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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Table III

Lysozyme antibodies produced with lysozyme-treated and 5× washed spores of *B. megaterium*. Precipitin reactions with the HCl-extract of Mg 21 spores

Lysozyme treatment of Mg 21 spores previous to extraction	Spore immune sera			
	Mg 11	Mg 17	Mg 19	Mg 45
yes	–	++++	++++	++++
no	–	–	–	++++

In Table II the results of the precipitin reactions obtained with the antianthrax horse serum (A8), used by TOMCSIK and SZONGOTT[6] when they first isolated the anthrax polysaccharide. Similar reactions were, however, obtained by using more than 10 of our anti-anthrax-polysaccharide rabbit sera, containing no yeast antibody. The yeast-gum was prepared with the method described by TOMCSIK[7] from the same strain of yeast as that used to prepare the G.F. medium.

Lysozyme as heterogenous antigen was fixed unexpectedly strongly on spores of *B. megaterium* treated with this enzyme to remove remnants of the vegetative cells. The spores were collected from a 24-h shaken potato-extract culture of *B. megaterium* in a phase when most of the spores were liberated and the sporangia dissolved. The spores were then centrifuged, resuspended in *M*/30 phosphate buffer at pH 7·0. An equal volume of 1:10,000 cryst. lysozyme (Mann) was added and the suspension incubated at 37°C for 30 min. The spores were completely freed from vegetative remnants by this process and were centrifuged and washed 5 times with distilled water and freeze dried. A heavy suspension prepared by resuspending the freeze dried material was injected 8 times intravenously in rabbits at intervals of 3 or 4 days to produce spore-antibodies.

Apart from type specific spore-antibodies, lysozyme antibodies were found in 4 out of 8 rabbit sera prepared with lysozyme treated spores of *B. megaterium* inspite of the five successive washings with distilled water. The 4 sera gave a fairly strong precipitation with 1:20,000 dilution of lysozyme and one of them reacted in a dilution of more than 1:1,000,000. The lysozyme antibodies were found to interfere with the precipitin reactions involving the hydrochloric acid extracts of whole spores or spore walls if the spores had been previously heated with lysozyme followed by five subsequent washings.

The only effective method of removing the lysozyme was found to be the extraction of the spore specific substance with antiformine and subsequent precipitation of the non-specific proteins with trichloracetic acid. This method yielded a 'peptide' which reacted only with the homologous spore serum.

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Zusammenfassung

Bakterielle Schüttelkulturen binden aus der Kulturflüssigkeit Hefeantigen, welches durch zweimaliges Waschen der Bakterien nicht entfernt werden kann. *B. megaterium*-Sporen werden durch Lysozymbehandlung von allen Resten der vegetativen Zellen befreit; sie binden das Enzym aber so stark, dass sie in Kaninchen, selbst nach fünfmaligem Waschen mit destilliertem Wasser, ausser Sporenantikörpern auch Lysozymantikörper produzieren.

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Measurement of Tissue pCO_2 in the Brain

Carbon dioxide, in itself a product of the metabolism of the nervous tissue and further brought to the tissue by the arterial blood, is of great importance for the normal function of the nerve cells. Among other things, it affects cell excitability and, on account of its potent effect upon local circulation, cell nutrition[1]. Since proper methods have hitherto been lacking, no quantitative data are available as to carbon dioxide tension in the brain tissue.

During the last two years, however, electrodes have been constructed for measurements of pCO_2 in liquids according to the principle first described by STOW *et al.*[2]. This principle implies a pH measurement in a thin layer of water outside a conventional pH electrode. This layer is allowed to come into gaseous equilibrium with a sample of unknown pCO_2 through a membrane, impermeable to ions. The pH is then altered in direct proportion to log pCO_2 of the sample.

Recently, a new type of electrode for continuous measurement of pCO_2 in liquids and tissue has been constructed[3] according to the above principle. This pCO_2 electrode contains a pH electrode with a plane glass membrane (diameter 3 mm) inserted into a plexiglass housing and touching with its lower end a 0·006 mm Teflon membrane. The pCO_2 electrode also contains a miniature calomel reference electrode in contact with a reference electrolyte (0·001 or 0·0001 *N* $NaHCO_3$). Because of the slight concavity of the glass membrane, a thin constant layer of reference solution is kept between the glass and the Teflon membranes. This arrangement provides a fast and sensitive electrode suited for recording rapid changes of pCO_2.

In the present paper, we have summarized our experience with the electrode described above when using it for continuous measurement of pCO_2 on the surface of the intact cerebral cortex of the cat. A detail report of the results will be published elsewhere[4].

The electrode was calibrated against saline solutions of known carbon dioxide concentrations at the temperature of the cortical surface in the experimental situation. Measurements on the cortex were carried out with the Teflon membrane of the electrode applied with a minimum of pressure directly onto the intact pial surface of the exposed cortex. Under Nembutal anaesthesia (40 mg/kg intraperitoneally), it was found that the cortical pCO_2 in a given area did not vary more than $\pm$ 1·5 mm Hg on repeated applications of the electrode provided the following conditions were controlled: cortical blood flow (measured by the outflow from the superior sagittal sinus[5]), cortical temperature, blood pressure, volume and

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