

strength, in 3 successive experiments with increasing length of Pxl-P treatment, were: hydroxylamine pretreated, 76, 70; 88, 82; 75, 64; NaCl-P pretreated, 52, 45; 35, 33; 29, 20.

Inhibition of reversal. Treatment with NH_2OH (hydrochloride 0.1M, pH 9, 1 h, 20°C) after Pxl-P prevented recovery of strength on subsequent transfer to NaCl-P alone at pH 7.5 (Figure 1); NH_2NH_2 (sulphate) had a similar effect.

Discussion. Pxl-P is a co-enzyme in a number of reactions involving amino-groups (see review by BANKS¹⁰) and is known to react with them. The hypothesis that the effect of Pxl-P on strength is brought about by rupture of azomethine linkages formed between an aldehyde and amino-group would explain the inhibition of recovery of strength by NH_2OH and NH_2NH_2 . It would also provide a reasonable explanation of the stabilizing effect of previous reduction with NaBH_4 . It is, however, not clear why pretreatment with NH_2OH or NH_2NH_2 , acting on a link of this nature, should have a stabilizing effect. BENSUSAN, MCKNIGHT and NAIDU¹¹, in contrast, report presumptive evidence of the disruption of such a link by NH_2NH_2 in a model compound (furfural-butylamine). The observation that NH_2OH and NH_2NH_2 have this effect is, however, of interest because they also protect skin against the weakening produced by lowered pH, as well as preventing recovery on return to neutral pH¹². NaBH_4 reduction also has a stabilizing effect against pH change, down to about 5¹³. These similarities between effects of Pxl-P and reduction of pH are compatible with the hypothesis that the same links are concerned.

Discussion has been confined to possible explanation of the results in terms of a single effect on collagen. This is likely to be an oversimplification: for example, it does not

readily explain the detachment of the epidermis produced by Pxl-P.

The fact that Pxl-P is a naturally occurring substance present in tissues perhaps adds interest to these observations, though normal concentrations in whole tissue are much lower than we used (e.g.¹⁴). Other naturally occurring substances with a similar effect are cysteine, glutathione¹ and homocysteine¹⁵ (but not reported in detail).

Résumé. Le traitement de la peau de la queue de jeunes rats avec du pyridoxal-5'-phosphate a réduit sa résistance (jusqu'à 70%). Cet effet est en grande partie réversible, la réversibilité pouvant toutefois être inhibée par l'hydrazine ou l'hydroxylamine: ces observations sont en accord avec l'hypothèse attribuant, pour une grande partie, la résistance de ce tissu à des liaisons azométhines, formées par la condensation de fonctions aldéhyde et amine.

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¹⁰ BARBARA E. C. BANKS, in *The Chemistry of the Amino Group* (Ed. S. PATAI; Interscience, New York 1968), p. 499.

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¹⁶ We are grateful to Miss CELIA COFFEY for technical assistance, and to the Medical Research Council of Great Britain for a grant.

Changes in Electrical Activity of the Cockroach *Blatta orientalis* L. Brain Induced by Anti-Lobster Brain Antibody

We demonstrated in mammals that the electrical activity of the brain and behaviour might be affected by anti-brain antibodies injected into the cerebral cavity¹. The enormous structural and functional complexity of the mammalian brain compels immunoneurological strategy to include some 'simple systems' in the study. It was considered, therefore, that the insect's brain, because of its relatively simple organization², represents a suitable model for the investigations of the biological properties of the anti-brain antibodies.

Materials and methods. One part of homogenized brain from lobster (*Palinurus vulgaris*) was mixed with 2 parts of complete Freund's adjuvant and injected s.c. into rabbits (0.8 ml of antigen-adjuvant mixture per animal). 20–30 days later each rabbit received i.p. 50 mg of lobster brain without adjuvant. The animals were bled at various time intervals, and the sera examined for the presence of anti-lobster brain antibodies by means of different precipitin reactions and complement fixation technique. Sera containing antibodies were pooled, immune γ -globulin fraction isolated³, dialyzed against physiological solution for insects⁴, and lyophilized. Normal rabbit γ -globulin was separated in an identical manner. Prior to use, normal and immune γ -globulins were dissolved separately in distilled water to a concentration of 40 mg/ml, and then 12 50% hemolytic units of guinea-pig complement were added to each solution.

The experiment was carried out on adult cockroaches (*Blatta orientalis* L.). After anaesthesia in carbon dioxide, the chitin cover from the frontal part of the head was removed, and steel or Ni-chrome bipolar and monopolar electrodes, having a tip of approximately 50 μ , were implanted in the brain as described previously⁵. In most instances the electrodes were inserted into the protocerebrum (Figure 1). Physiological solution, normal γ -globulin and immune γ -globulin were applied on the surface of the insect's brain with a glass micro-pipette. The electrical activity was recorded for at least 3 h using an 'Alvar' encephalograph.

Results and discussion. In spite of the wide phylogenetic distance between the lobster and the rabbit, 6 of 15 rabbits injected with the lobster brain in adjuvant devel-

¹ LJ. MIHAJLOVIĆ and B. D. JANKOVIĆ, *Nature* 192, 665 (1961); B. D. JANKOVIĆ, LJ. RAKIĆ, M. JANJIC, K. MITROVIĆ and J. IVANUŠ, *Path. europ.* 2, 87 (1966); B. D. JANKOVIĆ, LJ. RAKIĆ, R. VESKOV and J. HORVAT, *Nature* 218, 270 (1968).

² T. H. BULLOCK and G. A. HORRIDGE, *Structure and Function of the Nervous Systems of Invertebrates* (Freeman, San Francisco 1965), p. 816.

³ A. H. COONS, in *General Cytochemical Methods* (Ed. J. F. DANIELLI; Academic Press, New York 1958), p. 399.

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oped experimental allergic encephalomyelitis. This finding suggests that brains of vertebrates and invertebrates have some common antigens capable of inducing demyelinating disease, and the production of anti-brain antibodies which react with nervous tissue antigens of a large number of species.

The great majority of cockroaches tested with saline or normal γ -globulin lacking anti-brain antibodies did not exhibit apparent changes in electrical activity of the brain. On the other hand, 27 of 32 insects developed bioelectrical abnormalities following the application of anti-lobster brain antibody (Table). These changes were characterized by the appearance of continuous spike activity or by bursts of high voltage spikes in rather synchronous time intervals (Figure 2) without significant alteration in the background activity.

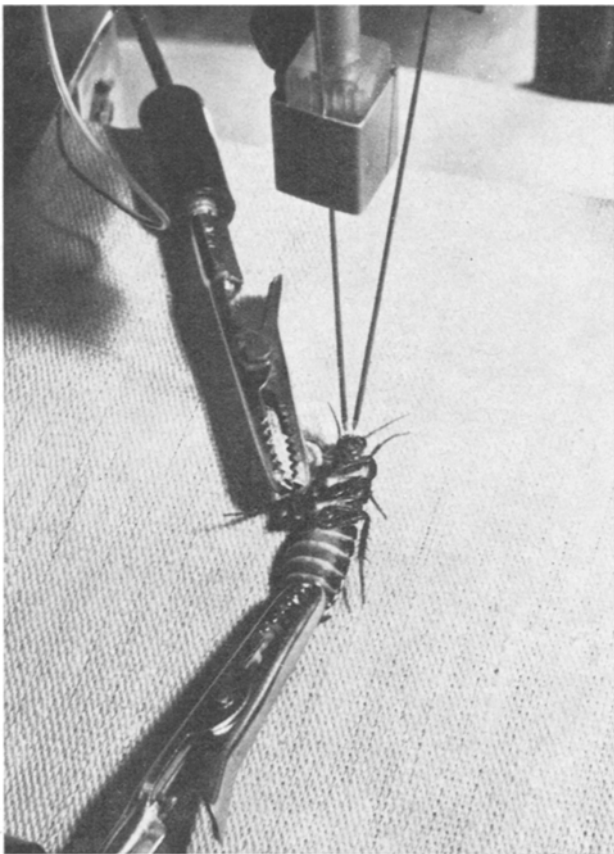


Fig. 1. The cockroach with electrodes inserted into the brain.

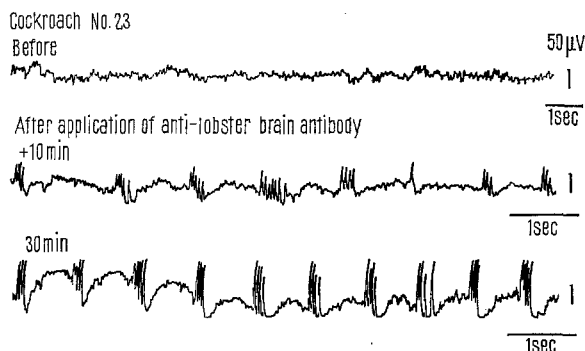


Fig. 2. Records showing changes in electrical activity of the cockroach brain after treatment with anti-lobster brain antibodies (immune γ -globulin).

Changes in electrical activity of the cockroach brain following application of anti-brain antibodies

Material applied on the surface of the brain	No. of insects tested	No. of insects with changed EEG activity
Physiological solution	48	2
Normal γ -globulin	18	3
Immune γ -globulin	32	27

Using potassium chloride microelectrodes, MAYNARD⁶ succeeded in registering extracellular potentials from the mushroom bodies of the cockroach brain. Normal axons of this insect do not give repetitive response on application of a long-lasting cathodal current⁷. Changes in electrical activity of the cockroach brain which occurred after the application of immune γ -globulin imply that an in vivo contact between anti-brain antibodies and brain antigens establishes or enhances conditions in which repetitive firing arises.

The mechanism by which an anti-brain antibody influences electrogenesis is still unknown. Since the activity of biologically active antigens (e.g. enzymes) can be altered in different ways by means of specific antibodies⁸, it follows that antibodies reacting with neural antigens may affect the biological properties of the neuron. We showed earlier, in an in vitro experiment, changes in the action potential of the giant axon of the lobster following application of anti-lobster nerve antibody⁹. Antibodies against soluble proteins of the squid axon alter the propagation of the membrane potential¹⁰, thus suggesting that the membrane proteins of the axon are the target for the activity of anti-neural antibodies¹¹. The in vivo combination of antibodies and nervous tissue antigens may also enhance or sustain the activity of some pharmacologically active substances¹². Another possibility would be that antibody acts on the resting membrane by affecting its ionic permeability. Obviously, there are several ways in which an anti-brain antibody can work and influence the function of the nerve cell¹³.

Zusammenfassung. Bei mit Langustenhirn immunisierten Kaninchen werden Anti-Hirn-Antikörper gebildet. Werden diese auf die Hirnoberfläche der Schabe *Blatta orientalis* L. gebracht, so kommt es zu erheblichen Veränderungen in der elektrischen Hirntätigkeit des Insekts.

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