Effect of Li^+ on mouse brain fumarase activity

Treatment	No. of mice	Body weight (g)	Fumarase (units/mg of protein)
Li_2CO_3	20	29.7 ± 0.8	1547 ± 29
Controls	20	28.8 ± 1.0	1414 ± 53

Each value represents the mean $\pm$ standard error of the mean.

were determined by the biuret method of GORNAL, BARDAILWL and DAVID[8], using crystalline bovine plasma albumin as standard and the specific activity of fumarase was expressed as units/mg of protein.

Results and discussion. The mean intakes of water or Li_2CO_3 solution throughout the period of experimentation were 6.3 and 6.4 ml/mouse/day, respectively. This volume is equivalent to 21.5 mg Li_2CO_3/kg body weight/day, which is in the range of the dose used in manic-depressive psychosis[1].

The final weights of mice and the brain fumarase specific activity are shown in the Table. Treatment with Li_2CO_3 does not influence the weights of the animals ($t = 0.703$, $P < 0.5$). A significant ($t = 2.201$, $P < 0.05$) increase of the specific fumarase activity was observed in Li^+ treated mice. Our experiments to demonstrate an in vitro effect of Li^+ were negative. Addition of Li_2CO_3 up to 0.5 mg/0.05 ml of the homogenate and incubation (37 °C, pH 7.4) for 15 min before fumarase determination did not change the enzyme activity.

FORN and VALDECASAS[9] reported the in vitro inhibition of rat and rabbit cerebral cortex adenyl cyclase by a wide range of Li^+ concentrations. On the other hand, the activation of fumarase by Li^+, as well as our previously reported effects of this ion on succinate dehydrogenase[5] (activation) and aconitase[6] (inhibition), were obtained after long term administration. These data suggest that the effects of lithium on those 3 enzymes of the Krebs cycle are indirect. The lack of knowledge of Li^+ mechanism of action precludes the exact evaluation of the role of these enzymes in manic-depressive psychosis.

Résumé. L'activité spécifique de la fumarase cérébrale des souris traitées pendant 132 jours au lithium (Li_2CO_3) a été déterminée. On a observé une activation significative de l'enzyme. Cependant, cet effet n'a pas été constaté in vitro.

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Effect of Carboxy- or Methemoglobinemia on Motor Conduction Velocity

There are several references to impairments of peripheral motoric nerve function after carbon monoxide (CO) intoxication: CO poisoning may produce peripheral neuropathy[1–10]; in dependence on CO partial pressure, the amplitude of action potential of isolated[11,12] or dissected[13] nerves decreased; and CO produces a retardation of the nerve conduction[3,13,14]. To determine whether the affection on the peripheral nerve is a hypoxic result only, we examined the motor conduction velocity of the N. ischiadicus after acute carboxy- or methemoglobinemia.

Methods. Male albino rats (outbred stock, about 200 g) in groups of 8–15 animals received s.c. injections of 0.5, 0.8, 2.4, and 12 mmol CO/kg or i.p. injections of 0.4, 0.8 and 1.2 mmol sodium nitrite ($NaNO_2$)/kg. Blood samples were taken after 30 min from the retro-orbital plexus. The rats were anaesthetized by hexobarbital (100 mg/kg i.p.) and the motor conduction velocity of N. ischiadicus determined according to the method of GLATZEL et al.[15]. Room temperature was 22 °C. The carboxyhemoglobin (CO-Hb) level was calculated from hemoglobin and CO level in blood. Hemoglobin was determined as cyanmethemoglobin. CO in blood was analysed according to the method of WENNESLAND[16] as modified by us[17,18]. The methemoglobin (Met-Hb) level in blood was assayed by the method of PFORDTE[19]. Student's t-test was used for statistical comparisons.

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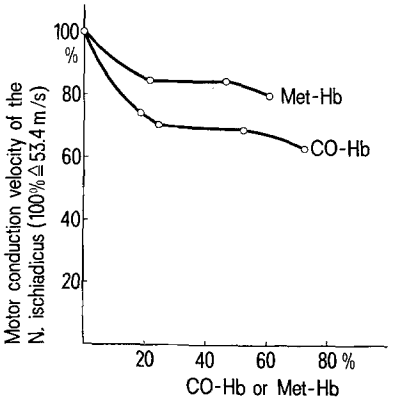
Results and discussion. The alteration of the motor conduction velocity depends on the impairment of hemoglobin function. Increasing carboxy- or methemoglobinemia to about 20% produces a distinct decrease, then to about 50% only insignificant changes and final a farther decrease of the motor conduction velocity (Figure).



Motor conduction velocity of the N. ischiadicus of rats following single injections of 0.5, 0.8, 2.4 and 12 mmol CO/kg (s.c.) or 0.4, 0.8 and 1.2 mmol $NaNO_2$/kg (i.p.).

But the extent of the retardation of nerve conduction in hypoxic conditions of the same degree is about twice as high following CO than $NaNO_2$ intoxication. This means that CO produced an additional effect on peripheral nerve function beside the hypoxic response induced by blockade of hemoglobin. A significant ($p < 0.001$) decrease of motor conduction velocity was observed already if a mean carboxyhemoglobinemia of 18.6% CO-Hb or in particular case of 13% CO-Hb was determined. It seems that the motor conduction velocity is a sensitive indicator for testing peripheral nerve after CO intoxication.

Zusammenfassung. Störung der Sauerstofftransportfunktion des Hämoglobins durch Carboxy- oder Methämoglobinämie vermindert die motorische Leitungsgeschwindigkeit am N. ischiadicus der Ratte. Das Ausmass der Verlangsamung der Nervenleitung ist nach gleichgrosser Blockade des Hämoglobins unter Kohlenmonoxideinfluss annähernd doppelt so gross wie unter Natriumnitriteinfluss.

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Ethyl-m-Aminobenzoate (MS-222) Anaesthesia in the Newt - Effect of D_2O, pH and Time of Day

Ethyl-m-aminobenzoate (metacain, MS-222) is a much used anaesthetic for cold blooded animals[1]. Although its mechanism of action is unknown, one likely possibility is that it inhibits transmitter release[2], and a reciprocal antagonism between ethyl-m-aminobenzoate and serotonin in several invertebrate species has been observed[3]. Recently it has been reported[4] that the rate of anaesthesia of the fish *Mollensia spec.* by various doses of MS-222 decreased markedly if 20–40% D_2O was substituted for water in the aquarium, and the results were attributed to the membrane-stabilizing properties of D_2O.

This interpretation is at variance with current theories of anaesthesia[5,6], and we have therefore examined the effect of D_2O on anaesthesia in aquatic amphibia. The urodeles, newts and salamanders, are particularly suitable for studies of this nature since metamorphic changes permit the examination of both water-and air-breathing forms. Further, the use of an aquatic animal and a water-soluble anaesthetic such as MS 222 permit the control of critical factors involved in the structure of water, which has been implicated in anaesthesia[5,6].

Materials. Ethy-m-aminobenzoate (MS 222) was obtained from Sandoz, Ltd. Basle, Switzerland. D_2O was obtained from the Isotope Separation plant of the Weizmann Institute (as a by-product of oxygen isotope separation). The protium content of this heavy water was determined by nuclear magnetic resonance and the deuterium content obtained by difference. The deuterium content ranged from 40%–97% and was diluted as described below.

Animals. Adult newts (*Triturus cristatus*) were previously maintained in a temperature-controlled room (22 ± 2°C) under constant light with continuous aeration for at least 6 months prior to the experiment. Larvae of the newt *Pleurodeles waltl* were raised in the laboratory from eggs under the above conditions and were 80–100 days old when used. The parthenogenic fish *Poecillia formosa* were obtained from Gossington Tropical Fisheries, Box 208, Delray Beach, Florida, and were maintained for at least 12 months in the same temperature-controlled room with continuous aeration and constant light.

Table I. Effect of D_2O on ethyl-m-aminobenzoate (MS 222) anaesthesia in the adult newt (*Triturus cristatus*)

	Anaesthesia time (sec)	Activity	Escapes
H_2O	–	7.2 ± 15.0	6.3 ± 15.0
H_2O + MS 222	747 ± 98	18.3 ± 5.8[a]	56.3 ± 38.7[a]
25% D_2O + MS 222	718 + 103	20.3 ± 7.0[a]	43.5 ± 20.7[a]

9 adult *Triturus cristatus* (6.2–9.4 g), raised in constant light and kept in Rehovot tapwater (RST) for 24 h before use. Activity and escape behaviour were determined during 5 min after transfer to a control tank of RST and during the first 5 min after subsequent transfer to freshly prepared solutions of MS 222 (133.3 mg/l). Each animal was tested twice in each experimental solution and the average time for anaesthesia in each condition was used to compute the group means expressed as mean ± S.D. [a] Indicates a significant difference from control, $p < 0.05$.

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