THE FILTRABILITY OF THE COMPONENTS OF ALEXIN

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Muir and Browning (1909) were the first to show that alexin is retained by the filter when fresh serum is passed through a Berkefeld candle. Several filtrations are generally required to remove all traces of the alexic activity, although when a fresh candle is employed for each filtration, the third or fourth filtrate is usually devoid of alexic power (Schmidt, 1914). It is not established whether or not the failure of later filtrates to haemolyse sensitised erythrocytes results from the removal by the filter of some one or of all of the known components of alexin from the serum. We have, therefore, studied the effect of Berkefeld filtration upon the alexic activity of fresh human serum with respect to the thermolabile elements, and the thermostable third and fourth components (Whitehead, Gordon and Wormall, 1925; Gordon, Whitehead and Wormall, 1926).

Experimental

The fresh human serum was passed through a series of twelve properly prepared Berkefeld filters[1], and the various filtrates were tested for the presence of the several components of alexin. Portions of the same fresh serum which were treated by heat (56° C. for $\frac{1}{4}$ hour), by zymin[2], and by ammonium hydroxide[3] and which alone were entirely without haemolytic power (see Table II), were used to test for the presence of the thermolabile elements, the third component and the fourth component respectively.

[1] The filters were washed thoroughly with hot, dilute sodium hydroxide followed by distilled water until the filtrate was no longer alkaline to phenolphthalein.

[2] Zymin was prepared in a manner similar to that described by Whitehead, Gordon and Wormall (1925). A cake of Fleischmann's yeast was shaken with 25 c.c. of absolute alcohol for $\frac{1}{2}$ hour, then with a like amount of absolute ether for the same period. To the residue was added 25 c.c. of physiological sodium chloride solution and the mixture was boiled for $\frac{1}{2}$ hour. The sediment obtained by centrifugation represented the zymin employed in this work. The serum was inactivated for the third component by adding an excess (25 per cent. by volume) of the zymin and incubating the mixture for 2 hours.

[3] Ammonium hydroxide of 1/6·5 normality was used to inactivate the fourth component of the fresh serum. Twenty-five per cent. by volume of the ammonia was added to the serum and the mixture incubated at 37° C. for $1\frac{1}{2}$ hours (Gordon, Whitehead and Wormall, 1926). After its *p*H was adjusted to about 7·5, such a serum was satisfactory to test for the presence of the fourth component.

RESULTS

It can be seen from the results shown in Table I that the haemolytic power of serum (S. 6/6) was lost after passage through the sixth Berkefeld filter. The sixth, ninth and twelfth filtrates of the serum were tested for the presence of the separate components of alexin (see Table III). The heat-labile components were absent from all, for none of these filtrates was activated by the addition of serum heated at 56° C. for $\frac{1}{4}$ hour. Both the third and the fourth components of alexin were, however, found in the sixth filtrate, for when a serum inactivated with respect to either of these elements was added, the haemolytic test was positive. Apparently the thermolabile elements which were supplied in either of these treated serums were the substances lacking from the sixth filtrate. A small amount of lysis was shown when either the zymin-treated serum or the ammonia-treated serum was added to the ninth filtrate. This result indicated the presence of both the third and the fourth components in the ninth filtrate. No component of alexin was demonstrable in the twelfth filtrate of the serum.

DISCUSSION

A relationship exists between the inactivation of the alexin of a serum by filtration and by heat. Those components of alexin which are most readily removed by filtration are the thermolabile elements. The third and the fourth components of alexin are removed only after prolonged filtration and likewise are inactivated only after prolonged heating or after heating at a temperature above that usually employed for the purpose of inactivation (56° C.). It is possible that the mechanism underlying the inactivation of fresh serum is the same whether the inactivation be accomplished by filtration or by heat, or, perhaps, by any other physical agency, such as prolonged agitation (Jacoby and Schütze, 1910; Schmidt, 1913).

Table I. *The effect of repeated filtration on the haemolytic power of normal serum* (S. 6/6). *To* 0·5 *c.c. of the serum dilutions indicated,* 0·1 *c.c. of sensitised sheep red cells was added and the mixture made up to* 1·5 *c.c. with* 0·85 *per cent. salt solution. After incubation at* 37° C. *for* 1 *hour, the tests were read for haemolysis.*

Serum dilution	Original normal serum	Haemolysis by filtrates				
		1st	3rd	6th	9th	12th
Undiluted	C	C	C	0	0	0
(1–5)	C	C	C	0	0	0
(1–10)	C	C	1+	0	0	0
(1–20)	C	C	0	0	0	0
(1–40)	3+	3+	0	0	0	0

C, complete haemolysis; 3+ to 1+, partial haemolysis; 0, no haemolysis.

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Table II. *Test for haemolytic activity of the treated serums* (S. 6/6) *employed for detecting the several components of alexin in the filtrates. One-tenth c.c. of sensitised sheep red cells and* 0·9 *c.c. saline were added to* 0·5 *c.c. of each treated serum.*

Treated serums	Haemolysis
0·5 c.c. of human serum heated at 56° C. for ½ hour	0
0·5 c.c. of human serum treated with ammonia*	0
0·5 c.c. of human serum treated with zymin†	0

* 0·75 c.c. of NH_4OH $N/6·5$ was added to 3·0 c.c. of fresh serum, the mixture was shaken and then incubated at 37° C. for 2 hours. The serum was adjusted to the *p*H of 7·5 with $N/4$ HCl.

† 0·75 c.c. of zymin was added to 3·0 c.c. of fresh serum and the mixture was incubated at 37° C. for 2 hours.

Table III. *Tests for the components of alexin in the filtrates of serum* (S. 6/6). *The tests were carried out by adding* 0·1 *c.c. of sensitised sheep red cells to the mixtures described.*

Test for	Mixtures	Haemolysis by filtrates* 6th	9th	12th
Thermolabile elements	0·2 c.c. filtrate + 0·2 c.c. heated serum + 1·0 c.c. saline	0	0	0
Third component	0·2 c.c. filtrate + 0·2 c.c. yeast-treated serum + 1·0 c.c. saline	C	1+	0
Fourth component	0·2 c.c. filtrate + 0·26 c.c.† ammonia treated serum + 0·94 c.c. saline	C	1+	0

* The first and third filtrates possessed all components of alexin (see Table I).

† 0·26 c.c. were used instead of 0·2 c.c. to account for the dilution by the ammonia added.

Conclusion

All of the known components of alexin can be removed from human serum by filtration through a series of Berkefeld candles. The thermolabile elements are the first to be removed and are not demonstrable in the serum after from three to six filtrations. The heat-stable constituents, the third and the fourth components, can still be detected after nine filtrations. All of the components of alexin are absent from the twelfth filtrate.

REFERENCES

Gordon, J., Whitehead, H. R. and Wormall, A. (1926). *Biochem. J.* **20**, 1028.
Jacoby, M. and Schütze, A. (1910). *Zeitschr. f. Immunitätsf.* **4**, 730.
Muir, R. and Browning, C. H. (1909). *J. Path. and Bact.* **13**, 232.
Schmidt, H. (1913). *J. Hygiene*, **13**, 291.
Schmidt, H. (1914). *Ibid.* **14**, 437.
Whitehead, H. R., Gordon, J. and Wormall, A. (1925). *Biochem. J.* **19**, 618.

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