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Isolation of Non-anticomplementary Human Immunoglobulin by *Cohn* Fractionation of Heated Plasma

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Summary: Heat treatment of human plasma at 56 °C eliminates an unstable immunoglobulin fraction. No anticomplementary activity is generated when the remaining heat-stable immunoglobulins are concentrated and purified by the *Cohn* alcohol method.

Gewinnung von nicht antikomplementärem Human-Immunglobulin durch Cohn-Fraktionierung von erhitztem Plasma

Zusammenfassung: Durch Erhitzen von Human-Plasma auf 56 °C wird eine unstabile Immunglobulinfraktion entfernt. Die im Plasma verbleibenden hitzestabilen Immunglobuline können durch *Cohn*'sche Alkoholfraktionierung isoliert werden, ohne daß während der Fraktionierung antikomplementäre Aktivität entsteht.

Introduction

Heat treatment of sera at 56 °C for 30 min, which is usually employed in serology to inactivate complement, causes partial aggregation of IgG. When the immunoglobulins in such inactive sera are analyzed by gel filtration, aggregated IgG molecules appear in the 19S fraction (1). Polymerization of immunoglobulins also occurs when sera are kept at 63 °C for 20 min at pH 7.0 (2).

In the present work, standard conditions have been established for the elimination of heat-labile immunoglobulins from human plasma. The resulting plasma is a convenient source for the production of immunoglobulin preparations without anticomplementary activity.

Material and Methods

Immunoelectrophoresis
Immunoelectrophoresis was performed according to the micromethod of *Scheidegger* (4) using antihuman rabbit sera (Medac, Hamburg).

Quantitative immunoelectrophoresis
Quantitative immunoelectrophoresis was carried out according to *Stephan* (5).

Immunoglobulin determination
The quantiplate of Messrs. Kallestad – distributed by Biotest, Frankfurt – was employed for determination of the immunoglobulins.

Purification of immunoglobulins
This was done according to the *Cohn* alcohol fractionation method as modified by *Nitschmann & Kistler* (6). The alcohol was eliminated by a 24-hours dialysis against 9 g/l NaCl.

Anticomplementary activity
This was determined by the method of *Kabat & Mayer* (7).

Immunoadsorption
Immunoadsorption by the heat-denatured proteins was carried out as follows: The precipitate from 1000 ml recalcified and heated citrate plasma was washed 3 times with 50 ml 9 g/l NaCl, and subsequently 3 times with 50 ml H_2O; each washing was followed by centrifugation. After lyophilisation of this material, antihuman rabbit serum was added (100 ml per 4 g lyophilized material) and stirred at 37 °C for 1 hour, then centrifuged. Quantitative immunoelectrophoresis was then performed.

Results and Discussion

Heat treatment of IgG

If heat treatment at 63 °C of human IgG is prolonged from 20 min to 40 min the polymeric fraction increases only slightly. Thus we did not polymerize the entire monomeric IgG-fraction at 63 °C, but only 50% of it (fig. 1). Furthermore, the monomeric fraction of immunoglobulin, heated at 63 °C for 40 min, shows the same low anticomplementary activity as the monomeric peak of the unheated starting material, whereas the polymeric fraction demonstrates an extremely high level of anticomplementary activity (fig. 2, tab. 1).

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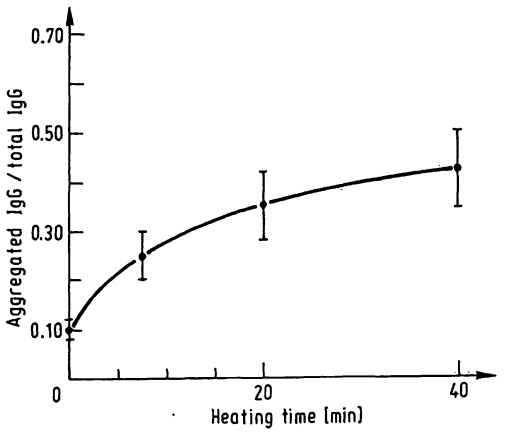

Fig. 1. Extent of aggregation of human immunoglobulin as a function of the time of heating at 63 °C.

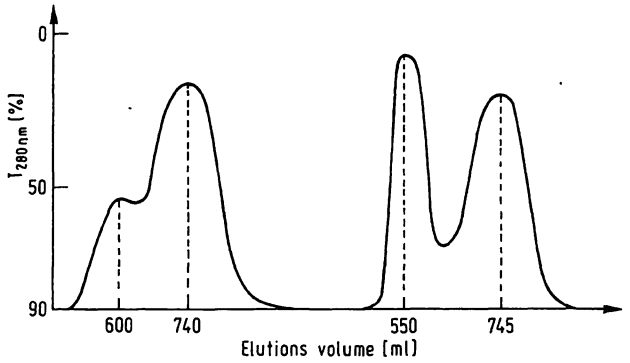

Fig. 2. Gel filtration of standard immunoglobulin on Sephadex G 150.
Left: normal immunoglobulin.
Right: immunoglobulin kept for 40 min at 63 °C.

Tab. 1. Anticomplementary activity of heated standard immunoglobulin.

Product	Anticomplementary activity (reciprocal titres) polymers*	7S IgG*
Standard immunoglobulin (unheated)	20	10
Standard immunoglobulin (40 min, 63 °C, pH 7.0)	2000	10

* Protein concentration: 25 g/l

Human plasma thus contains a fraction of immunoglobulins which are particularly heat-labile, and one may speculate that these immunoglobulins similarly aggregate when exposed to other denaturating conditions such as treatment in the *Cohn* fractionation procedure. This again would cause anticomplementary activity and in turn lead to the known problem of intravenous incompatibility of standard immunoglobulin (3). For this reason we tried to remove the heat-labile fraction by heating plasma.

Heat treatment of plasma

Human citrate plasma was heated at 56 °C for different times, and the immunoglobulins were purified by the *Cohn* method. The various IgG-preparations were tested for anticomplementary activity. The results are summarized in tab. 2. It is clear from the data that the longer the plasma is heated, the lower is the anticomplementary activity of the corresponding preparation.

The precipitate formed in recalcified plasma during the heating process (56 °C for 4 hours) consists mainly of IgG, IgA and IgM. This result was obtained by analyzing the immunoglobulin content of the supernatant (tab. 3). Approximately 20 % of the total immunoglobulins are eliminated by the heat treatment. When the precipitate is used as an immuno-adsorbent for antihuman rabbit sera, mainly antibodies against IgG, IgA and IgM are absorbed (fig. 3). This shows that, apart from the well known heat precipitation of fibrinogen, it is mainly immunoglobulins that are precipitated by heating plasma at 56 °C.

Tab. 2. Complement consumption of human immunoglobulin from heated plasma.

Heating time (h)	Complement (1:30) (ml/500 mg Immunoglobulin)	%
0	26	100
1/4	14	54
1/2	10	38
1	3	12
2	3	12
3	1	4
4	0	0

Tab. 3. Immunoglobulin concentration in heated recalcified plasma.

Product	IgG	IgA (mg/l)	IgM	Total (mg/l)
Unheated plasma	7750	1740	950	10440
Plasma kept for 4 h at 56 °C	6500	1280	750	8530

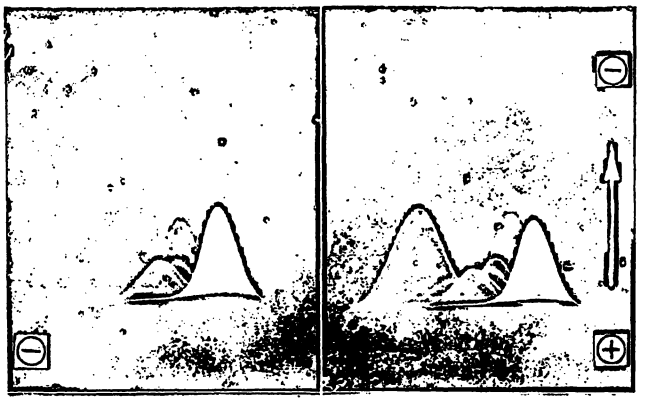

Fig. 3. Quantitative immunoelectrophoresis of human serum using antihuman rabbit serum.
Left: antihuman rabbit serum absorbed with precipitate of heat-treated plasma.
Right: unabsorbed antihuman rabbit serum.

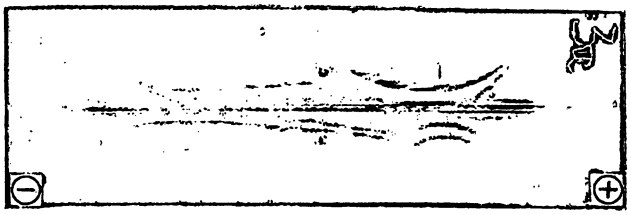

Fig. 4. Immunoelectrophoretic analysis of human serum (top) and IgG of heated plasma (bottom).

Tab. 4. Properties of IgG isolated from heated plasma.

pH-value	6.9
Protein concentration (g/l)	50
Purity (CAF) (%)	98.8
Immunoelectrophoretic analysis	Figure 4
7S fraction of immunoglobulins (%)	90
Anti streptolysin	600 I.U./ml
Anti rubella titre	1:512
Anticomplementary activity	Complement (1:30), 2 ml/500 mg immunoglobulin

From pooled plasma, kept at 56 °C for 4 hours, a 5 % immunoglobulin solution can be produced whose anticomplementary activity and antibody activity is equivalent to those of the commercial IgG-preparations for intravenous use (tab. 4). The question, whether an immunoglobulin preparation of this type will be suitable for treatment of antibody deficiency syndromes, has to be answered by thorough clinical investigations.

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References

1. Metzger, J.-J., Bourdieu, C., Rouze, P. & Houdayer, M. (1975) J. Immunol. Meth. *8*, 295–300.
2. Bier, O. G., Passos, H. C. & Siqueira, M. (1968) Immunology *14*, 291–298.
3. Barandun, S. (1964) Bibl. Haematol. (Basel) *17*, 35–37.
4. Scheidegger, J. J. (1955), Int. Arch. Allergy 7, 103–110.
5. Stephan, W. (1974), Quantitative zweidimensionale Immun-Elektrophorese auf Agarose-Gel. In: Serumproteine (Englhardt, A. & Lommel, H. eds.) S. 57–65, Verl. Chemie, Weinheim/Bergstraße.
6. Kistler, P. & Nitschmann, H. (1962) Vox Sang. 7, 414–424.
7. Kabat, E. A. & Mayer, M. M. (1964) Experimental Immunochemistry, 2nd Ed., chapt. 4, pp. 133–240 Charles C. Thomas, Springfield, Ill., USA.

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