at present makes it rather difficult to give any data on the theoretical side of the problem. The close agreement of protective and flocculative values indicates that the flocculation is a true toxin-antitoxin reaction. The agglutinative titer showed no correlation with the flocculative value.

The ratio of serum to toxin as shown by our tests indicates that the actual amount of toxin present in the unconcentrated filtrate of *Cl. welchii* is very small. This would mean that the low toxic qualities of such filtrates—low in comparison with other bacterial toxins—would not have to be ascribed to a relatively weak effect of the toxin but rather to a relatively small amount of toxin produced in broth cultures under conditions which we regard as optimal at the present state of our knowledge.

Summary. A flocculative reaction between the toxin of *Cl. welchii* (Type A) and its antitoxin is reported, which shows close correlation to the mouse-protective values of *Perfringens* antitoxins. Concentrated toxins are used as antigens.

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Calcium and Cephalin in Relation to the Clotting Power of Crystalline Trypsin.

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It is true[1] that crystalline trypsin[2] can clot ordinary citrated plasma and can activate prothrombin without added calcium. As these experimental facts appear to conflict with the statement of Northrop and Kunitz[3] that a trace of ionized calcium is necessary, we have reinvestigated the point minutely, with an enzyme preparation kindly supplied by the Rockefeller workers.

It was found (Table I) that the trypsin is much more active in the presence of added calcium salt and that excess of citrate can inhibit its action. Whereas trypsin alone requires amounts of the order of 1-2 mg to coagulate 1 cc of citrated dog plasma and the clots quickly undergo fibrinolysis, much smaller quantities (0.01-

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1 Eagle, H., *J. Gen. Physiol.*, 1937, **20**, 543.

2 Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1932, **16**, 267.

3 Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1935, **18**, 456.

TABLE I.

	Trypsin 1:1000	Distilled water	Cephalin 1:1000	$CaCl_2$ N/10	Citrated plasma	Clotting-times (sec.) at 38°C	
	cc	cc	cc	cc	cc	A	B*
1.	.5	.5	—	—	1.0	150	270
2.	.5	.25	.25	—	1.0	110	225
3.	.5	.25	—	.25	1.0	40	50
4.	.5	—	.25	.25	1.0	20	20
5.	—	.75	—	.25	1.0	365	
6.	—	.5	.25	.25	1.0	80	

*Trypsin solution boiled for 5 min.

0.02 mg) of enzyme, *synergized by calcium,* produce better clots which do not lyse in 24-48 hours.

The addition of cephalin to clotting systems containing the proteolytic enzyme results in a minor improvement of the thrombic activity developed. For its full demonstration, this requires the presence of calcium.

A minor loss of activity on boiling the crystalline trypsin solution[4] is restored by added cephalin and calcium. It is known, of course, that cruder enzyme preparations are thermolabile.

Lung extracts evince a thromboplastic action exactly analogous to that of dilute trypsin (*plus* cephalin). The partial retention of the activity in boiled lung extract has been explained on the basis of its phospholipid content.[5] We have a semi-quantitative confirmation of this in tests made with the isolated (total) lung P-lipids. Pending isolation, may we not regard the major, thermolabile factor in thromboplastic tissue extracts as analogous to a weak proteolytic enzyme?

In contrast to the view[1] that trypsin activates prothrombin directly, the new data indicate that it acts via the accepted mode of thrombin formation,[6] with its recognized dependence on calcium. It is necessary to revise the view that diffusible calcium ions are essential, in favor of the hypothesis that ionization (or 'orientation') of calcium can occur at colloidal surfaces where the close juxtaposition of prothrombin, cephalin and calcium permits of the thrombin reaction[6] even in the presence of moderate amounts of citrate, heparin, etc.

A correlation between coagulation and clot-retraction and fibrinolysis gains support from an enzyme theory.

We suggest the term "thromboplastic enzyme" for all proteases which can be shown to aid blood clotting in a manner analogous to a *weak* trypsin solution.

[4] Northrop, J. H., *J. Gen. Physiol.*, 1932, **16**, 367.

[5] Mills, C. A., *J. Biol. Chem.*, 1921, **46**, 135.

[6] Ferguson, J. H., *Am. J. Physiol.*, 1938, **123**, 341.