicken serum

A small amount of chicken serum was subjected to a preliminary fractionation process and yielded a sparingly soluble albumin fraction containing 0.6 % of trisaccharide and a readily soluble fraction containing 5 % of trisaccharide. Just as in the case of the other sera therefore the chicken serum albumin was divisible into a low carbohydrate (crystalalbumin) fraction and a high carbohydrate (seroglycoid) fraction. The pseudoglobulin fraction of the serum contained 4.6 % of trisaccharide.

DISCUSSION

The albumins of horse, ox, rabbit, chicken and human sera each behave characteristically when fractionated, the relative ease of precipitation of the components showing some variation, but in each case it has been possible to separate the albumin into two fractions of widely different properties. It is not claimed that completely pure proteins have been isolated, but there has been with each serum investigated a broad separation into a crystalalbumin fraction with a low polysaccharide content and a seroglycoid fraction containing considerable quantities of polysaccharide. In Table I are summarized the trisaccharide contents of the extreme fractions obtained.

Table I. *Trisaccharide contents of extreme fractions of various sera*

(Calculated as g. of galactose-mannose-glucosamine per 100 g. of protein)

Serum	Lowest value (crystalalbumin fraction)	Highest value (seroglycoid fraction)
Horse	0.05	10.0
Ox	0.15	8.2
Rabbit	0.3	7.8
Human	0.3	8.6
Chicken (preliminary fractions)	(0.6)	(5.0)

With chicken serum which had been subjected to only a preliminary fractionation the degree of purity of the fractions was less than in the other cases. The extent to which the fractionation of the albumin can be carried depends upon the amount of material and time available and also upon conditions of concentration, temperature, *pH* etc. Thus in the fractionation of one specimen of horse serum the filtrate after removal of the globulins was divided into two equal parts. From one half a small crystalline precipitate of albumin was collected and from the other a large amorphous precipitate. The first precipitate consisted of 2.3 g. of crystalalbumin containing 0.14 % of trisaccharide and the other of 7.9 g. containing 0.6 % of trisaccharide. Evidently the small precipitate was a much purer specimen of crystalalbumin than the larger.

Although, as already stated, the relative solubilities of the fractions vary with the different sera the same general properties are seen in the fractions obtained from the different animal species. In each case the crystalalbumin fraction with its low carbohydrate content had a higher optical rotatory power and was relatively colourless, whilst the seroglycoid fraction with its high content of trisaccharide had a lower rotatory power, was pigmented and, when isolated, was not coagulated by heat or precipitated by 2 % trichloroacetic acid.

Lustig & Ernst [1937] have recently examined the protein carbohydrate content of the sera of a number of animal species. Their results are quoted in terms of glucose and these have been roughly recalculated in terms of galactose-mannose-glucosamine (g.m.g.). In Table II are summarized Lustig & Ernst's results for whole serum and the results described in the present paper for various globulin preparations.

Table II. *Carbohydrate contents of sera and globulin fractions*

(Calculated as g. of g.m.g. per 100 g. protein)

Animal	Whole serum (Lustig & Ernst)	Globulin
Horse	2.4	3.0
Ox	3.2	3.3
Rabbit	2.4	3.9
Human	3.2	3.8
Chicken	5.0	4.6

Normal horse and ox serum albumin contain about 2% of trisaccharide. After making allowance for other glycoproteins, etc., this corresponds to about 20% of the albumin fraction being seroglycoid.

SUMMARY

1. Seroglycoid fractions have been obtained from human, horse, ox, rabbit and chicken blood serum.
2. Although the fractionations have varied to some extent with the different animals the general properties of the seroglycoid fractions are similar.
3. Besides containing considerable amounts of carbohydrate, seroglycoids differ from crystalbumins in their lower optical rotatory power and in not being coagulated by heat or precipitated by 2% trichloroacetic acid.

REFERENCES

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