in light and darkness. Preliminary observations indicate however that light causes first a stimulation and then an inhibition of O_2 fixation. The most striking difference between dark-and-light-aging occurs at the level of the latent period of the enzyme activity. In fresh chloroplasts, a 7 min average latent period was observed. After a 2 h chloroplast incubation in vitro, this period decreases to 66 and 5% in darkness and light, respectively. It appears that the impairment of the latent period in the light (and also to a lesser extent in darkness) reflects an advanced state of chloroplast aging which was already observed for morphological[2] and photochemical[7] parameters. In this connection, the effect of linolenic acid shows an interesting resemblance to aging. Indeed, increasing concentrations of linolenic acid accelerate the rate of polyphenoloxidase activity and diminish the latent period. Also, these results indicate a close interrelationship between inhibition of O_2 evolution and activation of polyphenoloxidase activity by a linolenic acid or aging treatment.

Thus, it appears that the increase of polyphenoloxidase activity during chloroplast aging and the discrepancy of the latent period behaviour, towards dark and light incubation of chloroplast, and, towards various linolenic acid concentrations, represent new parameters which must be taken into consideration in our study of aging of the photosynthetic apparatus in vitro[15].

Résumé. Un vieillissement in vitro de chloroplastes isolés d'épinard et un traitement par l'acide linolénique, à des concentrations croissantes, provoquent des inhibitions, comparables, de la photophosphorylation, de la capacité des plastides à se contracter et à dégager de l'O_2. De plus, ces deux traitements stimulent dans la même proportion l'activité des polyphénoloxydases. Ainsi, l'acide linolénique semble être l'un des facteurs responsables du vieillissement in vitro des chloroplastes.

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The Effects of Stimulation of the Olfactory Bulbs on the Serum Proteins of the Rat

In a previous paper[1] we found that bilateral excision or sectioning of the olfactory bulbs in the rat produced a decrease in the total serum proteins, albumin, and α- and β-globulins, while no change was observed in γ-globulin. These results led us to believe that stimulation of the olfactory bulbs could produce opposite effects. The present study has been effected to verify this hypothesis.

Materials and methods. 85 white rats of both sexes weighing from 140–220 g each and chosen from stock bred in our Institute, were used.

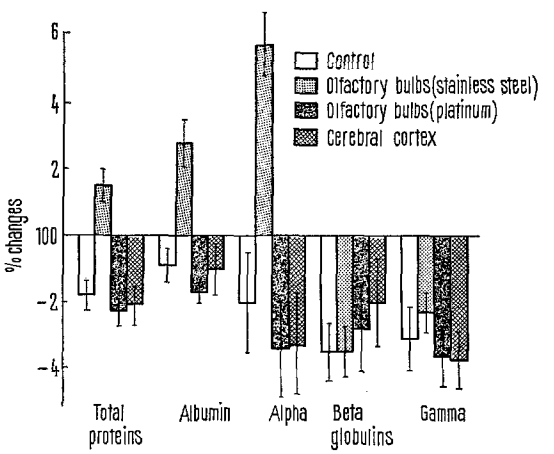
The animals were divided into 4 lots, as follows: a) bilateral insertion of stainless steel electrodes in both bulbs, no current being applied (control group – 14 subjects); b) stainless steel electrodes in both bulbs (electro-chemical stimulation – 22 subjects); c) platinum electrodes in both bulbs (6 subjects); d) bilateral insertion of stainless steel electrodes in the parietal cortex (electro-chemical stimulation – 7 subjects).

The electro-chemical stimulation was effected by means of stainless steel electrodes[2] of 0.3 mm in diameter, using a Nuclear Chicago Stimulator, model 7153, to provide monophase, square wave, direct current with an intensity of 1 mA for 10 sec.

In order to discard any possibility of the effects found being due to the lesions caused by the stainless steel electrodes instead of to the stimulation created by the iron ion deposit, platinum electrodes were also used, since the latter element does not produce a metallic ion deposit.

A stereotaxic apparatus, under visual control, was used to insert the electrodes to a depth of 1 mm in the parietal cortex and in the posterior part of the olfactory bulbs. The neutral electrode was placed in the stereotaxic apparatus close to the subject. Trepanation of the skull was performed under ether anesthesia in the area of the olfactory bulbs or in that corresponding to the parietal cortex. Total serum proteins and their subfractions were determined prior to operating and also 1, 3 and 5 h after stimulation.

The concentration of total serum proteins was determined by the biuret method, paper electrophoresis being used for that of the different subfractions. Tail sectioning



Changes in serum proteins 3 h after stimulation of the olfactory bulbs and cerebral cortex. Values are expressed in percentages of their initial value which is taken as 100%. Bars represent the mean ± S.E.

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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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