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THE DETECTION OF IMMUNE COMPLEXES BY ISOTACHOPHORESIS,(U)
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Soluble immune complexes (BSA - Rabbit IgG anti BSA) can be readily detected by means of analytical isotachophoresis. This particular technic has the advantages of being able to process microgram concentrations of reactants in lambda quantities within thirty minutes. Attempts will be undertaken to now investigate immune complexes containing microbiologic antigens such as bacterial toxins, viral polypeptides, etc.

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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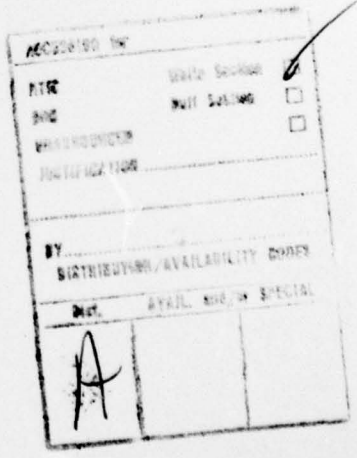

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The basic principle of isotachophoresis whereby ions are separated on the basis of net mobility has been known for many years and been described by several authors (1, 2). However, it was only in 1970 that Arlinger and Routs (3) and Svendsen and Rose (4) applied the technique to the fractionation of protein mixtures. The present report is the first to describe detection of soluble immune complexes by means of analytical isotachophoresis. Advantages of the technique include rapidity of analysis, i.e., approximately 30 minutes running time, and small sample size, as little as 2-10 µl of sample containing microgram concentrations of protein.

A standard quantitative precipitin test similar to that of Heidelberger and Kendall (5) was performed. Rabbit Ig(G) was prepared by passing rabbit antiserum for bovine serum albumin (BSA) over insolubilized protein A and then eluting the absorbed Ig(G) with 0.02 M glycine-HCl pH 2.5. After the pH of Ig(G) was readjusted to 7.3, increasing microgram amounts of BSA were mixed with a fixed concentration of rabbit anti-BSA Ig(G). Antibody-antigen mixtures were incubated at 37°C for 1 hour and at 4°C for 6 days. The incubation tubes were then centrifuged at 3000 g for 15 minutes and the precipitates and supernatants were collected for study.

Precipitates were washed three times with cold 0.05 M phosphate buffered saline (PBS), pH 7.3, and the pellets were redissolved in 0.1 N sodium hydroxide for spectrophotometric examination at 280 nm. A graph plotting optical density of the precipitate against the amount of antigen added is shown in Fig. 1. The equivalence point was established between 30 and 35 micrograms of BSA. Supernatant material taken from the area of marked antigen excess provided a source of soluble immune complexes.

Analytical isotachophoresis studies were performed with an LKB 2127 Tachophor apparatus. The separation chamber consisted of a 23 centimeter teflon capillary tube (internal diameter, 0.5 millimeters) maintained at a constant temperature of 20°C. The apparatus was equipped with a thermal detector and with an ultra violet detector set at 280 nm. The leading electrolyte, .005 M HCl-ammediol, pH 8.5 was supplemented with .04 A-4 methyl cellulose (Dow Chemical). The terminal buffer was 0.01 M episilon amino caproic acid (EACA) adjusted to pH 9.5 with Ba $(OH)_2$. A 0.01% solution of ampholine pH 3.5-10 was used as a non-u.v. absorbing spacer. Analyses of antigen alone, antibody alone, or of individual supernatants from mixtures of various proportions of antigen and antibody were performed at a constant current of 75 μA; the voltage increased from 3 kV to 20 kV during the running time of approximately 25 minutes per sample.

Figure 2 demonstrates net mobility profiles for: (a) rabbit Ig(G) alone, (b) BSA alone, (c) the supernatant at or near Ig(G)-BSA equivalence, (d) the supernatant from the zone of BSA excess and (e) the effluent after absorption of supernatant "d" on protein A. In the supernatant at antigen-antibody equivalence the total amount of Ig(G) is reduced due to loss in the insoluble precipitate and there is no evidence of uncombined BSA antigen. In supernatant from the zone of antigen excess a BSA peak is recognized as well as an intermediary peak between the BSA and the Ig(G) peaks. In more extreme areas of antigen excess, the supernatant shows increased concentrations of BSA and components in the intermediary peak. Passage of supernatant material over insolubilized protein A removed both the Ig(G) peak and the middle peak by binding the F(c) components.

Serological activity of samples analyzed by isotachophoresis is presented in Table I and lends support to the hypothesis that the newly formed intermediary peak was indeed an immune complex. The supernatant from the zone of antigen excess had complement fixing activity that could be removed by absorption with protein A, confirming the presence of F(c) containing components.

In summary, the combination of an antigen and its homologous antibody in the zone of antigen excess results in a net reduction in Ig(G) in the supernatant and the appearance of a soluble intermediary immune complex composed of BSA and Ig(G) anti-BSA. This complex containing Ig(G) is removed by passage over insolubilized protein A. In addition, unlike supernatants from the zone of BSA excess, supernatants of Ig(G) alone, BSA alone or of Ig(G)-BSA complexes formed at equivalence do not fix complement. Exposure of supernatants from the BSA excess zone to protein A removes complement fixing activity.

This is the first reported demonstration of the identification of soluble immune complexes by means of analytical isotachophoresis. It is hoped that the technique can be extended to the study of a wide variety of antigen and antibody systems to exploit the speed and convenience allowed by analytical isotachophoresis.

Table 1. Complement fixing ability of soluble supernatant specimens

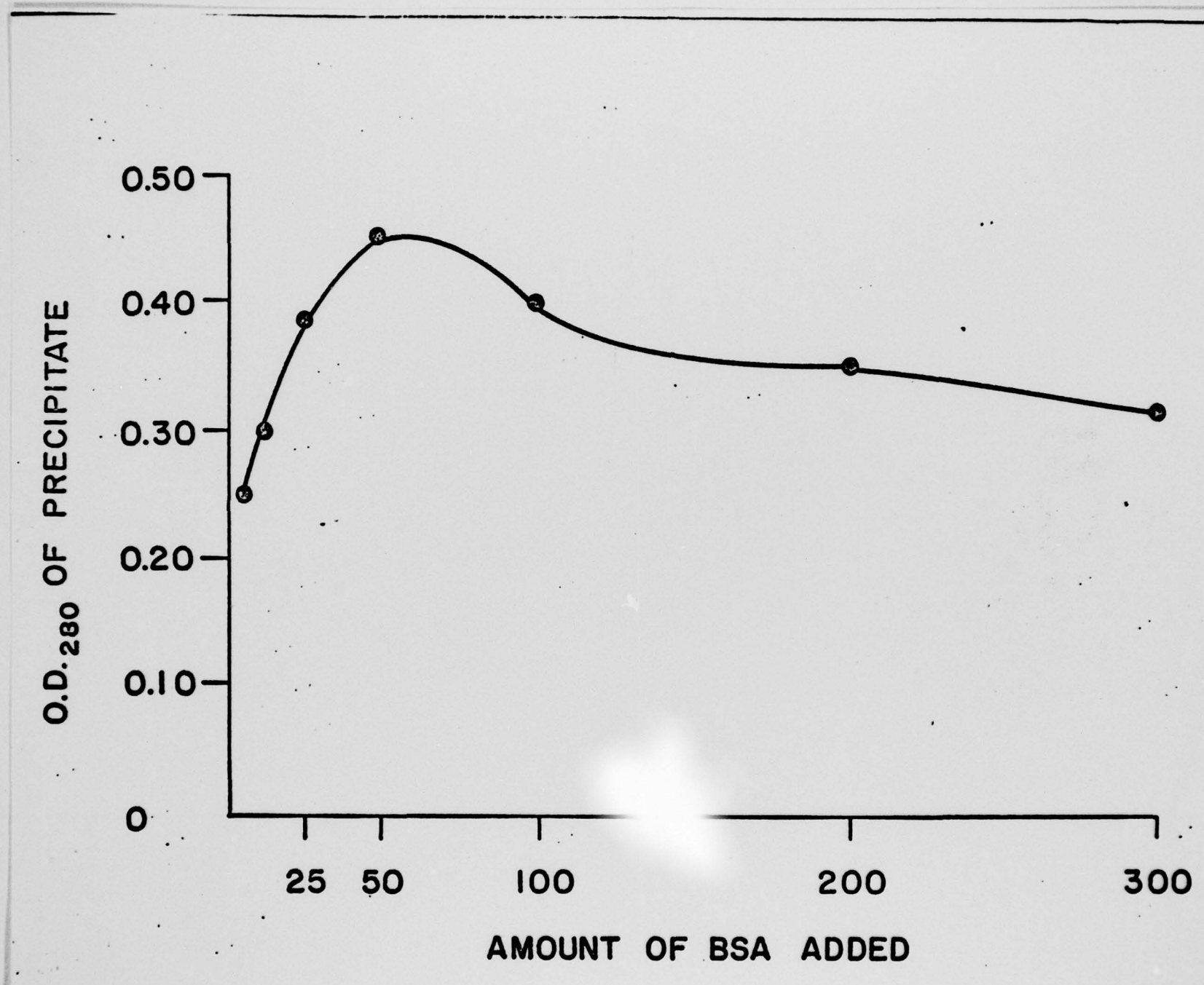
Supernatant specimen[a]	BSA added (μg)	Complement fixation titer
a	0	0
c	5	0
	12.5	0
	25	0
	50	1:8
	100	1:16
	150	1:32
d	200	1:32
e*	200	0

[a] Specimens correspond to those described in Fig. 2.

* Supernatant after adsorption on protein A.

Legend to Figure 1.

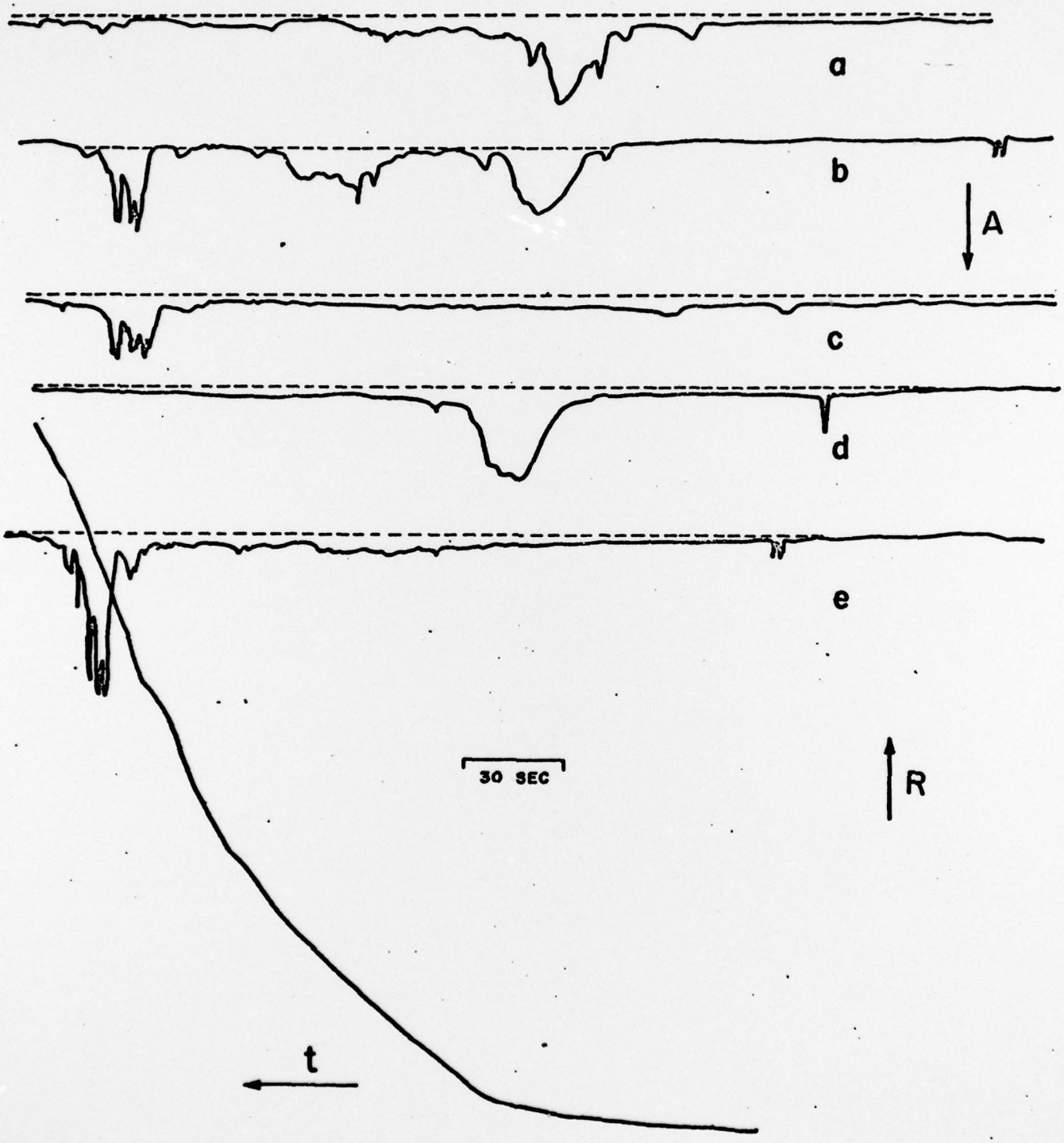
Precipitin curve of bovine serum albumin (BSA) with IgG anti-BSA.


0.50
0.40
0.30
0.20
0.10
0
O.D.280 OF PRECIPITATE
25
50
100
200
300
AMOUNT OF BSA ADDED

Legend to Figure 2.

Isotachopherograms of supernatants obtained from various points of the precipitin curve in Fig. 1. IgG alone is shown in (a) BSA in (b). At equivalence the total IgG is reduced and BSA is not detected. The supernatant (d) demonstrates both antigen BSA excess and a soluble immune complex and IgG. Following adsorption upon protein A, the F(c) containing components of (d) are removed and this is illustrated in (e).

A = Increasing UV absorption; R = increasing resistance; t = time.


a
b
A
c
d
e
30 SEC
R
t

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