Bound and trapped cyclic AMP before and after washing samples, as a function of quantity of Z-gel added

Volume Z-gel added (% total reaction volume)	Before washing		After washing	
	Bound (%)	Trapped (%)	Bound (%)	Trapped (%)
10	41.9	10.8	36.0	2.4
20	49.4	12.6	40.7	2.2
40	53.2	20.5	38.0	3.9
50	64.6	24.5	47.6	3.0
100	64.3	28.5	41.8	3.2
200	68.8	33.0	40.6	5.3

Values represent the means of duplicate samples.

precipitated with various amounts of Z-gel. The pellets were counted, washed, recentrifuged and recounted. The percent of antigen bound before and after washing with various quantities of Z-gel was then compared to the values expected for the amount of Scamp-TME employed [2].

To further evaluate the use of Z-gel precipitation, a dilution curve was prepared using samples containing 0.025 to 10.0 picomoles of unlabeled cyclic-AMP, along with a specific amount of Scamp-TME. The antigen-antibody complex was then precipitated with a 50% equivalent volume of Z-gel, washed with buffer and the activity obtained compared to that expected from a published standard curve obtained using ammonium sulfate precipitation [2].

Results and discussion. Additions of Z-gel to the reaction mixture produced increasing amounts of ^{125}I activity in the pellet, up to 50% of the reaction volume (Table). Further additions of Z-gel did not produce further increases in the amount of antigen-antibody complex precipitated. Z-gel produced large, well defined pellets at $800 \times g$ centrifugation for 10 min (cf. $3{,}000 \times g$ for 20 min required for ammonium sulfate). These reduced centrifuge requirements permit the handling of larger numbers of samples in the same time and the use of less elaborate centrifuge equipment. The Z-gel pellet showed no tendency to redisperse into the supernatant regardless of the volume employed. This physical stability of the pellet permitted reproducible decantation of large numbers of samples over a period of time. As can be seen from the Table, Z-gel caused significant amounts of free antigen to precipitate. This trapped free antigen increased with increasing amounts of added Z-gel. However, washing the pellet with sodium acetate buffer reduced this trapping to acceptable levels. Washing of the pellet following precipitation of the antigen-antibody complex resulted in a significant decrease in the activity present (Table). The extent of this reduction corresponded closely to the amount of trapped free cyclic-AMP removed by washing. That this reduction was not a result of the breakdown of the antigen-antibody complex was supported by the finding that the level of activity obtained for a 1 h reaction time (47% antigen binding at a 50% equivalent volume of Z-gel added, Table) compared favorably with that expected from published curves obtained with ammonium sulfate (50% antigen binding) [2].

The substitution of zirconyl phosphate gel for ammonium sulfate precipitation appears to be a feasible improvement of the STEINER radioimmunoassay of cyclic-AMP. The Z-gel produces a more stable and well defined pellet than that obtained with ammonium sulfate precipitation. Because of the larger pellet obtained, the potential for error during sample processing and handling is reduced.

Washing of the Z-gel pellet removes the potential for error from incomplete decantation of the supernatant which contains appreciable amounts of free labeled antigen. Residual supernatant remaining following washing of the Z-gel precipitate with sodium acetate buffer contains a much lower level of activity and it is therefore a diminished problem.

The optimum quantity of Z-gel to be used is in the range of 50% of the total reaction volume (250 µl of Z-gel per tube in the present procedure). This level represents an easily handled quantity which produces maximum antigen-antibody precipitation in a stable configuration with a minimum of free antigen binding.

Summary. An improvement in the technique for the radioimmunoassay of cyclic-AMP, wherein ammonium sulfate precipitation is replaced with zirconyl phosphate gel, is presented. This substitution produces a more stable pellet than that obtained with ammonium sulfate. This greatly reduces a potential source of error due to pellet instability.

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