Table 1. Effect of Crystalline Papain on Rabbit Anti-*p*-Azobenzoate Antibody*

Experiment No.	Sample	Papain used (mgm.)	$S_{20,w}$†	Conc. labelled *p*-iodobenzoate bound‡ ($M \times 10^6$)
1*A*	Normal γ-globulin	0	6·7	—
2*A*	Normal γ-globulin	16·5	3·4	0·06 ± 0·03
3*A*	Antibody γ-globulin	0	6·5	2·91 ± 0·07
4*A*	Antibody γ-globulin	1·7	3·6	2·66 ± 0·07
5*A*	Antibody γ-globulin	4·2	3·5	2·58 ± 0·03
6*A*	Antibody γ-globulin	16·5	3·5	2·17 ± 0·04
1*B*	Specifically purified antibody	0	7·0	5·07 ± 0·03
2*B*	Specifically purified antibody	0·5	3·5	4·75 ± 0·06

* Hydrolysis at 37° C. for 18–20 hr.; *p*H 7·0. Buffer contained sodium salts of phosphate (0·1 *M*), versene (0·002 *M*) and cysteine (0·01 *M*). 200 mgm. γ-globulin (series *A*) or 8 mgm. specifically purified antibody (series *B*) present in each mixture. Total volume 10 ml. in series *A*, 1·4 ml. in *B*. Papain added as an aqueous solution in two equal portions, at zero time and after 1½ hr.

† Sedimentation at 59,780 r.p.m. in 'Spinco' model *E* ultracentrifuge. Concentration of protein in sedimentation of series *A*, 10 mgm./ml. except for sample 1*A* (15 mgm./ml.); concentration in series *B*, 5·8 mgm./ml.

‡ Equilibrium dialysis in triplicate at 5·0 ± 0·1° C. with mean deviations shown. Free concentration of radioactive *p*-iodobenzoate after equilibration; in series *A*, $2{\cdot}74 \times 10^{-6}$ *M*; in series *B*, $4{\cdot}49 \times 10^{-6}$ *M*. Maximum deviation in free concentration in either series, 3 per cent.

In contrast with the large decrease in molecular size are the small changes in binding capacity. For samples 2*B* or 4*A*, which exhibited a single peak at 3·5 or 3·6 S., the concentration of hapten bound was more than 90 per cent as large as that for the corresponding untreated antibody preparation at the same free hapten concentration. Since the free hapten concentration was held constant, the results indicate that the decrease in the average binding constant or in the total number of binding sites was less than 10 per cent[6]. The use of larger amounts of papain resulted in somewhat greater decreases in binding capacity (up to 25 per cent) but most of the combining sites were evidently still intact.

Treatment with papain resulted in each case in complete loss of ability to precipitate with the ovalbumin–*p*-azobenzoate test antigen, as shown by tests made with a wide range of antigen concentrations. Control experiments, in which the papain was inactivated by treatment with 0·01 *M* ferricyanide prior to addition of antigen, showed that this failure to precipitate was due to the action of papain on the antibody (rather than on the antigen).

The results indicate that the rabbit antibody molecule can be degraded to a non-precipitating form of relatively low molecular weight with little effect on the specific combining sites. It appears also that measurements of binding capacity provide a convenient quantitative method for investigating the properties of these apparently univalent antibody fragments.

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Regeneration of Cellulose Acetate Membranes used for Zone Electrophoresis

The method described by Kohn[1] for the analysis of serum proteins by zone electrophoresis on strips of cellulose acetate membrane has been proved to be of great value. The development of this technique, as a micro-electrophoretic method[2], has emphasized the importance of cellulose acetate as a stabilizing medium. The sheets of cellulose acetate are relatively expensive compared with filter paper, and their higher cost may deter workers from using this excellent material. It has been found that the cellulose acetate membrane strip may be used several times after suitable treatment by the method to be described.

On completion of an analysis the used strip of cellulose acetate membrane with the stained bands of protein is placed in a shallow dish containing 200 ml. of buffer at *p*H 8·6 (veronal, sodium bicarbonate or sodium borate, ionic strength 0·05–0·1) to which is added 2·0–4·0 ml. trypsin solution. The trypsin solution contains 64 units of trypsin per ml. and is standardized by the method described by Coles[3]. The digestion of the protein occurs rapidly for quantities of protein in the range 100–200 μgm. and is complete at 37–39° C. in 2 hr.; but the digestion of 700 μgm. of protein, used with longer and wider strips, is not complete in 2 hr. and sometimes required periods of 16 hr. The longer digestion period is necessary to regenerate strips of cellulose acetate membrane which have been stored in a dry condition for some months after staining. After digestion of the protein, the cellulose acetate membrane strip is washed with distilled water and re-equilibrated in the buffer solution used for the electrophoretic separation. It is then ready for further use.

The cellulose acetate is quite stable under the conditions of regeneration: and in one instance a membrane strip has been regenerated four times without any apparent deterioration; this strip was dyed with lysamine green, bromphenol blue, nigrosine and finally bromphenol blue again for its use on the first, second, third and fourth occasions respectively.

In one instance 700 μgm. of serum protein had been separated into its component fractions by electrophoresis on a cellulose acetate membrane in a buffer solution to which copper sulphate had been added. The regeneration process, performed several months after the membrane had been dried, was not complete. The globulin fractions were digested by the trypsin; but the large stain corresponding to the albumin fraction was not removed. In this case, after treatment with trypsin, the membrane was washed with distilled water, then twice with ethyl alcohol and was finally equilibrated against benzene. The membrane appeared translucent and all traces of dye disappeared. The membrane was then washed with ethyl alcohol, followed by distilled water and finally re-equilibrated against the buffer solution unchanged. Its performance in use after regeneration was quite normal, and another sample of normal serum protein was fractionated successfully.

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