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PULMONARY OXYGEN TOXICITY: THE ROLE OF BIOGENIC AMINES, THE SYM--ETC(U)
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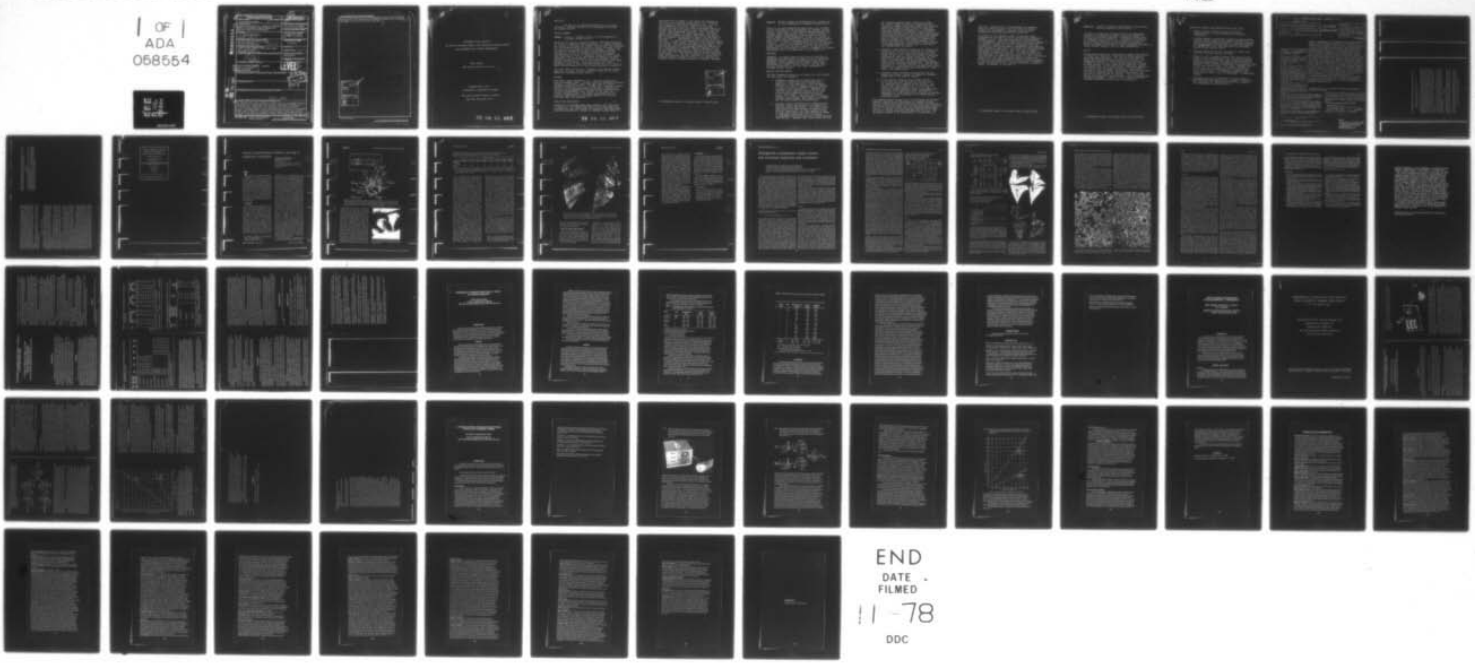

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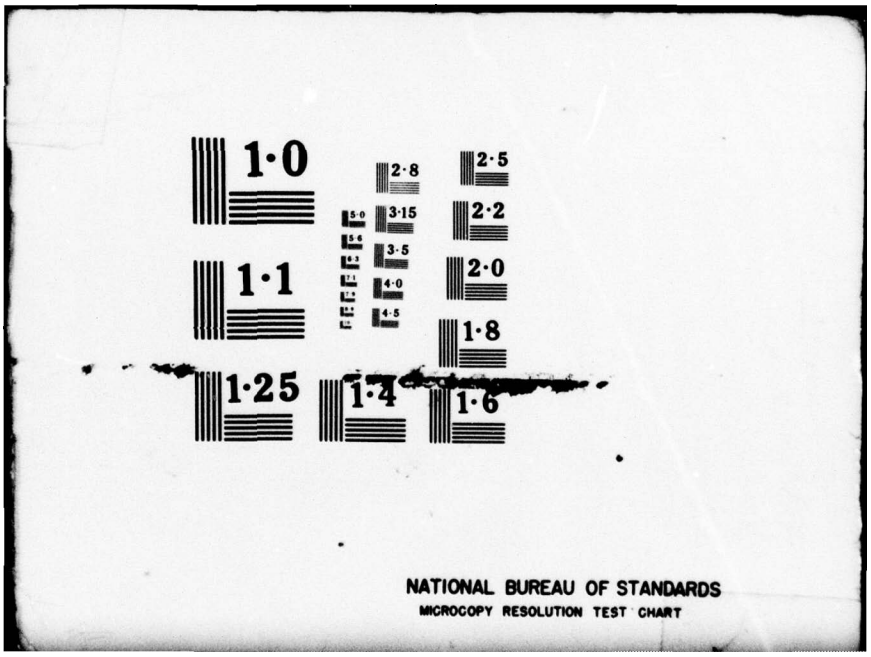
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This research project was conducted to evaluate the role of systemic factors in the pathogenesis of pulmonary oxygen toxicity. Studies were conducted on conscious adult male mongrel dogs. The following conclusions were reached: (1) Severe systemic hyperoxia has a significant aggravating effect in the pathogenesis of pulmonary oxygen toxicity; (2) Endogenous circulating and pulmonary tissue biogenic amines do not appear to have a direct relationship with the severity of lung damage by oxygen. (3) Surgical denervation of the lung does not protect it from toxic effects of oxygen. 4) Pathologic changes in the lungs do

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not appear to be the consequence of hemodynamic changes while animals b[illegible]
100% oxygen at 1 Ata or 2 Ata until they died.

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PULMONARY OXYGEN TOXICITY:
THE ROLE OF BIOGENIC AMINES, THE SYMPATHETIC NERVOUS SYSTEM, AND PULMONARY AND SYSTEMIC HEMODYNAMICS

FINAL REPORT

ONR CONTRACT N00014-75-C-0323 new

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PROFESSOR OF EXPERIMENTAL SURGERY

NEW YORK UNIVERSITY MEDICAL CENTER

NEW YORK, NEW YORK 10016

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OBJECTIVE

THE GOAL OF THIS RESEARCH PROJECT WAS TO EVALUATE THE ROLE OF SYSTEMIC FACTORS IN THE PATHOGENESIS OF PULMONARY OXYGEN TOXICITY.

PROJECT SUMMARY

PHASE I: LOCAL VS SYSTEMIC FACTORS IN THE PATHOGENESIS OF PULMONARY OXYGEN TOXICITY.

INJURY TO THE LUNG IS A SERIOUS HAZARD OF PROLONGED INHALATION OF OXYGEN AT INCREASED PARTIAL PRESSURES (*). OXYGEN TENSIONS ARE HIGHEST IN THE LUNG, AND SEVERAL INVESTIGATORS HAVE CONCLUDED THAT THE DAMAGING EFFECT OF OXYGEN IS DUE TO THE DIRECT ACTION OF THIS GAS ON THE ALVEOLAR CAPILLARY MEMBRANE. HOWEVER, THE EXISTENCE OF CONCOMITANT SYSTEMIC HYPEROXYGENATION RAISED THE POSSIBILITY THAT THE PULMONARY EFFECT OF HIGH OXYGEN PRESSURE RESULTED PARTLY OR COMPLETELY FROM A SYSTEMIC RATHER THAN LOCAL EFFECT. IT HAD BEEN SHOWN FOR EXAMPLE THAT NEUROSYMPATHETIC AND NEUROMUSCULAR ACTIVITY ARE REDUCED, ANIMALS EXPOSED CONTINUOUSLY TO HIGH OXYGEN TENSIONS DIED WITHOUT GROSS EVIDENCE OF LUNG DAMAGE. ALSO, STUDIES OF LUNG TISSUE METABOLISM UNDER IN VITRO CONDITIONS SHOWED THAT THIS TISSUE IS AMONG THE MOST RESISTANT TO THE POISONING EFFECTS OF OXYGEN (*).

THE FIRST PHASE OF THE STUDY, THEREFORE, WAS DIRECTED TOWARDS EVALUATION OF LOCAL VIS-A-VIS SYSTEMIC FACTORS IN THE PATHOGENESIS OF PULMONARY OXYGEN TOXICITY.

METHODS

EXPERIMENTS WERE PERFORMED ON FULLY CONSCIOUS UNMEDICATED ADULT MALE MONGREL DOGS. THIS CONDITION WAS CONSIDERED ESSENTIAL INASMUCH AS ANESTHETIC AGENTS HAVE BEEN SHOWN TO INHIBIT PULMONARY OXYGEN TOXICITY (*). A MODEL WITH A BRONCHO-CUTANEOUS FISTULA TO THE RIGHT DIAPHRAGMATIC LOBE MADE IT POSSIBLE TO MAINTAIN WIDE DIFFERENCES IN INSPIRED OXYGEN TENSIONS BETWEEN THE RIGHT DIAPHRAGMATIC LOBE, THE REST OF THE LUNG, AND SYSTEMIC ARTERIAL BLOOD. THE METHOD FOR CONSTRUCTING THE FISTULA IS DESCRIBED IN THE ATTACHED REPRINT OF AN ARTICLE PUBLISHED FROM THIS LABORATORY (*)

RESULTS AND CONCLUSIONS

STUDIES ON THE AFOREMENTIONED MODEL REVEALED THAT INHALATION OF OXYGEN AT 5 ATA THROUGH THE FISTULA TO THE ISOLATED RIGHT DIAPHRAGMATIC LOBE, AND THROUGH THE TRACHEA TO THE OTHER LOBES PRODUCED CHARACTERISTIC CHANGES OF PULMONARY OXYGEN TOXICITY.

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ADMINISTRATION OF OXYGEN TO THE TRACHEA AND NITROGEN TO THE FISTULA ALSO PRODUCED OXYGEN TOXICITY IN THE ENTIRE LUNG, INCLUDING THE RIGHT DIAPHRAGMATIC LOBE. THE Pa_{O_2} WAS APPROXIMATELY 2000 MMHG. INHALATION OF OXYGEN THROUGH THE FISTULA AND 10% OXYGEN IN NITROGEN THROUGH THE TRACHEA RESULTED IN NO DAMAGE TO ANY PORTION OF THE LUNG. THE Pa_{O_2} WAS APPROXIMATELY 800 MMHG. THUS, THE RESULTS SHOWED CONCLUSIVELY THAT UNDER HYPERBARIC CONDITIONS, REDUCTION OF SYSTEMIC HYPEROXIA DOES INHIBIT THE DEVELOPMENT OF PULMONARY OXYGEN TOXICITY EVEN IN THE PRESENCE OF VERY HIGH $P_{I_{O_2}}$. FURTHERMORE, PULMONARY OXYGEN TOXICITY WAS SEEN EVEN IN THE ABSENCE OF CONVULSIONS IN ANIMALS THAT INHALE OXYGEN THROUGH THE TRACHEA, AND NITROGEN THROUGH THE FISTULA. THE DATA WAS PRESENTED AND DISCUSSED AT THE NAVYWIDE WORKSHOP IN HIGH PRESSURE BIOMEDICAL RESEARCH IN 1971, THE FORUM ON FUNDAMENTAL SURGICAL PROBLEMS IN 1972 AND THE FASEB MEETING IN 1973. REPRINTS OF PUBLICATIONS IN WHICH OUR DATA WERE PRESENTED AND THE RESULTS DISCUSSED ARE ATTACHED(*). OUR RESULTS AGREED WITH THE FINDINGS OF OTHER INVESTIGATORS, WHO ALSO CONCLUDED THAT REDUCTION OF SYSTEMIC ARTERIAL OXYGENATION PROTECTS THE LUNG FROM THE POISONING EFFECT OF OXYGEN INHALED AT HYPERBARIC PRESSURES.

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PHASE II: BIOGENIC AMINES AND NEUROSYMPATHETIC INNERVATION IN THE PATHOGENESIS OF PULMONARY OXYGEN TOXICITY.

THOUGH IT HAS BEEN DEMONSTRATED CONCLUSIVELY THAT SYSTEMIC FACTORS CONTRIBUTE TO THE PATHOGENESIS OF PULMONARY OXYGEN TOXICITY, THE NATURE OF THESE FACTORS REMAINED OBSCURE. THE BIOGENIC AMINES WERE A MAJOR TARGET OF SUSPICION BECAUSE IT HAS BEEN SHOWN HYPOPHESECTOMY, CATECHOLAMINE INHIBITORS, CENTRAL NERVOUS SYSTEM DEPRESSANTS, AS WELL AS A VARIETY OF OTHER MEASURES AND PHARMACOLOGIC AGENTS WHICH REDUCE BIOGENIC AMINE ACTIVITY DIRECTLY INCREASED TOLERANCE TO HYPERBARIC OXYGEN (*). ALTHOUGH THE MECHANISMS OF ACTION HAVE NOT BEEN CLARIFIED, THERE IS CIRCUMSTANTIAL EVIDENCE THAT THE NEURO-HUMORAL AGENTS AFFECT THE LUNGS DIRECTLY OR INDIRECTLY BY INFLUENCING CELLULAR METABOLIC ACTIVITY, PULMONARY AND SYSTEMIC HEMODYNAMICS, OR BY THE FORMATION OF TOXIC PRODUCTS (*).

PROCEDURE

THEREFORE, THE SECOND PHASE OF THE STUDY WAS DIRECTED TO DETERMINING THE RELATIONSHIP BETWEEN LEVELS OF CIRCULATING ENDOGENOUS BIOGENIC AMINES AND PULMONARY OXYGEN TOXICITY, PULMONARY TISSUE CONCENTRATION OF BIOGENIC AMINES AND DEVELOPMENT OF PULMONARY OXYGEN TOXICITY, AND THE EFFECT OF TOTAL DENERVATION OF THE LUNG UNDER DEVELOPMENT OF PULMONARY OXYGEN TOXICITY.

RESULTS AND CONCLUSIONS

THE MOST NOTEWORTHY ASPECTS OF THE RESULTS OF THIS PHASE OF THE STUDY WERE THE FOLLOWING:

1) HYPERBARIC OXYGENATION WAS ASSOCIATED WITH AN INCREASE IN CIRCULATING CATECHOLAMINES ONLY IMMEDIATELY AFTER CONVULSION. EVEN PROLONGED EXPOSURE (10 HOURS) TO SUBCONVULSIVE O_2 TENSIONS (2 TO 2.5 ATA) DID NOT INCREASE CIRCULATING CATECHOLAMINE LEVELS SIGNIFICANTLY. SEROTONIN AND HISTAMINE LEVELS WERE NOT ALTERED IN THE CIRCULATION DURING HYPERBARIC OXYGENATION WITH OR WITHOUT CONVULSIONS. THE LUNGS FROM ANIMALS EXPOSED TO 10 HOURS OF SUBCONVULSIVE O_2 TENSIONS APPEARED GROSSLY NORMAL, AND SHOWED MINIMAL EDEMA AND CAPILLARY CONGESTION MICROSCOPICALLY.

2) PULMONARY TISSUE CONCENTRATIONS OF NOREPINEPHRINE WERE NOT FOUND TO BE RELATED TO THE SEVERITY OF PULMONARY DAMAGE FROM OXYGEN. FOR EXAMPLE, LUNGS FROM ANIMALS THAT WERE EXPOSED TO SUBCONVULSIVE DOSES OF OXYGEN UNTIL THEY WERE MORIBUND AS WELL AS LUNGS FROM ANIMALS WHICH WERE EXPOSED TO HIGHER O_2 TENSIONS AND CONVULSED UNTIL THEY BECAME STUPEROUS HAD HIGHER CONCENTRATIONS OF NOREPINEPHRINE THAN CONTROLS. HOWEVER, LUNGS FROM ANIMALS THAT DID

NOT CONVULSE APPEARED GROSSLY NORMAL AND SHOWED ONLY MINIMAL EDEMA AND CAPILLARY CONGESTION MICROSCOPICALLY, WHEREAS LUNGS FROM ANIMALS THAT CONVULSED SHOWED GROSS AREAS OF HEPATIZATION AND EDEMA AND CAPILLARY CONGESTION MICROSCOPICALLY. FURTHERMORE, LUNGS FROM ANIMALS THAT APPEARED IN GOOD CLINICAL CONDITION, AND WERE SACRIFICED 10 HOURS AFTER EXPOSURE TO SUBCONVULSIVE LEVELS OF OHP WERE INDISTINGUISHABLE BOTH BY GROSS AND BY MICROSCOPIC EXAMINATIONS FROM LUNGS OF ANIMALS THAT WERE EXPOSED TO SUBCONVULSIVE LEVELS OF OXYGEN UNTIL THEY BECAME MORIBUND. YET, THE 10 HOUR GROUP HAD A SIGNIFICANTLY LOWER PULMONARY TISSUE NOREPINEPHRINE CONCENTRATION THAN EITHER OF THE AFOREMENTIONED TWO GROUPS.

3) PULMONARY TISSUE SEROTONIN LEVELS DO NOT APPEAR TO BE QUANTITATIVELY RELATED TO THE SEVERITY OF PULMONARY OXYGEN TOXICITY. THE LUNGS FROM ANIMALS THAT CONVULSED DID NOT SHOW A SIGNIFICANT CHANGE IN SEROTONIN CONCENTRATION FROM THE CONTROL GROUP, BUT THE LUNGS FROM ANIMALS THAT WERE EXPOSED TO SUBCONVULSIVE LEVELS OF OXYGEN UNTIL THEY BECAME MORIBUND SHOWED SIGNIFICANTLY INCREASED CONCENTRATIONS. THE SEROTONIN CONCENTRATIONS IN ANIMALS EXPOSED TO 10 HOURS OF SUBCONVULSIVE O_2 TENSION WERE NOT INCREASED SIGNIFICANTLY. INDEED, THE CONCENTRATIONS WERE SIGNIFICANTLY LOWER THAN IN THE CONTROL GROUP.

4) PULMONARY TISSUE HISTAMINE CONCENTRATIONS DID NOT CHANGE SIGNIFICANTLY DURING THE COURSE OF HYPERBARIC OXYGENATION IN ANY GROUP OF ANIMALS.

5) TOTAL PULMONARY DENERVATION BY REIMPLANTATION OF THE LEFT LUNG DOES NOT PROTECT THE LUNG FROM THE DEVELOPMENT OF PULMONARY OXYGEN TOXICITY. ON THE CONTRARY, GROSS EXAMINATION OF THE LUNGS FROM ANIMALS THAT WERE EXPOSED TO O_2 AT 3 ATA, AND CONVULSED SHOWED MORE GROSS DAMAGE IN THE DENERVATED LUNG THAN IN THE INNERVATED LUNG. MICROSCOPIC EXAMINATION OF THESE LUNGS DID NOT SHOW ANY APPRECIABLE DIFFERENCE IN THE APPEARANCE OF THE INNERVATED AND DENERVATED LUNGS.

THE FOREGOING RESULTS SUGGEST THAT CATECHOLAMINES AND POSSIBLY SEROTONIN INFLUENCE THE PATHOGENESIS OF PULMONARY OXYGEN TOXICITY THROUGH SOME INDIRECT MECHANISM RATHER THAN DIRECTLY. INASMUCH AS DEATH IN ANIMALS THAT DID NOT CONVULSE WAS ASSOCIATED WITH STUPOR AND COMA RATHER THAN WITH SEVERE PULMONARY INSUFFICIENCY, IT APPEARS THAT THE MECHANISMS INVOLVED MAY BE ONES THAT AFFECT THE BRAIN PERHAPS EVEN MORE THAN THE LUNG. THE RECIPROCAL CHANGES IN THE CONCENTRATION OF CIRCULATING AND

PULMONARY TISSUE LEVELS OF CATECHOLAMINES AND SEROTONIN MAKES THE HYPOTHESIS ABOUT THE TOXIC EFFECT OF ADRENOCHROMES THAT ARE FORMED BY INCREASED CYCLIZATION OF EPINEPHRINE DURING OHP PARTICULARLY ATTRACTIVE.

THE RESULTS OF THE PRECEDING ASPECT OF OUR STUDY WERE PRESENTED AND DISCUSSED AT THE 5TH INTERNATIONAL HYPERBARIC CONGRESS IN 1973. THEY WERE PUBLISHED IN THE PROCEEDINGS
... OF THE CONGRESS; REPRINTS OF THESE PUBLICATIONS ARE ATTACHED (*). IN SUBSEQUENT STUDIES THE DISTRIBUTION OF THE CARDIAC OUTPUT TO THE RIGHT (INNERVATED) AND LEFT (DENERVATED) LUNG WAS MEASURED WITH RADIOACTIVE XENON. MEASUREMENTS WERE PERFORMED UNDER HYPERBARIC CONDITIONS. THIS ASPECT OF THE WORK REQUIRED DEVELOPMENT OF GEIGER MULLER TUBES THAT WOULD WITHSTAND ATMOSPHERIC PRESSURES UP TO 3 ATA. THE RESULTS SHOWED THAT THE DENERVATED LEFT LUNG RECEIVED 54% OF THE CARDIAC OUTPUT WHEN THE ANIMAL WAS BREATHING 100% OXYGEN AT 2 ATA. THE RESULTS TOGETHER WITH DATA ABOUT PULMONARY ARTERIAL PRESSURES IN ANIMALS WITH UNILATERAL PULMONARY DENERVATION WERE PRESENTED AT THE FORUM OF FUNDAMENTAL SURGICAL PROBLEMS OF THE AMERICAN COLLEGE OF SURGEONS IN 1973, AND WERE PUBLISHED IN SURGICAL
... FORUM; THE REPRINT IS ATTACHED (*)

(*) REFERENCES APPEAR IN ATTACHED COPIES OF PUBLICATIONS.

PHASE III: CHANGES IN PULMONARY HEMODYNAMICS DURING DEVELOPMENT OF PULMONARY OXYGEN TOXICITY.

THE DEARTH OF PUBLISHED INFORMATION ABOUT THE HEMODYNAMIC CHANGES IN THE GREATER AND LESSER CIRCULATIONS DURING THE EVOLUTION OF PROLONGED EXPOSURE TO HYPERBARIC OXYGEN LED US TO INVESTIGATE THIS QUESTION (*). THE EDEMA AND CONGESTION OF PULMONARY OXYGEN TOXICITY WAS ASCRIBED BY AT LEAST ONE GROUP OF INVESTIGATORS TO SEVERE SYSTEMIC HYPERTENSION DURING THE COURSE OF EXPOSURE TO OXYGEN HYPERBARIC PRESSURES (*). THESE FINDINGS, HOWEVER, HAVE NOT BEEN CORROBORATED.

METHOD

STUDIES WERE CONDUCTED ON CONSCIOUS MALE MONGREL DOGS EXPOSED TO OXYGEN UNTIL THEY DIED. ONE GROUP OF ANIMALS RECEIVED 96% OXYGEN AND NITROGEN AT 1 ATA, AND THE OTHER GROUP OF ANIMALS RECEIVED 90% OXYGEN AND NITROGEN AT 2.2 ATA. THE HEMODYNAMIC CHANGES THAT WERE NOTED IN THESE TWO GROUPS DIFFERED IN THAT THE 1 ATA GROUP SHOWED PERSISTENT INCREASE IN MEAN PULMONARY ARTERIAL PRESSURE AND REDUCTION IN HEART RATE WHEREAS THE 2.2 ATA GROUP SHOWED A PERSISTENT REDUCTION IN CARDIAC OUTPUT AND STROKE VOLUME WITH A SIMULTANEOUS INCREASE IN TOTAL PERIPHERAL AND PULMONARY VASCULAR EXISTENCES. LEFT ATRIAL PRESSURE WAS NOT INCREASED SIGNIFICANTLY AND EVIDENCE OF HEART FAILURE WAS WANTING. THE DATA AND THE RESULTS WERE PRESENTED, AND DISCUSSED AT THE 6TH INTERNATIONAL HYPERBARIC CONGRESS. A DRAFT OF A FULL LENGTH PAPER WHICH HAS BEEN SUBMITTED FOR PUBLICATION IS ENCLOSED(*).

(*) REFERENCES APPEAR IN ATTACHED COPIES OF PUBLICATIONS.

OVERVIEW OF RESULTS AND SUGGESTION FOR FUTURE STUDY.

1. SEVERE SYSTEMIC HYPEROXIA PLAYS A SIGNIFICANT AGGRAVATING ROLE IN THE PATHOGENESIS OF PULMONARY HYPERTENSION.

2. ALTHOUGH CHANGES IN ENDOGENOUS CIRCULATING AND PULMONARY TISSUE BIOGENIC AMINES DO NOT APPEAR TO BE RELATED TO THE SEVERITY OF LUNG DAMAGE BY OXYGEN THE RECIPROCAL CHANGES AND CATECHOLAMINES AND SEROTONIN MAY FAVOR THE FORMATION OF ADRENOCHROMES WHICH HAVE BEEN SHOWN TO HAVE POTENT EFFECTS ON THE LUNG AND BRAIN.

3. SURGICAL DENERVATION OF THE LUNG DOES NOT PROTECT THE LUNG FROM THE TOXIC EFFECTS OF OXYGEN.

4. OXYGEN AT 1 ATA IN ANIMALS EXPOSED UNTIL DEATH DID NOT CAUSE SYSTEMIC HYPERTENSION OR LEFT VENTRICULAR FAILURE. OXYGEN AT 2 ATA, ON THE OTHER HAND, PRODUCED EVIDENCE OF CONTINUOUS DEPRESSION OF LEFT VENTRICULAR FUNCTION AND MODERATE INITIAL SYSTEMIC HYPERTENSION THAT WOULD NOT CAUSE FAILURE OF A HEALTHY HEART. LEFT ATRIAL PRESSURES BOTH AT 1 ATA AND AT 2 ATA ROSE ONLY MINIMALLY AND NOT TO LEVELS WHICH WOULD BE ASSOCIATED WITH PULMONARY EDEMA IN THE HEALTHY LUNG. THE PATHOLOGIC CHANGES IN THE LUNGS DID NOT APPEAR TO BE THE CONSEQUENCE OF HEMODYNAMIC CHANGES.

5. CONTINUED STUDY OF THE FORMATION AND TOXIC EFFECTS OF ADRENOCHROMES UNDER CONDITIONS OF HYPEROXIA, APPEARS TO BE A LEAD WORTHY OF FURTHER EVALUATION FOR ITS ROLE IN THE PATHOGENESIS OF OXYGEN TOXICITY.

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SYSTEMIC HYPERBAROXIA IN THE PATHOGENESIS OF PULMONARY OXYGEN TOXICITY. Theobald Reich*, Joseph Tait*, and Toshio Suga*. (SPON: William M. Manger) N.Y.U. Med. Ctr., New York, N.Y. 10016

The role of systemic hyperbaroxia in the pathogenesis of pulmonary oxygen toxicity was studied in dogs with isolated broncho-cutaneous fistulas to the right diaphragmatic lobe. This model made it possible to vary systemic and alveolar oxygen tensions independently within a wide range. Studies were on conscious animals at 4.7 Ata for 5 hrs or until earlier death. Ventilation with 100% O_2 via both trachea and fistula (Group I, 7 animals, PaO_2 2903±86 mm Hg) produced macroscopic lung damage and convulsions in all animals with death before 5 hrs in 2; 100% O_2 via trachea and 10% O_2 + 90% N_2 via fistula (Group II, 11 animals PaO_2 1999±65) produced damage in all lobes in each animal, convulsions in 5 and death before 5 hrs in 2; 100% O_2 via fistula and 10% O_2 + 90% N_2 via trachea (Group III, 12 animals, PaO_2 795±82), and air via trachea and fistula (Group IV, 6 animals, PaO_2 574±23) produced no damage, no convulsions, and no deaths before 5 hrs.

Inasmuch as systemic hyperbaroxia results in lung damage even in lobes with low PaO_2, it is concluded that systemic hyperbaroxia plays a significant role in the pathogenesis of pulmonary oxygen toxicity.

(Supported by the J.A. Hartford Foundation and ONR Contract No. N00014-68-A-0292.)

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PATHOGENESIS OF PULMONARY OXYGEN TOXICITY

TOSHIO SUGA, M.D., JOSEPH L. TAIT, D.V.M., AND
THEOBALD REICH, M.D., F.A.C.S.

INHALATION OF OXYGEN at high partial pressures damages the lung. Inasmuch as oxygen tensions are highest in the lung it would seem that the damage results from direct action of oxygen on lung tissue (1). However, concomitant systemic hyperoxygenation raises the possibility that systemic factors also play a significant role. This is supported by findings that in *in vitro* tissue respiration studies lung tissue is one of the most resistant to the poisoning effects of oxygen and that in intact animals resistance to pulmonary oxygen toxicity can be increased by depressing neurosympathetic and neuromuscular activity (2,3).

In order to test whether oxygen at high partial pressures has a direct toxic effect on the lung, chronic lobar broncho-cutaneous fistulas were constructed in a group of adult male dogs to permit selective ventilation of different pulmonary segments with gases of varying oxygen concentrations.

METHODS

Broncho-cutaneous fistulas were constructed in a two stage operation. During the first operation, a polyethylene conduit was implanted between the right lower lobe bronchus and the subcutaneous

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From the Institute of Rehabilitation Medicine, and Department of Surgery, New York University School of Medicine, New York. *Supported by Office of Naval Research contract N00014-68-A-0292-0001 and the John A. Hartford Foundation.*

Reprint from Surgical Forum Volume XXI, 1970

tissue of the right chest wall. During the second operation, one month later, the skin over the conduit was excised and the bronchus at the deep end of the conduit was fenestrated. The fenestrated bronchus was isolated from the rest of the tracheo-bronchial tree by tightening a tourniquet which had been placed around the bronchus proximal to the polyethylene conduit at the initial operation. Arterial catheters to permit sampling of blood for gas analyses and a cuffed tracheostomy catheter to facilitate administration of breathing gas were also inserted.

Experimental dogs were studied in four groups in the full conscious state in a walk-in hyperbaric chamber at 4.7 atmospheres absolute (ATA) for up to five hr.

RESULTS AND CONCLUSIONS

In Group I five unmodified dogs exposed to 100% oxygen at 4.7 ATA (PaO_2 3100 ± 160, mean ± S.D.) developed gross signs of diffuse pulmonary oxygen toxicity (atelectasis not inflatable with air at 35 cm. H_2O pressure, congestion, consolidation). All experienced convulsions. Three dogs died in less than five hr. and two appeared moribund at five hr.

In Group II five modified dogs ventilated with 100% O_2 by way of both trachea and fistula (PaO_2 2850 ± 155) were affected as Group I.

In Group III six dogs ventilated with 100% oxygen through the fistula and 10% O_2 plus 90% N_2 through the trachea (PaO_2 790 ± 260 did not develop gross evidence of lung damage in any portion of the lung. None convulsed.

In Group IV seven dogs ventilated with 100% O_2 through the trachea and 10% O_2 plus 90% N_2 through the fistula (PaO_2 2020 ± 220) developed gross evidence of diffuse pulmonary damage predominantly but not exclusively in the oxygen ventilated segment. The extent of the damage grossly did not appear as severe as in Groups I and II. Four dogs had convulsions which appeared after a longer latent period than in Groups I and II. Lung damage was present in both dogs with and without convulsions.

The most frequently encountered signs of lung damage were congestion and patchy atelectasis seen grossly and on gelatin embedded whole lung sections (Gough-Wentworth). The bronchi of damaged lungs contained little or no blood. Microscopic examination generally confirmed congestion and patchy atelectasis but also revealed high incidence of endozoic pulmonary disease even in conditioned clinically healthy dogs with normal hemogram and chest x-ray.

In view of the finding that hyperbaric alveolar oxygen tensions were tolerated by the lung when systemic hyperoxia was not marked but damaged the lung when systemic hyperoxia was severe, it is concluded that systemic hyperoxia plays a significant role in the pathogenesis of pulmonary toxicity from hyperbaric oxygen.

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CHRONIC BRONCHOCUTANEOUS FISTULA IN THE DOG: A METHOD FOR CONSTRUCTION

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Chronic bronchocutaneous fistula in the dog: A method for construction

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The purpose of this report is to describe a technique for constructing a chronic bronchocutaneous fistula in the dog in order to allow ventilation of different lobes of the lung with different gases simultaneously in a conscious animal. The model was developed to facilitate assessment of local and systemic effects of hyperoxia in the pathogenesis of pulmonary oxygen toxicity; it makes possible high arterial with low alveolar oxygen tensions or, vice versa, low arterial with high alveolar oxygen tensions contemporaneously. The model is also suitable for study of local and systemic effects of other gases and aerosols on the lung.

METHODS

A bronchocutaneous fistula was established with the right diaphragmatic bronchus in a two-stage operation in five conditioned adult male mongrel dogs weighing 25 pounds or more. During the first stage, the animal was anesthetized with pentobarbital sodium (30 mg. per kilogram of body weight), the trachea was cannulated through the mouth with a cuffed tube, and a right thoracotomy was performed through the sixth intercostal space. Ventilation was maintained with air by using a positive pressure respirator. The ribs were spread, the diaphragmatic lobe bronchus was isolated between the origins of the bronchi of the apical and cardiac lobes, and two ¼ inch umbilical tapes were passed around it. The pulmonary artery was protected. When the length of the isolated diaphragmatic bronchus was less than 2 cm., the intermediate and cardiac lobes were excised, the latter to provide sufficient length of bronchus for the next step of the operation and the former to avoid its occlusion by the cannula to be inserted into the diaphragmatic bronchus during the second operation. A polyethylene conduit with a 1.8 cm. internal diameter (the casing of a 12 ml. disposable syringe was found convenient for this purpose) was then placed between the diaphragmatic bronchus and the subcutaneous tissue. The conduit was secured to the lateral aspect of the bronchus by means of the distal peribronchial tape, as illustrated in Fig. 1. The rib retractor was then removed and a 2 cm. segment of the seventh rib was resected to make way for the polyethylene conduit through the thoracic cage as laterally as possible. The limbs of the proximal peribronchial umbilical tape were passed through a No. 16 Fr. catheter which was brought out into the subcutaneous tissue adjacent to the polyethylene conduit. This improvised tourniquet was tightened during the second operation in order to isolate the diaphragmatic bronchus from the rest of the tracheobronchial tree. The chest was then closed, the skin was sutured over the conduit and tourniquet, and chest suction was applied until all air was removed. Penicillin was given daily for ten days.

The second operation, i.e., opening of the bronchocutaneous fistula was performed four

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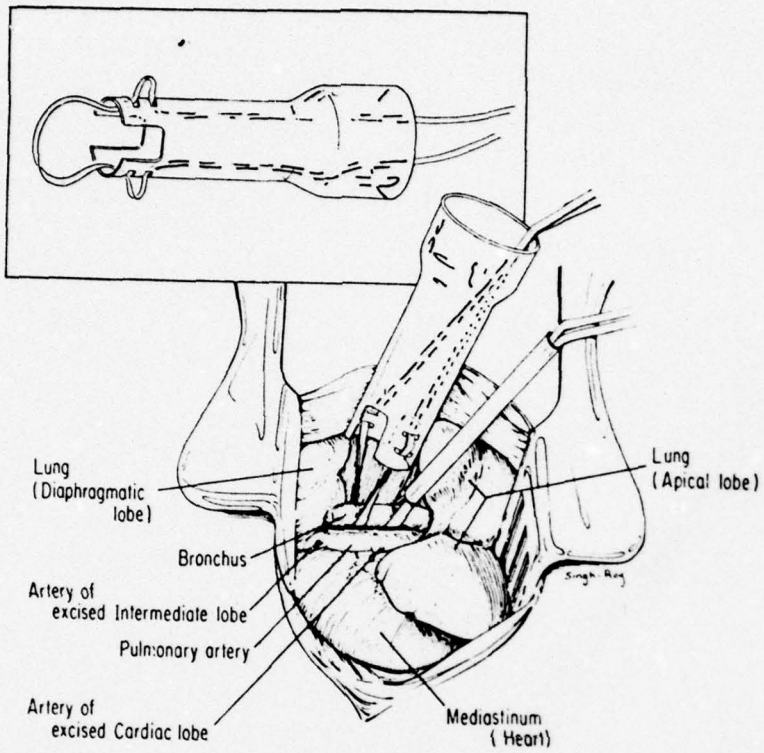

Fig. 1. Fixation of polyethylene conduit to right intermediate bronchus. Noncompressive, slip-proof attachment to the bronchus was accomplished with umbilical tape (¼ inch) passed through narrow slits in the conduit as illustrated. Both the conduit and the sheath of the tourniquet proximal to it were trimmed under the skin before its closure.

to five weeks later but only on animals in good health and with normal hemogram and chest x-ray findings (Fig. 2). By this time, scar tissue had sealed the conduit off from the pleural cavity. Light anesthesia was induced with thiopenthal sodium (25 mg. per kilogram of body weight), and the trachea was cannulated with a cuffed tube through a tracheostomy. Anesthesia was then continued with halothane. The skin and subcutaneous tissue over the superficial end of the polyethylene conduit was excised, and the bronchus at the deep end was identified by gentle traction on the umbilical tape fixing the conduit to the bronchus. This structure was fenestrated with electrocautery, and the tourniquet at its proximal end was tightened. Accurate identification of the bronchus prior to cautery was essential to avoid either perforation of the scar tissue

Fig. 2. Chest roentgenogram (right lateral) four weeks after implantation of polyethylene conduit into right chest.

Table I. *Arterial blood oxygen tensions during various levels of hyperoxia in dogs with chronic bronchocutaneous fistula: Average values for five animals*

	*Gas breathed**							
	T_a/F_a	T_a/F_o	T_o/F_a	T_o/F_o	T_A/F_A	$T_{N,O}/F_O$	$T_O/F_{N,O}$	T_O/F_O
Systemic arterial P_{O_2} (mm. Hg) Mean	86	101	367	547	542	807	1960	2801
±S.D.	±6	±12	±77	±52	±64	±225	±186	±179

*T_a T_O = air, 100 percent O_2 at 1 Ata via trachea. T_A T_O = air, 100 percent O_2 at 4.7 Ata via trachea. F_a F_o = air, 100 percent O_2 at 1 Ata (right diaphragmatic bronchus) F_A F_O = air, 100 percent O_2 at 4.7 Ata (right diaphragmatic bronchus). $T_{N,O}$ = 10 percent O_2 plus 90 percent N_2 via trachea at 4.7 Ata. $F_{N,O}$ = 10 percent O_2 plus 90 percent N_2 via fistula at 4.7 Ata.

wall of the fistula with ensuing pneumothorax or perforation of a major blood vessel with ensuing hemorrage. A No. 16 or 18 cuffed endotracheal catheter (as short as possible to minimize dead space) was then inserted into the diaphragmatic lobe. The polyethylene conduit was packed with gauze and the overlying skin was sutured around the endobronchial tube to hold it securely. The right carotid artery was catheterized to sample blood for gas tensions and pH. Three animals also had catheters passed into the right atrium through the external jugular vein. The catheters were filled with dilute heparin solution (20 mg. per liter of 0.9 percent saline) to keep them from clotting.

The effect of ventilating the trachea and fistula with gases of different oxygen concentrations on blood oxygen tensions was measured after the animals had recovered from anesthesia and were free from ataxia, shivering, hyperpenea, excitement, and hyperkinetic movements. The recovery period lasted approximately two to three hours. Animals were restrained with a Pavlov sling and a basket muzzle when necessary and exposed to the various breathing gases at 1 and 4.7 Ata (atmosphers absolute pressure) in a walk-in hyperbaric chamber at 20 to 25° C. The breathing gases were administered through semiclosed circuit anesthesia machines equipped with soda lime carbon dioxide absorbers on the exhalation side. The pop-off valve was set at 5 cm. H_2O in order to minimize atelectasis resulting from absorption of oxygen from the alveoli when 100 percent oxygen was administered.[2] Higher pop-off settings made the animals restless. The ventilating gases were administered in the following order: 1 Ata—air via both trachea and fistula (T_a/F_a), air via trachea and 100 percent oxygen via fistula (T_a/F_o), oxygen via trachea and air via fistula (T_o/F_a), and 100 percent oxygen via both trachea and fistula (T_o/F_o); 4.7 Ata—air via both trachea and fistula (T_A/F_A), 10 percent O_2 plus 90 percent N_2 through the trachea and 100 percent oxygen through the fistula ($T_{N,O}/F_O$), 100 percent O_2 through the trachea and 10 percent oxygen plus 90 percent N_2 through the fistula ($T_O/F_{N,O}$), and 100 percent O_2 via both trachea and fistula (T_O/F_O). Arterial blood specimens were drawn for gas analysis* after the animals had breathed the selected gas or gases for 15 minutes. Three animals also had mixed venous blood specimens drawn after breathing 100 percent O_2 via the trachea and 10 percent O_2 plus 90 percent N_2 via the fistula at 4.7 Ata.

Animals were put to death by a lethal dose of pentobarbital sodium 10 to 12 hours after fenestration of the bronchus. The lungs were excised and filled with 10 percent formalin in 0.1M neutral acetate buffer from a height of 35 cm. and prepared for examination by the Gough-Wentworth whole slice technique.[1] The appearance of these lungs was compared with lungs from ani-

*Blood P_{O_2} was measured with a Radiometer P_{O_2} electrode, type E 5036, fitted with a Teflon membrane. The electrode was calibrated with water in equilibrium with air at ambient pressure. Measurements were made at 1 and 4.7 Ata, as indicated in the text.

Fig. 3. Whole lung sections from dog with bronchocutaneous fistula one month after right intermediate and cardiac lobectomies and implantation of conduit. Specimens are from animal upon completion of protocol 12 hours after Stage II of operation. Except for scarring near the interlobar fissure and hilum on the right, both specimens are normal.

mals that were put to death one month after undergoing only stage one of the operation for a bronchocutaneous fistula.

RESULTS AND CONCLUSIONS

Average arterial blood oxygen tensions under the various experimental conditions are presented in Table I. Average mixed venous P_{O2} ($P\bar{v}_{O2}$) in three animals breathing 100 percent O_2 via the trachea and 10 percent O_2 plus 90 percent N_2 via the fistula at 4.7 Ata was 193 ± 67 mm. Hg.

Estimates of oxygen tensions in the inhaled gases are as follows: [$P_{I_{O_2}}$ (mm. Hg dry gas) = Ata × 760 × fraction O_2 in inhaled gas]: 1 Ata—air 160, 100 percent O_2 760, 4.7 Ata—10 percent O_2 plus 90 percent N_2 357, air 714, and 100 percent O_2 3572. Alveolar oxygen tensions are lower as a result of dilution by CO_2 ($P_{A_{CO2}}$ = 40 mm. Hg) and water vapor ($P_{A_{H_2O}}$ = 47 mm. Hg). Additional dilution by N_2 diffusing from the blood into the alveoli undoubtedly took place in the right diaphragmatic lobe

when the latter was ventilated with 100 percent O_2 and the rest of the lung with a N_2-containing gas. Although this "reverse diffusion" was not measured in the present study, the low Bunsen coefficient of N_2 (.012) suggests that the volume was small. By the same token, "reverse diffusion" of O_2 leading to increased P_{AO_2} in the right diaphragmatic lobe was a possibility when the latter was ventilated with 10 percent O_2 plus 90 percent N_2 and the rest of the lung with 100 percent O_2. Through the mixed venous P_{O_2} value in the present group of animals indicates that this did not take place, "reverse diffusion" of O_2 may take place in case of an increase in cardiac output, reduction in oxygen consumption or other causes for increased $P\bar{v}_{O_2}$.

Fig. 3 shows representative specimen of gelatin imbedded whole lung sections prepared by the Gough-Wentworth technique.[2] The sections are from an animal killed upon completion of the protocol about 12 hours after the bronchocutaneous fistula was opened. Except for scarring near the interlobar fissure and hilum, there is no evidence of congestion, consolidation, edema atelectasis, or other signs of disease.

SUMMARY

An operative technique has been developed for constructing an isolated bronchocutaneous fistula in the dog to allow ventilation of different lobes of the lung with different gases in a fully conscious animal. The model was developed to facilitate distinction between local and systemic effects of oxygen on the lung by making it possible to produce contemporaneously high systemic with low alveolar oxygen tensions and vice versa.

The report presents a description of surgical operative techniques and also values of systemic arterial blood oxygen tensions while fully conscious animals breathed gases of different oxygen tensions simultaneously through the trachea and fistula.

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Pathogenesis of pulmonary oxygen toxicity: role of systemic hyperoxia and convulsions

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REICH, THEOBALD, JOSEPH TAIT, TOSHIO SUGA, N. ERIC NAFTCHI, AND MARGARET DEMENY. *Pathogenesis of pulmonary oxygen toxicity: role of systemic hyperoxia and convulsions.* J. Appl. Physiol. 32(3): 374–379. 1972.—The effect of systemic hyperbaroxia in the pathogenesis of pulmonary oxygen toxicity was studied at 4.7 atmospheres absolute (Ata) for 5 hr or until earlier death in awake dogs with isolated chronic right diaphragmatic bronchocutaneous fistulas. Ventilation with 100% O_2 via both trachea and fistula (*group I*, 7 animals, Pa_{O_2} 2,903 ± 86 mm Hg) produced lung damage and convulsions in all animals and death before 5 hr in two; 100% O_2 via trachea and 10% O_2 + 90% N_2 via fistula (*group II*, 12 animals, Pa_{O_2} 1,999 ± 65) produced damage in all lobes in each animal, convulsions in five, and death before 5 hr in two of these; 100% O_2 via fistula and 10% O_2 + 90% N_2 via trachea (*group III*, 12 animals, Pa_{O_2} 795 ± 82), and air via trachea and fistula (*group IV*, 5 animals, Pa_{O_2} 574 ± 23) produced no macroscopic and significantly less microscopic damage, no convulsions, and no deaths. Thus, it is concluded that pulmonary oxygen toxicity can result rapidly from systemic hyperbaroxia alone, that convulsions are not necessary for lung to be damaged by OHP, and that the postcapillary pulmonary vessels are not involved primarily in the pathogenesis of this injury.

hyperbaric oxygenation; oxygen convulsions; alveolar hyperoxia; chronic bronchocutaneous fistula

THE SIGNIFICANCE of systemic hyperoxia in the pathogenesis of pulmonary damage by hyperbaric oxygen has been difficult to evaluate because of two major obstacles. First, marked systemic hyperoxia causes convulsions which alone may damage the lung (3) and second, until recently no satisfactory method existed to produce systemic hyperoxia without concomitant alveolar hyperoxia. Although systemic hyperoxia has been implicated by evidence that susceptibility to pulmonary oxygen toxicity is reduced by a variety of systemic biochemical and endocrine modifications or by construction of right-to-left shunts to reduce systemic oxygen tensions in the presence of high alveolar oxygen tensions, attenuation of seizure activity is concomitant with each of these modifications (1, 14, 16). Alveolar hyperoxia has been implicated by Penrod who concluded that atelectasis is an early deleterious effect resulting from absorption of oxygen from alveoli where it is trapped when bronchioles become occluded presumably from surface irritation by HPO (10). This investigator also demonstrated that simultaneous ventilation of one lung with oxygen and the other with nitrogen or helium at 5 atmospheres absolute (Ata) for 7 hr resulted in injury to the oxygen ventilated but not the inert gas ventilated lung (11). Unfortunately, neither the occurrence of convulsions nor the values of systemic oxygen tensions were reported in these studies performed on anesthetized animals.

In the present experiment the effects of high systemic oxygen tensions on the lung were studied in conscious dogs with chronic bronchocutaneous fistulas that enabled simultaneous ventilation of isolated lobes of the lung with gases containing oxygen at different partial pressures. By giving oxygen through the fistula and oxygen + nitrogen mixtures through the trachea, or vice versa, by giving oxygen through the trachea and oxygen + nitrogen through the fistula, it was possible to vary the systemic and alveolar oxygen tensions individually within wide limits (12).

METHODS

A bronchocutaneous fistula was established with the right diaphragmatic bronchus in adult male mongrel dogs that had been conditioned in our animal colony for 4–6 weeks, were dewormed, and were vaccinated against distemper, hepatitis, and leptospirosis. Special care was exercised to select only animals that showed good health, i.e., were vigorous, did not lose weight, consumed normal quantities of food, did not cough, had no nasal discharge, were afebrile, had normal hemogram, and normal roentgenogram of the chest.

The fistula was constructed in a two-stage operation essentially as described previously (12). During the first stage, a polyethylene conduit was implanted between the right diaphragmatic bronchus and the subcutaneous tissue of the right lateral chest wall. The cardiac and intermediate lobes were removed when necessary to provide sufficient length of diaphragmatic bronchus. The pulmonary and bronchial vessels to the remaining lobes were protected inasmuch as alteration of the blood supply may influence development of pulmonary oxygen toxicity (13). The second operation was performed 4–5 weeks later by which time scar tissue had sealed the polyethylene conduit from the pleural space. Again, only animals that had recovered good health, vigor, weight, for example, and had normal hemogram and no radiologic evidence of lung disease were

chosen for further study. Under light anesthesia induced with sodium thiopental and maintained with halothane, the skin over the conduit was excised, the diaphragmatic bronchus fenestrated with electrocautery, and intubated with a cuffed catheter, the tip and the balloon of which were coated with an anesthetic ointment. The right carotid artery was catheterized to facilitate sampling of blood for analysis of gas tensions and pH. In six animals, a catheter was also passed through the external jugular vein into the right atrium to facilitate sampling of mixed venous blood. The catheters were filled with heparin solution (20 mg/liter 0.9% saline) to keep them patent. Finally, a right-angle cuffed tracheostomy tube coated with anesthetic ointment was inserted into the trachea through a cervical tracheostomy.

Animals were permitted to recover from anesthesia until disappearance of ataxia, shivering, hyperpnea, excitement, and hyperkinetic movements. They were then taken into a walk-in hyperbaric chamber and placed in a harness in such a way that the animal was free to stand, sit, or lie, but not turn around. The animals were tranquil and exhibited no discomfort.

Tests were conducted at 4.7 Ata while the animals breathed $O_2 + N_2$ mixtures for 5 hr or less if the animal died. Ambient temperature was 20–25 C. Breathing gases were administered through semiclosed anesthesia machines equipped with soda lime-CO_2 absorbers on the exhalation side. Pressure at end expiration in the exhalation lines was set at 5 cm H_2O above ambient by a "pop-off" valve. This was done to minimize atelectasis due to absorption of O_2 from alveoli when 100% oxygen was administered (10). Higher pop-off pressures were not used because the animals became restless. Blood specimens (1.5 ml) were drawn after animals had breathed the selected gas or gases at 4.7 Ata for 20–30 min and 1.5–2 hr. In animals that had convulsions, the second samples were obtained during a quiescent period. The first samples were analyzed for pH, P_{CO_2}, and P_{O_2}; the later ones only for P_{O_2}.[1] Animals that survived exposure in the chamber were sacrificed alternately by an overdose of sodium pentobarbital or by anesthetizing the animal with pentobarbital and filling the lungs in situ with 10% formalin in 0.1 M neutral acetate buffer from a height of 35 cm. The excised lungs from the latter were photographed, tissue samples were taken for microscopic examination,[2] and the remaining lung was processed for examination by the Gough-Wentworth whole lung slice technique (5). The lungs from animals that received a lethal dose of barbiturate and from those that died in the chamber were inflated with air under 35 cm water pressure and photographed. They were then placed in a bucket containing 10% neutral formalin, filled with this fixative under 35 cm hydrostatic pressure, and examined the same way as the lungs fixed in situ. The results of the gross and microscopic examinations on test animals were compared with results of similar examination in five animals that had had only stage I operation 1 month earlier.

TABLE 1. *Arterial blood gas values, pH, and number of convulsions during various levels of systemic hyperoxia at 4.7 Ata in four groups of dogs with chronic bronchocutaneous fistula*

Group and No. of Animals Measured	Gas Breathed		Pa_{O_2}, mm Hg	$Paco_2$, mm Hg	pH	Avg No. of Seizures/ Animal
	Trachea	Fistula				
I (N=7)	100% O_2	100% O_2	2,903 ±86	28.1 ±1.6	7.50 ±0.1	11
II (N=10)	100% O_2	10% O_2 + 90% N_2	1,999 ±65	30.9 ±1.7	7.45 ±0.03	7*
III (N=7)	10% O_2 + 90% N_2	100% O_2	795 ±82	37.2 ±1.9	7.39 ±0.02	0
IV (N=6)	Air	Air	574 ±23.4	39.1 ±0.6	7.38 ±0.01	0

Values are means ± SEM. * Value for 5 animals that convulsed.

The groups of test animals studied and the number in each group that had blood gas values and blood pH measured are presented in Table 1.

All test animals that survived were placed on compressed air through both trachea and fistula just before decompression. Animals in *groups I* and *II* were brought to 3.7 Ata for 5 min, 1.9 Ata 5 min, 1.3 Ata 5 min, then to the surface. Animals in *groups III* and *IV* were decompressed stepwise at the following rate: 15 min each at 3.7 Ata, 2.8 Ata, 1.9 Ata, and 1.6 Ata; 30 min at 1.3 Ata; and then to the surface. There was no clinical evidence of dysbarism in any of the animals. It is not to be construed, however, that the aforementioned schedule for decompression is recommended as safe for general use.

RESULTS

Average values for arterial blood oxygen and carbon dioxide tensions, pH, and incidence of convulsions are summarized in Table 1. There was no statistically significant difference between Pa_{O_2} values after 15–30 min and after 1.5–2 hr breathing the selected gas or gases at 4.7 Ata.

All seven animals in *group I* convulsed and became stuporous rapidly. The earliest convulsions occurred after 33 min and the latest after 115 min of exposure. One animal died after 260 min and another after 295 min of hyperbaroxia. Surviving animals had dyspnea after decompression.

In *group II*, 5 of 12 animals convulsed, the earliest after 54 min and the latest after 255 min of hyperbaroxia. All animals with convulsions became stuporous and two died before decompression. Animals that had not convulsed remained lucid and ambulatory but developed moderate dyspnea upon decompression.

Individual arterial and venous blood gas and pH values for 10 animals in *group 2* are presented in Table 2. The results do not reveal significant differences between animals that convulsed and those that did not.

[1] pH, Pa_{O_2}, and Pa_{CO_2} were measured at 4.7 Ata with the following Radiometer instruments: thermostated (38 C) pH meter type 27 + PHA 927, P_{O_2} electrode type E 5046 using Teflon membrane, and P_{CO_2} electrode (Severinghaus) type E 5030. The apparatus was pressurized slowly about 1 hr before its use. The pH electrode was calibrated with standard buffers, the P_{O_2} electrode with water in equilibrium with air at 4.7 Ata, and P_{CO_2} electrode with 0.9% and 2.8% CO_2 gas standards. All calibrations were at 4.7 Ata.

[2] Specimens were obtained from the right diaphragmatic and apical lobes and the left diaphragmatic and intermediate lobes. All sections were stained with hematoxylin and eosin. Selected sections from *groups I* and *II* were stained with PAS and with Gram's stains.

TABLE 2. *Blood gas values and pH in 10 animals of group II* after 0.5 hr at 4.7 Ata*

Pa_{O_2}, mm Hg	$P\bar{v}_{O_2}$, mm Hg	Pa_{CO_2}, mm Hg	$P\bar{v}_{CO_2}$, mm Hg	pH_a	pH_v	Latency to Seizures,† min
Animals with convulsions						
2,203‡		29		7.48		112
1,820‡	191	38	53	7.42	7.32	260
2,280	184	26	41	7.52	7.40	123§
1,810‡	420	31	48	7.32	7.26	54§
2,282		27		7.47		98
Avg 2,079	265	30.2	47.3	7.44	7.33	
±SEM ±97	±63	±1.91	±2.8	±0.04	±0.03	
Animals without convulsions						
2,105				7.38		
1,833‡	137	32	44	7.43	7.35	
2,032		34		7.43		
1,812‡	170	29	37	7.46	7.42	
1,821	250	28	45	7.54	7.40	
Avg 1,921	186	30.8	42	7.45	7.39	
±SEM ±55	±27	±1.2	±2.2	±0.02	±0.02	

* 100% O_2 via trachea, 10% O_2 + 90% N_2 via fistula. † Time at 4.7 Ata before convulsions. ‡ Animals with microscopic pulmonary infection in trachea- or fistula-fed lobes. § Died before decompression.

TABLE 3. *Incidence of macroscopic and microscopic damage in trachea-fed (T) and fistula-fed (F) lobes of lung at various levels of hyperoxia at 4.7 Ata in dogs with bronchocutaneous fistula*

Group	I		II		III		IV		Control	
	T	F	T	F	T	F	T	F	T	F
No. of specimens with macroscopic congestion and atelectasis*	7	7	12	12	0	0	0	0	0	0
No. of specimens with microscopic damage:										
With infection	4	4	5	5	4	5	1	1	2	2
Without infection†	3	3	7	7	2	2	0	1	0	0
No. of specimens without microscopic damage (normal)	0	0	0	0	6	5	4	3	3	3
Total no. specimens examined	7	7	12	12	12	12	5	5	5	5

Groups as identified in Table 1. Controls had only stage I of operation for bronchocutaneous fistula as described in text (METHODS) 4 weeks before examination of lungs. Difference between incidence of microscopic damage in corresponding noninfected lobes in *groups II* (trachea-fed lobes 7/7, fistula-fed 7/7 and *III* (trachea 2/8, fistula 2/7) is significant at the 0.02 level. * After filling lung with air or fixing solution under 35 cm hydrostatic pressure. † Interstitial or alveolar edema, atelectasis, congestion, interstitial or alveolar hemorrhage.

The $P\bar{v}_{O_2}$ values in Table 2 are of additional interest because they indicate the range of $P_{A_{O_2}}$ in the right diaphragmatic lobe in *group II* animals. The estimated value of this parameter was 280 mm Hg.[3] This was probably exceeded only in the animal with a $P\bar{v}_{O_2}$ of 420. Under this reversal of the normal alveolar-capillary oxygen gradient, diffusion of oxygen in the right diaphragmatic lobe was undoubtedly from pulmonary arterial blood into the alveoli, thus raising the $P_{A_{O_2}}$ in this lobe to between 280 and 420 mm Hg.

Animals in *groups III* and *IV* experienced no convulsions. All remained lucid and ambulatory.

On gross examination, the lungs from *groups I* and *II* showed congestion and consolidation characterized by a

FIG. 1. Whole lung sections of oxygen damaged and normal lungs. *A:* specimen from *group I* (100% O_2 via both trachea and fistula to right diaphragmatic lobe) showing marked congestion of all lobes. *B:* specimen from *group II* (100% O_2 via trachea and 90% N_2 + 10% O_2 via fistula to RDL) showing marked congestion of all lobes. *C:* specimen from *group III* (90% N_2 + 10% O_2 via fistula to RDL and 100% O_2 via trachea) showing scarring of interlobar fissure and around fistula tract on right side. Lung tissue otherwise appears normal. *D:* specimen from *group IV* (compressed air via both trachea and fistula). Findings are as in *C*.

[3] Estimated $P_{A_{O_2}}$ (mm Hg) = 4.7 (Ata) × 760 (mm Hg/Ata) × $F_{I_{O_2}}$ (fraction O_2 in inhaled gas) − 40 ($P_{a_{CO_2}}$, mm Hg) − 47 ($P_{a_{H_2O}}$, mm Hg).

beefy red appearance of most of the lung. Portions of lung not inflatable with air under 35 cm hydrostatic pressure sank when placed in fixing solution. In *group II* there was no difference between the appearance of lungs from animals that had convulsed and those that had not. Lungs from *groups III* and *IV* were of normal color. Specimens examined before filling with formalin had normal consistency and were well aerated. The incidence of macroscopic findings is summarized in Table 3.

Representative whole lung sections are shown in Fig. 1. Specimens from *groups I* and *II* showed congestion and consolidation both in animals that convulsed and those that did not. The macroscopic lesions were either confluent involving one or more entire lobes or were distributed in large patches. It is of particular interest that in *group II* the right diaphragmatic lobe which was ventilated with 10% O_2 + 90% N_2 mixtures was also congested and consolidated not only in animals that convulsed but also in those that did not. Sections from *groups III* and *IV* were normal including, particularly, the right diaphragmatic lobe in *group III* which was ventilated with 100% O_2.

The microscopic findings are also summarized in Table 3 and illustrated in Fig. 2. In spite of fastidious care and selection of animals, clinically undetected microscopic pulmonary infection was found frequently in all groups, occurring in two of five control animals. There was no significant difference between the number of infected right diaphragmatic and left diaphragmatic or intermediate lobes in the same group or between the number of corresponding infected lobes in each group vs. the control. The lesions consisted of varying degrees of infiltration by acute inflammatory cells together with edema, atelectasis, congestion, and interstitial and/or intra-alveolar hemorrhage. Microabscesses and bacterial invasion were also found. Leukocytic infiltration in grossly normal and grossly damaged tissue in infected lobes was of the same intensity and was thought to have preceded exposure in the hyperbaric chamber (7). Congestion, edema, atelectasis, and hemorrhage associated with infected areas appeared indistinguishable from these findings in noninfected lungs. The lesions were distributed randomly, i.e., severest infection was not found predominantly in one group or in one lobe. Thus, in the infected lobes it was not possible to discern the etiology of edema, congestion, hemorrhage, and atelectasis, an

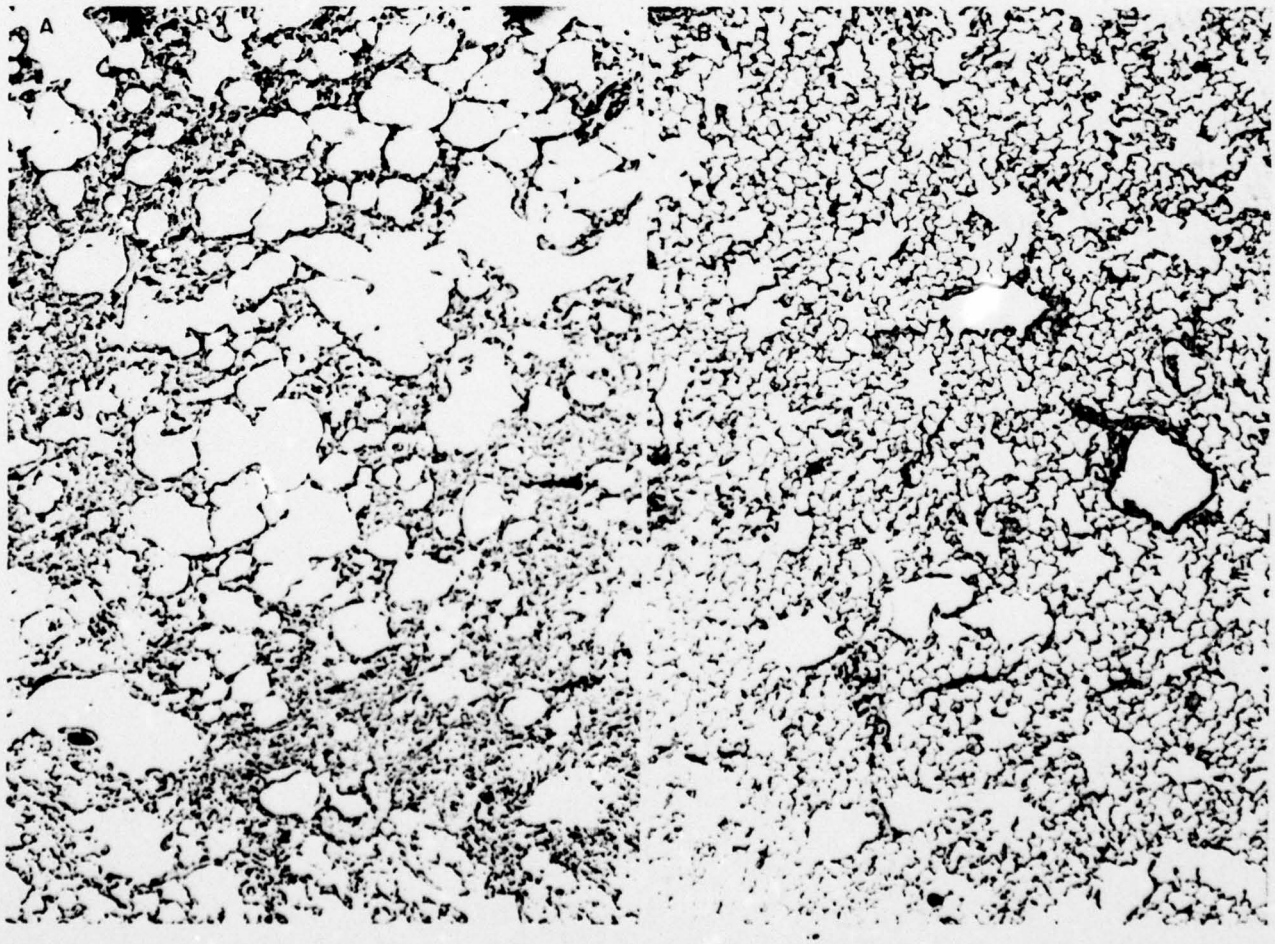

FIG. 2. Photomicrograph of specimens from right diaphragmatic lobes of dogs *A*: in *group III* showing essentially normal structure (hematoxylin and eosin, ×64). *B*: without convulsion in *group II* showing marked congestion, dilatation of capillaries, edema, dilatation of air sacs and alveoli, and atelectasis (hematoxylin and eosin, ×96).

infection or oxygen toxicity. However, statistical analysis of the incidence of these findings in noninfected lungs in *groups II* and *III*, which contained a sufficient number of noninfected specimens, showed that lesions were significantly more frequent in *group II* than in *group III* both in the fistula-fed right diaphragmatic lobes ($P < 0.02$) and in the trachea-fed, i.e., left diaphragmatic and intermediate lobes ($P < 0.02$). The severity of damage did not correlate with any measured parameter though there appeared to be more edema in *group II* that convulsed and there was less congestion in *group III* with microscopic lesions.

DISCUSSION

The results of the present experiment are noteworthy from the following points of view: they support the hypothesis that systemic hyperoxia plays an important role in the development of the pulmonary lesions of oxygen poisoning; they indicate that convulsive seizures are not necessary for the rapid development of such lesions; and they suggest that the postcapillary pulmonary vessels do not play a primary role in the pathogenesis of pulmonary oxygen toxicity. The first of these conclusions is based on the finding that the incidence of damage in the right diaphragmatic lobe was significantly higher in animals with severe systemic and only mild alveolar hyperoxia in this lobe (*group II*), than in animals with mild systemic but severe alveolar hyperoxia in the corresponding lobe (*group III*); the second, that in *group II* the lungs were damaged even in animals that did not convulse; and the third, that injury to the right diaphragmatic lobe in *group III* was significantly less frequent even though this lobe and therefore its pulmonary venous effluent was severely hyperoxic.

The mechanism by which high systemic oxygen tension produces pulmonary injury is not completely clear. The prevalent view appears to be that systemic hyperoxia causes formation of some extrapulmonary agent toxic to the lung but it has not been ruled out that at higher than normal tensions in the mixed venous blood, oxygen may act directly. Bean and his co-workers (3) regard the pulmonary effect of OHP as due in large part to the neuroendocrinogenic components of its CNS effect and convulsions and thus postulated a noxious extra-pulmonary agent. Our findings are that convulsions are not necessary for the rapid development of pulmonary oxygen toxicity. However, inasmuch as neuroendocrine and neurosympathetic activities may be stimulated even without seizure activity during hyperbaric oxygenation as a result of the reported CNS hypercarbia these findings do not refute Bean's hypothesis (9).

Another kind of extrapulmonary noxious agent was proposed by Kistler et al. (8). These investigators found that first to be injured by oxygen was the endothelium of the pulmonary capillaries not the alveolar epithelium. They therefore hypothesized that some blood-borne agent or agents were necessary for oxygen to poison the lung and speculated that these agents resulted from damage by oxygen to the red cells. While the marked congestion coupled with relatively little tracheobronchial and alveolar fluid found in this study is consonant with the view of Kistler et al. that pulmonary oxygen toxicity evolves from within outward, i.e., from the vascular endothelium to the alveolar epithelium, their localization of the initial injury to the pulmonary capillaries as well as their views of the nature of the injurious agent remain to be confirmed. The present results do not rule out the possibility that at elevated partial pressures oxygen itself affects the vasculature, i.e., that no other extra pulmonary agent is required, and that lung damage begins in the precapillary pulmonary vessels the normal content of which is venous blood containing oxygen at low tensions. In this regard, Winter et al. also observed that improved pulmonary tolerance to high alveolar oxygen tensions ($P_{I_{O_2}}$ 2–2.5 Ata) in dogs with right to left vascular shunts was associated with only minimal systemic hyperoxia ($P_{a_{O_2}} < 127 \pm 83$ mm Hg) and thus presumably normal $P_{\bar{v}_{O_2}}$ (14).

Wittner and Rosenbaum presented histologic evidence that hyperoxia provokes severe pulmonary arterial, arteriolar, and consequently venular constriction and postulated that this reduces pulmonary blood flow and thus contributes to pulmonary injury (15). Though these investigators found that neither histamine nor serotonin was responsible, it is not clear whether the effect of oxygen was direct or due to a systemic factor. Nevertheless, it appears from Wittner and Rosenbaum's observations, from the present finding of a paucity of exudate in the tracheobronchial tree and alveoli, and from finding similar to the latter by Behnke et al. (4), that increased postcapillary resistance is not of primary importance in the development of congestion and edema of pulmonary oxygen toxicity. It seems reasonable that were this not the case, the right diaphragmatic lobe (ventilated with 100% O_2 at 4.7 Ata) in *group III* would also have been damaged.

Present results while indicating the important role of systemic hyperoxia in the pathogenesis of oxygen toxicity do not rule out a deleterious topical effect, i.e., direct effect of oxygen on the alveolar epithelial surface. Studies on rats suggest that if exposure in our experiments had been continued for longer periods all lobes ventilated not only with OHP but also with compressed air (*group IV*) would have shown lesions of oxygen toxicity (16). Indeed, the most severe lung damage from oxygen has been recorded in animals breathing O_2 between only 0.7 and 3 Ata for up to several days. Below 0.45 Ata oxygen is tolerated for prolonged periods although the limit, if any exists, has not been established; above 3 Ata it causes convulsions and death before damage to lung tissue becomes advanced (2).

A high incidence of subclinical microscopic pulmonary infection in laboratory dogs has been reported in the more remote as well as the more recent past by other investigators also (6, 7). In our experience 6 weeks of "conditioning" commercially supplied dogs in a modern fastidiously serviced facility is insufficient assurance that even clinically, radiologically, and hemotologically healthy animals will be free from endozoic pulmonary infection. For this reason, histologic changes induced in the lung experimentally in the present study have been interpreted against this background and have included examination of control animals kenneled under the same conditions as experimental animals. The conclusion that the clear-cut macroscopic lesions in *groups I* and *II* in the present study resulted from hyper-

oxia is supported by findings that microscopic pulmonary damage in noninfected lungs was significantly higher in *group II* (mean Pa_{O_2} 1,999) than in *group III* (mean Pa_{O_2} 795) and also by the absence of evidence that hyperoxia at the high level found in *groups I* and *II* either stimulates overgrowth of pathogenic microorganisms and/or overproduction of microbial toxins or that it increases susceptability of tissues to such toxins.

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SYSTEMIC HYPERBAROXIA IN THE PATHOGENESIS OF PULMONARY OXYGEN TOXICITY. Theobald Reich*, Joseph Tait*, and Toshio Suga*. (SPON: William M. Manger) N.Y.U. Med.Ctr., New York, N.Y.10016

The role of systemic hyperbaroxia in the pathogenesis of pulmonary oxygen toxicity was studied in dogs with isolated broncho-cutaneous fistulas to the right diaphragmatic lobe. This model made it possible to vary systemic and alveolar oxygen tensions independently within a wide range. Studies were on conscious animals at 4.7 Ata for 5 hrs or until earlier death. Ventilation with 100% O_2 via both trachea and fistula (Group I, 7 animals, PaO_2 2903±86 mm Hg) produced macroscopic lung damage and convulsions in all animals with death before 5 hrs in 2; 100% O_2 via trachea and 10% O_2 + 90% N_2 fistula (Group II, 11 animals PaO_2 1999±65) produced damage in all lobes in each animal, convulsions in 5 and death before 5 hrs in 2; 100% O_2 via fistula and 10% O_2 + 90% N_2 via trachea (Group III, 12 animals, PaO_2 795±82), and air via trachea and fistula (Group IV, 6 animals, PaO_2 574±23) produced no damage, no convulsions, and no deaths before 5 hrs.

Inasmuch as systemic hyperbaroxia results in lung damage even in lobes with low PaO_2, it is concluded that systemic hyperbaroxia plays a significant role in the pathogenesis of pulmonary oxygen toxicity.

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PATHOGENESIS OF PULMONARY OXYGEN TOXICITY: I. THE ROLE OF CIRCULATING BIOGENIC AMINES

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INTRODUCTION

Administration of exogenous catecholamines particularly epinephrine has been shown to accelerate the development of oxygen toxicity. On the other hand, administration of adrenergic blocking agents decrease O_2 toxicity. This as well as other evidence that has been reviewed by Bean ('45) and Clark and Lambertsen ('71) indicate that hyperbaric oxygen (OPH) causes release of catecholamines and this may be involved in O_2 toxicity.

The purpose of the present study was to determine the quantity of circulating catecholamines, to measure the time course of changes – if any – in catecholamine levels, and finally to determine whether in addition to catecholamines the levels of other circulating biogenic amines, e.g. serotonin and histamine are changed during hyperbaric oxygenation.

METHODS

Studies were performed on conditioned adult male mongrel dogs of approximately equal size. The animals were trained to live in a large air tight plexiglass box fitted with inlet ports for breathing gas supply and outlet ports for escape of waste gas. The boxes had larger portholes covered with sleeves to enable insertion of the hands without allowing excessive gas leaks. The animals were exposed to various ambient conditions in these boxes. For the circulating biogenic amine studies and gas analysis blood was obtained from the right atrium and from the thoracic aorta. Each specimen was 9 ml. Two days prior to the study, the animals were anesthetized lightly with sodium thiopental and intravascular catheters were inserted through the neck and attached to the skin. Urine samples were collected through

an indwelling catheter inserted transuretherally just prior to the experiment.

Seven groups of six and one group of seven animals were studied. The animals were placed in a plexiglass box which was ventilated with air at 1 ATA for ½ hr then 100% O_2 at 1 ATA for ½ hr. Blood specimens were obtained at the ½ and 1 hr intervals.

Groups 1 and 2 were then given a 70:30::O_2:N_2 mixture and brought to 3.14 ATA (partial pressure O_2 2.2 ATA) and maintained at this level for three hours. Specimens were collected two hours after reaching the required pressure, and three hours thereafter. Finally the dogs were decompressed, allowed to breathe air for ½ hr and specimens collected. In Group 1 plasma was analyzed for norepinephrine (NE) and epinephrine (E), in Group 2 for serotonin and histamine.

Groups 3 and 4 were brought to 3.14 ATA 70:30::P_2:N_2 and kept at this level for ten hours. Specimens were collected four hours, seven hours, and ten hours after reaching 3.14 ATA. Plasma from animals in Group 3 was analyzed for NE and E and in Group 4 for serotonin and histamine.

Groups 5 and 6 were brought to 3.8 ATA 100% O_2 until they convulsed. Specimens were collected one hour after reaching 3.8 ATA, immediately after or during a convulsion, during the quiescent period following convulsion (approx. 10 min postictal) and after decompression and breathing air at 1 ATA. specimens in Group 5 were analyzed for NE and E, and Group 6 for serotonin and histamine.

Control Groups 7 and 8 breathed 100% O_2 at 1 ATA for three hours, then air at 1 ATA. Specimens were collected one hour and three hours after breathing 100% O_2 then : ½ hr after breathing air at 1 ATA. Specimens in Group 7 were analyzed for NE and E, and those in Group 8 for serotonin and histamine.

Urine specimens were collected whenever available and were analyzed for 5-hydroxindolacetic acid (5-HIAA).

The animals were sacrificed with an overdose of barbiturate. The lungs were removed, inflated with air at 35 cm hydrostatic pressure and examined for gross appearance. The tissue specimens were also removed for microscopic examination.

NE and E in plasma were assayed by the fluorometric method of Weil-Malherbe and Bone ('52) modified by Manager *et al.*, ('69). Free serotonin and histamine in plasma were assayed by the fluorometric method of Redlich and Glick ('69). 5-HIAA in urine was estimated by the method of Bartlett and Gilbert ('70).

Blood specimens were analyzed at depth (O_2 partial pressure 2.2 ATA) for PH, P_{O_2}, and P_{CO_2} values with a Radiometer instrument.

RESULTS

Average values for plasma biogenic amines in Group 1, 2, 3 and 4 are summarized in Table 1.

TABLE 1: Average Concentration of Circulating Biogenic Amines in Dogs During OHP ($P_{I_{O_2}}$ 2.2 ATA)

Groups and # of animals	Biogenic amines Concentration (mean ± S.E.M.) ng/ml plasma		AAP ½ hr	OHP 2 hr	OHP 3 hr	OHP 4 hr	OHP 7 hr	OHP 10 hr
I N = 7	Norepinephrine	A	1.2 ±0.4	1.1 ±0.4	2.7 ±1.0			
		V	1.6 ±0.4	1.9 ±0.7	1.6 ±0.6			
I N = 7	Epinephrine	A	0.6 ±0.2	0.9 ±1.2	2.2 ±1.5			
		V	0.8 ±0.2	1.0 ±0.4	2.0 ±0.7			
II N = 5	Serotonin	A	125 ±25.0	76 ±22.9*	92 ±16.1*			
		V	116 ±27.4	78 ±21.9	90 ±23.9			
II N = 6	Histamine	A	15 ±3.1	12 ±2.5	17 ±3.9			
		V	14 ±1.6	9 ±1.2	12 ±1.6			
III N = 6	Norepinephrine	A	1.26±0.49			3.1 ±0.7	2.8 ±0.8	5.0 ±1.8
		V	2.66±0.78			2.5 ±0.6	2.1 ±0.7	5.4 ±0.9
III N = 6	Epinephrine	A	0.85±0.35			2.1 ±1.2	2.4 ±1.2	2.6 ±1.2
		V	1.05±0.43			1.5 ±0.9	1.9 ±1.0	3.0 ±0.9
IV N = 5	Serotonin	A	55 ±4.1			42 ±5.7*	46 ±4.4*	52 ±8.1
		V	56 ±5.8			65 ±7.3	67 ±5.0	56 ±5.8
IV N = 6	Histamine	A	19 ±2.2			18 ±2.4	18 ±2.4	19 ±1.7
		V	21 ±2.3			21 ±2.2	19 ±2.1	20 ±2.1

A = arterial plasma, V = mixed venous plasma, AAP = air 1 ATA,
* = significantly different from control ($p < 0.05$)

The results show an increasing trend in arterial and mixed venous plasma of NE and E concentration during short (3 hr) and long (10 hr) hyperbaric oxygenation compared with control levels on air at 1 ATA. Arterial plasma serotonin levels decreased significantly ($p < 0.05$) after two hours exposure to OHP compared with levels on air at 1 ATA and remained low for the duration of the experiment. Mixed venous plasma serotonin levels decreased but not significantly. Histamine levels of arterial and mixed venous plasma did not change. Results of individual experiments in Groups 3 and 4 (10 hr OHP) are shown in Table 2.

All animals in Group 5 and 6 convulsed, the average time of onset of convulsion being 95 min. Circulating catecholamines increased significantly after convulsion compared with baseline values ($p < 0.05$). Serotonin and histamine levels were not altered. (Table 3).

TABLE 2: Concentration of Circulatory Biogenic Amines in Dogs After 10 Hours OHP

Dog	Catecholamines[1] AAP ½ hr A	Catecholamines[1] AAP ½ hr V	Catecholamines[1] OHP 10 hr A	Catecholamines[1] OHP 10 hr V	Serotonin AAP ½ hr A	Serotonin AAP ½ hr V	Serotonin OHP 10 hr A	Serotonin OHP 10 hr V	Histamine AAP ½ hr A	Histamine AAP ½ hr V	Histamine OHP 10 hr A	Histamine OHP 10 hr V
1	4.5	3.0	7.8	9.1	52	68	29	39	22	21	14	23
2	3.4	6.7	2.7	11.6	66	55	51	66	17	21	17	24
3	2.1	1.5	6.5	11.4	44	61	59	66	26	27	24	22
4	1.6	5.8	0.8	3.2	63	60	78	63	12	12	19	12
5	0.2	4.3	12.9	5.1	50	34	44	44	23	23	23	23
Mean[2]	2.4	4.3	6.1	6.6	55	56	52	56	20	20	19	21
± S.E.M.	0.7	0.9	2.1	2.1	4.1	5.8	8.1	5.8	2.4	2.4	1.9	2.2

AAP = Air 1 ATA, OHP = $P_{I_{O_2}}$ 2.2 ATA, A = Arterial plasma
V = Mixed venous plasma, [1] = Epinephrine + norepinephrine, [2] = ng/ml plasma

TABLE 3: Average Concentration of Circulatory Biogenic Amines in Dogs After O_2 Induced Convulsion.

Number of Dogs	Biogenic amines Concentration (means ± S.E.M.) ng/ml plasma		AAP ½ hr	OHP after convulsions
5	[1]Catecholamines	A	1.6 ±0.2	5.9 ±1.2 *
		V	2 7 ±0.3	7.2 ±2.96*
5	Serotonin	A	81 ±13	93 ±11
		V	75 ±14	73 ±16
5	Histamine	A	9.0 ±1.3	11 ±2.2
		V	10 ±2.6	13 ±3.9

1 = Epinephrine and norepinephrine, * = Different from control ($p < 0.05$)
A = Arterial plasma, V = Mixed venous plasma, AAP = Air 1 ATA
O = $P_{I_{O_2}}$ 3.8 ATA

In Groups 7 and 8 (controls, 100% O_2 1 ATA) there was no change in the plasma levels of NE and E compared with baseline. The decrease in plasma concentration of serotonin was not significant and there was no change in the plasma histamine levels.

Excretion of 5-HIAA in urine did not increase during OHP or convulsion. The average values of 5-HIAA in μg/mg creatinine for Groups 1, 2 and 3, 4 on air at 1 ATA was 1.22±0.29 and on OHP was 0.91±0.23. For Groups 5 and 6 on air at 1 ATA it was 2.23±0.71 and was changed to 1.27±0.43 after convulsion. There were five animals in each category.

Average arterial blood gas and pH values of animals exposed to OHP (O_2 partial pressure 2.2 ATA) for seven hours were: Pa_{O_2} 1398 mm Hg, Pa_{CO_2} 34 mm Hg and pH 7.42.

Grossly, all lungs appeared normal. Microscopically, all showed mild interstital edema and congestion. Hemorrhage and atelectasis were absent.

DISCUSSION

The results of this study, although at a preliminary stage, are of interest from several points of view. They indicate that OHP for three hours without convulsion is not a potent stimulus for increasing circulating catecholamine levels. However, prolonged inhalation of OHP or O_2 induced convulsions are associated with increased circulating catecholamine levels comparable with those reported by Manger, Wakim and Bollman ('59) in shocked dogs. The latter form of stress causes an increase in circulating catecholamines up to 10.6 μg/1.

The number of experiments in which animals were exposed for ten hours are not sufficient thus far to yield results that are statistically significant. The trend towards significant increase in circulating catecholamines in this group however, is apparent from Table 2 and is indicated by sequential analysis. It has been found (Reich and Demeny, '73) that lung tissue concentrations of catecholamines during continuous OHP for ten hours (2-2.5 ATA O_2) were not altered.

The present results are consistent with previously reported findings of increased neurosympathetic-sympathoadrenal activity during convulsive seizures (Bean, '45; Clark and Lambertsen, '71). Since the circulating catecholamine levels remained unchanged for at least three hours and then increased only insignificantly under OHP at subconvulsive levels, it appears that increased catecholamine levels in the blood were not present before convulsive seizure. Animals that convulsed, however, received oxygen at 3.8 ATA whereas animals that did not convulse received oxygen only at 2.2 ATA.

Serotonin concentration in the plasma was decreased significantly during prolonged OHP. As reported elsewhere at this symposium (Reich and Demeny, '74) lung tissue concentration was also decreased. Since the excretion of 5-HIAA in urine was not increased, it may be concluded that the reduction in serotonin concentration resulted either from an impairment in biosynthesis and/or from an alternate route of metabolism. It has been shown that tryptophan hydroxylase, the first enzyme in biosynthetic paths of serotonin is partially inhibited by O_2 at high partial pressures (Fischer and Kaufman, '72). Reduction in tissue serotonin after OHP was also observed by Buckingham et al., ('68) and was presumed to result from increased catecholamine activity and tissue damage in the lung. The present results are not consistent with this explanation since neither the circulatory levels of serotonin nor the urinary excretion of 5-HIAA increased during the course of the observation period.

The presence of reduced serotonin in the plasma and the lung simultaneously with increased circulating catecholamines is of particular interest. It has been shown recently that one of the mechanisms that protects catecholamines from oxidation to aminochromes is the formation of complexes between catecholamine and serotonin (VanderWende and Johnson, '70). These findings suggest the possibility that when serotonin levels are low aminochrome formation may be increased. The formation of aminochromes have been implicated by Houlihan *et al.*, ('70) as a causative factor in production of O_2 toxicity.

The role of histamine in the pathogenesis of pulmonary O_2 toxicity is obscure, both protective and accelerating effects having been described. The concomitant reduction of tissue histamine and serotonin and the fact that both of these biogenic amines are present in abundant quantities in mast cells suggest the possibility that a common mechanism may be operative in the decrease of both biogenic amines in the tissues. Additional information is needed to clarify the role of these biogenic amines in the development of O_2 toxicity in the lung.

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PATHOGENESIS OF PULMONARY OXYGEN TOXICITY. 2. EFFECT OF PULMONARY DENERVATION

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INTRODUCTION

The time course of changes in circulating catecholamines, serotonin, and histamine during prolonged inhalation of O_2 at high partial pressures were described by one of us earlier at this session in a preliminary report (Demeny, *et al.*, '74). In the present paper we wish to present our observations on the effect of pulmonary denervation on the lung tissue concentrations of norepinephrine (NE), serotonin and histamine and also on the effect of denervation in the pathogenesis of pulmonary O_2 toxicity.

METHODS

Studies were performed on conditioned adult male mongrel dogs. The left lung was denervated by reimplantation of the organ (Trummer, '65). Following six week convalescence, the animals were given Tylocine (20 mg/kg b.i.d.) for two weeks in order to minimize pulmonary infection. The animals were examined by bronchoscopy and chest x-rays during this two week period and only animals with normal findings, e.g. without stricture, ulceration or pneumonitis were studied further.

The animals were divided into three groups. Group C (control, four dogs) was anesthetized lightly with sodium pentobarbital and the anterior chest wall was raised by a trap-door shaped flap. Pieces of tissue were excised from both lower lobes and frozen in liquid nitrogen. After the death of the animal, the lungs were removed, all anastamoses inspected, and the organ inflated with air under 35 cm hydrostatic pressure and photographed. The lung was then filled with 10% formaldehyde in 1 M phosphate buffer (pH 7.4) under 35 cm hydrostatic pressure and processed for preparation of whole organ sections.

Group A (7 animals) were placed in a large air-tight plexiglass box equipped with portholes covered with sleeves to permit insertion of the hands and forearms without extensive leakage. The boxes were fitted with several inlet ports for gas supply and with escape ports for waste gas. They were flushed with 70:30::O_2:N_2 and pressurized in a walk-in chamber to 3.56 ATA so as to maintain the partial pressure of O_2 in the plexiglass box at 2.5 ATA. The temperature was 65°F. The concentration of O_2 in the plexiglass box was monitored continuously. These conditions were maintained until an animal had a convulsion. The pressure in the chamber was reduced to 2.86 ATA so as to maintain the partial pressure of O_2 in the plexiglass box at 2 ATA. Exposure was continued until the animal became moribund, breathing 6–8/min. Lung specimens were then removed and the lungs prepared for examination as in Group C. The specimens were obtained under pressure.

Group B (6 animals) were treated exactly as Group A except that the experiment was terminated after ten hours, approximately half the average survival time of Group A. Specimens were obtained under pressure as described for Group A.

None of the animals in Group A or B had convulsions while under 2 ATA of O_2. One animal in Group B had no convulsions and remained at 2.5 ATA of O_2 throughout the experiment.

Two animals in Group B had catheters inserted into the carotid artery two days prior to study to enable withdrawal of arterial blood for measuring Po_2, Pco_2 and pH during the exposure. During such procedure, the O_2 concentration in the box dropped 0 to 3% for about five minutes.

The assay techniques for the tissue concentrations of NE, serotonin, and histamine were according to a modified method of Sadavongvivad ('70).

Microscopic examination was by light microscopy of hematoxylin and eosin stained sections.

RESULTS

On gross examination, the lungs in all animals appeared normal. Adhesions between visceral and parietal pleura on the left side often made comparison between the surface appearance of the left and right lungs difficult. However, those portions of the left lung that were free of adhesions appeared pink and inflated readily. Transected portions of each lower lobe lung appeared grossly normal. There were no strictures at the tracheal or the pulmonary artery anastomoses as judged from the circumference at the anastomotic line in comparison to the circumference of the lumen just proximal and just distal. The anastomosis of the right atrium was covered with smooth shiny endothelium. There was no stricture of the pulmonary veins.

Microscopic examination of specimen from Group C (control) showed no difference between the right and left lower lobes. Both lungs appeared normal. Group A and B animals showed changes consistent

with early pulmonary O_2 toxicity. It was of particular interest that the lungs in Group A did not appear sufficiently damaged either grossly or microscopically to explain the animal's death.

The average tissue concentration of the biogenic amines in the innervated and denervated lungs in each of the groups is presented in Table 1 and results on each animal in Group A in Table 2.

TABLE 1: Average Biogenic Amine Content of Innervated and Denervated Lungs After Exposure to OHP[1,2]

Group	Side	Norepinephrine µg/g	Serotonin µg/g	Histamine µg/g
(A) 24± hr	R[4]	.228 ± .058	.522 ± .081	11.24 ± 2.38
Exposure	L[4]	.090 ± .019	.516 ± .076	12.42 ± 3.26
(B) 10± hr	R	.062 ± .022	.103 ± .061	11.38 ± 1.25
Exposure	L	.040 ± .014	.132 ± .032	10.58 ± 1.14
(C)[3]	R	.080 ± .008	.372 ± .023	17.06 ± .325
Control	L	.037 ± .021	.218 ± .111	18.81 ± .529

(1) 2.5 ATA till convulsion, then 2 ATA
(2) Significant differences identified in text (Results)
(3) Control group not exposed to OHP
(4) L = left lower lobe (denervated)
R = right lower lobe (innervated)

Denervation of the lung resulted in a significant reduction in its NE concentration ($p < .001$ right vs left Group C). The differences between the histamine concentrations and between the serotonin concentrations were not significant. Exposure to O_2 for ten hours (Group B) did not cause a significant change in the NE content of the lungs with respect to the control. The serotonin and histamine concentrations continued to show only insignificant differences between the right and left lung but both biogenic amines were reduced significantly in comparison with the corresponding lungs of the Group C (control).

Exposure to hyperbaric oxygen (OHP) until moribund (Group A) resulted in a significant increase in NE in the innervated lung compared with the corresponding lungs in both Groups C (control) and B ($p < .05$). The difference between the right and left lung was also significant ($p < .05$). The serotonin concentrations did not differ significantly between the right and left lung but were significantly higher than in the corresponding lungs in Groups C and B ($p < .01$).

Baseline Pa_{O_2} values in the two animals in Group B that had these measurements were 94 and 82, Pa_{CO_2} 38 and 39, and pH 7.39 and 7.42 respectively. The corresponding values after eight hrs of OHP were as follows: Pa_{O_2} 1240, 1340; Pa_{CO_2} 34, 36; and pH 7.42, 7.45.

TABLE 2: Biogenic Amine Content of Lungs After Lethal Exposure to OHP[1]

Animal No.	Side	Norepinephrine µg/g	Serotonin µg/g	Histamine µg/g
1	L[2]	.087	.296	8.60
	R[3]	.146	.268	10.50
2	L	.075	.418	6.50
	R	.124	.422	6.60
3	L	.153	.361	7.35
	R	.157	.380	7.50
4	L	0	.864	8.60
	R	.400	.944	9.70
5	L	.083	.556	6.00
	R	.498	.556	5.20
6	L	.093	.432	26.30
	R	.107	.515	16.30
7	L	.142	.685	23.60
	R	.162	.570	23.00
Average (±S.E.M.)	L	.090 ± .019*	.516 ± .076	12.42 ± 3.26
	R	.228 ± .058*	.522 ± .081	11.26 ±2.38

(1) 2.5 ATA till convulsion, then 2 ATA till death
(2) L = left lower lobe; denervated
(3) R = right lower lobe; innervated
* Significant difference between R and L ($p < .05$)

DISCUSSION

The evidence implicating the autonomic nervous system and biogenic amines in the pathogenesis of pulmonary O_2 toxicity has been discussed in comprehensive reviews of the subject by Bean ('45), Clarke and Lambertsen ('71) and other investigators. In essence, the evidence suggesting excessive sympathetic activity is derived from observations that pulmonary O_2 toxicity is retared by ganglionic blocking agents and made worse by stimulation of the superior cervical ganglion. Evidence implicating the parasympathetic

system is not as clear. For example, methacholine has been reported to exert a protective influence and atropine to abolish it. Yet, sectioning of the vagus nerves in the neck before exposure to O_2 in high partial pressures has been found to have a protective effect. The catecholamines, serotonin, and histamine have been implicated on basis of experiments in which the development of pulmonary O_2 toxicity is modified either by drugs that block these biogenic amines or by administration of exogenous biogenic amines. Except for experiments in which pulmonary O_2 toxicity was modified by stimulation of the superior cervical ganglion or by vagal interruption, other approaches have accompanying systemic effects which may themselves alter the effect of O_2 on the lung. Furthermore, changes in tissue catecholamine concentrations may have different effects in different tissues. Catecholamines accelerate the development of pulmonary O_2 toxicity yet the administration of monoamine oxidase inhibitors has an overall protective effect, presumably because these drugs prevent depletion of NE that occurs in the brain stem during hyperbaric oxygenation.

Thus, the present results are of interest because they indicate that denervation and depletion of catecholamines in the lung does not alter the early response of this tissue to the toxic effects of O_2 at high partial pressures in a conscious animal. Denervation prevents changes in catcholamine concentrations that occur during prolonged inhalation of O_2 but it does not modify changes in serotonin and histamine concentrations. These changes in pulmonary tissue concentration of biogenic amines are difficult to compare with changes in biogenic amines in other tissues. First, most measurements made by other investigators were made after animals were exposed to much higher pressures for shorter periods of time and convulsions probably affected the results. Rats breathing O_2 at 75 p.s.i. for 30 min for example, were found to have reduced catecholamine concentrations in the brain stem, and in the heart, increased serotonin concentration in the brain stem and decreased serotonin in the lung (Buckingham, Sommers and McNary, '68). In another study in rats, brain levels of NE and serotonin were unchanged after one hour of 100% O_2 at 4.95 ATA (Blenkarn, Schanberg and Saltzman, '69). The biomodal changes of NE and serotonin the present study i.e., early reduction followed by subsequent increase in concentration (in the denervated lung this holds true for serotonin only) may be explained possibly by inhibition of the enzymes tyrosine hydroxylase and tryptophan hydroxylase which are the rate limiting enzymes in the synthesis of catecholamines and serotonin respectively. When sufficiently low tissue concentrations of the biogenic amines are reached the net effect however, may be stimulation of the activities of these enzymes since prevailing evidence indicates that their activity is governed by the intracellular concentrations of these amines (Levitt, Spector, Sjoerdsma and Udenfriend, '65; Fischer and Kaufman, '72). Increased tissue concentrations of serotonin and NE in the innervated lungs of the moribund animals and serotonin in the denervated lung also

may have resulted from increased amounts of these biogenic amines in the circulation and perhaps from trapping of platelets in the lungs of the moribund animals. As was reported by us (Demeny *et al.*, '74), the absence of increased quantities of 5-HIAA in the urine suggests that serotonin was not degraded in appreciably increased quantities at any time. Increased glycol excretion in three dogs on the other hand suggests that increased degradation of catecholamines probably did occur.

The results of total denervation indicates that the innervation of the lung is not essential for the development of early pathological pulmonary changes during inhalation of hyperbaric oxygen. However, the results do not exclude the possibility that innervation may play a role in modifying the progression of the changes to full blown lesions of pulmonary oxygen toxicity. Furthermore, selective denervation (for example, severing the sympathetic nerves but preserving all other innervation of the lung) is technically extremely difficult and its effect has not been studied. It is conceivable that an imbalance of the activity of the autonomic nervous system supplying the lung may play a role in the development of the lesions of oxygen toxicity by the lung.

ACKNOWLEDGEMENT

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EFFECT OF OXYGEN AT HIGH PRESSURE ON CELLULAR FINESTRUCTURE: 1. STRIATED MUSCLE

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INTRODUCTION

Several studies have been made of the fine structural changes in skeletal and cardiac muscle accompanying acute and chronic hypoxia together with the changes resulting from training (Ou and Tenney, '70; Kraus and Kirsten, '69: Meerson *et al.*, '71; Bowers *et al.*, '71; Banister *et al.*, '71). Protection against hyperoxic (OHP) toxicity has even been afforded by acclimatization to hypoxia (Brashear and DeAtley, '72). However data on fine structural changes taking place in brain and particularly striated muscle under hyperoxic stress itself are relatively sparse (Banister, *et al.*, '72; Schnakenburg and Nolte, '70; Ballantine and Gutsche, '66).

During toxic reactions to hyperoxia (OHP) in the rat, there are obvious gross general convulsive movements of a large skeletal muscle mass and it seems desirable at this time to examine skeletal and cardiac muscle for fine structural changes accompanying convulsions of both a minor and severe nature precipitated by OHP.

MATERIAL AND METHOD

Animals

Male Sprague-Dawley rats (250–300 gms) were housed in groups of 5 in clean standard wire cages in a well-ventilated animal house and were maintained on a diet of standard rat lab chow and water *ad libitum*. Prior to the experiment, they were fasted for 14 hours. Thirty-five rats were randomly assigned to 4 groups: a) a normal group (N), sacrificed at sea level after no

HEMODYNAMICS OF THE GREATER AND LESSER CIRCULATION DURING DEVELOPMENT OF PULMONARY OXYGEN TOXICITY AT 1 Ata AND AT 2 Ata.

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A PORTABLE ELECTRONIC OXYGEN ANALYZER AND PRESSURE GAUGE FOR USE IN HYPERBARIC CHAMBERS

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INTRODUCTION

An electronic instrument for use in hyperbaric environments has been developed to measure the partial pressure and concentration of oxygen and the total ambient pressure. A prototype of the instrument is shown in Figure 1.

A block diagram of the instrument is shown in Figure 2.

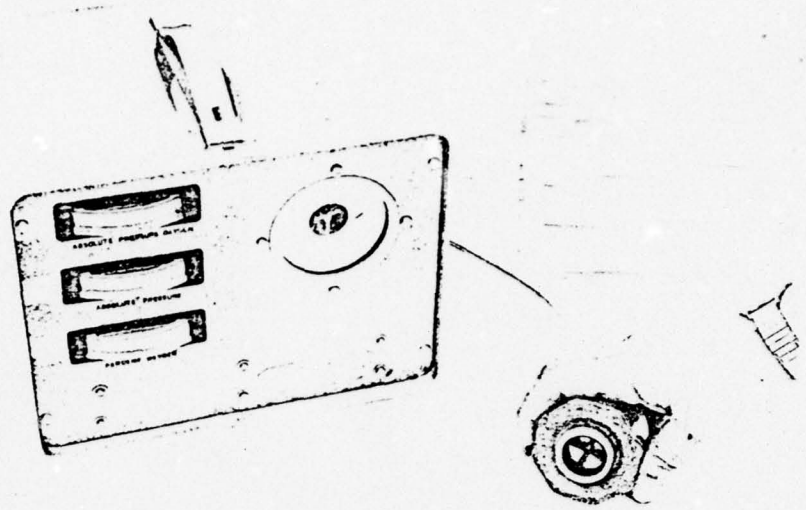
The pressure transducer is mounted permanently within the instrument cabinet. The O_2 sensor is attached to the cabinet by a flexible cable. When monitoring in a large space or volume, the O_2 sensor is allowed to remain in the cabinet. For small spaces or volumes the O_2 sensor is removed from the cabinet and inserted into the compartment being monitored.

Oxygen Sensor

The O_2 sensor is a self-generating O_2 battery [1] which is also called a "fuel cell" or "galvanic" sensor. It consists of a lead anode, a gold cathode, a FEP teflon diffusion barrier (membrane), a CsOH electrolyte bath, and an external load resistor. The gold cathode (the sensing electrode) is not involved in the reaction but serves as the site for the reduction of O_2 molecules to hydroxyl ions. The hydroxyl ions react with the lead "counter electrode" forming lead oxide and releasing electrons. The electron flow between anode and cathode, through an external load resistor, produces an output voltage which is directly proportional to the number of O_2 molecules reduced at the sensing electrode which is – in turn – proportional to the product of the permeation of O_2 through the FEP teflon

Fig 1. Prototype [illegible]

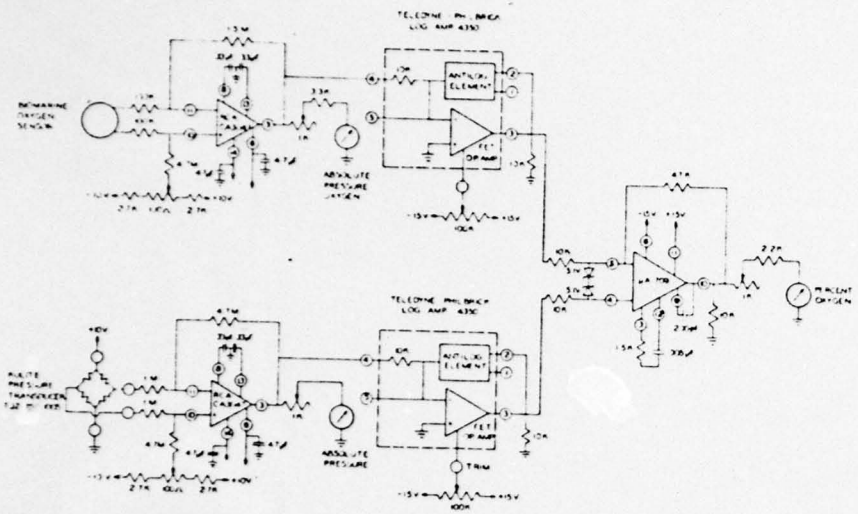
use in hyperbaric environments. This particular unit was specifically built to evaluate long term stability of the sensors, and contains a precision absolute pressure gauge which is not part of a regular instrument.



membrane and the O_2 pressure gradient across the teflon membrane. Since the sensor is designed to "use up" all of the O_2 which permeates the membrane, the P_{O_2} inside the membrane is equal to zero. Therefore, the output voltage is directly proportional to the P_{O_2} in the atmosphere external to the membrane.

This sensor has several advantages for application in a portable monitoring system. First, the instrument requires only a one point span calibration. This results from the fact that: when there is no O_2 available to go into the sensor, i.e. $P_{O_2} = 0$, the O_2 battery does not release electrons and generate an output voltage. This amounts, in effect, to a built-in zero. Secondly, compensation for the effects of temperature on the sensor is accomplished readily over a wide range of temperature. For example, at higher temperatures it is easier for O_2 molecules to diffuse across the membrane inasmuch as the permeation constant of the membrane increases with temperature. If the load resistance remained constant an erroneous value of P_{O_2} would be indicated. To prevent this error, the load resistor is connected in parallel with a thermistor, and this combination produces a load the resistance of which varies inversely with temperature. Thus, the product of current and resistance, i.e. the measured P_{O_2} remains

FIG 2: Block diagram of the O_2 analyzer and pressure gauge. The absolute air pressure and absolute O_2 pressure scales on the meters are linear (see Fig. 3). The O_2 concentration scale is logarithmic. In later models, antilog circuit elements convert the O_2 concentration scale to linear.



constant over the compensated wide temperature range. Thirdly, the sensor has a built-in failsafe signal because it must always indicate the P_{O_2} of air at ambient atmospheric conditions. If the membrane is accidently pierced, many more O_2 molecules will be admitted and the output will be increased greatly. If an electrical connection is broken, the output will be reduced to zero. In either event, it will be noted that the device is not in operating condition.

Several factors affect sensor life. The lead electrode is consumed in the sensor reaction. The sensor has enough lead to last for one year in 100% O_2 or five years in air at 1 ATA. Sensor life may be reduced also by bubbles within the cell and consequent reduction of the effective sensing area. This is overcome in the present design by external pressure on the membrane which tends to maintain the system free of bubbles. It should be emphasized that formation of bubbles will not lead to error as long as the instrument can be calibrated. Excessive evaporation of electrolyte will reduce sensor life. However, the vapor pressure of the liquid electrolyte is sufficiently low to minimize evaporation loss and keep the sensor in operating condition for five years. CO, and acidic gases such as fluorine, chlorine, and bromine will adversely affect sensor life. They will, with long exposure, react with the electrolyte. Precipitates

may form and deposit on the electrodes lowering cell output. These gases do not present a problem as long as their concentrations are low enough so that the atmosphere is breathable.

Performance of a typical O_2 sensor under hyperbaric conditions is as follows: error due to linearity – maximum ± 1% of full scale; temperature compensation error – maximum ± 5% of reading over temperature range 50 to 100 F (this can be reduced by use of specially matched thermistors); effects of humidity – accurate at relative humidities up to 100%; effect of pressure – can withstand a maximum pressure of 500 p.s.i. and can be decompressed at a maximum rate of 50 p.s.i. /min. Response time – 90% of full scale in less than 20–30 seconds. The response time is directly proportional to the membrane thickness. A thicker membrane is required as the operating pressure increases in order to produce a linear response curve as a function of pressure.

The O_2 sensor is commercially priced at approximately $200.00 each.

Pressure Transducer

The pressure transducer[2] contains a miniature silicon diaphragm on which a fully active four arm Wheatstone bridge strain gauge has been formed using solid state diffusion techniques. The diaphragm is "epoxied" to the rim of a miniature glass header to form a pressure capsule. Of the four piezoresistive gauge elements which comprise the bridge, two are near the center of the diaphragm and two are towards its periphery. When the diaphragm is deflected by pressure on the capsule, the ohmic resistance of the center gauge elements increases because they are subjected to tension. The peripheral gauge elements are simultaneously subjected to compression and their ohmic resistance decreases. Since each pair of gauge elements at the center and periphery are connected electrically as opposite arms of the bridge, the resulting unbalance produces an output of 100 mv nominal for 100 p.s.i. at an excitation of 10 volts DC or AC. This output is linear with applied pressure as shown in Figure 3.

The pressure capsule bearing the diaphragm is mounted into a four lead metal can with dimensions similar to a TO–5 transistor package. The top surface of the can has been replaced with a fine screen to allow the external ambient pressure to be communicated to the diaphragm.

The choice of this pressure sensor was based on its monolithic construction and small size which afford high mechanical ruggedness, low hysteresis and stable thermal characteristics. Tests pertinent to our application have confirmed the manufacturer's performance specifications. The combined error due to linearity and hysteresis is ± 0.5% of full scale. Sensitivity shift as a function of temperature in the range of our application is better than ± .005%/°F. Zero shift, due to the thermal expansion difference between the glass header and the silicon diaphragm is repeatable and amounts to ± .03% of full scale/°F. Repeatability of output is 0.10%

of applied pressure. Both elements are linear over the range 10 to 100 p.s.i.a.

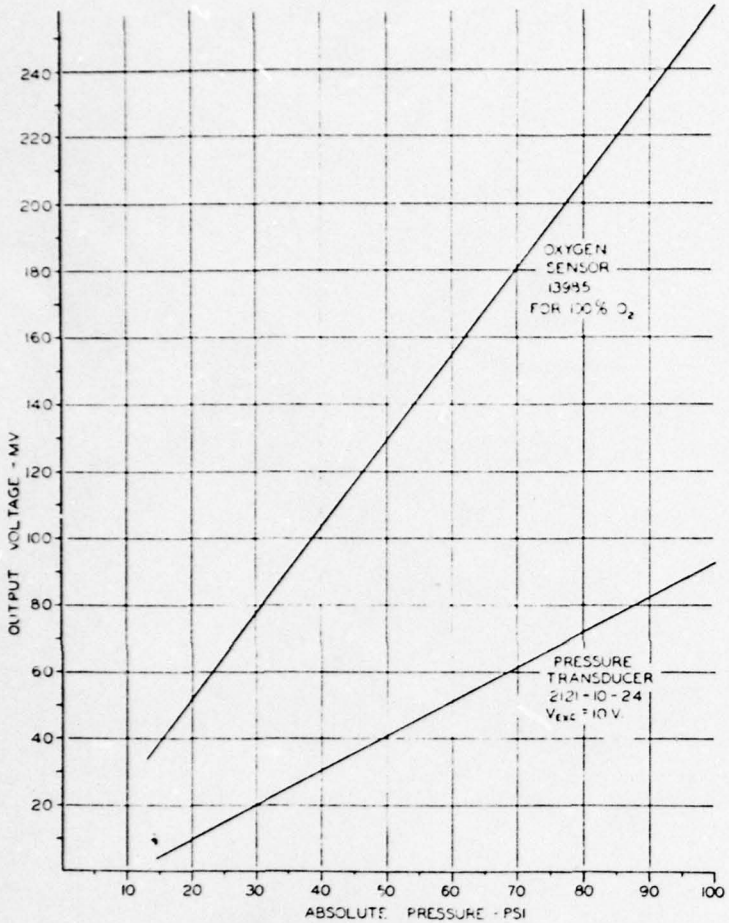


FIGURE 3 - DETECTOR OUTPUT AS A FUNCTION OF PRESSURE

of full scale. These results indicate that the *worst case error* for a standard pressure transducer used at a maximum operating pressure of 100 p.d.i. and a temperature range of 50 to 100°F, would be ± 2.35 p.s.i. at a full scale reading of 100 p.s.i. Of this 1.5 p.s.i. is due to the standard tolerance for zero shift and can be further reduced if required.

The diaphragm is rated to withstand a maximum pressure twice its rated operating pressure; and has a high natural frequency (65KHz).

The pressure transducer is commercially priced at approximately $100. each.

The power supply and electronic circuit components are solid state devices encapsulated for operation at pressures up to 100 p.s.i.a. No warm-up is required. The outputs from the O_2 sensor and pressure transducer are amplified and displayed directly on 0–1 milliampere DC meters. Both scales are linear. In addition, the logarithm of each transducer output is obtained with Philbrick logarithmic operational amplifiers. The logarithm of the ratio $\frac{\text{absolute } O_2 \text{ pressure}}{\text{total absolute pressure}} \times 100$ which is equal to the percent concentration of O_2, is obtained by subtracting the logarithmic outputs as shown in Figure 2. The percent O_2 concentration is also displayed on a 0–1 milliampere DC meter which has a logarithmic scale with range 5 to 100%. In order to perform the subtraction of the two logarithmic outputs, both linear transducer curves must be made to pass through a common origin by means of electrical offset and the amplification factors adjusted so that the percent O_2 scale reading will always be in the positive direction over the entire range. In a later model of the instrument, antilog circuit elements are used to convert the O_2 concentration scale to a linear one. The three meters, operational amplifiers, power supply, electronic components, and cabinet cost approximately $400. Parts for the entire instrument, including transducers cost $700.

Instrument Package

The model shown in Figure 1 operates from line voltage and has no on-off switches. Its line cord is terminated in an explosion-proof plug, which mates with an explosion-proof receptacle in the hyperbaric chamber. The receptacle is energized from the switch at the chamber control console. Small holes have been drilled into each meter case to permit pressure equalization.

The instrument shown in Figure 1 measures 8 in high by 10 in wide by 8 in deep and weighs 9 lb. An absolute pressure gauge was incorporated into this prototype model to facilitate evaluation of the long term stability of the sensors. With this gauge omitted, the instrument dimensions would be 6x6x6 in and, it would weigh 5 lb.

Utilization of the Instrument

This O_2 analyzer-gauge is designed to be taken into high pressure environments. A paramagnetic oxygen analyzer cannot be used in a hyperbaric environment without expensive modification because the glass dumb-bell which is required to rotate in the magnetic field would be crushed. The paramagnetic analyzer therefore has to be kept at 1 ATA and the pressure of the sampled gas also has to be reduced to 1 ATA before it enters the sampling chamber. The instrument would therefore be poorly suited for measuring small and low flow volumes. The fuel cell sensor has the following advantages: it can be exposed to high ambient pressures, it

has negligible "dead space", and it reads P_{O_2}, total ambient pressure, and percent O_2 directly. A rapid check on the calibration of the fuel cell can be performed against air at atmospheric pressure. A rapid check on the calibration of the pressure transducer is similarly performed against atmospheric pressure. The cost of parts of this single unit is only one-third the price of a paramagnetic system of comparable accuracy. The latter of course does not have capability for measuring the P_{O_2} or the absolute ambient pressure.

The instrument has proved valuable for measuring the O_2 tensions and concentrations in delivery systems, tents, ambient air, and exhaled gases.

FOOTNOTES

1. BioMarine Industries, Inc. (BMI) Devon, Pa., 19333.
2. Kulite Semiconductor Products, Inc., Ridgefield, N.J., 07657

64 Radiation Detectors and Sources in a Hyperbaric Chamber

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The hyperbaric environment creates an obstacle to use of radiation detection instruments and high intensity radiation sources. Systems with conventional vacuum or gas filled tubes are a hazard, and lithium drifted Si or Ge or ultrapure Ge detectors require cryogenic cooling. These difficulties were overcome by employing halogen quenched pancake type GM tubes and Cd–Te semiconductor gamma detectors. The GM tubes have .001" stainless steel windows and can operate up to 3 ATA. The Cd–Te detectors together with their preamplifiers have been operated up to 6 ATA. The power supply, scaler, and multi-channel analyzer are kept outside the chamber and are connected to the detectors within, through multipin connectors in the bulkhead. Cd–Te detectors operate at only 60 V, require no cooling, and can measure the gamma energy spectrum. Their sensitive volume, however, is much smaller than that of the GM tubes.

GM tubes were used to measure partition of the cardiac output between two lungs with 133–Xe. Cd–Te detectors have a 70% efficiency for 81 KEV gammas and will be used for measuring regional cerebral blood flow with 133–XE.

A modified radiologic instrument calibrator containing 137–Cs is used as a 130 C source in the hyperbaric chamber. The source is shielded by a mechanically operated absorber and interlock system and delivers up to 50 000 R/hour. The instrument is used to study the effect of gamma radiation on cell preparations under OHP.

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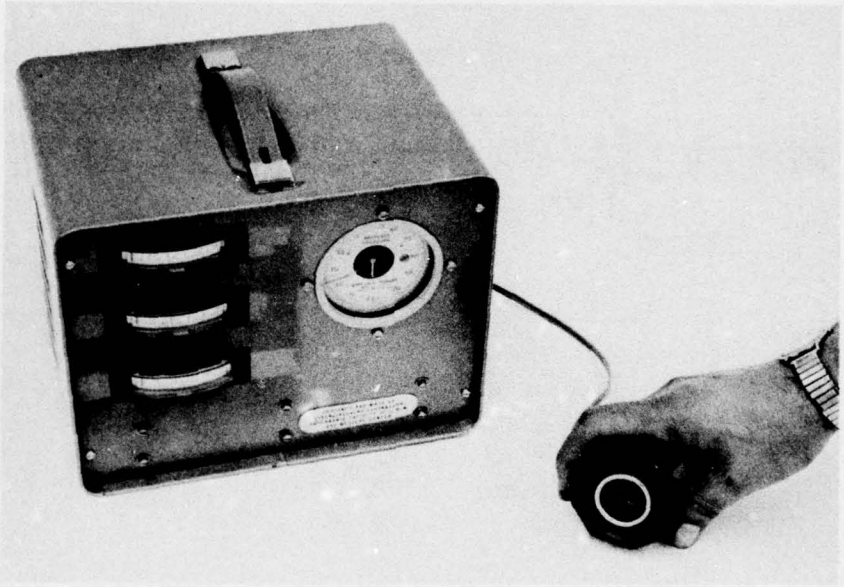
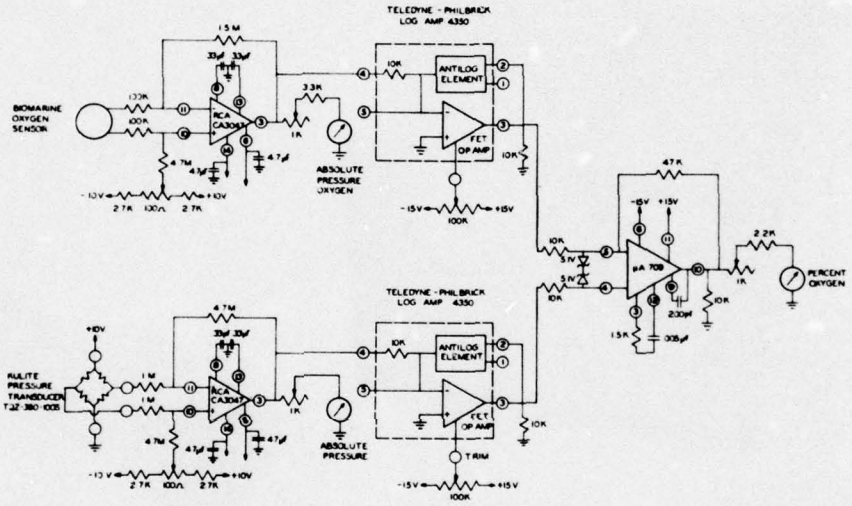
FIG 1: Prototype model of electronic O_2 analyzer and pressure gauge for use in hyperbaric environments. This particular unit was specifically built to evaluate long term stability of the sensors, and contains a precision absolute pressure gauge which is not part of a regular instrument.



membrane and the O_2 pressure gradient across the teflon membrane. Since the sensor is designed to "use up" all of the O_2 which permeates the membrane, the P_{O_2} inside the membrane is equal to zero. Therefore, the output voltage is directly proportional to the P_{O_2} in the atmosphere external to the membrane.

This sensor has several advantages for application in a portable monitoring system. First, the instrument requires only a one point span calibration. This results from the fact that: when there is no O_2 available to go into the sensor, i.e. $P_{O_2} = 0$, the O_2 battery does not release electrons and generate an output voltage. This amounts, in effect, to a built-in zero. Secondly, compensation for the effects of temperature on the sensor is accomplished readily over a wide range of temperature. For example, at higher temperatures it is easier for O_2 molecules to diffuse across the membrane inasmuch as the permeation constant of the membrane increases with temperature. If the load resistance remained constant an erroneous value of P_{O_2} would be indicated. To prevent this error, the load resistor is connected in parallel with a thermistor, and this combination produces a load the resistance of which varies inversely with temperature. Thus, the product of current and resistance, i.e. the measured P_{O_2} remains

FIG 2: Block diagram of the O_2 analyzer and pressure gauge. The absolute air pressure and absolute O_2 pressure scales on the meters are linear (see Fig. 3). The O_2 concentration scale is logarithmic. In later models, antilog circuit elements convert the O_2 concentration scale to linear.



constant over the compensated wide temperature range. Thirdly, the sensor has a built-in failsafe signal because it must always indicate the P_{O_2} of air at ambient atmospheric conditions. If the membrane is accidently pierced, many more O_2 molecules will be admitted and the output will be increased greatly. If an electrical connection is broken, the output will be reduced to zero. In either event, it will be noted that the device is not in operating condition.

Several factors affect sensor life. The lead electrode is consumed in the sensor reaction. The sensor has enough lead to last for one year in 100% O_2 or five years in air at 1 ATA. Sensor life may be reduced also by bubbles within the cell and consequent reduction of the effective sensing area. This is overcome in the present design by external pressure on the membrane which tends to maintain the system free of bubbles. It should be emphasized that formation of bubbles will not lead to error as long as the instrument can be calibrated. Excessive evaporation of electrolyte will reduce sensor life. However, the vapor pressure of the liquid electrolyte is sufficiently low to minimize evaporation loss and keep the sensor in operating condition for five years. CO_2 and acidic gases such as fluorine, chlorine, and bromine will adversely affect sensor life. They will, with long exposure, react with the electrolyte. Precipitates

may form and deposit on the electrodes lowering cell output. These gases do not present a problem as long as their concentrations are low enough so that the atmosphere is breathable.

Performance of a typical O_2 sensor under hyperbaric conditions is as follows: error due to linearity – maximum ± 1% of full scale; temperature compensation error – maximum ± 5% of reading over temperature range 50 to 100°F (this can be reduced by use of specially matched thermistors); effects of humidity – accurate at relative humidities up to 100%; effect of pressure – can withstand a maximum pressure of 500 p.s.i. and can be decompressed at a maximum rate of 50 p.s.i./min. Response time – 90% of full scale in less than 20–30 seconds. The response time is directly proportional to the membrane thickness. A thicker membrane is required as the operating pressure increases in order to produce a linear response curve as a function of pressure.

The O_2 sensor is commercially priced at approximately $200.00 each.

Pressure Transducer

The pressure transducer[2] contains a miniature silicon diaphragm on which a fully active four arm Wheatstone bridge strain gauge has been formed using solid state diffusion techniques. The diaphragm is "epoxied" to the rim of a miniature glass header to form a pressure capsule. Of the four piezoresistive gauge elements which comprise the bridge, two are near the center of the diaphragm and two are towards its periphery. When the diaphragm is deflected by pressure on the capsule, the ohmic resistance of the center gauge elements increases because they are subjected to tension. The peripheral gauge elements are simultaneously subjected to compression and their ohmic resistance decreases. Since each pair of gauge elements at the center and periphery are connected electrically as opposite arms of the bridge, the resulting unbalance produces an output of 100 mv nominal for 100 p.s.i. at an excitation of 10 volts DC or AC. This output is linear with applied pressure as shown in Figure 3.

The pressure capsule bearing the diaphragm is mounted into a four lead metal can with dimensions similar to a TO–5 transistor package. The top surface of the can has been replaced with a fine screen to allow the external ambient pressure to be communicated to the diaphragm.

The choice of this pressure sensor was based on its monolithic construction and small size which afford high mechanical ruggedness, low hysteresis and stable thermal characteristics. Tests pertinent to our application have confirmed the manufacturer's performance specifications. The combined error due to linearity and hysteresis is ± 0.5% of full scale. Sensitivity shift as a function of temperature in the range of our application is better than ± .005%/°F. Zero shift, due to the thermal expansion difference between the glass header and the silicon diaphragm is repeatable and amounts to ± .03% of full scale/°F. Repeatability of output is 0.10%

FIG 3: Outputs of the O_2 sensor and pressure transducer as a function of applied pressure. Both elements are linear over the range 10 to 100 p.s.i.a.

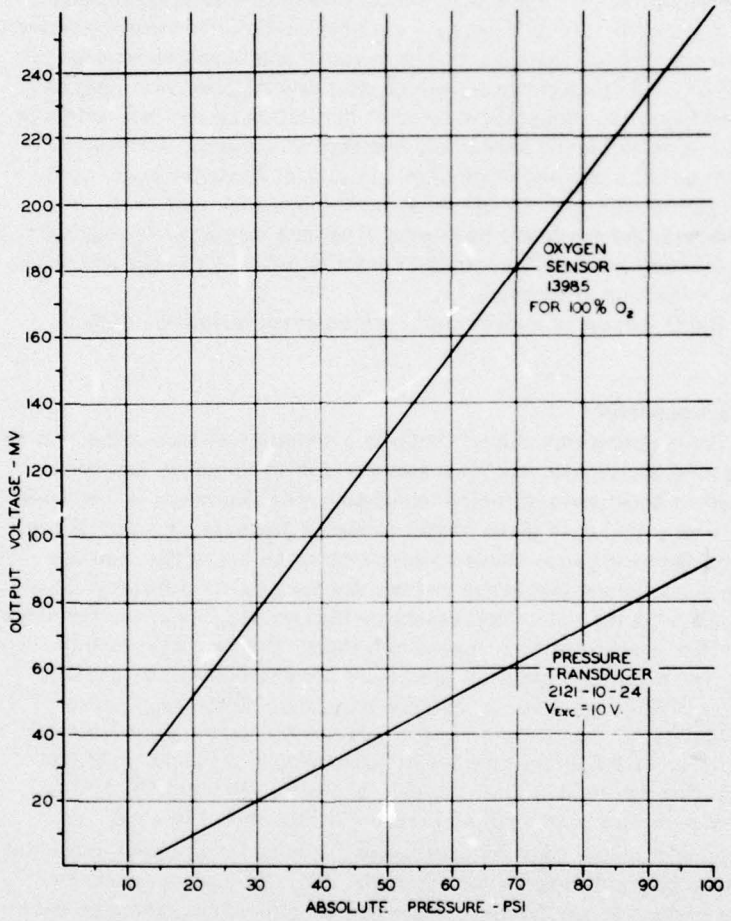


FIGURE 3 - DETECTOR OUTPUT AS A FUNCTION OF PRESSURE

of full scale. These results indicate that the *worst case error* for a standard pressure transducer used at a maximum operating pressure of 100 p.d.i. and a temperature range of 50 to 100°F, would be ± 2.35 p.s.i. at a full scale reading of 100 p.s.i. Of this 1.5 p.s.i. is due to the standard tolerance for zero shift and can be further reduced if required.

The diaphragm is rated to withstand a maximum pressure twice its rated operating pressure; and has a high natural frequency (65KHz).

The pressure transducer is commercially priced at approximately $100. each.

Electronic Circuit (Figure 2)

The power supply and electronic circuit components are solid state devices encapsulated for operation at pressures up to 100 p.s.i.a. No warm-up is required. The outputs from the O_2 sensor and pressure transducer are amplified and displayed directly on 0–1 milliampere DC meters. Both scales are linear. In addition, the logarithm of each transducer output is obtained with Philbrick logarithmic operational amplifiers. The logarithm of the ratio $\frac{\text{absolute } O_2 \text{ pressure}}{\text{total absolute pressure}} \times 100$ which is equal to the percent concentration of O_2, is obtained by subtracting the logarithmic outputs as shown in Figure 2. The percent O_2 concentration is also displayed on a 0–1 milliampere DC meter which has a logarithmic scale with range 5 to 100%. In order to perform the subtraction of the two logarithmic outputs, both linear transducer curves must be made to pass through a common origin by means of electrical offset and the amplification factors adjusted so that the percent O_2 scale reading will always be in the positive direction over the entire range. In a later model of the instrument, antilog circuit elements are used to convert the O_2 concentration scale to a linear one. The three meters, operational amplifiers, power supply, electronic components, and cabinet cost approximately $400. Parts for the entire instrument, including transducers cost $700.

Instrument Package

The model shown in Figure 1 operates from line voltage and has no on-off switches. Its line cord is terminated in an explosion-proof plug, which mates with an explosion-proof receptacle in the hyperbaric chamber. The receptacle is energized from the switch at the chamber control console. Small holes have been drilled into each meter case to permit pressure equalization.

The instrument shown in Figure 1 measures 8 in high by 10 in wide by 8 in deep and weighs 9 lb. An absolute pressure gauge was incorporated into this prototype model to facilitate evaluation of the long term stability of the sensors. With this gauge omitted, the instrument dimensions would be 6x6x6 in and, it would weigh 5 lb.

Utilization of the Instrument

This O_2 analyzer-gauge is designed to be taken into high pressure environments. A paramagnetic oxygen analyzer cannot be used in a hyperbaric environment without expensive modification because the glass dumb-bell which is required to rotate in the magnetic field would be crushed. The paramagnetic analyzer therefore has to be kept at 1 ATA and the pressure of the sampled gas also has to be reduced to 1 ATA before it enters the sampling chamber. The instrument would therefore be poorly suited for measuring small and low flow volumes. The fuel cell sensor has the following advantages: it can be exposed to high ambient pressures, it

has negligible "dead space", and it reads P_{O_2}, total ambient pressure, and percent O_2 directly. A rapid check on the calibration of the fuel cell can be performed against air at atmospheric pressure. A rapid check on the calibration of the pressure transducer is similarly performed against atmospheric pressure. The cost of parts of this single unit is only one-third the price of a paramagnetic system of comparable accuracy. The latter of course does not have capability for measuring the P_{O_2} or the absolute ambient pressure.

The instrument has proved valuable for measuring the O_2 tensions and concentrations in delivery systems, tents, ambient air, and exhaled gases.

FOOTNOTES

1. BioMarine Industries, Inc. (BMI) Devon, Pa., 19333.
2. Kulite Semiconductor Products, Inc., Ridgefield, N.J., 07657

MICROBIOLOGY AND INSTRUMENTATION

Rosasco, Buenos Aires: I would like to present our experience in the treatment of patients chronically ill with lepromatous leprosy. The patients have been treated from 1968 to date. Two hundred patients were treated with the following protocol: 60 min of 100% O_2 twice a day for three consecutive days, pressurized to 3 ATA in a single man chamber. Pure O_2 was used both for pressurization and for the breathing gas. A constant O_2 flow of 7 ℓ/min was used to ventilate the chamber. Ten patients have been followed for a 5 year period without recurrence. There are some slides here showing the patients before and after treatment. Some also recovered superficial sensibility in the skin. The remaining 190 patients are being observed with similar success.

Kindwall, Milwaukee: Dr. Rosasco, can you please review your treatment protocol for leprosy?

Rosasco, Buenos Aires: The patients receive the treatment for only three days, twice a day, in the chamber at 3 ATA of O_2.

Kindwall, Milwaukee: Were they not concomitantly being treated with other kinds of drugs?

Rosasco, Buenos Aires: In the next five years there was other treatment.

Kindwall, Milwaukee: Do you have a median age in terms of how long the disease was present? I assume that it had been chronic for many years.

Rosasco, Buenos Aires: Yes, it was.

Gottlieb, Fort Wayne: Dr. Rosasco, were these patients on sulphone therapy before, during or after treatment, or did you take them off drugs completely?

Rosasco, Buenos Aires: All patients have received sulphone before treatment. Afterwards it is very difficult to say whether they take sulphone because they form a dependency on some sort of drugs and for this reason it is impossible to be absolutely sure that the patient doesn't take sulphone or any other drugs after therapy.

Balentine, Charleston: Have you done any nasal blow studies or any mouse footpad assays of lepromas after treatment?

Rosasco, Buenos Aires: Yes, all patients are studied before treatment with the determination of the RDP in circulating blood. They have hard livers. The RDP after one chamber application is normal. All these patients have metabolic quotients studied by respirometry and they all have low level metabolic quotients. After treatment they are normal or just normal. All patients have been studied by baciloscopy and biopsy and all these remain negative in the next few years.

Balentine, Charleston: This is a very important part of this because some of the patients that have been on sulphone, for a long time afterwards, have very high nasal blow smears with acid fast bacilli even a year or so afterwards. I think it's worth pointing out that there is now an animal model, the armadillo, that can be infected with the mycobacterium leprae from

humans. The armadillo develops a disseminated disease without any immunosuppression. This might be a very valuable model to test this hypothesis in experimental animals with appropriate controls.

Gottlieb, Fort Wayne: As you know one of the big problems in any of the leprosy research has been the absence of an animal model and the gross inability to grow this organism in vitro. With the finding of the armadillo model about a year ago, I think it will open up the field quite extensively. I have a question for Dr. Demello: Why do you insist that there is a controversy on the use of OHP in the treatment of gas gangrene?

Demello, Minneapolis: On reviewing the literature and talking with some of the surgeons around our area, people at the Mayo Clinic, the University of Minnesota and various others, I find that some people still believe that treatment is ideal with just antibiotics and surgery. Quite a few of the doctors insist that OHP has more hazard for the patient than just surgery and antibiotics.

Gottlieb, Fort Wayne: The reason I ask is that in your model you said that you used Brummelkamp's procedure; three treatments the first day, two the second day and one the third day, but yet you were using 2 ATA of O_2. Brummelkamp used a pressure of 3 ATA. As I recall, from reviewing the literature, all the really successful studies in the treatment of gas gangrene whether with animal models or humans, have been done primarily at 3 ATA. When using 3 ATA of O_2 you have several beneficial effects: (1) decreasing the size of the gas pocket just by Boyle's Law, (2) increasing the O_2 tension and increasing rates of diffusion and (3) increasing effectiveness of O_2 on the organism itself. When you use 2 ATA of O_2, do you think that you can really extrapolate in general on the effectiveness of OHP in treating gas gangrene?

Demello, Minneapolis: Yes, we can. When we tried with 3 ATA there were greater problems with O_2 toxicity and the models did not work out too well since we were losing some of the animals to O_2 toxicity. Bringing it down to 2 ATA we could achieve the same beneficial effects. We keep saying that we are using the Brummelkamp and Boerema model just because of the schedule of the treatment. The only difference is the 2 ATA instead of three for the safety of the animals. We don't want to lose any of the animals because of O_2 toxicity, we want to stick to the infection and follow it.

Unsworth, Sidney: I'd like to continue with another question for Dr. Demello. May I suggest that perhaps you try 2.5 ATA O_2. It would seem a happy compromise. I think that you chose 1 kg rabbits and give them 5 mega Units of penicillin. If we take that to a 70 kg man, that's a rather large dose of penicillin. Why did you chose 5 mega Units per 24 hr for rabbits?

Demello, Minneapolis: We used the 5 million Units just to verify the effect of the maximum amount of the antibiotic that could be tolerated in the animal. For humans I think our schedule goes about 24 million Units in a day. Although we realize that this was very high, we still wanted to do it because it was the maximum dose tolerated by the animal.

Kindwall, Milwaukee: Dr. Demello, will you review again the method by which you administered the O_2. Did you use a monoplace chamber for the dogs?

Demello, Minneapolis: We had a special custom made metal box with a plexiglass front. We put that in our hyperbaric chamber and then we pressurized it and gave the animal O_2. This was to segregate the O_2 environment of the animals from the rest of the chamber.

Kindwall, Milwaukee: Essentially your O_2 delivery system was identical to that of a monoplace chamber, there was no mask involved or anything of the sort.

Demello, Minneapolis: That's right, no mask.

Kindwall, Milwaukee: In Amsterdam they use a large multiplace chamber masks and I think there is quite a difference in the amount of O_2 you really give depending on the efficiency of your mask. Brummelkamp did a lot of work with humans and he used 3 ATA. However, his mask may not have been that efficient. In our chamber we find that when we subtract water vapor, CO_2 and things of this sort, even with an excellent mask system, we have about 12.8% inefficiency. Then when we're at 50 ft we're really at 39 ft of pure O_2. When George Hart puts someone in a monoplace chamber at 2.5 ATA he might be really be approximating what is called 3 ATA in other systems. If you treated your dogs at 2 ATA you may have been approaching more closely 2.5 ATA in terms of Brummelkamp's experience. In other words, he may have been at 2.5 in terms of absolute O_2, so you may not be very much different there in terms of the arterial O_2 the patient was actually receiving. As far as the controversy about the treatment of gas gangrene with OHP: At two o'clock on Sunday morning I left the hospital after giving a treatment to a man who had been on antibiotics for some time. His urine was red and he was dying. The following morning his urine was clear. I don't think antibiotics could do that.

Hill, Duke: I have a couple of questions for Dr. Demello. It seems that there is still a lot of controversy among doctors on the clinical use of OHP for gas gangrene. I agree with you. First of all, I would like to know what the schedule of O_2 exposures was. I know there were three exposures at 2 ATA in the first 24 hr, but what was the exact schedule of exposures? We have found that many times, using OHP alone you can lose the effectiveness in treating gas gangrene if there's a long delay between the first and second exposures. The schedule of exposures even within the first 24 hr is extremely important since the infection can take off again. Secondly, I'd like to make a comment that the protection by antibiotics in experimental models and animals with gas gangrene has been noted many times. In fact, antitioxins and antibiotics in small experimental animals many times will protect and in our mouse model of gas gangrene actually penicillin would give you 100% survival, also antitoxin. This cannot be repeated in humans and this has been shown a number of times. I was a little concerned with the use of 100% mortality, because in other

words all of your control animals died. We found with our model of infection in mice that you could get an overwhelming gas gangrene infection with animals who died very rapidly. I don't think you could have stopped it with anything and OHP was much less effective. It would just simply prolong survival in these instances. Whereas, if you use what I think might be a little more biological a system where you go down to about 75% survival or anywhere between 50% and 80% you do get a very pronounced reduction in mortality using OHP, at least in mice. I might say that at 2 ATA and 3 ATA we got this reduction.

Demello, Minneapolis: As I mentioned in the introduction to my paper, not all animals respond to the gas gangrene infection equally. We had a problem with mice. In many of these mice, as the infection starts with their hind legs, they just chew their leg, slough it off and survive. Other mice were responding differently. Dr. Faiman and his group were having a problem infecting guinea pigs with gas gangrene. It seems that the experimental model that you are using and kind of organisms that you're using all have an effect on the infection. We found that mice and rats did not respond to infection very well, however, guinea pigs, rabbits and dogs responded very well. In using the spores we could calculate the number of organisms per cc to be injected that would produce an infection that would start to show at about 5 hr and in 12 hr you would have an active infection. We could calibrate it enough so that we could have death within 72 hr and thereby we control our infection much better. In all the controls, guinea pigs, rabbits and dogs, we had 100% mortality with a fulminating gas gangrene. We have a large number of animals and we schedule their chamber times. The first day we had the three treatments and we averaged a treatment about every 6 hr. Then the second day treatments are divided equally within the day, one in the morning and one in the afternoon. The third day, if they survived, they got the third treatment in the morning.

Hill, Duke: When was the first treatment given?

Demello, Minneapolis: The first treatment was between 5 – 12 hr after initiation of infection. We have about 20 animals so we have to schedule our chamber space accordingly. As I said, the infection will start about 5 hr, at 12 hr it's fulminating and we are between 5 and 12 hr for the first treatment.

Beerel, Buffalo: In the Proceedings there will be an article published, although it's not being presented now, on the treatment of 50 cases of gas gangrene within the last 5 yr. We have used 2.5 ATA for 2 hr, routinely, with excellent results. We have used more or less the same treatment schedule as Dr. Demello has indicated. The first three days we used treatment every 8 hr, if the treatment was 2 hr, it would be 6 hr in between. The next three days we use it every 12 hr in conjunction, of course, with approximately 20 million Units of penicillin in 24 hr.

Attar, Baltimore: I think some of the controversy about the treatment of gas

gangrene arose with Altemeier from Cincinnati who indicated that he had equally good results with his surgical and antibiotic treatment of gas gangrene. We have to admit that Dr. Altemeier is really the most experienced in this field since he has had tremendous past experience with it. I assume that they don't have a hyperbaric chamber. This is one of the reasons the controversy arose. At the University of Maryland we have had more than 20 cases of gas gangrene that were treated with OHP exactly according to Brummelkamp, i.e., a combination of OHP, minimal surgery and antibiotics and we have lost only two patients out of the 20 cases. I think the surgical results really indicate that to date there is nothing better than a compromise and it is a combination of OHP therapy, surgery and antibiotics throughout. I think the treatment has gone beyond the stage of experiment. There is already a clinical entity.

Hunt, San Francisco: I think one of the most exciting developments in hematology and infection recently has been the identification of the fact that although white cells can phagocytize bacteria, they can't kill them without O_2 in many instances. Today we've directed our attention to the effect of O_2 directly on the organism itself. I would hope by the next meeting that we'll have some information about what O_2 does to the white cell defense system which I think is probably equally, if not more, important.

Reich, New York: I'd like to ask our Russian colleagues what sort of instruments they use for measuring molecular and gaseous dissolved O_2 under pressure. I'm interested in both gaseous O_2 and dissolved O_2.

Mari, Vancouver for Utjamishev, Moscow: They have polarographic methods and they have some of their own equipment and some of it has been imported. If you want technical details I think Dr. Utjamishev will be able to send the information to you.

Beerel, Buffalo: I am particularly interested in Dr. Utjamishev's apparatus for tissue P_{O_2}'s. How accurate are they and how well can they be measured? I think this is an instrument that has plagued us by being quite unreliable and has not yet been fully developed in our country.

Mari, Vancouver for Utjamishev, Moscow: The degree of error in their polarographic electronic method is between 5% and 7%.

Nashimoto, Tokyo: I have a question for Dr. Niinikoski. What maximum pressure can your silastic tonometer tolerate? Do you use the apparatus under high pressures?

Niinikoski, Finland: The highest pressure at which we have measured gas tensions in animals has been 2.5 ATA. As I showed, the measurements were performed so that the assistant who was taking the samples in the glass capillaries and the animal were both pressurized. The glass capillaries were stored on crushed ice and sealed with wax on both ends before the measurement took place. The measurement took place after the decompression, at normal atmosphere. Hypoxic saline is just normal saline and nitrogen bubbles have been beaded through the saline solution.

Unknown Speaker: The method has no practical limitations on pressure

and it's error is essentially that of the measuring technique of the electrode.

Lundgren, Sweden: I would like to ask Dr. Niinikoski the reason for using anoxic saline. Wouldn't it do just as well with ordinary saline?

Niinikoski, Finland: We have been testing the system and have filled the tubing with normal saline equilibrated with air, that is to say, a P_{O_2} of 150 mmHg and it seems to function as well. But if we go into very low P_{O_2} conditions then we supply O_2 to the tissue and then we get artificial values. Of course, the system I'm using now, the hypoxic saline, extracts O_2 from the tissues but the amount it extracts, when a capillary sampling technique is used, is very small and is clearly smaller than the amount of O_2 supplied to the tissue.

Gottlieb, Fort Wayne: Dr. Longmuir, how do you visualize your beautiful technique being used in people?

Longmuir, Raleigh: We've found that the material is non-toxic in intact animals. It can be injected intravenously to make the animal up to one millimolar with respect to the material. This is far more than we need for fluorescence. All the tissues except the spleen take it up readily and the tissues fluoresce quite brightly. We've only just begun using this technique and we don't really have any good data to present on it yet. However, it does look as though we'll be able to measure the O_2 tension at least on the surface of the brain and other organs or tissues that can be exposed with a fairly high degree of accuracy in terms of P_{O_2}, both spatially and temporally. We can certainly discriminate to a half micron spatially, temporally the changes that occur are very rapid indeed. For deeper tissues we're developing the light pipe method so that we can put the UV light through a diacroic filter down the light pipe to the site that we want to measure or look at the distribution of O_2 concentration. Then, exciting at 366 mu and looking at 410, the 410 will come back up through the light pipe and straight through the diacroic filter this time and into our measuring system. We can use either a photomultiplier to look at the picture generally or we can attach it to the objective of a microscope and achieve the degree of discrimination that can be achieved with fiberoptics at least.

Youdin, New York: Dr. Longmuir, I think now we really can say that there is no single tissue of any given characteristic O_2 tension and there is no single cell of any characteristic O_2 tension. I'd like to just compare the methods we've been presenting here. The values that silastic tubing measures are average tissue P_{O_2} values which may have some clinical significance. I really believe that in clinical use the measurements of tissue gases under microscopic vision is not really very feasible and the method which gives an average P_{O_2} is actually needed. But, I think that this method of yours adds very much to the knowledge of O_2 supply in various parts of the tissues and cells. Assuming that the P_{O_2} at the site of the mitochondria is very low and there is some evidence from Silver's studies in England that it may be in the order of 1 mmHg or so, what is your guess as to what the P_{O_2} might be at the cell surface or just

underneath that?

Longmuir, Raleigh: I think perhaps my colleagues and collaborators who are already using the method might disagree with you that it's not quite practical yet. It's just that we don't have enough data to present here. On the question of P_{O_2}, we showed, 20 yr ago, that heart muscle preparations would respire at a constant rate down to something of the order of 0.02 mm. Presumably it's at that point that the NADH begins to be reduced and you see the increased fluorescence, by the Chance technique. Very likely the O_2 tension at the mitochondria in those living, unstained cells which are beginning to show NADH fluorescence, which we have to distinguish in our method by use of filters, have within the mitochondrium, certainly at the site of the cytochorme oxidase, an O_2 tension that has fallen somewhere way below 1 mm. The difficulty is that you can't really calibrate this technique directly because nobody knows what the O_2 tension inside cells is. From somewhat indirect measurements we can guess that the total gradient from outside to within the mitochondrium is in the order of 1.5 microns. When we come to whole tissue the picture becomes much more complicated because we have ground substance present between the cells and various other structures which seem to be relatively impermeable to O_2. We begin to get very much bigger gradients, of the order of several millimeters, when you start looking at a slice of tissue which may be just two cells thick. I think we can say that the O_2 tension within tissues is anywhere from arterial to zero and that the distribution is very complex. We clearly have a maxima near the arterial end of the capillaries and a minima somewhere between two cells, but this is not a uniform picture. We know that the mitochondria are not uniformly distributed, We have reason now to believe that the mitochondria aren't respiring at a constant rate all the time, but are fluctuating quite widely. There are obvious fluctuations in the O_2 supply with each breath and presumably with the opening and closing of capillaries and so on. In fact we're dealing with an extremely complex picture and if you want an analogy, your method measures the height of the tide and we're trying to look at all the ripples in the waves.

Gottlieb, Fort Wayne: Dr. Longmuir, your slides indicate that the nucleus may be anaerobic. Is it because the nucleus is anaerobic, that there was no fluorescence there, or is is that the dye is not getting across the nuclear membrane or that the histones are not binding your dye?

Longmuir, Raleigh: It seems that the nuclear membrane is impermeable to this material. We would expect the histones, in fact, to bind rather strongly if the material did get in. We find the partition coefficient between nuclear suspension and suspending fluid of the order of 10:1. It is extremely difficult to prepare nucleii that are not contaminated with significant amounts of microsomes which do have a high affinity for the material. We rather suspect that there is no para-nuturic acid at all in the cells and that produces the artifact of no fluorescence equalling quenched fluorescence

and that gives the apparently high O_2 content. It's probably some sort of mean of the O_2 tension at the surface of the nucleus.

Spencer, Seattle: Dr. Youdin, your device using the lead looks like a very interesting one that we all need in our hyperbaric chambers to monitor the atmosphere. You quoted that the cost would be about $700. Is this for parts alone and if we have our own shop are the plans available to build it? Are the components available on the market today or do you have to wait months for them?

Youdin, New York: Yes, you can build it. The pressure transducer that we use is made by Kulite. It's a standard pressure transducer. The O_2 sensor is a Biomarine standard unit. The only thing that is not standard is that the O_2 sensor being used requires three or four weeks to get the linear curve over the entire pressure range. Normally everyone is interested in as thin a membrane as possible so that you can get the fastest resolving time, but if you have a very thin membrane you don't get a linear characteristic at high pressure so you do have to wait about three or four weeks for that.

Youdin, New York: Dr. Spencer, I would like to know the smallest size bubble that you can detect with your device. How do you overcome the artifact of the acoustical coupling since those were quite substantial detectors? Do you fasten them in place or hand-hold them, or how do you manage that?

Spencer, Seattle: The acoustical coupling is usually done with a jelly. You can purchase acoustical gels that have a pretty good match or you can use just water. If you're in the water, the water itself works just as well. The coupling is not a large problem.

Youdin, New York: Do you take that sensor and put it right on the diver as he is in the water?

Spencer, Seattle: Yes, if there is moisture or liquid between the crystal and the skin, it works. I'm afraid we cannot tell you how small a bubble we can detect. We can only deduce from calculations that our engineers have made that we believe perhaps we can hear bubbles as small as 20 microns. However, data from the Symposium in Seattle just before this meeting, from one group at Duke producing calibrated bubbles, indicates that 200 microns is more reasonably what we can expect for diameter.

Jacobsen, Seattle: We don't have published data on this but we're presently using an in vitro set up where we're creating bubbles off a 1 mu neurological probe and we're detecting them with the ultrasonic doppler. We think we're in the range of a 1 micron bubble in an in vitro model. Other analytical work which I presented just a few days ago at the Symposium in Seattle provides speculation that we're probably detecting bubbles somewhere in the 50 micron range, possibly give or take 20 microns, because of estimates from the diffusion data. We are trying to determine where we are in relation to the time that we pick up the bubbles subsequent to the dye profile. I am supicious of the 200 micron estimate

and I think that we're probably somewhat smaller than that.

Hunt, San Francisco: Your resolving power still has to be somewhat larger than the other particles in blood doesn't it?

Spencer, Seattle: No, the resolving power would not be like a red cell. We're depending on the acoustical interface between a gas and a liquid and this is almost 100% reflection of the impinging sounds. As Dr. Jacobsen indicates, perhaps you can make a case for going down to smaller than a red cell. Certainly I think there are other reasons why we may not expect bubbles of that small size to be present.

Question from Unknown Speaker:

Youdin, New York: We felt that the present No-D schedules are conservative in the deep short time interval because we could exceed those exposures without producing bubbles and then of course bends is beyond that. The degree of conservativeness is only a matter of 5 min which, at those short times, perhaps is not a practical point as far as making any change is concerned.

Barr, Stockholm: I want to ask Dr. Youdin about the elegant design of the O_2 analyzer. I don't think you mentioned anything about the power supply. Is it driven by batteries? How do you solve the problem with pressure in the batteries and can it be used in the monoplace O_2 chamber?

Youdin, New York: This particular unit that I showed here uses a 110 volt supply. The cable is terminated in an explosion proof cable and in our chamber we have explosion proof receptacles so that this particular unit works from 110 volts. There is no switching because the receptical is actuated from the chamber console. We do have another model in which we use mercury batteries with the enclosed switches so that you can switch with no fear of sparking. That's perfectly all right because 110 volts is too much because we only need about 10 volts, so we can step down. The power supply is totally enclosed and batteries would not be a problem.

RADIOBIOLOGY

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