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Note on the relation of alexin to thrombin.By **THEO. C. BURNETT** (by invitation).

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The publishing of negative results is, as a rule, a questionable proceeding. Occasionally however, a negative result may have a positive bearing upon a subject, of more or less value, in which case the objection does not hold. It is for this reason I present the following note.

In reviewing the subject of coagulation some time ago, I was struck by a certain similarity between thrombin and alexin. Both are inactivated by a temperature of 56 degrees; both are derived from leucocytes;¹ thrombin consists of thrombokinase and calcium, while alexin consists of two parts, as is well known. It occurred to me that there might be a closer relation between the two; that they might in fact, be identical. It has probably occurred to many another, but in a hasty glance over the literature, I can find no mention of it. At first sight the idea seems absurd, for alexin is contained in serum that has been collected over a clot, and hence contains no thrombin. The alexic potency of a serum, however, increases by standing for some hours in contact with the clot, and it is conceivable that during that time thrombin is excreted by the leucocytes, but is not apparent because of the absence of fibrinogen wherewith to combine. Having occasion to prepare some thrombin for another purpose, I determined to test the matter.

The immune serum used was rabbit serum immunized against ox corpuscles. Tested with guinea-pig serum it had a potency of 1-800. (Ox blood was used on account of the ease with which material could be obtained at any time.) Most of the experiments were carried out with a 1-100 dilution, although similar results were obtained with higher dilutions.

The thrombin was prepared by Howell's method² and gave a

¹ It is generally conceded now that thrombin is derived from the platelets.

² Howell, W. H., *Am. Jour. Physiol.*, Vol. 26, 1910, p. 453.

coagulum with fibrinogen in a few minutes. As thrombin is made with 8 per cent. NaCl solution, it was diluted to approximate isotonicity, m/6. In order to still further control any possible osmotic effects from hypotonicity, solutions of m/5 and m/4 concentrations were also used to dilute the serum.

The system consisted of 1 c.c. of 1-100 immune serum, plus 0.1 c.c. washed ox corpuscles (5 per cent. emulsion), plus thrombin in amounts varying from 0.05 c.c. to 0.3 c.c. As a control one tube was always prepared with normal serum (alexin) instead of thrombin. The tubes were kept at a temperature of 36-37° C.

The results can be stated in a few words. The control tubes showed complete hemolysis in from fifteen to thirty minutes. In no instance was there a trace of hemolysis even after several hours, in the tubes containing thrombin. The corpuscles settled out of the solution and left a clear supernatant fluid, not even tinged with hemoglobin. Having obtained this negative result with thrombin, I then tried solutions of fibrinogen and of serum-globulin, in order to make the matter complete. Exactly the same results were obtained, and I think we may conclude that whatever alexin may be, it is certainly *not* identical with thrombin or the globulins.

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On the nature of the union of alexin with specific precipitates.

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In continuing studies on the nature of alexin fixation by mixtures of unformed proteins and their antisera, it occurred to the writer to examine whether the alexin fixation which is exerted by specific precipitates was subject to the same conditions that prevail in the case of sensitized cell complexes in their relations to the alexin fractions as first obtained by Ferrata. It is well-known, of course, that by dialysis, by dilution with weak acid in distilled water and by a number of other methods of globulin precipitation, the alexin or complement can be divided into two functional parts, one which comes down with the globulins, the