if they occurred in small amounts together with collagen. It is noticeable that the mixture of sugars in the lens capsule carbohydrate, glucose and galactose, are those which have been found in the 0.65 % of carbohydrate which it is difficult to remove from skin collagen (Schneider, 1937). The capsule also probably contains a small amount of hexosamine. Hydrolysates of whole capsule give a value of about 1 % and this might not be thought significant, but fractions prepared by enzyme digestion have been found to contain 2-4 % hexosamine, which makes it likely that a small amount is present in the capsule itself.

SUMMARY

1. Further analysis of ox lens capsule has failed to separate it into separate constituents.
2. It appears to be composed of a protein similar to collagen in many of its physical and chemical properties, but containing about 10 % of carbohydrate.
3. The carbohydrate consists mainly of glucose with a small amount of galactose.

I am greatly indebted to Dr D. M. Crowfoot, F.R.S., for the X-ray photograph of lens capsule and for her interpretation of it for me. I have pleasure in thanking Dr E. Bidwell for the preparation of collagenase.

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The Use of Radioactive Isotopes in Immunological Investigations

6. SOME OBSERVATIONS ON PRECIPITIN INHIBITION TESTS AND ON ANTIBODY VALENCE

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Landsteiner (1920) showed that the precipitation of azoproteins by their antisera could be partially or completely inhibited by simple substances structurally identical with, or very similar to, the compound which had been linked to the protein. This type of competitive inhibition has been used extensively by Landsteiner and his colleagues, and by many other immunologists, for the study of the serological specificity of conjugated proteins and other antigens (see the reviews by Marrack, 1938; Landsteiner, 1945; Boyd, 1947). It has also been shown that the immunological specificity of iodinated proteins is due to their 3:5-diiodotyrosine groups (Wormall, 1930), since 3:5-diiodotyrosine (and to a lesser degree the corresponding bromo-compound) specific-

ally inhibits the reaction between iodinated proteins and their antibodies; further evidence of this specific inhibitory action of 3:5-diiodotyrosine, and compounds of similar constitution and structure, was obtained by Jacobs (1932), and Snapper & Grunbaum (1936). Landsteiner suggested that the inhibitor specifically combined with the serologically active groups of the appropriate antibodies, thus preventing union of antibody and antigen to give precipitation. Proof of this union of specific inhibitor and antibodies was obtained by Marrack & Smith (1932) and Haurowitz & Breinl (1933). Further support was given by the observation that relatively simple azo dyes may (a) give precipitates with antisera to the corresponding dye-azoprotein,

and (b) cause anaphylactic shock when injected into guinea pigs which had been sensitized with the corresponding azoprotein.

Although serological inhibition tests have been extensively used for the qualitative study of precipitin and complement-fixation tests, few quantitative investigations have been made in this field (cf. however, Woolf, 1941). We have, therefore, studied the composition of precipitates separating from mixtures of iodinated proteins and their antibodies in the presence of varying amounts of specific inhibitor (3:5-diiodotyrosine). The results are discussed in relation to antibody valence.

The valence of antibodies, i.e. the number of specific combining groups or binding sites in the antibody molecule, has been widely studied, and two different views are held (for references see Boyd, 1947; Landsteiner, 1945; Grabar, 1950). Haurowitz, Boyd and others believe, that antibodies are univalent whilst Pauling and many other workers maintain that antibodies are bi- or multi-valent. The 'lattice theory' of the precipitin reaction (Marrack, 1938) requires that the antibody molecule should have at least two groups capable of combining with antigen, and Pauling and his colleagues have obtained evidence that most of the molecules of antibody are at least bivalent (cf. for example, Pauling, 1940; Pauling, Pressman & Ikeda, 1942; Pauling, Pressman & Campbell, 1944).

Antigen molecules are usually multivalent, and our iodinated protein antigens may contain up to sixty-five combining or determinant groups per molecule. Thus it seems probable, for reasons given later in this paper, that if a 'lattice structure' containing bi- or multi-valent antibody is precipitated in the presence of concentrations of specific inhibitor sufficient to cause only partial inhibition of precipitation, there should be a significant amount of the inhibitor in the precipitate. Investigations designed to test this possibility were therefore undertaken, and are described below. Brief accounts of some of the results have been given elsewhere (Banks, Francis, Mulligan & Wormall, 1949, 1950a).

EXPERIMENTAL

The non-radioactive iodinated serum globulins used for immunization and the ^{131}I -containing serum globulins used as test antigens were prepared as described (Banks, Francis, Mulligan & Wormall, 1950b). The preparation of other antigens is described below.

Iodinated rabbit serum proteins. 1% (w/v) KI solution (5.5 ml.) was treated with 22 $\mu\text{c.}$ of ^{131}I as NaI and I_2 was liberated by adding 0.1 N-KIO₃ (4 ml.) and then acidifying the mixture with HCl. The liberated I_2 was extracted with ether and the ethereal solution was added slowly to 2 ml. rabbit serum diluted with 4 ml. 2.5 N-NH₃. The ether was then rapidly removed *in vacuo* and the iodinated proteins were precipitated by adding acetic acid. The proteins were again dissolved at pH 7.5 and precipitated by addition of

acetic acid. This was repeated once more. The final precipitate was dissolved in 0.9% NaCl with the addition of 2 N-Na₂CO₃ to bring to pH 7.5. The non-radioactive iodinated rabbit serum proteins were prepared similarly; both preparations contained about 14% I.

3:5-Diiodotyrosine containing ^{131}I . This was prepared by the method of Harington (1928) with slight modifications introduced to reduce the loss of ^{131}I . A solution of 10% (w/v) KI (7.7 ml.) was added to 528 $\mu\text{c.}$ of ^{131}I (as NaI), followed by N-KIO₃ (5.5 ml.). A slight excess of N-HCl was then added, and the liberated I_2 was extracted with ether. The ethereal solution was then gradually added, with shaking, to a solution of tyrosine (0.25 g.) in NH₃ (sp.gr. 0.880) (5.5 ml.). When all the I_2 had been added the aqueous solution was separated and kept in a desiccator over conc. H₂SO₄ until practically all the NH₃ had been removed. The resulting brown sludge was dissolved in water (15 ml.) containing a few drops of N-NaOH, and the solution decolorized with charcoal. N-Acetic acid was then added to precipitate the 3:5-diiodotyrosine, which was then washed with ice-cold water and precipitated again, in crystalline form, by acidifying a slightly alkaline solution of the compound. The yield was 0.432 g. (72% of theory). With a thin-window bell-type counter, it gave 100 counts/ $\mu\text{g.}/\text{min.}$ For the inhibition tests a stock 0.02 M-solution, in 0.9% NaCl at pH 7.5, was prepared.

Antisera. These were obtained by the intramuscular injection of alum-precipitated antigens into rabbits (Proom, 1943). Fresh unfiltered antisera were used in the experiments recorded in Tables 1 and 3, and 'Berkefeld-filtered' and stored serum for the experiments recorded in Table 2 and Fig. 1 (cf. Banks *et al.* 1950b).

Precipitin tests. These were carried out as described by Banks *et al.* (1950b).

Precipitin-inhibition tests. These followed the general method of Landsteiner (1920), as used by Wormall (1930) for the inhibition of the iodinated protein antigen-antibody reaction by 3:5-diiodotyrosine. The inhibitor was added to the antiserum and the antigen solution was then added to the mixture. Where D₂O was used, it was added before the antigen. Preliminary tests were made with non-radioactive 3:5-diiodotyrosine to determine the amount of inhibitor required to give suitable partial inhibition of precipitate formation. Full details of these experiments are given with the tables or in the appropriate parts of the text.

Radioactivity determinations. These were made as described by Banks *et al.* (1950b), using side-window or bell-type thin end-window Geiger-Müller counters.

Molecular ratios. In calculating the molecular ratios, the value 165,000 was used for the molecular weight and 16.0% for the N content of antibodies and the mixed serum globulins. It is recognized that antibody molecules are not necessarily homogeneous, but in these investigations we were concerned with the average precipitating antibody molecules. The molecular weight of 3:5-diiodotyrosine is 433.

D₂O determinations. The heavy water samples were separated from the protein solutions by vacuum sublimation and condensation at about -70°, and their purity was checked by measurement of refractive index. The D₂O concentrations were then determined by the falling drop method (Barbour & Hamilton, 1926) using the apparatus described by Popják (1950).

Total iodine determinations. These were made by the method of Shahrokh (1943). Under the conditions of our experiments we have found this satisfactory for the determination of 10-100 $\mu\text{g.}$ of iodine, with an accuracy of $\pm 1 \mu\text{g.}$

RESULTS

Iodine content of precipitates separating from iodinated protein antigen-antibody mixtures containing 3:5-diiodotyrosine

In one typical experiment the precipitation of iodinated proteins by an antiserum to iodinated horse serum globulin was partially inhibited by 3:5-diiodotyrosine and the precipitates were analysed for antigen and inhibitor. In one set of mixtures the antigen was labelled with ^{131}I and in a parallel set the inhibitor only was radioactive. To exclude as far as possible interference due to species differences, the test antigen was prepared from rabbit serum proteins; the amount of antigen used was that required to give approximately maximal precipitation of antibody in the absence of inhibitor.

Samples (2 ml.) of the antiserum were treated with 3:5-diiodotyrosine (1 or 2 ml. of a 0.0025 or 0.01 M-solution in 0.9% NaCl), and 0.9% NaCl to make a total volume of 4 ml. The antigen solution (4 ml. of a 0.043% solution of ^{131}I -containing iodinated rabbit serum proteins or a 0.048% solution of a similarly prepared non-radioactive antigen) was added to each tube. The mixtures were stirred well and kept at 37° for 3 hr., followed by 15–18 hr. at about 4°. They were then centrifuged and the sides of each tube were washed with six drops of 0.9% NaCl, care being taken to avoid disturbing the precipitate. The precipitates were then dissolved in a few drops of approximately 0.5 N-NaOH, and ^{131}I and total I determinations made as described above. Control mixtures of normal rabbit serum plus radioactive antigen and/or inhibitor gave no precipitate.

In these experiments the amount of antigen precipitated in the mixtures containing radioactive antigen and non-radioactive inhibitor was 36–54% of that precipitated in the absence of inhibitor, and the corresponding range for the non-radioactive antigen series was 38–60% (Table 1). In the former series there was good agreement between the iodine contents of the precipitates as determined by ^{131}I and by total iodine. This suggests that all the iodine in the precipitates was due to antigen. More definite evidence of this was obtained from the analyses of precipitates from the mixtures containing radioactive diiodotyrosine and non-radioactive antigen, for these precipitates contained no detectable ^{131}I . The molecular weights of the antigen proteins are, however, about 150–400 times that of diiodotyrosine; these results with radio-diiodotyrosine of relatively low specific activity, and with precipitates which were not completely freed from supernatant solution, would not, therefore, exclude the possibility of the precipitate containing a few diiodotyrosine molecules per molecule of antigen or antibody.

In the experiment recorded in Table 1, the antibody:antigen ratio was not determined, but other experiments made with the same antiserum, but without inhibitor, gave ratios ranging from 4:1 (antigen excess) to 12:6 (antibody excess) for precipitates obtained with iodinated rabbit serum proteins as test antigen (assuming a mean molecular weight of 10^5 for the serum proteins), and a corresponding range of 1.5–5.0 with iodinated ovalbumin.

Table 1. *Iodine content of iodinated protein antigen-antibody precipitates from systems containing 3:5-diiodotyrosine*

Antigen: Iodinated rabbit serum proteins.

Antiserum: Rabbit antiserum to iodinated horse serum globulin.

Inhibitor: 3:5-Diiodotyrosine (DIT).

(Weights given are for precipitates from 2 ml. of antiserum. Other experimental details are given in the text.)

Component labelled with ¹³¹ I	DIT added (μg.)	Degree* of precipitation	Composition of precipitate		Amount of antigen as percentage of that added
			Iodine content		
			By ¹³¹ I (μg.)	Total I (μg.)	
Antigen	0	+ + ±	26.6	29.2	11.3
	1082	+ ±	15.2	16.0	6.4
	2165	+	13.9	14.2	5.9
	4330	±	12.1	12.4	5.1
	8660	tr.	10.6	10.2	4.5
DIT	0	+ + ±	0	30.9	11.6
	1082	+ ±	0†	18.3	6.9
	2165	+	0†	16.9	6.3
	4330	±	0†	13.8	5.2
	8660	tr.	0†	11.8	4.4

* Taken from a parallel set of precipitin tests made with one-twentieth the volumes of solutions used in the quantitative experiment. The readings given are those after 3 hr. at 37°, and the results are recorded as follows (in increasing degrees of precipitation): tr. (trace), ±, +, + ±, ++, +++.

† Counts within variation of background and therefore not quantitative, but indicate certainly less than 3 $\mu\text{g.}$ iodine.

Composition of iodinated protein antigen-antibody precipitates from systems containing 3:5-diiodotyrosine

In further inhibition experiments the washed specific precipitates were analysed for antigen and antibody.

Samples (0.5–3.0 ml.) of a rabbit antiserum to iodinated human serum globulin were treated with 0.01 M 3:5-diiodotyrosine (0.05, 0.083 or 0.133 ml./ml. antiserum) and then mixed with a solution of iodinated horse serum globulin containing ^{131}I (0.01–0.4 ml. of a solution containing 1.037 mg. N/ml./ml. antiserum). Sufficient 0.9% NaCl was added to make the total volume twice the original serum volume. The mixtures were kept at 37° for 1 hr. and then at 0–4° overnight; they were then washed twice with 0.25 ml. portions of 0.9% NaCl, dissolved in N-NaOH (0.5 ml.) and made up to 2.5 ml. Samples of these solutions (1–2 and 0.3 ml. respectively) were used for total N and radioactivity determinations.

Fig. 1. Total N in precipitates from the iodinated protein antigen-antibody system in the presence of 3:5-diiodotyrosine (inhibitor). Antigen, iodinated horse serum globulin. Antiserum, rabbit antiserum to iodinated human serum globulin. A, least inhibitor; C, most inhibitor. Further experimental details are given in Table 2.

The results show that, in some respects, the effect of adding the specific inhibitor is equivalent to reducing the amount of antibody available for combination with antigen. This causes not only a decrease in the amount of precipitate obtained (Fig. 1), but also, with most of the antigen-antibody mixtures examined, a decrease in the antibody: antigen ratio in the precipitate (Table 2). As increasing amounts of antigen are used, however, and the ratio tends towards its lower limit, this latter effect becomes less marked, and the inhibited and uninhibited systems ultimately give the same ratio.

It was not practicable to investigate fully the precipitation in inhibited systems containing very small amounts of antigen, since the amount of precipitate obtained was too small for complete analysis: for example, addition of 31 $\mu\text{g.}$ antigen produced, per ml. antiserum in an inhibited system, precipi-

tates containing only 2.5 and 6.5 $\mu\text{g.}$ antigen and antibody nitrogen respectively, compared with a precipitate containing about 84 $\mu\text{g.}$ nitrogen in the uninhibited system.

Table 2. *Composition of washed precipitates separating from iodinated protein antigen-antibody mixtures in the presence of 3:5-diiodotyrosine*

Antigen: Iodinated horse serum globulin (containing ^{131}I).

Antiserum: Rabbit antiserum to iodinated human serum globulin.

Inhibitor: 3:5-diiodotyrosine (DIT).

(The values in brackets are probably unreliable.)

Antigen added ($\mu\text{g. N/ml.}$ serum)	Antibody N : antigen N ratios of the precipitates			
	Unin- hibited (no DIT)	Partially inhibited		
		(A)*	(B)*	(C)*
10.4	8.1	—	—	—
20.8	5.9	—	—	—
31.1	—	2.7	2.6	(4.1)
62.3	2.9	1.9	1.9	2.3
156	1.7	1.3	—	1.4
208	1.4	1.0	1.1	1.4
311	1.1	(0.8)	1.1	1.2
415	1.3	1.1	1.2	—

* The concentrations of 3:5-diiodotyrosine in series (A), (B) and (C) were 2.5, 4.16 and $6.7 \times 10^{-4}\text{M}$, respectively.

The use of an additional tracer (heavy water) to measure the amount of supernatant solution adhering to serological precipitates

In many of our investigations we have sought to determine whether or not combined specific inhibitor is present in serological precipitates which separate from mixtures of antigen and antibody with sufficient inhibitor to effect only partial inhibition. The concentration of inhibitor needed for measurable inhibition is relatively large, and there is, therefore, a considerable amount of inhibitor in the supernatant solution which adheres to the precipitate. This makes it difficult for the amount of combined inhibitor to be determined. Serological precipitates can often be washed with cold 0.9% sodium chloride solution without serious loss of antigen or antibody, but it should not be assumed that this will also apply to any combined inhibitor in the complex. We decided, therefore, not to wash the precipitates in these particular inhibition tests, and we sought some method which would enable us to estimate the amount of adhering, as distinct from combined, inhibitor, in the unwashed precipitates. It was essential that any method adopted for this purpose should not interfere with the determination of antibody, antigen and total inhibitor in the precipitate, i.e. with total nitrogen, and, in the experiments described here, total iodine and ^{131}I determinations.

We found that our object could be achieved by using heavy water as an additional tracer. Deuterium oxide was added to the antigen-antibody-inhibitor mixture in amounts sufficient to render possible a determination of the deuterium oxide content of a measured fraction of the specific precipitate. From the results we calculated the amount of supernatant solution, and hence the uncombined inhibitor, associated with the precipitate; from this value and the nitrogen, iodine and ^{131}I contents, the amounts of antibody, antigen and combined inhibitor in the precipitate could be calculated. Preliminary experiments showed that, under the conditions of our experiments, a final concentration of 5% deuterium oxide in the mixture of antigen, antibody and inhibitor was suitable for this 'additional tracer' technique. A typical experiment was carried out as follows.

Samples (6 ml.) of a rabbit antiserum to iodinated horse serum globulin were mixed with NaCl solution containing heavy water (1 ml. containing 0.9% NaCl and 50% (w/v) D_2O) and ^{131}I -containing 3:5-diiodotyrosine solution (0.25, 0.75 or 2.0 ml. of 0.01M). The antigen (1 ml. of 0.6% iodinated human serum globulin) and sufficient 0.9% NaCl to give a total volume of 10 ml. were then added. The mixtures were kept at 37° for 3 hr. and then at $0-4^\circ$ overnight; the precipitates were separated by centrifuging, and the sides of the tubes were cleaned with filter paper, care being taken to avoid disturbing the precipitate. These unwashed precipitates were then dissolved in water containing a few drops of 0.5N-NaOH and the volume made up to 3 ml. Total N and ^{131}I determinations were made on 0.1 and 1.0 ml. samples respectively, and the D_2O contents of these solutions, and of the supernatant solutions separated from the precipitates, were also determined.

The results showed that the amount of combined inhibitor (3:5-diiodotyrosine) in the antigen-antibody complex was very small and probably zero (Table 3). The calculated molecular ratio of combined inhibitor:antibody was sometimes zero, and in no case was it greater than 1:20.

DISCUSSION

For the quantitative study of serological inhibition tests radio-isotopic tracers are particularly suitable. When the inhibitor has exactly the same composition and configuration as the determinant group of the antigen, the determination of antigen, antibody and inhibitor in mixtures containing all three is extremely difficult, and often impossible, by ordinary chemical methods. The analysis can readily be made, however, if either the antigen or the inhibitor contains a radioactive isotope; both may be labelled, with different radio-isotopes, but this is not usually necessary. In some of the experiments described here, for example, radioactive 3:5-diiodotyrosine and non-radioactive iodinated proteins were used. The possibility of exchange of ^{131}I between these two compounds in precipitin reactions carried out at about pH 7.5 can virtually be excluded, since exchange between iodide and either 3:5-diiodotyrosine or thyroxine is negligible at alkaline pH (Miller, Anderson, Madison & Salley, 1944; Frieden, Lipsett & Winzler, 1948).

When 3:5-diiodotyrosine is added, in amounts which only partially inhibit precipitation, to a mixture of iodinated protein antigen and its antiserum, the molecular ratio of antibody:antigen in the precipitate is usually less than that for the precipitate which separates in the absence of inhibitor. Woolf (1941) found that the addition of hapten effected a similar reduction in the antibody:antigen ratio of precipitates separating from azoprotein antigen-antibody systems. This is not surprising, since the action of the inhibitor can be attributed to its capacity to compete with the antigen for the reactive groups of the antibody. In effect, the inhibitor reduces the amount of available antibody in the solution, and with this change towards the excess antigen region there is a fall in the antibody:antigen ratio of the precipitates. When the con-

Table 3. *Composition of the unwashed precipitates separating from mixtures of iodinated proteins, their antibodies and 3:5-diiodotyrosine (inhibitor)*

Antigen: Iodinated human serum globulin.
Antiserum: Rabbit antiserum to horse serum globulin.
Inhibitor: 3:5-Diiodotyrosine (DIT) (containing ^{131}I).

(Experimental details are given in the text. All the amounts are given as $\mu\mu\text{mol./ml.}$ of antiserum added.)

Antigen added ($\mu\mu\text{mol.}$)	DIT added ($\mu\mu\text{mol.}$)	Composition of precipitate						
		Antibody ($\mu\mu\text{mol.}$)	Antigen ($\mu\mu\text{mol.}$)	Antibody : antigen (Mol. ratio)	DIT			'Combined' DIT : antibody (Mol. ratio)
					Total ($\mu\mu\text{mol.}$)	Free* ($\mu\mu\text{mol.}$)	'Combined' ($\mu\mu\text{mol.}$)	
7.03	0	12.55	3.85	3.3	0	0	0	—
	440	5.34	2.98	1.8	3.00	2.73	0.27	0.05
	1320	4.70	2.78	1.7	7.89	8.15	-0.26	0
	3520	3.44	2.17	1.6	15.10	15.04	0.06	0.017

* Determined by carrying out the precipitin reaction in a solution containing 5% D_2O , and measuring the D_2O content of the precipitate and supernatant solution.

centration of antigen is very high, however, we have found that the addition of even a very considerable excess of inhibitor does not lead to any significant change in the antibody:antigen ratio for the precipitate.

Competition between inhibitor and antigen apparently results in an equilibrium between flocculated antibody-antigen complexes and soluble complexes (antibody-inhibitor, and possibly, if antibody is bi- or multi-valent, antigen-antibody-inhibitor also). The flocculated particles might also contain inhibitor attached to some of the serologically active antibody groups, but no information on this point is apparently available in the literature. The results recorded here show that 'partially inhibited' precipitates obtained with the iodinated protein antigen-antibody system contain little or no inhibitor, and this observation appears to have an interesting bearing on antibody valence.

The antigen used in our experiments was undoubtedly multivalent, for it contained about sixty 3:5-diiodotyrosine residues per molecule, and furthermore it gave precipitates with antibody:antigen molecular ratios up to over 10:1 in the region of antibody excess in the absence of diiodotyrosine. If the antibody also is multivalent, and the precipitated complex has a 'lattice structure' (cf. Marrack, 1938) it seems highly probable that many of the antibody molecules in solution will have some of their valences 'blocked' by combination with diiodotyrosine molecules. Provided the remaining 'unblocked' valences are at least two in number, there seems no reason why these antibody-inhibitor complexes should not take part in lattice formation. If the antibody is bivalent and has only one valence blocked, or alternatively, if a few or even all but one of the valences of multivalent antibody are blocked, it seems reasonable to suppose that these antibody-inhibitor complexes should coprecipitate, as do Heidelberger's 'univalent or incomplete' antibodies (Heidelberger & Kendall, 1935; Heidelberger, Treffers & Mayer, 1940; cf. Kabat & Mayer, 1948). Thus with a multivalent antibody one might reasonably expect to find the specific inhibitor attached to the precipitates in these 'partially-inhibited' systems, as represented diagrammatically, for a bivalent antibody, in Fig. 2.

If any inhibitor is attached to the precipitated antibody, one might expect that the amount would be measurable, e.g. more than one molecule of inhibitor per twenty molecules of antibody, for it seems improbable that an insoluble antibody-antigen-inhibitor complex with a molecular ratio of, say, 20:11:1 would become soluble if one only of the antigen molecules were replaced by inhibitor. In most of our experiments with the 'partially-inhibited' iodoprotein serological system the amount of inhibitor in the precipitate was significantly less

than this. We feel justified, therefore, in suggesting that our observations support the view that the rabbit antibodies we have studied are univalent, i.e. each molecule has only one combining site for the diiodotyrosine group. On the basis of univalent antibodies, specific inhibition can readily be explained. Antigen and inhibitor compete for antibody, and where the antibody combining site is blocked by inhibitor, this antibody molecule is not able to precipitate, or be precipitated by, antigen.

Fig. 2. Hypothetical lattice formed by iodinated protein antigen and bivalent antibody in the presence of the specific inhibitor (3:5-diiodotyrosine).

Several authors have obtained evidence that precipitating antibodies are univalent (see, for example, Boyd, 1942; Haurowitz & Schwerin, 1942). There is also evidence of 'incomplete', 'partial' or 'blocking' antibodies (see, for example, Heidelberger & Mayer, 1940; Race, 1944; Morgan & Schütze, 1946) which are generally described as 'univalent'. Since, however, these 'partial' antibodies do not precipitate or agglutinate the appropriate antigens they are not comparable with those present in the rabbit antisera we have studied. Further support for the theory of univalence of precipitating antibodies was recently obtained by Porter (1950) in an interesting new approach to serological inhibition. By enzymic hydrolysis of rabbit antiovalbumin Porter obtained a specific inhibitor which combined with, but did not precipitate, the antigen. Only one molecule of

inhibitor was obtained from each molecule of antibody, and Porter concludes that this result is more in keeping with the occlusion theory of precipitation (Boyd, 1942) than the lattice theory.

In these inhibition tests with hydrolysed anti-ovalbumin, Porter (1950) found that ovalbumin-antiovalbumin precipitates separating in the presence of the specific inhibitor (hydrolysed antibody) contained no inhibitor, and this is rather surprising if, as is generally believed, antigens such as ovalbumin are multivalent. This conclusion was based, however, on the results of total nitrogen determinations and the assumption that all the antigen and nearly all the antibody were precipitated, and it would be difficult to justify these assumptions. A complete analysis of these precipitates, for antigen, antibody and inhibitor, would provide very interesting information, and we suggest that isotopic labelling could profitably be applied to this serological system.

SUMMARY

1. Using ^{131}I -labelled antigen or inhibitor, a quantitative study has been made of the iodinated protein antigen-antibody precipitin reaction in the

presence of the specific inhibitor, 3:5-diiodotyrosine.

2. The addition of inhibitor usually caused a fall in the antibody:antigen ratio of the precipitate, due apparently to the inhibitor reducing the concentration of free or effective antibody.

3. Some of these precipitin tests were carried out with mixtures containing 5% deuterium oxide. This made it possible for determinations to be made of the amount of supernatant solution, and hence the amount of free inhibitor, in the unwashed precipitates.

4. The amount of combined inhibitor in the precipitates was very small and sometimes immeasurable; at most, it was equivalent to one molecule of 3:5-diiodotyrosine per twenty molecules of antibody in the precipitate.

5. The results are discussed in relation to antibody valence, and it is suggested that they support the view that the precipitating antibodies studied are univalent.

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