Limitations. The use of a medium of much lowered conductivity or the case of very leaky membranes reduces accuracy of the measurement accordingly. Likewise, this method is difficult to apply to very dilute suspensions because the ratio $\varkappa/\varkappa_m$ approaches unity in this case. Shape complexity as found in red cells[10], for example, also impairs the straightforward application of Eq. 1, though not altogether inapplicable to spheroids or ellipsoids such as isolated mitochondria. With regard to other shape effects, a modification of the basic equation, e.g. by introducing an empirical parameter other than 1.5 into the exponent of Eq. 1, has been reported to be quite effective[1,11].

Summary. Supported by the fact of correspondence between the results of several independent techniques compared, we recommend here a conductometric method as a simple, nondestructive and reliable tool for determining the volume fraction of the suspensions of membrane-limited particles of biological relevance. It requires only conductivity measurements on a suspension and its medium[12].

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[10] H. Fricke, Phys. Rev. *24*, 575 (1924).

[11] R. E. Meredith and C. W. Tobias, in *Advances in Electrochemistry and Electrochemical Engineering* (Eds. P. Delahay and C. W. Tobias; John Wiley & Sons, Inc., New York 1962), vol. 2, p. 15.

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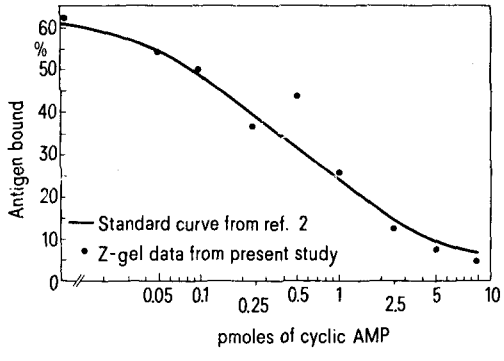
A Technical Improvement in the Radioimmunoassay of Cyclic-AMP

The radioimmunoassay of Steiner et al.[1] is a method which allows for the rapid measurement of cyclic-AMP in a large number of samples. The procedure involves the competition between isotopically labeled and unlabeled cyclic-AMP for specific antibody present in limiting amounts. As a result, the quantity of labeled antigen-antibody complex which is formed is inversely related to the amount of unlabeled antigen present. The quantity of unlabeled cyclic-AMP in a sample is then determined by comparison with a standard curve[2].

Separation of free from bound antigen has been achieved by precipitation of the complex with a 60% solution of saturated ammonium sulfate[2]. This suffers from the drawbacks that, owing to the small volume of the reaction mixture, the pellet obtained following centrifugation is very small and minute loses can cause significant variations. In addition, the ammonium sulfate precipitate is physically unstable. Thus, there tends to be a progressive redispersion of the pellet into the supernatant with standing, which can result in loss during the subsequent decantation. This potential source of error becomes especially important when a large number of samples are being processed, due to the longer time required.



Percent antigen bound as a function of the amount of unlabeled cyclic AMP present in the reaction mixture. Points: Z-gel precipitation of bound antigen-antibody complex, mean values of 2 experiments. Curve: standard curve using ammonium sulfate precipitation redrawn from reference[2] (with permission).

The present study was designed to test the feasibility of replacing the ammonium sulfate precipitation with zirconyl phosphate gel (Z-gel). The latter has been used for precipitation in the radioimmunoassay of carcinoembryonic antigen[3-5].

Materials and methods. The assay of cyclic-AMP followed the procedure outlined in the Schwarz-Mann technical bulletin[2] with the exception that Z-gel (Roche, Nutley, N.J.) was substituted for ammonium sulfate in the precipitation step. The labeled antigen was a cyclic-AMP derivative, succinyl cyclic-AMP tyrosine methyl ester [^{125}I], (Scamp-TME). The reaction was carried out as follows: 100 µl Scamp-TME was added to glass tubes containing 300 µl of sodium acetate buffer (pH 6.2). 100 µl of cyclic-AMP antiserum was then added to each tube. The reaction was allowed to proceed for 1 h at 0–4 °C. To determine the optimum quantity of Z-gel required to precipitate the cyclic-AMP-antibody complex, Z-gel additions, ranging from 10% to 200% of the total volume, were made. The tubes were then allowed to stand for 0.5 h on ice, centrifuged at $800 \times g$ for 10 min at 4 °C, decanted, swabbed, and the residual solids radioassayed in a crystal scintillation counter.

To determine if the Z-gel non-specifically precipitated unbound Scamp-TME, tubes containing only Scamp-TME in buffer were precipitated with varying amounts of Z-gel, centrifuged, decanted and counted. The pellet was then washed with 2.5 ml of sodium acetate buffer, centrifuged again and recounted. The second counting was then compared to the first count to ascertain the amount of Scamp-TME 'trapped' by the Z-gel.

To determine the stability of the antigen-antibody complex following Z-gel precipitation and washing, Scamp-TME + antisera were allowed to react and

[1] A. L. Steiner, D. M. Kipnis, R. Utiger and C. Parker, Proc. natn. Acad. Sci., USA *64*, 367 (1969).

[2] Schwarz/Mann Product Bulletin, Cyclic-AMP Radioimmunoassay Kit (Schwarz/Mann, Orangeburg, N.Y. 1972).

[3] H. J. Hansen, K. P. Lance and J. Krupey, J. clin. Res. *19*, 143 (1971).

[4] D. M. P. Thomson, J. Krupey, S. O. Freedman and P. Gold, Proc. natn. Acad. Sci., USA *64*, 161 (1969).

[5] T. M. Chu and G. Reynoso, Clin. Chem. *18*, 918 (1972).

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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