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Causes of Death in Animals Poisoned with Hydrogen Peroxide

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Numerous cases of fatal and nonfatal poisoning with hydrogen peroxide (HP) have been described [14]. The cause of death has been variously attributed to gas embolism [5-7], cardiac weakness [4], or respiratory failure [8]. However, the reported information on how HP affects the cardiovascular and respiratory systems is based solely on clinical findings which are often contradictory and do not give a clear picture of the pathological changes occurring in the body. On the other hand, attempts have been made over many years to use HP clinically for oxygenating blood in cases of asphyxia or hypoxia [9-11]. The purpose of the present study was to determine the exact causes of death in HP-poisoned animals.

MATERIALS AND METHODS

Randomly bred rats aged 9-10 months were used (n = 285). As the purpose of this study was to elucidate the causes of death in acute poisoning with HP, the animals were poisoned with the peroxide at the LD_{100} level (500 mg/kg) - a dose producing typical changes that arise in cases of HP intoxication. HP was injected subcutaneously as a 10% aqueous solution.

A PDM-3 instrument was used for measuring parameters of external respiration. Arterial pressure was measured in an iliac artery and venous pressure in a femoral vein, with graphic recording on an electrokymograph. The ECG was recorded in six leads (I, II, III, aVR, aVL, and aVF) using an EK2T-02 electrocardiograph. (Because the question of how ECGs should be interpreted in laboratory animals has not been resolved, the ECGs recorded in animal experiments for clinical purposes are difficult to interpret [12].) Stroke output and minute volume were calculated using an integral rheogram [3]. The gaseous composition of the blood (pO_2, pCO_2), its pH, and the percentage of oxyhemoglobin in it were determined in a Micro-Astroup analyzer (Radiometer). Arterial blood was sampled from an iliac artery and venous blood from a femoral vein (from the left paw before the HP injection and from the right paw after it). A pharmacological analysis was also performed

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TABLE 1. Time–Course of Stroke Output and Minute Volume after Hydrogen Peroxide Injection (Means±SEM; $n=14$)

	Baseline	Before respiratory arrest	After respiratory arrest
Stroke volume, ml	0.117±0.020	0.163±0.044*	0.189±0.021*
Minute volume, ml/min	58.05±6.94	39.37±2.99*	27.52±3.61*
Heart rate, beats/min	497±19.9	268±50.6*	155±26.4*

Note. Asterisk: $p<0.01$.

in an attempt to find possible ways of effective treatment of HP intoxication. The physiological data were treated statistically by Student's t test.

RESULTS

The clinical picture of HP poisoning was similar in all rats. Ten to fifteen seconds after the injection they ceased to move and began to gasp. Some rats developed tonic convulsions. Markedly increased intestinal peristalsis was observed. Respiratory arrest occurred at 1.5-3 min postinjection in most rats and at 10-15 min in a few animals. Cardiac activity persisted for 20-30 min after the respiratory arrest.

In rats that died 1.5-2 min postinjection, drastic falls in the frequency (from 125.0±6.0 to 26.3±11.7 breaths/min) and amplitude (from 14.0±1.8 to 10.0±1.2 relative units) of respiratory movements were noted. In the group of rats that died later, the breathing frequency decreased by an average of 36% at minutes 3-5 postinjection and then remained at this reduced level but fell sharply just before death. The amplitude of respiratory movements decreased progressively. For such rats, various distortions of the respiratory curve were observed. The most conspicuous change was a considerable prolongation of the expiratory phase, which occurred in 80% of the animals and was most likely due to contraction of the bronchi and a greatly increased resistance to expiration. This finding indicates that hypoventilation with markedly prolonged expiration occurs after HP administration.

Arterial pressure began to fall approximately 5 sec postinjection to reach 30% of the preinjection (baseline) level after 30 sec. By the 2nd minute, it had decreased by a further 25%. In contrast, no substantial changes in venous pressure were observed. Visual observations indicated that gas emboli in arteries and veins appeared in the greater circulation 15 to 20 sec postinjection, i.e., when the blood pressure had already begun to fall. This suggests that reduced tonus of blood vessels rather than gas embolism is at the basis of the hypotensive effect produced by HP.

Although electrical activity persisted in the heart for a considerable period after the respiratory arrest, HP elicited a number of strongly marked pathological responses from the heart. All rats exhibited pronounced bradycardia (157±15.5 beats/min at the time of respiratory arrest *vs* 475±14.4 before HP injection; $p<0.01$). The major ECG changes were those in conductivity. Before the respiratory arrest, the rats developed a first-degree arterioventricular (AV) block. In some rats, a sinoauricular block or arrhythmia was recorded. The first-degree AV block progressed to a second-degree AV block or to a subtotal AV block in some rats but in most rats it progressed straight to a complete AV block. Other ECG changes were also observed, notably the appearance of polytopic extrasystoles. An interesting and consistent finding was that while the RR intervals were usually irregular before respiratory arrest, after the arrest they became stable and ventricular contractions were more frequent - up to 370 per minute in some animals by the 20th min. The atrial rhythm remained constant in almost all rats (350-360 contractions/min). By minutes 15-20 after respiratory arrest, the atrial and ventricular rhythms became more or less equal while remaining independent. The observed disturbances in cardiac activity are associated with augmented parasympathetic innervation. An increase of the influence of the va-

TABLE 2. Effects of Hydrogen Peroxide on Gaseous Composition and pH of Rat Blood (Means±SEM; $n=12$)

	Arterial blood		Venous blood	
	baseline	after H_2O_2 injection	baseline	after H_2O_2 injection
pH	7.351±0.028	7.204±0.04*	7.397±0.028	7.264±0.06
pO_2, mm Hg	103.8±2.7	61.2±5.9**	40.5±1.4	48.5±2.0**
pCO_2, mm Hg	35.7±2.1	41.0±7.2	40.1±0.1	35.5±9.5
Hemoglobin, %	97.4±0.3	82.4±3.9**	74.8±2.4	75.3±2.7

Note. One asterisk: $p<0.05$; two asterisks: $p<0.01$.

gus nerve on the heart leads to the development of a sinoauricular block and to delayed conduction via the atrioventricular (AV) node. Moderate stimulation of the vagus nerve in mammals with a high heart rate may result in greater impairment of AV conduction in comparison with changes in the frequency of the sinus rhythm. The reduced automatism and conduction of the AV node weakens the inhibitory influences of the sinus pacemaker on the underlying foci of automatism, which may then become the sources of rhythmically arising excitation (of polytopic extrasystoles). The heightened frequency of ventricular contraction after respiratory arrest may be attributed to a gradual upward shift toward the AV node of the ectopic excitation foci in Purkinje's fibers.

Stroke volume after HP injection and particularly after respiratory arrest was greatly increased (Table 1). The development of cardiac blocks is known to involve a compensatory increase in cardiac output [7]. Moreover, HP has been shown to lower the total peripheral vascular resistance, which is inversely related to the magnitude of cardiac output [10]. Nevertheless, the increased stroke volume as a result of developing bradycardia is not sufficient to maintain the minute volume at a normal level.

Within the first minute after HP injection, acidosis developed in the arterial and venous blood of the rats (Table 2). Also, hypoxemia and hypercapnia were noted in the arterial blood and hyperoxia and hypocapnia in the venous blood.

The major roles in the pathogenesis of acute HP poisoning are played by two factors, namely extensive gas embolism and increased activity of the vagal nuclei. The administered HP passes through the interepithelial space and capillaries to enter the bloodstream, where it is broken down by catalase. On its way the HP irritates chemo- and mechanoreceptors as well as the afferent endings of the vagus. The resulting increase in the tonus of the vagal nuclei leads to reduced tonus of the vasoconstrictive centers, thereby producing a vasodilative effect. Simultaneously, the activity of the respiratory center is inhibited so that the main role in maintaining its tonus begins to be played by a humoral factor. The bronchial spasm arising under the impact of acetylcholine prolongs the expiratory phase and diminishes pulmonary ventilation. As the vagus activity increases, bradycardia and cardial blocks develop; the minute volume decreases. The gas emboli entering the arterial system bring about the development of an acute cor pulmonale, as is evidenced by ECG data. The ventilation/perfusion ratio decreases, which leads to a reduction in the pO_2 of the arterial blood and to the accumulation of carbon dioxide in it. Acidosis develops. The venous blood becomes enriched with oxygen entering from the gas bubbles and shows decreased pCO_2 values. In the presence of hypoventilation, the carbon dioxide depleted blood entering the carotid body inhibits the activity of the respiratory center, which results in respiratory arrest. Moreover, the gas emboli and oxygen that form during the decomposition of HP may elicit by themselves a number of nonspecific responses (paralysis, paresis, etc.).

Although the schematic description presented above does not, of course, cover all the pathological reactions that arise, it does provide guidelines for the selection of an optimal treatment for acute HP poisoning: atropine (0.15 mg/kg) or lobeline (14 mg/kg) injected intraperitoneally immediately after the HP injection prevented the death of 70 to 80% of rats and delayed it by 2 to 6 h in the rest.

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