This equation was derived by W. Carter, R. Scott, and M. Magat for natural rubber in toluene (1). Since toluene and benzene have similar  $\mu$  values for natural rubber, the equation was employed for the purpose of estimating molecular weight of the polymer in benzene. The molecular weight of the rubber is low; however, benzene would not extract polymer of high molecular weight-that is, gel rubber-even if it were present. A better method of extraction is needed. Since this is a mixed collection, some species are probably contributing more than the average content of 1.7 percent rubber.

An attempt was made to cure the soft, tacky, low-molecular-weight rubber using the "Peachy" cure and sulfur chloride. The cured products were of low moduli, and elongations did not exceed 200 percent. There was insufficient material for milling to incorporate curing agents.

Rubber extracted from sporophores of L. deceptiva was similar to that obtained from the mixed species. It too was cispolyisoprene (Fig. 1). The rubber content was low-0.16 percent on a dry weight basis. It was not characterized further.

The rubber extracted from ascocarps of Peziza was much tougher than that from Lactarius. Solubility in benzene was very low and deposition of polymer on the salt cakes was not uniform. Satisfactory films were not obtained, but the infrared spectrum (Fig. 1) suggests that this rubber is also cis-polyisoprene.

Fungi belonging to two of the major classes of fungi, Ascomycetes and Basidiomycetes, are able to synthesize rubber as cis-polyisoprene. Since fungi, phylogenetically, were derived from algaethat is, they are degenerate forms with loss of chlorophyll-the probability of rubber synthesis by species of this phylum is implied.

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# **Arsenic Tribromide Immersion** Liquids of High Index of Refraction

Immersion liquids of high index of refraction containing arsenic tribromide as the solvent, prepared in recent years (1, 2), have proved to be stable. In Table 1 are given the starting materials and their relative proportions by weight used to

Table 1. Arsenic tribromide immersion liquids. Composition is given in relative proportions by weight. Dispersion is measured by the difference in index between the *e* line of mercury (546 m $\mu$ ) and the *D* line of sodium (589 m $\mu$ ).

Compo- sition	<sup>n</sup> D (3	$\Delta n_{ m D} \times 10^{-3})$	Time (yr)	${\mathrm d}n/{\mathrm d}T$ (× 10 <sup>-4</sup> )	Disper- sion (× 10 <sup>-4</sup> )
6AsBr., 2S,				and and a second second second	
2As <sub>2</sub> S <sub>2</sub>	2.00	-2	11/4	6(1)	174 (I)
6AsBr <sub>3</sub> , 2Se,					
$2As_2S_2$	2.11	- 2	3	6	288
12AsBr <sub>3</sub> , 1S,					
$7As_2S_3$	2.07	- 1	$2\frac{1}{2}$		
6AsBr <sub>3</sub> , 2Se,					
$2As_2S_3$	2.04	-2	3		
14AsBr <sub>3</sub> , 3S,					
3As <sub>2</sub> S <sub>2</sub> , 2HgS	1.99	- 1	3	6	173
14AsBr <sub>3</sub> , 3S,					
3As <sub>2</sub> S <sub>3</sub> , 4HgS	2.01	- 3	3		
6AsBr <sub>3</sub> , 2S,					
1Se, $2As_2S_2$	2.02	- 1	$3\frac{1}{2}$	6	195
6AsBr <sub>3</sub> , 2S,					
$1$ Se, $2$ As $_2$ S $_3$	2.00	- 5	3		
$CH_{2}I_{2}$	1.74			7(4)	88 (1)

prepare the liquids, the indices of refraction of the liquids  $(n_{\rm D})$ , the change in index of refraction with time  $(\Delta n_{\rm D})$  $\times 10^{-3}$ ), and the temperature coefficients  $(\,\mathrm{d}n/\mathrm{d}T\,)$  and dispersion of some of the liquids (3). The liquids were prepared as described in an earlier paper (1). The disadvantages of the arsenic tribromide liquids have been listed in a more recent publication (2).

The liquid with an index of 2.11 is very viscous and dark red, but a thin film is light yellow. It is possible that even higher indices can be obtained with these mixtures.

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## Mechanism of

# Antibody-Antigen Reaction

Antiserums to yeast crystalline alcohol dehydrogenase (1) were produced in rabbits by subcutaneous injections of 10 mg at weekly intervals. The antibody partially inhibited the activity of the enzyme but did not inhibit liver alcohol dehydrogenase. The enzyme (antigen) reacted with the antibody to form a precipitate of antibody-antigen complex that nevertheless possessed enzymatic activity (2). Kinetic studies on the inhibition of the enzyme activity with increasing increments of antiserum (3) led to the formulation of the following concept.

When an antigen is first introduced into the body, it stimulates the formation of specific antibodies to special sites on its surface that possess the necessary configuration. If it is still present in the body or is again introduced into the animal at a time when antibodies are already present in the circulation, it reacts rapidly with these earlier antibodies to form an antibody-antigen complex (complex I). This complex, in which the configuration of the antigen and antibody are mutually altered, behaves as a new antigen. Specific antibodies are then formed against the new sites of altered configuration. Similarly, if the antigen is still present in the body, or upon the introduction of the antigen for a third time, the earlier antibodies react with it to form complex I, which then reacts with its specific antibody to form complex II, and this in turn behaves as a new antigen with a newly altered antigenic surface. Complexes III and IV, and subsequently others, may be formed in like manner. This process presumably continues with each introduction of the antigen until a stage is reached when the final complex ceases to be antigenic or fails to differ antigenically from the immediately preceding complex.

The concept just developed represents no departure from already accepted principles of immunology and protein chemistry. There are many examples of compounds, termed adjuvants, that act on some substances to influence their immunological reactivity (4). Furthermore, a staphylococcus toxin reacts with rabbit muscle extract to render it antigenic when it is injected into rabbits (5)

Proteins are capable of astonishing structural transformations. They can unfold to expose new groups, hydrophilic or hydrophobic as the case may be, depending on the environment (6). It is therefore to be expected that the structural configuration on the surface of the antibody-antigen complex must necessarily differ at certain sites from the original configurations of the individual antigen and antibody. It has already been shown that an antibody molecule does in fact increase in volume upon reacting with the antigen (7). Such an increase in volume is interpreted to signify an unfolding of the molecule with a structural modification of its surface configuration. The very fact that a precipitate is formed can readily be explained by the unfolding of both antibody and antigen so as to expose a large number of hydrophobic groups.

In support of this concept, it has been possible to show the following (8).

1) The subcutaneous injection of a thoroughly washed antibody-antigen complex into rabbits produced antiserum that reacted more rapidly with the complex than with the enzyme antigen itself. This reaction was measured by the rate of increase in turbidity and by the rate of inhibition of the enzymatic activity in each case, using the Beckman spectrophotometer at 340 mµ. The complex was prepared in all instances at the equivalence point. After an incubation of antiserum and antigen for 24 hours at 0°C, the mixture was centrifuged and washed three times with 5 ml of saline at 0°C.

2) It was also possible to show (Table 1) that one can absorb selectively and exhaustively with a particular complex and yet leave behind in the supernatant other antibodies that can react with the original antigen. Table 1 further shows that with one injection no antibody to a particular complex is detectable. With repeated injections, however, there is a simultaneous rise in the amount of antibody that reacts with the complex.

3) Table 2 shows in addition that one can absorb in a similar manner with two types of complexes and still detect antibodies left behind that react only with the original antigen. Furthermore, Table 2 shows that when absorption of antibody was done with the original antigen alone, the amount of antibody that precipitated was in accord with the sum of the antibody absorbed on the two complexes and that which was subsequently absorbed on the antigen. This indicated that the antigen formed a nucleus for the succeeding complexes, which in turn absorbed their respective antibodies.

According to this concept, the "univalent" or "incomplete" (9) antibody would have all the characteristics of an antibody to the complex. Furthermore, this concept readily explains certain anomalies recently observed in antibodyantigen reactions. It was reported that

Table 2. Antiserums that are completely absorbed with one antigen-antibody complex may nevertheless contain antibody for a second antigen-antibody complex; the supernatant fluid then contains residual antibodies that are reactive only with the simple antigen. The numerical values indicated in the antiserum code refer to the number of subcutaneous weekly injections of 10 mg of antigen given before serum was obtained. Serums No. 1 and 2 were absorbed by successive additions (I to IV) of 0.5 mg of the first complex and 0.3 mg for each subsequent addition (V to VI) of the second complex. The first complex was prepared from a rabbit serum obtained after nine injections of antigen, and the second complex was prepared from a rabbit after five injections of antigen. Serums No. 3 to 5 were similarly absorbed by 1.0 mg and 0.6 mg of the first and second complex, respectively. The first complex used in the last three experiments was prepared from a rabbit serum obtained after five weekly injections of antigen, and the second complex was prepared from a rabbit serum obtained after two injections of antigen. The amount used for absorption was dictated by the total amount of antibody present. Incubation time was 2 hours at 30°C, followed by 1 hour at 0°C at pH 8.25. The amount of antigen (enzyme) precipitated when antigen in slight excess was subsequently added (VII), or when it was added alone as in column 10, was calculated from the specific activity added minus the activity remaining in the supernatant. Normal serum was used as control.

Antibody absorbed (µg of protein/ml of antiserum)									
	Successive absorption (I to VII)								
Antiserum	First complex				Second complex		Antigen	Total	Antigen alone
	Ι	II	III	IV	V	VI	VII		
1 (D-2)	140	10	0	0	102	0	90	332	300
2 (A'-5)	100	90	60	0	156	0	118	524	580
3 (A-2)	670	195	0		0		815	1680	1550
4 (A-3)	80	195	0		160		1420	1855	
5 (F-7)	1170	322	0		180		1460	3132	3120

Rh antiserums fractionated by the electrophoresis convection method showed a considerable loss of total precipitating antibody when each fraction was measured separately. However, on recombination of the fractions, there was complete recovery of the antibody (10). It is apparent now that the different fractions contained limiting amounts of antibody to one or another complex. Conse-

Table 1. Presence in antiserums of antibodies to crystalline alcohol dehydrogenase that react uniquely with the simple antigen after complete absorption with an antigen-antibody complex. Antiserums were obtained from the same rabbit 1 week after the first, second, and third subcutaneous injections of 10 mg of enzyme as indicated in column 1. The complex used for the successive additions was prepared from the pooled serum of two rabbits, one receiving three and the other five weekly injections of 10 mg of enzyme. One milligram of complex was used for each successive absorption, and 0.06 mg of enzyme, representing a slight antigen (enzyme) excess, was used in the final step. Incubation time for each absorption was 24 hours at 0°C at pH 8.2. Normal rabbit serum was tested simultaneously under the same conditions as the immune serum and consequently served as control.

Weeklv	Antibody absorbed per milliliter of antiserum with successive additions $(I \text{ to } VI)$ of the same complex followed by addition of antigen $(\mu g/ml)$							
injections –			Cor		and the gradest			
	Ι	II	III	IV	V	VI	Antigen	
1st 2nd 3rd	0 440 820	0 410 620	320 510	220 380	180 270	0 0	240 780 440	

quently, only a portion of the antibody present in a fraction could be precipitated under the circumstances.

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I criticize not by finding fault but by a new creation.—MICHELANGELO.