98

MONOGRAPH

ON

HYDROGEN PEROXIDE

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HYDROGEN PEROXIDE

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HYDROGEN PEROXIDE

Summary

Since its discovery in 1818 by Louis-Jacques Thenard, hydrogen peroxide, due to its special chemical properties, has been the subject of much interest. In recent times it has attained a place of great importance in industry and has come to be recognized as an important intermediate in the metabolic pathways of the human body.

Very little information is available dealingg specifically with the absorption and metabolism of orally administered hydrogen peroxide. It is readily absorbed through the mucous linings of the mouth and large intestine, as reported by Ludewig (758, 761) and Urschel (1318), and although there are no reports available which specifically say so, it must surely be absorbed through the linings of the stomach and small intestine as well. Feeding studies (487, 621, 1097) and reports of accidental poisoning (170) lend support to this assumption. Catalase, which is found widely distributed in animal tissues, readily degrades H_2O_2, yielding water and oxygen (750, 760, 1161, 1318).

Hydrogen peroxide, when administered as a gastric lavage in concentrations of 0.5-3%, was found by Culmer et al. (248), to generally depress the acidity of the stomach contents and to stimulate mucous secretion by the stomach.

Wales et al. (1352) reported that male albino mice given 0.33 or 1% H_2O_2 in place of drinking water for up to 28 days before mating were able to sire normal litters, although there may have been some delay in parturition, and that the concentration, morphology and motility of the spermatozoa the mice produced was normal. Normal spermatozoa were also produced by albino rabbits given 0.33, 1, or 3% H_2O_2 for 6 weeks.

Kaufman (1161) reported that the injection of H_2O_2 into the whites of eggs did not impair the development of the embryo.

Schmidt (1151) reported that newborn mice given a single s.c. injection of 0.1 ml of 0.6% H_2O_2 and surviving at least 6 months displayed a tumor incidence of 20% with a latent period of 20 months and that mice given 3 such injections displayed a tumor incidence of 33% with a latent period of 15 months (control incidence 7-20%).

Using the S_2 sarcoma, the Ehrlich ascites carcinoma and the sarcoma 180, Schoeneich (1155) demonstrated that H_2O_2 can produce chromosomal aberrations in ascites tumors in mice and that this effect is positively correlated with the concentration of H_2O_2 injected.

According to Hauschild et al. (499), the whitish discoloration and

peculiar sensations resultant from topical application of H_2O_2 are not a caustic action as commonly believed, but are the result of the formation of gas bubbles within the tissue and the resultant temporary bloodlessness of the capillaries. Significant damage occurs only when the gas formation is so intense that it results in mechanical damage or prolonged inhibition of nutrient supply.

The effect of atmospheric exposure to H_2O_2 was tested by Punte et al. (1039), Oberst et al. (948), and Svirbely et al. (1250), using mice, rats, hamsters, rabbits and dogs. Symptoms commonly observed upon exposure, varying with the extent of exposure, were nasal irritation, inflammation and blinking of the eyes, slight gasping, loss of muscular coordination, minor H_2O_2 burns of the nose and paws, localized denudation, pulmonary congestion, gross opacities of the cornea, and in some cases death.

Solutions of 0.5 to 1.5% H_2O_2 given to male Holtzman rats in place of drinking water were reported by Shapiro et al. (1177) to induce extensive carious lesions and pathological changes in the periodontium, the intensity of the effect varying with the H_2O_2 concentration. Significant inhibition of body weight gain was also noted in these rats.

Several reports of acute toxicity studies of H_2O_2 have appeared in the literature, but in none of them was the peroxide administered orally. The i.p. injection of H_2O_2 (from 0.5 ml of 50% solution to 1.0 ml of 10% solution) into adult mice was reported by Dustin and Gompel (307) to result in the production of transient nuclear lesions in the intestine and thymus. In the treatment of 72 male Sprague-Dawley rats bearing 4-day-old Walker 256 carcinoma, Smith et al. (1214) found that the infusion or perfusion of the femoral artery with 40 mg H_2O_2/kg led to the occurrence of digital gangrene in about 30% of the animals. In experiments in which H_2O_2 was administered to rats, rabbits, cats, dogs and pigs intravenously and percutaneously, Hrubetz et al. (551) found that death resulting from such H_2O_2 administration is associated with gas embolism and that the degree of i.v. toxicity of H_2O_2 is an inverse function of the degree of local breakdown at the site of administration. Based on their results, the authors constructed a graph of the LD_{50} of i.v. H_2O_2 in rabbits with respect to the concentration of H_2O_2 injected. Symptoms of toxicity - shortness of breath, agitation, throwing of the head backward, spasms, increased peristalsis and repeated acts of defecating - were observed by Petrov (1003) following the i.v. administration of 1.2 ml/kg doses of 0.4-0.5% H_2O_2 in rabbits and 1-3% H_2O_2 in dogs. Pale, emphysematous lungs and the presence of considerable amounts of gas in the great veins and the right side of the heart were observed in those animals that died during the tolerance studies conducted by Loriniz et al. (750) of i.v. administration of H_2O_2 in rabbits and cats.

Five cases of non-fatal poisoning in which the victims drank 25-100 ml perhydrol (30% H_2O_2) were reported by Budagovskii et al. (170) to be characterized by sharp pains in the stomach and behind the sternum, breath retention, with a subsequent discharge of foam from the mouth

during vomital movements, a fleeting absence of conciousness, transitory motor and sensory impairment, a rise in temperature, microhemorrhaging in the conjunctiva and skin, and a moderate leucocytosis.

Five teams of researchers have published reports of short-term studies of H_2O_2 tolerance.

In a preliminary experiment conducted by Aoki and Tani (37), it was found that solutions of H_2O_2 given to mice, in place of drinking water, in concentrations in excess of 1% resulted in pronounced loss of body weight and death within 2 weeks. Solutions of 0.3 and 0.6% also caused loss in body weight. When the H_2O_2 solution was switched to water during the experiment, the weight loss was quickly recovered, indicating that the H_2O_2 exerted no fatal damage to the tissues of the internal organs.

Aoki and Tani (37) reported that treating 16 mice for 35 weeks with 0.15% H_2O_2 in place of drinking water resulted in the development of hydropic degeneration of hepatic and renal tubule epithelial tissues, necrosis, inflammation, and irregularity of tissue structure of the stomach wall, and hypertrophy of the lymphatic tissue of the small intestinal wall.

During the s.c. treatment of 30 young mice with 0.1 ml doses of 15% H_2O_2 twice daily for a period of 2 weeks, Lorincz et al. (750) observed in some animals brief embolic signs, generalized convulsions or paraplegia. Five of the mice died with embolic symptoms at various times during the experiment. In all cases the injection caused local s.c. emphysemas with small ulcers developing at the injection sites in about 25% of the animals.

Upon replacing the drinking water of rats with 0.25, 0.5, 2.5, 5, or 10% H_2O_2 for a period of 146 or 290 days, Romanowski (1097) found that the 0.25 and 0.5% solutions inhibited the growth and increased the mortality of the rats, and that the higher concentrations produced death in all cases within 43 days.

In a series of studies performed by Hankin (487) it was found that: replacing the drinking water of weanling rats with 0.45% H_2O_2 for a period of 3 weeks inhibited body weight gain; similar treatment of adult male rats for 6 weeks resulted in weight loss; female rats given 0.45% H_2O_2 for 5 months prior to mating, at which time they were switched to tap water, were able to produce normal litters; and that offspring of these rats exhibited inhibition of body weight gain when given the peroxide solution.

For a period of 3 weeks, Kawasaki et al. (621) treated groups of 8 male Wistar rats daily with 0.5 ml oral doses of 0, 0.12, 1.2, or 12% H_2O_2. The rats given 0.12% H_2O_2 exhibited normal growth, those given 1.2% displayed a slight growth inhibition, and those given 12% experienced swollen intestines immediately after dosing, with 4 of this group dying (treatment of this group was immediately discontinued).

In another study by Kawasaki et al. (622), male Wistar rats were given, by gastric intubation, 0.6, 1, 2, 3, or 6 mg H_2O_2/100 g body weight/day for 40 or 100 days. No adverse effects were observed in the animals given H_2O_2 in doses of 3 mg/100 g/day or less. The rats given 6 mg/100 g/day showed a significant drop in growth rate from the 20th day. The spleens of these rats showed significant increases in weight after 40 days, but not after 100 days. At termination of the experiment these rats exhibited decreases in hematocrit value, plasma protein concentration, and plasma catalase activity.

In the only long-term study available, Molnar (870) found that the s.c. injection of 0.01% H_2O_2 into 16 female mice in 0.5 ml doses 2 or 3 times per week from the age of 6 weeks to 14 months resulted in a considerable (24.5%) decrease in the average lifespan and an early loss of body weight.

HYDROGEN PEROXIDE

Chemical Information

I. Nomenclature

A. Common Names

1. Hydrogen peroxide
2. Hydrogen dioxide
3. Hydroperoxide

B. Chemical Names

1. Hydrogen peroxide

C. Trade Names

1. Albone

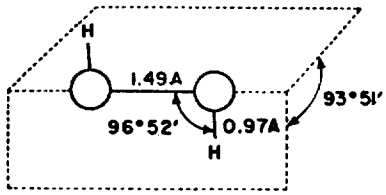
D. Chemical Abstracts Registry Number

Hydrogen peroxide	007722-84-1
Hydrogen peroxide solution	MX8007-30-5

II. Empirical Formula

H_2O_2

III. Structural Formula



IV. Molecular Weight

34.01

V. Specifications

Food Chemicals Codex

Assay	Not less than the labeled concentration or within the range stated on the label
Limits of Impurities	
Acidity (as H_2SO_4)	Not more than 300 ppm
Arsenic (as As)	Not more than 3 ppm
Heavy metals (as Pb)	Not more than 10 ppm
Iron	Not more than 0.5 ppm
Phosphate	Not more than 50 ppm
Residue on evaporation	Not more than 60 ppm
Tin	Not more than 10 ppm

VI. Description

A. General Characteristics

Anhydrous hydrogen peroxide is a colorless, bitter tasting liquid having an odor resembling ozone. It is caustic to the skin. Hydrogen peroxide is usually marketed as a solution in water in concentrations of 3-90% by weight.

B. Physical Properties

Distillable in high vacuum. Miscible with water; sol in ether; insol in petr ether; decomposed by many organic solvents. Further properties given in Table I.

TABLE I
Summary of Physical Properties

Percentage H_2O_2 (w/w)	100%	90%	50%	30%
Mole Fraction H_2O_2	1·0000	0·8266	0·3462	0·1850
Density (g/cc at 20°C)	1·4489	1·3930	1·1954	1·1110
Refractive Index[72] (Na "D" Line at 25°C) ..	1·4067	1·3980	1·3661	1·3519
Dielectric constant[72] (20°C)	74	76	83	83
Specific Conductivity (ohm^{-1} cm^{-1} × 10^{-6} at 25°C)	0·4	2·0	4·8	4·3
Viscosity[74] (Centipoises at 20°C)	1·24	1·27	1·17	1·08
pH	—	5·4	4·7	5·0
Surface Tension[74] (dyne/cm at 20°C)	80·4	79·3	75·7	74·3
Total vapour pressure[75,76] (mm Hg at 30°C)	3·1	5·2	18	24·5
Boiling Point (°C at 1 atmos.)	155·5	142	111	104·5
Freezing Point[77,78] (°C)	−1	−10·5	−50	−25·5
Heat of Vaporisation[78,79] (cal/g soln.)	300	327	424.	492
Heat of Decomposition[79] (cal/g soln. at 20°C)	690	616	335	200

C. Stability

Hydrogen peroxide may decompose violently if traces of impurities are present. Solutions of hydrogen peroxide gradually deteriorate and are usually stabilized by the addition of acetanilide or similar organic materials. Agitation or contact with rough surfaces, metals, or many other substances accelerates decomposition. Rapidly decomposed by alkalies, finely divided metals; the presence of mineral aid renders it more stable.

VII. Analytical Methods

The AOAC qualitative test for the presence of hydrogen peroxide in milk is as follows (1468):

a) Reagent - Dissolve 1 g V_2O_5 in 100 ml H_2SO_4

b) Test - Add 10-20 drops reagent to ca 10 ml sample and mix. Pink or red indicates H_2O_2.

Gilliland reported the development of a method for enzymatically detecting hydrogen peroxide at low levels in milk. It involved the use of horseradish peroxidase with o-dianisidine as a chromogenic hydrogen donor. The milk curd was precipitated at pH 4.5 with hydrochloric acid and the whey added to a mixture of peroxidase and chromogen. The amount of color developed (measured at 400 nm) as a result of the enzymatic reaction gave a measurement of the peroxide. The method detected levels of less than 1 mcg/milliliter in milk (431).

VIII. Occurrence and levels found in:

A. Plants

H_2O_2 is a metabolite normally produced and destroyed by a wide variety of plants.

B. Animals

H_2O_2 is known to occur in a wide variety of animals in extremely low concentrations as an intermediate in metabolic pathways.

C. Synthetics

No information available.

D. Natural Inorganic Sources

In a 1972 report, it was stated that atmospheric concentrations of up to 4 pphm of hydrogen peroxide were determined in early afternoon

hours at Hoboken, N.J., during moderate photochemical smog formation. At Riverside, Calif., concentrations as high as 18 pphm were detected during severe smog formation (407).

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage mg/kg	Measurement	Ref.
H_2O_2	Rat	-	ct	700	LD_{50}	1226
H_2O_2	Rat	-	iv	21	LD_{50}	1226

Mice

Dustin and Gompel reported that the i.p. injection of hydrogen peroxide (from 0.5 ml of 50% solution to 1.0 ml of 10% solution) into adult mice resulted in the production of intestinal lesions. Within 2 hours after injection, the intestinal wall showed a decrease in normal mitotic activity and pycnonecrotic nuclei appeared in the germinative region at the bottom of the glandular culs de sac. These changes reached their maximum between 4 and 6 hours and persisted, with the strongest doses, up to 24 hours. Similar nuclear lesions appeared in the thymus (307).

Rats

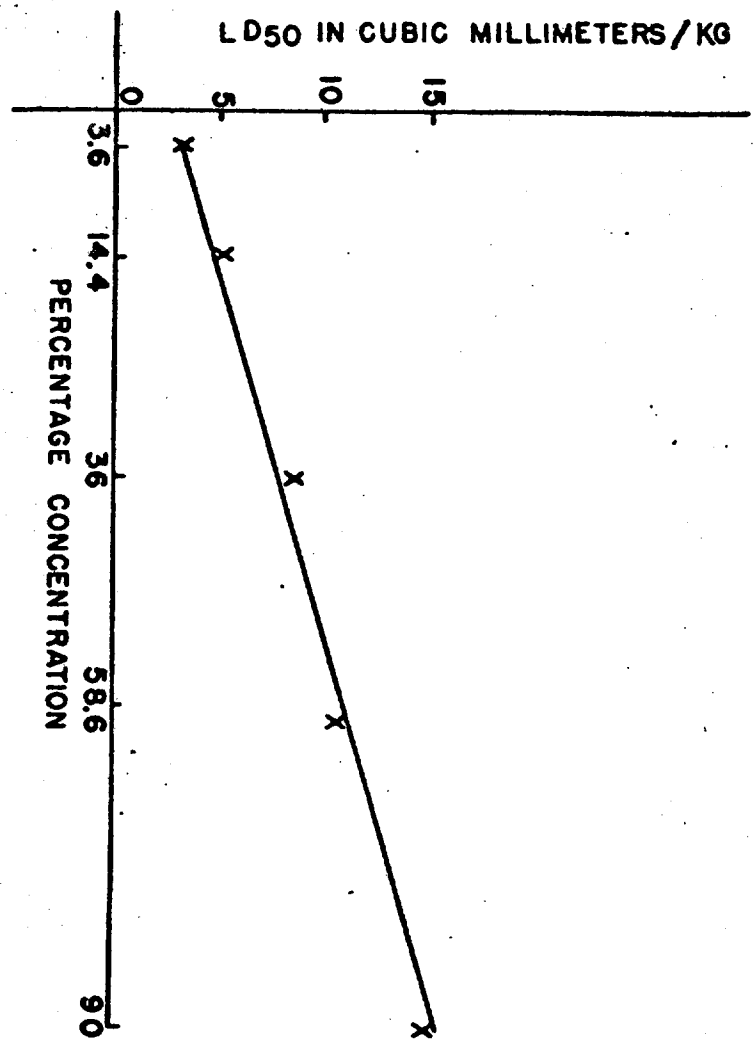
In studying the effect of hydrogen peroxide on tumor growth, Smith et al. treated 72 male Sprague-Dawley rats bearing 4-day-old Walker 256 carcmonia with 40 mg/kg H_2O_2 by isolated femoral artery perfusion or by femoral artery infusion, the drug being administered over a 15 minute period. While this treatment had no effect on tumor growth, it led to the occurrence of digital gangrene in about 30% of the animals (1214).

Rabbits

Hrubetz et al. studied the acute toxicity of hydrogen peroxide through intravenous injections into rabbits and percutaneous administration on the clipped backs of rabbits, rats, cats, dogs and pigs. The LD_{50} of i.v. H_2O_2 in rabbits, which was found to increase with dilution, is shown in Figure 1, and the results of percutaneous administration are presented in Table 1. Death was associated with gas embolism and this was probably the immediate cause. Degradation of the H_2O_2 releases O_2 into the tissues and blood stream, thereby producing the emboli. Studies with percutaneous administration indicate that the greater the concentration of the H_2O_2, the greater the local breakdown such that less material is available for the production of gas emboli in the blood stream, accounting for the lower toxicity on an embolic basis. In addition, i.v. tests have shown that the greater the concentration of H_2O_2 the greater

the degree of local embolism resulting in the mechanical blockage of the agent from further access to the general circulation. With lesser concentrations, the H_2O_2 becomes more widely distributed before degradation and the subsequent formation of emboli, resulting in more widespread damage (551).

FIGURE 1



Relation of intravenous LD50 in rabbits to the percent concentration of H_2O_2 solution injected.

Table 1

Species Differences in Percutaneous Toxicity of 90% H_2O_2

Animal	Dose cc./kg.	Deaths
Rabbit	0.5	6/12
Cat	2.0	0/2
Cat	3.16	0/2
Pig	2.0	2/5
Pig	1.0	0/1
Rat (white)	3.55	4/12
Rat (white)	4.0	9/12
Rat (black)	5.0	0/6
Rat (black)	6.0	2/6

In testing the acute toxicity of intravenous hydrogen peroxide, Petrov performed 8 experiments on 8 rabbits; 2 using 0.2% solution, 2 using 0.25%, 1 using 0.4%, 2 using 0.5%, and 1 using 1%. Dosages of 2 ml/kg were used, except for the 1% solution, of which only 1 ml/kg was used. The 0.2 and 0.25% solutions caused only a brief increase in respiration. The higher doses evoked a clearly toxic effect in the majority of cases, accompanied by an extreme shortness of breath, agitation, throwing of the head backward and a brief fit of spasms. After the spasms ended, respiration gradually slowed and was weakened to the point of paralysis. Of these 4 rabbits, 3 collapsed within 12 hours (1003).

Table 2 summarizes the results of tolerance studies conducted by Lorincz et al., of i.v. administration of hydrogen peroxide in rabbits. The animals surviving the injection recovered well and appeared normal throughout the remainder of the observation period. Post mortem examination of the animals dying during the experiments showed pale, emphysematous lumps and the presence of considerable amounts of gas in the great veins and right side of the heart (750).

TABLE 2

INTRAVENOUS HYDROGEN PEROXIDE TOLERANCES IN RABBIT

Animal	Weight Kg.	Strength H_2O_2 (cc. O_2 liberated per cc. by an excess of blood)	Needle Gauge	Duration of Injection	Injection Rate (O_2 equivalent as cc./kg./min.)	Symptoms of Gas Embolism
Rabbit No. 1	4.0	0.2	26	30 min.	0.12	None
Rabbit No. 2	4.5	1.0	26	15 min.	0.15	Mild
Rabbit No. 1	4.0	1.0	26	6 min.	0.16	Mild
Rabbit No. 1	4.0	0.5	24	10 min.	0.32	Mild
Rabbit No. 1	4.0	1.0	24	13 min.	0.64	Moderate
Rabbit No. 3	4.8	5.0	25	3 min.	0.70	Severe—death
Rabbit No. 4	3.5	3.3	25	12 min.	1.0	Severe—death
Rabbit No. 5	3.5	1.7	25	1 min.	1.3	Severe
Rabbit No. 4	3.5	3.3	25	½ min.	2.0	Very severe

Cats

Table 3 summarizes the results of tolerance studies, conducted by Lorincz et al., of i.v. administration of hydrogen peroxide in cats. The animals surviving the injection recovered well and appeared normal throughout the remainder of the observation period. Post mortem examination of the animals dying during the experiments showed pale, emphysematous lungs and the presence of considerable amounts of gas in the great veins and right side of the heart (750).

TABLE 3

INTRAVENOUS HYDROGEN PEROXIDE TOLERANCES IN CAT

Animal	Weight Kg.	Strength H_2O_2 (cc. O_2 liberated per cc. by an excess of blood)	Needle Gauge	Duration of Injection	Injection Rate (O_2 equivalent as cc./Kg./min.)	Symptoms of Gas Embolism
Cat No. 1	3.2	1.0	24	30 min.	0.34	None
Cat No. 1	3.2	1.0	24	30 min.	0.7	None
Cat No. 2	2.0	3.0	24	30 min.	1.5	Very mild
Cat No. 3	2.5	4.0	24	30 min.	1.6	Mild
Cat No. 4	2.5	5.0	24	30 min.	2.0	Moderate
Cat No. 5	3.3	4.0	24	6 min.	2.0	Severe—death
Cat No. 6	2.1	5.5	24	3 min.	2.6	Severe—death

Dogs

Following a series of more than 30 tests, Petrov reported that the i.v. injection of hydrogen peroxide into dogs in concentrations of 0.01 and 0.05% in doses of 1-2 ml/kg does not result in any significant changes. However, 0.3-2 ml/kg doses of a 1-3% solution evoked the following changes: slight shortness of breath lasting 10-15 minutes; increase in peristalsis and repeated acts of defecating; brief increase or decrease (0.3-0.7 degrees) in body temperature (1003).

Man

Budagovskii et al. reported 5 cases of accidental poisoning with perhydrol (30% H_2O_2). Three of the victims drank 25-30 ml, one swallowed 50 ml, and the fifth consumed close to 100 ml. Analagous clinical symptoms took place in each case, the severity being related to the amount consumed. All of the victims experienced sharp pains in the stomach and behind the sternum, breath retention, with a subsequent discharge of foam from the mouth during vomital movements, a fleeting absence of conciousness, transitory motor and sensory impairments, a rise in temperature, microhemorrhaging in the conjunctiva and skin, andd a moderate leucocytosis. A complete account of the fifth case is included in the report. In this case all symptoms disappeared within 3 weeks and no subsequent complications developed (170).

II. Short-Term Studies

Mice

In a preliminary experiment conducted by Aoki and Tani, special-grade 30% H_2O_2 was diluted with pure water and given to 3-week-old mice in place of drinking water. He concentrations of H_2O_2 given were 9, 4.5, 2.0, 1.0, 0.6, 0.3 and 0.15%. Concentrations in excess of 1% resulted in pronounced loss of body weight and death within 2 weeks. When the H_2O_2 solution was switched to water during the experiment, the weight loss was quickly recovered, indicating that the H_2O_2 exerted no fatal damage to the tissues of the internal organs. Some of the mice given 0.6% H_2O_2 exhibited a drop in growth rate to 80% three weeks later, but when the solution was replaced with water the body weight showed a favorable gain. With 0.3% H_2O_2, about 10% weight lose occurred within 1 week, and when the solution was changed to 1.0% the loss of body weight became more severe (37).

For a period of 35 weeks, Aoki and Tani replaced the drinking water of 16 mice, initially 30 days old, with an 0.15% aqueous H_2O_2 solution. Eight mice were maintained on regular drinking water as controls. From the 13th week on mice were sacrificed at 1-2 week intervals for histological examination of the liver, kidneys, spleen, small intestine and stomach. The body weight gain and macroscopic appearance of the treated animals showed no appreciable differences from the controls. Histological examination of 4 mice sacrificed in the 13th week revealed no pathological changes in any of the tissues studied. Small necrotic foci appeared scattered in the liver and wall of the stomach in the 16th week. In the 22nd week all of the organs studied showed pronounced changes, which became progressively more severe. By the 28th week hydropic degeneration f hepatic tissue and the epithelia of the renal tubules and hemosiderin formation in the spleen were prominent. The stomach wall also showed slight necrosis, inflammation, and irregularity of tissue structure. By the 35th week the lymphatic tissue of the small intestinal wall became hypertrophic (37).

Lorincz et al. treated 30 young mice, weighing approximately 15 g each, with 0.1 ml of 1.5% hydrogen peroxide in normal saline solution subcutaneously twice daily for a period of two weeks. At termination of the test period, sections of heart, lung, kidney, liver, spleen, gastrointestinal tract, pancreas, bone and mediastinal structures were subjected to histological examination. Several animals showed brief embolic signs immediately after the injections. Other commonly oobserved signs were brief generalized convulsions and paraplegia lasting for a few minutes. Five of the mice died with embolic symptoms at various times during the course of the experiments. In all cases the injections caused local s.c. emphysema, and small ulcers eventually developed at the injection sites in about 25% of the animals. Aseptic technic, however, was not followed in making the injections (750).

Rats

Romanowski et al. conducted two similar series of experiments in which male albino rats (unspecified number) were maintained on a regimen in which they received ad libitum in place of drinking water, a solution of 0.25, 0.5, 2.5, 5, or 10% hydrogen peroxide. Signs of toxicity were observed in all of the rats receiving H_2O_2. In the first series the control animals had a mean body weight after 290 days of 480 g and one death; those receiving 0.5% H_2O_2 obtained a mean body weight of 320 g and there were 8 deaths. In the second series after 146 days the corresponding figures were: controls 480 g and no deaths; with 0.25% H_2O_2, 452 g and one death; with 0.5% H_2O_2, 375 g and 2 deaths. A concentration of 0.25% H_2O_2 had no effect on the blood pressure, but 0.5% caused a slight increase. All of the rats given 2.5, 5, or 10% H_2O_2 died within 43 days (1097).

Hankin performed a series of tests on the effect of hydrogen peroxide on the growth and reproduction of rats. The animals. Osborne-Mendel strain rats, were maintained on a commercial dog food meal and received ad litibum as drinking water either tap water or an 0.45% solution of H_2O_2 (487).

During a 3-week test using 2 groups (unspecified number) of weanling male rats, the animals on tap water drank an average of 544 ml each, while those on H_2O_2 averaged only 282 ml each. The decreased amount of liquid intake was reflected in the total body weight; 156 g for those on tap water against 108 g for those on peroxide. No differences on the fresh weight of the testes, kidneys or spleen of the two groups were found (487).

In order to equalize any effects of limited intake of liquid, two groups (unspecified number) of male weanling rats were maintained for 3 weeks on a regimen in which the rats receiving tap water were limited to a quantity equal to that consumed by the rats receiving H_2O_2. At termination of the test, the average body weight was 116 g for those on peroxide and 104 g for those on tap water. Fresh and dry weights of the testes, kidneys, spleen and heart showed no differences between the two groups (487).

Three weanling female rats were given 0.45% H_2O_2 for 5 months, after which they were switched to tap water and were mated with normal males. Normal litters were produced. Six male litter mates were divided into 2 groups and given either peroxide or tap water for a period of 9 months. The animals given tap water reached a weight of 521 g, while the treated animals reached only 411 g. No other effects were noted (487).

Three older male rats with an average weight of 600 g were given 0.45% H_2O_2 for 6 weeks. At that time their average weight had fallen to 511 g. They were then given tap water and within 2 weeks had regained the lost weight (487).

To test the toxicity of hydrogen peroxide, Kawasaki, et al. divided 32 male istar rats into 4 groups and treated them daily with 0.5 ml oral doses of 0, 0.12, 1.2, or 12% H_2O_2. The group given 0.6 mg/day exhibited normal growth, while those given 6 mg/day displayed a slight inhibition. In the group given 60 mg/day, the intestines became swollen immediately after dosing, with four of the rats dying. The treatment of this group was terminated thereafter for those rats which remained alive and a normal growth curve was shown from the fourth day on. After 21 days, all of the animals were sacrificed and the weight of their organs determined. No significant changes were observed (621).

In another experiment performed by Kawasaki et al., groups of 9-12 male Wistar rats weighing about 70 g were treated, by gastric intubation, with 0.1 ml doses of hydrogen peroxide solutions in concentrations such that they received 0, 0.6, 1, 2, 3, or 6 mg H_2O_2/100 g body weight/day. After 40 days of such treatment alf of the animals were sacrificed, while the rest were continued on treatment for an additional 60 days. The rats given 6 mg/100 g/day showed a significant drop in growth rate from the 20th day, but the growth rates of all of the other groups were comparable to that of the controls. Upon determination of organ weights, only those rats given 6 mg/100 g/day showed any changes. Their spleens showed significant increases in weight after 40 days, but no differences from the controls were noticeable at termination of the study. No significant changes in weight were shown by the livers or kidneys of any of the groups. After 100 days of treatment the rats given 6 mg/100 g/day, but not those of any other group, exhibited decreases in hematocrit value, plasma protein concentration and plasma catalase activity (622).

III. Long-Term Studies

Mice

To test the effect of hydrogen peroxide administration on lifespan, Molnar gave 16 albino female mice subcutaneous injections of 0.01% H2O2 in 0.5 ml doses 2 or 3 times per week from the age of 6 weeks to 14 months; altogether 95 injections per animal. A second group of 16 female mice was given physiological sodium chloride injections in the same doses and a third group of 16 was maintained without treatments. As shown in Figure 2, the hydrogen peroxide administration caused considerable (24.5%) decrease in the average lifespan and an early loss of body weight (870).

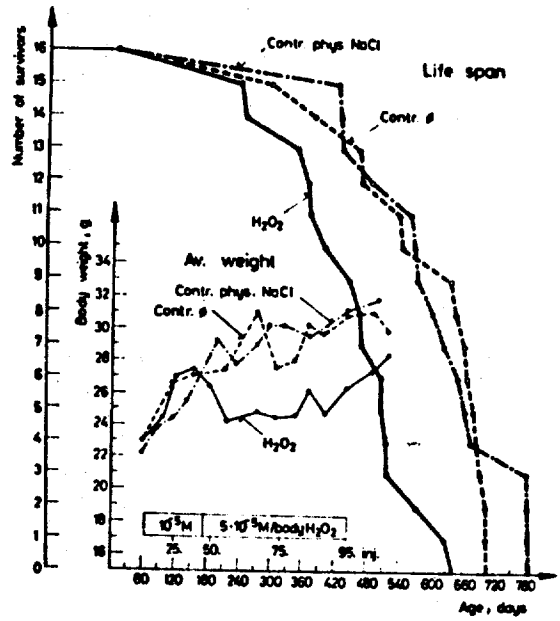


Fig. 2 The effect of prolonged microdoses of H_2O_2 on the lifespan and body weight of female albino mice. Average and maximum lifespans/day: 446.2 and 639 in the H_2O_2 group; 595 and 785 in the physiological NaCl group; 584.2 and 704 in the untreated group.

IV. Special Studies

Reproduction

In a study by Wales et al., 36 three-month-old male albino mice were divided into 3 groups of 12 and were given, in place of drinking water, 0.33, 1, or 3% H_2O_2. The group given 3% H_2O_2 refused to drink and after 5 days was removed from the experiment. The remaining 2 groups were each divided at random into 4 subgroups of 3 mice each for subsequent treatment as follows:

1. Two female mice were placed with each male on Day 7 and were allowed to mate. The pregnant females were removed before parturition. On Day 28 another pair of females were placed with each male;

2. Two females were placed with each male on Day 21;

3. Two females were placed with each male on Day 21, at which time the H_2O_2 solution was replaced by tap water;

4. The mice were killed on Day 21 and the epididymal spermatozoa examined. All females became pregnant within a few days and in each case healthy young were born in litters of normal size.

In subgroups 1 and 2, 1% H_2O_2 may have caused some delay in parturition, but this was questionable. Weight gain of the males during the first 3 weeks was equal in all subgroups. However, during the ensuing fortnight the mice in subgroups 1 and 2 gained somewhat less rapidly than those in subgroup 3. The concentration, morphology, and motility of the spermatozoa from subgroup 4 appeared normal (1352).

In an additional test by Wales et al., the drinking water of 3 albino rabbits was replaced by 0.33, 1, and 3% H_2O_2 solution. Examination of the semen at weekly intervals for 6 weeks revealed no changes in concentration, morphology or motility (1352).

Teratology

Kaufman (cited by Schumb et al. (1161)) reported that the injection of hydrogen peroxide into the whites of eggs did ot impair the development of the embryo.

Carcinogenicity

In a test conducted by Schmidt, hydrogen peroxide was injected s.c. once or 3 times into the backs of 649 newborn mice in a concentration of 0.6% in Ringers solution at a dose of 0.1 ml. As shown in Table 4, those mice given only one injection and surviving at least 6 months displayed a tumor incidence of 20%, with a latent period of 20 months. When 3 injections were given, the incidence of cancer rose to 33% and the latent period was reduced to 15 months. It was noted that the observed tumors did not appear directly in the injection area. Under ordinary conditions, C57B1 mice exhibit a spontaneous tumor frequency of 10-20%. AB strain mice maintained on a protein-rich feed presented a spontaneous mammary cancer rate below 5% and a spontaneous leucemia frequency of 2% (1151).

Table 4 Tumor Findings after Injection of Hydrogen Peroxide into Newborn Mice

Number of Animals	Strain	Number of Animals Evaluated (At least 6 mo. old) Male	Female	Still Alive	Findings	Latent Time	Dosage
17	AB	1			-		1x0.1 ml
27	AB		2		-		1x0.1 ml
55	AB				-		1x0.1 ml
42	AB				-		1x0.1 ml
40	AB				-		1x0.1 ml
77	AB	6	9		1 leucemia	18	1x0.1 ml
	C57B1				1 breast cancer	24	1x0.1 ml
	A/JAX						
26	X	3	6		1 leucemia + breast cancer	15	1x0.1 ml
	C57B1				1 lymphosarcoma of the thymus	20	1x0.1 ml
					1 leucemia	21	1x0.1 ml
					1 breast cancer	22	1x0.1 ml
19	AB	2	1		-		1x0.1 ml
303		12	18		6 tumor animals (20%)	20	
61	AB	3	5	2	1 breast cancer	14	3x0.1 ml
					1 breast cancer	17	3x0.1 ml
					1 double breast cancer	17	3x0.1 ml
					1 lung cancer	19	3x0.1 ml
21	AB	2		1	-		3x0.1 ml
29	AB		1		-		3x0.1 ml
29	AB	2	1		1 breast cancer	22	3x0.1 ml
31	AB		7		1 lung adenoma	10	3x0.1 ml
11	AB	1	4		1 hemangiosarcoma of the liver	15	3x0.1 ml
					1 breast cancer	16	3x0.1 ml
					2 breast cancer	22	3x0.1 ml
					1 leucemia	17	3x0.1 ml
52	AB	1			1 leucemia	12	3x0.1 ml
29.	AB	2	1	1	1 ovary tumor both sides	16	3x0.1 ml
21	AB	3	2		1 leucemia + lung adenoma	19	3x0.1 ml
62	AB	7			-		3x0.1 ml
346		21	21	4	14 tumor animals (approx. 33%)	15	
649		33	39	4	20	18	

In 1959, Peter Kovacic, although not the first to do so, presented a proposal suggesting that cancer may originate in the action of an agent, derived from either an artificial carcinogen or a spontaneous process, which directly or indirectly generates hydrogen peroxide in abnormally high concentrations. In short, Kovacic suggested that carcinogens act through the formation of the intermediary product hydrogen peroxide and that - is this substance that actually induces cancer (690, 691).

Since great advances have been made in the field of oncology since Kovacic made this proposal and this proposal is not currently accepted, it may be assumed that it has been disproven.

Mutagenicity

Schoeneich performed an experiment which demonstrated that hydrogen peroxide can produce chromosomal aberrations in ascites tumors in mice and that this effect is positively correlated with the concentration of H_2O_2 injected. The experimental material consisted of 3 hyperdiploid ascites tumor strains: the S_2 sarcoma, the Ehrlich ascites carcinoma and the sarcoma 180 which were grown in the inbred strain AB/Jena Gat. Hydrogen peroxide was injected i.p. 48 hours after implantation of the tumor and cytological preparation of the treated cells was carried out at various times thereafter. The data obtained are presented in Table 5 (1155).

TABLE 5

INDUCTION BY H_2O_2 OF CHROMATID ABERRATIONS IN THE S_2 SARCOMA, EHRLICH ASCITES CARCINOMA AND SARCOMA 180, AND ITS DEPENDENCE ON THE H_2O_2 CONCENTRATION APPLIED

Cells with all types of chromatid translocations, duplication deletions and (in favorable circumstances) deletions were scored. Cells with "gaps" only were excluded from scoring.

	Concentration (M)	*Number of animals*	*Number of cells scored*	*Metaphases with aberrations*	
				Number of metaphases	*%*
S_2 sarcoma	10^{-2}	4	600	72	12.0 ± 8.0[a]
	$5 \cdot 10^{-2}$	4	600	158	26.3 ± 7.2
	10^{-1}	4	600	179	29.8 ± 7.7
	$5 \cdot 10^{-1}$	3	450	201	44.7 ± 6.3
	Control	3	450	0	0
Ehrlich ascites carcinoma	10^{-2}	2	300	59	19.6 ± 4.8
	$5 \cdot 10^{-2}$	3	450	149	33.1 ± 11.3
	10^{-1}	3	450	119	26.4 ± 9.5
	$5 \cdot 10^{-1}$	1	100	38	38.0 ± 2.8
	Control	5	1000	2	0.2
Sarcoma 180	10^{-2}	2	300	13	4.3 ± 2.6
	$5 \cdot 10^{-2}$	2	300	48	16.0 ± 2.8
	10^{-1}	2	300	87	29.0 ± 5.2
	$5 \cdot 10^{-1}$	2	no mitoses		
	Control	2	300	0	0

[a] Standard error of the mean.

Caustic Action

Application of hydrogen peroxide to the skin or mucous linings of the body results in a whitish discoloration, accompanied by peculiar sensations - tickling and itching, which can sometimes increase to a stabbing pain. While it has long been and still commonly is believed that this phenomenon is a caustic action, research has been performed which refutes this idea. According to Hauschild et al. who studied this phenomenon in the skins, mucous membranes, and serous skins of dogs, cats, rabbits, guinea pigs, rats and mice with 1-30% H_2O_2 solutions, the whitishness observed upon H_2O_2 application is the result of the formation of gas bubbles within the tissue and the resultant temporary bloodlessness of the capillaries. Significant damage results only when the effect of H_2O_2 is so intensive that gas formation in the tissue results in mechanical damage or inhibits circulation such that the nutritive supply to the tissue is disturbed (499).

The validity of the report by Hauschild et al. (499) was in some measure corroborated through studies conducted by Ludewig (758, 760), in which he confirmed that H_2O_2 can penetrate the epidermis and mucous membranes and decompose in the underlying tissues, producing emphysemas and emboli as stated by Hauschild et al.

While Hauschild et al. (499), and several researchers whom they cited, reported that concentrations of H_2O_2 up to 30% exert no caustic action, Krop (699) has stated that 90% H_2O_2 can cause severe urns upon prolonged contact to the skin. No further information was given by Krop, so it is possible that the mechanisms involved were those reported by Hauschild et al.

Atmospheric Exposure

In testing the effect of atmospheric exposure of mice to hydrogen peroxide, Punte et al. exposed groups of white mice to aerosols of 90% H_2O_2 at the concentrations and for the times shown in Table 6. The mortality and survival times of the animals are also presented in Table 6. Upon exposure to the low concentrations the animals exhibited mild nasal irritation, blinking of the eyes, slight gasping, and a loss of muscular coordination, these symptoms generally disappearing within 30 minutes after discontinuation of treatment. Minor H_2O_2 burns were observed on the nose and paws of most animals. Exposure to higher concentrations resulted in similar, but more severe, symptoms. Premortal convulsions were observed in animals receiving lethal doses. The burns of the nose and paws incurred with non-lethal doses disappeared in less than a week, but the eye inflammation persisted for another week. At the end of the 2nd week the signs of primary irritation had disappeared from the eyes, except for 2 cases exposed to 19 mg/l for 10 minutes, in which the corneas showed gross opacities persisting throughout the test period. Pathological examination of the mice exposed to sublethal concentrations showed congestion of the lungs when sacrificed at various intervals over a period of 10 days after exposure. Four of the 20 mice

exposed to 5.2 mg/l showed some necrosis of bronchial epithelial cells. Mice which died during or within about an hour after exposure to 9.5-19 mg/l showed pulmonary congestion in most instances. Animals which survived these concentrations for several days or had been sacrificed after 5 and 8 weeks showed necrosis of bronchial epithelium. Gross opacities were present in the eyes of 4 animals exposed to the highest concentration (19 mg/l) and sacrificed after 8 weeks. Animals sacrificed 5 weeks after exposure to this concentration did not show gross eye lesions, but did show microscopic lesions. Microscopic lesions were also found in the eyes of the animals exposed to 9.4 mg/l eight weeks after exposure, while those examined 5 weeks after exposure to this concentration showed no significant changes (1039).

TABLE 6

Toxicity of 90% H_2O_2 Aerosol to Mice by Inhalation

Exposure Time min.	Analytical Concentration mg./l.	Mortality	Survival Time
5	3.6	0/10	---
5	4.2	0/10	---
5	5.2	0/10	---
5	5.2	0/10	---
5	9.4	1/10	6 days
10	13.2	5/10	*
10	13.4	1/10	6 min.
10	19.0	1/10	11 min.
15	11.8	5/10	5-50 min.
15	16.7	9/10	**

* 4/10 died between 12 and 120 minutes; a fifth died after 6 days
** 8/10 died between 11 and 48 minutes; a ninth died the next day

The inhalation toxicity of hydrogen eroxide vapor was tested by Oberst et al. through a series of experiments performed on mice, rats, dogs and rabbits (948).

In three separate 8-hour runs, six rats were exposed for 8 hours to a hydrogen peroxide concentration calculated to be 4000 mg/m^3 (2880 ppm) and were then killed at various times up to 14 days for pathological examination. No signs of intoxication were observed in the rats and no deaths resulted from the treatment. Post mortem examination of the trachea and lungs from all animals revealed severe congestion. Those animals killed during the first three days following exposure showed small localized areas of pulmonary edema without hemorrhage. In those animals killed between the 4th and 14th days, the majority of the lungs exhibited many

areas of alveolar emphysema in addition to the severe congestion. There were no signs of necrosis of the pulmonary mucosa. All other organs examined appeared normal (948).

In another test, 10 rats were exposed to a concentration ranging from 338 to 427 mg/m^3 (243 to 307 ppm) in each of 3 runs; one run persisted for 4 hours and the other 2 for 8 hours. After xposure, the animals were submitted for pathological examination at various times up to 14 days. The only outward evidence of toxic effects was scratching and licking. After 30 minutes the animals became quiet and slept most of the time. Pathological examinations disclosed results similar to those described in the previous test (948).

In testing the subacute inhalation toxicity, Oberst et al. exposed 23 rats to a concentration of 93 mg/m^3 (67 ppm), 10 mice to 79 mg/m^3 (57 ppm) and 10 mice to 107 mg/m^3 (77 ppm) six hours per day, five days per week, for 7, 3, and 4 weeks, respectively (a total of 30, 8, and 18 exposures, respectively). At the end of each week of exposure 1 or 2 rats were sacrificed for pathological examination. After two weeks of exposure, the animals showed profuse nasal discharge, the bottom of the feet became edematous, and an acute irritation developed in the region of the groin. During the fifth week of exposure there was a denudation of the hair around the nose, which was assumed to be a result of scratching by the animals in an effort to alleviate irritation. The exposure to hydrogen peroxide produced no significant changes in body weight gain. Mortality was increased greatly for the mice, but only slightly for the rats: 7 of 9 mice died after 8 exposures to 79 mg/m^3; 8 of 10 mice died after 18 exposures to 107 mg/m^3; 1 of 10 rats died after 30 exposures to 93 mg/m^3. Upon autopsy (of 13 rats), some of the organs showed congestion, but microscopic examination failed to reveal significant pathological changes (948).

Two dogs were exposed six hours a day, five days a week for six months to an average hydrogen peroxide vapor concentration of 10 mg/m^3 (7 ppm). Approximately once a month a sample of blood and urine from each dog was analyzed, the following determinations being made: whole blood - pH, sedimentation rate, oxygen content, nonprotein nitrogen level, red and white blood cell count, and hemoglobin; serum - bicarbonate, chloride, sugar, sodium, urea, calcium, phosphorus, and cholesterol; plasma - protein; urine (catheterized) - pH, specific gravity, albumin, sugar, and microscopic sediments. No signs of toxicity were observed until the 24th week, at which time the dogs began to sneeze occasionally after 5 hours exposure; lacrimation was also present. In the 25th week the dogs frequently refused food but ate again normally during the 26th and 27th weeks. By the 14th week the hair of both dogs showed bleaching, and some denuded areas were present over the back. The exposure to hydrogen peroxide produced no effect on body weight gain nor mortality. Sacrifice and examination of the dogs at termination of the test showed the skin was greatly thickened and there was marked loss of hair, but no destruction of hair follicles. The lung was the only internal organ which showed any abnormality. In this organ there were patchy areas of

atelectasis intermingled with emphysematous areas. There were some hyperplasia of the bronchial musculature and tortuosity of the bronchi. The small terminal bronchioles and respiratory bronchioles had hyperplastic muscular coats. Scattered throughout all areas of the lung tissue were small buds of red-staining tissue, and many of them were lined with cuboidal cells, which were most prominent in areas where the alveolar walls seemed to be fragmented. The musculature of the terminal or respiratory bronchioles seemed to have become hyperplastic, as if there had been a change in the connective stroma of the alveolar walls. Connective tissues and elastic tissue stain revealed these circular areas to be composed mainly of collagen, with occasional muscle cells and occasional strands of elastic tissue admixed. No significant changes in blood or urinary constituents were observed (948).

In a study by Oberst et al., eight black rabbits were exposed to hydrogen peroxide vapor at a concentration of 30 mg/m^3 (22 ppm) six hours per day, five days a week for a total of 60 exposures. Weekly ophthalmoscopic examination of the eyes revealed no significant changes (948).

The inhalation toxicity of hydrogen peroxide and ozone-hydrogen peroxide mixtures was tested by Svirbely et al. using mice, rats, and hamsters. The results of the tests are presented in Tables 7-10. A single 4-hour exposure to a hydrogen peroxide concentration of 16.1 ppm produced no deaths among 10 mice and 6 rats. The results of these tests suggest that the toxicity of mixtures of H_2O_2 and O_3 are more pronounced than that of either component separately. It was also found that some degree of tolerance against lethal doses of H_2O_2 and O_3 could be afforded by previous exposure to H_2O_2. In addition, prior exposure to O_3 was found to protect against the toxic effects of a mixture of H_2O_2 and O_3 (1250).

TABLE 7

Acute Inhalation Exposures (4 Hours) of Male Rats and Mice to Hydrogen Peroxide-Ozone Mixtures

Average chamber conc'n (ppm)		Species	Average weight (gm)	Mortality following exposure No. dead / No. exposed	
H_2O_2	O_3*			24 hrs	2 weeks
16.1	—	M	25	0/10	0/10
		R	220	0/5	0/5
—	1.1	M	25	0/45	Used for other studies
—	1.0	R	230	0/40	" " " "
16.0	0.90	M	27	3/10	
		R	252	1/5	1/5
7.8	0.96	M	27	2/10	2/10
		R	260	2/5	2/5
3.3	1.0	M	25	3/10	3/10
		R	272	0/5	0/5
1.5	0.92	M	26	0/10	0/10
		R	260	0/5	0/5

M—Mice, R—Rats.

* Exposure in large chamber, 100 cu. ft. capacity. The ozone used in this chamber was generated in a U. S. Air Ozonizer, Scottdale Ozone Company, Scottdale, Pa., as previously described.[11]

TABLE 8

Species Variation of Rodents in Single Inhalation Exposures to Hydrogen Peroxide and Ozone

Average chamber conc'n (ppm)		Species	Average weight (gm)	Duration of exposure (hours)	Mortality following exposure No. dead / No. exposed	
H_2O_2	O_3				24 hrs	2 weeks
24.8	1.5	H	112	4	0/10	0/10* (Group A)
		M	28	4	2/10	2/10
35.7	7.6	H	120	4	0/5	0/5* (Group B)
		M	26	4	5/10	5/10
—	44.6*	H	105	1½	0/7	(Group C)
—	16.9*	R	281	1	10/10	2 dead 10 min. before end of exposure 3 dead ½ hr after end of exposure 3 dead 1 hr after end of exposure 2 dead 1½ hrs after end of exposure

H—Hamsters, R—Rats, M—Mice.

* Large chamber, 100 cu. ft. capacity used for ozone exposure. Group A and B challenged about 6 weeks after end of observation period for 30 minutes with 35 ppm O_3.

1/10 and 3/10 hamsters in Group A died 3 and 6 hrs respectively after end of exposure. Surviving hamsters in Groups A, B, C, sacrificed 24 hours, 76 hours, and 6 days after end of exposure for pathologic observation.

TABLE 9

Responses of Mice Previously Exposed to Ozone When Challenged with Hydrogen Peroxide-Ozone Mixtures (4-Hour Exposures)

Exposure group	Chamber O_3 conc'n av. (ppm)	Recovery period (days)	Challenging dosages average chamber conc'n (ppm)		Mortality following exposure No. dead / No. exposed	
			H_2O_2	O_3	24 hrs	2 weeks
1	1.6	2	36.3	—	0/30	0/30
	Controls		37.4	—	0/10	0/10
2	1.6	3	—	5.7	0/30	0/30
	Controls		—	5.7	8/30	8/30
3	1.4	5–6	227.	—	7/25	14/25
	Controls		227.	—	5/25	22/25
4	1.6	4	31.5	5.6	0/30	0/30
	Controls		31.5	5.6	12/30	13/30

TABLE 10

Responses of Mice Previously Exposed to Hydrogen Peroxide when Challenged with Hydrogen Peroxide-Ozone Mixtures (4-Hour Exposures)

H_2O_2 chamber concentration (ppm)	Recovery period (days)	Challenging dosages average chamber conc'n (ppm)		Mortality following exposure No. dead / No. exposed	
		H_2O_2	O_3	24 hrs	2 weeks
5.6 (5 days)†	3	—	5.4	2/10	3/10
Controls		—	5.4	6/10	7/10
5.6 (5 days)†	3	78.1	—	0/10	0/10
Controls		78.1	—	0/10	0/10
5.6	4	—	5.6	4/10	4/10
Controls		—	5.6	7/10	7/10
16.3	2	—	5.8	9/10	10/10
Controls		—	5.8	9/10	10/10
46.2	3	—	5.8	1/10	1/10
41.4	2	—	5.6	3/10	4/10
Controls		—	5.6	8/10	8/10
40	7	113.	—	0/10	2/10
Controls		113.	—	1/10	4/10
40	8	194.	—	0/10	2/10
Controls		194	—	3/10	6/10
41	8	—	6.1	7/10	7/10
Controls		—	6.1	10/10	10/10
41	4	226.	—	1/10	5/10
*C H.(1/5 LC$_{50}$)	7	226.	—	1/10	1/10
Controls		226.	—	2/10	5/10

* C H. (Cumene Hydroperoxide) – 6 hour exposure to 100 ppm (inlet calculated concentration)

† Exposure 4 hours per day for 5 days at 5.6 ppm H_2O_2

Cariogenicity

The effect of hydrogen peroxide on the occurrence of dental caries in rats was studied by Shapiro et al. using 96 male Holtzman rats, weighing an average of 51.8 g. The rats were divided into 4 equal groups and given ad libitum as drinking fluid, for a period of 8 weeks, a solution of 0 (I), 0.5 (II), 1.0 (III), or 1.5% (IV) H_2O_2. Growth was significantly retarded in all of the treated rats, the degree of retardation being proportional to the peroxide concentration. At the end of the test period the average bodyy weights of the various groups were as follows: group I, 331.6 g; group II, 222.9 g; group III, 79.6 g, and group IV, 59.6 g (7 of these rats died). Histological examination revealed extensive carious lesions and pathological changes in the periodontium of all of the surviving rats of group IV and 15 of the rats in group III. Only seven of the 24 rats in group II had caries, and these were of a milder degree and were not accompanied by periodontal damage. A second test in which 2 groups of 10 rats each were given 1.0 or 1.5% H_2O_2 ad lib in the drinking water produced essentially the same results (1177).

Therapeutic Applications

The possible use of hydrogen peroxide as a source of extrapulmonary oxygen has been extensively investigated. It has been demonstrated that H_2O_2 can be an adjunctive source of oxygen for the anoxic or ischemic heart and can improve resuscitation in refractile arrhythmias or cardiac arrest. Intra-arterial infusion has been reported to reverse the atherosclerotic process, to be effective in the treatment of tumors, to potentiate the effect of irradiation on malignant tumors, to increase the localization of radioactive isotopes in malignant tumors, to protect against Clostridium welchii infections, and to promote wound healing. In addition to intra-arterial administration, other routes which have been tested include intravenous, cutaneous, subcutaneous, intra-oral, oral, and by enema (1318).

It must be mentioned, however, that the therapeutic administration of hydrogen peroxide, expecially i.v., has been shown to involve certain hazards, which range in degree from negligible to lethal. Methemoglobin formation and gas emboli are results often encountered upon rapid or extensive H_2O_2 administration. Toxic actions on blood tissue cells have also been reported. The extent of these effects has been correlated, inversely, to the blood catalase level. Gas embolism, which is apparently the most important of these effects, is discussed elsewhere. Methemoglobin formation and changes in blood tissue cells are observed only in animals with low blood catalase levels (man has a high level)or in cases where large or rapid doses of H_2O_2 are administered i.v., making these effects irrelevant to the toxicity of H_2O_2 as a food additive (459, 750, 355).

Biochemical Aspects

I. Breakdown

No information available

II. Absorption - Distribution

Ludewig reported that upon sublingual application of hydrogen peroxide to dogs, cats, and rabbits in concentrations of 3 to 30%, significant amounts of the H_2O_2 were absorbed, as evidenced by the presence of bubbles in the tongue and jugular veins (758).

The sublingual absorption of H_2O_2 in cats was verified by Ludewig in a later study using ^{18}O-labeled H_2O_2. It was found that within 58 minutes 33% of the ^{18}O of a 9% solution of H_2O_2 was present in the expired air; for a 19% solution the corresponding time was 34 minutes (761).

Perfusion of the large intestine of dogs with dilute H_2O_2 has been shown to elevate the oxygen content of the blood, indicating that the H_2O_2, or its degradation products, were absorbed by the intestine (1318).

III. Metabolism and Excretion

Although no articles were found which dealt directly with the metabolism of hydrogen peroxide, many authors have stated, as though it were a well-known fact, that H_2O_2 is readily broken down to water and O_2 by the enzyme catalase, which is found widely distributed in animal tissues (1161, 1318, 760, 750).

IV. Effects on Enzymes and Other Biochemical Parameters

Following tests on dogs and humans, Culmer et al. reported that hydrogen peroxide in solutions varying from 0.5 to 3% when used as a gastric lavage generally depress the acidity of the gastric contents, the extent of depression varying directly with the concentration of H_2O_2. Stimulation of mucous secretion by the stomach was also effected by H_2O_2. The effects on gastric secretion were most marked immediately after lavage, recovery occurring in from 24 hours to 15 days, depending on the individual susceptibility and the concentration of peroxide used. When the higher concentrations were employed, considerable discomfort was reported by the patients, and it was noted that H_2O_2 may be active in inducing hemorrhage from an ulcer (248).

V. Drug Interaction

No information available

VI. Consumer Exposure

The following tables were compiled in 1972 from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

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TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
HYDROGEN PEROXIDE NAS 0098	01	BAKED GOODS(R)	*	.00000	.00000
	03	OTHER GRAIN(R)	*	.00000	.00000
	04	FATS OILS(R)	*	.00000	.00000
	10	MEAT PRODS(R)	*	.00000	.00000
	20	GELATIN PUD(R)	*	.00000	.00000
	26	RECONST VEG(R)	*	********	********
	34	INS COF TEA(R)	*	********	********

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TABLE 11, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
HYDROGEN PEROXIDE NAS 0098	6/ 7	299,333	862,437	917,437			917,437

TABLE 13, PART A — POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
HYDROGEN PEROXIDE NAS 0098	01	BAKED GOODS(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	.000000	.000000	.000000
HYDROGEN PEROXIDE NAS 0098	03	OTHER GRAIN(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	.000000	.000000	.000000
HYDROGEN PEROXIDE NAS 0098	04	FATS OILS(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	.000000	.000000	.000000
HYDROGEN PEROXIDE NAS 0098	10	MEAT PRODS(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	.000000	.000000	.000000
HYDROGEN PEROXIDE NAS 0098	20	GELATIN PUD(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	.000000	.000000	.000000
HYDROGEN PEROXIDE NAS 0098	26	RECONST VEG(R)	*	0-5 MO.	**********	**********	**********
				6-11 MO.	**********	**********	**********
				12-23 MO.	**********	**********	**********
				2-65+ YR.	**********	**********	**********
HYDROGEN PEROXIDE NAS 0098	34	INS. COF TEA(R)	*	0-5 MO.	**********	**********	**********
				6-11 MO.	**********	**********	**********
				12-23 MO.	**********	**********	**********
				2-65+ YR.	**********	**********	**********
HYDROGEN PEROXIDE NAS 0098	ALL DATA GROUPS		6	0-5 MO.	.000000	.000000	.000000
	**********			6-11 MO.	.000000	.000000	.000000
	**********			12-23 MO.	.000000	.000000	.000000
	**********			2-65+ YR.	.000000	.000000	.000000

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Aoki, M., and Y. Tani: GROWTH AND HISTOPATHOLOGIC CHANGES IN MICE DRINKING HYDROGEN PEROXIDE SOLUTION INSTEAD OF WATER. Igaku to Seibutsugaku, Vol. 84, No. 3, pp. 159-162, 1972. Nagoya Women's College, Nagoya, Japan.

Hydrogen peroxide, which is widely used in food preparation for its bleaching and preservative action, has long been believed to be nontoxic since it decomposes into oxygen and water during the process of preparation or cooking, and in storage (1). In 1967, however, mass poisoning was caued by H_2O_2-treated noodles, which led to a discovery that hydrogen peroxide was retained in the food, and draw attention from the standpoint of food sanitation. The effect of hydrogen peroxide on animals has been studied by Takai (2), Kawasaki (3,4), and Katayama (5), but the periods of experiment in these studies were short, with 100 days as maximum. The authors gave H_2O_2 to mice for a prolonged period, and studied the growth and pathologic changes in the internal organs of the animals.

EXPERIMENTAL PROCEDURE

A solution containing H_2O_2 at a specific concentration was given to male dd mice in a water vessel, along with a sufficient amount of Clea (KUREA ?) CE-2 standard solid feed, and the animals were permitted to drink the solution instead of water upon demand.

As a preliminary experiment, special-grade 30% H_2O_2 was diluted with pure water and given to 3 weeks old mice at the height of the growing stage, upon demand. The concentration of H_2O_2 was changed from 9 to 4.5, 2.0, 1.0, 0.6, 0.3, and 0.15% in a descending order, and the concentration which resulted in normal, steady growth and produced no abnormal macroscopic change was chosen.

Experiment: Twenty-four, 30-day old dd mice were used. Six groups of mice, each consisting of 4, were placed in different cages. Four of them were experimental groups and the remaining two, the control. The experimental groups were given a 0.15% aqueous H_2O_2 solution in a vessel upon demand, and the amount remaining and the amount added were recorded at specific times daily and the average daily intake per animal was calculated. They were weighed twice weekly, and the experiment lasted for 35 weeks. From the 13th week from the beginning of experiment, the animals were killed by cutting the carotid artery at 1 - 2 weeks intervals, and the histopathologic changes in the liver, kidneys, spleen, small intestine, and stomach were studied.

RESULTS

Oral administration of a H_2O_2 solution at a concentration in excess of 1% to the mice caused pronounced loss of body weight and death within 2 weeks. In this experiment, the daily average intake by a young mouse weighing 8 - 13 g was 2.0 ml. When the H_2O_2 solution was switched to water during the experiment, the weight loss could be quickly recovered, which indicates that the high concentration of H_2O_2 exerted no fatal damage to the tissues of internal organs. Figure 1 shows the changes in body weight in some of the experimental cases. Some of the mice given a 0.6% solution indicated a drop of growth rate to 80% three weeks later, but when the solution was replaced with water, the

the body weight showed a favorable gain. With a 0.3% solution, about 10% weight loss occurred within 1 week, but when the solution was changed to a 1.0% solution, the loss of body weight became more severe.

Main Experiment: The body weight of the mice given a 0.15% aqueous H_2O_2 solution for 35 weeks showed no appreciable difference from that of the control or no macroscopic abnormality. The daily average intake per animal in this experiment was 3.95 ml (H_2O_2, 5.9 mg) of the solution and 5.0 g of solid feed whereas the control group consumed 5.45 ml of water and 5.85 g of solid feed.

The pathologic changes in the tissues of internal organs are shown in Table 1. Four mice displayed no morbid change in any of the tissues in the 13th week. Small nest-like necrotic foci scattered in the liver and the wall of the stomach in the 16th week. In the 22nd week, pronounced changes were found in all organs, i.e., the liver, kidneys, spleen, stomach, and the wall of the small intestine. The symptoms became aggravated progressively, and , by the 28th week, hydropic degeneration of hepatic tissue and the epithelia of the renal tubules, hemosiderin formation in the spleen were prominent. The wall of the stomach also showed slight necrosis, inflammation, and irregularity of tissue structure. By the 35th week, the lymphatic tissue of the small intestinal wall became hypertrophic.

DISCUSSION

The effect of H_2O_2 on the growth of animals has been studied by Katayama (5) and Kawasaki (3,4), but in their reports, they made no reference to pathologic changes in the tissues of internal organs. Kawasaki et al. gave H_2O_2 to rats at 0.6 or 6 mg per 100 g of body weight for 100 days, and observed slightly lower hematocrit value, plasma protein concentration, and plasma catalase activity only when 6 mg of H_2O_2 was forced into the stomach with a tube. In their paper, no reference was made to fistologic findings. Katayama et al. administered 40 or 80 mg of hydrogen peroxide to 10 mice for 10 weeks, but no abnormal change in the tissues of the liver, kidneys, and spleen was reported. In these experiments, H_2O_2 was given mixed in the feed, and the mode of administration is different from that employed by the present authors, i.e., an aqueous H_2O_2 solution was given orally upon demand. It is interesting to note that clear pathologic changes were found in the tissues when the solution was given for more than 16 weeks. The permissible level of H_2O_2 according to the Food Sanitation Law is 100 ppm for noodles and pasty products. In terms of the dose for a mature human individual weighing 50 kg, the dose used in this experiment is equivalent to 7.4 g per day, 74 kg of the food containing the permissible level of H_2O_2. When the authors determined the H_2O_2 contents of commercial food products sold in Nagoya by Umemoto's method (6) (table omitted), a large variety of products such as noodles, pasty products, and "tofu" (bean curd) were found to contain H_2O_2, and 12 out of 62 showed H_2O_2 contents higher than the prescribed level. This observation indicates that the effect of H_2O_2 taken in small quantities over a prolonged period needs to be examined, and it should be determined whether H_2O_2 is safe as a food additive.

CONCLUSION

A 0.15% H_2O_2 solution was orally given to mice upon demand for 35 weeks.

The daily average intake of H_2O_2 per animal was 5.9 mg. The body weight grew steadily, but some pathologic changes were found, e.g., hydropic degeneration and hemosiderin formation in the hepatic and splenic tissues, and changes in the kidneys, small intestine, and the wall of the stomach.

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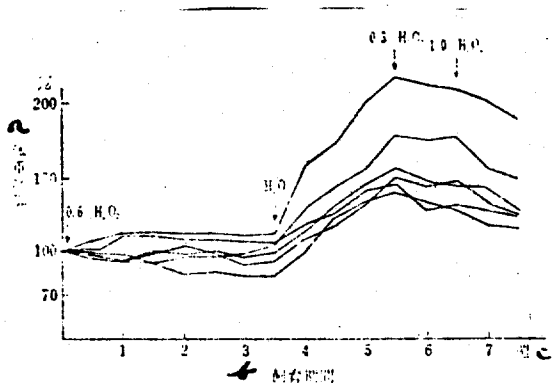

Figure 1. Changes in Body Weight in r Relation to H_2O_2 Concentration.

KEY: a, weight gain; b, time; c, weeks

TABLE 1. HISTOPATHOLOGIC CHANGES IN MICE GIVEN 0.15% H_2O_2 BY THE ORAL ROUTE

a	b	c	d	e	f	g	h
			肝	腎	脾	胃	小腸
13	4	1	1 異常なし	1 異常なし	1 異常なし	1 異常なし	1 異常なし
16	2	2	2 [illegible]	1 異常なし	1 異常なし	3 [illegible]	1 異常なし
22	1	3	3 [illegible]	2 [illegible]	2 [illegible]	4 [illegible]	3 [illegible]
21	1	3	3 [illegible]	3 [illegible]	1 異常なし	5 [illegible]	3 [illegible]
27	1	4	4 [illegible]	4 [illegible]	3 うっ血、ヘモジデリン沈着	2 ほとんど異常なし	2 変なし
28	1	3	3 [illegible]	4 [illegible]	3 うっ血、ヘモジデリン沈着	4 [illegible]	2 変なし
29	2	3	3 [illegible]	4 [illegible]	4 うっ血、ヘモジデリン沈着	1 [illegible]	2 変なし
34	2	5	5 [illegible]	4 [illegible]	3 うっ血、ヘモジデリン沈着	2 ほとんど異常なし	2 変なし
35	2	5	5 [illegible]	4 [illegible]	3 うっ血、ヘモジデリン沈着	5 [illegible]	4 [illegible]

HEADINGS: a, period of H_2O_2 ingestion (weeks); b, No. of animals; c, organ; d, liver; e, kidneys; f, spleen; g, stomach; h, small intestine.

Under heading d: 1, normal; 2, nest-like small necrotic foci; 3, partial hydropic degeneration; 4, hydropic degeneration of considerably high severity.

Under heading e: 1, normal; 2, partial degeneration of renal tubule epithelial cells; 3, slight formation of hyaline droplets in renal tubule epithelium; 4, mild hydropic degeneration of renal tubule epithelium

Under heading f: 1, normal; 2, slight proliferation of lymphatic follicles; 3, congestion and hemosiderin formation; 4, congestion, hemosiderin formation, and accumulation of neutrocytes in the wall

Under heading g: 1, normal; 2, nearly normal; 3, partial proliferation of cells in the wall; 4, cystic dilation of part of the glandular lumen;

5, degeneration of the epithelial cells of the covering; 6, mild necrosis, inflammation, and irregularity of tissue structure; 7, cellular infiltration in parts of the mucous membrane; 8, slight atrophy of the mucuous membrane

Under heading h: 1, normal; 2, no marked change; 3, hydropic change of the tips of intestinal pleural villi; 4, hypertrophy of lymphatic tissue.

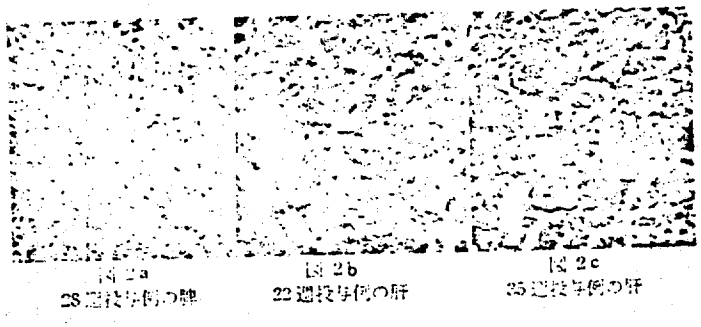

Figure 2a: Spleen, 28th week. Figure 2b. Liver, 22nd week; Figure 2c. Liver, 35th week.

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過酸化水素溶液を水のかわりに投与したハツカネズミの生育との組織変化

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食品の漂白と防腐のため広く使用されている過酸化水素 (H_2O_2) は，従来食品の加工，調理および貯蔵の過程において酸素と水に分解して無毒化されると考えられてきた[1]．しかし1967年 H_2O_2 処理麺類による集団中毒が発生し，食品中に H_2O_2 が残存することが明らかとなり，食品衛生学的見地から問題視されるようになった．H_2O_2 の動物生体におよぼす影響については高居[2]，川崎[3,4] 片山[5] の報告があるが，飼育期間は最大100日である．筆者はハツカネズミに H_2O_2 をなるべく長期間投与し，生育と内臓諸組織におよぼす病理学的変化を検討した．

方法

健康な dd 系ハツカネズミに給水器に入れた所要濃度の H_2O_2 を水の代りに投与し，クレア CE-2 標準固型飼料も充分量与えて任意に摂取させた．

まず予備実験として，体重増加の著しい生後3週間のハツカネズミに，特級30% H_2O_2 を純水で希釈して経口的に任意に摂取させた．H_2O_2 濃度は9，4.5，2.0，1.0，0.6，0.3，0.15%と，順次低濃度のものに変え，体重が順調な増加を示し，肉眼的にも何ら異常を認めない H_2O_2 の濃度を選定した．

実験：生後30日の dd 系ハツカネズミ24頭を使用し，4頭ずつ1組に入れて6群とし，4群を試験区，2群を対照区とした．試験群は0.15% H_2O_2 水溶液を給水器に入れて任意に摂取させ，毎日定刻に投与量と残存量を測定して摂取量を求め，1頭当りの1日平均摂取量を算出した．体重は週2回測定しながら35週間飼育した．一方実験開始より13週間経過した時から，1-2週間毎に動物の頸動脈を切って屠殺剖見し，肝，腎，脾，小腸，胃を病理組織学的に検索した．

結果

ハツカネズミに濃度1%以上の H_2O_2 溶液を経口投与すると体重は顕著に減少し，2週間以内に死亡する．この場合，体重8-13g の幼若動物の1日当りの平均摂取量は2.0ml であったが，実験中 H_2O_2 を水に変えれば，体重は速かに回

English Title for No. 7075: Growth and histopathologic changes in mice fed with hydrogen peroxide solution in stead of water. Mika Aoki and Yumiko Tani [Department of Home Economics, Nagoya Women's College, Nagoya] *Medicine and Biology*. 84(3): 159-162, March 10, 1972.

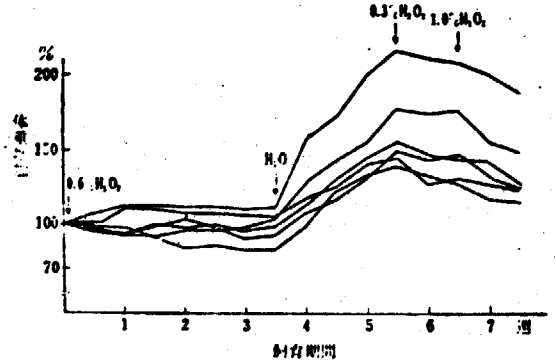

図1 H_2O_2 濃度変化による体重の消長

復し増加するため，内臓組織に致命的な損傷を受けていないものと推察した．実験例を図1に示す．0.6%溶液では3週間後，体重増加率は80%に減少するものがあるが，水に代えると著しく増加する．つぎに0.3%溶液を与えれば，1週

表1 0.15% H_2O_2 経口投与ハツカネズミの病理組織学的変化

H_2O_2 投与期間(週)	組織 / 動物数	肝	腎	脾	胃	小腸
13	4	異常なし	異常なし	異常なし	異常なし	異常なし
16	2	巣状小壊巣散在	異常なし	異常なし	壁細胞の部分的増加	異常なし
22	1	部分的に水腫様変性	尿管上皮細胞一部変性	リンパ濾胞やや増生	腺腔一部のう胞状拡張	小腸絨毛の尖端水腫様
24	1	部分的に水腫様変性	細尿管上皮軽度な硝子滴	異常なし	表層上皮細胞変性	小腸絨毛の尖端水腫様
27	1	かなりの水腫様変性	細尿管上皮やや水腫様変性	うっ血，ヘモジデリン沈着	ほとんど異常なし	著変なし
28	1	部分的に水腫様変性	細尿管上皮やや水腫様変性	うっ血，ヘモジデリン沈着	一部軽度壊死表層，組織の構造不規則	著変なし
29	2	部分的に水腫様変性	細尿管上皮やや水腫様変性	うっ血ヘモジデリン沈着，表層好中球集合	粘膜一部に細胞浸潤	著変なし
34	2	顕著な水腫様変性	細尿管上皮やや水腫様変性	うっ血，ヘモジデリン沈着	ほとんど異常なし	著変なし
35	2	顕著な水腫様変性	細尿管上皮やや水腫様変性	うっ血，ヘモジデリン沈着	やや粘膜萎縮	リンパ組織肥大性

間で体重は約10%減少し，さらに1.0%溶液に代えると著しく減少する．

本実験：0.15% H_2O_2 水溶液を与えて35週間飼育した場合の体重は対照群との間に顕著な差を認めず，肉眼的にも異常を認めなかった．本実験における1日1頭当りの平均摂取量は試験群において H_2O_2 溶液3.95 ml (H_2O_2 5.9 mg)，固型飼料5.0 g で，対照群は水5.45 ml，固型飼料5.85 g である．

内臓諸組織の病理学的変化は表1に示す．実験開始より13週間を経過したハツカネズミ4頭は，いずれの組織にも病学的変化なく，16週後に肝に部分的な巣状小壊死巣の散在と胃壁細胞の部分的な増加を認め，22週後には肝，腎，脾，胃，小腸壁などいずれの臓器にも明かな変化が現れた．その後これらの症状は次第に進行し，28週を経過すると肝組織の水腫様変性，腎細尿管上皮の水腫様変性，脾のヘモジデリン沈着等が顕著となり，胃壁細胞においても軽度な壊死，炎症，組織の不規則構造を認め，35週にはさらに小腸壁にもリンパ組織の肥大性をみるにいたった．

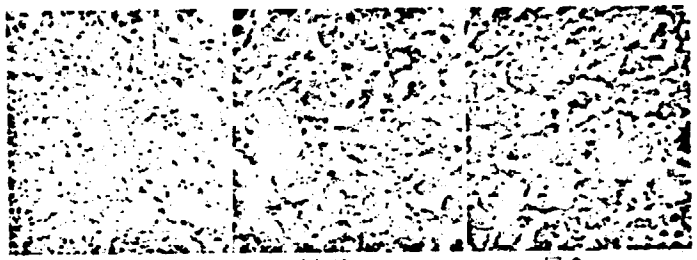

図2a 28週投与例の脾　図2b 22週投与例の肝　図2c 35週投与例の肝

考察

H_2O_2 の動物の成長におよぼす影響については片山[5]，川崎[3,4]の報告があるが，内臓諸組織の病理学的変化は認められていない．川崎らはダイコクネズミに体重160 g 当り6 mg または0.6 mg の H_2O_2 を投与して100日飼育し，ゾンデで胃腔内に6 mg の H_2O_2 を強制投与した場合のみヘマトクリット値，血漿蛋白濃度，血漿カタラーゼがわずかに低下するほか異常を認めていない．組織所見の報告はない．片山等はハツカネズミ10頭に H_2O_2 を40 mg または80 mg 投与して10週間飼育したが，肝，腎，脾に組織の異常を認めていない．これらは H_2O_2 を飼料に添加しての実験である．H_2O_2 を水溶液として経口的に任意に摂取させた筆者の方法とは異なるけれども，16週間以上投与すれば組織に明らかな病理的変化を認めたことは興味深い．食品衛生法による H_2O_2 許容量は麺類，練り製品で100 ppm，その他の食品については30 ppm であり，今回の H_2O_2 投与量を50 kg の成人に換算すれば1日当り7.4 g となり規準量の H_2O_2 を含有

する練り製品ならば74 kg の多量となる．一方筆者が名古屋市内で販売されている食品について梅本ら[6]の方法によって H_2O_2 の含量を測定したところ（表略）麩類，練り製品，豆腐など多くの食品に H_2O_2 が含有されるとともに，規準量を越えるものが検査した62品目中12品目ある．このようなことを考慮する時，さらに少量の H_2O_2 をより長期間投与し，その慢性毒性の有無を明確にした上，食品添加物としての適切性を検討すべきであると考えられる．

結論

ハツカネズミに0.15％ H_2O_2 溶液を経口的に任意に摂取せしめて35週間飼育した．1日1頭当りの H_2O_2 平均摂取量は5.9mgである．体重は順調に増加したが，肝，脾の組織にそれぞれ水腫様変性とヘモジデリン沈着を認めたほか，腎，小腸，胃壁細胞にも若干の病理学的変化を認めた．

組織標本の鑑定を賜った三重大学医学部病理学伊豆津公作教授に深謝します．

1）川城巌，藤井清次，岩尾裕之：食品添加物，恒星社厚生閣版 84 1967 —2）高居百合子，柳下義之，岩尾裕之：動物の生長におよぼす H_2O_2 の影響．栄養と食糧第17回学術講演会講演要旨 1969 —3）川崎近太郎，近藤雅臣，永納秀男，永山富雄：食品中に残存する過酸化水素について．食衛誌 9：241-247 1968 —4）川崎近太郎，近藤雅臣，竹内喜子，永納秀男，永山富雄：シロネズミの成長におよぼす H_2O_2 投与の影響．食衛誌 10：68-72 1969 —5）片山信，服部イク，太田秀夫，牧清雄，丹羽壮一：H_2O_2 経口投与におけるマウスの発育およびその臓器におよぼす影響．市邨学園短大自然科学研究会会誌 4：19-22 1971 —6）梅本進，桜原和子，天野慶之：水産ねり製品中の過酸化水素定量法ならびに市販品の過酸化水素残存量．食衛誌 9：139-146 1968

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Perhydrol Poisoning

In the literature, we were not able to find a complete clarification of problems dealing with the clinical study and treatment of peroral affections with perhydrol. The case of peroral poisoning with perhydrol, as described by Ts. V. Blokhaso (1968), which terminated in death, was not diagnosed during the lifetime of the victim. In a pathological-anatomical investigation there were established deep necrotic changes in the mucosa of the esophagus and of the stomach, lobar pneumonia, and a brain edema. We observed a group (5 persons) poisoning with perhydrol where it was taken accidentally instead of alcohol. Taking into consideration the peculiarities of clinical manifestations of intoxication, and the effectiveness of the treatment measures that had been employed, we decided to describe it.

S., 47 years old, 23.10.1969 (October 23, 1969), at 23.00 hours (o'clock), while he was in a state of slight intoxication, drank close to 100 ml of perhydrol; he immediately lost consciousness and fell. After 20 minutes he was brought to the hospital. In the admission room he complained of pains in the pharynx and behind the sternum (breastbone), salivation, nausea, and a general malaise. He was excited. Following emergency procedure, the patient was given 500 ml of cold milk and 500((? -- number not clear in text)) ml of a 0.25% solution of acetic acid; following this, he was placed in the hospital (infirmary). In the blood, at this time, there was disclosed a moderate leucocytosis (9000); the urine had no pathology.

At 24.00 (o'clock) vomiting started that was accompanied by a marked weakness, copious cold sweat, heart throbbing, and a drop in the AD (arterial pressure) to 80/40 mm Hg. Following the administration of noradrenalin and cordiamine, his "self-feeling" somewhat improved. The arterial pressure rose to 110/80 mm Hg; the pulse indicated 100 - 120 beats per minute; arrhythmia developed (on the EKG there were group ventricular extra-systoles) that lasted till morning. Bloody vomitus and pains in the throat increased; in connection with this, an inhalation of lidocaine was carried out; following this, the patient fell asleep.

At 4.00 24.10 the vomiting started once again with an admiture of mucous and blood. There were administered calcium chloride and a hypertonic solution of sodium chloride. At 4.15 the patient began to complain of a visual deterioration, pains in the area of the orbits and eyeballs; the reaction of the pupils to light was retained. With the purpose of relieving the pronounced excitation, dehydrobenzoperidol was administered; following this, the patient quieted down. The salivation diminished following an injection of atropine. A lumbar puncture was performed -- in the cerebrospinal fluid there was an increased albumin content.

At 7.10 the oculist disclosed fine hemorrhages on the conjunctiva, a plethora of veins in the fundus oculi, and a dilation of the pupils. The patient did not distinguish light from dark, even though the pupillary reactions had been retained. An assumption was expressed with regard to the presence of a gas embolism in the vessels of the cerebrum in the region of the fissura calcarina.

With a joint examination of the oculist and neuropathologist, at 8.00, the following was observed.

The pupils react well to light; nevertheless, the patient does not see the lamp light at a close distance; he does not react when objects are brought close to the eyes. A reaction has been retained to sound irritants,

but it is not always adequate; upon being asked to open his eyes, he opens his mouth, and so forth. On the left, there are positive pyramidal (corticospinal) signs, and the deep reflexes are increased. On the feet -- there is a dorsal flexion upon provoking reflexes of the sole; the knee-heel test was uncertain. There was a psychomotor excitation. There were prescribed vasodilators (theophylline, papaverine, and so forth) and cardiac stimulants, antibiotics, vitamins, lasix, a physiological solution, administered intravenously by the drop method, and a 5% solution of glucose up to 2 liters per day. Hourly diuresis of 60 - 70 ml.

At 23.00 the patient unexpectedly turned blue (cyanotic), and there were manifestations of motor restlessness, dyspnea, an onset of coughing with a foamy sputum, and spasms of the lower extremities. In the lungs there were copious, different-caliber rales. Following a suctioning off from the trachea and bronchi of the foamy liquid, the respiration improved, and the cyanosis disappeared. For the relief of the excitation there were administered 2 ml of gibernal, 1 ml of dologran, and 2 ml of pipolphen. The patient rapidly quieted down and slept during the night, even though with interruptions.

25.10 -- the patient continued to complain about a complete absence of vision, dyspnea, a marked salivation, an excruciating burning pain in the nasopharynx, behind the sternum and in the epigastrium. Roentgenoscopy of the organs in the chest and esophagus: the pharyngeal lumen is constricted (edema of the soft tissues), the esophagus is without any abnormalities, the shadow of the mediastinum is within the norm, the diaphragm is mobile, and in the lower sections are seen fine, focal (lumber) pneumonic shadows. The detoxication and symptomatic therapy continued; penicillin was administered intramuscularly. At 15.00, an epileptoid seizure developed that was cut short by means of administering 2 ml of sevenal, 2 ml of pipolphen,

and 1 ml of gibernal. At 20.00 there took place fleeting visual hallucinations; for the rest,the neurologic symptoms remained as before.

By morning, at 26.10, vision was restored, and he began to distinguish newsprint. He answers questions willingly; orientation is insufficient, does not remember numbers, incidents, and so forth. Critical judgment is absent. The reaction of the pupils to light is lethargic; slight bilateral ptosis; convergence weakness. Tendon reflexes are uniform; the abdominal ones are flaccid; particularly on the right, there are no pathological reflexes. Fine hemorrhages in the sclera and conjunctiva. The faucial mucosa is whitish, and the posterior wall of the pharynx is hyperemic and edematous. Pulse of 76 bears per minute, rhythmic. The arterial pressure is 120/80 mm Hg. The heart tones are weakened. 1 tone at the apex is dull. Periodically, cough with a foamy sputum. In the lungs, against the background of an intensified vesicular respiration, occasional dry rales. Hypersalivation. Tongue coated with a gray deposit. Stomach moderately painful in the epigastric region. Liver and spleen are not enlarged.

Analysis of the blood: Hb 13.9 g %; erythrocytes, 3,950,000; leucocytes, 14,400, band cells, 6%, segmented cells, 78%, lymphocytes, 10%, monocytes, 6%; ESR (erythrocyte sedimentation rate, 28 mm per hour; thrombocytes, 190,000; reticulocytes, 4%; residual nitrogen, 50 mg %; cholesterol, 208 mg %; bilirubin, 0.13 mg %; sugar, 77 mg %; potassium, 20.5%; sodium, 322 mg %; calcium, 102 ((? -- number not clear in text)) mg %; chlorides, 585 mg %; sialic acid, 0.198; G Shch T (glutamic oxaloacetic transaminase), 265 units; PVT (pyruvic transaminase), 109 units; total albumin in the plasma, 8.06 g %; including albumins, 49.6%, alpha$_1$-globulins, 14.5%, alpha$_2$-globulins, 13%, beta-globulins, 8.9%, gamma-globulins, 14%; prothrombin, 45%. The osmotic resistance of the erythrocytes, maximum, 0.32, minimum, 0.48; thrombo test No. 6; coagulability of the blood, accord-

ing to Duke, 4 minutes, 50 seconds; duration of bleeding, according to Mass-Magro, 2 minutes ; recalcification time, 60 seconds; alkaline reserve, 95 milliequivalents/liter; Torn's test, 161/50.

Urine analysis: specific gravity, 1019, acid reaction; albumin, 0.254%; no sugar; in the residue, occasional hyaline casts; 3 - 4 leucocytes in the visual field and occasional erythrocytes; diastase, 64 units; uropepsin, 8 mg/hour; reaction for urobilin sharply positive; daily discharge of 17-ketosteroids, 12.4 mg. Analysis of the sputum: mucopurulent; reaction for blood, positive; erythrocytes cover the entire visual field; in places, there are seen blood veins,and many leucocytes.

Taking into account the possibility of the addition of a secondary infection, and that of the esophagal structure, treatment was continued with antibiotics (tetran-"C" in an amount of 250 mg 2 times per day), prednisolone, and antihistamine preparations. He received copious drinks, polyvitamins (B_1, B2, B_6, B_{12}, PP, A, C); infusions of a 40% solution of glucose, oxygeno-therapy; for the night -- soporifics, bromine preparations. Careful oral cavity hygiene; respiratory calisthenics.

On the 5th day, the visual acuity for each eye was 1 D. Memory improved for current events. Pains continue in the throat and ears that intensify while swallowing; scratchy feeling behind the sternum; cough. Urination difficult. Extensive ecchymoses in the areas where the tourniquet was applied to the shoulders. In the lower sections of the lungs, a few light small babbling rales.

By the 6th day the condition improved considerably. Patient is well oriented in time and space; thinking logical. Slight cough is observed with a foamy sputum. Rales are not heard in the lungs. Pulse and arterial pressure within the normal range. Stomach not painful. Slight reduction in the abdominal reflexes. No symptoms of dysuria.

In the days following, the "self-feeling" continued to improve. No subsequent complications, even though up to 13.11 troubled with a feeling of tickling in the throat; huskiness of the voice, and slight pains behind the sternum while swallowing; objectively, however, on the side of the internal organs, no substantial deviations were observed. At 4:11, during roentgenoscopy of the organs in the chest, esophagus, stomach, and intestine, no pathology was evidenced. The body temperature up to 2:11 was subfebrile, and subsequently -- normal. The EKG showed no abnormalities. The blood indices likewise came down to normal. The mucosa of the oral cavity and of the nasopharynx was slightly hyperemic; tongue clean, moist. Stomach soft, no pain.

15:11 -- discharged in good condition.

In other cases,the poisoning was less severe and depended on the amount of perhydrol consumed. In the victim who had swallowed 50 ml of perhydrol, the poisoning was of moderate severity, and in the remaining three, who had drunk 25 - 30 ml, it took a light course. In each of them, there took place analogous clinical symptoms, even though they were pronounced to a lesser degree.

In all the victims, there were observed sharp pains in the stomach and behind the sternum, breath retention, with a subsequent discharge of foam from the mouth during vomital movements, a fleeting absence of consciousness, and also, transitory motor and sensory impairments, a rise in the temperature, microhemorrhaging in the conjunctiva and skin, and a moderate leucocytosis. In 4 of the patients no paralysis was noted, nor pareses, nor pneumonia, nor pronounced cardiovascular disturbances.

Taking into account that perhydrol decomposes rapidly upon interacting with alkalis and organic substances, with the separation of oxygen, one may believe that the affection of the central nervous system is due to

gas microembolism. It is only through this, in our opinion, that one may explain the diversity of the local symptoms. In individual cases there may develop visual disturbances of the central type, epileptoid seizures, and other motor disturbances, and also, Korsakoff's syndrome, hemipareses, and so forth.

The pathogenesis of lobar pneumonia, apparently, is associated with a burn of the mucosa in the respiratory passages, and the hemorrhagic syndrome -- with the toxic influence of perhydrol on the liver and vessel walls,and with an increase in the permeability of the latter. This was attested to by the reduction in the prothrombin indices and by the presence of hemorrhages at the places of injection. In conclusion, it is necessary to underscore that a correctly timed, intensive, complex treatment, which is directed to the restoration of hemodynamics and to the removal of the intoxication, and also, the application of massive doses of antibiotics in conjunction with corticosteroids, prove to be effective even in serious cases of peroral perhydrol poisoning.

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Отравление пергидролем

В ЛИТЕРАТУРЕ нам не удалось найти полного освещения вопросов клиники и лечения пероральных поражений пергидролем. Описываемый Ц. В. Блохасо (1968) случай перорального отравления пергидролем, закончившийся смертью, при жизни пострадавшего распознан не был. При патологоанатомическом исследовании были установлены глубокие некротические изменения слизистой пищевода и желудка, очаговая пневмония и отек мозга. Нами наблюдалось групповое (5 человек) отравление пергидролем при случайном приеме его вместо алкоголя. Учитывая особенности клинических проявлений интоксикации и эффективность применявшихся лечебных мероприятий, мы решили его описать.

С., 47 лет, 23.10.1969 г. в 23.00, будучи в состоянии легкого опьянения, выпил около 150 мл пергидроля, сразу потерял сознание и упал. Через 20 минут был доставлен в больницу. В приемном покое жаловался на боли в глотке и за грудиной, слюнотечение, тошноту и общее недомогание. Был возбужден. В порядке экстренной помощи больному дано 500 мл холодного молока и 500 мл 0,25% раствора уксусной кислоты, после чего он был помещен в стационар. В крови в это время выявлен умеренный лейкоцитоз (9000), моча без патологии.

В 24.00 началась рвота, которая сопровождалась резкой слабостью, обильным холодным потом, сердцебиением и падением АД до 80/40 мм рт. ст. После введения норадреналина и кордиамина самочувствие несколько улучшилось, АД повысилось до 110/80 мм рт. ст.; пульс 100—120 ударов в минуту, появилась аритмия (на ЭКГ групповые желудочковые экстрасистолы), продолжавшаяся до утра. Кровянистая рвота и боли в горле нарастали, в связи с чем проведена ингаляция лидокаином, после которой больной уснул.

В 4.00 24.10 вновь началась рвота с примесью слизи и крови. Введены хлористый кальций и гипертонический раствор хлористого натрия. В 4.15 больной стал жаловаться на ухудшение зрения, боли в области орбит и глазных яблок; реакция зрачков на свет была сохранена. С целью снятия сильного возбуждения введен дегидробензперидол, после чего больной успокоился. Слюнотечение уменьшилось после инъекции атропина. Сделана люмбальная пункция — в ликворе повышено содержание белка.

В 7.10 окулист выявил мелкие кровоизлияния на конъюнктиве, полнокровие вен глазного дна, расширение зрачков. Больной не различает свет и тень, хотя зрачковые реакции сохранены. Было высказано предположение о наличии газовой эмболии сосудов головного мозга в области fissura calcarina.

При совместном осмотре окулиста и невропатолога в 8.00 отмечено следующее.

Зрачки хорошо реагируют на свет, вместе с тем больной не видит света лампы на близком расстоянии, при приближении предметов к глазам не реагирует. На звуковые раздражители реакция сохранена, но не всегда адекватна: на просьбу открыть глаза открывает рот и т. д. Слева положительные пирамидные знаки, глубокие рефлексы повышены. На стопах — дорзальное сгибание при вызывании подошвенных рефлексов; коленно-пяточная проба неуверенная. Психомоторное возбуждение. Назначены сосудорасширяющие (диафиллин, папаверин и др.) и сердечные средства, антибиотики, витамины, лазикс, внутривенно капельно физиологический раствор и 5% раствор глюкозы до 2 л в сутки. Почасовой диурез 60—70 мл.

В 23.00 больной неожиданно посинел, появились двигательное беспокойство, одышка, приступ кашля с пенистой мокротой, судороги нижних конечностей. В легких обильные разнокалиберные хрипы. После отсоса из трахеи и бронхов пенистой жидкости дыхание улучшилось, цианоз исчез. Для снятия возбуждения введено 2 мл гибернала, 1 мл долоргана и 2 мл пипольфена. Вскоре больной успокоился и ночь, хотя и с перерывами, спал.

25.10 больной продолжал жаловаться на полное отсутствие зрения, одышку, сильное слюнотечение, мучительное жжение в носоглотке, за грудиной и в эпигастрии. Рентгеноскопия органов грудной клетки и пищевода: просвет глотки сужен (отек мягких тканей), пищевод без особенностей, тень средостения в норме, диафрагма подвижная, в нижних отделах видны мелкие очаговые пневмонические тени. Продолжалась дезинтоксикационная и симптоматическая терапия, внутримышечно вводился пенициллин. В 15.00 развился эпилептиформный припадок, который был купирован введением 2 мл севенала, 2 мл пипольфена и 1 мл гибернала. В 20.00 имели место кратковременные зрительные галлюцинации, в остальном неврологическая симптоматика оставалась прежней.

К утру 26.10 зрение восстановилось, стал различать газетный шрифт. На вопросы отвечает охотно, ориентировка недостаточная, не помнит числа, места происшествия и т. д. Критика отсутствует. Реакция зрачков на свет вялая, легкий двусторонний птоз, слабость конвергенции. Сухожильные рефлексы равномерные, брюшные — вялые, особенно справа, патологических рефлексов нет. Мелкие кровоизлияния в склеры и конъюнктивы. Слизистая зева белесовата, задняя стенка глотки гиперемирована, отечна. Пульс 76 ударов в минуту, ритмичный. АД 120/80 мм рт. ст. Тоны сердца ослаблены. I тон у верхушки приглушен. Периодически кашель с пенистой мокротой. В легких на фоне усиленного везикулярного дыхания единичные сухие хрипы. Гиперсаливация. Язык обложен серым налетом. Живот умеренно болезнен в эпигастральной области. Печень и селезенка не увеличены.

Анализ крови: Hb 13,9 г%, э. 3 950 000, л. 14 400, п. 6%, с. 78%, лимф. 10%, мон. 6%, РОЭ 28 мм в час, тромбоцитов 190 000, ретикулоцитов 4%, остаточного азота 50 мг%, холестерина 208 мг%, билирубина 0,13 мг%, сахара 77 мг%, калия 20,5 мг%, натрия 322 мг%, кальция 10,[illegible] мг%, хлоридов 585 мг%, сиаловой кислоты 0,198, ГЩТ 265 ед., ПВТ 109 ед.; всего белка в плазме 8,06 г%, в том числе альбуминов 49,6%, α_1-глобулинов 14,[illegible]%, α_2-глобулинов 13%, β-глобулинов 8,[illegible]%, γ-глобулинов 14%; протромбина 45%. Осмотическая резистентность эритроцитов максимальная 0,32, минимальная 0,4[illegible], тромботест № 6; свертываемость крови по Дуке 4 мин. 50 сек., длительность кровотечения по Мас—Магро 2 мин., время рекальцификации 60 сек.; щелочный резерв 95 мэкв/л; проба Торна 161/50.

Анализ мочи: удельный вес 1019, реакция кислая, белка 0,254‰; сахара нет; в осадке единичные гиалиновые цилиндры, 3—4 лейкоцита в поле зрения и единичные эритроциты; диастаза 64 ед., уропепсин 8 мг/час; реакция на уробилин резко положительная; суточное выделение 17-кетостероидов 12,4 мг. Анализ мокроты: слизисто-гнойная, реакция на кровь положительная, эритроциты покрывают все поле зрения, местами видны прожилки крови, много лейкоцитов.

Учитывая возможность присоединения вторичной инфекции и стриктуры пищевода, продолжали лечение антибиотиками (тетран-«С» по 250 мг 2 раза в сутки), преднизолоном, антигистаминными препаратами. Получал обильное питье, поливитамины (B_1, B_2, B_6, B_{12}, PP, A, C), вливания 40% раствора глюкозы, оксигенотерапию, на ночь — снотворные, препараты брома. Тщательный туалет полости рта, дыхательная гимнастика.

На 5-й день острота зрения на каждый глаз 1 D. Улучшилась память на текущие события. Сохраняются боли в горле и ушах, усиливающиеся при глотании, царапание за грудиной, кашель. Мочеиспускание затрудненное. Обширные экхимозы в местах наложения жгута на плечах. В нижних отделах легких немногочисленные мелкопузырчатые хрипы.

К 6-му дню состояние значительно улучшилось. Больной хорошо ориентируется во времени и пространстве, мышление логичное. Отмечается небольшой кашель с пенистой мокротой. В легких хрипы не выслушиваются. Пульс и АД в пределах нормы. Живот безболезнен. Легкое снижение брюшных рефлексов. Дизурических явлений нет.

В последующие дни самочувствие продолжало улучшаться. Никаких поздних осложнений не было, хотя до 13.11 беспокоили чувство першения в горле, осипость голоса и небольшие боли за грудиной при глотании, объективно же со стороны внутренних органов существенных отклонений не отмечалось. 4.11 при рентгеноскопии органов грудной клетки, пищевода, желудка и кишечника патологии не выявлено. Температура тела до 2.11 была субфебрильная, в дальнейшем — нормальная. ЭКГ без особенностей. Показатели крови так

пришли к норме. Слизистая полости рта и носоглотки слегка гиперемирована, язык чистый, влажный. Живот мягкий, безболезненный.

15.11 выписан в хорошем состоянии.

В других случаях отравление было более легким и зависело от количества выпитого пергидроля. У пострадавшего, выпившего 50 мл пергидроля, отравление было средней тяжести, у остальных трех, выпивших 25—30 мл, протекало легко. У каждого из них имели место аналогичные клинические симптомы, хотя и в меньшей степени выраженные.

У всех пострадавших отмечались резкие боли в животе и за грудиной, задержка дыхания с последующим выделением пены изо рта при рвотных движениях, кратковременная утрата сознания, а также преходящие двигательные и чувствительные нарушения, повышение температуры, микрогеморрагии в конъюнктиву и кожу, умеренный лейкоцитоз. У 4 больных не отмечалось параличей и парезов, пневмоний и выраженных сердечно-сосудистых расстройств.

Учитывая, что пергидроль быстро разлагается при взаимодействии со щелочами и органическими веществами с выделением кислорода, можно думать, что поражение центральной нервной системы обусловливается газовой микроэмболией. Только этим, по нашему мнению, можно объяснить пестроту локальной симптоматики. В отдельных случаях могут возникнуть расстройства зрения по центральному типу, эпилептиформные припадки и другие двигательные расстройства, а также корсаковский синдром, гемипарезы и т. д.

Патогенез очаговой пневмонии, по-видимому, связан с ожогом слизистой дыхательных путей, а геморрагический синдром — с токсическим воздействием пергидроля на печень и стенки сосудов и повышением проницаемости последних. На это указывало снижение показателей протромбина и наличие кровоизлияний в местах инъекций. В заключение необходимо подчеркнуть, что своевременное интенсивное комплексное лечение, направленное на восстановление гемодинамики и снятие интоксикации, а также применение массивных доз антибиотиков в сочетании с кортикостероидами оказываются эффективными даже в тяжелых случаях перорального отравления пергидролем.

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Hydrogen Peroxide as a Depressant of Gastric Acidity*

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A GREAT amount of work has been done in search of methods for decreasing the acidity of the gastric contents. One of the substances which has been used for this purpose is hydrogen peroxide. Petri (1) in 1908 reported that large quantities of a dilute solution (300 c.c. of a 0.5% solution) introduced into an empty stomach markedly lowered the acidity of the gastric contents and recommended its use in the treatment of hyperacidity. Since then a number of clinicians (2, 3, 4, 5, 6 and 7) have reported that dilute hydrogen peroxide is a means of reducing the secretion of gastric acid and of increasing the secretion of mucus. All agreed that acid secretion was reduced, but not all recommended its therapeutic use. The degree of inhibition of acid reported varied. Hurst (8) in 1931 reported the use of hydrogen peroxide for relieving the achlorhydria or hypochlorhydria seen in chronic gastritis. He used solutions varying from 0.013% to 0.095% as a gastric lavage. It is to be noted that Hurst used hydrogen peroxide in much more dilute solutions than did the others. He supposed that it was an effective mechanical means of dislodging the excessive mucus which was present in chronic gastritis. He suggested that excessive mucus prevented acid secretion by blocking the mouths of the acid-secreting glands.

For some time one of us (A.J.A.) has been interested in studying experimentally the effects on gastric secretion of various agents when applied to the gastric mucosa of animals. Our review of the literature indicated that the effect of hydrogen peroxide on gastric secretion should be studied under controlled laboratory conditions. This we have done; in addition we have extended our studies to include a few human subjects.

METHODS

Five Pavlov pouch dogs and three dogs with intact stomachs were used. All were in good health at the time the experiments were initiated. Five clinic patients were studied; the clinical diagnosis was duodenal ulcer in three, and gastric ulcer in one; one had a normal stomach insofar as could be determined by physical findings and laboratory tests, but he complained of dyspepsia and epigastric distress.

The secretion of the pouch dogs was collected by the orthodox method. In the dogs with intact stomachs the gastric contents were aspirated by means of a number 32 French colon tube which was passed each time a sample was to be withdrawn. We first tried using a retained Rehfuss tube; it worked well during the control tests, but after hydrogen peroxide had been administered there was so much mucus on subsequent aspirations that the small tube invariably became occluded. In the human subjects a Rehfuss tube was used.

Two methods of stimulating the gastric secretion were used. Histamine was used in all three groups of subjects; in each case 1.0 mgm. of ergamine acid phosphate was injected subcutaneously. In addition, on the dogs with pouches, tests were made in which the stimulus was a test meal.

In the dogs with pouches when histamine was used as the stimulus, a control test started with the injection of the histamine, the gastric samples being collected several times during a period of 90 minutes. At the end of 90 minutes another milligram of histamine was injected and the collection of juice continued. In the dogs with intact stomachs and the human subjects, only one test period of 90 minutes was employed. In the case of the dogs with pouches when a meal was used as a stimulus, the secretion was collected for a period of eight hours after feeding.

In the case of each subject a number of control tests were made to establish the normal response of the individual to the stimulus being used.

RESULTS

The complete data are too bulky to be presented. Therefore we shall give a representative protocol of one of the experiments and will then summarize the results obtained in the remainder.

The effect of a single application of 3.0% hydrogen peroxide on the histamine response: Dog No. 1 had a Pavlov pouch. On injecting 1.0 mgm. of histamine the pouch secreted an average of 14.0 c.c. of gastric juice containing 53 mgm. of free HCl and 63 mgm. of total acid in 90 minutes. After the necessary control tests, the pouch was washed with 100 c.c. of 3.0% hydrogen peroxide (see Table I). Beginning 15 minutes later the secretion of the pouch was collected for two 30 minute periods. During this time the secretion was mucoid in nature, amounted to 18.2 c.c., and contained no free acid, but had a slight amount of total acid. Then 1.0 mgm. of histamine was injected subcutaneously. In 90 minutes 23.2 c.c. of secretion were collected containing only 6.00 mgm. of free HCl and 21.60 mgm. of total acid. Then the injection of histamine and collection of secretion were repeated with the results shown in Table I. The results of all these particular experiments on Dog No. 1 are included in Table II. The results show clearly that washing the pouch with 3.0% hydrogen peroxide markedly depressed the secretory response to histamine. The juice was rich in mucoid secretion.

In the five dogs in which this experiment was performed, the free HCl secretion was reduced on the average 65.8% after the first injection of histamine and 56.5% after the second. The volume of mucoid secretion obtained varied widely, but was increased in all.

Following a single treatment of the pouch with

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hydrogen peroxide, the dogs were injected daily with histamine to determine the time at which the secretory response returned to normal. The time of recovery varied widely. One dog had not returned to normal in 29 days. One returned to normal in 5 days, one in 9 days, one in 10 days, and one in 12 days. One secreted hypernormally at 12 days, one at 15 days, and another at 18 days.

The effect of repeated applications of 3.0% hydrogen peroxide on the histamine response: In two of the dogs whose response to histamine had returned to normal in from five to nine days after a single application of hydrogen peroxide, 3.0% hydrogen peroxide was applied to the pouch twice daily, morning and evening, for 15 days. The response to histamine was determined on the third, fifth, eighth, eleventh, and fifteenth days. The secretion of free and total acid was reduced markedly in both dogs. In one dog the free acid was reduced by 96% (almost abolished), the total acid being reduced 83%. In the other the free acid was reduced 77% and the total acid 46%. The volume of secretion, chiefly mucoid, was reduced 23% in one and increased 48% in the other. From the first dog, on some days, a clear rather dilute mucoid secretion devoid of free acid (pH 4.2) was obtained.

The effect of a single application of various concentrations of hydrogen peroxide on the histamine response was studied in the three other dogs with pouches. It was found that a 0.5% solution was effective in reducing the secretion of acid, the average varying from 12% to 66% in the total acid and from 32% to 83% in free acid in the different dogs. The results, as might be anticipated, showed that the stronger the concentration the greater the reduction in acid secretion. Concentrations of 0.5, 1.0, 1.5, 2.0, and 3.0% were employed.

The results with a meal as the stimulus: After the pouches had returned to normal and suitable response to the test meal had been obtained, the hydrogen peroxide was applied and the meal fed. The secretion of the pouch after the meal was collected for eight hours.

The data are shown in Table III. The results are exceedingly variable, much more so than the results obtained with histamine as a stimulus. This should be expected because the response to the stimulus of a meal is normally more variable than the response to histamine. The variation may be about 25% from day to day in the same dog. Further, after the first hour or two following the application of the peroxide and feeding, acid secretion starts to increase. The amount of secretion varies in the same and different dogs. It must be recalled that the histamine response occurs only during a period of from one to one and one-half hours. It is to be noted that a 3% solution was required to produce a definite reduction of the free acid in two of the three animals in series 2; and in series 1, dog No. 2 showed little decrease even to 3.0%.

In one dog (No. 8) a 2% solution was effective. Dog No. 11 manifested a definite increase in acid secretion after the application of each of the concentrations of peroxide except the 3% solution. This is the animal that showed the most marked hypernormal response to histamine during the recovery of the pouch from the effect of 3% peroxide.

TABLE I

The effect of a single application of 3.0% hydrogen peroxide on the histamine response

Time	Vol. in c.c.	C.U. Free Acid	mgm. Free Acid	C.U. Total Acid	mgm. Total Acid	Remarks
9:25						Pouch washed with 100 c.c. of 3.0% hydrogen peroxide. Started collecting.
9:40						
10:10	12.0	-0-	-0-	½	0.12	
10:40	6.2	-0-	-0-	4	0.62	
	18.2				0.74	
10:40						Injected 1.0 mgm. histamine.
10:55	2.8	-0-	-0-	8	0.84	
11:15	7.5	8	2.25	25	6.75	
11:25	3.0	18	2.10	68	7.50	
11:40	3.3	18	1.65	35	4.29	
11:55	2.6	-0-	-0-	18	1.82	
12:10	4.0	-0-	-0-	4	0.40	
	23.2		6.00		21.60	
12:10						Injected 1.0 mgm. histamine.
12:25	5.8	-0-	-0-	3	1.74	
12:40	5.7	15	2.85	40	8.55	
12:55	3.9	25	3.51	53	7.41	
1:10	2.6	15	1.30	43	4.16	
1:25	2.2	8	0.66	25	1.98	
1:40	2.8	-0-	-0-	15	1.40	
	23.0		8.32		25.24	

Dogs with normal stomachs. Three dogs with intact stomachs were treated with 100 c.c. of 3% hydrogen peroxide after several control responses to histamine had been obtained. The peroxide solution was withdrawn after three minutes. The secretion of free acid was reduced from 95% to 100%, and the total acid was reduced from 81% to 90%. The secretion of mucus was so copious that a colon tube had to be used to aspirate the gastric contents. The dogs were nauseated and retched after the aspiration of the peroxide. One of the dogs on being treated again on the following day showed a large amount of blood in the gastric contents.

This depression of secretion was anticipated. The greater viscidity of the secretion probably was due to stimulation of the mucosa of the pyloric antrum in addition to that of the fundus. Nausea and vomiting did not occur in the dogs with Pavlov pouches for several reasons. The area to which the peroxide was applied was smaller. The nauseating effects of the stomach tube were absent. In the animals with whole stomachs some of the hydrogen peroxide undoubtedly passed into the duodenum. Three per cent peroxide obviously was too irritating for the stomach of one of the animals.

TABLE II

	1st Injection of Histamine					2nd Injection of Histamine				
		Free acid		Total acid			Free acid		Total acid	
	Vol.	MxCU	mgm.	MxCU	mgm.	Vol.	MxCU	mgm.	MxCU	mgm.
Controls										
1	15.4	130	63.47	148	69.90	8.7	177	37.10	193	40.71
2	10.0	128	37.74	150	42.80	8.2	133	32.69	143	36.13
3	12.9	128	46.43	145	58.80	10.1	130	39.05	138	43.90
4	17.7	133	64.17	143	79.98	15.7	138	68.92	145	75.69
Averages	14.0	130	52.95	146	62.87	10.7	144	44.44	155	49.11
Using 3% hydrogen peroxide										
1	15.5	20	4.55	38	12.15					
2	16.9	38	15.09	53	23.54					
3	19.2	53	22.61	65	31.51	18.2	88	42.32	103	52.27
4	23.2	18	6.00	68	21.60	23.0	25	8.32	58	25.24
5	13.2	20	4.62	53	16.85	15.5	55	19.54	80	34.84
6	16.5	25	7.31	50	16.76	15.6	40	16.13	65	30.31
7	13.0	63	22.12	88	32.76	21.0	88	47.25	118	68.42
8	9.5	15	3.42	38	9.95	3.5	0	00.00	26	2.34
9	15.1	38	11.94	58	21.01	13.7	33	5.80	48	15.06
10	3.5	25	5.54	48	12.00	4.1	25	3.55	48	7.03
11	15.3	35	10.33	55	11.85	16.1	43	15.52	65	27.20
12	8.5	38	4.83	55	9.83	9.7	78	24.87	95	16.96
Averages	14.6	32	9.37	56	18.32	14.0	48	18.33	70	27.97
Per cent of change from control averages	Inc. 4.3	Dec. 75.4	Dec. 81.4	Dec. 61.6	Dec. 70.8	Inc. 30.8	Dec. 66.6	Dec. 58.7	Dec. 54.8	Dec. 43.0

Vol. — volume in c.c.
MxCU — maximum clinical units
mgm. — milligrams of free or total acid in terms of HCl
Inc. — increase
Dec. — decrease

HUMAN SUBJECTS

Because 3.0% hydrogen peroxide was found to be markedly irritating to humans, 1.0% and 2.0% solutions usually were employed. A 3.0% solution was used in the "normal" individual in a few tests. Histamine was used as a stimulus. The entire content of the stomach was removed every ten minutes for 90 minutes after the injection of histamine. From three to five control responses to histamine were obtained prior to the commencement of treatments with hydrogen peroxide. The stomach was lavaged with from 100 to 300 c.c. of the peroxide which was withdrawn after three to five minutes. This procedure was repeated two or three times a week, until four to twenty treatments had been given.

In one patient with duodenal ulcer in which only 1.0% peroxide was used, the acid secretion after five treatments was increased by about 35% in spite of the

formation of considerable mucus. Because the lavage caused discomfort and because the symptoms were augmented, the treatment was discontinued. In a second duodenal ulcer patient similarly treated, the free acid secreted was decreased by 45% and the total

TABLE III

	Vol.	Free acid		Total acid	
		MxCU	mgm.	MxCU	mgm.
1st series 3%					
Dog No. 1	30.9	85.2	96.7	77.6	90.2
Dog No. 2	5.5	10.8	11.5	3.6	6.4
2nd series ½%					
Dog No. 1	1.8	9.9	10.1	2.2	9.7
Dog No. 8	34.4	62.3	74.2	21.6	60.8
Dog No. 11	19.2	23.2*	49.7*	21.6*	31.9*
1%					
Dog No. 1	21.7	27.2	45.4	19.4	38.6
Dog No. 8	45.3	61.6	77.4	20.7	65.6
Dog No. 11	10.4*	33.9*	181.7*	12.2*	95.8*
1½%					
Dog No. 1	54.1*	43.8	27.7	36.6	10.5
Dog No. 8	4.6	32.6	36.8	11.7	26.0
2%					
Dog No. 1	10.5	24.0	27.2	23.1	11.9
Dog No. 8	21.2	57.9	68.3	25.2	63.8
Dog No. 11	13.6	21.4*	32.5*	14.8*	25.3*
3%					
Dog No. 1	10.7*	52.0	58.8	44.0	49.2
Dog No. 11	32.9*	67.8	63.8	52.7	46.1

The figure shows the average per cent of change from the control response; those marked with an asterisk represent percentage increases, the others decreases.

acid by 18%; this patient secreted much mucus. Because the symptoms were not decreased, the treatment was discontinued. The stomach of the third patient with duodenal ulcer was lavaged eight times with 1.0% and 2.0% solution of peroxide. Five tests were performed during a three week period. Discomfort from the lavage occasionally was reported. The two per cent solution of peroxide decreased the free acid values by about 90% and the total acid values by about 30%. The symptoms of this patient disappeared with this regime without other medication or dietary changes. In the patient with a gastric ulcer, 1.0% solution of peroxide increased the secretion of both acid and mucus; 2.0% caused a decrease of about 50% in the acid values. The treatment was discontinued because the patient's symptoms were not relieved after nine treatments during a period of four weeks, and because there so frequently was considerable blood in the gastric contents following hydrogen peroxide. In the individual with a "normal" stomach, concentrations of peroxide varying from 1.0% to 3.0% were used. Twenty treatments were given and nine tests were done during this time. More patients were not used because of the discomfort and bleeding experienced by those we did treat in this way.

The results of the tests confirmed the observations on the dogs, that 3.0% solution of peroxide reduced acid values in response to histamine to a greater extent than the 1.0% solution. In the patient with gastric ulcer and in one of three patients with duodenal ulcer a 1.0% solution of hydrogen peroxide increased the acid values of the stomach contents after histamine. In the other two patients with duodenal ulcer either 1.0% or 2.0% solution of hydrogen peroxide reduced the acid values after histamine. In the individual with a "normal" stomach 1.0% to 3.0% solution of hydrogen peroxide lowered the acid values in response to histamine, the stronger solution being the more effective.

DISCUSSION OF RESULTS

The preceding results indicate that in general the lavaging of the stomach with hydrogen peroxide solution (½-3%) produced a decrease in the acid of the gastric contents when either a meal or subcutaneous injection of histamine was used to stimulate acid secretion. This decrease was observed in the per cent of acid in the gastric contents and in the total milligrams of acid, both free and total, in each case. The stronger the solution of hydrogen peroxide used the greater was the decrease of acid response. This decrease varied widely in different subjects, and also, from time to time, in the same subject. However, one of the dogs and two of the five human subjects consistently showed increases in acid secretion rather than decreases. The mucous secretion always was augmented.

At times there was a complete absence of free acid (but no instances of complete absence of total acid) in response to histamine stimulus. This was observed four times in dog No. 1, once in dog No. 2, twice in dog No. 8, twice in dog No. 6, twice in dog No. 9, and twice in dog No. 12. However, it was the exception rather than the rule. This type of secretion was observed by Ivy (9) and Dawson (10) in the gastritis produced in Pavlov pouch dogs by X-rays.

While no chemical analyses for content of mucus were made, it was obvious that generally an increase in mucoid or mucus secretion paralleled a decrease in acidity. This observation certainly was true in those tests in which no free acid was found. In several instances when there was no free acid, determinations of the chloride in the secretion were made; and it was found that the chloride content was no higher than that which is normal for mucus or mucoid secretion.

Other investigators have reported using 300 c.c. of a 0.5% solution for gastric lavage of peptic ulcer patients. Petri, Poly, Hall, Kelling, and Dienst were enthusiastic in regard to the use of hydrogen peroxide in the treatment of peptic ulcer because the secretion of acid is reduced and that of mucus is increased. Hall stated, however, that it did not seem to benefit cases with active ulcer. Winternitz found that it decreased acidity for only short intervals, and discarded its use. Nielson also observed a depression of free acid with a return to normal values or nearly normal values in 24 hours. Hurst used dilute solutions (0.013-0.095%) in chronic gastritis, observing an increase in acid secretion.

Our results show that to obtain a significant reduc-

tion in acid secretion in response to histamine or a meal, stronger concentrations of peroxide (one and two per cent) must be employed. Even then stimulation, as observed by Hurst with very weak solutions, may occasionally occur. A three per cent solution is too irritating. When the weaker solutions are used the return of the secretory response to normal is relatively rapid. In the case of a "susceptible" stomach, however, a 0.5% solution applied every other day will reduce acid secretion significantly. However, we suspect that it might cause active ulcers to bleed; for the gastric ulcer patient showed bleeding with one and two per cent solutions, and one of the dogs with "normal" stomach showed marked bleeding after 3%. Although it would be desirable to have some simple and innocuous method for reducing the secretion of acid by the stomach, we do not feel that the use of hydrogen peroxide can be recommended without considerable reservation.

CONCLUSIONS

1. Hydrogen peroxide in solutions varying from one-half to three per cent when used as a gastric lavage may depress the acidity of the gastric contents. This may be true even when using histamine as the gastric secretory stimulus. However, in an occasional dog and human subject a one or two per cent solution may induce a stimulating effect. The individual susceptibility varies widely, a three per cent solution being required to depress significantly acid secretion to histamine in some individuals.

2. The stronger the solution of peroxide used, the greater is its effect in depressing the acidity.

3. Hydrogen peroxide influences the volume of gastric secretion in response to a stimulus. The volume of secretion may be either increased or decreased. The volume changes do not correlate with the changes in acidity.

4. Hydrogen peroxide stimulates the secretion of mucus by the stomach.

5. The effects of hydrogen peroxide on gastric secretion are most marked immediately after lavage. Recovery occurs in from 24 hours to 15 days, depending on the individual susceptibility and the concentration of peroxide used.

6. Hydrogen peroxide is not a satisfactory means of depressing gastric acidity in patients. It may be active in inducing hemorrhage from an ulcer. When used in the most effective concentrations it causes too much discomfort, and its effect in concentrations which do not cause discomfort cannot be predicted.

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Action of Hydrogen Peroxide on Cell Mitosis in the Mouse Intestine

P. Dustin and C. Gompel

Research undertaken in very different directions indicate that radiation-like mitotic poisons, or more or less a certain number of them, acts on cell mitotis by an oxidation reaction. It is by oxidation of the -SH to S-S that Bacq and his collaborators[1] have explained the diverse biological effects of yperite (mustard gas) and its derivatives (vesication, Lundsgaard effect). It is by means of the quionones that the radiation-like di- and triphenol cause cellular destruction in the germinative zone of the mouse intestine.[2] In short, it is by an analagous enough mechanism (oxidation of -SH functions or others indispensable to the cell mitosis) that one tends to explain certain effects of the ionizing radiation. The oxidation would have taken place in this case by means of H_2O_2, beginning with the fundamental HO discharged by the radiation. It is known that hydrogen peroxide also produces the Lundsgaard effect which seems to characterize the muscular action of a great member of mitotic poisons (3, 4). The toxic effect of H_2O_2 and a concentration excessive in oxygen, has recently been studied on a level with several enzymic systems, especially those carriers ~~by~~ of groups -SH (5, 6).

It is important then to know if the hydrogen peroxide itself will not be a mitotic poison. We used the cell mitosis of the adult mouse intestine as material. The solutions of H_2O_2, prepared extemporaneously, by dilution of the solution to 100 volumes, were injected into the abdominal cavity. Only the solutions exceeding 50% will give rise to a release of oxygen such that will kill the animal in a few seconds on a account of mechanical reasons.

The most diluted solutions decompose slowly enough in the peritoneum, and more than 24 hours after their injection, the gaseous bubbles are still visible. We made use of chemically-pure hydrogen peroxide from three different sources with coincident results. The effective doses tolerated by the animal varied between 0.5 cubic cm. from a 50% solution and 1 cubic cm. from a 10% solution.

The intestinal lesions are visible after two hours, reaching their maximum between 4 and 6 hours and persisting with the strongest doses up to 24 hours. Close to the normal cell mitosis but slightly diminished in number, pycnonecrotic nuclei appeared in the germinative region at the bottom of the glandular culs de sac. These pycnonecrotic nuclei arise from intermetotic nuclei and the thymonucleic fragments thus formed are expelled toward the glandular opening, and then included in the epithelium with the cells from which they progressively migrated, towards the center of the intestinal villus. The figures are typically those of a radiation-like effect of moderate importance. The cell mitosis in progress did not seem altered. The action of the hydrogen peroxide has a morphological pecularity: it is the large variability of the lesions from one gland to another.

On a level with the germinative centers of the lymphoid tissue, and still more in the cortical thymic, there is massive destruction of cells (by pycnonecrosis) after the first hours. It is a question of partly an "alarm reaction", sufficiently intense to destroy the greatest part of the cortical thymic in 24 hours (thymus inverted 48 hours after).

During the entire experimental period, the general state of the animals, aside from all abdominal destension, is quite bad.

One might be astonished that a substance as unstable as H_2O_2 has the time to exercise such prolonged effects. We have noted its relatively slow decomposition, which can be explained by inhibition of the peroxidase by the great excess of H_2O_2 (7). The cytological observations typically "radiation-like", seemed-to bear the simplest proof that this modality of cellular destruction is linked to the oxidation of components essential to the cell mitosis. If this hypothesis is accurate, it would be necessary to find again the same changes in animals placed under a high pressure atmosphere of O_2, which we propose to do. However, theproblem of pycnonecrosis of germinal cells is still not resolved because it would be necessary to know that which is particularly sensitive to oxidation. If it is true what numerous proof indicate, that it is a question of functions, -SH belonging to one or several enzymes, it is remarkable then that recent experiments carried out on the same material, by the intraperitoneal injection of monoiodoacetic and monoiodoacetamide and, only showed very discreet mitotic changes (metaphasic). These

poisons from the -SH do not cause the radiation-type lesions. It is not at all unlikely that a metal belonging to an enzyme would be oxidized. This hypothesis could apply to the radiation-like action of acridines, which can combine with biologically important metals (8). Finally, a more direct effect on the thymonucleoprotein nucleus should not be excluded: Robertson and coll. (9) have in effect showed that H_2O_2 reduces in an irreversible manner, the viscosity of thymonucleohistone solutions. If one remembers that yperite (10) as well as the rays (11, 12) have a similar biological effect, one will be less surprised that hydrogen peroxide had the radiation-like effect that we have described.

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Action du peroxyde d'hydrogène sur les mitoses intestinales de la Souris.

par P. Dustin et C. Gompel.

Des recherches entreprises dans des directions très différentes indiquaient que les poisons mitotiques radiomimétiques, ou tout au moins un certain nombre d'entre eux, agissaient sur la mitose par une oxydation. C'est par l'oxydation des -SH en S-S que Bacq et ses collaborateurs (1) ont expliqué les diverses actions biologiques de l'ypérite et de ses dérivés (vésication, effet Lundsgaard). C'est par l'intermédiaire des quinones que les di- et triphénols radiomimétiques amènent des destructions cellulaires dans la zone germinative de l'intestin de la Souris (2). C'est enfin par un mécanisme assez analogue (oxydation de fonctions -SH ou autres indispensables à la mitose) que l'on tente d'expliquer certains effets des radiations ionisantes. L'oxydation aurait lieu dans ce cas par l'intermédiaire de la formation de H_2O_2 à partir des radicaux HO libérés par les radiations. On sait que le peroxyde d'hydrogène produit lui aussi l'effet Lundsgaard qui paraît caractériser l'action musculaire d'un grand nombre de poisons mitotiques (3, 4). L'action toxique de H_2O_2 et d'une concentration excessive en oxygène, a récemment été étudiée au niveau de plusieurs systèmes enzymatiques, notamment ceux porteurs de groupes -SH (5, 6).

Il importait donc de savoir si le peroxyde d'hydrogène ne serait pas lui-même un poison mitotique. Nous avons utilisé comme matériel les mitoses intestinales de la Souris adulte. Les solutions de H_2O_2, préparées extemporanément par dilution de solution à 100 volumes étaient injectées dans la cavité abdominale. Seules les solutions dépassant 50 p. 100 donnèrent lieu à un dégagement d'oxygène tel qu'il tua l'animal pour des raisons mécaniques en quelques secondes.

Les solutions plus diluées se décomposent assez lentement dans le péritoine, et plus de 24 h. après leur injection, des bulles gazeuses sont encore visible. Nous nous sommes servis de peroxyde d'hydrogène chimiquement pur, de trois provenances différentes, avec des résultats superposables. Les doses efficaces et tolérées par l'animal varient entre 0,5 cm³ d'une solution à 5 p. 100 et 1 cm³ à 10 p. 100.

Les lésions intestinales sont visibles dès la 2e heure, atteignent leur maximum entre 4 et 6 heures, persistent, avec les plus fortes doses, jusqu'à 24 heures. A côté de mitoses normales mais en nombre apparemment un peu diminué, des pycnonécroses apparaissent dans la région germinative, au fond des culs de sac glandulaires. Ces pycnonécroses proviennent de noyaux intermitotiques, et les fragments thymonucléiques ainsi formés sont tantôt éliminés vers la lumière

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glandulaire, tantôt inclus dans l'épithélium avec les cellules duquel ils migrent progressivement vers la pointe des villosités. Les images sont typiquement celles d'une action radiomimétique d'importance moyenne. Les mitoses en cours ne paraissent pas altérées. L'action du peroxyde d'hydrogène a une particularité morphologique : c'est la grande variabilité des lésions, d'une glande à une autre.

Au niveau des centres germinatifs du tissu lymphoïde, et encore plus dans la corticale thymique, des destructions massives de cellules (par pycnonécrose) prennent place à partir des premières heures. Il s'agit sans doute en partie d'une « réaction d'alarme » : elle est suffisamment intense pour détruire la plus grande partie de la corticale thymique en 24 h. (thymus inversé 48 h. après).

Pendant toute la période expérimentale, l'état général des animaux — indépendamment de toute distension abdominale — est assez mauvais.

On pourrait s'étonner qu'une substance aussi instable que H_2O_2 ait le temps d'exercer des effets aussi prolongés. Nous avons signalé sa décomposition relativement lente, ce qui peut s'expliquer par une inhibition des peroxydases par le grand excès de H_2O_2 (7). Les observations cytologiques, typiquement « radiomimétiques », paraissent apporter une des preuves les plus simples que cette modalité de destruction cellulaire est liée à l'oxydation de composants nécessaires à la mitose. Si cette hypothèse est exacte, il faudrait retrouver les mêmes altérations chez des animaux placés dans une atmosphère d'O_2 à forte tension, ce que nous proposons de faire. Cependant, le problème de la pycnonécrose des cellules germinatives n'est pas encore résolu, car il faudrait savoir ce qui est particulièrement sensible à l'oxydation. S'il est vrai que de nombreux arguments indiquent qu'il s'agirait de fonctions —SH appartenant à un ou plusieurs ferments, il est cependant remarquable que des expériences récentes, poursuivies sur le même matériel, par l'injection intrapéritonéale d'acide monoiodoacétique et de monoiodoacétamide, n'aient montré que des altérations mitotiques (métaphasiques) très discrètes. Ces poisons des —SH n'amènent pas de lésions radiométiques. Il pourrait se faire qu'un métal appartenant à une enzyme soit oxydé. Cette hypothèse pourrait s'appliquer à l'action radiomimétique des acridines, qui peuvent se combiner à des métaux biologiquement importants (8). Enfin, une action plus directe sur les thymonucléoprotéines du noyau ne saurait être exclue : Robertson et coll. (9) ont en effet montré que H_2O_2 réduisait de façon irréversible la viscosité de solutions de thymonucléohistone. Si l'on se rappelle que l'yprite (10) ainsi que les rayons (11, 12) ont une action biochimique semblable, on sera moins surpris que le peroxyde d'hydrogène ait l'action radiomimétique que nous avons décrite.

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PERIPHERAL BLOOD CHANGES IN HUMANS AND EXPERIMENTAL ANIMALS FOLLOWING THE INFUSION OF HYDROGEN PEROXIDE INTO THE CAROTID ARTERY*

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The lethal effect of ionizing irradiation on tissue can be greatly augmented by increasing the oxygen concentration at the time of their exposure.[1-9] On this premise, studies were initiated in this institution in 1961 to determine the efficacy of hydrogen peroxide as an oxygen source when infused intraarterially immediately before and during irradiation therapy in the management of malignant tumors. The findings have been encouraging, and preliminary reports have been published.[10-14]

Coincidentally, peripheral blood studies were completed on all patients receiving hydrogen peroxide adjuvant therapy. Some increase was noted in one or more formed blood elements; the most consistant changes occurring in those patients being infused into the common or internal carotid artery. The following is a preliminary report on the peripheral blood changes observed in experimental animals and in humans being infused intraarterially with hydrogen peroxide.

MATERIALS AND METHODS

To March 1964, 137 patients have been studied under the hydrogen peroxide infusion protocol. Of these, 33 have been treated for head and neck malignancies, 13 of whom had hydrogen peroxide infused into the common or internal carotid artery. In 11 of the 13 cases, a marked change has been observed in the peripheral blood picture. Two of the other 104 patients demonstrated a significant change in peripheral blood (both patients were being treated for carcinoma of the bladder). Animal studies were subsequently initiated to confirm these observations:

Experimental animal protocol. Female, albino rabbits weighing 6 to 8 pounds were used in duplicate studies conducted with 34 animals divided into three groups: Group I was comprised of 14 animals and, a solution was infused into the right common carotid (all catheters used for animal infusions were PE-10 polyethylene) of 10.0 ml hydrogen peroxide (0.12 per cent) in "Ionosol-T".‡ Group II, comprised of 11 animals, was infused with 10.0 ml "Ionosol-T" only into the right common carotid artery. Group III, which was made up of 9 animals, was infused with 10.0 ml hydrogen peroxide (0.12 per cent) in "Ionosol-T" into the right femoral artery.

Surgical procedures were as follows: Animals were anesthetized with 1.0 to 3.0 ml pentobarbital sodium, 60 mg/ml administered I.V. into the marginal ear vein. The area over the right carotid artery was shaved, a 2-cm incision made, the artery exposed, and the catheter inserted into the lumen of the artery and secured with a purse-string suture. The infusion was accomplished over a 13- to 15-minute period.

Peripheral blood studies consisted of WBC, reticulocyte, differential, platelet,§ and microhematocrit. These were conducted before infusion and every 24 hours afterwards for 7 to 20 days postinfusion.

Human studies. In the human series studied (137 patients), the catheter varied in size and location, depending upon the tumor site. To March 1, 1964, 33 patients have been treated for malignant tumors of the head and neck, 13 of whom had the catheter placed in either the common or internal carotid artery. In the other 20, the catheter was placed into the external carotid via either the superficial temporal or superior thyroid artery. For the most part, the remaining 104 patients have been treated for malignancies involving the liver, bladder, ovary, pancreas, endometrium, colon or cervix, with

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† From the Chas. A. Sammons Research Division, Baylor University Medical Center, Dallas, Texas.

‡ Abbott Laboratories, Chicago, Illinois.

§ Platelet counts by phase contrast and Rees-Ecker methods were performed sometimes simultaneously and sometimes independently by two separate laboratories.

the catheters passed retrograde through the femoral artery to the appropriate level in the aorta.

In the head and neck area, the infusion of 250 ml of 0.12 to 0.24 per cent hydrogen peroxide with 25 to 50 mg "Priscoline"* in an isotonic solution was accomplished 5 days per week during the course of irradiation therapy. The blood studies listed above were conducted routinely before, during and daily (but for varying extended periods) following therapy.

RESULTS

Animal studies. The animals infused with 10.0 ml of 0.12 per cent hydrogen peroxide in "Ionosol-T" into the right common carotid artery showed an increase in circulating platelets (fig. 1) within the first 24-hour period. The time of maximum response was between 24 to 72 hours; maximum counts varied from 600,000 to 900,000 platelets with an average preinfusion count between 200,000 to 300,000. The counts generally returned to approximately the preinfusion levels between the fifth and eighth day.

In these animals, an increase in circulating reticulocytes has been noted from a preinfusion level of 1.0 to 2.0 per cent to 8.0 to 15.0 per cent between the fourth and seventh day postinfusion. All components return to a value approximating the preinfusion levels by 15 days postinfusion. Animals in the two control groups ("Ionosol-T" infused into the right carotid artery or 0.12 per cent hydrogen peroxide infused into the femoral artery) showed no significant change in their respective peripheral blood pictures during the time of observation.

Two animals in the group being infused with hydrogen peroxide into the carotid artery showed no marked change in the peripheral blood counts. These animals were sacrificed and autopsied; the catheter was found to be extending into the external carotid artery.

Preliminary data from Fe^{59} studies indicate an increase in clearance and reappearance rates in those animals infused into the carotid artery with hydrogen peroxide.

In six animals, blood was drawn from the external jugular vein immediately after being infused with 0.12 per cent hydrogen peroxide in "Ionosol-T" into the common carotid artery.

* Ciba Pharmaceutical Co., Summit, New Jersey.

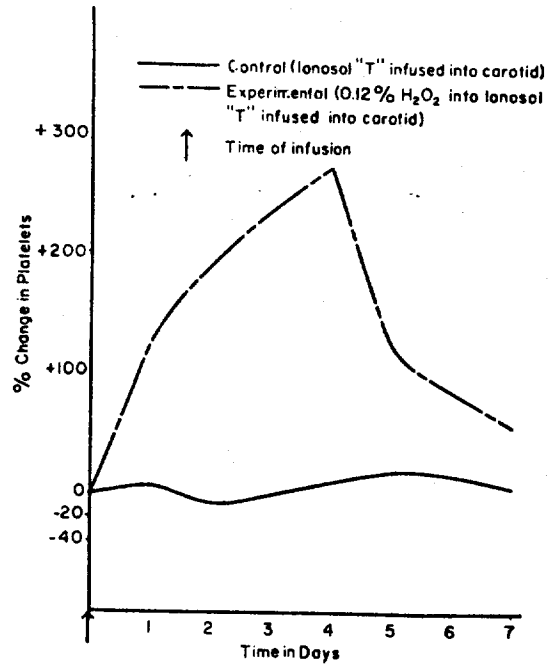

FIG. 1. Platelet data from rabbits infused with either 10.0 ml of 0.12 per cent hydrogen peroxide in "Ionosol-T", or "Ionosol-T" only into the right common carotid artery.

The blood thus withdrawn was injected I.V. into the femoral vein of six noninfused recipients. A rise in platelets following the pattern of the hydrogen peroxide-infused animals was noted in four animals, although the order of magnitude was somewhat less.

Human studies. An increase in one or all formed elements of the perpiheral blood picture has been noted in 11 of 13 patients being infused with 0.12 per cent hydrogen peroxide in isotonic solution into the common or internal artery. One patient of 20 being infused with the same material (identical concentrations and rate) into the external carotid artery and two of 104 patients being infused with the solution into the aorta showed a significant increase in blood platelets.

Six patients have received a single infusion of 250 ml of 0.12 per cent hydrogen peroxide with 50 mg "Priscoline" into the common carotid artery. Three have shown an increase in circulating platelets of more than 100 per cent. In one patient, the platelet count (phase method) increased from 220,000 to 567,000. The count began to drop by the eighth postinfusion day and the patient was reinfused; the

platelet count increased after the second infusion to about the original peak value. The count remained elevated for the period of time the patient was followed—18 days.

Three case histories are related in the following text.

CASE REPORTS

Patient S-1. This patient (WF-43) had a malignant schwannoma (neurofibrosarcoma) involving the V, VII, VIII, IX, X, XI, and XII cranial nerves, and the right side of the brain stem and posterior fossa. A no. 5 French polyvinyl catheter was placed into the right internal carotid artery. The patient was infused 18 times in 25 days with 250 ml of 0.12 per cent hydrogen peroxide in 5.0 per cent dextrose in water. As can be seen in figure 2, the platelet count rose from 500,000 to more than 1,200,000; the reticulocytes increased from 1.2 per cent to approximately 9.0 per cent, which was followed by a slight drop. At 105 days postinfusion, a moderate elevation is still discernible.

Patient S-2. The patient (WM-42) had an epidermoid carcinoma of the maxillary antrium. A no. 5-French polyvinyl catheter was inserted into the right common carotid artery. The patient was infused 16 times in 24 days with 250 ml of 0.12 per cent hydrogen peroxide in "Ionosol-T." Figure 3 depicts the results. A total rise in platelet count was noted from 210,000 to 818,000/cmm, and a rise in total WBC from 9,900 to 36,980/cmm was observed. The hematocrit did not change appreciably; however, an increase in reticulocytes from 0.6 to 3.2 per cent was noted. Sixty days following the first infusion, the WBC was 24,800 and the platelet count 268,000/cmm.

Patient F-1. This patient (WF-43) had widely metastatic carcinoma of the breast. During a 14-month period, she had a right radical mastectomy, receiving 60 mgm "Thio-TEPA" followed in 30 days by 3,850 r Co^{60} radiotherapy. Six months later, she underwent a bilateral oopherectomy, followed in 2 months by 3.6 millicuries of P^{32} and testosterone. She was admitted to the hospital in the terminal phase of her disease 6 months later. At the time of admission, her peripheral blood picture was as follows: WBC—2,000; platelet count—7,500; reticulocytes—0.6 per cent. A no. 5-French polyvinyl catheter was passed into the right common carotid artery via the superificial temporal artery. She was given seven infusions of 0.12 per cent hydrogen peroxide in "Ionosol-T" in 9 days. As can be seen in figure 4, following the first two infusions, the platelet count rose to 212,000/cmm with no remarkable change in either reticulocytes or WBC. Treatment was discontinued for two days, during which time the platelet count fell to 94,000/cmm. Five more infusions were given thereafter, during which

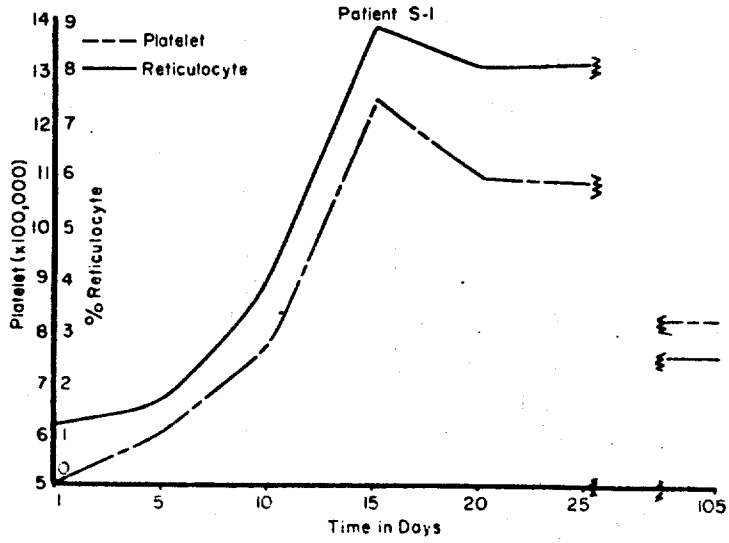

FIG. 2. Platelet and reticulocyte response in a 43-year-old woman infused with 0.12 per cent hydrogen peroxide in 5 per cent dextrose in water into the right internal carotid artery.

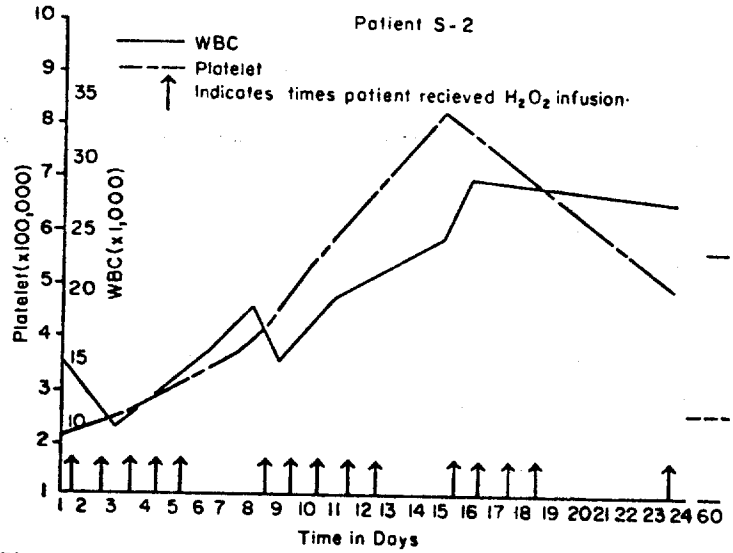

FIG. 3. Platelet and WBC response in a 42-year-old man infused with 0.12 per cent hydrogen peroxide in "Ionosol-T" into the right common carotid artery.

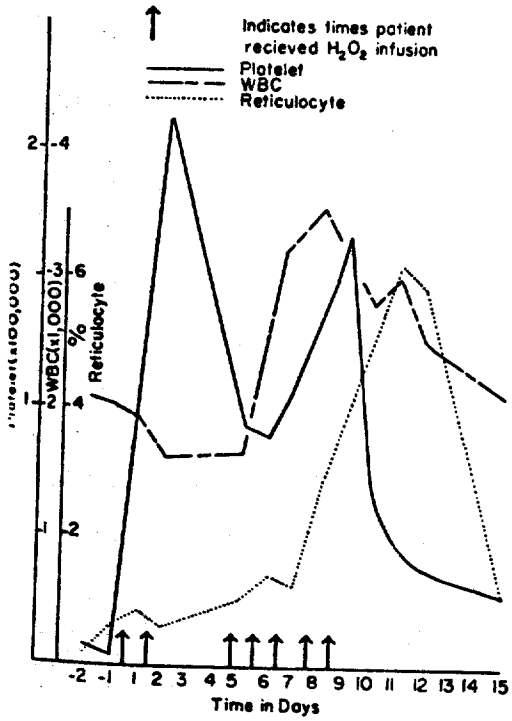

FIG. 4. Platelet, WBC and reticulocyte response in a 43-year-old woman infused with 0.12 per cent hydrogen peroxide in "Ionosol-T" into the right common carotid artery.

time the platelet count rose to 164,000/cmm, with the WBC at 3,800/cmm. Over the next 5-day period, the reticulocyte count continued to increase to 5.8 per cent. Five days after the last infusion, the patient expired. On that day the platelet count had dropped to 50,000/cmm; WBC to 2,400 cmm; reticulocyte count to 2.0 per cent.

SUMMARY

1. An increase in circulating platelets and reticulocytes has been observed in rabbits following the infusion of hydrogen peroxide in "Ionosol-T" into the common carotid artery.

2. An increase in one or more of the formed elements in the peripheral circulation (platelets, reticulocytes, or white blood cells) has been noted in 11 of 13 patients being infused with a solution of hydrogen peroxide into either the internal or common carotid artery. In one of 20 patients being infused into the external carotid, and two of 104 patients being infused into the aorta, a significant increase in platelet levels was measured.

Studies are in progress to determine the nature and mechanisms of the observed stimulation and to delineate any humoral factors which might be involved. A relationship between a possible humoral factor and "erythropoietin" is being investigated.[15-22]

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11

Hydrogen Peroxide in the Urban Atmosphere

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Hydrogen peroxide was measured in the atmospheres of Hoboken, N. J. and Riverside, Calif. At Hoboken, concentrations up to 4 pphm of hydrogen peroxide were determined in early afternoon hours during moderate photochemical smog formation. At Riverside, concentrations as high as 18 pphm were detected during severe smog formation. The concentration of hydrogen peroxide paralleled that of total oxidant.

In the presence of sunlight and oxides of nitrogen (NO_x), hydrocarbons react to form new products, some of which are called oxidants. The most commonly investigated photochemically produced oxidants in the urban atmosphere are ozone (O_3), nitrogen dioxide (NO_2), and peroxyacetylnitrate (PAN).

Recent laboratory studies show that another oxidant, peroxybenzoylnitrate (*1*), forms when systems containing aromatic hydrocarbons and NO_x are irradiated; however, this oxidant has not been found in the atmosphere. Other laboratory studies show that the photolyses of aldehydes (*2, 3, 4*) yield organic and inorganic hydroperoxides. Altshuller, Cohen, *et al.* (*2*) investigated the photolysis of propionaldehyde and found that ethyl hydroperoxide was a product. These workers also identified methyl hydroperoxide as a product in the photo-oxidation of acetaldehyde. Purcell and Cohen (*4*), studying the photo-oxidation products of formaldehyde (HCHO), found hydrogen peroxide (H_2O_2). Earlier work by Carruthers and Norrish (*5*) and later work by Horner and Style (*6*), concerning the photo-oxidation of HCHO, showed no H_2O_2. The products found in these studies were formic acid, CO, CO_2, and hydrogen; however, this work unlike the work of Purcell and Cohen was done at relatively high HCHO concentrations. Purcell and Cohen worked in the

low concentration range of 1–30 ppm (v/v) whereas the others used millimeter partial pressures of HCHO.

Bufalini and Brubaker (7) studied the carbon fragments and oxidant formation resulting from the photo-oxidation of HCHO at two different wavelengths (3660 and 3130 A), with and without added NO_2 in the system. The only oxidant found when 12 ppm of HCHO in air was photo-oxidized at 3660 A was H_2O_2. When HCHO was photo-oxidized at 3660 A in the presence of NO_2, O_3 was also found. The maximum H_2O_2 concentration was that observed when no NO_2 was present. When HCHO was irradiated at 3130 A, 16 times more H_2O_2 was observed compared with irradiating at 3660 A. With added NO_2 and 3130 A radiation, only half as much H_2O_2 was found.

Aldehydes are primary and secondary pollutants in the urban atmosphere, and since laboratory studies indicate that they photo-oxidize to form peroxides, the presence of peroxides in the atmosphere seems evident.

In the early 1950's Haagen-Smit (8) stated that the oxidizing effect of smog resulted from the combined action of NO_2 and O_3 and peroxides. However no quantitative or qualitative study of hydroperoxides in the urban atmosphere has been reported.

Whether or not H_2O_2 is present in the urban atmosphere and, if it is, the concentration at which it exists are studied here.

Experimental

Hydrogen peroxide was determined in laboratory irradiated systems, in irradiated air samples containing auto exhaust collected at the entrance of the Lincoln Tunnel, and in ambient air samples at Hoboken, N. J., and

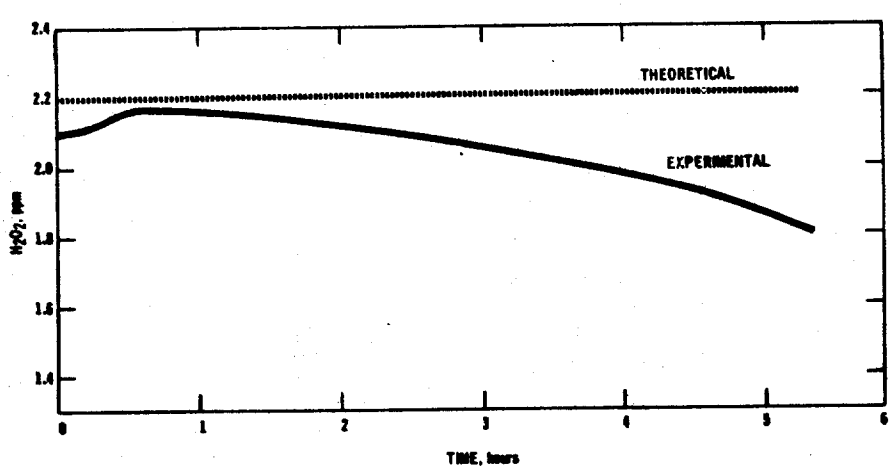

Figure 1. Stability of hydrogen peroxide in a FEP Teflon bag

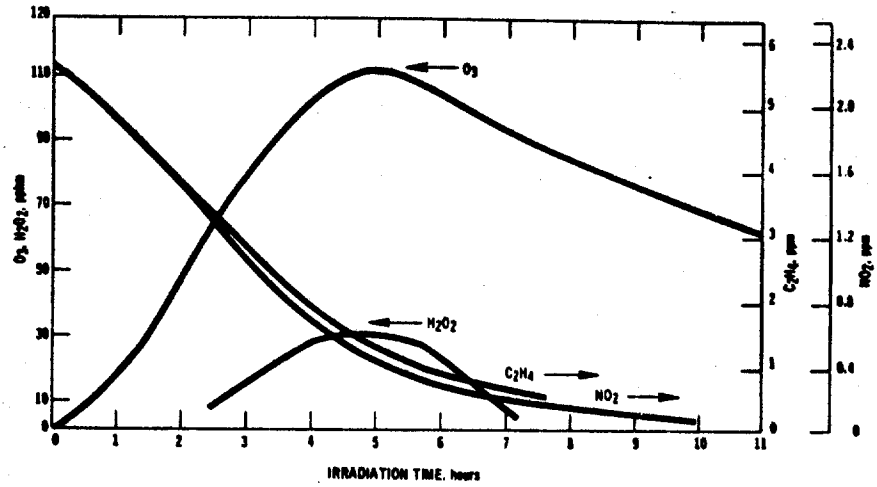

Figure 2. Irradiation at 3660 A of ethylene with nitrogen dioxide

Riverside, Calif. The specific method developed by Cohen and Purcell (*9*) was used; here titanium IV and 8-quinolinol react with H_2O_2 to form a colored complex with absorption maximum at 450 mμ. There was no interference in the method for urban atmospheric levels of NO, NO_2, O_3, PAN, SO_2, and hydrocarbons.

Hydrogen peroxide concentrations in FEP Teflon bags were determined by the titanium IV–8-quinolinol and the catalyzed 1% potassium iodide colorimetric method (*10*).

At the New Jersey site O_3 was monitored with the Regener type instruments (*11*). In the laboratory it was determined with an instrument that measured the chemiluminescence from the ethylene-O_3 reaction (*12*).

Total oxidant readings in California were found on a Mast ozone instrument. In the low pphm concentration range of H_2O_2 the Mast instrument does not respond; in the ppm range of H_2O_2 the response is low and erratic.

Nitrogen dioxide was measured using the Saltzman method (*13*). Nitric oxide was oxidized to NO_2 and subsequently determined as NO_2.

Hydrocarbons were separated on a Porapack Q column and detected with a flame ionization detector.

All chemicals and gases were reagent grade and were used without purifying further.

Laboratory irradiations were conducted in a chamber fitted with 36 GE F-42-T6 blacklamps; the energy maximum was at 3660 A. Temperature in the chamber was maintained at 25 ± 2°C; Teflon FEP bags were used as reaction vessels in the chamber.

The light intensity of the chamber was measured as the rate of NO_2 photolysis in nitrogen (*14*). The first-order dissociation constant in the FEP Teflon bags was found to be 0.36 min^{-1}. The stability of H_2O_2 in FEP bags was investigated by injecting microliter quantities of a standardized 22.9% H_2O_2 solution into a metered stream of air while the bag was being filled. The theoretical concentration of peroxide was calculated from the amount of liquid injected and the volume of air used. The stability of H_2O_2 is shown in Figure 1. The concentrations observed experimentally were lower than the calculated theoretical concentration. This discrepancy was probably caused by destruction of H_2O_2 on the bag wall. The initial increase in H_2O_2 noted over the first half hour possibly resulted from desorption of H_2O_2 from the wall. When a bag is initially being filled, the surface–volume ratio is large, and some H_2O_2 apparently condenses on the wall. As the bag is filled, the surface–volume ratio decreases and H_2O_2 comes off the wall and into the gas phase.

Laboratory systems containing hydrocarbons and NO_x in air were irradiated and analyzed for oxidants. Four hydrocarbons that produced large amounts of HCHO per mole of reacted hydrocarbon were 1,3,5-trimethylbenzene, propylene, 1-butene, and ethylene. Hydrogen peroxide was detected in all four systems. Ozone was the major oxidant in these systems. Figure 2 shows the fate of a mixture of 5.5 ppm of ethylene (C_2H_4) and 2.2 ppm of NO_2 irradiated at 3660 A for 11 hours. The O_3

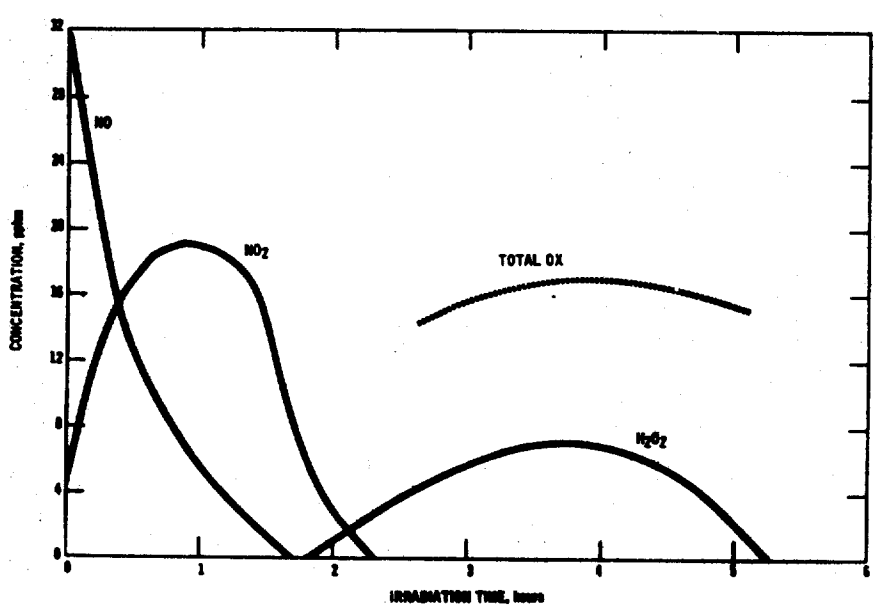

Figure 3. Lincoln Tunnel air sample irradiated in sunlight

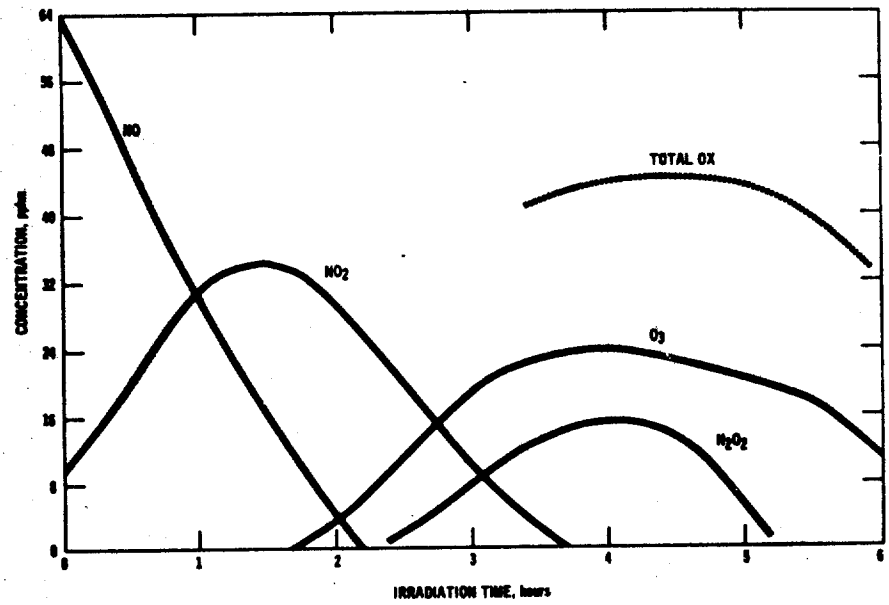

Figure 4. Lincoln Tunnel air sample with nitric oxide, irradiated in sunlight

and H_2O_2 maximum concentrations occurred at almost the same time after 5 hours of irradiation.

During the summer of 1970, the Environmental Protection Agency had one of its mobile instrument trailers stationed in Hoboken, N. J. Air was collected in large plastic bags during the early morning hours at the time of heavy traffic at the New Jersey entrance to the Lincoln Tunnel. These bags of air were protected from sunlight and returned to the instrument trailer for irradiation and analysis. The irradiation was performed by exposing the bags to sunlight atop the trailer.

Before irradiation, one bag contained 6.0 ppm of CO, 4.1 ppm of methane, and 1.9 ppm of non-methane hydrocarbons; the results from irradiating this bag are shown in Figure 3. The total NO_x was 36 pphm, with 29.5 pphm as NO. After 1 hour of irradiation the NO_2 maximum was reached; after 2 hours all of the NO and most of the NO_2 had disappeared. Total oxidant maximum and H_2O_2 maximum were observed after 3¾ hours of irradiation.

To determine the effect of increasing the ratio of NO_x to hydrocarbon (NO_x/HC) on the formation of oxidant and rate of hydrocarbon reaction, more NO was injected into a sample of collected air like that used in Figure 3. The NO concentration was increased by a factor of 2; the results of irradiating the altered sample are shown in Figure 4. The NO_2 maximum was reached 30 minutes later than that for the unaltered air

sample. After 3 hours of irradiation the system still contained NO_2. Most of the NO_x was lost from these systems during the irradiation with the nitrogen going to organic nitrates and nitric acid (*15*). Ozone and H_2O_2 maximums occurred after 4 hours of irradiation. The maximum H_2O_2 concentration in the altered system was twice H_2O_2 concentration in the unaltered system.

The ambient atmosphere at the mobile instrument site in Hoboken, N.J. contained up to 4 pphm of H_2O_2 on a day with high solar radiation and apparent photochemical smog formation. Hydrogen peroxide was observed between 12:00 A.M. and 2:00 P.M. On days when solar radiation was low because of cloud cover, no H_2O_2 was observed.

In August 1970 the urban atmosphere at Riverside, Calif. was sampled for H_2O_2 during days of photochemical smog formation. On the sixth, during a severe smog episode, concentrations of oxidant as high as 65 pphm were measured by the Mast ozone instrument (Figure 5). Hydrogen peroxide reached a maximum of 18 pphm during the episode at about the time the total oxidant was at its maximum.

Moving of the polluted air mass and changing of photochemical smog formation are seen in the changes in H_2O_2 concentrations at Riverside. Figure 6 shows the H_2O_2 concentration as a function of time of day at the Riverside site for three different days. On August 7 moderate to heavy smog buildup was observed for the air mass that had moved eastwardly over Riverside. Maximum total oxidant observed was about 30

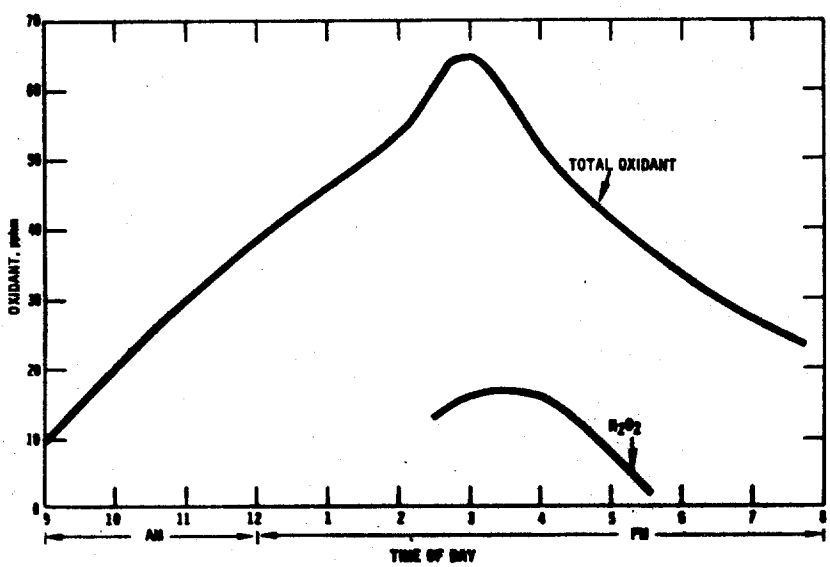

Figure 5. Measured oxidant at Riverside, Calif. (August 6, 1970)

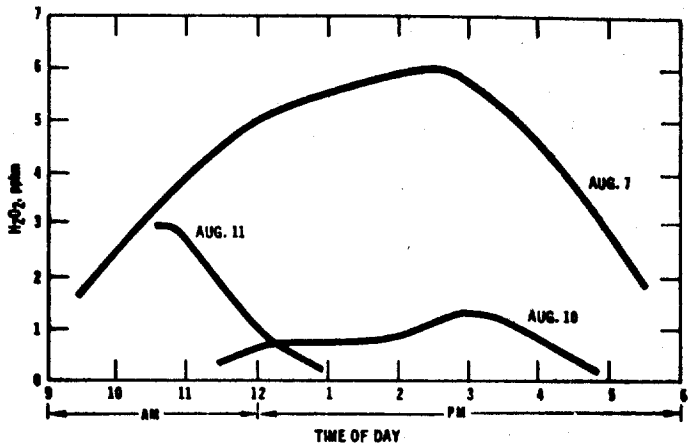

Figure 6. Hydrogen peroxide concentrations at Riverside, Calif. (August 1970)

pphm between 2:00 and 3:00 P.M. A maximum concentration of 6 pphm of H_2O_2 was found. The polluted air mass that formed west of Riverside on August 11 never reached the sampling site because of a wind direction change. Less photochemical smog on August 10 was indicated by increased visibility and lower oxidant readings; the maximum oxidant was observed about 3:00 P.M. when 1 pphm of H_2O_2 was observed.

Discussion

Analyses for H_2O_2 in laboratory-irradiated systems, in irradiated air samples including auto exhaust, and in the ambient polluted atmosphere have shown that H_2O_2 is present.

The formation of H_2O_2 is explained by a reaction sequence involving the photolysis of HCHO, nitrous acid, and NO_2. The photodissociation of HCHO is described by two processes (*16, 17, 18*):

$$HCHO \xrightarrow{h\nu} H + HCO \text{ and } HCHO \xrightarrow{h\nu} H_2 + CO$$

The first process is less important at longer wavelengths. The hydrogen atoms produced by it produce a hydroperoxy radical (Equation 1).

$$H + O_2 \xrightarrow{M} HO_2 \tag{1}$$

The hydroperoxy radical abstracts a labile hydrogen from a hydrocarbon or an aldehyde and forms H_2O_2.

$$HO_2\cdot + RCHO \rightarrow RCO + H_2O_2 \tag{2}$$

When CO, NO_x, and water vapor are present, hydrogen atoms form by the following sequence of reactions:

$$NO + NO_2 + H_2O \rightarrow 2\,HNO_2 \quad (3)$$

$$HNO_2 \xrightarrow{h\nu} OH + NO \quad (4)$$

$$NO_2 \xrightarrow{h\nu} O + NO \quad (5)$$

$$O + RH \text{ or } RCHO \rightarrow OH + R \text{ or } RCO \quad (6)$$

$$OH + CO \rightarrow CO_2 + H \quad (7)$$

Hydrogen peroxide is then produced by Equations 1 and 2.

The H_2O_2 concentration curves in Figures 3 and 4 indicate that H_2O_2 does not form when NO is present. This is explained by the reaction

$$HO_2 + NO \rightarrow NO_2 + HO \quad (8)$$

which is analogous to the oxidation of NO by RO_2, previously given as part of the overall reaction mechanism of photochemical air pollution (*19*).

The authors believe this is the first time H_2O_2 has been quantitatively and qualitatively identified in the urban atmosphere. Organic hydroperoxides probably are also formed in the urban atmosphere, but at lower concentrations.

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Enzymatic Determination of Residual Hydrogen Peroxide in Milk [1, 2]

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Abstract

A method for enzymatically detecting hydrogen peroxide at low levels in milk was developed. It involved the use of horseradish peroxidase with *o*-dianisidine as a chromogenic hydrogen donor. The milk curd was precipitated at pH 4.5 with hydrochloric acid and the whey added to a mixture of peroxidase and chromogen. The amount of color developed (measured at 400 mμ) as a result of the enzymatic reaction gave a measurement of the peroxide. The method detected levels of less than 1 μg/milliliter in milk.

The hydrogen peroxide and catalase treatment of milk for cheese making has received considerable attention in recent years. It has been shown to reduce the microbial load of the milk sufficiently for cheese making. On the other hand, it has been suggested that peroxide may remain in sufficient amounts to be inhibitory to the lactic streptococci used as starter cultures (3, 9, and 11). The starch-iodine method is usually employed to check the treated milk for residual peroxide. Even though a negative test is obtained for the starch iodine method, inhibition of lactic streptococci in such milk has been reported (9 and 11). According to Subramanian and Olson, the lower limit which can be detected by this method in milk is 10 μg/milliliter (9). This indicates the need for a more sensitive test.

There are several methods in addition to the starch-iodine test for detecting or measuring hydrogen peroxide in milk (1, 6, and 8), but none of these is capable of quantitating levels lower than 10 μg/milliliter. According to Munday (8), the Arnold-Mantzel method, involving vanadic acid as indicator, is the most sensitive for milk. This procedure can detect less than 80 ppm hydrogen peroxide in milk. Amin and Olson (1) have developed a spectrophotometric method using titanium tetrachloride which can be used to quantitate peroxide in milk at concentrations of 33-500 μg/milliliter.

Luck (6) mentioned the possible use of peroxidase and benzidine as a qualitative test for the peroxide in milk. Peroxidase with various hydrogen donors has been used to quantitate peroxide in fluids or liquids other than milk (4, 5, 7). The hydrogen donor used in these methods is usually a colorless compound which develops color when oxidized in the reaction:

$$H_2O_2 + AH_2\ (\text{donor}) \xrightarrow{\text{Peroxidase}} H_2O + A\ (\text{oxidized donor}).$$

Such reactions can be measured colorimetrically and the amount of hydrogen peroxide determined by comparing the amount of color developed with a standard curve.

This paper presents data obtained by using horseradish peroxidase to measure residual hydrogen peroxide in milk.

Methods

Reagents. All reagents were prepared with glass-distilled water using acid-cleaned glassware. The hydrogen peroxide (30%) was obtained from Allied Chemical Co., Morrison, N. J., and was diluted to the desired concentration with distilled water or milk just before each assay. Horseradish peroxidase (Nutritional Biochemicals Corp.) was dissolved in distilled water at a concentration of 1 mg/milliliter. The stock peroxidase solution was then filter-sterilized and stored at 5 C until used. A 1:100 dilution of this stock solution was used in the assays. One per cent *o*-dianisidine (Nutritional Biochemicals Corp.) in methanol was used as the chromogen for the hydrogen peroxide-peroxidase reaction.

Preparation of standard curve. Standards containing 1, 2, 3, 4, and 5 μg H_2O_2/milliliter were prepared in 0.01 M acetate buffer (pH 4.5). Ten milliliters of each standard were then diluted with an additional 10 ml of the buffer. The assay was accomplished by adding 5 ml of each diluted peroxide standard to sep-

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arate test tubes containing 1 ml peroxidase (0.01 mg/milliliter) and 0.1 ml *o*-dianisidine solution. The blank consisted of 5 ml 0.01 M acetate buffer plus 1 ml peroxidase and 0.1 ml *o*-dianisidine. The contents of each tube were mixed well and incubated at 23-26 C. After exactly 10 min of incubation, 0.2 ml of 4 N HCl was added to each tube to stop the reaction and stabilize the color. Five minutes after adding HCl the optical density of each standard was measured at 400 mμ in a Spectronic 20 colorimeter. The standard curve was constructed by plotting the optical density readings against micrograms of hydrogen peroxide.

Hydrogen peroxide determination in milk. Milk samples were adjusted to room temperature before adding the desired amount of H_2O_2. Ten milliliters of the milk sample, or a dilution thereof, were immediately adjusted to pH 4.5 with 0.1 N HCl. If the milk sample were diluted before analysis, 0.01 N HCl was used to adjust the pH. Two milliliters of 0.1 M acetate buffer (pH 4.5) were added and the mixture diluted to 20 ml with distilled water. The samples were then filtered through Whatman no. 42 filter paper to remove the curd. Centrifugation of the samples at 3,400 × *g* for 10 min may also be used to remove curd. To have the filtrate for each sample serve as the blank, 5 ml of the filtrate for each sample were added to a tube containing 1 ml distilled water and 0.1 ml *o*-dianisidine solution, then adding another 5 ml of the filtrate to a tube containing 1 ml peroxidase solution and 0.1 ml *o*-dianisidine. The assay tubes were then incubated 10 min at room temperature (23-26 C), 0.2 ml of 4 N HCl added to each tube, and the optical density determined after an additional 5 min of incubation. This was deemed necessary, to eliminate interference of the milk solids in determining the amount of color developed by the reaction of the H_2O_2, peroxidase, and chromogen. This would be especially true for milk containing a low level of H_2O_2, requiring that it be assayed without dilution.

Results

Standard curve. The stock hydrogen peroxide (30%) was titrated with a standardized solution of sodium thiosulfate to determine the exact concentration. The solution was found to be 30.6% H_2O_2. Based on this concentration, standards were prepared and subjected to the enzymatic analysis. The standard curve thus obtained is shown in Fig. 1. Each point on this curve represents an average of seven assays.

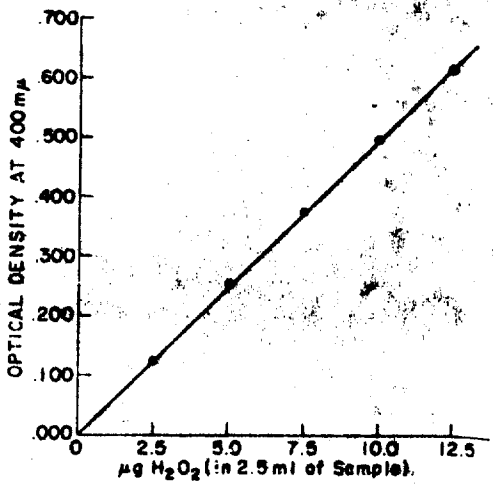

FIG. 1. Standard curve for peroxidase-*o*-dianisidine determination of hydrogen peroxide.

Hydrogen peroxide determination in reconstituted nonfat milk solids. Nonfat milk solids was reconstituted in distilled water to contain 10% solids, steamed (100 C) for 30 min, and stored in the refrigerator until used in an assay. The reconstituted milk was adjusted to room temperature and hydrogen peroxide added to the desired concentrations. Immediately after adding peroxide the samples were mixed well, diluted if necessary, and assayed. Table 1 shows the results from a series of such experiments. The per cent recovery was lowest in the samples to which 1 μg/milliliter was added. The amount of recovery increased as the amount of added peroxide increased. This apparently was due to greater destruction of the lower levels of peroxide by milk components, indicating a higher rate of destruction as the ratio of milk solids to added peroxide increased.

The type of milk, as well as the heat treatment to which it was subjected, influenced the

TABLE 1. Measurement of hydrogen peroxide in reconstituted nonfat milk solids.

Hydrogen peroxide		
Added	Measured*	Recovered
(μg/ml)		(%)
1	0.36	36.0
2	0.95	47.5
3	1.47	49.0
4	2.14	53.5
5	2.77	55.5
30	23.48	78.5
300	235.21	78.5

* Average of four to 14 replicates.

per cent recovery of added peroxide. This was shown in a series of experiments involving pasteurized homogenized whole milk and raw skimmilk obtained from the North Carolina State University dairy plant. The raw skimmilk was divided into two portions; one portion was used as a raw sample and the other was steamed (100 C) for 30 min. Reconstituted milk (steamed 30 min) was included for comparison. Hydrogen peroxide (30 μg/milliliter) was added to each sample and the assay immediately performed in the usual manner. A summary of results obtained is presented in Table 2. These results are averages of five replicates for each type of milk. Recoveries from reconstituted milk and heated skimmilk were about equal, being lowest for raw skimmilk. Pasteurized whole milk was not as detrimental to the recovery of added hydrogen peroxide as was raw skimmilk.

The hydrogen peroxide content of raw and heated skimmilk from the same lot was adjusted to 300 μg/milliliter and assayed at various time intervals. A portion of the sample was diluted and five replicate aliquots assayed immediately. The original samples were incubated at room temperature and assayed in the same manner after 15 and 30 min. The results are shown graphically in Fig. 2. These curves show that less peroxide was detectable in each sample as the incubation time increased. The hydrogen peroxide in the raw skimmilk dissipated more than that in the heated skimmilk. This was probably due to inactivation of lactoperoxidase or catalase in the milk by the heat treatment to which the heated sample was subjected before adding peroxide.

Discussion

Horseradish peroxidase has been used to quantitate low levels of peroxide in systems other than milk (4-7). The sensitivity of lactic starter cultures attributed to traces of hydrogen peroxide remaining in hydrogen peroxide–catalase-treated milk (3, 9, 11) indicates the need for a more sensitive test for peroxide, to determine when milk can be used without adversely affecting growth of starter culture. The starch-iodine method has been reported to detect levels of peroxide in milk as low as 10 μg/milliliter; however, it lacks sufficient sensitivity to ensure that milk does not contain levels which may be inhibitory for growth of starter cultures (9). Use of peroxidase in conjunction with *o*-dianisidine appears to provide a method of detecting hydrogen peroxide in milk at levels of 1 μg/milliliter or lower.

Because hydrogen peroxide is subject to decomposition by organic matter, it is impossible to accurately quantitate the total amount which has been added to milk at any given time. Attempts to construct a standard curve using milk resulted in a plot which neither paralleled the curve for standards prepared in water nor passed through the origin. This might indicate decomposition of peroxide in milk before adding peroxidase, as well as different amounts of decomposition at each level of added peroxide. Subsequent experiments revealed that the per cent recovery increased as the amount of added peroxide increased. In determining the hydrogen peroxide concentration in a sample of milk, therefore, it is possible to measure only the residual peroxide and not the amount originally added. The foregoing was the basis for developing the standard curve in water rather than in milk.

TABLE 2. Efficiency of recovery of hydrogen peroxide in various types of milk.

Type of milk	Hydrogen peroxide Added	Measured	Recovered
	(μg/ml)		(%)
Reconstituted, NFMS	30	22.5	75
Whole milk	30	16.6	55
Raw skimmilk*	30	5.9	20
Heated skimmilk*	30	23.3	78

*Same lot of skimmilk.

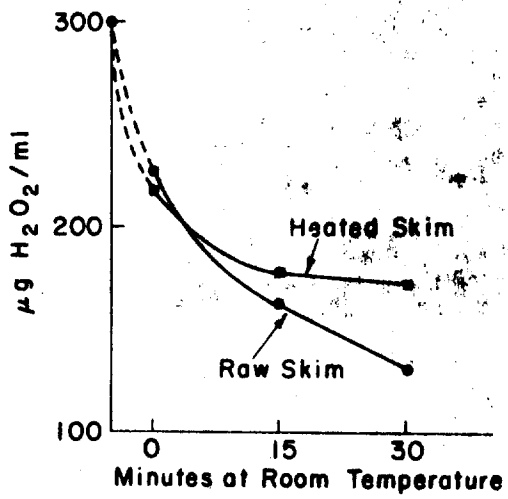

FIG. 2. Decomposition of hydrogen peroxide in raw and heated skimmilk.

The fact that the standard curve formed a straight line which passed through the experimentally determined points for each standard, as well as the origin of the graph, indicated no detectable destruction of peroxide in the water or buffer before adding the enzyme in the assay. On the other hand, since it is evident that low levels of hydrogen peroxide added to milk are very unstable and decompose rapidly, it would be impossible to prepare a valid standard curve from data in which milk was used to prepare the standards. By preparing standards in water, satisfactory stability of hydrogen peroxide can be maintained during measurement. It must be realized, however, that the peroxide measured in milk is dependent on the content of reactive components as well as the length of time the peroxide has been added.

Our results support those of Amin and Olson (1, 2) wherein the stability of hydrogen peroxide in milk was shown to be dependent to a certain extent on the composition and heat treatment of the milk. Some of the decomposition in raw skimmilk was apparently due to enzymatic action, since heating (100 C for 30 min) the milk before adding hydrogen peroxide resulted in reduced decomposition. The milk fat may have been responsible for the lower recovery in the pasteurized whole milk than in the nonfat milk samples. Our results also indicate that the amount of decomposition in milk tends to reach a point of equilibrium.

Acknowledgments

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Hydrogen peroxide

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Toxic action of hydrogen peroxide on the blood tissue cells

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The discovery of hydrogen peroxide dates back to 1818, thanks to Louis-Jacques Thenard, the celebrated French chemist, who obtained it by the well known reaction of barium dioxide with sulphuric acid:

$BaO_2 + H_2SO_4$ forms $BaSO_4 + H_2O_2$

It is a compound endowed with special chemical properties, derived primarily from its power of oxidation. Remember that the exidizing action of hydrogen peroxide is produced by subtraction of hydrogen ions and electrons from substances coming into contact with it, according to the reaction:

$H_2O_2 + 2H+ + 2e$ forms $2H_2O$.

But in the presence of a highly oxidizing substance, the character of the reaction changes, and the peroxide behaves as a reducer:

$H_2O_2 + O_2$ forms $2O_2 + 2H+ + 2e$.

It is precisely these chemical properties which make hydrogen peroxide such a complex substance from the toxicological point of view. Its action on the organic tissues is not yet completely known. We are led to think that peroxide can form, particularly under certain circumstances, in the heart of the cells of living organisms, in particular under the influence of ionizing radiations.

From the industrial point of view, hydrogen peroxide assumes remarkable importance owing to its use in chemical propellants for rockets. One example of these liquid propellants is that based on the hydrogen peroxide-permanganate system. Under pressure, the permanganate catalyzes the reaction of fission of the oxygenated water in the following manner:

$5H_2O_2 + 2MnO^-4 + 6H^+$ forms $2\ Mn++ + 8H_2O + 5O_2$.

Another use is in the preparation of epoxides and glycols from unsaturated hydrocarbons and natural fats. It is also used in the manufacture of plastifiers and solvents for polyvinyl resins; for bleaching fabrics, hair, ivory, silk; for felting horsehair and restoring paints by oxidation of black lead sulphide to white lead sulphate.

The concentrations in which it is used vary from 3% (as an antiseptic) to 90% (in propellants).

The industrial preparation of hydrogen peroxide is done by distillation in a vacuum of peroxy disulphuric, or dipersulphuric acid, obtained electrolytically. The reaction is as follows: $H_2S_2O_8 + 2H_2O$ forms $2H_2SO_4 + H_2O_2$.

Another method is based on the oxidation of isopropyl acid by organic compounds such as the alkyl anthraquinones.

As we can see, hydrogen peroxide is a substance of great scientific and practical interest. Its chemical properties and industrial applications of ever widening scope, require a thorough knowledge of its action and its toxicity to the animal (in) the organs or tissues most directly involved. Among these, the blood tissue is of particular importance, by reason of the richness of its enzymatic patrimony (we need only mention the presence of the catalases, enzymes directly involved in the defense of the organism), also by reason of the facility of observation to which its cellular elements lend themselves in the variety of function.

In regard to the toxicological action of hydrogen on the blood, studies were made by Granata and Broussard (1963-1964-1965) and by Granata, De Angelis, Piscaglia and Faraci (1967). In the course of these studies, several interesting aspects of the toxicology of hydrogen peroxide were revealed. We need only mention the cytophasic activity triggered by the toxic agent at the level of the blood cells, and manifesting itself in the form of phenomena of phagocytosis involving all the cellular elements of the blood, from the red corpuscles to the platelets and the leucocytes. We have as a matter of fact, described cases of neutrophile, monocytary, eosinophile and lymphocytary erythrophagia; granulocytary and monocytary thrombocytophagia; neutrophile, lymphocytary and monocytary neutrophagia; lymphocytary thrombocytophagia; neutrophile lymphophagia; basophile neutrophagia; basophile lymphophagia, etc. This singular phenomenon of leucocytes participating in a reciprocal phagocytosis was called "adelphophagia" (Granata and Broussard). Subsequent research demonstrated, among other things, that peroxide is in a position to act on the blood tissue even in a very highly diluted state; we have observed cases of cytophagia in the dose of one drop per ml of blood of 1% peroxide. T he action of peroxide is favored by an acid or slightly alkaline pH, and retarded by a highly alkaline pH.

Other studies have envisaged the importance of erythrocytary catalase

on the determinism of the cytophagic phenomenon, and have verified that the enzyme has no noteworthy influence in one direction or the other.

To the detriment of the red corpuscles, we have also spotted a hemolytic action which manifests itself primarily at concentrations below 2.50% or above 3.25%. The red cell, on the contary, resists at concentrations of peroxide lying between these figures.

Even on the platelets, hydrogen peroxide has a toxic effect which manifests itself by interesting phenomena. We frequently observe a transformation of the thrombocytes into macro and mega-thrombocytes (patelets whose dimensions reach, and sometimes exceed, those of an erythrocyte). Furthermore, we find thrombocytes disposed in a ring around leucocytary cells, formations which probably represent prephagocytary stages.

When treating blood in vitro with hydrogen peroxide in a dilution of 12%, at the dose of 0.1 - 0.2 - 0.3 ml per 4 ml of blood, Granata et coll. obtained morphological, volumetrical and dyschromic modifications of the plate elements, with alteration either of the chromometer or the hyalometer. In certain cases we are witnesses to the fusion of many elements together, giving rise to "plate skeins." These "skeins" result from an interlacing of filaments which assume a basophile coloration and are derived from the fusion of the plate elements. Their size can even reach that of one or two monocytes. The "skeins" are sometimes disposed in a ring around the leucocytary cells (see fig. 1). The phenomenon of plate fusion in skeins is produced more easily in treated whole blood than in blood washed by means of an isotonic solution.

The studies made will emphasize the interest of the toxicological phenomena determined by hydrogen peroxide at the level of the blood tissue. Once again the hematic cells have proved to be truly indicated for the study of the toxicological mechanisms, especially because they permit demonstration of certain particular aspects of the toxic mechanism.

The results reproted above come from research which was designed to examine morphological and chromophile modifications in the hematic cells treated "in vitro" with hydrogen peroxide at different concentrations. Among other things we compared the results obtained with samples of whole blood and samples of blood washed with physiological serum, in order to distinguish on the one hand the direct action of the toxin on the hematic cells, and on the other, the role of plasma in the determinism of the cyto-toxic phenomena observed.

Methodology

1. We first prepared the following dilutions of hydrogen peroxide: 1%, 3%, 6%, 10%, 12%, 24%.

2. Venous blood was taken from healthy adults under the usual conditions of sterility and minimal heparinization. The blood was kept at the temperature of 25°C for the entire time of the experiment. This temperature, as shown by the prior experiments (Cranata) makes it possible to keep the cells alive for a period of at least 2 hours. Then a part of the blood sample was washed in an isotonic, saline physiological solution, all precautions being taken to prevent removing the leucocytary layer.

The whole blood and the washed blood were then distributed separately at the rate of 4 ml per test tube.

3. The research, for each experiment, bore on four samples of blood from a given subject, to wit:

a) 4 ml of untreated whole blood

b) 4 ml of whole blood treated with hydrogen peroxide

c) 4 ml of blood washed with isotonic physiological saline solution with no toxic addition

d) 4 ml of washed blood treated with hydrogen peroxide.

4. The quantities of peroxide successively used in the various experiments were the following:

a)	dilution	of	hydrogen	peroxide to	1%,	0.1	ml	per ml	of blood
b)	"	"	"	"	1%,	0.2	"	"	"
c)	"	"	"	"	3%,	0.1	"	"	"
d)	"	"	"	"	6%,	0.1	"	"	"
e)	"	"	"	"	6%	0.2	"	"	"
d)	"	"	"	"	10%,	0.1	"	"	"
e)	"	"	"	"	12%,	0.1	"	"	"
f)	"	"	"	"	10%,	0.05	"	"	"

The same quantity of peroxide was used simultaneously for whole blood and washed blood.

5. The peroxide must be mixed with the blood in small quantities, shaking the tube very gently; the latter must be tall enough to retain the foam forming on addition of the peroxide.

6. With a glass rod, every ten minutes for two hours, we take a drop of blood from each tube and spread it on an object glass according to the usual technique. The rubbing is allowed to dry in the air. The colora-

tion is then produced with the panoptic method of Pappenheims, and the observation made with the immersion lens.

Results

The toxic effect of hydrogen peroxide on blood cells is certain, but it differs according to the presence or absence of plasma. Most of the phenomena observed are actually different under the two conditions. There are phenomena which occur at any dilution of the toxin, and others which, on the contrary, are linked to well-defined concentrations.

The principal observations made in the course of this study are the following:

1. The blood cells treated after washing in physiological serum retain their tinctorial characteristics in the panoptic coloration of Pappenheim.

But treated cells of whole blood show modifications of chromatism, reversible in the majority of cases, which manifests itself more or less precociously and last for a longer or shorter time according to the concentration of toxin used. Tn general, the red corpuscles acquire a grayish, or even a bluish-green color.

The nuclei of the neutrophile granulocytes gradually lose their coloration, which shifts from a light violet to a greenish then greyish color, (see fig. 1). Their cytoplasm often appears rimmed with granulations, and hyperchromic. In other cases it is / degranulated.

The eosinophile granulocytes, as they lose nuclear coloration, retain that of the cytoplasmic granulations, which only rarely show phenomena of dyschromia.

The basophile granulocytes have a characteristic behavior insofar as their granulations are concerned (see below), but they follow the chromatic modffications of the other granulocytes, while the coloration of their granulations remains unchanged, and may even be said to be accentuated.

The large lymphocytes show a reduction in nuclear chromatism, less marked, however, than in the granulocytes. Their cytoplasmic basophilia is enchanced, and sometimes it is possible to observe a variation in the state of solubility of theccytoplasmic substances, with formation of basophile granulations visible in the space left empty by the nucleus. The phenomenon is also visible in the monocytes, which behave in a manner similar to that of the lymphocytes, but which lose the color of the nucleus more easily.

The small lymphocytes are less frequently dyschromic, and the coloration of the nucleus is much more resistant.

The thrombocytes are the most resistant cells from the point of view of coloration, even at the largest doses of the toxin. We frequently find masses of plates, normally and even highly colored, beside leucocytes with clearly hypo- and dyschromic nuclei.

2. In treated whole blood, anisocytosis is very frequent. After a time which varies according to the concentration of the toxin (in general from 15 to 40 minutes), spherocytary red cells appear, for all toxin dilutions used. The spherocytes are hyperchromic and dyschromic, and often show, granulations on their surface, or along their perimeter, which in most cases have a purplish-violet shade. These granulations are also visible on erythrocytes that are not completely spherical; they are disposed around cell lesions of the vacuolar or crater type, or on the periphery of the element.

The spherocytes and their granulations are a function of the concentration of the hydrogen peroxide, and of the time of action of the toxin on the blood; they are numerous in a 12% dilution, and very rare at 1%. Their frequency can be represented by a curve; at the start of the treatment they are rate, then they become more and more numerous, then diminish until they disappear. The higher the concentration of the perioxide, the more the peak of the curve moves toward the initial period of the observation.

Fig. 1 page 111 Human venous blood treated in vitro with 0.1 ml of 6% hydrogen peroxide per ml. Rubbing after 60 minutes of treatment. Panoptic Pappenheim coloration. The neutrophile granulocytes have an abnormal shade and above all a low-intensity coloration with dyschromia of the nucleus. The cytoplasms however, are hyperchromic.

The color of these spherocytes is more intense than that of the other erythrocytes. Sometimes they contrast by an unmistakeably pink color (these could be called: "pink cells") in the midst of the other greenish-gray hematias. It is even possible to observe spherocytes without granulations.

With 12% peroxide, and only on the blood of certain subjects who probably have particularly fragile hematies, it is possible to see erythrosytes of normal volume or even larger than the norm, with much smaller granulations, much finer than those we have described previously, and having a weakly basophile coloration.

Many hematias, particularly in the presence of 6% peroxide, have remained hypochromic, with reduction of their consistency and sometimes augmentation

of their volume, the adhesion of the cell to the slide (the plate) being increased owing to the reduction in consistency of the element (see fig.2). The alterations of the spherocytary type mentioned above have never been observed when the treatment was carried out on washed blood, whatever the peroxide concentration used.

Fig. 2, page 112 Human venous blood treated in vitro with 0.2 ml of 12% hydrogen peroxide per ml. Smear after 90 minutes of treatment. Panoptic Pappenheim treatment. We observe, in the center of the figure, three spherocytes with small peripheral granulations of a purplish-violet color.

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3. With hydrogen peroxide dilutions of 3 to 24%, and on whole blood, we note remarkable alterations in the lymphocytes, The nucleus often appears pycnotic and dysmorphic. We find elements (particularly at the dilution of 24%) having an aspect with two, three and even six lobes. Sometimes the nucleus has convexities. More rarely we observe elements with a double nucleus.

4. The monocytes, particularly in the presence of 6% peroxide, and on treated whole blood, assume a very irregular nuclear morphology with vacuoles in the cytoplasm and in the nucleus.

5. Basophile granulocytes, particularly at the highest concentrations, of the toxin, show a displacement of the basolphile granulations toward the cytoplasm, and the periphery of the cell (see fig. 3).

The phenomenon is present in whole blood as well as washed blood.

6. The plates tend to form masses and to increase in volume as they are transformed into macro- and mega-thrombocytes. At the higher peroxide concentrations we sometimes observe a central disposition of the chromomer, swelling of the cell with tendancy to fusion of the cellular elements with each other. The plate phenomena appear particularly evident when we use hydrogen peroxide at 12%. Around the 39th or 40th minute of the treatment, masses of 5 elements or more form, showing complex morphological modifications, phenomena of dyschromia and turgescence. Successively, the elements merge together, becoming transformed into an interlacing of filaments assuming a basophile coloration. This is how what we call "skeins" are formed. The phenomenon of "plate fusion into skeins" previously reported by Granata et coll. (1967) is produced with greater case in treated whole blood. In washed blood, however, the phenomena of plate enlargement and chromomer

centralization are more frequent. The plate "skeins," as well as the plates, can assume a perileucocytary position, particularly around neutrophile granulocytes and monocytes (fig. 4).

Fig. 3 page 113 Venous human blood treated in vitro with 0.1 ml of 12% hydrogen peroxide per ml. Smear after 40 minutes of treatment. Panoptic Pappenheim coloration. Basophile granulocyte with most of the granulations shifted toward the cytoplasm. Note the dyschromia and the low-intensity coloration of the nucleus.

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7. The cytophagic phenomena, already spotted by Granata and Broussard (1963), were confirmed in the course of these studies. They appeared much more frequently and obviously, in the samples of blood treated with peroxide after washing with isotonic solution.

The presence of the plasma, consequently, makes it more difficult to measure cytophagia. The frequency of these phenomena, as a matter of fact, is proportional to the concentration of perioxide used, and we note this most clearly at 10-12%.

The leucocytes of the blood smears made after 24 or 48 hours of treatment show serious degenerative modifications, particularly in the case of washed blood.

This should probably be attributed to either the absence of plasma a natural protective medium, or, as we have seen, to a greater intensity of the cytophagic processes. We know, as a matter of fact, that the phagocytary cells are exposed in turn to lysis processes, consecutive on the absorption of the phagocyte elements and to the products of their intracellular catabolism (Granata).

Fig. 4 page 114 Venous blood (human) treated in vitro with 0.1 ml of 12% hydrogen peroxide per ml. Smear after 90 minutes of treatment. Panoptic Pappenheim coloration. Plate skeins of perileucocytary rosettes.

Discussion and conclusions

Analysis of the results obtained make it possible to make the following deductions:

1. Hydrogen peroxide provokes alterations throughout all the lines of blood cells. The most important toxicological phenomena relating to the red

corpuscles are: the appearance of anisocytosis; the formation of spherocytes, the appearance of granulations, particularly on the spherocytes; the dyschromic variations.

In respect of the lymphocytary cells: augmentation of the basophilia and granular transformation of the cytoplasm; dyschromic alterations of the nucleus, particularly in the case of the large lymphocytes; morphological alterations of the caryoplasm (projections, abnormal lobulation, double nucleus).

In respect of the monocytes: augmentation of cytoplasmic basophilia, dyschromic and morphological alterations of the caryoplasm.

In respect of the neutrophile granulocytes: dyschromic alterations of the nucleus, degranulization and vacuolization of the cytoplasm.

In respect of the eosinophile granulocytes: alterations similar to those of the neutrophiles. The eosinophile granulations, however, retain their normal, or almost normal coloration, even where the nucleus is discolored.

In respect of the basophile granulocytes: alterations similar to those of the neutrophiles. The basophile granulations tend to lose their nuclear disposition, and to shift into the cytoplasm and toward the periphery of the cell.

In respect of the plates: morphological alterations with formation of macro- and mega-thrombocytes, perileucocytary disposition of the elements, and tendency to confluence (phenomenon of plate fusion in skeins).

2. Confirmation of the phenomena of hematic cytophagia, particularly obvious at the highest concentrations of the peroxide, and in blood treated by washing the cells by means of physiological serum.

3. The plasma plays an important role in the determinism, and in the incidence of the various cellular, toxicilogical phenomena due to hydrogen peroxide. We can affirm, principally, that the plasma favors the appearance of anisocytosic alterations of the hematias, and in particular the formation of the spherocytes. Furthermore it makes the formation of granulations, in the hematias themselves, easier. The absence of plasma, on the contrary, favors the manifestation of cytophagic phenomena. Even the behavior of the plates is different according to whether the blood is treated whole, or washed. In the first case, as a matter of fact, we observe the phenomena of plate fusion in skeins, and their perileucocytary fusion. In the second case, on the contrary, it is easier to verify the alterations in volume of the elements.

Another interesting piece of information on the subject of plasma results

from the fact that, while the nuclei of the leucocytes become dyschromic and even achromic if the blood is treated whole, they will, on the contrary retain their normal coloration in washed blood. The same erythrocytes which, in the first case, are clearly dyschromic, will, in the second case, even if their color is not perfectly normal, nevertheless show a coloration which closely approaches the acidophile shade.

All of this lends plausibility to the hypothesis that plasma is involved directly in the toxicological mechanism of hydrogen peroxide. It is probable that the plasma components react with formation of compounds which are toxic to the cells, so that plasma permits the fission or transformation of the peroxide into derivatives endowed with high toxicity, as soon as they have penetrated into the cells. Cytophagia, on the contary, is facilitated more by the absence of plasma. The latter consequently, seems to exert a defensive cellular action capable of inhibiting the phenomenon, perhaps even in favor of a rapid transformation of the peroxide. This leads us to think that only the direct penetration of the toxin, and probably its intracellular fision, are in a position to trigger the cytophagic phenomenon. This observation confirms the hypothesis of an enzymatic and bio-electric mechanism of phagocytosis itself.

As we see, the toxic action of hydrogen peroxide on the blood tissue involves all the components of the blood, including the plasma itself.

The chemical reactions intervening at the level of the plasma and the cells, and causing numerous phenomena affecting the vitality, the functions and the morphology of the blood elements, are particularly interesting. In this respect we should underline the facility which peroxide has in splitting, with formation of ions and exchanges of electrons. The importance of these chemical phenomena on the intracellular structures and on the enzymatic balance, leads us to link the mechanism of the toxic action of hydrogen peroxide to profound alterations in the ionic state and the enzymatic function of the elements, i.e. to consider it as a bioclectric-enzymopathic mechanism.

The results obtained reveal the existence of numerous problems concerning the toxicity and the biochemical activity of peroxide. These problems, by the relations which they maintain with the phenomena of intra-organic hydrolysis determined by ionizing radiations acquire a very present interest.

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Action toxique du peroxyde d'hydrogène sur les cellules du tissu sanguin.

Par

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La découverte du peroxyde d'hydrogène remonte à 1818, grâce à Louis-Jacques Thénard, célèbre chimiste français, qui l'obtint par la réaction bien connue du bioxyde de baryum avec l'acide sulfurique : $BaO_2 + H_2SO_4 \rightarrow BaSO_4 + H_2O_2$.

Il s'agit d'un composé doté de propriétés chimiques particulières, dérivant surtout de son pouvoir d'oxydation. Il faut rappeler que l'action oxydante du peroxyde d'hydrogène se produit par soustraction d'ions hydrogène et d'électrons aux substances venant à son contact, selon la réaction :

$$H_2O_2 + 2H^+ + 2e \rightarrow 2H_2O.$$

Mais en présence d'une substance fortement oxydante le caractère de la réaction se modifie et le peroxyde se comporte en réducteur :

$$H_2O_2 + O_2 \rightarrow 2O_2 + 2H^+ + 2e.$$

Ce sont précisément ces propriétés chimiques qui font du peroxyde d'hydrogène une substance complexe du point de vue toxicologique. Son action sur les tissus organiques n'est pas encore complètement connue. On est amené à penser que le peroxyde peut se former notamment, dans certaines circonstances, au sein des cellules des organismes vivants, en particulier sous l'influence des radiations ionisantes.

Du point de vue industriel, le peroxyde d'hydrogène prend une importance remarquable du fait de son emploi dans les propulseurs chimiques pour fusées. Un exemple de ces propulseurs liquides est celui fondé sur le système peroxyde

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d'hydrogène-permanganate. Sous pression, le permanganate catalyse la réaction de scission de l'eau oxygénée de la façon suivante :

$$5H_2O_2 + 2MnO^-_4 + 6H^+ \rightarrow 2Mn^{++} + 8H_2O + 5O_2.$$

Un autre emploi est celui de la préparation d'époxydes et de glycols à partir des hydrocarbures non saturés et des graisses naturelles. Il est utilisé en outre dans la fabrication de plastifiants et de solvants des résines polyvinyliques; pour blanchir les tissus, décolorer les cheveux, les ivoires, la soie; pour feutrer le crin et pour restaurer les peintures par oxydation du sulfure de plomb noir en sulfate de plomb blanc.

Les concentrations auxquelles il est employé, varient de 3 p. 100 (comme antiseptique) à 90 p. 100 (dans les propulseurs).

La préparation industrielle de peroxyde d'hydrogène se fait par distillation sous vide de l'acide peroxydisulfurique, ou dipersulfurique, obtenue par voie électrolytique. La réaction est la suivante : $H_2S_2O_8 + 2H_2O \rightarrow 2H_2SO_4 + H_2O_2$.

Une autre méthode se fonde sur l'oxydation de l'acide isopropylique par des composés organiques comme les alkylanthraquinones.

Comme on le voit, le peroxyde d'hydrogène est une substance d'un haut intérêt scientifique et pratique. Ses propriétés chimiques et ses applications industrielles de plus en plus étendues exigent une connaissance approfondie de son action et de sa toxicité pour l'animal, les organes ou tissus les plus directement intéressés. Parmi ceux-ci le tissu sanguin a une importance particulière, en raison de la richesse de son patrimoine enzymatique (il suffit de rappeler la présence des catalases, enzymes directement intéressées à la défense de l'organisme), en raison aussi de la facilité d'observation à laquelle se prêtent ses éléments cellulaires aux multiples fonctions.

A propos de l'action toxicologique du peroxyde d'hydrogène sur le sang, des recherches ont été effectuées par Granata et Broussard (1963-1964-1965) et par Granata, De Angelis, Piscaglia et Faraci (1967). Au cours de ces recherches ont été mis en évidence quelques aspects intéressants de la toxicologie du peroxyde d'hydrogène. Il suffira de rappeler l'activité cytophagique, déclanchée par l'agent toxique au niveau des cellules sanguines et se manifestant sous forme de phénomènes de phagocytose, intéressant tous les éléments cellulaires du sang, des globules rouges jusqu'aux plaquettes et aux leucocytes. On a en effet décrit des cas d'érythrophagie neutrophile, monocytaire, éosinophile et lymphocytaire; de thrombocytophagie granulocytaire et monocytaire; de neutrophagie neutrophile, lymphocytaire et monocytaire; de thrombocytophagie lymphocytaire; de lymphophagie neutrophile; de neutrophagie basophile; de lymphophagie basophile, etc. Ce phénomène singulier de leucocytes participant à une phagocytose réciproque a été appelé « adelphophagie » (Granata et Broussard). Des recherches ultérieures ont démontré entre autres choses que le peroxyde est en mesure d'agir sur le tissu sanguin, même à des dilutions très élevées; on a observé des cas de cytophagie à la dose d'une goutte par ml de sang de peroxyde à 1 p. 100. L'action du peroxyde est favorisée par un pH acide ou légèrement alcalin et retardé par un pH fortement alcalin.

D'autres recherches ont visé l'importance de la catalase érythrocytaire sur le déterminisme du phénomène cytophagique et ont vérifié que l'enzyme n'a une influence notable ni dans un sens ni dans l'autre.

Au détriment des globules rouges, on a relevé en outre une action hémolytique qui se manifeste surtout à des concentrations inférieures à 2,50 p. 100 ou supérieures

à 3,25 p. 100. L'hématie résiste par contre à des concentrations de peroxyde comprises entre ces deux taux.

Même sur les plaquettes, le peroxyde d'hydrogène a une action toxique qui se manifeste par des phénomènes intéressants. On observe fréquemment une transformation des thrombocytes en macro- et méga-thrombocytes (plaquettes dont les dimensions atteignent et quelquefois dépassent celles d'un érythrocyte). En outre on trouve des thrombocytes disposés en couronne autour des cellules leucocytaires, formations qui représentent vraisemblablement des stades préphagocytaires.

En traitant du sang in vitro avec du peroxyde d'hydrogène à la dilution de 12 p. 100 à la dose de 0,1 - 0,2 - 0,3 ml pour 4 ml de sang, Granata et Coll. ont obtenu des modifications morphologiques, volumétriques et dyschromiques des éléments plaquettaires, avec altérations soit du chromomère, soit du hyalomère. Dans certains cas on assiste à la fusion de nombreux éléments entre eux, donnant lieu aux « écheveaux plaquettaires ». Ces « écheveaux » résultent d'un entrelacement de filaments, qui prennent une coloration basophile et dérivent de la fusion des éléments plaquettaires. Leur dimension peut même atteindre celle d'un ou deux monocytes. Les « écheveaux » se disposent parfois en couronne autour des cellules leucocytaires (voir la fig. 1). Le phénomène de la *fusion plaquettaire en écheveaux* se produit plus facilement dans le sang traité *in toto*, que dans le sang lavé au moyen d'une solution isotonique.

Les recherches effectuées soulignent l'intérêt des phénomènes toxicologiques déterminés par le peroxyde d'hydrogène au niveau du tissu sanguin. Encore une fois les cellules hématiques se sont avérées vraiment indiquées pour l'étude des mécanismes toxicologiques, surtout parce qu'elles permettent de mettre en évidence certains aspects particuliers du mécanisme toxique.

Les résultats rapportés ci-dessus proviennent de recherches ayant pour but d'examiner les modifications morphologiques et chromophiles des cellules hématiques traitées « in vitro » avec du peroxyde d'hydrogène à différentes concentrations. Entre autres, on a comparé entre eux les résultats obtenus avec des échantillons de sang *in toto* et des échantillons de sang lavé avec du sérum physiologique et cela afin de distinguer d'une part l'action directe du toxique sur les cellules hématiques, de l'autre le rôle du plasma dans le déterminisme des phénomènes cyto-toxiques constatés.

Méthodologie.

1° On a préparé préalablement les dilutions suivantes de peroxyde d'hydrogène : 1 p. 100; 3 p. 100; 6 p. 100; 10 p. 100; 12 p. 100; 24 p. 100.

2° Du sang veineux a été prélevé chez des adultes sains dans les conditions habituelles de stérilité et d'héparinisation minimale. Le sang a été maintenu à la température de 25°C pour toute la durée de l'expérience. Cette température, comme l'ont montré les expériences antérieures (Granata), permet de maintenir vitales les cellules pour une période d'au moins 2 heures. Puis une partie du sang prélevé a été lavé dans une solution saline physiologique isotonique, toutes précautions prises pour ne pas enlever la couche leucocytaire.

Le sang *in toto* et le sang lavé ont été ensuite répartis séparément à raison de 4 ml par tube à essai.

3° La recherche a porté, pour chaque expérience sur quatre échantillons du sang d'un même sujet à savoir :

a) 4 ml de sang *in toto* non traité;
b) 4 ml de sang *in toto* traité par peroxyde d'hydrogène;
c) 4 ml de sang lavé avec une solution saline physiologique isotonique sans apport toxique;
d) 4 ml de sang lavé et traité par peroxyde d'hydrogène.

4° Les quantités de peroxyde successivement employées dans les différentes expériences ont été les suivantes :

a)	dilution du peroxyde d'hydrogène à			1 p. 100,	0,1 ml par ml de sang.		
b)	—	—		1 —	0,2 ml	—	—
c)	—	—	—	3 ,	0,1 ml	—	—
d)	—	—	—	6 ,	0,1 ml	—	—
e)	—	—	—	6 ,	0,2 ml	—	—
d)	—	—	—	10 ,	0,1 ml	—	—
e)	—	—	—	12 ,	0,1 ml	—	—
f)	—	—	—	10 ,	0,05 ml	—	—

La même quantité de peroxyde a été employée simultanément pour le sang *in toto* et pour le sang lavé.

5° Le peroxyde doit être mélangé au sang par petites quantités en agitant le tube très délicatement; celui-ci doit être suffisamment haut pour retenir la mousse qui se forme à l'adjonction du peroxyde.

6° Avec une baguette en verre on prélève, toutes les 10 minutes pendant 2 heures, une goutte de sang dans chaque tube et on l'étale sur une lame porte-objet selon la technique usuelle. On laisse le frottis dessécher à l'air. La coloration est ensuite effectuée avec la méthode panoptique de Pappenheim et l'observation se fait au moyen de l'objectif à immersion.

Résultats.

L'action toxique du peroxyde d'hydrogène sur les cellules sanguines est certaine, mais elle diffère selon la présence ou non du plasma. La plupart des phénomènes observés sont en effet différents dans les deux conditions. Il y a des phénomènes qui se produisent à n'importe quelle dilution du toxique et d'autres qui, au contraire, sont liés à des concentrations bien déterminées.

Les constatations principales faites au cours de cette étude sont les suivantes :

1° Les cellules sanguines traitées après lavage en sérum physiologique gardent leurs caractéristiques tinctoriales à la coloration panoptique de Pappenheim.

Par contre les cellules du sang traité *in toto* présentent des modifications du chromatisme, dans la plupart des cas, réversibles, qui se manifestent plus ou moins précocement et durent plus ou moins longtemps selon la concentration du toxique employé. En général, les globules rouges prennent une couleur grisâtre ou bien une teinte vert-bleue.

Les noyaux des granulocytes neutrophiles perdent graduellement leur coloration qui passe du violet-clair à une couleur verdâtre puis grisâtre (voir la fig. 1). Leur cytoplasme se présente souvent bourré de granulations et hyperchromique. Dans d'autres cas, il est dégranulé.

Les granulocytes éosinophiles, tout en perdant la coloration nucléaire, gardent celle des granulations cytoplasmiques, qui ne montrent que rarement des phénomènes de dyschromie.

Les granulocytes basophiles ont un comportement caractéristique pour ce qui concerne leurs granulations (*vide infra*), mais ils suivent les modifications chromatiques des autres granulocytes, en maintenant toutefois inaltérée et on dirait même accentuée la coloration de leurs granulations.

Les grands lymphocytes présentent une diminution du chromatisme nucléaire, moins marquée toutefois que dans les granulocytes. Leur basophilie cytoplasmique est augmentée et parfois il est possible d'observer une variation de l'état de solubilité des substances cytoplasmiques, avec formation de granulations basophiles visibles dans l'espace laissé libre par le noyau. Le phénomène est visible aussi dans les monocytes, qui ont un comportement semblable à celui des lymphocytes, mais qui perdent plus facilement la couleur du noyau.

Les petits lymphocytes sont moins fréquemment dyschromiques et la coloration du noyau est beaucoup plus résistante.

Les thrombocytes sont les cellules les plus résistantes du point de vue de la coloration, cela même aux doses les plus élevées du toxique. On trouve fréquemment des amas de plaquettes normalement et même fortement colorées à côté de leucocytes à noyaux nettement hypo- et dyschromiques.

2° Dans le sang traité *in toto*, l'anisocytose est très fréquente. Après un délai variable selon la concentration du toxique (en général de 15 à 40 mn), des hématies sphérocytaires apparaissent et cela pour toutes les dilutions du toxique utilisées. Les sphérocytes sont hyperchromiques et dyschromiques et souvent ils présentent

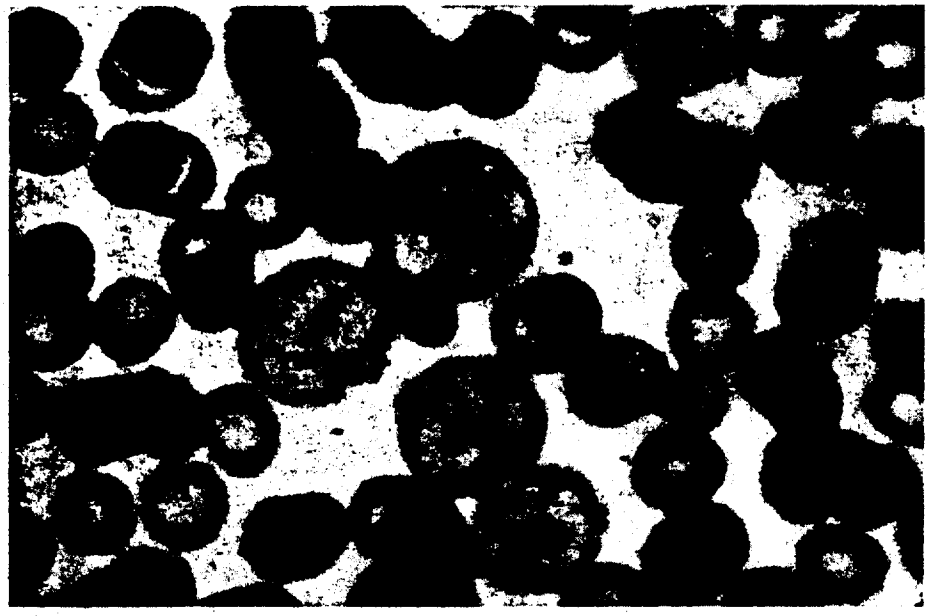

Fig. 1. — *Sang veineux humain traité in vitro* avec 0,1 ml de peroxyde d'hydrogène à 6 p. 100 par ml. Frottis après 60 mn de traitement. Coloration panoptique de Pappenheim. Les granulocytes neutrophiles présentent une teinte anormale et surtout une coloration peu intense avec dyschromie du noyau. Par contre les cytoplasmes sont hyperchromiques.

sur leur surface ou le long de leur périphérie des granulations qui dans la plupart des cas ont une teinte violet-pourpre. Ces granulations sont visibles aussi sur des érythrocytes non complètement sphériques; elles sont disposées autour de lésions de la cellule de type vacuolaire, ou à cratère, ou bien à la périphérie de l'élément.

Les sphérocytes et leurs granulations sont fonction de la concentration du peroxyde d'hydrogène et de la durée de l'action du toxique sur le sang; ils sont nombreux à la dilution 12 p. 100 et ils sont très rares à celle 1 p. 100. Leur fréquence peut être représentée par une courbe : au début du traitement ils sont rares, ensuite ils deviennent de plus en plus nombreux puis ils diminuent jusqu'à disparaître. Plus la concentration du peroxyde est élevée et plus le sommet de la courbe se déplace vers la période initiale de l'observation.

La couleur de ces sphérocytes est plus intense que celle des autres érythrocytes. Souvent ils tranchent par une couleur franchement rosée (on pourrait les appeler : « cellules rosées ») au milieu des autres hématies gris-verdâtres. Il est même possible d'observer des sphérocytes sans granulations.

Avec le peroxyde à 12 p. 100 et seulement sur le sang de certains sujets ayant probablement des hématies particulièrement fragiles, il est possible de voir des érythrocytes de volume normal, ou même supérieur à la norme, avec des granulations, beaucoup plus petites, plus fines que celles que nous avons décrites et présentant une coloration faiblement basophile.

De nombreuses hématies, surtout en présence de peroxyde à 6 p. 100, sont restées hypochromiques, avec diminution de leur consistance et quelquefois augmentation de leur volume, l'adhérence de la cellule à la lame étant accrue du fait de la réduction de consistance de l'élément (voir fig. 2). Les altérations de type sphérocytaire mentionnées ci-dessus n'ont jamais été observées lorsque le traitement était effectué sur le sang lavé, quelle que fût la concentration du peroxyde employé.

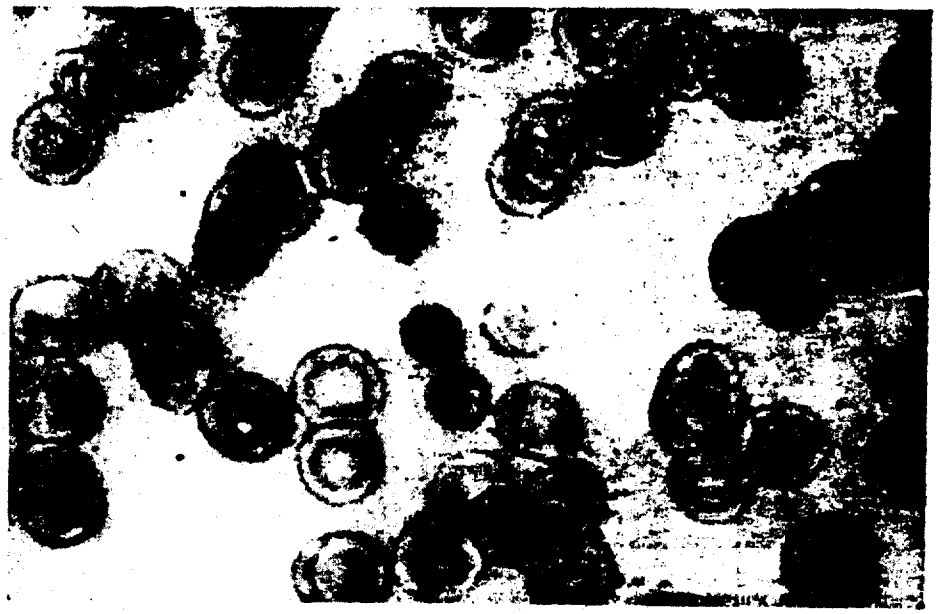

Fig. 2. — *Sang veineux humain traité in vitro* avec 0,2 ml de peroxyde d'hydrogène à 12 p. 100 par ml. Frottis après 90 mn de traitement. Coloration panoptique de Pappenheim. On observe, au centre de la figure, trois sphérocytes avec de petites granulations périphériques de couleur violet-pourpre.

3° Avec les dilutions de peroxyde d'hydrogène de 3 à 24 p. 100 et sur le sang *in toto*, on observe des altérations remarquables des lymphocytes. Le noyau se présente souvent pycnotique et dysmorphique. On trouve des éléments (surtout à la dilution de 24 p. 100) présentant un aspect à deux, trois et même six lobes. Quelquefois le noyau présente des bosses. Plus rarement on observe des éléments avec un double noyau.

4° Les monocytes, surtout en présence de peroxyde à 6 p. 100, et sur le sang traité *in toto*, affectent une morphologie nucléaire très irrégulière avec des vacuoles dans le cytoplasme et le noyau.

5° Les granulocytes basophiles, surtout aux concentrations les plus élevées du toxique, montrent un déplacement des granulations basophiles vers le cytoplasme et la périphérie de la cellule (voir fig. 3).

Le phénomène est présent dans le sang traité *in toto* ainsi que dans le sang lavé.

6° Les plaquettes tendent à former des amas et à augmenter de volume en se transformant en macro- et méga-thrombocytes. Aux concentrations de peroxyde les plus élevées, on observe quelquefois une disposition centralisée du chromomère, gonflement de la cellule avec tendance à la fusion des éléments cellulaires entre eux. Les phénomènes plaquettaires se présentent de façon particulièrement évidente lorsqu'on utilise du peroxyde d'hydrogène à 12 p. 100. Vers la 30e ou 40e minute du traitement, des amas se forment de 5 éléments ou davantage qui présentent des modifications morphologiques complexes, des phénomènes de dyschromie et de turgescence. Successivement les éléments se fondent entre eux, en se transformant dans un entrelacement de filaments qui prennent une coloration basophile. De

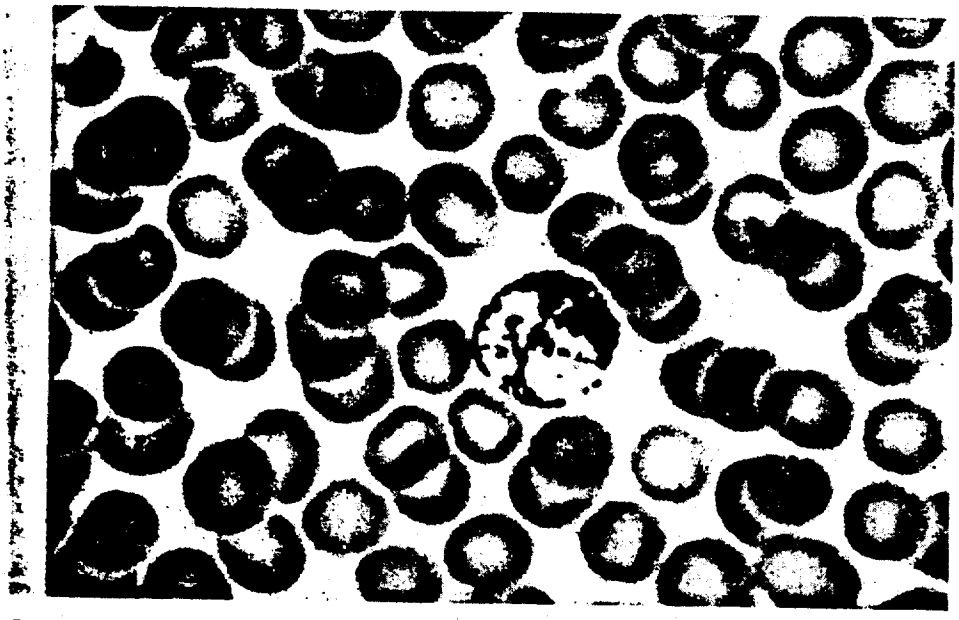

Fig. 3. — *Sang veineux humain traité in vitro* avec 0,1 ml de peroxyde d'hydrogène à 12 p. 100 par ml. Frottis après 40 mn de traitement. Coloration panoptique de Pappenheim. Granulocyte basophile avec la plupart des granulations déplacées vers le cytoplasme. On remarque la dyschromie et la coloration peu intense du noyau.

cette façon se constituent ce que nous avons appelé *écheveaux*. Le phénomène de la « fusion plaquettaire en écheveaux », déjà relevé par Granata et Coll. (1967) se produit avec plus de facilité dans le sang traité *in toto*. Dans le sang lavé par contre les phénomènes d'agrandissement des plaquettes et de centralisation du chromomère sont plus fréquents. Les « écheveaux » plaquettaires, de même que les plaquettes, peuvent prendre une position *périleucocytaire*, surtout autour des monocytes et des granulocytes neutrophiles (fig. 4).

7° Les phénomènes cytophagiques, déjà relevés par Granata et Broussard (1963), ont été confirmés au cours de ces recherches. Ils sont apparus bien plus fréquents et manifestes dans les échantillons de sang traités par le peroxyde après lavage avec solution isotonique.

La présence du plasma rend, par conséquent, plus difficile la détermination de la cytophagie. La fréquence de ce phénomène est en effet proportionnelle à la concentration du peroxyde employé et on l'observe le plus nettement à 10-12 p. 100.

Les leucocytes des frottis de sang effectués après 24 ou 48 h de traitement présentent de graves modifications dégénératives, surtout s'il s'agit de sang lavé.

Cela doit probablement être rapproché soit de l'absence du plasma, milieu protecteur naturel, soit, comme on l'a vu, d'une plus grande intensité des processus cytophagiques. On sait, en effet, que les cellules phagocytaires s'exposent à leur tour à des processus de lyse, consécutifs à l'absorption des éléments phagocytés et aux produits de leur catabolisme intracellulaire (Granata).

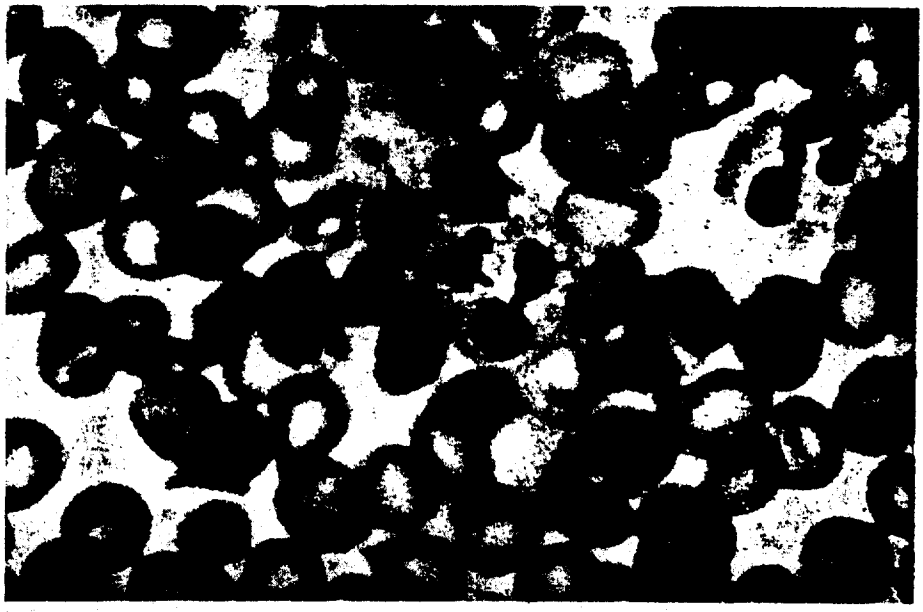

Fig. 4. — *Sang veineux humain traité in vitro* avec 0,1 ml de peroxyde d'hydrogène à 12 p. 100 par ml. Frottis après 90 mn de traitement. Coloration panoptique de Pappenheim. Écheveaux plaquettaires en rosette périleucocytaire.

Discussion et conclusions.

L'analyse des résultats obtenus permet de formuler les déductions suivantes :

1° Le peroxyde d'hydrogène provoque des altérations sur toutes les lignées des cellules sanguines. Les phénomènes toxicologiques les plus importants concernant les globules rouges sont : l'apparition d'anisocytose; la formation de sphérocytes; l'apparition de granulations, surtout sur les sphérocytes; les variations dyschromiques.

En ce qui concerne les cellules lymphocytaires : augmentation de la basophilie et transformation granulaire du cytoplasme; altérations dyschromiques du noyau, surtout chez les grands lymphocytes; picnose nucléaire; altérations morphologiques du caryoplasme (bosses, lobulation anormale, double noyau).

En ce qui concerne les monocytes : augmentation de la basophilie cytoplasmique, altérations dyschromiques et morphologiques du caryoplasme.

En ce qui concerne les granulocytes neutrophiles : altérations dyschromiques du noyau, dégranulation et vacuolisation du cytoplasme.

En ce qui concerne les granulocytes éosinophiles : altérations semblables à celles des neutrophiles. Les granulations éosinophiles maintiennent toutefois leur coloration normale ou presque, même là où le noyau est décoloré.

En ce qui concerne les granulocytes basophiles : altérations semblables à celles des neutrophiles. Les granulations basophiles tendent à perdre leur disposition nucléaire et à se porter dans le cytoplasme et vers la périphérie de la cellule.

En ce qui concerne les plaquettes : altérations morphologiques avec formation de macro- et mégathrombocytes, disposition périleucocytaire des éléments et tendance à la confluence (phénomène de la *fusion plaquettaire en écheveaux*).

2° Confirmation des phénomènes de cytophagie hématique, particulièrement évidents aux concentrations les plus élevées du peroxyde et dans le sang traité par lavage des cellules au moyen de sérum physiologique.

3° Le plasma joue un rôle important dans le déterminisme et dans l'incidence des divers phénomènes toxicologiques cellulaires dus au peroxyde d'hydrogène. On peut affirmer principalement que le plasma favorise l'apparition des altérations anisocytosiques des hématies et en particulier la formation des sphérocytes. En outre il rend plus facile la formation de granulations dans les hématies mêmes. L'absence du plasma favorise par contre la manifestation des phénomènes cytophagiques. Même le comportement des plaquettes est différent selon que le sang est traité *in toto* ou lavé. Dans le premier cas on observe en effet les phénomènes de la *fusion plaquettaire en écheveaux* et de leur disposition périleucocytaire. Dans le deuxième cas il est plus facile par contre de vérifier les altérations de volume des éléments.

Une autre donnée intéressante au sujet de l'action du plasma résulte du fait que, tandis que les noyaux des leucocytes deviennent dyschromiques et même achromiques si le sang est traité *in toto*, dans le sang lavé au contraire ils conservent leur coloration normale. Les mêmes érythrocytes qui dans le premier cas sont nettement *dyschromiques*, dans le deuxième, même si leur coloris n'est pas parfaitement normal, présentent toutefois une coloration qui approche de près la teinte acidophile.

Tout cela donne du crédit à l'hypothèse que le plasma est intéressé directement dans le mécanisme toxicologique du peroxyde d'hydrogène. Il est probable que les composants plasmatiques réagissent avec formation de composés toxiques à l'égard des cellules ou que le plasma permet la scission ou la transformation du peroxyde en dérivés doués d'une toxicité élevée, une fois qu'ils ont pénétré dans les cellules. La cytophagie par contre est facilitée davantage par l'absence du plasma. Ce dernier semble par conséquent exercer une action de défense cellulaire capable d'inhiber le phénomène, peut-être même à la faveur d'une transformation rapide du peroxyde. Cela fait penser que seules la pénétration directe du toxique et probablement sa scission intra-cellulaire sont en mesure de déclencher le phénomène cytophagique. Cette observation confirme l'hypothèse d'un mécanisme enzymatique et de nature bioélectrique de la phagocytose elle-même.

Comme on le voit, l'action toxique du peroxyde d'hydrogène sur le tissu sanguin intéresse tous les composants du sang, y compris le plasma lui-même.

Les réactions chimiques qui interviennent au niveau du plasma et des cellules et qui sont la cause de nombreux phénomènes affectant la vitalité, les fonctions et la morphologie des éléments sanguins, sont particulièrement intéressantes. Il faut souligner à ce propos la facilité du peroxyde à se scinder avec formation d'ions et échanges d'électrons. L'importance de ces phénomènes chimiques sur les structures intracellulaires et sur l'équilibre enzymatique nous amène à rattacher le méca-

nisme d'action toxique du peroxyde d'hydrogène à de profondes altérations de l'état ionique et de la fonction enzymatique des éléments, c'est-à-dire à l'envisager comme un mécanisme *bioélectrique-enzymopathique*.

Les résultats obtenus mettent en évidence l'existence de nombreux problèmes concernant la toxicité et l'activité biochimique du peroxyde. Ces problèmes, par les relations qu'ils entretiennent avec les phénomènes de l'hydrolyse intraorganique déterminés par les radiations ionisantes, acquièrent une vive actualité.

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Hydrogen Peroxide Ingestion and the Growth of Rats

SEVERAL recent communications have dealt with the capacity of ingested hydrogen peroxide to affect the regression of implanted tumours in rats. Holman[1] has claimed that a 0·45 per cent solution of hydrogen peroxide given to rats in lieu of tap-water caused regression of certain tumours; the opposite conclusion has been stated by Green and Westrop[2] and by Ghadially and Wiseman[3]. Accordingly, it seemed important to determine the effect of hydrogen peroxide on normal rats.

In one experiment, two groups of weanling male rats of the Osborne-Mendel strain developed at this Station were given a commercial dog food meal, and either tap-water or 0·45 per cent hydrogen peroxide *ad libitum* for a three-week period. During the test period, the animals on tap-water consumed an average of 544 ml. each, whereas those on peroxide averaged only 282 ml. each. The decreased amount of liquid intake was reflected in the total body-weight; 156 gm. for those on tap-water against 108 gm. for those on peroxide. No differences in the fresh weight of the testes, kidneys or spleen of the two groups were found.

In order to equalize any effects of limited intake of liquid, another group of male weanling rats was offered 0·45 per cent hydrogen peroxide in lieu of tap-water for a three-week period. Daily consumption was recorded, and a comparable group of male weanlings was given an amount of tap-water equal to the liquid consumed by the animals on peroxide. This regimen was also continued for a three-week period. It was observed that the group of rats on tap-water were continually seeking water, whereas those on peroxide did not act in a similar manner. The average body-weight was 116 gm. for those on peroxide and 104 gm. for those on tap-water. An attempt was made to see if ingestion of peroxide affected the fresh weight of some of the internal organs. Calculated in terms of percentage of body-weight, no important differences were found in the testes, kidneys, spleen or heart. The dry weights of these same tissues, calculated as percentage of body-weight, also failed to show differences.

Three weanling females were placed on a 0·45 per cent hydrogen peroxide regimen and maintained on it for five months. They were then given tap-water instead and were mated with normal males. Normal litters were produced. Six male litter mates were divided into two equal groups: one received peroxide while the other received tap-water. The animals were maintained on their respective regimens for nine months. The only noticeable effect was a difference in body-weight; an average of 521 gm. for those on tap-water against 411 gm. for those on peroxide. Three older males with an average weight of 600 gm. were given 0·45 per cent hydrogen peroxide for six weeks. At that time their average weight had fallen to 511 gm. They were then given tap-water and within two weeks had regained the lost weight.

Weanling rats offered the hydrogen peroxide *ad libitum* failed to gain weight normally. Although it had previously been shown that larger rats fed peroxide failed to gain weight normally[2], this phenomenon has not previously been noted for weanlings. Ghadially and Wiseman[3] attributed the loss of weight to diminished food intake; but, as noted above, this result may also be caused by a diminished liquid intake. Furthermore, long-term feeding of peroxide did not appear to affect reproduction in female rats.

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THE "CAUSTIC" EFFECT OF HYDROGEN PEROXIDE

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(Pharmacological Institute (Director: Prof. Dr. F. Hauschild) and Pathological Institute (Director: Prof. Dr. H. Bredt) of the Karl-Marx University (Leipzig) (Received at the Editorial Office: September 15, 1958) (4 illustrations).

Schüler, a dentist, who for more than 30 years successfully employs solutions containing high percentages of hydrogen peroxide on numerous patients, recently emphasizes that even 30-35% hydrogen peroxide does not have a caustic effect (35).

Induced by this communication, we checked the literature available to us and found that until now, this property of hydrogen peroxide (h.p.) gave rise to very discrepant statements.

Evidently, it is still generally believed that h.p. has a caustic effect (Eichholtz, 155; Kobert, 1902; Lewin, 1893; Møller, 1953; Sollmann, 1950, and others, see (1)(3)(6)(7)(11)(14)(15)(18)(24)(27)(29-32)(36)(39)(41)(42); at first glance, this opinion seems to be credible on the basis of the following facts:

After solutions containing high percentages of h.p. have been applied to human skin or mucous membrane, there appears a whitish discoloration (allochromasia) of the respective area similar to cauterization with hydrochloric acid or phenol which depends upon the local and individual reactivity; in addition, there is felt a peculiar tickling and itching which sometimes can increase to a stabbing pain (v.Berde, 1926; Gabrylowicz, 1890; Jessen, 1904, and other investigators, see (14)(23)(26)(28)(29)(33)(34)(39). Furthermore, various local lesions have been described which were observed after h.p. had

been applied to intact surfaces (v. Berde, 1926; Herold, 1908; Huss, 1902; Lorth, 1930). A considerably smaller number of investigators (v. Brücke and Goldbach, 1943; Schüler, 1955; Stöhr, 1868; Peter, 1927, and others) have published articles in which they point out that the whitish discoloration is capable of reversion or that no real caustic effect is attributable to h.p., however, these studies have apparently not attracted much attention.

Hence, the experiments described below had the purpose of elucidating the problem which principle is to be held responsible for the whitish discoloration capable of reversion and in which manner a potential local lesion can be caused by the application of concentrated h.p. solutions.

METHODOLOGY

We treated skins, mucous membranes, and serous skins of dogs, cats and rabbits, guinea pigs, rats, and mice with 1-30% h.p. solutions using "Perhydrol" of the Merck Company.

The duration of a single h.p. application was gradually changed from a few seconds to 4 hours, and the time period from the end of the effect to the sampling and fixation of the tissue was gradually changed from a few seconds to 72 hours.

After numerous preliminary experiments, we predominantly used tongues, hearts, and hindpaws of white rats. If necessary, the animals were narcotized with urethane and in the case of the experiments on the tongues and the hearts, artificial respiration was administered to the animals after preceding tracheotomy. For human skin treated in situ with h.p., we were able to use freshly prepared specimens taken during the operation. Moreover, corneous lamellae in the region of the palm over the metacarpal capitula were cut off with a razor blade and either in the native state or fixed and stained, served for the microscopical evaluation of horizontal- and cross-sections.

The animal tissues we took blood-warm were fixed in Susa solution, the human skin tissues were fixed in formalin, and all tissues were embedded in

paraffin. Because of technical difficulties during the microtomy, methyl-benzoate - celloidin had to be used for the skins and paws. In addition, we prepared frozen sections of the isolated skin of the paws which together with the cross-sections prepared after decalcification of the paws made it possible to arrive at a reliable evaluation.

As dyeing methods, we chose hematoxylin - eosin (H. E.). Elastica stain, van Gieson's stain, and the trichrome stain modified according to Goldner. We prepared several gradual sections, if necessary, also serial sections.

For this experimentation, we used over 200 animals, 20 specimens taken during the operation, altogether 2,000 histological sections, and we experimented on ourselves many times.

RESULTS OF THE EXPERIMENTS

Regarding all tissues treated with h.p. (such as skins, mucous membranes, serous skins, our observations were in principle the same. Different were only the h.p. concentrations required and concerning the reactions, their extent, their temporal course, and the threshold of their perception, all these differences depending upon the properties of the various tissues:

The whitish discoloration of the tissues is clearly recognizable as an emphysema even upon observation with the naked eye and upon palpation. The appearance of gas bubbles in the vessels can be readily observed after application of 1% h.p. solution to the serosa; then, the lumen of the smallest vessels is for the most part totally filled with gas, and gas bubbles of different size migrate with the blood in the draining veins.

Tongue -- The tongues of cats and dogs were especially suited for following a rapid spreading of gas which occurred after a short h.p. effect,which first remained in the vessel, and which more or less uniformly inflated the tongue to form a bulge. Emphysematous bulges of medium size reverted within a few hours, vessels inflated with gas gradually filled with blood and after a

temporary hyperemia, the tongue regained its original appearance. Extended necroses developed when the h.p. effect was repeated several times before the reversion of these phenomena or was continued after the first application until the tongue seemed to be inflated to form a spongious structure (Fig. 1c).

When specimens of tongues of rats were taken immediately after a short-term h.p. effect, the full-size cross-section showed histologically conspicyous large, partly round tissue recesses which were limited by muscles, by connective tissue, or in some cases by endothelium (Fig. 1b) and which have to be regarded as a consequence of the gas collecting in tissues and vessels. Upon strong magnification, we perceived smaller cavities which were arranged focally or like beads, which were located under the epithelium, and which partly protruded into the stratum germinativium (Fig. 1d).

In the case of intensively treated tongues, the emphysema was so extended that most of the time, it was impossible to differentiate the tissue structures. Merely the epithelial stratum which had become smaller through the dilation allowed us to distinguish larger intraepithelial cavities and blistery detachments of the stratum corneum (Fig. 1d). With respect to the tongues of rats which had been treated with h.p. for a short period of time and of which we had taken specimens after 24 hours and 48 hours, we observed a partial loosening of the tissue structures, sporadic cavities, occasionally small group necroses in the stratum germinatinum with indications of locally limited inflammation and blistery conversion of cell aggregates situated above this stratum. We could not find these changes when we studied the sections of tongues taken 72 hours after the treatment.

Experimentation on additional mucous membrane regions essentially gave the same results which in part agreed with those obtained by other investigators (von Brucke and Goldbach, 1943; Honsell, 1900). However, the tongue

offers a larger range for examination than the mucous membrane as we believe for the following reasons: In contradistinction to more aboral parts of the digestive tract, the tongue comes in contact with higher percentages of h.p., also in the practice, i.e., when treatments within the oral cavity are carried out (Andresen, 1907; Jessen, 1904; Peter, 1927; Schuler, 1955, 1956). Complications of the conditions, such as additional effects because of pressure increase in cavities and effects of hydrochloric acid, did not take place, and the visual control was not made more difficult.

Heart -- The h.p. effect on the epicardium offered the advantage that the observation of the vessel was not disturbed by inflation of loose connective tissue and that the function of the organ in situ was especially well observable.

When one lets fall some drops of 5-10% hydrochloric acid on the epicardium, one obtains a brownish discoloration of the heart muscle, the coronary vessels seemed to be filled, taking on a black-bluish color, and the exhausted heart stopped after a few uncoordinated contractions; by contrast, after applying h.p., we observed a rapidly increasing whitish coloration corresponding to the spreading of the emphysema. The coronary vessels as well as the vestibules, the contractions of which were greatly stimulated, were filled with bright red blood and numerous gas bubbles. The whitish discoloration gradually reverted via a gray shade so that within a very short time, the heart seemed to be again totally normal in appearance and function.

Too intensive a h.p. effect was capable of producing stasis in the ventricles filled with great amounts of spume and resulting in gaseous pulmonal embolism. The outer walls of the ventricles were permeated by bubbles predominately situated in the subepicardium, depending upon the intensity of the treatment and the strength of the walls; in the section, the septum seemed to be unchanged. Hearts which had been taken two hours after the

effect, histologically showed isolated serous cavities, occasionally small hemorrhages in the myocardium and marginality of the leukocytes in some veins, signifying the stasis. At this instant, the epicardium was only torn when the emphysemas were especially pronounced (Fig. 2).

Figures 1a to 1d

Tongue of the Rat (H. E., microscopic magnification; Figs. 1a-1c, 12 times; Fig. 1d, 63 times) Fig. 1a, normal; Figs. 1b and 1d, after 1 min. effect of 30% H_2O_2; Fig. 1c, after 5 min. effect of 30% H_2O_2.

Figs.1a and 1b on p. 54

Figs. 1c and 1d on p. 55

Figure 2

Ventricle of the Heart of the Rat, Cross-Section (H. E., microscopic magnification, 8 times).

Immediately after 30 sec. effect of 10% H_2O_2 on the epicardium.

Epidermis -- To evaluate macroscopically the sequence of h.p. effects on the intact epidermis, it is best to use human skin which according to individual sensitivity and regional structure makes possible distinction among three types of the "whitishness";

1) Upon the epicutaneous effect of 20 to 30% h.p. solution, a snow-white discoloration of the skin at the volar side of the hand was especially distinctly observable (Fig. 3a); this discoloration started from the cristae and disappeared within one to two hours. As the histological sections showed, this phenomenon was based on the embedding of very small gas bubbles in the upper layers of the stratum corneum (Figs. 3b-3d).

2) At the same time, there occurred a phenomenon which could be more distinctly observed at the skin of the abdomen and the volar side of the forearm, i.e., a discharge followed by arterial hyperemia which lasted only a few minutes and apparently can be conceived as a gaseous embolism within the most peripheral vessels.

3) A prolonged effect of Perhydrol resulted in clearly visible and palpable gas bulges which were located at greater depth. In the histological section, the human abdominal skin epicutaneously treated with h.p. (Fig. 4c) correspondingly showed medium-sized roundish cavities which were dispersed in the corium and predominantly disposed around the sudoral glands and their draining ducts. Here and there, a locally limited swelling of the collagenous connective tissue was found below blistery detachments of the epidermis.

Prolonged effect of Perhydrol on the shaved dorsal and abdominal skins of adult rats made it possible to obtain emphysematous bulges which could reach the size of a plum, but were able to revert fast. Any necroses and disturbances in the hair growth were not observable in the post-observation period.

Figures 3a - 3 d

Human Skin (Finger, Palm) (microscopic magnification, Figs. 3b-3d, 63 times), Fig. 3a, index finger after 5 minutes effect of 30% H_2O_2; Fig. 3b, horizontal section: corneous lamella, non-treated; Fig. 3c like 3b except for preceding H_2O_2 effect in situ (30 min., 30% H_2O_2); Fig. 3d is a cross-section to 3c.

In situ evaluation of tissues which had been treated with h.p. seemed important to us since wrong conceptions as to the reversibility of phenomena can sometimes be based on the observation of tissues which have been removed from their connexus and deprived of their normal blood supply and catalase activity (Solbrig, 1949; Wolpers, 1948). Moreover, it had to be taken into account

that a prolonged period of time can elapse until histologically identifiable changes have formed after cauterizations (for example, by sublimate or formalin).

For the histological experiments, we predomonantly used the hindpaws of rats, the skin of which is relatively similar to human skin, hence,their depilation becomes unnecessary so that their integrity is not endangered. Immersion of the paws into h.p. solutions produced emphysemas which gradually revert without leaving any significant changes. In case the paws after treatment were so emphysematous that they felt as if one touches air bulges, the reversion took place via an edematous stage.

When we took specimens of the paws immediately after the treatment, we got the impression that the large gas bubbles were predominantly located in the deep layers above the phalanges (Fig. 4b).

Figures 4a - 4c

Cross-Sections of Skins (H.E., microscopic magnification for Figs. 4a and 4b, 25 times, for Fig. 4c, 63 times). Fig. 4a depicts a normal, non-treated hindpaw of the rat; Fig. 4b, hindpaw of the rat, immediately after 30 min. effect of 30% H_2O_2; Fig. 4c, human abdominal skin (specimen taken during operation), immediately after 30 min. effect of 30% H_2O_2 in situ.

Figs. 4a and 4b on p. 58

Fig. 4c on p. 59

When we had treated skins with 20-30% h.p. solutions for 30-60 minutes and examined them after 24, 48 and 72 hours after the treatment, we were unable to find histologically any reliable indications for the formation of cauterization sequences. Only in a few preparations did we observe cellular reactions with a small participation of leukocytes, which reactions are also known as the consequence of subcutaneous oxygen insufflations (Leconte and Demarquai) and are predominantly located subepithelially and in some cases,

focally and intraepithelially. In a part of the cases in which especially extended emphysemas had been formed, thehistological section showed in spots detached and thus, necrotic epithelial regions below which a serous-leukocytoid exudate was found. In addition, we observed a serous imbibition of the tissue and a fibrinous coagulum in the veins.

Discussion of the Results

An isolated consideration of single results obtained after the h.p. effect could give the impression as if h.p. has a caustic effect and thus leads to cauterization or escharosis, however, a summary of the present results leads to the following conceptions:

When we treated the intact skin or mucous membrane of animals with high percentages of h.p. solutions, we were able to demonstrate histologically that gas bubbles of different size appear in the treated region of the tissue and its vessels. Thereby and by the removal of the blood from the capillaries, a whitishness arises which gradually reverts with increasing resorption or with the disappearance of the gas. Depending upon the thickness and strength of the covering layer, the gas located in the uppermost layers can lead to a scission of epithelium aggregates which, for example, in the human epidermis can impart a slight roughness after a chronic h.p. effect. The gas bubbles which occur in the various layers are, in general, resorbed without causing any damages which are detectable macroscopically or histologically.

When the gas development in the tissue is too intensive, mechanically caused lacerations with small hemorrhages can occur, especially when very tender tissue structures and excessive percentages of h.p. are involved or the intervals between treatments are too small. In such cases, it may happen that under certain circumstances, the tissue elements which are surrounded by gas and have a reduced catalase activity cannot be nourished sufficiently and

cannot be protected against the protein coagulating effect of the h.p., consequently, they become necrotic and acquire an increased sensitivity toward h.p. and other influences. Simultaneously, the start of circulatory disturbances provides an explanation for the fibrous coagulation in the veins, the serous imbibition of the tissue, and the information mentioned at the beginning with regard to local damages after h.p. application unless these phenomena are attributable to admixtures, such as for example, the addition of acids for reasons of stabilization (v. Berde, 1926; Lorth, 1930; Stöhr, 1868).

It can be accepted that the tickling which is generally felt can be attributed to the spreading of the gas bubbles (v. Brücke and Goldbach, 1943); sometimes, this tickling has been also observed after the insufflation of molecular oxygen.

On the basis of the present experiments, one has to have reservations when defending the occasionally used practice according to which high percentages of h.p. (5-30%) are applied to mucous membranes for therapeutic or diagnostic purposes. Although a cauterization in the proper sense does not take place, mere mechanical tissue destructions can occur, caused by the great amounts of oxygen gas formed in the tissue and in addition, large regions may remain without sufficient blood supply for a prolonged period of time. For the same reasons, the rinsing of the oral cavity with high percentages of h.p. which Schüler (35) recommended is to be regarded as a potentially dangerous practice.

However, as has been shown elsewhere (Ludewig, 1958), considerable quantities of gaseous (and also atomic?) oxygen can be introduced into the tissues and the blood stream without danger over a long time when appropriate forms and concentrations of hydrogen peroxide are used for epicutaneous application.

Later on, we will discuss elsewhere the problem of the resorption- and scission mechanism of h.p. which has not been satisfactorily solved and which falls outside the scope of this experimentation.

The "bleaching effect" of h.p. cannot be a cause for the whitishness of the skin which we have described at the beginning because the treated spot has resumed its original color at the latest within a few hours, and this happens also upon repreated application. Evidently, the pigments of the normal skin located in the basal epithelial layers and below have not been reached by a sufficient concentration of undecomposed h.p. A genuine bleaching effect can only develop when exerted on pigment containing structures which are poor in or free from catalase and easily accessible to the h.p.; hair is an example of such structures.

Summary

The opinion still frequently held that concentrated hydrogen peroxide has a caustic effect cannot withstand experimental scrutiny. The assertion that hydrogen peroxide has a caustic effect cannot be justified by whitish discoloration and disagreeable or painful sensations after Perhydrol treatment of the skin as well as by the fact that sometimes tissues are damaged.

Many experiments on animals, preparation of specimens taking from humans, many experiments on ourselves, and a great number of histological sections made it possible to observe that the whitishness after the hydrogen peroxide effect which is capable of reversion is based on gas bubbles embedded in the tissue and a temporary bloodlessness of the capillaries and that this whitishness is not attributable to an irreversible damage (cauterization). Significant damages were only observed when the effect of hydrogen peroxide was so intensive that very extended emphysemas and local inhibitions of circulation caused disturbances in the nutritive supply to limited tissue regions.

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Über die „ätzende" Wirkung von Wasserstoffperoxyd

Von
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Mit 4 Textabbildungen

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SCHÜLER, der in der zahnärztlichen Praxis an umfangreichem Patientengut hochprozentige Wasserstoffperoxydlösungen über mehr als 30 Jahre erfolgreich anwendet, weist neuerlich nachdrücklich darauf hin, daß selbst 30—35%iges Wasserstoffperoxyd nicht ätzend wirkt[35].

Angeregt durch diese Mitteilung konnten wir feststellen, daß in der uns zugängigen Literatur bisher über diese Eigenschaft des Wasserstoffperoxyds (WPO) sehr unterschiedliche Angaben gemacht werden.

Die offenbar allgemein noch vorherrschende Ansicht, daß WPO ätzend wirke (EICHHOLTZ 1955, KOBERT 1902, LEWIN 1893, MÖLLER 1953, SOLLMANN 1950 u.a., siehe [1,3,6,7,11,14,15,18,24,27,29–32,36,39,41,42]) mag auf Grund folgender Tatsachen zunächst glaubhaft erscheinen:

Nach Aufbringen hochprozentiger WPO-Lösungen auf menschliche Haut oder Schleimhaut entsteht in Abhängigkeit von lokaler und individueller Reaktionsbereitschaft, ähnlich der Verätzung mit Salzsäure oder Phenol, eine weißliche Verfärbung des betreffenden Bezirkes, sowie ein eigentümliches Kribbeln und Jucken, das sich mitunter zu stechendem Schmerz steigern kann (v. BERDE 1926, GABRYLOWICZ 1890, JESSEN 1904, sowie andere Autoren, siehe [14,23,26,28,29,33,34,39]). Zudem werden verschiedentlich lokale Schädigungen nach WPO-Applikation auf unverletzte Oberflächen beschrieben (v. BERDE 1926, HEROLD 1908, HUSS 1902, LORTH 1930). Eine wesentlich kleinere Anzahl von Autoren dagegen weist in offenbar wenig beachteten Arbeiten (v. BRÜCKE u. GOLDBACH 1943, SCHÜLER 1955, STÖHR 1868, PETER 1927 u. a., siehe [4,9,12,23,34,43]) darauf hin, daß die Weißfärbung rückbildungsfähig ist oder dem WPO keine echte Ätzwirkung zukomme.

Die im folgenden beschriebenen Untersuchungen hatten daher den Zweck, die Frage zu klären, worauf die rückbildungsfähige Weißfärbung des behandelten Gewebes beruht und auf welche Weise eventuell eine lokale Schädigung durch Anwendung konzentrierterer WPO-Lösungen zustande kommen kann.

Methodik

Wir behandelten Haut, Schleimhaut und seröse Häute verschiedener Regionen von Hunden, Katzen und Kaninchen, Meerschweinchen, Ratten und Mäusen mit WPO-Lösungen in Konzentrationen von 1—30%, ausgehend vom „Perhydrol" der Fa. Merck.

Die Dauer der einmaligen Einwirkung des WPO wurde von wenigen Sekunden bis zu 4 Std, die Zeitspanne von Einwirkungsende bis Gewebsentnahme und Fixierung von einigen Sekunden bis zu 72 Std abgestuft.

Nach zahlreichen Vorversuchen verwendeten wir vorwiegend Zunge, Herz und Hinterlaufpfoten weißer Ratten. Soweit erforderlich, wurden die Tiere mit Urethan narkotisiert und bei den Versuchen an Zunge und Herz nach vorheriger Tracheotomie künstlich beatmet. In situ WPO-behandelte Menschenhaut konnten wir frischen Operationspräparaten entnehmen. Außerdem wurden mit der Rasierklinge Hornlamellen im Bereich der Hohlhand über den Metacarpalköpfchen abgetragen und nativ oder fixiert und gefärbt in Flach- und Querschnitten mikroskopisch beurteilt.

Die Fixierung der lebensfrisch entnommenen tierischen Gewebe erfolgte in Susa-Lösung, die der Menschenhaut in Formalin, die Einbettung in Paraffin. Wegen der technischen Schwierigkeiten bei der Mikrotomschnittführung mußten Häute und Pfoten über Methylbenzoat-Celloidin gebracht werden. Zusätzlich wurden Gefrierschnitte der isolierten Pfotenhaut hergestellt, die in Verbindung mit den nach Entkalkung der Pfoten gewonnenen Querschnitten eine zuverlässige Beurteilung ermöglichten.

Als Färbemethoden wählten wir Hämatoxylin-Eosin, Elastica-van Gieson- und die Trichrom-Färbung, modifiziert nach GOLDNER. Von jedem Präparat fertigten wir mehrere Stufenschnitte, gegebenenfalls auch Serienschnitte an.

Diese Untersuchungen wurden an über 200 Tieren, 20 Operationspräparaten, insgesamt etwa 2000 histologischen Schnitten und in zahlreichen Selbstversuchen durchgeführt.

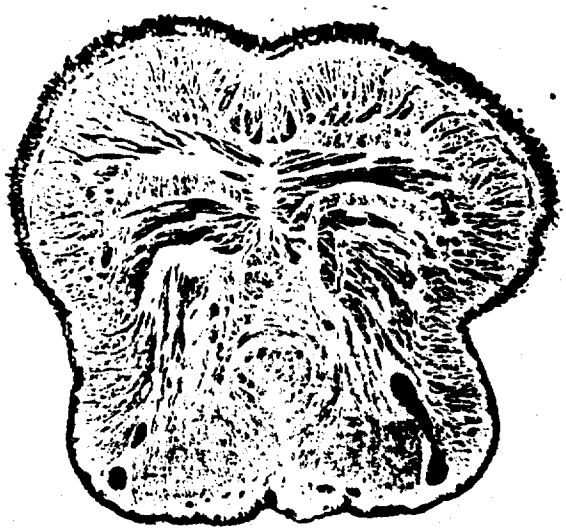
Versuchsergebnisse

Wir konnten an allen mit WPO behandelten Geweben (Haut, Schleimhaut, seröse Häute) grundsätzlich die gleichen Beobachtungen machen. Unterschiedlich waren nur die erforderlichen WPO-Konzentrationen, Ausmaß, zeitlicher Ablauf und Erkennbarkeit der Reaktionen in Abhängigkeit von den Eigenschaften der verschiedenen Gewebe:

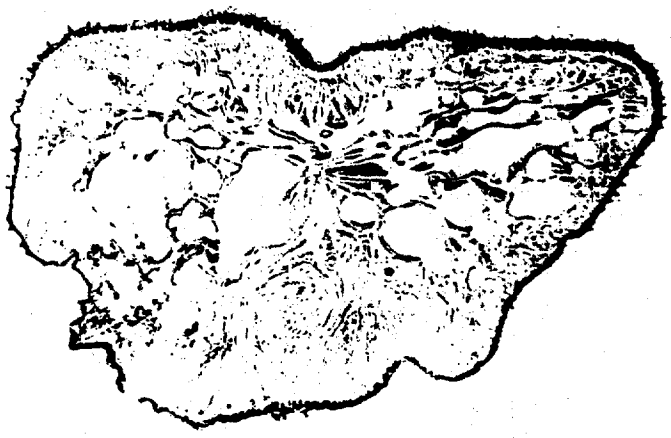
Die *weißliche Gewebsverfärbung* ist oft bereits mit bloßem Auge und durch Palpation eindeutig als Emphysem zu erkennen. Das Auftreten von *Gasblasen* in den Gefäßen kann bereits nach Aufbringen von 1%iger WPO-Lösung auf die Serosa beobachtet werden; das Lumen der kleinsten Gefäße ist dann zumeist ganz mit Gas ausgefüllt, in den ableitenden Venen wandern Gasblasen verschiedener Größe mit dem Blutstrom.

Zunge. Besonders gut war an der Zunge von Katzen und Hunden nach kurzer WPO-Einwirkung eine rasche Gasausbreitung zu verfolgen, die sich zunächst an den Gefäßverlauf hielt und die Zunge mehr oder weniger gleichmäßig polsterförmig auftrieb. Mittelgroße Emphysempolster bildeten sich binnen einiger Stunden wieder zurück, gasgeblähte Gefäße füllten sich allmählich mit Blut und nach vorübergehender Hyperämie nahm die Zunge ihr ursprüngliches Aussehen wieder an. Wurde die WPO-Einwirkung vor Rückbildung dieser Erscheinungen öfters wiederholt, oder schon beim erstenmal so lange fortgesetzt, bis die Zunge zu einem schwammigen Gebilde aufgebläht erschien (Abb. 1c), entwickelten sich ausgedehnte Nekrosen.

Zu intensive WPO-Einwirkung konnte zum Stillstand der mächtig schaumgeblähten Ventrikel bei pulmonaler Gasembolie führen. Die Ventrikelaußenwände waren in Abhängigkeit von der Behandlungsintensität



a



b

Abb. 1 a u. b

Abb. 1 a—d. Rattenzunge (H. E., mikroskopische Vergrößerung: a—c 12fach, d 63fach). a normal, b und d nach 1 min Einwirkungsdauer von 30% H_2O_2; c nach 5 min Einwirkungsdauer von 30% H_2O_2

An unmittelbar nach kurzfristiger WPO-Einwirkung entnommenen Rattenzungen fielen histologisch am Übersichtsquerschnitt größere, zumeist rundliche Gewebsaussparungen auf, die muskulär, bindegewebig oder vereinzelt auch endothelial begrenzt waren (Abb. 1b) und die als Folge der Gasansammlung in Geweben und Gefäßen angesehen werden müssen. Bei starker Vergrößerung erkannte man herdförmig oder perlschnurartig angeordnete kleinere Hohlräume, die unmittelbar unter dem Epithel, teilweise ins Stratum germinativum hineinragend, gelegen waren (Abb. 1d).

An intensiv behandelten Zungen war die Ausdehnung des Emphysems so stark, daß die nähere Differenzierung der Gewebsstruktur größtenteils unmöglich wurde. Lediglich an der durch Dehnung verschmälerten Epithelschicht waren größere intraepitheliale Hohlräume und blasige Abhebungen der Hornschicht erkennbar (Abb. 1c). An den kurzfristig mit WPO behandelten und nach 24 und 48 Std entnommenen Rattenzungen beobachteten wir eine teilweise Auflockerung der Gewebsstrukturen, vereinzelte Hohlraumbildungen, gelegentlich kleine Gruppennekrosen im Stratum germinativum mit Merkmalen örtlich begrenzter Entzündung und blasige Umwandlung darüber gelegener Zellverbände. Diese Veränderungen konnten wir an den Schnitten der 72 Std nach der Behandlung entnommenen Zungen nicht mehr finden.

Die an weiteren Schleimhautbereichen durchgeführten Versuche brachten — teilweise in Übereinstimmung mit anderen Untersuchern (v. Brücke u. Goldbach 1943, Honsell 1900) — im wesentlichen gleichartige Ergebnisse. Aus folgenden Gründen erschien uns aber die Zunge als das geeignetste Untersuchungsfeld für die Schleimhaut: Die Zunge kommt im Gegensatz zu aboralen Teilen des Verdauungstraktes auch in praxi — bei Behandlungen innerhalb der Mundhöhle (Andresen 1907, Jessen 1904, Peter 1927, Schüler 1955, 1956) — mit hochprozentigen WPO-Lösungen in Berührung. Komplikationen der Verhältnisse wie durch zusätzliche Beeinflussung infolge Drucksteigerung in Hohlräumen, Salzsäurewirkung, Erschwerung der Sichtkontrolle entfielen.

Herz. Die WPO-Einwirkung auf das *Epikard* bot den Vorteil, daß die Gefäßbeobachtung nicht durch Aufblähung lockeren Bindegewebes gestört wurde und die Organfunktion in situ besonders gut zu beobachten war.

Während einige Tropfen 5—10%iger Salzsäure, auf das Epikard geträufelt, zu einer bräunlichen Verfärbung des Herzmuskels führten, die Koronargefäße schwarzblau gefüllt erschienen und nach wenigen unkoordinierten Kontraktionen das erschlaffte Herz still stand, wurde nach Aufbringen von WPO eine rasch zunehmende, der Ausbreitung des Emphysems entsprechende Weißfärbung beobachtet. Die Herzkranzgefäße waren ebenso wie die Vorhöfe, deren Kontraktionen kräftig angeregt wurden, mit hellrotem Blut und zahlreichen Gasblasen angefüllt. Die weißliche Verfärbung bildete sich über eine graurote Tönung allmählich zurück, so daß das Herz innerhalb kürzester Zeit in Aussehen und Funktion wieder völlig normal erschien.

und Wandstärke mit vorwiegend subepikardial gelegenen Blasen durchsetzt, das Septum erschien im Schnitt unverändert. Herzen, die 2 Std nach der WPO-Einwirkung entnommen worden waren, zeigten

Abb. 1 c u. d

histologisch vereinzelt serös gefüllte Hohlräume, gelegentlich kleinere Blutungen im Myokard und als Zeichen der Stase Randständigkeit der

Leukocyten in einigen Venen. Das Epikard war zu diesem Zeitpunkt nur dann zerrissen, wenn die Emphyseme besonders stark ausgeprägt waren (Abb. 2).

Epidermis. Zur *makroskopischen* Beurteilung der WPO-Einwirkungsfolgen auf die intakte Epidermis eignete sich am besten die *menschliche* Haut, die je nach individueller Empfindlichkeit und regionärbedingter Struktur 3 Arten der „Weißfärbung" unterscheiden ließ:

Abb. 2. Rattenherz-Ventrikel. Querschnitt (H. E., mikroskopische Vergrößerung 8fach). Unmittelbar nach 30 sec Einwirkungsdauer von 10% H_2O_2 auf das Epikard

1. Bei epicutaner Einwirkung von 20 bis 30%iger WPO-Lösung war besonders deutlich an der volaren Seite der Hand eine schneeweiße, oberflächliche Hautverfärbung erkennbar (Abb. 3a), die zumeist von den Cristae ausging und innerhalb von 1—2 Std verschwand. Wie die histologischen Schnitte zeigten, beruhte diese Erscheinung auf der Einlagerung kleinster Gasblasen in den obersten Lagen des Stratum corneum (Abb. 3b—d).

2. Gleichzeitig trat — deutlicher an der Haut des Bauches und der volaren Unterarmseite zu sehen — eine von arterieller Hyperämie gefolgte Abblassung auf, die nur wenige Minuten bestand und offenbar als Gasembolie innerhalb der peripheren Gefäße aufgefaßt werden kann.

3. Nach längerer Einwirkung von Perhydrol entstanden deutlich sicht- und tastbare, tiefer gelegene Gaspolster. Im histologischen Schnitt zeigte die mit WPO epicutan behandelte menschliche Bauchhaut (Abb. 4c) dementsprechend im Corium verstreute mittelgroße rundliche, vorwiegend um die Schweißdrüsen und ihre Ausführungsgänge angeordnete Hohlräume. Stellenweise fand sich unter blasigen Abhebungen der Epidermis eine örtlich begrenzte Quellung des kollagenen Bindegewebes.

Bei längerer epicutaner Einwirkung von Perhydrol waren an der rasierten Rücken- und Bauchhaut erwachsener Ratten rasch rückbildungsfähige Emphysempolster zu erzielen, die bis zu Pflaumengröße annehmen konnten. In der Nachbeobachtungszeit konnten makroskopisch keine Nekrosen und keine Störungen im Haarwachstum festgestellt werden.

Es schien uns wichtig, mit WPO behandelte Gewebe *in situ* zu beurteilen, da aus ihrem Zusammenhang gelöste und ihrer normalen Durchblutung und Katalaseaktivität beraubte Gewebe mitunter zu falschen Vorstellungen über die Reversibilität der Erscheinungen führen können (SOLBERG 1949, WOLPERS 1948). Außerdem war zu berücksichtigen, daß bis zur Ausbildung histologisch faßbarer Veränderungen nach Verätzungen (z. B. durch Sublimat oder Formalin) längere Zeit verstreichen kann.

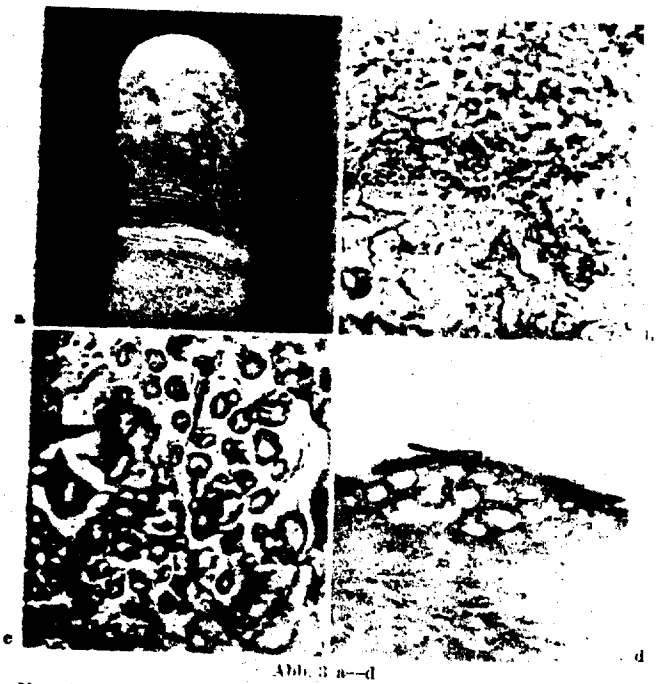


Abb. 3 a—d

Abb. 3 a—d. Menschliche Haut (Finger, Hohlhand). (mikroskopische Vergrößerung b—d [illegible]) a Zeigefinger nach 5 min. Einwirkungsdauer von 30% H_2O_2; b Flachschnitt: Hornhautlamellen unbehandelt; c wie b, jedoch nach vorangehender H_2O_2-Einwirkung in situ (30 min 30% H_2O_2); d Querschnitt zu c

Für die *histologischen* Untersuchungen verwendeten wir vorwiegend die Hinterlaufpfoten von Ratten, da deren Haut der menschlichen verhältnismäßig ähnlich ist und sich eine Depilation erübrigt, so daß ihre Integrität nicht gefährdet war. Die durch Eintauchen der Pfoten in WPO-Lösungen erzeugten Emphyseme bildeten sich allmählich zurück, ohne nennenswerte Veränderungen zu hinterlassen. Waren die Pfoten nach der Behandlung so emphysematös, daß sie sich insgesamt luftkissenartig anfühlten, erfolgte die Rückbildung zumeist über ein Ödemstadium.

Histologisch gewannen wir an den unmittelbar nach der Behandlung entnommenen Pfoten den Eindruck, daß die großen Gasblasen vorwiegend in den tiefen Schichten oberhalb der Phalangen lokalisiert waren (Abb. 4b).

An Häuten, die 30—60 min mit 20—30%igen WPO-Lösungen behandelt worden waren, fanden sich 24, 48 und 72 Std nach der Einwirkung histologisch keine sicheren Anhaltspunkte für die Ausbildung von Verätzungsfolgen. Lediglich in einzelnen Präparaten beobachteten

Abb. 4 a u. b

Abb. 4a—c. Haut-Quer[illegible] (H.E.), mikroskopische Vergrößerung: a und b 25fach, c 63fach. a normale, unbehandelte R[illegible]aterhaut-Pfote; b Rattenhinterhaut-Pfote unmittelbar nach 30 min Einwirkungsdauer von [illegible] H_2O_2; c menschliche Bauchhaut (Operationspräparat), unmittelbar [illegible] 30 min Einwirkung in situ von 30 % H_2O_2

wir celluläre Reaktionen mit geringer Leukocytenbeteiligung, die auch als Folge subcutaner Sauerstoffinsufflationen bekannt sind (LÉCONTE u. DEMARQUAI) und vorwiegend subepithelial und vereinzelt herdförmig auch intraepithelial gelegen waren. In einem Teil der Fälle, in denen durch extreme WPO-Einwirkung besonders ausgedehnte Emphyseme entstanden waren, zeigten sich im histologischen Schnitt stellenweise

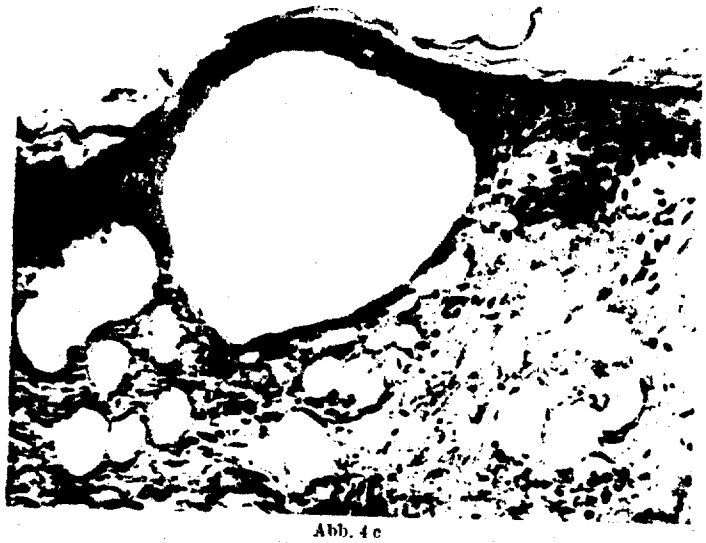


Abb. 4 c

abgehobene und dadurch nekrotische Epithelbezirke, unter denen sich serös-leukocytäres Exsudat befand. Zudem beobachteten wir eine seröse Durchtränkung des Gewebes und Fibringerinnsel in den Venen.

Besprechung der Versuchsergebnisse

Während eine isolierte Betrachtung einzelner Befunde nach Wasserstoffperoxyd-Einwirkung den Anschein erwecken könnte, WPO wirke ätzend, führe also zur Korrosion oder Verschorfung, kommt man bei einer Zusammenfassung der vorliegenden Ergebnisse zu folgenden Vorstellungen:

Wie wir histologisch veranschaulichen konnten, treten unter Einwirkung hochprozentiger WPO-Lösungen auf intakte tierische Haut oder Schleimhaut unterschiedlich große Gasblasen im behandelten Gewebsbereich und seinen Gefäßen auf. Hierdurch und durch Verdrängung des Blutes aus den Capillaren entsteht eine Weißfärbung, die sich mit zunehmender Resorption bzw. mit dem Verschwinden des Gases allmählich zurückbildet. Das in den obersten Schichten befindliche Gas kann,

je nach Dicke und Festigkeit der bedeckenden Lage, zur Absprengung von Epithelzellverbänden führen, die beispielsweise an der menschlichen Epidermis nach chronischer WPO-Einwirkung als leichte Aufrauhung der Haut imponieren kann. Die in den verschiedenen Schichten auftretenden Gasblasen werden im allgemeinen resorbiert, ohne makroskopisch oder histologisch nachweisbare Schädigungen zu verursachen.

Ist die Gasentwicklung im Gewebe zu intensiv, so kann es zu mechanisch bedingten Zerreißungen mit kleinen Blutungen kommen, besonders dann, wenn es sich um sehr zarte Gewebsstrukturen und zu hohe WPO-Konzentrationen handelt oder die Behandlungsintervalle zu klein sind. In solchen Fällen können unter Umständen die gasumgebenen, an Katalaseaktivität geminderten Gewebselemente nicht mehr ausreichend ernährt, vor der eiweißkoagulierenden Wirkung des WPO geschützt und daher nekrotisch werden, womit eine gesteigerte Empfindlichkeit gegen WPO und andere Einflüsse Hand in Hand geht. Gleichzeitig einsetzende Zirkulationsstörungen, erklären die Fibringerinnsel in den Venen, die seröse Durchtränkung des Gewebes und auch die eingangs erwähnten Mitteilungen über lokale Schädigungen nach WPO-Anwendung, sofern nicht Beimischungen, z.B. Säurezusätze aus Stabilisierungsgründen anzuschuldigen sind (v. BERDE 1926; LORTH 1930; STÖHR 1868).

Das zumeist empfundene Kribbeln ist annehmbar auf Ausbreitung der Gasblasen zurückzuführen (v. BRÜCKE u. GOLDBACH 1943) und wird auch zuweilen nach Insufflation molekularen Sauerstoffs beobachtet.

Nach den vorliegenden Untersuchungen ist das mitunter geübte Aufbringen größerer Mengen hochprozentigen Wasserstoffperoxyds (5–30%) auf Schleimhäute zu therapeutischen und diagnostischen Zwecken nur mit Vorbehalten vertretbar, obgleich es nicht zu einer eigentlichen Verätzung kommt. Wohl aber können rein mechanisch Gewebsdestruktionen durch das im Gewebe in großen Mengen entstehende Sauerstoffgas auftreten und außerdem große Gebiete für längere Zeit ohne ausreichende Blutversorgung bleiben. Das Spülen der Mundhöhle mit hochprozentigem WPO, wie es von SCHÜLER[35] empfohlen wird, ist aus dengleichen Gründen nicht als gefahrlos anzusehen.

Dagegen ist es möglich, wie dies auch an anderer Stelle (LUDEWIG 1958) gezeigt wurde, durch *epicutane* Applikation von Wasserstoffperoxyd in geeigneter Form und Konzentration gefahrlos und über lange Zeit beträchtliche Mengen gasförmigen (und auch atomaren?) Sauerstoffs in die Gewebe und die Blutbahn zu bringen.

Auf die den Rahmen der Arbeit überschreitende und auch noch nicht befriedigend geklärte Frage nach dem Resorptions- und Spaltungsmechanismus des WPO wird später an anderer Stelle eingegangen werden.

Die „bleichende Wirkung" des WPO kann an der eingangs beschriebenen Weißfärbung der Haut nicht ursächlich beteiligt sein, da die behandelte Stelle — auch bei häufigerer Anwendung — spätestens innerhalb einiger Stunden ihre normale Farbe wieder angenommen hat. Offenbar werden die in den basalen Epithelschichten und darunter liegenden Pigmente der normalen Haut von unzersetztem WPO nicht in hinreichender Konzentration erreicht. Eine echte Bleichwirkung kann nur an katalasearmen oder -freien, dem WPO leicht zugänglichen, pigmenthaltigen Strukturen — z.B. an den Haaren — zur Entfaltung kommen.

Zusammenfassung

Die vielfach noch vertretene Ansicht, konzentriertes Wasserstoffperoxyd wirke ätzend, kann einer experimentellen Überprüfung nicht standhalten. Weiße Verfärbungen und unangenehme oder schmerzhafte Empfindungen nach Perhydrol-Behandlung der Haut sowie die Tatsache, daß es mitunter zu Gewebsschädigungen kommen kann, berechtigen nicht zu der Behauptung, daß Wasserstoffperoxyd ätzt.

Es konnte in zahlreichen Tierversuchen, an menschlichen Operationspräparaten, in vielen Selbstversuchen und an einer großen Zahl von histologischen Schnitten beobachtet werden, daß die rückbildungsfähige Weißfärbung nach Wasserstoffperoxydeinwirkung auf Einlagerung von Gasblasen im Gewebe und auf einer flüchtigen Blutleere der Capillaren und nicht auf irreversibler Schädigung (Ätzung) beruht. Nennenswerte Schäden wurden nur dann beobachtet, wenn die Einwirkung von Wasserstoffperoxyd so intensiv war, daß es über die Ausbildung allzu ausgedehnter Emphyseme und lokaler Zirkulationsbehinderung zu Ernährungsstörungen begrenzter Gewebsbezirke kam.

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Medical Laboratories Research Report No. 75

The Cause of the Increasing Intravenous Toxicity Of 90% Hydrogen Peroxide With Progressive Dilutions

ABSTRACT

OBJECT.

In connection with evaluation of the possible health hazards of aviation fuels to man based on their toxicity to animals, it became necessary to account for the greater intravenous toxicity of the same amount of hydrogen peroxide when introduced in a diluted form.

RESULTS.

Studies of the intravenous and percutaneous toxicity of 90% hydrogen peroxide have shown that death is associated with gas embolism and that this is probably the immediate cause. The peroxide penetrates the skin intact and then undergoes breakdown within the tissues as well as within the blood stream. The degree of breakdown must be related to the catalase activity of the tissues and of the blood.

With intravenous injection, the toxicity in rabbits is predictably increased with the dilution. Studies with percutaneous administration indicate that the greater the concentration of the agent, the greater the local breakdown, so that less material is available for the production of gas emboli in the blood stream and this would account for the lower toxicity on an embolic basis. In fact, when the agent is injected intravenously in the rabbit's ear, there is a visible difference in the amount of local breakdown caused by the concentrated and the dilute forms.

Further proof is offered by a study of the circulatory blocking effect of the oxygen bubble masses when the peroxide is injected into the jugular vein at a point below the site of an infusion of normal saline. The higher the concentration of the agent, the greater the local breakdown and the greater the blocking effect. With successive dilutions, the blocking effects were diminished; the signs of toxicity, as evidenced by convulsions, were increased; and there were more deaths. Finally, with each concentration, there is reached a dosage which is too small to produce blocking, signs of toxicity, or death.

CONCLUSIONS.

1. Hydrogen peroxide, percutaneously as well as intravenously, produces death which is associated with gas embolism.

2. The intravenous toxicity of hydrogen peroxide is an inverse function of the degree of local breakdown at the site of administration.

3. The explanation for the dependence of toxicity on dilution has physical basis in that the local reaction, with its liberation of oxygen nd formation of bubble masses obstructing blood flow, allows less of the ydrogen peroxide to enter the general circulation and exercise its systemic r lethal embolic effects.

Medical Laboratories Research Report No. 75

The Cause of the Increasing Intravenous Toxicity Of 90% Hydrogen Peroxide with Progressive Dilutions

I. INTRODUCTION.

A. Object.

As a part of a study of the health hazards of aviation fuels on the basis of animal toxicity, to study the cause of the increasing intravenous toxicity of 90% hydrogen peroxide with progressive dilutions.

B. Authority.

Authorized by the Chief Chemical Officer under Project No. 4-61-14-002, Health Hazards of Military Chemicals, Test Program No. T1, Cml C Research and Development Program for fiscal year 1950.

II. HISTORICAL.

Lorincz, et al., (1) have reported that the resistance of the cat and the rabbit to intravenous 3% hydrogen peroxide was limited by the development of gas embolism. Studies in Toxicology Branch have shown that death is associated with gas embolism and that this is probably the immediate cause.

III. THEORETICAL AND EXPERIMENTAL.

A. Materials and Equipment.

The hydrogen peroxide used in this experiment was obtained from the Buffalo Electro-Chemical Company. The density for the 85-90% compound is reported to be 1.394 at 18°C.

All dilutions of the agent were made with distilled water. Intravenous injections were made into the marginal ear veins of rabbits except for the study of the circulatory blocking effects when the injections were made into the jugular vein. Percutaneous administration was carried out on the clipped backs of rabbits, rats, cats, dogs and pigs.

B. Procedure and Results.

With intravenous injection (ear vein), the toxicity in rabbits is predictably increased with the dilution. For example, the LD50 for the 90% was 15 cu. mm./kg., while for the 4% solution it was 3.2 cu.mm./kg. The inverse ratio, within limits, of the LD50 to the percentage concentration is illustrated in Table 1 and in Figure 1.

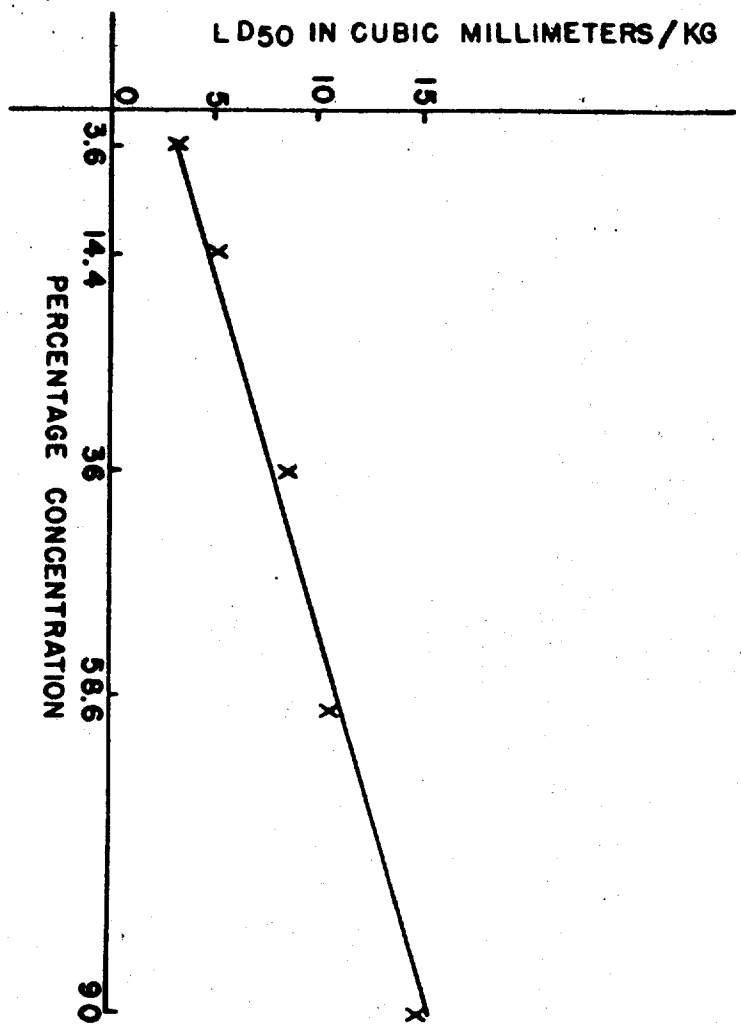
Table 1

Effect of Dilution on Toxicity of Hydrogen Peroxide (H_2O_2) Intravenously in Rabbits

Dose cu.mm./kg.	Dilution	Percent H_2O_2	Deaths	LD50
5.0	None	90.0	0/6	
10.0	"	"	0/6	
12.6	"	"	2/6	
15.0	"	"	3/6	15.0
15.8	"	"	4/6	
20.0	"	"	6/6	
25.0	"	"	2/2	
	3.2-5 cc.			
8.0	with H_2O	58.6	2/6	
10.0	"	"	2/6	12.6
12.6	"	"	4/6	
15.8	"	"	4/6	
	2-5 cc.			
7.9	with H_2O	36.0	3/12	
8.9	"	"	5/9	8.9
10.0	"	"	5/6	
	0.79-5 cc.			
2.0	with H_2O	14.4	0/6	
3.2	"	"	1/6	
4.0	"	"	3/6	5.0
5.0	"	"	3/6	
6.3	"	"	6/6	
	0.2-5 cc.			
2.0	with H_2O	3.6	0/6	
2.5	"	"	3/6	
3.2	"	"	3/6	3.2
4.0	"	"	4/6	
5.0	"	"	5/6	

In casting about for a plausible explanation for this finding, some of the results with percutaneous administration seem pertinent and require consideration at this point. They suggest that, grossly, the greater the local reaction, the lower the systemic toxicity. The local effect of the percutaneous administration to rats is illustrated in Fig.2. The bulging of the skin is produced by the accumulation of oxygen as the peroxide undergoes breakdown beneath the skin. The gas in the tissues can be aspirated and shown to be oxygen by the glowing splinter method even 24 hours after the administration of the agent. In the case of the rat where the skin of the back is loosely attached, this bulging is more pronounced and localized than in other animals.

FIGURE 1



Relation of intravenous LD50 in rabbits to the percent concentration of H_2O_2 solution injected.

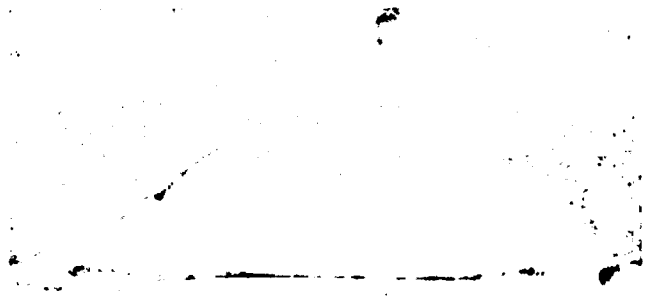


FIGURE 2

Percutaneous H_2O_2 (90%) applied to the clipped back of the rat. Note the bulging of the skin due to accumulated oxygen.

FIGURE 3

Intravenous H_2O_2 (90%)

Rabbit ear showing veins before the injection.

FIGURE 4

Intravenous H_2O_2 (90%)

Rabbit ear after the injection into the lateral vein. Arrow indicates the site of injection.

There is considerable species variation in the toxicity by the percutaneous route. Table 2 illustrates these differences.

Table 2

Species Differences in Percutaneous Toxicity of 90% H_2O_2

Animal	Dose cc./kg.	Deaths
Rabbit	0.5	6/12
Cat	2.0	0/2
Cat	3.16	0/2
Pig	2.0	2/5
Pig	1.0	0/1
Rat (white)	3.55	4/12
Rat (white)	4.0	9/12
Rat (black)	5.0	0/6
Rat (black)	6.0	2/6

The high susceptibility of the rabbit to embolism is well known (2). A factor contributing to the variation among species may be the differences in the levels of tissue and blood catalases. Lorincz, et al., (1) reported a wide variation in the catalase activity of the blood of various animals.

It seems then that after some breakdown at the surface, remaining intact peroxide penetrates the skin, and a portion continues to breakdown, liberating oxygen intracutaneously, subcutaneously and intramuscularly. Consequently, less of the material is available for entering the blood stream to breakdown further and release the oxygen which gives rise to subsequent embolic effects.

The differences in the amount of local breakdown caused by the concentrated and the dilute forms is readily visible when the agent is administered intravenously in the rabbit's ear. Sufficient pressure is produced by the rapid reaction to force oxygen bubble masses into the surrounding tributary vessels and into the adjacent tissues, producing a peculiar and characteristic blanching. The more concentrated the agent, the greater the breakdown within the immediate area and also the lower the general toxicity. Figures 3 and 4 show the veins of a rabbit's ear before and after injection. It can be seen that the accumulated oxygen bubble masses are forced in all directions from the site of the injection.

Whole blood rapidly reacts with 90% hydrogen peroxide, in vitro, to form a tremendous amount of foam composed of oxygen bubbles held together by a fibrin-like film and forming a tenacious mass. This film may be coagulated protein resulting, at least in part, from the heat of reaction liberated when the peroxide breaks down. Such an interpretation has been reported by Licurzi (3).

In order to test the theory that these oxygen bubble masses produce a circulatory blocking effect, a series of experiments were performed on rabbits in which the effects of the intravenous injection of hydrogen peroxide on the flow of an intravenous saline infusion were determined. The jugular vein, while being infused with normal saline, was injected with hydrogen peroxide in varying concentrations at a point central to the site of the saline introduction, i.e., downstream to the saline infusion. The results are summarized in Table 3.

Table 3

Circulatory Blocking Effects of Intravenous Hydrogen Peroxide Upon Intrajugular Saline Infusions

Dilution	Dose as 90% H_2O_2 cu.mm./kg.	Effect upon Infusion Flow			Effect on Rabbit	
		Stopped	Slowed	None	Convulsions	Deaths
None (90%)	10		1/1		1/1	0/1
"	5	1/2	1/2		1/2	0/2
"	2		2/2		0/2	0/2
				Total	2/5	0/5
	Weighted Average 4.8					
1:6	7.9		1/2	1/2	2/2	2/2
"	6.3	1/3	2/3		3/3	2/3
"	4.0		3/3		1/3	0/3
"	2.0			2/2	1/2	0/2
				Total	7/10	4/10
	Weighted Average 5.8					
1:25	5.0		3/5	2/5	5/5	4/5
1:25	2.5			2/2	0/2	0/2
				Total	5/7	4/7
	Weighted Average 4.3					

With 5 cu. mm./kg. of the 90%, there was stopping or slowing in all cases, whereas with 5 cu. mm./kg. of the 1:25 dilution, there was only slowing or or continued flow. With 2 cu. mm./kg., the 90% produced slowing, but with successive dilutions there was no local blocking. Thus, the higher the concentration of the agent, the greater the local breakdown and the greater the blocking effect. With successive dilutions, the blocking effects were diminished; the signs of toxicity, as evidenced by convulsions, were increased; and there were more deaths. Finally, with each concentration, a dosage is reached which is too small to produce blocking, signs of toxicity, or death.

On the basis of the results in Table 3, Table 4 was constructed representing an estimate, arrived at by inspection of the dosage of hydrogen peroxide in cubic millimeters that would be necessary to produce blocking or slowing in 50% of the injections.

Table 4

Estimates of the Cubic Millimeters of 90% Hydrogen Peroxide Necessary to Produce Blocking or Slowing in 50% of Intrajugular Injections

Dilution	Block or Slowing in 50% cu.mm./kg.	LD50 cu.mm./kg.	Ratio of LD50 to Blocking 50
None	< 2	>10	>5
1:6	$>2, <4$	6	2
1:25	4-5	4	1

With no dilution the LD50 dose is five times that required to produce block. With dilution the blocking dose increases while the lethal dose decreases, reducing the ratio to about 2. With further dilution the dose required for blocking rises and the LD50 dose falls to a ratio of one. Thus, the more concentrated the agent, the greater the local breakdown, the smaller the dose necessary to cause blocking, and the larger the dose necessary to cause death. As the dilution is increased, the local breakdown becomes less, it requires more to produce blocking or slowing, and more of the unchanged form is available to produce systemic effects. Thus, with each successive dilution, the killing dose of the agent becomes smaller as indicated by the decreasing LD50 and the smaller ratio of the LD50 to the blocking effect.

IV. CONCLUSIONS.

1. Hydrogen peroxide (90%), by percutaneous as well as intravenous administration, produces death which is in each case associated with embolism.

2. There is a species difference in the susceptibility to the agent administered percutaneously.

3. The intravenous toxicity of 90% hydrogen peroxide is an inverse function of the degree of local breakdown at the site of the administration.

4. The explanation for the inverse relation between the dilution and the toxicity of hydrogen peroxide has a physical basis in that the more oxygen that is liberated and fixed at the site of the injection, the less is available for subsequent release in the blood stream to produce embolic phenomena, while the intravascular bubble masses, if big enough, mechanically hinder further access of hydrogen peroxide to the general circulation.

V. RECOMMENDATIONS.

1. Intravenous toxicity data on hydrogen peroxide should be interpreted in the light of these findings.

2. It is essential in reporting such data to give the dilutions used and to make comparisons at like dilutions.

3. To fully characterize the intravenous toxicity of hydrogen peroxide, it is necessary to use a range of dilutions.

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Studies on Hydrogen Peroxide contained in Commercial Food

(The manuscript was received on April 3, 1968)

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(For a brief English synopsis, refer to the original text on p.241.)

Introduction

Hydrogen peroxide which is widely used in the bleaching of foodstuff including kneaded food producs such as noodles is reported to be decomposed by oxygen and water and leaves nothing harmful whatsoever (1). Accordingly, hydrogen peroxide is one of these items for which there are no standards as to its use.

It has become clear recently, however, that a considerable amount of hydrogen peroxide is left in the food products sold on the market and much discussion has been carried out on the effect of residual hydrogen peroxide upon human bodies (2). In view of the fact that hydrogen peroxide is used not only in kneaded food products but also in many other kinds of foodstuff, it is believed that its effect is considerable. Thus, there is need for a reexamination of hydrogen peroxide which is used as an additive to food. Therefore, its stability in food products, its toxicity and its sterilizing effect, etc. were examined.

Experimental Method

1) Sample Food:

Kneaded food products sold on the market, 44 different kinds (including 40 "kamabokd" fish food, three kinds of "naruto," and one kind of "hampen") and 25 noodle products sold on the market (including 18 kinds of "udon", two kinds of spaghetti, one kind of "soba" and four kinds of "lamen") were used. In the experimental preparation of "kamaboko" fish cakes, the ground meat without the addition of a binder was used after it was boiled.

2) Qualitative and Quantitative Determinations of Hydrogen Peroxide:

For each sample, two thin pieces were prepared. A vanadium sulfate (3) solution was dropped on one and a titanic sulfate solution was dropped on the other. If hydrogen peroxide exists in the sample, the portions where these reagents were dropped would change to yellow. As for the hydrogen peroxide content in the samples, 10 g of the sample was collected and 10 g was added to the same per second and, after it was smashed in a glass mortar, distilled water was added to it to make the entire volume 200 ml. Determinations were carried out by using the iodine method. Along with this method, the peroxide method was also used (4, 5). Since there was no difference between the two methods, it was decided to mainly use the iodine method.

(Insert Table 1 on p. 242 H_2O_2 contents in various commercial foods.)

3) Toxicity Test:

Wistar-strain male rats purchased on the market, whose body weight was approximately 50 grams, were used. As feed, oriental solid feed MF was used and the experimental rats were raised by allowing them to freely take the feed and water in a constant-temperature chamber whose temperature was set at 22 degC. plus/minus one degC. Thirty-two rats were classified into four test groups. In each group, a 0.12%, 1.2%, and 12% hydrogen peroxide solution was given at the rate of 0.5 ml. per rat, forcefully through the mouth, after the measurement of the body weight between nine and 10 o'clock in the morning. The state of the body weight growth was observed for a period of 21 days. The control was given 0.5 ml of water.

4) Determination of Sterilizing and Decomposition-preventive Effect

By using colitis germs, staphilococcus and enteritis biblio, the sterilizing effect of hydrogen peroxide was measured in accordance with the phenolic acid co-efficient measuring method. As for the decomposition preventive test, "kamaboko" fish cake was prepared from the minced fish meat and the products were classified into two groups,one with an addition of hydrogen peroxide and

the other with no addition of the same.

Both samples were placed quietly in an incubator at the temperature of 37 degC and the total number of bacteria after 48 hours was compared. For the measurement of the total number of bacteria, a certain fixed quantity of a smashed sample was used and, after it was diluted by the 10-time dilution method, it was carried out by the plane culturing method. For the measurement of colitis germs, an EMB culture medium was used.

Experimental Result

1. Hydrogen Peroxide existing in Kneaded food Products and Noodle Products Sold on the Market

Regarding 44 kinds of kneaded food products sold on the market, including 25 kinds of noodle products, a qualitative test was carried out on hydrogen peroxide. On the case of four kneaded food products and 16 noodle products, a positive reaction was observed. A pseudo-positive reaction was observed in seven kneaded food products (including those which developed a brownish yellow color in a period of one hour). In the case of these samples, hydrogen peroxide was determined. As a result, it was clarified that a large number of food products have a residue of hydrogen peroxide in a considerable quantity as shown in Table 1.

All of the kneaded food products in which a large amount of hydrogen peroxide was found were of the "naruto" kind and no hydrogen peroxide was found in the "kamaboko" fish meat cake which was used in the current experiment.

In the case of noodle products, on the other hand, it became clear that a high concentration of hydrogen peroxide exists in a large number of cases. In the case of "udon" (Japanese noodle), it became clear that the rate of detection and the remaining concentrations were high.

The above-described result is indicative of the fact that not only in the kneaded food products but also in the noodle products, hydrogen peroxide is left. From this, it can be assumed that hydrogen peroxide is left in food if hydrogen

peroxide was used for the purpose of bleaching and decomposition prevention.

2. Thermal Stability of Hydrogen Peroxide

It has already become clear that hydrogen peroxide is left in a large number of food products which are sold on the market. At the time when these foods are actually prepared for eating, such a treatment as heating is given them in many cases. Therefore, the effect of heat upon hydrogen peroxide contained in food products was examined.

(Insert Figure 1 on p. 243 Effect of homogenized kamaboko (boiled fish paste) on the heat stability of H202.)

First, kamaboko minced fish meat was caused to co-exist or not caused to co-exist in a solution containing 1600 ppm of hydrogen peroxide. These were heated to a temperature of 100 (translator's note: degrees C.?) and the amount of residual hydrogen peroxide was measured at various times.

As is shown in Figure 1, there was observed a tendency for the stability of hydrogen peroxide to increase at the time when kamaboko minced meat was caused to co-exist. As factors for such an increase in stability, it is possible to consider the effect of protein, starch, and amino acids which are constained in the kamaboko. Therefore, the stability was examined by causing gluten and glutamic acid to co-exist. However, only a slight effect was observed in this case.

On the other hand, it was discovered that phosphate which is used as a stabilization agent (7) for hydrogen peroxide was contained in kamaboko as a binding agent. Thus, it was believed to stem from the effect of the phosphate. Therefore, kamaboko was prepared from minced meat without an addition of any binding agent and the decomposition of hydrogen peroxide at the time when it was smashed was examined at various times.

(Insert Figure 2 on p. 243. Effect of homogenized kamaboko (boiled fish-paste) on the heat stability of H202.)

The result (which is shown in Figure 2) showed that there was a slight

stabilization effect but, as compared with the case where a binding agent was added, it was clear that stability was reduced.

From these results, it is assumed that such high polymer substances as protein and starch which are contained in the kamaboko somewhat affect the stability of hydrogen peroxide. However, it can be stated that the phosphate which co-exists plays an important role.

(Insert Figure 3 on p. 243. Effect of homogenized noodle on the heat stability of H202.)

Regarding the thermal stability of hydrogen peroxide which is left in the noodle products, the effect of the addition of it in a smashed state was examined as in the case of kamaboko. As a result, it was clarified that the hydrogen peroxide is stabilized by the co-existence of smashed items as shown in Figure 3. A result which is similar to this was reported to have been observed even in the boiling treatment of "udon" (Japanese noodle) which has an addition of hydrogen peroxide (6).

3. Effect of mastication upon hydrogen peroxide which is left in food products.

The hydrogen peroxide which is left in food products is not completely decomposed and, in addition, some of these food products are eaten in their raw state like kamaboko. Therefore, it is correct to assume that the hydrogen peroxide which is left behind is taken into living bodies. Therefore, an examination of the effect upon hydrogen peroxide contained in food products was carried out. Twenty (20) g. of "naruto" in which the residual amount of hydrogen peroxide was known was given to three test recipients and, after 20 mastications, hydrogen peroxide contained in the masticated food was determined.

As shown in Table 2, it was made clear that from 60% to 70% of hydrogen peroxide still remains even after mastications and that no complete decomposition is effected by such mastications.

(Insert Table 2 on p. 244 Effect of mastication on H2C2 content in kamaboko (boiled fish paste).)

4. Effect of Ascorbic Acid upon Hydrogen Peroxide contained in Food

It has been clarified that the hydrogen peroxide which is left in the food remains stable at the time of cooking and mastication. Now, the effect of a chemical treatment and a reducing agent, in particular, was examined. As a reducing agent, ascorbic acid which is approved as a food additive like hydrogen peroxide was used.

Kamaboko whose hydrogen peroxide content was known was cut into pieces, each weighing 10 gl, and the same was immersed in a 1% ascorbic acid solution, and the amount of hydrogen peroxide which was left was measured at various times.

(Insert Figure 4 on p. 244. Effect of ascorbic acid on H202 content in sliced kamaboko (boiled fish paste).)

As is shown in Figure 4, the amount of residual hydrogen peroxide was reduced at the beginning of the immersions, with almost no change being observed thereafter. This is indicative of the fact that, in the test pieces, only the hydrogen peroxide which exists on the cut surface which is in direct contact with the ascorbic acid solution was decomposed and that the hydrogen peroxide which exists in the interior without being contacted by the ascorbic acid solution was not affected at all.

On the other hand, when an ascorbic acid solution was added to these samples smashed along with "sea sands," it was found that the same was decomposed almost in a fixed quantity. (Refer to Figure 5) This phenomenon was also shown (Table 3) in the case where ascorbic acid was made to co-exist at the time when the effect of mastication was examined in the previous dase. It can be stated that, in this manner, it exists in an extremely unstable state in food products as compared with the reducing agent.

5. Toxicity Test

Hydrogen peroxide contained in food in a state of high instability against a chemical treatment such as one using ascorbic acid remains comparatively stable under heating and by mastication. Since these food products are offered on the kitchen tables without going through such a chemical treatment, it is believed that the hydrogen peroxide, if it exists in the food, could easily enter the digestive organs of humans.

(Insert Figure 6 on p. 244 Effect of H202 on the growth of Rats)

Therefore, the toxicity of hydrogen peroxide was examined by a test on the growth of rats. T he variations in the body weights of rats in the groups which were administered 0.6 mg. of hydrogen peroxide per day, 6.0 mg. per day and 60 mg. per day were measured for a period of 21days and were compared with those of the control group. The result which is described in Figure 6 was obtained.

(Insert Table 6 on p. 245 Effect of H202 treatment on bacterial growth in Kamaboko (boiled fish paste).)

In other words, a considerably high rate of growth was shown in the group administered 0.6 mg. per day as in the control, and only a slight delay in growth was observed in the group administered 6.0 mg. per day. In the group which was administered 60 mg. per day, the intestinal part was swollen up immediately after such administration, with four rats falling to die. The administration of the same was terminated thereafter for those rats which remained alive. In this case, a normal growth curve was shown from the fourth day on. After 21 days, all rats were killed and the weight of their organs was examined. The result obtained is shown in Table 4. No special change was observed in all of the groups.

(Insert Table 3 on p.244 Effect of mastication with the addition of ascorbic acid on H202 content in kamaboko (boiled fish paste.))

(Insert Table 4 on p. 245 Effect of administration of H202 on the body

and organ weights of rats.)

(Insert Table 5 on p.245 Sterilizing effect of H202 to some food poisoning bacteria.)

6. Sterilizing and Decomposition Preventive Effect of Hydrogen Peroxide

It has become clear that hydrogen peroxide has a sterilizing effect (8). It has been reported that the producer oxygen which is produced by the decomposition of hydrogen peroxide serves as a sterilizing force (9).

It was necessary to examine whether the hydrogen peroxide which has been stabilized by the addition of a stabilizing agent as in the food products has a similar effect. Therefore, a sterilizing test was carried out by using colitis germs, staphilococcus aureus and enteritis biblio which are related to food products in the case where pyrophosphoric acid was added as a stabilization agent and relative to food with an addition of hydrogen peroxide (and?) pyrophosphoric acid. In this case, an examination was also carried out on the relationship between the time of contact between the bacterium used and the hydrogen peroxide and the sterilizing effect. These results are shown in Table 5 in a summary form.

A comparatively low concentration of hydrogen peroxide was found to have an effect on the colitis germs and enteritis and it has an especially powerful sterilizing effect upon enteritis biblio. However, its effect upon stabhilococcus aureus was somewhat small as compared with these.

The above result was exactly the same as in the case where no pyrophosphoric acid was added. This is indicative of the fact that the addition of a stabilization agent has no effect upon the sterilizing effect of hydrogen peroxide.

Next, the effect of hydrogen peroxide upon an increase in the number of colitis germs in the case colitis germs were experimentally caused to adhere to kneaded food products or when the number of raw bacteria was increased.

By using fresh kamaboko products, those which were impregnated with hydrogen peroxide (678 to 783 ppm) obtained by immersing the same in a 0.23% hydrogen solution for a period of 30 minutes and those which were not impregnated with hydrogen peroxide were left one night in a room (10 degC to 20 degC) and in a 30 degC incubator, and the number of raw bacteria was measured.

In addition, the kamabokos which were immersed in a colitis germ suspension solution were classified into two groups. After one of them had been immersed in a hydrogen peroxide solution, it was left overnight in an incubabor.

The results obtained above are summarized in Table 6. By the presence or absence of hydrogen peroxide in kamaboko, there is a great difference in the number of raw bacterias, thus indicating the fact that hydrogen peroxide is effective in the preservation of kamaboko. Exactly the same result was obtained in the case of colitis germs and hydrogen peroxide was found to be one of the useful means for making the compositions of kneaded fish meat products suitable.

Observations

Regarding 44 kinds of kneaded food products which were sold on the market, the residual amount of hydrogen peroxide contained was measured. As a result of such a measurement, hydrogen peroxide was found in four kinds of these food products, with the highest value being 2470 ppm. Of these positive test bodies all of those showing a high degree of hydrogen peroxide contents were of the "Naruto" kind. It is believed to be significant that a large amount of hydrogen peroxide was detected in these products where the state of bleaching was considered important from the standpoint of commercial values.

In the present experiment, a large amount of hydrogen peroxide was not discovered in kamaboko, the result being completely different from what is described in literature (2). Regarding this, it is believed that the residual amounts become different for different manufacturing districts as stated in the said journal. In the current experiment, the "kamaboko" products manu-

factured in the Kansai district were used as the test bodies, with a result that only a small amount of hydrogen peroxide was left in these products.

Regarding the wrapped noodle products, on the other hand, those in which hydrogenperoxide was left were quite numerous, and many of them showed quite a high content of the same.

From the above results, it is a fact that a considerable amount of hydrogen peroxide is left in the kneaded food products and noodle products which are sold on the market, and it can be stated that it is a problem which should be examined from the standpoint of food hygiene.

It is not clear in what state such residual hydrogen peroxide exists in the food. However, at least in its relationship with co-existing food components, it is stable under the application of heat and it also exists in such a state that it is not easily decomposed by mastication. Accordingly, it is believed that the theory to the effect that hydrogen peroxide is decomposed with water and oxygen at the time of processing and cooking, thereby leaving nothing harmful (1) (which is based on the results of experiments concerning thermal stability and mastication or the result of a measurement of residual amounts in the food products sold on the market) should be corrected.

In view of the fact that hydrogen peroxide remains in food in a comparatively stable state, it is believed to enter the human body quite easily at the time when such food is taken in. Accordingly, an examination of the toxicity of hydrogen peroxide should be carried out. However, no clear-cut report is found in this respect. In the continuous administration of 0.6 mg per day for a period of 21 days in this experiment, absolutely no abnormality was found in the growth and internal organs of rats, thereby indicating the possibility that hydrogen peroxide does not have such an acute toxicity.

Since the method of administration was an indirect method of administration into the intestines, it is assumed that the values are still higher when

the conversion is made as the residual amount of hydrogen peroxide in food, if the hydrogen peroxide in food undergoes some kind of decomposition before it reaches the interior of the stomach after it has been cooked and swallowed down by humans.

However, these results are the results which were obtained in a short-term test after all. Unless the result of a long-term test on its toxicity is taken into consideration, no conclusion can be drawn regarding the toxicity or use limits of hydrogen peroxide. Hydrogen peroxide is often used not only for bleaching purposes but also for decomposition prevention and sterilization purposes at the same time. For example, it has been reported that hydrogen peroxide is effective in the preservation of wrapped noodle products (10). Actually, wrapped noodle products which have been treated with hydrogen peroxide for this purpose are being sold on the market at present.

It belongs to the realm of public knowledge that hydrogen peroxide is used for sterilizing purposes (6). In our experiment, it was observed that it has a powerful sterilizing effect on colitis germs, staphilococcus aureus and enteritis biblio, which become problems in terms of food hygiene. It was clarified that it was especially effective on enteritis biblio. This is indicative of the fact that the hydrogen peroxide treatment given to food is a useful means for sterilization and decomposition prevention from the standpoint of food hygiene.

According to the compositional specifications of the Law of Food Hygiene, the kneaded fish-meat products are stipulated to be "negative to colitis germs." It can be considered to be a useful means for meeting such a specification. If by a treatment using hydrogen peroxide or by its residual amount in this manner, bacterial pollution (smear) is prevented and the preservative character of food is raised, it can be stated that the use of hydrogen peroxide within the limits which are confirmed from the standpoint of safety is a useful measure from the standpoint of food hygiene. This is assumed to be related to the fact

that the detection of residual chlorine in the service water supply or in the pool is considered to be a guarantee of its safety. Accordingly, it will be necessary to provide standards for its content within the limits which take into consideration both safety and effectiveness.

In the determination of its use standards, the toxicity of hydrogen peroxide should be taken into consideration. The toxic test in our experiment was carried out for a short period of time and, moreover, the standards for the administration of hydrogen peroxide were made borad. Therefore, it is impossible to set up accurate standards. Concerning this problem, it is believed that detailed amounts of administration should be set up and that a long-term toxicity test should be carried out. In connection with the establishment of standards, further, the calculation of standards for infants should also be carried out since noodle products, etc. are often used as ablactation food for infants.

Summary

It was found that some of the kneaded food products and noodle products which are sold on the market contain a residual amount of hydrogen peroxide. Since the hydrogen peroxide which is added to these foods is considerably stable under the application of heat and by mastication, it is anticipated that the hydrogen peroxide which is contained in food is taken into living bodies.

As the result of an examination of the effect of hydrogen peroxide upon the growth of rats by administering the same for a period of 21 days on a continuous basis, the administration of 0.6 mg per 100 g. had almost no effect upon the increase of body weight and the weight of internal organs.

The result of an examination of the sterilizing effect of the same upon colitis germs, staphilococcus aureus and enteritis bilblio showed that a high degree of sterilization was found on all of the bacteria. An especially powerful sterilizing effect was found on enteritis biblio.

Based on the aforementioned results, the observation was made on the significance of hydrogen peroxide as a food additive and on its use standards.

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References and Footnotes:

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食品中に残存する過酸化水素について

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Studies on Hydrogen Peroxide contained in Commercial Food

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It was found that an extremely high concentration of hydrogen peroxide was contained in many commercial products of boiled fish-pastes and packed noodles which have been usually treated with hydrogen peroxide for bleaching and sterilizing. Hydrogen peroxide contained in these foods was stable for heating or mastication, but it was easily decomposed by adding ascorbic acid.

The toxic effect of hydrogen peroxide on rats was tested by observing the growth rate of rats and the changes of their organ weights. From the results, it was shown that any significant changes were not observed in the growth rate and organ weights of used rats at the dose of 0.6 mg day for 21 days but some toxic effects were observed at the dose of 6.0 mg day. Antibacterial test using *E. coli*, *Staph. aureus* and *V. parahaemolyticus* showed that hydrogen peroxide had strong antibacterial action against these strains, especially *V. parahaemolyticus*.

Hydrogen peroxide has been commonly used as one of food additives for bleaching and sterilizing the foods, but no limitation was set up for the amount of use in this case. From the above results, it was discussed that the limitation for its residual amount after the processing must be set up from the standpoints of public health.

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緒言

ねり製品，めん製品などの食品の漂白を目的としてひろく使用されている過酸化水素は食品の加工調理の際，酸素と水に分解され，なんらの有害物を残さないものとされている[1]．したがって過酸化水素は現在食品添加物中使用規準のない品目に入れられている．しかし，最近多くの市販ねり製品中に過酸化水素が多量残存していることが明らかとなり，残存過酸化水素の人体に及ぼす影響について論じられている[2]．過酸化水素がねり製品のみならず，各種食品に使用されている現状から一般に与える影響が大きいと考えられ，食品添加物としての過酸化水素を再検討する必要があるので食品中の安定性，毒性および防腐殺菌効果などについて検討することにした．

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実験方法

1) 試料食品——市販ねり製品44種（かまぼこ：40種，なると：3種，はんぺん：1種）および市販めん製品25種（うどん：18種，そば：1種，スパゲッティ：2種，ラーメン：4種）を用いた．実験的にかまぼこを調製する場合は，結着剤を入れていないすり身を購入し，煮沸した．

2) 過酸化水素の定性および定量——1試料につき2枚の薄片を調製し，一方に1％硫酸ハナンウム溶液[3]，他方に硫酸第二チタン溶液を添加した．試料中に過酸化水素が存在すれば，これらの試薬を滴下した部位が黄色ないしかっ色に呈色する．試料中の過酸化水素含量は試料を10gとり，これに海砂10gを加え乳パチ中で磨砕後蒸留水を加えて全量を200mlとし，ヨウ素法により定量した．この方法と並行して Peroxidase 法[4,5] を用いたが両者の結果に差がなかったので，おもにヨウ素法を用いた．

Table 1. H_2O_2 Contents in Various Commercial Foods

Sample No.	Commercial foods	H_2O_2 (ppm)	Sample No.	Commercial foods	H_2O_2 (ppm)
1	"Naruto"	2470	36	"Kamaboko"	0
2	〃	499	37	〃	0
3	〃	24	38	〃	0
4	〃	0	39	〃	0
5	〃	0	40	〃	0
6	"Hanpen"	0	41	〃	0
7	"Kamaboko"	11	42	〃	0
8	〃	0	43	〃	0
9	〃	0	44	〃	0
10	〃	0	45	Packed Noodle	1208
11	〃	0	46	〃	853
12	〃	0	47	〃	363
13	〃	0	48	〃	309
14	〃	0	49	〃	305
15	〃	0	50	〃	261
16	〃	0	51	〃	243
17	〃	0	52	〃	238
18	〃	0	53	〃	212
19	〃	0	54	〃	162
20	〃	0	55	〃	34
21	〃	0	56	〃	16
22	〃	0	57	〃	12
23	〃	0	58	〃	9
24	〃	0	59	〃	0
25	〃	0	60	〃	0
26	〃	0	61	〃	0
27	〃	0	62	〃	0
28	〃	0	63	〃	16
29	〃	0	64	Spaghetti	85
30	〃	0	65	〃	0
31	〃	0	66	"Chuka-soba"	0
32	〃	0	67	〃	0
33	〃	0	68	〃	0
34	〃	0	69	〃	0
35	〃	0			

3) 急性試験 ―― 体重約 50 g の市販 Wister 系雄ラットを用いた。飼料としてオリエンタル社の粉末MFを用い，22 ± 1 ℃の室内で飼育した。飼料および水を自由に摂取させ飼育した。ただし32匹を4試験群とした。各群にそれぞれ0.12%，1.2%および12%過酸化水素溶液を1匹あたり 0.5 ml すつ毎日午前9時～10時に体重測定後経口的に強制投与し，21日間の発育状態を観察した。対照群は 0.5 ml の水を投与した。

4) 殺菌，防腐効果の測定 ―― 過酸化水素の殺菌力は大腸菌，ブドウ球菌，腸炎ビブリオを用い有効酸性菌数測定法により測定した。防腐試験はまり身からかまぼこをえらび，過酸化水素添加と無添加とした。両試料を37℃恒温器中に保存し，48時間後の総菌数を比較した。総菌数測定は試料磨砕物の一定量をとり10倍希釈法により希釈後平板培養法により行なった。大腸菌数の測定はEMB培地を用いて行なった。

実験結果

1. 市販ねり製品，めん製品中に残存する過酸化水素

市販ねり製品44種およびめん製品25種について過酸化水素定性試験を行ない，ねり製品4種，めん製品16種に

陽性反応，ねり製品7種に疑陽性反応（1時間後に黄かっ色を呈したもの）がみられたので，これらの試料について過酸化素を定量した．その結果 Table 1 に示すように試料中にはかなりの量の過酸化水素が残存している製品のあることが明らかとなった．ねり製品中多量の過酸化水素が検出されたものはすべてなるとであり，本実験に使用したかまぼこにはほとんど検出されなかった．これに対してめん製品では多くの試料に高濃度の過酸化水素が残存していることが明らかとなり，とくにうどんでは検出率および残存濃度が高いことが判明した．以上の結果はねり製品のみならずめん製品にも過酸化水素が残存していることを示し，このことから漂白，防腐を目的として過酸化水素を使用した食品はいずれも食品中に過酸化水素が残存しているものと予想しなくてはならない．

2. 過酸化水素の熱安定性

市販食品中に過酸化水素が残存しているものが多数あることはすでに明らかとなったが，これら食品が実際に調理される場合加熱などの処理が行なわれることが多いので，食品中の過酸化水素が熱によりどのような影響を受けるかを検討した．まず，1600 ppm の過酸化水素を含む溶液にかまぼこ磨砕物を共存させたものと，させないものを100 に加熱し，残存過酸化水素量を経時的に測定した結果 Fig. 1 のごとくかまぼこ磨砕物を共存させ

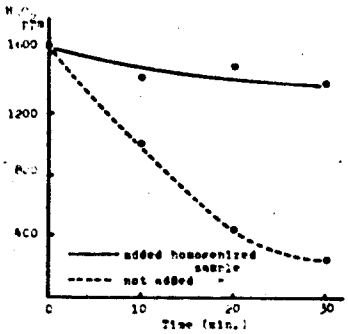

Fig. 1. Effect of homogenized Kamaboko (boiled fish-paste) on the heat stability of H_2O_2 ($Na_4P_2O_7$ added)

ときの過酸化水素の安定性が増大する傾向がみられた．このように安定性が増加する原因として，かまぼこ中のタンパク質，ペプトン，アミノ酸などの影響が考えられるのでグルテン，グルタミン酸などを共存させて安定性を検討したが，わずかに効果がみられたにすぎなかった．一方，すでに過酸化水素の安定剤[2]として使用されているリン酸塩がかまぼこ中に結着剤として含有されていることから，リン酸塩の影響によるものと考えられたので，結着剤無添加のすり身からかまぼこを調製し，これを磨砕したときの過酸化水素の分解を経時的に検討した．その結果 Fig. 2 に示すようにわずかに安定効果はみられたが結着剤添加の場合と比較して明らかに安定性が減少した．これらの結果から，かまぼこ中のタンパク質，ペプトンなどの高分子物質も若干過酸化水素の安定性に影響していると考えられるが，共存するリン酸塩が大きな役割を果たしているものといえる．

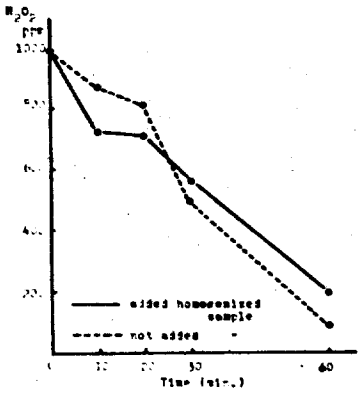

Fig. 2. Effect of homogenized Kamaboko (boiled fish-paste) on the heat stability of H_2O_2

一方，めん類に残存する過酸化水素の熱安定性についてかまぼこと同様その磨砕物の添加による影響について検討したが，Fig. 3 のように磨砕物共存により過酸化水素が安定化されることが判明した．これと同様な結果は過酸化水素添加うどんの蒸煮処理においてもみられることが報告されている[6]．

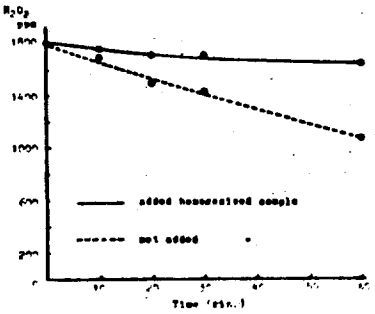

Fig. 3. Effect of homogenized noodle on the heat stability of H_2O_2

3. 食品中に残存する過酸化水素に対するそしゃくの影響

食品中に残存する過酸化水素が調理の際にも完全に分解されず，またかまぼこのように生食されるものもある

ことから，残存する過酸化水素が生体に摂取されることが当然予想される．そこでまず，そしゃく時食品中の過酸化水素がどのような影響を受けるかについて検討した．過酸化水素残存既知のかまぼこ 20 g を 3 人の試験者に与え，20回そしゃく後それぞれのそしゃく物について過酸化水素を定量したところ，Table 2 のように 60～

Table 2. Effect of Mastication on H_2O_2 Content in Kamaboko (boiled fish-paste)

Experiment No.	Before mastication (ppm)	After mastication (ppm)
I	821	639
II	876	595
III	680	459

70％の過酸化水素がそしゃく後も残存し，この程度のそしゃくでは完全に分解されないことが明らかとなった．

4. 食品中に残存する過酸化水素に対するアスコルビン酸の影響

食品中に残存する過酸化水素が調理時あるいはそしゃく時においても安定であることが明らかとなったが，化学的処理とくに還元剤によりどのような影響を受けるかを検討した．還元剤としては過酸化水素と同様食品添加物として認可されているアスコルビン酸を使用した．過酸化水素残存既知のかまぼこを 10 g の切片とし，これを 1％アスコルビン酸溶液に浸漬し，経時的に残存過酸化水素量を測定したところ Fig. 4 に示すように浸漬初

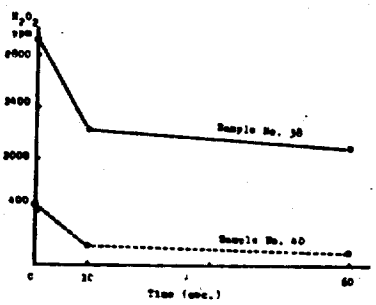

Fig. 4. Effect of ascorbic acid on H_2O_2 content in sliced Kamaboko (boiled fish-paste)

時においては残存過酸化水素量は減少したが，その後はほとんど変化しなかった．このことは試料切片においてアスコルビン酸溶液と直接接触する切断面に存在する過酸化水素のみが分解され，アスコルビン酸溶液と接触しない内部に存在する過酸化水素はなんら影響を受けないことを示している．一方，これらの試料を海砂とともに磨砕したものにアスコルビン酸溶液を添加するとほとんど定量的に分解されることが判明した(Fig. 5)．この現象

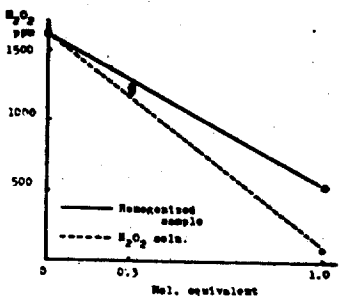

Fig. 5. Effect of ascorbic acid on H_2O_2 content in homogenates of H_2O_2 treated Kamaboko (boiled fish-paste)

Table 3. Effect of Mastication with the Addition of Ascorbic Acid on H_2O_2 Content in Kamaboko (boiled fish-paste)

Experiment No.	Before mastication (ppm)	After mastication with AsA* (ppm)
I	872	0
II	876	0
III	680	0

* Ascorbic acid

は前記のそしゃくによる影響を検討した際，アスコルビン酸を共存させた場合にも示され (Table 3)，このような還元剤に対してはきわめて不安定な状態で食品中に存在しているものといえる．

5. 毒性試験

アスコルビン酸などの化学的処理に対してきわめて不安定な食品中の過酸化水素も，熱あるいはそしゃくに対して比較的安定であり，通常このような化学的処理を経ないでこれらの食品が食用に供せられることから，もし

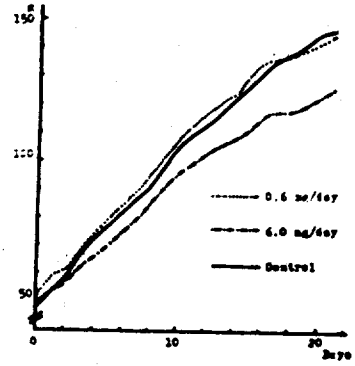

Fig. 6. Effect of H_2O_2 on the growth of rats

Table 4. Effects of Administration of H_2O_2 on the Body and Organ Weights of Rats

Group of test animals	Initial body weights (g)	Final body weights (g)	Body weights increases (g)	Liver weights (g)	Liver wt./Body wt.	Spleen weights (g)	Spleen wt./Body wt.	Kidney weights (g)	Kidney wt./Body wt.
Control	47.9	145.7	97.8	7.8	0.053	0.66	0.0046	1.34	0.0092
H_2O_2 0.06mg/day	52.4	144.8	92.4	7.6	0.053	0.59	0.0041	1.40	0.0097
H_2O_2 6.0mg/day	49.5	127.0	77.5	7.5	0.051	0.78	0.0061	1.28	0.0098

食品中に過酸化水素が残存すれば容易に消化管内に食品とともに入るものと考えられる．そこで過酸化水素の毒性をラットの発育試験により極めた．過酸化水素 0.6 mg/day, 6.0 mg/day および 60 mg/day 投与群の体重変化を 21 日間にわたり対照群と比較したところ Fig. 6 に示すような結果が得られた．すなわち，0.6 mg/day 投与群は対照と同様正常な発育を示し，6.0 mg/day 投与群はわずかに発育の遅延をみた．60 mg/day 投与群では投与直後腹部が膨満し，4 日で死亡したが，生存しているものについてその後投与を停止したとき 4 日後から正常な発育曲線を示した．21 日後全ラットを殺し，その臓器重量について極めた結果 Table 4 のごとく，いずれの投与群においても特別な変化は認められなかった．

Table 5. Sterilizing Effect of H_2O_2 to Some Food Poisoning Bacteria

Incubation time (min)	*E. coli*				*Staph. aureus*				*Vibrio parahaemolyticus*			
H_2O_2 concentration (%)	2.5	5	10	15	2.5	5	10	15	2.5	5	10	15
0.03	−	−	−	−	−	−	−	−	−	−	−	−
0.3	−	−	−	−	−	−	−	−	+	+	−	+
3.0	+	+	+	+	−	−	−	−	+	+	+	−
6.0	+	+	+	+	−	−	−	−	+	+	+	−
8.0	+	+	+	+	−	−	−	+	+	+	+	+
9.0	+	+	+	+	−	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	+	+	+	+

+ Growth inhibition
− No inhibition

6. 過酸化水素の殺菌，防腐効果

過酸化水素の殺菌力を有することはすでに明らかであり[8]，過酸化水素が分解して生ずる発生期の酸素による強化力が殺菌力となる[9]と報告されている．しかし，食品のように安定剤が添加され安定化された過酸化水素が同様な効力を有するかどうかを極める必要があるので，安定剤としてピロリン酸塩を添加した場合および過酸化水素ピロリン酸付加物について食品に関係ある大腸菌，ブドウ球菌，腸炎ビブリオを用いて殺菌試験を行なった．この際使用菌と過酸化水素の反応時間の殺菌力とのどのような関係にあるかをあわせて極めた．これらの結果をまとめて Table 5 に示した．過酸化水素は大腸菌，腸炎ビブリオには比較的低濃度で効力を有し，とくに腸炎ビブリオには強い殺菌性を示したが，ブドウ球菌に対してはこれらに比較して効果はさ[illegible]であった．この結果はピロリン酸塩を添加しない場合と全く同様であり，安定剤の添加は過酸化水素の殺菌力に影響しないことが判明した．つぎに実際にねり製品を用い，生菌数の増加あるいは人為的に大腸菌を付着させた場合の大腸菌数の増加におよぼす過酸化水素の効果を極めた．新しく調

Table 6. Effect of H_2O_2 Treatment on Bacterial Growth in Kamaboko (boiled fish paste)

Incubating conditions (15 hrs)	H_2O_2* content (ppm)	Viable cell (/g)	*E. coli*** MPN (/10 g)	*E. coli*** EMB medium (/g)
Room (12°C)	0	5 × 10^2	1.8 × 10^4 +	4 × 10^8
	555	25	5	10
Incubator (30°C)	0	1.2 × 10^8	1.8 × 10^4 +	3 × 10^7
	555	0	79	5

* Sliced samples (10 g) were soaked in 0.3% H_2O_2 solution for 30 min
** Control and H_2O_2 treated samples were soaked in 20 ml of *E. coli* cultures for 10 min

製したかまぼこを0.23%過酸化水素溶液に30分間浸漬した過酸化水素含有かまぼこ (678～783 ppm) と不含のものとを室内 (10°～20°) と30°ふ卵器に一夜放置した後，生菌数を測定した．また，かまぼこを大腸菌懸濁液に浸漬したものを二つに分け，一方を過酸化水素溶液に浸漬させたのちふ卵器および室内に一夜放置した．以上の結果をまとめると Table 6 に示すように，過酸化水素がかまぼこに存在するか否かにより，その生菌数には大きな違いがあり，かまぼこの保存に過酸化水素が有効であることが示された．また，大腸菌数に関しても全く同様の結果となり過酸化水素処理は魚肉ねり製品の成分規格に適合させるための有利な手段のひとつであることを示している．

考　　察

市販ねり製品44種について過酸化水素残存量を測定した結果，そのうち4種について過酸化水素が検出され，最高値は 2470 ppm を示した．これら陽性検体のうち過酸化水素残存量の高いものはいずれも"なると"であり，商品価値上とくに白状態が重視される本品において多量の過酸化水素が検出されたことは意味あることと考えられる．本実験においては"かまぼこ"からは多量の過酸化水素は検出されておらず文献[7]に記載された結果と全く異なったものとなったが，これは同じく記載されているように産地により残存量に違いがあり，本実験には関西地方の"かまぼこ"を検体として用いた結果少量の過酸化水素しか残存していないという結果となったものと考えられる．一方，包装めん類については過酸化水素の残存しているものが高率に検出され，しかも相当高い含有量を示すものが多かった．以上のことから市販ねり製品，めん製品に相当量の過酸化水素が残存していることは事実であり，食品衛生上検討すべき問題であることが確認されたものといえる．このように食品中に残存している過酸化水素がどのような状態で存在しているかについては不明であるが，少なくとも共存する食品成分との関係において熱に対して安定であり，またそしゃくに対しても容易に分解されない状態で存在しているものといえる．したがって熱安定性に関する実験およびそしゃく実験などの結果あるいは市販食品の残存量測定結果から知り，調理の際過酸化水素が水と酸素に分解され，なんらの有害物を残さないという解説[8]は訂正されるべきであると考えられる．過酸化水素が比較的安定な状態で食品中に残存することから，これら食品が実用に供せられた場合当然生体内に入るものと考えられる．したがって過酸化水素の毒性についての検討が行なわれなければならないが，これに関しては明確な記載はあまり見当たらない．本実験において行なった 0.6 mg/day，21日間連続投与においてはラットの発育および内臓所見には全く異常は認められず，過酸化水素がそれ程急激な毒性を有しているものとは考えられない．また，投与方法は胃内への直接投与であることから，食品中の過酸化水素が調理，そしゃくされ嚥下後胃内に到達するまでにある程度の分解をうけるものとすれば，食品中の残存過酸化水素量として換算する際，さらに上回った値になることが予想される．しかしながら，これらの結果はあくまで短期間の毒性試験から得た結果であり，慢性毒性試験の結果をまたなければ過酸化水素の毒性あるいは使用限度について結論を下すことはできない．過酸化水素が食品の漂白を目的として用いられると同時に防腐，殺菌の目的で用いられることが多い．たとえば，包装めん製品の保存に過酸化水素が有効であることが報告され[9]，実際にこの目的で過酸化水素処理された包装めん製品が市販されている．過酸化水素が殺菌作用を有することはすでに周知のことであるが，本実験において食品衛生上問題となる大腸菌，ブドウ球菌，腸炎ビブリオに対して強力な殺菌作用を有することが認められとくに腸炎ビブリオに有効であることが明らかとなったが，このことは食品の過酸化水素処理が防腐，殺菌のみならず食品衛生上有用な手段であることを示すものである．食品衛生法の成分規格では魚肉ねり製品は"大腸菌群陰性"と規定しているが，この規定に合致させるためにも有利な手段であるといえる．このように過酸化水素による処理あるいはその残留によって細菌汚染を防ぎ，また食品の保存性を高めうるならば安全性の確認された限度内での使用はむしろ食品衛生上有益な措置であるともいえる．このことは上水やプールにおいて残留塩素の検出されることが安全性の保証とされていることにつながる問題と考えられる．したがって安全性と有効性が考慮された限度内で含量基準を設ける必要がある．使用基準の設定についてはまず過酸化水素の毒性が考慮されなくてはならないが，本研究での毒性試験は短期間であり，しかも過酸化水素の投与基準を大幅にとってあるため正確な基準を設定することはできない．この問題に関してはさらに詳細な投与量を規定し，長期の毒性試験が必要であると考えられる．また基準設定の場合めん製品などは離乳食に用いられる例が多いので乳幼児を対照とした基準量の算出が考慮されなくてはならない．

要　　約

市販ねり製品，めん製品に高濃度の過酸化水素が残留するものがかなりあることが判明した．これらの食品に添加された過酸化水素は熱あるいはそしゃくに対してもかなり安定であることから食品中の過酸化水素が生体内に摂取されることが予想された．ラットの発育におよぼす過酸化水素の影響を21日間連続直接投与により検討した結果 0.6 mg/100 g では体重増加，臓器重量にはとんど影響が認められなかった．大腸菌，ブドウ球菌，腸炎ビブリオに対する殺菌効果を検討した結果，いずれの菌種にも高度の殺菌性を示したが，とくに腸炎ビブリオには強力な殺菌性を示した．

以上の結果から過酸化水素の食品添加物としての意義と使用基準について考察した．菌株を分与された大阪大学微生物病研究所堀野恒三郎教授，千葉大学腐敗研究所藤巻喜久夫教授に謝意を表します．また，本研究に協力いただいた昭和42年度衛生化学教室特別実習生に感謝します．

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Kawasaki-'69
Bib No. 622

Kawasaki, C.*, M. Kondo*, T. Nagayama*, Y. Takeuchi*, and H. Nagano** : EFFECT OF HYDROGEN PEROXIDE ON THE GROWTH OF RATS. Shokuhin Eiseigaku Zasshi, Vol. 10 (2), pp. 68-72, 1969.

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INTRODUCTION

In the previous study (1), the effect of hydrogen peroxide on the growth of white rats was observed for 3 weeks. In that experiment, hydrogen peroxide was directly injected into the stomach through a tube. The study showed the effect of hydrogen peroxide itself, but not the effect of hydrogen peroxide contained in food products. The dose of hydrogen peroxide also varied widely from group to group, and the period of observ ation was short. In the present study, these liminations were modified. Hydrogen peroxide was given at 4 different doses. It was introduced directly into the stomach through a tube in one type of experiment, whereas in the other experiment, hydrogen peroxide phosphate was mixed in the feed. The experiment was continued for 3 months, and the animals were killled at the end of experiment. The growth, the catalase activity, the hematocrit value, etc. were examined, and the following results were obtained.

EXPERIMENTAL PROCEDURE

1. EXPERIMENTAL ANIMALS AND THEIR CARE AND FEEDING

In the oral administration of hydrogen peroxide, male Wistar rats weighing about 70 g were individually kept in metal mesh cages in a room at a constant temperature of 22 ± 1°C. Each group consisted of 9 - 12 animals. Forty days later, one half of the animals of each group were killed, and major organs were weighed. The rest of the animals were kept alive for additional 60 days and given Oriental MF solid feed and water upon demand. Groups of white rats weighing about 90 g, each consisting of 6, were given hydrogen peroxide phosphate containing feed for 90 days.

2. MODE OF ADMINISTRATION

a) Injection of Hydrogen Peroxide Directly into the Stomach through a Tube

Commercial, 30% special-grade hydrogen peroxide solution was diluted with distilled water, to hydrogen peroxide concentrations of 0.6 - 6 mg/ml. The solutions were introduced into the stomach through a tube at 0.6, 1, 2, 3, and 6 mg per 100 g of body weight at a specific time every morning after the animals were weighed. The dose of 1 mg/day/100 g b.w. is equivalent to an intake of 200 g of kamaboko (fish cake) containing 2,500 ppm of hydrogen peroxide by a human individual weighing 50 kg. The control received water instead of the hydrogen peroxide solution by the oral route for 100 days.

b) Oral Dministration of Hydrogen Peroxide in the Form of a Hydrogen Peroxide Phosphate Containing Feed

Experimental feed was prepared by adding 1% hydrogen peroxide phosphate additive, which had been diluted with filter paper powder (Takeda Pharmaceutical Co.) to Oriental MF powder feed. The control received a feed containing 1%

filter paper powder, instead of hydrogen peroxide, twice daily for 30 days, and weighed. Thirty days later, the feed intake was no longer calculated but the animals were weighed at 5-day intervals for 90 days.

3. WEIGHING OF MAJOR ORGANS

The thoracic region of one half of each group which received hydrogen peroxide directly through a tube was opened under ether anesthesia on the 40th day and the rest were operated on the 100th day. The blood was collected from the heart with an injection syringe as completely as possible, thereby killing the animals. During autopsy, the liver, the kidneys, and the spleen were removed, washed with physiological saline solution, dried with filter paper, and weighed. The procedure was repeated on all the animals which received the experimental feed, on the 90th day.

4. DETERMINATION OF FEED INTAKE

Fifty to sixty g portions of feed were placed in glass vessels and given to the animals individually. The amount left uneaten and the amount spilled outisde the vessel were weighed, and subtracted from the initial quantity.

5. OTHER PROCEDURES

Heparin-treated blood was centrifuged at 3,500 rpm for 15 minutes, and, after the hematocrit value was determined, the plasma was separated and its catalase activity was determined by Dille's method (2). The plasma protein was determined with Hitachi protein meter.

EXPERIMENTAL RESULTS

1. DIRECT INJECTION OF HYDROGEN PEROXIDE INTO THE STOMACH WITH A TUBE

1) EFFECT ON THE GROWTH OF RATS

Figure 1 plots growth curves showing thechanges in body weight by average when hydrogen peroxide was given at 0.6, 1, 2, 3, and 6 mg/day/100 g body weight. The 6 mg group showed a drop in growth rate from the 20th day, the difference from the control being statistically significant at 5% level. However, other groups showed no appreciable difference from the control.

2) EFFECT ON ORGAN WEIGHT

One half of each group were killed on the 40th day and the rest on the 100th day, and their organs were weighed. The results are shown in Tables 1 and 2. At 6 mg/100 g of body weight, the spleen showed an increase in weight 40 days later at 5% significance level. However, no difference from the control was noted 100 days later. No appreciable change was shown by the liver or kidneys of any of the groups.

3) HEMATOCRIT VALUE, PLASMA PROTEIN CONCENTRATION, AND PLASMA CATALASE ACTIVITY

Table 3 shows the average body weights, hematocrit values, plasma protein

Table 1. Effects of Oral Administration of H_2O_2 Solution on the Body and Organ Weight of Rats (after 40 days)

Group of test animals	Initial body weight	Final body weight	Body weight gain	Liver weight	Liver wt. / Body wt.	Kidney weight	Kidney wt. / Body wt.	Spleen weight	Spleen wt. / Body wt.
	g	g	g	g	%	g	%	g	%
1	69.2	[illegible]	162.5	16.22	4.43 ± 0.10	1.70	0.73 ± 0.06	0.53	0.23 ± 0.04
2	70.8	[illegible]	157.2	16.85	4.75 ± 0.22	1.73	0.76 ± 0.05	0.57	0.25 ± 0.07
3	70.8	[illegible]	151.2	16.18	4.70 ± 0.04	1.75	0.79 ± 0.06	0.52	0.21 ± 0.04
4	70.6	[illegible]	153.2	[illegible]	4.80 ± 0.11	1.78	0.75 ± [illegible]	0.60	0.25 ± 0.03
5	65.5	[illegible]	165.5	[illegible]	4.55 ± 0.28	1.72	0.75 ± 0.07	0.70	0.30 ± 0.05
6	68.8	198.2	129.4	[illegible]	4.06 ± 0.38	[illegible]	0.79 ± 0.10	0.71	0.38 ± 0.05

The values shown in the table indicate the average of 4-6 rats ± the standard deviation

Table 2. Effects of Oral Administration of H_2O_2 Solution on the Body and Organ Weight of Rats (after 100 days)

Group of test animals	Initial body weight	Final body weight	Body weight gain	Liver weight	Liver wt. / Body wt.	Kidney weight	Kidney wt. / Body wt.	Spleen weight	Spleen wt. / Body wt.
	g	g	g	g	%	g	%	g	%
1	69.2	323.0	263.8	12.14	3.64 ± 0.11	2.20	0.66 ± 0.09	0.60	0.18 ± 0.06
2	70.8	321.2	253.4	12.73	3.91 ± 0.14	2.35	0.72 ± 0.04	0.65	0.20 ± 0.02
3	70.8	326.6	255.8	12.42	3.82 ± 0.29	2.20	0.67 ± 0.04	0.62	0.19 ± 0.03
4	70.0	342.2	272.2	11.89	3.44 ± 1.96	2.28	0.67 ± 0.15	0.64	0.19 ± 0.03
5	65.5	333.6	268.1	10.60	3.19 ± 1.13	2.18	0.70 ± 0.07	0.58	0.18 ± 0.01
6	68.8	307.3	238.5	11.69	3.58 ± 0.50	1.97	0.64 ± 0.05	0.50	0.17 ± 0.03

The values shown in the table indicate the average of 5-6 rats ± the standard deviation

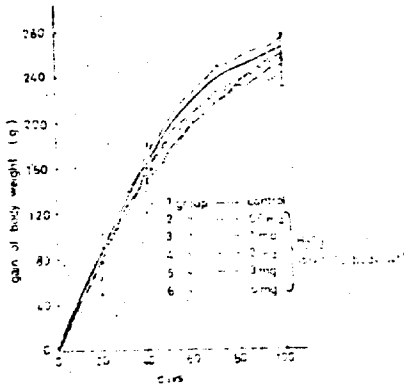

Fig. 1. Effect of H_2O_2 on the growth of rats. Oral administration of H_2O_2 solution

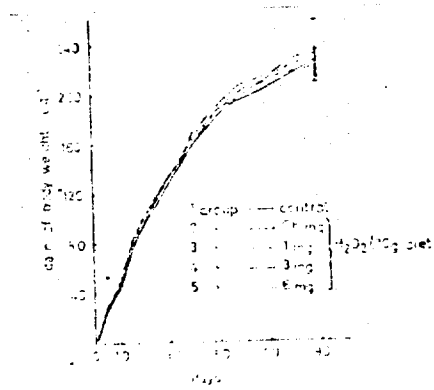

Fig. 2. Effect of H_2O_2 on the growth of rats (feeding on diet containing H_2O_2)

concentrations, and plasma catalase activities of the animals killed on the 100th day. The hematocrit value, plasma protein concentration, and plasma catalase activity of the 6 mg/day/100 g group were lower than the control, at a significance level of 5%. No significant difference was shown by any other group.

II. ADMINISTRATION OF HYDROGEN PEROXIDE IN THE FORM OF HYDROGEN PEROXIDE PHOSPHATE CONTAINING FEED

1) EFFECT ON THE GROWTH

Figure 2 shows growth curves of the groups given the feeds containing 0.6, 1, 3, and 6 mg/20 g of hydrogen peroxide. There was no difference between the groups.

2) EFFECT ON ORGAN WEIGHT

All the animals were killed on the 90th day, and their organs were weighed. Tables4 shows that no significant difference in the weight of organs, the liver, kidneys, and spleen, was noted between the groups.

3) FEED EFFICIENCY AND INTAKE

Table 5 shows the feed intakes and feed efficiencies of the different groups during the period of 30 days from the onset of experiment. The feed intake was an average of 20 g in all groups, and the feed efficiency was also of no significant difference among the groups.

4) HEMATOCRIT VALU, PLASMA PROTEIN CONCENTRATION, AND PLASMA CATALASE ACTIVITY

Table 6 shows the average body weights, hematocrit values, plasma protein concentrations, and plasma catalase activities of the animals killed at the end of the experiment. No difference is noted between the groups.

Table 3. Effects of Oral Administration of H_2O_2 Solution on the Blood Hematocrit Value, the Plasma Protein Value and the Plasma Catalase Activity

Group of test animals	Final body weight	Hematocrit value	Protein value	Catalase activity
	g	%	%	unit/ml
1	323.0	47.88 ± 1.93	6.65 ± 0.22	6.78 ± 0.68
2	321.2	47.15 ± 2.42	6.67 ± 0.22	7.36 ± 1.40
3	326.6	49.28 ± 2.18	6.86 ± 0.37	7.09 ± 1.72
4	312.2	49.71 ± 2.04	6.56 ± 0.21	7.60 ± 1.12
5	313.6	50.20 ± 2.80	6.80 ± 0.33	5.34 ± 1.16
6	307.3	41.42 ± 2.68	6.25 ± 0.29	5.87 ± 0.69

The values shown in the table indicate the average of 3-6 rats ± the standard deviation

Table 4.- Effects of H_2O_2 Added to Diet on the Organ Weight of Rats

Group of test animals	Initial body weight	Final body weight	Body weight gain	Liver weight	Liver wt. / Body wt.	Kidney weight	Kidney wt. / Body wt.	Spleen weight	Spleen wt. / Body wt.
	g	g	g	g	%	g	%	g	%
1	91.0	313.8	222.8	12.94	4.11 ± 0.35	2.38	0.76 ± 0.04	0.64	0.20 ± 0.02
2	93.2	315.5	222.3	12.83	4.07 ± 0.13	2.60	0.83 ± 0.08	0.72	0.23 ± 0.02
3	91.4	312.2	220.8	11.38	3.64 ± 0.21	2.32	0.75 ± 0.05	0.56	0.18 ± 0.02
4	90.3	320.0	229.7	11.57	3.56 ± 0.22	2.42	0.75 ± 0.11	0.58	0.18 ± 0.03
5	91.0	324.2	233.2	10.54	3.25 ± 0.35	2.34	0.73 ± 0.06	0.68	0.21 ± 0.02

The values shown in the table indicate the average of 5-6 rats ± the standard deviation

Table 5. Efficiency of Diet Containing H_2O_2 (for 30 days)

Group of test animals	Initial body weight	Final body weight	Body weight gain	Total diet intake	Diet efficiency
	g	g	g	g	%
1	91.0	226.2	135.3	592.9	22.8
2	93.2	225.3	132.1	606.9	21.8
3	91.4	220.7	129.3	594.7	21.6
4	90.3	224.7	134.4	590.3	22.8
5	91.0	235.2	144.2	638.3	22.6

The values shown in the table indicate the average of 5-6 rats

Table 6. Effects of H_2O_2 Added to Diet on the Blood Hematocrit Value, the Plasma Protein Value and Plasma Catalase Activity

Group of test animals	Final body weight	Hematocrit value	Protein value	Catalase activity
	g	%	%	unit/ml
1	313.8	49.62 ± 3.80	6.56 ± 0.53	5.43 ± 1.47
2	315.5	48.07 ± 2.43	6.72 ± 0.24	6.78 ± 1.21
3	312.2	50.42 ± 2.05	6.70 ± 0.46	7.50 ± 0.97
4	320.0	49.48 ± 1.70	6.70 ± 0.10	7.13 ± 1.83
5	324.2	49.72 ± 1.68	6.72 ± 0.17	5.62 ± 0.95

The values shown in the table indicate the average of 5-6 rats ± the standard deviation

DISCUSSION

Hydrogen peroxide is widely used as a bleaching agent and preservative for food preparation. Since it is retained in the food (1), it has been a subject of concern whether the hydrogen peroxide ingested as an ingredient

of food could exert a harmful effect onliving organisms. In the previous study, the effect of hydrogen peroxide on the growth of rats was examined in an attempt to determine its toxicity, but the experiment for a short period revealed that it exerted no harmful effect on the growth or organ weight at a dose equivalent to the amount of hydrogen peroxide retained in the food, but it retarded the growth of the animals at a dosage of 6 mg/day. In this study, hydrogen peoxide was introduced both by direct injection into the stomach by the use of a gastric tube and by an indirect route by way of food to whihh it was added, at varying doses and the period of observation was extended to 100 days.

When hydrogen peroxide was directly introduced into the stomach, the growth was retarded at 6 mg/day/100 g of body weight, just as in the previous study. The groups given at less than 3 mg/day/100 g body weight showed no difference at more than 5% significance level from the control. The 6 mg/100 g b.w. group also indicated the effect of hydrogen peroxide on their organs, the hematocrit value, and theplasma catalase activity. It seemed that hydrogen peroxide at a high concentration exerts harmful effects on living organism. These results, however, do not necessarily indicate the effect of the hydrogen peroxide remaining in food. In terms of the amount consumed by a human individual weighing about 50 kg, the dosage of 6 mg/day is equivalent to 3 g/day. The concentration of residual hydrogen peroxide in a commercial kneaded foot product and noodles is about 500 ppm, and even in "naruto" (fish cake) which contains the largest amount of hydrogen peroxide among various food products only contains 3,000 ppm. Supposing that a human individual weighing 50 kg consumes 200 g of food which contains hydrogen peroxide at such a high concentration, the amount of hydrogen peroxide taken is equivalent to 600 mg/day. Thus, the above dosage, 6 mg/day/100 g body weight, is equivalent to the daily consumption of 200 g of food which contains 15,000 ppm. Such situation is unthinkable, and if the effect of hydrogen peroxide at this high concentration is ever be pursued, it would be from a different angle, not in terms of the effect of the hydrogen peroxide contained in food.

In the experiment using hydrogen containing feed, no harmful effect was not d even at any of the concentrations from 0.6 - 6 mg/day. Unlike the forced injection through a tube, the toxicity was less manifest, probably due to its gradual ingestion in a small amount at a time along with other food constituents.

As the indicators of the toxicity of hydrogen peroxide, changes in the body weight, organ weight, hematocrit value, and plasma catalase activity were also exa ned. It may be necessary to examine the catalase activities in other tissues, but , at the present stage, the need is not strong.

As stated above, the hydrogen peoxide retained in general food products hardly exhibits toxicity, and, within the range of the content in commercial food products, its toxicity does not seem to be of serious concern. However, if the concentration is in excess of 1,000 ppm, the palatability of the food is said to deteriorate. This not only lowers the commercial value of the food, but also produces a physiological discomfort. Therefore, the content of hydrogen peroxide should be limited to a certain level, while its preservative power be taken into consideration. The permissible limit of hydrogen peroxide content should be determined based on the residual concentration in the food and food preservation.

CONCLUSION

1. Hydrogen peroxide was directly injected into the stomach at 0.6 - 6 mg/day/100 g body weight and changes in body weight were followed for 100 days. The 6 mg/day/100 g of body weight group indicated a slight increase in body weight from the 20th day, the difference from the control being significant at 5% level, but other groups showed no appreciable difference. The organ weights on the 40th day showed no notable change, except for a slight increase shown by the spleen of the 6 mg/day/100 g bo.w. This group, however, showed no appreciable difference on the 100th day. Changes inhematocrit value and plasma protein concentration were shown by the 6 mg group. The 6 and 3 mg groups indicated changes in catalase activity.

2. When hydrogen peroxide was added to the feed, the growth curve of even the 6 mg group showed no difference from the control. There was no significant difference in feed intake or efficiency, major organ weights, hematocrit value, plasma protein concentration, or plasma catalase activity.

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報 文

シロネズミの成長におよぼす過酸化水素投与の影響

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Effect of Hydrogen Peroxide on the Growth of Rats

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The toxic effects of hydrogen peroxide on the growth rate of rats were observed for 100 days, and at the end of experiments, the organ weights, blood hematocrit values, plasma protein values and plasma catalase activities of rats administered hydrogen peroxide were compared with those of control animals. Two kinds of administration method, oral administration by catheter and feeding with diet containing hydrogen peroxide, were employed in this experiment.

In the case of oral administration by catheter, it was shown that no changes were observed at the doses of less than 3 mg/day/100 g body wt., although considerable changes were observed in the hematocrit values, protein values and catalase activities at the dose of 6 mg/day/100 g body wt.. However, in the case of feeding on diet containing hydrogen peroxide, no significant effects were observed at the all doses used in this work, less than 6 mg/day.

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緒 言

前報[1]において，シロネズミの成長におよぼす過酸化水素投与の影響を3週間観察した結果について報告した．しかし，この結果は胃ゾンデによる直接胃腔内投与によるものであり，したがって過酸化水素そのものの影響を示し，食物に添加された過酸化水素の影響を反映しているとはいえない．また，各群への過酸化水素の投与量も人体換算，その他の理由にもとづいたので，今回はこれらの点を考慮した実験を行なった．すなわち，過酸化水素投与法を二通りとし，投与量としては，従来の胃ゾンデによるものの以外に，過酸化水素リン酸緩衝液を添加した飼料を用いた．このような方法を用いて，3か月にわたるシロネズミの成長実験および実験終了後に得たものについてのカタラーゼ活性，ヘマトクリット値その他について検討を行ない，2, 3の知見を得たので報告する．

実験方法

1. 実験動物および飼育法

経口投与法による過酸化水素の投与実験に関しては，体重70 g前後のWister系雄シロネズミを1群9~12匹として，22±1°の恒温飼育室で1匹ずつ金網製の飼育かごに入れて飼育した．40日後，各群とも半数の動物を殺し，主要臓器重量を測定し，残りの動物はさらに60日間飼育した．飼料として，オリエンタルMF固形飼料を用い，飼料および水は任意に摂取させた．また，過酸化水素リン酸緩衝液添加飼料を用いた実験には，体重

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90g前後のシロネズミを1群6匹として，90日間飼育した．

2. 試料投与法

a) 胃ゾンデによる直接胃腔内投与

試料液は市販特級30%過酸化水素水を蒸留水で希釈し，過酸化水素0.6～6mg/mlの過酸化水素水を調製して，体重100gあたり過酸化水素として0.6, 1, 2, 3および6mgを毎朝定時に体重測定後，胃ゾンデを用いて胃腔内に投与した．なお，1mg day/100g body wt.の過酸化水素投与量は体重50kgのヒトが，過酸化水素2,500ppm含有かまぼこ200gを毎日摂取することに相当する．また，対照として過酸化水素水の代りに水を同様に経口投与して，100日間実験した．

b) 過酸化水素リン酸塩付加物添加飼料投与

サリエンタルMF粉末飼料に，過酸化水素リン酸塩付加物(武田薬品工業株式会社製)をロ紙粉で希釈して1%添加し，飼料20gあたり過酸化水素として0.6, 1, 3および6mg含有するように調製して試験飼料とした．また，対照として，過酸化水素を含まないロ紙粉を同様に1%添加したものを飼料とし，30日間2日毎に飼料摂取量および体重を測定した．なお，30日以後，飼料摂取量は測定せず，5日毎に体重のみ測定し，90日間実験した．

3. 主要臓器重量の測定

胃ゾンデによる直接胃腔内投与群については試料投与開始後40日にて各群とも半数の動物を，また100日にて残りの全動物をエーテル麻酔下腹部を開き，心臓より注射器にてできるだけ血液を採取して死に至らしめ，剖検下肝臓，腎臓および脾臓を摘出し，生理食塩水で洗浄後，ロ紙で水分を除き秤量した．過酸化水素リン酸塩付加物添加飼料投与群については，90日にて全動物を同様に処理し，臓器を秤量した．

4. 飼料摂取量の測定

50～60gの試料を秤量して，ガラス製の飼料入れに入れて個別に与え，2日毎に食べ残した量およびこぼした量を秤量し，はじめに与えた量より差し引いて飼料摂取量とした．

5. その他

ヘパリン処理した血液を3,500rpm, 15分遠沈し，ヘマトクリット値を測定したのち，血しょうをとり，Dille[2]の方法によりカタラーゼ活性を測定した．なお，血しょうタンパク量の測定は日立タンパク計を使用した．

実験結果

I. 胃ゾンデによる直接胃腔内投与

1) シロネズミの成長におよぼす影響

過酸化水素を0.6, 1, 2, 3および6mg day/100g body wt.投与したときの体重変化を平均体重増加曲線であらわしたところ，Fig. 1に示すような結果が得られた．すなわち，6mg投与群は経口投与開始後20日頃より体重増加量が減少し，対照と比較して5%有意水準で差が認められた．しかし，3mg以下の投与群においては差は認められなかつた．

2) 主要臓器重量におよぼす影響

投与開始後40日にて各群とも半数の動物を，また100日後に残りの全動物を殺し，その臓器重量を測定したところ，Table 1, 2に示すように，6mg day/100g body wt.投与した場合，40日後における脾臓重量がやや大き

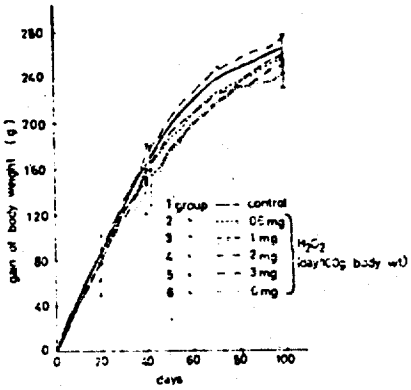

Fig. 1. Effect of H_2O_2 on the growth of rats (Oral administration of H_2O_2 solution)

Table 1. Effects of Oral Administration of H_2O_2 Solution on the Body and Organ Weight of Rats (after 40 days)

Group of test animals	Initial body weight	Final body weight	Body weight gain	Liver weight	Liver wt. / Body wt.	Kidney weight	Kidney wt. / Body wt.	Spleen weight	Spleen wt. / Body wt.
	g	g	g	g	%	g	%	g	%
1	69.2	231.7	162.5	10.32	4.43±0.40	1.70	0.73±0.06	0.53	0.23±0.04
2	70.8	228.0	157.2	10.85	4.75±0.22	1.73	0.76±0.05	0.57	0.25±0.07
3	70.8	222.0	151.2	10.48	4.70±0.04	1.75	0.79±0.06	0.52	0.24±0.04
4	70.6	223.8	153.2	11.38	4.80±0.41	1.78	0.75±0.04	0.60	0.26±0.03
5	65.5	231.0	165.5	10.50	4.55±0.28	1.72	0.75±0.07	0.70	0.30±0.05
6	68.8	198.2	129.4	9.10	4.66±0.38	1.54	0.79±0.10	0.74	0.38±0.05

The values shown in the table indicate the average of 4-6 rats ± the standard deviation

Table 2. Effects of Oral Administration of H_2O_2 Solution on the Body and Organ Weight of Rats (after 100 days)

Group of test animals	Initial body weight	Final body weight	Body weight gain	Liver weight	Liver wt. / Body wt.	Kidney weight	Kidney wt. / Body wt.	Spleen weight	Spleen wt. / Body wt.
	g	g	g	g	%	g	%	g	%
1	69.2	333.0	263.8	12.14	3.64±0.11	2.20	0.66±0.09	0.60	0.18±0.06
2	70.8	324.2	243.4	12.73	3.94±0.14	2.35	0.72±0.04	0.65	0.20±0.02
3	70.8	326.6	255.8	12.42	3.82±0.29	2.20	0.67±0.04	0.62	0.19±0.03
4	70.0	342.2	272.2	11.80	3.44±1.96	2.28	0.67±0.15	0.64	0.19±0.03
5	65.5	313.6	248.1	10.60	3.39±1.13	2.18	0.70±0.07	0.58	0.18±0.04
6	68.8	307.3	238.5	11.00	3.58±0.50	1.97	0.64±0.05	0.50	0.17±0.03

The values shown in the table indicate the average of 5-6 rats ± the standard deviation

Table 3. Effects of Oral Administration of H_2O_2 Solution on the Blood-Hematocrit Value, the Plasma-Protein Value and the Plasma-Catalase Activity

Group of test animals	Final body weight	Hematocrit value	Protein value	Catalase activity
	g	%	%	unit/ml
1	333.0	47.88±1.93	6.66±0.22	6.78±0.68
2	324.2	47.17±2.42	6.65±0.22	7.36±1.40
3	326.6	49.28±2.18	6.86±0.37	7.00±1.72
4	342.2	49.74±2.04	6.56±0.31	7.60±1.12
5	313.6	50.20±2.80	6.80±0.33	5.34±1.10
6	307.3	44.43±2.68	6.25±0.29	5.87±0.69

The values shown in the table indicate the average of 3-6 rats ± the standard deviation

く，5% 有意水準で差が認められた．しかし，100 日後においては差は認められなかった．肝臓，腎臓においては，いずれの群も差は認められなかった．

3) ヘマトクリット値，血しょうタンパク濃度および血しょうカタラーゼ活性

Table 3 は投与開始後 100 日にてと殺した動物の平均体重，ヘマトクリット値，血しょうタンパク濃度および血しょうカタラーゼ活性である．6 mg day/100 g body wt. 投与した場合，ヘマトクリット値，血しょうタンパク濃度，血しょうカタラーゼ活性ともに差が低く，5% 有意水準で差が認められた．また，3 mg day/100 g body wt. 投与群においてはカタラーゼ活性が差が低く，5% 有意水準で差が認められた．しかし，他の群においてはいずれの場合も差は認められなかった．

II. 過酸化水素リン酸塩付加物添加投与

1) シロネスミの成長におよぼす影響

過酸化水素として 0.6, 1, 3 および 6 mg/20 g diet 投与したときの体重変化を平均体重増加曲線であらわしたところ，Fig. 2 に示すように各群とも差は認められなかった．

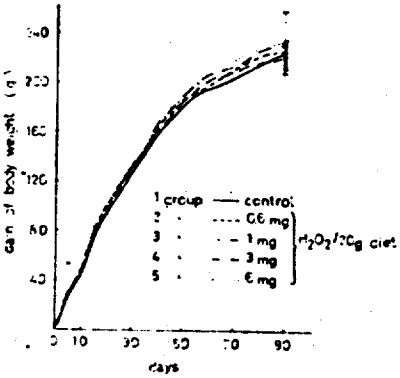

Fig. 2. Effect of H_2O_2 on the growth of rats (Feeding on diet containing H_2O_2)

2) 主要臓器重量におよぼす影響

投与開始後90日にて全動物をと殺し，その臓器重量を測定したところ，Table 4 に示すように肝臓，腎臓および脾臓のいずれにおいても群間に差は認められなかった．

3) 飼料摂取量および飼料効率

Table 5 は投与開始後30日間における飼料摂取量および飼料効率を示したものである．飼料摂取量は各群とも平均 20 g であり，飼料効率においても群間に差は認められなかった．

4) ヘマトクリット値，血しょうタンパク濃度および血しょうカタラーゼ活性

Table 6 は実験終了後と殺した動物の平均体重，ヘマトクリット値，血しょうタンパク濃度および血しょう中のカタラーゼ活性を示したものである．いずれにおいても群間に差は認められなかった．

考　察

食品の漂白あるいは防腐の目的で過酸化水素がひろく用いられているが，食品中で過酸化水素が残存するりこ

Table 4. Effects of H_2O_2 Added to Diet on the Organ Weight of Rats

Group of test animals	Initial body weight	Final body weight	Body weight gain	Liver weight	Liver wt. / Body wt.	Kidney weight	Kidney wt. / Body wt.	Spleen weight	Spleen wt. / Body wt.
	g	g	g	g	%	g	%	g	%
1	91.0	313.8	222.8	12.94	4.11 ± 0.35	2.38	0.76 ± 0.04	0.64	0.20 ± 0.02
2	93.2	315.5	222.3	12.83	4.07 ± 0.13	2.60	0.83 ± 0.08	0.72	0.23 ± 0.02
3	91.4	312.2	220.8	11.38	3.64 ± 0.21	2.32	0.75 ± 0.05	0.56	0.18 ± 0.02
4	90.3	320.0	229.7	11.37	3.56 ± 0.22	2.42	0.75 ± 0.11	0.58	0.18 ± 0.03
5	91.0	324.2	233.2	10.54	3.25 ± 0.35	2.34	0.73 ± 0.06	0.68	0.21 ± 0.02

The values shown in the table indicate the average of 5-6 rats ± the standard deviation

Table 5. Efficiency of Diet Containing H_2O_2 (for 30 days)

Group of test animals	Initial body weight	Final body weight	Body weight gain	Total diet intake	Diet efficiency
	g	g	g	g	%
1	91.0	226.2	135.3	592.9	22.8
2	93.2	225.3	132.1	606.9	21.8
3	91.4	220.7	129.3	594.7	21.6
4	90.3	224.7	134.4	590.3	22.8
5	91.0	235.2	144.2	653.3	22.6

The values shown in the table indicate the average of 5-6 rats

Table 6. Effects of H_2O_2 Added to Diet on the Blood-Hematocrit Value, the Plasma-Protein Value and Plasma-Catalase Activity

Group of test animals	Final body weight	Hematocrit value	Protein value	Catalase activity
	g	%	%	unit/ml
1	313.8	49.62 ± 3.80	6.56 ± 0.53	5.43 ± 1.47
2	315.5	48.67 ± 2.43	6.72 ± 0.24	6.78 ± 1.21
3	312.2	50.42 ± 2.05	6.70 ± 0.46	7.30 ± 0.97
4	320.0	49.48 ± 1.70	6.70 ± 0.10	7.13 ± 1.83
5	324.2	49.72 ± 1.68	6.72 ± 0.17	5.62 ± 0.95

The values shown in the table indicate the average of 5-6 rats ± the standard deviation

ことから，これらの食品を摂取した方の生体に与える影響が問題となっている．本報において，過酸化水素の毒性をしらべる方法の1つとして，シロネズミの成長にどのような影響を与えるかを検討したが，短期間の飼育実験から，食品残留程度の過酸化水素投与ではネズミの発育ないしは臓器重量に全く影響はなかったが，6 mg/day の投与で発育遅延がみられることがわかった．本実験において，投与量の幅をさらに細かくとり，投与法としては，胃ゾンデによるもの以外に，過酸化水素リン酸塩付加物を添加した飼料を用いて，100 日間にわたる観察を行なった．

過酸化水素を直接胃腔内に投与した場合，6 mg/day/100 g body wt. 投与群において，前回同様発育遅延がみられたが，3 mg/day/100 g body wt. 以下の投与群では，対照と比較して5%有意水準では認められなかった．また，内臓外見，ヘマトクリット値，血しょうカタラーゼ活性などにおいても，6 mg/day/100 g body wt. 投与群において影響をおよぼし，高濃度の過酸化水素投与は生体に影響を与えるものと思われる．しかし，これらの投与方法は過酸化水素を直接胃腔内に投与したものであり，食品中に残留する過酸化水素の影響を示しているとはいいがたい．また，6 mg/day/100 g body wt. の過酸化水素投与量を体重50 kg のヒトに換算すると，3 g/day の投与量となる．一方，市販品の製品，ふん製品中の過酸化水素残存量は 500 ppm 程度であり，たかだか最も残存量の多かった"なると"でさえ，3,000 ppm 程度であるが，したがって，このような高濃度残存する食品を，体重 50 kg のヒトが毎日 200 g 食するとして，600 mg/day の過酸化水素を摂取することになり，上記の 6 mg/day/100 g body wt. の投与量は 15,000 ppm 含有食品を毎日 200 g 摂取することに相当する．したがって，このような大量の過酸化水素が含有されることは実際ではあるえないが，もしこの影響をさらに詳細に検討するとしても，食品残留過酸化水素の影響とは別の観点から追求されるべきものと思われる．

一方，過酸化水素添加飼料を用いた実験において，0.6～6 mg/day のいずれの過酸化水素投与量でも全く影響はあらわれず，胃ゾンデによる強制投与における場合と異なり，毒性はあらわれ難いことが明らかとなった．これは食品成分そのものに過酸化水素が少量ずつ徐々に摂取されたために影響を受けにくかったものと思われる．

以上の過酸化水素強制投与実験および飼料添加実験で，毒性指標の指標として，体重増加，臓器重量以外にヘマトクリット値，血しょうカタラーゼ活性などを参考にした．この血しょうカタラーゼ活性については，血しょう以外の組織などにおける活性も検討する必要があると思わ

れるが，現時点ではそれほど重要視する必要はないと考えられる．

このように，一般食品中に残留する程度の過酸化水素については，ほとんど毒性は認められず，海藻食品の残留量程度の範囲においては，一応必要以上に毒性を考慮する必要はないと思われる．しかし，残留濃度が 1,000 ppm 以上になると味覚に変調をきたすなど，食品としての価値はもちろん，生理的な不快感を与えるので，食品中の過酸化水素残存量は当然規定しなくてはならないが，一方では過酸化水素添加による効果の点を無視することはできず，今後食品中の残存過酸化水素濃度と食品保存との関連を検討したうえで，残存量の規定を考慮した方がよいように思われる．

結　　論

1) 胃ゾンデにより，過酸化水素 0.6～6 mg/day/100g body wt. を直接胃管内投与し，100 日間その体重変化量を測定した結果，6 mg/day/100 g body wt. 投与群では実験開始後20日頃より体重増加量が減少し，対照と比較して5% 有意水準で差が認められたが，3mg/day/100g body wt. 以下の投与群では差は認められなかった．また，投与開始後 40 日における臓器重量は 6 mg/day/100 g body wt. 投与群において，肝臓がやや大きく差が認められたが，肝臓および腎臓では変化はなかった．また，100 日後においてはいずれの場合も差は認められなかった．ヘマトクリット値，血しょうタンパク濃度は 6 mg/day/100 g body wt. 投与群で差が認められ，カタラーゼ活性は 6 mg/day/100 g body wt., 3 mg/day/100 g body wt. 投与群において差が認められた．他の群においてはいずれの場合も差は認められなかった．

2) 飼料に過酸化水素を添加して摂取させたばあい，6 mg/day 投与群においても対照と変わらぬ成長を示し，飼料摂取量，飼料効率，主要臓器重量，ヘマトクリット値，血しょうタンパク濃度および血しょうカタラーゼ活性とも群間に差は認められなかった．

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The Toxicity and Health Hazards of Rocket Propellants

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Rockets and other guided missiles require fuels which deliver extremely large amounts of energy to small combustion chambers in short periods of time. This requires combustible materials of high energy and reactivity coupled with powerful oxidizers. In many instances these fuels and oxidizers and their decomposition products are poisonous, and conditions attending their use may present hazards to personnel. Thus it becomes important for persons concerned with these materials to be educated in the nature of the hazards, how to prevent injury, and how to administer first aid if exposure occurs. The following report was prepared by a member of the research staff of the Army Chemical Corps Medical Laboratories, which conduct toxicological investigations in this field for the Armed Forces. It deals with the following propellants: (a) Fuels: ammonia, aniline, alcohols (ethyl, furfuryl, methyl), hydrazine, and JP-4. (b) Oxidizers: hydrogen peroxide, liquid oxygen, and red fuming nitric acid.

Introduction

TOXICITY may be defined as the inherent ability of a chemical to produce injury once it gains access to the body. Hazard may be defined as the likelihood of toxic injury prevailing during the handling and use of the chemical. From these definitions it may be seen that a chemical is not necessarily hazardous despite great toxicity because under the usual circumstances of handling the chemical the likelihood of toxic exposure may not arise. Also, a chemical of low or moderate toxicity may be extremely hazardous if the circumstances of its handling provides adequate opportunity for toxic injury.

Fundamentally, the best means of dealing with a poison is to avoid it. Often this is not possible, of course, and other means must be sought. Nevertheless, avoidance is, in fact, the basis for what is known as a program of "preventive medicine" applied by officials responsible for the safety of personnel who handle poisonous materials in some way. Avoidance or prevention of poisoning may take many forms or be achieved in one or more ways: use of protective clothing and equipment; adequate ventilation of facilities; safety-minded design of all equipment associated with the use, handling, and storage of toxics with the view of reducing the likelihood of toxic exposure. Hence, a concerted effort to reduce or eliminate hazard by supervisors, safety officers, engineers, operators, and others is required to devise and maintain safe handling procedures and other protective measures. An essential part of such a program is the education of all potential handlers in the hazard and its control, in self aid and first aid. Truly, the "ounce of prevention" being "worth a pound of cure" is an understatement. The link in a chain of safety precautions which fails is of no comfort to the victim of poisoning; hence all links must be sound. The nature and extent of preventive procedures are determined by the likelihood of exposure under the conditions of handling and use, by the potency of the poison, by the nature of its poisonous effect, and by the manner in which it gains access to the body (that is, skin contact, inhalation, etc.).

In the event of failure of the precautions at any point, removal of the poison from personnel or from equipment is the next step; actually, removal of personnel from contaminated spaces may be indicated. This implies advance provision for decontamination procedures and equipment effective for the particular poison, such as eyewashes and neutralizers. However, almost without exception, adequate water supplies for showers, eye baths, and sprinkler sprays at storage and using sites must be provided. In some instances provisions for whole body immersion in water may be highly desirable as well. Filters or canisters in respiratory protective devices should be changed often, preferably after each operation, to prevent accumulation of hypergolic mixtures. In general, consumption of alcoholic beverages by these exposed to toxic chemicals should be discouraged, and steady users of alcohol should be given other employment. Smoking should be discouraged while handling toxic materials, since this offers an insidious means for ingestion or inhalation of the toxic material from a cigarette or pipe contaminated with the hands.

Modern rocketry and guided missiles in general require fueling systems capable of delivering large amounts of energy to a small location, in variable but short periods of time. This means combustibles of high energy and reactivity coupled with powerful oxidizers. In many instances the fuels and oxidizers, and their decomposition products, may be toxic and the conditions attending their use may be hazardous. Thus, it behooves all concerned to become thoroughly acquainted with the nature and extent of the hazard, how to avoid it, and what to do in the event of accidental exposure of personnel. The following discussion is intended to present a sketch of the toxicity of several fuels and oxidizers in common use today, with suggestions on prevention of toxic exposures and on treatment of poisoning.

I Fuels

Ammonia (1, 2, 3)[2]

Ammonia used as a component of propellant mixtures is a concentrated solution of ammonia in water, producing a strong alkali. Its fumes are extremely irritating to the respiratory tract and to the eye. The inhalation hazard of am-

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[2] Numbers in parentheses refer to References on page 236.

monia is low by virtue of the irritant effects of ammonia fumes so that voluntary exposure to amounts which would produce injury is quite unlikely.

The principal hazard from ammonia is in the contact of liquid with the skin and eye. Such contact may result in serious alkali burns unless promptly and vigorously acted upon—prompt and vigorous flushing with large amounts of water should generally suffice for the prevention of any serious injury. Handlers of ammonia who run the risk of liquid splash should be equipped with protective eyeglasses, suitable rubber gloves, aprons, and boots. Provisions for drenching showers should be made at all handling sites. After treatment with water, exposed parts of the body may be given a supplementary treatment of sponging with diluted (5 per cent) acetic acid (or vinegar) solution. This may be particularly desirable when there has been any appreciable delay in the application of water to an exposed site inasmuch as ammonia, being a strong base, may produce deep penetrating burns by virtue of soluble alkali metaprotein formation.

Aniline (2, 4, 5, 6)

Aniline is an oily liquid, normally colorless, but which darkens on exposure to light and air; it is flammable and has a burning taste and characteristic mild aromatic odor. It is a weak base forming salts with mineral and other acids and is miscible with organic solvents but only sparingly so with water. Its vapor density is about 3, its boiling point about 185° C, and its vapor pressure about 15 mm of mercury at approximately 80° C. Vapors of aniline do not present a toxic hazard by inhalation at ordinary temperatures because of the low volatility of aniline. However, at higher temperatures hazard may develop so that it is necessary to have respiratory protection. The maximum allowable concentration (MAC) of aniline vapors is fixed at 5 parts per million of air for an 8-hour daily exposure. It is a skin irritant. Although irritation and ulceration of the urinary bladder have been reported in the chronically exposed, it is no longer believed that bladder tumors are caused by aniline (8).

The principal toxic hazard from aniline is by way of the skin as the portal of entry to the body. Symptoms of poisoning by aniline may be conveniently separated into two classes: (a) Its effect upon the central nervous system gives rise to headache, weakness, difficulty in breathing or dyspnea, convulsions, and psychic disturbances. (b) The action of aniline upon hemoglobin of the blood gives rise to methemoglobin formation resulting in characteristic cyanosis or blueness of the fingernail beds, lips, and mucous membranes. It is claimed by some that methemoglobin formation by aniline is enhanced by ingestion of alcohol. Chronic exposures to aniline may give rise to eruptions upon the skin. The diagnosis of aniline poisoning is usually based upon a history of exposure and upon the characteristic intense blueness or cyanosis which occurs. Recently, it has been discovered that urinary excretion of a substance which combines with the "sulfonamide reagents" may be detected in exposed personnel sometime prior to the appearance of cyanosis or methemoglobin in the blood (6a). Periodic examination of the urine of aniline workers may well turn out to be a very useful index of the extent of exposure. Methemoglobin levels in the blood of 15 per cent or higher of the total hemoglobin is readily detectable by cyanosis and it is frequently accompanied by albumin and aminophenol in the urine. Lethargy and dyspnea occur at levels of 30 to 40 per cent methemoglobin, and 60 to 70 per cent methemoglobinemia generally is accompanied by a semistuporous condition of the victim. A simple test of suspected methemoglobinemia is as follows: venous blood shaken in air in the test tube becomes a cherry red in the absence of methemoglobin; in contrast, it maintains its dark bluish-purple hue in the presence of appreciable amounts of methemoglobin. This is a presumptive test and should always be subsequently confirmed by spectroscopic and other examination of the blood.

For those handling aniline, goggles, gloves, aprons, and rubber boots should be provided. Adequate respirators should be provided whenever there is reason to believe that high vapor concentration may prevail at a given operation. Since aniline is quite flammable, fireproof clothing should be provided. Clothing saturated with aniline should be removed immediately and suitably laundered, including the use of a dilute acetic-acid solution. Skin contaminated with aniline should be promptly washed with copious amounts of water followed by sponging with 5 per cent solution of acetic acid (or vinegar). Contamination of the eye should likewise be treated by washing with large amounts of water. The need for removal of contaminated clothing and adequate laundering cannot be overemphasized inasmuch as in addition to being a skin irritant, aniline is absorbed through the skin, as indicated earlier, and improperly laundered clothing may serve as a source of chronic poisoning.

Medical measures for the treatment of severe aniline intoxication should include intravenously administered methylene blue to reduce the methemoglobinemia. Facilities for administration of 100 per cent oxygen inhalation should be provided; in severe poisioning this may be life-saving.

Alcohols

Ethyl alcohol (2, 7, 8). Ethyl alcohol (ethanol, grain alcohol, alcohol) is a colorless flammable volatile liquid having a sweet, ethereal odor and a burning taste in its undiluted form. It is freely miscible with water and many organic solvents. Absolute (100 per cent) alcohol has a boiling point of 78.4° C, a vapor pressure of 40 mm of mercury at 25° C, and a vapor density of 1.59. It forms explosive mixtures with air within the following limits: upper 19 per cent and lower 3.28 per cent. 95 per cent ethyl alcohol, the most widely used grade, is somewhat different in its physical constants than the 100 per cent concentration; however, the differences are not great.

Although ethyl alcohol is toxic by inhalation, 1000 parts per million of air are considered safe for an 8-hour daily exposure, although irritation of the eyes and of the upper respiratory tract are often seen at this concentration; this concentration is not readily reached in open spaces. The principal toxic hazard is encountered by ingestion. The ingestion of 300 to 400 cc of ethyl alcohol within one hour by a nonhabituated drinker may result fatally. Denaturing agents often are the principal causes of severe intoxication with what is otherwise labeled alcohol and imbibed as potable ethyl alcohol.

Personnel protective measures consists of providing adequate ventilation wherever ethyl alcohol is to be used in enclosed spaces, and the removal of grossly contaminated clothing. If intoxicating amounts of alcohol have been ingested or inhaled, simple removal of the subject from the atmosphere and prevention of further ingestion may suffice to insure recovery. In the event that the subject is stuporous or comatose, medical assistance should be sought. Medical treatment should be directed to the maintenance of adequate hydration of the subject by the administration of suitable parenteral fluids and the administration of central nervous system stimulants as required such as amphetamine, caffeine, and picrotoxin.

Furfuryl alcohol (9). Furfuryl alcohol is a yellowish liquid, turning brown or dark red on exposure to light and air. It explodes violently with mineral acids and with some strong organic acids. It is freely miscible with water in all proportions and with most organic solvents. Its vapor density is slightly greater than three and its vapor pressure is 2 mm of mercury at 30° C. The inherent toxicity of furfuryl alcohol is not very great and its inhalation hazard is quite small owing to its low vapor pressure.

Skin absorption is slight and there is very little effect on skin and eyes. However, it is hazardous by virtue of the fact

that it may be ingested. In large amounts it has the usual effects of the alcohols upon the central nervous system. No special precautions for handling and use need to be observed except that individuals must be cautioned against ingestion. Contamination of the skin and eye may be adequately treated by flushing with adequate amounts of water. Contaminated clothing should be removed and washed to remove a source of skin irritation.

Medical treatment for the severely poisoned revolves around supportive measures for central nervous system depression which is common to the treatment of all alcohol poisoning, and around combatting dehydration and acidosis.

Methyl alcohol (2, 7, 8). Methyl alcohol (wood alcohol, methanol) is a colorless, flammable liquid which is volatile and has an odor very similar to that of ethyl or grain alcohol. Like ethyl alcohol, it is freely miscible with water and many organic liquids. Like ethyl alcohol, it is a very widely used solvent for many substances in many processes. In the pure state, methyl alcohol has a boiling point of 64.5° C, a vapor pressure of 96 mm mercury at 20° C, and forms explosive mixtures at the following concentrations: upper 36.5 per cent and lower 6.7 per cent. Methyl alcohol vapors have a density of 1.11.

Methyl alcohol may be toxic by ingestion, by skin absorption, and by inhalation. It is perhaps responsible for the great majority of cases of severe poisoning by ethyl alcohol to which it has been added as a denaturant. Exposure to vapors may produce the same train of symptoms as those due to ethyl alcohol inhalation, namely, signs commonly considered to be those of inebriation. In addition, nausea, vomiting, headache, and irritation of the respiratory tract may be seen. The vapor concentration of 200 parts per million of air are generally believed to be safe for 8-hour daily exposures. Larger amounts produce dizziness, ataxia, severe abdominal pain, convulsions, blindness, brain edema, and death. Blindness has followed ingestion of as little as 10 cc, although susceptibility varies greatly. It is believed that doses of 100–250 cc or less may result in death if taken by ingestion. Smaller doses often repeated may accumulate and may result in irreversible injury over a period of time. For this reason, periodic surveys of the atmospheric contamination at plant sites should be carried out.

Personnel protective measures should include adequate skin protection against contact, as well as adequate ventilation of all enclosed spaces. All personnel coming in contact with it, or likely to come in contact with it, should be thoroughly informed concerning the hazards of methyl alcohol.

Cases of exposure should be immediately removed from the atmosphere containing the alcohol, or if the accident has been by skin contact, clothing should be removed and the skin washed with copious amounts of water. If by ingestion, the subject should be made to vomit; this may be life-saving if ingestion has been recent. In addition, gastric lavage should be carried out. As with ethyl alcohol, measures should be instituted to prevent severe dehydration and circulatory collapse; coma should be treated as in ethyl alcohol poisoning.

Hydrazine (10, 11, 12)

Hydrazine is a colorless liquid with characteristic though not intense ammoniacal odor. Its fumes and vapors react violently with acids and flame and with other oxidizing agents. Hydrazine mixes with water and alcohols in all proportions. Its vapor pressure is about 18 mm of mercury at 30° C, and its boiling point is about 113.5° C. Its low vapor pressure is deceptive in that its vapors present a definite explosion hazard. Hydrazine presents a toxic hazard by inhalation, by skin contact, and by ingestion. In concentrations of 2000 parts per million or less, hydrazine vapors are quite toxic. However, at such concentrations, the vapors are quite irritating and this fact serves to limit acute exposures to high concentrations. Lower concentrations may produce irritation of the respiratory tract, and irritation and edema of the eyes. These effects do not necessarily provide adequate warning since they may not develop for some hours after exposure. Severe irritation of the eyes and even temporary blindness is possible after prolonged exposure. Effects of prolonged exposure usually include actions in the respiratory tract and upon the liver and very likely the kidney. The highest concentrations producing little or no injury to animals exposed over long periods of time are in the neighborhood of 5 parts per million.

Skin contact may result both in typical alkali burns which may be quite severe and in systemic intoxication. The latter is principally characterized by extensive fatty infiltration of the liver, kidney damage, and sometimes anemia. Protective clothing and equipment should consist of goggles, gloves, and fire-resistant protective clothing, and boots. It should be noted that rags saturated with hydrazine may inflame spontaneously if left lying around. Drenching showers for extensive skin exposure should be provided. In addition to water, 5 per cent acetic-acid solution baths or vinegar should be provided for soaking of exposed skin parts. The eye should be washed liberally with water.

Medical treatment for hydrazine burns follows that for burns of other origin. Treatment of liver injury should include a diet rich in protein, carbohydrate, and vitamins, and kidney injury should be treated as renal failure of similar extent and nature from other causes, as indicated by kidney tests.

JP-4 Fuel

JP-4 fuel is a jet engine fuel composed principally of aliphatic hydrocarbons and less than 25 per cent aromatic hydrocarbons. In odor and in appearance it very closely resembles such materials as kerosene and is a flammable volatile liquid distilling between 80 and 250° C.

JP-4 fuel is generally harmless when applied to the skin if it is promptly removed. However, if it is allowed to remain in contact with the skin for a long time, erythema and vesicles may develop on the skin. If the exposures are repeated and prolonged, the skin may be necrotized. The most frequently seen effects upon the skin are those of the drying by solvent action on the natural oils of the skin. There is little danger of systemic toxicity by skin penetration. However, if lead compounds are present in the fuel, lead intoxication may result by skin penetration. Vapor inhalation of 1000 parts per million or more produces symptoms closely resembling those of ethyl alcohol, although the symptoms are usually much more severe. Headache, nausea, vomiting, convulsions, prostration, cyanosis, coma, and even death have been reported. Chronic exposure is said to produce a form of intoxication with very vague symptomatology. Injury to the lungs has been observed when the fuel has been siphoned and portions aspirated accidentally. The symptoms after ingestion resemble those after inhalation except that they may be delayed in onset.

Personnel protective measures should include suitable skin covering, adequate ventilation of closed spaces, and respiratory protective devices such as those needed for gasoline. Removal of personnel to the open air after exposure should be carried out, and contaminated clothing after spillage should be promptly removed. The treatment for severe poisoning should be similar to that resulting from ethyl alcohol poisoning.

II Oxidizers

Hydrogen peroxide (13, 14)

Hydrogen peroxide (90 per cent) is a colorless liquid which decomposes spontaneously with the evolution of oxygen. Decomposition is accelerated by shaking, by impurities, or by

contact with large surfaces. It is a powerful oxidizing agent which combines explosively with combustible materials. It is miscible with water in all proportions.

Contact with the skin causes blanching of the area with a burning sensation. The skin effects disappear rapidly if the contact is very brief; however, severe burns may result from prolonged contact. Skin penetration is not a source of systemic poisoning. The inhalation of vapors produces redness and burning of the eyes and irritation of the nose and throat. Lacrimation, running nose, and excessive throat secretions result. Tissue damage by the vapors is not ordinarily encountered except after prolonged or repeated exposures; however, if hydrogen peroxide is present in the form of a fine mist or aerosol, tissue damage can result in the eye, trachea, and in the lungs. In experimental animals, a particularly insidious eye effect has been observed, namely, opacities of the cornea which appear several weeks after exposure. Hence, there should be periodic, frequent eye and upper respiratory examinations by the plant physician.

Personnel handling 90 per cent hydrogen peroxide should be provided with goggles or face masks and suitable skin protection in the form of gloves, aprons, and other clothing. Clothing of combustible materials such as wool and cotton should not be worn inasmuch as these materials may ignite spontaneously on contact with 90 per cent hydrogen peroxide; hence, safety clothing should be provided which should be made of polyvinyl chloride, polyethylene, or neoprene. Where there is a possibility of exposure to aerosol or fine mist, respirators and eye protection should be provided. In the event of gross contamination, safety showers should be used immediately. The eye should be promptly washed with large amounts of water. Serious skin injury should be treated as any burns of similar extent.

Liquid oxygen (15)

Liquid oxygen is a heavy, odorless and very slightly bluish liquid with a boiling point of −183° C at atmospheric pressure. It is explosive in contact with easily oxidizable materials; sometimes explosive mixtures are formed by combination of liquid oxygen with organic materials, which may be detonated by slight shock. Liquid oxygen as such is not toxic and is not irritating. Its hazard arises from its active support of combustion and its temperature. It should be stored in such a manner that it does not come in contact with flames or flammable materials, and where adequate ventilation can be provided. Accumulation of highly concentrated oxygen pockets should be avoided because cotton, wool, or other similar materials stored in such atmospheres may burn very violently upon ignition. Accidental spillage should be minimized to avoid injury by the coldness of the liquid, which can produce serious burns on contact with the skin or other portions of the body. Eyeglasses and gloves and other suitable skin protective equipment should be used by personnel handling liquid oxygen. Contaminated portions of the body should be treated as any other burned area and temperature effects minimized by soaking exposed parts in water.

Red Fuming Nitric Acid (8, 16, 17)

Red fuming nitric acid is a clear liquid ranging in color from yellow to reddish brown, strongly fuming, and extremely corrosive. The liquid is generally 87 to 92 per cent nitric acid with varying amounts of water and oxides of nitrogen, which are mainly nitrogen tetroxide. The fumes are yellowish red due to the nitrogen oxides, and are suffocating and poisonous; the fumes also contain nitric-acid vapors. Red fuming nitric acid is a powerful oxidizer readily attacking most organic materials. It reacts explosively with many organic materials such as aniline, xylidine, and furfuryl alcohol. It is precisely this property which makes it desirable for propellant use.

Red fuming nitric acid produces yellow staining of the skin after very brief contact; however, if contact is for any period, it is apt to result in a very severe burn. However, red fuming nitric acid may also produce poisoning by inhalation of the fumes. Fumes may arise from the decomposition of the acid itself, and it is for this reason that white fuming nitric acid has hazards similar to red fuming nitric acid. Decomposition of the acid to produce the fumes may be favored by contact with metals, organic materials, and possibly by the interaction of the oxides among themselves and with oxygen. Toxicologically, the most important nitrogen oxides are nitric oxide, nitrogen dioxide, and nitrogen tetroxide. Color or odor of the fumes cannot be relied upon primarily as a warning for toxic exposure. Of the three oxides, nitrogen dioxide and nitrogen tetroxide are probably the most important toxicologically, having severe effects upon the upper and lower respiratory tracts. In addition, they produce methemoglobin in the blood. Concentrations of 50 to 150 parts per million in the inspired air are said to be without discomfort; nonetheless, they may be extremely dangerous for even short periods. A safe concentration for 8-hour daily exposures should probably be 5 parts per million or less.

Shortly after an exposure, coughing, rapid asthmatic breathing, nausea, vomiting, and fatigue may be observed. There may be a large increase in the platelet count in venous blood, sometimes amounting to 60 per cent to 100 per cent. The blood pressure may fall and polycythemia may appear in the blood with resulting increase in hemoglobin content (no methemoglobinemia). The chest signs may become more severe with pain in the chest, spasmodic coughing, difficulty in breathing, these signs appearing several hours after the exposure. Fatal pulmonary edema and bronchopneumonia may appear. The variety in signs and symptoms is so great that all concerned with the handling of red fuming nitric acid and white fuming nitric acid should be alert to any unexplained symptomatology. Chronic exposure to oxides of nitrogen is said to produce wearing down and decay of the teeth, pulmonary emphysema, and irritation of the respiratory tract. Sometimes ulcerations of the nose or mouth are seen.

Personnel protective measures should begin at the preplacement medical examination. At this time the circulatory and respiratory system should receive special attention with the view to eliminating personnel who have bronchitis, asthma, or diseases of the heart. Frequent physical examinations should be carried out with special attention to the respiratory tract and the state of the teeth. Frequent surveys of the atmospheric contamination by oxides of nitrogen should be carried out. Adequate protective clothing such as gloves, aprons, and protection for the eyes should be provided. The only adequate protection against nitrous fumes in enclosed spaces is the so-called self-contained oxygen mask, inasmuch as the common gas-mask canisters do not provide adequate protection. It cannot be overemphasized that time is of the essence in skin, eye, or ingestion episodes, and that any delay may result in disaster. Drenching showers or immersion baths in the vicinity of any operation should be provided to deal with splash accidents. Where possible, bicarbonate of soda should be added to the baths for washing contaminated skin areas. Skin or eye contamination should be vigorously and promptly treated by flooding with large amounts of water. Washing should be carried out for at least 15 minutes. Contaminated clothing should be promptly removed and medical attention obtained without delay. Ingested nitric acid should be diluted by drinking water liberally. After the acid has been so diluted, vomiting may be encouraged; however, passing a stomach tube or vomiting may be a hazardous procedure in itself since the stomach may rupture if it has been severely burned. Hence, the decision for this should be left to the physician.

Casualties from inhalation of nitrous fumes should be placed in bed for 24 hours, even though the initial signs may not be alarming. Oxygen should be administered to combat the development of pulmonary edema as well as to fully oxy-

genate the blood. This should be done by nasal catheter if necessary until all danger from anoxia or pulmonary edema has passed. Artificial respiration should be administered cautiously in the event that the lung has been damaged. Respiratory depressants and cardiac stimulants should be avoided unless there are clear-cut signs of heart failure. Venesection for relief of pulmonary edema should be carried out with great caution if there are signs of impending circulatory collapse. Intercurring infectious complications such as pneumonia should be treated with antibiotics. Contamination of the eye should receive very prompt and vigorous attention inasmuch as seconds may count in the prevention of severe eye damage. Prompt washing with large amounts of water and with water containing 1 to 2 per cent sodium bicarbonate should be vigorously carried out until a physician arrives.

The Combustion Products of Fuels and Oxidizers

The hazard of combustion products of fuels and oxidizers which have been discussed in the past paragraphs may be summed up as follows:

(a) Little is known concerning the identity of the intermediary oxidation products of the rocket combustion of fuels and oxidizers mentioned in the foregoing paragraphs. However, the principal combustion products of toxicological interest in this discussion are believed to be carbon monoxide and the oxides of nitrogen, the latter wherever nitrogenous fuels and/or nitrogen containing oxidizers are used. Much is known concerning the toxicity of carbon monoxide; hence, it is not necessary to dwell in detail here upon the hazards of carbon monoxide. It should suffice here to indicate that adequate ventilation will provide necessary relief from exposures and will eliminate further hazard; artificial respiration with oxygen for the severely poisoned is necessary. When the halogens are an important part of the fuel system, hydrogen halides, viz., chlorides, must be considered in the picture of hazard. Similarly if sulfur is present, the oxidation products of sulfur, such as sulfur dioxide, sulfur trioxide, and sulfuric acid must likewise be considered. However, the fumes from the latter are generally so irritating as to preclude voluntary exposure of personnel to dangerous amounts.

(b) The ideal approach to the problem of combustion products is adequate, periodic, atmospheric surveys, not only of a quantitative nature for specific expected products but also of a qualitative nature in an attempt to determine unknown toxic contaminants in the air. This is particularly true where new fuels and oxidizers are being developed; this is to the end that hazard may be eliminated or that atmospheric contamination may be reduced to safe levels.

Discussion

Certain general remarks in the introduction were necessarily sketchy. These may now be extended to emphasize certain aspects of the problem of dealing with toxic materials.

The prevention of injury is of paramount importance in dealing with hazardous chemicals, just as it is in dealing with explosives, electricity, or disease. A prime requisite for a successful program of prevention is the proper training of all concerned in the inherent dangerous properties, including toxic potential—the subject of this report—and in the proper use of procedures and devices established beforehand which are intended to prevent injury. All concerned must be convinced that the dangers of the propellant chemicals described in the preceding passages are not exaggerated. At the same time, however, it should be stressed to all that these materials can be handled safely, just as explosives handlers and electrical workers, to cite two common examples, deal safely with no less hazardous situations daily. This emphasizes that handlers must respect but not fear that which they handle, and this in turn means that each supervisor must be thoroughly familiar with the psychological make-up of each member of his team in order to prevent (a) callousness and carelessness in the overconfident and incredulous, and (b) almost paralysing fear, which in itself may make for accident proneness, in the overcautious or timid. A daily appraisal of the fitness of all team members is of great value.

Concerning general protective measures, it is axiomatic that suitable personal protective equipment, in addition to safety devices on materiel, be provided as indicated in connection with each chemical in the preceding pages. However, the versatility and effectiveness of water in maintaining safety in the presence of a very wide variety of toxic hazards are not generally stressed as much as they deserve. Its ubiquity and general usefulness for other purposes—hence, already procured and provided—adapt it admirably as an indispensable item among those in the first line of protection. Hence, it must be available for dispensing by failure-proof mechanisms, which need not be more complex than common hose lines, showers, tanks, or vats for whole body immersion, eye-wash basins, and other means, as local requirements dictate and local capabilities permit. Respiratory protective devices must obviously be tested frequently, since certain parts may lose effectiveness not apparent on inspection. Canisters, filters, and the like used against one chemical hazard should be discarded to render impossible use against another, so as to prevent opportunity for forming dangerous mixtures with the first. It is, in any case, of great importance that the presence of protective gear of any kind not foster relaxation of vigilance or encourage a false sense of security. An indispensable part of preventive efforts is what may be termed "good housekeeping," viz., scrupulous attention to prompt elimination of contaminated sites, however small, be they in the laboratory, plant, or storage shed. Together with good personal hygiene in prompt removal of hazardous materials from skin and clothes, good housekeeping can go far in preventing the insidious growth of toxic situations. Smoking should be discouraged where it offers a means for ingestion or inhalation of toxic materials.

In the event of failure of preventive efforts, i.e., an accident which exposes personnel, speed in application of first aid and self aid is critical in minimising injury from these propellant materials. This fact cannot be overemphasized, and in general drenching of body parts exposed to liquid or removal of the subject from a fume- or vapor-contaminated atmosphere must be carried out without the slightest delay. This is especially important in connection with eye contamination with red fuming nitric acid, with urgency in treating skin exposure to liquid red fuming nitric acid being a close second. Literally, in this case, seconds count if injury is to be successfully averted or progression of injury prevented. Though chemical facts may argue for the use of a chemical antidote, the time available for any successful treatment of liquid splash injury by red fuming nitric acid is so short that failure to apply readily available water by seconds, while searching for sodium bicarbonate solution, may spell disaster. All concerned must be convinced that self-aid procedures exist which, when promptly and properly applied, are not less, but rather more effective, for all their simplicity, than elaborate measures applied too late. Like considerations apply no less forcibly to first aid, properly and promptly administered. As nearly concurrently as possible, medical attention should be sought.

When compelling circumstances require exposure in spite of known hazard, such as unprotected entry into a contaminated atmosphere, risks are taken when they are clearly outweighed by the known consequences of not taking the risks. Conversely, those concerned in dealing with new chemicals of suspected, though unknown, hazard should not expose themselves or others unjustifiably. In this connection, competent toxicological and medical advice should be sought.

(Continued on page 236)

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STUDIES ON THE PARENTERAL ADMINISTRATION OF HYDROGEN PEROXIDE * † ‡

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Introduction

At times the condition of the pulmonary system is such that the effective administration of oxygen to patients by inhalation methods is impossible. To combat oxygen-want under these circumstances, resort to other methods of administration has been attempted. Among these methods, the intravenous administration of oxygen has been used clinically on several occasions with reportedly good therapeutic results. In 1902, Mariani (1) injected oxygen intravenously into a patient dying of tuberculosis. The pulse and respiration improved. The treatment, however, was not repeated and the patient died the next day. Tunnicliffe and Stebbing (2) in 1916 treated 3 very ill patients with intravenous oxygen. They stated that 600 to 1,200 cc. per hour can be given, that dyspnea and cyanosis are rapidly relieved, and that the beneficial results of the intravenous injection of oxygen were certainly more lasting than could apparently be explained by the mere relieving of cyanosis. Singh and Shah (3) in 1940 gave intravenous oxygen to 6 patients with severe pulmonary disease. All 6 showed clinical improvement as was indicated by raised blood pressure and improved pulse. They concluded by stating, "In man about 10 to 20 cc. per minute of oxygen can be administered intravenously. . . . This amount of oxygen does not appear to be considerable, but still a distinct clinical improvement follows its administration."

Ziegler (4) in 1941 devised a more practical apparatus for the continuous administration of intravenous oxygen at rates varying from 200 to 1,000 cc. per hour for periods as long as nine hours. He observed that cyanosis cleared, the pulse rate decreased, blood pressure increased if hypotension was present, and the mental state improved. He also reported that in patients in shock or with heart failure the blood pressure increased and the pulse and respiratory rates decreased. One pa-

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tient with severe pulmonary edema was relieved after twelve hours following administration of intravenous oxygen for five hours. He also stated that the clinical improvement in patients, "seems to be all out of proportion to the small amount of oxygen administered." He never observed symptoms of gas embolism, and used the criteria of definite increases in pulse rate and respiration as indicative that too much oxygen was being given.

Recently Jacobi et al. (5) successfully treated 3 patients in severe progressive acute secondary traumatic shock by the method of Ziegler. They also induced irreversible toxic shock in dogs by the intravenous injection of a saline extract of dog muscle. Three untreated and 3 plasma treated control animals died, while 5 dogs given 100 cc. of oxygen per hour intravenously for ten to fifteen hours survived.

Experimental injection of oxygen intravenously in animals has been extensively studied by several investigators (6, 7, 8, 9). On the whole, however, results have not been encouraging because of marked embolic phenomena. Grodins, Ivy, and Adler (10) in 1943 gave oxygen intravenously to dogs under nembutal anesthesia at rates varying from 0.21 cc. to 3.2 cc. per kilogram per minute. They concluded that the anoxemia from the anesthesia was not corrected but actually aggravated by the treatment since the measured arterial oxygen in most animals was reduced. Reduction of blood pressure and increased respiratory rates were also noted. They suggested that the anoxemia was increased because of a reduction in the pulmonary capillary bed by multiple gas emboli, and concluded that intravenous oxygen in the form of gas bubbles is impractical from a therapeutic viewpoint.

The reasons for the disparity between the clinical and animal observations are not clear. Possibly the presence of terminal states or shock in the human cases is important. Also, the larger size of the human organism plus a factor of species difference in susceptibility to gas embolism may in part account for the difference.

The limiting factor in the administration of oxygen by vein is the formation of gas emboli. If the oxygen is administered as small bubbles, the tolerance is increased because the bubbles dissolve more rapidly. Hydrogen peroxide decomposes into water and gaseous oxygen, a reaction which is much hastened by the presence of catalase in human blood. Since the hydrogen peroxide is in solution, molecules of it are widely separated from each other by water. The gaseous oxygen produced by decomposition of the substance, therefore, should be in the form of minute bubbles. As a result of their small size, the oxygen bubbles should dissolve very rapidly and have less tendency to produce gas embolism. The experiments recorded in this paper were performed to test the validity of this premise, and to determine the value of hydrogen peroxide as an easily administered and controlled form of intravenous oxygen therapy.

The intravenous administration of hydrogen peroxide has been recorded in the literature on two occasions. In 1920, Oliver and Murphy (11), during an epidemic in Mesopotamia, treated 24 individuals with very severe influenzal pneumonia with intravenous hydrogen peroxide. They administered approximately 0.6 per cent hydrogen peroxide over fifteen-minute intervals, which is equivalent to intravenous oxygen given at a rate of about 600 cc. per hour. They noted slower, steadier and deeper respirations following the injections, and also a drop in pulse rate and marked general clinical improvement. The mortality in the series was 49 per cent as compared to 80 per cent in a similar untreated group. In the fatal cases autopsies failed to reveal any evidence of gas embolism. In the cases successfully treated the fever dropped by crisis a few hours after administration of intravenous hydrogen peroxide. The authors considered the beneficial results largely the result of oxidation of circulating toxins.

In 1944, Siderova (12) reported the use of intravenous hydrogen peroxide in animals as an antidote against potassium cyanide poisoning. He successfully administered doses of 1 to 3 cc. per kilogram of 1 to 2 per cent hydrogen peroxide against 2.4 times the lethal dose of potassium cyanide given subcutaneously in dogs. In general, the treatment was less effective in rabbits than in dogs. He concluded that the effect was owing in part to the oxidizing and methemoglobin-forming properties of hydrogen peroxide.

EXPERIMENTAL METHODS AND RESULTS

Preliminary experiments on the effect of hydrogen peroxide on blood were performed. Addition of an equal volume of 3 per cent hydrogen peroxide in normal saline solution to human blood was found not to alter the hematocrit, microscopic appearance or differential picture as compared with a similar sample to which only normal saline solution had been added. While human blood retained its bright red color and foamed vigorously on the addition of 3 per cent hydrogen peroxide, dog blood foamed less vigorously and rapidly turned a dark brown color. Even with very small amounts of hydrogen peroxide this color change was evident. Bloods from a few other animal species were similarly treated with hydrogen peroxide. Rabbit, cat, mouse, and rat bloods remained bright red like human blood while chicken blood turned dark, as did that of the dog. A mixture of half dog and half human blood remained bright red.

Determinations of quantitative catalase activity (Kat. f.) on various bloods were undertaken following a modification of von Euler and Josephson's procedure (13) suggested by DuBois (14).

Thirty-five cubic centimeters of 0.02 normal hydrogen peroxide, 10 cc. of M/15 sodium phosphate buffer pH 6.8, and 4 cc. of distilled water were mixed and placed in an ice water bath at 4 C. One cubic centi-

meter of a diluted blood sample (prepared by diluting 0.10 cc. of blood to 10 cc. with water) was then added to the mixture and allowed to react for two minutes. Five cubic centimeters of the mixture was then pipetted into 10 cc. of 1 normal sulfuric acid and the remaining hydrogen peroxide titrated with 0.002 normal potassium permanganate. Blank titrations were performed with 1 cc. of water substituted for the blood solution.

Calculations were based on the formula,

$$\text{Kat. f.} = \frac{K}{\text{Gm. enzyme preparation}}$$

where K is the monomolecular reaction velocity constant,

$K = (1/t) \log (C_o/C_t)$,

C_o — initial hydrogen peroxide concentration,

C_t — hydrogen peroxide concentration at end of time t.

The ratio (C_o/C_t) in this case is equivalent to the ratio of the volumes of potassium permanganate solution in the blank over that in the unknown titrations. Duplicate determinations were done on each sample.

Results of these determinations are given in table 1 for oxalated blood samples not over seventy-two hours old. It is evident from this table that considerable individual and wide species variation in blood catalase activity exists. Becht (15), in 1919, first called attention to this fact through a rather crude method involving the determination of the total volume of oxygen liberated from a solution of hydrogen peroxide by measured quantities of various bloods. He noted that human, cat, and horse bloods have very much greater catalytic power than dog, goat, and sheep bloods. No mention, however, is made of the differential effect of hydrogen peroxide on the various bloods.

The catalase determinations provide the explanation for the different effects of hydrogen peroxide upon the blood of various animal species. If the catalase content of blood is high, hydrogen peroxide is rapidly decomposed to water and gaseous oxygen, which foams in the bright red blood. If the catalase content of blood is low, the peroxide decomposes slowly, changing hemoglobin into methemoglobin probably both directly and also through the action of nascent oxygen formed by the reaction between blood peroxidase and hydrogen peroxide.

An attempt was made to increase the catalase activity of the blood of a dog weighing 8 kg. by injecting intravenously 100 cc. of centrifuged human erythrocytes over a thirty-minute period. Blood samples were drawn before, and fifteen minutes after, the injection. The Kat. f. before injection was 0.3 and the hematocrit reading 33 per cent. After the injection the Kat. f. was 1.2 and the hematocrit reading 24 per cent, which indicates considerable hemolysis. When hydrogen peroxide was added to the latter sample only slight darkening occurred. Hence, the

critical Kat. f. level to protect blood against excessive formation of methemoglobin by hydrogen peroxide is somewhere around one. Incidentally, after two days the dog recovered entirely from the effects of the transfusion of human red cells even though extensive hematuria occurred during the first day.

TABLE 1

CATALASE ACTIVITY DETERMINATIONS ON VARIOUS BLOODS

Sample	Kat. f.	Av. Kat. f.
Human adult No. 1	25.4 26.6	26.0
Human adult No. 2	30.4 30.3	30.4
Human adult No. 3	35.9 36.3	36.1
Human adult No. 4	22.5 22.3	22.4
Human adult No. 5	24.6 24.8	24.7
Human infant—5 months	20.1 19.2	19.7
Cat. No. 1	27.0 27.0	27.0
Cat. No. 2	15.8 16.0	15.9
Rabbit No. 1	12.4 13.0	12.7
Rabbit No. 2	15.3 15.8	15.6
Rat	6.9 7.1	7.0
Mouse	6.9 6.9	6.9
Dog No. 1	0.3 0.3	0.3
Dog No. 2	0.3 0.3	0.3
Young rooster	0.02 0.015	0.02

Tolerance studies of intravenous administration of hydrogen peroxide in several animals were undertaken. Normal saline solutions of pure 30 per cent hydrogen peroxide (Superoxol) in various dilutions were used. The strengths of the solutions will be expressed in terms of the volume oxygen equivalent, keeping in mind that a 3 per cent solution of hydrogen peroxide yields approximately ten volumes of oxygen

per cubic centimeter. Light to moderate pentothal or nembutal anesthesia was used in almost all instances to keep the animals quiet.

In the dog, ten volume hydrogen peroxide injected intravenously at a rate approximately equivalent to 1 cc. of oxygen per kilogram per minute, within five minutes led to an intense brownish "cyanosis" without associated dyspnea or signs of gas embolism. This "cyanosis" slowly cleared over a three day period and undoubtedly represented methemoglobinemia.

In an unanesthetized young rooster (approximately 750 Gm.), 1.5 cc. of ten volume hydrogen peroxide given intravenously over a three-minute period led to deep brownish cyanosis and death. The oxygen capacity of the blood at death was measured as 5.9 volumes per cent, using the Van Slyke (16) manometric method.

In contrast to the hydrogen peroxide tolerance limitation based chiefly on methemoglobin formation in the dog and chicken, it was found that phenomena owing to gas embolism limited the tolerance in the rabbit and cat. The following sequence of events was quite regularly observed in these animals during progressive slow gas embolization, either from intravenous hydrogen peroxide or oxygen administration.

1. Tachypnea—ultimately reaching rates exceeding 100 per minute.
2. Pallor.
3. Accentuation of heart tones.
4. Early cyanosis.
5. Tinkling sound over precordium owing to the presence of tiny intracardiac gas bubbles.
6. Continuous loud rumbling to-and-fro murmur of intracardiac gas.
7. Development of slow, deep, irregular, gasping respiration rapidly leading to complete apnea in the inspiratory phase.
8. Sudden weakening of pulse and bradycardia.
9. Cardiac arrhythmias.
10. Severe cyanosis.
11. Sudden, rapid fall in blood pressure.
12. Pupillary dilatation and urinary and fecal incontinence.
13. Complete cessation of heart action and respiration.

The signs of gas embolism are classified as mild up to number five in sequence, as moderate between five and seven, and as severe after seven. A characteristic feature of the sequence was the sudden development of each additional symptom. It is also of interest to note that the blood pressure and pulse remained essentially unchanged until terminal stages were reached. Although in several animals the retinal vessels were observed for gas bubbles, none were ever seen. On autopsy at no time could gas be detected in the left side of the heart or systemic circulation despite the presence at times of tremendous amounts of gas in the right heart. The most remarkable feature of the

entire process, however, was the rapid and complete recovery of the animals even at near terminal stages when administration of either the hydrogen peroxide or oxygen was stopped.

Since posture and injection site are important factors in the tolerance to gas embolism as first emphasized by Van Allen, Hrdina and Clark (17), all injections were given in the femoral vein with the animals in a horizontal position.

In table 2 the results are summarized of tolerance studies of intravenous administration of hydrogen peroxide in rabbits and cats. For

TABLE 2

INTRAVENOUS HYDROGEN PEROXIDE TOLERANCES IN CAT AND RABBIT

Animal	Weight Kg.	Strength H_2O_2 (cc. O_2 liberated per cc. by an excess of blood)	Needle Gauge	Duration of Injection	Injection Rate (O_2 equivalent as cc./Kg./min.)	Symptoms of Gas Embolism
Rabbit No. 1	4.0	0.2	26	30 min.	0.12	None
Rabbit No. 2	4.5	1.0	26	15 min.	0.15	Mild
Rabbit No. 1	4.0	1.0	26	6 min.	0.16	Mild
Rabbit No. 1	4.0	0.5	24	10 min.	0.32	Mild
Rabbit No. 1	4.0	1.0	24	13 min.	0.64	Moderate
Rabbit No. 3	4.8	5.0	25	3 min.	0.70	Severe—death
Rabbit No. 4	3.5	3.3	25	12 min.	1.0	Severe—death
Rabbit No. 5	3.5	1.7	25	1 min.	1.3	Severe
Rabbit No. 4	3.5	3.3	25	½ min.	2.0	Very severe
Cat No. 1	3.2	1.0	21	30 min.	0.34	None
Cat No. 1	3.2	1.0	24	30 min.	0.7	None
Cat No. 2	2.0	3.0	24	30 min.	1.5	Very mild
Cat No. 3	2.5	4.0	21	30 min.	1.6	Mild
Cat No. 4	2.5	5.0	21	30 min.	2.0	Moderate
Cat No. 5	3.3	4.0	24	6 min.	2.0	Severe—death
Cat No. 6	2.1	5.5	21	3 min.	2.6	Severe—death

purposes of comparison, a few tolerance measurements of intravenous oxygen gas were done in cats, with the results as given in table 3.

The data presented in these tables seem to indicate that: (1) a considerable species difference in susceptibility to gas embolism exists between the rabbit and cat; (2) considerable individual variation in susceptibility to gas embolism exists; (3) the more dilute solutions of hydrogen peroxide less readily cause severe signs of gas embolism; and (4) in the cat, oxygen can be administered intravenously in the form of hydrogen peroxide at least twice as fast as in the form of gaseous oxygen without producing severe embolic signs.

The animals surviving these experiments, after recovering from anesthesia, appeared normal in all respects and remained well during several weeks of observation. On autopsy, all animals dying during the experiments showed pale, emphysematous lungs and the presence of considerable amounts of gas in the great veins and right side of the heart only.

Chronic studies on the parenteral administration of hydrogen peroxide were carried out in mice. With lethal doses given subcutaneously (0.3 cc. of a 3 per cent solution per 25 Gm. mouse), death occurred from gas embolism as demonstrated by the symptomatic course and autopsy findings. Apparently a large part of the hydrogen peroxide enters the vascular system before being decomposed by catalase.

TABLE 3

INTRAVENOUS OXYGEN TOLERANCE IN THE CAT

Animal	Wgt. Kg.	Needle Gauge	Injection Rate of O_2 (cc./Kg./min.)	Duration	Embolic Symptoms
Cat No. 7	3.3	24	0.3	30 min.	Mod. severe
Cat No. 8	1.9	27	0.52	30 min.	Mod. severe
Cat No. 4	2.5	26	0.6	15 min.	Moderate
Cat No. 4	2.5	26	0.8	13 min.	Severe
Cat No. 4	2.5	24	0.8	30 min.	Severe
Cat No. 4	2.5	24	1.2	4 min.	Severe – death

In a series of 30 young mice (approximately 15 Gm. each), maximum tolerated doses of 0.1 cc. of 1.5 per cent hydrogen peroxide in normal saline solution were given subcutaneously twice daily over a period of two weeks. Several animals showed brief embolic signs immediately after the injections. In addition to the general picture of gas embolism already discussed, another commonly observed sign was paraplegia lasting for a few minutes. An occasional animal also exhibited brief generalized convulsions. Five mice died with embolic symptoms at various times during the course of the experiments.

Locally, subcutaneous emphysema was present in all cases after the injections and small ulcers eventually developed at the injection sites in about 25 per cent of the animals. Aseptic technic, however, was not followed in making the injections. No further apparent ill effects, other than those temporarily caused by embolic phenomena, were observed as a result of the injections in the surviving mice. In a control group of 5 mice given similar injections of an equal volume of normal saline alone, however, ascites and omental edema were observed. Histologic sections were taken of various organs of some of these animals, including heart, lung, kidney, liver, spleen, gastrointestinal tract, pancreas, bone and mediastinal structures. No tissue differences between the hydrogen peroxide and the saline-treated mice were noted with the exception of the edema as mentioned previously and local superficial skin ulcerations at the injection sites in some of the peroxide treated animals. Histologically, however, the animals dying of gas embolism showed distended pulmonary alveoli and occasional small hemorrhagic areas in the lungs and brain.

Arterial analyses of blood gas, using the Van Slyke manometric apparatus and methods, were made on 2 cats under pentothal anes-

thesia, who were treated with intravenous hydrogen peroxide for hypoxia induced by administration of 90 per cent nitrous oxide and 10 per cent oxygen by means of a face mask. The results given in table 4 indicate that hypoxia, rather than being relieved by the administration of even relatively small amounts of hydrogen peroxide, is actually aggravated even though in interpreting the data one makes allowance for blood loss incident to sampling. Gas embolization of the pulmonary capillary bed, as suggested by the work of Grodins et al. (10), probably in large part accounts for these results.

TABLE 4

EFFECT OF I.V. HYDROGEN PEROXIDE ON ARTERIAL BLOOD GASES IN HYPOXIA

Cat	Wgt. Kg.	Treatment	Duration of Treatment	Blood Sample	Volumes Per Cent		Symptoms
					Oxygen	CO_2	
I	1.5	90% N_2O and 10% oxygen inhalation	7 min.	7 cc.	14.55	56.40	Trace of cyanosis
		Above plus I.V. H_2O_2 in 8.4 cc. N saline = 0.4 cc. of O_2/Kg./min.	21 min.	7 cc.	8.32	45.23	Cyanosis but no embolic signs
		I.V. H_2O_2 discontinued	10 min.	7 cc.	9.53	33.74	Pallor and cyanosis
II	2.1	90% N_2O and 10% oxygen inhalation	16 min.	7 cc.	17.50	41.92	None
		Above plus I.V. H_2O_2 in 8 cc. N saline = 0.4 cc. of O_2/Kg./min.	15 min.	7 cc.	16.20	43.17	None
		H_2O_2 injection increased to be = 1 cc. of O_2/Kg./min.	15 min.	7 cc.	6.90	49.03	Cyanosis and mod. embolic signs

In two cats the effect of intravenous administration of hydrogen peroxide in carbon monoxide poisoning was studied. Illuminating gas was administered by means of a face mask until the respiratory rhythm changed, and then was discontinued. Arterial blood samples of 5 cc. were then taken at intervals before and after a period of intravenous administration of hydrogen peroxide. Analyses for oxygen, carbon monoxide and carbon dioxide were made on these samples by the Van Slyke manometric methods.

From the results given in the graphs in figures 1 and 2, it is evident that intravenous administration of hydrogen peroxide had but little effect in accelerating the elimination rate of carbon monoxide. Thus

in figure 1, during the thirty-five-minute period prior to the administration of hydrogen peroxide, the carbon monoxide of the arterial blood decreased from 10.49 to 5.85 volumes per cent and oxygen increased from 3.38 to 8.53 volumes per cent. Then in the ensuing thirty-five minutes during which the peroxide had been given, carbon monoxide dropped further to 3.21 and oxygen increased to 10.33 volumes per cent.

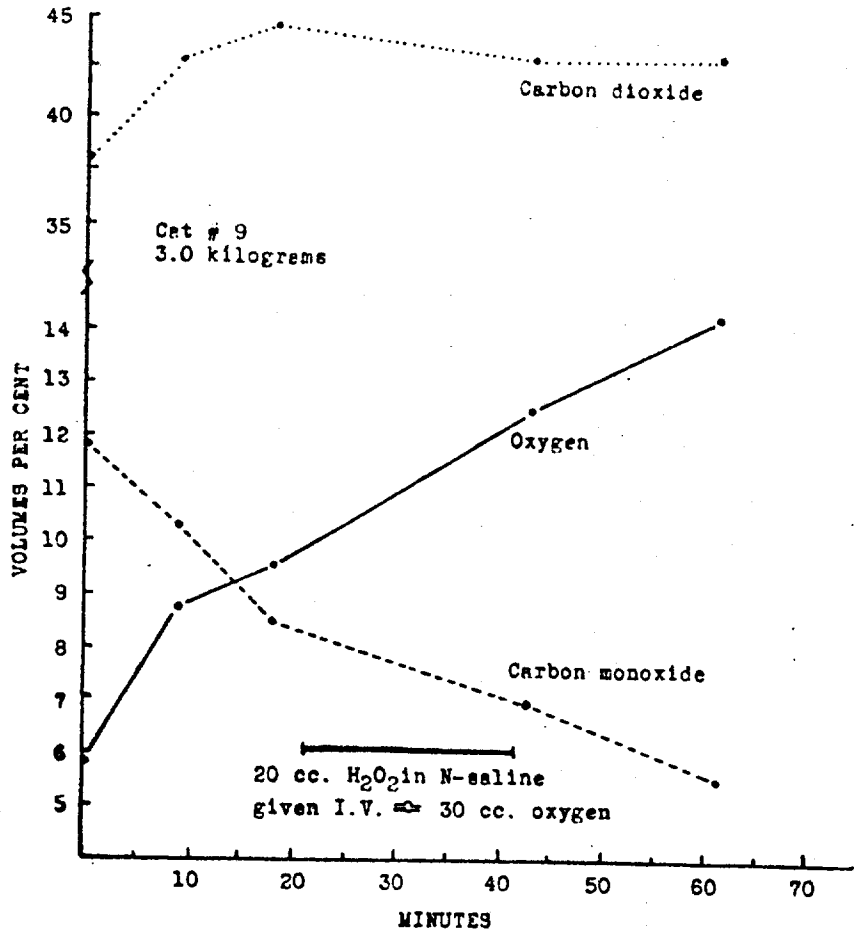

FIG. 1. Effect of intravenous hydrogen peroxide in carbon monoxide poisoning.

Similarly in figure 2, in the twenty minutes prior to intravenous injection of hydrogen peroxide, the carbon monoxide of the arterial blood dropped from 11.80 to 8.42 volumes per cent, and oxygen increased from 5.87 to 9.56 volumes per cent. In the following twenty minutes during which the peroxide was given, carbon monoxide fell further to 6.94 and oxygen increased to 12.54 volumes per cent. Then after another twenty-minute interval, carbon monoxide was 5.44 volumes per cent and oxygen was 14.24 volumes per cent.

In both animals during the administration of hydrogen peroxide, respiration became less labored. No signs of gas embolism occurred and both cats made an uneventful recovery from the experiments. It is perhaps significant that in these experiments the intravenous administration of hydrogen peroxide did not increase hypoxia as in the previous investigations.

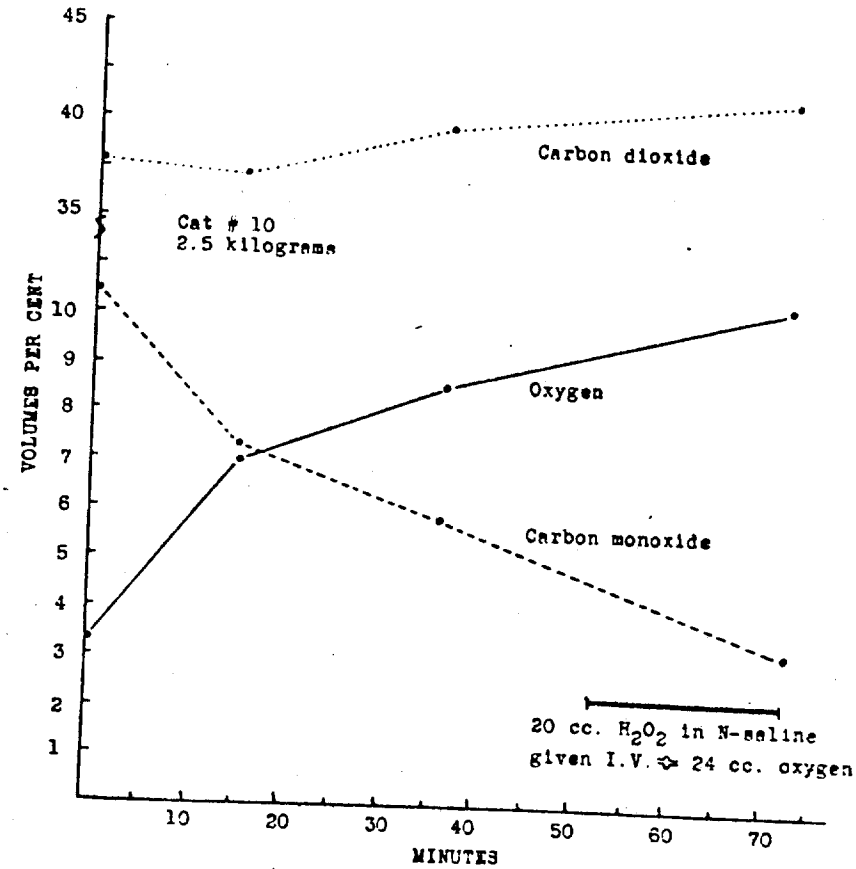

FIG. 2. Effect of intravenous hydrogen peroxide in carbon monoxide poisoning.

Finally, preliminary observations on the effects of intravenous administration of hydrogen peroxide in cats affected by several types of chemical toxic shock and hemorrhage were made. Five animals were used and shock was induced in one animal each by intravenous injections of peptone, histamine, procaine and sodium nitrite, and by sudden 30 per cent hemorrhage. Hydrogen peroxide was administered at rates equivalent to 1 cc. of oxygen per kilogram per minute. In all cases of chemical shock, after the administration of hydrogen peroxide the blood pressure rose about 20 mm. of mercury from previous systolic levels below 65 mm. of mercury. The effect, however, could not be sustained and lasted for only a very few minutes. In sodium nitrite shock the

administration of hydrogen peroxide led to increasing further methemoglobinemic "cyanosis." In the case of shock resulting from massive hemorrhage, the intravenous administration of hydrogen peroxide had no effect on the blood pressure, while later injection of the removed blood promptly restored the blood pressure to normal.

DISCUSSION

This investigation indicates that in animals with sufficient blood catalase, hydrogen peroxide can be given intravenously with effects similar to those following intravenous administration of oxygen. Significantly larger amounts of oxygen, however, are tolerated in the form of hydrogen peroxide. The method of administration of intravenous hydrogen peroxide is quite simple and can be readily controlled.

In animals very little therapeutic value could be found for intravenous treatment with hydrogen peroxide. It must be recalled, however, that in animals similar poor results are reported for intravenous administration of oxygen, while in man clinically beneficial results are recorded. It, therefore, seems indicated that further cautious clinical experiments might be carried out with intravenous administration of hydrogen peroxide substituted for intravenous administration of oxygen.

SUMMARY

The effect of hydrogen peroxide on the blood of various animals was observed and quantitative determinations of blood catalase activity were made. No deleterious effects were observed other than formation of methemoglobin in those species with very low blood catalase levels.

Tolerance observations on intravenous administration of hydrogen peroxide were made. In the dog and chicken, hydrogen peroxide tolerance was limited by formation of methemoglobin, while in the rabbit and cat by gas embolism. The rabbit was more susceptible to gas embolism than was the cat.

Oxygen in the form of hydrogen peroxide could be administered intravenously in cats twice as fast as in the form of oxygen gas without producing severe embolic signs.

Observations were made in mice on the chronic administration of hydrogen peroxide given subcutaneously twice daily for two weeks. Other than for immediate temporary embolic phenomena and an incidence of local skin ulcers in 25 per cent, essentially no deleterious effects were observed.

Hypoxia was induced in 2 cats by inhalation of 90 per cent nitrous oxide. Small amounts of hydrogen peroxide given intravenously aggravated rather than reduced the hypoxia as shown by arterial blood gas analyses.

Intravenous therapy with hydrogen peroxide for carbon monoxide poisoning, hemorrhage, and chemical toxic shock in 7 cats produced no noteworthy benefit.

Although hydrogen peroxide given intravenously has little or no value in the cat, further investigation of its use in the treatment of certain hypoxic and shock states in man may be indicated.

We wish to express our sincere appreciation for the encouragement and valuable guidance of Professor E. M. K. Geiling throughout the course of this investigation.

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Intraoral Application of Hydrogen Peroxide

As shown in a previous paper and since confirmed by clinical work, hydrogen peroxide (HPO) is resorbed by intact animal and human skin, which makes it possible to introduce oxygen by this means into tissues and circulation and influence cyanoses locally.

Based on these facts and in view of the fact that HPO in concentrations up to 30% is successfully used in the oral cavity for therapeutic or diagnostic purposes, the question was of interest if a clinically significant HPO resorption occurs from the oral cavity, with what dangers this treatment is fraught, and how to explain the general effects observed on numerous patients after rinsing of the mouth with hydrogen peroxide.

According to the available literature, it had to be assumed that HPO is not resorbed by the intact skin, and from mucous and serous membranes only when HPO is introduced into "closed body cavities." Especially for the mucous membrane of the mouth a possibility of HPO resorption and thereby the danger of gas embolism by intraoral HPO application are strongly negated.

Method

The tests were performed on 3 dogs and 17 cats in Chloralose-urethane anesthesia (130 mg or 90 mg kg i.p.resp.) as well as on 5 rabbits of both sexes in urethane anesthesia (1.4 mg/kg.s.c.).

The respiration was transferred via a Y-tube in the trachea to a Marey capsule and further via a lever to Russ-Kymo, so that aspirations caused deflections down and expiration, up. The bloody reading of the arterial blood pressure was performed on the left art. carot. com. via a mercury manometer, with coagulation prevented by counter-infusion of a sodium carbonate solution according to Trendelenburg.

The arterial blood samples for the gas determination were taken under anaerobic cauteries from the right a. femoralis. The O_2 and CO_2 content as

well as the total capacity of the blood were determined manometrically in duplicates according to van Slyke and Neil or to Sendray (micro method) with the help of Na-Aescin.

The application of hydrogen peroxide (in concentrations of from 3-30% starting with Perhydrol Merck) was performed, after pressureless tamponing of the throat with paraffin coated surgical cotton, by application of a small cotton ball drenched with HPO underneath the tongue. After removal of the cotton ball, the area was rinsed with physiological NaCl solution.

For the insufflation of O_2, CO_2 and N_2 into the right v. jug. ext., we used a well tightened system consisting of a 20 ccm syringe with an electrically motivated permanent transfusion apparatus, a threeway stopcock, a no. 20 needle and the necessary hose connections.

Results

After sublingual application of HPO in concentrations of 3-30%, gas bubbles of different sizes were observed in the tongue and jugular veins which quickly flowed towards the heart - beyond the time of application. (fig. 1 and 2). The time between the beginning of the effect and the appearance of visible gas bubbles was subject to major individual fluctuations, but it became shorter and shorter with increasing HPO concentrations and usually took only a few seconds with 30% HPO. The flowing of gas bubbles into the jugular veins could also be observed after application of HPO to other areas of the oral mucous membrane and on the vertically position cat.

When 30% HPO was permitted to act for several minutes on the tongue, the sublingual vessel were in places tautly filled with gas; also, numerous emphysematic areas developed. (fig.1a) While these cushions regressed in a short time, gas remained diffuse shining and plapable in the depth of the tongue for some length of time. If the HPO application was not too intense and not repeated for less than a week, the tongue resumed its original

appearance after temporary hyperemia (fig. 1b).

Intravenous oxygen insufflation and sublingual HPO application have generally identical effects on blood pressure and respiration of all test animals, with rabbits reacting with greatest sensitivity. A short time after the appearance of visible gas bubbles, in the jugular veins (fig.2), the respiration slowly increased; under the conditions of our tests, the amplitudes increased considerably, while the frequency showed mostly only a slight increase. (fig.3). In some tests, irregular snatches of breath showed in between. No noticeable changes of respiration were achieved with HPO below 3%, while definitive reactions could be brought about with 30% HPO within the shortest time (fig. 3).

As expected, uniform relations between the amount of oxygen insufflated per time unit, the HPO concentration, and the increase of respiration were not obtained in our test arrangement. To give an idea of the magnitude of the amount of gas entering the body after intra oral application of HPO, the case of a cat weighing 2.6 kg should be mentioned in whose case the intravenous insufflation of 6.5 ccm oxygen within 5 min (=0.5 ccm/kg min) caused approximately the same changes of respiration, blood pressure, and blood gas conditions as were caused by 5 min sublingual effect of 2 ccm 30% HPO.

The arterial blood pressure remained constant with small amounts of inflowing gas or rose slightly, while under the influence of larger amounts of gas or too fast insufflation it generally rell or became very labile.

Generally similar effects could be achieved by intravenous insufflation of N_2 or CO_2. Corresponding to the coefficients of absorption, significantly smaller amounts of N_2 and considerably larger amounts of CO_2 were endured.

Further increase of the parapulmonary oxygen supply by either insufflation or HPO application lead to the death of the animal with steep drop of blood pressure and sudden stoppage of breathing. In the autopsy of these

cases, we found a right ventricle bloated by light red bloody foam and still beating irregularly; in the large vein trunks near the heart and the pulmonary artery we found numerous gas bubbles. The left ventricle which only contained traces of dark reddish blue blood had stopped in the systole. The lungs showed macroscopically and histologically numerous dys- and atelectatic areas, limited overbloating and hemorrhagia. (Fig.4)

By intravenous oxygen insufflation as well as by sublingual HPO application, the oxygen saturation of the arterial blood was lowered compared to sufficiently constant initial values, while the CO_2 content rose correspondingly. With clearly increased respiration, the oxygen content of the arterial blood in our tests is 4.7-68.6% lower and the CO_2 content 6.9-67.9% higher, dependent on the sensitivity of the animal, then the corresponding values prior to the HPO application.

When large sublethal amounts of oxygen were introduced, the naked eye could see that the blood in the v. jug. ext. had become light red, that in the carotis tube dark blue-red. The values for blood gas returned faster to their initial condition than the respiration. These changes of respiration and blood gas values after intravenous oxygen insufflation or sublingual HPO application occurred also in animals which had been vagotomized on both sides prior to the test.

Discussion

At first, it may seem illogical to bring undiluted Perhydrol into contact with the healthy mucous membrane of the mouth for clinical purposes, since it is generally thought that HPO in high concentration has an etching action, leading to blistering and scabbing. This opinion which looses much of its credibility after comparison of figs. 1a and 1b, could be contradicted by corresponding observations and histologic determinations.

Peter had already shown in 1927 that the white discolorization of HPO

treated human oral mucous membrane is due to the incorporation of minute gas bubbles. V. Brucke and Goldbach came to quite similar results in 1943 on the intestinal mucous membrane, and in 1954, Gross reported about an incident where local emphysema had appeared instead of after intra-oral application of HPO. Hauschild, Ludewig, and Muehlberg showed recently in animal tests that the white discolorization of the tongue after HPO rinses represents emphysema which can regress without leaving anyohistologically recognizable changes. Damage possible after too intensive or too frequent HPO application can be explained by the fact that the tissues may be robbed of their protective catalase activity and that stasis in oxygen filled capillaries and the long lasting gas infiltration may lead to limited nutritional disturbances. The tissue damage described by Huss and Berde after PHO application may possibly be explained now by these facts.

Since we saw gas bubbles streaming toward the heart after intraoral application of HPO (fig.2), it was obvious to think of the comparison to intravenous oxygen insufflation, the advantages and dangers of which have been frequently examined and discussed. We therefore compared in our tests the reactions occurring after intra-oral HPO application with those we were able to observe on the same animal material after insufflation of gas into the vena jugularis externa as Gaertner, Nysten, Stuerz, and Muller had done.

The effort to arrive at a uniform opinion about the respiratory, blood gas, and circulatory reactions caused by intravenous gas supply based on a comparison of authoritative publications must turn out unsatisfactory because numerous factors - frequently not reported or reportable - lead to non-comparable test conditions. Decisive are, e.g.: Size of animal and vein, therefore size and number of O_2 bubbles insufflated within a unit of time, depth of anesthesia, tonus of diaphragm, and sensitivity characteristic to a given species or individual, like the fact that the sensitivity toward pulmonary embolism increases from dog via cat to rabbit. In addition, we had to

consider in our tests the catalase activity which is 0.3 in the dog, but 21 in the cat while at the same time subject to certain variations within the same species, even in the same animal.

The reported observations and comparison results lead effortlessly to the conclusion, that the circulatory, repsiratory, and blood gas changes after intraoral HPO application and after intravenous oxygen insufflation have the same origin: in both cases, O_2 bubbles, visible to the naked eye and of comparable size, flow towards the heart. As the diameter of the capillaries in the human lung alveoles measures approx. 10 μ, and as, according to Curtillet, gas bubbles can never enter through vessels with a diameter of less than 30 μ, it can be expected that numerous lung capillaries become clogged during bubble absorption. The increase of the flow resistance caused by this leads to increased pressure in the small circulation, which in turn causes, aside from gradual use of reserve capillaries, the opening of intra-pulmonary shunts, so that enough blood can flow to the left ventricle to maintain the minute volume. Through this functional right-left shunt, venous blood can directly enter into the main circulation. This causes a change of the arterial environment - the arterial hypoxemia and the pH displacement are less important than the specific action of the CO_2 increase - which stimulates the respiration, mainly in the direction of an increase of amplitude which attempts to balance the acid-base equilibrium by eliminating the CO_2. Without this protective mechanism, it could hardly be explained that oxygen and nitrogen with their relatively small coefficient of absorption (The coefficient of absorption of the blood at 37°C and 760 mm Hg gas pressure are: O_2 - 0.0223; CO_2- 0.520; N_2 - 0.0119) can be insufflated intravenously for some length of time without dangerous impairment of the circulation. Overfast insufflation of larger amounts of oxygen leads to displacement of the pulmonary stem, preventing the functioning of the reserve

capillaries and the different anastomoses, so that the left ventricle beats empty. In contrast, during moderate O_2 insufflation for too long a time, or HPO application, it appears that there is an exhaustion of the pulmonary and cardiac protective mechanisms and reserve powers.

The existence of interpulmonary shunts was postulated by Hochrein on the basis of physiological considerations and anatomically proven by v. Hayek on man, and by Castigli on different mammals. The knowledge about the functional and pathogenic importance of such shunts, however, is still very fragmentary.

The main circuit of the pulmonary circulation, formed by the capillaries of the alveolary septs, can if needed be circumvented by secondary circuits and shunts, so that up to about 20% of the blood of the a. pulmonalis - in the presence of atelectases even up to 50% - can be withdrawn from the pulmonary gas exchange. V. Hayek even proved histologically that lung artery and vein are connected with each other via the so called block arteries and the bronchial network surrounding the smaller bronchi. Since the filling of this bronchial vein network is related to the width of the enclosed bronchus and thereby to the air supply of the corresponding lung segment, the suggestion is given that the above mentioned dys- or atelectases are caused by the compression of bronchi by the overcrowded bronchial vein network. - Pulmonary and bronchial arteries which, according to Rivier show already wide diameter anostomoses near the hilus, are interconnected via the extra-muscular closable block arteris. The flow direction in these anastomoses depends largely upon the pressure conditions in the connected arteries. The broncho-pulmonary flow direction is reversed when - as is possible in multiple pulmonary embolism - a sufficiently large increase of pressure takes place in the pulmonary circulation, when the embolic closure lie peripherally to the mouth of the block arteries, and when the block arteries are intact.

A. and v. pulmonalis have another shunt in the so-called giant capillary network of the pleura, whose importance for the general circulation consists in the fact that, due to the width of the giant capillaries, the blood is able to flow into the veins against a lower resistance.

It can be assumed that differential sensitivity against multiple pulmonary embolism is largely due to individual formation and efficiency of the reserve capillaries and shunts. It has also to be considered that large diameter intrapulmonar a.v.-anastomoses are the common route for gas bubbles from the right to the left ventricle. Prinz metall and coworkers were able to prove on several animals that glas balls of 100-250 μ diam can occasionally pass the pulmonary circulation. Cain mentions similar suppositions concerning the transport of tumor cells and the transfer of fat droplets during fat embolism.

The danger of a peripheral arterial embolism after inexpert intraoral HPO application is therefore also present when the foramen ovale is closed.

The hemorrhages in the lung during gas embolism which we observed in agreement with Schaefer and Goepfert, like the localized muscle figer damage in the right ventricle wall, as described by Meesen, can be tentatively explained by the increased pressure in the pulmonary arterial system caused by intrapulmonary obstruction.

After observing, in agreement with Dick, that the reinforced respiration persists longer than the alterations of the arterial blood gas values caused by the embolism, we think we are warranged in assuming as its cause the persistance (hysteresis) of the stimulation of the respiratory center.

Gollwitzer and Pinotti had already made the observation on dogs 15 yrs ago that the respiratory volume remains elevated for several minutes after completion of CO_2 breathing, ven though the reaction of the arterial blood is displaced toward the alkaline side. In ingenious tests and interpretations, the authors proved that the continuation of the increased respira-

tory activity is not due to the continued hematogenous carbonic acid stimulus, nor is it due to local CO_2 retention in the respiratory center of the glomus caroticum, and that the excitability of the respiratory center is lowered rather than increased. Rather, the continued increased respiratory activity was proven to be caused by the fact that the respiratory center remained at an increased state of activity. This endurance of the central excitation which has been named, after a proposal of Bethes, hysteresis of the respiratory center is definitively connected with the chemical excitation of the respiratory center(delayed inactivation of intermediate compounds, like acetyl choline, or delayed regeneration of ionic shifts?), but not in any proven way with the reflectoric excitation of the respiratory center by the chemo receptors of the glomus caroticum.

For reasons of clarity, the reflex phenomena connected with pulmonary embolism were not included in this discussion, especially since embolization after vagotomy lead to the same changes as in intact animals. But our test results on healthy, in part very embolism-resistant animals which, in addition, were in deep anesthesia during the tests, and who except for one cat - were treated in horizontal position, should not be immediately transferred to conscious sitting or standing patients. With improper intraoral HPO application it would be easily possible, that, due to overly large gas bubble influx,excessive reflex occurrances - like the pulmonary release reflex encountered by Schwiegk, which we did not need to anticipate in the case of the cat - may lead to collapse or even death.

Hormonal reactions also interfere in this interplay of numerous reflectoric, hemodynamic, and chemical processes during pulmonary empolism. Little is known at present about in how far histamine, adrenaline, and serotonine, liberated by the multiple embolism, participate in the "switch operation" of the intra-pulmonary shunts and in the appearance of pathologically-anatomically recognizable changes in lung and heart. According to our

observations, HPO is resorbed by the mucous membranes of the mouth, but the oxygen that is delivered to the organism by this means, the permissible total of which is here, in any case, less than 1/10 of the requirement at rest, is retained in the form of bubbles by the lung as by a filter. Corresponding results are reported by v. Bruecke and Goldbach, who, similar to Buchheim and Assmuth, infused HPO into the small intestine and thereby introduced oxygen through the intestinal wall into the circulation. Corresponding to the lung in intraoral HPO application, the liver acted as the filter in enteric HPO infusion in a massive gas embolism of the portal vein. A parapulmonary oxygen therapy with HPO is therefore impossible either via oral cavity or via intestinal mucous membrane. It is the more peculiar that intravenous oxygen insufflations, intravenous HPO injections, as well as oral rinses with undiluted Perhydrol, and peroral doses of low-percentage HPO are judged more acceptable by clinical experts than should be expected from corresponding animal tests. - Schueler who in more than 30 years has treated many thousands of patients with Perhydrol mouth rinses, observed remarkable "stimulating effects on nervous system and circulation." If such opinions are not attributed to suggestive effects, the earlier reports might be the basis for the hypothesis that the CO_2 retention may lead transitorily to a dearly payed for change of blood distribution favoring heart and brain, that maybe even the coronary blood supply may be improved by a (subclinical) pulmonary embolism.

We come to the following practical conclusions: In oral rinses with concentrated HPO which cause strong salivation, the HPO is thereby diluted and at times its resorption interfered by catalase containing material like blood and puss, but in practical application more extensive mucous membrane areas that are better able to resorb the HPO come in contact with this material than in our tests. If the patient follows Schueler's advise and

puts undiluted Perhydrol in his mouth and rinses with compressed lips, the HPO resorption, the mechanism of which we do not yet completely understand, may be considerably enhanced by the increased pressure inside the oral cavity, according to classical conceptions of "closed cavities," with the resulting danger comparable to oxygen insufflation into the veins of the neck. (It should be considered that 1 ml 30% HPO may liberate approx. 100 ccm O_2). For this reason and the possibilities of local disturbances of enzymes and nutrients mentioned by Hauschild, Ludewig, and Muehlberg, undiluted Pherhydrol should be used only at intervals of several days, in smallest amounts, and only for the treatment of limited mucous membrane areas with open mouth and for no more than 1 min. When used in this way, the undesirable side-effects of intraoral HPO treatment would recede behind the advantages of local oxygen enrichment.

Summary

Based on previous research as well as diagnostic and therapeutic successes with intra-oral application of undiluted Perhydrol, the question was examined if a clinically significant resorption of H202 by the oral mucous membranes takes place.

By sublingual application of HPO in concentrations from 3-30% concentrations to dogs, cats, and rabbits, the same changes were precipitated as were observed after intravenous insufflation of oxygen:

1) Transport of well visible gas bubbles in the draining veins.
2) Increased respiratory activity: considerable increase of the amplitudes, minimal changes of frequency.
3) For the duration of sufficient passage of blood through the lungs, no increased lowering of the arterial blood pressure.
4) Arterial hypoxemia and CO_2 retention.
5) Macroscopically and microscopically detectable lung damage (hemorrhagia, dys- and atelectases).

In the course of continued Perhydrol effect, limited, initially reversible emphysema of the mucous membranes were formed, ending in death by pulmonary gas embolism.

The theoretical connections of these phenomena and their consequences in practical work are discussed.

Literature

(no titles given)

Fig. 1 a and b Underside of tongue, dog (Test no. 26) a) After 3 min reaction time with 30% H202; Hearth-shaped and peripheral emphysema, gas bubbles in the sublingual veins b) 12 hrs later: Embhysema resorbed, light hyperemia

Fig. 2 Vena Jugularis sin., dog (test No. 26) In intraoral application of H202, numerous gas bubbles of different sizes migrate to the heart in the upper layers of the blood stream.

Fig. 3 Rabbit (German Gross-Silber No.160, 2.4 kg, urethane narcosis (1.4 g/kg s.c.) From above to below: Arterial blood gas values (Total capacity: 14.14 vol.%), respiration with indication of frequency, blood pressure in the a. carotis, _____sublingual effect of H202 (30%) time record: 1 min

Fig. 4 a and b Rabbit (German Gross-Silber, test no. 16) a) normal lung tissue (formalin fixed)11E dyed. Microsc. magnif. 25X b) Dys-telecatic area of the same lung, hearth-shaped over-bloating of alveoli and alveolar ducts after intra-oral application of hydrogen peroxide.

(Translated by Carl Demrick Associates,Inc/CG/t)

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Aus dem Pharmakologischen Institut der Karl-Marx Universität Leipzig
(Direktor: Prof. Dr. med. F. Hauschild)

Zur intraoralen Anwendung von Wasserstoffperoxyd

Von

Reinhard Ludewig

Mit 4 Textabbildungen

(Eingegangen am 21. Januar 1959)

Wie wir in einer vorangehenden Arbeit zeigen konnten[41] und wie indessen auch von klinischer Seite bestätigt wurde (Sprung u. Ludewig[65]), wird Wasserstoffperoxyd (WPO) von tierischer sowie von menschlicher intakter Haut resorbiert, so daß man auf diesem Wege Sauerstoff ins Gewebe und die Blutbahn einbringen und Cyanosen lokal beeinflussen kann.

Auf Grund dieser Feststellungen und im Hinblick auf die Tatsache, daß WPO in Konzentrationen bis zu 30% zu therapeutischen und diagnostischen Zwecken auch in der Mundhöhle erfolgreich angewandt wird (Andresen[2], Berg[5], Jessen[35], Peter[52], Scholtz[58], Schüler[59], Wendel[71]), interessierte uns die Beantwortung der Fragen, ob auch von der Mundhöhle aus eine klinisch bedeutsame WPO-Resorption stattfindet, mit welchen Gefahren diese Anwendungsweise behaftet ist und wie sich die von Schüler[59c] an zahlreichen Patienten beobachteten Allgemeinwirkungen nach Mundspülung mit Perhydrol erklären.

Nach der von uns übersehenen Literatur mußte vordem angenommen werden, daß WPO von der intakten Haut aus nicht (Magnus[43]) und von mucösen und serösen Häuten nur dann in beträchtlichem Umfange resorbiert wird, wenn man WPO in „geschlossene Körperhöhlen" einbringt (v. Bruns[9], Kühner[36], Meyer u. Gottlieb[46], Petri[53]). Speziell für die Mundschleimhaut wird die Möglichkeit einer WPO-Resorption und damit die Gefahr einer Gasembolie durch intraorale WPO-Applikation ausdrücklich in Abrede gestellt (Schüler[59a]).

Methodik

Die Versuche wurden an 3 Hunden und 17 Katzen in Chloralose-Urethannarkose (130 mg bzw. 90 mg/kg i.p.) sowie an 5 Kaninchen beiderlei Geschlechts in Urethannarkose (1,4/kg, s.c.) durchgeführt.

Die *Atmung* wurde über eine in die Trachea eingebundene Y-Kanüle auf eine Mareysche Kapsel und weiter mittels Hebel auf Ruß-Kymo übertragen, so daß Inspirationen Ausschläge nach unten, Exspirationen solche nach oben hervorriefen.

Die blutige Messung des arteriellen *Blutdrucks* erfolgte in der linken Art. carot. com. über ein Quecksilbermanometer, wobei die Blutgerinnung durch Gegeninfusion einer Natriumcarbonatlösung nach TRENDELENBURG verhindert wurde.

Die arteriellen Blutproben zur *Blutgasbestimmung* wurden unter anaeroben Kautelen aus der rechten A. femoralis entnommen. Der O_2- und der CO_2-Gehalt sowie die Totalkapazität des Blutes wurden manometrisch in Doppelbestimmungen nach VAN SLYKE u. NEIL[64] bzw. nach SENDROY[62] (Mikromethode) unter Verwendung von Na-Aescin (LUDEWIG u. RÖMISCH[42]) ermittelt.

Die Applikation von Wasserstoffperoxyd (in Konzentrationen von 3–30%, ausgehend vom Perhydrol der Firma Merck) erfolgte nach druckloser Tamponade des Rachens mit paraffinierter Watte durch Auflegen eines WPO-getränkten kleinen Wattebausches auf die Zungenunterseite. Nach Entfernung dieses Wattebausches wurde jeweils mit physiologischer Kochsalzlösung nachgespült.

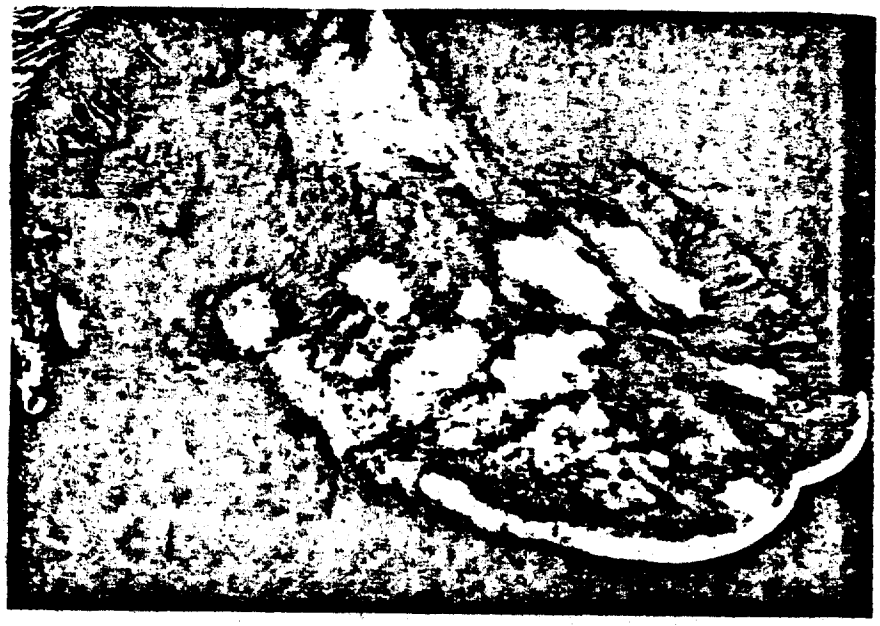
Zu den in die rechte V. jug. ext. verabreichten Insufflationen von O_2, CO_2 und N_2 wurde ein gut abgedichtetes System verwendet, das aus einer 20 cm³ Rekordspritze in einem elektrisch betriebenen Dauertransfusionsgerät, einem Dreiwegehahn, einer 20er Kanüle und den erforderlichen Schlauchverbindungen bestand.

Ergebnisse

Nach sublingualer Applikation von WPO in Konzentrationen von 3–30% wurden in den Zungen- und Jugularvenen Gasblasen unterschiedlicher Größe beobachtet, die dem Herzen rasch – über die Applikationsdauer hinaus – zuströmten (Abb. 1 u. 2). Die Zeitspanne vom Einwirkungsbeginn bis zum Auftreten sichtbarer Gasblasen war großen individuellen Schwankungen unterworfen, jedoch wurde sie mit steigender WPO-Konzentration immer kürzer und betrug bei Anwendung von 30%igem WPO zumeist nur einige Sekunden. Das Einströmen von Gasblasen in die Jugularvenen konnte auch nach WPO-Applikation auf die übrigen Bereiche der Mundschleimhaut und an der vertikal gestellten Katze beobachtet werden.

Wurde dem 30%igen WPO Gelegenheit gegeben, über mehrere Minuten auf die Zunge einzuwirken, so waren die sublingualen Gefäße streckenweise strotzend mit Gas gefüllt; zudem bildeten sich zahlreiche Emphysembezirke aus (Abb. 1a). Während sich diese Polster bald zurückbildeten, war in der Tiefe der Zunge noch längere Zeit Gas diffus durchschimmernd und palpabel. War die Anwendung des WPO nicht zu intensiv und wurde sie vor einer Woche nicht wiederholt, so nahm die Zunge nach vorübergehender Hyperämie ihr ursprüngliches Aussehen wieder an (Abb. 1b).

Intravenöse Sauerstoffinsufflation und sublinguale WPO-Anwendung zeigten im wesentlichen gleichartige Wirkung auf *Blutdruck* und *Atmung* aller Versuchstiere, wobei Kaninchen am empfindlichsten reagierten. Kurz nach dem ersten Auftreten gut sichtbarer Gasblasen in den Vv. jug. (Abb. 2) nahm die Atemtätigkeit allmählich zu, indem bei unseren Versuchsbedingungen die Amplituden sich erheblich vergrößerten, während die Frequenz zumeist nur eine geringe Steigerung erfuhr (Abb. 3). In



a

b

Abb. 1a u. b. Zungenunterseite. Hund. Vers. Nr. 261. a Nach 5 min Einwirkungsdauer von 90 %igem H_2O_2: herdförmige und randständige Emphysembildung, Gasblasen in den sublingualen Venen. b 12 std später: Emphysome rückgebildet, leichte Hyperämie.

einigen Versuchen schalteten sich unregelmäßig Schnappatmungszüge zwischen. Mit WPO-Konzentrationen unter 3% waren keine faßbaren Atemveränderungen mehr zu erzielen, während mit 30%igem WPO

Abb. 2. V. jug. ext. sin., Hund (Vers. Nr. 26). Unter intraoraler Anwendung von H_2O_2 wandern zahlreiche Gasblasen unterschiedlicher Größe in den oberen Schichten des Blutstromes zum Herzen.

innerhalb kürzester Zeit stets deutliche Atemreaktionen ausgelöst wurden (Abb. 3).

Einheitliche Relationen zwischen der pro Zeiteinheit insufflierten Sauerstoffmenge, der angewandten WPO-Konzentration und dem Zuwachs der Atemgröße waren erwartungsgemäß bei unserer Versuchsanordnung nicht gegeben. Um jedoch eine Vorstellung von der Größenordnung der einströmenden Gasmenge nach intraoraler WPO-Anwendung zu vermitteln, sei beispielsweise eine 2.6 kg schwere Katze angeführt, bei der die intravenöse Insufflation von 6.5 cm^3 Sauerstoff innerhalb von 5 min (= 0.5 $cm^3 \cdot kg \cdot min$) annähernd die gleichen Veränderungen von Atmung, Blutdruck- und Blutgasverhältnissen auslöste wie sie durch 5 min sublinguale Einwirkung von 2 cm^3 30%igen WPOs hervorgerufen wurden.

Der arterielle Blutdruck blieb bei kleinen Mengen einströmenden Gases unverändert oder stieg leicht an, während er unter dem Einfluß größerer Gasmengen oder bei zu schneller Insufflation im allgemeinen abfiel oder sehr labil wurde.

Im wesentlichen gleichartige Ergebnisse ließen sich durch intravenöse Insufflationen von N_2 oder CO_2 erzielen. Dem Absorptionskoeffizienten entsprechend wurden vom N_2 wesentlich kleinere und vom CO_2 erheblich größere Mengen vertragen (vgl. auch M. MÜLLER[17]).

Weitere Steigerung der parapulmonalen Sauerstoffzufuhr durch Insufflation oder WPO-Anwendung führte unter steilem Blutdruckabfall

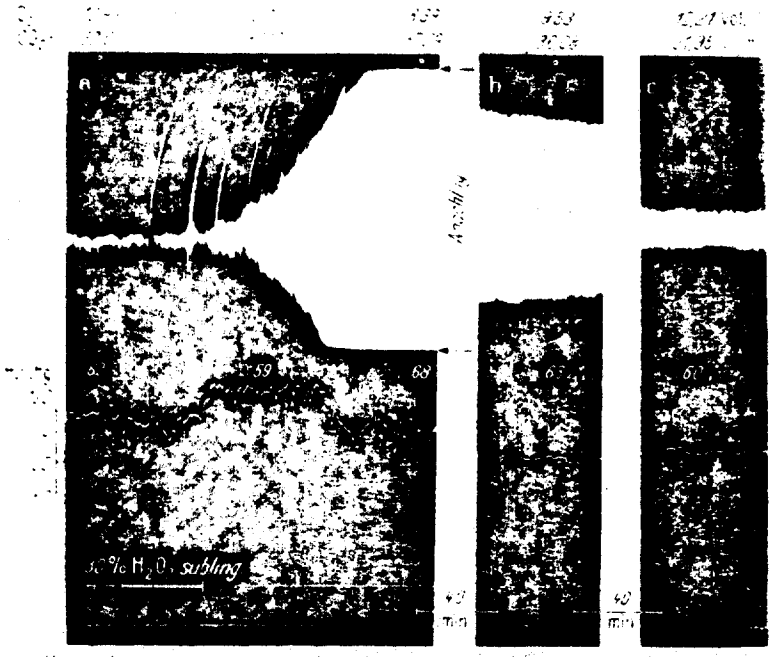


Abb. 3. Kaninchen, Herz-Lunge-Gleichsilber, Nr. 16, 2,4 kg; Urethan-Na-Kose (1,4 g/kg) [illegible]; von oben nach unten: Arterielle Blutgaswerte (Totalkapaz.: 11,11 Vol. %), Atmung mit Frequenzangaben, Blutdruck in der Aorta, [illegible] sublinguale Einwirkung von H_2O_2 (30 %), Zeitschreibung: 1 min

und plötzlichem Atemstillstand rasch zum Tode des Tieres. In diesen Fällen zeigte sich bei der Obduktion ein von hellrotem Blutschaum geblähtes, noch unregelmäßig schlagendes rechtes Herz; in den Coronargefäßen sowie in den großen, herznahen Venenstämmen und der A. pulmonalis fanden sich zahlreiche Gasblasen. Das linke Herz, in dem sich nur noch Reste dunkelblauroten Blutes fanden, stand in Systole still. Die Lungen ließen makroskopisch und *histologisch* zahlreiche dys- und atelektatische Bezirke, umschriebene Überblähungen und Hämorrhagien erkennen (Abb. 4)[*].

Durch intravenöse Sauerstoffinsufflation wurde ebenso wie durch sublinguale WPO-Anwendung die *Sauerstoffsättigung* des arteriellen

* Der Hochschulfilmbildstelle unserer Universität und Herrn Dr. MÜLLER danke ich für die Mithilfe bei Anfertigung der Photographien und der histologischen Präparate.

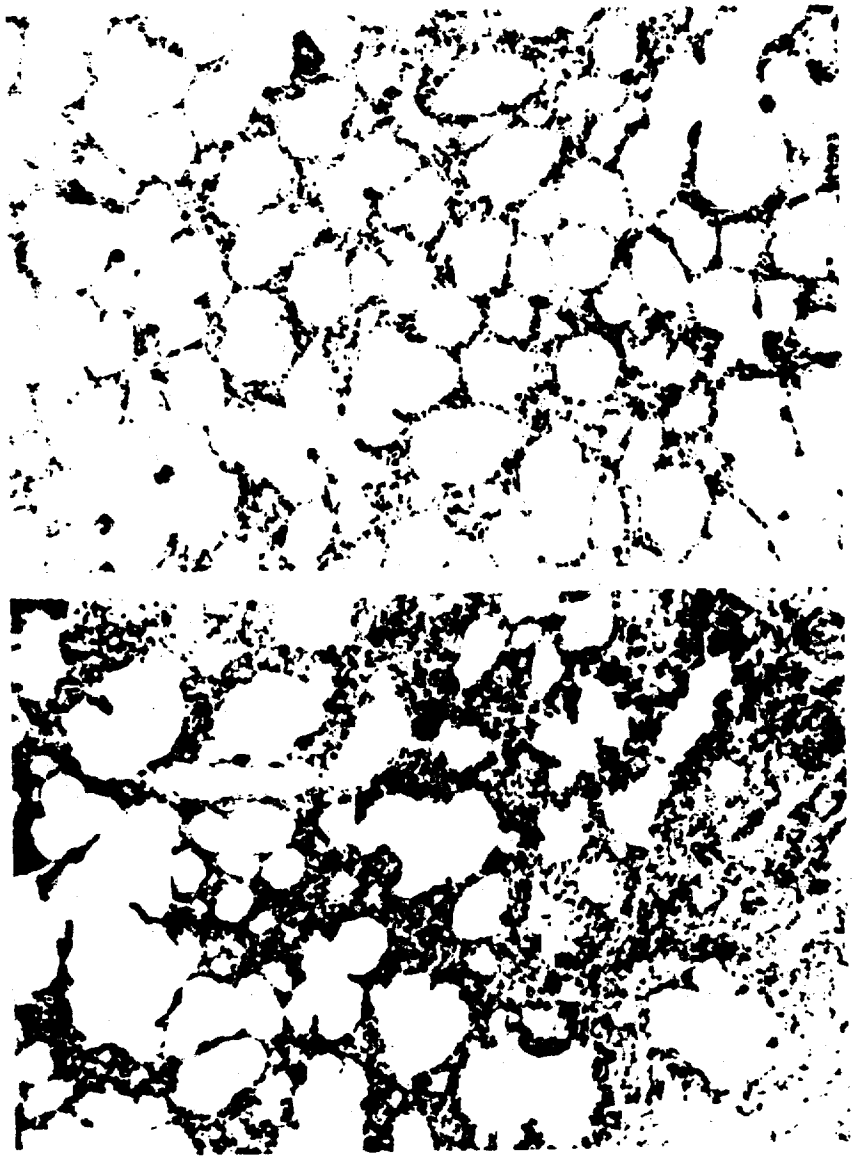


Abb. 4a u. b. Kaninchen (Deutscher Großsilber, Vers. Nr. 16). a) Normales Lungengewebe (formalinfixiert, H.E.-gefärbt; mikrosk. Vergr. 25fach). b) Dystelektatischer Bezirk der gleichen Lunge, herdförmige Überblähungen von Alveolen und Alveolargängen nach intraoraler Anwendung von Wasserstoffperoxyd (s. Text).

Blutes gegenüber hinreichend konstanten Ausgangswerten herabgesetzt, wogegen der CO_2-Gehalt entsprechend anstieg. Während deutlich erhöhter Atemtätigkeit lag in unseren Versuchen der Sauerstoffgehalt des

arteriellen Blutes je nach Empfindlichkeit des Tieres um 4.7–58.6% niedriger und der CO_2-Gehalt um 6.9 bis zu 67.9% höher als der entsprechende Blutgasgehalt vor der Insufflation bzw. WPO-Anwendung.

Bei Zufuhr großer subletaler Sauerstoffmengen war mit bloßem Auge bereits sehr gut zu beobachten, daß das Blut in der V. jug. ext. hellrot, in der Carotiskanüle dagegen dunkelblaurot wurde. Die Blutgaswerte erreichten früher als die Atemtätigkeit ihren Ausgangswert. Die geschilderten Veränderungen der Atmung und der Blutgaswerte nach intravenöser Sauerstoffinsufflation oder sublingualer WPO-Anwendung traten auch an den Tieren auf, die vorher beiderseits vagotomiert worden waren.

Diskussion

Zunächst mag es abwegig erscheinen, unverdünntes Perhydrol zu klinischen Zwecken überhaupt mit der gesunden Mundschleimhaut in Berührung zu bringen, da noch vielfach die Ansicht besteht, hochprozentiges WPO wirke *ätzend*, führe also zu Korrosion oder Verschorfung. Diese Auffassung, die schon bei vergleichender Betrachtung der Abb. 1a und 1b an Glaubwürdigkeit verliert, konnte jedoch durch entsprechende Beobachtungen und histologische Untersuchungen widerlegt werden.

Peter[58] hatte bereits 1927 gezeigt, daß die Weißfärbung WPO-behandelter menschlicher Mundschleimhaut auf der Einlagerung kleinster Gasblasen beruht. 1943 kamen v. Brücke u. Goldbach[8] an der Darmschleimhaut zu ganz entsprechenden Ergebnissen und 1953 berichtete Gross[21] über einen Zwischenfall, bei dem nach intraoraler Anwendung von WPO statt Verätzungen lokale Emphyseme aufgetreten waren. Tierexperimentell wurde kürzlich von Hauschild, Ludewig u. Muhlberg[27] gezeigt, daß die an der Zunge durch WPO-Bespülung erzeugte Weißfärbung Emphysemen entspricht, die sich zurückbilden können, ohne wesentliche histologisch faßbare Veränderungen zu hinterlassen. Die nach zu intensiver oder zu auch wiederholter WPO-Anwendung möglichen Schädigungen sind darauf zurückzuführen, daß die behandelten Gewebsstrukturen ihrer schützenden Katalase aktivität beraubt werden und daß es infolge der Stase in sauerstoffgefüllten Capillaren und der lang anhaltenden Gasinfiltration zu umschriebenen Ernährungsstörungen kommen kann. Wahrscheinlich lassen sich auf diese Weise nachträglich auch die von Hüssl und v. Berdel beschriebenen Gewebsschädigungen nach WPO-Anwendung erklären.

Da wir nach intraoraler WPO-Anwendung Gasblasen dem Herzen zuströmen sahen (Abb. 2), lag der *Vergleich mit der intravenösen Sauerstoffinsufflation* nahe, deren Nutzen und Gefahren vielfach untersucht und diskutiert wurden. Wir verglichen daher unter unseren Versuchsbedingungen die nach intraoraler WPO-Anwendung auftretenden Reaktionen mit denen, die wir am gleichen Tiermaterial nach Insufflation von Gas in die Vena jugularis externa, in die auch Gaertner[19], Nysten[50], Stuerz[66] und Müller[17] insufflierten, beobachten konnten.

Die Bemühung, durch Vergleich maßgeblicher Veröffentlichungen zu einer einheitlichen Ansicht über die im Gefolge intravenöser Gaszufuhr auftretenden Atmungs-, Blutgas- und Kreislaufreaktionen zu kommen, muß deshalb unbefriedigend

verlaufen, weil zahlreiche — oft nicht angegebene oder anzugebende — Faktoren miteinander *unvergleichbare Versuchsbedingungen* schaffen. Ausschlaggebend sind beispielsweise: Tier- und Venengröße bzw. Größe und Zahl der innerhalb einer Zeiteinheit insufflierten O_2-Blasen (BOURNE u. SMITH[7]), Injektionsort (LORINCZ und Mitarbeiter[40]), Narkosetiefe (MATTHES[44], SCHWIEGK[61], WHITTERIDGE[74]), Zwerchfellton (HESS[30]) und an Species und Individuum gebundene Empfindlichkeit so nimmt z. B. die Empfindlichkeit gegen Pulmonalembolie vom Hund über die Katze zum Kaninchen zu (GUNDERMANN[24], LORINCZ und Mitarbeiter[40], SINGH[63], SCHAEFER u. GÖPFERT[57], WHITTERIDGE[74]). Bei unseren Versuchen muß zudem noch die Katalaseaktivität in Betracht gezogen werden, die beim Hund etwa bei 0,3 und bei der Katze bei ca. 21 liegt (LORINCZ und Mitarbeiter[40]) und die auch innerhalb der gleichen Species, ja sogar an ein und demselben Tier gewissen Schwankungen unterworfen ist (WALTER u. HELLER[70]).

Die mitgeteilten Beobachtungen und Vergleichsergebnisse lassen zwanglos den Schluß zu, daß den nach intraoraler WPO-Anwendung und intravenöser Sauerstoffeinblasung auftretenden Kreislauf-, Atmungs- und Blutgasveränderungen die gleiche Ursache zugrunde liegt: In beiden Fällen strömen dem Herzen mit bloßem Auge sichtbare O_2-Blasen vergleichbarer Größe zu. Da der Durchmesser der Capillaren in den Lungenalveolen (des Menschen) nur etwa 10 μ beträgt (HAYEK[28d]) und nach CURTILLET[11] Gasblasen nie durch Gefäße treten können, deren Durchmesser kleiner als 30 μ ist, tritt erwartungsgemäß für die Dauer der Blasenabsorption ein Verschluß zahlreicher Lungencapillaren ein. Die dadurch hervorgerufene Zunahme des Strömungswiderstandes führt zur Drucksteigerung im kleinen Kreislauf, die ihrerseits neben allmählichem Infunktiontreten von *Reservecapillaren* (EULER[18], GAIDA[20], HACHMEISTER[25]) die Eröffnung *intrapulmonaler Kurzschlußwege* hervorruft, so daß dem linken Herzen zur Aufrechterhaltung des Minutenvolumens noch genügend Blut zufließen kann. Durch diesen funktionellen Rechts-Links-Shunt kann venöses Blut direkt in den großen Kreislauf übertreten. Die dadurch bedingte Änderung des arteriellen Blutmilieus — die arterielle Hypoxämie (BOURNE u. SMITH[7], DICK[15], GRODINS und Mitarbeiter[23], HALMAGYI[26], LORINCZ und Mitarbeiter[40], NIDEN u. AVIADO[48], SCHAEFER u. GÖPFERT[57], WESTON u. KAREL[73]) und die p_H-Verschiebung treten hinter der spezifischen Wirkung der Kohlensäurezunahme zurück (BÜCHER[10], LAQUEUR u. VERZAR[38], REIN[55]) — bedingt eine Anregung der Atemtätigkeit, die sich vorwiegend in der Amplitudenvergrößerung ausdrückt und mit der versucht wird, durch Ausscheidung von Kohlensäure das Säure-Basen-Gleichgewicht auszubalanzieren. Ohne diese Schutzmechanismen wäre es bei den verhältnismäßig kleinen Absorptionskoeffizienten des Sauerstoffes und des Stickstoffs* auch kaum erklärbar, daß diese Gase intravenös über längere Zeit ohne lebensgefährliche Beeinträchtigung des Kreislaufs (M. MÜLLER[47]) eingeblasen

* Die Absorptionskoeffizienten des Blutes sind bei 37°C und 760 mm Hg Gasdruck für O_2: 0,0223, CO_2: 0,520, N_2: 0,0119.

werden können. — Zu schnelles Einströmen großer Sauerstoffmengen führt zur Verlegung des Pulmonalisstammes, so daß die Reservecapillaren und die verschiedenen Anastomosen gar nicht erst in Funktion treten können und das linke Herz leer schlägt. Dagegen scheint bei zu lang anhaltender mäßiger O_2-Insufflation bzw. WPO-Anwendung eine Erschöpfung der pulmonalen (HACHMEISTER[25]) und kardialen (SCHUMACHER u. JUHN[60]) Schutzmechanismen und Reservekräfte einzutreten.

Die Existenz *intrapulmonaler Kurzschlußwege* wurde von HOCHREIN[31] auf Grund physiologischer Überlegungen postuliert und durch v. HAYEK[28] am Menschen sowie von CASTIGLI[13] an verschiedenen Säugetieren anatomisch nachgewiesen. Die Vorstellungen über die funktionelle und pathogenetische Bedeutung derartiger Kurzschlüsse sind derzeit jedoch noch sehr lückenhaft (CAIN[12]).

Der Hauptschluß des Lungenkreislaufes, der durch die Capillaren der Alveolarsepten gebildet wird, kann im Bedarfsfalle über Kurz- und Nebenschlüsse umgangen werden (v. HAYEK[28]), so daß bis zu etwa 20% von der Blutmenge der A. pulmonalis — bei Vorliegen von Atelektasen sogar bis zu 50% (BERGGREN[6]) — dem pulmonalen Gasaustausch entzogen werden kann. v. HAYEK[28a] wies histologisch nach, daß Lungenarterie und -vene über sog. Sperrarterien und das die kleinen Bronchien umgebende bronchiale Venennetz miteinander in Verbindung stehen. Da die Füllung dieses bronchialen Venennetzes mit der Weite des umschlossenen Bronchus und dadurch mit der Luftversorgung des zugehörigen Lungenbezirkes in Zusammenhang steht (v. HAYEK[28a]), liegt die Vermutung nahe, daß die oben erwähnten Dys- und Atelektasen die Folgen von Bronchuskompressionen durch überfüllte bronchiale Venennetze sind. — Auch Pulmonal- und Bronchialarterien, die nach RIVIER[56] bereits in Hilusnähe weitlumige Anastomosen aufweisen, sind über die besonders muskelstarken, verschließbaren Sperrarterien miteinander verbunden (v. HAYEK[28b,c,d]). Die Durchströmungsrichtung in diesen Anastomosen hängt weitgehend von den Druckverhältnissen in den verbundenen Arterien ab (CAIN[12], DÜTSCH[16], v. HAYEK[28c], LAPP[27]). Die bronchopulmonale Strömungsrichtung wird umgekehrt, wenn — wie bei multipler Lungenembolie möglich — eine ausreichende Druckerhöhung im Lungenkreislauf eintritt, wenn die embolischen Verschlüsse peripher der Sperrarterien-Mündung sitzen und wenn die Sperrarterien intakt sind (CAIN[12]).

A. und V. pulmonalis besitzen in dem sog. Riesencapillarnetz der Pleura außerdem noch einen Nebenschluß, dessen Bedeutung für den Gesamtkreislauf darin liegt, daß durch die Weite der Riesencapillaren das Blut gegen einen geringeren Widerstand in die Venen strömen kann (v. HAYEK[28c,d]).

Es ist anzunehmen, daß von der individuellen Ausbildung und Funktionstüchtigkeit der Reservecapillaren und Kurzschlußwege die unterschiedliche Empfindlichkeit gegen multiple Lungenembolie weitgehend bestimmt wird. Auch muß in Betracht gezogen werden, daß weitlumige, intrapulmonale a.v.-Anastomosen der vielgesuchte Weg sind, den Gasblasen vom rechten zum linken Herzen wandern können. So konnten PRINZMETALL und Mitarbeiter[54] an verschiedenen Tieren nachweisen, daß Glaskugeln von 100—250 μ Durchmesser den Lungenkreislauf mitunter passieren können. Ähnliche Vermutungen äußert CAIN[12] bezüglich der Verschleppung von Tumorzellen und des Übertretens von Fett-Tröpfchen bei der Fettembolie.

Die Gefahr einer peripheren arteriellen Embolie nach unsachgemäßer intraoraler WPO-Anwendung ist also auch dann gegeben, wenn das Foramen ovale geschlossen ist.

Die in der Lunge auch von uns bei Gasembolie — in Übereinstimmung mit SCHAEFER u. GÖPFERT[57] — beobachteten Hämorrhagien sind annehmbar ebenso wie die von MEESEN[45] beschriebenen, vorwiegend in der rechten Herzkammerwand lokalisierten Muskelfaserschädigungen hauptsächlich auf die im Gefolge intrapulmonaler Stauung auftretende Druckerhöhung im Pulmonalarteriensystem zurückzuführen.

Wenn wir in Übereinstimmung mit DICK[15] beobachten konnten, daß die verstärkte Atemtätigkeit länger anhält als die durch die Embolie hervorgerufenen Veränderungen der arteriellen Blutgaswerte, so glauben wir als Ursache dafür die *Nachdauer (Hysteresis) der Erregung des Atemzentrums* annehmen zu dürfen.

GOLLWITZER u. PINOTTI[22] hatten schon vor 15 Jahren an Hunden die Beobachtung gemacht, daß das Atemvolumen nach Beendigung einer Kohlensäurebeatmung mehrere Minuten noch erhöht bleibt, obwohl sich die Reaktion des arteriellen Blutes nach der alkalischen Seite verschiebt. In scharfsinnig angelegten Untersuchungen und Überlegungen konnten die Autoren zeigen, daß die Fortdauer der erhöhten Atemtätigkeit weder auf dem Fortbestehen eines hämatogenen Kohlensäurereizes noch auf einer örtlichen Kohlensäureretention im Atemzentrum oder im Glomus caroticum beruht und daß die Erregbarkeit des Atemzentrums eher herabgesetzt als gesteigert ist. Als Ursache für die Fortdauer der erhöhten Atemtätigkeit konnte vielmehr ein Verharren des Atemzentrums in einem erhöhten Tätigkeitszustand nachgewiesen werden. Diese Nachdauer der zentralen Erregung, die nach einem Vorschlag BETHES als Hysteresis des Atemzentrums bezeichnet wird, ist maßgebend an die chemische Erregung des Atemzentrums (verspätete Inaktivierung von Übertragerstoffen — wie Azetylcholin — oder verspätete Zuckbildung von ionalen Verschiebungen?) nicht jedoch nachweisbar an die reflektorische Erregung des Atemzentrums von den Chemoreceptoren des Glomus caroticum aus gebunden.

Aus Gründen der Übersicht wurden die unter Lungenembolie auftretenden *Reflexvorgänge* bisher in die Diskussion nicht einbezogen, zumal die von uns herangezogenen Kriterien auch nach Vagotomie durch die Embolisierung im wesentlichen die gleichen Veränderungen erfahren wie an intakten Tieren. Immerhin aber dürfen die Untersuchungsergebnisse von gesunden, teilweise sehr embolieresistenten Tieren, die zudem bei den Versuchen tief narkotisiert waren und — bis auf eine Katze — in horizontaler Lage behandelt wurden, nicht ohne weiteres auf den wachen, sitzenden oder stehenden Patienten übertragen werden. Bei unsachgemäßer intraoraler WPO-Anwendung wäre es nämlich leicht möglich, daß infolge allzu großen Gasblasseneinstromes überschießende Reflexvorgänge — beispielsweise der von SCHWIEGK[41] gefundene Lungenentlastungsreflex, mit dem wir bei der Katze nicht zu rechnen brauchten (HENZE[29], AMANN und Mitarbeiter[1]) — zum Kollaps oder gar zum Tode führen.

In das Zusammenspiel zahlreicher reflektorischer, hämodynamischer und chemischer Vorgänge bei der Lungenembolie greifen auch *hormonelle Reaktionen* noch ein. Inwieweit durch die multiple Embolie freigesetztes Histamin, Adrenalin und Serotonin (HALMAGYI[26], HESS[30], JAHN[34],

WESTERMANN[72], WHITTERIDGE[74]) auch an der „Weichenstellung" (CAIN[12]) der intrapulmonalen Umgehungswege und an der Entstehung der pathologisch-anatomisch nachweisbaren Veränderungen in der Lunge und am Herzen beteiligt sind, ist vorerst wenig bekannt.

Nach unseren Beobachtungen wird WPO von der Mundschleimhaut zwar resorbiert, doch der auf diese Weise dem Organismus zugeführte Sauerstoff, dessen zulässige Gesamtmenge hier ohnehin unter $^1/_{10}$ des Ruhebedarfs liegt, wird in Blasen von der Lunge wie von einem Filter zurückgehalten. Von ganz entsprechenden Ergebnissen berichten v. BRÜCKE u. GOLDBACH[8], die in Anlehnung an BUCHHEIM[11] und ASSMUTH[5] WPO in den Dünndarm infundierten und damit Sauerstoff durch die Darmwand in den Kreislauf brachten. Wie bei intraoraler WPO-Anwendung die Lunge, so wirkte bei dieser enteralen WPO-Infusion die Leber als Filter, so daß eine massive Gasembolie der Pfortader eintrat. Eine *parapulmonale Sauerstofftherapie* läßt sich mit WPO also weder von der Mundhöhle noch von der Darmschleimhaut aus durchführen. Umso eigentümlicher ist es, daß von klinischer Seite intravenöse Sauerstoffeinblasungen (LEMAIRE[39], TUNNICLIFFE u. STEBBING[68]), intravenöse WPO-Injektionen (OLIVER u. MURPHY[51], WAGNER[69]) ebenso wie Mundspülungen mit unverdünntem Perhydrol (SCHÜLER[59]) und perorale Gaben von niederprozentigem WPO (HOFMOKL[32], NOWIKOFF[49], TRAUGOTT[67]) wesentlich günstiger beurteilt werden, als entsprechende Tierversuche das erwarten lassen (LORINCZ und Mitarbeiter[40]). — SCHÜLER[59c]), der in über 30 Jahren viele Tausend Patienten mit Perhydrolmundspülungen behandelt hat, beobachtete auffallend „stimulierende Wirkungen auf Nervensystem und Kreislauf". Wenn man derartige Angaben nicht auf Suggestivwirkung zurückführen will, so könnte auf Grund vorstehender Überlegungen die Hypothese aufgestellt werden, daß die in der dargestellten Weise zustande kommende Kohlensäureretention vorübergehend zu einer teuer erkauften Blutverteilungsänderung zugunsten des Herzens und des Gehirns (REIN[55]) führt, daß vielleicht sogar die Kranzgefäßdurchblutung als Folge der (subklinischen) Lungenembolie verbessert wird (ECKARDT[17]).

Für die *Praxis* kommen wir auf Grund der mitgeteilten Tatsachen zu nachstehenden Schlußfolgerungen: Wohl wird bei Mundspülungen mit hochprozentigem WPO, durch das eine starke Salivation ausgelöst wird, das WPO verdünnt und mitunter durch Anwesenheit katalaschaltigen Materials — wie z. B. Blut und Eiter — an der Resorption behindert, dafür aber kommen in praxi viel ausgedehntere, resorptionstüchtige Schleimhautbereiche mit dem WPO in Berührung als das bei unseren Versuchen der Fall war. Nimmt der Patient nach den Empfehlungen SCHÜLERS unverdünntes Perhydrol in den Mund und spült mit geschlossenen Lippen, so wird die WPO-Resorption, deren Mechanismus

wir im einzelnen noch nicht kennen, vermutlich durch die in der Mundhöhle eintretende Drucksteigerung nach den klassischen Vorstellungen von den „geschlossenen Hohlräumen" noch wesentlich verstärkt und damit eine Gefahr heraufbeschworen, die der einer Sauerstoffinsufflation in die Halsvenen vergleichbar ist. (Man bedenke, daß 1 ml 30%igen H_2O_2 etwa 100 cm³ O_2 abzuspalten vermag.) Aus diesem Grunde und wegen der von HAUSCHILD, LUDEWIG u. MÜHLBERG[27] aufgezeigten Möglichkeiten lokaler Ferment- und Ernährungsstörungen sollte unverdünntes Perhydrol nur in mehrtägigen Abständen, in kleinsten Mengen und nur zur Behandlung eng begrenzter Schleimhautbezirke bei geöffnetem Mund und nicht länger als 1 min angewandt werden. Auf diese Weise dürften unerwünschte Nebenwirkungen bei intraoraler Anwendung hochprozentigen Wasserstoffperoxyds hinter dem Nutzen lokaler Sauerstoffanreicherung zurücktreten.

Zusammenfassung

Auf Grund vorangehender Arbeiten sowie diagnostischer und therapeutischer Erfolge mit der intraoralen Anwendung unverdünnten Perhydrols wurde die Frage untersucht, ob von der Mundschleimhaut eine klinisch bedeutsame Wasserstoffperoxyd-Resorption stattfindet.

An Hunden, Katzen und Kaninchen wurden durch sublinguale Applikation von Wasserstoffperoxyd in Konzentrationen von 3–30% die gleichen Veränderungen hervorgerufen wie sie nach intravenöser Insufflation von Sauerstoff beobachtet werden konnten:

1. Transport gut sichtbarer Gasblasen in den ableitenden Venen.
2. Gesteigerte Atemtätigkeit: beträchtliche Zunahme der Amplituden, geringe Frequenzänderungen.
3. Für die Dauer hinreichender Blutpassage durch die Lungen kein stärkerer Abfall des arteriellen Blutdruckes.
4. Arterielle Hypoxämie und CO_2-Retention.
5. Makroskopisch und mikroskopisch nachweisbare Lungenschädigungen (Hämorrhagien, Dys- und Atelektasen).

Im Verlaufe fortgesetzter Perhydroleinwirkung kam es zur Ausbildung umschriebener, anfänglich noch rückbildungsfähiger Schleimhautemphyseme und zum Tod durch pulmonale Gasembolie.

Die theoretischen Zusammenhänge dieser Erscheinungen und die für die Praxis sich ergebenden Konsequenzen werden besprochen.

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AN INTEGRATED CONCEPT OF CARCINOGENIC-ANTICARCINOGENIC ACTION[1]

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Recently, a theory has been formulated (Kovacic, 1960a) which presents a unified picture of the initial step of carcinogenesis, applicable to a wide variety of carcinogens. According to the proposal, cancer originates in the action of an agent, derived from either an artificial carcinogen or a spontaneous process, which directly or indirectly generates hydrogen peroxide in abnormally high concentration. Possible routes whereby such a situation might arise include the inhibition of catalase, catalytic conversion of oxygen to hydrogen peroxide, and decomposition of body water by high energy radiation. Attack by hydrogen peroxide upon the cellular constituents is considered (Kovacic, 1960b) the subsequent stage in the sequence of reactions leading to the cancerous condition. This approach emphasizes the importance of the hydrogen peroxide hypothesis which had been advanced previously by various investigators without wide acceptance. Moreover, for the first time a reasonable theory is provided which correlates in a specific manner a wide array of structurally different carcinogens in relation to the initiation of cancer.

This communication presents an integrated view of carcinogenic-anticarcinogenic action based primarily upon these theoretical considerations, in addition to certain previously reported concepts and investigations.

Three types of cellular response (Haddow, 1947) appear likely in connection with increased levels of hydrogen peroxide. Cellular changes induced by slight increases could be overcome readily in the direction of normal cell regeneration. Exceptionally high concentrations would result in destruction of the cell as a reproductive entity. However, intermediate levels might well effect reversion to a simpler state capable of growth and division—the condition designated as malignancy.

It is likely that the cancer cell is susceptible to attack by a number of different routes. Certain antagonistic agents appear to act as antimetabolites; this category will not be treated in this communication. Anticarcinogens, at least those discussed, will be considered as functioning by either (1) increasing hydrogen peroxide concentration or (2) eliminating excess hydrogen peroxide.

Anticarcinogenic Action by an Increase in Hydrogen Peroxide Concentration

Let us first briefly discuss the mechanism which is proposed (Kovacic, 1960a) for the initiation of cancer, the unifying aspect being the generation of hydrogen

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peroxide. A possible route advanced in the case of aromatic hydrocarbons, aromatic amines, phenols, and azobenzenes entails the conversion of oxygen to hydrogen peroxide through the catalytic action of a metabolite such as an *o*-quinone or a quinonimine. As a more preferred alternative, increases in hydrogen peroxide are considered to result from catalase inhibition by reaction of the carcinogen with enzyme protein accompanied by coordination with iron. Tumor-inducing agents placed in this category include urethan, nitrogen mustards, ethylenimines, ethylene oxides, sulfonic acid esters of diols and *o*-aminophenols, as well as *o*-quinones—a class not yet reported to be carcinogenic. Finally, high energy radiation is known to generate hydrogen peroxide by decomposition of water.

A baffling paradox of oncology exists in the demonstration (Haddow, 1935; Haddow et al., 1948; Greenstein, 1954) that almost all of these same compounds or types of compounds are anticarcinogenic. If the premise is valid that these agents induce cancer by generating excessive amounts of hydrogen peroxide, it may well be that their anticarcinogenic action also is intimately related to the peroxide-forming property. Should this be true, the paradox would be resolved. How might it be possible for increased concentrations of hydrogen peroxide both to induce and combat cancer? An essential component of the interpretation is the thesis advanced by Holman (1956) that ". . . malignant cells—which are already deficient in catalase and abnormally sensitive to oxygen—are so easily killed by overoxidation. . . ." Support for this conclusion was provided by the demonstrated ability of hydrogen peroxide (Hollcroft and Lorenz, 1952; Makino and Tanaka, 1955; Worrall, 1956; Holman, 1957) to destroy cancerous tissue selectively. It is interesting that fetal liver is similar to hepatoma in its extremely low catalase content (Greenstein, 1954) and that agents which are antagonistic to cancer also damage (Skipper, Bennett and Wheeler, 1952) the most actively dividing normal cells. Consistent with the exposition presented thus far is the large reduction (Haddow et al., 1948) in the inhibitory effects of anticarcinogens by use of a high protein diet, as well as the anticancer action (Griffin, Clayton and Baumann, 1949) of the same type of diet, presumably by competition of diet protein with catalase protein for combination with the carcinogen-anticarcinogen.

Since many anticarcinogens also induce cancer, the anticancer property of hydrogen peroxide can be taken as an indication that it may also be carcinogenic. It is significant that hydrogen peroxide is effective in producing melanotic tumors in ***Drosophila melanogaster*** (Glass and Plaine, 1953; Plaine, 1955a, 1955b; Plaine and Glass, 1955). Similarly, inhibition of neoplasia by 9,10-phenanthrenequinone (Powell, 1951) supports the prediction that *o*-quinones will be added to the list of carcinogenic agents.

Chemotherapy with these various types of compounds, as well as with ionizing radiation, has in general been disappointing—a story of relapse and limited applicability. In light of the theoretical interpretations, this would indicate that increased concentrations of hydrogen peroxide are not completely effective due perhaps to the survival of resistant strains of cancer cells, or else to the generation by peroxide of new malignant cells as the established growths are eliminated. It should follow from this same reasoning that initiation of cancer by hydrogen peroxide would involve a certain degree of simultaneous inhibition. In fact, a number of investigators have observed precisely this type of refractory condition on application of carcinogens (Haddow et al., 1948; see Vesselinovitch, 1958). Numerous other factors involving high degrees of specificity would also be expected to play a role in the carcinogen-anticarcinogen balance.

Anticarcinogenic Action by Elimination of Excess Hydrogen Peroxide

On the basis of the hydrogen peroxide hypothesis, compounds capable of combining readily with hydrogen peroxide or its derived radicals would be classified

as potential cancer preventives. Substances reported as anticarcinogens (Greenstein, 1954; Skipper and Bennett, 1958) which may well function in this manner are mercaptans, maleic acid and aldehydes. Once the neoplastic condition is established, compounds in this category would act only to prevent the further generation of cancerous cells from normal ones. It should be pointed out that these substances or their metabolites can also be included in the alternative proposal which appears to be of wider scope.

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A PROPOSED ROLE FOR HYDROGEN PEROXIDE IN CARCINOGENESIS†

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According to a recent proposal (Kovacic, 1959), cancer may originate in the action of an agent, derived from either an artificial carcinogen or a spontaneous process, which directly or indirectly generates hydrogen peroxide in abnormally high concentration. Since this concept is applicable to a wide variety of carcinogens, it presents a broadly unified picture of the initiation phase of carcinogenesis. Plausible routes leading to high levels of peroxide were discussed in the preceding publication. The present report is concerned with the possible mode of action of hydrogen peroxide in its assigned role as the actual carcinogenic agent in the cases previously described.

There are three principal lines of evidence which buttress the proposition that hydrogen peroxide plays a key role in at least certain types of carcinogenesis.

1. Glass and Plaine (1953) made the very significant discovery that hydrogen peroxide produces melanotic tumors in *Drosophila melanogaster* (Plaine and Glass, 1955; Plaine, 1955a, 1955b).

2. Hydrogen peroxide is reported to be anti-carcinogenic (Hollcroft and Lorenz, 1952; Makino and Tanaka, 1955; Worrall, 1956; Holman, 1957; Sugiura, 1958).

3. Hydrogen peroxide possesses mutagenic properties (Demerec, 1953; Plaine, 1955a; for other references see Schumb, Satterfield and Wentworth, 1955a).

Since many carcinogens, such as ionizing radiation, nitrogen mustards and epoxides, possess mutagenic and cancer-inhibiting properties, these same characteristics in the case of hydrogen peroxide can be taken as additional evidence to strengthen the hypothesis.

It is worthy of mention that inorganic and organic hypochlorites, which are chemically similar to hydrogen peroxide, both enhance and inhibit tumor growth (Laszlo, Burk and Wight, 1959). The free radical decomposition of hypochlorites is discussed by Walling (1957).

In the present context, it is reasonable to assume that the cancer cell properties of low catalase content (Greenstein, 1954a) and high peroxide concentration (Rondoni and Cudkowicz, 1953) represent a continuation of the conditions which are critical for establishment of the malignancy.

With these concepts in mind, let us now consider the possible action of hydrogen peroxide in its effecting the transformation of a normal cell to a cancerous one, once the peroxide is present in abnormally high concentration. Existing evidence will be presented to support the contention that hydrogen peroxide, in the form of derived radicals, induces cancer by forming a template of simpler structure.

Cell Morphology and Histology

The idea of a common, or quite similar, mode of origin (Haddow, 1946–1947; Walpole et al., 1954) is consistent with the rather close resemblance between cancer cells regardless of the method of their generation. As Greenstein (1954b) pointed out ". . . tumors resemble each other more than they do normal tissues, or more than normal tissues resemble each other. . . ." A large number of investigators have noted that a wide variety of carcinogens induces a similar sequence of histological changes during the conversion of a normal cell to a malignant one

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ing that the decrease in viscosity continues even after termination of irradiation (Taylor, Greenstein and Hollaender, 1948). The degradation has been attributed (Weiss, 1952; Conway, 1954) to attack by radicals derived from peroxides, hydrolysis of labile phosphate esters, and loss of hydrogen bonding. In 1950 Dickey advanced the hypothesis that the fundamental biological effect of irradiation is a free-radical promoted, depolymerization of deoxyribosenucleic acid. Similarly, drastic reductions in the viscosity of DNA mixed with hydrogen peroxide were obtained on short exposure to ultraviolet light (Butler and Smith, 1950; Smith and Butler, 1951). With irradiation alone, depolymerization also occurs, but the rate is considerably less than that in the presence of the peroxide (Hollaender, Greenstein and Jenrette, 1941). Butler and Conway (1953) studied the degrading action of hydrogen peroxide irradiated with ultraviolet light on DNA and simple model compounds. Evidence was obtained for the following modes of reaction: cleavage of the phosphate-sugar link, fission of the bond between sugar and base, and oxidation of the sugar and base moieties.

The depolymerizing action of hydrogen peroxide in the various systems has been attributed by Butler and coworkers to intermediate formation of the highly reactive hydroxyl and hydroperoxyl radicals.

If carcinogenesis involves template simplification, the hydroxyl radical may be only one of a number of reagents, including other radicals (Park, 1947 1950; Jensen, 1950; Butler, 1950, Greenstein, 1954d; Hirsch, 1956), capable of effecting this transformation. As an additional example, on the basis of a decrease in solution viscosity, nitrogen mustard appears to degrade nucleic acid (Butler et al., 1952; Greenstein, 1954d).

Role of Protein

It is not known with certainty whether or not protein is a crucial part of the template structure. If protein is intimately concerned, then similar considerations might be applied, in relation to simplification of the template, as were discussed in the case of nucleic acid. An attractive possibility would involve oxidative cleavage of disulfide crosslinks in the protein portion of the cell. Normally these crosslinks, which are part of the cystine structure, can be reversibly cleaved to cysteine residues. A reasonable oxidative route in the cancerous condition which may be of increased importance is the conversion to cysteinesulfinic acid and cysteic acid moieties. The significant point is the irreversible cleavage of crosslinks which are important in determining cell characteristics. A mass of evidence indicates that cancer protein has undergone changes of this or similar nature. Mason (1958) theorized that the associated protein exerts a protective action on nucleic acid, and that this effect is lost during carcinogenesis. In addition, interference by the carcinogen with normal association of nucleic acid with protein has been suggested (Haddow, 1957)

If protein does not constitute a vital part of the template, then the transformations discussed in the following sections can be considered a secondary consequence of cancer induction. In any case the experimental data provide additional support for the hydrogen peroxide hypothesis although it is recognized that other interpretations are possible.

Sulfur-containing constituents.—Many investigators have proposed that the sulfur-containing functional groups of cellular protein are concerned in some manner with the carcinogenic process. Shapiro and Eldjarn (1955a) previously suggested the irreversible oxidation of vital disulfide linkages as an important step in the mechanism of radiation damage (also see Eyring and Bowers, 1952) and, moreover, demonstrated (1955b) the conversion of cystamine to 2-aminoethanesulfinic acid and taurine by treatment with hydrogen peroxide. As a closer analogy, the oxidation in vitro with hydrogen peroxide of cystine to cysteic acid has been reported by Schöberl (1933).

Previously, statements concerning the amino acid composition of normal and

cancerous tissue have in most instances stressed their similarity. However, certain well-established differences in amino acid content may perhaps result from the high level of hydrogen peroxide which is deemed important in carcinogenesis, and which is reported (Rondoni and Cudkowicz, 1953) to exist in cancer cells. In a significant investigation, Greenstein and Leuthardt (1944) determined the cystine and cysteine content of extractable proteins derived from normal and neoplastic rat tissues. Although the analyses for total sulfur were essentially identical in the two cases, marked differences were noted in cystine, as well as cysteine, content. The values for the cancer transplants were as much as 40 to 50 percent below those for normal adult liver. In related work (Roberts et al., 1949), there were indications of a slight decrease in the level of cystine on conversion of normal skin to carcinoma.

A considerable amount of evidence from a number of laboratories points to a reduction in the sulfhydryl content of various protein constituents in the cancerous condition. From a study involving enzyme systems, Rondoni and Barbieri (1950) found that carcinogenic polynuclear compounds, such as benzpyrene and dibenzanthracene, inhibited the autolytic function of proteolytic enzymes, whereas noncarcinogenic hydrocarbons were without effect. The activity of these enzymes is intimately related to the presence of free sulfhydryl groups. Barron and coworkers (1949) have reported a number of interesting findings in this area of research. They demonstrated that this same type of enzyme, after inactivation by irradiation, could be reactivated to varying degrees by treatment with reducing agents. However, as the radiation dose was increased the destruction became increasingly irreversible. The latter result was interpreted by Shapiro and Eldjarn (1955a) on the basis of irreversible oxidation of the mercaptan groups beyond the disulfide state.

It is well established that a drop in serum sulfhydryl content occurs with the onset of neoplasia. For example, this effect has been observed for irradiation with x-rays and also for administration of nitrogen mustards (Shacter and Shimkin, 1950). Likewise, Wood and Kraynak (1953) noted a significant decline in the serum or plasma sulfhydryl content which persisted for a considerable time after intravenous injection of colloidal benzpyrene. Although it is known that cancer serum contains a subnormal amount of albumin, Schoenbach and his colleagues (1950) found that the decreased sulfhydryl levels still pertained even after correction for the reduction in albumin.

In an analysis for glutathione involving a comparison with normal rat liver, Greenstein (1942) reported a 24 percent lower content for the transplanted rat hepatoma, and Kinosita (1938) a value 11 percent less in the case of the primary hapatoma. In another investigation, evidence was obtained (Rondoni and Boretti, 1947) for a decrease in the SH-content of water-soluble liver protein after intravenous injection of benzpyrene.

A number of the investigators cited have interpreted these results on the basis of destruction of sulfhydryl groups by oxidation, or, more commonly, by combination with the carcinogen.

Also pertinent to this discussion is the work of Ghosh and Lardy (1952) entailing alkali-treatment of acetone powders derived from various tissues. They reported that such treatment yielded elemental sulfur in considerably greater amount from tumorous as compared with normal tissue, and, on this basis, suggested a structure difference in the sulfur-containing proteins derived from the two sources. It is interesting that thiolsulfonic esters, products formed by partial oxidation of disulfides with hydrogen peroxide, are considered to yield sulfur as an intermediate product from their interaction with potassium sulfide (Connor, 1948).

Molecular weight.—The occurrence of an irreversible oxidative cleavage of disulfide crosslinks would result in a decrease in the average molecular weight of the protein. Attack by hydrogen peroxide at other protein bonds also appears possible (Schumb, Satterfield and Wentworth, 1955c). It is well established that

the concentration in blood of proteinlike materials, which are not precipitated by heat or protein precipitants, increases in the blood of cancer patients (Winzler and Smyth, 1948). This condition is presumably caused by a rise in the level of low molecular weight proteins, proteoses or polypeptides (Greenstein, 1954e).

Excess hydrogen peroxide present in the body would also be expected to degrade other constituents, e.g., carbohydrate (Schumb, Satterfield and Wentworth, 1955d).

Coagulation.—The concept of a lower degree of crosslinking in cancer protein is consistent with the observation of Rondoni (1941), and also of Bassi and Bernelli-Zazzera (1954), that soluble cancer protein is more difficult to coagulate than corresponding normal protein. The same salt-soluble protein fraction was investigated for cancerous, normal and regenerating liver. There was a regular decrease in the case of precipitation by heat with continued intake of *p*-dimethylaminoazobenzene, with a change in the opposite direction occurring in the case of regenerating liver. Furthermore, various investigators (Huggins, Cleveland and Jensen, 1950; Glass, Boyd and Dworecki, 1951) have reported that blood serum from a tumor-bearing host is more difficult to coagulate as compared with normal serum. In this connection, it is significant that Huggins and Jensen (1949) cite a definite correlation between the presence of sulfhydryl groups and turbidity formation.

Electrophoresis.—High concentrations of hydrogen peroxide would be expected to form acidic products, or products of increased polarity, not only from protein, but also from other body constituents, e.g., carboxylic acids from carbohydrates. There is general agreement among various investigators that soluble tumor protein possesses an electrophoretic pattern characterized by an increase in the proportion of faster-migrating components and a decrease in slower-moving ones, as compared with the corresponding normal protein (Sorof and Cohen, 1951; Eldredge and Luck, 1952; Hoffman and Schechtman, 1952; also see Ambrose, James and Lowick; 1956; Straumfjord and Hummel, 1959). This finding was interpreted by Sorof and Cohen (1951) as indicating a lower average isoelectric point for the soluble cancer protein. In addition, one of the components present in increased amounts was found to be a mucoprotein having an isoelectric point lower than pH 4 (Petermann and Hogness, 1948; Mehl, Golden and Winzler, 1949). It is very interesting that the acid protein gives a test reaction with toluidine blue similar to that characteristic of high molecular weight sulfuric acid esters (Petermann and Hogness, 1948).

Both an increase in polarity and a decrease in the molecular weight of cancer protein could account for the observed results in the electrophoresis experiments.

Related Considerations

Mutation.—Since many cancer-producing compounds are also mutagenic, it appears reasonable to classify carcinogenesis as an example of a mutation process in light of the concepts discussed. In many cases, both types of change may well proceed by a similar process: intermediary formation of hydrogen peroxide in high concentration. On this common basis, carcinogenesis would be regarded as producing a mutation characterized by a greater degree of dislocation.

Summary

It is believed that cancer may originate in the action of an agent which directly or indirectly generates hydrogen peroxide in abnormally high concentration. The subsequent stage is considered to be the formation of a template of simpler structure. Existing evidence is presented to support the proposed mode of action of hydrogen peroxide

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(Rhoads, 1946; Farber, 1956; Haddow, 1957; Korpássy, 1959). Furthermore, Duryee et al., (1960) remarked on the striking similarity between human and amphibian neoplasms.

Energy Considerations

The evidence indicates quite conclusively that the cancer cell is at a lower, more favorable energy level than the normal cell. For one thing, it has a simpler, more primitive structure; i.e., the tumor cell is less differentiated. Also, the rate of growth is faster; it can grow at the expense of the normal cell. It is clear, however, that the normal cell does not revert spontaneously to the more favorable energy level — apparently an energy barrier must be overcome (Schrödinger, 1944; Haddow, 1946-1947). Hydrogen peroxide possesses a high energy content (Schumb, Satterfield and Wentworth, 1955b), probably sufficient to provide the necessary energy of activation.

The foregoing considerations point to a gain in entropy when a normal cell is converted to a cancerous one (Rondoni, 1955; Flory, 1953). A more favorable entropy level for the template might be achieved in various ways, all of which would result in a simplification of structure: depolymerization; reduction in the degree of cross-linking; decrease in the number of strands comprising the helices; reduction in inter-chain forces, e.g., hydrogen bonding; or selective destruction of those nucleic acids concerned with specialized functions.

Role of Nucleic Acid

There is widespread agreement that nucleoproteins play an important role in the hereditary process, as well as in general cellular organization and function. Therefore, in connection with the hypothesis of template simplification, it is logical to focus primary attention on these vital cellular constituents. A number of investigators (e.g., Haddow, 1951; Levan, 1956; Hellström, 1959) have observed changes in chromosomal structure on application of carcinogens or in the transformation of normal to cancerous tissue. Recently, infectious DNA (dooxyribonucleic acid) was isolated (DiMayorca et al., 1959) from polyoma virus derived from leukemic mice (Stewart, Eddy and Borgese, 1958) and shown to be cancer inducing. Previously, Hays et al., (1957) and also Latarjet et al., (1958) reported cancer induction on injection of nucleic acid obtained from leukemic mice. Although the precise structural changes have not yet been elucidated, the evidence indicates that the DNA's from normal cells and from the corresponding cancer cells differ in certain of their properties (Polli and Semenza, 1955a, 1955b, 1956; Villa et al., 1955; Polli, 1957; Polli and Shooter, 1958; Polli et al,. 1959; Sloan-Kettering Report, 1957–1959; DiMayorca et al., 1960). It might well be that a relatively small alternation in the DNA ". . . would lead to enormous differences in the functional elements synthesized at this locus" (Greenstein, 1954c).

Conflicting reports exist in the literature concerning the effect of hydrogen peroxide on nucleic acid. In some cases a marked depolymerization was noted, whereas in others there was essentially no change (Taylor, Greenstein and Hollaender, 1948; Krejci, Sweeny and Hambleton, 1949; Butler and Conway, 1950; Conway and Butler, 1952; Yamafuji, Hiramaya and Miyata, 1956). Butler and Conway (1950) suggested that the difference in behavior may be due to the presence in the sensitive samples of catalysts capable of converting hydrogen peroxide to active radicals.

Various workers (Scholes, Stein and Weiss, 1949; Butler and Smith, 1950; Butler and Conway, 1950) have demonstrated that the degrading action of hydrogen peroxide is greatly enhanced by the addition of ferrous ions, even for preparations of nucleic acid insenstitive to hydrogen peroxide alone (Conway and Butler, 1952). Many investigators (e.g., Sparrow and Rosenfeld, 1946; Taylor, Greenstein and Hollaender, 1948; Scholes, Stein and Weiss, 1949) have reported the degrading action of ionizing radiation on nucleic acid. It is interest-

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HYDROPEROXIDASE[1] - DISTRIBUTION IN THE SKIN AND TRANSEPIDERMAL PENETRATION[2] OF HYDROGEN PEROXIDE FOLLOWING EPICUTANEOUS APPLICATION[3]

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Studies by Hauschild et al. have shown that the epicutaneous application of high-percentage hydrogen peroxide solutions, in contrast to the prevailing opinion, does not lead to corrosion but merely simulates the latter by the deposition of gas bubbles in the tissue and its capillaries. In other animal-experimentaland clinical papers statements regarding the method of action of externally applied hydrogen peroxide have been made (Ludewig, Ludewig and Goerisch, Huebner et al., Riede, Hoefs and Ludewig, Francke and Ludewig) and good clinical results have been obtained (Sprung and Ludewig, Sprung, Sprung et al., Hoefs, Gartmann and Hoefs, Schroth, Zimmerman, Bethmann, Elsasser and Neudel, Gartmann and Hoefs, Herwig, Nawka, Schilling, Wehner, Preusser, Schmidt). However, the question that is taken up in this paper has remained unanswered thus far, namely, whether hydrogen peroxide (H_2O_2) can penetrate the epidermis detectably undecomposed or whether, by virtue of the distribution of hydroperoxidase, there is a quantitative splitting of H_2O_2 even before the corium is reached.

1.

In the following, catalase and peroxidase are combined under the heading of hydroperoxidase (c.f. Theorell as well as Chance), when both properties of these enzymes are involved, which are frequently difficult to differentiate. The term catalase, aside from citations in the literature, is used only when that part of the enzyme is to be characterized which can split O_2 off from hydrogen peroxide. Accordingly, peroxidase is understood to be the enzyme which transfers oxygen from the peroxide to the acceptors, without having the ability to liberate molecular oxygen.

2.

We prefer the somewhat more general concept of penetration to the idea of permeation which is more frequently associated with membranes, and expand our discussion of the problem only peripherally to resorption, since in the present paper we are dealing primarily with penetration of the epidermis and the local oxygen supply and only secondarily with the possibility of the penetration of unsplit H_2O_2 into the circulation.

3.

This publication constitutes one part of the author's inaugural disseratation.

The usual procedure for demonstrating the transparency of the skin to a particular substance by chemical determinations in the blood and substances excreted from the body, pharmacological or toxicological effectiveness or labeling with stable or radioactive isotopes could not be used in the present study. H_2O_2 that would enter the tissues or circulation would have already gone beyond the point of detection because of catalytic decomposition.

The fact that we had not managed in our preliminary experiments to find H_2O_2 following epicutaneous application of high-percentage of H_2O_2 solutions in a subepithelial tissue macerate was subject to three different interpretations:

1. H_2O_2 does not penetrate the epidermis, as stated by Magnus as well as Rieger et al.
2. H_2O_2 penetrates the corium only in amounts which could not be detected in our tissue macerates.
3. H_2O_2 is broken up more rapidly in the tissue than the determination could be made.

Since it was suspected that H_2O_2 penetration is very significantly affected by fermentative fission, it seemed to us to be important to clarify the situation regarding hydroperoxidase distribution in the skin.

As we had to conclude in conjunction with Leonhardi, there are only old and not very satisfactory data on the skin as far as localization of this most active of all enzymes: Sexsmith et al. were satisfied with detecting catalase in human and animal skin without providing any greater detail as far as localization is concerned. Hermann, who like Loele, found peroxidase in formalin-fixed sections claimed that this enzyme was found in the epithelium, especially in the region of the stratum lucudum. Wohlgemuth et al., Szoerenyi, Yamasaki as well as Melczer, on the basis of titrimetric enzymatic determinations with tissue homogenisates, concluded that catalase as well as peroxidase are uniformly distributed through all the layers of the skin. According to these views, and also in conjunction with an unfounded

statement by Eichholtz, which claims that the epithelium contains catalase, one would expect that external application of H_2O_2 would lead to fission that would be linked to intensive development of gas on the skin. Burns, Honsel as well as Mankiewicz, however, have already pointed out that there is no fission of H_2O_2 on the skin. It was therefore necessary to study the distribution of hydroperoxidase with a method that would allow a more precise location. It seemed to us that the histotopochemical determination of unfixed frozen sections would be particularly suitable because this technique, aside from localization of the enzyme, makes it possible to ascertain how much H_2O_2 has penetrated the tissue. It was our feeling that the penetration of H_2O_2 could be interrupted by freezing with liquid nitrogen and only determining the distribution of H_2O_2 under the microscope with the aid of enzyme-chemical reactions following treatment of histological native sections during thawing and recording them photographically.

METHOD

In order to determine enzyme distribution and the penetrating ability of H_2O_2, we used the following types of skin:

Frog: Web, belly skin

Rat: Belly skin, rear extremities, tongue.

Cat: Foot-pad skin, tongue.

Man: Skin (taken from cadavers or fresh operation preparations[1)] from the soles of the feet, belly, thumb (digital pulp), axilla, scalp.

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1 We are indebted to Professor G. Holle, Director of the Department of Pathology of Karl Marx University and Dr. L. Cossel for providing the cadaver skin and Dr. H. Uebermuth, Director of the Surgical Clinic of Karl Marx University, as well as Dr. W. Wehner for the skin from operational preparations.

For location of the enzyme, frozen sections were prepared using CO_2 or N_2. The frozen native sections were initially coated with the reagents listed below under the microscope and photographed at certain time intervals. To have a control, "plucked" preparations of the skins as well as mucles, nerves and connective tissue fibers and subcutaneous fatty tissue were coated according to the same principle with the reagent and subjected to histochemical comparison.

For determination of catalase, we used H_2O_2 solutions ($10^{-6}...10^{-2}$ mole), in an albumin-glycerine preparation that is conventionally employed in histology. After a few minutes, adhesive gas bubbles developed above structures containing catalase, thus allowing a rough orientation with respect to the location of the enzyme.

To detect peroxidase, we used the benzidine reaction according to Prenant, which had shown itself to be valuable in preliminary tests in accordance with the modifications of De Robertis et al., Kreibisch et al., as well as Loele. The results were checked by using some reagents that are employed for detection of peroxidase: alpha-naphthol and (text missing) phenylene diamine (Loele, Dupoy, Storch), leuco-acid-fuchsin (lison), patent blue (Fautrez), (word blurred) (Schoenbein), diaminofluorene (Reigl). The H_2O_2 concentration in the reagent was set so that the less sensitive pseudoperoxidase, which is contained primarily in the blood, would not change the benzidine into benzidine blue.

The enzyme specificity was ensured by pretreatment of the sections with hydroxylamine ($10^{-3}...10^{-2}$ mole) or by heating the preparation to 120°C for 20 minutes. In all of the versions of the experiment, checks with solutions of pure horseradish peroxidase and catalase[2]) were carried out.

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[2] We are indebted to Dr. Schaefer and Dr. Rose of VEB Arzneimittelwerk Dresden for supplying the pure enzymes.

To determine H_2O_2 penetration, the following procedure is suitable:

H_2O_2 is placed on the skin (previously cleaned with water at body temperature) either as 3-30% aqueous solution or in the form of a 3-20% emulsion (lanette base). Very hairy skin was shaved 24 hours prior to the start of the test, carefully without using any aids. In situ, the areas of skin exposed to the H_2O_2 on anesthesized animals were removed during the H_2O_2 action. Cold fixation took place by rapid plunging of the treated piece of skin in liquid nitrogen. While the H_2O_2 solutions were soaked up immediately prior to freezing, the H_2O_2 emulsion could be left on the skin until it was placed in the nitrogen, since it could be removed easily and cleanly as a frozen plate after the tissue had hardened. The frozen, roughly rectangular pieces of skin were then placed in a special holder designed for this purpose[1] and cut with a microtome at right angles to the surface of the skin (narrow (word blurred) corresponding to the thickness of the skin -- to the knife blade). The thickness of the sections was 40 to 60 microns. By moving the microtome knife from side to side and cleaning it constantly, it was possible reliably to eliminate transferring impurities containing H_2O_2 to the cutting surface.

During cutting, cooling with CO_2 or N_2 was regulated so that the frozen object maintained a consistency suitable for cutting. The frozen sections were carefully coated with the reagent on precooled slides under a microscope; the reagent quickly froze on the section. During the gradual thawing

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1 The holder was made by the master mechanic in our department, Mr. Teubner, to whom we are grateful.

Some of the histochemical studies were performed at the Department of Biology of VEB Arzneimittelwerk Dresden. For his understanding support and kind generosity, making it possible for us to do our work, we are grateful to Prof. R. Thren and the capable assistance of C. Holfeld and G. Traenkner.

process, at places where H_2O_2 was present an enzyme-chemical color reaction or gas bubble formation occurred, as was determined by means of photomicrography, before the microscopic image disappeared within a few minutes because of diffusion of the dye that was formed or wandering of the gas bubbles. The pictures were taken with a single lens reflex camera (Exakta-Varex) and a Miflex attachment. The proof of permeation of H_2O_2 in these frozen sections was carried out by analogy with the detection of enzymes described above and according to AVI-Dor et al, also with the benzidine reaction. For this purpose, the benzidine solution, containing protein-glycerine, had added to it pure horseradish peroxidase instead of H_2O_2, so that the H_2O_2 could be localized in the tissue by means of benzidine blue. By adding the pure catalase, the presence of H_2O_2 in the section was revealed also by the development of gas bubbles, which adhered sufficiently long at the place where they were formed due to the viscosity of the reagent mixture. To the extent that it was possible, the results were also checked by using titanium sulfuric acid.

Aqueous benzidine-peroxidase solution was also used to determine the penetration of undecomposed H_2O_2 in the vessels of frog web skin: the vessels of the rear extremities of male edible frogs were perfused according to Laewen-Trendelenburg. The reagent was added immediately prior to the epicutaneous application of 5-10% H_2O_2 solutions to the frog Ringer solution that was used for profusion or injected interlamellarly.

In order to clarify the subepidermal H_2O_2 fission by affecting gas bubble formation histologically by developing artificial, structurally bound catalase deposits, we produced inflammation with terpeneol. Rats received injections of 0.05 or 0.01 ml terpeneol beneath the shaved belly skin or in the skine of the rear paws. The injection was made as far as possible from the deposit. 72 hours after the injection, the skin of the injected area was brought in contact for 10 to 20 minutes with 10-30% H_2O_2 solutions. Follow-

ing removal of most of the H_2O_2, the tissue area was excised over a wide area, fixed in formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin.

As the model for the propagation of H_2O_2 in catalase-rich tissue, freshly removed rat livers were used. These organs were placed for more than 3 minutes in a 6% H_2O_2 solution -- partly after 5 minutes of pretreatment with 1 mole hydroxylamine solution -- and then subjected to the usual histological sectioning and staining procedures (embedding in paraffin, hemotoxylin-eosin staining).

RESULTS

A. Hydroperoxidase Distribution

Epidermis: catalase could not be found in any layer of the epidermis. In only a few preparations, individual gas bubbles were observed on the surface of the epithelium and in the region of the openings of the sweat glands (Figure 1).

The benzidine-peroxidase reaction was also negative in the stratum corneum, but it was positive in the layers of the stratum germinativum. Intensity of the blue coloration increased gradually from the only slightly tinted stratum granulosum to the stratum basale (Figures 2 and 3).

The partially horny surface epithelium of the tongues of our experimental animals showed neither catalase nor peroxidase activity (Figure 4).

Control tests with benzidine blue obtained from a positive peroxidase reaction showed that a color reaction in the area indicated above to be peroxidase-negative is quite possible from the dye technique standpoint. (See also Figure 7).

Corium: the boundary between the epidermis and the corium was clearly demarked by the positive outcome of the catalase reaction within the epithelium (Figure 1 and 3). The development of gas bubbles and the formation of benzi-

dine blue indicating peroxidase proceeded mainly from the vessels and the muscle fibers, while in agreement with the results of "plucked" preparations of the nerve, connective tissue fibers and fatty tissue, no significant hydroperoxidase activity was found in general. In those sections that had been pretreated with hydroxylamine or by heating, the reactions to catalase and peroxidase were absent.

B. Detection of the Penetration of Hydrogen Peroxide

After only a few minutes of epicutaneous action of 5-30% solutions, H_2O_2 could already be found localized in the epidermis. In accordance with the enzymes contained in the reagent, complementary images were obtained to a certain extent in conjunction with the previously indicated hydroperoxidase detection in the untreated skin (Figures 1 to 3): the layers of the epidermis that had been found to be unstained or free of gas bubbles in the detection of hydroperoxidase distribution were tinted blue after the epicutaneous application of H_2O_2 or were covered by gas bubbles (Figure 6). It was only after high-percentage H_2O_2 had been allowed to act for several hours on cadaver skin or following previous infiltration of the skin with hydroylamine that a positive outcome of the reaction to H_2O_2 could be observed in the corium as well (Figure 7).

Rat skins, in which inflammation had been produced by terpeneol injections, were recognized after epicutaneous H_2O_2 action by the relatively superficially located infiltrates and indicated emphysema (Figures 9 and 10). In the control skins which had not been modified by inflammation, on the other hand, pronounced emphysema appeared only after equal or longer lasting action of H_2O_2 in the area of the lower-lying, catalase-containing structures (e.g., musculature) (Figure 8).

Following epicutaneous H_2O_2 action on very hairy animal or human skin, the intracutaneously developing gas bubbles were found mainly between the hair follicles. Applying an emulsion containing H_2O_2 produced much more pronounced

emphysema on the belly skin of rats than was produced by simple painting of an emulsion of the same H_2O_2 concentration (See Figure 11 and 12).

Vertical sectioning of frog web skin permitted localization of emphysema following epicutaneous action of H_2O_2 histologically beneath the epidermis. In the vessels that were located closest to the epidermis, following epicutaneous application of 5-10% H_2O_2 solutions, H_2O_2 could be detected within a few seconds if the vessels had been perfused with Ringer solution containing benzidine-peroxidase or the reagent had been applied between the lamellae of the web (Figure 13).

In rat livers that were used for comparison as models, gas bubbles developed near the surface when the organs were placed in the H_2O_2 solutions. If this effect was preceded by preliminary treatment with hydroxylamine the gas bubbles were located almost exclusively in the interior or the organ.

DISCUSSION

A. Hydroperoxidase Distribution

The detection of catalase distribution in the skin is complicated by the fact that suitable histochemical straining techniques for detecting this enzyme have not yet been found, and none are expected to be found.

Nishiyama et al. (1953) attempted to localize the catalase histochemically in various organs. They proceeded on the basis of the fact that catalase can break down monoethylperoxide to acetaldehyde, which they then detect with Schiff's reagent. By using pure enzymes, we were able to prove that the same reaction is accelerated by peroxidase and even proceeds relatively rapidly in contact with air and with formation of acetaldehyde[1]. This method is there-

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[1] We would like to thank the chemist at our department, Dr. R. Schmiedel, for preparing the monoethylperoxide.

fore not usable for specific detection of catalase. Methods that are based on the fact that lenco-dyes are offered as oxygen acceptors can likewise not be used because the catalase always exhibits peroxidatic activity.

Since it is only the splitting off of molecular oxygen from hydrogen peroxide that is specific as far as the definition of catalase is concerned and is also particularly significant for the present problem, we were forced to study this gas bubble formation in horizontally-located histological sections. This method, in contrast to the detection of peroxidase, allows only a relatively coarse orientation, but it is possible to reach the conclusion which is of great interest in this connection that the epithelium of the skin and mucous membranes is practically inactive from the catalytic standpoint and therefore H_2O_2 cannot be hindered in its penetration by enzymatic fission. Moreover, the results obtained by this method have allowed a corrected interpretation of the quantitative catalase determinations of Wohlgemuth et al., Szoerenyi, Yamasaki as well as Melcher, on which the previously valid views concerned catalase content of skin are based.

The authors have divided the skin roughly into three layers by two horizontal cuts and homogenized them. They were able to find no significant difference in comparing the titrimetrically determined catalase activities, in agreement with their own subsequent studies. From this they drew the erroneous conclusion that all layers of the skin, including the epidermis, have approximately the same degree of catalase activity. However, since a coarse horizontal cut beneath the epithelium involves mainly the richly vascularized papillar bodies and is added to the homogenisate of the topmost layer, the richness of blood catalase in the quantitative determination conceals the shortage of catalase in the epithelium.

The benzidine reaction used for histochemical peroxidase detection was considered by us to be particularly suitable for a number of reasons: the method is the best tested and most widely used one; the reaction begins rela-

tively rapidly and is quite simple and sensitive in comparison to others which mostly have several stages. In addition, it offered the possibility of detecting even traces of H_2O_2 with the same reaction principle without using a dye bath in a horizontally located section by coating the native preparation with a reagent. For this purpose, only pure peroxidase could be added to the reagent mixture instead of H_2O_2.

The results indicate that oxygen fromthe H_2O_2 that enters the epidermis can be carried by peroxidases in the area of the stratum germinitivum to acceptors if the H_2O_2 penetrates the hydroperoxidatically inactive stratum cornium.

B. Proof of Hydrogen Peroxide Penetration

The benzidine reaction used to detect hydrogen peroxide in histological sections, aside from the reasons given already, was also particularly suitable because the benzidine blue which results contrasts better against the intrinsic color of the native section than do other reagents such as titanium sulfuric acid or peroxytitanic acid, because the indicator has at least the same sensitivity as more specific reagents and because it can be demonstrated at the same time with the positive outcome of the reaction that a structure that remains uncolored in the peroxidase test can theoretically be colored by benzidine blue. Since the indicator for molecular oxygen does not work in this way and since control tests with titanium sulfuric acid or with catalase solution provided corresponding results (although they were less photogenic), the specificity of benzidine reaction for H_2O_2 was ensured for the requirements established.

Because it was to be feared from the methodological standpoint that after treatment of the surface of the skin, any H_2O_2 that was still adhering would be carried to the surface of the section during cutting and thus could give erroneous evidence of penetration, we performed preliminary tests with dye

solutions and emulsions. Observing the necessary precautions, we excluded these sources of error. As Figure 5 shows, the H_2O_2, which was in contact with the skin for only 2 seconds and was then removed, all else being equal, remained as a minor residue on the surface. When H_2O_2 was found in the epidermis after various periods of time, the color (in agreement with the fact that the catalytic splitting can begin only beneath the epidermis) initially did not cross the boundary between the epidermis and the corium. Such a boundary simply could not have been maintained in the event of disadvantageous contamination with H_2O_2.

Cold fixing with liquid nitrogen had an uncommonly strong effect as far as making the cutting of the object (frozen to the hardness of glass) was concerned, and allowed only relatively thick sections to be cut. It had to be done, however, since all other methods of fixation were too slow and too indifferent as far as the splitting rate and the water solubility of H_2O_2 were concerned. As we were able to determine (in agreement with Shikamma et al.), in our experimental arrangement as well there was practically no hydroperoxidase inactivation caused by the temperatures that we used, which were so low (about -196°C).

The detection of nondecomposed H_2O_2 in the corium was possible using this method only after previous restriction of hydroperoxidase activity or after extreme periods of action of high-percentage H_2O_2 on cadaver skin. However, with respect to catalase distribution and our histological findings (see Figure 8, for example), this led to the view that H_2O_2, after penetration of the non-vascular epidermis, is transported relatively rapidly by intercellular or vasal means and is split only when it comes in contact with catalase-containing structures in the corium or in deep layers with liberation of molecular oxygen. To confirm this view, inflammatory infiltrates were more suitable than the relatively rapidly resorbable catalase solutions, in which the hydroperoxidase was bound corpuscularly and had to be held at the desired

point in some way. It was histologically demonstrable that the splitting of oxygen in the living skin occurs primarily through the infiltrate and in areas in which no significant gas formation could be otherwise observed in the control animals.

This experimental arrangement with respect to the animals corresponds to the clinical observations of Peter, who recommended the application of H_2O_2 to the gums to limit subepithelial infiltrates and abscesses. Only recently, Coppes (1962) has also pointed out that the white discoloration produced by H_2O_2 in infected mucous membranes in man is particularly clearly evident.

In order to convince ourselves that it is the distribution of enzyme activity and not the morphological development of the tissue penetrated by the H_2O_2 which is critical for the localization of gas bubbles, the action of H_2O_2 on isolated liver partly pretreated with hydroxylamine was compared. In this relatively homogeneous model, it was clear that $H2O_2$ can also penetrate the tissues richest in catalase with emphysema formation, but it wanders through catalase-poor (here, artificially inactivated) regions but without gas bubble formation and liberates O_2 only in the depths of homogenous, but catalytically active, tissue by means of enzymatic fission.

The gas bubble that is produced by the contact of H_2O_2 with a catalase-rich structure protects (for the duration of its existence) against further action and splitting of H_2O_2 at the same point and thus against catalase inactivation (see below).

The proof that the gas bubbles that are partially observed in the capillaries owe their origin to an intravasal H_2O_2 fission could not be found in warm-blooded animals due to the relatively high tissue and blood catalase activity. It was possible, however, in the catalytically less active frog web skin whose vessels are readily available for direct observation and which showed lesser (but completely comparable to those of warm-blooded animals) reactions with respect to the subepithelial development of gas

bubbles following epicutaneous H_2O_2 application.

While it has not yet been decided whether or not the penetration of water into the skin takes place transepidermally or through the supplementary formations (Rothman), our histochemical and histological results indicated that the "preformed paths" of the skin are not preferred by the H_2O_2. It is even possible that they are nearly closed to the H_2O_2; since locking air bubbles are imbedded in the epithelial hair root scalp, since gas bubbles can form in perspiration under H_2O_2 action, and since the concavities present at the outlets of preformed pathways can be viewed as centers for the formation of bubbles in gaseous solutions according to the ideas of Glasser. At these points, there must be therefore even less possibility of wetting and penetration of H_2O_2 than is the case for other aqueous solutions (Impens, Winternitz, Calvery, Rothman). We were also able to observed under the incident-light microscope that the gas bubbles that appear on human skin following the epicutaneous application of H_2O_2 were localized primarily in the regions of such concavities.

It was mainly the hair root scalp that was involved in the rat belly skin because the sweat glands (according tothe data of Tennet and Hieronymi and our own observations) are scarcely formed in the rat.

Since the air that was found in the preformed pathways could be dispelled by application of salves, according to Calvery et al., Rothman, and others, penetrating substances incorporated in sales could produce resorption, to a large extent through massage. In the case of H_2O_2 as well, it was possible to demonstrate clearly both macroscopically and histologically in conjunction with the spread of emphysema that the absorption can be increased by applying a H_2O_2-containing salve. The same amount of H_2O_2 salve led to much less emphysema formation after simple coating of the skin and particularly if the skin was given an appropriate massage before or after application (see Figure 11 and 12).

RESULTS

In order to answer the question of whether epicutaneously applied hydrogen peroxide can penetrate the epidermis or whether a quantitative splitting of H_2O_2 because of the hydroperoxidase activity of the epithelium takes place even before the corium is reached, it was necessary first of all to clarify the nature of the distribution of the enzyme in the skin. Using native frozen sections, the catalase was localized by means of an H_2O_2 containing reagent and peroxidase was localized with benzidine reaction. It was found that the epidermis shows no catalase activity. Peroxidase, on the other hand, is found in the stratum germinativum, while the stratum cornium is peroxidase-free. In the corium and the deeper layers of tissue the blood vessels and musculature clearly show positive nerves, connective tissue fibers and fatty tissues but merely indicate positive or negative hydroperoxidase reactions.

Using a histotopichemical technique whose details have been described and discussed briefly, it was shown by means of photomicrographs that H_2O_2 can in fact penetrate the epidermis and enter the corium. Penetration occurs with disregard of the "preformed pathways" and is primarily transepidermal. The location of the emphysema that is created by enzymatic fission of the H_2O_2 that has penetrated is dependent primarily on catalase distribution in treated areas of tissue. The gas bubbles that appear in the capillaries of frog web skin following epicutaneous H_2O_2 application may occur intracapillarly due to the penetration of H_2O_2.

SUMMARY

In conjunction with the therapeutic use of high-percentage hydrogen peroxide solutions it has been determined histotopichemically that H_2O_2 penetrates the epidermis without being decomposed and can penetrate the corium. The penetration occurs with disregard of the "preformed pathways", primarily

transepidermally. The localization of the emphysema which is formed in the enzymatic decomposition of the H_2O_2 which has penetrated, is primarily a function of the catalase distribution in the tissue area treated.

EXPLANATION OF THE PLATES

Plate VIII

Figure 1: Skin of human sole, determination of catalase (10 X).

Figure 2: Skin of human sole, peroxidase detection (16X).

Figure 3: Skin of human sole, hydroperoxidase detection (10X).

Figure 4: Cat's tongue, hydroperoxidase detections (10X).

Figure 5: Skin of human sole, detection of H_2O_2 residue following 2 seconds of epicutaneous application of H_2O_2, 10% (native; 16X).

Figure 6: Skin of human sole, detection of H_2O_2 after 20 minutes of epicutaneous action of H_2O_2, 10% (native; 16X).

Figure 7: Skin of human sole, (cadaver), detection of H_2O_2 after 180 minutes of epicutaneous action of H_2O_2, 20% (native; 10X).

Figure 8: Rat's paw, 30 minutes after epicutaneous action of 10% H_2O_2 (emphysema formation, especially in the lower-lying area of the diagonally striated musculature) (H.-E.; 10X).

Figure 9: Rat's paw. Inflammation with terpeneol: 30 minutes after epicutaneous action of 10% H_2O_2 (formation of emphysema in the superficial area of the inflammatory infiltrate ((H.-E.; 16X).

Figure 10: Rat belly skin, terpeneol inflammation: 10 minutes after epicutaneous action of 20% H_2O_2 (emphysema formation by infiltrate) (H.-E.; 10X).

Figure 11: Rat belly skin, after application of a 10% H_2O_2 emulsion (length of action: 5 minutes) (H.-E.; 10X).

Figure 12: Rat belly skin, after application (5minutes) of a 10% H_2O_2 emulsion (H.-E.; 10X).

Figure 13: Frog web skin, transillumination, intravasal H_2O_2 detection after 10 seconds of epicutaneous action of 10% H_2O_2 (native; 16X).

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Aus dem Institut für Pharmakologie und Toxikologie der Karl-Marx-Universität Leipzig
(Direktor: Prof. Dr. F. HAUSCHILD)

Hydroperoxidase[1])-Verteilung in der Haut und transepidermale Penetration[2]) von Wasserstoffperoxid nach epikutaner Applikation[3])

Von R. LUDEWIG

Mit Tafel VIII

(Eingegangen am 4. November 1963)

Aus Untersuchungen von HAUSCHILD und Mitarb. hatte sich ergeben, daß die epikutane Anwendung hochprozentiger Wasserstoffperoxidlösungen im Gegensatz zur vorherrschenden Ansicht nicht zu Verätzungen führt, sondern solche durch Einlagerung von Gasblasen in das Gewebe und seine Kapillaren nur vortäuscht. In weiteren tierexperimentellen und klinischen Arbeiten wurden Aussagen über die Wirkungsweise (LUDEWIG, LUDEWIG und GÖRISCH, HÜBNER und Mitarb., RIEDE, HÖFS und LUDEWIG, FRANCKE und LUDEWIG) und die gute klinische Brauchbarkeit (SPRUNG und LUDEWIG, SPRUNG, SPRUNG und Mitarb., HÖFS, GARTMANN und HÖFS, SCHROTH, ZIMMERMANN, BETHMANN, ELSASSER und NEUDEL, GARTMANN und HÖFS, HERWIG, NAWKA, SCHILLING, WEHNER, PREUSSER, SCHMIDT) äußerlich angewandten Wasserstoffperoxids gemacht. Unbeantwortet war bisher jedoch die im folgenden bearbeitete Frage, ob Wasserstoffperoxid (H_2O_2) nachweisbar unzersetzt die Epidermis durchdringen kann oder ob auf Grund der Hydroperoxidaseverteilung mit einer quantitativen Spaltung des H_2O_2 noch vor Erreichen des Koriums zu rechnen ist.

1) Unter *Hydroperoxidase* werden im folgenden Katalase und Peroxidase zusammengefaßt (vgl. THEORELL sowie CHANCE), wenn es sich um beide, oft kaum voneinander zu trennende Eigenschaften dieser Enzyme handelt. Die Bezeichnung Katalase wird — außer beim Zitieren von Literatur — nur dann verwendet, wenn jener Fermentanteil charakterisiert werden soll, der aus Wasserstoffperoxid O_2 abspalten kann. Unter Peroxidase wird entsprechend das Ferment verstanden, das den Sauerstoff von Peroxiden auf Akzeptoren überträgt, ohne die Fähigkeit zu besitzen, molekularen Sauerstoff freizusetzen.

2) Wir ziehen den etwas allgemeiner aufzufassenden Begriff der *Penetration* dem häufiger auf Membranen bezogenen der *Permeation* hier vor und erweitern unsere Fragestellung nur am Rande auf die *Resorption*, da es bei der vorliegenden Arbeit in erster Linie auf die Durchdringung der Epidermis und das lokale Sauerstoffangebot und erst in zweiter Linie auf die Möglichkeit des Eindringens ungespaltenen H_2O_2 in den Kreislauf ankommt.

3) Dieser Veröffentlichung liegt ein Teil der Habilitationsschrift des Verfassers zugrunde.

Das übliche Vorgehen, die Durchlässigkeit der Haut für eine bestimmte Substanz durch chemischen Nachweis in Blut und Körperausscheidungen, durch die pharmakologische bzw. toxikologische Wirksamkeit oder durch Markierung mit stabilen oder radioaktiven Isotopen zu demonstrieren, war zur Bearbeitung der vorliegenden Fragestellung nicht brauchbar. In das Gewebe oder den Kreislauf gelangtes H_2O_2 hätte sich dem Nachweis vorzeitig durch katalatische Zersetzung entzogen.

Die Tatsache, daß es uns in Vorversuchen nicht gelungen war, nach epikutaner Anwendung hochprozentiger H_2O_2-Lösungen im subepithelialen Gewebsmazerat H_2O_2 nachzuweisen, ließ 3 Deutungen zu:

1. H_2O_2 durchdringt die Epidermis nicht — wie von MAGNUS sowie RIEGER und Mitarb. behauptet.
2. H_2O_2 dringt bis ins Korium nur in Mengen vor, die in unseren Gewebsmazeraten nicht mehr erfaßt wurden.
3. H_2O_2 wird im Gewebe schneller gespalten, als der Nachweis gelang.

Da zu vermuten war, daß die H_2O_2-Penetration sehr wesentlich durch fementative Spaltung beeinflußt wird, erschien es uns wichtig, zunächst Klarheit über die Hydroperoxidaseverteilung in der Haut zu gewinnen.

Wie wir in Übereinstimmung mit LEONHARDI feststellen mußten, existieren für die Haut über die Lokalisation dieser aktivsten aller Fermente nur ältere, wenig befriedigende Angaben: SEXSMITH und Mitarb. begnügten sich mit dem Nachweis von *Katalase* in menschlichen und tierischen Häuten, ohne nähere Angaben über die Lokalisation zu machen. HERMANN, der *Peroxidase* in Anlehnung an LOELE an formalinfixierten Schnitten nachwies, vertrat die Auffassung, dieses Ferment sei im Epithel, besonders im Bereiche des Stratum lucidum, anzutreffen. WOHLGEMUTH und Mitarb., SZÖRENYI, YAMASAKI sowie MELCZER kamen auf Grund titrimetrischer Fermentbestimmungen an Gewebshomogenisaten zu der Überzeugung, daß Katalase bzw. Peroxidase gleichmäßig über sämtliche Schichten der Haut verteilt sei. Nach diesen Ansichten — ebenso wie im Hinblick auf eine unbegründete Angabe von EICHHOLTZ, nach der das Epithel Katalase enthält — müßte bei äußerlicher Anwendung von H_2O_2 eine mit intensiver Gasentwicklung auf der Haut verbundene Spaltung erwartet werden. BRUNS, HONSELL sowie MANKIEWICZ hatten aber bereits darauf hingewiesen, daß eine Spaltung von H_2O_2 auf der Haut nicht stattfindet. Es war daher erforderlich, die Verteilung der Hydroperoxidasen mit einer Methodik zu untersuchen, die eine exaktere Lokalisation erlaubte. Dazu schien uns die histotopochemische Untersuchung unfixierter Gefrierschnitte deshalb besonders geeignet, weil diese Technik außer der Fermentlokalisation noch die Möglichkeit bot, ins Gewebe eingedrungenes H_2O_2 zu erfassen. Es war unsere Absicht, das Vordringen von H_2O_2 durch Einfrieren mit flüssigem Stickstoff zu unterbrechen und erst nach Anfertigung

histologischer Nativschnitte während des Auftauens die Verteilung des H_2O_2 unter dem Mikroskop mit Hilfe fermentchemischer Reaktionen sichtbar zu machen und photographisch festzuhalten.

Methodik

Zur Ermittlung der Enzymverteilung und der Penetrationsfähigkeit von H_2O_2 verwendeten wir folgende Häute:

Frosch: Schwimmhaut, Bauchhaut.
Ratte: Bauchhaut, Hinterläufe, Zunge.
Katze: Sohlenballenhaut, Zunge.
Mensch: Der Leiche oder frischen Operationspräparaten[1]) entnommene Haut von Fußsohle, Bauch, Daumen (Fingerbeere), Axilla, Kopfschwarte.

Zur Fermentlokalisation wurden Gefrierschnitte unter Verwendung von CO_2 oder N_2 angefertigt. Die gefrorenen Nativschnitte wurden anschließend unter dem Mikroskop mit den unten genannten Reagentien überschichtet und in bestimmten Zeitabständen photographiert. Zur Kontrolle wurden Zupfpräparate der Haut sowie von Muskulatur, Nerven, Bindegewebsfasern und subkutanem Fettgewebe nach dem gleichen Prinzip mit Reagens überschichtet und zum histochemischen Vergleich herangezogen.

Zum *Nachweis der Katalase* dienten uns H_2O_2-Lösungen (10^{-6} ... 10^{-2} mol), zu deren Verdünnung teilweise die in der Histologie gebräuchliche Albumin-Glyzerin-Zubereitung verwendet wurde. Über katalasehaltigen Strukturen entwickelten sich einige Minuten am Ort der Entstehung haftende Gasblasen, die eine grobe Orientierung über die Lokalisation des Fermentes ermöglichten.

Zum *Nachweis der Peroxidase* benutzten wir die *Benzidin*reaktion nach PRENANT, die sich in Vorversuchen gegenüber den Modifikationen von DE ROBERTIS und Mitarb., KREIBISCH und Mitarb. sowie LOELE überlegen gezeigt hatte. Die Ergebnisse wurden durch Anwendung einiger anderer für den Peroxidasenachweis gebräuchlicher Reagentien kontrolliert: α-Naphthol und p-Phenylendiamin (LOELE, DUPOY, STORCH), Leukosäurefuchsin (LISON), Patentblau (FAUTREZ), Guajakol (SCHÖNBEIN), Diaminofluoren (FEIGL). Die H_2O_2-Konzentration im Reagens wurde jeweils so eingestellt, daß die unempfindlichere Pseudoperoxidase, die vorwiegend im Blut enthalten ist, das Benzidin nicht in Benzidinblau überführte.

Die Fermentspezifität wurde durch Vorbehandlung der Schnitte mit Hydroxylamin (10^{-3} ... 10^{-2} mol) oder durch Erhitzen der Präparate auf 120 °C über 20 Min. gesichert. Für alle Versuchsanordnungen wurden Kontrollen mit Lösungen reiner Meerrettichperoxidase und Katalase[2]) durchgeführt.

Zum *Nachweis der H_2O_2-Penetration* eignet sich folgendes Vorgehen:

H_2O_2 wurde entweder als 3—30%ige wäßrige Lösung oder in Form einer 3—20%igen Emulsion (Lanettebasis) auf die vorher mit körperwarmem Wasser gereinigte Haut aufgebracht. Stark behaarte Häute wurden 24 Std. vor Versuchsbeginn vorsichtig — ohne Anwendung von Hilfsstoffen — rasiert. In situ dem H_2O_2 ausgesetzte Hautbezirke narkotisierter Tiere wurden noch während der H_2O_2-Einwirkung entnommen. Die Kältefixation erfolgte durch rasches Ein-

1) Herrn Prof. Dr. G. HOLLE, Direktor des Pathologischen Instituts der Karl-Marx-Universität, und Herrn Doz. Dr. med. habil. L. COSSEL danken wir für die Überlassung der Leichenhaut und Herrn Prof. Dr. H. UEBERMUTH, Direktor der Chirurgischen Klinik der Karl-Marx-Universität, sowie Herrn Dr. W. WEHNER für die Haut von Operationspräparaten.

2) Für die Überlassung der Reinfermente sind wir Herrn Dr. SCHÄFER und Herrn Dr. ROSE, VEB Arzneimittelwerk Dresden, dankbar.

bringen des behandelten Hautstückes in flüssigen Stickstoff. Während die H_2O_2-Lösungen unmittelbar vor dem Einfrieren abgetupft wurden, konnte die H_2O_2-Emulsion beim Einbringen in den Stickstoff noch auf der Haut belassen werden, da sie als gefrorene Platte nach Erhärten des Gewebes sich mühelos und sauber abheben ließ. Das eingefrorene, etwa rechteckige Hautstück wurde anschließend in eine für diese Zwecke geeignete Haltevorrichtung[1]) gespannt und mit dem Schlittenmikroton senkrecht zur Hautoberfläche (schmale, der Hautdicke entsprechende Kante zur Messerschneide) geschnitten. Die Schnittdicke betrug 40—60 μm. Durch seitliche Verschiebung und laufende Reinigung des Mikrotommessers wurde eine Verschleppung H_2O_2haltiger Verunreinigungen auf die Schnittfläche nachweisbar vermieden.

Während des Schneidens mußte die Nachkühlung mit CO_2 bzw. N_2 so geregelt werden, daß das gefrorene Objekt eine zum Schneiden ausreichende Konsistenz behielt. Die Gefrierschnitte wurden auf vorgekühlten Objektträgern unter dem Mikroskop mit dem sofort am Schnitt festfrierenden Reagens vorsichtig überschichtet. Während des allmählichen Auftauens setzte an Orten der H_2O_2-Anwesenheit die fermentchemische Farbreaktion bzw. die Gasblasenbildung ein, die mikrophotographisch festgehalten wurde, bevor sich das mikroskopische Bild durch Diffusion des gebildeten Farbstoffes oder Abwandern der Gasblasen innerhalb weniger Minuten verwischte. Die Aufnahmen wurden mit einer Spiegelreflexkamera (Exakta-Varex) und Miflexzusatzgerät angefertigt. Der Nachweis permeierten H_2O_2 in diesen Gefrierschnitten erfolgte in Analogie zu dem vorn geschilderten Fermentnachweis und in Anlehnung an AVI-Dor und Mitarb. ebenfalls mit der Benzidinreaktion. Zu diesem Zwecke wurde der eiweiß-glyzerinhaltigen Benzidinlösung statt H_2O_2 reine Meerrettichperoxidase zugesetzt, so daß sich H_2O_2 im Gewebe durch Benzidinblau lokalisieren ließ. Bei Zusatz von reiner Katalase zeigte sich die Gegenwart von H_2O_2 im Schnitt außerdem durch Entstehung von Gasblasen, die infolge der Viskosität des Reagensgemisches ausreichend lang am Orte ihrer Bildung hafteten. Soweit möglich, wurden die Ergebnisse auch durch Verwendung von Titanschwefelsäure kontrolliert.

Wäßrige Benzidin-Peroxidase-Lösung diente auch dazu, an der Froschschwimmhaut das Eindringen unzersetzten H_2O_2 in Gefäße nachzuweisen: Die Gefäße der hinteren Extremitäten männlicher Eskulenten wurden nach Läwen-Trendelenburg durchströmt. Das Reagens wurde unmittelbar vor der epikutanen Applikation 5—10%iger H_2O_2-Lösungen der zur Durchströmung verwendeten Frosch-Ringer-Lösung zugesetzt oder interlamellär injiziert.

In der Absicht, durch Anlage künstlicher, strukturgebundener Katalasedepots auch die subepidermale H_2O_2-Spaltung durch Beeinflussung der Gasblasenbildung histologisch zu verdeutlichen, erzeugten wir Terpentinölentzündungen. Dazu erhielten Ratten unter die rasierte Bauchhaut oder in die Haut der Hinterlaufpfoten Injektionen von 0,05 oder 0,1 ml Terpentinöl. Der Einstich erfolgte möglichst weit entfernt vom Depot. 72 Std. nach der Injektion wurde die Haut der untersuchten Region für die Dauer von 10—20 Min. mit 10—30%igen H_2O_2-Lösungen in Kontakt gebracht. Nach gründlicher Entfernung des H_2O_2 wurde der Gewebsbezirk in weitem Umkreis exzidiert, in Formalin fixiert, in Paraffin eingebettet, geschnitten und mit Hämatoxylin-Eosin gefärbt.

Als Modell für die Propagation von H_2O_2 in katalasereichem Gewebe wurden frisch entnommene Rattenlebern verwendet. Diese Organe wurden über 3 Min. in 6%ige H_2O_2-Lösungen ein-

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1) Die Haltevorrichtung wurde in dankenswerter Weise vom Mechanikermeister unseres Instituts, Herrn Teubner, angefertigt.

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gebracht — teilweise nach 5 Min. Vorbehandlung mit 1 mol Hydroxylaminlösung — und anschließend dem üblichen histologischen Schnitt- und Färbeverfahren (Paraffineinbettung, Hämatoxylin-Eosin-Färbung) unterzogen.

Ergebnisse

A. Hydroperoxidaseverteilung

Epidermis: Katalase konnte in keiner Epidermisschicht nachgewiesen werden. Nur in einigen Präparaten wurden vereinzelte Gasblasen an der Epitheloberfläche und im Bereich von Schweißdrüsenausführungsgängen beobachtet (Fig. 1).

Die Benzidin-*Peroxidase*-Reaktion verlief im Stratum corneum ebenfalls negativ, dagegen fiel sie in den Schichten des Stratum germinativum positiv aus. Die Intensität der Blaufärbung nahm von dem nur andeutungsweise tingierten Stratum granulosum bis zum Stratum basale allmählich zu (Fig. 2 und 3).

Das teilweise verhornte Oberflächenepithel der Zungen unserer Versuchstiere ließ weder Katalase- noch Peroxidaseaktivität erkennen (Fig. 4).

Kontrollversuche mit dem aus einer positiven Peroxidasereaktion hervorgegangenen Benzidinblau zeigten, daß eine Farbreaktion in den oben als peroxidasenegativ gekennzeichneten Bereichen färbungstechnisch durchaus möglich ist (vgl. auch Fig. 7).

Korium: Die Grenze zwischen Epidermis und Korium wurde deutlich durch den positiven Ausfall der Katalasereaktion unterhalb des Epithels markiert (Fig. 1 und 3). Die Entstehung der Gasblasen und die Peroxidase anzeigende Bildung von Benzidinblau gingen vorwiegend von Gefäßen und Muskelfasern aus, während in Übereinstimmung mit Ergebnissen an Zupfpräparaten Nerven, Bindegewebsfasern und Fettgewebe im allgemeinen keine nennenswerte Hydroperoxidaseaktivität sichtbar werden ließen. An Schnitten, die mit Hydroxylamin oder durch Erhitzen vorbehandelt worden waren, blieben die Reaktionen auf Katalase und Peroxidase aus.

B. Wasserstoffperoxid-Penetrations-Nachweis

H_2O_2 konnte bereits nach wenigen Minuten epikutaner Einwirkung 5—30%iger Lösungen in der Epidermis lokalisiert werden. Entsprechend den im Reagens enthaltenen Fermenten entwickelten sich gewissermaßen Komplementärbilder zu dem vorangehend gezeigten Hydroperoxidasenachweis in der unbehandelten Haut (Fig. 1—3): Die beim Nachweis der Hydroperoxidaseverteilung ungefärbten bzw. gasblasenfreien Epidermisschichten waren nach epikutaner Anwendung des H_2O_2 blau tingiert bzw. von Gasblasen überlagert (Fig. 6). Erst nach mehrstündiger Einwirkungsdauer von hochprozentigem H_2O_2 auf Leichenhaut oder nach vorangehender Infiltration der Haut mit Hydroxylamin konnte ein positiver Ausfall der Reaktionen auf H_2O_2 auch im Korium beobachtet werden (Fig. 7).

Rattenhäute, an denen durch Terpentinöl-Injektionen Entzündungen hervorgerufen worden waren, ließen nach epikutaner H_2O_2-Einwirkung über den relativ oberflächlich liegenden Infiltraten Emphyseme erkennen (Fig. 9 und 10). An den *nicht* entzündlich veränderten Kontrollhäuten traten ausgeprägte Emphyseme nach gleich oder länger dauernder Einwirkung von H_2O_2 dagegen erst im Bereiche der tiefer liegenden, katalasehaltigen Strukturen (z. B. Muskulatur) auf (Fig. 8).

Nach epikutaner H_2O_2-Einwirkung auf stark behaarte tierische oder menschliche Haut waren die intrakutan auftretenden Gasblasen vorwiegend zwischen den Haarfolikeln angeordnet. — Durch Einmassieren einer H_2O_2-haltigen Emulsion wurden an der Rattenbauchhaut wesentlich stärker ausgedehnte Emphyseme erzielt als durch einfaches Aufstreichen einer Emulsion gleicher H_2O_2-Konzentration (vgl. Fig. 11 und 12).

Am Vertikalschnitt der Froschschwimmhaut waren Emphyseme nach epikutaner Einwirkung von H_2O_2 histologisch bereits unterhalb der Epidermis zu lokalisieren. In den der Epidermis zumeist dicht anliegenden Gefäßen konnte nach epikutaner Applikation von 5—10%igen H_2O_2-Lösungen binnen weniger Sekunden H_2O_2 nachgewiesen werden, wenn die Gefäße mit benzidin-peroxidasehaltiger Ringerlösung durchströmt oder das Reagens zwischen die Schwimmhautlamellen appliziert wurde (Fig. 13).

In Rattenlebern, die als Modelle zum Vergleich herangezogen wurden, zeigten sich oberflächennahe Gasblasen, wenn die Organe in H_2O_2-Lösungen eingebracht wurden. Ging dieser Einwirkung eine Vorbehandlung mit Hydroxylamin voraus, so waren die Gasblasen fast ausschließlich in der Tiefe des Organs lokalisiert.

Diskussion

A. Zur Hydroperoxidaseverteilung

Die Darstellung der *Katalase*-Verteilung in der Haut wurde dadurch erschwert, daß geeignete histochemische Färbetechniken zum Nachweis dieses Fermentes bisher noch nicht bekannt geworden und wohl auch nicht zu erwarten sind.

Nishiyama und Mitarb. (1953) haben den Versuch gemacht, die Katalase histochemisch in verschiedenen Organen zu lokalisieren. Sie gingen dabei von der Tatsache aus, daß Katalase Monoäthylperoxid zu Azetaldehyd abbauen kann, den sie mit Schiffschem Reagens nachwiesen. Wir konnten uns durch Verwendung von Reinfermenten davon überzeugen, daß die gleiche Reaktion auch durch Peroxidase beschleunigt wird und unter Azetaldehydbildung beim Kontakt mit Luft sogar spontan verhältnismäßig rasch abläuft[1]). Diese Methode ist also zum spezifischen Nachweis von Katalase unbrauchbar. Verfahren, die darauf beruhen, daß Leukofarbstoffe als Sauerstoffakzeptoren angeboten werden, sind ebenfalls nicht verwendbar, weil der Katalase stets auch peroxidatische Aktivität anhaftet.

1) Die Herstellung des Monoäthylperoxids verdanken wir dem Chemiker unseres Instituts, Herrn Dr. R. Schmiedel.

Da lediglich die Abspaltung molekularen Sauerstoffs aus Wasserstoffperoxid definitionsgemäß für die Katalase spezifisch und für die vorliegende Fragestellung auch besonders bedeutsam ist, waren wir gezwungen, die beschriebene Gasblasenbildung am horizontal liegenden histologischen Schnitt heranzuziehen. Diese Methode vermittelt zwar im Vergleich zum Nachweis der Peroxidase nur eine relativ grobe Orientierung, es konnte aber mit ihr der in diesem Zusammenhang interessierende Nachweis erbracht werden, daß das Epithel der Haut und der Schleimhaut katalatisch praktisch inaktiv ist und demzufolge H_2O_2 nicht durch enzymatische Spaltung an der Penetration hindern kann. Außerdem erlaubten die mit dieser Methodik gewonnenen Ergebnisse eine korrigierende Interpretation der quantitativen Katalasebestimmungen von WOHLGEMUTH und Mitarb., SZÖRENYI, YAMASAKI sowie von MELCZER, auf die sich die bisher gültigen Ansichten vom Katalasegehalt der Haut stützten.

Die Autoren hatten Haut durch 2 Horizontalschnitte grob in 3 Schichten zerlegt, diese homogenisiert und beim Vergleich der titrimetrisch ermittelten Kataloseaktivitäten — in Übereinstimmung mit eigenen Nachuntersuchungen — keine nennenswerte Differenz finden können. Sie zogen daraus den Fehlschluß, *allen* Schichten der Haut, also auch der Epidermis, sei annähernd gleiche Kataloseaktivität zuzuschreiben. Da jedoch bei einem grob geführten Horizontalschnitt unterhalb des Epithels zumeist der gefäßreiche Papillarkörper miterfaßt und ins Homogenisat der obersten Schicht aufgenommen wird, verdeckt dessen Blutkatalasereichtum bei der quantitativen Bestimmung den Katalasemangel des Epithels.

Die zum histochemischen *Peroxidase*-Nachweis verwendete Benzidinreaktion hat sich uns aus verschiedenen Gründen bewährt: Die Methode gilt als die besterprobte und weitestverbreitete; die Reaktion setzt verhältnismäßig rasch ein, sie ist im Vergleich zu anderen, meist mehrstufigen Verfahren ausreichend empfindlich und einfach. Außerdem bot sie die Möglichkeit, mit dem gleichen Reaktionsprinzip ohne Verwendung eines Färbebades am horizontal liegenden Schnitt durch Überschichten des Nativpräparates mit Reagens auch H_2O_2 in Spuren nachzuweisen. Zu diesem Zweck mußte dem Reagensgemisch lediglich reine Peroxidase statt H_2O_2 zugesetzt werden.

Die Ergebnisse lassen den Schluß zu, daß Sauerstoff des in die Epidermis eintretenden H_2O_2 durch Peroxidasen im Bereich des Stratum germinativum auf geeignete Akzeptoren übertragen werden kann, wenn H_2O_2 das hydroperoxidatisch inaktive Stratum corneum durchdringt.

B. Zum Wasserstoffperoxid-Penetrations-Nachweis

Die zum Wasserstoffperoxidnachweis am histologischen Schnitt verwendete Benzidinreaktion war, außer den bereits genannten Gründen, deshalb auch hier besonders geeignet, weil das entstehende Benzidinblau besser als andere Reagentien — z. B. Titanschwefelsäure bzw. Pertitansäure — gegen die Eigenfarbe der Nativschnitte kontrastierte, weil der Indikator mindestens die gleiche Empfindlichkeit wie spezifischere Reagentien besitzt und weil mit dem positiven Ausfall der Reaktion zugleich demonstriert werden konnte, daß eine beim Peroxidasenachweis ungefärbte Struktur prinzipiell mit Benzidinblau tingierbar ist. Da der Indikator auf molekularen Sauerstoff nicht in dieser Weise anspricht und da Kontrollversuche mit Titanschwefelsäure oder mit Katalaselösung als H_2O_2-Indikator entsprechende, wenn auch weniger photogene Ergebnisse lieferten, war für die gestellten Anforderungen die Spezifität der Benzidinreaktion auf H_2O_2 gesichert.

Weil methodisch zu befürchten war, daß nach der Behandlung der Hautoberfläche noch anhaftendes H_2O_2 beim Schneiden auf die Schnittfläche verschleppt und so eine Penetration vorgetäuscht worden sein konnte, führten wir Vorversuche mit farbigen Lösungen und Emulsionen durch. Bei Beachtung der sich daraus ergebenden Kautelen war diese Fehlerquelle auszuschließen. Wie Fig. 5 zeigt, verblieb H_2O_2, das nur für 2 Sek. mit der Haut in Kontakt gebracht und dann

ceteris paribus entfernt worden war, als kleiner Rest an der Oberfläche. Wenn nach unterschiedlichen Einwirkungszeiten H_2O_2 *in der Epidermis* nachgewiesen werden konnte, so überschritt die Färbung — übereinstimmend mit der Tatsache, daß die katalatische Spaltung erst unterhalb der Epidermis beginnen kann — zunächst doch nicht die Grenze zwischen Epidermis und Korium. Eine solche Grenze wäre bei nachträglichem Einschleppen von H_2O_2 nicht ohne weiteres eingehalten worden.

Die Kältefixation mit flüssigem Stickstoff erschwerte das Schneiden der glashart gefrorenen Objekte ungemein und ließ nur die Herstellung verhältnismäßig dicker Schnitte zu. Sie mußte aber trotzdem durchgeführt werden, da alle anderen Fixationsmethoden im Hinblick auf die Spaltungsgeschwindigkeit und die Wasserlöslichkeit des H_2O_2 zu langsam und zu indifferent sind. Wie wir in Übereinstimmung mit Shikama und Mitarb. auch für unsere Versuchsanordnung feststellen konnten, tritt eine Hydroperoxidase-Inaktivierung durch die hier vorherrschenden niedrigen Temperaturen (etwa —196 °C) praktisch nicht in Erscheinung.

Der Nachweis von unzersetztem H_2O_2 *im Korium* gelang uns mit dieser Methode nur nach vorangehender Hemmung der Hydroperoxidaseaktivität oder nach extremen Einwirkungszeiten von hochprozentigem H_2O_2 an der Leichenhaut. Das aber führte im Hinblick auf die Katalaseverteilung und unsere histologischen Befunde (als Beispiel s. Fig. 8) zu der Vorstellung, daß H_2O_2 nach Penetration der gefäßlosen Epidermis verhältnismäßig rasch auf interzellulärem oder vasalem Wege abtransportiert und erst beim Kontakt mit katalasehaltigen Strukturen im Korium oder in tieferen Schichten unter Freisetzung molekularen Sauerstoffs gespalten wird. Zur Bestätigung dieser Annahme eigneten sich besser als relativ rasch resorbierbare Katalaselösungen entzündliche Infiltrate, in denen die Hydroperoxidase korpuskulär gebunden und am gewünschten Ort einigermaßen zu halten ist. Damit war histologisch demonstrierbar, daß in der lebenden Haut die Abspaltung von Sauerstoff vorwiegend über dem Infiltrat und in Bezirken erfolgt, in denen am Kontrolltier sonst keine nennenswerte Gasbildung zu erkennen war.

Dieser Versuchsanordnung am Tier entsprechen die klinischen Beobachtungen von Peter, der die Applikation von H_2O_2 auf das Zahnfleisch zur Abgrenzung subepithelialer Infiltrate und Abszesse empfohlen hat. Erst kürzlich wies auch Coppes (1962) wieder darauf hin, daß die durch H_2O_2 verursachte weiße Verfärbung an *entzündeten* Schleimhäuten des Menschen besonders deutlich hervortritt.

Um uns davon zu überzeugen, daß für die Lokalisation der Gasblasen tatsächlich die Verteilung der Fermentaktivität und nicht die morphologische Beschaffenheit des vom H_2O_2 durchdrungenen Gewebes ausschlaggebend ist, wurde die Einwirkung von H_2O_2 auf isolierte, teilweise mit Hydroxylamin vorbehandelter Lebern zum Vergleich herangezogen. An diesem verhältnismäßig homogenen Modell war zu veranschaulichen, daß H_2O_2 auch in die katalasereichsten Gewebe unter Emphysembildung eindringen kann, katalasearme — hier künstlich inaktivierte — Regionen aber ohne Gasblasenbildung durchwandert und erst in der Tiefe gleichartigen, jedoch katalatisch aktiven Gewebes durch enzymatische Spaltung O_2 freisetzt.

Die beim H_2O_2-Kontakt mit einer katalasereichen Struktur entstehende Gasblase schützt für die Dauer ihres Bestehens vor weiterer Einwirkung und Spaltung des H_2O_2 an gleicher Stelle und damit vor Katalase-Inaktivierung (s. später).

Der Nachweis, daß die teilweise in den Kapillaren beobachteten Gasblasen ihre Entstehung einer *intravasalen* H_2O_2-Spaltung verdanken können, war an Warmblütlern wegen der verhältnismäßig hohen Gewebs- und Blutkatalaseaktivität nicht zu erbringen. Er gelang aber an der katalatisch weniger aktiven Froschschwimmhaut, deren Gefäße der direkten Beobachtung gut zugängig sind und die in bezug auf die subepithelisle Entstehung von Gasblasen nach epikutaner H_2O_2-Applikation geringere, aber mit Warmblütlern durchaus vergleichbare Reaktionen gezeigt hatte.

Während bisher noch nicht entschieden wurde, ob das Eindringen von Wasser in die Haut transepidermal oder über die Anhangsgebilde erfolgt (ROTHMAN), lassen unsere histochemischen und histologischen Ergebnisse erkennen, daß die „präformierten Wege" der Haut vom H_2O_2 nicht bevorzugt werden. Es ist sogar wahrscheinlich, daß sie für das H_2O_2 nahezu verschlossen sind, da in den epithelialen Haarwurzelscheiden sperrende Luftblasen eingelagert sind, da sich im Schweiß unter H_2O_2-Einwirkung Gasbläschen bilden können und da die an den Austrittsstellen der präformierten Wege vorhandenen Konkavitäten nach den Vorstellungen von GLASSER als Zentren für die Bildung von Blasen in gashaltigen Lösungen angesehen werden müssen. An diesen Stellen ist dann mit einer Benetzung und mit einem Eindringen von H_2O_2 noch weniger zu rechnen, als das für andere wäßrige Lösungen bekannt ist (IMPENS, WINTERNITZ, CALVERY, ROTHMAN). Wir konnten im Auflichtmikroskop auch beobachten, daß die bei epikutaner Applikation von H_2O_2 auf der menschlichen Haut entstandenen Gasblasen vorwiegend im Bereiche solcher Konkavitäten lokalisiert waren.

An der Rattenbauchhaut kommen vorwiegend die Haarwurzelscheiden in Betracht, weil die Schweißdrüsen nach den Angaben von TENNET sowie HIERONYMI und eigenen Beobachtungen bei der Ratte kaum ausgebildet sind.

Da sich die in den präformierten Wegen befindliche Luft durch Einmassieren von Salben verdrängen läßt, können nach CALVERY und Mitarb., ROTHMAN und Mitarb. u. a. penetrationsfähige, in Salben inkorporierte Substanzen durch Massage in größerem Umfange zur Resorption gebracht werden. Auch für H_2O_2 ließ sich makroskopisch und histologisch an der Emphysemausdehnung deutlich demonstrieren, daß die Aufnahme durch Einmassieren einer H_2O_2-haltigen Salbe gesteigert werden kann. Die gleiche Menge der H_2O_2-Salbe führte nach einfachem Bestreichen der Haut zu wesentlich geringerer Emphysembildung, und zwar auch dann, wenn die Haut vor und oder nach der Applikation einer entsprechenden Massage ausgesetzt wurde (vgl. Fig. 11 und 12).

Ergebnisse

Zur Beantwortung der Frage, ob epikutan appliziertes Wasserstoffperoxyd die Epidermis durchdringen kann oder ob auf Grund der Hydroperoxidaseaktivität des Epithels eine quantitative Staltung des H_2O_2 noch vor Erreichen des Koriums erfolgt, war es zunächst erforderlich, die Enzymverteilung in der Haut aufzuklären. An nativen Gefrierschnitten wurde die Katalase durch ein H_2O_2-haltiges Reagens und die Peroxidase mit der Benzidinreaktion lokalisiert. Es wurde festgestellt, daß die Epidermis keine katalatische Aktivität besitzt. Peroxidase dagegen findet sich im Stratum germinativum, während das Stratum corneum peroxidasefrei ist. Im Korium und den tieferen Gewebsschichten zeigten Blutgefäße und Muskulatur deutlich positive Nerven, Bindegewebsfasern und Fettgewebe dageben nur *angedeutet* positive oder negative Hydroperoxidasereaktionen.

Mit einer histotopochemischen Technik, deren Einzelheiten kurz beschrieben und diskutiert werden, wurde mikrophotographisch dargelegt, daß H_2O_2 die Epidermis unzersetzt penetrieren und ins Korium vordringen kann. Die Penetration erfolgt unter Vernachlässigung der „präformierten Wege" vorwiegend transepidermal. Die Lokalisation der durch enzymatische Spaltung penetrierten H_2O_2 entstehenden Emphyseme ist vorwiegend von der Katalaseverteilung im behandelten Gewebs-

bereich abhängig. Die in den Kapillaren der Froschschwimmhaut nach epikutaner H_2O_2-Applikation auftretenden Gasblasen können intrakapillar durch Eindringen von H_2O_2 entstehen.

Zusammenfassung

Im Zusammenhang mit der therapeutischen Anwendung hochprozentiger Wasserstoffperoxidlösungen wird histotopochemisch nachgewiesen, daß H_2O_2 die Epidermis unzersetzt penetrieren und ins Korium vordringen kann. Die Penetration erfolgt unter Vernachlässigung der „präformierten Wege" vorwiegend transepidermal. Die Lokalisation der Emphyseme, die sich bei der enzymatischen Spaltung des penetrierten H_2O_2 bilden, ist vorwiegend von der Katalaseverteilung im behandelten Gewebsbereich abhängig.

Резюме

В связи с лечебным применением высокопроцентных растворов пероксиси водорода доказывается гистохимическим путем, что H_2O_2 может проникнуть — не разложившись — через эпидермис и попасть в corium. Проникновение происходит при пренебрежении «преформированных путей» преимущественно через эпидермис. Локализация эмфизем, образующихся при ферментативном расщеплении проникнутого H_2O_2, зависит преимущественно от распределения каталазы в обработанной области ткани.

Summary

In connexion with the therapeutic use of strong hydrogen peroxide solutions it is demonstrated histotopochemically that H_2O_2 is able to penetrate the epidermis undisintegrated, advancing into the corium. The penetration takes place transdermically for the most part, neglecting 'preformed ways'. The localization of the emphysemas generated in the process of enzymatic decomposition of penetrated H_2O_2 depends for the most part on catalase distribution in the tissue area treated.

Résumé

En liaison avec l'application thérapeutique des solutions du péroxyde d'hydrogène à haute teneur, l'auteur a constaté histotopochimiquement que H_2O_2 peut pénétrer l'épiderme et avancer dans le corium sans être indécomposé. Cette pénétration se fait — en négligeant les "voies préformées" — surtout transépidermalement. La localisation des emphysèmes qui se forment à l'occasion de la dissociation du H_2O_2 pénétré dépend principalement de la distribution de la catalase dans le tissu traité.

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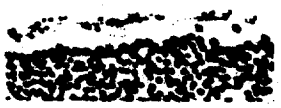

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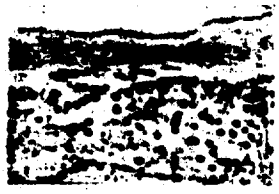

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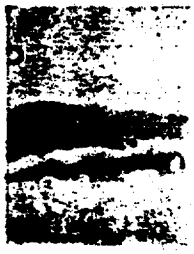

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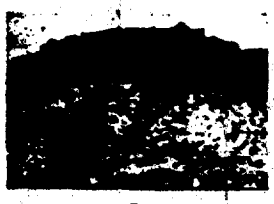

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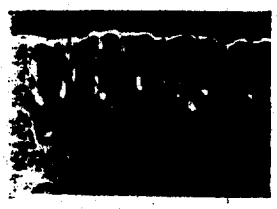

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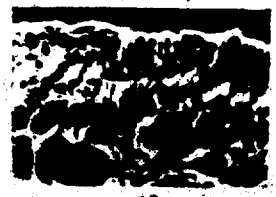

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Tafelerklärung

Tafel VIII

Fig. 1. Menschliche Sohlenhaut, Katalasenachweis (mikroskopische Vergr. 10fach).

Fig. 2. Menschliche Sohlenhaut, Peroxidasenachweis (mikroskopische Vergr. 16fach).

Fig. 3. Menschliche Sohlenhaut, Hydroperoxidasenachweis (mikroskopische Vergr. 10fach).

Fig. 4. Katzenzunge, Hydroperoxidasenachweis (mikroskopische Vergr. 10fach).

Fig. 5. Menschliche Sohlenhaut, Nachweis von H_2O_2-Resten nach 2 Sek. epikutaner Einwirkung von H_2O_2, 10%ig (nativ; mikroskopische Vergr. 16fach).

Fig. 6. Menschliche Sohlenhaut, Nachweis von H_2O_2 nach 20 Min. epikutaner Einwirkung von H_2O_2, 10%ig (nativ; mikroskopische Vergr. 16fach).

Fig. 7. Menschliche Sohlenhaut (Leiche), Nachweis von H_2O_2 nach 180 Min. epikutaner Einwirkung von H_2O_2, 20% (nativ; mikroskopische Vergr. 10fach).

Fig. 8. Rattenpfote, 30 Min. nach epikutaner Einwirkung von 10%igem H_2O_2 (Emphysembildung besonders im tiefliegenden Bereich der quergestreiften Muskulatur) (H.-E.; mikroskopische Vergr. 10fach).

Fig. 9. Rattenpfote, Terpentinölentzündung: 30 Min. nach epikutaner Einwirkung von 10%igem H_2O_2 (Emphysembildung im oberflächlich liegenden Bereich des entzündlichen Infiltrates) (H.-E.; mikroskopische Vergr. 16fach).

Fig. 10. Rattenbauchhaut, Terpentinölentzündung: 10 Min. nach epikutaner Einwirkung von 20%igem H_2O_2 (Emphysembildung über Infiltrat) (H.-E.; mikroskopische Vergr. 10fach).

Fig. 11. Rattenbauchhaut, nach Aufstreichen einer 10%igen H_2O_2-Emulsion (Einwirkungsdauer 5 Min.) (H.-E.; mikroskopische Vergr. 10fach).

Fig. 12. Rattenbauchhaut, nach Einmassieren (5 Min.) einer 10%igen H_2O_2-Emulsion (H.-E.; mikroskopische Vergr. 10fach).

Fig. 13. Froschschwimmhaut, Durchsicht, intravasaler H_2O_2-Nachweis nach 10 Sek. epikutaner Einwirkung von 10%igem H_2O_2 (nativ; mikroskopische Vergr. 16fach).

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Abhandl. Deut. Akad. Wiss., Berlin, Kl. Chem., Geol. Biol. 1964(7):549-552.

Demonstration of ^{18}O in Expirated Air and Blood during sublingual action of ^{18}O - tagged hydrogen peroxide

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Summary:

The intraoral application, especially the sublingual application of hydrogen peroxide (H_2O_2) in contrast to the epicutaneous application, leads very rapidly to the formation of large gas bubbles in the veins carrying blood away. An investigation was undertaken to determine whether the gas supplied is exhaled through the lung in this manner to the blood stream, and whether the oxygen from the H_2O_2 also reaches the systemic circulation after lung passage. To answer this question H_2O_2 was tagged with the stabile isotope ^{18}O. By mass spectrometrical determination of the relative frequency of ^{18}O in oxygen from expirated air and blood, it could be shown in cats, that within comparatively short time a considerable part (in our experiments within less than one hour almost a third) of oxygen, which would be released with complete decomposition of the available H_2O_2, is exhaled through the lung. Although in the systemic circulation no gas bubbles could be observed, the tagged oxygen originating from the H_2O_2 could also be traced in arterial blood. Simultaneously blood gas analyses of van SLYKE nevertheless showed that the oxygen saturation of the arterial blood under sublingual H_2O_2 application decreased. Methodology and significance of the results are briefly discussed.

1. Introduction

In a previous paper (1) we were able to show, that hydrogen peroxide (H_2O_2) after intraoral application in contrast to epicutaneous application can rapidly lead to the formation

of large gas bubbles in the veins carrying blood away, and thus can cause pulmonary gas embolisms. With regard to our investigation results on the distribution and effect of intravasal insufflated oxygen (2) the questions arose, whether the oxygen introduced through intraoral H_2O_2-application, which is brought into the blood stream is again exhaled through the lung, and whether oxygen from H_2O_2 reaches the systemic circulation after passage through the lung. To answer these questions it was necessary to tag the oxygen in H_2O_2 with the stabile isotope ^{18}O and then the ^{18}O concentration in the expirated air as well as in the arterial blood to determine mass-spectrometrically [1]. Such a procedure offered at the same time the possibility, to demonstrate in an exact manner, that the rise which was observed after the effect of H_2O_2 on the skin, or rather oxygen saturation in the venous blood by penetration of the oxygen from the H_2O_2 is caused by and cannot just be explained alone by decrease in utilisation or the opening of arterial-veinal anastomoses. This investigation has another practical significance, because H_2O_2 is also used in dentistry intraorally in solutions.

2. Methodology

Due to the expense of these experiments with tagged hydrogen peroxide, we were able to perform our experiments on only two cats after several preliminary experiments with untagged H_2O_2 and with ^{18}O - tagged O_2. Involved were a 3.4 kg female (experiment 1) and a 2.6 kg male (experiment 2). The cats were anesthesized with 90 mg/kg Chloralose and 360 mg/kg Urethane. Measured amounts of a 9 or 19% H_2O_2 solution with an ^{18}O - concentration of 4.03 or 3.96 atomic % were slowly dripped upon the slightly concave tongue underside, so that no significant disturbances in breathing and circulation occurred. In the first experiment the effect was

1) We thank the Institute For Physical Separation of Materials of the Academy of Sciences of Berlin (Dir.: Prof. Dr. J. Mühlenpfordt), especially Dr. G. HÜBNER, together with Dr. GORISCH, Institute for Pharmacology and Toxicology of the Karl-Marx-University, who assisted in performing the experiments, for the very expensive tagging of H_2O_2 through glow discharge.

left alone until the end of the experiment, whereas in the second experiment the application was distributed between two portions. The interval was introduced through rinsing the tongue underside with physiological common salt. Recording of the blood pressure in the left A. carotis occurred by means of the Trendelenburg counterflow apparatus and mercury-manometer. Recording of breathing occurred by means of tracheal cannuli with branching to Marey's capsule on a Russ-kymograph. Directly on the tracheal cannula was a tightly fastened 1.5 cm beneath the cricoid a low-resistance breathing valve, whose inhalation side was connected through a rubber hose with the exterior air and whose exhalation side was connected to a silica-gel-cartridge with breathing bags through a cold trap (ca. -- 70^0 C). Samples of expirated air were continuously decanted during the experiment from the breathing bags into evacuated special glass containers for mass-spectrometrical determination of the relative frequency of ^{18}O in oxygen. We checked the content of O_2 and CO_2 in air breathed by HALDANE'S method. We examined the blood samples taken during the experiment from the left A. femoralis and after completion of the experiment from the right heart cavity for their O_2- and CO_2- content according to the manometric method of van SLYKE or SENDROY using a modification by LUDEWIG and ROMISCH (3) as well as by HOLFELD (4). At the same time the relative frequency of ^{18}O in blood oxygen was determined in a few of these blood samples. Calculation of the oxygen amount present in the breathed air, or in blood and derived from the H_2O_2 was done according to the principle of isotope-dilution-analysis. For technical details concerning collection, decanting of samples as well as analysis and its evaluation, refer to HUBNER, LUDEWIG, GORISCH, BIRKENFELD (5); HUBNER, LUDEWIG, and GORISCH (6); GORISCH, HUBNER and LUDEWIG (7).

3. Results

As may be seen from Table 1, almost one third of that oxygen quantity which is exhaled would be released on complete

resorption and splitting of H_2O_2, by sublingual application of 1.5 ml 9% H_2O_2 within 58 minutes or of 0.1 ml 19% H_2O_2 within 34 minutes. The expirated air collected within the first five minutes after onset of the effect, already reached an ^{18}O - concentration corresponding to the average value. Within the interval lasting 78 minutes between two H_2O_2 - effects in the second experiment (see Table 1, No. 2a, b) no more tagged oxygen was exhaled, while in the first 7 minutes after renewed H_2O_2 -input an exhalation of ^{18}O-tagged oxygen could be demonstrated again.--- In the exhaled carbon dioxide no increase in ^{18}O-amount could be determined. Under the H_2O_2-effect a significant increase in ^{18}O-concentration in the arterial blood was measured, even though neither through behavior of blood pressure and breathing nor subsequently by section was it possible to show the presence of gas bubbles in the systemic circulation. In agreement with our earlier observations, the oxygen saturation of the arterial blood gradually decreased. The CO_2 -content increased correspondingly. Part of the oxygen separated from the H_2O_2 was rediscovered in the veinal blood at the end of the experiment.

4. Discussion

Even though our main experiments were performed on only two cats, the question we posed, as to exhalation of the oxygen was clearly answered. In addition to the high measurement accuracy, the findings that the tagged oxygen in the peroxide link exchanges neither with water nor with air and that the entire oxygen formed during the decomposition of H_2O_2 in hydros solution stems from the peroxide, proved to be especially advantageous (8,9,10,11,12).

A part of the oxygen reaching the veins passes the lung capillaries and reaches systemic circulation. However, since we could never observe gas bubbles in the left heart and in the arteries, it must be assumed, that the oxygen rediscovered in the arterial blood was combined or dissolved at the latest before entry into the left heart. Under no circumstances may it be speculated from this result, that

one could in this manner improve the oxygen supply of the organism. Examination of the manometrically determined blood gas values alone shows that the oxygen supply really becomes worse. In earlier experiments we could show, that the cause for this is to sought in a disturbance of the gas exchange within the lung triggered by pulmonary gas embolism. A part of the separated oxygen which was not sought by us in this connection (as previous histological investigations have shown) is stored in the tongue tissue itself and could be utilized there if the need arose.---The fact, that the relative frequency of ^{18}O in CO_2 was not raised, does not exclude a utilization at all, since the oxygen separated from the H_2O_2 can enter only in the carbon dioxide through the body water. If the oxygen amount separated from the H_2O_2 is even so great, that emphesema can form, it does not suffice to effect a measurable isotope displacement in body water (13).

Sublingual Action						Expirated Air			
Experiment no.	H_2O_2-content of solution in %	^{18}O concentration in atomic %	Amt. in ml	Action time in min.	max. fissionable O_2 in ml	O_2 rediscovered in expirated air		^{18}O portion in atomic %	
						in ml	in %	before H_2O_2 input	under H_2O_2 action
1	9	4.02	1.5	58	46.5	12.8	27.5	0.205 ± 0.001	0.225 ± 0.004
2a	19	3.96	0.1	34	6.6	2.0	30.3	0.204 ± 0.002	0.210 ± 0.003
2b	19	3.96	0.35	18	23.3	1.6	6.9	0.204 ± 0.002	0.219 ± 0.00[illegible]

Blood Gases								
Experiment no.	O_2 saturation % of arterial blood		CO_2-Volume% in arterial blood		^{18}O Atom % in arterial blood		^{18}O % in veinal blood at end of experiment	O_2 in m rediscovered in tota blood a end of experiment
	before H_2O_2 input	after H_2O_2 action	before H_2O_2 input	after H_2O_2 action	before H_2O_2 input	after H_2O_2 action		
1	92.4	57.8	61.8	68.3	0.205	0.260		
2a	89.5	77.7	56.3	59.7	0.205	0.217	0.400	0.8
2b	61.2	35.5	62.3	66.7	0.205	0.219	0.258	0.15

Table 1: Demonstration of ^{18}O in expirated air and blood during sublingual action of ^{18}O - tagged H_2O_2.

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Nachweis von ^{18}O in Exspirationsluft und Blut während sublingualer Einwirkung ^{18}O-markierten Wasserstoffperoxids

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Zusammenfassung:

Da die intraorale, insbesondere die sublinguale Anwendung von Wasserstoffperoxid (WPO) im Gegensatz zur epikutanen Applikation sehr rasch zum Auftreten größerer Gasblasen in den ableitenden Venen führt, wurde untersucht, ob das auf diese Weise in die Blutbahn eingebrachte Gas über die Lunge wieder abgeatmet wird und ob dem WPO entstammender Sauerstoff nach Lungenpassage auch in den großen Kreislauf gelangt. Zur Beantwortung dieser Fragestellung wurde WPO mit dem stabilen Isotop ^{18}O markiert. Durch massenspektrometrische Ermittlung der relativen Häufigkeit des ^{18}O im Sauerstoff von Exspirationsluft und Blut konnte an Katzen gezeigt werden, daß innerhalb verhältnismäßig kurzer Zeit ein beträchtlicher Anteil (in unseren Versuchen innerhalb von weniger als 1 Stunde fast 1/3) des Sauerstoffs, der bei vollständiger Zersetzung des angebotenen WPOs frei würde, über die Lunge abgeatmet wird. Obwohl im großen Kreislauf keine Gasblasen beobachtet werden konnten, war dem WPO entstammender, markierter Sauerstoff auch im arteriellen Blut nachweisbar. Gleichzeitig durchgeführte Blutgasanalysen nach van SLYKE ließen jedoch erkennen, daß die Sauerstoffsättigung des arteriellen Blutes unter der sublingualen WPO-Anwendung abfiel. Methodik und Bedeutung der Ergebnisse werden kurz diskutiert.

1. Einleitung

In einer vorangehenden Arbeit / 1 / hatten wir zeigen können, daß Wasserstoffperoxid (WPO) nach intraoraler Anwendung im Gegensatz zur epikutanen Applikation rasch zum Auftreten größerer Gasblasen in den ableitenden Venen führen und somit pulmonale Gasembolien hervorrufen kann. Im Hinblick auf unsere Untersuchungsergebnisse über Verbleib und Wirkung intravasal insufflierten Sauerstoffs / 2 / erhoben sich daher die Fragen, ob der durch intraorale WPO-Anwendung in die Blutbahn eingebrachte Sauerstoff über die Lunge wieder abgeatmet wird und ob dem WPO entstammender Sauerstoff nach Lungenpassage auch in den großen Kreislauf gelangt. Zur Beantwortung dieser Fragen war es erforderlich, den Sauerstoff im WPO mit dem stabilen Isotop ^{18}O zu markieren und dann die ^{18}O-Konzentration in der Exspirationsluft sowie im arteriellen Blut massenspektrometrisch zu ermitteln[1]. Ein solches Vorgehen bot zugleich die Möglichkeit, exakt zu beweisen, daß der nach Einwirkung von WPO auf Haut bzw. Schleimhaut beobachtete Anstieg der Sauerstoffsättigung im abströmenden Venenblut durch Eindringen dem WPO entstammenden Sauerstoffs verursacht wird und nicht allein mit Senkung der Sauerstoff-Utilisation oder Eröffnung arterio-venöser Anastomosen erklärbar ist.
Eine praktische Bedeutung kommt der Bearbeitung dieser Fragestellung deswegen noch zu, weil WPO auch in Form hochprozentiger Lösungen intraoral zahnärztlich angewandt wird.

2. Methodik

In Anbetracht der Kostspieligkeit der aufwendigen Versuche mit markiertem Wasserstoffperoxid konnten wir unsere Untersuchungen nach mehreren Vorversuchen mit nichtmarkiertem WPO und mit ^{18}O markiertem O_2 nur an 2 Katzen durchführen. Es handelte sich dabei um ein weibliches 3,4 kg (Versuch 1) und ein männliches 2,6 kg schweres Tier (Versuch 2).

1) Die sehr kostspielige Markierung des H_2O_2 auf dem Wege der Glimmentladung verdanken wir dem Institut für physikalische Stofftrennung der Akademie der Wissenschaften zu Berlin (Dir.: Prof. Dr. J. Mühlenpfordt), insbesondere Herrn Dr. G. HÜBNER, der uns zusammen mit Herrn Dr. GÖRISCH, Institut für Pharmakologie und Toxikologie der Karl-Marx-Universität, bei der Durchführung der Versuche unterstützt hat.

Die Katzen wurden mit 90 mg/kg Chloralose und 360 mg/kg Urethan i. p. narkotisiert. Abgemessene Mengen einer 9 bzw. 19%igen WPO-Lösung mit einer ^{18}O-Konzentration von 4,03 bzw. 3,96 Atomprozent wurden auf die leicht konkav gelagerte Zungenunterseite so langsam aufgetropft, daß keine größeren Störungen von Atmung und Kreislauf auftraten. Im ersten Versuch wurde die Einwirkung bis zum Versuchsende belassen, während im zweiten Versuch die Applikation auf zwei Portionen verteilt wurde. Das Intervall wurde eingeleitet durch Spülung der Zungenunterseite mit physiologischer Kochsalzlösung.

Die Registrierung des Blutdruckes in der linken A. carotis erfolgte mittels Trendelenburgscher Gegenstromapparatur und Quecksilber-Manometer, die der Atmung über Trachealkanüle und Abzweig zur Mareyschen Kapsel auf einem Ruß-Kymographen. Unmittelbar an der etwa 1,5 cm unterhalb des Krikoids fest eingebundenen Trachealkanüle befand sich ein widerstandsarmes Atemventil, dessen Einatmungsseite über einen weitlumigen Gummischlauch mit der Außenluft und dessen Ausatmungsseite über eine Kältefalle (ca. - 70°C) und eine Silikagel-Patrone mit Atembeuteln in Verbindung stand. Proben der Exspirationsluft wurden während des Versuches laufend zur massenspektrometrischen Bestimmung der relativen Häufigkeit von ^{18}O im Sauerstoff aus den Atembeuteln in evakuierte Spezial-Glasgefäße abgefüllt. Den Gehalt der Atemluft an O_2 und CO_2 kontrollierten wir nach der Methode von HALDANE. Die während des Versuches aus der linken A.femoralis und nach Versuchsende aus der rechten Herzkammer unter anaeroben Kautelen entnommenen Blutproben untersuchten wir auf ihren O_2- und CO_2-Gehalt nach der manometrischen Methode von van SLYKE bzw. SENDROY in einer Modifikation von LUDEWIG und RÖMISCH / 3 / sowie HOLFELD / 4 /. Gleichzeitig wurde in einzelnen dieser Blutproben die relative Häufigkeit von ^{18}O im Blutsauerstoff bestimmt.
Die Berechnung der in der Atemluft bzw. im Blut vorhandenen aus dem WPO abgespaltenen Sauerstoffmenge erfolgte nach dem Prinzip der Isotopen-Verdünnungsanalyse.

Technische Einzelheiten des Auffangens, Abfüllens der Meßproben sowie der Analyse und ihrer Bewertung siehe bei HÜBNER, LUDEWIG, GÖRISCH, BIRKENFELD / 5 /; HÜBNER, LUDEWIG und GÖRISCH / 6 /; GÖRISCH, HÜBNER und LUDEWIG / 7 /.

3. Ergebnisse

Wie aus der nachstehenden Tab. 1 zu ersehen ist, wird bei sublingualer Anwendung von 1,5 ml 9%igem WPO innerhalb von 58 Minuten oder von 0,1 ml 19%igem WPO innerhalb von 34 Minuten fast 1/3 derjenigen Sauerstoffmenge abgeatmet, die bei vollständiger Resorption und Spaltung des WPOs frei würde. Die innerhalb der ersten 5 Minuten nach Einwirkungsbeginn gesammelte Exspirationsluft erreichte bereits die dem Mittelwert entsprechende ^{18}O-Konzentration. Innerhalb des 78 Minuten langen Intervalls zwischen zwei WPO-Einwirkungen im zweiten Versuch (vgl. Tab. 1, Nr. 2a, b) wurde kein markierter Sauerstoff mehr abgeatmet, während sich in den ersten 7 Minuten nach erneuter WPO-Gabe wieder eine Abatmung von ^{18}O markiertem Sauerstoff nachweisen ließ. - Im abgeatmeten Kohlendioxid war keine Vermehrung des ^{18}O-Anteils festzustellen.
Unter der WPO-Einwirkung wurde eine starke Zunahme der ^{18}O-Konzentration im arteriellen Blut gemessen, obwohl weder durch Verhalten von Blutdruck und Atmung noch anschließend bei der Sektion Gasblasen im großen Kreislauf nachzuweisen waren. In Übereinstimmung mit unseren früher schon gemachten Beobachtungen sank die Sauerstoffsättigung des arteriellen Blutes allmählich ab. Der CO_2-Gehalt stieg entsprechend an. Ein Teil des aus dem WPO abgespaltenen Sauerstoffs wurde bei Versuchsende noch im venösen Blut wiedergefunden.

4. Diskussion

Obwohl unsere Hauptversuche nur an zwei Katzen durchgeführt wurden, war die von uns gestellte Frage nach der Abatmung des angebotenen Sauerstoffs eindeutig zu beantworten. Als besonders vorteilhaft erwiesen sich neben der großen Meßgenauigkeit die Tatsachen, daß in der Peroxidbrücke markierter Sauerstoff weder mit dem Wasser noch mit der Luft

austauscht und daß der gesamte bei der Zersetzung von WPO in wäßriger Lösung entstehende Sauerstoff dem Peroxid entstammt / 8, 9, 10, 11, 12 /.
Unsere Ergebnisse zeigen, daß ein überraschend großer Anteil des mit der sublingualen WPO-Applikation angebotenen Sauerstoffs ähnlich wie intravenös insufflierter O_2 über die Lungen abgeatmet wird. Ein Teil des in die Venen gelangten Sauerstoffs passiert die Lungenkapillaren und gelangt in den großen Kreislauf. Da wir im linken Herzen und in den Arterien jedoch niemals Gasblasen beobachten konnten, muß angenommen werden, daß der im Arterienblut wiedergefundene Sauerstoff spätestens vor Eintritt in das linke Herz physikalisch gebunden oder gelöst wurde. Keinesfalls darf aus diesem Ergebnis aber die spekulative Folgerung gezogen werden, man könne auf diese Weise die Sauerstoffversorgung des Organismus verbessern. Wie sich allein bei Betrachtung der manometrisch ermittelten Blutgaswerte zeigt, wird die Sauerstoffversorgung eher verschlechtert. In früheren Versuchen konnten wir zeigen, daß die Ursache dafür in einer durch pulmonale Gasembolie ausgelösten Störung des Gasaustausches in der Lunge zu suchen ist. Ein Teil des abgespaltenen, von uns in diesem Zusammenhang nicht gesuchten Sauerstoffs wird - wie vorangegangene histologische Untersuchungsergebnisse zeigten - im Zungengewebe selbst eingelagert und könnte dort im Bedarfsfalle utilisiert werden. - Die Tatsache, daß die relative Häufigkeit von ^{18}O im CO_2 nicht erhöht war, schließt eine Utilisation keineswegs aus, da der aus dem WPO abgespaltene Sauerstoff nur über das Körperwasser in das Kohlendioxid eintreten kann. Wenn die aus dem WPO abgespaltene Sauerstoffmenge auch so groß ist, daß sich Emphyseme bilden können, so reicht sie doch bei weitem nicht aus, um eine meßbare Isotopenverschiebung im Körperwasser zu bewirken / 13 /.

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Vers. Nr.	Einwirkend sublingual: H_2O_2-Gehalt d.Lösg. in %	^{18}O-Kons. in Atom %	Menge in ml	Einwirkungszeit in min	max. abspaltbares O_2 in ml	Exspirationsluft: in Exspirationsluft wiedergef. O_2 in ml	in %	^{18}O-Anteil in Atom %: vor H_2O_2-Gabe	unter H_2O_2-Einwirkung
1	9	4,03	1,5	58	46,5	12,8	27,5	0,205 ± 0,001	0,225 ± 0,004
2a	19	3,96	0,1	34	6,6	2,0	30,3	0,204 ± 0,002	0,210 ± 0,003
2b	19	3,96	0,35	18	23,3	1,6	6,9	0,204 ± 0,002	0,219 ± 0,004

Blutgase

Vers. Nr.	O_2-Sättig.% des art. Blutes: vor H_2O_2-Gabe	nach H_2O_2-Einwirkg.	CO_2-Vol % in art. Blut: vor H_2O_2-Gabe	nach H_2O_2-Einwirkg.	^{18}O Atom % in art. Blut: vor H_2O_2-Gabe	unter H_2O_2-Einwirkg.	^{18}O% im venösen Blut bei Versuchsende	im Gesamt-Blut b. Versuchsende wiedergef. O_2 in ml
1	92,4	57,8	61,8	68,3	0,205	0,260	0,400	0,8
2a	89,5	77,7	56,3	59,7	0,205	0,217	-	-
2b	61,2	35,5	62,3	66,7	0,205	0,219	0,258	0,15

Tab. 1: Nachweis von ^{18}O in Exspirationsluft und Blut während sublingualer Einwirkung ^{18}O-markierten Wasserstoffperoxids.

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BBIOLOGICAL ASPECTS OF AGEING AND THE CONCEPT OF OLOGICAL CATHODE PROTECTION*

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"*Can biologists afford to ignore the dimension of electrons?*" — Albert Szent-Györgyi

SUMMARY

A series of tentative experiments was carried out to investigate some aspects of the analogy between biological and non-biological ageing. Prolonged microdoses of H_2O_2 (as a purely oxidizing compound) decreased the lifespan and body weight of mice. So did positive electric charging, while continuous negative charging produced a comparatively longer lifespan and higher body weight on average. It also gave relative resistance against X-radiation. Positive electric charging is supposed to act as an electrochemical oxidant, while negative charge may protect biomolecules against spontaneous oxidative damage, as technological cathode protection counteracts the corrosion of metals.

Ultimately, in ageing molecules electrons fall from higher energy levels to lower ones, driven by the greater total electronegativity of the newly linked atoms, as compared to the original linkages of lesser total electronegativity.

The ageing of inanimate and living systems seems to be analogous:

(a) in thermodynamic spontaneity[1-6];

(b) in the oxidative character of most of the molecular end-products, including oss-linkages[7-16];

(c) in the central role of intermediate free radicals[10-18]; and

(d) in the ageing retarding and radio-protective effectiveness of chemical ntioxidants in both systems[19-27].

Experiments were carried out to investigate some further aspects of this nalogy: whether biological ageing will be affected (1) by a chemical oxidant, H_2O_2;) by the application of the anticorrosive cathode protection used in technology; d of its opposite procedure, positive electric charging; and (3) whether these ectrical treatments will affect radiation damage.

* This work is based on the author's paper "An interpretation of ageing with regard to thermo-namics and material structure as a basis for Biological Cathode Protection" read at the Conference the European Biology Section of the International Association of Gerontology, Leeds, 1970.

METHODS

1. Sixteen albino female mice were each given hydrogen peroxide subcutaneously in doses of 0.5 ml, in a concentration of 0.01%, *i.e.* a body concentration of about $5 \cdot 10^{-5}$ M, dissolved in physiological sodium chloride, twice or three times a week from the age of 6 weeks to 14 months; altogether 95 injections per animal. One of two control groups of 16 female mice received physiological sodium chloride injections in the same doses; the other control group received no treatment whatever. Times of death and changes in average body weights were registered.

2. Three groups of 16 albino male mice were kept in insulated cages with metal baseplates from the age of 6 weeks until they expired. One group was charged with negative, the other with positive, electricity, *i.e.* their baseplates were connected with the negative and positive pole of a supply source, respectively, while the opposite pole was grounded. 1.2 V was applied until the age of 220 days, and 110 V thereafter. The charge could be measured on the animals' surface with a quadrant electrometer. The control cage was placed close between the "negative" and "positive" cages. The lifespans and the average body weights were recorded.

In this experiment a modified Olsen–Everitt test[28] was also performed, *i.e.* the breaking-up times of tail collagen fibres taken from the mice at the age of 480 days, hooked-up and loaded with a weight of 2.54 g in a concentrated urea solution at a temperature of 37°C, were recorded and compared to those of untreated 80-day-old animals. A separate group of untreated females served as control.

3. Three groups of 25 albino male mice were kept in their respective insulated cages charged negatively, positively and not charged (control) in the same manner as in the former experiment, from the age of 6 weeks. The voltage was 280 V. Each group was subjected to whole-body X-radiation in doses of 250 R when 90-days old, 300 R when 120-days old, and 250 R when 190-days old. (Voltage, 130 kV; intensity, 12 mA; filter, aluminium, 3 mm thick; distance, 150 cm; durations 109, 118 and 109 min.) Electric charge was maintained throughout irradiation too. Survival times and average body weights were registered, and white blood cell counts were taken on the 3rd and 5th day after the 2nd irradiation from each group.

RESULTS

1. Small and prolonged doses of H_2O_2 caused considerable (24.5%) decrease in the average lifespan and an early loss of body weight (Fig. 1).

2. The negatively charged animals showed a marked increase in average lifespan and body weight as compared to those charged positively (Fig. 2). Unfortunately, the control group could not be evaluated, because the animals — with one exception — expired at the age of 1 year, owing to unidentified damage.

The modified Olsen–Everitt test (Fig. 3) indicated that 50% of the fibres of the negatively charged animals should be regarded as "biologically younger" than those of the controls and positives.

3. Although the life-shortening effect of X-rays was working in all groups,

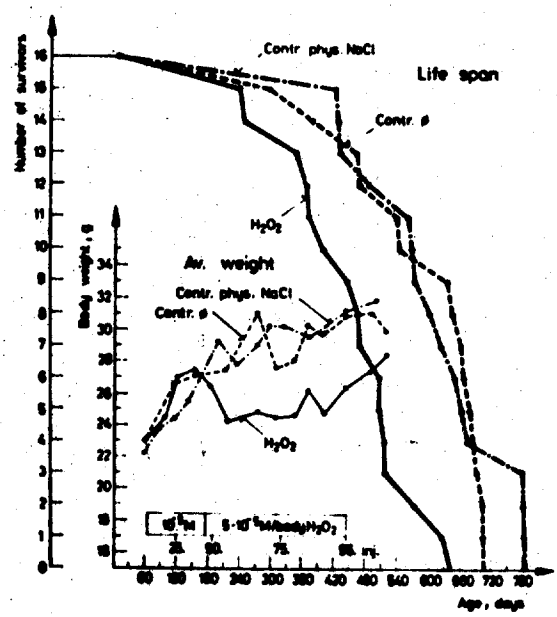


Fig. 1. The effect of prolonged microdoses of H_2O_2 on the lifespan and body weight of female albino mice. Average and maximum lifespans/day: 446.2 and 639 in the H_2O_2 group; 595 and 785 in the physiological NaCl group; 584.2 and 704 in the untreated group.

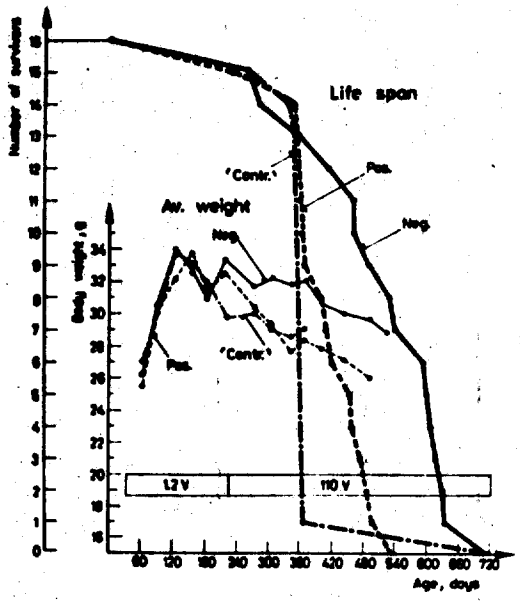


Fig. 2. The effect of positive and negative electric charging on the lifespan and body weight of male mice. Average and maximum lifespans in days: 505.7 and 713 in the negative group; 401.8 and 530 in the positive. Untreated controls could not be evaluated.

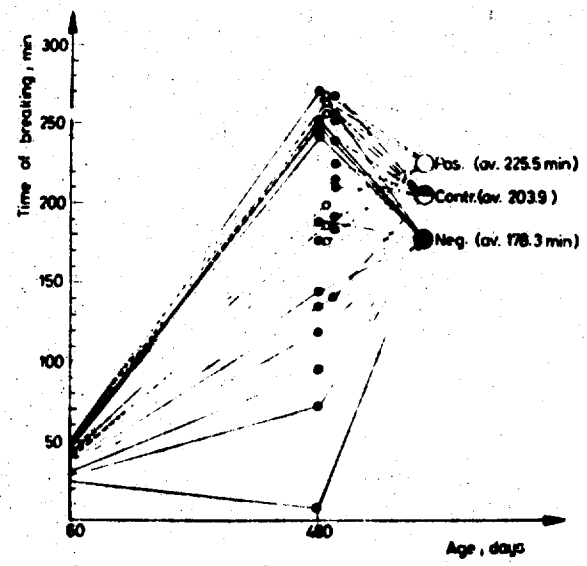


Fig. 3. Modified Olsen-Everitt test (for details see text). Half of the tail collagen fibres of 480-day-old, negatively charged, mice should be regarded as "biologically younger" than those of the positively charged and control animals of the same age.

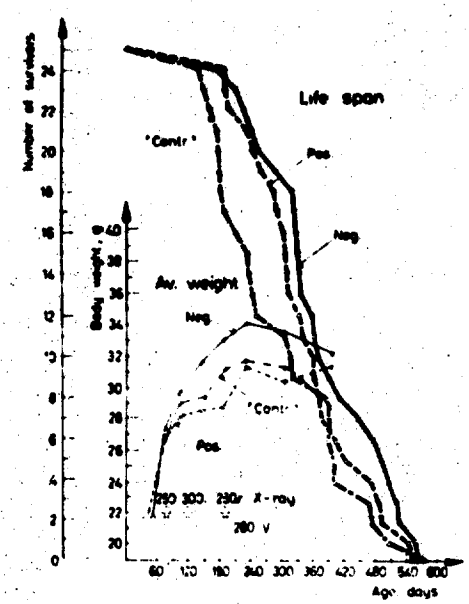


Fig. 4. Effect of negative and positive electric charge on the lifespan and body weight of ~~X-irradiated~~ male mice. Average and maximum lifespans in days: 359.7 and 564 in the negatives; 340.3 and 554 in the positives.

negative charging gave a relative resistance to it (Fig. 4). The average weight curves begin to diverge at an early stage, as in the previous experiment, and again, the highest body weights were found in the negative group, and the lowest in the positive one.

White blood cell counts (Fig. 5) averaged more than twice as much (7130) in the negatives as in the positives (3180), and nearly twice as much as in the controls (3950).

DISCUSSION

In experiment *1* the primary molecular damages caused by the artificially introduced H_2O_2 must be of a purely oxidative character owing to the free radicals ($\dot{O}H$, $H\dot{O}_2$) liberated from it. Its nonspecific destructive effect, manifest in the decrease of lifespan and body weight, is a general one, and may ultimately be traced back to massive cell loss.

In experiments *2* and *3* animals were electrically charged. Negative charging of a conductor obviously means that its surface is overflowing with a mass of free electrons, while positive charging draws off electrons from the surface. In the former case, owing to a concentric, centripetal "electron pressure", free radicals within the body may be neutralized by the incoming electrons, while physiological positive ions presumably remain unaltered owing to their low electron affinity. So, *negative charging is supposed to protect the biomolecules against spontaneous oxidation*, essentially in the same way as technological cathode protection counteracts the corrosion of metals, while positive charging acts like chemical oxidants. This may explain the consistent difference in some characteristic parameters (lifespan, body weight, breaking time of collagen fibres, post-radiation survival, white blood cell count) between the negatively and positively charged animals.

Repeated experiments on larger amounts of material (and in the electric

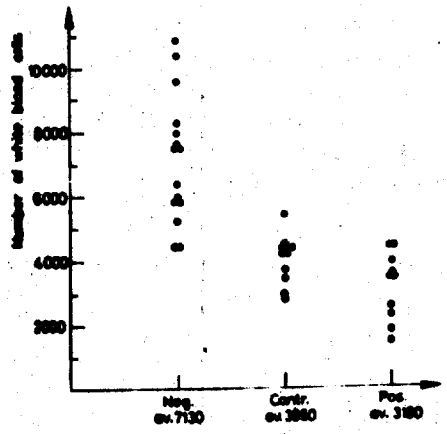


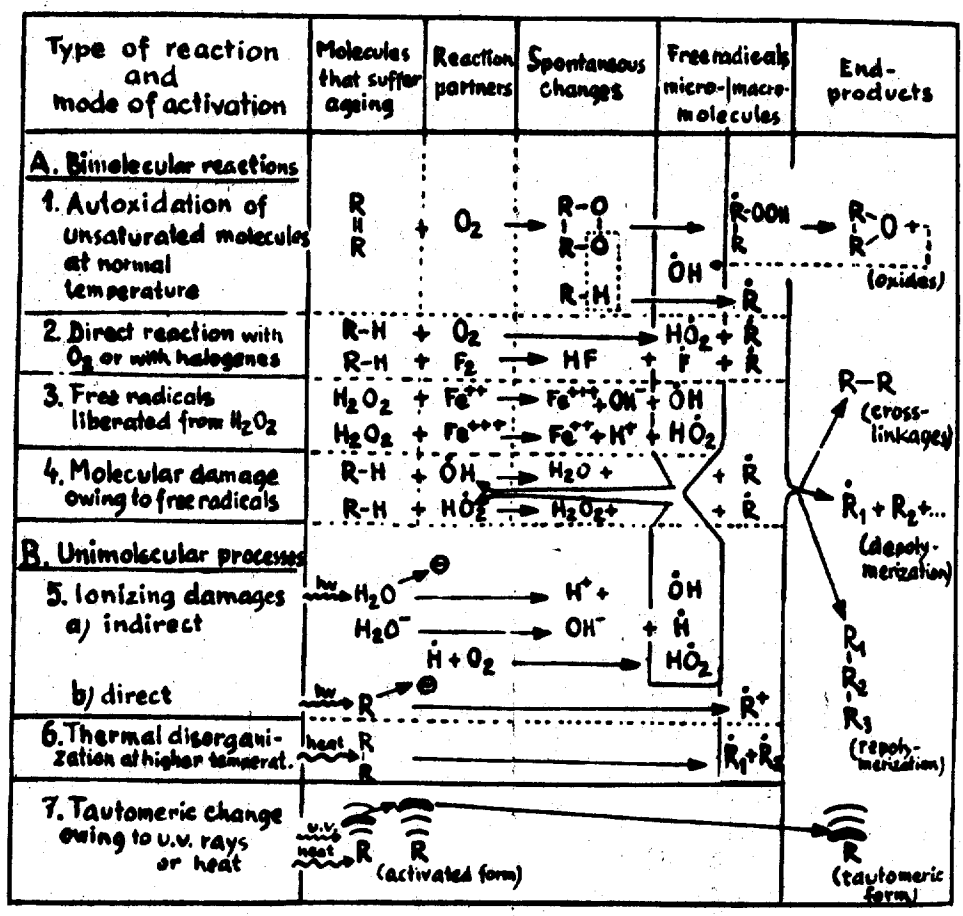
Fig. 5. White blood cell counts of X-radiated, negatively, positively charged and uncharged male mice (for details see text).

models with uniform voltages below 10 V) are required to confirm these tentative results, and will be conducted accordingly to establish how far "biological cathode protection" can effectively retard senescence and act as a radio-protective means.

What are those subbiological mechanisms of ageing supposedly affected by this electric model? In principle, *energetically unstable molecular systems* cannot be conserved for a long time at normal temperature, because they *suffer spontaneous, irreversible chemical changes towards the free energy minimum state, i.e. they age.* This goes not only for native biomolecules such as proteins, nucleic acids, lipids, polysaccharides, *etc.* both *in vitro* and *in vivo*, but also for inanimate systems such as corroding metals, or synthesized macromolecules, colloids, rubber, plastics, lacquers, dyes, fats, *etc.* For instance, heat, ultraviolet or ionizing rays, oxidizing compounds or free radicals may well induce DNA mutations[22] just as they accelerate the ageing of

TABLE 1

SOME CHARACTERISTIC PATTERNS OF MOLECULAR AGEING



rubber, by means of fundamentally similar mechanisms, *e.g.* by producing oxidative cross-linkages.

The prerequisite of all these apparently different processes of molecular ageing is the free energy content of the system. Chemical energy takes its origin ultimately from photosynthesis and is represented by the kinetic energy of all linking and especially of π electrons moving in molecular orbitals. Various forms of activation energy (thermal, light, ultraviolet or ionizing effects) play the part of the trigger in these processes. They may occur either *bimolecularly*, in the presence of reaction partners of great electron affinity (oxygen, fluorine, chlorine, *etc.*, or free radicals such as $\dot{O}H$, $H\dot{O}_2$, *etc.*) *when oxidation of the metastable molecule always occurs*; or *unimolecularly*, as in the case of *direct ionization, thermal disorganization or tautomeric changes* (Table I). *In both types of spontaneous reaction electrons "fall" from higher energy levels to lower ones*, and the energy difference will be released. Ultimately, the *common motive force* in every spontaneous chemical reaction *is the greater total electronegativity of the* atoms that form the new, *i.e. oxidized, linkages* as compared to the lower total electronegativity in the original ones.

This very same thermodynamic principle also holds good for the ageing of the whole living organism. In Nature only spontaneous, irreversible processes take place with absolute certainty — and so does biological ageing. If it were directly programmed, in other words, if ageing itself needed energy, it would be vulnerable and bound to fail according to statistical probability, *e.g.* by the spontaneous mutations of the genes carrying this "programme". But on the contrary, *molecular turnover and mitoses, by working against the spontaneous damage that constitutes ageing, may be conceived as an "antiageing programme"*[29].

ACKNOWLEDGEMENTS

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INHALATION TOXICITY OF NINETY PER CENT HYDROGEN PEROXIDE VAPOR

Acute, Subacute, and Chronic Exposures of Laboratory Animals

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SINCE the use of 90% hydrogen peroxide (H_2O_2) is becoming more and more prevalent, the potentialities of its vapor toxicity as a health hazard must be recognized. Little information was found in the literature concerning the toxicity and irritating effects of the vapor by inhalation. Leber and Luft [1] reported that in an accidental exposure of men to the vapor of 90% H_2O_2 there was an increased flow of saliva and a scratchy feeling of the throat, with inflammation of the respiratory passage. Masterman and Wiseman [2] concluded from their clinical observations that there is little reason to expect serious lung damage in men exposed to white fumes given off by 90% H_2O_2 when brought in contact with a catalytic material, such as a heavy metal or one of its salts. Suitta [3] stated that nasal irritations in man may become chronic after repeated exposure to H_2O_2 vapor.

EXPERIMENTAL STUDIES

In view of the meager information on the health hazards of 90% H_2O_2 vapor and the increasing importance of the liquid agent in industry, further studies were warranted. Inhalation toxicities and eye irritation were studied in laboratory animals under the following conditions.

(*a*) *Acute Inhalation Toxicity.*—Rats were exposed eight hours to H_2O_2 vapor, varying in concentrations from 338 to 427 mg./m.^{3} (243 to 307 ppm).

(*b*) *Subacute Inhalation Toxicity.*—Rats were exposed six hours a day, five days a week for six weeks to H_2O_2 vapor at an average concentration of 93 mg./m.^{3} (67 ppm). Two groups of mice were exposed in like manner to the vapor at average concentrations of 79 mg./m.^{3} (57 ppm) and 107 mg./m.^{3} (77 ppm), respectively.

(*c*) *Chronic Inhalation Toxicity.*—Two dogs were exposed six hours a day, five days a week for six months to an average vapor concentration of 10 mg./m.^{3} (7 ppm).

(*d*) Rabbits were exposed six hours a day, five days a week over a three-month period to a level of 30 mg./m.^{3} (22 ppm) of H_2O_2 vapor for studying the irritating effects on the eyes.

From the Chemical Corps Medical Laboratories.

METHOD AND PROCEDURE

1. *Acute Inhalation Toxicity Tests (Rats) to Saturated Vapors.*—Test 1 (Nominal Chamber Concentrations): A 0.01 cubic meter glass jar with a metal top was used as a gassing chamber. H_2O_2* was dispersed as a saturated vapor by passing dried air at a rate of 2 liters per minute through a bubbler containing the compound at 25 C. The concentration of the H_2O_2 vapor in the chamber was not determined by analysis but was calculated from the difference in weight of the bubbler before and after dispersion and the total volume of air passed through the liquid.

In three separate eight-hour runs six rats were exposed for eight hours and were then killed at various times up to 14 days for pathological examination.

Test 2 (Concentration by Chemical Analysis): To a 0.76 cubic meter gassing chamber was attached a train of three dispersal bubblers connected in series. Each was filled with glass beads and contained 10 ml. of liquid 90% H_2O_2. Room air was drawn through the train of bubblers and the chamber at a rate of 0.05 cubic meter per minute, giving, after equilibrium time, an approximately saturated vapor concentration. The concentration of the H_2O_2 in the chamber was determined by drawing samples of air through a stock solution of titanium sulfate reagent.† The color intensity was read in a Klett-Summerson colorimeter using a No. 54 filter (540 mμ). Standards for comparison were prepared from the titanium sulfate reagent mixed with H_2O_2 solutions of various concentrations.

To determine whether or not slippage occurred from the collection bubbler, a second bubbler was connected in series with the first. No color developed in this bubbler; therefore, only one collection bubbler was used in subsequent tests.

Ten rats were exposed in each of three runs. One run extended over a four-hour period and the other two over eight-hour periods. After exposure, animals were submitted for pathological examination at various times up to 14 days.

2. *Subacute Toxicity Tests (Rats and Mice).*—A dynamic flow gassing chamber having a capacity of 0.44 cubic meter was used. The agent was dispersed in the chamber by drawing room air through a horizontal porous clay cylinder inserted loosely in a larger glass cylinder containing H_2O_2. The combined cylinders were rotated at 12 rpm, and the air flow was 0.150 cubic meter per minute. To determine the concentration of the agent in the chamber, samples of air were drawn from the chamber at a rate of approximately 1 liter per minute through 20 ml. of distilled water in each of two collection bubblers connected in series. The time of sampling usually varied from 10 to 60 minutes, the total volume of air being measured by means of a wet-test meter. The amount of agent present in the combined collection sample was determined by addition of 10 ml. of 5% KI and 30 ml. of 25% sulfuric acid. This was followed by 3 drops of 1 N ammonium molybdate, which catalyzed the reaction and released free iodine. The stoppered flask which contained these solutions was placed immediately in the dark for five minutes to allow the reaction to be completed. The iodine was titrated with standardized $Na_2S_2O_3$, starch solution being used to detect the end-point. The H_2O_2 concentration in milligrams per cubic meter was calculated by the following formula: $\frac{\text{ml. } Na_2S_2O_3 \times N \times 17}{\text{vol. of air sample in m.}^3}$ where N is the normality of $Na_2S_2O_3$.

The temperature in the gassing chamber varied from 26 to 29 C. during the exposure of the animals. A group of 23 rats (10 reserved for mortality studies and 13 for pathology) and 40 mice were exposed to an average H_2O_2 vapor concentration of 93 mg./m.^{3} (rats) and 79 and 107 mg./m.^{3} (mice) 6 hours daily, five days a week, and over a period of approximately six weeks (180 hours of exposure). A group of 10 rats served as controls. At the end of each week of exposure one or two rats were killed for autopsy. All rats were weighed twice a week. Mice were not weighed.

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* The liquid H_2O_2 received for this study from the Naval Research Laboratory, Washington, D. C., contained 90% H_2O_2 as determined by chemical analysis.

† The titanium sulfate reagent was made by digesting TiO_2 with 100 ml. of concentrated H_2SO_4 for three days in a sand bath at 150 to 160 C. The solution was filtered through a sintered glass filter and diluted to four volumes with water for the stock solution. Ti^{++++} is oxidized by H_2O_2 to pertitanic acid (H_2TiO_4) which has a yellow color and is stable for six hours.[4]

3. *Chronic Tests (Dogs).*—A dynamic flow gassing chamber with a capacity of 0.76 cubic meter was used. The concentration was established in the chamber by passing dry air at a constant rate through a bubbler containing H_2O_2 maintained at 25 C. The vapors from the bubbler were mixed with room air in a mixing bowl attached to the chamber, and the mixture was drawn through the gassing chamber.

The chamber concentration was determined by the titanium sulfate method described in Test 2 under acute inhalation toxicity tests. The concentration was checked by the same analytical method used in the subacute toxicity tests. The results of the two methods were in good agreement.

The chronic test was conducted on two dogs by exposing them to an average vapor concentration of 10 mg./m.^{3} six hours per day, five days per week, over a six-month period for a total of 126 exposures. The six-hour exposure period was measured from the moment air containing H_2O_2 vapor was turned into the chamber until it was turned off. The animals were not removed until the chamber was free of the vapor. A third dog, serving as a control, was kept in an open cage near the gassing chamber.

Approximately once a month blood and urine from each dog were analyzed. The blood was drawn under oil from the anterior cephalic vein; a portion of it was heparinized in a separate tube. The urine specimen was a catheterized sample. The following determinations were made:

Whole Blood: pH, sedimentation rate, oxygen content,[5] nonprotein nitrogen level,[6] red and white blood cell count, and hemoglobin.[7]

Serum: bicarbonate,[8] chloride,[9] sugar,[10] sodium,[11] urea,[12] calcium,[13] phosphorus,[13] and cholesterol.[14]

Plasma: protein.[15]

Urine (catheterized): pH, specific gravity, albumin (Robert's reagent), sugar (Benedict's reagent), and microscopic sediments.

4. *Effect of Vapor on Eyes of Rabbits.*—Eight black rabbits were placed in a constant flow gassing chamber having a capacity of 0.76 cubic meter and a vapor concentration of about 30 mg./m.^{3} (22 ppm). These rabbits were exposed six hours a day, five days a week for a total of 60 exposures. The concentration in the chamber was established by the same method as that described for the chronic exposure test on dogs. The eyes were examined with an ophthalmoscope once a week for possible corneal damage. Two control rabbits were kept under the same conditions, except that they were not exposed to H_2O_2 vapors.

RESULTS

1. *Acute Inhalation Toxicity Tests on Rats (Saturated Vapor).*—Test 1: The nominal vapor concentration of the chamber was calculated to be 4,000 mg./m.^{3} or 2,880 ppm. No signs of intoxication were observed in the rats after eight hours of exposure in a chamber to which saturated vapors of H_2O_2 were introduced. In three separate eight-hour exposures there were no deaths.

The trachea and lungs from all animals exhibited severe congestion. Those animals killed during the first three days following exposure showed small localized areas of pulmonary edema without hemorrhage. In those animals killed between the 4th and 14th days, the majority of the lungs exhibited many areas of alveolar emphysema in addition to the severe congestion. There were no signs of necrosis of the pulmonary mucosa. All other organs examined appeared normal.

Test 2: In this test the concentration of the H_2O_2 vapor in the chamber was determined by chemical analysis. The concentration in three runs varied from 338 to 427 mg./m.^{3} (243 to 307 ppm) instead of the calculated value of 4,000 mg./m.^{3} (2,880 ppm) obtained in Test 1.

The only outward evidence of toxic effects was shown by scratching and licking. After 30 minutes the animals became quiet and slept most of the time. There were

no deaths resulting from either the single four-hour exposure or the two eight-hour exposures. Pathological examination of the animals showed results similar to those described in Test 1.

2. *Subacute Toxicity Tests on Mice (Analytical Concentration, 79 and 107 Mg./M.³ or 57 and 77 Ppm) and Rats (93 Mg./M.³ or 67 Ppm).*—The rats and mice showed profuse nasal discharge after two weeks of exposure. The bottom of the feet became edematous, and an acute irritation was noted in the region of the groin. During the fifth week of exposure there was a denudation of the hair around the nose, which was probably due to scratching by the animals in an effort to alleviate irritation.

TABLE 1.—*Subacute Exposures of Mice to Chamber Concentrations of Ninety Per Cent H_2O_2 Vapor*

Weeks Exposed, No.	Exposures, per Week, No.	Accumulative Exposure Time, Hr.	Average Weekly Analytical Concentration		Accumulative Mortality Rate
			Mg./M.³	Ppm *	
Group 1					
1	2	12	85	61	0/9
2	3	30	88	60	3/9
3	3	48	69	50	7/9
Average			79	57	
Total †					
3	8	48	..	..	7/9
Group 2					
1	3	18	88	63	1/10
2	5	48	105	75	5/10
3	5	78	96	69	5/10
4	5	108	137	99	8/10
Average			107	77	
Total †					
4	18	108	..	..	8/10

* Factor of 0.719 × milligrams was used to calculate ppm.
† Accumulative experimental data.

The exposed rats gained in body weight, varying from 40 to 80 gm., during the course of experimentation, while the controls gained from 30 to 50 gm. The difference in weight gain for the two groups was statistically insignificant.

In the first of two groups of mice exposed, seven out of nine ‡ animals died after eight exposures to an average concentration of 79 mg./m.³ (57 ppm), as shown in Table 1. In the second group at an average concentration of 107 mg./m.³ (77 ppm), 5 out of 10 mice died after 8 exposures, and 8 out of 10 mice died after 18 exposures. There were no deaths in the control group. The mortality rate for the exposed rats (Table 2) was considerably less than that for the exposed mice. For the former, 30 exposures to an average concentration of 93 mg./m.³ (67 ppm) resulted in the death of only 1 rat out of 10.

Thirteen rats were autopsied following various numbers of exposures (Table 3). All organs were examined grossly, and tissue sections were made of lungs, trachea,

‡ The 10th mouse in this group disappeared unaccountably from the cage.

liver, kidneys, spleen, and cornea. The organs either were normal or showed some congestion. Microscopic examination of these tissues failed to reveal significant pathological changes.

3. *Chronic Inhalation Toxicity Tests on Two Dogs (Average Analytical Concentration, 10 Mg./M.³ or 7 Ppm, Table 4).*—No toxic signs were observed in the

TABLE 2.—*Subacute Exposures of Rats to Chamber Concentrations of Ninety Per Cent H_2O_2 Vapor*

Weeks Exposed, No.	Exposures, per Week, No.	Accumulative Exposure Time, Hr.	Average Weekly Analytical Concentration		Accumulative Mortality Rate
			Mg./M.³	Ppm *	
1	2	12	85	61	0/10
2	3	30	83	60	0/10
3	5	60	55	40	1/10
4	5	90	87	63	0/10
5	5	120	105	75	0/10
6	5	150	96	69	0/10
7	5	180	137	99	0/10
Average			93	67	
Total † 7	30	180	..	..	1/10

* Factor of 0.719 × milligrams was used to calculate ppm.
† Accumulative experimental data.

TABLE 3.—*Gross Observations on Organs from Rats Killed After Subacute Exposures to Ninety Per Cent H_2O_2 Vapor*
(Consult Table 2)

Week Ends in Which Rats Were Submitted for Autopsy	Rats Submitted for Autopsy, No.	Results on Examination of Organs				
		Trachea	Lung	Spleen	Liver	Kidney
1st	2	Congestion* Normal	Congestion	Normal	Normal	Normal
2d	1	Normal	Slight congestion	Normal	Normal	Congestion
3d	2	Normal	Slight congestion	Normal	Normal	Congestion* Normal
4th	1	Normal	Slight congestion	Normal	Congestion	Congestion
5th	1	Congestion	Slight congestion	Normal	Normal	Normal
7th	5	Congestion	Congestion	Normal	Congestion Normal	Normal Congestion

* Same degree as controls.

dogs up to and including the 23d week of exposure. In the 24th week the dogs began to sneeze occasionally after five hours in the chamber; lacrimation was also present. The lacrimal secretions and sneezing persisted for a short time after the dogs were removed from the chamber following an exposure. In the 25th week the dogs frequently refused food but ate again normally during the 26th and 27th weeks.

By the 14th week the hair of both dogs showed bleaching, and some denuded areas were present over the back. Frequently the animals were observed sniffing

the H_2O_2 vapors at the chamber intake. This action was more prominent at about the 20th week and persisted until the end of the test. The control dog showed no abnormality of any kind.

The initial weight of one test dog was 13 kg. This animal gained 0.3 kg. by the end of the experiment. The other test dog weighed 17 kg. initially and gained 0.2 kg. during the six months of exposure. The control dog gained 0.4 kg. during the same period, the initial weight being 10 kg. The maximum weight increase

TABLE 4.—*Weekly Average Chamber Concentration of Ninety Per Cent H_2O_2 Vapor Used During Subacute Exposures to Dogs**

Weeks Exposed, No.	Exposures per Week, No.	Accumulative Exposure Time, Hr.	Average Weekly Concentration	
			Mg./M.³	Ppm
1	5	30	8	6
2	5	60	11	8
3	4	84	10	7
4	5	114	10	7
5	5	144	9	6
6	5	174	14	10
7	5	204	12	9
8	5	234	12	9
9	5	264	11	8
10	5	294	10	7
11	5	324	10	7
12	5	354	5	4
13	5	384	7	5
14	4	408	7	5
15	5	438	7	5
16	5	468	11	8
17	5	498	9	6
18	5	528	11	8
19	5	558	9	6
20	5	588	10	7
21	5	618	12	9
22	5	648	11	8
23	4	672	10	7
24	4	696	10	7
25	5	726	9	6
26	5	756	12	9
Average			10	7
Total †				
26	126	756	..	..

* Two dogs.
† Accumulative experimental data.

for the exposed dogs was 0.5 to 0.9 kg. during the course of the experiment, but some weight was lost toward the end of the experiment, giving a lower over-all weight increase.

There were no deaths resulting from a six-month exposure to 10 mg./m.³ (7 ppm) of H_2O_2 vapors.

At the conclusion of the six-month exposure period the animals were killed for study of pathology. The skin of the exposed dogs was greatly thickened, and there was a marked loss of hair. Microscopic examination showed that the hair follicles were not destroyed. The examination of the organs of the thoracic and abdominal cavities revealed that the tissue changes were confined almost entirely to the lungs. In this organ there were patchy areas of atelectasis intermingled with emphysematous areas. There were some hyperplasia of the bronchial musculature

and tortuosity of the bronchi. The small terminal bronchioles and respiratory bronchioles had hyperplastic muscular coats. Scattered throughout all areas of the lung tissue were small buds of red-staining tissue, and many of them were lined with cuboidal cells, which were most prominent in areas where the alveolar walls seemed to be fragmented. The musculature of the terminal or respiratory bronchioles seemed to have become hyperplastic, as if there had been a change in the connective stroma of the alveolar walls. Connective tissues and elastic tissue stain revealed these circular areas to be composed mainly of collagen, with occasional muscle cells and occasional strands of elastic tissue admixed. The control dog exhibited no histologic changes on autopsy examination.

No significant changes from normal in blood and urinary constituents were observed during these tests.

4. *Effect of Vapor on Eyes of Rabbits (Average Analytical Concentration, 30 Mg./M.³ or 22 Ppm for 60 Exposures).*§—No significant changes as the result of these exposures were noted by ophthalmoscopic examination of the eyes. The eyes of the control group appeared identical with those of the exposed animals.

COMMENT

It is clearly demonstrated that the vapor of 90% H_2O_2, in concentrations of 7 ppm or more, will produce lung irritation in animals if the exposure time is long enough. Even single exposures to maximum concentrations of the vapor for four to eight hours produced evidence of respiratory irritation in rats, even though there were no apparent signs of intoxication during the exposure period. Prolonged daily exposures to vapor concentration levels ranging from 50 to 99 ppm produced death in mice within as few as five exposures. Rats exposed under the same conditions and concentration levels showed some congestion of the lungs. The two dogs which were exposed daily for a six-month period to a low vapor concentration (7 ppm) showed evidence of chronic irritation. The agent produced some external irritation, as it was noted that the dogs scratched themselves to the extent that several areas on the body were denuded. Autopsy revealed definite abnormality confined chiefly to the respiratory tract as a result of the irritating effects of the vapor. The skin was greatly thickened although no destruction of hair follicles was present. Eyes of the rabbits did not appear to be damaged in any way by the vapor after 12 weeks of daily exposures.

The lowest concentration level studied over an extended period of time was 7 ppm, which produced evidence of chronic irritation in dogs. On the basis of this information it is believed that a 90% H_2O_2 vapor concentration of less than 4 ppm (one-half of the lowest concentration level studied) probably would have little or no harmful effects in man if he were to be exposed daily to the vapor for long periods of time. Relatively short exposures, several hours a day, several times a week, to levels of 7 ppm probably would not under these conditions produce serious or harmful effects.

In this report one finds an excellent example of a discrepancy occurring in the chamber concentration values when the determinations are made by a nominal method. A nominal method gives only the theoretical concentration in the chamber

§ There were no unusual toxic signs except for a slight serous discharge from the nose. The black hair of the rabbits was bleached to a light brown color. There were no deaths.

if the vapor remains dispersed in the air. The vapors of some agents are removed from the air during passage through the chamber by either absorption, adsorption, decomposition, condensation, or by a combination of these or other factors. A comparison of nominal with analytical values shows that about 90% of the H_2O_2 was removed from the air; consequently animals exposed to the vapor received only about one-tenth of the expected concentration. This shows the unreliability of nominal calculations and points out the need for analysis to obtain reliable data.

In this connection a vapor that is so readily removed from air by one of several means (absorption, adsorption, decomposition, or condensation) may not be as hazardous as one remaining fully dispersed under most conditions. This would produce a condition in which a continually decreasing concentration would be occurring exclusive of dilution by air to the extent that eventually a comparatively safe concentration would be established. On the other hand, should such a vapor leak continuously into an area under poor conditions of ventilation, the hazard undoubtedly would be serious.

SUMMARY

Rats exposed eight hours to H_2O_2 vapor in concentrations varying from 338 to 427 mg./m.^{3} (243 to 307 ppm) showed no outward toxic signs other than scratching and licking themselves. There were no deaths. Pathological examination revealed a congestion of the trachea and lungs. Small localized areas of pulmonary edema without hemorrhage and areas of alveolar emphysema were present.

Rats and mice exposed six hours a day, five days a week for six weeks to H_2O_2 vapor at an average concentration of 93 mg./m.^{3} (67 ppm) for rats and 79 and 107 mg./m.^{3} (57 and 77 ppm), respectively, for two groups of mice showed external body irritation, profuse nasal discharge, and bleaching of the hair. More than 50% of the mice were dead after eight exposures. Only 1 rat out of 10 died during 30 exposures. Rats, on autopsy, showed no marked abnormality other than congestion of the respiratory tract.

Two dogs exposed six hours a day, five days a week for six months to an average vapor concentration of 10 mg./m.^{3} (7 ppm) developed external body irritation, sneezing, lacrimation, and bleaching of hair. The pathology showed the skin was greatly thickened, but no destruction of hair follicles was present. The lung was the only internal organ which showed any abnormality resulting from the irritating effects of the vapor. No significant changes in blood or urinary constituents were observed during these tests.

Rabbits exposed six hours a day, five days a week over a three-month period to 90% H_2O_2 vapor at an average concentration of 30 mg./m.^{3} (22 ppm) did not develop any eye injury. The hair became bleached, and some irritation was noted around the nose.

The maximum allowable concentration of 90% H_2O_2 vapor is less than 7 ppm.

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Petrov-'40
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ON THE PROBLEM OF INTRAVENOUS ADMINISTRATION OF HYDROGEN PEROXIDE TO HORSES, DOGS, AND RABBITS[1]

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Farmakologiya i Toksikologiya 3, No. 5 (1940)

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Among the methods of oxygen therapy, one means of administering oxygen directly into the blood has great significance, the possibilities for which have already been indicated by Histen and Demarkej (cited in Komarova).

We conducted our own experiment in working out a simpler and more convenient means of administering oxygen directly into the blood, in which hydrogen peroxide was used as a substance which easily yields oxygen.

The majority of authors of textbooks on pharmacology indicate the impossibility of using H_2O_2, due to possible embolism. Cases of embolism are described during administration of large amounts of H_2O_2 into the pleural cavity for the purpose of flushing it out. Injection of H_2O_2 deep into wounds presents the same dangers.

Concerning the problem of the intravenous administration of hydrogen peroxide for therapeutic purposes, we found no discussion of it in the literature available to us, with the exception of the work of Vishenin, who successfully administered a 0.5% solution of hydrogen peroxide intravenously to treat acute poisoning in dogs.

Regarding the subcutaneous administration of hydrogen peroxide for therapeutic purposes, there is in the literature only the single report of Gutira and Marek (Goshestvenskiy).

The purpose of our work was to clarify the possibility of using hydrogen peroxide intravenously and to establish the therapeutic and toxic concentrations for several animal species.

Procedure

The experiments were conducted on horse, dogs, and rabbits.

For the experiments, we used control preparations of Perhydrol or an official solution of hydrogen peroxide. The solutions were prepared *ex tempore* in a 0.9 physiological solution, first heated to 35-37°.

The hydrogen peroxide solution was administered to the horses in the *v. jugularis*, and to the dogs and rabbits in the ear vein. The duration of hydrogen peroxide administration was 3-7 minutes for the horses and from 0.5 to 3 minutes for the rabbits.

After hydrogen peroxide administration, systematic observations were made of the animals, recording all changes, mainly in temperature, pulse rate, respiration, and general state. In several experiments, the percentage of hemoglobin content was studied. Examination was done at 10 and 30 minutes, and 1, 1.5, 3, 4, 5, 6, 24, and 48 hours

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[1] This paper was presented on Feb. 19, 1939 at the Scientific Conference of the Chkalov Agricultural, Zoological, and Veterinary Institute.

after H_2O_2 administration. Hemoglobin was determined in 6 horses and 3 dogs, at 1, 3-4, and 6 hours after administration.

Experiments on the Horses

17 observations were made on 15 animals, of which 15 observations were made on horses with glanders and 2 on healthy horses.

The most typical of the experimental results are presented in Table 1.

To illustrate the changes in temperature, pulse rate, and respiration, we present Figs. 1 and 2.

Table 1. Results of Experiments on Intravenous Administration of Hydrogen Peroxide to Horses

# of Animal	Sex & Age	Deg. of Fatness	Wt., kg	Dose & Conc. of H_2O_2 Solution: Gen. Dose, cc	Dose/kg wt, cc	Conc., %	Dur.n of Admin., min.	Brief Clinical Data
1	Mare, 14 years	Mod.	350	700	2	0.004	7	No deviation from the norm
2	Mare, 20 years	Less than mod.	385	770	2	0.01	3	Brief, 0.5° increase in temperature after 10 min.
3	Stallion, 10 years	Mod.	265	530	2	0.25	3	Short-term slowing of pulse (by 18 beats) & temp. inc. of 0.4° after 30 minutes
4	Mare, 10 years	Mod.	300	600	2	0.5	3	After 5 min., shortness of breath lasting 30 min.; temp. inc. of 0.4° after 30 min.
5	Gelding, 13 years	Mod.	320	1600	5	0.5	5	Shortness of breath after 40 min.
6	Mare, 14 years	Mod.	350	350	1	1	3	Shortness of breath after 30 min.
7	Mare, 17 years	Mod.	350	700	2	1	5	Shortness of breath after 1.5 hr; brief, 0.5° temp. dec. after 10 min.; muscular tremors & slightly depressed state aft. 1 hr.

# of Animal	Sex & Age	Deg. of Fatness	Wt., kg	Dose & Conc. of H_2O_2 Solution: Gen. Dose, cc	Dose/kg wt, cc	Conc., %	Dur.n of Admin., min.	Brief Clinical Data
8	Stallion,	Mod.	270	350	1.3	2	3	Immediately after administration, the horse collapsed; spasms after 3 min.; severe shortness of breath after 1 hr.; increase in pulse rate, increase in peristalsis, and repeated acts of defecation. The horse got up after 35 min., emp. dec. of 1.3° after 1 hr., depressed state after 2.5 hr.

Temperature, pulse, and respiration data were noted only for a period of 3-4 hours, since changes under the influence of hydrogen peroxide are short-term, and in the majority of cases, disappear after 1 hour.

Analyzing the data obtained, it may be seen that hydrogen peroxide in concentrations of 0.004, 0.01, and 0.25% in 2-cc doses per kg wt do not evoke, in the majority of horses, any changes, with the exception of an increase in temperature of 0.4-0.5° (horses # 3 and 5) and a slowing of the pulse (horse # 5).

With an increased concentration of the hydrogen peroxide solution to 0.5-1% in a 1-2 cc dose per kg, shortness of breath is observed which lasts from 0.5 to 1.5 hours. In several cases, an increase of 0.7-0.9° in body temperature and muscular tremors were observed.

Hydrogen peroxide in 2-3% solution in 1-1.5 cc doses per kg wt evoke the following symptoms: 1) a severe shortness of breath which begins at the instant of H_2O_2 administration and continues for 20

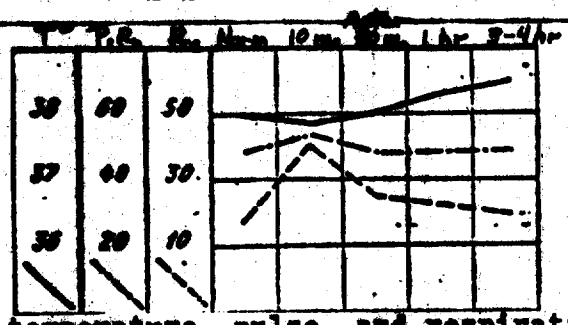

Fig. 1. Curve of temperature, pulse, and respiration during intravenous administration of hydrogen peroxide to a horse in a 1% solution in doses of 1cc/kg wt (experiment # 10).

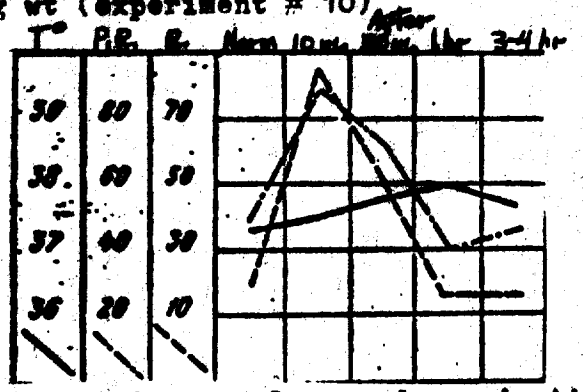

Fig. 2. Curve of temperature, pulse, and respiration during intravenous administration of hydrogen peroxide to a horse in a 2% solution in 1.5-cc doses/kg wt (experiment # 16).

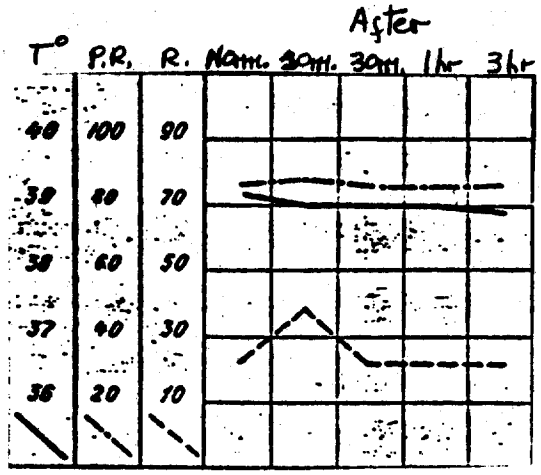

Fig. 3. Curve of temperature, pulse, and respiration during intravenous administration of hydrogen peroxide to a dog in a 1% solution in 2-cc doses/kg wt (experiment # 15).

minutes to 1 hour; 2) immediately after H_2O_2 administration with increased shortness of breath, the horses suddenly fall down (horse # 8); 3) depressed state for 40 minutes to 2.5 hours; 4) brief attack of spasms (horse # 8), replacing the depressed state; 5) increase in peristalsis and repeated acts of defecation; 6) increase in pulse rate, etc.

The study of percentage of hemoglobin content in 6 horses did not yield any deviations from the norm.

During intravenous administration of hydrogen peroxide, no differences in action were established in the healthy horses or the ones with glanders.

Experiments on Dogs

In all, more than 30 observations were made; the most typical of the experiments are presented in Table 2.

Table 2. Results of Experiments on Intravenous Administration of Hydrogen Peroxide to Dogs

# of Animal	Sex	Deg. of Fatness	Wt., kg	Dose		Conc. of H_2O_2 Solution		Dur.n of Admin., min.	Brief Clinical Data
				Gen. dose, cc	Dose/kg wt, cc	Conc., %			
1	M	Good	14	14	1	0.01		1	No deviation from norm
2	M	Good	15	22.5	1.5	0.5		2	Same
3	F	Mod.	11.4	3.4	0.3	1		0.5	Brief inc. in respiration
4	F	Mod.	11.4	5.7	0.5	1		0.5	Brief dec. in pulse rate after 30 min.
5	M	Good	13.5	13.5	1	1		1	Inc. in peristalsis, frequent acts of defecation, 0.7° inc. in body temp. after 10 min.

# of Animal	Sex	Deg. of Fatness	Wt., kg	Dose & Solution: Gen. dose, cc	Dose/kg wt, cc	Conc. of H_2O_2 Solution, Conc., %	Dur.n of Admin., min.	Brief Clinical Data
6	M	Good	13.5	17.5	1	1	1	0.3° inc. in temp. after 10 min.
7	M	Good	5.2	5.2	1	1	1	No deviation
8	M	Good	14	28	2	1	2	Inc. in peristalsis, defecation
9	M	Mod.	11.5	23	2	1	2	Slight shortness of breath after 15 min.
10	M	Mod.	6	12	2	1	1	No deviation
11	M	Mod.	10	20	2	1	5	Same
12	M	Mod.	0.3	0.3	1	2	1	Shortness of breath after 1 hour
13	M	Good	12	24	2	2	4	Shortness of breath after 20 min., 1.4° inc. in temp. after 1 hr
14	M	Mod.	5.5	11	2	2	2	Shortness of breath after 25 min., inc. in pulse rate & peristalsis, and repeated acts of defecation, 0.4 inc. in temp. and depressed state

As is evident from Table 2, hydrogen peroxide in concentrations of 0.01 and 0.05% in doses of 1-2 cc/kg wt does not cause any deviation from the norm during intravenous administration to dogs.

Hydrogen peroxide in a 1% solution and 0.3-2 cc doses/kg wt evoked the following changes: 1) slight shortness of breath lasting 10-15 minutes and 30 minutes at maximum; 2) increase in peristalsis and repeated acts of defecation; 3) brief increase or decrease (0.3-0.7°) in body temperature.

With the administration of the 2% solution in 1-2 cc doses/kg wt, as a rule, shortness of breath occurs which begins immediately after administration and lasts from 10 minutes to 1 hour and 20 minutes. In one case, an increase in body temperature is noted, as well as an increase in peristalsis and repeated acts of defecation.

A 3% solution of hydrogen peroxide in 1-2 cc doses. kg wt evokes analogous symptoms (shortness of breath, depressed state, increase in peristalsis and pulse rate, etc.), but the last is expressed more strongly than in the experiments with the 2% concentration.

The hemoglobin studies, which were done on 3 dogs, yielded no deviations from the norm.

We present an illustration of changes in temperature, pulse rate, and respiration in Fig. 7.

Experiments on Rabbits

Eight experiments in all were conducted on eight rabbits: 2 using 0.2% solution, 2 using 0.25%, 1 using 0.4%, 2 using 0.5%, and 1 using 1%.

The results of the experiments are as follows: 1) for 0.2-0.25% hydrogen peroxide concentrations (in doses of 2 cc/kg wt), only a brief increase in respiration is observed; 2) hydrogen peroxide in 0.4-0.5%

solution (in 2-cc doses) and in 1% solution in a dose of 1 cc/kg wt evoked a clearly toxic effect in the majority of cases, accompanied by an extreme shortness of breath, agitation, throwing of the head backward, and a brief fit of spasms. After the spasms ended, respiration gradually slowed and was weakened to the point of paralysis. Of 4 rabbits in the given group, 3 collapsed (after 4, 9 minutes and 12 hours).

Conclusion

The data presented indicate that with an increase in concentration of H_2O_2 solutions, the characteristic symptoms in horses and dogs are shortness of breath, increase in peristalsis, and repeated acts of defecation, more rarely spasms of muscular tremors (the latter only in the horses), and in the rabbits, shortness of breath and characteristic throwing of the head backward. It must be mentioned that the sensitivity of the different animals to the intravenous administration of H_2O_2 is unique: the rabbits have a greater sensitivity than the dogs or horse.

If the data from our experiments are compared with data from the intravenous administration of oxygen (Grin'kov, Komarov), we obtain a complete analogy.

According to Tsyganov's data, with the introduction of oxygen into the veins of dogs, an increase of 60-120% in respiration is observed, representing a reactive phenomenon, accompanied by removal of an excess of oxygen from the organism. A similar mechanism of action evidently takes place during the intravenous administration of H_2O_2.

Other symptoms of H_2O_2 action in rabbits (throwing the head backward, spasms preceding death) are evidently explained by a vascular embolism, which Teshni indicates for subcutaneous administration of H_2O_2 to rabbits. As for changes in temperature, intestines, heart, and so on, the mechanism of this action still remains unclear, in our opinion.

In conclusion, it must be mentioned that at the present time, using established concentrations and doses of H_2O_2, investigators in our department administer 0.5, 1, and 2% solutions intravenously to dogs in certain poisoning cases, and not once have complications been seen.

Results

1. Therapeutic doses of hydrogen peroxide (intravenous administration for 1 kg wt of the animal) may be considered as follows: a) for horses, 2-5 cc of 0.5% solution and 1-2 cc of 1%; b) for dogs, 1-2 cc of 0.5, 1, and 2% solutions; c) for rabbits, up to 2 cc of 0.2-0.25% solution.

2. Toxic doses of hydrogen peroxide for 1 kg wt are: for horses, 1-1.5 cc of 2-3% solution; for dogs, 1-2 cc 3% solution, and for rabbits, up to 2 cc of 0.1-0.5% solution or higher. In horses and dogs, the indicated concentrations and doses evoke symptoms of mildly toxic action, while in rabbits the action is accompanied by death in the majority of cases.

3. The rabbits have a greater sensitivity to intravenous H_2O_2 administration than do horses or dogs.

4. The techniques of intravenous hydrogen peroxide administration are simple and may replace cumbersome means of intravenous

oxygen administration.

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ФАРМАКОЛОГИЯ И ТОКСИКОЛОГИЯ

1940 — ТОМ III — ВЫП. 5

К ВОПРОСУ О ВНУТРИВЕННОМ ВВЕДЕНИИ ПЕРЕКИСИ ВОДОРОДА ЛОШАДЯМ, СОБАКАМ И КРОЛИКАМ [1]

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Среди методов кислородной терапии большое значение может иметь способ введения кислорода непосредственно в кровь, на возможность которого указано еще Нистеном (Nisten) и Демаркей (Demarkej) (цит. по Комарову).

Мы поставили своей задачей разработать более простой и удобный способ введения кислорода непосредственно в кровь, для чего использовали перекись водорода как вещество, легко отдающее кислород.

Большинство авторов учебных пособий по фармакологии указывает на невозможность применения H_2O_2 внутривенно вследствие возможной эмболии. Описаны случаи эмболии и при введении больших количеств H_2O_2 в плевральную полость с целью промывания. Те же опасности представляет инъекция H_2O_2 в глубину раны.

Что касается вопроса о внутривенном введении перекиси водорода с терапевтической целью, то в доступной нам литературе указаний мы не нашли, за исключением работы Мишенина, который с успехом применял внутривенно 0,5% раствор перекиси водорода для лечения острых сероводородных отравлений у собак.

Относительно подкожного применения перекиси водорода с терапевтической целью в литературе имеются только отдельные указания Гутира и Марек (Сошественский).

Цель нашей работы заключается в том, чтобы выяснить возможность применения перекиси водорода внутривенно и установить терапевтические и токсические концентрации для некоторых видов животных.

Методика

Опыты проводились на лошадях, собаках и кроликах.

Для опытов мы пользовались проверенными препаратами пергидрола или официнального раствора перекиси водорода. Растворы готовились ex tempore на 0,9 физиологическом растворе, предварительно прогретом до 35—37°.

Раствор перекиси водорода вводился лошадям в v. jugularis, собакам и кроликам — в ушную вену. Продолжительность введения перекиси водорода составляла у лошадей 3—7 минут, а у кроликов от 0,5 до 3 минут.

После введения перекиси водорода за животными устанавливалось систематическое наблюдение с регистрацией всех изменений, главным образом, со стороны температуры, пульса, дыхания и общего состояния; в некоторых опытах исследовалось процентное содержание гемоглобина. Обследование проводилось через 10, 30 минут, 1, 1.5, 3, 4, 5, 6, 24 и 48 часов после введения H_2O_2. Гемоглобин определялся у 6 лошадей и 3 собак через 1, 3—4 и 6 часов после введения H_2O_2.

Опыты на лошадях

Проведено 17 наблюдений на 15 животных, из них: 15 наблюдений на лошадях-маллеинщиках, а 2 — на здоровых лошадях.

Наиболее типичные результаты опытов приведены в табл. 1.

Для иллюстрации изменений со стороны температуры, пульса и дыхания приводим рис. 1 и 2.

[1] Работа доложена 19.II.1939 г. на научной конференции Чкаловского агро-зоо-ветинститута.

Таблица 1. Результаты опытов по внутривенному введению перекиси водорода лошадям

№ животного	Пол и возраст животного	Упитанность	Вес в кг	Доза и концентрация раствора H_2O_2			Продолжительность введения в минутах	Краткие клинические данные
				общая доза раствора в см³	доза на 1 кг веса в см³	концентрация в %		
1	Кобыла, 14 лет	Средняя	350	700	2	0,004	7	Отклонений от нормы не было
2	» 20 »	Ниже среднего	385	770	2	0,01	3	Непродолжительное повышение температуры через 10 минут на 0,5°
3	Жеребец, 10 »	Средняя	265	530	2	0,25	3	Кратковременное замедление пульса (на 18 ударов) и повышение температуры через 30 минут на 0,4°
4	Кобыла, 10 »	»	300	600	2	0,5	3	Через 5 минут одышка, продолжающаяся 30 минут, повышение температуры через 30 минут на 0,4°
5	Мерин, 13 »	»	320	1 600	5	0,5	5	Одышка в течение 40 минут
6	Кобыла, 14 »	»	350	350	1	1	3	Одышка в течение 30 минут
7	» 17 »	»	350	700	2	1	5	Одышка в течение 1,5 часа. Кратковременное понижение температуры через 10 минут на 0,5°. Мышечное дрожание и слабо угнетенное состояние в течение 1 часа
8	Жеребец, 10 »	»	270	350	1,3	2	3	Тотчас после введения лошадь упала; судороги в течение 3 минут. Сильная одышка в течение 1 часа, учащение пульса, усиление перистальтики и повторяющиеся акты дефекации. Лошадь встала через 35 минут. Понижение температуры через 1 час на 1,3°, угнетенное состояние в течение 2,5 часа

Данные температуры, пульса и дыхания отмечены только в пределах 3—4 часов, так как изменения под влиянием перекиси водорода кратковременны и в большинстве случаев исчезают по прошествии 1 часа.

Анализируя полученные данные, можно видеть, что перекись водорода в концентрации 0,004, 0.01 и 0,25% при дозе 2 см³ на 1 кг веса не вызывает у большинства животных никаких изменений, за исключением непродолжительного повышения температуры на 0,4—0,5° (лошади № 3 и 5) и замедление пульса (лошадь № 5).

С повышением концентрации раствора перекиси водорода до 0,5—1% при дозе 1—2 см³ на 1 кг наблюдается одышка, продолжающаяся от 0,5 до 1,5 часа. В некоторых случаях отмечены повышение температуры тела на 0,7—0,9° и мышечное дрожание.

Перекись водорода в 2—3% растворе при дозе 1—1,5 см³ на 1 кг веса вызывает следующие симптомы: 1) сильную одышку, которая начинается в момент введения H_2O_2 и продолжается в течение 20 минут

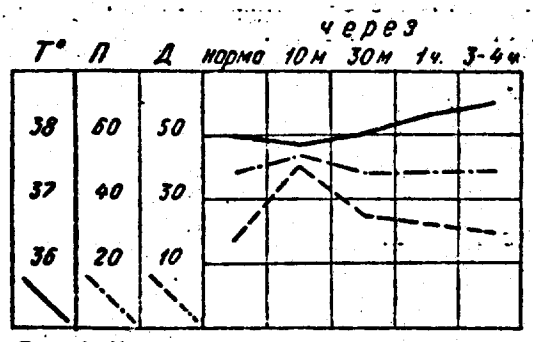

Рис. 1. Кривая температуры, пульса и дыхания при внутривенном введении перекиси водорода лошади в 1% растворе в дозе 1 см³ на 1 кг веса (опыт № 10)

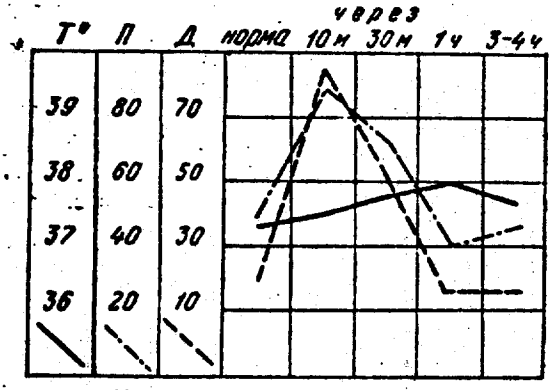

Рис. 2. Кривая температуры, пульса и дыхания при внутривенном введении перекиси водорода лошади в 2% растворе в дозе 1,5 см³ на 1 кг веса (опыт № 16)

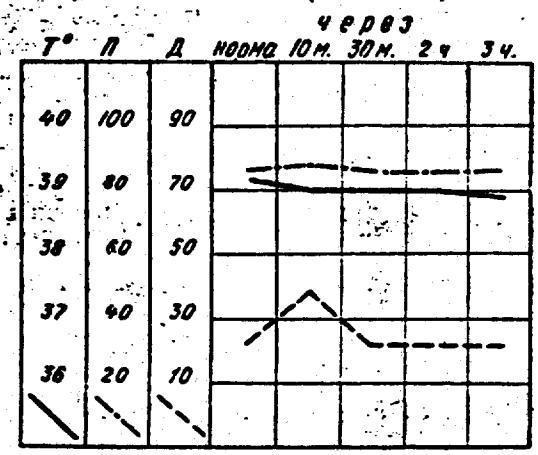

Рис. 3. Кривая температуры, пульса и дыхания при внутривенном введении перекиси водорода собаке в 1% растворе в дозе 2 см³ на 1 кг веса (опыт № 15)

и до 1 часа; 2) тотчас после введения H_2O_2 при нарастающей одышке лошади внезапно падают (лошадь № 8); 3) угнетенное состояние в течение 40 минут и до 2,5 часа; 4) непродолжительный приступ судорог

(лошадь № 8), сменяющийся угнетенным состоянием; 5) усиление перистальтики и повторяющиеся акты дефекации; 6) учащение пульса и др.

Исследование процентного содержания гемоглобина у 6 лошадей никаких отклонений от нормы не дало.

При внутривенном введении H_2O_2 никакой разницы в действии у здоровых лошадей и лошадей-маллеинщиков не установлено.

Опыты на собаках

Всего было проведено больше 30 наблюдений; наиболее типичные опыты приведены в табл. 2.

Таблица 2. Результаты опытов по внутривенному введению перекиси водорода собакам

№ животного	Пол животного	Упитанность у	Вес в кг	Доза и концентрация раствора H_2O_2			Продолжительность введения в минутах	Клинические данные
				общая доза раствора в см³	доза на 1 кг веса в см³	концентрация в %		
1	Самец	Хорошая	14	14	1	0,01	1	Отклонений от нормы не было
2	»	»	15	22,5	1,5	0,5	2	То же
3	Самка	Средняя	11,4	3,4	0,3	1	0,5	Кратковременное учащение дыхания
4	»	»	11,4	5,7	0,5	1	0,5	Кратковременное замедление пульса через 30 минут
5	Самец	Хорошая	13,5	13,5	1	1	1	Усиление перистальтики, частые акты дефекации, повышение температуры тела через 10 минут на 0,7°
6	»	»	13,5	13,5	1	1	1	Через 10 минут повышение температуры на 0,3°
7	Самка	»	5,2	5,2	1	1	1	Отклонений не было
8	Самец	»	14	28	2	1	2	Усиление перистальтики, дефекация
9	Самка	Средняя	11,5	23	2	1	2	Слабая одышка в течение 15 минут
10	Самец	»	6	12	2	1	1	Отклонений не было
11	»	»	10	20	2	1	5	То же
12	»	»	9,3	9,3	1	2	1	Одышка в течение 1 часа
13	»	Хорошая	12	24	2	2	4	Одышка в течение 1 часа 20 мин., повышение температуры через 1 час на 1,4°
14	Самка	Средняя	5,5	11	2	3	2	Одышка в течение 25 минут, учащение пульса, усиление перистальтики и повторяющиеся акты дефекации, повышение температуры на 0,4° и угнетенное состояние

Как видно из табл. 2, перекись водорода в концентрации 0,01 и 0,05% при дозе от 1 до 2 см³ на 1 кг веса при внутривенном введении собакам не вызывает никаких отклонений от нормы.

Перекись водорода в 1% растворе в дозе от 0,3 до 2 см³ на 1 кг веса вызывает следующие изменения: 1) слабую одышку, продолжающуюся в течение 10—15 минут и максимум 30 минут; 2) усиление перистальтики и повторяющиеся акты дефекации; 3) непродолжительное повышение или понижение температуры тела на 0,3—0,7°.

При введении 2% раствора в дозе 1—2 см³ на 1 кг веса, как правило, наступает одышка, которая начинается тотчас после введения и

продолжается в течение от 10 минут до 1 часа 20 мин. В 1 случае отмечены повышение температуры тела, усиление перистальтики и повторяющиеся акты дефекации.

3% раствор перекиси водорода в дозе 1—2 см³ на 1 кг веса вызывает аналогичные симптомы (одышка, угнетенное состояние, усиление перистальтики, учащение пульса и др.), но последние выражены сильнее, чем в опытах с 2% концентрацией.

Исследование гемоглобина, которое проводилось у 3 собак, никаких отклонений от нормы не дало.

Для иллюстрации изменений со стороны температуры, пульса и дыхания приводим рис. 3.

Опыты на кроликах

Всего поставлено 8 опытов на 8 кроликах, из них: с 0,2% раствором — 2 опыта; 0,25% — 2; 0,4% — 1; 0,5% — 2; 1% — 1 опыт.

Результаты опытов сводятся к следующему: 1) при концентрациях перекиси водорода 0,2—0,25% (в дозе 2 см³ на 1 кг веса) наблюдается только кратковременное учащение дыхания; 2) перекись водорода в 0,4—0,5% растворе (в дозе 2 см³) и в 1% растворе в дозе 1 см³ на 1 кг веса вызывает в большинстве случаев явно токсическое действие, сопровождающееся сильной одышкой, возбуждением, запрокидыванием головы назад, непродолжительным приступом судорог. После прекращения судорог дыхание постепенно замедляется и ослабляется вплоть до паралича. Из 4 кроликов данной группы 3 пали (через 4,8 минуты и 12 часов).

Заключение

Приведенные данные показывают, что с увеличением концентрации растворов H_2O_2 наиболее характерными симптомами у лошадей и собак являются одышка, усиление перистальтики, повторяющиеся акты дефекации, реже наблюдаются судороги или мышечное дрожание (последние — только у лошадей), а у кроликов — одышка и характерное запрокидывание головы назад. При этом следует отметить, что чувствительность различных животных к внутривенному введению H_2O_2 неодинакова: кролики обладают большей чувствительностью, чем собаки и лошади.

Если сопоставить данные наших опытов с данными по внутривенному введению кислорода (Гриньков, Комаров), то получаем полную аналогию.

По данным Цыганова, при введении в вену кислорода у собак наблюдается учащение дыхания на 60—120%, представляющее реактивное явление, способствующее удалению из организма избытка кислорода. Подобный механизм действия, повидимому, имеет место и при внутривенном введении H_2O_2.

Другие симптомы действия H_2O_2 у кроликов (запрокидывание головы назад, судороги, предшествующие смерти) объясняются, повидимому, эмболией сосудов, на что указывает Кешни при подкожном введении H_2O_2 кроликам. Что касается изменений со стороны температуры, кишечника, сердца и пр., то механизм этого действия нам остается пока неясным.

В заключение следует отметить, что в настоящее время, пользуясь установленными концентрациями и дозами H_2O_2, работники нашей кафедры применяют собакам 0,5—1—2% растворы внутривенно при некоторых отравлениях и при этом ни разу не видели случаев осложнений.

Выводы

1. Терапевтическими дозами перекиси водорода при внутривенном введении на 1 кг веса животного можно считать следующие: а) для лошади 2—5 см³ 0,5% раствора и 1—2 см³ — 1%; б) для собаки 1—2 см³ 0,5—1—2% раствора; в) для кролика до 2 см³ 0,2—0,25% раствора.

2. Токсическими дозами перекиси водорода на 1 кг веса являются: для лошади 1—1,5 см³ 2—3% раствора; для собаки 1—2 см³ 3%, а для кролика до 2 см³ 0,4—0,5% раствора и выше, причем у лошадей и собак указанные концентрации и дозы вызывают симптомы слабо токсического действия, а у кроликов в большинстве случаев действие сопровождается смертельным исходом.

3. Кролики обладают большей чувствительностью к внутривенному введению H_2O_2, чем лошади и собаки.

4. Техника внутривенного введения перекиси водорода проста и может заменить громоздкий способ внутривенного введения кислорода.

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ON THE INTRAVENOUS INJECTION OF HYDROGEN PEROXIDE TO HORSES, DOGS AND RABBITS

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Chair of Pharmacology (Chief — S. G. Sidorova) of the Chkalov Agro-Zoo-Veterinary Institute

It is pointed out by most of modern authors that because of its rapid decomposition hydrogen peroxide should not be injected intravenously to avoid embolie of the heart and cerebral vessels.

The purpose of the present study is to elucidate the possibility of intravenous injection of hydrogen peroxide and to determine the therapeutic and toxic concentrations for certain animal species.

The following are the results obtained in horses, dogs and rabbits.

1. As therapeutic doses of hydrogen peroxide upon intravenous injection we may consider the following ones.

a) for the horse, 2—5 c.c. 0.5% solution and 1—2 c.c. 1% solution.

b) for the dog, 1—2 c.c. 0.5—1 — 2% solution.

c) for the rabbit, up to 2 c.c. 0.2—0.25% solution.

2. Toxic doses of hydrogen peroxide are 1—1.5 c.c. 2—3% solution per 1 kg body weight (horse); 1—2 c.c. 3% solution per 1 kg body weight (dog), and up to 2 c.c. 0.4—0.5% solution or more per 1 kg body weight (rabbit).

3. The rabbits are more susceptible to intravenous injection of hydrogen peroxide than horses or dogs.

4. The technics of intravenous injection of hydrogen peroxide is very simple and may well replace the cumbersome method of intravenous injection of oxygen.

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THE INHALATION TOXICITY OF AEROSOLS OF 90% HYDROGEN PEROXIDE

by

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The Inhalation Toxicity of Aerosols of 90% Hydrogen Peroxide

ABSTRACT

OBJECT.

The object of the work reported in this paper was to determine the toxicological effects produced in mice exposed to 90% hydrogen peroxide (H_2O_2) dispersed as an aerosol.

RESULTS.

Groups of mice were exposed for 5-15 min. to an aerosol atmosphere produced from 90% H_2O_2 at levels of concentrations ranging from approximately 4 mg./l. to 19 mg./l. Symptoms were noted. Gross and microscopic tissue studies were made. Particle sizes were determined using the cascade impactor.

CONCLUSIONS.

1. An aerosol atmosphere produced from 90% H_2O_2 can cause significant pulmonary irritation and congestion in mice exposed for 5 min.

2. Irritating effects to eyes and nose would preclude the likelihood of prolonged exposure to high aerosol concentrations (10 mg./l. of 90% H_2O_2) which might be lethal.

3. Survivors of exceptionally high concentrations (9-19 mg./l. of H_2O_2) may develop corneal damage several weeks after exposure.

RECOMMENDATIONS.

Skin, eye, and respiratory protection for personnel is indicated when the circumstances of handling 90% H_2O_2 could result in an aerosol exposure.

Medical Laboratories Research Report No. 189

The Inhalation Toxicity of Aerosols of 90% Hydrogen Peroxide

I. INTRODUCTION.

A. Object.

The object of the work reported in this paper was to determine the toxicological effects produced in mice exposed to 90% hydrogen peroxide (H_2O_2) dispersed as an aerosol.

B. Authority.

Authorized by the Chief Chemical Officer under Project 4-61-14-002, Health Hazards of Military Chemicals, Test Program No. T15, Cml C Research and Development Program for fiscal year 1952.

II. HISTORICAL.

The possible expanding technological applications of 90% H_2O_2 of interest to the Armed Forces have necessitated an evaluation of its health hazards.

Skin contact with the liquid is a significant hazard. Relatively small amounts of liquid in the eye can cause severe corneal damage with resultant visual handicap or permanent blindness (1). Saturated 90% H_2O_2 vapors for 8 hours, although not lethal will cause slight pulmonary edema and localized emphysema in rats (2). Under operational conditions involving only vapors, no major respiratory hazard would be offered by a single acute exposure. This conclusion is in general agreement with that cited in other reports on concentrated H_2O_2 (1, 3).

It has been demonstrated qualitatively that aerosols of 90% H_2O_2 have a high order of toxicity by inhalation in rats (4). However, no controlled quantitative studies involving aerosol toxicity by inhalation and the hazard which it might present have been reported. Therefore, this toxicity test was undertaken to evaluate the inhalation hazard associated with the use of 90% H_2O_2 which might produce an accidental spray or aerosol.

III. EXPERIMENTAL.

A. Materials.

The 90% H_2O_2 used in this study was obtained from the U. S. Naval Engineering Experiment Station, Annapolis, Maryland.

B. Exposure and Analytical Procedures.

The aerosol was dispersed by passing dry air through 3 parallel "vaponefrin" glass nebulizers containing 90% H_2O_2. The aerosol thus generated was diluted in a mixing chamber before entering a 20 l. glass exposure chamber. The airflow through the latter was approximately 30 l./min.

Two series of H_2O_2 exposures were carried out in the chamber, one ranging from 3.6-5.2 mg./l. for 5 min. and one ranging from 9.4-19.0 mg./l. for 5-15 min. In each exposure 10 white mice weighing about 20 g. each were used. The disperser was started with the animals in the chamber and sampling of the chamber atmosphere was initiated 4 min. later when the chamber concentration had reached equilibrium (5). At the termination of the exposure period the chamber was aerated with fresh air for at least 30 min. before the animals were removed to observation cages. The mice were observed during exposure and then daily until sacrificed (2 days-8 weeks) for pathological studies.

Samples for colorimetric analysis of H_2O_2 (6) concentrations were collected by 2 midget impingers in series, each containing 10 ml. of titanium sulfate reagent.

The cascade impactor was used to characterize the aerosol with respect to particle size distribution.

C. Results.

1. Toxicity.

Results of exposing mice 5 min. to aerosols of 90% H_2O_2 indicate that concentrations of the order of 3.6 to 5.2 mg./l. of H_2O_2 are sublethal. When the concentration was increased to 9.4 mg./l. the lethality range was reached with death occurring 6 days following exposure. At concentrations ranging from 12 to 19 mg./l. for 10 and 15 min. exposures, the survival time was reduced in the majority of animals to less than an hour. These results are shown in Table I.

TABLE I

Toxicity of 90% H_2O_2 Aerosol to Mice by Inhalation

Exposure Time min.	Analytical Concentration mg./l.	Mortality	Survival Time
5	3.6	0/10	---
5	4.2	0/10	---
5	5.2	0/10	---
5	5.2	0/10	---
5	9.4	1/10	6 days
10	13.2	5/10	*
10	13.4	1/10	6 min.
10	19.0	1/10	11 min.
15	11.8	5/10	5-50 min.
15	16.7	9/10	**

* 4/10 died between 12 and 120 minutes; a fifth died after 6 days
** 8/10 died between 11 and 48 minutes; a ninth died the next day

Chamber temperatures varied from 26° C. to 28° C. The mass-median particle size of the aerosols as determined by the cascade impactor was approximately 3.5 microns.

With reference to analytical samples, it may be pointed out that no H_2O_2 was found in the second impinger in any experiment.

2. Symptoms.

The symptoms of the animals during exposure to the low concentrations consisted of a mild nasal irritation, blinking of the eyes, slight gasping, and a loss of muscular coordination. These symptoms generally disappeared within 30 min. after removal from the aerosol atmosphere. Minor H_2O_2 burns were observed on the nose and paws of most animals. The symptoms of the animals during exposure to the higher concentrations were similar to those exhibited at the lower concentrations except that they were more severe.

The animals which died as a result of exposure to the aerosol went through a short convulsant period before death. In survivors the burns of the nose and paws disappeared in less than a week, but the eye inflammation persisted for another week. At the end of the second week the signs of primary irritation had disappeared from the eyes, except for 2 cases exposed to 19 mg./l. for 10 min. in which the corneas showed gross opacities, which persisted for the remainder of the observation period (6 weeks).

3. Pathology.

The mice exposed to the sublethal range of concentrations (3.6-5.2 mg./l.) showed congestion of the lungs when sacrificed at various intervals over a period of 10 days after exposure. Four of the 20 mice exposed to 5.2 mg./l showed some necrosis of bronchial epithelial cells.

Animals which died during or within about an hour after exposure to 9.4-19 mg./l. showed pulmonary congestion in most instances. However, animals which survived these concentrations for several days or had been sacrificed after 5 and 8 weeks showed necrosis of bronchial epithelium.

The mode of death was not determined. The possibility of oxygen embolism should be kept in mind.

Gross opacities were present in the eyes of 4 animals exposed to the highest concentration (19 mg./l.) and sacrificed after 8 weeks. Animals sacrificed 5 weeks after exposure to this concentration did not show gross eye lesions, but did show microscopic lesions.

Microscopic lesions were found in the eyes of the animals exposed to 9.4 mg./l. 8 weeks after exposure, while those examined 5 weeks after exposure to this concentration showed no significant changes.

These findings indicate that there is an insidious and slowly developing corneal damage subsequent to exposure to high aerosol concentrations of H_2O_2. No cataracts were seen in the mice, and no eye lesions were seen in control mice or in those exposed at the lower levels.

IV. DISCUSSION.

During preliminary experiments using H_2O_2 aerosol a number of animals died with subcutaneous accumulations of O_2 on the dependent portions of the body (swelling, blistering, and congestion of digital portions of the limbs). This

corresponds to those times when the droplet size of the aerosol was too large and it collected in small pools on the chamber floor. When the droplet size was reduced below 4 μ there was no accumulation of liquid H_2O_2 on the floor and only minor burns were observed on the nose and paws of the animals.

An attempt was made to determine the LCt50 of H_2O_2 aerosol for a short exposure time. This study was discontinued when it became evident that H_2O_2 concentrations would have to be increased to levels which would be improbable under operational conditions. The vapor pressure (3) of 90% H_2O_2 is such that saturated vapors could contain 3-5 mg./l. of H_2O_2 and local high aerosol concentrations would be reduced rapidly to the vapor phase.

While exposures to saturated 90% H_2O_2 vapors for 8 hours caused slight pulmonary edema and localized emphysema, exposure to low aerosol concentrations in addition to the vapors for 5 minutes showed congestion of the lungs and significant pulmonary irritation when the animals were sacrificed over a period of 10 days after exposure. Animals which survived high concentrations of the aerosol showed necrosis of bronchial epithelium and in some cases severe corneal damage when sacrificed after 5 and 8 weeks.

It is unlikely that an operational situation could produce a persistent aerosol concentration of H_2O_2. However, the animal experiments reported herein indicate that particulates of H_2O_2 with vapors for 5-min. exposures can cause pulmonary irritation and congestion. The particle sizes in these studies were in the range that penetrate the upper respiratory tract to the bronchial regions.

V. CONCLUSIONS.

1. An aerosol atmosphere produced from 90% H_2O_2 can cause significant pulmonary irritation and congestion in mice exposed for 5 min.

2. Irritating effects to eyes and nose would preclude the likelihood of prolonged exposure to high aerosol concentrations (10 mg./l. of 90% H_2O_2) which might be lethal.

3. Survivors of exceptionally high concentrations (9-19 mg./l. of H_2O_2) may develop corneal damage several weeks after exposure.

VI. RECOMMENDATION.

Skin, eye and respiratory protection for personnel is indicated when the circumstances of handling 90% H_2O_2 could result in an aerosol exposure.

VII. ACKNOWLEDGMENT.

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Effects of Hydrogen Peroxide on Normal and Hypertensive Rats

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Introduction

Moore, Murray and *Huston*[1] showed that the respiration of heart and kidney from hypertensive rats was lower than that of tissue from normal animals. *Liberman*[2] reported that inhalation of oxygen had an ameliorating effect on certain aspects of clinical hypertension. Little is known about the systemic pharmacology of hydrogen peroxide although influences on tumour formation have been explored[3-9]. It was thought of interest, therefore, to investigate the effect of hydrogen peroxide on the blood pressure and longevity of normal and hypertensive animals.

Method

Male albino rats were made hypertensive by removal of one kidney, subcutaneous implantation of a 20 mg. pellet of desoxycorticosterone acetate (DCA) and administration of 1% sodium chloride in the drinking water. Blood pressures were determined under light pentobarbital anesthesia by means of a photoelectric tensometer[10]. Two series were run at different times, so there are two sets of data. Percentage strengths of hydrogen peroxide used were 10, 5, 2.5, 0.5 and 0.25. The hydrogen per-

* Rats were made hypertensive by subcutaneous implantation of desoxycortisosterone acetate pellets, removal of one kidney and administration of 1% sodium chloride as the drinking fluid. Hydrogen peroxide solution, (0.5% and 0.25%) administered orally had the effect of reducing the hypertension and increasing the survival rate.

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oxide was administered in the drinking solution beginning 7 days after the operation in the first series and 12 in the other.

The groups studied were as follows:

1. Normal rats receiving (a) water, (b) 10% H_2O_2, (c) 5% H_2O_2, (d) 2.5% H_2O_2, (e) 0.5% H_2O_2, (f) 0.25 H_2O_2.

2. DCA-implanted rats receiving (a) 1% saline, (b) 2.5% H_2O_2 in saline, (c) 0.5% H_2O_2 in saline, (d) 0.25% H_2O_2 in saline.

The blood pressures and weights were recorded weekly for two months and thereafter less frequently.

Results

The 10, 5 and 2.5% H_2O_2 solutions were toxic and all the animals died within 43 days. However, even with the toxic level of 2.5% H_2O_2 the blood pressure of the hypertensive animals so treated was lower than that of untreated hypertensive animals.

0.25% H_2O_2 had no effect on the blood pressure of normal animals but 0.5% caused a slight increase. Both these strengths showed evidence of some toxicity as demonstrated by body weight and mortality. In the first series the normal animals had a mean body weight after 290 days of 480 g. and one death; those receiving 0.5% H_2O_2 had a mean body weight of *320* g. and there were 8 deaths. In the second series after 146 days the corresponding figures were: normals 480 g. and no deaths; with 0.5% H_2O_2, *375* g. and 2 deaths; with 0.25% H_2O_2, *452* g. and one death.

It will be seen from Fig. 1 and 2 that both strengths of peroxide had a marked effect in reducing the blood pressure and prolonging the life of hypertensive animals. In series 1 (Fig. 1) all the untreated hypertensive animals had died within 63 days whereas in the group receiving 0.5% H_2O_2 3 of original 10 were living after 243 days. In the second series (Fig. 2) all the untreated animals had died within 59 days whereas 8 of 10 survived to 146 days with 0.5% and with 0.25% H_2O_2.

Both strengths of hydrogen peroxide reduced the blood pressure of the DCA implanted animals. In series 1, the surviving untreated animal had a blood pressure of 205 mm. at 52 days whereas at that time the mean of the pressures of the 7 treated animals was 159. The mean pressure of the surviving members of the latter group rose to 168 at 243 days. In the second series the surviving untreated hypertensive animal had a mean pressure of 205 mm. at 58 days. The nine surviving animals receiving 0.5% H_2O_2 had a mean pressure of 130 mm. at a similar period. The mean pressure rose to 153 mm. after 146 days (8 animals). The ten animals receiving 0.25% had a mean pressure at 58 days of 122 mm. This rose to 156 mm. after 146 days (8 animals).

Since it was noted in the first series that the several groups differed in fluid intake a record was kept in the second series during the first month of the experiment. The normal animals ingested about 1000 ml. 3

Figure 1

Effect of 0.5 % hydrogen peroxide on the blood pressure of DCA implanted rats

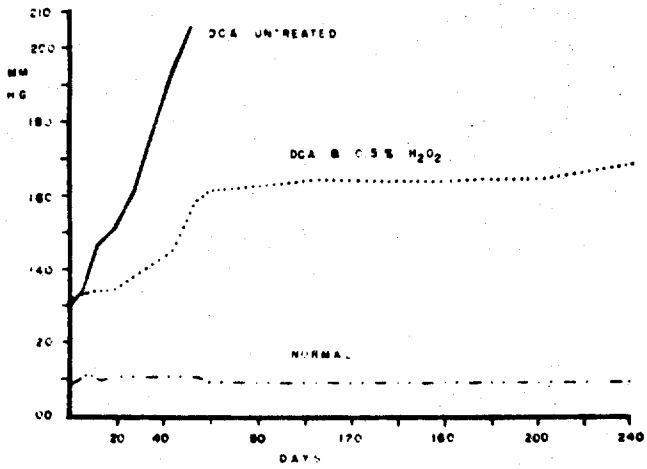
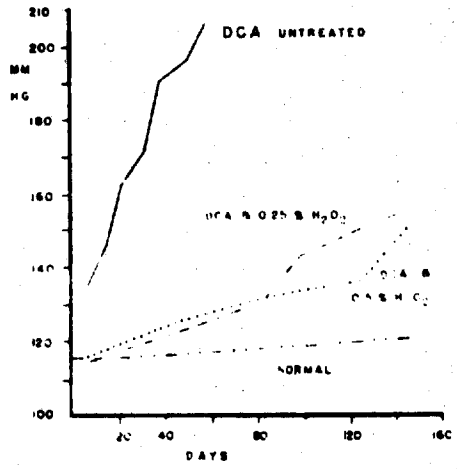


Figure 2

Effect of 0.25 % and 0.5 % hydrogen peroxide on the blood pressure of DCA implanted rats



days per 10 rats. The hypertensive untreated animals drank approximately twice this amount. The DCA-implanted animals receiving 0.5 % H_2O_2 drank about 800 ml. 3 days per 10 rats; and those receiving 0.25 % H_2O_2 drank about 1500 ml. 3 days per 10 rats. Since DCA-implanted animals receiving H_2O_2 drank a smaller amount of fluid they would be ingesting a smaller amount of sodium chloride. This might influence the degree of hypertension

Discussion

It is unknown what role the reduced oxygen consumption of the hypertensive heart and kidney reported by *Moore, Murray* and *Huston*[1] plays in the pathogenesis of experimental hypertension. However, since oxygen utilization is reduced, it seems possible that an oxygen releasing agent such as hydrogen peroxide might modify the hypertension. The lower blood pressure and reduced mortality of the hypertensive animals appears to indicate that this is the case. The mechanism is obscure. Little data are available on the fate of orally ingested hydrogen peroxide. Since the solution releases oxygen on contact with organic material it would be expected that a high proportion of the oxygen would be expended in reaction with the intestinal contents. Systemic effects might therefore be due to absorption of small amounts of hydrogen peroxide or of oxidation products of ingested food or a result of other changes in nutritional conditions.

Hypertensive animals drink about twice the amount of fluid that normal animals do. The reduced fluid intake of hypertensive animals receiving hydrogen peroxide may have influenced the development of the hypertension. It is unknown whether the decreased salt intake reduced the hypertension or whether the modification of the hypertension tended to return towards normal the fluid intake.

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Experiments on the Carcinogenic Effect of Hydrogen Peroxide and on the Mechanism of Ray Carcinogenesis

Hydrogen peroxide forms during the roentgen irradiation of water as well as of water containing tissues and tissue liquids. Several authors assign decisive importance to H_2O_2 in the effect of irradiation. It is surprising, however, that the proof of the carcinogenic effect of hydrogen peroxide has not yet been brought. The following article will therefore report on some tests connected with this question. In connection with it, the results of a series of tests to the mechanism of ray carcinogenesis will be reported.

Test material and method

Hydrogen peroxide (Perhydrol/Merck) was injected S.C. once or several times into the backs of newborn mice, preferably of the AB strain, in a dilution of 0.6% in Ringer solution at a dosis of 0.1 ml.

In other tests, we mixed normal rat serum with Perhydrol. After completion of the decomposition of the serum catalase (end of foam and bubble formation after approx. 2½ hrs.), the serum was administered in the same way.

In the second part of the tests, adult mice were totally irradiated with 750 r (250 KV, 0.5 mm CU). Plasma (heparin addition), serum, erythrocyte sediment, and G4 filtrate of liver and spleen of the irradiated animals were generally obtained 8 hrs. later and injected s.c. once to three times at a dosis of 0.1 - 0.4 ml into newborn mice of the strains C57Bl/6,C57Bl, AB,X, and A/JaX. The technique of filtrate preparation from organs was the same as in our previous tests on the cell-free transmission of tumors. In another test series, the serum from adult rats after total irradiation with 1000 r was injected into newborn mice. In other tests, the serum of irradiated mice was, prior to the injection, heated to 60degC (30 min) or boiled for a short time or dialized for about 16 hrs for the removal of low molecular substances. In the case of repeated injections, we usually waited 2-3 days after the first injection, until the baby mice had somewhat recovered. All animals were observed until their spontaneous death, subsequently dissected, and all find-

ings suspected to be tumorous examined histologically.

Results and discussion

Table 1 shows the first test series with hydrogen peroxide.

Hydrogen peroxide formed by roentgen irradiation as essential component of the radiation effect has been discussed by numerous authors. Ebert traces the change of sensitivity of tissues towards X-rays to differences of the oxygen present which in turn depends upon the amount of H202 formed by the irradiation. Warburg and his coworkers also attribute decisive importance to hydrogen peroxide in the damaging of cancer cells by X-rays, and traces their special sensitivity to low catalase content. Holzer and Frank add to ascites cells such H202 concentrations as are, according to Warburg, formed by glycolysis inhibiting X-ray doses. They observed a lowering of the glycolysis and the NAD concentration. According to Pantlitschko and Seelich, the glycolysis of Ehrlich ascites cells is completely inhibited by H202 at a concentration of $1.5.10^{-4}$N. This inhibition can be canceled by nicotinamide.

Rondoni has considered hydrogen peroxide as essential for the cancer formation; he assumes that several hydrophilic groups of the albumen molecules are destroyed by H202 which leads to a "physical aggregation" of those specific proteins which take part in the cell division. Haddow represents similar concepts. According to Covacic, H202 assumes generally a central position in carcinogenesis. According to his hypothesis, cancer is caused by all agents which lead directly or indirectly to the formation of H202 in abnormally high concentrations. The next step is supposed to be the formation of a matrix of simple structure with the participation of nucleic acid.

Van Duren and coworkers refer in a report on the carcinogenic effect of epoxides, lactones, and peroxy compounds to their importance as possible carcinogenic intermediates in the metabolism of aromatic hydrocarbons and as environmental carcinogens. Williams also suspects the formation of hydroperoxides in the intermediary metabolism of aromatic hydrocarbons. Their presence in cigaret smoke is

also considered likely. Their presence is also known in autoxidized fats and oils which play an important role in human nutrition. Carcinogenic activity has been proved for 6. β- hydroperoxi- Δ^4 -cholestene-3-one in mice and also for 1-hydroperoxi-1-vinylcyclohexene-3.

The above mentioned hypothetical discussions about the role of H202 in cancer which represent only a selection make the still missing experimental clarification of a possible carcinogenic activity of the substance most desirable.

Since it was already clear that H202 would be very fast decomposed after injection by the action of the catalase, we administered a sublethal dosis in the hope to achieve a higher H202 concentration than primarily formed after X-ray treatment, at least for a short time. In the case of a causal participation of H202 in the X-ray induced carcinogenesis, an increase in the tumor yield compared to the results in table 1 should be expected with higher H202 concentrations. In these H202 tests, we used exclusively AB mice which exhibit a far lower spontaneous tumor rate than C57Bl mice whose spontaneous tumor frequency - mainly leucemia and lymphosarcoma - is given as 10-20%. The tumor stress of the AB strain had previously been examined by us on more than 800 animals. We found however, that after changing to an especially protein-rich feed, the mammary cancer rate has since been considerably reduced and is sure to lie below 5%; among 1321 fully grown females of our breed, we could find at our last visit only 2 animals with breast cancer. The spontaneous leucemia frequency was recently about 2%. After injection of H202, it rose to 10% of all our test animals, the mammary cancer rate of the tested females, to 28%.

In separate evaluation of the tests with single or triple H202 injection, the percentage of tumor animals is increased from 20 to 33%, while the average latent period was shortened from 20 to 15 months.

We therefore consider the increased tumor yield in table 1 to be the result of the H202 injection.

It should be remembered in this context that the activity of the hydrogen peroxide is limited to moments because of the very fast decomposition by catalase.

We also want to mention the important fact that the observed tumors did not appear directly in the injection area, which should indicate an indirect mechanism. However, the comparatively large amount of oxygen after the decomposition of the peroxide by the catalase involves the whole subcutaneous tissue of the baby mouse; the mouse appears bloated a short time after the injection. The short reaction period of the nascent hydrogen peroxide which appears to exceed all other known carcinogens, confers upon H202 a certain special position among the by now approximately 400 chemical substances with carcinogenic effects. We think it probable that the oxygen in statu nascendi with its special reactivity leads to the formation of a toxic oxidation product which may be considered the actual active agent.

To obtain more information about this, we mixed hydrogen peroxide in vitro with rat serum which, compared to complete blood and tissue contains relatively little catalase and injected the serum after substantial destruction of the added H202 about 2½ hrs later also into newborn mice.

The results are shown in table 2

Unfortunately, the evaluation of this test is complicated by the different nature of the test animals. Here too, the breast cancer frequency is noticeable: Spontaneous mammary carcinomas appear only exceptionally in C57Bl mice. Altogether, however, the tumor yield is lower than after direct administration of H202. The dosage in this series was 0.1 ml in single application, where 0.6-1.2% Perhydrol was mixed 1:1 with rat serum.

It can be assumed that the tumor yield after H202 injection could be considerably increased if it were possible to delay the decomposition by catalase. We therefore performed a series of tests where hydrogen peroxide was injected after preliminary administration of 3-amino-1,2,4-triazole. Aminotriazole is

known as a blocking agent for catalase. The results of these tests will be reported at a later time.

Starting point for the tests of the second test series for radiation carcinogenesis was a publication by Mathe and Bernard. These two authors transferred the plasma of adult C57Bl/6 mice after total body irradiation with 600-750 r to 74 new born animals of the same strain. Among 11 surviving animals, 4 died, according to their reports, of leucemia, one mouse of reticular cell sarcoma, and 4 ofepithelial tumors. For the interpretation of this statistically significant increase in the tumor rate of this strain which has since been essentially confirmed on rats after irradiation with Co^{60} in somewhat different test arrangement, we think there are principally 3 possibilities:

1) The irradiation might cause substances with cardinogenic properties to form from low molecular weight innate compounds.

2) an activation of latent infections with tumor virus types (polyoma virus, leucemia virus) due to irradiation induced RES damage might be feasible,

3) the severe X-ray irradiation might, by the alteration of the nucleic acid metabolism, lead to the formation of an endogenous cancer induct with virus-like properties which enters the blood plasma from the body cells.

A decision on these 3 possibilities could only be made by experimental means. For this purpose - in addition to assurance about the initial results- the following tests were performed which are compiled in table 3.

Table 3 indicates that in none of the test series with irradiated animals as starting material was a convincing increase of the tumor rate achieved when compared with control animals that had been treated with serum from unirradiated mice. We mainly injected serum from irradiated animals after various pre-treatments, as the heparin addition necessary for the obtaining of plasma was badly tolerated by the babies. We consider it unlikely, however, that the lacking agreement of our results with those by Mathe and Bernard are due

to this fact. The majority of our test animals belonged also to the B57Bl/6 strain. In single tests, especially with the untreated serum of irradiated mice, we found an accumulation of tumor animals which, even though it cannot be statistically assured in the collective evaluation of all single series, demands reserve in considering the tests as completely negative. Because of insufficient unambiguity and the more or less negative total result, we omitted the publication of these tests which were started together with R. Huber in Berlin-Buch and date several years back. The results of our tests with hydrogen peroxide which have an inner connection with this posing of the problem made it worth while to publish this short tabulary introduction which also shows the distribution of the tumors within the single series with identical starting material for comparison and juxtapostion.

Headings for Table 1 on following page

number of animals

2. strain

3. Number of animals evaluated

4. Male

5. Female

6. still alive

7. findings

8. latent time

9. Dosage

Table 1 Tumor Findings after Injection of hydrogen Peroxide into newborn Mice

1	2	3		6	7	8	9
		4	5				
17	AB	1			-	18	1x0.1 ml
27	AB		2		-		"
55	AB				-		"
42	AB				-		"
40	AB				-		"
77	AB	6	9		1 leucemia	18	"
	C57Bl				1 breast cancer	24	"
	A/JAX						
26	X	3	6		1 leucemia + breast cancer	15	"
	C57Bl				1 lymphosarcoma of the thymus	20	"
					1 leucemia	21	"
					1 breast cancer	22	"
19	AB	2	1		-		"
303		12	18		6 tumor animals (20%)	20	
61	AB	3	5	2	1 breast cancer	14	3x0.1 ml
					1 breast cancer	17	"
					1 double breast cancer	17	"
					1 lung cancer	19	"
21	AB	2		1	-		"
29	AB		1		- [illegible]		"
29	AB	2	1		1 breast cancer	22	"
31	AB		7		1 lung adenoma	10	"
11	AB	1	4		1 hemangiosarcoma of the liver	15	"
					1 breast cancer	16	"
					2 breast cancer	22	"
					1 leucemia	17	"
52	AB	1			1 leucemia 1 leucemia	12	"
29	AB	2	1	1	1 ovary tumor both sides	16	"
21	AB	3	2		1 leucemia #lung adenoma	19	"
62	AB	7			-		"
346		21	21	4	14 tumor animals (approx.33%)	15	

Table 2

Tumor frequency after injecting newborn mice with normal rat serum pretreated with H_2O_2

number of animals prepared	strain	number of evaluated animals (minimum age 6 months)	findings	latent time
70	X,AB	15	1 breast cancer	21
	C57Bl		1 lung adenoma	14
			1 likely leucemia	17
30	C57Bl	5	1 breast cancer	14
			1 breast cancer	23
35	AB,C57Bl	10	1 lung adenoma	9
	X		1 breast cancer	19
			1 sarcoma in the lung	9
60	C57Bl	18	-	
	A/JAX, AB		18	
54	C57Bl,AB	10	1 leucemia	18
			1 breast cancer	15
			1 lymphosarcoma in abdominal cavity	16
249		67	10 tumor animals (15%)	16

Table 3

Test results after injection of serum, plasma, and organ filtrates from lethally irradiated animals into newborn mice in different test arrangements

1. number of animals prepared 2. strain 3. dead at minimum age of 6 months 4. findings 5. 1st dose 6. 2nd dose 2rd dose

1	2	3	4	5	6
normal mouse serum(controls)					
39	C57Bl,X	18	2 leucemias	0.1	0.2
67	C57Bl/6,X	5	1 leucemia	0.1	0.2
106		23	3 leucemias (apppox.13%)		
Plasma irradiated mice(+ heparin)					
57	C57Bl/6	-	dead within 24 hours	0.3	
14	C57Bl/6	6		0.03	0.01 0.1
25	C57Bl/6	-	dead within 24 hours	0.2	
47	C57Bl/6	-	"	0.1	
143		6	-		
Serum, irradiated mice (750 r)					
21	C57Bl/6	6	polymorphicell sarcoma in mesenterium	0.2	0.1
14	C57Bl/6,AB	2	1 leucemia	0.2	0.1
28	C57Bl,AB	5	1 lymphosarcoma of the thymus 1 lymphosarcoma of the mesenterial lymph nodes 1 leucemia + uterine adeno cancer	0.1	0.2
25	C57Bl,AB	6	lymphosarcoma of the thymus 1 leucemia 1 lung cancer	0.1	0.2
27	C57Bl,AB	6	-	0.1	0.2
38	C57Bl/6,AB C57Bl C57	11 13	- 1 leucemia after 3 months)	0.1 0.2	0.2 0.1
8	C57Bl/6	-	-		0.1
10	C57Bl/6	7	-	0.2	0.1
13	C57Bl/6	1	-	0.2	0.2 0.1
225		57	8 tumor animals (+) leucemia after 3 months) = 14%		
serum, irradiated mice, heated to 60°C					
50	C57Bl/6 C57Bl,X	15	1 leucemia	0.1	0.2
25	C57Bl/6	8	-	0.1	0.2
58	C57Bl/6,X	5	1 doubtful leucemia	0.1	0.2
37	C57Bl,X	20	1 leucemia 1 leyer cancer	0.1 0.1	0.2 0.2

1	2	3	4	5	6
170		48	3 tumor animals = 6%		
Serum, irradiated mice, heated to 100°C					
13	C57Bl/6	4	-	0.1	0.2
6	C57Bl/6	4	-	0.1	0.2
8	C57Bl/6	2	1 leucemia	0.1	0.2
27		10	1 tumor animal (approx.10%)		
Serum, irradiated mice after dialysis					
38	X,AB,C57Bl	9	1 leucemia	0.1	0.2
22	C57Bl/6,AB	7	-	0.1	0.2
38	X	8	1 round cell sarcoma in abdominal cavity	0.1	0.2
25	C57Bl,C57Bl/6,X	7	-	0.1	0.2
123		31	2 tumor animals		
34 spleen	filtrate, irradiated mice				
26	AB, X	6	1 leucemia suspicion	0.3	
53	C57Bl,C57Bl/6	11	1 leucemia	0.3	
	AB		1 breast cancer		
79		17	2 tumor animals		
34 liver filtrate, irradiated mice					
59	C57Bl/6	11	-	0.3	
98	C57Bl/6,AB C57Bl	12	-	0.3	
157		23	-		
Erythritocyte sediment, irradiated mice					
35	AB,C57Bl A/JAX	1	-	0.1	0.2
38	C57Bl,AB	2	-	0.05	0.1
44	C57Bl/6	16	2 leucemias	0.05	
28	C57Bl/6	-	-	0.1	
145		19	2 tumor animals		
Serum, irradiated mice (1000 r)					
38	C57Bl/6	15	-	0.1	0.2
32	C57Bl/6	8	2 questionable leucemias	0.1	0.2
33	C57Bl/6,X	4	-	0.1	0.2
					0.2
43	C57Bl/6	19	1 sarcoma in the liver 1 leucemia	0.1	0.2
61	C57Bl,X	11	4 leucemia(after 4 months)	0.1	0.2
					0.2
25	C57Bl,C57Bl/6	10	-	0.1	0.2
232		67	2 tumor animals (+1 leucemia after 3 months)		

(Translated by Carl Demrick Associates,Inc/CG/t)

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Aus der Forschungsstelle für Experimentelle Onkologie
der Deutschen Akademie der Wissenschaften zu Berlin in Potsdam-Rehbrücke
(Direktor: Dr. med. habil. F. SCHMIDT)

Versuche zur cancerogenen Wirkung von Wasserstoffperoxid und zum Mechanismus der Strahlencancerogenese

FERDINAND SCHMIDT

(Eingegangen am 25. 1. 1964)

Zusammenfassung

Es wird über eine Tumorausbeute von 28% nach Injektion von Wasserstoffperoxid bei 72 mindestens 6 Monate überlebenden Mäusen berichtet. Bei getrennter Auswertung der Serien, in denen die Säuglinge eine oder drei Injektionen an H_2O_2 erhielten, stieg die Tumorausbeute von 20 auf 33%; außerdem ergab sich dadurch eine Verkürzung der Latenzzeit von 20 auf 15 Monate.

Wasserstoffperoxid nimmt im Vergleich zu den etwa 400 bisher bekannten chemischen Stoffen mit cancerogener Wirksamkeit eine gewisse Sonderstellung nicht nur wegen seiner einfachen Struktur ein, sondern vor allem wegen der Kürze der Einwirkungsdauer der nativen Substanz, die diejenige aller anderen chemischen Cancerogene übertreffen dürfte.

Eine Erhöhung der Mamma-Carcinomhäufigkeit wurde auch nach Injektion von Rattennormalserum erzielt, das vorher in vitro mit Wasserstoffperoxid behandelt worden war.

Eine überzeugende Bestätigung der Befunde französischer Autoren (MATHÉ und BERNARD) über eine cancerogene Wirkung des Plasmas mit Letaldosen totalbestrahlter Mäuse gelang in veränderter Versuchsanordnung mit Serum an sehr viel größerer Tierzahl nicht.

Weitere Versuche zur Erhöhung der Turmorausbeute und zur Aufklärung des vermutlich indirekten Wirkungsmechanismus der Turmorentstehung nach H_2O_2-Injektion sind im Gange.

Wasserstoffperoxid entsteht u. a. bei der Röntgenbestrahlung von Wasser sowie von wasserhaltigen Geweben und Gewebsflüssigkeiten. Bei der Strahlenwirkung wird dem H_2O_2 von mehreren Autoren entscheidende Bedeutung zuerkannt. Überraschenderweise steht jedoch der Nachweis der cancerogenen Wirkung von Wasserstoffperoxid noch aus. Im folgenden soll deshalb über Versuche zu dieser Frage berichtet werden. Im Zusammenhang damit werden die Ergebnisse einer Reihe von Versuchen zum Mechanismus der Strahlencancerogenese mitgeteilt.

Versuchsmaterial und Methodik

Wasserstoffperoxid (Perhydrol/Merck) wurde bei neugeborenen Mäusesäuglingen, vorwiegend des AB-Stammes, nach Verdünnung auf 0,6% mit Ringerlösung in einer Dosis von 0,1 ml s.c. am Rücken einmal oder mehrmals injiziert.

In weiteren Ansätzen mischten wir Rattennormalserum mit Perhydrol. Nach Zersetzung des H_2O_2 durch die Serumkatalase (Aufhören der Schaum- und Bläschenbildung nach etwa 2½ Std.) wurde das Serum in gleicher Weise s. c. appliziert.

Im zweiten Teil der Versuche wurden erwachsene Mäuse mit 750 r (250 KV, 0,5 mm Cu) totalbestrahlt. Plasma (Heparinzusatz), Serum, Erythrozytensediment und G4-Filtrat von Leber und Milz der bestrahlten Tiere wurde in der Regel 8 Std. später gewonnen und in einer Dosis von 0,1 – 0,4 ml bei neugeborenen Mäusen der Stämme C57Bl/6, C57Bl, AB, X und A/JAX ein- bis dreimal s. c. injiziert. Die Technik der Filtratherstellung aus Organen war die gleiche wie in unseren früheren Versuchen zur zellfreien Tumorübertragung (z. B. [1]). In einer Versuchsreihe wurde auch das Serum erwachsener Ratten nach Totalbestrahlung mit 1000 r bei neugeborenen Mäusen injiziert. In weiteren Ansätzen wurde das Serum bestrahlter Mäuse vor der Injektion auf 60 °C (30 min) erhitzt bzw. kurz aufgekocht oder zur Entfernung niedermolekularer Stoffe etwa 16 Std. dialysiert. Bei mehrmaliger Injektion warteten wir in der Regel nach der 1. Injektion 2 – 3 Tage, bis sich die Säuglinge etwas erholt hatten. Alle Tiere wurden bis zum spontanen Ableben verfolgt, anschließend seziert und alle tumorverdächtigen Befunde histologisch untersucht.

Ergebnisse und Diskussion

Die Ergebnisse der ersten Versuchsreihe mit Wasserstoffperoxid zeigt Tab. 1.

Durch Röntgenbestrahlung entstehendes Wasserstoffperoxid als essentielle Komponente der Strahlenwirkung wird von zahlreichen Autoren diskutiert: EBERT [2] führt die Veränderungen der Sensibilität von Geweben gegenüber Röntgenstrahlen auf Unterschiede im vorhandenen Sauerstoff zurück, die ihrerseits von der Menge des durch die Bestrahlung gebildeten H_2O_2 abhängen. Auch von WARBURG und Mitarbeitern [3], [4] wird dem Wasserstoffperoxid bei der Schädigung von Krebszellen durch Röntgenstrahlen eine entscheidende Bedeutung beigemessen und ihre besondere Empfindlichkeit auf den niedrigen Katalasegehalt zurückgeführt. HOLZER und FRANK [5] setzten zu Asciteszellen jene H_2O_2-Konzentrationen zu, die nach WARBURG bei glykolysehemmenden Röntgendosen entstehen. Sie sahen ein Absinken der Glykolyse und der NAD-Konzentration. Auch nach PANTLITSCHKO und SEELICH [6] wird die Glykolyse von EHRLICH-Asciteszellen durch H_2O_2 in einer Konzentration von $1{,}5 \cdot 10^{-4}$ n vollständig gehemmt. Die Hemmung kann durch Nikotinsäureamid aufgehoben werden.

Im Hinblick auf die Krebsentstehung hält RONDONI [7], [8] seit langem das Wasserstoffperoxid für wesentlich; er nimmt an, daß durch H_2O_2 einige hydrophile Gruppen der Eiweißmoleküle zerstört werden, die zu einer „physikalischen Aggregation" speziell der Proteine führen, die an der Zellteilung mitwirken. Ähnliche Auffassungen vertritt HADDOW [9]. Nach KOVACIC [10] nimmt H_2O_2 generell bei der Cancerogenese eine zentrale Stellung ein. Nach seiner Hypothese entsteht Krebs durch alle Agentien,

Tabelle 1. Tumorbefunde nach Injektion von Wasserstoffsuperoxid bei neugeborenen Mäusesäuglingen

angesetzte Tierzahl	Stamm	auswertbare Tierzahl (Mindestalter 6 Monate) ♂	♀	noch am Leben	Befunde	Latenzzeit (Monate)	Dosis
17	AB	1			—		1 × 0,1 ml
27	AB		2		—		"
55	AB				—		"
42	AB				—		"
40	AB				—		"
77	AB	6	9		1 Leukämie	18	"
	C57Bl				1 Mamma-Ca	24	"
	A/JAX						
26	X	3	6		1 Leukämie + Mamma-Ca	15	"
	C57Bl				1 Lymphosarkom des Thymus	20	"
					1 Leukämie	21	"
					1 Mamma-Ca	22	"
19	AB	2	1		—		"
303		12	18		6 Tumortiere (= 20%)	20	
61	AB	3	5	2	1 Mamma-Ca	14	3 × 0,1 ml
					1 Mamma-Ca	17	"
					1 zweifaches Mamma-Ca	17	"
					1 Lungen-Ca	19	"
21	AB	2		1	—		"
29	AB		1		—		"
29	AB	2	1		1 Mamma-Ca	22	"
31	AB		7		1 Lungenadenom	10	"
11	AB	1	4		1 Hämangiosarkom der Leber	15	"
					1 Mamma-Ca	16	"
					2 Mamma-Ca	22	"
					1 Leukämie	17	"
52	AB	1			1 Leukämie	12	"
29	AB	2	1	1	1 beiderseits Ovarialtumor	16	"
21	AB	3	2		1 Leukämie + Lungenadenom	19	"
62	AB	7			—		"
346		21	21	4	14 Tumortiere (~ 33%)	15	

die direkt oder indirekt zur Bildung von H_2O_2 in abnorm hoher Konzentration führen. Als nächste Stufe wird unter Beteiligung von Nukleinsäure die Bildung einer Matrize von einfacher gebauter Struktur betrachtet.

VAN DUUREN und Mitarbeiter [11] weisen in einer Untersuchung über die cancerogene Wirksamkeit von Epoxiden, Laktonen und Peroxyverbindungen auf deren Bedeutung als mögliche cancerogene Zwischenprodukte im Stoffwechsel aromatischer Kohlenwasserstoffe und als Milieu-Cancerogene hin. Die Entstehung von Hydroperoxiden im Intermediärstoffwechsel von aromatischen Kohlenwasserstoffen vermutet auch WILLIAMS [12]. Ihre Anwesenheit im Zigarettenrauch wird ebenfalls für wahrscheinlich gehalten [13]. Ihr Vorhandensein ist ferner bekannt in autoxidierten Fetten und Ölen, die in der menschlichen Ernährung eine wichtige Rolle spielen [14], [15]. Cancerogene Wirksamkeit ist für 6,β-Hydroperoxy-Δ^4-cholesten-3-on bei Mäusen nachgewiesen [16], ebenso für 1-Hydroperoxy-1-vinylcyclohexen-3 [11].

Die angeführten hypothetischen Erörterungen zur Rolle des H_2O_2 beim Krebs, die lediglich eine Auswahl darstellen, lassen die noch ausstehende experimentelle Klärung einer etwaigen cancerogenen Aktivität der Substanz (s. [17], [18]) als dringend wünschenswert erscheinen.

Da von vornherein klar war, daß das H_2O_2 durch die Katalase nach der Injektion sehr schnell zersetzt werden würde, applizierten wir eine subletale Dosis in der Hoffnung, dadurch wenigstens für kurze Zeit eine höhere Konzentration an H_2O_2 zu erreichen als sie nach Röntgenbestrahlung primär entstehen dürfte. Im Falle einer ursächlichen Beteiligung des H_2O_2 an der strahleninduzierten Cancerogenese war bei höherer H_2O_2-Konzentration eine Erhöhung der Tumorausbeute im Vergleich zu den in Tabelle 1 zusammengestellten Ansätzen zu erwarten. Wir verwendeten zu den H_2O_2-Versuchen fast ausschließlich AB-Mäuse, die eine wesentlich niedrigere Spontantumorrate aufweisen als C57Bl-Mäuse, deren Spontantumorhäufigkeit — die vor allem Leukämien und Lymphosarkome betrifft — mit 10—20% angegeben wird [19]. Die Tumorbelastung des AB-Stammes wurde von uns früher an mehr als 800 Zuchttieren untersucht [20]. Wir konnten jedoch die Feststellung machen, daß durch die erfolgte Umstellung auf ein besonders eiweißreiches Futter (Nährkonzentrat der VEB Küssnerwerke Berlin-Weißensee) zumindest die Mamma-Ca-Rate seither wesentlich zurückgegangen ist und mit Sicherheit weniger als 5% beträgt; unter 1321 voll erwachsenen AB-Weibchen unserer Zucht konnten wir bei der letzten Visite nur 2 Tiere mit Mamma-Ca finden. Die Spontanleukämiehäufigkeit lag seinerzeit bei etwa 2%. Nach Injektion von H_2O_2 stieg sie auf 10% aller Versuchstiere, die Mamma-Ca-Rate der Versuchsweibchen auf 28%.

Bei getrennter Auswertung der Ansätze mit einmaliger bzw. dreimaliger H_2O_2-Injektion erhöhte sich der Prozentsatz der Tumortiere von 20 auf 33%, während sich die durchschnittliche Latenzzeit von 20 auf 15 Monate verkürzte.

Wir betrachten demnach die in Tab. 1 zusammengestellte erhöhte Tumo ausbeute als Effekt der H_2O_2-Injektion.

Es ist bei dieser Tumorausbeute zu bedenken, daß die Einwirkungsmöglic keit des Wasserstoffperoxids infolge der sehr raschen Zersetzung dur Katalase sich fast nur auf Augenblicke beschränkt.

Wichtig erscheint uns ferner die Feststellung, daß die beobachteten G schwülste nicht unmittelbar im Bereich der Injektionsstelle auftraten, w auf einen indirekten Wirkungsmechanismus hindeuten dürfte; allerdin wird durch die vergleichsweise große Sauerstoffmenge nach Zersetzung d Peroxids durch Katalase das gesamte Subkutangewebe des Säuglings Mitleidenschaft gezogen; der Säugling erscheint kurz nach der Injektion w aufgeblasen. Die kurze Einwirkungszeit des nativen Wasserstoffperoxid die vermutlich diejenige aller bisher bekannten Cancerogene übertrifft, gi dem H_2O_2 eine gewisse Sonderstellung unter den inzwischen annähern 400 bisher bekannten chemischen Stoffen mit krebserzeugender Wirksam keit. Wir halten es für möglich, daß der entstehende Sauerstoff in sta nascendi infolge seiner besonderen Reaktionsfreudigkeit zur Bildung ein toxischen Oxydationsprodukts führt, das als eigentlich wirksames Agens betrachten ist.

Um darüber näheren Aufschluß zu erhalten, mischten wir Wasserstof peroxid in vitro mit Rattenserum, das im Vergleich zum Vollblut u Gewebe nur relativ wenig Katalase enthält und injizierten das Serum na weitgehender Zerstörung des zugesetzten H_2O_2 etwa 2½ Stunden spät ebenfalls bei neugeborenen Mäusesäuglingen.

Die Ergebnisse zeigt Tab. 2.

Leider ist die Auswertung dieses Versuchs infolge der Verschiedenartigke der verwendeten Versuchstiere erschwert. Auffällig ist jedoch auch hier d Mamma-Ca-Häufigkeit; spontane Mamma-Carcinome treten bei C57B Mäusen nur in Ausnahmefällen auf. Insgesamt ist jedoch die Tumorau beute niedriger als nach direkter Applikation von H_2O_2. Die Dosis betr in diesen Serien bei einmaliger Applikation 0,1 ml, wobei 0,6—1,2%ig Perhydrol 1:1 mit Rattenserum gemischt wurde.

Es ist anzunehmen, daß die Turmorausbeute nach Injektion von Wasse stoffperoxid noch beträchtlich gesteigert werden könnte, wenn es gelän die Zersetzung durch Katalase zu verzögern. Wir haben deshalb eine Rei von Versuchen angesetzt, in denen Wasserstoffsuperoxid erst nach vo heriger Applikation von 3-Amino-1,2,4-triazol injiziert wurde. Aminotri zol ist als Katalaseblocker bekannt. Über die Ergebnisse dieser Versuc wird später berichtet werden.

Ausgangspunkt der Versuche der zweiten Versuchsserie zur Strahlenca cerogenese war eine Publikation von Mathé und Bernard [21]. Diese beid Autoren übertrugen das Plasma erwachsener C57Bl/6-Mäusen nach Ga körperbestrahlung mit 600—750 r auf 74 neugeborene Tiere des gleiche

Tabelle 2

Tumorhäufigkeit nach Injektion von mit H_2O_2 vorbehandeltem Rattennormalserum bei neugeborenen Mäusesäuglingen

angesetzte Tierzahl	Stamm	auswertbare Tierzahl (Mindestalter 6 Monate)	Befunde	Latenzzeit (Monate)
70	X, AB C57Bl	15	1 Mamma-Ca 1 Lungenadenom 1 wahrscheinliche Leukämie	21 14 17
30	C57Bl	5	1 Mamma-Ca 1 Mamma-Ca	14 23
35	AB, C57Bl X	19	1 Lungenadenom 1 Mamma-Ca 1 Sarkom in der Lunge	9 19 9
60	C57Bl A/JAX, AB	18	—	
54	C57Bl, AB	10	1 Leukämie 1 Mamma-Ca 1 Lymphosarkom im Bauchraum	18 15 16
249		67	10 Tumortiere (15%)	16

Stammes. Unter 11 überlebenden Tieren starben nach ihren Angaben 4 mit Leukämie, eine Maus mit Retikulumzellsarkom und 4 mit epithelialen Tumoren. Zur Interpretation dieser statistisch signifikanten Erhöhung der Tumorrate des verwendeten Stammes, die inzwischen im Prinzip an Ratten nach Bestrahlung mit ^{60}Co in etwas abweichender Versuchsanordnung von anderer Seite [22] bestätigt wurde, kamen nach unserer Ansicht in erster Linie 3 Möglichkeiten in Frage:

1. Durch die Bestrahlung könnten aus niedermolekularen körpereigenen Stoffen Substanzen mit cancerogener Wirkung entstehen, wobei in erster Linie an Peroxide zu denken war,

2. durch die strahlenbedingte Schädigung des RES wäre eine Aktivierung latenter Infektionen mit Tumorvirusarten (Polyoma-Virus, Leukämievirusarten) denkbar,

3. die starke Röntgenbestrahlung könnte durch Veränderung des Nukleinsäurestoffwechsels zur Bildung eines endogenen Krebsinduktes mit virusähnlichen Eigenschaften [23], [24] führen, der aus den Körperzellen in das Blutplasma übertritt.

Die Entscheidung über diese 3 Möglichkeiten war nur experimentell zu treffen. Diesem Zweck — neben der Sicherung der Ausgangsbefunde — sollten die folgenden Versuche dienen, die in Tab. 3 zusammengestellt sind.

Tabelle 3

Versuchsergebnisse nach Injektion von Serum, Plasma und Organfiltraten letalbestrahlter Tiere in verschiedener Versuchsanordnung bei neugeborenen Mäusen

angesetzte Tierzahl	Stamm	im Mindestalter von 6 Monaten tot	Befunde	1. Dosis	2. Dosis 3. Dosis
Mäusenormalserum (Kontrolle)					
39	C57Bl, X	18	2 Leukämien	0,1	0,2
67	C57Bl/6, X	5	1 Leukämie	0,1	0,2
106		23	3 Leukämien (~ 13%)		
Plasma bestrahlter Mäuse (+ Heparin)					
57	C57Bl/6	—	innerhalb von 24 Stunden tot	0,3	
14	C57Bl/6	6		0,03	0,01 0,1
25	C57Bl/6	—	innerhalb von 24 Stunden tot	0,2	
47	C57Bl/6	-	innerhalb von 24 Stunden tot	0,1	
143		6	—		
Serum bestrahlter Mäuse (750 r)					
21	C57Bl/6	6	polymorphzelliges Sarkom im Mesenterium	0,2	0,1
14	C57Bl/6, AB	2	1 Leukämie		0,1
28	C57Bl, AB	5	1 Lymphosarkom des Thymus 1 Lymphosarkom der mesenterialen Lymphknoten	0,2 0,1	0,1 0,2
25	C57Bl, AB	6	1 Leukämie + Adeno-Ca des Uterus 1 Lymphosarkom des Thymus 1 Leukämie 1 Lungen-Ca	0,1	0,2
27	C57Bl, AB	6	—	0,1	0,2
38	C57Bl/6, AB C57Bl	11	—	0,1	0,2

41	C57Bl AB	13	1 Leukämie (nach 3 Monaten)	0,2	0,1
8	C57Bl/6	7	—	0,2	0,1
10	C57Bl/6	7	—	0,2	0,2
13	C57Bl/6	1	—	0,2	0,1
225		57	8 Tumortiere (+ 1 Leukämie nach 3 Monaten) = 14%		
Serum bestrahlter Mäuse, erwärmt auf 60 °C					
50	C57Bl/6, C57Bl, X	15	1 Leukämie	0,1	0,2
25	C57Bl/6	8	—	0,1	0,2
58	C57Bl/6, X	5	1 fragliche Leukämie	0,1	0,2
37	C57Bl, X	20	1 Leukämie 1 Leyer-Ca	0,1	0,2 0,2
170		48	3 Tumortiere = 6%		
Serum bestrahlter Mäuse, erhitzt auf 100 °C					
13	C57Bl/6	4	—	0,1	0,2
6	C57Bl/6	4	—	0,1	0,2
8	C57Bl/6	2	1 Leukämie	0,1	0,2
27		10	1 Tumortier (~ 10%)		
Serum bestrahlter Mäuse nach Dialyse					
38	X, AB, C57Bl	9	1 Leukämie	0,1	0,2
22	C57Bl/6, AB	7	—	0,1	0,2
38	X	8	1 Rundzellensarkom im Bauchraum	0,1	0,2
25	C57Bl, C57Bl/6, X	7	—	0,1	0,2
123		31	2 Tumortiere		
G4-Milzfiltrat bestrahlter Mäuse					
26	AB, X	6	1 Leukämieverdacht	0,3	
53	C57Bl, C57Bl/6, AB	11	1 Leukämie 1 Mamma-Ca	0,3	
79		17	2 Tumortiere		

Tabelle 3 (Fortsetzung)

angesetzte Tierzahl	Stamm	im Mindestalter von 6 Monaten tot	Befunde	1. Dosis	2. Dosis 3. Dosis
G4-Leberfiltrat bestrahlter Mäuse					
59	C57Bl/6	11	—	0,3	
98	C57Bl/6, AB, C57Bl	12	—	0,3	
157		23	—		
Erythrozytensediment bestrahlter Mäuse					
35	AB, C57Bl, A/JAX	1	—	0,1	0,2
38	C57Bl, AB	2	—	0,05	0,1
44	C57Bl/6	16	2 Leukämien	0,05	
28	C57Bl/6	—	—	0,1	
145		19	2 Tumortiere		
Serum bestrahlter Ratten (1000 r)					
38	C57Bl/6	15	—	0,1	0,2
32	C57Bl/6	8	2 fragliche Leukämien	0,1	0,2
33	C57Bl/6, X	4	—	0,1	0,2
43	C57Bl/6	19	1 Sarkom in der Leber	0,1	0,2
61	C57Bl, X	11	1 Leukämie 4 Leukämien (nach 3 Monaten)	0,1	0,2
25	C57Bl, C57Bl/6	10	—	0,1	0,2
232		67	2 Tumortiere (+ 1 Leukämie nach 3 Monaten)		

Tab. 3 geht hervor, daß mit keiner der getesteten Versuchsanordnungen Verwendung von Ausgangsmaterial bestrahlter Tiere eine überzeugende Erhöhung der Tumorrate erzielt wurde, wenn man die Kontrolltiere mit Serum unbestrahlter Mäuse als Vergleich heranzieht. Wir injizierten hauptsächlich Serum bestrahlter Tiere nach verschiedenartiger Vorbehandlung, wobei der zur Plasmagewinnung erforderliche Heparinzusatz von den Säugetieren schlecht vertragen wurde (s. Tab. 3). Wir halten es jedoch für unwahrscheinlich, daß die fehlende Übereinstimmung der Resultate unserer Versuche mit den Ergebnissen von MATHÉ und BERNARD [21] darauf zurückzuführen ist. Der größte Teil unserer Versuchstiere gehörte ebenfalls dem Stamm C57Bl/6 an. In einzelnen Ansätzen, speziell mit dem unbehandelten Serum bestrahlter Mäuse, war eine starke Häufung von Tumortieren festzustellen, die sich zwar bei zusammenfassender Auswertung aller Einzelserien nicht statistisch sichern läßt, aber doch zur Zurückhaltung mahnt, die Versuche als völlig negativ zu betrachten. Wegen dieser unzureichenden Eindeutigkeit und des mehr oder weniger negativen Gesamtergebnisses unterblieb auch die Veröffentlichung dieser gemeinsam mit R. HUBER[1] in Berlin-Buch begonnenen, schon mehrere Jahre zurückliegenden Versuche. Die Resultate unserer Versuche mit Wasserstoffperoxid, die mit dieser Problemstellung in innerem Zusammenhang stehen, ließen indessen eine kurze tabellarische Anführung, aus der auch die Verteilung der Tumoren innerhalb der Einzelserien mit gleichem Ausgangsmaterial ersichtlich ist, zur vergleichenden Gegenüberstellung ratsam erscheinen.

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Summary

F. SCHMIDT: Experiments on the carcinogenic effect of hydrogen peroxide and the mechanism of ray cancerogenesis

A 28 per cent tumour yield after hydrogen peroxide injection on 72 mice at a minimu survival of 6 months is reported. Separate evaluation of experiments, in which t animals received one or three H_2O_2 injections, gave an increase in tumour yield fro 20 to 33 per cent; besides, the latent period was reduced from 20 to 15 months. In co parison to the approximately 400 hitherto known chemical substances with carcin genic effects hydrogen peroxide occupies a certain extra position, not only for i simple structure, but above all for the short duration of action of the native su stance, which certainly surpasses that of auy other chemical cancerogen.

An certain increase in breast cancer frequency has also been achieved after injectio of rat normal serum, which was previously treated with hydrogen peroxide in vitro.

The findings of french authors relative the carcinogenic effect of the plasma with let doses of totally irradiated mice in modified experimental arrangement with serum on a much higher number of animals could not be confirmed convincingly.

Further experiments to increased tumour yield and to examine the presumably i direct mechanism of tumour formation after H_2O_2 injection are in progress.

Резюме

Ф. Шмидт: Опыты по канцерогенному действию перекиси водорода и механизму лучевого канцерогенеза

Авторы сообщают о 28%ном выходе опухоли после инъекции перекиси водо рода у 72 мышей переживущих на не менее 6 месяцев. При раздельной оцени серий опытов, в которых животные получили одну или три инъекции H_2O выход опухоли оказался повышенным от 20 до 33%, и кроме того наблюдалось сокращение скрытого периода от 20 до 15 месяцев. Среди приблизительн 400 известных до сих пор химических веществ с канцерогенным действие перекись водорода занимает особое место не только из-за ее простой структуры а прежде всего из-за краткости времени воздействия нативного вещества которое, должно быть, превосходит соответствующие величины всех других химических канцерогенов.

ыло достигнуто повышение частоты рака молочной железы также после ъекции нормальной сыворотки крыс, предварительно обработанной пере-сью водорода in vitro.

блюдения французских авторов о канцерогенном действии плазмы с леталь-ми дозами полностью облученных мышей при модифицированном порядке ытов с сывороткой, со значительно большим количеством животных убеди-льно не подтверждались.

роводят дальнейшие опыты по повышению выхода опухоли и для выяснения редположительно косвенного механизма образования опухоли после инъек-и H_2O_2.

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The induction of chromosomal aberrations by hydrogen peroxide in strains of ascites tumors in mice

The question of mutagenic activity of hydrogen peroxide has frequently been discussed, especially in connection with radiation-induced mutagenesis[1-3,7,11,12]. The experiments that have been done up to now in order to test the compound as to its capability of inducing chromosomal aberrations have given no conclusive evidence for such an activity (summaries in MICHAELIS AND RIEGER[4], SOBELS[7]).

In contrast the application of H_2O_2 to strains of ascites tumor in mice resulted in the induction of chromosomal structural changes.

The experimental material consisted of 3 hyperdiploid ascites tumor strains: the S_2 sarcoma, the Ehrlich ascites carcinoma and the sarcoma 180 which were grown in the inbred strain AB Jena Gat. Inoculum size was 0.2 ml in all experiments. H_2O_2 was injected intraperitoneally 48 h after implantation of the tumor. Cytological preparation (technic according to MICHAELIS *et al.*[5]) of the treated cells was carried out at various times thereafter. Metaphases were used for the scoring of chromosomal structural changes. Less than 1% of the cells of untreated tumors contained spontaneous chromatid aberrations.

Various concentrations of H_2O_2 (injected amount in all cases 1 ml) were injected in the first experimental series, and the tumors were checked 48 h thereafter. The results, summarized in Table I, show that H_2O_2 is able to induce chromosomal aberrations in the test material. The aberrations were exclusively on chromatid basis (chromatid aberrations). The radiomimetic effectivity was positively correlated with the concentration of H_2O_2 injected.

The frequency of aberrations induced by one and the same concentration of H_2O_2 varied considerably from animal to animal. This is in contrast to similar experiments with alkylating agents (SCHÖNEICH[6]). Independent of the size of the inoculum the tumors varied from passage to passage and from animal to animal with respect to the number of red blood cells. This variation in the number of red cells may give rise to different concentrations of catalase in the ascites fluid and is suspected to be responsible for the pronounced variation in the frequency of chromatid aberrations induced by H_2O_2. As compared with normal cells the catalase concentration in ascites tumor cells is extremely low (WARBURG[8-10]). Scoring of red blood cells, with the aim of finding correlations between their number and the aberration frequencies, is now being done in order to prove or disprove this working hypothesis.

In a second series of experiments 1 ml of 10^{-1} *M* H_2O_2 was injected, and the cells were fixed at different intervals after treatment in order to follow the distribution of aberrations in time and to find the aberration maximum (Table II). During the first hours after treatment a pronounced mitotic depression was observed which disappeared after 20–24 h. Scoring of aberrations in cells of fixations made earlier than 19 h after injection of the compound proved difficult since the number of cells in mitosis was extremely low. 10–12 h after treatment the first cells with aberrations were found. The number of cells with aberrations increased up to 24 h after treatment, and this percentage was found to be practically constant for all fixations up to 72 h. With later fixations the percentage of aberrant metaphases decreased.

TABLE I

INDUCTION BY H_2O_2 OF CHROMATID ABERRATIONS IN THE S_2 SARCOMA, EHRLICH ASCITES CARCINOMA AND SARCOMA 180, AND ITS DEPENDENCE ON THE H_2O_2 CONCENTRATION APPLIED

Cells with all types of chromatid translocations, duplication deletions and (in favorable circumstances) deletions were scored. Cells with "gaps" only were excluded from scoring.

	Concentration (M)	*Number of animals*	*Number of cells scored*	*Metaphases with aberrations*	
				Number of metaphases	%
S_2 sarcoma	10^{-2}	4	600	72	12.0 ± 8.0[a]
	$5 \cdot 10^{-2}$	4	600	158	26.3 ± 7.2
	10^{-1}	4	600	179	29.8 ± 7.7
	$5 \cdot 10^{-1}$	3	450	201	44.7 ± 6.3
	Control	3	450	0	0
Ehrlich ascites carcinoma	10^{-2}	2	300	59	19.6 ± 4.8
	$5 \cdot 10^{-2}$	3	450	149	33.1 ± 11.3
	10^{-1}	3	450	119	26.4 ± 9.5
	$5 \cdot 10^{-1}$	1	100	38	38.0 ± 2.8
	Control	5	1000	2	0.2
Sarcoma 180	10^{-2}	2	300	13	4.3 ± 2.6
	$5 \cdot 10^{-2}$	2	300	48	16.0 ± 2.8
	10^{-1}	2	300	87	29.0 ± 5.2
	$5 \cdot 10^{-1}$	2	no mitoses		
	Control	2	300	0	0

[a] Standard error of the mean.

Fig. 1. Metaphase chromosomes of an untreated S_2 sarcoma cell (2000 ×).

Fig. 2. Metaphase chromosomes of an S_2 sarcoma cell treated with H_2O_2 and fixed 48 h after treatment. Numerous chromatid aberrations of various types (2000 ×).

TABLE II

THE RATE OF INDUCED CHROMATID ABERRATIONS AT VARIOUS TIMES AFTER INJECTION OF 1 ml OF 10^{-1} *M* H_2O_2

Interval between treatment and fixation (h)	*Number of animals*	*Number of cells scored*	*Metaphases with aberrations*	
			Number of metaphases	%
8	2	300	0	0
10	2	300	4	1.3 ± 1.06
12	2	300	6	2.0 ± 1.8
14	2	300	16	5.3 ± 2.6
16	1	150	25	16.6
18	2	300	49	16.3 ± 7.4
20	3	500	104	20.8 ± 12.9
22	2	300	49	16.3 ± 4.3
24	2	200	52	26.0 ± 3.6
26	2	300	83	27.6 ± 2.1
28	2	300	82	27.3 ± 3.4
30	2	300	85	28.3 ± 2.5
36	2	300	78	26.0 ± 2.5
48	4	600	179	29.8 ± 7.7
54	2	350	92	26.3 ± 4.9
72	2	300	105	35.0 ± 8.1
96	2	350	61	17.4 ± 2.6
Control (144 h after tumor inoculation)	1	300	1	0.6

The number of aberrations per damaged cell was low in the fixations up to 24 h. Damaged cells contained 1–2 aberrations. In later fixations the number of aberrations per damaged cell increased, and in fixations 48 h after treatment a high number of chromosomes was found to be engaged in aberrations (Fig. 2).

These results offer unequivocal evidence for the capacity of H_2O_2 to induce chromatid aberrations in the tumors tested. Whether this effect is due to the physiological pecularities of the tumor cells or represents a general phenomenon characteristic of all animal cells is the object of further experiments.

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Induction of Dental Caries and Pathological Changes in Periodontium of Rat with Hydrogen Peroxide and Other Oxidizing Agents

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In preliminary studies in this laboratory it was observed that immature rats fed a stock ration and administered hydrogen peroxide in the drinking water as a 1 or 1.5 per cent solution developed extensive carious lesions and pathological changes in the periodontium. These effects were noted as early as the third week of peroxide administration and were well advanced after an 8-week period. They did not occur, however, in rats fed a similar diet with hydrogen peroxide omitted from the drinking water. The present series of experiments was undertaken to confirm and extend these earlier observations.

MATERIALS AND METHODS

Experiment I. Effects of graded levels of hydrogen peroxide in the drinking water on the teeth and periodontium of rats.—Ninety-six male rats of the Holtzman strain were selected at an average body weight of 51.8 gm. (range 50–55 gm.) and were divided into four comparable groups of 24 animals each. The four groups were provided a stock ration* and drinking fluid ad libitum but differed as follows: group I received distilled water as the source of drinking fluid, whereas groups II, III, and IV were provided with distilled water to which H_2O_2 had been added in amounts sufficient to make a ½, 1, and 1.5 per cent solution, respectively.† The rats were placed in metal cages with raised screen bottoms (2 animals per cage) and were weighed weekly during the course of the experiment. Surviving animals were sacrificed after 8 weeks. Their heads and submaxillary glands were removed and fixed immediately in an 80 per cent alcoholic Bouin's (picric-acetic-formalin) solution. The heads were dacalcified in 10 per cent nitric acid in 10 per cent formalin and washed with a saturated lithium carbonate solution. They were then dehydrated and infiltrated in the routine manner, imbedded in 50 per cent nitrocellulose, sectioned at 20 μ thickness, and stained with hematoxylin and eosin, Mallorys' anilin blue collagen stain, and periodic acid. The salivary glands were imbedded in paraffin, sectioned at 7 μ, and stained with sematoxylin and eosin.

Experiment II. Comparative effects of inanition and hydrogen peroxide administered in the drinking water on the teeth and periodontium of rats.—Fifty male rats of the

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* Wayne Lab Blox, Allied Mills, Inc., Chicago, Illinois.

† The pH of these solutions was 6.55, 6.42, and 6.30, respectively.

Holtzman strain were selected at an average body weight of 42.6 gm. (range 39–46 gm.) and were divided into five comparable groups of 10 animals each. All rats were fed a diet similar in composition to that employed in Experiment I. However, whereas the diet in Experiment I was in the form of hard pellets or blocks approximately $\frac{5}{8}$ inch wide, 1 inch long, and $\frac{3}{8}$ inch high, that administered in the present experiment was provided as a finely ground powder. Group A received this diet ad libitum; groups B and C were fed the diet at levels of 7.5 and 6 gm. per day, respectively. All three of the above groups were administered distilled water ad libitum. Groups D and E were fed ad libitum but were given hydrogen peroxide in the drinking water as a 1 and 1.5 per cent solution, respectively. Animals were placed in individual metal cages and were weighed weekly during the course of the experiment. Surviving animals were sacrificed after 8 weeks, and histologic sections were prepared of the teeth, periodontal tissues, and submaxillary glands, as indicated above.

Experiment III. Effects of dietary supplements of zinc peroxide, potassium permanganate, and sodium perborate on the teeth and periodontium of rats.—Subsequent to the experiments indicated above, preliminary studies were conducted on the effects of oxidizing agents other than hydrogen peroxide on the induction of dental caries and periodontal disease in the rat. Twenty-four male rats of the Holtzman strain were selected at an average body weight of 43.2 gm. (range 40–45 gm.) and divided into four comparable groups of 6 animals each. One group (group a) was fed the powdered diet employed in Experiment II; the others (groups b, c, and d) were fed the powdered diet supplementd with 2 per cent zinc peroxide, 2 per cent potassium permanganate, and 2 per cent sodium perborate, respectively. Distilled water was provided ad libitum to all groups. The experimental procedure was similar to that employed in the previous experiments. After 8 weeks of ad libitum feeding, animals were sacrificed, and histologic sections were prepared of the teeth and periodontal tissues.

RESULTS

Experiment I.—Growth was significantly retarded in all rats which received hydrogen peroxide in their drinking water, the degree of retardation being proportional to the peroxide concentration. At the time of sacrifice the average body weight of rats in the various groups was as follows: group I, 331.6 ± 6.9 gm.;* group II, 222.9 ± 15.0 gm.; group III, 79.6 ± 4.8 gm.; and group IV, 59.6 ± 7.3 gm. Seven of the rats in group IV died during the course of the experiment; the remaining animals in this group and all those in the other groups survived. In agreement with preliminary findings, hydrogen peroxide when incorporated in the drinking water as a 1 or 1.5 per cent solution resulted in extensive carious lesions and pathological changes in the periodontium after an 8-week period of administration. All surviving animals in group IV and 15 of the 24 rats in group III showed such changes. Seven out of 24 rats in group II also had caries, but the latter were mild. No periodontal damage was observed in this group. The control rats in group I exhibited neither carious nor periodontal damage (Figs. 1, 2 and 7).

The carious lesions started at the tip of the cusp and extended through the dentin to the pulp (Figs. 3, 4). In the more severely affected animals, complete destruction of the

* Including standard error of the mean.

FIG. 1.—Section of jaws, control animal (group I). Mallory's stain. 20μ, ×10

FIG. 2.—Section of molar distal root area of control animal (group I), showing dentin, pulp, periodontal ligament, and alveolar process. Note resorption area on root surface. Stain hematoxylin

FIG. 3.—Section of jaws of experimental animal receiving 1.5 per cent hydrogen peroxide in drinking water (group IV). Note caries in lower first molar and tips of cusps of upper molars. Note extent of pulp necrosis. Mallory's stain. 20μ, ×10.

FIG. 4.—Lower first molar of experimental animal receiving 1.5 per cent hydrogen peroxide in drinking water (group IV). Note pulp and periapical necrosis. Carious areas at the tip of cusp. Mal-

crown and necrosis of the pulp, with fragmentation of the tooth, were observed. Caries occurred in the molars of both mandible and maxilla, being more prevalent in the lower jaw than in the upper. It was most marked in the first molar, although the second molars and to a lesser extent the third were also affected. Some of the damage to the periodontium appeared to be directly related to the caries (Figs. 3, 4), while other effects, such as resorption of cementum, dentin, and alveolar bone, gingival hyperplasia, and necrosis of the gingiva, appeared to be independent of the carious process. The periodontal changes varied in severity from gingival hyperplasia to necrosis of gingival papillae (Fig. 5). Resorption of alveolar crest cementum and dentin was also noted in some animals at the lingual margin of the upper incisor teeth (Fig. 6). It is of interest that small areas of cementum resorption, although less extensive than the above, also occurred on the distal aspect of the root surface of control rats (group I) (Fig. 2). These areas sometimes penetrated the dentin layer but were usually confined to the cementum.

Resorption of the lamina dura and supporting bone was frequent in peroxide-treated

FIG. 5.—Interdental gingival papilla in molar area of animal receiving 1.5 per cent hydrogen peroxide in drinking water (group IV). Note necrosis of interdental papilla. Mallory's stain. 7μ, ×30.

animals both in the interradicular space and on the mesial and distal root surfaces along the whole length of the root. Destruction of periodontal ligament fibers associated with inflammatory changes and ulceration was also noted. Early changes in the gingival margin (Fig. 8) were demonstrated as a loss of collagen fibrils with an infiltration of inflammatory cells, primarily plasma cells and lymphocytes. The pattern of arrangement of the collagen fiber bundles was altered in the experimental animals, with the free gingival fibers and alveolar crest fibers depleted or absent. The epithelium on the buccal aspect was edematous, with hyperkeratosis and parakeratosis. The pocket epithelium appeared unaffected.

Atrophic changes were observed in the secretory cells of the submaxillary glands of rats in groups III and IV (Fig. 10). These changes were not present in the rats of group I (Fig. 9) and were either absent or present to a minimal degree in rats of group II.

Experiment II.—In agreement with the earlier experiment, growth was significantly retarded in rats administered the 1 and 1.5 per cent hydrogen peroxide solutions

FIG. 6.—Upper incisor of animal receiving 1.5 per cent hydrogen peroxide in drinking water (group IV). Note resorption of lingual alveolar crest and of dentin. Also note destruction of periodontal ligament fibers. Mallory's stain. 20μ, ×35.

(groups D and E), although to a lesser extent than in the former experiment.* Growth was also retarded in rats fed the restricted diets. The average body weight of rats in the various groups at the time of sacrifice was as follows: group A, 304.0 ± 9.3 gm.; group B, 133.2 ± 1.6 gm.; group C, 106.5 ± 1.2 gm.; group D, 131.0 ± 5.4 gm.; and group E, 85.1 ± 4.5 gm. Only 1 of the 10 rats in group E died during the course of the experiment. All other rats in this and the other groups survived.

Histologically, no significant differences were observed between the rats given hydrogen peroxide in the present experiment (groups D and E) and the corresponding groups in Experiment I (groups III and IV). The incidence and severity of caries and periodontal damage were comparable in the two experiments. Dental caries and pathological

* The precise cause of the smaller weight increment of peroxide-treated animals in Experiment I is not known. It is possible that, as a result of the gingival changes in the peroxide-treated rats, the "hard" pelleted ration fed in Experiment I caused more trauma and pain than the powdered ration employed in Experiment II, with the result that food consumption (and gain in body weight) were less for the peroxide-treated rats in Experiment I.

FIG. 7.—Gingival margin of control animal (group I). Note normal arrangement and distribution of epithelium and connective-tissue fiber bundles of periodontal attachment apparatus. Mallory's stain. 20μ, ×30.

changes in the periodontium and submaxillary glands were not observed in rats fed the restricted diets (groups B and C). Group A was comparable in all respects to the normal controls (group I) of the former experiment.

Experiment III.—Growth was significantly retarded in all rats administered the zinc peroxide, potassium permanganate, or sodium perborate supplements. After 8 weeks of feeding, the average body weight of rats in the various groups was as follows: group a, 308.6 ± 8.6 gm.; group b, 174.0 ± 11.1 gm.; group c, 179.7 ± 8.4 gm.; and group d, 227.4 ± 12.1 gm. Dental caries with pulp necrosis similar to that obtained with hydrogen peroxide at a 1 or 1.5 per cent level in the drinking water was observed in the rats fed the zinc peroxide and sodium perborate supplements (groups b and d). In addition, rats fed the latter supplements exhibited marked resorption of the whole root surface, with only a thin dentinal layer remaining at the time of sacrifice (Fig. 11). Periodontal damage and caries with pulp necrosis did not occur in rats fed the potassium permanganate supplement (group c). One hundred per cent of the latter series, however, had surface caries.

FIG. 8.—Gingival margin of animal receiving 1.5 per cent hydrogen peroxide in drinking water (group IV). Note edema, parakeratosis, and slight hyperkeratosis in epithelium. Note especially destruction of free gingival collagen fiber bundles. Mallory's stain. 20μ, ×30.

FIG. 9.—Submaxillary gland of control animal (group I). Stain hematoxylin and eosin. 7μ, ×250

FIG. 10.—Submaxillary gland of animal receiving 1.5 per cent hydrogen peroxide in drinking water (group IV). Note pyknosis of all cellular elements. Stain hematoxylin and eosin. 7μ, ×250.

DISCUSSION

Present findings indicate that hydrogen peroxide when administered as a 1 or 1.5 per cent solution in the drinking water induces dental caries in the rat. The carious lesions start at the tip of the cusp and extend through the dentin to the pulp. In the more severely affected animals, complete destruction of the crown and necrosis of the pulp with fragmentation of the tooth occur. The type of caries induced differs significantly in origin and apppearance from the "occlusal," "fissure," and "smooth-surface" caries more commonly observed in the rat.[1-3] It is of interest that the caries occurred despite the fact that the rats were fed a "non-cariogenic" stock ration, on which caries were never observed in the absence of hydrogen peroxide treatment. This is the first report, so far as is known to the present investigators, in which caries have been induced in rats fed a stock ration adequate for normal growth and reproduction without the special dietary modifications required for caries production in the past.

It is felt that the production of caries on such a diet constitutes a potentially useful

FIG. 11.—Molar tooth of the animal receiving 2 per cent zinc peroxide in the food ration. Note dentin resorption along total outline of root, with destruction of periodontal ligament fibers, loss of interradicular bone, and necrosis of pulp. Mallory's stain. 7μ, ×30.

"tool" for the study of anticaries agents that is superior in a number of respects to the use of animals whose caries develop as a consequence of the administration of very specialized diets differing radically from that commonly ingested by man. Present findings also suggest the desirability of further studies on the possible role of oxidizing agents of bacterial, salivary, or food origin in the etiology of caries and periodontal disease in man.

Findings of the present experiments are not to be interpreted as indicating that hydrogen peroxide and other oxidizing agents as used clinically have a deleterious effect. The dosages employed and the length of time that the oxidizing agents were administered in the present experiments are never encountered under clinical conditions. On the contrary, there is some evidence that oxidizing agents at low levels in the diet may actually inhibit caries formation (as was reported by Hein, Wisotzky, and Quigley,[4] with a supplement of 0.3 per cent sodium carbonate peroxide in the diet) or have no effect at all (as was observed by Hein and Wisotzky[5] with low levels of potassium permanganate).

No data are available as to the mechanisms whereby hydrogen peroxide and the other oxidizing agents employed in the present experiment exert their deleterious effect. Changes in the oral flora induced by these materials may be implicated in the findings obtained. In view of the pathological changes induced by oxidizing agents in the submaxillary glands, it may be that resulting modifications in salivary flow or secretion contributed to the observed results. Goldhaber[6] recently observed that hyperoxia induced by the introduction of 95 per cent O_2 and 5 per cent CO_2 into the atmosphere of tissue cultures of young mouse calvaria consistently resulted in rapid and marked bone resorption. It is possible that the administration of hydrogen peroxide and other oxidizing agents in excessive amounts induces hyperoxia at the cellular level, which may in turn contribute to the findings noted.

SUMMARY

Immature rats fed a non-cariogenic ration and administered hydrogen peroxide in the drinking water as a 1 or 1.5 per cent solution developed extensive carious lesions and pathological changes in the periodontium and submaxillary glands after an 8-week period of administration. Similar effects were noted when zinc peroxide and sodium perborate were incorporated at a 2 per cent level in the ration. The significance of these findings is briefly discussed. These dental and periodontal changes appeared to be independent of weight loss brought about by inanition.

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EFFECTS OF INCREASED OXYGEN TENSION ON TUMOR RESPONSE TO AN ALKYLATING AGENT

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It has been well established that the response of many normal and neoplastic tissues to ionizing radiation is enhanced under conditions of increased tissue oxygen tension. Because many of the biologic effects of the alkylating agents have been shown to be similar to those produced by irradiation, it seemed pertinent to study the effects of increased oxygen tension on the tumor response to an alkylating agent. The administration of oxygen under pressure has been the standard method of attempting to achieve an increase in tissue oxygen tension during irradiation or antitumor chemotherapy. A simple method for increasing tissue oxygen tension is the intra-arterial administration of hydrogen peroxide. The purpose of our experiment was to study the effects of this increase in tissue oxygen tension on the tumor response to regional chemotherapy with an alkylating agent.

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Male Sprague-Dawley rats bearing a 4-day-old Walker 256 carcinoma in the right hind limb musculature were used in this study. These rats were treated with triethylene thiophosphoramide (TSPA) in a dose of 4 mg./kg. and hydrogen peroxide in a dose of 40 mg./kg., administered by previously described techniques of isolated femoral artery perfusion and by femoral artery infusion (1). In all cases the drugs were administered over a 15 min. period. Control groups were given each drug alone. A further group of rats was given this dose of hydrogen peroxide by these routes and estimations of tumor oxygen tensions were made with a Beckman model 160 micropolarographic probe.

The administration of hydrogen peroxide by these techniques raised the oxygen tension in the center of the tumor from an average normal level of 110 mm. Hg to 260 mm. Hg in the groups which were perfused and to 305 mm. Hg in the animals treated by infusion. The results obtained are presented in Table I.

Table I—Survival Time and Limb Damage in Rats Bearing the Walker 256 Carcinoma and Treated with TSPA and Hydrogen Peroxide

TREATMENT	NUMBER OF RATS	SURVIVAL (DAYS) MEAN	SURVIVAL (DAYS) RANGE	LIMB DAMAGE
Infusion TSPA + hydrogen peroxide	45	45.0	28–64	24.2%
Infusion TSPA	58	36.0	25–60	
Infusion hydrogen peroxide	36	25.3	18–39	25.0%
Untreated controls	102	21.6	17–32	
Perfusion TSPA + hydrogen peroxide	36	43.7	26–60	22.2%
Perfusion TSPA	53	38.9	28–53	4.9%
Perfusion hydrogen peroxide	36	24.6	20–40	29.4%

The incidence of metastases and metastatic pattern was the same in all groups. The administration of hydrogen peroxide by these methods was not an innocuous procedure, as observed by the occurrence of digital gangrene in 30% of the animals.

These results demonstrate that hydrogen peroxide will potentiate the antitumor effect of TSPA on the Walker 256 carcinoma, presumably by raising the tumor oxygen tension.

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Enhanced Toxicity of Ozone-Hydrogen Peroxide Mixtures

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Concern over enhanced toxicity of air pollutant mixtures has led to the experimental demonstration that hydrogen peroxide (H_2O_2) in concentrations of a few ppm killed animals after a brief exposure when inhaled with a nonlethal concentration (1 ppm) of ozone (O_3). It was possible also to demonstrate a moderate degree of protection against otherwise lethal doses of H_2O_2, by a single prior exposure to H_2O_2. Moreover, cross tolerance against the organic peroxide, cumene hydroperoxide was afforded also by a prior exposure to H_2O_2. Particularly noteworthy, was a tolerance produced by a prior exposure to O_3 to mixtures of H_2O_2 and O_3, despite the fact that such mixtures normally show enhanced toxicity.

TOXICOLOGIC studies on air pollutants in this laboratory in the past have involved single substances. Emphasis has been centered particularly on ozone[1, 2, 3] because of its dominant role in oxidant smog and because of many conflicting viewpoints on its toxicity that have appeared in the literature.[4]

The toxicity of ozone *per se* has been established for small animals.[5] It was further demonstrated that the observed toxicity could not be attributed to the presence of nitrogen oxides that may accompany ozone production.[1] An extreme range of physiologic response, from the rapidly lethal effects of ozone under added stress of exercise,[5] to the marked tolerance to acute effects developed from brief exposure to relatively low concentrations of ozone,[1, 6] were found in rats and mice. Experimental evidence in another laboratory has confirmed the above observations concerning ozone toxicity and tolerance.[6, 7] Respiratory distress, generally noted in laboratory animals exposed to ozone, has also been reported in welders using the inert-gas-shielded arc welding process[8] and in exposed human subjects.[9, 10]

Although considerable progress has been made concerning the toxicity of ozone alone, with regard to its lethal effects, tolerance and pulmonary irritation, reports of acute smog incidents have pointed to the distinct possibility that both direct and indirect effects of air pollutants on health may arise not only from single pollutants but also from combinations as well. Accordingly a systematic study was initiated involving the interactions of common air pollutants, chiefly the important respiratory irritants, with ozone, in order to ascertain whether or not the degree of injury observed in exposed animals could be attributed to a possible potentiation of combinations of these irritants.

The present studies involved testing the injurious nature of the reaction of hydrogen peroxide-ozone mixtures. Kinetic studies in aqueous solutions have indicated that hydrogen peroxide may be associated with ozone and its reaction product with water and organic substances to generate free radicals, HO and HO_2, as well as organic free radicals.[11] Prior tests for possible ozone contaminants, such as oxides of nitrogen, hydrogen peroxide, and free radicals (HO_2, OH, HO_3, O_4, etc.), in a specially constructed mass spectrometer failed to reveal significant amounts of these substances, however, but the possibility of free radicals and/or hydrogen peroxide could not be ruled out owing to the relative lack of sensitivity of the instrument.[5] This investigation included therefore, (1) the determination of acute and subacute lethal effects of such mixtures, (2) the "spread" of tolerance, observable with ozone, to hydrogen peroxide, and (3) the cross tolerance to either component following brief exposures to nonlethal levels of hydrogen peroxide or ozone.

Methods

Rats (Wistar-derived), mice (Swiss-derived), and hamsters used in these experiments were

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adult, male animals obtained from commercial sources and maintained in this laboratory for several weeks on a ration of Rockland Rat Diet prior to study. The basic diet and water were available to the animals at all times except when the exposures were in progress.

Exposures, with approximately one air change per minute, were routinely carried out in a glass chamber of 18.5 liters capacity, as previously described.[8] A large chamber of 100 ft^3 capacity, used exclusively for ozone inhalation studies,[8] was utilized on several occasions. The vapor air mixtures used in these studies were based on volume per cent.

Varying concentrations of ozone-air mixtures were prepared by diluting metered amounts of ozone, generated by passing three liters of air per minute through a plastic-dielectric ozonizer of a low-current-density type, with measured amounts of compressed air. The air used for the mixtures was scrubbed by passage through a fritted bubbler containing potassium dichromate in concentrated sulfuric acid, followed successively by columns of a mixture of soda lime plus silica gel and Drierite.[4] Details concerning the construction and chemical analyses of the output of the ozonizer, as well as the absence of organic impurities in the scrubbed compressed air have been discussed in another report from this laboratory.[8] Ozone determinations were made by the alkaline-potassium iodide method of Smith and Diamond[12] as modified by Byers and Saltzman.[13]

The hydrogen peroxide-air mixtures were prepared by diluting the vapors, generated by the passage of either water-pumped nitrogen or scrubbed compressed air at specified rates through a bubbler containing approximately 12 ml of 90% H_2O_2, at room temperature, with scrubbed air, to give a final flow rate of about 20 liters per minute. This dilution rate was chosen because pilot studies, without animals, had indicated that assay values for the concentration of hydrogen peroxide in the chamber sampled at the outlet were about 50% lower than values at the inlet if one air change in four minutes was passed into the chamber in contrast to a difference around 8% with one air change in one or even two minutes. When animals were present in the chamber a similar discrepancy in analytic values at the sampling sites with one air change in four minutes had been noted with ozone.[8] The rate of air flow for dilution undoubtedly plays an important role in the analyses of substances that are catalytically destroyed during passage through the chamber not only at the surface of the animals but also by absorption, adsorption, decomposition, condensation, or a combination of these and other factors. Desired levels of hydrogen peroxide in the exposure chamber could not be obtained with Superoxyl (30% H_2O_2) even at elevated temperatures but were satisfactorily obtained with 90% H_2O_2. The measurement of concentrations of hydrogen peroxide in the air mixtures was based on the development of a stable yellow color with stock solution of titanium sulfate reagent.[14, 15] This reaction was found to be specific for hydrogen peroxide only since no color development was observed with varying amounts of ozone.

Hydrogen peroxide-ozone mixtures were prepared by diluting metered amounts of these components, generated by methods outlined in preceding sections, with scrubbed air to a rate around 20 liters per minute. Concentrations of hydrogen peroxide in the mixtures were determined directly with the titanium sulfate reagent. As hydrogen peroxide was found to interfere with ozone analysis by its reaction via the alkaline-iodide reagent, air samples of the mixtures were first passed through a bubbler containing a mixture of 4% CrO_3 and 2.8% $KMnO_4$ in distilled water (this scrubbing process removed the hydrogen peroxide but no ozone) and then into a second bubbler containing the alkaline-iodide reagent for ozone analysis. In order to allow for a correction blank for traces of oxidants from the first bubbler (liberating free iodine from the alkaline-iodide reagent), scrubbed compressed air was drawn into the two bubblers under similar sampling procedures employed for analyses of the mixtures. Pilot studies, without animals, indicated that satisfactory analytical determinations could be obtained for the components either individually or in combination. Analyses of these mixtures showed no rapid reaction between ozone and hydrogen peroxide in the vapor state, as previously stated (15).

Inhalation exposures of small laboratory animals to varying concentrations of the components of the mixtures, either singly or in combination, were generally limited to a single, four-hour period. Analyses of the inlet and outlet concentrations of vapor-air mixtures from the chamber were made at periodic intervals. Although differences of 10–20% between samples at the two sites previously were reported for ozone determinations,[8] they were more pronounced with hydrogen peroxide, especially when larger animals (rats) were exposed. The discrepancy between analytic and calculated concentration for hydrogen peroxide has been discussed in a report from another laboratory.[14]

Observations concerning the lethal effects of the mixtures in the animals were noted during and after exposure. Tissues from animals that

died from the exposures or were killed subsequently with intraperitoneal injections of nembutal were prepared for routine histopathologic examination.

Results of Exposures

The mortality data observed among mice, rats, and hamsters after single, 4-hour exposures to hydrogen peroxide and ozone individually or in mixtures are presented in Tables I and II. Mice were found to be more sensitive test animals than rats for assaying the toxicity of a series of admixtures in which the ozone concentration was maintained around one ppm while the H_2O_2 concentration for individual experiments was decreased successively from 16.6 to 1.5 ppm (Table I). Similarly in Table II, hamsters were shown to be more resistant to such mixtures, and also to higher levels of ozone than was indicated by previous reports.[6]

Examination of the data on the mortality in control groups shown in Tables III and IV yields greater information on the susceptibility of mice to single, 4-hour exposures to ozone or hydrogen peroxide alone. In such exposures, ozone becomes lethal to small rodents at 5–6 ppm. At 78 ppm H_2O_2 alone is not lethal to mice on 4-hour exposures; at 113 ppm, one of 10 mice died within 24 hours and 4 of 10 died within the two-week observation period (Table IV). At 194 and 226 ppm H_2O_2 the mortality among mice was somewhat greater. It is notable that deaths due to

TABLE I

Acute Inhalation Exposures (4 Hours) of Male Rats and Mice to Hydrogen Peroxide-Ozone Mixtures

Average chamber conc'n (ppm)		Species	Average weight (gm)	Mortality following exposure No. dead / No. exposed	
H_2O_2	O_3*			24 hrs	2 weeks
16.1	—	M	25	0/10	0/10
		R	220	0/5	0/5
—	1.1	M	25	0/45	Used for other studies
—	1.0	R	230	0/40	" " " "
16.0	0.90	M	27	3/10	
		R	252	1/5	1/5
7.6	0.96	M	27	2/10	2/10
		R	260	2/5	2/5
3.3	1.0	M	25	3/10	3/10
		R	272	0/5	0/5
1.5	0.93	M	26	0/10	0/10
		R	260	0/5	0/5

M—Mice, R—Rats.

* Exposure in large chamber, 100 cu. ft. capacity. The ozone used in this chamber was generated in a U. S. Air Ozoniser, Scottdale Ozone Company, Scottdale, Pa., as previously de-[illegible]

TABLE II

Species Variation of Rodents in Single Inhalation Exposures to Hydrogen Peroxide and Ozone

Average chamber conc'n (ppm)		Species	Average weight (gm)	Duration of exposure (hours)	Mortality following exposure No. dead / No. exposed	
H_2O_2	O_3				24 hrs	2 weeks
24.8	1.5	H	112	4	0/10	0/10* (Group A)
		M	28	4	2/10	2/10
35.7	7.6	H	120	4	0/5	0/5* (Group B)
		M	26	4	5/10	5/10
	44.6*	H	105	1½	0/7	(Group C)
—	16.9*	R	280	1	10/10	2 dead 10 min. before end of exposure 3 dead ½ hr after end of exposure 3 dead 1 hr after end of exposure 2 dead 1½ hrs after end of exposure

H—Hamsters, R—Rats, M—Mice.

* Large chamber, 100 cu. ft. capacity used for ozone exposure. Group A and B challenged about 6 weeks after end of observation period for 70 minutes with 38 ppm O_3.

1/10 and 3/10 hamsters in Group A dead 3 and 6 hrs. respectively after end of exposure. Surviving hamsters in Groups A, B, C, sacrificed 29 hours, 76 hours, and 6 days after end of exposure for pathologic observation.

TABLE III

Responses of Mice Previously Exposed to Ozone When Challenged with Hydrogen Peroxide-Ozone Mixtures (4-Hour Exposures)

Exposure group	Chamber O_3 conc'n av. (ppm)	Recovery period (days)	Challenging dosages average chamber conc'n (ppm)		Mortality following exposure No. dead / No. exposed	
			H_2O_2	O_3	24 hrs	2 weeks
1	1.6	2	36.3	—	0/30	0/30
	Controls		37.4	—	0/10	0/10
2	1.6	3	—	5.7	0/30	0/30
	Controls		—	5.7	8/30	8/30
3	1.4	5–6	227.	—	7/25	14/25
	Controls		227.	—	5/25	22/25
4	1.6	4	34.5	5.6	0/30	0/30
	Controls		34.5	5.6	12/30	13/30

TABLE IV

Responses of Mice Previously Exposed to Hydrogen Peroxide when Challenged with Hydrogen Peroxide-Ozone Mixtures (4-Hour Exposures)

H_2O_2 chamber concentration (ppm)	Recovery period (days)	Challenging dosages average chamber conc'n (ppm)		Mortality following exposure No. dead / No. exposed	
		H_2O_2	O_3	24 hrs	2 weeks
5.6 (5 days)†	3	—	5.4	2/10	3/10
Controls		—	5.4	6/10	7/10
5.6 (5 days)†	3	78.1	—	0/10	0/10
Controls		78.1	—	0/10	0/10
5.6	4	—	5.6	4/10	4/10
Controls		—	5.6	7/10	7/10
16.3	2	—	5.8	9/10	10/10
Controls		—	5.8	9/10	10/10
46.2	3	—	5.8	1/10	1/10
41.4	2	—	5.6	3/10	4/10
Controls		—	5.6	8/10	8/10
40	7	113.	—	0/10	2/10
Controls		113.	—	1/10	4/10
40	8	194.	—	0/10	2/10
Controls		194.	—	3/10	6/10
44	6	—	6.1	7/10	7/10
Controls		—	6.1	10/10	10/10
44	4	226.	—	1/10	5/10
*C.H.(1/5 LC_{50})	7	226.	—	1/10	3/10
Controls		226.	—	1/10	5/10

* C. H. (Cumene Hydroperoxide)—6-hour exposure to 100 ppm (inlet calculated concentration).

† Exposure 4 hours per day for 5 days at 5.6 ppm H_2O_2.

H_2O_2 were in general, more delayed than was the case with lethal exposures to ozone.

Respiratory distress, indicated by spasmodic breathing and characteristic of ozone intoxication, was observed in a majority of the animals exposed to mixtures of hydrogen peroxide-ozone. Recovery from the respiratory distress generally occurred in hamsters within 24 hours following a 4-hour exposure to 9–12 ppm O_3; however, hamsters exposed to 40 ppm O_3 for about one hour still exhibited labored breathing 72 hours following the end of exposure.

Histopathologic examination of organs of sacrificed mice and rats exposed to lethal or near-lethal concentrations of ozone showed intense pulmonary edema, congestion of interstitial areas, massive extravasation of red blood cells into alveoli and mobilization of macrophages. Other changes included recent congestion in the liver and kidney as well as a depletion of lipoid from adrenal cortices. It was not possible by histopathologic examination to differentiate between alterations in tissues characteristic of ozone itself, and possible ozone contaminants such as oxides of nitrogen, free radicals or other oxidants.

In other inhalation studies, edema, desquamation of tracheal and bronchial epithelium, engorgement of lung parenchyma, a moderate degree of engorgement of the liver and depletion of cortical lipids were generally observed in rats and mice exposed to mixtures of hydrogen peroxide (13–25 ppm) and ozone (1.2–2.9 ppm). The extent of the pathologic changes depended on duration of survival. Exposure to these mixtures appeared to produce histologically visible edema sooner than from ozone alone.

Histopathologic examination of various organs of hamsters exposed to 44.6 ppm of ozone (Table II), indicated that primarily the lungs were affected. Edema was noted 29 hours post exposure: the fluid was stringy and apparently of a low protein content, and partial desquamation of the bronchial epithelium was present. Observations 76 hours post exposure included absence of edema, regeneration of bronchial epithelium, thickening and infiltration with chronic inflammatory cells of the ductal, as well as adjacent alveolar walls, a marked hyperplasia of the alveolar lining cells, marked dilation of alveolar ducts and periplural alveoli.

The histologic picture, 6 days post exposure, showed disappearance of acute dilatation of the ducts and regression in the peribronchiolar and periductal monocytic infiltration. The bronchiolar and ductal walls were slightly puckered and tortuous, and resembled those found in long-term ozone exposures[3] except for lack of fibrosis.

Cross Tolerance

Previous studies in this laboratory[2,8] have demonstrated that a marked tolerance to normally acute lethal exposures to ozone develops in rodents following initial exposures to relatively low ozone concentrations. This is illustrated by exposure group 2 in Table III where all the preexposed mice survived a subsequent challenge dose of ozone which killed 8 of 30 control animals receiving no preexposure. Respiratory distress, characteristic of ozone exposures, was still observed in the preexposed animals during and following the challenge dose.

The data in Table III indicate that a tolerance similar to that found for ozone may also be established against lethal levels of H_2O_2 alone (Group 3) or against mixtures of H_2O_2-O_3 (Group 4). This capacity of O_3 to cross-protect

animals against subsequent exposures to H_2O_2 is remarkable, but is all the more remarkable in protecting animals against a mixture of H_2O_2-O_3 which in itself is a highly potentiating toxic mixture as shown in Table I. Other responses to exposure, however, such as hyper-excitability, eye irritation, emaciation and delayed deaths, generally observed in mice exposed to very high levels of H_2O_2, still occurred and were not suppressed by the tolerance following a challenging dose of the components, either singly or in admixture.

Preexposure to H_2O_2

Table IV presents mortality data on groups of mice preexposed to H_2O_2 and subsequently challenged by normally lethal exposures to either ozone or hydrogen peroxide. Five days exposure at 5.6 ppm H_2O_2 and single exposures to 40 ppm H_2O_2 resulted in marked tolerance to subsequent challenge doses of ozone. A single exposure to 5.6 ppm H_2O_2 conferred somewhat less tolerance; a single exposure to 16 ppm H_2O_2 resulted in no tolerance. This apparent anomaly is probably related to the difference in recovery period after preexposure in these two experiments.

A single preexposure at about 40 ppm H_2O_2 yielded a certain, perhaps equivocal, degree of tolerance to subsequent challenge doses of H_2O_2 (113–226 ppm).

A comparable preexposure to an organic peroxide, cumene hydroperoxide, at one-fifth of its LC_{50} provided a measure of tolerance to lethal concentrations of H_2O_2.

Histopathologic examination of the lungs of mice exposed during these studies and killed 7 to 16 days after the last exposure were similar to those reported for mixtures of H_2O_2 and O_3.

Preexposure to H_2O_2-O_3 Mixture

Two groups of hamsters were exposed to mixtures of H_2O_2 and O_3 in the experiments reported in Table II (Group A and Group B). All these hamsters survived these exposures and subsequently were challenged 7 to 8 weeks later with 48 ppm O_3.

Group A, preexposed to the mixture at lower concentrations, showed a moderate amount of edema six hours after the challenge exposure. At 29 hours post challenge, findings were similar to those previously described for hamsters exposed to ozone. A very pronounced cellular reaction in peribronchial and periductal tissue was evident in hamsters sacrificed 76 hours post challenge.

The higher concentration of preexposure mixture was given Group B. At 29 hours post challenge there was no edema, but evidence of peribronchiolar reaction was seen. The cellular reaction (76 hours post challenge) of peribronchial and periductal tissues was comparable to that observed in the above group.

These findings in hamsters again stress that two separate sites of action, or possibly two separate mechanisms of tissue damage may be involved in ozone intoxication. Although preexposure to ozone may decrease the amount of edema in the lung from a challenge dose, only slight reduction in cellular reaction was noted in the bronchiolar and ductal tissues.

Comment

Observations, based on mortality and histopathologic examination of lungs of mice, a species found to be slightly more sensitive than rats, but far more susceptible than hamsters in these inhalation studies, suggest that the toxicity of mixtures of hydrogen peroxide-ozone was more pronounced than that of either component separately. Hydrogen peroxide in concentrations 3.3 ppm and above enhanced the toxic effects of levels of ozone (around one ppm) normally not acutely injurious in mice; concentrations of 7.8 ppm H_2O_2 and above increased the toxicity of ozone in rats. Pulmonary edema, characteristic of moderately high levels greater than 2 ppm ozone was not found in mice exposed to levels of H_2O_2 around 226 ppm, but some hyperplasia of the bronchial epithelium was observed 12 days later. Exposures to mixtures of H_2O_2 (13–25 ppm) and ozone (1.2–2.9 ppm) appeared to accelerate the production of histologically visible edema and periductal cellular reaction more than that seen from exposure to ozone alone.

Histopathologic examination of the lungs of ozone-exposed animals indicated that two sites or mechanisms of tissue damage appear to be involved in the response, whereby a marked tolerance to acute lethal effects of ozone developed in rodents following initial exposure to relatively low ozone concentrations. Although pre-exposure to ozone decreased the amount of pulmonary edema following a challenging dose, little reduction in the cellular reaction of peribronchial and periductal tissue was noted. Moreover, respiratory distress, indicated by labored breathing and characteristic of ozone intoxication, was still observed in the "tolerant" animals. It should be emphasized here that this tolerance does not prevent the development of fibrotic changes in the lungs produced from daily repeated exposures to low levels of ozone.[3] We have no explanation for the mechanism of the enhanced toxicity of the H_2O_2-O_3 mixture.

A tolerance comparable to that found for ozone was generally observed in mice challenged with high doses (above 113 ppm) of H_2O_2 following pre-exposure around 1/5 LD_{50} level of H_2O_2 (40 ppm). H_2O_2 provided also a cross-tolerance to O_3 in mice, and to an organic peroxide, cumene hydroperoxide. This cross tolerance afforded by substances possessing a common chemical grouping, namely peroxide, suggests a common mechanism of action. Because of the well known capacity of peroxides to denature protein, an immuno-chemical mechanism may account for at least part of the observed tolerance; there is some indication from biochemical studies that adaptive enzymes may be involved. Substances such as serotonin, adrenalin or histamine or other factors may also be involved. The "spread" of tolerance to other pulmonary irritants should be studied further.

The question of whether possible ozone contaminants, such as free radicals or other oxidants, affect the toxicity of ozone in laboratory animals, will have to be investigated by more sensitive analytic procedures, and histochemical or enzymatic tests, since histopathologic examination failed to resolve the issue.

Summary

1. Hydrogen peroxide in concentrations of 3.3 ppm and above enhanced the toxic effects of usually noninjurious doses of ozone (around one ppm) in mice; concentrations of 7.8 ppm and above of H_2O_2 were required in rats.

2. A moderate degree of protection to lethal challenging doses of hydrogen peroxide, comparable to the tolerance developed by ozone for itself, developed after initial exposure of mice around 0.2 LD_{50} level of H_2O_2.

3. A cross-tolerance to the acute lethal effects of ozone can likewise be developed in mice after repeated exposure to low levels of H_2O_2 or a single dose about 0.2 of the lethal dose. An initial exposure to approximately 0.2 LD_{50} of H_2O_2, or to an organic peroxide, cumene hydroperoxide, also afforded tolerance to a challenging dose of hydrogen peroxide. A tolerance to ozone was developed in hamsters, although this species is more resistant to the toxic effects of ozone than mice and rats. The development of tolerance to a mixture of H_2O_2 and O_3 with potentiated toxicity, from a prior exposure to ozone seems particularly noteworthy.

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Cardiovascular Effects of Hydrogen Peroxide: Current Status

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Dallas, Texas

IN AN ATTEMPT TO FIND A BETTER METHOD for oxygenating tissues which would compare with hyperbaric oxygenation, an intravascular technique of administering oxygen in a regional or systemic system,[1-3] employing dilute solutions of hydrogen peroxide (H_2O_2) has been evaluated. Hydrogen peroxide is rapidly degraded to oxygen and water by catalase and peroxidases, enzyme systems present in excess quantities in the blood. Following H_2O_2 decomposition in biologic fluids, it was noted that more oxygen was being recovered from such fluids than one would expect from 100 per cent saturation with oxygen at 1 atm. Experiments revealed this to be a simple supersaturation of a liquid with a gas.[4] Hydrogen peroxide releases dissolved oxygen equivalent to that found in solutions under oxygen at 3-8 atmosphere pressure. H_2O_2 administration does not require lung transport. It can be given continuously over long periods of time, it can be administered by a single physician without expensive equipment and large teams, and it avoids compression-decompression hazards, as well as central nervous system and pulmonary toxicity. Hydrogen peroxide has been demonstrated to be an adjunctive source of oxygen for the anoxic or ischemic heart and can improve resuscitation in refractile arrhythmias or cardiac arrest.[1,5,6] Intra-arterial infusion of H_2O_2 has been noted to reverse the atherosclerotic process,[7,8] potentiate the effect of irradiation on malignant tumors,[9,10] increase the localization of radioactive isotopes in malignant tumors,[11,12] protect against *Clostridium welchi* [illegible] tions,[13] promote wound healing[14] an[illegible] as an excellent source of regional o[illegible] tion without significant systemic to[illegible]

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CARDIAC EFFECTS OF HYDRO[illegible] PEROXIDE

Direct Tissue pO_2 Measurements (Myocardium)

Direct pO_2 measurements taken i[illegible] bit myocardium during (1) the int[illegible] arterial infusion of dilute H_2O_2 into the coronary arteries, and (2) the direct application of dilute H_2O_2 to the epicardium demonstrate the relative increase in myocardial pO_2 during the intra-arterial infusion of H_2O_2 to the coronary arteries (Fig. 1) and by the direct diffusion (Fig. 2) respectively.[1]

Oxygenation of the Ischemic or Anoxic Myocardium of Small Animals: Tracheal Occlusion

The myocardium of rabbits was rendered anoxic by tracheal cross-clamp.[1] These animals were divided into three groups of ten animals each: in Group 1, H_2O_2 was infused into the coronary arteries; in Group 2, it was perfused intrapericardially; in Group 3 (controls), the carrier solutions were administered by each of the two routes, but without H_2O_2. In Groups 1 and 2, animals treated with 0.12-0.72 per cent H_2O_2, the arterial blood pressure fell to 0 between 12-15 minutes in contrast to six minutes for the controls, and the arterial pH fell to 7.0 from 10 to 15 minutes in Groups 1 and 2 in contrast to six minutes for Group 3. Cardiac activity in Groups 1 and 2 remained relatively normal and neither nodal rhythm nor cardiac arrest occurred within 120 minutes of observation in contrast to Group 3 where arrhyth-

mias and nodal rhythm appeared in an average of five minutes and cardiac arrest from 10-16 minutes in all animals. All animals in Group 3 developed cardiac arrest, and, following failure of standard resuscitative methods, most could be revived by adding H_2O_2 to the heart by whichever route of administration of carrier solution was being employed.

Coronary Artery Ligation

In another group of rabbits, the myocardium was rendered anoxic and ischemic by ligation of coronary artery branches singly or in combination.[1] Because of the nature of rabbit myocardium, it was impossible in 20 animals to produce a standard ischemic lesion which was reproducible from animal to animal with regard to ECG or blood pressure changes. This made it necessary to evaluate the reversal of ischemic myocardial changes by regional applications of H_2O_2 within a given animal rather than by comparison of the effect on a standard lesion in a large number of animals. Following ligation of a variety of coronary arteries including the circumflex, the anterior descending, and the right coronary (or a combination of these), dilute H_2O_2 (range 0.06-0.72 per cent) was infused antegrade into the coronary arteries by a slow drip or perfused inside the pericardium at the time of ligation. Specific ECG abnormalities associated with myocardial ischemia, such as ST segment elevation, elevation or depression of the T wave, nodal block, and ventricular tachycardia or fibrillation, could be readily and often repeatedly reversed in the animals by application of the H_2O_2. A blood pressure drop associated with cardiac arrhythmias could also be reversed, but less consistently (Fig. 3-4).

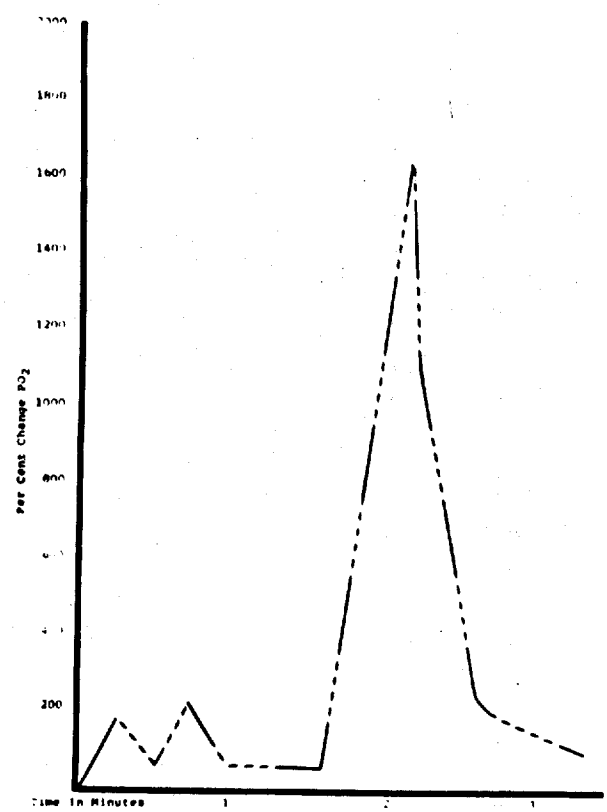


FIGURE 1: Changes in myocardial oxygen tension following coronary artery infusion of 0.36 per cent hydrogen peroxide.

Myocardial Protection during Ischemia in Large Animals

To evaluate the effect of H_2O_2 in protecting a thicker ischemic myocardium, where diffusion is a more important factor, pigs weighing 40-80 kg underwent ligation of the right coronary artery and 50 per cent of the anterior descending vessel. Two groups of ten pigs each were studied: Group 1 to evaluate the effect of intrapericardial H_2O_2 perfusion during coronary artery ligation, and Group 2 to serve as controls.[5,6]

In Group 1, two plastic No. 190 polyethylene catheters were sutured into the pericardium—one for inflow and the other for outflow. The epicardium was perfused with a Harvard constant-infusion pump, and a steady flow of Hank's solution with 0.06 per cent H_2O_2 at 37° was constantly infused into the pericardium at the rate of approximately 400 ml/hour. In the ten control animals, the same experiment was conducted with carrier solution alone without H_2O_2.

In group 2, ventricular fibrillation occurred between two and 32 minutes in all but one animal (average time [illegible] min), (Table 1). Resuscitation by cardiac massage and defibrillation was succe[illegible]d in seven animals, but failed in two. [illegible] return of regular sinus rhythm, mea[illegible] blood pressure remained less than the [illegible]al control value of 100 mm Hg in [illegible] but three animals, the average being 3[illegible] mm Hg mean arterial blood pressure [illegible] animals developed ventricular fib[illegible] several times and could be resuscita[illegible] but they never maintained normal [illegible]rial blood pressure. Survival time ranged from two minutes to two hours, the average being 20 minutes. Myocardial tissue pO[illegible] values were much lower than original control values in all cases. Necropsy examina-

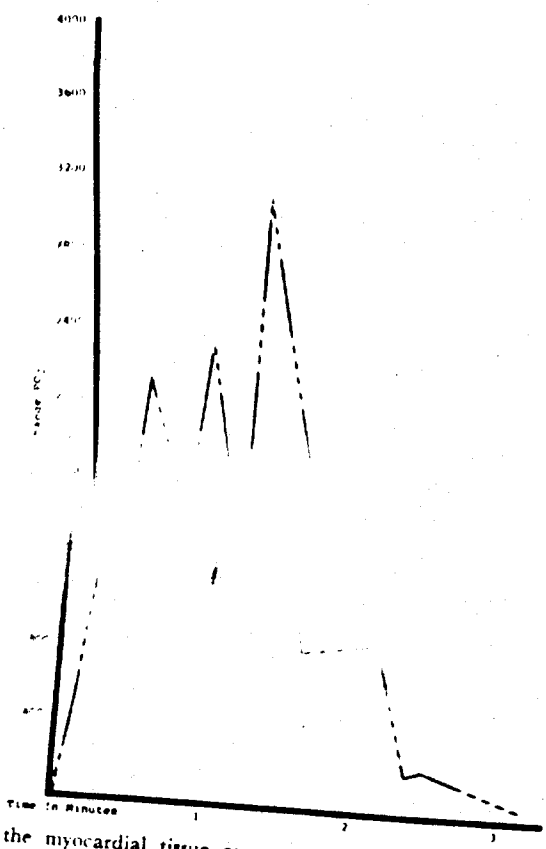


FIGURE 2: Changes in the myocardial tissue oxygen tension following the direct epicardial application of 0.36 per cent hydrogen peroxide.

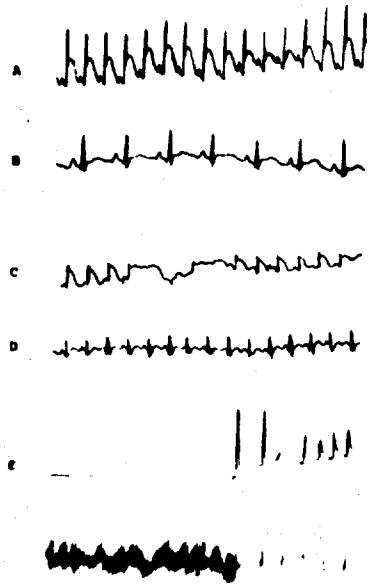
tion of all pigs revealed a large area of gross infarction over the lower right and left ventricles, with distal infarction in the septum.

In the Group 1 animals treated with 0.06 per cent H_2O_2 in Hank's solution, five animals never developed ventricular fibrillation; in the other five, onset occurred between five and 30 minutes (average time 20 min). Resuscitation by cardiac massage and electrical defibrillation was successful in all cases. Nine animals survived three hours and were sacrificed. One expired at two hours (Table 2). All maintained normal blood pressure (mean value 100 mm Hg) and normal to slightly elevated tissue pO_2 in comparison to the control preligation values. The infarcted area was in a similar location to that of control animals, but it was much less extensive and limited primarily to the subendocardial area and the distal septum.

In all control animals, after a terminal episode when no blood pressure or cardiac activity was obtainable by standard resuscitative methods, dilute H_2O_2 solutions were added to the myocardium. In four animals, resuscitation was successful despite massive infarctions and systemic acidosis.

Although this method of application is limited by the diffusion of H_2O_2 through the thick myocardium, and although the septum does not appear to be protected by the epicardial application, the improved protection of the treated animals, when compared to controls, appeared significant. No toxicity was demonstrated during therapy.

Employing the same standard coronary artery ligation, other experiments were performed to evaluate coronary artery and right atrial infusion of dilute hydrogen peroxide. These animals received some protection and did better than the control group in which carrier solutions alone were used; however, the duration of protection was much less than those treated by intrapericardial perfusion of H_2O_2.



[illegible] 3. Electrocardiographic changes in rab[illegible] with ischemic myocardium during application [illegible] dilute hydrogen peroxide. A 5 minutes post-[illegible] of circumflex. Note elevation of S-T seg-[illegible] B 2 minutes after infusion of 0.48 per [illegible] hydrogen peroxide in 5 per cent dextrose in wa[illegible] C 8 minutes post-ligation of proximal and [illegible] descending circumflex branches of L. coro-[illegible] Note elevation of S-T segment; D 30 sec-[illegible] after direct application of 0.24 per cent hy-[illegible] peroxide; E Note fibrillation; arterial pressure zero to arrow. At this point direct application of 0.24 per cent hydrogen peroxide to myocardium produced resumption of beat and a rise of arterial pressure to 50 mm/Hg.

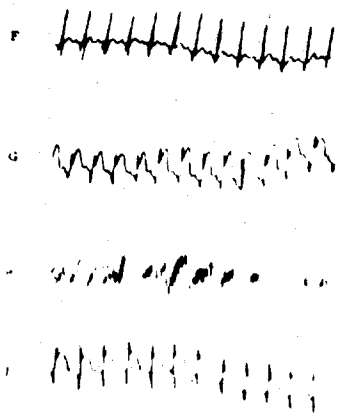


FIGURE 4. Electrocardiographic changes [illegible] bits with ischemic myocardium during [illegible] of dilute hydrogen peroxide. F Control ECG, with the chest open and the animal breathing [illegible] per cent oxygen. BP 140/110; G ECG ta[illegible] 2 minutes after ligation of the distal descendi[illegible] circumflex branch of the left coronary arter[illegible] BP 85/70; H Fibrillation 3.5 minutes after liga[illegible] BP 0; I ECG 5 minutes after direct applic[illegible] of 0.36 per cent hydrogen peroxide and 20 [illegible]utes after the beginning of fibrillation. BP 90[illegible]

Dimethyl sulfoxide (DMSO) was evaluated as an agent which theoretically might increase diffusion of the H_2O_2 through the thick myocardium and also counteract the vasoconstriction effect of oxygen on the small arterioles. Although the initial results of combining 10 per cent DMSO with .06 per cent H_2O_2 in rabbit hearts undergoing ischemia appeared beneficial.[8] No significant advantage could be observed when employed in larger porcine hearts.[8,15]

Effect of H_2O_2 on Totally Ischemic Hearts During Aortic Cross-Clamping

In an effort to evaluate the effect of H_2O_2 on the totally ischemic heart of large animals with thick ventricles, dogs,* calves, and goats were placed on cardiopulmonary bypass and the aorta was cross-clamped for one to two hours.[5,6,16] Hydrogen peroxide was applied directly to the epicardium, perfused retrograde through the coronary sinus, infused through the coronary arteries, or instilled intraventricularly (or combinations of these methods were used). Experiments were carried out at normothermia to evaluate the effectiveness of H_2O_2 as the only source of oxygen for the heart. Twenty-five control animals (ten dogs, five goats, and ten calves) were studied in similar fashion for comparative analysis. All animals were anesthetized with endotracheal halothane and placed on total cardiopulmonary bypass, and the aortas were cross-clamped 60-120 minutes. The hemi-azygos vein was ligated at its entry to the coronary sinus, providing a totally anoxic heart except for the oxygen supplied by H_2O_2. The hearts were vented through the left and right ventricular apexes for decompression. After removal of the aortic cross-clamp, the coronary arteries were again perfused with blood, and sufficient time was allowed for normal cardiac rhythm to return without artificial resuscitation. H_2O_2 application (0.06-0.24 per cent) began when the aortic cross-clamp was placed.

The hearts of all animals fibrillated within 30 minutes, most fibrillating between five and 15 minutes. None of the control animals could be resuscitated after one hour of aortic cross-clamping, and most could not be resuscitated after 30 minutes. Average time of successful resuscitation in dogs was after 20 minutes of aortic cross-clamping; for calves and goats, it was approximately 40 minutes. In the treated animals, after one hour of aortic cross-clamping, reversal to regular sinus rhythm was fairly consistent with most methods of H_2O_2 application. Beyond this, however, protection was sporadic. Most hearts required direct-current defibrillation and some required calcium and epinephrine injections into the aortic route to reverse ventricular fibrillation to regular sinus rhythm (Table 3). Coronary artery infusion for over 60 minutes was not protective when employed alone, and when combined with

*Since dogs do not have adequate quantities of catalase for H_2O_2 decomposition, this enzyme was added in all canine experiments. This is expensive and involves an additional variable which makes the dog an undesirable experimental animal for H_2O_2 investigation. Rabbits, pigs, goats and humans appear to have adequate amounts of catalase for regional H_2O_2 application.

TABLE 1—EFFECTS OF PERICARDIAL PERFUSION OF H_2O_2 AND CONTROL PIGS AFTER CORONARY ARTERY LIGATION

	Control (Carrier Sol. Only)	Treated H_2O_2
Incidence of ventricular fibrillation	9/10	5/10
Average onset time of ventricular fib.	12 min	20 min
Reversibility of ventricular fib.	7/9	10
Av. no. shocks to reverse ventricular fib.	5	1-2
Normal mean arterial blood pressure	4/10	9/10
Myocardial tissue oxygen tension	Less than control	Normal or greater than control

TABLE 2—SURVIVAL OF PIGS TREATED BY PERICARDIAL PERFUSION OF H_2O_2 AFTER CORONARY ARTERY LIGATION

Hrs.	Control	H_2O_2
0*	2	0
1	5	0
2	1	1
3	2	9

*Initial ventricular fibrillation fatal.

epicardial application, the results were no better. Coronary sinus retrograde perfusion alone also failed to protect the heart. Epicardial application and coronary sinus perfusion protected 50 per cent of the animals, some tolerating 100 minutes of aortic cross-clamping. The best protection was achieved with epicardial application, coronary sinus perfusion, and intraventricular instillation of H_2O_2.

Despite reversion to regular sinus rhythm, most of the animals could not maintain circulation off cardiopulmonary bypass, indicating only partial protection by the H_2O_2 and failure to support the whole heart during this length of ischemia at normothermia. Five calves maintained normal blood pressure when aortic cross-clamping lasted only 60 minutes, and these were long-term survivors of two weeks or more. Two dogs maintained normal blood pressure off cardiopulmonary bypass when aortic cross-clamping lasted less than 45 minutes, a significantly longer time than in control dogs. The long-term survivors showed no evidence of H_2O_2 toxicity.

Myocardial tissue pO_2 levels in the H_2O_2-treated animals were equal to or greater than control levels during the ischemic phase. In the control groups, these values fell much below the original control values, in some animals reaching zero.

The epicardium appeared white after H_2O_2 oxygenation, but color returned on perfusion of the coronary arteries with blood. The opened hearts of the survivors had normal ventricular and atrial walls with occasional brownish areas in the septum. The hearts with prolonged ischemia, which failed to resume normal rhythm, showed moderate superficial edema, grossly normal atria, and normal ventricular walls two-thirds through their thickness from the epicardial surface. From there to the endocardial surface, the muscle appeared blue and ischemic except in the animals treated by intraventricular instillation of H_2O_2. In these hearts, the myocardium appeared normal. In the animals not treated by coronary artery or coronary sinus perfusion, the septum appeared bluish-black in areas, suggesting ischemia. The micro-

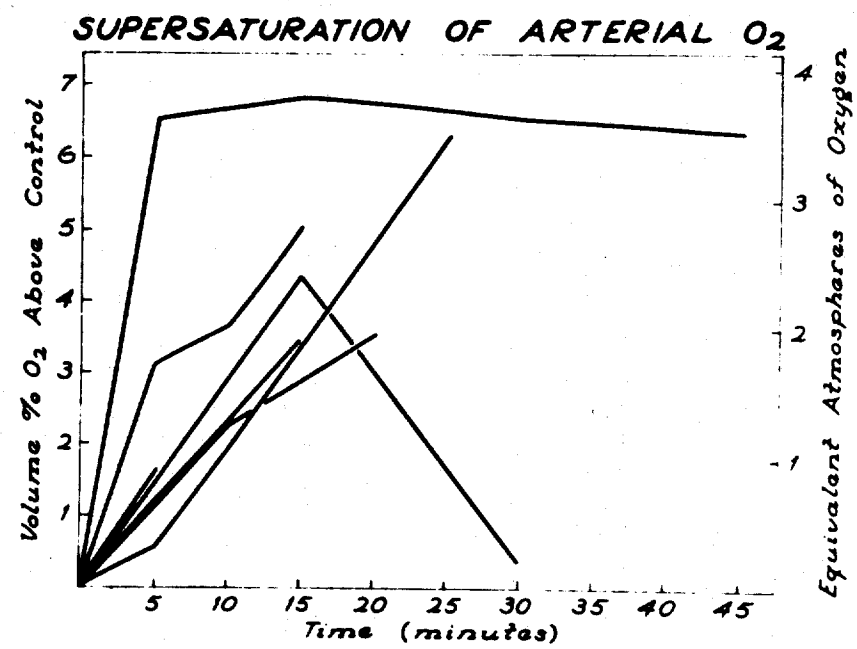


FIGURE 5: The increase in left atrial oxygen content following nebulization of hydrogen peroxide and concentrations from 1 to 6 per cent. Each line represents a different rabbit treated with a different concentration from 1 to 6 per cent.

scopic findings were consistent with the gross pathologic appearance.

Hydrogen peroxide appears to prolong the safe period of myocardial ischemia, but not consistently beyond 60 minutes. Survivors showed no evidence of H_2O_2 toxicity. The diffusion factors in the thick myocardium suggest that epicardial application or coronary artery infusion alone cannot protect the myocardium for long periods of time. Coronary artery infusion alone may carry the hazard of vasospasm despite administration of vasodilator agents such as papaverine. As the myocardium becomes thicker, problems of oxygen diffusion become more difficult. The conduction mechanism is not well protected by epicardial application alone, but H_2O_2 seems to have some value when given intraarterially or retrograde by coronary sinus perfusion. The intraventricular H_2O_2 seems to aid diffusion through the thick-walled myocardium as well as through the septum. The absence of blood flow through the heart to remove any waste products may be a problem in H_2O_2 application. Certainly other factors, such as carbon dioxide transport and acidosis, contribute to prolonged inadequate protection by H_2O_2 alone. This suggests that H_2O_2 may be an important adjunct in the treatment of myocardial ischemia and anoxia, but its application may be limited in total support of the bloodless heart.

Clinical Trial

Because of the apparent advantages in myocardial support during anoxia, H_2O_2 was used to treat a 60-year-old white female for vascular collapse of unknown etiology, unresponsive to the conventional methods of resuscitation.[8] A cardiac catheter was passed through the right brachial artery to the root of the aorta. ECG and blood pressure were monitored. Within one minute after the infusion of 0.12 per cent H_2O_2, the ECG reverted from a nodal to a regular sinus rhythm, and the mean arterial pressure increased from 35 to 70 mm Hg within three minutes. Within ten minutes after the infusion was stopped, the ECG again reverted to nodal rhythm and the blood pressure dropped. This sequence was repeated five times during a 12-hour period, reversal of ECG abnormalities and hypotension being achieved with each infusion of H_2O_2. The patient eventually expired when H_2O_2 therapy was stopped.

Assisted Systemic Oxygenation with Hydrogen Peroxide

Dilute H_2O_2 was administered to rabbits by three different techniques, to supply oxygen systematically: (1) by peritoneal perfusion,[8] (2) by rectal perfusion and (3) by ventilation with an H_2O_2 aerosol.[17]

Peritoneal Perfusion of Hydrogen Peroxide

For studies on peritoneal perfusion H_2O_2, rabbits were anesthetized with sodium pentobarbital, intubated, and placed on IPPB (intermittent positive pressure breathing) apparatus with 100 per cent oxygen in order to maintain a constant ventilation rate and 100 per cent arterial oxygen saturation. A midline incision was then made to expose the portal vein, and a flanged PE-90 catheter was secured in the vein with a purse-string suture and withdrawn to the flange so as not to obstruct venous flow. A dialysis kit (Baxter K-128) was secured in the peritoneum and the wound closed. The peritoneum was perfused with warm dilute H_2O_2 solutions (0.12-0.48 per cent), and blood samples were collected periodically from the portal vein.

The oxygen content of the portal vein blood during dialysis approximated that in the arterial blood. The carbon dioxide content of the venous blood could be adjusted by varying the pH of the buffer system of the H_2O_2. In the pilot studies, the carbon dioxide could not be adjusted to a degree necessary for systemic maintenance; however, this may be possible after further investigation.

Rectal Perfusion of Hydrogen Peroxide

Studies on rectal perfusion of H_2O_2 were conducted in dogs, and, as in the rabbits, a catheter was placed in the portal vein for blood sampling. The large bowel was perfused via the rectum with dilute H_2O_2, and

blood samples were collected from the portal vein during perfusion. As in peritoneal perfusion, the oxygen content of the portal vein blood approximated that of 100 per cent saturated arterial blood. No attempt has been made to adjust carbon dioxide by this procedure.

Ventilation with an Hydrogen Peroxide Aerosol

Rabbits anesthetized with sodium pentobarbital were intubated and placed on 100 per cent oxygen on a Bird respirator. The chest was opened between the fourth and fifth intercostal spaces, the pericardium slit, and a flanged PE-90 catheter secured into the left atrium by a purse-string suture. The chest was closed and the animal allowed to breathe 100 per cent oxygen for 30 minutes before aerosol H_2O_2 was given. Concentrations of H_2O_2 from 1 per cent to 6 per cent in normal saline were nebulized as an aerosol.

After nebulization therapy, the left atrial blood of these animals was found to be "supersaturated" with oxygen containing quantities equivalent to that expected with oxygen administration at 3 atm. When this value was exceeded, small bubbles began to appear in the samples. Figure 5 shows the effects of different H_2O_2 concentrations. An abrupt drop in atrial pO_2 occurred in the animal given H_2O_2 by aerosol, but it was felt that this animal developed bronchospasm. The 1 per cent aerosol, which was least irritating, provided as good arterial oxygen concentration as the higher concentrations.[7]

Vascular Effects of Hydrogen Peroxide

Effect of Intra-arterial Hydrogen Peroxide on Arteriosclerosis

Kann, Mengel, Hochstein and others have shown that the formation of lipid peroxides is one sequela of exposure to oxygen at high pressure. We have noted a consistent reduction in the severity of atherosclerotic disease in the infused area of patients receiving intra-arterial hydrogen peroxide. Pilot experiments have been performed in our laboratory to investigate this observation.[7,8]

(1) Gross, histologic and chemical evaluation of aortas taken at post mortem from patients treated for extended periods with intra-arterial hydrogen peroxide.

Several patients who have been infused intra-arterially with hydrogen peroxide as an adjunct to irradiation therapy in the management of their malignant disease have undergone post mortem examination. During the necropsy the catheter was left in place, the aorta was split longitudinally and the tip of the catheter marked. Sections were prepared from the aorta immediately above and below the catheter tip for comparative histologic evaluation by Oil Red O and H and E stains. Weighed sections of the vessel were then prepared for total lipid analysis. The components (cholesterol, cholesterol esters, phospholipids, triglycerides and free fatty acids) were separated by thin-layer chromatography. The material was taken from the thin-layer chromatogram and quantitated by gas chromatography.

In all cases studied to date, the patients received hydrogen peroxide infusion alone as an adjunct to other modes of therapy for a variety of conditions over extended periods of time ranging from 4-16 weeks. During this time, the individuals received daily infusions of 250 ml of hydrogen peroxide in "Ionosol-T" with a peroxide concentration ranging from 0.36 per cent to 0.48 per cent. Upon gross examination, the segment of the aorta being infused was found to be different from the area not being infused. This difference was marked by a decrease in the number and severity of atheromatous plaques and an increase in flexibility and elasticity of the vessel. The thoracic aorta contained numerous atheromatous plaques, some of which are eroding into the lumen. Ordinarily one expects to find an increase in number and severity of the atheromatous lesions from the thoracic to the abdominal aorta. In these patients, where the catheter was in the abdominal aorta below the renal ar-

teries, there was a lack of raised lesions from the point of the catheter to the bifurcation, and the iliacs were relatively free of gross disease except for some intimal and subintimal fibrosis. Histologic evaluation by Oil Red O stained sections showed a decrease in total subintimal lipid deposits. When weighed samples of the vessels were extracted and total lipids determined, it was found that approximately a 50 per cent reduction in total lipids (10-40 per cent decrease in cholesterol and 20-50 per cent decrease in cholesterol esters) had occurred in the area being infused with hydrogen peroxide.

(2) *In vitro* studies of human aortas incubated with dilute hydrogen peroxide in saline or incubated in saline under oxygen at increased pressure.

Aortas were procured at post-mortem from a number of adults who had expired for a variety of reasons, but who had received no hydrogen peroxide during their hospital course. The tissue from several aortas was cut into 5 mm squares, pooled, washed 15 times in cold saline and divided into a number of 15 gm samples. These samples were divided into three parts; one sample was incubated in 100 ml of a 0.36 per cent hydrogen peroxide in saline, the second sample was incubated in 100 ml of saline under oxygen at 2,4 and 6 atmospheres absolute pressure, and the third sample was incubated in 100 ml of saline in room air normal pressure, the latter serving as a control. After two hours of incubation, the tissue was removed by two filtrations, the lipid extracted from the resulting fluid and analyzed as described earlier.

The relative quantities of lipid in the fluid following incubation of human aorta in 0.36 per cent hydrogen peroxide or saline under oxygen at 6 atmospheres absolute pressure were significantly higher than the controls. A slightly higher total lipid value was achieved with hydrogen peroxide when compared to oxygen at 6 atmospheres absolute. Only a trace of lipid was detected in samples incubated in saline at normal pressures.

(3) *In vivo* studies of changes in total serum in humans following the intra-arterial infusion of hydrogen peroxide.

Venous blood samples were collected from the cubital vein immediately before and after the infusions of .24-.48 per cent H_2O_2 into the abdominal aorta with a total time lapse between the two samples of 20-30 minutes. Serum lipid determinations were carried out as described earlier. It was noted that the total amount of increase in lipids following the infusion seems to be related to both severity of disease and concentration of hydrogen peroxide infused.

Clinical Trial

A 59-year-old woman with severe basilar artery insufficiency secondary to inoperable

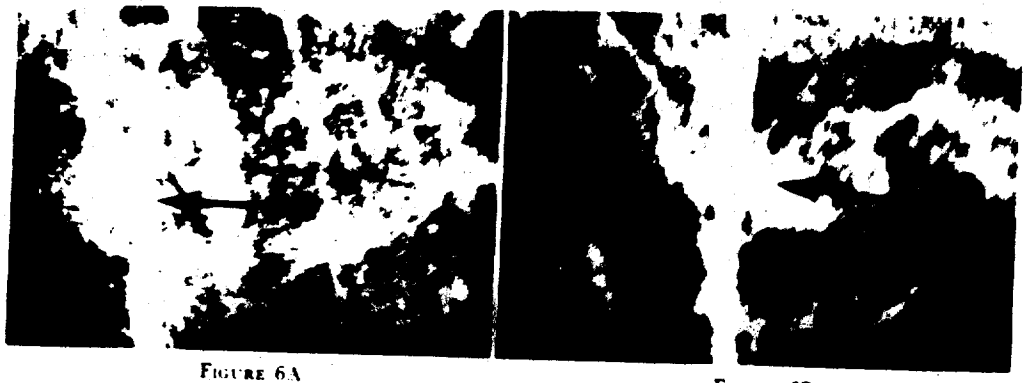


FIGURE 6A FIGURE 6B

FIGURE 6: Right vertebral arteriogram performed by retrograde innominate artery injection before (A) and after (B) intra-arterial H_2O_2 therapy demonstrates apparent diminution in the arteriosclerotic stenosis of the mid-vertebral artery.

arteriosclerotic stenosis of her mid right vertebral artery, whose symptoms were progressive on heparin therapy, was treated by intra-arterial H_2O_2 in October 1966. Her left vertebral artery was congenitally small and both carotid arteries were patent. An indwelling catheter was placed retrograde into the innominate artery through a branch of the thyrocervical trunk. Over 29 days, she was given 100 treatments, each consisting of .12 per cent H_2O_2 in 5 per cent D and W and 12 mg of tolazoline (Priscoline) at a rate of 18 ml/min.

Her symptoms, which were progressive, appeared to stabilize and possibly improve on early evaluation. The arteriogram following H_2O_2 therapy, in contrast to that obtained before treatment, revealed an apparent decrease in the arteriosclerotic stenosis (Fig. 6a, b) and less delay in the vertebral artery filling time when compared to the carotid artery. A long-term follow-up will be necessary to evaluate the neurologic and arteriographic result.[11]

Potentiation of Effect of Irradiation Therapy on Malignant Tumors by Intra-arterial Hydrogen Peroxide

One of the basic radiobiologic concepts dealing with the lethality of ionizing radiation is concerned with the oxygen concentration in a tissue at the time of its exposure to radiation. It has been known that the slope of the radiosensitivity curve increases steeply as the oxygen concentration increases above anoxia, and at high oxygen concentration reaches a maximum sensitivity, which is difficult to exceed by increasing the amount of oxygen present. Due to the innate anoxic character of neoplastic tissue and the fact that the greatest increase of radiosensitivity is seen at the lower oxygen levels, the oxygen effect is more pronounced in some tumor tissue than in the corresponding or adjacent normal tissue. Therefore, the radiotherapeutic ratio can be favorably influenced by increasing the oxygen in the region of the tumor. Various groups have used hyperbaric oxygen to achieve this result in the treatment of malignant tumors. Our group has used intra-arterial hydrogen peroxide in a regional system to achieve the same result.[10,19,21]

A catheter is introduced into the artery supplying the neoplasm under direct surgical exposure and left in place for several weeks. At the present time, the following three areas are used: the external carotid artery is preferably used for treatment of head and neck malignancies, the brachial or femoral arteries for extremity tumor therapy and the abdominal aorta retrograde through the femoral artery for therapy of malignant disease in the abdomen and pelvis. The infusions and standard protracted external irradiation to an expected 100 per cent tolerance dosage is given daily. The irradiation therapy is carried out during the latter portion of the infusion and the infusion is terminated following the radiation.

Care is taken with infusion of solutions of hydrogen peroxide to avoid severe vessel spasm and bubbling. With careful individualization and experience, all significant complications such as infection, clotting, etc., have virtually been eliminated.

From 1961 through 1965, 131 patients with malignancies have been treated using this technique. We have selected patients who in our opinion had less than a 10 per cent chance of surviving three years if the accepted forms of therapy had been employed. Although the number of patients are small and the number of variables great, the 44 per cent three-year survivals in this group of patients with advanced disease appears significant. Specifically, we are encouraged with the results to date in the head and neck series, advanced cervix and ovary, recurrent carcinoma of the colon and carcinoma of the bladder. The results in the carcinoma of the bladder appear specifically significant when considered in the light of our dosage of 4500 rads in four weeks to the entire pelvis.

Preferential Localization of Radio-isotopes in Malignant Tissue by Intra-arterial Hydrogen Peroxide

It has been observed that when certain

isotopes were given intra-arterially following the intra-arterial infusion of hydrogen peroxide, the isotope localized preferentially in the area of the malignant tumor in the infused field.[11,12,22,23] These studies were extended and conducted in rats, using the Walker 256 carcinosarcoma; in rabbits, using the VX-2 carcinoma; and in humans having a variety of malignancies.

Utilizing HELA cells and KB cells has allowed us to demonstrate selective uptake of radioactive iodinated blood albumin (RISA) under the direct influence of hydrogen peroxide. The process is reversible, such that the application of hydrogen peroxide and its withdrawal prior to the addition of the isotope reveals virtually no uptake of the large molecule isotope. Similar, but as yet incomplete studies using the same modality under hyperbaric conditions of 2 to 6 atmospheres demonstrate the same phenomena, and this also appears to be reversible. The mechanism is unknown as yet, but the selective reaction of normal cells to tumor cells appear to be in a ratio of approximately 1:5. In addition, the effective half-life of the isotope is prolonged because of the considerable increase in time that the isotope remains inside the cell. Autoradiographic studies using the electron microscope at 40,000 x show the distribution to be predominantly at the cell wall, and within the nucleus.

Subsequent studies utilizing VX-2 symmetrical hind leg tumors in rabbits, catheterizing both iliac arteries individually, flushing the left with 7 ml of carrier solution, 10 microcuries of RISA, and following with 3 ml of carrier solution show little or no concentration of RISA at 48, 72, or 96 hours. The right leg was infused in the same manner with the exception of .12 per cent hydrogen peroxide concentration in the carrier solution. In contrast to the control, the right leg showed considerable concentration of the isotope within the tumor at 24, 48, 72, and even 96 hours. Forty animals were tested this way and all demonstrated the same phenomenon.

Retrospective type human scanning studies were then carried out. Seventy-eight scans have been done. The procedure used entailed doing a percutaneous puncture of either the femoral or the carotid artery with a 19-gauge Cournand needle. A catheter is then threaded through the needle until the tip is at the desired level, 200 [illegible] of from .12 per cent to .48 per cent hydrogen peroxide in Ionosol T with dextro[illegible] infused, the infusion is then stopped [illegible] 250 microcuries of RISA is injected i[illegible] the catheter. Following this, 50 ml of [illegible] peroxide solution is infused. The proced[illegible] is then completed and the catheter a[illegible] needle removed. Scans are done at 24, [illegible] 72, and occasionally 96 hours. The procedure is carried out completely on an outpatient basis.

Malignancies of the brain, extremity, thorax, abdomen and pelvis have been studied. No positive localization has occurred in lesions other than malignant tumors. In 78 scans, three false negative and one false positive scans have resulted. The false negative scans represented two cases of pulmonary metastasis and probably demonstrate an inability to infuse adequately this area as yet. One false negative scan was a poorly vascularized recurrent carcinoma of the thyroid. The false positive scan occurred in a patient with an extremity rhabdomyosarcoma. Pelvic metastases were diagnosed by the scan, but at surgery, these were not found.

Peripheral Blood Changes Following Infusion of Hydrogen Peroxide Into the Carotid Artery

Peripheral blood studies on patients receiving adjunctive H_2O_2 therapy demonstrated an increase in one or more of the formed blood elements when the H_2O_2 was infused in the internal or common carotid arteries. This phenomenon was not observed with H_2O_2 infusion in other arteries.[24]

A threefold increase in circulating platelets and reticulocytes has been observed in rabbits following the infusion of hydrogen peroxide in "Ionosol-T" into the common

carotid artery in contrast to the increase in controls. The maximum increase was 24 hours after infusion. A threefold increase in one or more of the formed elements in the peripheral circulation (platelets, reticulocytes, or white blood cells) has been noted in 11 of 13 patients being infused with a solution of hydrogen peroxide into either the internal or common carotid artery. Studies are in progress to determine the nature and mechanisms of the observed stimulation and to delineate any humoral factors which might be involved. A relationship between a possible humoral factor and "erythropoietin" is being investigated.

The Effect of Intra-arterial Hydrogen Peroxide on Clostridium Perfringens Infection

The success of hyperbaric oxygen on aerobic "gas gangrene" infections prompted the use of regional intra-arterial H_2O_2 in the therapy of these infections.[13] Clostridial myositis was produced in 48 rabbits by injection of a *Clostridium perfringens*-epinephrine preparation into the left thigh. Twenty-four of these rabbits were treated with intermittent infusions of 0.18 per cent hydrogen peroxide in normal saline with tolazoline hydrochloride (Priscoline). Twenty-four control rabbits were treated with intermittent infusions of a normal saline with tolazoline hydrochloride (Priscoline). The hydrogen peroxide-treated rabbits had a mortality of 45.9 per cent compared with a 87.6 per cent mortality in the control group. These mortality figures suggest that intra-arterial hydrogen peroxide may be of benefit in the treatment of clostridial myositis.

Acceleration of Wound Healing by Intra-arterial Hydrogen Peroxide

During the early studies in the treatment of patients with intra-arterial hydrogen peroxide and irradiation therapy, it was found that the wounds would heal at a much faster rate and with less scar formation. As a result of this, wounds that were refractory to conventional modes of therapy were treated with intra-arterial hydrogen peroxide either alone or in combination with intra-arterial antibiotics.[14] The techniques used were daily or twice daily infusions of intra-arterial hydrogen peroxide, with or without intra-arterial antibiotics in 25 patients. All refractile wounds were healed promptly by this method. Several amputations were avoided. Further investigation of tensile strength, scar formation and role of healing in calibrated wounds in animals should be performed.

Summary

Investigation of dilute solutions of hydrogen peroxide as an improved method of oxygenating tissues over hyperbaric oxygenation has been conducted. Hydrogen peroxide given intra-arterially in the regional system or directly to the exterior of an organ is decomposed into oxygen and water and produces oxygen tensions ranging from 3 to 8 atmospheres pressure regionally. This does not require lung transport, can be given continuously over long periods of time, can be administered by a single physician without expensive equipment and large teams and avoids decompression and compression hazards, as well as pulmonary and CNS toxicity of hyperbaric oxygenation. Hydrogen peroxide has been demonstrated to be an adjunctive source of oxygen for the anoxic or ischemic heart and can improve resuscitation in refractile arrhythmias or cardiac arrest. Intra-arterial infusion has been noted to reverse the atherosclerotic process, potentiate the effect of irradiation on malignant tumors, increase the localization of radioisotopes in malignant tumors, protect against *Clostridium welchii* infections, promote wound healing and serve as a safe, left source of regional oxygenation without significant systemic toxicity. Further investigation in all areas is necessary to establish the quantitative value and limitations of H_2O_2.

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THE SPERMICIDAL ACTIVITY OF HYDROGEN PEROXIDE *IN VITRO* AND *IN VIVO*

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SUMMARY

The effect of hydrogen peroxide on the motility of bull, fowl, dog, ram, mouse, rabbit and human spermatozoa has been studied *in vitro*. Although 3 p.p.m. peroxide had a small but significant action on bull and fowl spermatozoa, those of the other species were more resistant, and 30–300 p.p.m. was usually required to produce substantial spermicidal effects, and even 3000 p.p.m. failed to immobilize rabbit spermatozoa completely.

Ram, dog and rabbit spermatozoa were rendered more susceptible by washing, and the seminal plasma was found to decompose hydrogen peroxide. The agent, in rabbit seminal plasma at least, is probably catalase.

Hydrogen peroxide given orally to mice and rabbits did not affect either the spermatozoa or fertility.

The toxicity of hydrogen peroxide to mammalian spermatozoa [Guenther, 1907; Mann & Leone, 1953; Mann, 1954] is of particular interest since, apart from its possible value as a contraceptive, hydrogen peroxide is known to be produced by spermatozoa under aerobic conditions [Tosic & Walton, 1950] and is responsible for the detrimental effect of the oxygenation of mammalian semen which may occur during transport and handling [Macleod, 1943*a*; Prince & Almquist, 1948; Van-Demark, Salisbury & Bratton, 1949]. Marine spermatozoa are also susceptible to peroxide, and the harmful action of X-rays on these and other spermatozoa may be due, at least partially, to the formation of peroxide in the medium [Evans, 1947; Barron, Gasvoda & Flood, 1949].

According to Holman [1957], rats implanted with adenocarcinoma can be successfully treated by replacing their drinking water with dilute solutions of hydrogen peroxide, and this therapy has also been tried on human patients suffering from tumours. If, as these observations suggest, orally administered hydrogen peroxide were to find its way into the tissues it might be expected to have an adverse effect on spermatozoa in the epididymis. Although, perhaps, undesirable in the treatment of cancer, this could provide a means of contraception.

The work reported in this paper was undertaken to determine the spermicidal level of hydrogen peroxide for a number of species and to study the effect on fertility of orally administered hydrogen peroxide.

MATERIALS AND METHODS

Collection of semen

Bull and rabbit semen was collected by artificial vagina [White, 1954], dog and fowl semen by massage [Burrows & Quinn, 1939]. Rams with a surgical fistula into one vas deferens were used so that on electrical ejaculation epididymal spermatozoa could be obtained from the fistula and the ejaculated semen from the other testis. The epididymal spermatozoa were adjusted to the same concentration as the ejaculated cells by adding calcium-free Krebs–Ringer–phosphate–fructose diluent (0·132M–NaCl, 0·005M–KCl, 0·001M–KH_2PO_4, 0·001M–$MgSO_4.7H_2O$, 0·010M–Na_2HPO_4 and 100 mg/100 ml. fructose) [Umbreit, Burris & Stauffer, 1949]. Normal human ejaculates were provided by a sterility clinic. Mouse epididymal spermatozoa were obtained by dissection immediately after killing the animals and suspended in a small volume of diluent.

Metabolic and motility studies

Commercial 100 vol. (30 %) hydrogen peroxide was checked by iodometric titration and diluent added to give solutions containing 3000·0, 300·0, 30·0, 3·0, and 0·3 p.p.m. H_2O_2. Diluent without added peroxide was used as a control.

Semen was mixed in small tubes with 10–20 parts diluent for the motility studies and with 5–10 parts diluent for the metabolic experiments. For some tests the spermatozoa were first washed, the semen diluted 1:10, and then centrifuged at 1500 rev./min (approx. 300 g) for 10 min. The supernatant was then replaced with an equal vol. diluent and the spermatozoa were dispersed and recentrifuged. The second supernatant was removed and the volume adjusted to that of the original semen. The washed suspension was then treated in the same way as the unwashed semen.

For the motility studies drops of sperm suspensions were examined at ½, 1, 1½ and 2 hr under a low-power microscope and motility was scored by the system of Emmens [1947]. Full motility was rated as 4 and complete immotility as zero. Since quarter-grades were frequently used, however, the total score over the experimental period has been multiplied by 4 to avoid fractions. These values are referred to subsequently as the motility index [Emmens, 1948].

For the metabolic studies at 37° C, aliquots of the spermatozoal suspensions were deproteinized with 5 % $ZnSO_4.7H_2O$ and 0·3N–$Ba(OH)_2$ initially and after 2 hr. Fructose was estimated by the method of Mann [1948] and lactic acid by the technique of Barker & Summerson [1941].

Statistical analysis

The significance of the *in vitro* studies has been assessed by the analysis of variance and calculation of the median effective dose, i.e. the concentration of peroxide causing a 50 % reduction in motility or metabolism. In calculating the median effective dose the data over the region of useful information have been transformed by expressing the response to peroxide as a percentage of the control and then converting to angles [Claringbold, Biggers & Emmens, 1953]. The region of useful information varied for each species, but in all cases included three levels of peroxide which by suitable conversion gave coefficients for log dose of −1, 0 and 1.

Estimation of peroxide breakdown

The iodometric method of Sumner & Somers [1947] was used for the estimation of catalase activity in seminal plasma and washed spermatozoa.

Plasma was obtained by centrifuging the semen and decanting the supernatant which was examined under the microscope to be sure it was free of cells. Usually a 1:4 dilution of plasma with distilled water was found suitable for the estimation. The spermatozoa were suspended in 10 vol. diluent, washed twice and added to the peroxide in as small a volume as possible.

Oral administration of peroxide

Thirty-six 3-month-old male albino mice were caged singly and divided at random into three groups of twelve. The drinking water was then replaced by 0·33, 1 and 3% hydrogen peroxide respectively, and the solutions changed twice weekly to avoid any decrease in activity of the peroxide. The mice were fed the standard laboratory diet *ad lib.* and were weighed at the start of the experiment and at weekly intervals.

The mice on the highest level of peroxide (3%) refused to drink and after 5 days were removed from the experiment as they had lost about 20% of their body weight.

The remaining two groups were each divided at random into four subgroups of three mice for subsequent treatments as follows:

Subgroup 1. Two female mice were placed with each male on the 7th day (day 7) after replacing the drinking water by peroxide solution. The mice mated within a few days and the pregnant females were removed before parturition. On day 28 another pair of females was placed with each male.

Subgroup 2. Two female mice were placed with each male on day 21.

Subgroup 3. As for subgroup 2, except that the peroxide solution was replaced by tap water when the females were placed with the males. This was done in case any effect on fertility was due to the consumption of peroxide by the females.

Subgroup 4. The mice were killed on day 21 and the epididymal spermatozoa examined.

The drinking water of three albino rabbits was also replaced by 0·33, 1 and 3% peroxide and the semen examined at weekly intervals for 6 weeks.

RESULTS

Motility studies

The effect of 0·3, 3·0, 30·0, 300·0 and 3000·0 p.p.m. hydrogen peroxide on the motility of bull, fowl, dog, ram, rabbit, human and mouse spermatozoa is shown in Table 1.

Separate analyses of variance were first performed for each species using the motility indices of each ejaculate as unit observation. Bartlett's test showed that the residual variances were homogeneous for the bull, fowl, ram, dog and rabbit ($\chi^2_6 = 8{\cdot}98$, $P > 0{\cdot}05$).

An overall analysis of variance for these species (Table 2) was therefore undertaken using the mean motility indices which were first transformed as described in the section on statistical analysis.

Table 1. *Effect of hydrogen peroxide on motility index of spermatozoa* in vitro

(Mean values are given with number of replications in parentheses after species.)

	Bull (9)		Fowl (9)		Dog (4)		Ram (5)				Rabbit (4)			Man (9)	Mouse (5)
							Ejaculated		Epididymal						
lrogen oxide p.m.)	Un-washed	Washed	Un-washed	Washed	Un-washed	Washed	Un-washed	Washed	Un-washed	Washed	Un-washed	Washed	Washed plasma	Un-washed	Un-washed
0	50	42	61	60	47	41	56	53	57	51	56	52	56	42	35
0·3	52	43	61	60	48	41	54	53	57	51	59	52	58	41	33
3·0	41	34	48	48	48	45	52	47	58	51	58	52	57	41	33
30·0	9	6	1	1	40	9	22	7	35	19	58	34	54	28	32
300·0	0	0	0	0	2	0	2	0	3	0	45	2	32	0	4
000·0	0	0	0	0	0	0	0	0	0	0	9	0	7	0	0
(p.p.m.)	8·0	7·6	5·0	5·0	57·4	22·1	33·8	8·6	42·7	33·2	797·0	45·3	476·0	31·0	89·0

The slopes for the different species and treatments did not differ significantly and the median effective dose (M.E.D.) of peroxide for each species (see Table 1) was calculated and the variance of each M.E.D. estimated from the error term. The 12 degrees of freedom associated with the M.E.D.'s were partitioned using the mean variance of the M.E.D.'s as error term in the analysis which is summarized in Table 3.

Table 2. *Summary of overall analysis of variance for the transformed data of Table 1, excluding results for man and mouse*

(The error mean square is shown at the foot of the variance ratio column.)

Source of variation	D.F.	Variance ratio
Between means	12	1·6
Average slope	1	491·5**
Between slopes	12	0·5
Error	13	77

** $P < 0{\cdot}01$.

Table 3. *Summary of analysis of the M.E.D.'s*

Source of variation	D.F.	Variance ratio
Species differences	4	729·9**
Overall effect of washing	1	230·3**
Species/washing interaction	4	37·7**
Ram: ejac. *v.* epid. sperm	1	75·8**
Ram: washing/sperm interaction	1	37·4**
Rabbit: effect of plasma	1	327·8**
Error	13	0·00157

** $P < 0{\cdot}01$.

In the preliminary analysis the residual variance proved heterogeneous for man and mouse when compared with the other species and the data were not included in the overall analysis. For each of these species the individual ejaculate motility indices were transformed into angles, the M.E.D. calculated from the common mean slope and the variance of the M.E.D. estimated from the error mean square.

There are large species differences in tolerance to hydrogen peroxide. Bull and fowl spermatozoa are the most susceptible; unwashed dog, ram, human and mouse spermatozoa are less affected, and about five to ten times the concentration of hydrogen peroxide is needed to depress motility. The rabbit spermatozoa were by far the most resistant of those studied, about 100 times as resistant as bull and fowl spermatozoa.

The overall effect of washing spermatozoa was to increase the toxicity of hydrogen peroxide, but this varied with each species. Thus, washing did not affect the susceptibility of bull and fowl spermatozoa, but dog, ram and especially rabbit spermatozoa were rendered less resistant to peroxide. If rabbit spermatozoa were suspended in their own plasma after washing, their resistance to peroxide was increased almost to that of the unwashed cells.

Ejaculated ram spermatozoa were less resistant than epididymal ones and although washing increased the spermicidal activity of peroxide in both cases, the effect was greater on ejaculated spermatozoa.

Since the human semen was normally several hours old at the start of the tests, three fresh ejaculates were obtained and used immediately on collection and again after standing 5 hr at room temperature. The age of the semen did not modify the results.

When hydrogen peroxide was present in concentrations sufficient to depress motility, its effect was usually complete within the first half hour and the ½ and 2 hr motility scores were almost identical. Only in bull spermatozoa at 3 and 30 p.p.m. and ram spermatozoa at 30 p.p.m. peroxide was there a gradual decrease in motility during the 2 hr period.

Metabolic studies

Table 4 shows the mean rate of fructose breakdown and lactic acid accumulation by bull spermatozoa in the presence of 0·3, 3·0, 30·0 p.p.m. hydrogen peroxide. The M.E.D.'s of hydrogen peroxide calculated from these two series of metabolic studies are not significantly different nor do they differ significantly from the M.E.D. calculated from the motility studies.

Estimation of peroxide breakdown by semen

In view of the resistance of rabbit spermatozoa to peroxide, especially in the presence of seminal plasma, attempts were made to detect and estimate the breakdown

Table 4. *Effect of hydrogen peroxide on fructose breakdown and lactic acid production by bull spermatozoa*

(Values are the mean of six and seven ejaculates respectively.)

Hydrogen peroxide (p.p.m.)	Fructose breakdown (μg/10^8 sperm/2 hr)	Lactic acid production (μg/10^8 sperm/2 hr)
0	274	258
0·3	270	255
3·0	195	191
30·0	17	35
M.E.D.	4·5 p.p.m.	3·9 p.p.m.

of hydrogen peroxide by rabbit semen. The capacity of rabbit seminal plasma to decompose peroxide could be readily demonstrated but varied considerably with different ejaculates. The mean weight of hydrogen peroxide broken down by 1 ml. rabbit seminal plasma when mixed with 0·01 M-H_2O_2 at 0° C for 3 min was 220 mg (range 95–353 for eight ejaculates, S.E. = 36); the mean monomolecular K value (K_s) [Sumner & Somers, 1947] was 0·13 (range 0·06–0·23, S.E. = 0·02). The breakdown of peroxide was completely inhibited by boiling the plasma for 5 min, by 10^{-3}M sodium azide and by 10^{-4}M sodium cyanide; 10^{-5}M sodium cyanide caused about 70% inhibition. Sodium fluoride (10^{-3}M) usually decreased activity by about 30%. The results for rabbit spermatozoa were also variable. The mean weight of hydrogen peroxide decomposed by 10^9 cells in 3 min was 81 mg (range 40–153 for six ejaculates, S.E. = 17) and the mean K_s = 0·09 (range 0·03–0·22, S.E. = 0·03).

Since ram and dog spermatozoa were also more susceptible to hydrogen peroxide on washing, ram seminal plasma, dog sperm-fraction plasma and dog prostatic fluid were each checked for their capacity to decompose peroxide.

Ram seminal plasma and dog sperm-fraction plasma had some activity, but it was too low to be estimated accurately. Dog prostatic fluid was completely inert.

Oral administration of peroxide

Table 5 shows the number and size of the litters and the number of days to parturition after placing non-lactating female mice with the males receiving peroxide. As the mean gestation period of non-lactating mice in the laboratory colony is $20 \pm 1{\cdot}0$ days the approximate time of conception can be estimated by subtracting this figure from the number of days to parturition.

Table 5. *Fertility of mice receiving hydrogen peroxide orally*

Subgroup	Days on peroxide*	Peroxide (%)	No. of litters†	Days to parturition (± S.E.)	Litter size (± S.E.)
1	7	0·33	6	23·3 ± 1·7	5·3 ± 0·6
	7	1·00	6	30·0 ± 1·7	5·3 ± 0·8
2	21	0·33	6	25·2 ± 0·6	5·3 ± 0·7
	21	1·00	6	33·5 ± 2·0	5·8 ± 0·5
3‡	21	0·33	6	24·5 ± 0·7	6·5 ± 0·4
	21	1·00	6	23·0 ± 0·3	5·0 ± 0·6
1	28	0·33	6	23·5 ± 0·4	7·0 ± 0·4
	28	1·00	6	24·8 ± 0·4	5·2 ± 0·6

* Prior to placing females with males. † Maximum possible was six.
‡ Peroxide replaced by water after females placed in cages.

All females became pregnant within a few days and in each case healthy young were born in litters of normal size. In subgroups 1 and 2, 1% peroxide appeared to cause some delay in parturition. The effect was, however, small and inconsistent and may well have been caused by extraneous factors.

All males receiving 0·33 and 1% peroxide increased in body weight, and there was no significant difference in gain between these groups during the first 3 weeks. During the subsequent fortnight the mice in subgroups 1 and 2 gained weight less rapidly than those in subgroup 3, the mean gains and standard errors being $1{\cdot}04 \pm 0{\cdot}14$ and $4{\cdot}42 \pm 1{\cdot}06$ g respectively. Since the peroxide of subgroup 3 was replaced by normal drinking water on the 21st day, it seemed possible that the lower concentrations of peroxide, as well as the 3%, caused some reduction in the rate of gain in body weight.

The concentration, morphology and motility of the mouse spermatozoa from subgroup 4 and the spermatozoa of the three rabbits receiving peroxide appeared quite normal.

DISCUSSION

Although 3 p.p.m. hydrogen peroxide had small but significant effects on bull and fowl spermatozoa in the present study, those of other species were more resistant and 10–100 times this concentration (i.e. 30–300 p.p.m.) was usually required to produce substantial spermicidal effects over a 2 hr period and even 1000 times (i.e. 3000 p.p.m.) failed to immobilize rabbit spermatozoa completely. Exogenous hydrogen peroxide may not, therefore, be quite as spermicidal as is generally believed although when produced endogenously it may be more active (Macleod, 1943b

Tosic & Walton, 1950]. In the case of human spermatozoa, for instance, it is not nearly as toxic as diethyldithiocarbamate which usually abolishes motility completely at a concentration of 15 p.p.m. under similar conditions [White, 1955, 1956]. Nevertheless, hydrogen peroxide is more active than the constituents of most contact contraceptives; it is also cheap, readily available and harmless to tissues generally, but apparently has not been used as a spermicide. The results presented here, however, indicate that hydrogen peroxide would not be effective as an oral contraceptive.

The spermatozoa and fertility of human subjects should not be affected by oral peroxide therapy. The absence of such a side effect is highly desirable, but this finding suggests that peroxide may be broken down before reaching the tissues and thus brings the whole rationale of the cancer treatment into question. It may be noted that saliva [Nickerson, Kraus & Perry, 1957] and erythrocytes [Sumner & Somers, 1947] both contain enzymes that decompose hydrogen peroxide, and that Green & Westrop [1958] have been unsuccessful in treating rat tumours with hydrogen peroxide.

The concentrations of peroxide required to reduce motility, fructolysis and lactic acid production of bull spermatozoa are very similar; and all three effects probably stem from the oxidation of essential sulphydryl groups in enzymes of the glycolytic cycle [Mann & Leone, 1953].

There can be little doubt that the high resistance of unwashed rabbit spermatozoa to hydrogen peroxide is due chiefly to breakdown by the seminal plasma. All the peroxide in the tubes (even at 3000 p.p.m.) would be expected to be decomposed by rabbit seminal plasma within 3 min. Since this property is destroyed by boiling, and inhibited by cyanide, azide and fluoride, the agent is probably catalase [Keilin & Hartree, 1936], rather than pyruvate which Tosic & Walton [1950] have also shown to be capable of eliminating peroxide.

Ram and dog spermatozoa were also rendered more susceptible to peroxide by washing and some breakdown by the seminal plasma could be demonstrated. In view of this, unwashed ejaculated spermatozoa might have been expected to be less affected by peroxide than were epididymal spermatozoa. In actual fact, however, the reverse is true for the ram. Fowl and bull seminal plasma seem to be completely devoid of catalase activity, and this explains why no increase in spermicidal effect was found on washing. The low catalase content of the spermatozoa of some species has also been noticed by other workers and no doubt is an important factor in the toxic action of peroxide [Mann, 1954; Tosic & Walton, 1950].

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