less than DNA value at day 3 of lactation. All hormones stimulated lactogenesis but GH was less effective than the other 2 hormones. It was concluded that ovarian hormones (EB + P) play a dominant role in preparturient growth, whereas GH and HCA play dominant roles in "lactational growth" of rat.

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Role of THAM in Protecting Mice Against Convulsive Episodes Caused by Exposure to Oxygen Under High Pressure. (28065)

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Recently Sanger *et al.*(1) and Bean(2) have shown that tris (hydroxymethyl)aminomethane (tris or THAM) protects rats and mice against the toxic effects of oxygen under high pressure (OHP). Both groups of investigators ascribe the protective properties of THAM to its buffering capacity and the maintenance of the cerebral pCO_2 and acid-base balance. To support this view, Sanger *et al.*(1) reported that decreasing the buffering capacity of THAM by neutralization with HCl decreased its protective properties against the toxic effects of OHP.

However, data from microbial experimentation(3-6) suggest possible alternate mechanisms for the protective action of THAM. Using *Lactobacillus arabinosus* MacLeod and Onofrey(3) showed that K^+ competitively reversed the antibacterial action of various amines including THAM. Decreasing the pH of triethanolamine completely eliminated its toxic action; these data imply that a similar effect would occur also if the pH of the other amines was decreased. Provasoli *et al.*(4,5) reported the involvement of K^+ in reversing THAM toxicities in marine and fresh water algae. It should be mentioned that the toxicity of THAM for *Volvox globator* could not be counteracted by Ca, Mg, Na, K, or trace metals(5). Packer *et al.*(6) concluded that these latter data imply that in some organisms THAM might interfere with certain organic metabolites.

The above data are interpreted to mean that the protective action of THAM against the toxic effects of OHP may not be due entirely to its buffering abilities but to its capacity to interfere with K^+ and/or organic metabolites. This investigation was designed to study the possible reversing effects of K on THAM protection against the toxic action of OHP, and possibly to gain new insights into the mechanism of oxygen toxicity.

Method. Young female Swiss mice 20-30 g were used as experimental animals. The test animals were administered 1 cc, i.p., of the appropriate test solution. The animals were placed in a "mouse house"—a multi-

tiered, circular, plexiglass compartmentalized cage capable of housing 6-8 mice. Each compartment houses one mouse. There were sufficient holes in each compartment to permit air circulation. This cage was placed in a pressure chamber which contained 10-15 g of powdered potassium superoxide for absorption of CO_2; air circulation in the chamber was accomplished by means of a fan. The experiments herein described were carried out in an environment of 100% oxygen at 45 psig (4 atm absolute). The time from injection to attainment of full pressure was approximately 25-30 minutes. The animals were observed continually during their exposure. Toxicity was measured as the time of onset of the first detectable sign of seizure activity as measured from the time full pressure was attained. This seemingly indefinite endpoint was chosen because of the extreme variability of response of these animals to OHP. The variability in the symptoms of oxygen toxicity in humans was stressed by Donald(7). Some animals had foreleg tremor as the first sign of seizure activity whereas other animals had hindleg tremor, head tremor or a combination of these as the first detectable signs of CNS effects of OHP; other animals never revealing any apparent overt symptoms of CNS involvement would suddenly manifest mild or violent whole-body seizures. Control animals as well as experimental animals showed rapid and vigorous forepaw activity. These are interpreted as escape movements since they appeared purposeful and since similar activity was observed in animals at increased pressures in an air atmosphere. Due to the variability of response of these animals to OHP it was not possible to use any one behavior pattern as an endpoint for all animals.

The test solutions consisted of 0.3 M THAM, 0.01 M NaCl, 0.01 M KCl, 0.3 M THAM in 0.01 M NaCl, 0.3 M THAM in 0.01 M KCl. Many experiments were performed as paired experiments in which 4 control animals and 4 THAM-treated animals were placed simultaneously in the pressure chamber. Similar experiments were performed when comparing THAM-Na-treated mice with mice receiving THAM-K. Experiments were performed in which the observers were unaware of the treatment of the animals.

TABLE I. Effect of K^+ and Na^+ on Reversing THAM Protection Against Oxygen Toxicity.

Treatment	No. of Mice	Avg seizure time in sec
Untreated	29	1042 ± 482*
THAM	29	1701 ± 882‖
KCl	8†	976 ± 125
NaCl	8†	1037 ± 317
THAM-KCl	31‡	1125 ± 430
THAM-NaCl	23‡	919 ± 480§

* Standard deviation.

† One animal did not have any apparent seizure during a 38 minute exposure.

‡ Four animals did not have any apparent seizure during exposures lasting for 45, 38, 33, 30 min respectively.

§ One animal required 2300 sec before signs of seizure activity became apparent.

‖ The difference between the means of THAM-treated and control animals is significant; $p<.01$. The difference between the means of THAM-treated and THAM-KCl-treated animals is significant; $p<.01$.

The data summarized in Table I reveal a significant difference between time of onset of seizure activity in THAM-treated animals as compared to untreated mice; THAM-treated animals require a longer period of exposure to OHP before onset of seizure activity. However, as seen by the variance, there is an overlap in the time required before onset of seizure activity. The protective properties of THAM are not very pronounced at 4 atm. The data of Sanger *et al.* support this conclusion: they have shown that as the pO_2 is increased the protective effect of THAM decreases. The relatively rapid onset of seizure activity in some of the THAM-treated animals leads one to wonder how fast these same animals would have succumbed had they not been treated with THAM.

The fact that addition of small quantities of potassium chloride to THAM (Table I) reduced the time of exposure to OHP for onset of seizure activity, coupled with the fact that there is no significant difference in convulsion time between untreated mice and mice treated with THAM-KCl, supports the hypothesis that THAM exerts a protective action against the toxic effects of OHP by interfering with cation function. This argument is further augmented by the knowledge that in these experiments the buffering ca-

pacity of THAM was not altered. These data imply that the buffering abilities of THAM and thereby the maintenance of the cerebral acid-base balance and pCO_2 may not be the primary mechanism by which THAM protects against oxygen toxicity. A surprising finding was that this reversal of THAM protection apparently was not specific for potassium, since low concentrations of sodium ions also appear to have similar reversing properties. It is interesting to note that both cations had a common anion; thus the question arises as to whether the reversal of THAM protection may not be due to the presence of the chloride anion.

Bean(2) used solutions of THAM in NaCl and found protection in rats at 80 psig. Extrapolation of the data of Sanger *et al.*(1) to 80 psig reveals that 0.3 M THAM would not protect mice against the CNS effects of OHP. We were unable to obtain protection when 1.0 cc of 0.3 M THAM was given to six 40 g, male mice 30 minutes before exposure to oxygen at 75 psig.

Discussion. The data presented do not indicate how these ions interfere with or affect the functioning of THAM in protecting against oxygen toxicity. More detailed experimentation, requiring brain perfusion studies with various THAM-Na and THAM-K solutions under increased oxygen pressures, would be required before a definitive statement could be made concerning the possible role of these ions in counteracting THAM protection. The possible involvement of other cations(3,8,9) in reversing THAM's protective effect requires exploration.

The reason(s) for the relatively long time required for some control animals to convulse, presumably while under identical conditions as the other control animals, are unknown; this may be a reflection of biological variation. Bean (personal communication) also noted this variability of response to OHP. One possible explanation of the large variance seen in the THAM-treated animals centers around THAM's ability to produce a hypoglycemia. Based on the observation of Marks(10), Sanger *et al.*(1) argue that hypoglycemia should decrease exposure time to OHP in order to produce seizure activity. Therefore, hypoglycemia should reduce THAM's effectiveness as a protecting agent. Perhaps the THAM-treated animals that responded rapidly to OHP in our experiments had a relatively more pronounced hypoglycemia than the other THAM-treated animals and therefore were more sensitive to oxygen-induced convulsions. By an extension of this line of reasoning it should be possible to increase the effectiveness of THAM protection by preventing the hypoglycemia.

Although the data reported suggest that THAM may exert its protective action to oxygen toxicity by interfering with cation metabolism, they do not rule out the possible direct inhibition by THAM of neuronal utilization of organic metabolites. The fact that THAM is readily and rapidly diffused intracellularly coupled with the data available on *Volvox globator*(5) certainly adds to this possibility. Also, Mahler(11) has pointed out that THAM might interact in certain enzyme systems.

It should be mentioned that THAM-treated animals are quite depressed, *i.e.*, they are slightly less active than untreated animals. This depression becomes more marked as concentration of THAM is increased. Whether or not this depression is due to the induced hypoglycemia and/or interference with enzyme functioning is not known. We have found that a mixture of 0.6 M THAM and 0.15 M KCl is a fatal combination. In one experiment 4 of 6 animals died within 3 hours after receiving an i.p. injection of 1.0 cc of this solution: animals given either 1.0 cc 0.6 M THAM or 0.15 M KCl survived. In a repeat of this experiment 3 of 6 THAM-KCl-treated animals died within 24 hours.

During our experimentation it was noted that THAM-treated animals did a considerable amount of deep gasping. What effect this maneuver may have in preventing lung damage and contributing to the protective effect of THAM on the lungs, as reported by Bean, is difficult to assess. Penrod(12) has shown that preventing atelectasis by intermittent inflation of the lungs during exposure to oxygen prevents lung damage. Gasping

may contribute to the delay of onset of atelectasis.

Summary. THAM, 0.3 M, protected mice from the CNS symptoms of oxygen toxicity at 4 atm. This was shown by the prolonged time required before onset of seizure activity. The same concentration of THAM did not protect at 6 atm. Addition of low concentrations of KCl and NaCl appeared to reverse this protection. A mixture of THAM, 0.6 M, and 0.15 M KCl is lethal to mice.

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Effect of Oxamate on Oxidation of Specifically Labeled Glucose by Ehrlich Ascites Carcinoma Cells.* (28066)

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Oxamic acid, a compound structurally related to pyruvic acid, has been demonstrated to be a potent inhibitor of lactic dehydrogenases derived from beef heart muscle(1), from rabbit skeletal muscle(2), and from several tumors(2). With Ehrlich ascites tumor cells under aerobic conditions, oxamic acid inhibits uptake of glucose, inhibits glycolysis of glucose to lactic acid, stimulates consumption of oxygen with glucose substrate, has no significant effect on endogenous respiration, and restores the glucose-inhibited respiration (Crabtree effect) to the endogenous rate. The concentrations of oxamic acid required for reversal of the Crabtree effect parallel approximately those needed for inhibition of glycolysis(2).

A stimulation in expired $C^{14}O_2$ with Ehrlich ascites carcinoma cells occurs when the substrate glucose-U-C^{14} concentration is reduced below 0.0025 M. This phenomenon has been ascribed to a release of the Crabtree effect(3); at the same glucose concentrations consumption of oxygen is also increased(4). We have noted (unpublished data) a profound stimulation of expired $C^{14}O_2$ in presence of oxamate (0.01 M, 0.03 M, 0.06 M) with whole Ehrlich ascites carcinoma cells incubated aerobically with glucose-U-C^{14} (0.01 M) for 2 hours. To assess the participation of the hexose monophosphate shunt and the Krebs tricarboxylic acid cycle in this oxamate induced stimulation, the fates of glucose-C^{14} substrates labeled in the 1 position and in the 6 position were studied.

Materials and methods. For all experiments, oxamic acid was converted to the sodium salt. The glucose substrate (0.01 M) was labeled with either glucose-1-C^{14} or glucose-6-C^{14} purchased from commercial sources.

The Ehrlich ascites carcinoma cells were harvested after 7 to 10 days of culture in the

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