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Abstract: This final year resulted in a pulling together of all five years' work and will culminate in many publications during the next two years. The following studies were performed and publications are in preparation:

1) Indolamine 2,3 dioxygenase has been found in human lung; this enzyme has potential as an important oxygen radical scavenger, 2) MK421, is a non-sulfhydryl group angiotensin-converting-enzyme inhibitor which was found not to alter adrenergic responsiveness, 3) Neuron-specific-enolase and 5-HT immunoreactive lung neuroendocrine cell populations are not the same in the fetal monkey lung implying that either development rates are not the same or more than one population is present, 4) a possible genetic relationship between cytochrome P-450 enzyme induction and oxidative stress has been established implying that the susceptibility to oxygen toxicity may be inherited as well as environmental, 5) Selenium and vitamin E deficiency may cause a decrease of the medial thickness in small pulmonary arteries implying that regulation of smooth muscle reactivity may be related to levels of organic hydroperoxides, lipid peroxidation, lipoxygenase or cyclooxygenase products, or a change in platelet activation status, 6) The smallest subunit of Lipid A, Lipid X (mol.wqt. 711) has been characterized and causes all of the physiological effects on the pulmonary circulation seen with complete endotoxin, and fnally 7) Venous dispersion of lung 5-HT uptake kinetics using the bolus injection technique were different when trace doses were superimposed on constant background concentrations.

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Lung Metabolism, Function, and Morphology During Hyperoxic and Hyperbaric Exposure

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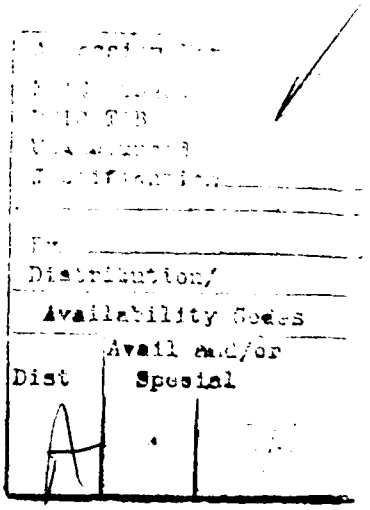

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Objecties

The objectives for year 05 were four-fold:

I. Pulmonary oxygen toxicity in hamsters.

II. The role of angiotensin in modulating physiologic rseponse to chronic changes in inspired oxygen tension.

III. The effects of inspired O_2 on cardiopulmonary responses to regulatory peptides.

IV. Serotonin and trytophan and their role in oxygen toxicity.

Additionally, we continued and completed studies from previous years. Specifically we did the following studies which are either still in progress or will be soon completed:

A. We have developed an *in vitro* model which allows us to quantitate changes in endothelial cell functions, eg. 5-HT uptake.

B. The role of endotoxin in pulmonary vasular responses and the relation to oxygen toxicity.

C. More fully describe the role of oxidants as pulmonary vasodilators.

Summary of accomplishments:

The results of these studies can be summarized as follows:

I. POSSIBLE GENETIC RELATIONSHIP BETWEEN CYTOCHROME P-450 ENZYME INDUCTION AND OXIDATIVE STRESS. Susceptibility to oxygen toxicity was studied in C3H/HeJ and DBA/2J mice. Mice responsive to hepatic enzyme induction by aromatic hydrocarbons (C3H/HeJ, Ah^b/Ah^b) were more sensitive to the toxic effects of 100% oxygen exposure than were genetically unresponsive mice (DBA/2J, Ah^d/Ah^d). DBA/2J mice survived significantly longer exposure periods, with less lung damage. In C3H/HeJ mice, lung and liver cytochrome P-

450 enzyme levels increased during 100% oxygen exposure (maximum levels at 72-96 hours), and subsequently decreased prior to death. No changes were seen in cytochrome P-450 levels in oxygen-toxic DBA/2J mice. Metabolic pathways involving cytochrome P-450 enzymes may play a role in oxidative damage due to the generation of oxygen radicals. The genetic responsiveness of mice to hepatic enzyme induction may play a role in susceptibility to oxidative stress.

II. CHRONIC INHIBITION OF ANGIOTENSIN CONVERTING ENZYME (ACE) WITH MK421 IN NORMOTENSIVE, NORMOXIC, GUINEA PIGS. Effects of the converting enzyme inhibitor MK421 (the ethyl ester of N-[(S)-1-carboxy-3-phenylpropyl]-L-Ala-L-Pro) on pressor responses to A-I, A-II, epinephrine and norepinephrine were studied in anesthetized normotensive guinea pigs. Acute administration of MK421 (100 µg/kg) either intravenously or subcutaneously produced a significant decrease (-46 ± 6%) in mean arterial blood pressure (MABP) with a nadir of 40 min and duration of 2-6 hrs. The pressor response to A-I (140 pM/kg) was significantly reduced from 35 ± 4 mmHg (Control) to 11 ± 3 mmHg (post-MK421). Guinea pigs treated chronically (72 hrs) with MK421 (20 µg/kg/min, sq) also had significantly ($p<0.05$) lower MABP (36 ± 2 mmHg) than untreated controls (52 ± 44 mmHg). Chronic treatment also produced a significant shift to the right in the pressor response curve to A-I with the highest dose of A-I producing a ΔMABP = 64 ± 3 mmHg in controls vs a ΔMABP = 31 ± 2 mmHg in treated animals. MK421 did not alter pressor responses to A-II or the catecholamines. These data demonstrate that MK421 produces hypotension in normotensive, normoxic, guinea pigs without altering adrenergic responsiveness. Pulmonary vascular data not analyzed at this time.

III. a) A NON-ADRENERGIC RELAXANT RESPONSE OF ISOLATED GUINEA-PIG PULMONARY ARTERY TO FIELD STIMULATION. In order to determine if a non-adrenergic relaxant response exists in the guinea-pig pulmonary arteries, we examined frequency-response curves to field stimulation on isolated segments of the proximal and distal main pulmonary artery and right and left main branches taken from male albino guinea-pigs. In the absence of treatments, field stimulation (10v, 1 msec) produced a frequency dependent (1-32 hz) contractile response, which was markedly reduced by pretreatment of animals with reserpine (5 mg/kg, 16-20 hours) and blocked by treatment of tissues with phenoxybenzamine (PBZ; 10-5M,15 min.) or tetrodotoxin (TTX, 10-6M, 30 min.). Relaxant responses were examined on tissues from reserpine treated animals, which were contracted with PGF2a in the presence of PBZ to about 25% of the barium maximum. Field stimulation produced a frequency-dependent (0.2 - 8hz) relaxant response which was blocked by pretreatment of tissues with TTX, but not with propranolol (10-6M, 1 hour) alone or in combination with theophylline (10-4M, 15 min.), an antagonist of some purinergic relaxant responses. It is concluded that a nervous , non-adrenergic relaxant response to field stimulation exists in the guinea-pig extralobar pulmonary artery and is not mediated through beta adrenergic or theophylline and sensitive purinergic receptors.

III. b) NEURON SPECIFIC ENOLASE AND SEROTONIN DISTRIBUTION IN THE FETAL RHESUS MONKEY LUNG BY IMMUNOCYTOCHEMISTRY. The distribution of neuron specific enolase (NSE) and serotonin (5-HT) have been investigated in fetal rhesus monkey (Macaca mulatta) lungs by the modified peroxidase-anti-peroxidase (PAP) method of Sternberger. Fetuses were delivered by C-section at 135-145 days gestational age. Lung was removed immediately and samples

fixed in Bouins and paraffin embedded. Five and 10u sections were cut and processed for PAP according to Polak and Van Noorden. Dilutions for NSE and 5-HT were 1:4000, overnight. The distribution of the cells positive to the two antibodies was different. NSE-like activity was confined to single cells of the epithelium of large intrapulmonary bronchi and peri-bronchial glands. 5-HT-like activity is found both in single cells and in clusters of cells (NEBs) of a few larger intrapulmonary bronchi and in the peripheral airways. This could imply that at least two distinct populations of neuroendocrine-like cells, as identified by these antibodies, exist in the fetus of this species.

IV. HUMAN LUNG APPEARS TO HAVE INDOLEAMINE 2,3-DIOXYGENASE ACTIVITY; A POTENTIAL OXYGEN RADICAL SCAVENGER. Indoleamine 2,3-Dioxygenase (IDO), first described by Hayaishi, can use superoxide (O_2-) as a substrate to cleave the pyrrole ring of tryptophan (T) in the conversion to formylkynurenine (FK). This enzyme differs catalytically, molecularly, immunologically and in its substrate specificity and distribution from tryptophan 2,3 dioxygenase (TPO). TPO is liver based and only uses O_2 to cleave the pyrrole ring from T. Because IDO has been found in the lung of many species other than man and can use O_2-, we investigated whether it exists in man. Nine samples of fresh human lung were obtained at surgery and reactions run aerobically. The reults (expressed as umol kynurenine per mg lung tissue protein) as determined by high performance liquid chromatograhy (HPLC were: 1) Baseline, 0.71 (median); 2) with T, 1.53; 3) with T and xanthine and xanthine oxidase as superoxide generators, 2.85; 4) T plus superoxide dismutase as a competitive O_2- scavenger, 1.61. These data (1<2,3,4,; 3>1,2,4; and 2=4) imply IDO exists in man; its significance as O_2- radical scavenger and inducibility is being studied.

Previous years objectives:

A. 1) IMPACT OF VENOUS DISPERSION OF LUNG SEROTONIN UPTAKE KINETICS. Two indicator dilution methods (A and B) were used to evalute the nonlinear transport kinetics for serotonin (5HT) uptake in isolated dog lungs. In A, a bolus injection produced a range of capillary 5HT concnetrations (C) which included Km. In B, boluses containing trace doses of 5HT were superimposed on a series of constant background C's, including Km. Bronikowski et al. (Math.Biosci. 61:237, 1982) predicted on theoretical grounds that venous dispersion would result in Km and Vmax values from Method A which were lower than those obtained by Method B by the ratio of C at the sampling site (which is measurable) to the end capillary C (which is not measurable) while Vmax/Km should be the same by both methods. Since the kinetic parameters determined from B are independent of venous dispersion, comparison of the two methods should provide estimates of end capillary C's in Method A, and of venous dispersin. Method A resulted in Km, Vmax and Vmax/Km values which were 0.66, 0.63, and 1.03 times those of Method B, respectively. Thus, the results are consistent with model prediction and indicate that in this system venous dispersion resulted in C's at the sampling site whch were about 35% lower than end capillary C's. The standard deviation in post-capillary transit times was about 1.8 seconds.

A. 2) ENHANCEMENT BY PHENTOLAMINE OF RESPONSES TO 5-HT AFTER TACHYPHYLAXIS. The dose-response curve to 5-HT in guinea-pig isolated pulmonary arteries exhibits two contractile phases (Fed. Proc. 41: 1649, 1982). The first phase (I) is blocked by methysergide (MSG) and undergoes tachyphylaxis. MSG does not block phase II of the 5-HT dose-response

curve. Phentolamine (P) changes the shape of the 5-HT curve such that a single contractile phase is prevalent. In order to examine the mechanism of this effect, we studied the action of P on responses to 5-HT in the proximal half of the main pulmonary artery (PM) and the extralobar left main branch (LB), both suspended as rings in isolated tissue baths. Guinea pigs were treated with reserpine (5 mg/kg, i.p.) 16-25 hr before each experiment. Exposure of the arteries to 10^{-3}M 5-HT followed by a 30 min wash period resulted in total loss of phase I of the 5-HT dose-response curve. Partial loss of phase II was also observed and was larger in the LB than in the PM. Exposure to P, 10^{-5}M, during the wash period resulted in partial restoration of contractile responses to 5-HT. The enhancement by P was blocked by concomitant exposure to MSG, 10^{-8}M. The effect of MSG was larger in the LLB segment. MSG alone did not alter the dose-response effects of 5-HT after tachyphylaxis. The results suggest that P acts to reduce desensitization of MSG-sensitive 5-HT receptors.

B. LIPID X (LX), A MONOSACCHARIDE SUBUNIT OF LIPID A REPRODUCES THE PATHOPHYSIOLOGICAL EFFECTS OF ENDOTOXIN (LPS). LX, a monosaccharide subunit of lipid A, found in the membranes of certain E. coli mutants whose preliminary structure was first reported in J. Biol. Chem. 256:10690, 1981, now has been further characterized by K. Takayama, L. Anderson and C. Raetz (J. Biol. Chem., in preparation). They found this to be a monosaccharide containing 2-hydroxymyristic acids (structural details to be reported at Am. Soc. Biol. Chem. 1983) with molecular weight 711. Using the lung-lymph (L-L) sheep model of Staub, we studied the physiological effects of this moiety on the pulmonary circulation and compared these effects to LPS. An accumulative dose effect of 40 µg/kg LX was studied in these preliminary investigations. P_{PA} pressure showed a transient rise after the first two 10 µg/kg doses which

was sustained (>300%) similar to LPS after the 3rd (20 µg/kg). L-L flow increased >200% in phase 1 and 150-300% in phase 2. Permeability by lymph/plasma oncotic ratios implied increased transport of proteins during phase 2. These findings demonstrate that LX is the smallest subunit of LPS able to reproduce the pathophysiology of LPS.

C. 2-BUTANONE PEROXIDE AND t-BUTYL HYDROPEROXIDE CAUSE THE OXIDATION OF GLUTHATHIONE AND OTHER CELLULAR SULFHYDRYL GROUPS. We examined the effects of these agents on pulmonary vascular reactivity in 13 anesthetized dogs. 2-butanone peroxide decreased the pulmonary vascular resistance attained after 15 minues of hypoxia (F_1O_2 10%) in 7 dogs from 6.3±0.4 to 3.4±0.3 mm Hg/L/min ($p<.01$ while leaving systemic arterial pressure unchanged. t-butyl hydroperoxide decreased the 15 minute hypoxic pulmonary vascular resistance in 6 dogs from 6.3±0.9 to 3.7±0.6 mm Hg/L/min ($p<0.1$). The mechanism by which these agents cause pulmonary vasodilatatin is not certain but might involve the oxidation of sulfhydryl groups in enzymes or membranes. Becuase 2-butanone peroxide given intravenously did not produce systemic hypotension, unlike t-butyl hydroperoxide and the drugs currently available for the clinical treatment of pulmonary hypertension, further studies of its mechanism of selective action are indicated.

1982 Presentations:

FASEB: 1) Closed circuit animal chamber for studying the effects of oxygen at variable concentrations. Janet C. Gonder, Peter S. Thorne, Edwin N. Lightfood, J.A. Will.

2) Changes in inspired oxygen modify the homedynamic response to angiotensin in awake sheep. A.M. Nielsen, D.F. Erichsen, and J.A. Will.

3) Pharmacologic attenuation of hypoxia-induced arterial hypertrophy in rat lungs. Inge M. Keith, James A. Will, and E. Kenneth Weir.

International Symposium on Pulmonary Hypertension, Vienna, Austria:

1. Pulmonary vasodilators in Experimental Chronic Pulmonary-Hypertension. Bull. Europ. de Physiopathologie Respiratorie 18: 4,p. 91, 1982. J.A. Will, I. Keith, E. Weir.
2. Oxidants cause pulmonary vasodilation. Bull. Europ. de Physiopathologie Respiratorie 18: 4, p. 93, 1982. J.A. Will, A.M. Nielsen, J.W. Eaton, and E.K. Weir.

American Heart Association:

A new group of pulmonary vasodilators. E. Kenneth Weir, Lucy J. Lindquist, Elliot Chesler, James A. Will and John W. Eaton.

European Society of Clinical Respiratory Physiology, Cefalu, Sicily, Italy:

Angiotensin converting enzyme activity not oxygen-dependent in chronic hyoxia. J.A. Will, A.M. Nielsen, and D.F. Erichsen.

Oxidants: A new group of pulmonary vasodilators. James A. Will, and E. Kenneth Weir.

The interaction of serotonin and hypoxia on the pulmonary circulation. J. A. Will, I.M. Keith, E. Burt Olson, Jr., Jacob Chacko and E. Kenneth Weir.

Manuscripts published:

Brown, M.J., D.F. Erichsen, R. Helgerson, and J.A. Will. A modification for preparing the chronic lung lymph fistula in sheep. J. Appl. Physiol: Respirat. Environ. Exercise Physiol. 52(6, 1664-1666, 1982

J.A. Will. Neuroendocine and metabolic factors in pulmonary circulatory control. Advances in Shock Research, 8:13-20, 1982.

Manuscripts in press:

Keith, I.M. and J.A. Will. Dynamics of the neuroendocine cell-regulartoy peptide system in the lung. Exper. Lung Res.

Will, J.A., I.M. Keith, C.K. Buckner, J. Chacko, E. Burt Olson, Jr, E. Kenneth Weir. Serotonin and the Lung. In: The Endocrine Lung in Health and Disease. W.B. Saunders.

Weir, E.K., and J.A. Will. Oxidants: a new group of Pulmonary vasodilators. European J. Clinical Resp. Physiology.

Manuscripts submitted:

J.A. Will, I.M. Keith J. Chacko, E. Burt Olson, Jr. Anatomic evidence that serotonin is a pulmonary vasoconstrictor in hypoxia and modulator of pulmonary arterial medial thickness of rats. Eper. Lung Res.

Manuscripts in revision for resubmission:

D.F. Erichsen, C. Malcorps, M. Brown, R.A. Proctor, J.R. Starling and J. A. Will. Endotoxin-induced alterations in pulmonary endothelial permeability. Norepinephrine removal, and hemodynamics in awake sheep. (Journal of Applied Physiology)

R. Rodriguez, C.Malcorps, J.A. Will, E.N. Lightfoot. Non-parametric determination of the distribution of tansit times in the presence of early recirculatin from sampled indicator-dilution. (Research in Basic Cardiology.

Manuscripts in preparation:

D.F. Erichsen, J.A. Will and R.H. Demling. Effect of hyperoxia on hemodynamics, permeability and amine removal in awake sheep.

D.F. Erichsen, C. Juratsch, M. Brown and J.A. Will. Acute pulmonary artery hypertension produced by distension of the main pulmonary artery compared with acute hypoxia in awake sheep

D.F. Erichsen, C. Malcorps, and J.A. Will. Adverse reaction to the injection of premixed autogenous blood and indocyanine green in awake sheep.

Nielsen, A.M., D.F. Erichsen, and J.A. Will. Changes inspired oxygen modify the hemodynamic response to angiotensin in awake sheep.

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ANIMAL USE STATEMENT

All animal studies and preparations used in the experiments outlined in this report have been designed within the guidelines for the CARE AND USE OF LABORATORY ANIMALS. Permission and supervision of such studies has been approved by RARC, the appropriate commission at this University.