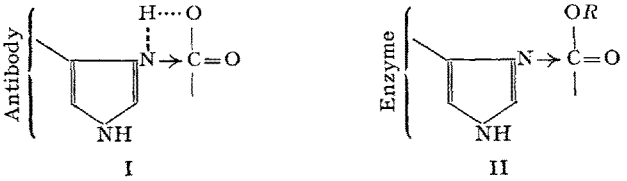
Antigen-antibody Interaction at Specific Binding Sites: a Mechanism Involving Iminazole

The view that antigen-antibody interaction at specific combining sites is primarily of the acid-base type[1], is consistent with thermodynamic data[2–4], salt effects[5], and the fact that antigen-antibody complexes are completely dissociated in strongly acid or alkaline solutions. SINGER and CAMPBELL[2], who studied the effect of pH on antigen-antibody equilibria, concluded that specific interaction was occurring between an ionised carboxyl in the antibody and a complementary protonated basic group (BH^+) in the antigen. Subsequent work [6–8], though broadly compatible with this picture, implies conversely that the basic group (B) may be attached to the antibody rather than to the antigen. TOZER, CAMMACK, and SMITH[9] have reported that antigen-antibody complexes undergo reversible dissociation in aqueous carbon dioxide at pH 5·0. In the light of the foregoing, these results would be consistent with specific interaction between a carboxyl and a basic group (B) the nature of which should permit its reversible protonation in the region of pH 5. The basic nitrogen (pK 5·6–7·0) of iminazole uniquely approaches this requirement, since other protein basic groups have appreciably higher pK values[10].

If one assumes that B is the iminazole nucleus of a histidine unit at a specific combining site in the antibody, the mechanism of primary binding with antigen may be depicted by the partial structure I in which the strongly donating iminazole is coordinated to an acceptor carboxyl group in the antigen.



I II

The iminazole nucleus has a characteristic tendency to interact with acyl groups in such a manner, as is evident in the facility with which simple iminazoles promote acyl transfer[11], and catalyse the hydrolysis of carboxylic esters[12,13]. The dimerisation of chymotrypsin is believed to be due to iminazole-carboxyl interaction[14]. Chymotrypsin, and certain other enzymes, appear to bind and hydrolyse their acyl substrates[15,16] by interaction with iminazole at the enzyme active site. On this basis an analogy[17] between antigen-antibody and substrate-enzyme interaction at specific sites may be drawn in terms of the structures I and II respectively.

The iminazole hypothesis accounts particularly well for the ease with which aqueous carbon dioxide effects dissociation of antigen-antibody complexes. This may now be ascribed to the combined effects of favourable pH, low ionic strength, and formation of a complex between iminazole and carbon dioxide which displaces the carboxyl group from the binding site. The formation of iminazole-carbon dioxide complexes has been proposed by ROUGHTON and BOOTH[18] to account for the peculiar catalytic effect which iminazoles exert on the carbon dioxide-water equilibrium. The role of combined histidine in the absorption of carbon dioxide by hemoglobin at physiological pH (Bohr effect), appears to be a similar phenomenon[19]. Bonding between iminazole, at the specific site of the antibody, and a carboxyl group would account for the haptenic properties of many aromatic carboxylic acids[8]. Antigen-antibody complexes have been found to undergo dissociation in the presence of aqueous carboxylic acids[20–22]. ISLIKER has suggested[23], in the case of phthalic acid, that dissociation may be the result of interaction between the carboxyl and the antibody combining site.

NISONOFF and PRESSMAN[24] have found that acetylation of antibody with acetic anhydride does not markedly affect the specific combining region. These facts are consistent with the implication of iminazole, since the iminazole nucleus, unlike other protein basic groups would not yield a stable acetyl derivative under these conditions[25,26].

PORTER[27] early showed that the iminazole groups were chemically reactive in antibody globulins, but not in the corresponding complexes with antigen. Iminazole groups are also particularly susceptible to the effects of irradiation[14,28]. Antibody molecules are inactivated by such treatment[29–34], though they appear to be protected by the

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presence of antigen. The rapid loss of anaphylactic properties which accompanies irradiation inactivation is interesting[31]. In the light of the iminazole hypothesis, this suggests that interaction of antigen with histidine iminazole at a specific combining site in the antibody, and release of protein-bound histamine by antigen[35–37] may have a common origin.

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Résumé

L'auteur propose un mode de liaison entre les molécules d'antigène et d'anticorps au moyen d'un centre réactif spécifique résultant de l'interaction d'un groupe récepteur carboxylique de l'antigène avec le groupe iminazole d'une unite particulière histidine de l'anticorps. Plusieurs faits viennent donc confirmer l'hypothèse selon laquelle nous suggérons une analogie entre les interactions antigène-anticorps et les interactions de certains enzymes et de leurs substrats.

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Fixation of Certain Heterogenous Antigenic Substances on Bacterial Cells and Endospores

The GLADSTONE and FILDES medium (G.F.)[1] has been widely used for many years for the culture of various bacteria. No report has been made on disadvantages due to the fact that it contains a casein derivative and a yeast-extract, both of which are antigenic substances.

The simple process of washing the cells of *B. megaterium* grown on G.F.-agar twice was sufficient to eliminate adhering yeast and casein from the cells and no yeast antibodies were found in any of the 40 sera prepared in rabbits for the purpose of typing[2]. TOMCSIK AND BAUMANN-GRACE[3], however, observed disturbing cross-reactions with *B. cereus* in the precipitation reaction when the supernatant fluid from a 24-h broth culture in G.F.-casein-yeast medium was used. A study of the cross reactions obtained with culture fluids and with various fractions of the medium alone revealed that some of the *B. cereus* immune sera contained yeast antibodies as shown in Table I.

It can be seen that 3 sera (C2, C3, C14) cross reacted with supernatant of the C3 strain of *B. cereus* and that 4 sera (C3, C14, C21, C27) contained yeast antibodies. In the complete casein-yeast medium, therefore, the sum of the specific cross-reactions and those due only to presence of yeast antibodies was observed. Although heterogenous

Table I

Precipitin reactions with sera of rabbits immunized with shaken cultures of several *B. cereus* strains in casein-yeast medium

Antigen		Immune sera					
Culture	Medium	C 2	C 3	C9	C 14	C 21	C 27
C 3	Casein-yeast*	+++	++++	±	+++	+++	+++
C 3	Casein*	+++	++++	–	++++	–	–
–	Yeast-extract**	–	++++	–	++++	++++	++++
–	Casein**	–	–	–	–	–	–

* Undiluted supernatants from fluid cultures.
** Same concentration as that recommended for the Gladstone-Fildes medium.

antibodies were not found in any of 40 rabbit sera prepared with *B. megaterium* cells grown on G.F. agar, 6 out of 13 sera prepared from *B. cereus* prepared in shaken broth cultures were found to contain antibodies against the yeast in the medium. Since both the cells of *B. megaterium* and those of *B. cereus* were washed twice after harvesting, it must be assumed that the yeast antigen from the medium is fixed more firmly to the bacterial cells in liquid shaken cultures than on agar medium. The amount of yeast fixed, however, was not sufficient to interfere with or to be demonstrable by the agglutination reaction and the specific cell wall reaction.

It occurred to us that failure to recognise the presence of yeast antibodies in their rabbit sera, probably led CAVE-BROWN-CAVE *et al.*[4] to the erroneous postulation of two polysaccharides in anthrax. Our own experiments[5] showed that, provided yeast is omitted from the medium, only one polysaccharide, the one which we defined as cell-wall polysaccharide, could be isolated from the G.F. culture fluid. This polysaccharide corresponded to that described by other workers, being a galactose-glucosamine-peptide complex which gave a specific precipitation reaction only with anthrax polysaccharide sera and a few related sera from *B. cereus* strains. If yeast extract was present in the medium, however, the impure polysaccharide fraction reacted not only with the anthrax sera but also with an unrelated serum of *B. cereus* (C27) known to have a high content of yeast antibodies. By precipitation with $Ba(OH)_2$, as described by CAVE-BROWN-CAVE *et al.*, two polysaccharides could be isolated, one a mannan, serologically identical with the yeast polysaccharide, and the other the typical anthrax polysaccharide.

Table II

Precipitin reactions of a pure and of an impure anthrax polysaccharide contaminated with yeast gum

Polysaccharide-antigens 1:10,000 dilution	Sera	
	A 8 (horse)	C 27 (rabbit)
1 Pure anthrax polysaccharide from yeast free medium . . .	++++	–
2 Impure anthrax polysaccharide from yeast containing medium	++++	++++
3 $Ba(OH)_2$ insoluble part of 2 .	±	+++
4 Yeast gum	–	++++

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