Effect of Crystallized Trypsin of Increased Enzymic Activity in Trypsin Tube Tests *

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Armour Laboratories are now producing crystallized trypsin of increased enzymic activity and their most recent product has a potency of 2,500 units/mg.

During the development of the "concurrent trypsin method" the trypsin used had a potency of only 1,000 units/mg., and the trypsin concentration of 0.48%, recommended as being optimal in the serum-cell mixture, therefore corresponded to an enzymic activity of 480,000 units%. As stressed in the original publication, the "concurrent method" is sensitive only within a fairly narrow range of trypsin concentration, but it was not made clear that this critical concentration is in fact dependent upon the potency of the enzyme used, since what is important is that the enzymic activity in the incubated mixture must be maintained between 300,000 and 500,000 units%. The strength of the stock trypsin solution must therefore be varied according to the potency of the crysallized trypsin reagent (see Table).

* Addendum to "Studies on concurrent sensitization and trypsinization with the development of a simple trypsin tube test for routine Rhesus grouping and as a screening test for incomplete antibodies" (*J. clin. Path.*, 1957, **10**, 236) and "Direct compatibility testing" (*J. clin. Path.*, 1958, **11**, 311).

	Crystallized Trypsin (Armour Laboratories)		
	1,000 units	2,500 units	3,000 units
Stock trypsin solution (g.%)	3.00	1.25	1.00

It should be carefully noted that if the 2,500 units/mg. crystallized trypsin is used at the concentration advised in the original paper the test becomes insensitive and weak antibodies will be missed. Furthermore with a stronger antibody (such as would be used for Rh grouping), although positive reactions will be obtained, the agglutinations will be much weaker and less clear-cut than they should be.

The trypsin-saline method has now been used for the routine Rh grouping of almost 8,000 cases with excellent results.

It offers certain very definite advantages, namely, (1) simplicity and speed of performance; (2) considerable economy of grouping serum; (3) more sensitive, reliable, and clear-cut results than albumin methods. Rouleaux formation is no problem and the obvious distinction between the positive and negative tubes considerably lessens the chances of "false positive" readings being recorded by less experienced workers; (4) grouping sera can be used suitably diluted (usually 1/8 to 1/10) so that D^u samples either do not react or give weak microscopic agglutination.

All negatively reacting samples are screened by the Coombs technique and possible D^u samples further investigated.