EFFECT OF EXOGENOUS SUPEROXIDE DISMUTASE AND 1,4-DIAZOBICYCLO-[2.2.2]OCTANE ON THE RESISTANCE OF MICE TO ACUTE OXYGEN POISONING

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A single intravenous injection of a purified preparation of superoxide dismutase (1000 units) simultaneously with catalase (0.5 mg) or without it into mice did not protect the animals against the toxic action of 100% oxygen under a pressure of 5 atm. Intraperitoneal injection of 1,4-diazobicyclo[2.2.2]octane (6 mg) under these conditions prolonged the preconvulsive period and increased the survival rate of the mice. It is suggested that singlet oxygen is formed during hyperoxia.

KEY WORDS: *superoxide dismutase*; 1,4-*diazobicyclo*[2.2.2]*octane; catalase; singlet oxygen*; *hyperoxia.*

In biological systems a superoxide anion (O_2^-) may be formed and the rate of its generation is increased with an increase in the partial pressure of oxygen [11]. The O_2^- anion is detoxicated by superoxide dismutase (SOD), the protective action of which in hyperoxia has been demonstrated in model systems with various biological objects [3]. It has been suggested that SOD can function directly as a quencher of singlet oxygen ($^1O_2$) but this has recently been denied [13]. Meanwhile SOD blocks the nonenzymic reaction of dismutation of O_2^-, leading to the formation of $^1O_2$ and thereby prevents damage to the cell components by this more reactive form of active oxygen. The writers showed previously that repeated exposure to oxygen under high pressure causes a decrease in SOD activity in erythrocytes [4] and the brain [5].

The object of this investigation was to study the effect of SOD and of 1,4-diazobicyclo-[2.2.2]octane (DABCO), a synthetic quencher of $^1O_2$, on the resistance of mice to acute oxygen poisoning. Since there is yet no direct method of detecting $^1O_2$ under in vivo conditions, such an approach is the only way of establishing the role of singlet oxygen in the cell damage caused during hyperoxia.

EXPERIMENTAL METHOD

Noninbred albino mice weighing 20-25 g, exposed in pure oxygen at a pressure of 5 atm for 1 h, or for 70 min in the case of administration of DABCO, were used. The pressure in the pressure chamber was raised in the course of 20 min; decompression lasted 15 min. During exposure, the pressure chamber was ventilated with pure oxygen. The resistance of the animals to hyperoxia was estimated by means of two criteria: the time of appearance of strong clonic convulsions after the pressure had reached the assigned level and the survival rate of the animals, which was determined 2 h after exposure to oxygen. If the mice did not develop convulsions during the experiment, the duration of the animal's stay in the pressure chamber was conventionally regarded as the preconvulsive period. Animals of the control and experimental groups were exposed simultaneously in the pressure chamber..

The enzyme preparations were dissolved in 0.9% NaCl with 0.005 M potassium phosphate buffer, pH 7.8, and injected into the caudal vein (in a volume of 0.4 ml) immediately before the animal was placed in the pressure chamber. Control mice of one group received an injec-

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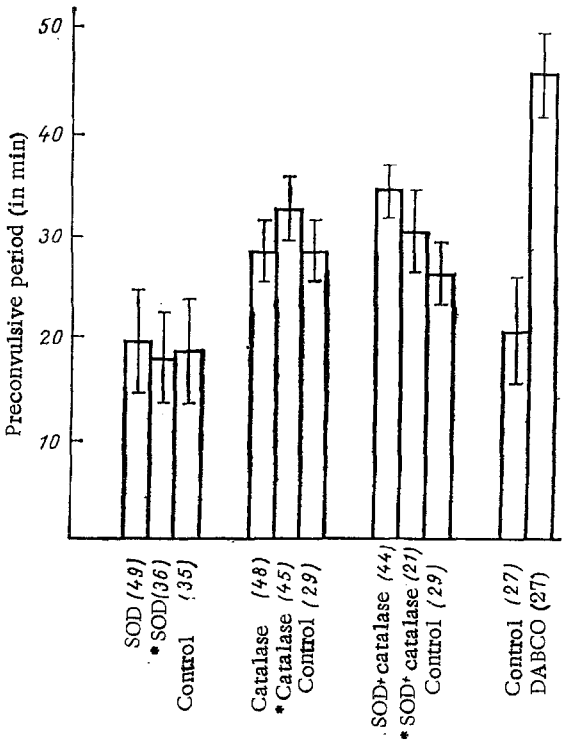

Fig. 1. Effect of SOD and DABCO on length of preconvulsive period in mice exposed to the action of oxygen at 5 atm. Here and in Fig. 2: asterisk indicates groups of animals receiving injection of solution of enzymes denatured by boiling. Number of animals in each group shown in parentheses.

tion of NaCl solution, the other group received a solution of enzyme inactivated by boiling. DABCO (Aldrich Chemical Company, Inc., USA) was dissolved in 0.9% NaCl and injected intraperitoneally (in a dose of 6 mg in a volume of 0.5 ml) before the animal was placed in the pressure chamber. In this case the control group of mice received 0.9% NaCl by intraperitoneal injection.

SOD was isolated from bovine blood [12] with an additional stage of purification of the enzyme, precipitated with ammonium sulfate (90% saturation) on a Sephadex G-75 column (2.5 × 100 cm). The enzyme was eluted by 0.1 M KCl with 0.005 M potassium phosphate buffer, pH 7.8, and kept in ammonium sulfate solution (90% saturation). Before use the enzyme was dialyzed against 0.9% NaCl with 0.005 M potassium phosphate buffer, pH 7.8, for 12 h. In an adrenalin autooxidation system [1] the preparation had an activity of 6200 units/mg protein. On electrophoresis in polyacrylamide gel [10] with Coomassie-250 dye or with nitro-BT, under conditions of photochemical O_2^- generation [9], two protein bands, both possessing SOD activity, where detected in the preparation. To determine SOD activity in mouse blood the hemoglobin was removed by treatment with chloroform and ethanol [12]. The catalase preparation (Reakhim Mark B) had an activity, after Aebi [6], of $1.4 \cdot 10^7$ $M^{-1} \cdot sec^{-1}$. For a single injection, 1000 units SOD and 0.5 mg catalase were used.

EXPERIMENTAL RESULTS AND DISCUSSION

Injection of SOD did not prolong the preconvulsive period or increase the survival rate of the mice during exposure to hyperbaric oxygen (Figs. 1 and 2). The absence of protective action of SOD in hyperoxia can be attributed to various causes: a) spatial isolation of the injected enzyme from the sources of O_2^- generation, b) the relative unimportance of O_2^- in the pathogenesis of oxygen poisoning, c) the toxic action of H_2O_2, a product of the superoxide dismutase reaction, d) an inadequate dose of enzyme injected.

The blood SOD activity in the batch of mice investigated was 466.2 ± 59.0 units/ml. Assuming that the blood volume of a mouse is 1.5 ml [2], the dose of enzyme injected was about 150% of the total blood SOD content. Exogenous protein is known to be inactivated in the

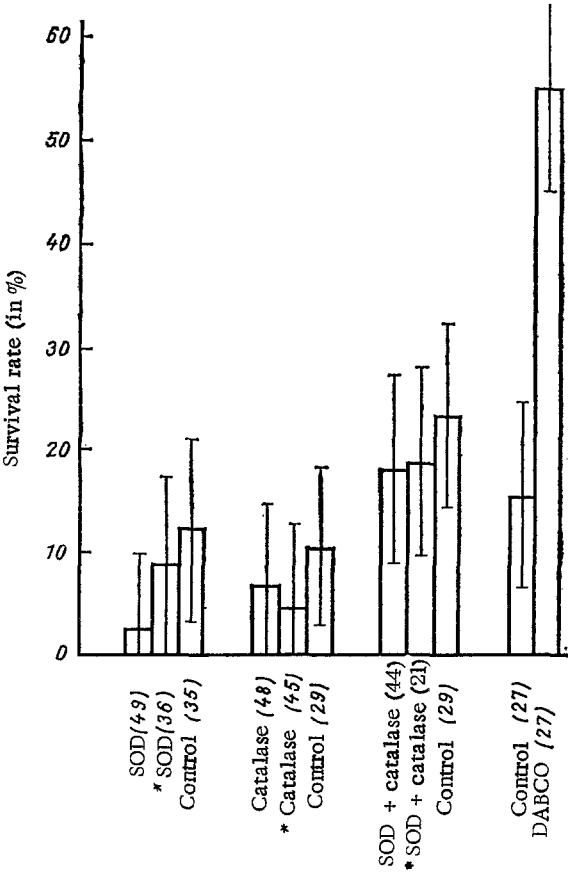

Fig. 2. Effect of SOD and DABCO on survival of mice exposed to the action of oxygen at 5 atm.

blood stream and taken up by the tissue cells. The circulation time of most enzymes in the blood [7] is considerably greater than that of SOD. Small doses of SOD are known [15] to have a radioprotective effect.

It follows from Figs. 1 and 2 that injection of catalase simultaneously with SOD also was ineffective, so that any adverse action of SOD into the more rapid formation of H_2O_2 can be ruled out.

Since the nervous system is primarily affected by the toxic action of oxygen in acute hyperoxia the absence of an effect of SOD was evidently attributable to the function of the blood–brain barrier, which prevented the exogenous enzyme from reaching the points of O_2^- generation in the nerve cells. The facts described above do not rule out the possible role of O_2^- in the mechanism of oxygen toxicity in animals. It has been shown that SOD has a protective action under hyperoxic conditions against the toxic action of paraquat, a substance causing lung damage through increased generation of O_2^- [8], but according to the morphological data, injection of SOD does not prevent lung damage in chronic hyperoxia [8]. In intact animals kept in an atmosphere of air exogenous SOD had no appreciable protective action [8, 15, 16].

The formation of $^1O_2$ in biological systems has not been confirmed by direct methods. However, the ability of quenchers of $^1O_2$ to inhibit certain oxidative processes in such systems has generally been taken [3] to be an indication of the role of $^1O_2$ in them. Interaction between $^1O_2$ olefins leads to the formation of peroxides and, under certain conditions, it initiates autooxidation of unsaturated lipids [17]. Quenchers of $^1O_2$ have been shown to have a marked inhibitory action in aqueous media on oxidative injury to liposomes, microsomes, and mitochondria [3]. DABCO is a water-soluble quencher of $^1O_2$. The effectiveness of its action has been demonstrated in various model systems [3, 14]. A single injection of DABCO considerably increases the resistance of mice to the toxic action of hyperbaric oxygen (see Figs. 1 and 2). This suggests that, under the conditions of hyperbaric oxygenation used, $^1O_2$ is formed in the tissues of the mice. However, since the pharmacological action of DABCO in

vivo is unknown, the effect observed cannot be regarded as incontrovertible evidence of a role of $^1O_2$ in the mechanism of oxygen toxicty.

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EFFECT OF AN ATHEROGENIC DIET ON AGE DIFFERENCES IN CHOLESTEROL BIOSYNTHESIS IN THE RAT LIVER

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After intraperitoneal injection of radioactive sodium acetate into rats of two age groups (6-8 and 28-32 months) the dynamics of cholesterol biosynthesis in the liver was observed to be slower in the older animals. The specific liver cholesterol activity of the older rats was lower at the maximum of uptake of the label than in the younger rats. An atherogenic diet for 20 days (0.25 g cholesterol/100 g body weight) led to an increase in the total cholesterol content but to inhibition of its biosynthesis in the liver, and this effect was most marked in the younger rats. Continued administration of cholesterol depressed its biosynthesis still more, especially in the older animals.

KEY WORDS: *liver*; *cholesterol biosynthesis*; *age*.

The metabolism of cholesterol and its esters is intimately connected with the metabolism of certain hormones and vitamins [5]. The role of cholesterol in pathology is determined by

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