Ethanol and acetaldehyde do not increase the blood-brain and blood-retinal barrier permeability to sodium fluorescein

M. E. Hillbom and T. M. T. Tervo

Research Laboratories of the State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10 (Finland), and Department of Anatomy, University of Helsinki, SF-00170 Helsinki 17 (Finland), 19 January 1981

Summary. No extravasation of sodium fluorescein from capillaries of the cerebral cortex and retina could be demonstrated in rats during severe nonlethal ethanol and/or acetaldehyde poisoning. The results do not exclude the possibility that repeated intoxications may gradually open the blood-brain and blood-retinal barriers, but do indicate the absence of an effect during acute intoxication.

Although it is well-known that chronic alcoholics frequently have radiological brain atrophy and dementia, tissue culture studies prove that such concentrations of ethanol as are present during nonlethal human intoxications do not damage nerve cells[2,3]. Some indirect effects of ethanol may lie behind the common clinical findings. One possibility is disruption of the blood-brain barrier (BBB) and consequent gradual damage to the brain.

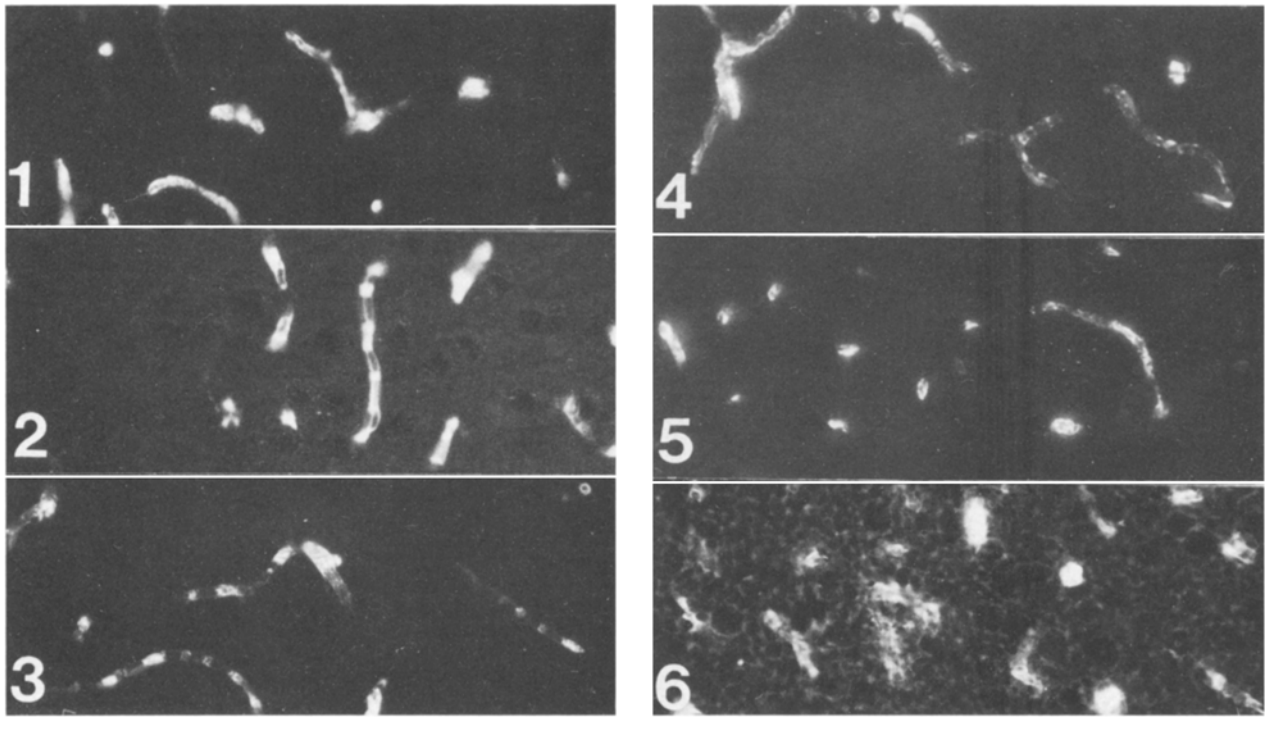
Several studies suggest that the transport of various substances through the BBB is influenced by the presence of even small concentrations of ethanol[4-7]. We therefore investigated whether ethanol or its metabolite acetaldehyde, when present in blood in concentrations compatible with clinical intoxication, damage the capillary endothelium in the cerebral and retinal vessels so that lipid-insoluble particles could pour into the corresponding parenchymas. We used sodium fluorescein, which has a low mol. wt (376 dalton) and small diameter (Stokes'radius 5.5 Å), to investigate the problem. The tracer has previously been shown to be suitable for such a study[8,9].

Material and methods. 27 male 3-month-old Sprague-Dawley rats were used for the experiments. 7 of them received single i.p. injections of ethanol (3, 5, 6 or 7 g/kg of 2 M solution in saline) and 2 were given ethanol (5 g/kg daily of 2 M solution in tap water) via a stomach tube for 1 or 3 days. Acetaldehyde (0.05, 0.1 or 0.2 g/kg of 1 M solution in saline) was injected into the femoral veins of 10 rats and 4 of them were also pretreated with a single dose of ethanol (5 or 6 g/kg i.p.). 8 control animals received an equal volume of saline i.v.

Cyanamide (Sigma, Missouri), an aldehyde dehydrogenase inhibitor known to increase rat blood acetaldehyde levels markedly during ethanol metabolism[10,11], was given to 2 ethanol, 1 acetaldehyde and 1 control rat. It was always injected i.p. diluted in saline solution 10 min before the other substances were administered, the dose being 0.03 g/kg.

Sodium fluorescein (Merck, Germany) was used as an i.v. tracer. It was injected (0.48 mg/kg of 2% w/v solution) in saline into the femoral vein 5 min before decapitation. The rats were killed either 15 min or 2 or 5 h after ethanol or acetaldehyde administration. All the i.v. injections were given under light ether anesthesia unless the animal was so severely intoxicated by ethanol that this was not needed.

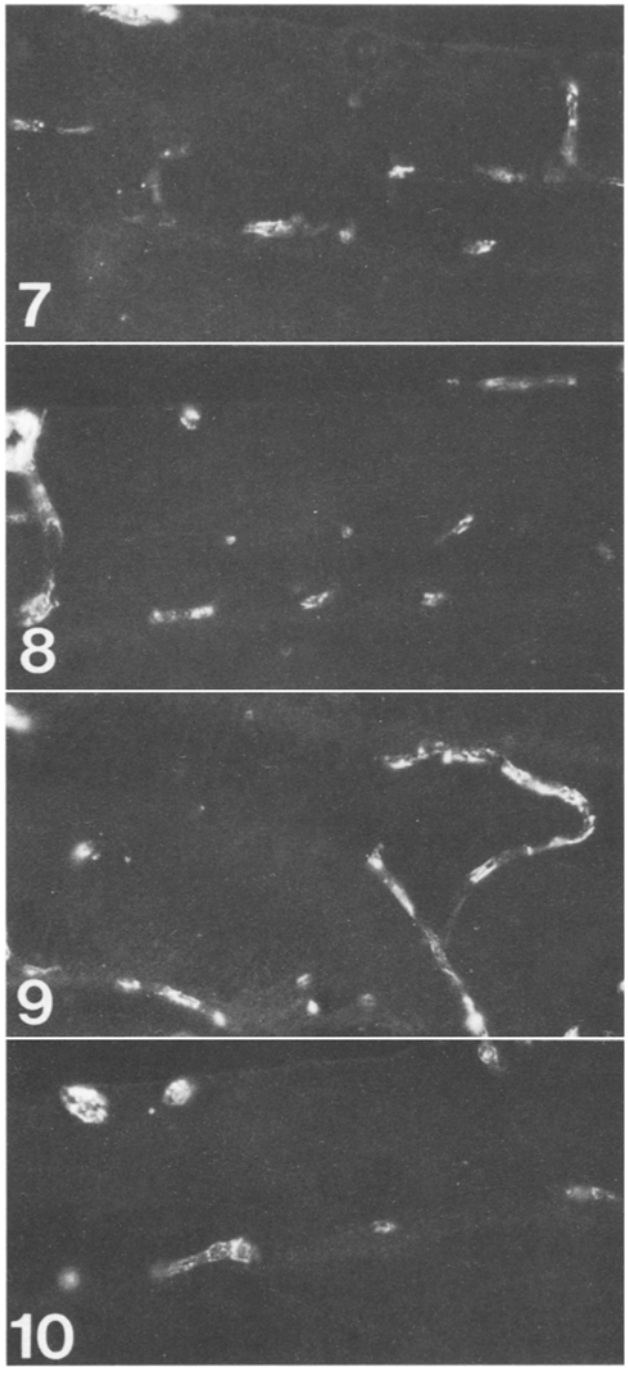
After decapitation the skull was opened, several pieces from the parietal cerebral cortex were excised and the eyes enucleated. The tissues were immediately frozen in liquid nitrogen and thereafter freeze-dried at −45 °C in a vacuum



Figures 1-6. Distribution of sodium fluorescein in the rat parietal cerebral cortex. Treatments: *1* Ethanol 7 g/kg for 15 min. *2* Ethanol 5 g/kg + cyanamide for 3 days. *3* Acetaldehyde 0.1 g/kg for 15 min. *4* Acetaldehyde 0.1 g/kg and cyanamide for 2 h. *5* Negative control (saline). *6* Positive control. Urea (2 M solution in saline) was injected into the carotid artery 10 min before i.v. injection of the tracer.

of 10^{-4} mm Hg for 1 week[9]. After embedding the tissues in paraffin wax, 5-µm thick sections were cut and mounted on glass slides and deparaffinized with xylene. The sections were viewed with a Leitz Ortholux fluorescence microscope equipped with an HBO 200 mercury lamp and a TK 150 dichroic mirror. The primary filters were BG 38, BG 12 and a KP 500 interference filter, while a Leitz K 510 served as a secondary filter.

Results. Regardless of the various doses of ethanol used, the tracer substance was always found inside the capillaries of both brain and retinal tissues. However, leakage of the tracer from arachnoideal vessels into superficial parts of the cerebral cortex was constantly observed (figs 1–10). Neither single i.v. acetaldehyde injections nor i.v. acetaldehyde just after i.p. ethanol broke the BBB or the BRB. The situation remained the same when rats were pretreated with cyanamide. Even if we gave both cyanamide and ethanol daily for 3 days the tissue barriers remained impermeable to sodium fluorescein.



Figures 7–10. Distribution of sodium fluorescein in the rat retina. Treatments: *7* Ethanol 7 g/kg for 15 min. *8* Ethanol 5 g/kg + cyanamide for 3 days. *9* Acetaldehyde 0.1 g/kg and cyanamide for 2 h. *10* Negative control (saline).

Control experiments revealed that aortic blood ethanol concentrations varied between 30 and 160 mmoles/l in rats given 3–7 g/kg of ethanol i.p. Aortic blood acetaldehyde levels[12] did not exceed 0.01 mmoles/l simultaneously, unless cyanamide was given. On the other hand, when 0.1 or 0.2 g/kg of acetaldehyde was slowly injected into the femoral vein, aortic blood acetaldehyde concentrations of 0.3–6.0 mmoles/l were found.

Discussion. It is believed that the BBB is not opened by blood ethanol concentrations present during nonlethal intoxications[13]. However, Rössner demonstrated that the permeability of the barrier to Evans blue (2% solution in saline i.v.) is increased shortly after i.v. injections of ethanol (1 or 3 g/kg of 10% solution) and remains so for a couple of days thereafter[4]. For example, 6 h after administration of 1 g/kg of ethanol to mice a 15% increase in extravasation of Evans blue was found.

The major difference between that study and the present one is the route of ethanol administration. Our method better simulated the normal clinical situation of intoxication since we gave ethanol either p.o. or i.p. An i.v. injection of a 10% ethanol solution, although not lethal, certainly results in a much higher concentration of ethanol in the blood of cerebral capillaries for a short period of time than an i.p. injection does. Other experiments have shown that intracarotid injections or infusions of hyperosmotic solutions including ethanol damage the BBB[14–16].

Our results confirm previous work reviewed by Kalant[17] and indicate that even nearly lethal blood ethanol levels do not open the BBB to sodium fluorescein. Basseches and DiGregorio reported that phenobarbital was found in higher concentrations in CSF, if an i.v. dose of 0.4 g/kg of ethanol preceded its administration[7]. Since the maximal arterial blood ethanol concentration must have been far less than 100 mmoles/l in their experiment, the effect was probably not due to damage of the BBB.

As far as we know effects of acetaldehyde on the permeability of the BBB have not been reported. Although very low concentrations, if any, of acetaldehyde are usually found in the human blood during normal ethanol metabolism[10,18], acetaldehyde intoxications may occur in connection with use of aversive drugs, for example. We used doses of acetaldehyde that were near the LD_{50}[19], but could not observe signs of breakdown of the blood-tissue barriers studied.

In conclusion, neither acute nonlethal ethanol nor acetaldehyde intoxication is followed by breakdown of the BBB or the BRB. Nevertheless, it remains to be excluded that repeated severe intoxications can gradually open the barriers. One preliminary experiment showed no major effect on the BBB either during ethanol intoxication or withdrawal[20], but another suggested that there may be one[21]. Consequently, further experiments are needed to elucidate the chronic effects.

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Some properties of *Bufo bufo* tyrosinase during development

M. Miranda[1], D. Botti, M. Di Cola and A.M. Ragnelli

Institute of General Biology, University of L'Aquila, via Assergi 6, I-67100 L'Aquila (Italy), 5 January 1981

Summary. Tyrosinase expression during *Bufo bufo* development has been investigated. Until stage 19, only 1 electrophoretic band is detectable, but at a later stage (25) 3 bands appear. The K_m for L-3,4-dihydroxyphenylalanine (L-dopa) was also determined.

Vertebrate tyrosinase (EC 1.14.18.1) is expressed in some neural crest derivatives; melanocytes and mosaic pigment cells such as erythrophores, melanophores and iridophores, which, according to Bagnara et al.[2], originate from a common stem cell. Transdetermination of some other neural crest derivatives, such as autonomic nervous system cells and chromaffin cells, may result in tyrosinase expression[3,4]. The compartmentation of this enzyme within melanosomes of the specialized melanogenic cells may assume a role in the regulation of L-tyrosine and L-dopa partition between melanogenesis and catecholaminosynthesis[2,5].

During amphibian development tyrosinase is expressed after neural induction, when a new synthesis of enzyme occurs[5]; information is available about enzyme properties and isozyme pattern in *Rana pipiens* skin[6,7]. The aim of the present work is to provide information about some kinetic properties of embryo tyrosinase, and the disc-gel electrophoresis pattern of the enzyme extracted from *Bufo bufo* embryo melanosomes during development.

Experimental. 50 ml of *Bufo bufo* embryos, from the same ovoposition which occurred in the laboratory, at the stages 8 and 25 of development[8], were washed thrice with 0.5 M sucrose, resuspended with 30 ml of the same medium and homogenized by hand with a Potter homogenizer by 4 pestle strokes. The homogenate was centrifuged for 5 min at 650 × g, and the supernatant was collected and recentrifuged as above; 2 volumes of this supernatant were layered on a sucrose discontinuous density gradient consisting of 1.8 M sucrose (1 vol.) and 1.4 M sucrose (1 vol.), and centrifuged for 120 min at 73,000 × g. Premelanosomes were recovered at the bottom of the tube, as ascertained by electron microscopy and tyrosinase detection (figs 1A and

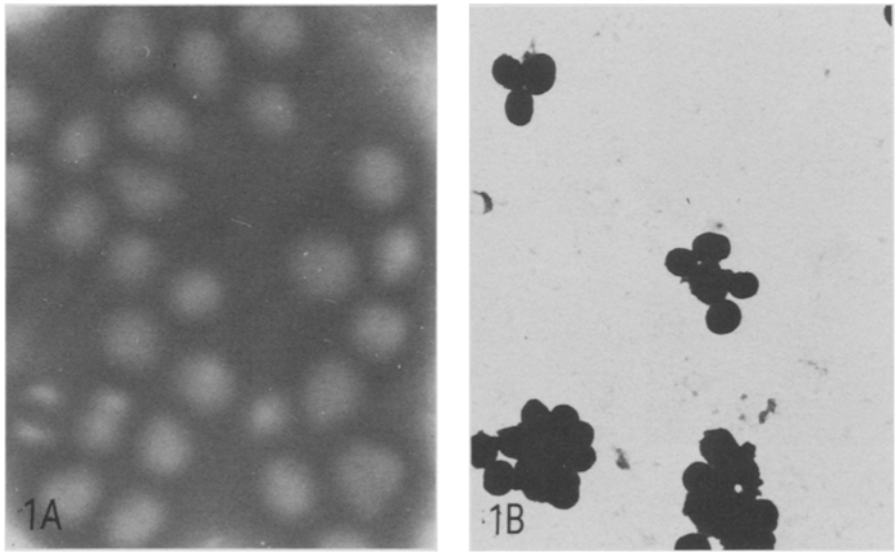

Figure 1. Electron micrographs of negatively stained *Bufo bufo* embryo melanosomes. *A* Premelanosomes from stage 8. × 20,000. *B* Late melanosomes from stage 14. × 12,750.