Changes in serum proteins 1 h after electro-chemical stimulation of the cerebral cortex and the olfactory bulbs

	No. of animals	Total proteins	Albumin	Globulins		
				α	β	γ
Control	8	99.2 ± 0.40 [a]	99.9 ± 0.53	99.7 ± 1.83	96.9 ± 1.09	98.0 ± 1.14
Stimulation of cortex	8	98.5 ± 0.60	99.7 ± 0.83	99.8 ± 1.35	95.7 ± 2.29	98.1 ± 1.26
Stimulation of olfactory bulbs	8	102.5 ± 0.50 $P < 0.001$	104.3 ± 1.38 $P < 0.01$	105.1 ± 1.35 $P < 0.05$	96.4 ± 1.32	95.9 ± 1.87

[a] Mean $\pm$ S.E. Values are expressed in percentages of their initial value which is taken as 100%. P = degree of significance resulting from comparison of other lots with control. P values are shown only when the difference is significant.

provided the blood which was left to clot before isolating the serum by centrifugation.

Results and discussion. The results obtained 3 h after stimulation can be seen in the Figure. The total serum proteins, albumin and α-globulin increased significantly ($P < 0.001$) in electro-chemically stimulated subjects – i.e. an opposite effect was produced to that previously observed through lesion or excision of the olfactory bulbs.

The β-globulin was unaffected by electro-chemical stimulation, whereas in former subjects with excized bulbs it had decreased significantly.

Nor was the γ-globulin altered by electro-chemical stimulation of the bulbs, which was only to be expected, since the excision of the same had not affected it previously either.

Lots a) (controls), c) (platinum electrodes) and d) (electro-chemical stimulation of parietal cortex) chowed a drop in total serum proteins and subfractions which was attributed to blood extractions previous to stimulation.

Other 3 lots of rats were also used to effect determinations 1 and 5 h after stimulating the olfactory bulbs, to try to establish the time at which the changes began and the length of their duration. The results found 1 h after electro-chemical stimulation were similar to those found 3 h after the same operation, as can be observed in the Table. 5 h after stimulation, the values returned to normal, having lost all significant statistical differences. MORIMOTO[3], on applying electric stimulation to another part of the nervous system (hypothalamus), also found passing changes in albumin and globulins[3].

The lack of increase in total serum proteins, albumin and α-globulin on stimulating the cerebral cortex indicates that the changes found through stimulating the bulbs were not due to a non-specific stimulatory effect of the nervous tissues; while the fact that the use of platinum electrodes did not produce an increase either indicates that such effects were not due to lesion of the olfactory bulbs but to their stimulation[4].

Resumen. La estimulación electroquímica bilateral de bulbos olfatorios con corriente directa de 1 mA durante 10 segundos en ratas, produce aumento de concentración de proteínas séricas totales, albúmina y globulina α.

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The Effect of Aspartate on the Electroretinogram of the Vertebrate Retina

In some cold-blooded vertebrates, the electroretinogram (ERG) in response to high-intensity flashes exhibits the *a*-wave, the P III in Granit's classification, consisting of 2 separate negative deflections (a_1 and a_2) which have different properties[1]. The first negative deflection (a_1) has been called the late receptor potential (late RP) by BROWN et al.[2], and the current for *a* is produced in the inner segment, but the permeability change is at the outer segment[3,4]. As described first by FURUKAWA and HANAWA[5], sodium L-aspartate was demonstrated to have a strong and highly specific inhibitory effect on the *b*-wave of the toad retina, and they succeeded in keeping the amplitude of an ERG, which consisted solely of the P III component, constant over 3 h. In this experiment, we found that L- and D-aspartate are appropriate agents for the isolation of the late RP.

Material and method. Throughout this study 70 eyes of the bullfrog (*Rana catesbiana*) were used. The isolated retina deprived of the pigment epithelium was dissected from the dark-adapted animals and was sandwiched between 2 acryl resin plates, each of which had a hole of 6 mm diameter in middle, as described previously[6]. The retina was initially immersed for 30 min in CONWAY's[7] solution containing 26 mM glucose. After a stable control amplitude of ERG was established, the solution on both sides of the retina was replaced by a test solution containing various concentrations of aspartate or glutamate[8].

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The bathing solutions, isotonic and pH 7.8, were stirred throughout the experiment by a stream of a mixture of 98% O_2 and 2% CO_2. The pre-amplifier and cathode ray oscilloscope recorded the difference of electrical potential between the solution on the scleral side and that on the vitreal side of the retina. All the experiments were carried out at room temperature between 15 and 20 °C. All the preparations were stimulated from the vitreal side by a light flash which was produced by a xenon flash lamp.

Results and discussion. The oscilloscope traces reproduced in Figure 1, A show the light-evoked responses which were obtained with the higher stimulus intensity (20 Joules). The upper response was recorded from the retina which had been immersed in Conway's for 30 min. The initial biphasic deflection was the positive and negative phases of the early RP which was generated without any detectable latency. The negative second phase of the early RP was followed immediately by an *a*-wave consisting of 2 separate negative deflections with a time to the maximum peak of approximately 50 msec. The lower record is the second response obtained from the same retina 30 min after the application of 5 m*M* L-aspartate in CONWAY's solution. It is interesting to note that there was no noticeable change in the shape or amplitude of the early and late RP, but the a_2- and *b*-wave completely disappeared. Previously, it was shown by MURAKAMI and KANEKO[9], who used microelectrode techniques, that the P III was composed of 2 components: the distal component which was generated by the receptors, and the proximal which arose from the inner nuclear layer. SILLMAN et al.[10] have recently presented evidence that the observed potential consists exclusively of the receptor response after treatment of the retina with sodium aspartate. The above-mentioned result, therefore, clearly indicates that the a_1- (late RP) and a_2-wave recorded with external electrodes represent the distal and proximal component of P III, respectively.

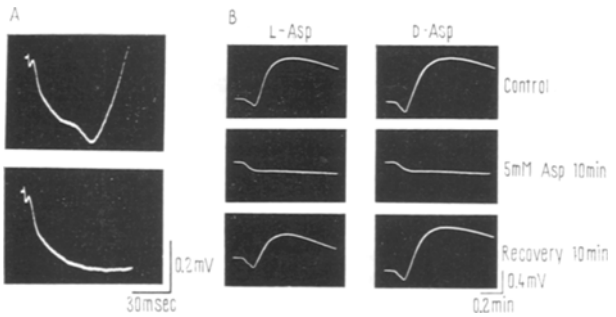

Fig. 1. The effect of 5 m*M* L- and D-aspartate on the frog ERG. Amplification condenser-coupled, with time constant 0.3 sec, and the polarity of records was such that an upward deflexion indicates positivity of the vitreal side of the preparation. A) The initial portion of the ERG evoked with an intense photic stimulation (20 Joules). Upper record was obtained from the retina immersed in CONWAY's and lower one was in 5 m*M* L-aspartate CONWAY's. Both records from same retina. B) The effect of aspartate on the *b*-wave. Both L- and D-aspartate abolished completely the *b*-wave within 10 min, and this effect was reversible.

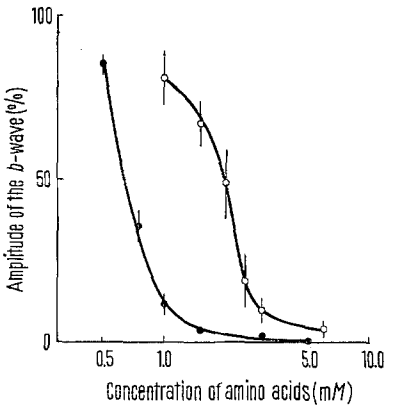

Fig. 2. Log concentration-response curves of the frog retina for L-aspartate (●—●) and L-glutamate (○—○). The *b*-wave amplitude was measured from the trough of the *a*-wave to the peak of the *b*-wave and expressed as a percentage of that in CONWAY's. Each point is an average of 5 experiments.

The changes of the ERG caused by the application of L-aspartate were always reversible. It is of interest to note that D-aspartate and L-glutamate are also effective in the isolation of the late RP. Figure 1, B shows the negative component of the ERG isolated in Conway's solution containing 5 m*M* L- and D-aspartate. In both cases, almost complete recovery of the *b*-wave was observed when the preparation was returned to the aspartate-free solution. However, the recovery rate after treatment with D-aspartate was more rapid than that with L-aspartate. After both surfaces of the retina were exposed to a test solution containing aspartate or glutamate, the amplitude of the *b*-wave gradually declined, and then stabilized to a new steady state value within a period of approximately 15 min. Figure 2 represents experimental log dose-response curves for L-aspartate and L-glutamate. A parallel shift is observed in the curves of these 2 compounds along the log-dose axis, though glutamate is less active than aspartate. The Hill coefficient n_H for the decrement of response amplitude from its original value, computed as described by CHANGEUX and PODLESKI[11], is approximately 2.3.

The structural homologues of aspartate, such as homoserine, threonine, GABA, asparagine and succinate, and NH_4Cl were not effective. The complete mechanism for the aspartate action on the ERG remains to be clarified, but it may be suggested that 2 carboxyl groups and 1 amino group contained in the aspartate molecule are of special importance for the suppression of the a_2- and *b*-wave, and that the effect of aspartate is not caused by an enzymatic mechanism in the retina, but is associated with a reversible binding of aspartate to the 'active sites' located somewhere in the structures responsible for the a_2- and *b*-wave.

Zusammenfassung. Es wird gefunden, dass nach Applikation des zur Perfusionsflüssigkeit zugefügten L-Aspartats auf die isolierte Froschretina eine auffallende Hemmung der a_2- und *b*-Welle auftritt, dies jedoch ohne sichtbare Veränderung der Form und Amplitude des «early and late receptor potential».

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