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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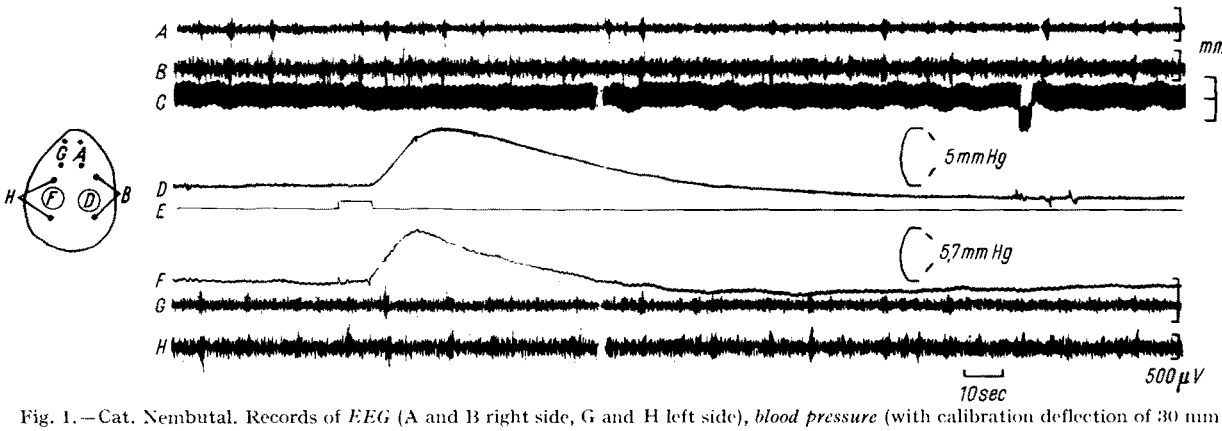

Fig. 1.—Cat. Nembutal. Records of *EEG* (A and B right side, G and H left side), *blood pressure* (with calibration deflection of 30 mm Hg) (C), and *cortical* pCO_2 from two electrodes placed upon right (D) and left (F) suprasylvian gyrus. The signal (E) marks period during which a bag containing 6·0% carbon dioxide in oxygen was attached to an endotracheal tube, allowing the cat to take 3–4 breaths of the gas mixture. Note almost identical rapid increase and slow decline of pCO_2 in right and left hemisphere. There were no reactions from the blood pressure or the EEG of this small amount of carbon dioxide.

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 *cerveau isolé* 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 (*v. 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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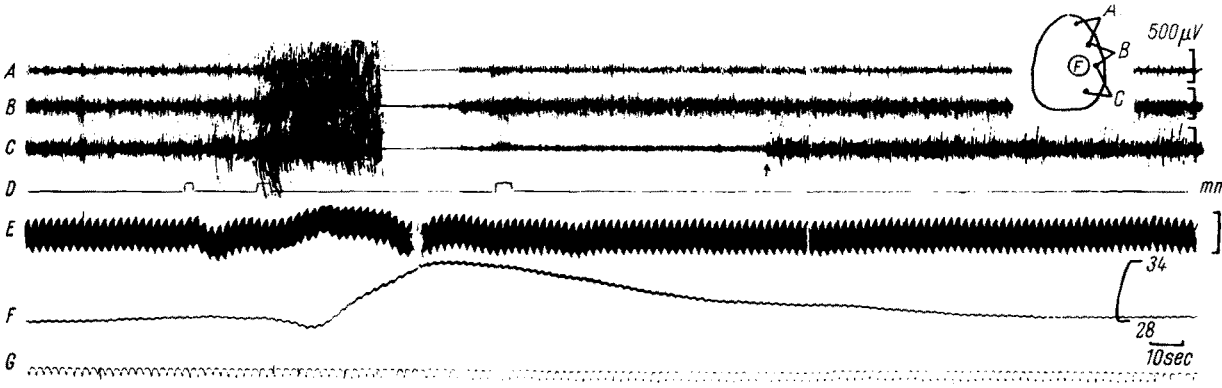

Fig. 2.—Cat. Nembutal. Cervical sympathetic nerves sectioned. Flaxedil and artificial respiration. Records of *EEG* (A, B and C; arrow in C indicates resetting of amplification to original value), *blood pressure* (E), and cortical pCO_2 (F) measured by an electrode placed upon the right suprasylvian gyrus. First signal on line D marks intravenous injection of 2 cm³ of a 5% Metrazol solution. The second signal indicates period of handclaps which starts an epileptic seizure lasting about 40 s. During the seizure there is an initial decrease in pCO_2 which is explained by an increase in blood pressure (and cortical blood flow which was not measured). There follows a marked and longlasting period on increased pCO_2 which outlasts the seizure for about 4 min. The calculated values of cortical pCO_2 (mm Hg) are low due to hyperventilation.

pCO_2 during Metrazol induced attacks showed that there is a marked and long-lasting increase of pCO_2 (Fig. 2) which must be due to a considerable increase of the production of carbon dioxide during the seizure, during which, furthermore, the cortical blood flow is so much greater than normally.

The results so far obtained show that constant conditions for measurements of cortical pCO_2 may be achieved experimentally and that, in principle, the pCO_2 electrode described, when placed upon the intact cerebral cortex, reacts according to expectations. We have explored the possibility of measuring cortical pCO_2 after removal of the pia, or some of the gray matter. This lead to unstable conditions during which the pCO_2 of the injured surface was observed to increase. Therefore we have chosen surface measurements and we have interpreted the values obtained as representing a mean cortical pCO_2. At present, however, there is no information available as to, e.g. diffusion conditions of carbon dioxide in the brain. Exact knowledge of the capillary anatomy of the cat's cerebral cortex is also lacking. Such information is necessary before a theoretical evaluation of the data obtained can be carried out. A further analysis of the measuring conditions is under way.

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Zusammenfassung

Der mittlere pCO_2 in der Gehirnrinde von Katzen wurde mittels einer neuen Elektrode kontinuierlich registriert. Es wird ein kurzer Bericht über die Messbedingungen, nebst Beispielen von pCO_2-Veränderungen bei verschiedenen Funktionszuständen, gegeben.

Mast Cells in Bronchial Connective Tissue of Man

Importance of such Cells in Allergic Tissue Injury

This report concerns researches conducted on bronchobiopsies taken on the orifice of the middle lobe bronchus fixed with formalin Merck 10% and stained with toluidine blue at pH 6·5. Counting of mast cells was made in 20 fields using ocular $\times 10$ and objective $\times 100$.

Normal subjects (5 cases): Mast cells were numerous (45 ± 10): fusiform, circular, oval. Part of them ('normal' cells) showed cytoplasm thronged with violet metachromatic granules and intergranular cytoplasm extremely scarse or not demonstrable (Fig. 1 *A*); the others ('abnormal' cells) had quite different aspect, i.e. light degranulation disruption and scattering of the granules. Proportion of 'normal' cells (NC) to 'abnormal' ones (AC) was always NC:AC > 1. Sections treated with hyaluronidase (37°C for 18 h) revealed the opposite proportion owing to prevalence of degranulated cells with distinctly visible violet granules surrounded by amorphous metachromatic substance, which appeared dissolved in the cytoplasm thus assuming a purple-red staining (Fig. 1 *B*). Most of the mast cells clearly revealed their nucleus because of the intense degranulation provoked by the enzyme.

Inflammatory injuries (5 cases): The number of mast cells was not far from the average observed in normal cases. A more marked tendency to degranulation and scattering of the granules in the ground substance of the connective tissue (Fig. 1 *CD*) was ascertained.

Treatment with dexamethasone (5 cases affected with aspecific bronchitis): Corticosteroid (Deltafluorene Lepetit) was administered orally 3–4·5 mg/day after preliminary bronchobioptical examination. Histological control after a treatment of at least 10 days showed a numerical reduction of mast cells (under 15). Identifiable cells presented a reduced diameter degranulation and small violet granules surrounded by amorphous intergranular substance metachromatically very light-red stained. There were also some disrupted cells with granules scattered in the connective tissue and mast cells presenting ortochromatic granules (Fig. 1 *EF*).

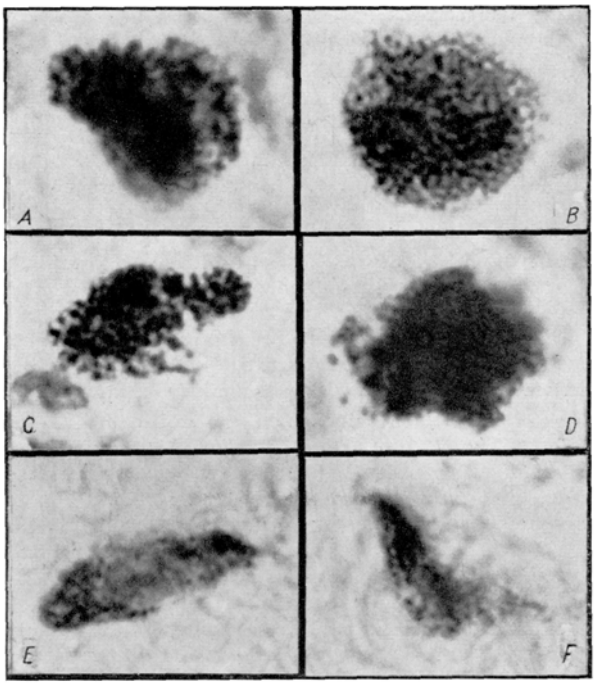

Fig. 1.—(*A*) Normal mast-cells. (*B*) Mast-cells after treatment with hyaluronidase. (*C*) Partial degranulation (bronchitis). (*D*) Partial disruption (bronchitis). (*E–F*) Damage following treatment with dexamethasone (Magnification $\times 2000$).

Serial sections of same biopsies incubated for 18 h at 37°C in hyaluronidase showed both a further reduction of granules' diameter and the amorphous intergranular substance dissolved in the cytoplasm thus appearing purple-red stained.

Allergic injuries (12 cases): Biopsies were taken from patients affected with bronchial asthma free of any secondary pathological complications. Counting of mast cells in remission phase of asthma gave a high number (35 ± 5), whereas the result in biopsies taken in full asthma attack was under 10: in several cases a careful examination of many histological sections was necessary before finding a single mast cell. Morphological and metachromatic changes were noticed in all examined cases both in remission phase and in full asthma attack: proportion of 'normal' cells (NC) to 'abnormal' ones (AC) in the former case was always NC:AC < 1 with a still more marked diversion from rule in the latter. The rare mast cells observed in biopsies taken in full asthma attack all exhibited a very intense degranulation and disruption with granules scattered in the ground substance of the connective tissue.