feet, have not yet been adequately examined. A new survey is now being planned which will link the higher Kermanshah valleys with the alluvium of the Khuzestan plain, by traverses along various tributaries of the Karkheh River. *If* incipient agriculture had its earliest beginnings at these lower levels, to be shifted upslope rather abruptly just after 11,000 years ago because of an onset of desiccation, the traces of "incipient" settlements at the lower altitudes should certainly appear during such an intensive survey.

References and Notes

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24 March 1961

Electrophoretic Analysis of Immobilization Antigens of Paramecium aurelia

Abstract. The isoelectric points of the immobilization antigens A, B, and D of strain 51 of *Paramecium aurelia* (variety 4) have been determined to be 4.0, 3.9, and 4.3, respectively.

When paramecia are placed in homologous antisera, an immobilization reaction takes place which may result in the death of the animals if they are exposed to sufficiently high concentrations. Beale (*1*) and Preer and Preer (*2*) have demonstrated that the immobilization antigens are associated with the cilia and body wall. Preer (*3*) has isolated the immobilization antigens and has shown that they constitute the major soluble protein in the cilia. Investigations by Sonneborn (*4*) have shown that within a given strain there can be produced a series of alternative serotypes whose individuals contain one of a number of specific antigens. Previous genetic analysis has revealed the interaction of the cytoplasm, genes, and environment in determining the antigen expressed [see Beale (*5*) for a detailed review].

Transformation from one serotype to another may occur spontaneously or may be induced by a variety of conditions (*4*). It is, however, possible to obtain and maintain large quantities of animals of a pure serotype under specified conditions.

Although each serotype within the homozygous strain 51 may readily be distinguished serologically, certain cross reactions occur. Preer (*6*) has reported the occurrence of groups into which the antigens may be placed dependent upon their cross reactions. For example, 51 A and 51 B show a weak cross reaction, while neither cross reacts with 51 D.

In light of the genetic control over antigen production and specificity, it is of interest to gain an understanding of the structural differences that exist on a molecular level as a direct reflection of the differences that exist among the respective genes. This report is concerned with the electrophoretic properties of the proteins as the first step in characterizing chemically the structural differences between certain of the immobilization antigens which can be produced by the homozygous strain 51.

The electrophoretic behavior of the immobilization antigens A, B, and D of strain 51 of *Paramecium aurelia* (variety 4) was studied with a Perkin-Elmer model 38 Tiselius apparatus. The electrophoresis buffers employed in all runs were 0.1 ionic strength and were prepared by the method described by Miller and Golder (*7*) with a *p*H range of 2.6 through 11.5. The specific antigens were isolated by Preer's technique (*3*). In order to facilitate comparison, mixtures, rather than the individual proteins were subjected to electrophoresis. Mixtures were prepared so that they consisted of two parts of A and one part of B; other mixtures consisted of two parts of A and one part of D. One component was made twice as concentrated as the other to aid in identification. Identification was confirmed by taking samples from their respective zones in the Tiselius cell and identifying them serologically.

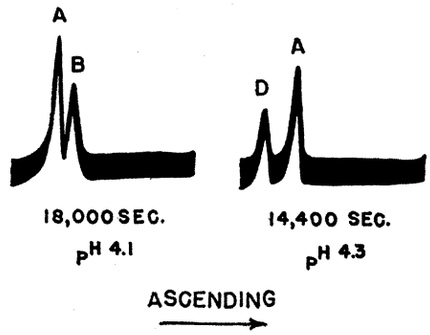

Fig. 1. Tracings of the ascending electrophoretic patterns of 2:1 mixtures (stock 51) of antigens A and B, and A and D, respectively.

Figure 1 represents tracings of the electrophoretic patterns of mixtures of A and B, and A and D. Mobilities were calculated, and complete curves for A and D appear in Fig. 2. The isoelectric points of A, B, and D were determined to be 4.0, 3.9, and 4.3, respectively. The isoelectric point of antigen A is in agreement with the estimate made by Preer from paper electrophoresis (*8*).

Antigens A and B separated only after prolonged electrophoresis at *p*H values near their respective isoelectric points, demonstrating a close similarity in their net charges. This similarity is reflected by the closeness of their isoelectric points. A and D, on the other hand, showed separation at all *p*H values run between 2.6 and 11.5. The electrophoretic behavior parallels the ammonium sulfate solubilities and immunological relationships reported by Preer (*7*). As previously mentioned, antigens A and B cross react, while neither shows any cross reactivity with antigen D. Antigens A and B possess the same ammonium sulfate solubility, while antigen D exhibits a higher ammonium sulfate solubility.

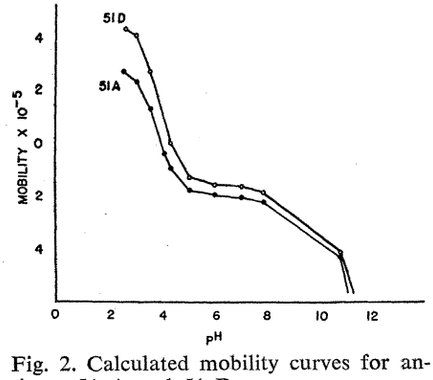

Fig. 2. Calculated mobility curves for antigens 51 A and 51 D.

Bishop and Beale (*9*) working with variety 1 of *P. aurelia*, have reported different electrophoretic mobilities on starch gel for three different isolated antigens, G, D, and T.

The differences in immunological relationships, ammonium sulfate solubilities, and isoelectric points suggest that the antigens differ in their amino acid sequence. However, the possibility that the variations result only from differences in the gross pattern of folding cannot be ruled out. Studies designed to settle this point are now being undertaken. It is hoped that light will be shed on the general problem of the genetic control of protein specificity (*10*).

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Excitation and Inhibition of Neuronal Firing in Visual Cortex by Reticular Stimulation

Abstract. The frequency of action potentials of about one-third of the neurons sampled in the striate cortex of awake rabbits was clearly modified by mild stimulation of the reticular core of the brain stem. Reticular stimulation often brought about enhancement of firing in units activated by light, while it usually had the contrary effect upon light-inhibited units.

A behavioral study (*1*) has demonstrated that the threshold of tachistoscopic discrimination can be lowered by electrostimulation of the brain-stem tegmentum, thus indicating that reticular activation facilitates some central process of vision. The purpose of the present investigation (*2*) was to study how reticular activation affects single neurons in the visual cortex. In acute experiments, these cells have already been shown to be influenced by stimulation of diffuse projection nuclei of the thalamus (*3*). However, the use of chronic animals is more desirable for such investigations than the use of any kind of acute preparation, in which lesion of tissues or the action of some drug may obscure effects attributable to the experimental variables.

The experiments were performed on New Zealand white rabbits. Steel microelectrodes, made according to Green's technique (*4*), were utilized for recording action potentials of single units. Each animal was surgically prepared under anesthesia, at least 1 day before any experiment. A tubular steel implant, fitting in a trephine hole in the skull, was cemented to the bone after the underlying dura had been removed. Two electrodes for stimulation were introduced through another hole and implanted in the mesencephalic tegmentum. The skin was sutured around the electrode leads and the metal implant. The lumen of the latter was filled with warm mineral oil and sealed with a threaded cap.

For an experimental session the animal was put in a hammock, and the mineral oil in the implant was replaced by a 4-percent solution of agar at 40°C which solidifies at body temperature, thus eliminating respiratory movements of the brain. To drive a microelectrode for recording, a special hydraulic micropositioner, resembling in some features the one developed by Hubel (*5*), was used (Fig. 1). A hollow adapter, screwed to the implant, is the base to which a nylon cylinder is fastened. The microelectrode is set in the center of a nylon piston riding inside and is electrically coupled to the input of an amplifying system through a cathode follower. A vent in the side of the adapter maintains the space between piston and agar at atmospheric pressure, while the cylinder is filled with oil above the piston and connected to a micrometer-syringe by polyethylene tubing. When the syringe is advanced, the microelectrode tip traverses the agar and penetrates the cortex. The preparation permits repeated punctures in each animal for a number of days.

Diffuse binocular light was used (about 1070 lux at the corneas), lasting 1 sec or more. The animals, with eye atropinized, were in the dark between stimuli. Reticular stimulation was applied in trains, usually 250 msec long, of 300-cy/sec pulses. Intensity, normally ranging between 50 and 150 μa, was always chosen so that it was insufficient to cause any kind of motor reaction. The position of all brain-stem electrodes was histologically verified.

One hundred units of the striate cortex were studied. It is assumed that the majority of records were extracellular and of cell-body spikes. The spontaneous rate of unit impulses varied considerably, ranging from 0 to 54 spikes per second, but most units exhibited slow (median, 1.47 spikes per second) and irregular firing. Grouped or clustered activity was seen, though it was far less common than it is in thalamic units under the same experimental conditions (*6*). According to their reactions to light, all units were classified as Jung *et al.* have done with cortical cells in the cat (*7*): 13 units showed an increment of firing rate at the onset of illumination (B-type of Jung); 14 were inhibited at the "on" of the light and showed an "off" discharge (D-type); and 21 other units reacted with increments of discharge both at "on" and "off" of the light (E-type). One-half of all units did not show any definite reactions to long dif-

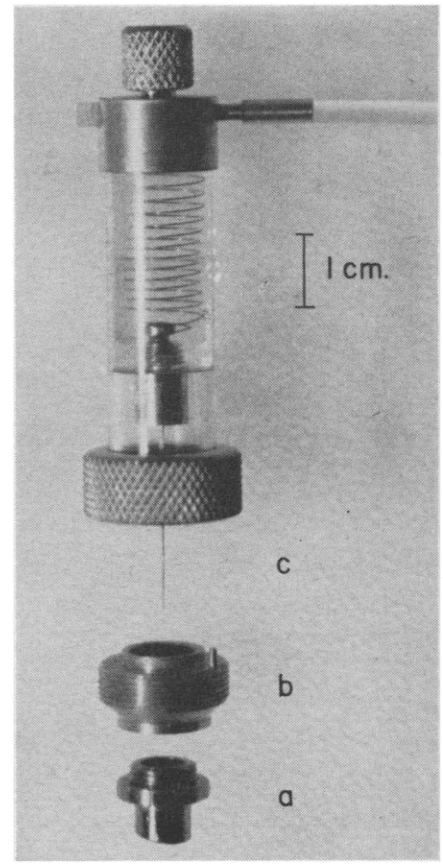

Fig. 1. Micropositioner. (*a*) Implant; (*b*) adapter; (*c*) microelectrode. The aluminum top of the cylinder is connected to the input.