(2 mg.kg^{-1}) given before the 'test phase' did not statistically reduce the time spent drinking of animals treated with diazepam (4 mg.kg^{-1}) before the 'shocks-phase'.

Discussion. A reduction of the central levels of catecholamines seems to induce a memory deficit[4]. Furthermore, baclofen – as GABA-like drugs do – reduces catecholamines turn-over[5,6]. It would be tempting to relate the increase of the time spent drinking elicited by baclofen to an alteration of the memory processes induced by its activities on central catecholamines. However, under our experimental conditions, it does not seem that baclofen alters learning or memory processes but more likely impairs the transfer of the conditioned suppression from the drug to saline. This dissociation appears to be asymmetrical, since conditioned suppression transferred from water to drug.

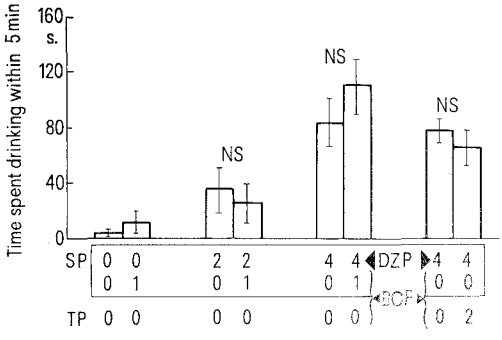

Fig. 2. 'Test phase'. Effects of baclofen given before either the 'shocks phase' (SP) or the 'test phase' (TP) on diazepam effects on the drinking inhibition. DZP, diazepam; BCF, baclofen; NS, no statistically significant difference between baclofen treated and non treated rats. Vertical bars represent SEM.

Baclofen did not modify diazepam-induced amnesia: baclofen (given before the 'shocks phase') did not potentiate the effect of diazepam on the drinking inhibition, nor (given before the 'test phase' did it elicit a reduction of the increased drinking time induced by diazepam. These data do not allow one to relate the amnesia – or an eventual dissociation of learning responsible for such an effect – induced by diazepam to those of its biochemical effects also exerted by baclofen or GABA-like drugs[5–7].

Finally, these data seem not to support a strong relationship between the GABA-enhanced receptor activity induced by benzodiazepines[1,7] and their amnesic effect. The induction of dissociation of learning seems a property of drugs – anticholinergic drugs particularly[8] – which are known to elicit in man a state of confusion. Accordingly, the results obtained with baclofen, under our experimental conditions, suggest that this drug may induce in man a state of confusion.

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Lipoperoxide Formation in the Retina in Ocular Siderosis

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Summary. Insertion of iron nail into the vitreous cavity provoked the formation of lipoperoxide in the retina. In accord with the increase in lipoperoxide in the retina, ERG began to decrease. In vitro experiment using isolated retina, lipoperoxide was found to be increased in the presence of ferric or ferrous ions, while it was inhibited by adding antioxidants or ethylenediamine tetraacetate. From these results, direct cause of retinal degeneration in siderosis could be ascribed to the formation of lipoperoxide by iron-ions liberated from the piece of iron, resulting into the degeneration of the visual cell layers of the retina.

Inspite of many experimental and clinical studies on ocular siderosis, the mechanism of retinal degeneration in this case has never been well elucidated. Taking into account of our previous finding that the formation of lipoperoxide in the retina is a possible cause for the retinal degeneration when rabbit is exposed to high concentration of oxygen[3], as well as the reports that iron or iron compound provokes the lipoperoxide formation in the tissues or cell organelles[4–9], we reached a hypothesis that the formation of lipoperoxide could be the direct cause for the retinal degeneration in the ocular siderosis. The present paper deals with experimental data on the formation of lipoperoxide in the retina caused by an iron nail in vivo or iron-ions in vitro.

Animals used were albino rabbits weighing 2–3 kg. For in vivo experiment, one eye was used for the experiment and the other for the control. After the instillation anesthesia using 0.4% benoxinate hydrochloride, the conjunctiva and Tenon's capsule were incised for about 5 mm in length in the region of the pars plana in the upper temporal quadrant and the sclera was exposed. An iron nail ($\varnothing$ 0.8 × 8 mm) sterilized was inserted carefully into the vitreous cavity through the sclera. The conjunctiva was closed by a suture and antibiotic ointment was applied.

For ERG measurements, one or two drops of 0.4% benoxinate hydrochloride were used as a topical anesthetic. After 20 min of dark adaptation, ERG was recorded with time constant, 0.03 sec, using xenon light stimulus of 2.0 jouls. The change in ERG before and after the insertion of an iron nail was followed with time until enucleation. Amplitudes of a- and b-waves were summed and the ratio values between the experimental and control were calculated.

Lipoperoxide in the retina was determined by thiobarbituric acid (TBA) method[10]. The procedure is similar to

Effects of antioxidants and EDTA on lipoperoxide formation by ferrous ions

Additions	Lipoperoxide value
Fe^{2+}	10.46 ± 1.36
Fe^{2+} + glutathione	5.49 ± 1.49
Fe^{2+} + riboflavin tetrabutyrate	3.06 ± 0.57
Fe^{2+} + α-tocopherol	2.54 ± 0.41
Fe^{2+} + EDTA	1.30 ± 0.09

Concentrations of additions are: $FeCl_2$, 1×10^{-5} *M*; glutathione, 5×10^{-3} *M*; riboflavin tetrabutyrate, 5×10^{-3} *M*; α-tocopherol, 5×10^{-3} *M*; EDTA, 1×10^{-3} *M*. Lipoperoxide value was represented in terms of nmoles of malonaldehyde, which were calculated from the value for tetraethoxypropane treated by TBA method described in the text. Means of 4 experiments and standard deviation are given.

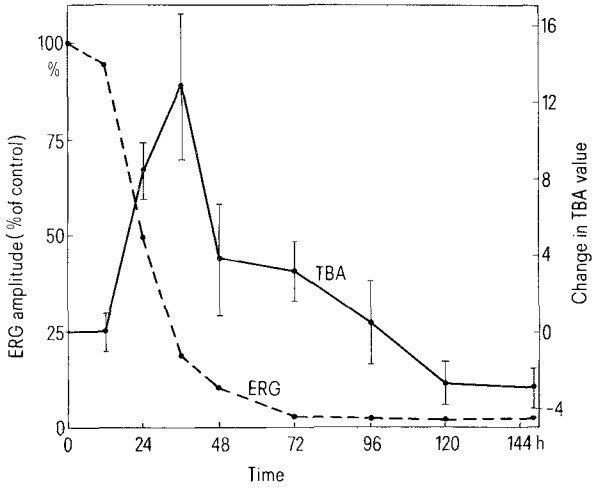

Fig. 1. Changes in ERG and TBA values in the retina of rabbit caused by an iron nail insertion. Experimental conditions, see text. The amplitudes of ERG (a- and b-waves) were represented by percent of mathematical means, obtained by multiple measurements for at least 5 animals. TBA values were represented by mathematical means of difference between the data obtained from at least 5 animals with and without an iron nail insertion. Bars represent standard deviation calculated from the difference (σ_D).

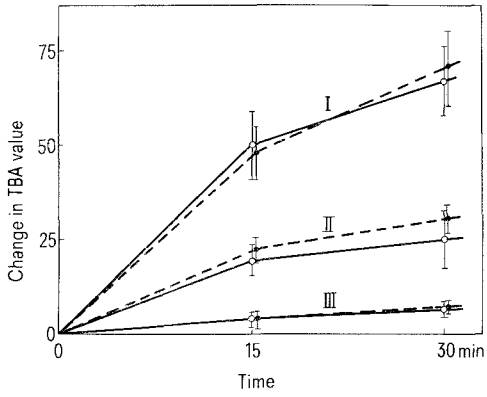

Fig. 2. Lipoperoxide formation with ferrous or ferric ions. Open circles, ferrous chloride; solid circles, ferric chloride. Final concentrations of iron ions are 10^{-4} *M* (I), 10^{-5} *M* (II) and 10^{-6} *M* (III). TBA values were represented by mathematical means of difference between the data obtained from at least 5 retina suspensions with and without iron ions. Bars represent standard deviation calculated from the difference (σ_D).

that reported by NISHIGAKI et al.[11]. The eye of an animal was enucleated and the retina was taken for each measurement. After recording wet weight, the tissue was homogenized in 1.0 ml of cold 0.9% NaCl aqueous solution. The homogenate was transferred to a centrifuge tube and 0.5 ml of 0.9% NaCl was added. To the homogenate, 5.0 ml of TBA reagent (the mixture of equal volumes of 0.67% TBA aqueous solution and glacial acetic acid) were added, and heated at 95 °C in an oil bath for 1 h. After cooling with tap water, 5.0 ml of chloroform were added, shaken vigorously and centrifuged at 3,000 *g* for 10 min. The supernatant was centrifuged further at 10,000 *g* for 10 min, and clear supernatant obtained was subjected to absorbance measurement at 532 nm.

In Figure 1, the change in the amount of lipoperoxide with time is shown. It began to increase 12 h after the insertion, reached the maximum at 36 h and began to decrease. In accord with the increase in the amount of lipoperoxide, ERG amplitude began to decrease, becoming non-recordable at 72 h after the insertion. This indicates that ferric or ferrous ions liberated from an iron nail, provoked the peroxidation of unsaturated fatty acids in the retina, resulting in the degeneration of protein in situ, which naturally injures the retinal functions such as ERG amplitude.

To verify the provocation of lipoperoxide in the retina by ferric or ferrous ions, in vitro experiments were carried out. A detached retina was suspended in 0.9 ml of 0.9% NaCl solution with or without 0.1 ml of $FeCl_3$ or $FeCl_2$ solution at different concentration (final concentration, $10^{-6} \sim 10^{-4}$ *M*) and at different time (0 ~ 30 min). Figure 2 shows the rate of lipoperoxide formation by ferric or ferrous ions. It is obvious that both ferric and ferrous ions are effective in provocation of lipoperoxide and the effects depend on their concentration.

To confirm the effect of ferric or ferrous ions to provoke lipid peroxidation, the effects of antioxidants were examined. As shown in the Table, both glutathione and α-tocopherol prevented the lipid peroxidation. Riboflavin 2′, 3′, 4′, 5′-tetrabutyrate, which was reported to decompose the lipoperoxide as well as to act as antioxidant[12], effectively suppressed the level of lipoperoxide. Remarkable effect of EDTA could be ascribed to the formation of iron-chelate.

Recently WEISS and GRAF[13] reported that the highly unsaturated fatty acid in the retina in experimental siderosis decreased significantly. This might be explained by the formation of lipoperoxide from unsaturated fatty acid as demonstrated in the present experiments.

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