Yeast gum was prepared with the method described by Tomcsik and Szongott when they first isolated the antianthrax polysaccharide. Similar reactions were, however, obtained by using more than 10 of our anti-anthrax-poly saccharide rabbit sera, containing no yeast antibody. The yeast-gum was prepared with the method described by Tomcsik from the same strain of yeast as that used to prepare the G.F. medium. Spores were then centrifuged, resuspended in enzyme to remove remnants of the vegetative cells. The spores were collected from a 24-h shaken potato-extract medium; they were centrifuged and washed 5 times with distilled water and then treated with lysozyme to remove remnants of the vegetative cells. The spores were liberated and the sporangia dissolved. The spores were then centrifuged, resuspended in a protein-free fluid, and washed spores were collected from a 24-h shaking period. The spores were obtained by using more than 10 of our anti-anthrax-poly saccharide rabbit sera, containing no yeast antibody. The yeast-gum was prepared with the method described by Tomcsik 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 crystalline 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.

This work was supported by a grant from the Swiss Federal Labour Office (Bern).

Joyce B. Baumann-Grace and J. Tomcsik

Institute for Hygiene and Bacteriology, University of Basle (Switzerland), April 20, 1959.

Zusammenfassung

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

**Table 111**

<table>
<thead>
<tr>
<th>Lysozyme treatment of Mg 21 spores previous to extraction</th>
<th>Spore immune sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>Mg 11</td>
</tr>
<tr>
<td>no</td>
<td>-</td>
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</tbody>
</table>

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

Lysozyme antibodies produced with lysozyme-treated and 5 × washed spores of B. megaterium. Precipitin reactions with the HCl extract of Mg 21 spores were strongly positive on spores of B. megaterium obtained by using more than 10 of our anti-anthrax-poly saccharide rabbit sera, containing no yeast antibody. The yeast-gum was prepared with the method described by Tomcsik from the same strain of yeast as that used to prepare the G.F. medium.

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rate of respiration, and EEG. Variations in \( pCO_2 \) under such conditions between different cortical areas were found to be less than \( \pm 3 \) mm Hg. The presence of larger vessels under the electrode did not influence this variation. A change in cortical blood flow brought about by, e.g., an increase of blood pressure, immediately gave a lower value of cortical \( pCO_2 \), and a higher value following a decrease of pressure. The response time of the electrode was found to be very short, so that a few breaths of an increased concentration of carbon dioxide in the inspired air gave an increased cortical \( pCO_2 \) within seconds (Fig. 1).

In six experiments in which the cortical \( pCO_2 \) was related to the \( pCO_2 \) of arterial and venous blood, the cortical value was constantly found to exceed the arterial one (samples from the femoral artery) by 10–20 mm Hg, and the venous one (samples from the superior sagittal sinus) by 4–6 mm Hg. Samples from the femoral vein regularly showed somewhat lower values than did those from the sinus. This relationship requires further investigation. In some experiments, artificial respiration after curarization was carried out. Variations in rate of respiration were followed by rapid alterations, so that hyperventilation decreased, and hypoventilation increased the cortical \( pCO_2 \). Within certain limits, it was possible to set the cortical \( pCO_2 \) at a predetermined value simply by varying the respiratory rate.

Changes in cortical \( pCO_2 \) were also recorded during induction of changes in the electrical activity of the cortex as measured by surface EEG leads. Some of these observations were carried out in unanaesthetized \textit{cerebral} preparations under controlled circulatory and respiratory conditions. An increase of cortical \( pCO_2 \) was recorded when an ‘arousal reaction’ was induced by electrical stimulation of the brain stem reticular formation. This change in \( pCO_2 \) had a duration of about the same length as the desynchronization of the EEG pattern. Previously it has been demonstrated that a typical arousal reaction is accompanied by an increase of the cortical blood flow which is due to a local vasodilatation\(^6\). This increase tends in itself to lower the cortical \( pCO_2 \) (r.s.). The present finding of an increased cortical \( pCO_2 \) in arousal therefore supports the hypothesis that the aroused state implies an augmented cortical metabolism\(^7\).

Another state of increased cortical metabolism is represented by the epileptic seizure\(^8\). Records of cortical

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