The alleged transformation of serum albumin into serum globulins

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Earlier experimenters have claimed that serum albumin can be converted into serum globulin by the action of simple reagents in vitro, usually by alkali (14, 17) or heat (18). Bechhold (3) stated that it can be accomplished by salts of heavy metals and by light. These earlier claims seem very improbable in view of the amino acid contents (1, 9-12), the results of formol titrations (15), and sulfur and carbohydrate determinations (4) made on albumin, globulin, and "artificial globulin." A true conversion is especially unlikely in view of the work of Fanconi (6), who found that the "artificial globulins" behaved, as judged by anaphylactic reactions, simply like more or less racemized albumin.

More recently Fischer has published in several places (7, 8) a new claim of the transformation of albumin to globulin by means of heparin (antiprothrombin). He states (8), "Albumin-heparin compounds behave as genuine globulins, and we are justified in considering them as identical with the globulins." He also says, "Heparin acts on the principle of the 'unregelmăssige Reihe,' that is, greater amounts inhibit, and smaller promote the flocculation. The euglobulin goes into solution with a small amount of heparin; the pseudoglobulin needs a larger amount of heparin than the euglobulin for solution."

The last part of the latter statement is contradicted by conclusions drawn by Reiner and Kopp (16) in 1928, "...gegen die Annahme, dass das Heparin der Stoff ist, der unter physiologischen Verhältnissen das Globulin peptisiert."

It is with Fischer's first statement that we have chiefly to do here. The criteria of identity given by Fischer are all physical characteristics, and it seemed of interest to apply also the more
Albumin to Globulin Transformation

delicate and specific methods of immunology; for if the artificial globulin is identical with the natural product, then it should give identical immunological reactions.

We have found that the addition of heparin to a solution of pure crystalline serum albumin produced no flocculation at all, over a wide pH range and even with added electrolytes; also that the albumin solution to which heparin had been added behaved immunologically like albumin. We did find, however, that the addition of heparin to an albumin that had been precipitated only once or twice, and not freed of ammonium sulfate by careful dialysis against buffers, caused the protein to flock out, as stated by Fischer. This product was soluble in saline, and was precipitated by half saturation with ammonium sulfate; however, it still reacted to the same degree as albumin with an antialbumin precipitating serum, and entirely failed to react with an antiglobulin serum. The same was found to be true of its reaction with an appropriately adsorbed old antiserum obtained by injecting whole horse serum, showing that the failure of the "artificial globulin" to react like a globulin was not due to any immunological difference between the purified proteins and the proteins as they exist naturally in horse serum. It would seem, then, that the product obtained by the reaction of heparin with serum albumin can hardly be said to be identical with serum globulin.

EXPERIMENTAL

Preparation of Proteins—Serum albumin and serum globulin were prepared from fresh horse serum by the methods described by Adair and Robinson (2). After four crystallizations, the albumin under toluene was dialyzed in cellophane 4 days in the cold against daily changes of the buffer mixture described by these authors. This was followed by a similar dialysis against daily changes of distilled water. The final solution contained 3.33 mg. of nitrogen per ml., corresponding to a protein content of about 2 per cent. It was sterilized by Berkefeld filtration, and part of it rendered isotonic by the addition of NaCl.

The serum globulin was precipitated four times, then dialyzed

Similarly, Henriques and Klausen (13) have found that the addition of heparin to serum did not increase the amount of chemically demonstrable globulin.
in the ice box, against the buffer solution described by Adair and Robinson, for 6 days, the buffer solution being changed each day, and toluene being always present. It was then dialyzed under the same conditions against 0.85 per cent NaCl solution for 4 days. The final solution contained 4.91 mg. of N per ml. corresponding to a protein content of about 3 per cent. It was sterilized by Berkefeld filtration.

Later, serum albumin was prepared from horse sera, which had been stored some time in the ice box, by removing the globulins by half saturation with ammonium sulfate, and inducing precipitation in the filtrate by the cautious addition of 0.2 N H$_2$SO$_4$. The resulting precipitate was dissolved in distilled water, and in some cases further purified by precipitation by the addition of sufficient saturated ammonium sulfate solution, in which case the precipitate was redissolved and dialyzed overnight against distilled water. These preparations all gave similar results, and will be designated as crude serum albumin.

Preparation of Antisera—Two rabbits were immunized against the isotonic albumin solution and two against the globulin, and the pooled sera from each pair used for the tests. Each rabbit received seven intraabdominal injections of 4 ml. at weekly intervals.

Reactions of antisera are displayed in Table I. It is indicated by the cross reactions of unadsorbed sera, and confirmed by untabulated adsorption tests, that the globulin preparation contained some albumin. This in itself urges the necessity for immunological tests on such preparations before they are used for accurate chemical or physical quantitative determinations from which far-reaching deductions are drawn. In the present instance the antialbumin precipitin was easily removed from the antiglobulin or anti-horse serum by adding 0.3 ml. of albumin solution per ml. of serum, keeping at 37° for 30 minutes, and centrifuging.

Addition of Heparin to Albumin—Small amounts of successive dilutions of heparin solution were added to the crystalline albumin solution which had been brought to pH 5 by the addition of dilute HCl or H$_2$SO$_4$. Though other hydrogen ion concentrations than pH 5 were investigated, no precipitate formed, in contrast to the ease with which it was obtained with the crude albumin solution.
The precipitate formed in the heparin-crude albumin mixture, when dissolved in an original volume of saline, was tested against the anti-horse serum and the adsorbed antiglobulin sera. The results appear in Table II.

### TABLE I

**Precipitin Reactions of Antisera**

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>Albumin</th>
<th>Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antigen dilutions</td>
<td>Antigen dilutions</td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8</td>
<td>1 2 3 4 5 6 7 8</td>
</tr>
<tr>
<td>601-2 (anti-albumin)</td>
<td>+ + + + + + 0 0 0</td>
<td>+ + + + + + 0 0 0</td>
</tr>
<tr>
<td></td>
<td>p p p p p p 0 0 0</td>
<td>p p p p p p 0 0 0</td>
</tr>
<tr>
<td>603-4 (anti-globulin)</td>
<td>+ + + + + + 0</td>
<td>+ + + + + + 0</td>
</tr>
<tr>
<td></td>
<td>p p p p p p 0 ± 0</td>
<td>p p p p p p 0 ± 0</td>
</tr>
<tr>
<td>603-4 (adsorbed with albumin)</td>
<td>⊥ 0 0 0 0 0 0 0 0</td>
<td>+ + + + + + 0</td>
</tr>
<tr>
<td></td>
<td>0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>191 (anti-horse serum)</td>
<td>+ + + + 0 0 0 0</td>
<td>+ + + + 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>p p p p 0 0 0 0</td>
<td>p p p p 0 0 0 0 ± 0 0 0</td>
</tr>
<tr>
<td>191 (adsorbed with albumin)</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td>+ + + + 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>602 (normal serum)</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

+ signifies positive interfacial ("ring") test read after 30 minutes; p, positive sedimentation test read after 18 hours in the cold; A signifies antigen control; S, serum control. Dilutions are given in powers of 3; thus 6 means a dilution of 1:3^6 = 1:729. ± signifies a weakly positive interfacial or sedimentation reaction.

It is seen that the albumin-heparin compounds behave immunologically exactly like albumin, and not at all like globulins. The diminished titer of Serum 191 with the redissolved albumin-heparin flocculi finds acceptable explanations in the fact that the
crude albumin alone did not react in as high dilution as the purified albumin, and in the probability that not all of the crude albumin in solution was precipitated by the heparin.

Were further confirmation desired, immune sera could be produced with albumin-heparin compounds. However, inasmuch as our purified albumin did not flocculate with heparin, and as crude albumin doubtless contains some globulin, there are objections to the use of either mixture as an immunizing agent. Serum globulin is a more active antigen than serum albumin and its presence in crude albumin could be expected rather regularly to give rise to antiglobulin of appreciable titer—perhaps even higher in potency than the concurrently produced antialbumin. In such cases, the embarrassment to a confident interpretation of the reactivities of such sera would be considerable.

### TABLE II

**Precipitin Reactions of Albumin-Heparin Compounds**

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>Albumin + heparin (no precipitate)</th>
<th>Crude albumin + heparin (precipitate, dissolved)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilutions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 A 8</td>
<td>1 2 3 4 5 A</td>
</tr>
<tr>
<td>603-4 (adsorbed</td>
<td>0 0 0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>with albumin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>191 (anti-horse</td>
<td>+ + + + 0 0 0 0 0 0 0 0</td>
<td>+ + + 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>serum)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>191 (adsorbed</td>
<td>0 0 0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>with albumin)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ signifies positive interfacial ("ring") test read after 30 minutes; p, positive sedimentation test read after 18 hours in the cold; A signifies antigen control; S, serum control. Dilutions are given in powers of 3; thus 6 means a dilution of $1:3^6 = 1:729$. ± signifies a weakly positive interfacial or sedimentation reaction.

DISCUSSION

It is evident that from the older point of view, which considered the serum proteins as definite chemical individuals, the chemical and immunological evidence available is sufficient to rule out the
suggestion that any of the "artificial globulins" so far reported can be identical with natural globulin. Fischer, however, states (8), "This consideration agrees very well with the modern conception (Sørensen) which considers the proteins as reversible dissociable systems of components." It may be well to quote Sørensen's point of view in his own words (19). He says: "These substances [i.e. proteins] are represented by the ordinary formula $A_xB_yC_z \ldots$, where $A$, $B$, $C$, etc. indicate entire complexes, mainly polypeptides, whereas the subjoined indices, $x, y, z$, etc. indicate the numbers of the said complexes contained in the whole component system."

It is not wholly obvious that a series of albumin-heparin compounds, each with its own dissociation constant, meets the conditions of Sørensen's concept. But in any case, it follows that if these proteins are, as Sørensen maintains, made up of a system of reversibly dissociable components, then globulin must contain a component or components not present in albumin. Consequently, (a) the combination of albumin with an extraneous substance other than the group or groups characterizing globulin—and, from the immunological data, it seems unlikely that heparin is that group—or (b) the action of a reagent which might be supposed to rearrange these components, could hardly transform albumin into globulin. The composition of heparin is apparently still undecided, but it is thought to be a glycuronic acid derivative; in any case the very small amount that suffices to produce the changes observed by Fischer would seem to preclude any possibility that heparin is the source of any amino acid, or additional amount of amino acid, present in globulin but not in albumin.

The immunological evidence likewise shows that the "artificial globulins" did not contain certain antigenic determinants present in natural globulin, and, in the case of the albumin-heparin compounds, still contained the specific determinants found in albumin, but absent from globulin. This proves that the albumin-heparin compounds are not identical with globulin. It indicates, but does not absolutely prove, as the chemical evidence seems to do, that it would be impossible to make globulin from albumin in vitro by a simple reagent. For though it seems unlikely that an antigenic specificity could be conditioned by a different reversible linking
through residual valencies of the same components (one would expect the components in either case to arrange themselves into the state of equilibrium appropriate to the conditions obtaining in the blood and tissues of the injected animal, and consequently produce identical antigenic stimuli), we cannot state this definitely, in view of our extreme ignorance of what constitutes an antigenic determinant in a natural protein.

The observations of Carpenter and Hucker (5) who apparently found antigenic specificity in caseins of different molecular weights, do not definitely argue against this point of view, for it is not certain that the three forms were all made up from the same components, or even if so, that the transformations there involved really fall into the class of reversible transformations considered by Sørensen.

To those who might urge that our isolated proteins are artificial and so could behave differently than proteins in their natural state, we draw attention to the abundant evidence that this does not seem to be true in the immunological sense. Comparison of immune sera produced by properly isolated and by natural proteins gives little or no indication that antigenic specificity has been altered by processing.

**SUMMARY**

Heparin-serum albumin compounds prepared from several samples of albumin, including one which had been crystallized four times, were investigated immunologically and found to behave like unaltered albumin, and not at all like globulin. This is shown to be in line with chemical analyses of albumin and globulin made by other workers, indicating that albumin-heparin compounds are not identical with globulin. The interpretation of this from Sørensen's conception of proteins as reversible dissociable systems of components is considered.

Even though antigenic specificity may be profoundly influenced by certain minor changes in the protein molecule, we urge, as has been done before, that the developing technology of immune reactions has much to offer the chemist who wishes information concerning the purity and intimate nature of the protein compounds with which he deals.
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