Is the Action of Calcium in the Coagulation of Blood Stoichiometric or Catalytic?[1]

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By determining the amount of thrombin formed from known quantities of calcium, one is able to conclude whether the action of the latter is catalytic or stoichiometric. A simple method to control accurately the concentration of calcium is to add known amounts to plasma obtained from blood decalcified by passage through Amberlite[2] according to the procedure recently described (*4*). Thrombin may be satisfactorily estimated by allowing a plasma to clot and then (after the thrombin has been inactivated by the normal antithrombin of the plasma) determining the amount of prothrombin remaining.

TABLE 1

QUANTITATIVE RELATIONSHIP BETWEEN PROTHROMBIN ACTIVATION AND THE CALCIUM CONCENTRATION IN HUMAN PLASMA

Mixture	(cc.)	Clotting time of mixture (sec.)	Prothrombin remaining in serum after 1 hr. incubation	
			Time* (sec.)	Per cent of normal (approximate)
Amberlite plasma	0.4			
1 Thromboplastin	0.1	90	11	100
Calcium chloride, 0.0006 M	0.1			
Amberlite plasma	0.4			
2 Thromboplastin	0.1	13½	35	10
Calcium chloride, 0.0025 M	0.1			
Amberlite plasma	0.4			
3 Thromboplastin	0.1	12½	105	2
Calcium chloride, 0.005 M	0.1			

* To 0.1 cc. of serum were added 0.1 cc. of thromboplastin, 0.1 cc. of 0.02 M calcium chloride, and 0.1 cc. of fibrinogen or fresh human plasma treated with tricalcium phosphate. The preparation of reagents and the general procedure have been described previously (*2*).

The results summarized in Table 1 clearly suggest that the relation of calcium concentration to thrombin production is stoichiometric. When the calcium chloride concentration in plasma is 0.00015 M (Mixture 1) or lower, very little prothrombin is changed to thrombin in one hour, even though other conditions, including an excess of thromboplastin, are optimum. The thrombin formed, although very small in amount, coagulates all the fibrinogen because it acts enzymatically. Were the action of calcium catalytic, it would similarly convert all the prothrombin to thrombin. It is only when the calcium concentration of plasma is 0.0012 M (Mixture 3) that nearly all of the prothrombin is consumed or converted.

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[2] Amberlite IR 100 is a phenol-formaldehyde resin which functions as a cation exchanger.

These findings supply another link in the chain of evidence that the reaction or reactions that bring about the production of thrombin from the prothrombin complex, thromboplastin and calcium, are chemical and not enzymatic. That thromboplastin acts stoichiometrically was indicated by the work of Mertz, Seegers, and Smith (*1*) and recently confirmed by the writer working with hemophilic blood (*5*).

Calcium is closely associated with prothrombin, but the solution of this relationship is contingent upon a fuller understanding of the composition of prothrombin. The concept of the writer (*3*) that it is a complex and not a unitary substance has recently found clinical confirmation (*6*). The writer has studied one family in which three members have a congenitally reduced concentration of one of the components, designated as "B", and recently he has discovered another family in which two brothers have a congenital lowered level of a second principle, which has been named component A. Much study will be required before one can determine how these factors interact, but from available data it appears fairly certain that the reactions follow the law of mass action, and that none of these factors can be considered accelerators or activators in an enzymatic or catalytic sense.

References

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The Action of the Endotoxin of *Trypanosoma cruzi* (KR) on Malignant Mouse Tumors[1]

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This communication is essentially a verification of the findings of Roskin and Kluyeva (*2*) in producing the substance designated as KR, an extract of the lysed cells of *T. cruzi*, which has a lytic effect on certain malignant tumors.

A culture of *T. cruzi* was obtained which originated from authentic cases of Chagas' disease.[2] Following the Russian prescription, this was allowed to grow on a sterile medium containing rabbit blood at a temperature of 22–24° C. In a period of 14–19 days large colonies of organisms were obtained.

Only minor changes were made in the original Russian procedures in preparing the extract of the endotoxin. Lysis was accomplished by covering with pyrogen-free distilled water and allowing to stand at refrigerator temperatures overnight. Metaphen at a final concentration of 1:10,000 was used to ensure additional protection against bacterial contamination.

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[2] Grateful acknowledgment is made to J. A. Aeschlimann and R. Schnitzer, of Hoffman-La Roche, Inc., Nutley, New Jersey, and to Malcolm Soule, University of Michigan, for cultures designated as 2 and 3. (When the two cultures were combined in one, the designation is 23. Culture 3 was used in practically all our work, unmixed with 2.)