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THE CARBON DIOXIDE RESPONSE CURVE OF THE DOG AT SEA LEVEL AND AT ALTITUDE

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FOREWORD

This report was submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Physiology in the Graduate Division University of California, Berkeley, California.

The experiments described in this report were completed during the years 1953-1956, while the author was enrolled in the United States Air Force Institute of Technology program.

Compilation and revision of this report were completed under Project 7164, "Biomedical Criteria for Aerospace Flight," Task 716404, "Physiological Criteria for Altered Environments," supported by the Biomedical Laboratory, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio.

Professor Nello Pace is gratefully acknowledged for his encouragement and guidance on this report. The author is also indebted to Professor Ralph H. Kellogg for many helpful suggestions concerning instrumentation and to Professor Franklin M. Henry for advice concerning statistical methods. Dr. L. G. C. E. Pugn contributed substantially from his accumulated knowledge concerning the use of the micro Scholander gas analyzer under field conditions.

This report has been reviewed and is approved.

WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory

ABSTRACT

Carbon dioxide response curves were measured in three trained dogs before, during, and after an 18-day sojourn at 12,470 ft altitude. The effects of sedation on CO_2 sensitivity were studied in two dogs, using scopolamine and scopolamine-thiopental. In addition, tests were made for the presence of an hypoxic drive component in resting ventilation at altitude by measuring the effect on ventilation of a sudden change in alveolar O_2 tension from 55 mm Hg to 110 mm Hg. Reductions occurred in resting alveolar O_2 and CO_2 tensions to 53 and 71% of sea level control, respectively. On the average, CO_2 sensitivity was increased during the first 10 days to 160% of sea level control. The CO_2 response curve was shifted horizontally to the left by about 8 mm Hg. This decrease in CO_2 threshold was statistically significant ($P < 0.01$). Sedation increased the variability of the results. However, there was some evidence that CO_2 sensitivity was reduced by sedation. These effects were more pronounced at altitude than at sea level. Tests for an hypoxic drive component showed a transitory reduction in alveolar ventilation occurring 1 minute after the increase in alveolar O_2 tension. In subjects under sedation with scopolamine-thiopental, 40% of resting ventilation was attributable to peripheral chemoreflexes. Tests done during the 4th to 11th days after returning to sea level were similar to the prerun control results, indicating no residual effect of the changes induced by altitude.

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I. Introduction

When CO_2 is added to the inspired gas, an increase occurs in pulmonary ventilation. Plots of this stimulus-response relationship are designated as CO_2 response curves. Alveolar P_{CO_2} is usually considered to be the independent variable (x-axis), in accordance with the findings of Haldane and Priestly (61), and ventilation the dependent variable (y-axis). Although the curves obtained by various investigators have varied somewhat, because of the different criteria used, all CO_2 response curves have a basic similarity because they are all measures of the same phenomenon. Some investigators have used alveolar ventilation ratio (58, 134), considering the control ventilation as equal to one, while others have used respiratory minute volume (80), or alveolar ventilation/m^2 of body surface (26) as the index of response. Still others have measured respiratory frequency (87), tidal volume (98), or phrenic action potentials (160, 161) as the index of response to increased inspired P_{CO_2}. The stimulus has been variously defined in terms of arterial P_{CO_2} (26), alveolar P_{CO_2} (120), inspired P_{CO_2} (132), arterial pH (79), or cerebrospinal fluid (CSF) pH (148).

Hasselbalch and Lindhard (63) showed in 1911 that the CO_2 response curve of man was altered at an altitude of about 10,000 ft, and that the alteration persisted even though alveolar P_{O_2} was raised to normal sea

level value. They found that for a given increase in ventilation, a smaller increase in alveolar P_{CO_2} was required at altitude. Lindhard (94) in the same year, showed that the CO_2 response curve was elevated by concurrent O_2-lack. He also investigated the effects of drugs and found the response curve to be greatly elevated by strychnine and markedly depressed by morphine, with chloral causing less depression than morphine.

Gray reviewed the published data on various aspects of respiratory control mechanisms in 1950 and attempted to integrate and describe all known respiratory phenomena quantitatively through a series of empirically derived equations, known collectively as the multiple factor theory (57, 58). He suggested that CO_2 threshold be defined as that value of alveolar P_{CO_2} beyond which increases in P_{CO_2} are accompanied by increases in ventilation. CO_2 sensitivity was clearly differentiated from CO_2 threshold, and defined as the slope of the stimulus-response line when CO_2 was added to the inspired gas and the resulting increase in pulmonary ventilation was plotted as a function of alveolar P_{CO_2}.

Subsequently Nielsen and Smith (116) showed that there is a powerful (multiplicative) interaction between CO_2 and acute severe hypoxia. Their results showed that the effects of hypoxia and increased P_{CO_2} are not simply additive, as Gray had assumed. They also demonstrated a fixed threshold of alveolar P_{CO_2} in acute

severe hypoxia.

When the present experiments were started, Rahn and his associates (130, 132, 134) had shown that the respiratory response to added CO_2 was augmented in altitude sojourners, and that this increased responsiveness continued when alveolar P_{O_2} was raised to normal sea level values, confirming the earlier limited observations of Hasselbalch and Lindhard. Kellogg, et al. (80) extended these observations. At that time there was doubt as to whether the slope of the stimulus-response curve was increased at altitude, but the horizontal shift of the curve to the left was clearly established. Subsequently, Kellogg and his coworkers have repeatedly demonstrated an increase in the slope of the CO_2 response curve in sojourners at 14,200 ft (78, 79), and the same result was found by Milledge (105) in mountain climbers at 19,000 ft.

During the early stages of my experiments there was doubt that the animals could be sufficiently well trained that meaningful respiratory data could be obtained. Suskind has since reported the results of respiratory gas exchange studies in trained dogs (155). Gilfillan, et al. in 1958 measured the CO_2 response curves of trained dogs in connection with their studies of chemoreceptor mechanisms (55). They reported that the CO_2 responses of intact dogs were qualitatively similar to those of man but evidently did not perform detailed analyses. In the present studies preanesthetic

sedation was initially used on the assumption that it would produce tractable subjects without producing appreciable depression of the respiratory response to CO_2. Although it was subsequently found that the dogs could be satisfactorily trained, the sedation experiments were continued.

The objectives of these studies were: 1) to quantitatively define the CO_2 response curve of the dog before, during, and after a sojourn at an elevation of 12,500 ft; 2) to define the time course of respiratory adaptation during the first 10 days at altitude; 3) to determine the importance of O_2-lack in maintaining resting pulmonary ventilation; 4) to study the effects of preanesthetic sedation on the CO_2 response curve of the dog, at sea level and at altitude.

The experiments were performed during the period from July 26, 1956 through September 19, 1956. The sea level experiments were done at Berkeley, California, elevation = 250 ft., and the altitude experiments were done at the Barcroft Laboratory of the White Mountain Research Station, elevation = 12,470 ft. Recorded environmental parameters were as follows: at sea level, P_B = 751.1 mm Hg, $\pm$2.6 (standard deviation), range = 746.3 - 756.1 mm Hg, dry bulb room temperature = 21.3 C, $\pm$1.8, range = 19.0 - 25.5 C; at altitude, P_B = 487.8 mm Hg, $\pm$1.8, range = 484.5 - 489.9 mm Hg, dry bulb temperature = 21.2 C, $\pm$1.3, range = 19.0 - 24.2 C. The sea level control experiments were performed before the altitude sojourn and again at sea level following the altitude exposure, with the subjects awake and under preanesthetic

sedation. During the first ten days at altitude CO_2 response curves were determined daily on awake animals. CO_2 response curves on sedated subjects were measured during the following seven days at altitude and the measurements were repeated on the unsedated animals on the eighteenth and final day of the altitude sojourn. Details concerning the subjects are given in TABLES I, II, III, and XI.

II. Methods and Materials

A. Introduction

Respiratory experiments were performed at sea level and at altitude on three male dogs, two mongrels and a purebred beagle. Each dog served as his own control. To minimize possible metabolic effects the animals were fasted for 12 hr prior to the experiments. Experiments on a given subject were performed at about the same time of day on successive days. The experiments were performed in an isolated room (65, 125).

1. Description of a Typical Experiment

The dog stood quietly in the modified Pavlov harness for at least 10 min before the experiment began (see Fig. 1). When he appeared to be in a steady state a rubber face mask with respiratory valve was attached to his muzzle. Fig. 2 shows the method of attachment.

Short lengths of one inch inside diameter respiratory tubing connected the respiratory valve outlet to a gas meter and the inlet to a six liter spirometer. A manifold and valve system was used to fill the spirometer from any one of a bank of gas cylinders, containing mixtures of CO_2 and air. In the altitude experiments O_2 percentages were adjusted to provide alveolar P_{O_2}'s slightly above normal sea level values.

After the dog was fitted with the mask he breathed room

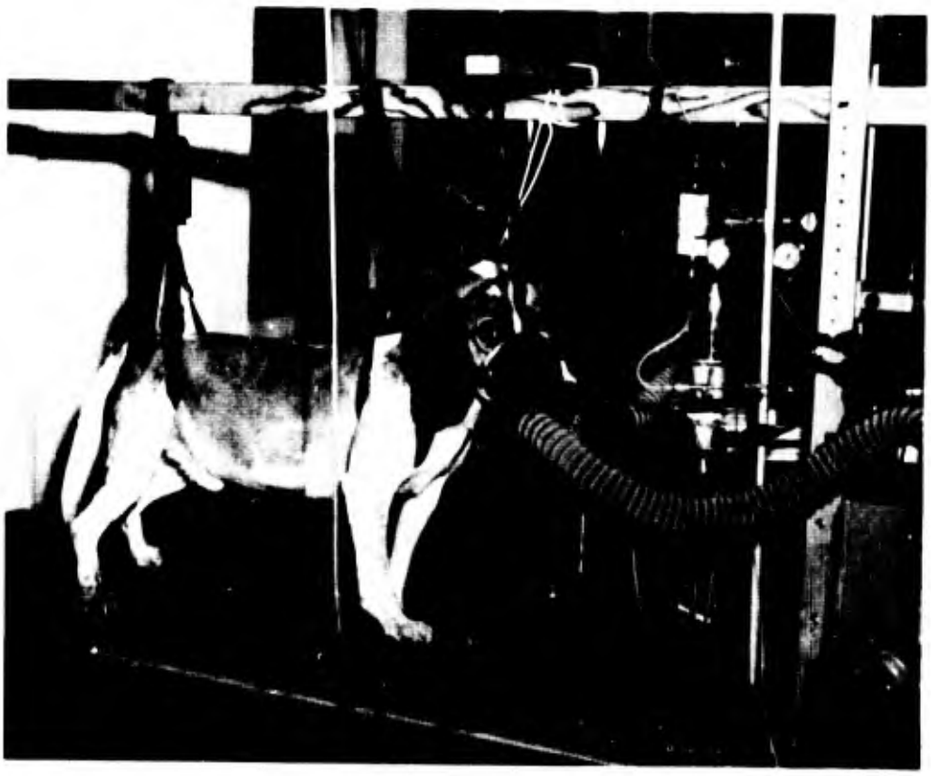

Fig. 1. Purebred beagle restrained in Pavlov harness. Adjustable rubber band supports for respiratory tubing at ends of crossbar; snap-swivel cord for restraining dog's head, leg restraint straps.

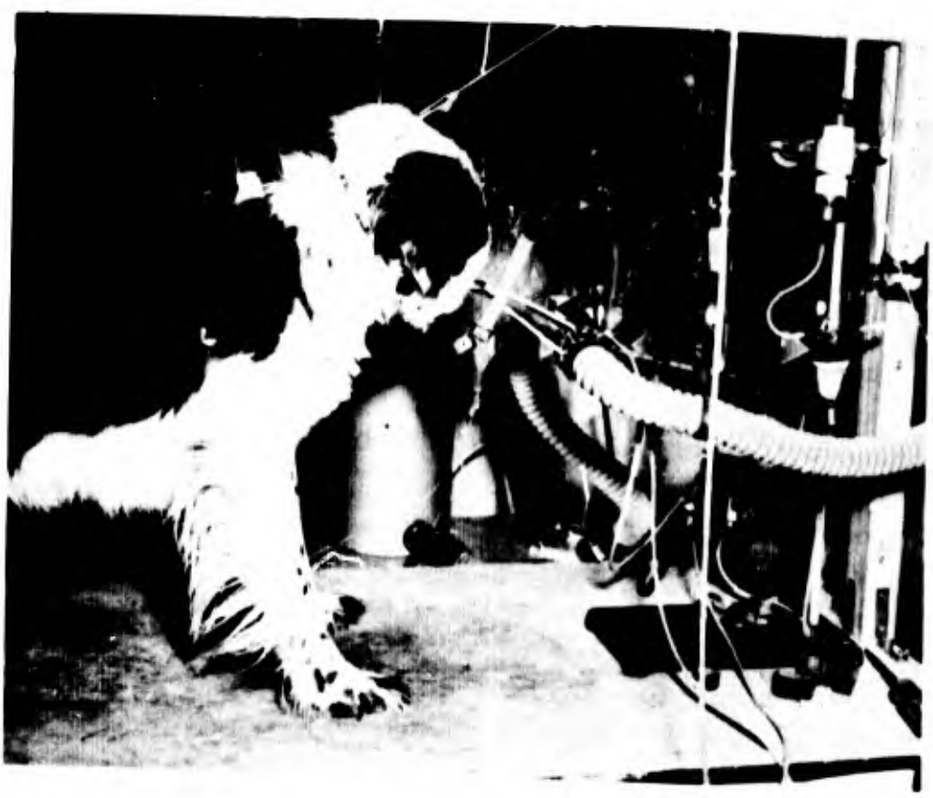

Fig. 2. Method of attaching face mask to dog's muzzle and respiratory valve to rubber mask.

air for a 10 min baseline period. The three-way valve was then turned and the subject breathed compressed air for five minutes. At five minute intervals the inspired CO_2 was increased successively to 1, 2, 4, and 6% (plus an additional step of 8% at altitude). Step increments of inspired CO_2 were used to facilitate the attainment of steady state conditions since each increase was a relatively small change from the preceding period. The subject breathed the highest CO_2 concentration for 10 min so that an estimate could be made of the maximal respiratory response at that concentration.

A specially built system of the Rahn-Otis-Nielsen type collected alveolar gas samples from each breath. The alveolar gas flowed through O_2 and CO_2 analyzers in a continuous flow system. The volume of gas required for the analyzers was constant and negligibly small.

Beckman O_2 and critical orifice CO_2 analyzer readings were recorded each minute for alveolar P_{O_2} and P_{CO_2}, respectively. Respiratory frequency was measured by a pressure sensitive digital counter. Respiratory minute volume was measured every minute with a gas meter. Other data recorded during each experiment included: gas meter outlet temperature, room temperature, barometric pressure, water bath temperature, calibration data on the gas analyzers, and observations on the behavior of the subjects. Micro Scholander analyses provided primary standard values of O_2 and CO_2 concentrations.

Fig. 3 shows a general view of a dog and the equipment used in a typical experiment. The same equipment used at Berkeley was transported to the Barcroft Laboratory of the White Mountain Research Station (33), and used for the altitude studies.

B. Techniques

1. Training of Dogs

Dogs were trained to wear face masks and to serve as subjects for respiratory experiments in which they breathed increasingly higher inspired P_{CO_2} gas mixtures. Attempts to train purebred litter mate beagles were only partially successful. Selected mongrel animals were easier to train than purebred beagles. This observation confirms unpublished results of Hemingway (64). Training was more successful when the dog was restrained in a modified Pavlov harness and introduced to the total equipment complex at the outset. Observations relating to training and selection of the subjects are given in Tables I and II. Both positive and negative reinforcement techniques were employed in training the dogs to wear face masks. Punishment (electric shock) appeared to be more effective than reward (food).

2. Sedation

There are inherent difficulties in isolating and measuring

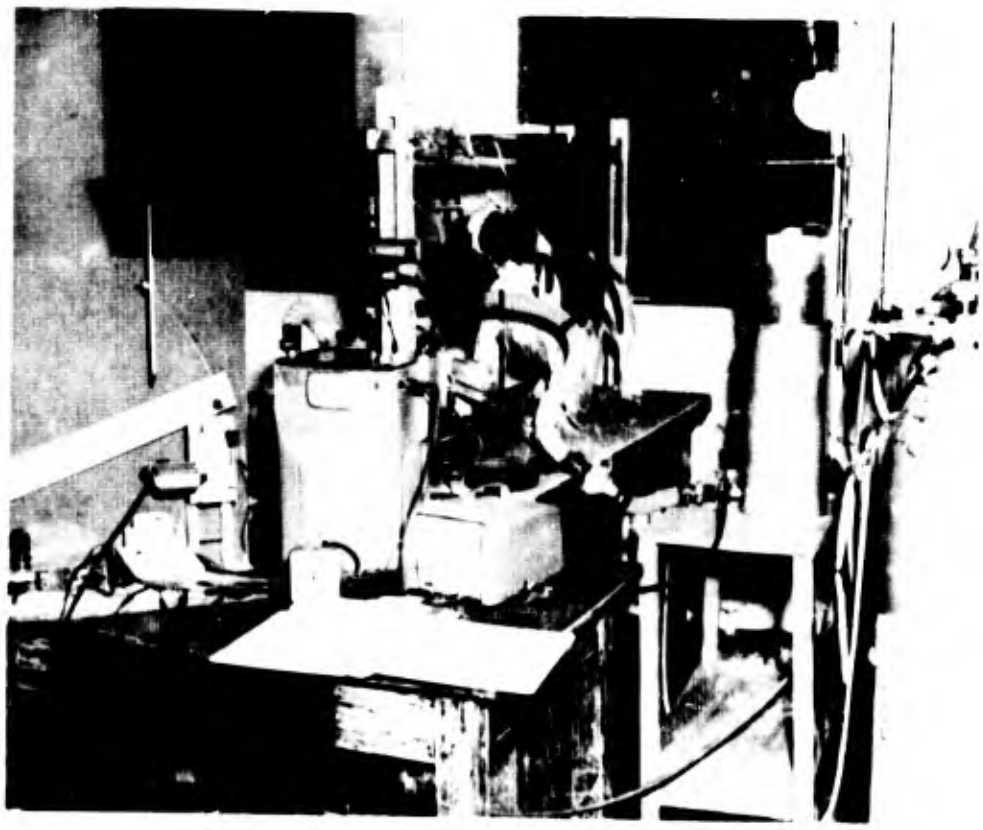

Fig. 3. General view of experimental complex, showing: Beckman O_2 analyzer; critical orifice CO_2 analyzer; respiratory frequency counter; modified gas meter; spirometer; gas cylinders; and other details.

TABLE I. Training record of purebred beagles and summary of other data

Name or No.	Sex	Type	Whelped	Delivered	Started Training	Rating Score[a]	Avian Dist. Vac. I.C.H.V.[b]	Observations & remarks
88-A	♂	Beagle	8-24-53	1-24-54	1-30-54	1	10-26-53	Sacrificed 3-16-54; liver & bladder histology; frank distemper "break" on vaccination.
88-B	♂	Beagle	8-24-53	1-24-54	1-30-54	1	10-26-53	Died: 3-1-54.
88-C	♂	Beagle	8-24-53	1-24-54	2-1-54	1	10-26-53	Died: 7-23-56, complications following surgery.
E-51	♂	Beagle	7-30-55	1-31-56	2-1-56	2	9-7-55 1-27-56	Used for CO_2-response experiments.
D-49	♂	Beagle	7-24-55	1-31-56	2-1-56	0	9-3-55 1-27-56	Eliminated from training 5-7-56; completely intractable.

[a] Rating Scale - usability of experimental animals:

5 = Excellent
4 = Good
3 = Fair
2 = Poor
1 = Borderline
0 = Impossible, no experiments done.

[b] Infectious Canine Hepatitis Vaccine.

TABLE II. Training record of mongrel dogs and summary of other data

Name or No.	Sex	Type	Screened	Started Training	Rating Score[a]	Observations & remarks
Rusty	♂	Mongrel-Collie	5-1-56	5-5-56	4	Used for CO_2-response experiments.
Wags	♂	Mongrel-Setter	5-1-56	5-6-56	5	Used for CO_2-response experiments.
	♂	Dalmation	7-24-56			Mask doesn't fit dog.
210-56	♂	Mongrel	7-27-56			Panicked at sight of mask, did not tolerate being placed on table.
205-56	♂	Mongrel	7-27-56			Muzzle too small for mask.
207-56	♂	Mongrel	7-27-56			Not suitable for further training - temperamental.
212-56	♂	Doberman Pinscher	7-27-56			Probably deaf.
213-56	♂	Mongrel	7-27-56			Unfriendly - fights restraining harness.

[a] Rating Scale - usability of experimental animals:

5 = Excellent
4 = Good
3 = Fair
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1 = Borderline
0 = Impossible, no experiments done.

respiratory effects of sedatives and anesthetics, as distinct entities different from the respiratory changes that occur in the intact animal with acclimatization to high altitude. Therefore it was evident that the experimental measurements should be made on intact animals. However, at an early stage of the investigation there was doubt that it would be possible to train the animals.

A brief survey of the literature was made to select suitable drugs having minimal effects on respiratory center sensitivity to CO_2 (6, 10, 56, 73, 104, 150). Preliminary experiments were performed using analgesic doses of Carbromal and several of the Rauwolfia derivatives. Carbon dioxide response curves were obtained from subjects sedated with scopolamine hydrobromide and with a two drug combination of scopolamine and thiopental sodium (Pentothal, Abbott). Preanesthetic doses were used: scopolamine = 0.004 mg/kg; thiopental = 0.8 mg/kg, injected iv. The sedated animals were conscious and they responded readily to visual and auditory stimuli. Experiments on sedated subjects were continued even though it became apparent that the dog training program would be successful.

C. Equipment

1. Introduction

Much of the equipment had to be specially built. One basic problem was to devise a method for accurately measuring small respiratory gas volumes. The sampling of alveolar gas was another problem area which also involved the criteria for respiratory valves. Fig. 4 shows the functional relationships of the principal components.

2. Rubber Latex Face Mask for Dogs

In 1927 Blalock described a light weight rubber mask which evidently was excellent for measuring respiratory minute volumes in dogs (13). Although Blalock's mask had been manufactured commercially, no similar item was available at the time of this study.

Other animal masks were described in the literature. Gaddum developed a mask made from a tin can partially filled with plaster of Paris and coated with wax (54); Endres described a mask made of fine sheet rubber which covered the animal's snout and was secured by rubber bands around the neck (46); Chiodi, et al. used leather masks with lanolin and rubber bandages to make an airtight seal (27). None of these masks appeared to be well adapted to the particular requirements of this investigation.

Dr. Max Kleiber, of the University of California at Davis, had developed a mask for measurements of respiratory minute volumes

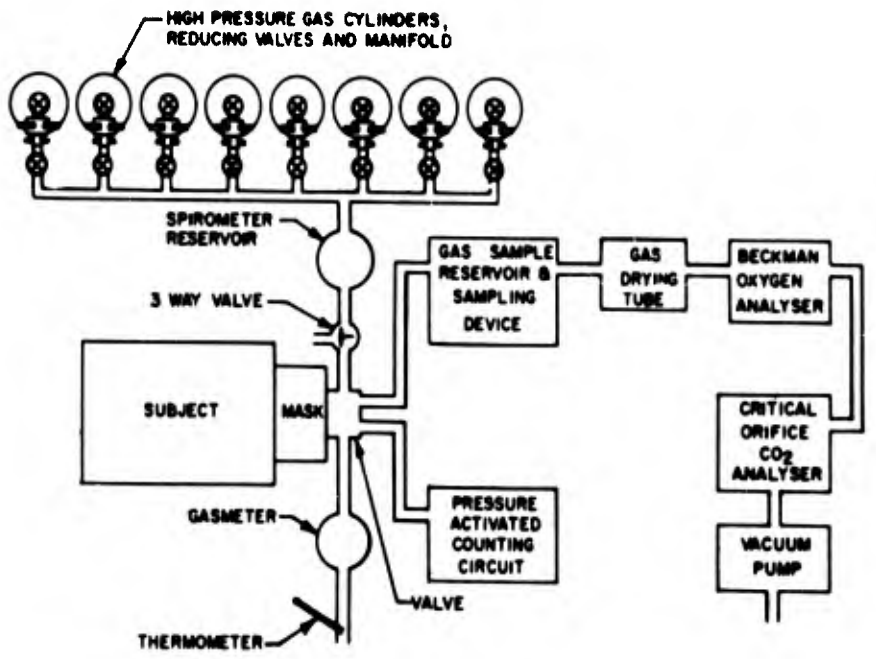

Fig. 4. Schematic diagram of experimental complex.

of sheep. Although Dr. Kleiber's mask was not suitable for use with dogs, I obtained much valuable information from his group. Their techniques were used for making inflatable sealing bladders.

In a personal communication Hemingway described a mask for measuring gaseous exchange in trained dogs (64). His mask was made of a rubber inner tube which completely covered the dog's head and neck. No data were given, but from the diagrams it appeared that the mask had a large dead space.

A useful reference on latex techniques is available from the British Natural Rubber Development Board (17). Supplies and detailed information can be obtained from many plastics supply houses and hobby shops. The essential steps in fabricating a rubber latex face mask for dogs were as follows:

(a). The dog was surgically anesthetized and intubated with an endotracheal catheter.

(b). After the dog's muzzle had been coated lightly with petroleum jelly his muzzle was closely wrapped with quick setting orthopedic plaster bandages.

(c). After about 30 min. the cast was removed, allowed to become thoroughly dry, and trimmed with a fine toothed saw. The inside surface was given several coats of orange shellac followed by several coats of clear lacquer to seal the porous surface.

(d). The inside surface of the finished mold was coated

lightly with white Vaseline. The mold was supported in a suitable container, partially filled with damp fine sand.

(e). The supported mold was filled to the top with casting plaster. After the plaster had cured for about 24 hrs. the model separated readily from the mold. A model and mold are shown in Fig. 5.

(f). After overnight drying in an oven at about 100 F., the model of the dog's muzzle was given several coats of orange shellac to reduce its porosity. Several coats of lacquer were then applied to the surface of the model to produce a smooth surface.

(g). The sealing bladder, made as described subsequently, was positioned near the base of the model so that it completely covered the corners of the dog's lips when the mask was in use. The proper location was determined by comparing the plaster model with the dog and marking the model with a wax pencil for future reference.

(h). A standard soft rubber respiratory valve mouthpiece was mounted on the nose end of the model. The mouthpiece was shaped to the model and held in place with cord wound diagonally across the flat base of the plaster model. The cord gave the completed mask greater strength and also held the sealing bladder in its proper position.

(i). The model was placed on its flat base, on a square of clean glass and anchored in place with a few drops of melted paraffin.

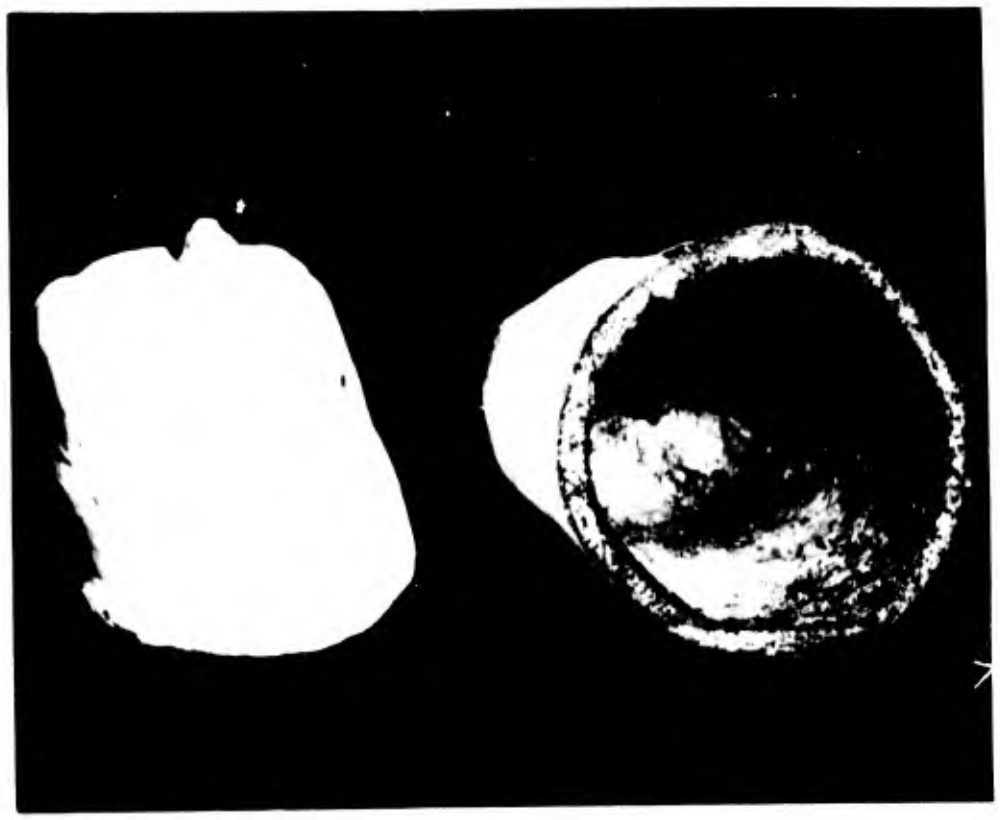

Fig. 5. Completed plaster model of dog's muzzle and mold used to cast it.

(j). Liquid latex was applied to the entire surface of the model with a soft paint brush. The latex extended out about one inch onto the surface of the glass.

(k). Successive thin uniform coats of latex were applied until a thickness of about one-fourth inch was achieved. The whole mask was then spiral wrapped with cotton cord to give additional strength to the finished product and several additional coats of liquid latex were applied to seal in the reinforcing cord.

(l). A supporting Lucite[1] framework was fastened around the mask to provide rigidity in the area of the sealing bladder and the mouthpiece and to provide a means of attaching the mask to the dog. The frame can be seen clearly in Fig. 6. Some difficulty was encountered with this design as the Lucite hooks broke off on several occasions. Aluminum would probably be more satisfactory for the framework.

(m). Several additional coats of liquid latex were applied over the supporting framework to integrate the entire structure.

(n). The completed mask was peeled from the supporting glass surface and separated from the model after being allowed to dry for 24 hr following the final coat of latex.

(o). The flat ridge formed at the glass surface was

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[1]Registered trademark for methyl methacrylate, a clear transparent, thermoplastic resin. Also known as Plexiglas or Perspex.

Fig. 6. Completed rubber latex dog mask, showing details of Lucite frame, attachment hooks, and Schroeder valve of pneumatic sealing bladder.

trimmed off using heavy scissors. The inner and outer surfaces of the mask were dusted liberally with zinc oxide powder to accelerate vulcanization of the latex and to prevent the adherence of foreign objects. With reasonable care the completed mask will easily give several years of constant use.

The sealing bladder was made from a bicycle inner tube of the high pressure type and its associated Schroeder valve. Proper fit was assured by tying the bladder in position directly onto the plaster model of the dog's head while the bladder was being cemented. An automobile tire cold-patching kit was used to join the free ends of the bladder. When the mask was in use the bladder was slightly inflated with a bicycle tire pump. The pneumatic bladder compensated for small differences in muzzle diameter among the experimental animals.

Leak tests were made with a standard laboratory BMR apparatus. The slope of the oxygen consumption curve was determined with the spirometer bell finely balanced; then a 60 g weight was added to the bell of the spirometer and the measurement was repeated. If there had been any leakage, the apparent O_2 consumption would have increased after the weight was added to the bell. The mask-to-dog seal was tested several times with all three of the animals used as subjects in the CO_2-response tests. All tests showed no measurable leakage. Fig. 7 shows the results of a typical leak test.

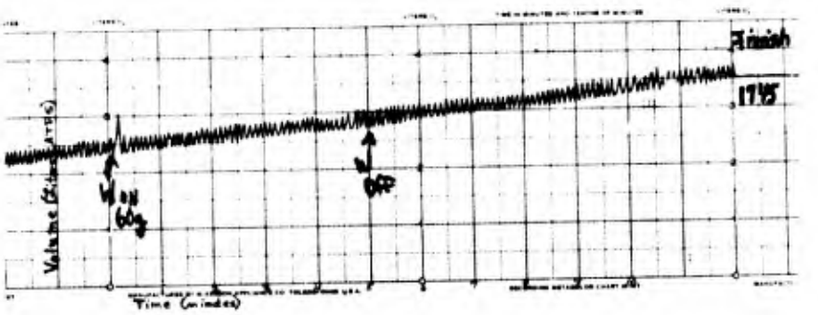

Fig. 7. Result of a typical test for leaks, showing the oxygen consumption curve of a dog before, during, and after addition of a 60 g weight to the bell of the spirometer.

Table III shows respiratory dead space for each subject, including the dead space of the mask and the Von Döbeln modified valve. The respiratory dead space of each animal was determined for both O_2 and CO_2 by measuring alveolar P_{O_2} and P_{CO_2} with the dogs breathing room air at rest, at sea level, for a ten minute period. Mixed expired air was collected in a well balanced spirometer and analyzed for P_{O_2} and P_{CO_2}. Respiratory dead space was computed from the Bohr formula (15).

A recent report by Nims and Worth (117) describes the fabrication of a Fiberglas mask which completely covers the dog's head. The Nims and Worth mask is basically similar to the full head mask described by Hemingway in a personal communication in 1956 (64). These investigators used several of the same techniques that were described in the preceding pages, viz., plaster head molds made of orthopedic plaster bandages and an inflatable sealing bladder. From the descriptions given it would appear that this mask might have a relatively large dead space. They presented conclusive evidence that their mask did not leak. The Nims and Worth mask was developed completely independently and reported in 1961 whereas the mask described in this report was built in 1955. The mask described in the present paper had a weight of 237 g, the Nims and Worth mask weighed 525 g.

TABLE III. Dead space measurements of subjects. Values are from one determination in each case.

Subject	Dead space-O_2 (liters, BTPS)	Dead space-CO_2 (liters, BTPS)
Wags	0.102	0.094
Rusty	0.056	0.055
Lunk	0.063	0.059

3. Household Service Gas Meter

Experiments were done to evaluate the characteristics of portable dry test and wet test gas meters, using a carefully balanced, 120 liter, chain-compensated Collins gasometer as a primary standard. The tests showed that: a) calibration of the dry test meter, as described by Newcomer (113), decreased rather than increased the accuracy of the meter, b) in all cases the accuracy of the uncalibrated dry test meter was as good as that of the wet test meter, c) at high flow rates (about 100 liters/min) the accuracy of the wet test meter fell off markedly.

The dry test meter, as supplied by the manufacturer, was not suitable for making respiratory minute volume measurements in dogs because the minimum measurable gas volume was about 1.4 liters, therefore attempts were undertaken to further modify it. The gas meter cover was removed and the totalizing gear train was disengaged from the central shaft worm gear and removed from the meter housing. A stiff copper wire was attached to the central shaft, and a circular scale, drawn on transparent plastic, was centered over the central shaft. With this new scale, the resolving power of the device was greatly increased so that one could read directly to 0.01 of a complete revolution (0.047 liters). The meter was further modified by adding a simple electrical switch, so that each complete revolution of the dial pointer actuated a digital counting circuit. The

modified meter is shown in Fig. 8. Gas volume measurements showed perfect agreement between the modified uncalibrated gas meter and the Collins gasometer.

Because there is no water seal in the dry test gas meter, its use in measuring expired gas volumes has been criticized on the grounds that one cannot be certain the volume measured represents gas saturated with water vapor at ambient temperature (31). To determine whether this criticism was valid, numerous test collections of expired gas were made and the question was examined both experimentally and theoretically. It was concluded that if the meter outlet temperature were near or above body temperature the gas volumes measured would not be saturated (30, 66, 111, 157). Such conditions did not exist in any of the measurements made during the present studies.

4. Respiratory Valves

Performance data on some commercially available respiratory valves are shown in Table IV. The Siebe-Gorman (double Douglas) valve was selected for use in this study because it was lightweight and durable. The standard valve was first modified by installing sampling taps.

Preliminary measurements on dogs, breathing room air at rest, gave alveolar O_2 values that were high and alveolar CO_2 values that were low, showing that the dog's average tidal volume

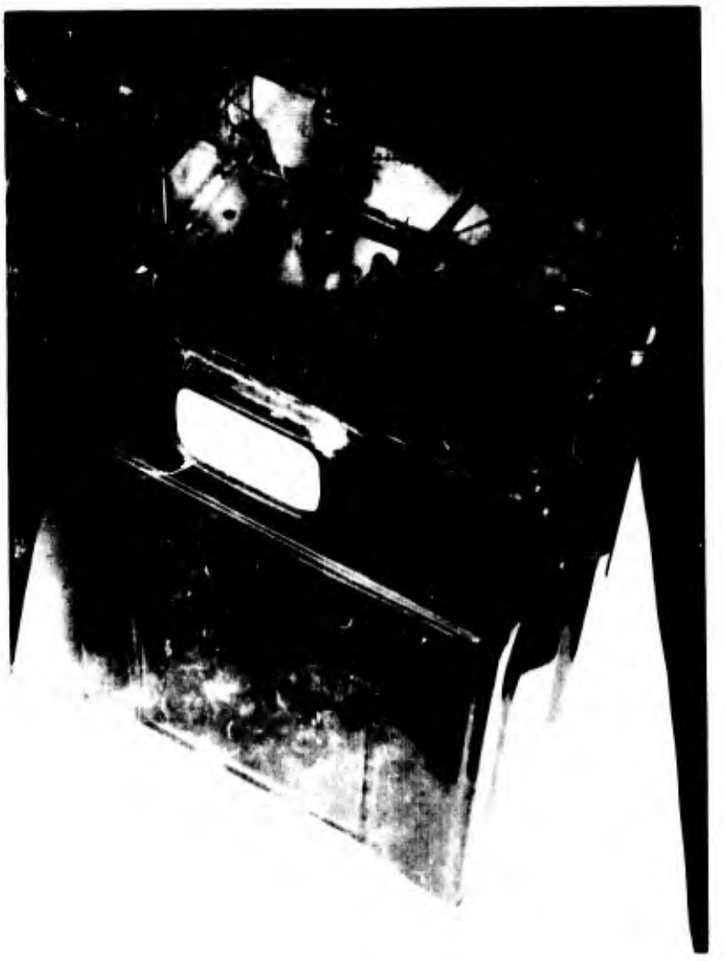

Fig. 8. Modified household service gas meter, showing; Lucite cover and sealing gasket; pointer and scale; counting switch; color coded electrical sockets; and other details.

TABLE IV. Comparative data showing resistance of respiratory valves to air flow at various flow rates. Pressure differential across valve measured in mm H_2O pressure. Valves arranged in order of increasing resistance at a flow rate of 100 liters/min.

Valve or Device	Flow Rate (Liters/Min) 10	50	100	150	250	Opening Pressure	Dead Space[a]	Ref[b]
Bannister and Cormack Type I	...	...	...	...	8	...	70 ml	(5)
Bannister and Cormack Type II	...	...	...	...	11	...	10 ml	(5)
Bailey	1.4	2.3	5.6	12.5	...	...	...	(31)
Henderson-Haggard	1.0	2.9	6.2	9.5	...	21.9	...	(31)
Collins "J"	2.1	3.9	6.3	7.2	...	0.2	...	(31)
Sadd	2.1	5.7	10.6	16.4	...	12.3	...	(31)
Rudolph-Robinson (Inspiratory)	2.0	9.0	19.0	...	...	...	...	
Siebe-Gorman (Inspiratory)	3.0	11.0	22.0	...	...	...	38 ml	
Connell	4.8	14.3	24.9	35.2	...	18.7	...	(31)
Siebe-Gorman (Expiratory)	4.5	13.0	26.5	...	...	...	38 ml	
Sadd	7.2	17.1	27.1	37.6	...	...	...	(31)
Rudolph-Robinson (Expiratory)	3.0	13.5	28.0	...	...	...	...	
Siebe-Gorman	...	...	...	38.0	...	...	40 ml	(5)
Standard Douglas (Mica Flap)	...	...	...	54.0	...	...	55 ml	(5)
Rudolph-Robinson	...	...	...	...	50.0	...	84 ml	(76)
Dry-Test Gasometer	*	25.0	63.5	...	...	*	...	
Hansen[c] (Inspiratory)	10.5	55.0	96.0	...	...	...	7.5 ml	
Hansen[c] (Expiratory)	6.0	42.5	134.0	...	...	...	7.5 ml	

[a] Geometric dead space of complete valve, including mouthpiece tube.

[b] Source of data, if other than author's experiment.

[*] Pressure difference too small to measure with water manometer.

[c] Special valve for respiratory measurements on dogs, constructed by Mr. J. T. Hansen, Dept. of Physiology, Univ. of Calif., Berkeley, Calif.

was not great enough to adequately flush the valve's dead space. Geometric dead space of valve and mouthpiece tube equalled 38 ml.

The valve was next modified by adding a thin metal diaphragm halfway between inspiratory and expiratory valves, as suggested by Von Döbeln (159). Fig. 10 shows this modification schematically. Subsequently a one-eighth inch thickness of liquid rubber latex was added to each side of the diaphragm and vulcanized. The latex assured a gastight diaphragm, increased its durability, and further decreased the dead space without increasing the valve's resistance to air flow. Measured geometric dead space of the modified valve and mouthpiece tube was 25 ml. Applying Von Döbeln's results to this valve gives a value for functional dead space of about 5 ml.

Table V shows data comparing alveolar O_2 and CO_2 values in a dog, breathing quietly at rest, with and without the Von Döbeln modification. All alveolar gas samples were taken with the same Rahn-Otis type sampling device and analyzed by methods to be described subsequently. Alveolar O_2 and CO_2 partial pressures shown in Table V, and in all subsequent data, were calculated in accordance with formulae shown in Appendix C. It is apparent, from these results that the Von Döbeln modification was very effective in reducing the functional dead space of the Siebe-Gorman valve. Dead space of the unmodified valve was about 20% of average tidal volume while the dead space of the modified valve was less than 5% of average tidal volume.

TABLE V. Comparison of alveolar P_{O_2} and P_{CO_2} values, Rahn-Otis end tidal alveolar sampler, Siebe-Gorman valve, with and without Von Döbeln partition. Values shown are: mean, ± average deviation, (number of consecutive observations). Observations were made at one minute intervals with subject breathing room air, at rest. Subject: Wags.

	Without partition 18 July 1956	Without partition 19 July 1956	With partition 26 July 1956[a]
Alveolar P_{O_2} (mm Hg)	124.7, ± 1.5, (10)	128.3, ± 2.4, (10)	113.8, ± 1.8, (10)
Alveolar P_{CO_2} (mm Hg)	14.9, ± 0.8, (10)	11.2, ± 3.9, (10)	27.3, ± 1.4, (10)
Tidal volume (ml, BTPS)	205.0, ± 7.0, (8)	209.0, ±10.0, (10)	257.0, ±17.0, (10)

[a] Partition consisted of thin brass shim stock, latex reinforcement was added later. Geometric dead space of this valve and mouthpiece tube equalled 38 ml. According to data of Von Döbeln, effective dead space would be approximately 8 ml.

5. Summary and Conclusions

Procedures used in fabricating a rubber latex face mask for dogs were described in detail. Tests showed no measurable leakage. A household service dry test gas meter was modified for measuring respiratory gas volumes in dogs. The modification procedure was described. Improved measurements of alveolar P_{O_2} and P_{CO_2} in resting dogs were obtained using a double Douglas valve which had been modified according to the method of Von Döbeln, so that the dead space of the valve was about five per cent of average tidal volume.

D. Analytical Techniques

1. Gas Analyzers

Dr. Ralph H. Kellogg suggested the use of a critical orifice continuous CO_2 analyzer and made many valuable suggestions which resulted in an improved instrument. Dr. J. Mead had previously reported the successful use of such a device in student laboratories (103). The analyzer built for these studies is shown in Fig. 3.

Sonic orifices were prepared by fire polishing short lengths of Hg thermometer Pyrex glass tubing. The differential manometer was constructed so that it could be rotated around its zero point. The magnetic attraction between a small Alnico permanent

magnet and its keeper was used to keep the manometer at any desired angle of inclination from the horizontal. This arrangement facilitated very fine adjustments of manometer sensitivity. The manometer scale was permanently marked to read directly in units of per cent CO_2.

Volume air flow through the critical (sonic) orifice varies with temperature, which affects the velocity of sound. Mass flow also varies directly with absolute pressure and inversely with temperature, since they affect air density (164). Stability and accuracy of the analyzer were improved by introducing copper coils upstream from the two orifices, to facilitate thermal equilibration. The orifices, copper coils, and associated polyethylene tubing were immersed in a water bath at room temperature.

My observations support Mead's conclusion that the principle source of instability and drift is the heat liberated by the reaction of CO_2 with Ascarite.[2] The efficiency of the CO_2 absorbing chamber was improved by a perforated gas distribution tube. It was found that Caroxite[3] was not as satisfactory as Ascarite. Performance of the analyzer was improved if the CO_2 absorbing chamber was cleaned and repacked daily with a fresh chemical charge. Measurements

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[2]Commercial name for CO_2 absorbent consisting of NaOH fused on asbestos fibers.

[3]Commercial name for CO_2 absorbent consisting of NaOH on asbestos fibers, with an indicating dye that changes color as CO_2 is absorbed and NaOH is converted to Na_2CO_3.

of response times showed that the response to 6% CO_2 was 99% complete in 2 min.

The critical orifice analyzer was calibrated before and after each experiment with gases whose CO_2 concentrations were determined to a precision of ±0.02% absolute (average deviation) by repeated analyses with a Scholander micro gas analyzer. I found it necessary to apply a calibration curve to each experiment, since the use of a single calibration per day gave average deviations and ranges approximately three times greater than the values reported by Mead who found that a single calibration was adequate for an entire day. This difference was probably due to the greater CO_2 loading of the Ascarite in the present studies. Simple statistical analysis of data from 195 pre- and post-test calibrations showed that the uncertainty (average deviation) in calibrated critical orifice analyzer readings was ±0.04% with a range of ±0.16% CO_2.

Greater accuracy of the analyzer could probably be achieved by applying more precise manometric techniques. In particular, the use of a tapered plug in the fluid well can compensate for compressibility, which is a non-linear function of column height (23). Improvement in performance would also undoubtedly result from better temperature control since the height of the liquid column is a function of fluid density which in turn is temperature dependent.

The O_2 analyzer used in these studies was a Beckman

Model C2P, paramagnetic analyzer (124), with rapid response characteristics, response from 0-100% being complete in 10 sec. The paramagnetism of O_2 varies with temperature and the Model C2P is equipped with a thermostatically controlled heating device that automatically compensates for temperature variations in sample gas temperatures between 10-40 C. Calibration of the Beckman analyzer was checked daily on gases of known composition.

Scholander micro gas analyses (146) were used as primary standard values of O_2 and CO_2 percentages. Modifications suggested by Dr. L. G. C. E. Pugh improved the performance of the device, particularly under the field conditions prevailing at the Barcroft Laboratory. Stopcocks with keepers were placed below the reagent stoppers and kept closed except when more reagent was added. This modification completely eliminated the use of Scholander's empirical correction for the coefficient of elasticity. Problems of unstable readings due to fluctuating room temperatures were solved by replacing the standard leveling bulb by a Hg pump, made from a hypodermic syringe and mounted inside the water bath. An improved gas transfer pipette was devised from a 20 ml hypodermic syringe, a 3-way stainless steel stopcock, and an 18 gauge needle with a polyethylene sleeve tip. Accuracy achieved with the micro Scholander analyzer in routine use was $\pm 0.040\%$ O_2 and $\pm 0.030\%$ CO_2 (average deviation).

Table VI shows the results of independent analyses of

TABLE VI. Comparison of independent analyses of unknown gas mixtures by micro Scholander and critical orifice-Beckman analyzers. Values given are: mean, ± average deviation, (number of determinations).*

Cylinder No.	Per Cent O_2		Per Cent CO_2	
	Micro Scholander	Beckman	Micro Scholander	Critical Orifice
OG-29454	21.06, ±0.04, (6)	21.03, ±0.02, (3)	0.073, ±0.03, (6)	0.025, ±0.006, (3)
OG-48462	20.51, ±0.01, (4)	20.47, ±0.02, (3)	1.090, ±0.02, (5)	1.043, ±0.018, (3)
OG-48460	20.99, ±0.07, (4)	21.05, ±0.00, (2)	2.312, ±0.02, (5)	2.258, ±0.022, (3)

* O_2: Avg difference between $\bar{X}$'s = ±0.04%, CO_2: Avg difference between $\bar{X}$'s = ±0.05%.

unknown gas samples by two different methods; micro Scholander and critical orifice CO_2 and Beckman O_2 continuous flow analyses. It is apparent that these methods were of comparable precision.

2. Alveolar Gas Sampling Techniques

The alveolar gas sampling device of Fenn, Rahn, and Otis (48,133) has been widely used since 1949. Although the gas sample stream may be analyzed continuously, the sampling is performed discontinuously.

Fig. 9 is a schematic diagram of the Rahn-Otis alveolar gas sampler. The device operates in the following manner. During inspiration, negative pressure in the mask causes the balloon to inflate with gas from the last portion of expired gas from the previous expiration. When expiration occurs the positive mask pressure causes the balloon to collapse, forcing gas out of the balloon at precisely the time when the gas passing by the sampling tube orifice contains a high proportion of dead space gas. The balloon thus acts as a very sensitive valve, preventing dead space gas from entering the sampling tube, since its rate of emptying is very much greater than the rate at which the gas sample is being drawn into the pump. Most of the gas from the collapsing balloon passes back into the expired air stream. Thus, in theory, the sampler supplies a steady stream of end tidal gas to the analyzers. In practice, the subject's respiration must be observed and the device must be adjusted periodically to prevent too rapid collapse

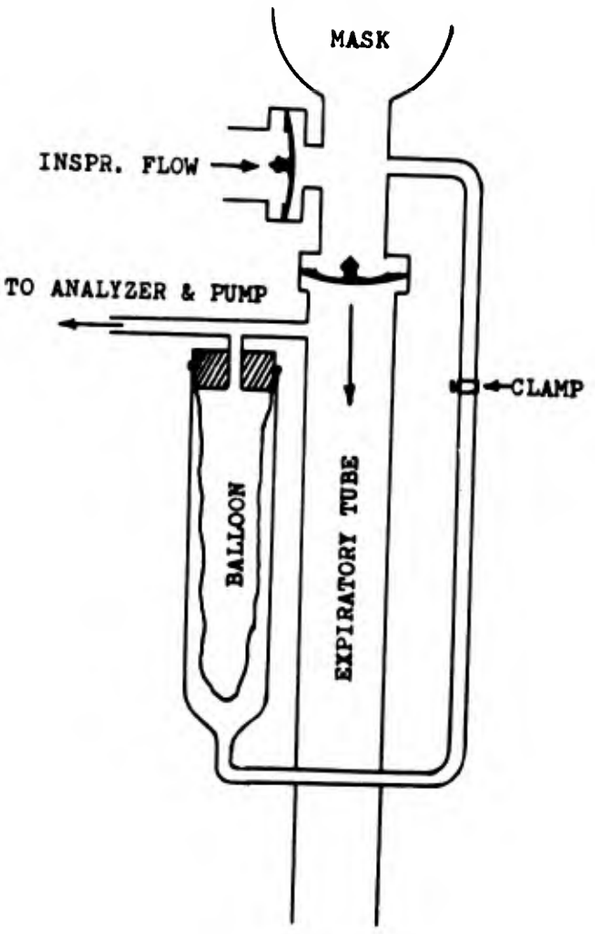

Fig. 9.. Schematic diagram of Rahn-Otis end tidal alveolar gas sampler. Redrawn from Rahn and Otis (133).

of the balloon. When the balloon collapses too rapidly, dead space gas is drawn into the sampling line, and the resulting values are erroneously low for P_{CO_2} and high for P_{O_2}. Too rapid collapse of the balloon can result from a sticking expiratory valve due to the accumulation of saliva. The emptying rate of the balloon is controlled by adjusting the screw clamp shown in Fig. 9.

Alveolar gas values obtained with this device are nearly always lower for P_{CO_2} and higher for P_{O_2} than the corresponding values obtained with the classical Haldane-Priestley end-expiratory method. Rahn and his co-workers have presented evidence that the alveolar gas obtained by their method is more representative of average alveolar gas than that obtained by the Haldane-Priestley method. They have suggested that the ultimate criterion for judging if a sampling device gives representative average alveolar gas is to compare the P_{CO_2} in alveolar gas with P_{CO_2} in mixed arterial systemic blood (129). This criterion was not applied to any of the devices used with dogs in the present study.

Initially, efforts to develop a method for obtaining continuous alveolar gas samples from dogs were limited to the use of Rahn-Otis end tidal samplers. A thin-walled rubber balloon of 40 ml capacity seemed to be satisfactory, in contrast to a balloon of 9.1 ml volume, which was too small to prevent dead space gas from entering the sampling line during expiration.

Although the Rahn-Otis sampling technique was more successful with the Von Döbeln modified valve, continuing difficulties were encountered. I attempted to improve the technique for alveolar air sampling in dogs by developing a special device. Fig. 10 shows a schematic diagram of this device, which was based in part on ideas previously described by Fenn, Rahn, and Otis (48) and by Nielson and Smith (116).

Negative or positive pressure within the respiratory valve caused the tambour to move down or up, making or breaking the current to a solenoid valve which controlled the flow of gas to the balloon. During expiration the solenoid valve remained closed, therefore no gas entered the sampling line from the expired air stream and the gas for the analyzers was aspirated from the balloon. When inspiration occurred the solenoid valve opened, filling the balloon with gas from the last portion of the previous expiration. The Hg cup was adjusted so that sampling occurred only during active inspiration, hence there was no possibility of the sample becoming contaminated with dead space gas. The balloon had to be of sufficient volume that it would furnish gas to the analyzers during the period when the solenoid valve was closed, i.e., during apnea and expiration. In order to prevent possible damage to the analyzers it was necessary to use a balloon that was considerably larger than a conventional Rahn-Otis balloon so that it definitely did <u>not</u> empty completely with each cycle.

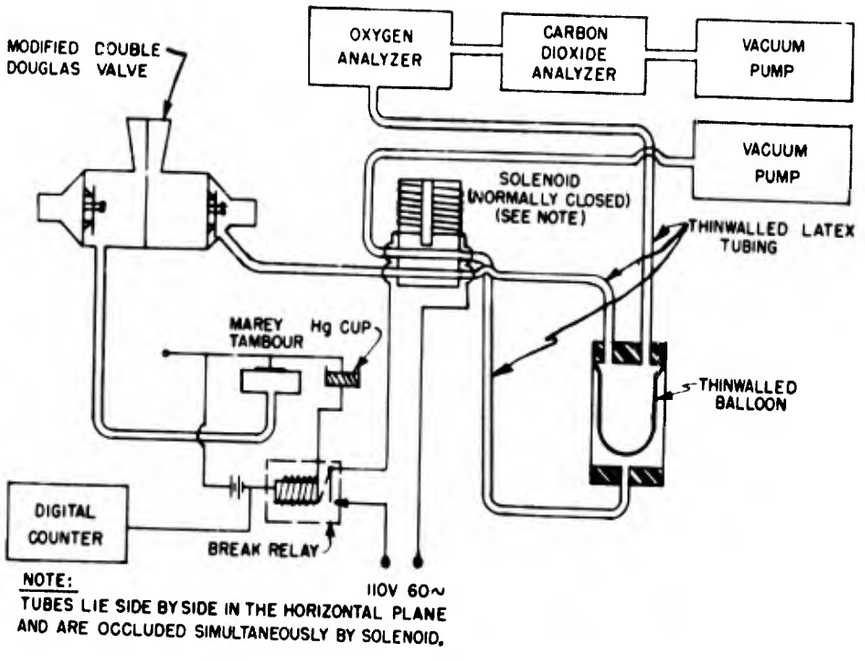

Fig. 10. Schematic diagram of electromagnetic device for automatic end tidal gas sampling (116, 133).

Hence the gas being aspirated through the analyzers was a mixture of end tidal gas from several expirations and the device did not reflect breath to breath variations in alveolar P_{O_2} and P_{CO_2}. This fact was of no importance in the present studies since the objective was to obtain representative data on alveolar P_{O_2} and P_{CO_2} once each minute.

Tests were conducted to compare performance characteristics of Rahn-Otis and electromagnetic sampling devices. No appreciable differences were found.

The electromagnetic sampler did not require the periodic adjustments that were necessary for successful operation of the Rahn-Otis sampler. The Rahn-Otis sampler has one decided advantage over the electromagnetic sampler shown in Fig. 10. In this continuous flow analysis system, the sample balloon can become exhausted if the subject enters a period of apnea, with consequent damage to the analyzers. This difficulty occurred several times during the altitude experiments, when transient apnea occurred after the inspired P_{O_2} was raised from normal ambient pressure (91 mm Hg) to approximately sea level value (148 mm Hg).

3. Summary and Conclusions

A modified critical orifice CO_2 analyzer was used to provide continuous analyses of end-expiratory alveolar gas samples. Several modifications were made to the design reported by Mead,

including an easily adjustable differential manometer with a direct reading scale. Calibration curves were applied to all raw values for per cent CO_2. The absolute accuracy of the calibrated instrument was $\pm 0.04\%$ CO_2 (average deviation). Increased accuracy of the instrument could be obtained by applying precise manometric techniques.

The end tidal gas sampler of Fenn, Rahn, and Otis was used with trained dogs breathing room air. This sampler required close monitoring and periodic adjustments to insure proper emptying of the balloon. A modified electromagnetic sampler was constructed, similar in many respects to a Rahn-Otis sampler, but using a solenoid valve to prevent the entry of dead space gas to the sampling line, as suggested by Nielsen and Smith.

Several modifications were made to the standard Scholander micro gas analyzer. Stopcocks, added to the reagent filling tubes, eliminated the empirical correction for the coefficient of elasticity. A Hg pump, mounted in the water bath, solved the drift problem encountered under field conditions. An improved gas transfer pipette was developed using a hypodermic syringe. Analyses for O_2 and CO_2 by Beckman and critical orifice analyzers, respectively, were comparable in accuracy to micro Scholander analyses.

III. Results

A. Typical Experiments

Many respiratory physiologists have emphasized the necessity for using fully trained, healthy subjects in order to obtain meaningful data on humans. The experimental problems of variability in respiratory parameters are at least as great when one uses trained dogs as subjects. Technical difficulties made it impossible to perform an equal number of experiments on each subject. Therefore, the results will be presented showing individual subjects' responses wherever practicable. Figs. 11 and 12 show the results from two typical experiments, at sea level and at altitude, respectively. For comparative purposes, respiratory volumes are shown in two ways: as alveolar ventilation and as respiratory minute volume. These Figs. show data acquired during a 10 min recovery period on room air. Since recovery data were not obtained in all tests the results will not be presented.

Alveolar P_{O_2} was maintained at, or above, its normal sea level value during both altitude and sea level experiments. The values for inspired P_{CO_2} and P_{O_2} shown in Figs. 11 and 12 are essentially correct for all experiments since all altitude experiments were performed with the same set of gas mixtures and another set was used for all sea level experiments. The only variable with respect

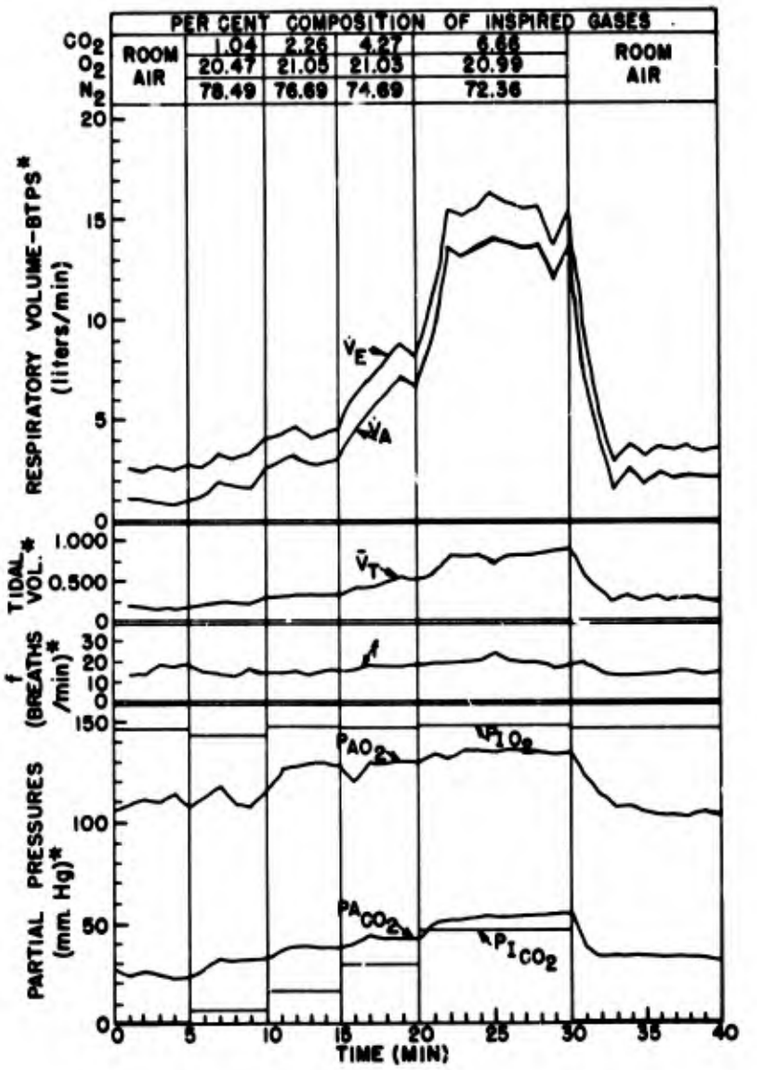

Fig. 11. Results of a typical CO_2-response test at sea level. Both time and inspired P_{CO_2} are independent variables. Dependent variables include: respiratory minute volume; alveolar ventilation; tidal volume; respiratory frequency; alveolar P_{O_2}; and alveolar P_{CO_2}. Per cent composition of inspired gas mixtures is shown at the top, and inspired P_{CO_2} and P_{O_2} in mm Hg pressure are shown at the bottom. Subject: Wags. Date: Sept. 13, 1956.

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* During the first six minutes of this test, tidal volume was less than twice the dead space of the animal. These data are not included in the values shown in Tables VII and IX and Fig. 13.

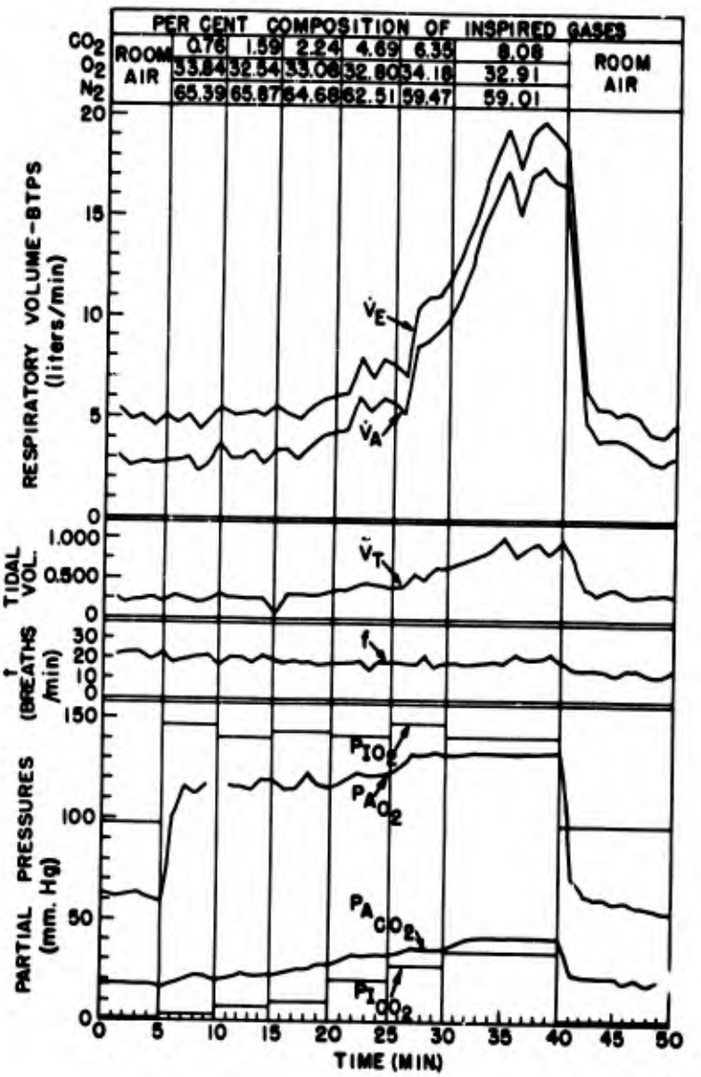

Fig. 12. Results of a typical CO_2-response test at altitude. Four different gas mixtures were used at sea level but six were used at altitude. One of the extra gas mixtures used at altitude provided an alveolar P_{O_2} that was slightly above normal sea level value without any added CO_2. The data collected during this period (minutes five to ten) provided a measure of the hypoxic drive component of resting alveolar ventilation. Subject: Wags. Date: Aug. 29, 1956.

to inspired P_{CO_2} and P_{O_2} was daily variations in local barometric pressure.

B. Respiratory Parameters in Awake Dogs Breathing Room Air at Sea Level and at Altitude

Tables VII and VIII present data obtained from thirty three experiments on three dogs. The dogs were judged to be in resting steady states, breathing either room air or compressed air. Table VII presents sea level data and Table VIII shows results from experiments performed at the Barcroft Laboratory during the first ten days at altitude. Both these tables show the results on individual animals. Tables IX and X present group averages for the three dogs, at sea level and at an altitude of 12,470 ft, respectively. Fig. 13 shows a graphical comparison of these data on both an individual and a group basis, as per cent of sea level control value. Standard errors of the means are plotted as percentages of the respective means. Standard errors of the means varied from less than one per cent for alveolar P_{O_2} to about eight per cent for alveolar ventilation.

Room temperature was an uncontrolled variable in these experiments, hence panting occurred occasionally. Respiratory rates of over 100 breaths/min and tidal volumes which approached

TABLE VII. Respiratory data from experiments on three dogs at sea level, awake, judged to be in resting steady states, breathing room air or compressed air. Data points were eliminated where tidal volume was equal to or less than twice the dead space, or where respiratory frequency was equal to or greater than forty breaths per minute. All gas volumes converted to BTPS. Values given are; mean, ± one standard error of the mean, (number of determinations).

Parameter	Subject		
	Wags	Rusty	Lunk
Alveolar P_{O_2} (mm Hg)	112.9, ±0.8, (13)	115.9, ±1.1, (8)	99.6, ±0.7, (28)
Alveolar P_{CO_2} (mm Hg)	27.1, ±0.5, (13)	21.0, ±0.7, (8)	33.6, ±0.4, (28)
Alveolar ventilation (liters/min)	3.10, ±0.19, (13)	3.33, ±0.22, (8)	2.31, ±0.15, (28)
Tidal volume (liters)	0.255, ±0.006, (13)	0.193, ±0.012, (8)	0.281, ±0.014, (28)
Respiratory frequency (breaths/min)	20.3, ±0.1, (13)	24.7, ±1.0, (8)	11.0, ±0.7, (28)

TABLE VIII. Respiratory data from experiments on three dogs at the Barcroft Laboratory, awake, judged to be in resting steady states, breathing room air. Data points were eliminated where tidal volumes were equal to or less than twice the dead space, or respiratory frequencies were equal to or greater than forty breaths per minute. All gas volumes converted to BTPS. Values given are; mean, ± one standard error of the mean, (number of determinations).

Parameter	Subject		
	Wags	Rusty	Lunk
Alveolar P_{O_2} (mm Hg)	57.7, ±0.7, (41)	61.3, ±1.0, (25)	55.6, ±0.4, (12)
Alveolar P_{CO_2} (mm Hg)	19.9, ±0.4, (41)	16.1, ±1.1, (25)	22.0, ±0.3, (12)
Alveolar ventilation (liters/min)	3.02, ±0.14, (41)	3.30, ±0.22, (25)	2.29, ±0.22, (12)
Tidal volume (liters)	0.265, ±0.006, (41)	0.196, ±0.005, (25)	0.205, ±0.011, (12)
Respiratory frequency (breaths/min)	19.5, ±1.2, (41)	24.0, ±1.7, (25)	15.9, ±0.7, (12)

TABLE IX. Group average data on resting alveolar P_{O_2} and P_{CO_2} ; tidal volume; respiratory frequency; and alveolar ventilation. Three dogs, awake, at sea level, breathing room air or compressed air. Data points were eliminated where tidal volume was equal to or less than twice the dead space, or where respiratory frequency was equal to or greater than forty breaths per minute. All gas volumes corrected to BTPS.

Parameter	Mean	Standard Error	Range
Alveolar P_{O_2} (mm Hg)	109.5	±5.0	94.1 -119.3
Alveolar P_{CO_2} (mm Hg)	27.2	±3.6	18.7 - 37.1
Alveolar ventilation (liters/min)	2.91	±0.31	1.12 - 4.22
Tidal volume (liters)	0.243	±0.026	0.150- 0.429
Respiratory frequency (breaths/min)	18.7	±4.0	7.0 - 29.0

TABLE X. Group average data on resting alveolar P_{O_2} and P_{CO_2}; tidal volume; respiratory frequency; and alveolar ventilation. Three dogs, awake, at the Barcroft Laboratory, breathing room air. Data points were eliminated where tidal volume was equal to or less than twice the dead space, or where respiratory frequency was equal to or greater than forty breaths per minute. All gas volumes corrected to BTPS.

Parameter	Mean	Standard Error	Range
Alveolar P_{O_2} (mm Hg)	58.2	±1.7	49.2 -68.0
Alveolar P_{CO_2} (mm Hg)	19.3	±1.7	13.2 -24.9
Alveolar ventilation (liters/min)	2.87	±0.30	1.30 - 5.51
Tidal volume (liters)	0.222	±0.023	0.150- 0.358
Respiratory frequency (breaths/min)	19.8	±2.3	10.0 -37.0

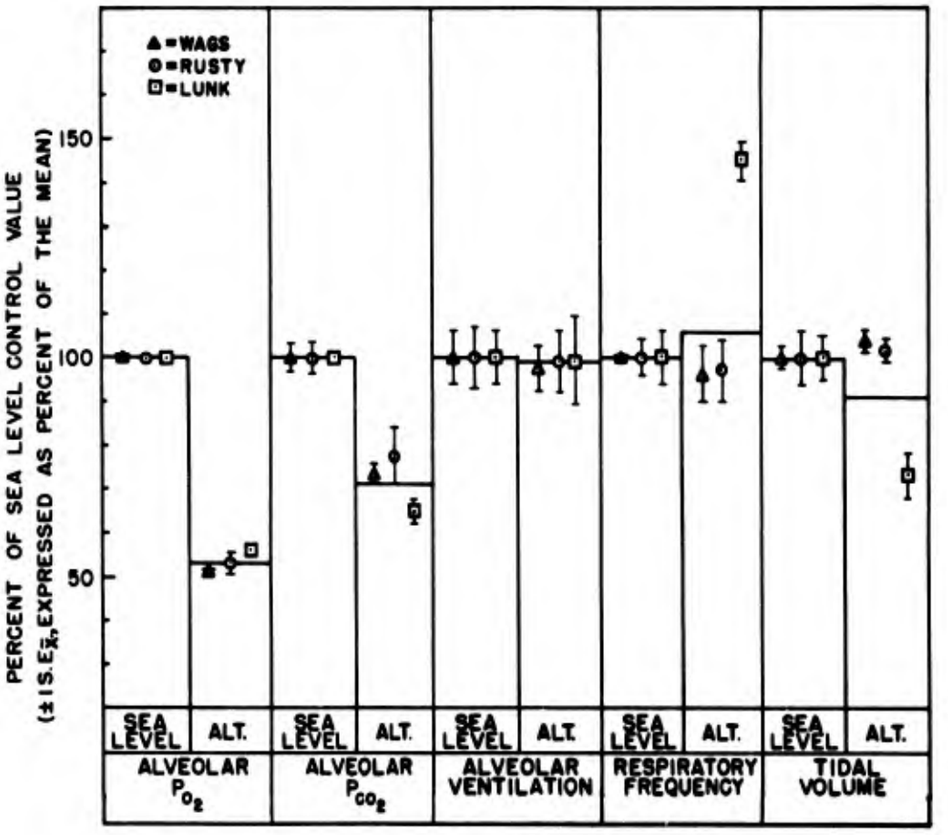

Fig. 13. Changes in respiratory parameters at altitude as compared to sea level. Animals at rest, breathing room air, awake. Data expressed as per cent of sea level control value. Vertical lines show plus or minus one standard error of the mean, expressed as per cent. Bar graph shows group means, with mean values for each subject superimposed.

anatomical dead space volumes were recorded. Under such conditions it was impossible to obtain representative alveolar air samples. Because of the panting problem, data points were eliminated where tidal volumes were equal to or less than twice the dead space of the animal, or where respiratory frequencies were equal to or greater than 40 breaths/min. This limitation applied primarily to the room air data.

It is apparent from inspection of Fig. 13 that both alveolar P_{O_2} and P_{CO_2} are greatly lowered in the dog at altitude. The lowered alveolar P_{O_2} of course merely reflects the primary variable in the experiments; i.e., the decreased barometric pressure. None of the animals showed a change in resting alveolar ventilation. In two of the three animals, resting tidal volume and respiratory frequency did not change as a result of ten days' exposure to an altitude of 12,470 ft. In the third dog, tidal volume was significantly lower and respiratory frequency was significantly higher at altitude.

The results shown in Fig. 13 and Tables IX and X were obtained by pooling the data from repeated experiments on each subject under identical conditions. It was apparent from observations made during the experiments that there was a great deal of random variation in the respiratory data on dogs breathing room air. For this reason no attempt was made to analyze the altitude data in terms of elapsed time to determine whether alveolar P_{O_2}, alveolar P_{CO_2},

alveolar ventilation, respiratory frequency, or tidal volume may have varied in a systematic manner with elapsed time at altitude.

The variation in respiratory baseline measurements on awake dogs is illustrated in Fig. 14 which shows the results of two tests on each subject, awake, at altitude, on the twelfth day. Measurements were made each minute for a 10 min period with the dog breathing room air at rest. Results are expressed as per cent of sea level control. An analysis was made to see whether respiratory baseline measurements varied as a function of time of day. No relationship was found. The technique of pooling data from repeated experiments on each animal under identical conditions gave a larger sample and reduced the probability that the mean values would be distorted by random variations.

Several criteria have been suggested for judging whether or not altitude acclimatization has been achieved. One of the earliest of these criteria is the maintenance of body weight in mature animals (154). Table XI shows mean body weights of the subjects as determined from ten daily measurements at sea level and from fourteen daily measurements at altitude. Analysis of the body weight data on a day by day basis showed no change during the eighteen day altitude sojourn.

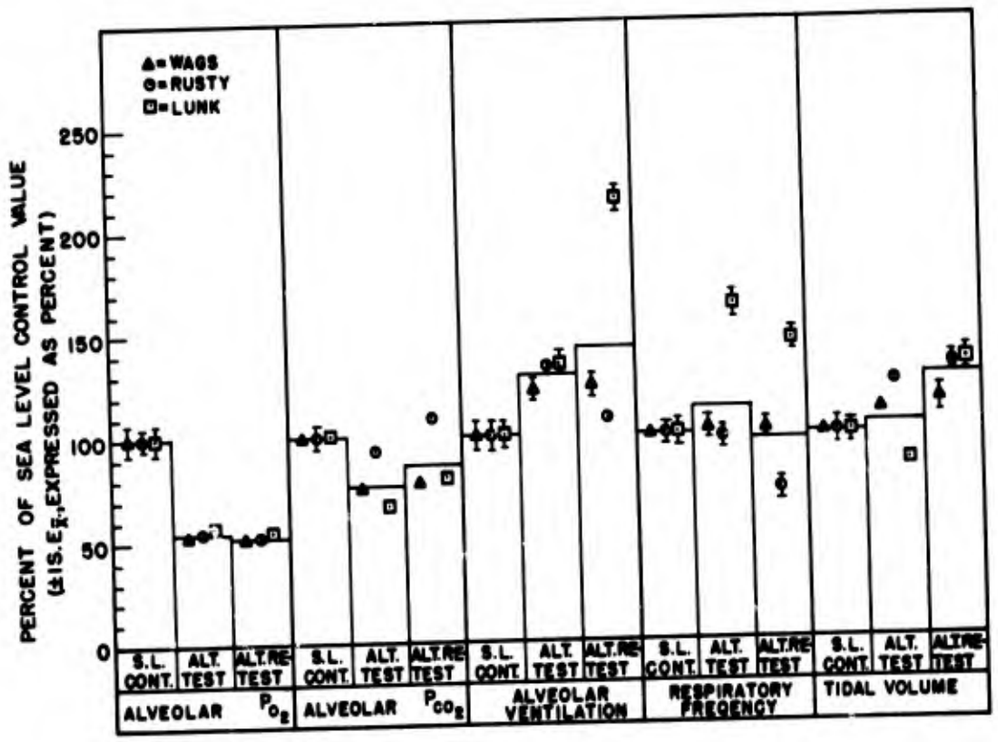

Fig. 14. Variability of respiratory measurements on three awake dogs, breathing room air, at rest. Results are shown where baseline measurements were repeated on each animal on the same day, (twelfth day at altitude). Mean values expressed as per cent of sea level control. Bar graph shows group averages with values for individual subjects superimposed. Uncertainty shown as plus or minus one standard error of the mean, as per cent.

TABLE XI. Body weights of three subjects, mean, ± standard deviation, (number of daily determinations).

Subject	Fasting body weight (kg)	
	Sea level	Altitude
Wags	17.7, ±0.3, (10)	17.8, ±0.2, (14)
Rusty	15.5, ±0.8, (10)	15.4, ±0.4, (14)
Lunk	12.3, ±0.4, (10)	12.2, ±0.4, (12)

C. Carbon Dioxide Sensitivity Tests on Awake Dogs

The word "awake" is used throughout this report to indicate "unsedated" dogs, although the sedated dogs were not asleep since they responded readily to auditory or visual stimuli. Fig. 15 summarizes the results of CO_2 sensitivity tests on three awake dogs at sea level and at altitude. Data on each animal were pooled from multiple tests under identical conditions, using the values recorded for the last two minutes on each gas mixture. Fig. 15 shows alveolar ventilation plotted against simultaneously determined alveolar P_{CO_2} for three subjects, with straight lines connecting the group average values. Uncertainty in the data on each subject is shown as plus or minus one standard error of the mean for both alveolar ventilation and alveolar P_{CO_2}. The consistent individual differences between the three subjects are readily apparent. Each curve appears to have two components; a flat portion in which increases in alveolar P_{CO_2} produce essentially no change in alveolar ventilation; and a more or less straight line portion in which increases in alveolar P_{CO_2} are accompanied by corresponding increases in alveolar ventilation.

Alveolar P_{CO_2} threshold may be considered as the point on the CO_2 response curve beyond which any further increase in alveolar P_{CO_2} produces a corresponding increase in alveolar ventilation. By this definition, it appears from inspection of Fig. 15 that alveolar P_{CO_2} threshold was lowered by about 6-7 mm Hg pressure at altitude.

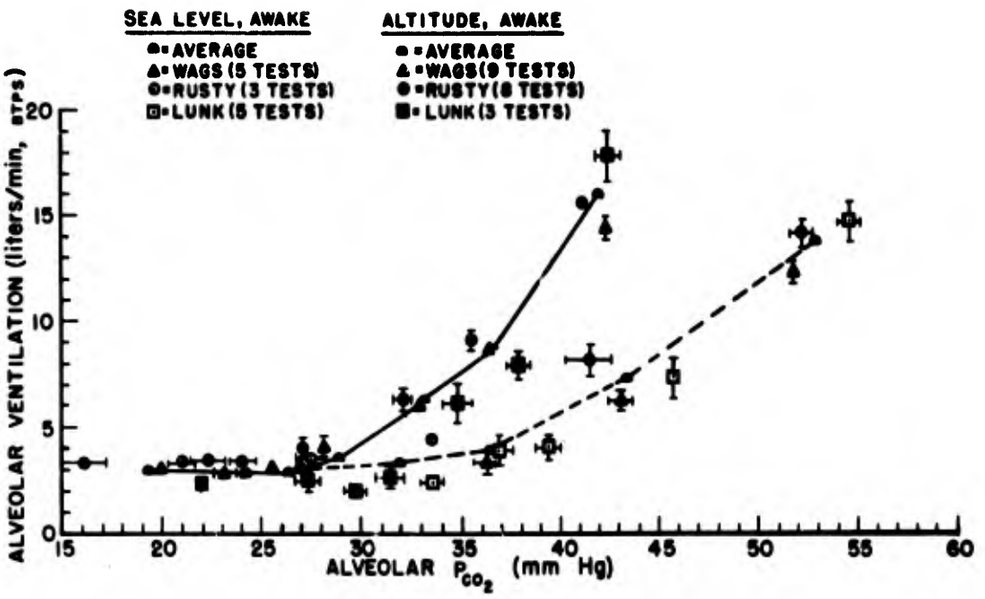

Fig. 15. Alveolar ventilation as a function of alveolar P_{CO_2}, at sea level and at altitude. Three dogs, awake. Inspired P_{CO_2} was increased in step increments and held constant for five minute periods. Measurements made during the last two minutes on each gas mixture were pooled for each subject. Values shown are means, plus or minus one standard error of the mean.

It also appears that both altitude and sea level CO_2 response curves are concave upward with the altitude curve having a more pronounced upward concavity.

Carbon dioxide sensitivity may be defined as the slope of the CO_2 response curve when CO_2 is added to the inspired gas mixture and the resulting alveolar ventilation is plotted against simultaneous values of alveolar P_{CO_2}. Carbon dioxide sensitivity is thus defined in terms of unit increase in alveolar ventilation per unit increase in alveolar P_{CO_2}. From the results expressed in Fig. 15 it appears that there is an increase in CO_2 sensitivity in the awake dog at altitude. Statistical techniques were applied to these data to permit a more quantitative interpretation of the results. The results of the statistical analyses are presented below.

D. The Effects of Preanesthetic Sedation on the CO_2 Sensitivity of Dogs

Two different drugs were used: scopolamine and a two drug combination of scopolamine plus thiopental. These drugs were intended to produce preanesthetic sedation (56), not anesthesia. Dosages were as follows: scopolamine = 0.004 mg/kg; thiopental = 0.4 cc, 2%, freshly prepared solution/kg (0.8 mg/kg), injected iv. The subjects

were conscious throughout each test and responded readily to auditory or visual stimuli. Two tests were performed on each subject with each drug(s) at sea level and at altitude during days eleven through sixteen. The data were pooled for each subject. Fig. 16 shows the group average curves at sea level for the three subjects; awake, under scopolamine sedation, and under scopolamine plus thiopental sedation. Data points for each individual are also plotted, with uncertainty shown as plus or minus one standard error of the mean. Fig. 17 shows the same information for the same three subjects tested at altitude.

Apparently Fig. 16 shows that scopolamine alone had no effect on either CO_2 threshold or CO_2 sensitivity at sea level. The two drug combination of scopolamine plus thiopental may have reduced the CO_2 sensitivity but there was no effect on CO_2 threshold.

Fig. 17 indicates that CO_2 sensitivity was reduced at altitude in the sedated animals as compared to tests of the same subjects awake. The two drug combination of scopolamine plus thiopental was apparently much more effective as a respiratory depressant. Effects on CO_2 threshold are not well defined. The altitude sedation curves are for two subjects. The altitude tests were not completed on one subject because of a lack of time available for the experiments.

Fig. 18 shows group average values for awake and sedated subjects at sea level and at altitude to facilitate visual comparison of

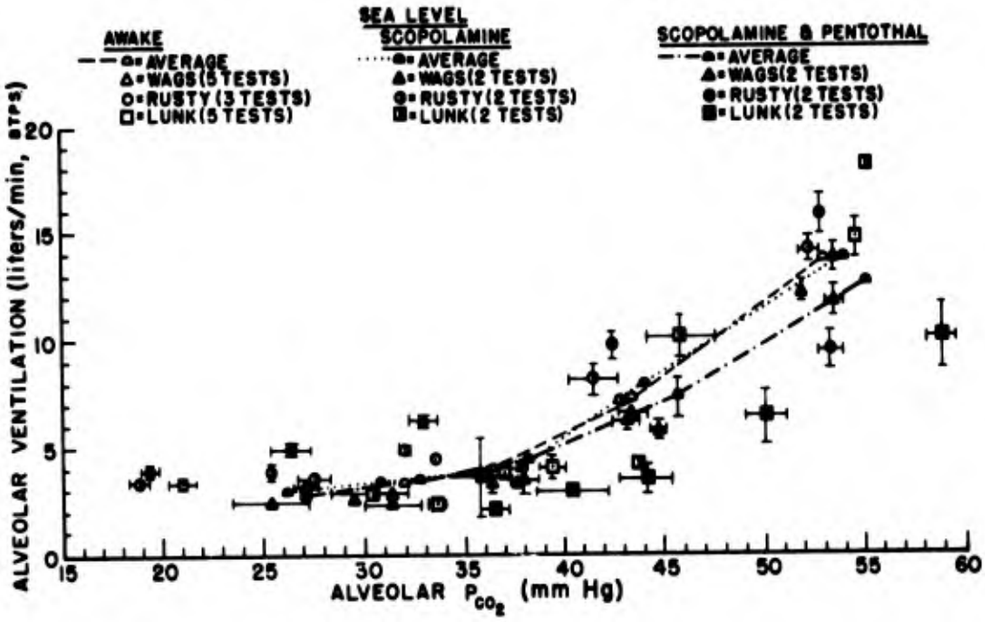

Fig. 16. Alveolar ventilation as a function of alveolar P_{CO_2}. Data points, plus or minus one standard error of the mean, are shown for three dogs under the following conditions; awake (control); under scopolamine sedation; under scopolamine and thiopental sedation. All experiments performed at sea level.

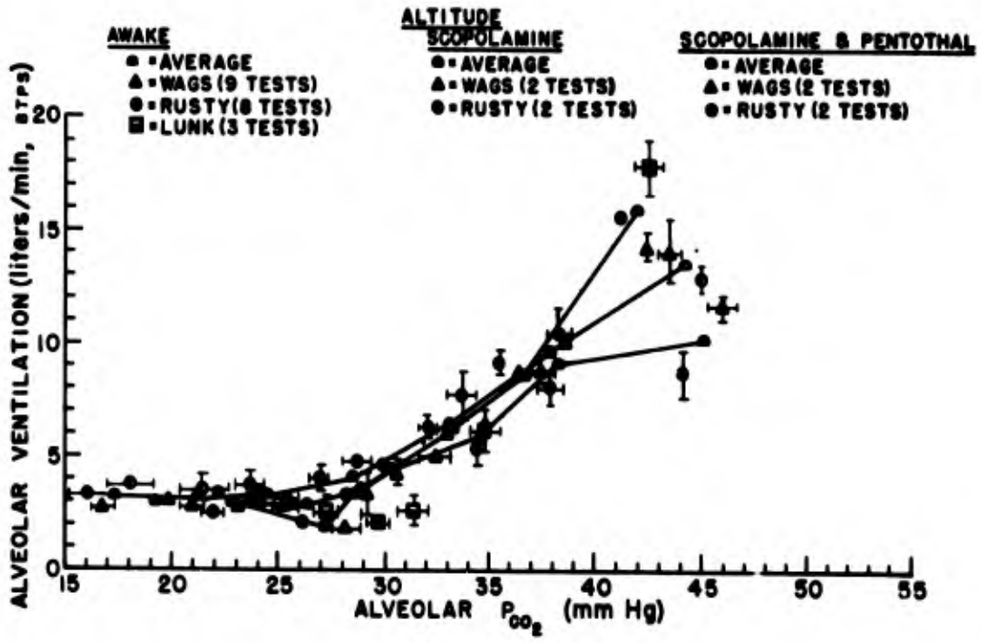

Fig. 17. Alveolar ventilation as a function of alveolar P_{CO_2}. Data points are shown for group averages and for individual dogs. Uncertainty in measurements on individual animals shown as plus or minus one standard error of the mean. All experiments were performed at altitude and included the following conditions: awake; scopolamine sedation; scopolamine plus thiopental sedation. Solid lines connect group mean data points.

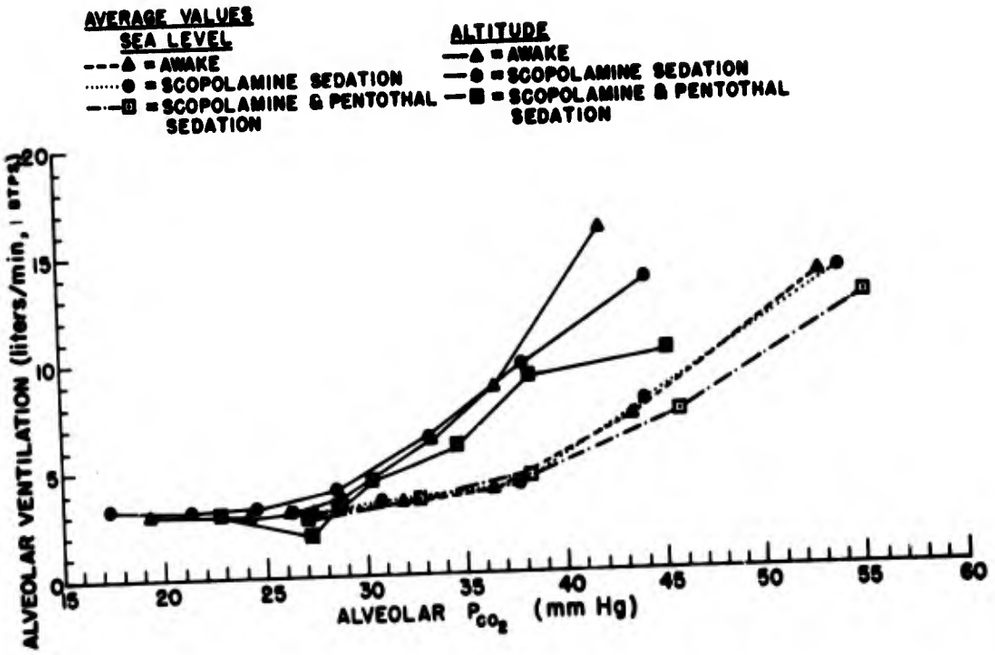

Fig. 18. Alveolar ventilation as a function of alveolar P_{CO_2}. Average values are shown for a group of three dogs at sea level. The altitude sedation curves are for two dogs. The animals were tested under three conditions; awake, under scopolamine sedation, and under scopolamine plus thiopental sedation.

the results under the various experimental conditions.

Several experiments were performed using sedative doses of drugs other than scopolamine or the scopolamine-thiopental combination. Too few experiments were done to warrant many comparisons with the other results. However, results from one experiment on one dog, surgically anesthetized with a dosage of 0.5 cc/kg sodium pentobarbital (Veterinary Nembutal, Abbott) are shown in Fig. 19. These results are presented to illustrate the decrease in CO_2 sensitivity that is usually referred to clinically as respiratory depression. The respiratory depression seen in clinical anesthesia is a completely different phenomenon from the effects that were encountered with preanesthetic sedation in these studies.

E. Carbon Dioxide Sensitivity; Defined as the Slope of the Least Squares Straight Line Relating Alveolar Ventilation to Alveolar pCO_2.

Figs. 15-18 show, qualitatively, that changes occurred in both CO_2 threshold and sensitivity at sea level and at altitude. However, to permit a quantitative estimate of these changes the data were fitted to least squares straight lines. Statistical procedures used were those described by Snedecor (151) and Spiegel (153). Statistical formulae are shown in Appendix C, 6.

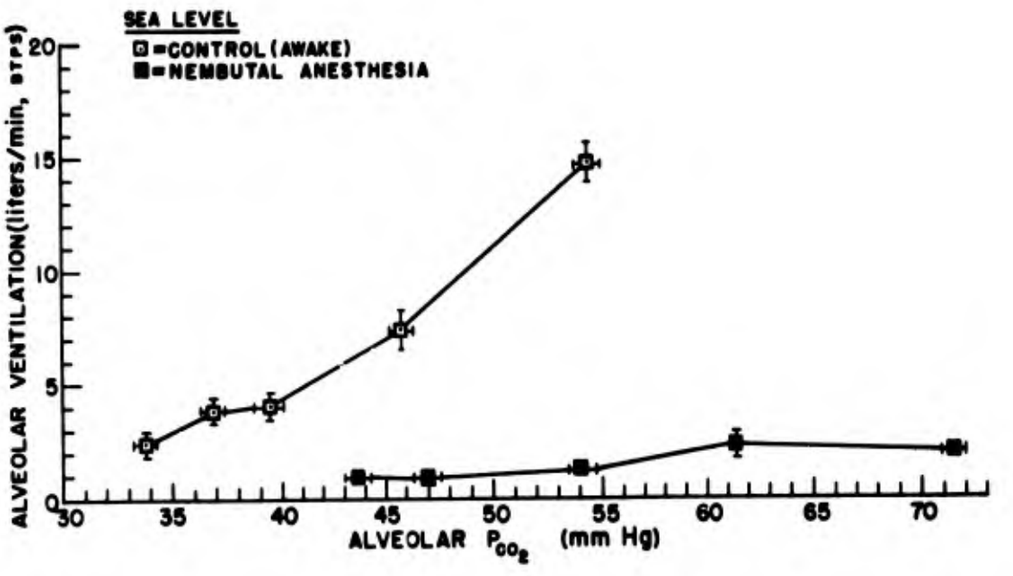

Fig. 19. Alveolar ventilation as a function of alveolar P_{CO_2}. Data points, plus or minus one standard error of the mean, are shown for one subject under the following conditions; awake (control); under surgical anesthesia by sodium pentobarbital (0.5 cc Veterinary Nembutal/kg). Subject: Lunk (purebred beagle).

Because of the uncertainty in the measurements of alveolar P_{CO_2} in the dogs at low tidal volumes, the flat portions of the CO_2 response curves (in which alveolar P_{CO_2} apparently increased by about 10 mm Hg with no concomitant increase in alveolar ventilation) were disregarded. The point of inflection of each curve was determined by applying the t-test to successive pairs of data points from pooled data on each subject. Starting with room air, the t-test was applied to successive pairs of data points to test for a possibly significant increase in alveolar ventilation ($P < 0.05$).

This procedure defined the two values of alveolar ventilation which bracketed the point beyond which further increases in alveolar P_{CO_2} caused increased alveolar ventilation. When the two values of alveolar ventilation had been thus defined for each subject, the arithmetic mean of the two corresponding alveolar P_{CO_2} values was then defined as the alveolar P_{CO_2} threshold. In fitting the least squares straight lines to the data, only those points were considered which fell above the statistically defined alveolar P_{CO_2} threshold.

Fig. 20 shows values of CO_2 sensitivity plotted against time in days; before, during, and after the altitude sojourn. The bar graph on the right side suggests that CO_2 sensitivity in the post-altitude tests was slightly above its pre-altitude level in awake dogs, but the differences were very small compared to the increase in sensitivity during the altitude sojourn. The post-altitude tests were

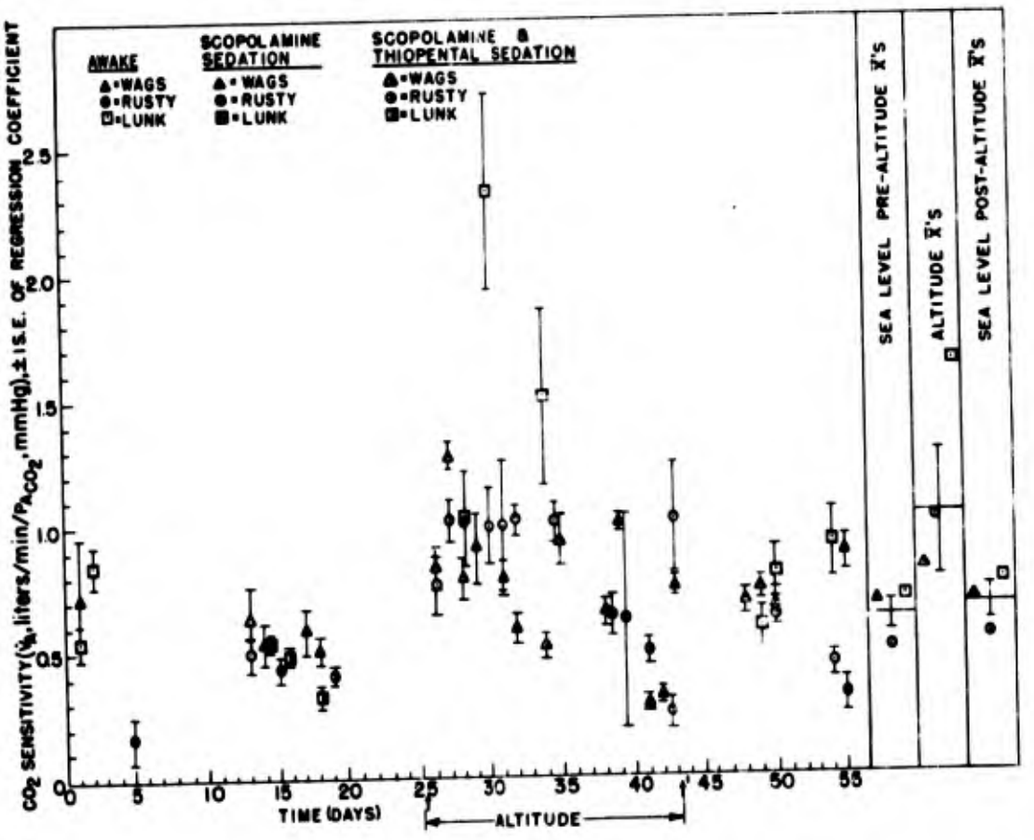

Fig. 20. CO_2 sensitivity as a function of time. Data points are the slopes (regression coefficients) of least squares straight lines fitted to individual experiments. Uncertainty shown as plus or minus one standard error of the regression coefficient.

done during the 4th through 11th days following return to sea level.

Tabular values of CO_2 sensitivity are shown in Tables XII - XV. Tests for goodness of fit showed that in nearly all cases the "CO_2 response curves" were satisfactorily fitted with straight lines. Of the 33 experiments done on awake dogs, only one was not satisfactorily reduced to a straight line, whereas in the 19 experiments on sedated subjects five were not satisfactorily fitted. This is largely due to the greater variability caused by the drugs.

Fig. 21 summarizes the data on CO_2 sensitivity. The group averages show that at sea level scopolamine reduced CO_2 sensitivity to the same degree that scopolamine plus thiopental did. In the awake dogs, CO_2 sensitivity during the first ten days of the altitude sojourn averaged 160% of the sea level control values. At altitude, scopolamine lowered CO_2 sensitivity, as compared to unsedated animals at altitude, and CO_2 sensitivity was reduced to a greater extent by the two drug combination. While these changes seem to follow a consistent pattern, none of the group mean values differ significantly from sea level control by t-test.

F. Alveolar P_{CO_2} at a Constant Alveolar Ventilation

Fig. 22 shows alveolar P_{CO_2} at a constant alveolar ventilation

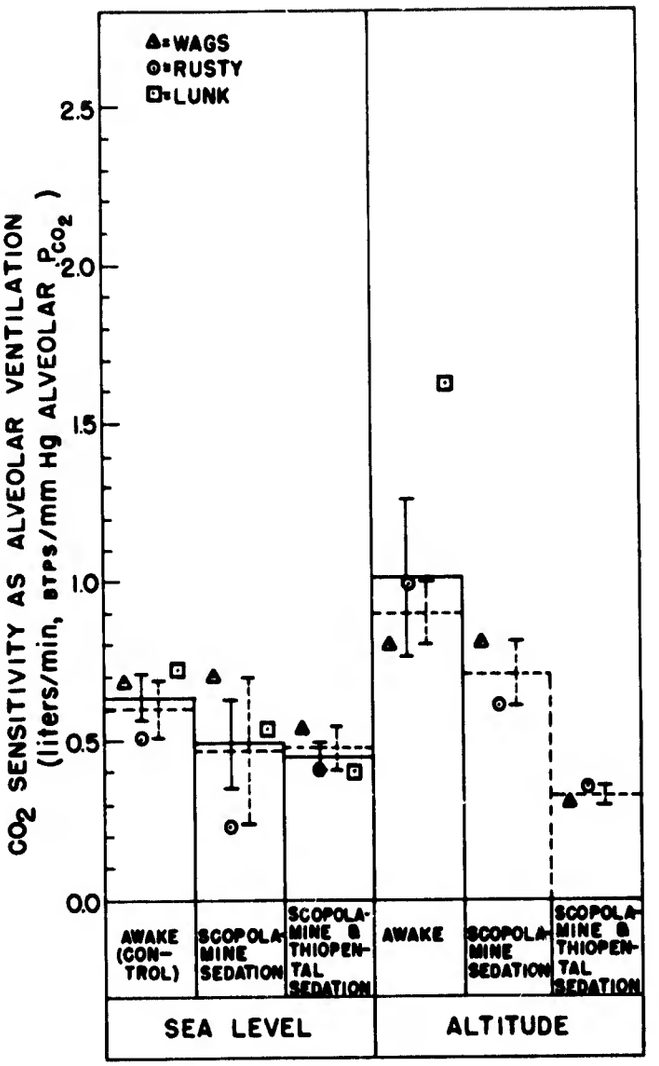

Fig. 21. CO_2 sensitivities as determined from regression coefficients of least squares regression lines. Bar graph shows group average values, values for individual animals are superimposed. Uncertainty shown as plus or minus one standard error of the mean. CO_2 sensitivity expressed in units of alveolar ventilation (liters/min, BTPS) per mm Hg alveolar P_{CO_2}. Solid lines show values for three subjects. Dotted lines show values for two subjects.

TABLE XII. CO_2 sensitivities as determined from the slopes of least squares regression lines, fitted to data from individual CO_2 response experiments at sea level, subjects awake. $\hat{S}_b$ = standard error of the regression coefficient, modified for small samples.

Subject	Date	Time#	CO_2 Sensitivity $\dot{V}_A/P_{A_{CO_2}}$ (liters/min/mm Hg)	$\hat{S}_b$	Goodness of Fit, Test for Linearity t	d.f.
Wags	26 July 56	t-24	0.717	0.238	3.015	2
	7 Aug 56	t-12	0.626	0.134	4.672*	2
	11 Sept 56	t'+5	0.676	0.051	13.132**	2
	12 Sept 56	t'+6	0.733	0.038	19.443**	2
	13 Sept 56	t'+7	0.669	0.057	11.696**	2
	$\overline{X}$, Sea Level		0.684			
Rusty	7 Aug 56	t-12	0.486	0.071	6.870**	4
	13 Sept 56	t'+7	0.624	0.031	19.803**	2
	17 Sept 56	t'+11	0.433	0.046	9.350*	2
	$\overline{X}$, Sea Level		0.514			
Lunk	26 July 56	t-24	0.539	0.073	7.378***	6
	27 July 56	t-23	0.841	0.082	10.197***	6
	12 Sept 56	t'+6	0.576	0.080	7.916***	6
	13 Sept 56	t'+7	0.792	0.108	7.351***	6
	17 Sept 56	t'+11	0.911	0.138	6.602*	2
	$\overline{X}$, Sea Level		0.732			
Group $\overline{X}$			0.643			

* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$
# t = Arrival day at Crooked Creek Laboratory
t' = Last day at altitude

TABLE XIII. CO_2 sensitivities as determined from the slopes of least squares regression lines, fitted to the data of individual CO_2 response experiments at altitude, subjects awake. $\hat{S}_b$ = standard error of the regression coefficient, modified for small samples.

Subject	Date	Time#	CO_2 Sensitivity, $\dot{V}_A/P_{ACO_2}$ ($\dot{V}_A$, liters/min/mm Hg pressure)	$\hat{S}_b$	Goodness of Fit, Test for Linearity t	d.f.
Wags	20 Aug 56	0.81	0.834	0.079	10.459***	6
	21 Aug 56	1.85	1.272	0.053	24.049**	2
	22 Aug 56	3.00	0.779	0.077	10.139***	6
	23 Aug 56	3.78	0.904	0.139	6.490***	6
	25 Aug 56	5.64	0.772	0.069	11.356***	6
	26 Aug 56	6.68	0.581	0.057	10.127***	6
	28 Aug 56	8.85	0.512	0.053	9.684***	6
	29 Aug 56	9.75	0.930	0.097	9.542***	6
	6 Sept 56	17.53	0.737	0.039	19.044***	6
	$\overline{X}$, Altitude		0.813			
Rusty	20 Aug 56	0.98	0.761	0.122	6,231**	4
	21 Aug 56	2.02	1.059	0.071	14.986***	4
	22 Aug 56	3.07	1.015	0.013	9.819***	4
	24 Aug 56	4.79	0.989	0.151	6.552**	4
	25 Aug 56	5.77	0.989	0.258	3.835*	4
	26 Aug 56	6.81	1.122	0.062	17.973***	4
	29 Aug 56	9.81	1.049	0.075	14.023***	4
	6 Sept 56	17.65	1.100	0.224	4.898**	4
	$\overline{X}$, Altitude		1.011			
Lunk	23 Aug 56	3.13	1.071	0.194	5.523**	4
	24 Aug 56	5.06	2.324	0.394	5.898**	4
	28 Aug 56	8.68	1.500	0.349	4.298*	4
	$\overline{X}$, Altitude		1.632			
Group $\overline{X}$			1.152			

* = P < 0.05

** = P < 0.01

*** = P < 0.001

\# = Days from arrival at Crooked Creek

TABLE XIV. CO_2 sensitivities as determined from the slopes of least squares regression lines, fitted to data from individual CO_2 response experiments, subjects sedated by scopolamine. $\hat{S}_b$ = standard error of regression coefficient, modified for small samples.

AT SEA LEVEL

Subject	Date	Time#	CO_2 Sensitivity, $\dot{V}_A/P_{A_{CO_2}}$ (liters/min/mm Hg)	$\hat{S}_b$	Goodness of Fit, Test for linearity t	d.f.
Wags	8 Aug 56	t-11	0.528	0.079	6.665*	2
	18 Sept 56	t'+12	0.868	0.074	11.651**	2
	$\bar{X}$		0.698			
Rusty	30 July 56	t-20	0.157	0.090	1.734	2
	18 Sept 56	t'+12	0.306	0.073	4.172	2
	$\bar{X}$		0.232			
Lunk	8 Aug 56	t-11	0.537	0.030	17.709**	2
Group $\bar{X}$, (3)			0.489			
Group $\bar{X}$, (2)			0.465			

AT ALTITUDE

Wags	1 Sept 56	t+13	0.641	0.049	13.110***	4
	2 Sept 56	t+14	0.995	0.029	33.833***	4
	$\bar{X}$		0.818			
Rusty	1 Sept 56	t+13	0.626	0.085	7.355**	4
	2 Sept 56	t+14	0.612	0.422	1.449	2
	$\bar{X}$		0.619			
Lunk	(No experiments done at altitude.)					
Group $\bar{X}$, (2)			0.718			

* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$
# t = Arrival day at Crooked Creek Laboratory

TABLE XV. CO_2 sensitivities as determined from the slopes of least squares regression lines, fitted to data from individual CO_2 response experiments, subjects sedated by scopolamine plus thiopental. $\hat{S}_b$ = standard error of the regression coefficient, modified for small samples.

AT SEA LEVEL

Subject	Date	Time#	CO_2 Sensitivity, $\dot{V}_A/P_{A_{CO_2}}$ (liters/min/mm Hg)	$\hat{S}_b$	Goodness of Fit, Test for Linearity t	d.f.
Wags	11 Aug 56	t-8	0.580	0.091	6.355**	4
	12 Aug 56	t-7	0.501	0.048	10.523***	4
	$\bar{X}$		0.541			
Rusty	9 Aug 56	t-10	0.427	0.046	9.209***	6
	13 Aug 56	t-6	0.395	0.027	14.622***	6
	$\bar{X}$		0.411			
Lunk	9 Aug 56	t-10	0.484	0.033	14.454***	6
	12 Aug 56	t-7	0.318	0.042	7.505***	6
	$\bar{X}$		0.401			
Group $\bar{X}$, (3)			0.451			
Group $\bar{X}$, (2)			0.476			

AT ALTITUDE

Wags	4 Sept 56	t+16	0.293	0.157	1.869	2
	5 Sept 56	t+17	0.322	0.161	1.997	2
	$\bar{X}$		0.307			
Rusty	4 Sept 56	t+16	0.492	0.046	10.603***	6
	5 Sept 56	t+17	0.236	0.062	3.799**	8
	$\bar{X}$		0.364			
Lunk	(No experiments done at altitude.)					
Group $\bar{X}$, (2)			0.335			

* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$
# t = Day of Arrival at Crooked Creek Laboratory

of 8.0 liters/min. The arbitrary value of 8.0 liters/min was chosen as a reference value because it was near the middle of most of the CO_2 response curves. This comparison of sea level and altitude responses to CO_2 takes into account the change in CO_2 threshold as well as the change in CO_2 sensitivity. Fig. 22 shows that the altitude shift in the CO_2 response curve was essentially complete when the first experiments were performed, a few hours following arrival at the Barcroft Laboratory. Tables XVI - XIX present the tabular data which are summarized in Fig. 22 and 23.

The group average data on the right side of Fig. 22 again suggest the possibility that some residual "altitude shift" was still present during the 4th through 11th day at sea level following the altitude sojourn.

Fig. 23 summarizes the data on alveolar P_{CO_2} at a fixed value of alveolar ventilation and shows that the chief effect of the sedation was to increase the variation in the results, both at sea level and at altitude. The two drug combination of scopolamine plus thiopental evidently caused more variability than did scopolamine alone since the standard error of the mean (2 dogs) is four times greater with the two drug combination than with scopolamine alone, both at sea level and at altitude. Inspection shows that alveolar P_{CO_2} at an alveolar ventilation of 8.0 liters/min in awake dogs did not differ significantly from that in sedated dogs, either at sea level or at

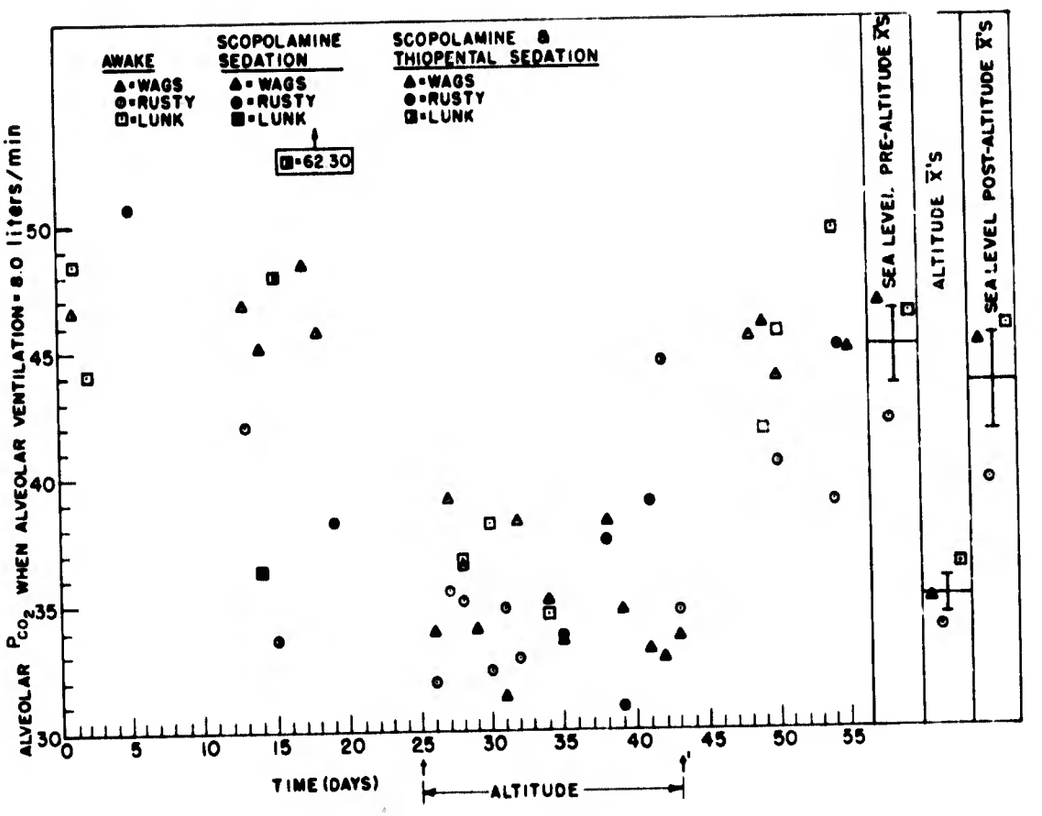

Fig. 22. Alveolar P_{CO_2} at an alveolar ventilation rate of eight liters per minute (BTPS), as a function of time. Alveolar P_{CO_2} determined from the least squares equation for each experiment.

TABLE XVI. Values of alveolar P_{CO_2} when alveolar ventilation equals 8.0 liters/min, as determined from the least squares equations for individual CO_2 response experiments, at sea level, subjects awake.

Subject	Date	Time (days)*	$P_{A_{CO_2}}$ (mm Hg)
Wags	26 July 56	t-24	46.57
	7 Aug 56	t-12	46.75
	11 Sept 56	t'+5	45.50
	12 Sept 56	t'+6	45.93
	13 Sept 56	t'+7	43.79
	$\overline{X}$, Sea Level		45.71
Rusty	7 Aug 56	t-12	42.02
	13 Sept 56	t'+7	40.36
	17 Sept 56	t'+11	38.94
	$\overline{X}$, Sea Level		40.44
Lunk	26 July 56	t-24	48.38
	27 July 56	t-23	44.06
	12 Sept 56	t'+6	41.75
	13 Sept 56	t'+7	45.60
	17 Sept 56	t'+11	49.65
	$\overline{X}$, Sea Level		45.89
$\overline{X}$, Group			44.01

* t = Day of arrival at Crooked Creek Laboratory
t' = Last day at altitude

TABLE XVII. Values of alveolar P_{CO_2} when alveolar ventilation equals 8.0 liters/min, as determined from the least squares equations for individual CO_2 response experiments, at altitude, subjects awake.

Subject	Date	Time (days)*	$P_{A_{CO_2}}$ (mm Hg)
Wags	20 Aug 56	0.81	33.88
	21 Aug 56	1.85	39.14
	22 Aug 56	3.00	36.49
	23 Aug 56	3.78	33.92
	25 Aug 56	5.64	31.26
	26 Aug 56	6.68	38.16
	28 Aug 56	8.85	35.14
	29 Aug 56	9.75	33.47
	6 Sept 56	17.53	33.73
	$\overline{X}$, Altitude		35.02
Rusty	20 Aug 56	0.98	31.93
	21 Aug 56	2.02	35.54
	22 Aug 56	3.07	35.05
	24 Aug 56	4.79	32.30
	25 Aug 56	5.77	34.82
	26 Aug 56	6.81	32.84
	29 Aug 56	9.81	33.71
	6 Sept 56	17.65	34.73
	$\overline{X}$, Altitude		33.86
Lunk	23 Aug 56	3.13	36.63
	24 Aug 56	5.06	38.12
	28 Aug 56	8.68	34.47
	$\overline{X}$, Altitude		36.41
$\overline{X}$, Group (3)			35.10
$\overline{X}$, Group (2)			34.44

* = From arrival at Crooked Creek

TABLE XVIII. Values of alveolar P_{CO_2} when alveolar ventilation equals 8.0 liters/min, as determined from the least squares equations for individual CO_2 response experiments, subjects sedated by scopolamine.

Subject	Date	Time*	$P_{A_{CO_2}}$ (mm Hg)
AT SEA LEVEL			
Wags	8 Aug 56	t-11	45.14
	18 Sept 56	t'+12	44.93
	$\bar{X}$		45.04
Rusty	30 July 56	t-20	50.70
	18 Sept 56	t'+12	44.99
	$\bar{X}$		47.84
Lunk	8 Aug 56	t-11	36.23
Group $\bar{X}$, (3)			43.04
Group $\bar{X}$, (2)			46.44
AT ALTITUDE			
Wags	1 Sept 56	t+13	38.23
	2 Sept 56	t+14	34.67
	$\bar{X}$		36.45
Rusty	1 Sept 56	t+13	37.41
	2 Sept 56	t+14	30.90
	$\bar{X}$		34.16
Lunk	(No experiments done at altitude.)		
Group $\bar{X}$, (2)			35.31

* t = Arrival day at Crooked Creek Laboratory
t' = Last day at altitude

TABLE XIX. Values of alveolar P_{CO_2} when alveolar ventilation equals 8.0 liters/min, as determined from the least squares equations for individual CO_2 response experiments, subjects sedated by scopolamine plus thiopental.

Subject	Date	Time (days)*	$P_{A_{CO_2}}$ (mm Hg)
AT SEA LEVEL			
Wags	11 Aug 56	t-8	48.35
	12 Aug 56	t-7	45.73
	$\bar{X}$		47.04
Rusty	9 Aug 56	t-10	33.57
	13 Aug 56	t-6	38.22
	$\bar{X}$		35.89
Lunk	9 Aug 56	t-10	47.91
	12 Aug 56	t-7	62.30
	$\bar{X}$		55.10
Group $\bar{X}$, (3)			46.01
Group $\bar{X}$, (2)			41.47
AT ALTITUDE			
Wags	4 Sept 56	t+16	33.22
	5 Sept 56	t+17	32.81
	$\bar{X}$		33.02
Rusty	4 Sept 56	t+16	38.98
	5 Sept 56	t+17	44.46
	$\bar{X}$		41.72
Lunk	(No experiments done at altitude.)		
Group $\bar{X}$, (2)			37.37

* t = Arrival day at Crooked Creek Laboratory

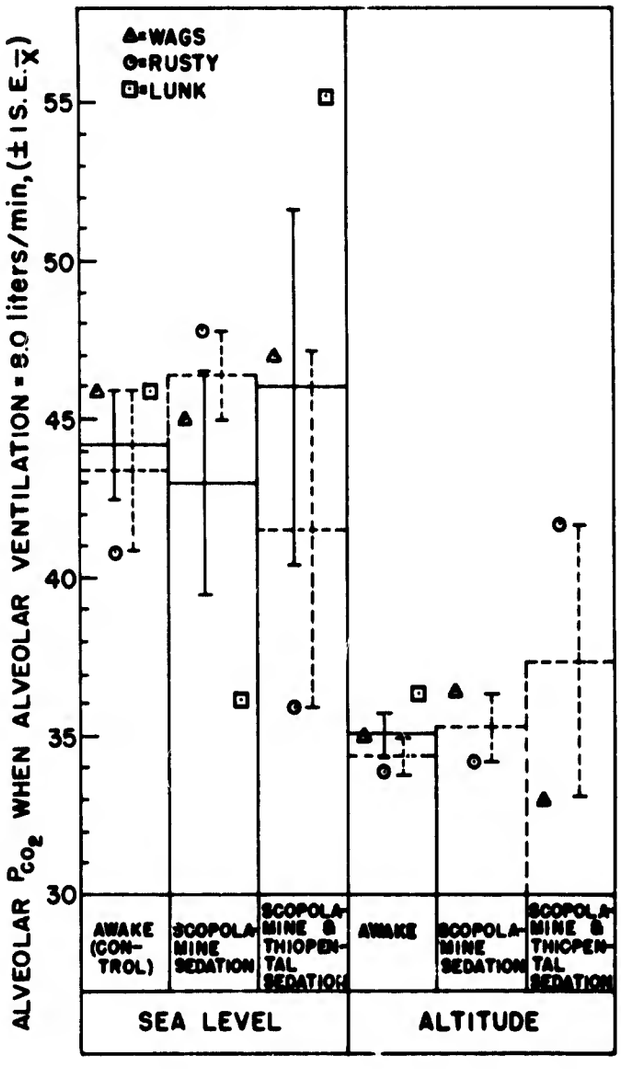

Fig. 23. Alveolar P_{CO_2} at a constant alveolar ventilation of 8.0 liters/min, as determined from the least squares equations for each experiment. Uncertainty shown as plus or minus one standard error of the mean. Solid lines show data for a group of three subjects, dotted lines show values for a group of two subjects.

altitude. The difference between sea level and altitude values in awake dogs was highly significant by t-test ($P < 0.01$).

G. Hypoxic Drive Component of Resting Alveolar Ventilation

Tests were made for the presence of an hypoxic drive component in the resting alveolar ventilation at altitude of awake dogs during the first ten days. Resting ventilation was measured with the animal breathing room air (control) followed by measurements made while the subject breathed a gas mixture that provided an alveolar P_{O_2} of 110 mm Hg, without any increase in inspired P_{CO_2}. The pooled data from these experiments are shown in Fig. 24. The same tests were repeated during days eleven through eighteen at altitude with the animals under sedation. Data from the last two minutes on each gas were used for this Fig., which shows that there was no hypoxic component in resting alveolar ventilation in awake dogs at altitude. Resting alveolar ventilation did not vary significantly from control when the subjects breathed room air while under sedation by scopolamine alone or by scopolamine plus thiopental. However, in subjects under scopolamine plus thiopental sedation, resting alveolar ventilation was reduced to 60% of control value when alveolar P_{O_2} was raised to sea level value.

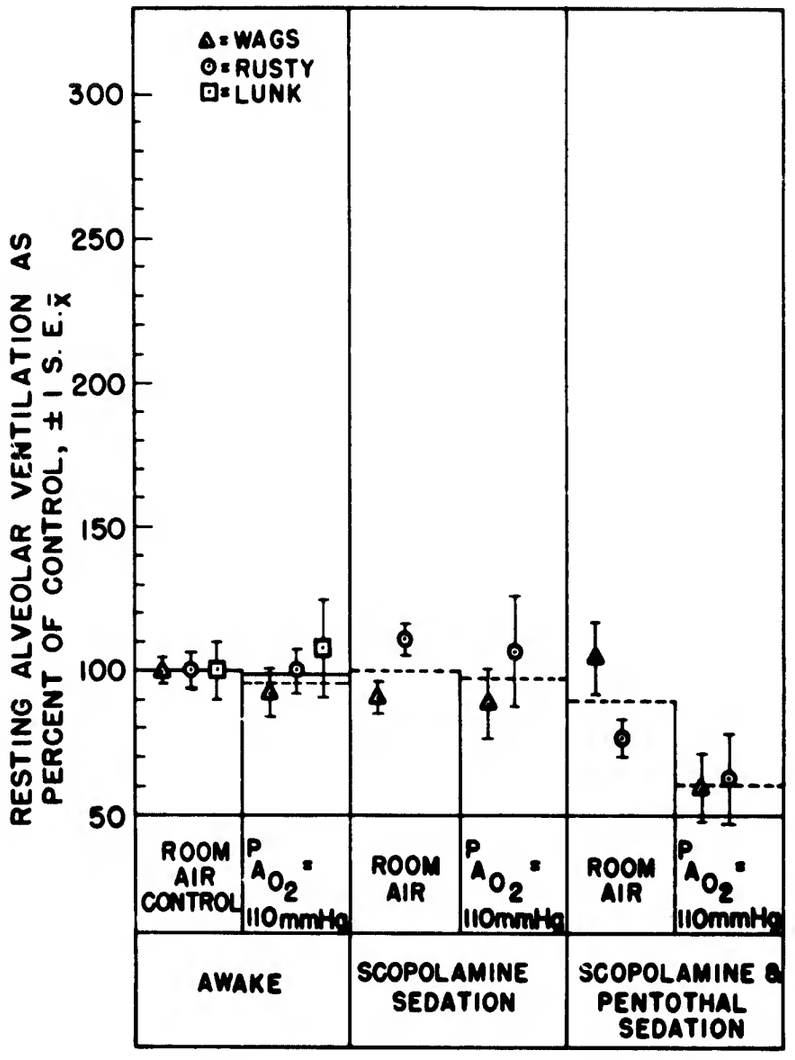

Fig. 24. Hypoxic drive component of resting alveolar ventilation at altitude. Resting alveolar ventilation, expressed as per cent of control, is shown with subject breathing either room air or an enriched O_2 mixture (without added CO_2) under the following conditions: awake; under scopolamine sedation; under scopolamine plus thiopental sedation. Control value is alveolar ventilation while awake dog breathes room air at altitude. Bar graph shows group averages with individual values superimposed.

Fig. 25 and 26 show alveolar ventilation on a minute by minute basis. It is apparent that there is a fall in alveolar ventilation at the end of the first minute after alveolar P_{O_2} is raised from 55 to 110 mm Hg, in awake dogs and in dogs sedated by scopolamine. In dogs sedated by scopolamine plus thiopental there is also a transient fall in alveolar ventilation, but the low point occurs at the end of the second minute. These transient changes are of course obscured when only the "steady state" values recorded during minutes four and five are considered.

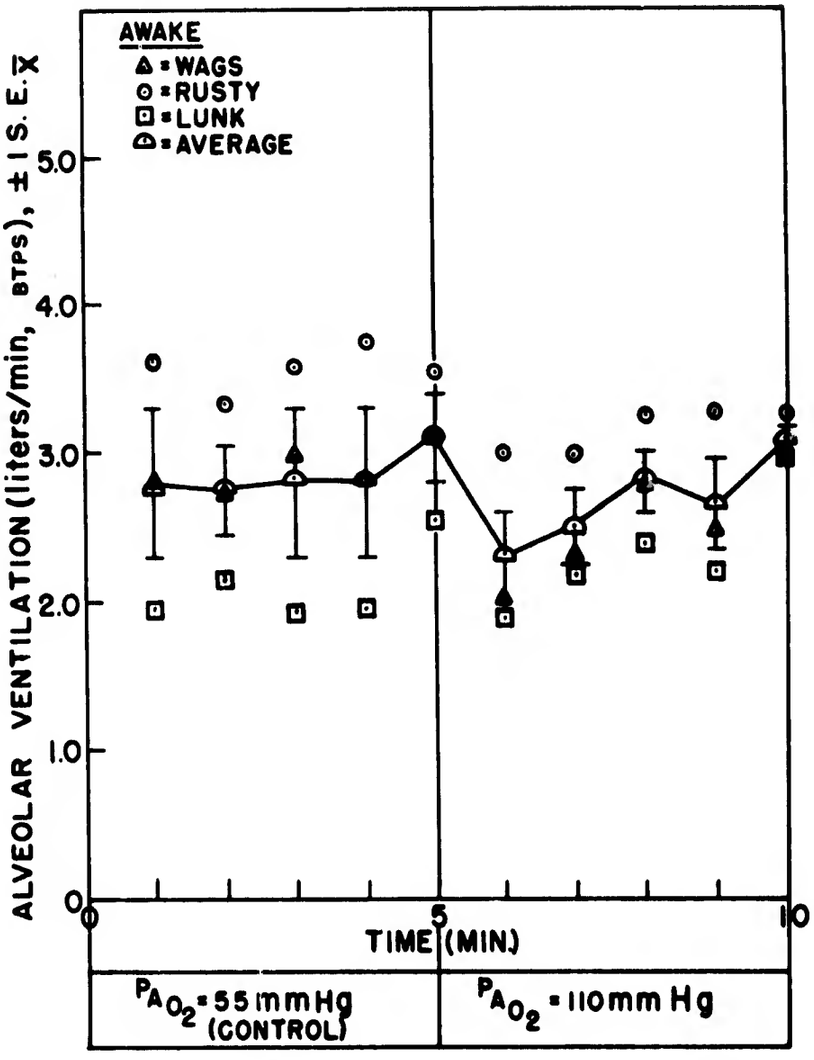

Fig. 25. Resting alveolar ventilation as a function of time, following acute elevation of alveolar P_{O_2} from chronic hypoxic to normal sea level value. Subjects awake.

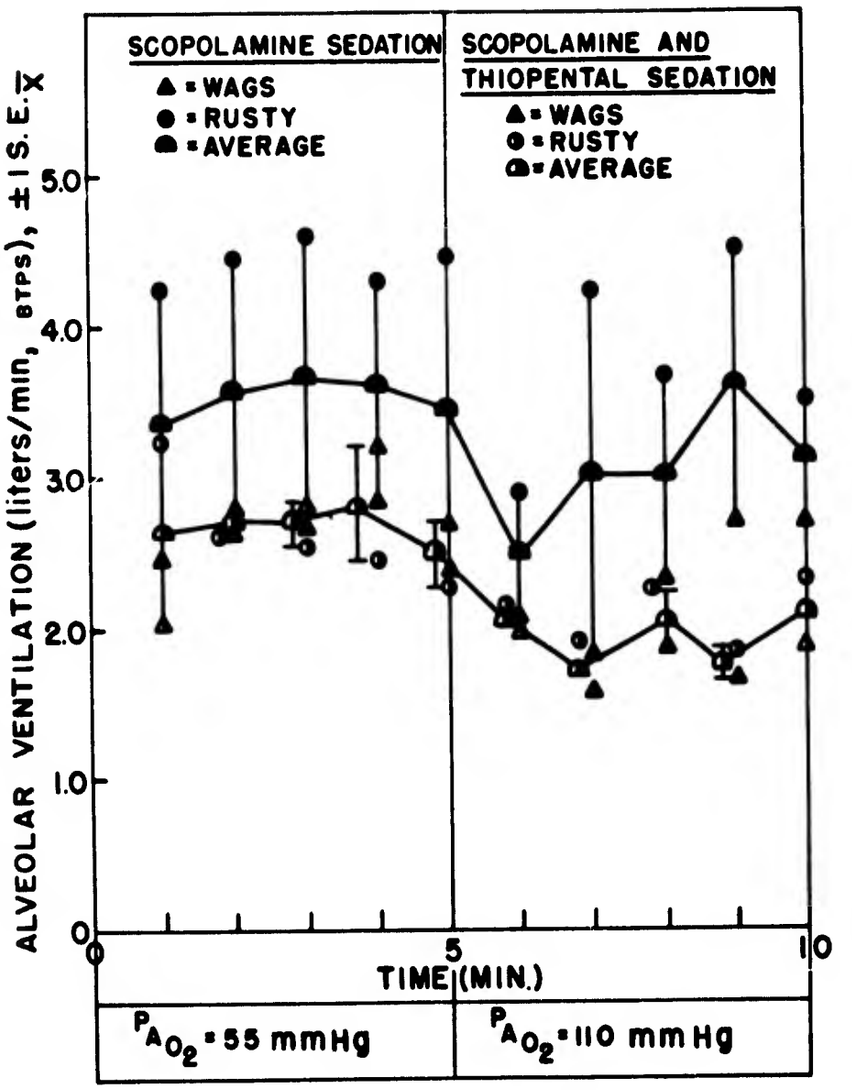

Fig. 26. Resting alveolar ventilation as a function of time, following acute elevation of alveolar P_{O_2} from chronic hypoxic to normal sea level value. Subjects under preanesthetic sedation by scopolamine and by scopolamine-thiopental.

IV. Discussion

A. Typical Experiments

Initially, respiratory minute volume was used as the index of response to increased inspired P_{CO_2} gas mixtures. However, one of the dogs showed a marked tendency to pant while breathing room air, even at room temperatures of about 20 C, which resulted in very high resting minute volumes. Therefore alveolar ventilation rate was used as the index of response rather than minute volume of expired gas, since alveolar ventilation rate takes into account individual differences in dead space and respiratory frequency, and presumably represents that portion of the respiratory minute volume that is effective in gas exchange at the alveolar membrane. Alveolar ventilation was calculated as follows:

$$\dot{V}_A = \dot{V}_E - f(V_D)$$

where:

$\dot{V}_A$ = alveolar ventilation rate, liters/min, BTPS

$\dot{V}_E$ = volume of air expired/min, liters, BTPS

f = respiratory frequency, breaths/min

V_D = volume of dead space, liters, BTPS, assuming that respiratory dead space remained constant. This assumption appears to be reasonable for the anatomical dead space (7, 9, 11, 50, 59, 123, 141), although the evidence shows that virtual or physiological dead space increases linearly with increasing tidal volumes above

the resting level (34, 59, 141, 165), particularly in heavy exercise. Respiratory dead space data in Table III show close agreement in the results for O_2 and CO_2 dead space. The absolute values are in reasonably good agreement with the results of Severinghaus and Stupfel who measured respiratory dead space in anesthetized dogs (149).

It should be emphasized that in the present experiments the inspired O_2 concentrations were increased at altitude to provide alveolar P_{O_2}'s slightly above normal sea level values. It was assumed that this procedure would minimize the reflex hypoxic ventilatory drive from peripheral chemoreceptors and that the breathing responses to increased P_{CO_2} would represent primarily the respiratory center responses (143). The positive interaction between hypoxia and hypercapnia in causing increased pulmonary ventilation is well known (3, 69, 70, 94, 95, 105, 116, 118). Evidence concerning the reflex stimulation of respiration by CO_2 acting on the chemoreceptors during normoxia seems conflicting (8, 38, 47, 51, 52, 71, 93, 144). While it would seem to be axiomatic that the chemoreceptors are stimulated by increased H^+ concentration of arterial blood (87), and thus would be stimulated by increased inspired P_{CO_2} through its effect in lowering arterial blood pH, Bjurstedt (12) concluded that during hypoxic hyperventilation in anesthetized dogs, increased P_{CO_2} depressed peripheral chemoreceptor activity and, conversely,

increased alkalinity of arterial blood potentiated the chemoreceptor drive. There is also some evidence that chemoreceptor reflexes play a more dominant role in the control of respiration during anesthesia than in the normal intact subject (143). Evidently there have not been any experiments concerned with the relative importance of centrogenic and chemoreflex control of breathing with subjects under preanesthetic sedation.

It was of interest to know whether the breathing responses measured in the present studies represented steady state equilibria. Simple statistical analyses showed that the alveolar P_{CO_2} during minutes four and five was 97.8%, ±1.2% (standard error) of the value during minutes nine and ten at sea level, alveolar ventilation during minutes four and five was 98.0%, ±3.2% of the value during minutes nine and ten at sea level, and the corresponding values at altitude were 98.9%, ±7.4% for alveolar P_{CO_2} and 96.7%, ±3.6% for alveolar ventilation. The subjects breathed 6% CO_2 at sea level and 8% CO_2 at altitude for 10 min. Although these data do not prove that steady state responses were obtained, they do show that the same relative responses were obtained at sea level and at altitude. Limited analyses of respiratory gas exchange (R) values showed that plateau values were achieved by the fourth minute on each gas mixture. Padget (121) concluded that a steady state response to 6% CO_2 was reached after 10 min. White, et al. (163) concluded that steady state

responses were obtained after about 10 min of breathing 6% CO_2 and that there were no differences between the responses to CO_2 in air or CO_2 in O_2. Grodins, et al. (60) questioned these findings and indicated that at least 30 min were required for steady state responses in breathing 5-6% CO_2 in air. Grodins, et al. cited previous work by Adolph, Nance, and Schilling (1), which showed that steady state responses were not obtained after 30 min of breathing 4.5% CO_2 in air. Thirty minutes or more was also required for a return to pre-test equilibrium values. Nielsen (114) used 45 min periods with subjects breathing 7.5% CO_2. All the foregoing results were obtained on human subjects. Although the response curves obtained in the present studies did show the high frequency components mentioned by Grodins, et al. (60), my findings seem to be more consistent with the results of Padget (121) and of White, et al. (163), and less in agreement with the results of Grodins, et al. (60), Adolph, Nance, and Schilling (1), and Nielsen (114). However, all the aforementioned experiments used acute, single-step exposures to high inspired P_{CO_2} whereas my experiments used progressive step increases, so it is difficult to make comparisons. In the present studies the same per cent response was achieved in both sea level and altitude experiments; therefore, the results were not influenced by the question of whether a true steady state equilibrium was achieved.

B. Respiratory Parameters in Dogs Breathing Room Air at Sea Level and at Altitude

Jourdanet was one of the first investigators to make systematic measurements of respiratory parameters in high altitude dwellers (75). Many early workers made observations on the rate, depth, and respiratory minute volume in sojourners at high altitudes (45, 53, 112, 158, 167). One of the principal findings was that the amount of change varied a great deal with different individuals.

The limitations inherently associated with the measurement of alveolar P_{O_2} and P_{CO_2} in awake dogs breathing room air have already been discussed. Despite my best efforts to solve the alveolar gas sampling problem, there may have been some dilution by dead space gas, particularly when the subjects were breathing room air. Average tidal volumes were 243 ml at sea level and 222 ml at altitude (BTPS), with average respiratory dead space, as determined by Bohr formula, equal to 72 ml (BTPS).

Rahn has suggested that the best criterion for judging the validity of "alveolar" samples is to compare the alveolar P_{CO_2} with simultaneously determined arterial P_{CO_2} (129). This criterion was not applied during this investigation. However, Suskind (155) made simultaneous measurements of alveolar and arterial P_{CO_2} and alveolar P_{O_2} in dogs trained to wear masks and lie supine, breathing air. From nine experiments on awake animals, she reported simultaneous arterial P_{CO_2} and alveolar P_{CO_2} of 30.4 and 29.3 mm Hg,

respectively, with corresponding alveolar P_{O_2} of 108.4 mm Hg. Suskind used a Rahn-Otis sampler. The agreement between alveolar and arterial P_{CO_2} seems reasonably good. It would appear that the special sampler used in my studies was at least as good as a conventional Rahn-Otis sampler. My data on absolute values of average alveolar P_{CO_2} and P_{O_2} in dogs breathing room air at sea level are 2 mm Hg lower for P_{CO_2} and 1 mm Hg higher for P_{O_2} as compared to the results of Suskind. The differences could be accounted for by assuming that some dilution of the alveolar samples by dead space gas did in fact occur during the present studies. Rahn showed that in man the erect position is associated with an apparent hyperventilation which lowers the resting alveolar P_{CO_2} by about 4 mm Hg as compared to the supine position (129). Suskind's dogs were trained to lie supine, in contrast to the normal standing position of the animals in the present studies. The difference in observed alveolar P_{CO_2} in the resting dog as reported by Suskind, in comparison with results from the present studies, is consistent with the assumption that a shift in alveolar P_{CO_2} also occurs in the dog with the erect posture.

Determinations of R values by Rahn-Fenn isopleth (131), using the average values for alveolar P_{O_2} and P_{CO_2} shown in Tables VII - X, showed that all values were low. At sea level, values for three subjects were: 0.78, 0.65, and 0.68; at altitude: 0.57, 0.52, and 0.58. Using the group average values from Tables IX and X the

corresponding R values were 0.68 at sea level and 0.55 at altitude. Comparisons were made, on data from individual experiments, on a minute by minute basis to see whether the reasons for the low R values could be determined. No explanation was found but it was apparent that the R values were consistently low, that they did not result from previous hyperventilation, and that surgical anesthesia had no effect. No explanation was found for the altitude R values being lower than sea level. Evidently some systematic error occurred throughout the experiments with the result that either O_2 or CO_2 values were falsely low. The possibility that the apparent systematic error may have affected the results cannot be denied.

However, most of the conclusions are based on changes rather than on absolute values. The fact that the altitude R values were consistently lower than sea level R values is a troublesome aspect at best. A decrease in R values at altitudes of about 10,000 ft in humans has been reported by Hasselbalch and Lindhard (63), Hetherington, Luft, and Ivy (67), and Rahn and Otis (132). However, these reported decreases averaged about 0.05 units whereas the altitude values are about 0.10 units lower in the present instance.

With reference to Fig. 13, the reductions in alveolar P_{O_2} and P_{CO_2} at altitude agree closely with predicted values from the nomogram of Peters and Van Slyke (126) for man acclimatized to an altitude of 12,500 ft: predicted alveolar P_{O_2} = 57% of sea level

value, observed alveolar P_{O_2} = 53% of sea level value; predicted alveolar P_{CO_2} = 71% of sea level value, observed alveolar P_{CO_2} = 71% of sea level value. The nomogram of Peters and Van Slyke also shows a predicted increase in alveolar ventilation in acclimatized man at 12,500 ft to 140% of sea level value. The observed mean value for alveolar ventilation in dogs was 100% of sea level value. However, measurements made on the twelfth day at altitude (see Fig. 14) showed mean alveolar ventilation to be 145% of sea level control. This apparent anomaly requires some comment. As Kellogg clearly pointed out (77), the reduction in resting alveolar P_{CO_2} that characterizes altitude acclimatization can only be brought about through an increase in alveolar ventilation, which initially results in CO_2 being eliminated from the lungs at a rate which exceeds its rate of production in the body. The classical hypothesis was that the resulting respiratory alkalosis is compensated through increased renal excretion of buffer base and a new equilibrium is established in which blood pH returns to normal with a lowered plasma CO_2 buffering capacity, so that in the altitude acclimatized state the increased alveolar ventilation is maintained primarily by the respiratory center under the stimulus of CO_2 (28, 29, 154, 158). It would appear that my findings of reduced alveolar P_{CO_2} with no change in alveolar ventilation probably indicate erroneous alveolar ventilation data. The standard error of this measurement was about 8% both at sea

level and at altitude. Dead space values enter into the calculation of alveolar ventilation, and the anatomical dead space values were based on one determination for each subject. It should be noted that the determination of resting alveolar ventilation at altitude (Fig. 13) is based on measurements made during the first ten days at altitude, which obscures any progressive change with elapsed time which may have occurred.

Although some workers have reported no increase in resting alveolar ventilation in humans acclimatized to moderate altitude (132), it is generally assumed that an increase in ventilation while breathing air is one of the characteristic changes in man as a result of acclimatization to altitude and there is much supporting evidence for this viewpoint (2, 28, 39, 120). However, Chiodi (26) calculated alveolar ventilation per m^2 of body surface using physiological rather than anatomical dead space and found that, in permanent residents, the increase in resting alveolar ventilation was statistically significant only at 14,000 ft altitude. Hurtado, et al. reported similar results (74). Chiodi also studied respiratory minute volume, alveolar ventilation and alveolar P_{CO_2} in 37 long-term residents at 4,000 ft elevation. He found that the increases in respiratory minute volume and alveolar ventilation were not statistically significant, but the decrease in alveolar P_{CO_2} was, presumably showing that alveolar ventilation tends to return to normal levels in the long-term resident

at moderate altitudes, following a transient period of increased alveolar ventilation in which a lower equilibrium value of alveolar P_{CO_2} is established, see also (120).

C. Altitude Acclimatization

The physiology of organisms acutely or chronically exposed to the effects of mountain altitudes has been studied since the year 1660, when Robert Boyle published his classic studies on the rarefaction of air (16). However, there still is no general agreement on a precise definition of what constitutes altitude acclimatization. Monge has studied the records of the early Spanish colonists in the Andes and has concluded that in both man and domestic animals fertility is drastically reduced during several generations (108, 109). Reduced fertility is reported to occur at the relatively moderate altitude of 10,000 ft. Extensive studies of reproduction in rodents have been carried out at the White Mountain Laboratories. Krum, working at the Barcroft Laboratory, found that second generation female rats of the Long-Evans strain produced normal sized litters, but only 31% of the offspring survived to the age of six months (84); changes in lactation and in the composition of the mother's milk were found. Mice of the A strain were reared at 10,150 feet but could not

be reared at all at an altitude of 12,470 feet (119). These examples are cited to illustrate the point that altitude acclimatization includes a broad complex of changes in physiological mechanisms and that altitude acclimatization may mean different things to different investigators.

Campbell proposed a number of criteria for judging whether or not subjects could be considered altitude acclimatized (18-21). Among these criteria were: a) normal fertility, b) maintenance of normal body weight in mature subjects and normal growth rates in immature subjects, c) no loss of appetite, d) a feeling of general well-being. In the present studies the period at altitude was too short to permit any evaluation of fertility. The data from the present 18 day sojourn showed no change in body weights. Changes in body weight might have become evident if the altitude exposure had been continued for a longer period. No changes were observed in appetites of the subjects. Part of the normal routine was to permit each subject a 15-20 min period for unrestrained outdoor exercise following each experiment before he was returned to his cage. The dogs' behavior during these periods showed no indication of any adverse effects of altitude. In fact, all observations of the subjects' behavior indicated a feeling of general well-being in all three dogs during the entire altitude sojourn. It is well established that the dog is very resistant to mountain sickness (112).

D. Carbon Dioxide Sensitivity Tests on Awake Dogs

It has been known for many years that CO_2 added to the inspired gas causes an increase in the volume of air breathed and that a greater increase occurs in subjects at mountain altitudes than at sea level, see Hasselbalch and Lindhard, 1911, (63). Although this phenomenon has been studied extensively, various investigators have not been in complete agreement as to the meaning of their results.

Among those who have studied the augmented ventilatory response to added inspired CO_2 in the intact human during altitude acclimatization, Rahn and his co-workers (130, 134), Houston and Riley (72, 139), Milledge (105) and others have interpreted their results to mean that the increased ventilation is largely a reflection of the lowered CO_2 buffering capacity of the plasma. Others (116) have maintained that the increased response cannot be entirely explained on this basis and that there is a separate independent effect of altitude which somehow acts to increase pulmonary ventilation at altitude, presumably by increasing the responsiveness of the respiratory centers to CO_2. Nielsen (114) produced a lowered alkaline reserve by administering ammonium chloride at sea level so that his subjects then showed a reduced CO_2 buffering capacity comparable to that of the altitude acclimatized man. Added inspired CO_2 produced comparable shifts in "threshold" but not in "sensitivity index" in those subjects, and he therefore maintains that the lowered bicarbonate

reserve of the plasma cannot satisfactorily explain the increased ventilatory response to CO_2 at altitude.

Others who have studied various aspects of this phenomenon during acute (41, 62) and chronic (80) exposures to low O_2 tension have concluded that the respiratory center may show an increased responsiveness to added CO_2 but that the lowered CO_2 buffering capacity of the plasma is probably the most important part of the mechanism. Rahn, et al. (134), Pace (120), Kellogg (78, 79), Rahn and Otis (132), Kellogg, Vaughan and Badger (82), and Kellogg, Reed and Todd (81) found a shift to the left and increases in slope of the CO_2 response curve during altitude acclimatization at altitudes of 9,500 - 14,000 ft. Dejours, et al. (39) concluded that oxygen lack is the primary factor in respiratory changes in man during altitude acclimatization, and that any change in respiratory center sensitivity to CO_2 is unlikely. Although questions as to the relative importance of centrogenic and chemoreflex mechanisms in respiratory control are related to the present problem, detailed discussions of these questions are beyond the scope of the present investigation. The experiments did not unequivocally rule out the possibility that chemoreflex stimulation was a factor.

Gillfillan, et al. (55) measured the carbon dioxide response curves of awake dogs at altitude and at sea level during the course of their experiments on peripheral chemoreceptor mechanisms. They

concluded that the responses of the dog are similar to those of man, although detailed analyses were evidently not done.

Dill and Zamcheck measured the respiratory responses of two human subjects to increased P_{CO_2} during exposure to acute hypoxia (41). Although they did not draw any conclusions as to the CO_2 sensitivity of the respiratory centers, it is interesting to note that they obtained completely different results from their two subjects, emphasizing the importance of individual differences.

Recent reports by Chiodi state that respiratory response to inhaled CO_2 was greater in newcomers and less in residents at high altitude, than was found in normal subjects at sea level, (24, 25, 26). It is difficult to reconcile this finding with current hypotheses concerning the mechanisms of respiratory acclimatization, although Rahn, et al. (134) postulate that the decreased respiratory response of permanent residents could be due to the greater CO_2 buffering capacity of the permanent residents because of their known increase in circulating hemoglobin.

There appears to be general agreement that the CO_2 response curve of man is shifted to the left during altitude acclimatization. However, few attempts have been made to quantitatively define the simultaneous increase in the slope of the CO_2 response curve. For example, Rahn, et al. (134), in their 1953 Mt. Evans study, calculated that the slopes of the CO_2 response curves were 0.40, 0.485, and

0.521, respectively, for elevations of 550, 9,500 and 14,100 ft, but they evidently made no attempt to determine whether these changes in slope were significant, and their "curves" appear to be a series of parallel straight lines. They plotted alveolar ventilation ratio against alveolar P_{CO_2} to determine whether the observed phenomena fitted Gray's general equation for alveolar ventilation (58): $V_aR = K(pCO_2) - 15$; and interpreted their results in terms of counter-clockwise rotation of the response curves around a fixed negative value of alveolar ventilation ratio. This interpretation requires that the response lines be extrapolated far beyond the points that are subject to experimental verification, a procedure that is inherently open to question (35). Furthermore, one might ask what possible physiological meaning can be attributed to an alveolar ventilation ratio 15 times less than zero?

E. Carbon Dioxide Sensitivity: Defined as the Slope of the Least Squares Straight Line Relating Alveolar Ventilation to Alveolar pCO_2

Lindhard studied the effects of increased inspired P_{CO_2} on pulmonary ventilation during normoxia, hypoxia, and hyperoxia in 1911 (94). He defined "ventilation quotient" as alveolar ventilation per minute/mm Hg alveolar P_{CO_2}, and defined "excitability" of the

respiratory center to CO_2 as ventilation quotient, expressed as per cent of control, but evidently did not differentiate "CO_2 threshold" from "CO_2 sensitivity".

Gray suggested that CO_2 sensitivity be defined as the slope of the stimulus-response curve, and that CO_2 threshold be defined as the value of alveolar P_{CO_2} beyond which increases in alveolar P_{CO_2} are accompanied by increases in alveolar ventilation (58).

Kellogg, et al. (80) concluded that an increase in the slope of the CO_2 response curve indicated a change in the motor or central part of the respiratory control mechanism, possibly due to generalized increase of respiratory center activity or facilitation of impulses to the respiratory muscles. Conversely, the horizontal shift of the curve to the left was interpreted as being caused by a change in the sensory side of the mechanism. In their 1957 report on human sojourners at 12,500 ft elevation, Kellogg, et al. (80) concluded that ". . . the present observations show little or no change in sensitivity during acclimatization to chronic hypoxia." Subsequently, Kellogg and his associates have repeatedly demonstrated an increase in the slope of the CO_2 response curve of human sojourners (78, 79) mostly at elevations of 14,200 ft, and similar results have been reported by others (105), also on humans and at 19,000 ft elevation.

The present studies were undertaken partly for the purpose of accomplishing critical evaluation of the question of whether the

slope of the CO_2 response curve changes significantly in dogs during acclimatization to an altitude of 12,500 ft. In order to define changes in slopes quantitatively, the data from each experiment were fitted to straight lines by the method of least squares.

Figs. 20 and 21 show individual test and average values of CO_2 sensitivity, respectively. Although CO_2 sensitivity was increased in awake dogs at altitude to 160% of the sea level value (group average) and the other observed changes appeared to be consistent, none of the observed changes was statistically significant. It is true that the group of subjects was small (three dogs for the awake group, two dogs for the altitude sedated groups) and that statistical significance is difficult to establish with such a small group. However, it is also true that the shift of the curve to the left (see Fig. 23) in the same subjects was statistically significant ($P < 0.01$), and that the most obvious effect of sedation in this measurement was to increase the variability of the results. The inescapable conclusion is that in dogs, at an altitude of 12,500 ft, the sensory side of the respiratory control mechanism plays the dominant role. The results of Rahn, et al. (134), Milledge (105), Pace (120), and Kellogg and associates (78, 79, 81, 82), provide strong evidence that the slope of the CO_2 response curve does increase progressively in human sojourners at altitudes above 12,500 ft.

It appears that the increase of the slope of the CO_2 response curve is of marginal importance at 12,500 ft, and that the slope

increases progressively with increasing altitudes. Since the principal environmental factor encountered in higher elevations is progressively greater hypoxia, one possible mechanism might be that the heightened activity of the peripheral chemoreceptors causes a long-lasting elevation of respiratory center activity and/or facilitation of impulses to the respiratory muscles, possibly through a mechanism of reverberating internuncial neurons analagous to that of the reticular activating system of the brain stem. This effect might persist for some hours following acute interruption of the hypoxic stimulus, so that "increased CO_2 sensitivity" would be observed when CO_2 enriched gas mixtures (which provide sea level alveolar P_{O_2}'s) are administered acutely. According to this proposed scheme, the increased CO_2 sensitivity at altitudes above 12,500 ft is only a special case of the well known positive interaction between hypoxemia and hypercapnia. Although of an anecdotal nature, the frequently encountered difficulty in going to sleep at altitude suggests the possibility that some such mechanism may be present, perhaps even involving the activating mechanisms of the reticular formation of the brain stem itself.

Data from the present studies showed that the CO_2 response curves were concave upwards, with the upward concavity being more pronounced at altitude. Similar results were reported from sea level CO_2 response experiments by Lin[illegible]94), Hasselbalch and Lindhard (63), Lambertsen, et al. (89), and from both sea level and

altitude experiments (normal alveolar P_{O_2}) by Kellogg, et al. (80). Because the upward concavity in the response curves of the dogs was so apparent (see Fig. 15), tests of goodness of fit were made on the least squares equations from each experiment. In most cases the fit was satisfactory, showing that the data may be satisfactorily treated without allowing for the apparent fact that CO_2 sensitivity is not linear but increases slightly with increasing alveolar P_{CO_2}. It has been generally assumed that the CO_2 response curve is linear (26, 58, 95, 96, 97). However, statistical tests for the nonlinearity have apparently not been reported. Lambertsen (87) suggests that the slight curvature may result from small errors in direct sampling of alveolar gas, due to contamination of the sample by dead space gas; obviously such errors are larger at low tidal volumes than at large tidal volumes. However, Kellogg, et al. (80) presented limited evidence that this explanation does not suffice. It would seem that there could be a real physiological basis for this phenomenon. For example, a positive feed-back mechanism might exist whereby afferent impulses from stretch receptors of the chest wall or from the respiratory muscles, impinging on the respiratory center would increase its activity or facilitate impulses to the respiratory muscles, so that with progressively higher ventilatory volumes, increases in alveolar P_{CO_2} result in progressively greater increases in ventilation. It is clear that O_2-lack plays no part in this phenomenon, and that the

effect is small.

Lloyd, Jukes, and Cunningham (96), Cunningham, et al. (36), and Lloyd and Cunningham (95) have studied the relationships between pulmonary ventilation and alveolar P_{CO_2} and P_{O_2}. They express the relationship between ventilation and alveolar P_{CO_2} in the form of a linear equation: $\dot{V} = S(P_{ACO_2} - B)$; S is thus equal to "$CO_2$ sensitivity" and B is "related to CO_2 threshold", since it is the zero ventilation intercept when $\dot{V}$ (y-axis) is plotted against alveolar P_{CO_2} (x-axis). Their method of reducing and plotting the data is similar to the method I employed in that "CO_2 sensitivity" is expressed numerically as the slope of the stimulus-response line. These investigators use the reduced major axis rather than the least squares straight line to describe the relationship between pulmonary ventilation and alveolar P_{CO_2}. The reduced major axis, as discussed by Kermack and Haldane (83), has the advantage that no assumptions are made as to which of the variables under consideration is independent or dependent. In my experiments there was just as much variability in alveolar P_{CO_2} values as in alveolar ventilation (see Figs. 15 - 17). Therefore, it would seem reasonable to suppose that most of the variations were in fact due to biological variability. There is no question that the reduced major axis would have been a more appropriate analytical technique for quantitating the relationship between alveolar ventilation and alveolar P_{CO_2}. I did not learn about this technique until after

the data had been reduced using the least squares method. However, the overall conclusions drawn from the studies would certainly not have been different if the reduced major axis had been used rather than the least squares straight line. The reduced major axis technique has been used recently by Milledge (105) and by Kellogg (79) to quantitatively describe their investigations of CO_2 response curves during altitude acclimatization. Other examples of attempts to describe and predict respiratory phenomena through the use of mathematical equations include Gray (57, 58), Grodins, et al. (60), Loeschke (97), and Lambertsen, et al. (90).

F. Threshold Alveolar P_{CO_2}

As judged from the apparent points of inflection of the CO_2 response curves, alveolar P_{CO_2} threshold was lowered in the awake dogs at altitude by about 8 mm Hg (see Fig. 15). Since it has already been shown that the increase in slope of the CO_2 response curve was small, the data on alveolar P_{CO_2} at an alveolar ventilation of 8.0 liters/min (see Figs. 22 and 23) provide further evidence that CO_2 threshold was lowered. The decrease was about 9 mm Hg, as estimated from Fig. 23 (ignoring the increase in slope of the stimulus-response line). In reducing the data, threshold alveolar P_{CO_2} values

for each subject were quantitatively defined at sea level and at altitude, as previously explained. Alveolar P_{CO_2} thresholds determined in this fashion showed no consistent pattern of change, probably because the statistical method employed tended to obscure the meaningful changes. The determination of threshold values is an inherently difficult experimental problem, particularly so in the case of the CO_2 stimulus to respiration, because of the number of necessarily uncontrolled variables that may cause small changes in ventilation in the awake subject. Furthermore, the present experiments were not designed primarily to measure P_{CO_2} threshold.

Nielsen and Smith (116), in their study of the effects of increased inspired P_{CO_2} during acute severe hypoxia, clearly demonstrated an alveolar P_{CO_2} threshold of about 30 mm Hg. This threshold, which they interpreted to be the threshold of the respiratory center, was the same in various degrees of acute hypoxia as in normoxia. To the extent that "B" in the formulation of Lloyd and Cunningham (95) represents P_{CO_2} threshold, the data of Kellogg (79) on human sojourners at the Summit Laboratory of the White Mountain Research Station, show quantitative evidence of a decrease in P_{CO_2} threshold of about 10 mm Hg at 14,200 ft altitude. Similar results were reported by Milledge (105) who found a decrease in "B" of about 15 mm Hg at 19,000 ft elevation. Milledge compared his data on "B" and arterial plasma bicarbonate values in acclimatized subjects with similar data

on the relationship between "B" and plasma HCO_3^- in subjects who were in metabolic acidosis from ingested ammonium chloride (36). He concluded that the observed reduction in "B" at altitude could be accounted for by the decrease in plasma HCO_3^-. The classical observations of Dill, Talbott, and Consolazio (40), which showed a decrease in plasma CO_2 buffering capacity of the altitude acclimatized man have been reconfirmed many times.

Recently Kellogg has questioned certain aspects of the traditional ideas concerning the changes in acid-base balance during altitude acclimatization. His experiments showed that "The alteration in chemical regulation of breathing, or, more specifically, the fall in B (zero ventilation intercept) as well as the rise in S (slope of the CO_2 response curve) which occurs during acclimatization to altitude cannot be explained simply in terms of acid-base changes, which are not sufficient even to compensate for the respiratory alkalosis." (79). He also postulated that ". . . the adequate stimulus produced by CO_2 inhalation may be the effective pH in some region which is readily penetrated by CO_2 and less readily by other acids and which tends to change its acid-base characteristics, perhaps by active transport across its limiting membranes, in such a way as to restore its own pH to normal within a few days (i.e., faster than the kidney restores the pH of the blood) despite continued depression of its P_{CO_2}." (79).

Subsequently, Severinghaus, et al. (147, 148) studied the

normoxic CO_2 response curves of four men during an 8 day sojourn at 12,500 ft. They measured arterial and cerebrospinal fluid (CSF) pH at sea level and on the 2nd and 8th days at altitude, and calculated CSF and arterial pH for each value of alveolar P_{CO_2} during the CO_2 response tests. CSF bicarbonate was reduced by 4-5 mEq/liter within 1-2 days at altitude, but blood standard bicarbonate was only reduced by 1 mEq/liter at the end of a week. Their results thus confirmed Kellogg's hypothesis and they concluded that ". . . medullary respiratory chemoreceptor drive, initially reduced at altitude by hyperventilation alkalosis, is restored to normal during acclimatization by reduction in CSF HCO_3^-, the incremental ventilatory drive being supplied by peripheral chemoreceptors. The blood-CSF barrier appears to respond to the initial hyperventilation alkalosis by actively reducing CSF HCO_3^-; the data suggest that CSF pH is thus regulated by active transport by the blood-CSF barrier." (148).

If these results are confirmed, a major contribution to our understanding of the mechanisms of respiratory control will have been made. In this regard, recent remarks of Schmidt seem appropriate (142). Bleich, Berkman, and Schwartz studied the response of CSF to sustained hypercapnia in 35 dogs, exposed continuously to 12% CO_2 in 21% O_2 for periods up to 5 days (14). Their findings ". . . provide no evidence that the pH of cerebrospinal fluid is protected during respiratory acidosis in the fashion recently proposed for other acid-

base disturbances [ref. to Severinghaus, et al. 196? paper]." I do not wish to arbitrate this controversy, but I would point out that concentrations of CO_2 greater than 10% at one atmosphere of pressure are generally conceded to be intolerable or acutely toxic (58, 88); therefore, the manifestations observed by Bleich, Berkman, and Schwartz might profitably be considered in studying the mechanisms of acute toxicity of CO_2, particularly those observations made after 24 hr and 5 days of continuous exposure. Furthermore, without going into detailed discussions of Donnan equilibria, the Nernst equation, and transmembrane potential differences, it appears that the results of Bleich, Berkman, and Schwartz do not refute the findings of Severinghaus, et al. (147, 148).

Severinghaus, et al. (148) used CSF from lumbar punctures and assumed that the CSF so obtained was essentially identical to cisternal CSF. But experiments by Fisher and Christianson (49), on man during hyperventilation and recovery, reportedly showed that changes in pH, CO_2, and HCO_3^- were rapid in cisternal fluid, although less in magnitude than the changes in arterial blood, whereas changes in lumbar CSF were minimal and slow, lagging behind cisternal changes by 10-20 min.

Recent work has focused attention on superficial chemosensitive areas on the medulla (92, 98, 106, 107). The subject was reviewed by Winterstein in 1961 (166). Robin, et al. (140) concluded

that the acid-base relations of CSF probably do not reflect the acid-base balance of the respiratory center. It appears that conclusive evidence concerning the role of superficial chemoreceptors on the medullary surface, and their relationships to CSF acid-base dynamics and normal respiratory control mechanisms has not yet been presented (86).

G. The Effects of Preanesthetic Sedation on the CO_2 Response Curves of Dogs

Most early attempts to evaluate the effects of drugs on respiratory control mechanisms were based on measurements of respiratory rate, depth, and minute volume of resting ventilation before and after administration of the drug. Any alteration in respiration that was produced by drugs also affected arterial P_{CO_2}, tending to offset the drug effects. Therefore, the early studies had a marked tendency to underestimate the respiratory effects of various drugs.

Subsequently the techniques were improved by administering enriched CO_2 gas mixtures to the test subjects and comparing the respiratory responses before and after administration of the drugs. Lindhard studied the effects of drugs on human responses to increased inspired P_{CO_2} in 1911 (94). In three human subjects he found marked

depression of the slope of the curve with morphine (0.015 g), less depression with chloral (2.0 g) and greatly increased CO_2 response with strychnine (0.006 g nitrate of strychnine). He plotted his results as ratios of changes to initial values, hence all his observations were based on apparent changes of the slopes of the stimulus-response curves, since all curves had a common origin. Prescott, et al. (128) showed that in equianalgesic doses, many of the synthetic substitutes were fully comparable to morphine in their respiratory depressant effects. The work of Prescott, et al. was reported in 1949, and at that time it was widely believed that some of the synthetic derivatives (meperidine in particular) were markedly less depressing to the respiratory center in comparison to morphine, which was known to be a respiratory depressant.

Using the CO_2 inhalation technique, Dripps and Dumke (43) found a constant and progressive diminution in respiratory response to 10% CO_2 in O_2 in decerebrate dogs and cats with the following drugs: ether, chloralose, morphine, barbital, pentobarbital and thiopental. Respiratory minute volume with the subjects breathing room air was decreased by all the above drugs, except ether which produced an increase.

Experiments of this type have obvious limitations since alveolar P_{CO_2} and arterial pH must be taken into consideration, particularly since it is doubtful that steady-state responses are

usually achieved within the time allotted to the experiments (44).

Recently, refinements have been introduced to provide continuous analysis of alveolar P_{CO_2} and arterial pH. Von Euler and Söderburg have used curarized animals, maintained a constant respiratory minute volume by means of a pump, and used phrenic action potentials as the index of response to increased inspired CO_2 (160, 161). Lambertsen and his associates (85, 89, 91, 99) have developed a system for maintaining a constant elevated arterial CO_2 tension through continuous analyses of expired air. Any change in arterial CO_2 tension serves as a signal for compensation in the composition of the inspired gas mixture.

Dripps and Severinghaus, in a 1955 review of this field of investigation reached the following conclusion ". . . narcotics and general anesthetic agents depress the respiratory center, the degree of depression varying directly with the depth of narcosis . . ." (44).

Nearly all of the experimental work on the effects of anesthetic and analgesic drugs has been concerned with dosages that are used in conjunction with surgical anesthesia. Nevertheless, the results of these investigations provide a basis for evaluating the results from the present studies, in which dosages were adjusted to produce preanesthetic sedation in the customary surgical classification of stages and planes of anesthesia (56).

A detailed discussion of the mechanism of action of anesthetics

is beyond the scope of this report, but it would appear that cerebral hypoxia is one common denominator for all general anesthetics, narcotics and analgesics. The present studies suggest that analgesic doses of scopolamine and scopolamine plus thiopental had greater depressant effects on CO_2 sensitivity in dogs at altitude than at sea level. These results can be explained by assuming that the altitude adapted dog (although breathing gas mixtures that provided sea level equivalent alveolar P_{O_2}) still had some degree of oxygen deficiency and this deficiency acted synergistically with the analgesics to produce a greater degree of cerebral hypoxia with a given drug dosage. The obvious implication is that for a given degree of analgesia, smaller dosages of drugs are required at altitude than at sea level. This interpretation is consistent with the paradoxical finding that it was more difficult, at altitude, to conduct the experiments on sedated animals than on unsedated animals. The sedated animals showed unpredictable and exaggerated responses to auditory stimuli, particularly with scopolamine-thiopental sedation. Evidently analgesic doses of scopolamine or scopolamine-thiopental may exert their effects on respiratory control by reducing the activity of the respiratory centers. In view of the known idiosyncratic effects of scopolamine (56), and the recent demonstrations of species and individual differences in the intermediary metabolism of drug conjugates (22), no firm conclusions can be drawn from the present data. It should be noted that none of the observed

changes associated with the administration of drugs was statistically significant. The only observed effect of the drugs on the "CO_2 threshold" or sensory side of the respiratory control mechanism was an increase in variability of the results, this increased variability being twice as great with scopolamine-thiopental as with scopolamine alone, both at sea level and at altitude (see Fig. 23). Conversely, the variability of the results in the effects of drugs on slope or "CO_2 sensitivity", was twice as great with scopolamine alone as with scopolamine-thiopental, both at sea level and at altitude (see Fig. 21). The reasons for these differences are not immediately apparent.

H. Ventilatory Response to Increased Inspired CO_2 in the Awake Dog as a Function of Elapsed Time at Altitude

Estimates of the length of time required for acclimatization to mountain altitudes range from a few days (80) to a few generations (109), depending on the criteria used. Most of the evidence seems to indicate that in man the greatest change in the respiratory parameters occurs within a few days, with smaller adjustments continuing for as long as a month (42, 130, 132, 158).

Quantitative data concerning the rate of respiratory adaptation to altitude in dogs have not been reported so far as I am aware.

Originally I intended to measure the CO_2 response curve of each subject every day for the first ten days at altitude, and then to repeat the measurements again on the twentieth day. I assumed that the changes would be relatively large during the first few days. The measurements on the twentieth day were intended to show whether slow changes were still occurring, and the period between the tenth and twentieth days at altitude was reserved for experiments on the effects of analgesia, when the animals presumably would have been in comparatively stable altitude acclimatized states insofar as respiratory parameters were concerned.

It was not possible to carry out all the experiments that were planned: three tests were performed on one subject, nine on another, and eight on the third subject. Least squares straight lines were fitted to the data from each experiment. Fig. 20 shows the values for CO_2 sensitivity for each subject as a function of time at altitude, and Fig. 22 shows alveolar P_{CO_2} at an alveolar ventilation of 8.0 liters/min (which is an indirect measure of CO_2 threshold) in the same manner. Unsuccessful attempts were made to relate the changes in CO_2 sensitivity to elapsed time at altitude, using various statistical techniques. It is evident from inspection of Fig. 22 that the CO_2 response curve was shifted to the left by about 10 mm Hg, at the time of the first test, within a few hours after arrival at the Barcroft Laboratory (12,500 ft elevation), and that no further changes occurred thereafter. It should

be noted that the dogs were transported from Berkeley to Crooked Creek Laboratory (10,000 ft elevation) via Sonora Pass (9,000 ft elevation) in the Sierra Nevada Mountains on the preceding day and spent the night at the Crooked Creek Laboratory. Thus at "time zero" for the altitude exposure at 12,500 ft, the subjects had already been exposed to altitudes above 8,000 ft for 18-20 hr.

The present results showing that the altitude shift of the CO_2 response curve was complete in less than 24 hr are in agreement with recent results of Severinghaus, et al. (147, 148) who found that the reduction of CSF HCO_3^- was complete within 1-2 days in humans after arrival at 12,500 ft altitude. It would appear that altitude chamber experiments would be the logical experimental method for quantitatively defining the time course of respiratory adaptation to moderate altitudes since most of the change seems to occur within the first 24 hr of exposure.

There is considerable evidence that the dog is markedly more tolerant of respiratory alkalosis than is man (41, 115, 138). The extent to which respiratory alkalosis induced by panting may have influenced the subjects' breathing responses to added CO_2 is not known. It is quite possible that some of the variability observed could be attributed to this factor.

I. Hypoxic Drive Component of Resting Alveolar Ventilation

Experiments were done to determine if a certain degree of hypoxia contributes to the resting ventilation in the dog at altitude. Ventilatory volume was measured each minute in each subject while he breathed room air for 5 min (control). The measurements were then repeated for another 5 min while the subject breathed a gas mixture that provided an alveolar P_{O_2} of 110 mm Hg pressure without any added CO_2. This same sequence was repeated with the subjects under sedation by scopolamine and by scopolamine plus thiopental. Fig. 24 shows the results of these experiments, where each data point is the mean of at least two experiments performed under identical conditions, using the values recorded during the 4th and 5th minute in each condition.

It is apparent from inspection of Fig. 24 that, when group average results in the steady state are considered, raising the alveolar P_{O_2} to sea level value caused a large reduction in resting alveolar ventilation only in subjects under sedation by scopolamine-thiopental. This is the only apparent change that occurred, and it is not statistically significant ($0.10 > P > 0.05$) by t-test, but there are only two subjects in the group.

Marshall and Rosenfeld (102) described the depression of respiration by oxygen in patients whose respiratory centers were already depressed by anesthesia. The phenomenon is well known (44)

and is usually ascribed to some change in the sinoaortic chemoreceptors caused by oxygen, so that chemoreceptor activity is greatly reduced or abolished. Under conditions of surgical anesthesia complete apnea may ensue following the administration of oxygen-rich gas mixtures.

In the present instance, the marked reduction in alveolar ventilation which occurred after alveolar P_{O_2} was raised to sea level value appears to indicate that in the subjects under preanesthetic sedation by scopolamine plus thiopental about 40% of alveolar ventilation was being maintained by hypoxic stimuli when room air was breathed. It also follows from these average results that there was essentially no hypoxic drive component of resting alveolar ventilation in the awake subjects during the first 10 days at altitude, and none in subjects under sedation by scopolamine alone.

This interpretation may be an oversimplification according to the views of Dejours, et al. (39). According to these authors, the effects of O_2 must be measured within a 20 sec period following a single breath of O_2. The respiratory response within this period supposedly represents a relatively pure chemoreceptive effect, free from complicating compensatory factors such as increased acidity of the blood due to increased saturation of hemoglobin. Dejours, et al. studied ventilatory control in three human subjects during the 16th to 19th days at an altitude of 11,850 ft and concluded that an hypoxic

drive via the chemoreceptors was present, that it was constant, and that it was responsible for at least 50% of the ventilation. While they did not exclude the possibility of a "CO_2-H^+ stimulus" they concluded that it was unlikely that such a stimulus was of any greater importance at altitude than at sea level.

Kellogg, et al. (80) found that respiratory minute volume was 12% higher in humans breathing room air at an altitude of 12,470 ft as compared to volumes measured with the subjects breathing a gas mixture that provided sea level equivalent alveolar P_{O_2}. It appears that there are two distinct possibilities that must be considered in explaining the results of the present "steady state" studies on dogs: a) the dog differs from man in that there is no hypoxic stimulus, or b) the measurements were not precise enough to detect the hypoxic component.

Fig. 25 shows alveolar ventilation values on a minute by minute basis in awake dogs at altitude. At the end of the first minute, following the elevation of alveolar P_{O_2} from 55 to 110 mm Hg, alveolar ventilation is reduced and the reduction is possibly statistically significant ($P < 0.05$). This change, which seems to support the results of Dejours, et al. (39) is completely obscured when only the steady state values are considered. Similar results are apparent from the data on sedated dogs, as shown in Fig. 26. The transitory decrease in alveolar ventilation of the dog, following acute elevation of alveolar P_{O_2} from 55 to 110 mm Hg is smaller than the corresponding change

reported by Dejours, et al. (39) for humans at 11,850 ft. But direct comparisons may be misleading because of differences in techniques. A transitory reduction of ventilation in dogs breathing 100% O_2 at sea level was reported by Watt, Dumke, and Comroe (162). Experiments by Fitzgerald, Zajtchuk, and Perkins (51, 52), in which the carotid chemoreceptors of anesthetized dogs were perfused with blood of known pH, P_{CO_2}, and P_{O_2} tend to support Dejours, et al. If the results of Severinghaus, et al. (147, 148) are correct, the greatest chemoreceptor drive should occur within the first 24 hr following arrival at altitude. Data from the present studies were not complete enough to permit a determination as to whether the hypoxic drive was greater during the first few days. The data of D' Angelo (37) are pertinent in this regard. He studied respiratory metabolism in human subjects at 8,000 - 10,000 ft simulated altitude in a low-pressure chamber. Respiratory volumes became augmented, generally between the 2nd to 4th hr of exposure, while urinary pH rose linearly after the 1st hr to reach an alkalotic plateau in about 4.5 hr.

J. Deacclimatization

There are many reports which show that some of the altitude adaptive mechanisms persist for periods of a few days to several

months when the altitude acclimatized individual returns to sea level (119, 120, 136, 137, 156). For example, Monge holds to the view that the altitude acclimatized man is at a disadvantage at sea level. He cites historical evidence such as the loss of battles by high altitude dwellers when they fought in the lowlands and, also, present day failures of athletes who dwell at high altitudes to duplicate their altitude performances when they compete at sea level (110). On the other hand, Balke and Wells (4), Luft (100, 101), and others have presented evidence to show that altitude acclimatization increases man's tolerance to acute hypoxia and muscular exercise and the effect persists for about eight weeks following return to sea level.

With regard to various respiratory modifications characteristic of the altitude acclimatized state, return to sea level is accompanied by a reversal of the changes within a matter of days (72) or weeks (42, 81, 135). An early observation showed that a man continued to hyperventilate for at least a month following a sojourn on Pike's Peak (145).

I originally intended to repeat the sea level experiments on dogs immediately following their return to Berkeley from an eighteen day sojourn at the White Mountain Research Station, to investigate the question of whether deacclimatization effects could be demonstrated in respiratory parameters. As previously mentioned, the first post-altitude tests were conducted on the fourth day following return to sea

level. Figs. 20 and 22 suggest that the sea level CO_2 response curves were slightly shifted to the left and elevated during the 4th through 11th day at sea level following return from altitude. However, these changes are so small as to be almost negligible. A larger number of tests on more subjects would be required to fully evaluate the deacclimatization effects. Presumably the greatest changes occur within one or two days at sea level. It is by no means certain that man and dog are identical in their mechanisms of respiratory adaptation to altitude (29). Nearly all of the evidence that shows altitude-induced respiratory changes persisting for weeks or months after return to sea level is derived from observations on man.

V. Summary and Conclusions

Carbon dioxide response curves were measured in three trained dogs; before, during, and after an 18 day altitude sojourn at 12,470 ft. The effects of preanesthetic sedation on ventilatory response to added CO_2 were also studied in two of the subjects, at sea level and at altitude, using scopolamine and scopolamine-thiopental. In order to minimize the effects of peripheral chemoreceptor drive, the gas mixtures provided alveolar P_{O_2}'s equal to normal sea level values. Tests were made for the presence of an hypoxic drive component in resting alveolar ventilation at altitude by administering gas mixtures that acutely provided sea level equivalent alveolar P_{O_2}'s, without added CO_2.

End tidal alveolar gas samples were collected by a special electromagnetic device and analyzed continuously for O_2 and CO_2 by Beckman and critical orifice gas analyzers, respectively. Carbon dioxide response curves were determined by plotting alveolar P_{CO_2} (x-axis) against simultaneous alveolar ventilation (y-axis). The stimulus-response data were satisfactorily fitted to least squares straight lines. CO_2 sensitivity was defined as the slope of the line. Alveolar P_{CO_2} at a fixed value (8.0 liters/min) of alveolar ventilation provided an indirect measure of CO_2 threshold. A reduction in alveolar ventilation, following acute elevation of alveolar P_{O_2} from 55 to 110 mm Hg pressure, was used to measure the hypoxic drive com-

ponent of resting alveolar ventilation at altitude.

Group averages for the first 10 days at altitude, with the subjects breathing room air, showed reductions in resting alveolar P_{O_2} and P_{CO_2} to 53% and 71% of sea level control, respectively, as expected. Respiratory frequency, tidal volume, and alveolar ventilation were unchanged at altitude.

On the average, CO_2 sensitivity was increased during the first 10 days at altitude to 160% of sea level control. However, the increase was not statistically significant. CO_2 threshold was reduced, i.e., the response curve was shifted horizontally to the left, by about 8 mm Hg alveolar P_{CO_2}. As measured by the reduction in alveolar P_{CO_2} at a fixed rate of alveolar ventilation, the decrease in CO_2 threshold was statistically significant ($P < 0.01$) by t-test.

The shift of the response curve to the left and the increase in CO_2 sensitivity were essentially complete when the first experiments were performed, a few hours after arrival at the Barcroft Laboratory, and no further changes occurred thereafter. However, the dogs had already been exposed to altitudes above 8,000 ft for about 18 hr when they arrived at the altitude laboratory.

The most obvious effect of sedation was an increase in the variability of the results. The data showed limited evidence that CO_2 sensitivity was reduced by preanesthetic sedation, with the same dosages producing greater effects at altitude than at sea level.

Sedation had no consistent effect on CO_2 threshold.

Tests for an hypoxic drive component in resting alveolar ventilation at altitude showed a marked transitory reduction of ventilation at the end of the first minute following acute elevation of alveolar P_{O_2} from 55 to 110 mm Hg pressure. In dogs under pre-anesthetic sedation by scopolamine-thiopental 40% of resting alveolar ventilation was attributable to peripheral chemoreflexes.

Data from tests during the 4 th through 11 th days at sea level, following the altitude sojourn, showed only a slight suggestion that residual altitude changes were still present in both CO_2 sensitivity and CO_2 threshold.

The changes in the CO_2 response curve of dogs, during the first 10 days at an altitude of 12,470 ft, involve primarily a shift to the left with a small and inconsistent steepening of the stimulus-response line. Ventilatory responses of the dog to increased inspired CO_2 are similar to those reported by others for man. The differences that were observed may be partly due to the dog's greater tolerance to respiratory alkalosis and hypoxia.

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VII. Appendices

A. Analyses of Compressed Gas Cylinders* - Summary of Data (Dry Gas Basis)

Nominal % CO_2	Tank No.	Type Analyzer	Per Cent O_2			Per Cent CO_2		
			Mean	Avg Deviation	N	Mean	Avg Deviation	N
(As of 2 Sept 1955, Barcroft Laboratory)								
0	OG-21160	Micro Scholander	34.31	±0.05	3	0.13	±0.04	3
1	USN-1846461	" "	33.21	±0.03	3	1.17	±0.03	3
2	G-48417	" "	33.40	±0.05	3	2.37	±0.04	3
4	OG-46005	" "	32.80	±0.05	4	4.69	±0.05	5
4	OG-13662	" "	32.59	±0.09	4	4.72	±0.01	4
6	OG-41755	" "	34.11	±0.02	4	6.40	±0.03	4
6	WNGO-58	" "	34.18	±0.04	4	6.35	±0.01	4
6	USA-3053981	" "	33.88	±0.02	3	6.39	±0.04	3
8	G-48528	" "	33.25	±0.08	5	8.58	±0.08	5
8	OG-40200	" "	33.27	±0.04	4	8.54	±0.06	4
8	G-43995	" "	33.33	±0.02	3	8.68	±0.06	3

* Includes data on cylinders used in a previous study (80).

Appendix A, continued

Nominal % CO_2	Tank No.	Type Analyzer	Per Cent O_2 Mean	Per Cent O_2 Avg Deviation	Per Cent O_2 N	Per Cent CO_2 Mean	Per Cent CO_2 Avg Deviation	Per Cent CO_2 N
(As of 13 Dec 1955, Berkeley)								
0	OG-29454	Micro Scholander	21.06	±0.04	6	0.07	±0.03	6
1	OG-48462	" "	20.51	±0.01	4	1.09	±0.02	5
2	OG-48460	" "	20.99	±0.07	4	2.31	±0.02	5
4	G-44803	" "	21.13	±0.03	4	4.66	±0.02	5
6	KCO-13727	" "	20.69	±0.04	4	6.30	±0.02	4
6	HSC-220280	" "	20.80	±0.01	4	6.30	±0.02	4
(As of 15 May 1956, Berkeley)								
4	OG-44139	Micro Scholander	21.08	±0.04	4	4.66	±0.01	4
6	OG-41701	" "	20.58	±0.03	5	6.34	±0.01	5
(As of 20 May 1956, Barcroft Laboratory)								
2	OG-29705	Micro Scholander	33.08	±0.02	3	2.24	±0.01	3
4	OG-44073	" "	32.70	±0.07	3	4.64	±0.02	3

Appendix A, continued

Nominal % CO_2	Tank No.	Type Analyzer	Per Cent O_2 Mean	Per Cent O_2 Avg Deviation	Per Cent O_2 N	Per Cent CO_2 Mean	Per Cent CO_2 Avg Deviation	Per Cent CO_2 N
(As of 10 Aug 1956, Berkeley)								
0	OG-29454	Critical Orifice & Beckman	21.03	±0.02	3	0.03	±0.01	3
1	OG-48462	" "	20.47	±0.02	3	1.04	±0.02	3
2	OG-48460	" "	21.05	±0.00	2	2.26	±0.02	3
4	OG-41701*	" "	21.03		1	4.27	±0.03	3
6	OG-21160**	" "				6.57	±0.01	?
(As of 5 Sept 1956, Barcroft Laboratory)								
0	OG-41766	Critical Orifice & Beckman	33.84	±0.00	3	0.76	±0.00	3
1	OG-48529	" "	32.54		1	1.59	±0.04	4
8	OG-41713	" "	32.91	±0.00	4	8.08	±0.05	4
(As of 12 Sept 1956, Berkeley)								
2	OG-40286	Critical Orifice & Beckman	22.79	±0.00	3	2.27	±0.01	3
6	OG-21160	" "	20.99	±0.00	3	6.65	±0.02	3

* Refilled with 4% CO_2, 7 Aug 1956.

** Refilled with 6% CO_2, 19 June 1956.

B. Manufacturer's Analyses and Record of Compressed Gas Cylinders Used in CO_2 Sensitivity Studies on Dogs

Nominal % CO_2	Tank No.	Manufacturer's Analyses % O_2	% CO_2	% N_2	Remarks
(Sea Level Experiments)					
0	OG-29454	(Compressed Air)			Empty 11 Aug 1956
0	OG-	(Compressed Air)			
1	OG-48462				
2	OG-48460	21.1	2.2	76.7	
4	OG-44139	21.1	4.4	74.5	Empty 5 Aug 1956
4	OG-41701				In Service 9 Aug 1956
6	OG-21160				
(Altitude Experiments)					
0	OG-41766	34.2	0	65.8	
1	OG-48529	33.5	1.1	65.4	
2	OG-29705	34.2	2.2	63 6	
4	OG-46005	33.6	4.4	62.0	
4	OG-44073	33.6	4.4	62.0	Used only for calibrating critical orifice analyzer
6	WNGO-58	34.3	6.3	59.4	
8	OG-41713	33.6	8.5	57.9	

C. Sample Calculations, Formulae, and Conversion Tables

(All pressures expressed in units of mm Hg)

1. Alveolar P_{O_2}

$$P_{A_{O_2}} = \text{Beckman } O_2 \frac{(P_B - 52)}{(P_B - \text{Asp.})}$$

where: $P_{A_{O_2}}$ = Alveolar O_2 partial pressure

Beckman O_2 = O_2 partial pressure (dry gas), as measured by the Beckman O_2 Analyzer from an end-tidal gas sampler.

P_B = Barometric pressure.

Asp. = Pressure drop across the Beckman analysis cell.

52 = A constant, equal to saturation vapor pressure of water at 39 C (68).

Dog's body temperature assumed to be constant at 39 C (152).

2. Alveolar P_{CO_2}

$$P_{A_{CO_2}} = F_{A_{CO_2}} (P_B - 52)$$

where: $P_{A_{CO_2}}$ = Alveolar CO_2 partial pressure.

$F_{A_{CO_2}}$ = Decimal fraction of CO_2 (dry gas) contained in alveolar gas sample, as measured by the critical orifice CO_2 analyzer.

3. Gas volume conversion, ATPS to BTPS (31).

$$V_{BTPS} = V_{ATPS} \left[\frac{273 + 39}{273 + \text{gas temp. C}} \right] \left[\frac{P_B - P_{H_2O_t \text{ gas temp.}}}{P_B - P_{H_2O} \text{ at } 39C} \right]$$

where: $P_{H_2O_t}$ gas temp. = Vapor pressure of water at observed gas temperature, C.

P_{H_2O} at 39C = Vapor pressure of water at dog's body temperature of 39 C, assumed constant = 52 mm Hg.

4. Calculated Gas Volume Conversion Factors, V_{ATPS} to V_{BTPS}, Combined Pressure and Temperature Factors for Dog at P_B = 760 mm Hg.* $V_{BTPS} - V_{ATPS}$ (Factor)

Gas Temperature C	Factor
18.2	1.126
18.4	1.126
18.6	1.125
18.8	1.122
19.0	1.121
19.2	1.121
19.4	1.119
19.6	1.118
19.8	1.118
20.0	1.117
20.2	1.115
20.4	1.114
20.6	1.114
20.8	1.112
21.0	1.111
21.2	1.111
21.4	1.109
21.6	1.108
21.8	1.107
22.0	1.106
22.2	1.104
22.4	1.103
22.6	1.101
22.8	1.100
23.0	1.100
23.2	1.098
23.4	1.097
23.6	1.096
23.8	1.095
24.0	1.094
24.2	1.093
24.4	1.092
24.6	1.091
24.8	1.090
25.0	1.089

* Differences between the standard P_B for a given altitude and the observed P_B may be ignored (31).

5. Calculated Gas Volume Factors, V_{ATPS} to V_{BTPS}, Combined Pressure and Temperature Factors for Dog at P_B = 487 mm Hg. (Barcroft Laboratory, White Mountain High Altitude Research Station).

Gas Temperature C	Factor
18.2	1.160
18.4	1.160
18.6	1.158
18.8	1.157
19.0	1.156
19.2	1.154
19.4	1.153
19.6	1.151
19.8	1.151
20.0	1.149
20.2	1.148
20.4	1.146
20.6	1.146
20.8	1.145
21.0	1.143
21.2	1.143
21.4	1.141
21.6	1.138
21.8	1.137
22.0	1.136
22.2	1.135
22.4	1.133
22.6	1.132
22.8	1.131
23.0	1.129
23.2	1.128
23.4	1.127
23.6	1.125
23.8	1.124
24.0	1.121
24.2	1.121
24.4	1.119
24.6	1.118
24.8	1.117
25.0	1.115

6. Statistical Formulae (Derivations of some of the formulae are given by Spiegel (153), using the method of partial differentiation.)

a. Equation of Least Squares Straight Line

(1). Type Equations

(a). If X is the Independent Variable

$$Y = a_i x + a_o$$

$$a_i = \frac{(N)(\Sigma XY)-(\Sigma X)(\Sigma Y)}{(N)(\Sigma X^2)-(\Sigma X)^2}$$

$$a_o = \frac{(\Sigma Y)(\Sigma X^2)-(\Sigma X)(\Sigma XY)}{(N)(\Sigma X^2)-(\Sigma X)^2}$$

where: a_i = the slope of the straight line whose equation is $Y = a_i x + a_o$,

a_o = the Y intercept,

and N = number of observations.

(b). If Y is the Independent Variable

$$X = b_i Y + b_o$$

$$b_i = \frac{(N)(\Sigma XY)-(\Sigma X)(\Sigma Y)}{(N)(\Sigma Y^2)-(\Sigma Y)^2}$$

$$b_o = \frac{(\Sigma X)(\Sigma Y^2)-(\Sigma Y)(\Sigma XY)}{(N)(\Sigma Y^2)-(\Sigma Y)^2}$$

where: b_i = the slope of the straight line whose equation is $X = b_i Y + b_o$,

b_o = the X intercept,

and N = number of observations.

(c). Standard Error of Estimate of Y on X = Sy.x; see Spiegel (153).

Let Y_{est} = the value of Y for given values of X as estimated from $Y = a_i x + a_o$. A measure of scatter about the regression line of Y on X is then given by

$$Sy.x = \sqrt{\frac{\Sigma (Y-Y_{est})^2}{N}}$$

which is called the standard error of estimate of Y on X.

Or $$Sy.x^2 = \frac{\Sigma Y^2 - a_o \Sigma Y - a_i \Sigma XY}{N}$$

and $$Sy.x = \sqrt{\frac{\Sigma Y^2 - a_o \Sigma Y - a_i \Sigma XY}{N}}$$

$$= \sqrt{\frac{\Sigma Y^2 - b_o \Sigma Y - b_i \Sigma XY}{N}}$$

(d). Modified Standard Error of Estimate of Y on X for Small Samples = $\hat{S}y.x$.

$$\hat{S}y.x = \sqrt{\frac{\Sigma Y^2 - a_o \Sigma Y - a_i \Sigma XY}{N-2}}$$

$$= \sqrt{\frac{\Sigma Y^2 - b_o \Sigma Y - b_i \Sigma XY}{N-2}}$$

(e). Sample Standard Error of the Regression Coefficient = $\hat{S}_b$; see (151).

$$\hat{S}_b = \hat{S}_{y.x} \Big/ \left[\Sigma x^2\right]^{\frac{1}{2}}$$

where: $$\Sigma x^2 = \Sigma X^2 - \frac{(\Sigma X)^2}{N}$$

$\hat{S}y.x$ = the standard error of estimate of Y on X.

(f). A test of significance of regression coefficient (a_i) is given by the t ratio

$t = a_i / \hat{S}_b$; d.f. = N-2, see (151).