COMPARATIVE PHYSIOLOGY OF RESPIRATORY MECHANISMS

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A Study of the Effect of Carbon Dioxide Inhalation on the Decerebrate Duck

by

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ABSTRACT

The respiratory response to CO_2 inhalation was investigated on decerebrate Aylesbury ducks. Co_2 was administered via a low resistance, low dead space perspex valve and tracheotomy tube. The ducks remained in good condition with normal postural and righting reflexes for several days. On inhaling a new CO_2-air mixture it took about 10 minutes to achieve a new steady state of ventilation. The ventilatory minute volume rose proportionately with the inspired CO_2 concentration and the percentage increase ventilation was comparable with decerebrate hens and with man. Inspired CO_2 concentration above 6% caused depression of respiration. CO_2 inhalation had little effect on respiratory frequency; ventilation was increased mainly by increased tidal volume; the relation between ventila-:tion and tidal volume was linear up to four times the resting ventilation.

The R.Q. change on CO_2 inhalation found in the duck was different from that seen in man, but similar to that of hens. The quantity of CO_2 eliminated in the duck was similar to that found in most studies in mammals, but the quantity of CO_2 taken up was about ten times larger.

The basic pattern of the effect of CO_2 inhalation in stimulating respira-:tion seems similar in ducks (diving birds), hen (non-diving birds) and man. But in the ducks much larger quantities of CO_2 were lost from the inspired CO_2-air mixtures than observed in most studies on the mammals.

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INTRODUCTION

Comparative studies of the mechanism of respiratory control began nearly a century ago in 1870, when Paul Bert made observations on the resistance to asphyxia of the domestic duck after submersion. From his studies in both mammals and birds he established the fact that the duck is possessed of an outstanding resistance to submersion. His experiments gave a mean of 11 minutes 17 seconds before death in ducks as compared to only 3 minutes 31 seconds in the hens. After having excluded the exist-:ence of anatomical factors either in the circulatory or respiratory systems he attributed this difference to the larger volume of blood in the ducks. Although he did not give any conclusive evidence for the mechanism of this resistance to submersion his work led to many studies on the control of respiration in diving animals.

Frances Huxley (1913a, b) in an extensive study on conscious, anaesth-:etized and decerebrate ducks demonstrated the reflex nature of submersion apnoea. She also found that apart from submersion, straightening of the neck or dorsiflexion of the head upon the neck produced apnoea in the duck. This cessation of respiration was shown to be a postural reflex as extirpa-:tion of the labyrinths or section of the cervical nerve roots prevented apnoea. Huxley (1913c) also demonstrated that the reflex stoppage of breath-:ing on submersion was accompanied by slowing of the heart which was abolished by the action of atropine. This suggested that the efferent path of this reflex was the vagus nerve and that adaptations of the circulatory system might be responsible in some ways for the resistance of the duck to asphyxia by submersion.

It is well known, however, that in mammals CO_2 is a respiratory

stimulant. The respiratory centre is extremely sensitive to the slightest increase or diminution of the partial pressure of CO_2 in the alveolar air. From experiments in which CO_2 was added to inspired air Haldane & Priestley (1905) found that in man a rise of 0.2% of CO_2 in the alveolar air, corresponding to an increase of 1.4 mm in the pCO_2 was sufficient to double the rate of alveolar ventilation.

When a duck dives, the postural reflexes come into play and breathing stops. It was not understood why CO_2, which has a strong stimulatory effect on mammalian respiration, could not overcome this postural apnoea. These contradictory factors led Orr & Watson (1913) to investigate the effect of CO_2 on the respiration of the duck. They found that administration of CO_2 by tracheotomy tube not only did not stimulate, but actually depressed respiration in the duck, whether conscious, anaesthetized with ether or decerebrate. They suggested that the presence of CO_2 in the inspired air acts as an inhibitory influence either by slowing of the respiratory movement or causing complete apnoea according to the percentage of CO_2 present. They observed that generally 20% CO_2 produced complete apnoea, sometimes broken by asphyxial struggles, 10% CO_2 reduced the frequency of respiration by over 50% and 5% CO_2 reduced the frequency by about 30%. The amplitude of respiration was usually increased, but not to such a degree as to compensate for the decreased rate of respiration.

Dooley & Köppányi (1929) suggested that CO_2, when inhaled, might cause irritation of the respiratory passage and produce depression of respiration without the necessity of assuming any central action. This fact was confirmed by them when they injected CO_2 into the humeri in ducks by a method of continuous insufflation. In ducks and other birds the humeri are connected with the lungs and trachea through the cervical air sacs.

A duck thus insufflated with a current of air or pure O_2 showed first a slowing and shortly afterwards a cessation of respiration. The administra-:tion of CO_2 did not produce even a temporary slowing of respiration. On the other hand, if apnoea was produced by insufflation with O_2 or air, the administration of CO_2 interrupted the apnoea and caused exaggerated respira-:tory efforts. So, the claim of Orr & Watson that CO_2 is a depressant of the respiratory centre in the duck was rejected.

The interruption of postural apnoea in certain cases may well be due to CO_2 stimulation, although Dooley & Koppányi (1929) had shown experi-:mentally that CO_2 as a rule did not interrupt postural apnoea, for ducks can be killed by maintaining them in an apnoeic position. The period of exaggerated breathing succeeding postural apnoea was taken as additional evidence of CO_2 stimulation.

In his study of sensitivity of respiration of CO_2 in diving mammals such as beaver and muskrat, Irving (1938) demonstrated that CO_2 had less effect on respiration, heart rate, blood pressure and muscle blood flow than on non-diving animals. In his experiments on conscious seals, addi-:tion of CO_2 to the inspired air frequently depressed the volumes of pulmon-:ary ventilation and did not regularly cause an increase until more than 5% CO_2 was administered. This seemed to him relatively insensitive com-:pared to the degree of sensitivity of CO_2 on human respiration as indicated by the observations of Haldane & Priestley (1905). Thus, he came to the conclusion that unlike the non-diving mammals the breathing of these diving mammals are less sensitive to CO_2. It seemed that respiratory control mechanisms of diving animals were typically mammalian and differed only in a quantitative way from the land animals.

Measurement of pulmonary gas exchange and ventilatory response to

CO_2 were made in harbour seals breathing air, 4%, 6% and 10% CO_2 through a Ruben respiratory valve by Robin, Murdaugh, Pyron, Weiss & Stores in 1963. Exposure to each mixture was maintained for 10-15 minutes before gas collection to permit the achievement of a relatively steady state. They found that the seal had a higher PA CO_2 (48 ± 6 mm Hg) and a lower PA O_2 (88 ± 9 mm Hg) compared to man and a lower ventilatory response to CO_2 both in terms of slope and intercept of CO_2 response curves.

Hiestand & Randall (1941) attempted to locate the site of sensitivity to CO_2 in the respiratory tract of the muscovy duck. They noted that application of weak acids and other noxious material to the nasopharynx caused apnoea, similar to the administration of CO_2. After applying cocaine to the same site, administration of CO_2 produced 'vigorous' stimulation of respiration. Thus, they were able to show that inhibitory receptors or nerve endings exist in the upper respiratory tract which are stimulated by CO_2.

The fact that the vagus nerve is the efferent nerve in the reflex physiological adjustments of respiration and circulation which occur in response to submersion in diving mammals has been known for a long time. Richet (1899) and Huxley (1913c) had demonstrated the absence of these reactions in vagotomised and atropinised birds. Recently Andersen (1963) demonstrated that the trigeminal nerve was an additional afferent pathway for this reflex in the duck. The ophthalmic division appears to be the most important branch of the trigeminal nerve in this respect, followed by the mandibular portion. The maxillary branch, however, could not be shown to serve such a function. He was thus tempted to speculate that sensory messages from the beak region normally inhibit the respiratory centre of the duck. Information about the structure of these receptors is, however,

still lacking.

Andersen & Lövö (1964) made quantitative studies on the effect of CO_2 on the respiration of conscious ducks, breathing through a tracheal cannula. Their results showed that the respiratory minute volume invariably rose when the duck inhaled gas mixtures containing CO_2 concentrations between 0.03-6.5 vol. per cent. In 2 out of their 5 experiments the minute volume was reduced on raising the inspired CO_2 concentration of 9% and above. In their experiments however, the ducks breathed each gas for about 5 minutes and only 2 minutes were allowed before the collection of a sample. These birds were conscious and it seems very probable that they would need considerable restraint which would affect their pulmonary ventilation.

Johnston & Jukes (1966) measured the ventilatory response of decerebrate hens to inhaled CO_2-air mixtures. A similar pattern of the relationship between steady state pulmonary ventilation and inhaled CO_2 in the hen and that found for unanaesthetized man and other mammals suggested a common mechanism for CO_2 acting as a respiratory stimulant.

It seems that the conclusions that can be drawn from these previous studies are:

(1) that CO_2 stimulates respirations in all mammalian species, diving as well as non-diving,

(2) that perhaps the response of pulmonary ventilation in inspired CO_2 in divers may not be the same quantitatively as in non-diving mammals and birds,

(3) the resistance to asphyxia by submersion in ducks may be due to a

strong postural reflex and to reflex inhibition of the respiratory centre from the CO_2-sensitive receptors in the nasopharyngeal region.

However, in view of the fact that ducks are capable of enduring long periods of apnoea during diving, a study of the progressive effects of inhalation of CO_2 on the ventilation and on body stores of CO_2 was undertaken.

Carbon dioxide is present in the body mainly in the form of carbonic acid, bicarbonate and carbamino compounds. The various stores of CO_2 in the body can be considered to be in the lungs, in the blood, in soft tissues and in bones. The whole body CO_2 store is thought to be in the order of 120 l. in an adult man (Farhi & Rahn, 1955). The CO_2 stores of the blood are small compared to the CO_2 stores of the tissues (soft tissues and bone). Human blood is known to take up, in the neighbourhood of 40 mm Hg tension, about 0.05 ml% CO_2 for each mm change of tension (Haldane & Priestley, 1935; Roughton, 1964). Adolph, Nance & Shiling (1929) in their experiments on cats estimated that body tissues as a whole had a CO_2 capacity of 6-25 times that of blood. Shephard (1955b) deduced that during the first minute of experimental hypercapnia there was a rapid rise of CO_2 tension in the pulmonary venous blood, and an increase in CO_2 content (2.2-2.3 ml/100 ml serum) which declined rapidly to half this value. He concluded that after the first minute the major proportion of the retained CO_2 is distributed amongst the remaining body tissues.

Changes in the CO_2 stores contribute in a positive or a negative way to the normal metabolic gas exchanges: study of alterations in the CO_2 stores provides an assessment of the capacity of the gas store. The CO_2 store of the body can be increased by inhalation of CO_2 or by hypoventilation as in the depression of the respiratory centre during intravenous

anaesthesia by sodium pentothal in man and dog (Suskind & Rahn, 1954). On the other hand, it can be decreased by passive or active hyperventila-:tion.

Acute changes in ventilation affect the gas exchange largely by altera-:tion in the CO_2 output, oxygen consumption tending to remain constant (Rahn & Otis, 1949). An increase or decrease of CO_2 body store is indica-:ted by a reduction or an increase, respectively, of the respiratory quotient (R.Q.) from the steady state value.

The time taken by the body to come into equilibrium with a change in the alveolar CO_2 tension varies slightly in different species, but in most of those studied a new steady-state is reached within 1-2 hrs, although it may take days to establish a complete equilibrium. It is obvious that this attainment of equilibrium with the inspired CO_2 is important in the accurate assessment of CO_2 stores. The results of previous work indicated that in mammals the CO_2 storage capacity of the whole body varies from 1.5-3.8 cc/kg body weight/mmpCO_2 in experiments lasting for 20-100 minutes (Farhi & Rahn, 1963). The variation in the results may be associated with the equilibrium period and the fact that different tissues of the body may have different CO_2 dissociation curves and might not act as a single com-:partment.

Vance & Fowler (1960) in their hyperventilation studies in man found that the slope of the CO_2 stores output was not a single exponential function of time. This indicated different sites of CO_2 stores in the body with varying slopes of CO_2 dissociation. It was, therefore, unlikely that the whole body mass would act as a single compartment of CO_2 stores. Freeman & Fenn (1953) obtained large slopes of CO_2 dissociation curves

(11.6 cc/kg/mm CO_2) in rats equilibrated with 10% CO_2 in air for 6-28 days. This demonstrated the presence of a large store of CO_2 in the tissues including bone from which CO_2 is released very slowly.

The difference in the structure of the respiratory apparatus in birds (Hazelhoff, 1951) to that of mammals and the fact that communications exist between the air sacs and the bones (Sturkie, 1954; Salt & Zeuthen, 1960) might make a difference in their CO_2 store capacities. Studies of the change of respiratory gas exchange on CO_2 inhalation in the duck (a diver) and in the hen (non-diver) were made for comparison.

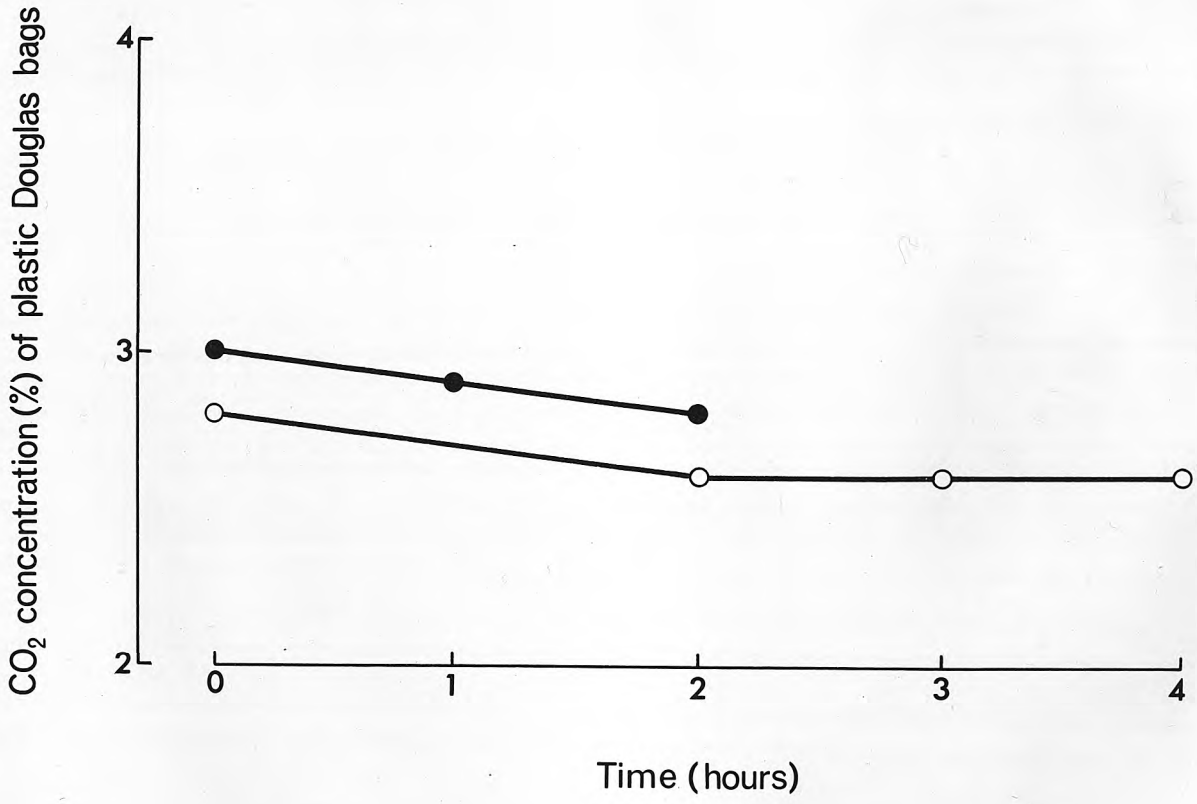


Fig. 1. Rate of CO_2 loss from plastic Douglas-bags.

METHODS

Twenty-one experiments were performed on 14 Aylesbury ducks, (♀). The body weight of the ducks varied between 1.9-3.5 kg with a mean weight of 2.77 kg.

Two experiments were performed on 2 hens (brown Leghorns) weighing 2.0 kg and 3.6 kg.

I. Operative procedure

A. Preparation

1. The birds were routinely fed once daily in the morning; they received no food on the morning of operation.

2. General anaesthesia

Nembutal (Pentobarbitone Sodium B.P., Abbott Laboratories, Ltd.) 15-20 mg/kg was injected into a wing vein. During the course of the operation ether was given, when necessary, on an open mask to maintain a sufficient depth of anaesthesia to prevent reflex movements.

B. Operations

1. Tracheostomy

Commonly a vein was seen running along the trachea; this was ligatured. A transverse incision was made between 2 tracheal cartilages in the lower end of the trachea, a 'Y' polythene tube was inserted which had an internal diameter of 5 mm, this was only slightly smaller than that of the trachea. The skin incision was sutured over the tracheal cannula. The insertion of the cannula low down in the neck allowed the inspired air to by-pass the carbon-dioxide sensitive receptors in the upper respiratory tract (Hiestand

& Randall, 1941).

2. Decerebration

A longitudinal incision was made on the dorsum of the skull through the skin. A transverse incision in the periosteum was then made along the superior nuchal line and the periosteum reflected laterally on each side. A triangular piece of bone was removed by means of a bone-nibbler, leaving a central strip of bone in the midline over the saggital sinus. This procedure pre-:vented the risk of damaging the fragile sinus which would cause consider-:able haemorrhage and also reduced the operation time by eliminating the need to identify and ligature the sinus. The dura mater was incised and the cerebral hemisphere on each side was removed by suction. Haemorrhage as a rule was slight. The cavity was then filled with 2 small pieces of cellulose gauze and a small cotton wool plug. The skin incision was then stitched together and ligatured. After some early experiments the brain was removed, fixed in formalin, and examined to confirm that decerebration was complete. One ml. of Strypen (Penicillin 250,000 units and Streptomyin 0.25 g, May & Baker) was routinely injected intramuscular-:ly.

C. Post operative care and nutrition

Normally, the ducks sat very quietly until about six hours after decerebration, then they tended to become more alert and responded to noise. They could stand up by the second or the third day. The only signs of neurological damage seen in some birds were weakness of the legs and inability to stand up.

One experiment on each duck was performed on the day after decere-:bration and sometimes further experiments were carried out up to 6 days

after operation, thus care and nutritional upkeep were essential. After decerebration the ducks were kept in a pen in the laboratory(Temp. 18.5-23.5°C)

To reduce the risk of sepsis 1ml. of Strypen administered on the 1st day was occasionally followed by further daily injections of the 2nd and 3rd days. The tracheal tubes were cleared of mucus and secretions regularly, usually 3-4 times daily.

To supply the basic calorific and water requirements on the day of decerebration, glucose 1g/hr (4.2Cal/hr) and 2 ml./kg/hr water (Dicker & Haslam, 1966) i.e., 4g glucose in approximately 25 ml. water were given by means of an oesophageal tube. On the second day at the end of the experiment if the duck was kept for further experiments, it was given Complan (a dried milk food with a supplement of oil, protein, vitamins and mineral, Glaxo Laboratories, Ltd., Calorific value 450 Cal/100 g), in water, instead of glucose.

II. Apparatus used

The carbon-dioxide-air mixtures were prepared by mixing air, pumped through an air-rotameter by an electric pump, with CO_2 passed through a CO_2-rotameter from a cylinder. The CO_2 was delivered from a 2 lb. cylinder through a needle valve with a pressure guage. To obtain a CO_2 concentration fairly close in value to the precalculated concentration it was found essential to warm the reducing valve and also to ensure that the cylinder was full enough to indicate a pressure of approximately 50 kg/cm^2. The CO_2 - air mixtures were collected in 100 l. capacity plastic Douglas-bags fitted with 2 way taps. It was found advisable not to fill them com-:pletely, as the high pressure in the bags when connected to the respiratory

valve tended to keep the inspiratory flap of the valve open. The plastic Douglas-bags had low resistance to inflation as they were made of light plastic material and the CO_2 loss from them during the course of the experiment was slight; the percentage loss during the first hour was 0.10% which compared favourably to a loss of 0.05% in 15 minutes of 40 l. of ex-:pired air (4% CO_2) collected in a 200 l. capacity conventional Douglas-bag (Shephard, 1955a), (Fig. 1).

The CO_2% of the CO_2 mixtures was analysed with the Lloyd-Haldane gas analysis apparatus (Lloyd, 1958). In some experiments the percentage of both CO_2 and O_2 in the mixtures was analysed and in some cases the CO_2 content of the room air was also analysed several hours after the start of the experiment. This prevented errors in the respiratory quotient calcula-:tion which might arise if the CO_2 of the room air rose significantly during the course of the experiment. The maximum CO_2 concentration ever found in room air was 0.20%. Duplicate analyses were performed for each sample and an agreement within 0.02% or very rarely 0.03% of each other was acceptable.

The bag of prepared CO_2-air mixture, varying between 2-6% CO_2 was connected to the inspiratory side of the respiratory valve via a three-way tap and rubber tubing. The internal diameter of the tubing was 2.2 cm and the connections were made as short as possible with no sharp bends or kinks. The specially made perspex respiratory valve used had a dead-space of 3 cc and a very low resistance (Johnston & Jukes, 1966), the com-:bined resistance of the 'Y' tube and the respiratory valve was 0.20 cm H_2O at a flow rate of 8 l./min. The valve had to be heated during the experiment to about 41°C to prevent the flaps sticking due to condensation of water vapour. The expiratory end of the valve was also connected to a Douglas-bag via a

3-way tap and rubber tubing similar to the inspiratory end.

The CO_2 and O_2 concentration of the expired air collected in these bags was analysed and in some cases the volume was measured by a wet-type gas meter. The gas volumes were reduced to body temperature and standard pressure. Average body temperature of ducks and hens was $41^{o}C$.

III. Conduct of experiments

These experiments involved decerebrate ducks and hens breathing either room air or CO_2-air mixtures and collection of the expired air. For this the following factors were considered particularly important.

1. Low dead-space of the tracheal cannula and respiratory valve.
2. Low resistance of the system.
3. Accurate gas analysis.
4. Accurate measurement of ventilatory volumes.

The methods by which these possible sources of error were minimised as far as possible are described in the appropriate sections.

Preparations for the experiments consisted of filling the Douglas-bag with a precalculated concentration of CO_2-air mixture, checking the CO_2% with the Lloyd-Haldane gas analyser.

The respiratory valve was warmed for about 30 minutes before the experiment started. The birds tracheal cannula was connected to the respiratory valve and it breathed room air for at least 30 minutes in most cases before collection of the first sample of expired air was started.

The resting collection periods varied from 20-30 minutes. The CO_2 and O_2 percentages of the resting collection were analysed and the volume measured with a wet gas meter. The respiratory frequency was counted from observation of either the chest wall movements or the movements of the respiratory valve.

Two different types of experiments were carried out:

Group I. After collecting a resting sample of expired air while the bird breathed room air, the bird inspired successively different concentrations of CO_2 ranging from 2-9%, each for a period of 30 minutes. The collect-:ion of expired air for each concentration of gas inspired began after breathing that particular concentration for 10 minutes (except Experiment 19, see results). The ventilatory volume changes due to inhalation of different concentrations of CO_2-air mixtures were measured and the respiratory frequency was counted.

Group II. After collecting the first sample of expired air while the bird breathed room air, the bird inhaled one particular concentration of the CO_2-air mixture for a period of 60-90 minutes. Accordingly, 2 or 3 consecutive 30 minute collections of expired air were made during this period of CO_2 inhalation. In every case, collection of the expired air started one minute after the start of CO_2 inhalation.

The CO_2 and O_2 percentages of the expired air were analysed.

In some birds the same procedure as above was carried out but at the end of CO_2 inhalation expired air was collected continuously for up to 1 hour after CO_2-air inhalation ceased while the bird breathed room air.

CO_2 and O_2% in each sample was analysed and the R.Q. of the successive collection periods over the duration of the whole experiment was calculated. The ventilatory volume for each period was measured, allowance being made for the volume of air used in the gas analysis.

The respiratory frequency was counted as above and the tidal volume calculated from the minute volume. For these experiments the amounts of CO_2 accumulated by the bird during the inhalation of CO_2 and the amount of CO_2 eliminated in subsequent breathing of room air were calculated.

Control series

Two ducks were studied. One breathed room air for 100 minutes and the other for 120 minutes. In the first experiment expired air was collected for consecutive 20 minute periods and in the second for consecutive 30 minute periods. The CO_2 and O_2 percentages of the expired air was measured for all samples and the R.Q. calculated. This gave a measure of the varia-:tion in R.Q. which could be expected in any duck during the period equival-:ent to the usual duration of the experiments.

During the experiments the birds generally sat quietly and very little restraint was necessary. Most of the birds were disturbed by sharp, loud noises but a few did not even react to such noises. Much struggling by the ducks was sometimes observed during the first few minutes of administration of high concentrations of CO_2 (5-9%) but they soon settled down.

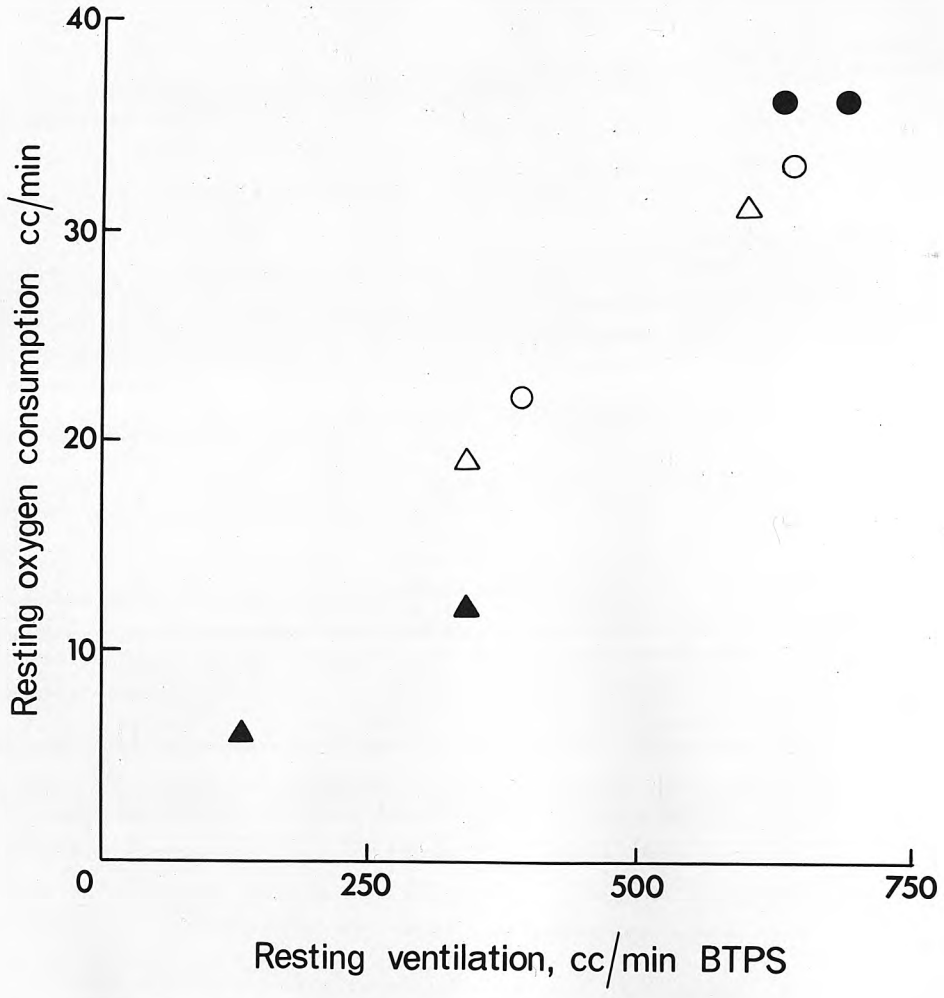


Fig. 2. Relation between resting ventilation and oxygen consumption in decerebrate ducks.

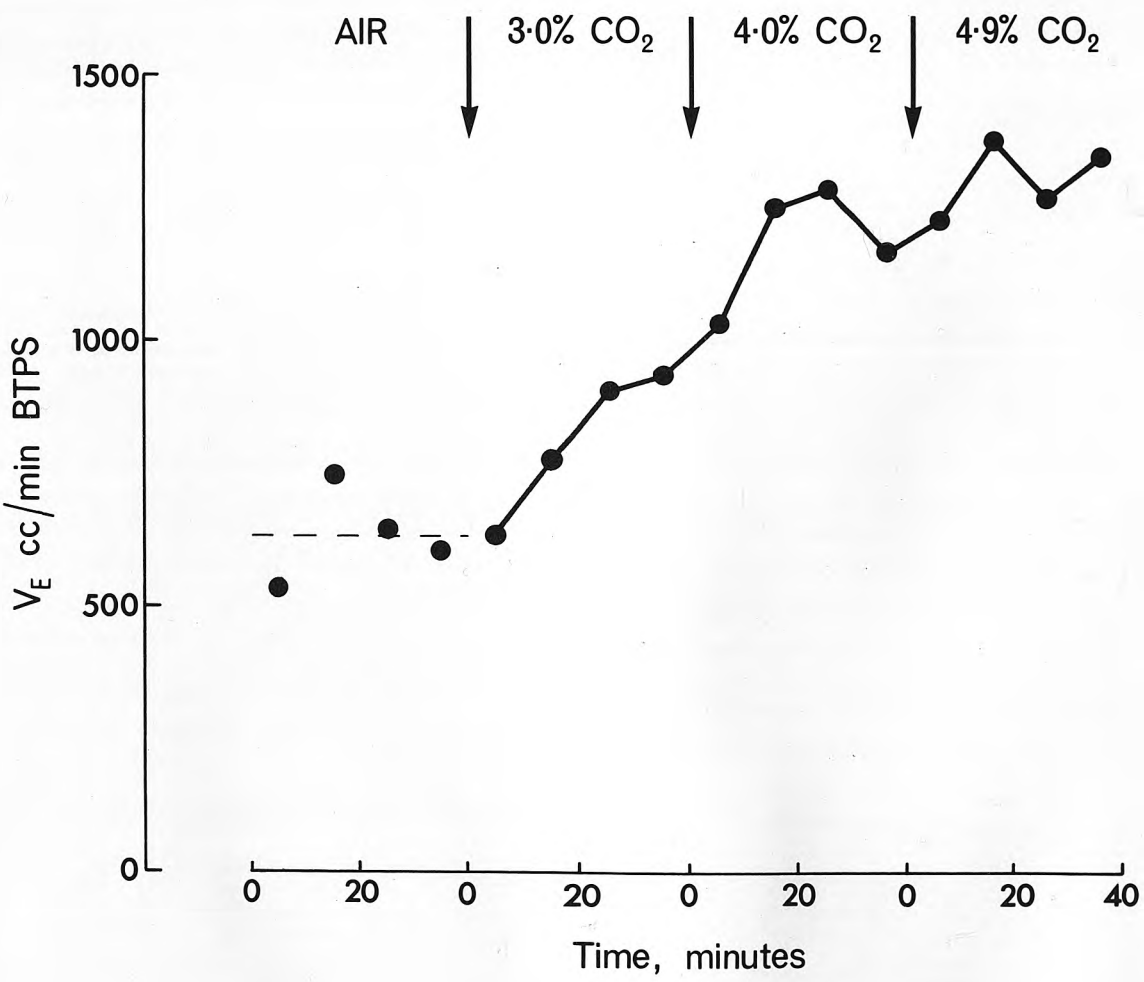


Fig. 3. Time taken for the ventilation to reach a new steady state on increasing the inspired CO_2 concentration.

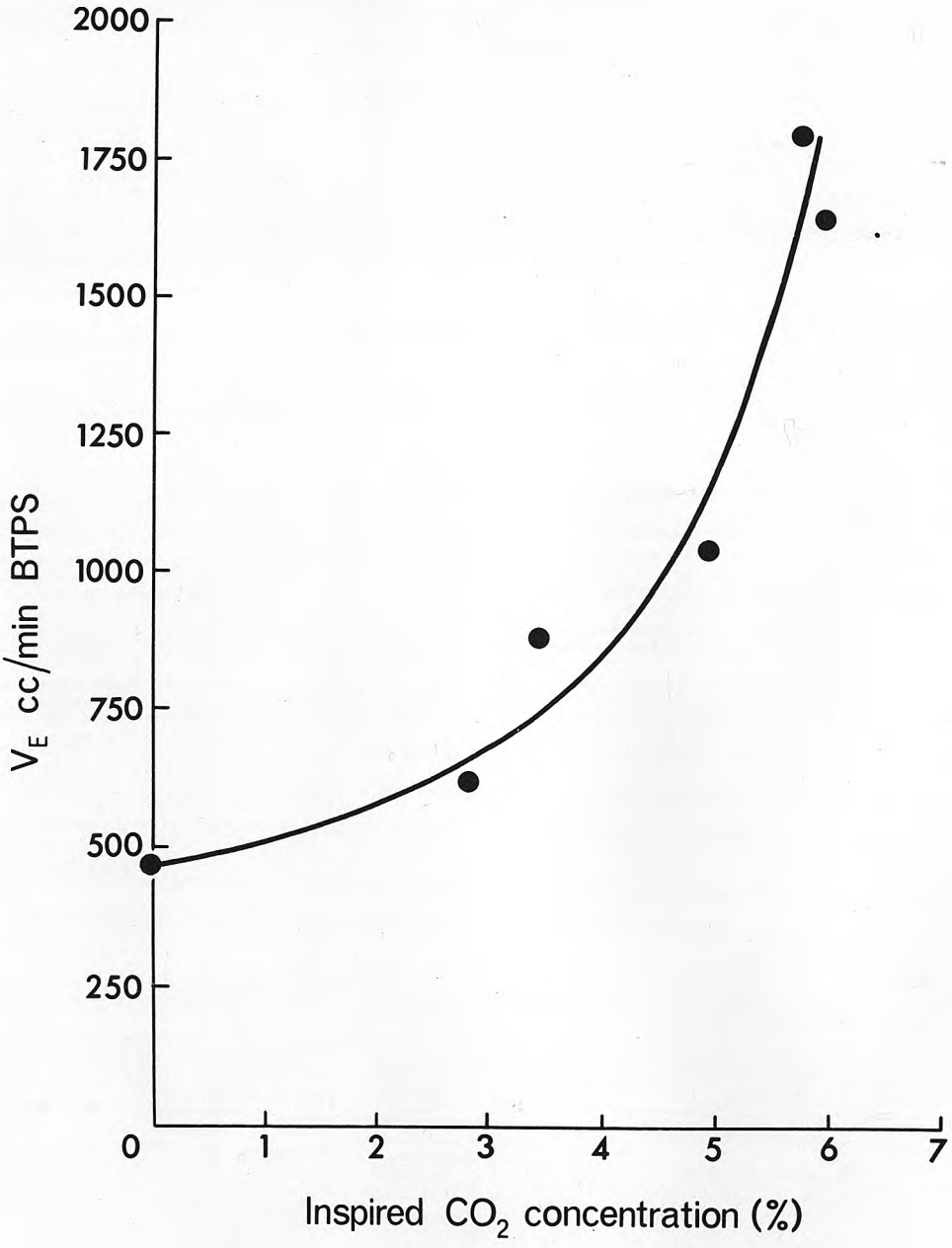


Fig. 4. Ventilatory responses to inhalation of CO_2 in the decerebrate duck (Experiment 1.).

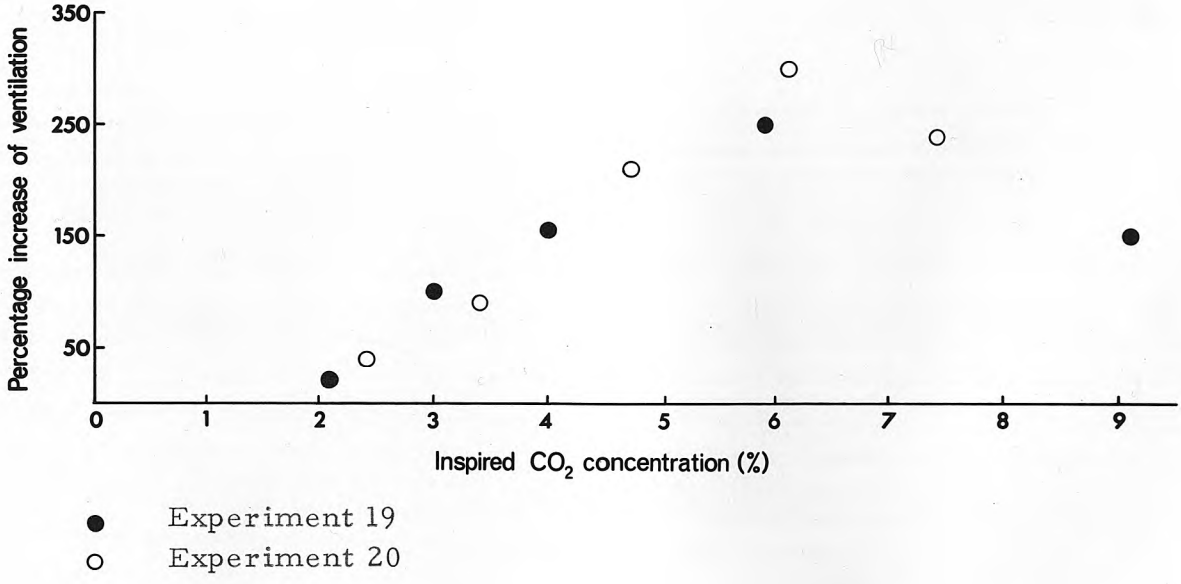


Fig. 5. The respiratory response of ducks breathing 2-9% CO_2.

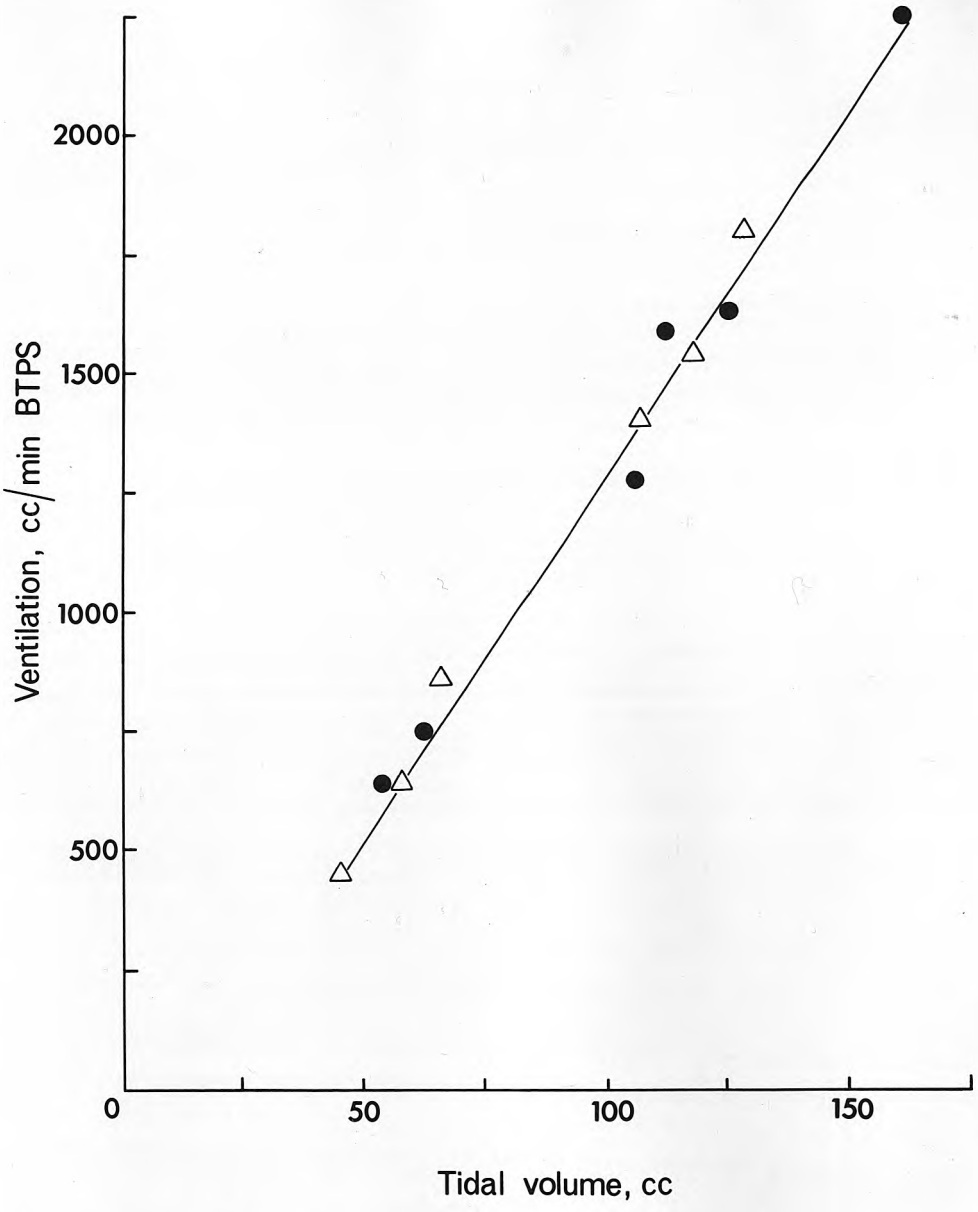


Fig. 6. Relation between pulmonary ventilation and tidal volume in 2 ducks breathing 2-9% CO_2.

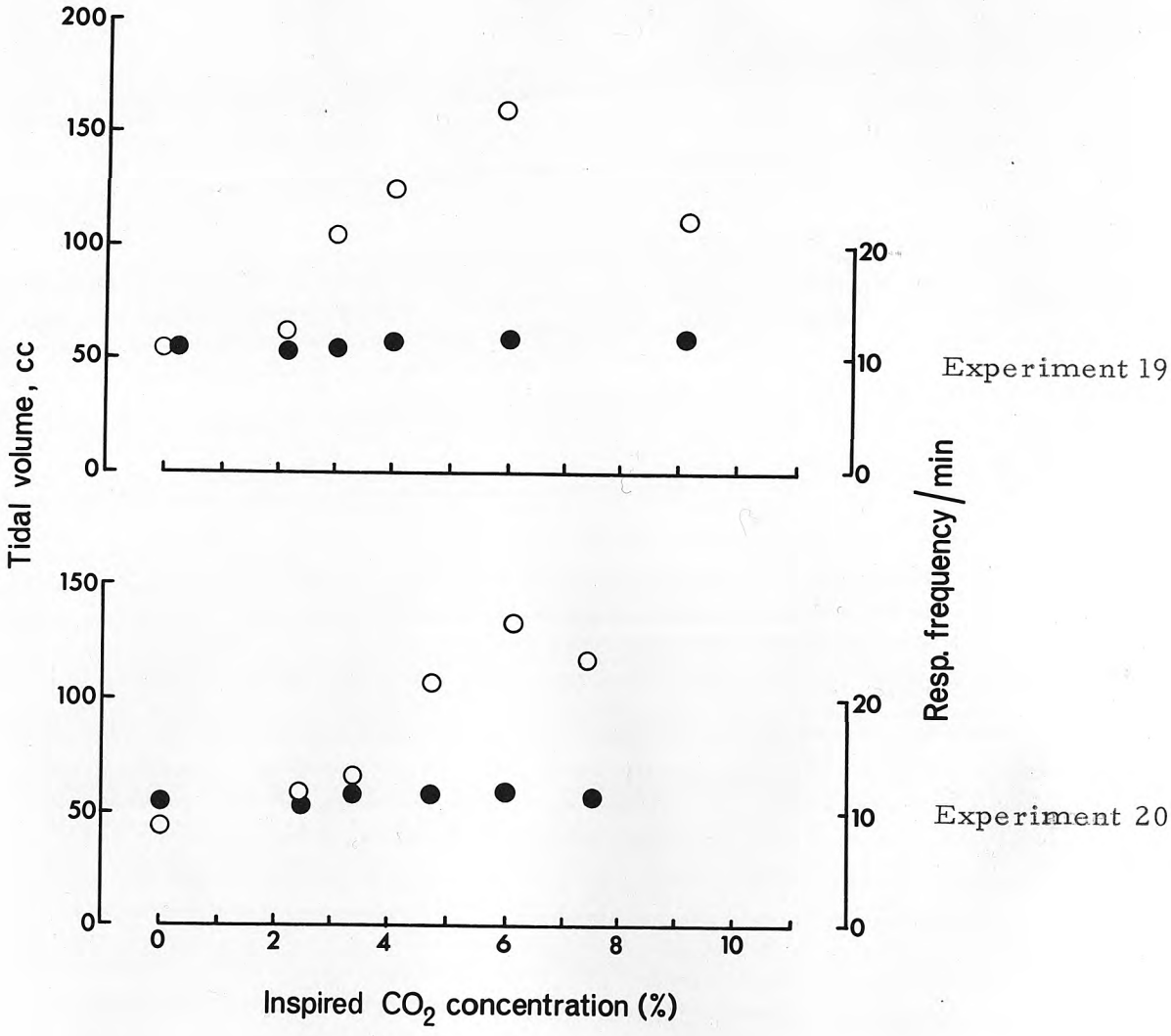


Fig. 7. The effect of CO_2 on the tidal volume and respiratory frequency.

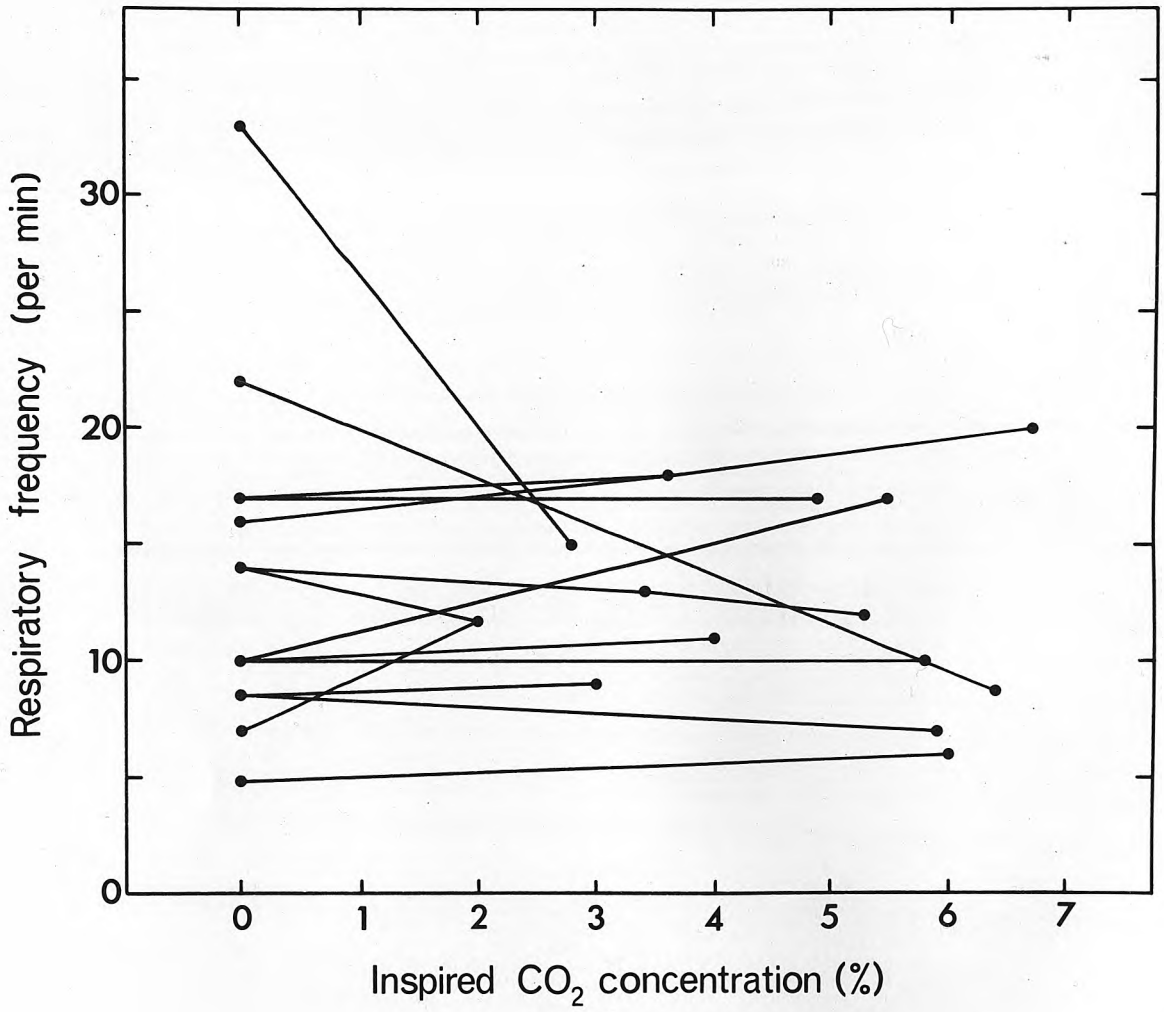


Fig. 8. The effect of 30 minutes inhalation of CO_2 on the respiratory frequency of the decerebrate duck.

Table I. Some resting respiratory data for the decerebrate duck.

	Ventilation cc/min BTPS	Respiratory frequency per min	Tidal volume cc	CO_2 production cc/min BTPS	O_2 consumption cc/min BTPS
Range	133 - 691	5 - 17	26 - 69	4 - 26	6 - 36
Mean	487	12	46	17	24
S.D.	± 174	± 4	± 12	± 8	± 11
N	10	19	10	8	8

RESULTS

I. The respiratory response to carbon dioxide

A study of the resting ventilatory values in decerebrate ducks showed a wide variation from one duck to another (Table 1).

The ducks which were less alert the day after decerebration had lower values for pulmonary ventilation and correspondingly lower oxygen uptake: a linear relationship between the ventilation and oxygen consumption was seen for all levels of resting ventilation studied (Fig. 2).

All data for the experiments are listed in Appendix 1.

A. The relationship between inspired CO_2 concentration and pulmonary ventilation.

Experiment 21 (Fig. 3) was designed to determine how long ducks took to reach a new respiratory equilibrium after they started to inspire an increased concentration of CO_2. Expired air was collected in successive 10 minute samples, particular care being taken to ensure that the duck was not disturbed and that all measurements were made with the duck at rest, if the duck struggled further measurements were postponed for 10 minutes. The successive increases in the percentage of inspired CO_2 were small so that the resulting increases in pulmonary ventilation were also small. Fig. 3 shows that in the first 10 minutes following an increase in inspired CO_2 the ventilation rose to a value near, but lower than, the 3 successive 10 minute periods. These latter 3 periods did not show any consistent variation and it could be assumed that a new steady state had been achieved

after the first 10 minutes. Fig. 3 also shows that as the percentage of inspired CO_2 rose there was a correspondingly greater effect on pulmon-:ary ventilation.

Experiment 1 (Fig. 4) shows the ventilatory response to increased inspired CO_2, in this case each concentration was inspired for 30 minutes and the samples were collected for the last 20 minutes of each 30 minute period. The curve obtained for the relationship between the inspired CO_2 concentration and ventilation is comparable to that obtained for the decere-:brate hen (Johnston & Jukes, 1966).

The increase in ventilation with increasing inspired CO_2 concentra-:tions up to 6% agreed well with the results of Andersen & Lövö (1964) in the duck. It was found that increasing the inspired CO_2 concentration slightly further reduced the ventilatory response. This was in contrast to the observation of Andersen & Lövö (1964) in which the minute volume con-:tinued to increase until the inspired CO_2 concentration was raised to 10%. The collection of expired gas volumes in their study was made in the last 3 minutes of 5 minutes inhalation of each concentration of CO_2 by which time a new respiratory equilibrium to an increased inspired CO_2 concentra-:tion is not likely to be reached.

In experiments 19 and 20 in which the ducks breathed CO_2-air mixtures ranging from 2-9%, the respiratory response was reduced when the inspired CO_2 concentration exceeded 6% (Fig. 5). In these two experiments 30 minute collections were made one minute after starting to breathe each concentration of CO_2.

This reduction in ventilatory response on raising the inspired CO_2 concentration to above 6% in the duck seems different from that in man. The experiments of Haldane & Priestley (1905) on themselves, performed in an airtight chamber, demonstrated an increase of ventilation with increased inspired CO_2 concentrations of up to 7.66%. Dripps & Comroe (1947) studied the respiratory response to inhalation of 7.6 and 10.4% CO_2 in oxygen and found average minute volume increases of 51.5 l./min and 76.36 l./min respectively. However, in this study the inhalation of CO_2 was continued until the minute volume did not vary more than 10% during four 30 second periods or until the 'subject became definitely uncomfortable'. The subjects were allowed to have a 5-10 minutes' rest breathing room air after each procedure.

B. The effect of increased CO_2 concentration on the depth and frequency of respiration.

In the decerebrate duck the increase in minute volume following CO_2 inhalation was mainly brought about by an increase in the tidal volume: the respiratory frequency tended to remain unchanged or changed only moderately. The failure of respiratory frequency to increase until high concentrations of CO_2 are inhaled has been noted in man by Haldane & Priestley (1905). These results were obtained from rebreathing experiments where the concentration of CO_2 was changing continuously. Bancroft & Margaria (1931) made observations on the effect of CO_2 in steady state experiments and found that the rate of respiration increased along with an increase of total ventilation. This observation agrees with the data of Hey, Lloyd, Cunningham, Jukes & Bolton (1966) which also show that the respiratory frequency increases with an increase of pulmonary ventilation during CO_2 inhalation or exercise.

In experiments 19 and 20 (Fig. 6) with increasing inspired CO_2 concentration varying from 2-9%, the relation between pulmonary ventila-:tion and the tidal volume remained linear up to 300% increase in ventilation observed.

In man, Hey et al. (1966) found that during CO_2 inhalation the tidal volume reached a maximum at a pulmonary ventilation of about 55 l./min and up to this level of ventilation (450% above resting) the plot of ventilation against tidal volume was linear. Thus, it seems both in man and duck on CO_2 inhalation, the depth of respiration increases in parallel with pulmonary ventilation up to high levels of ventilation.

Although inhalation of CO_2 did not much affect the respiratory frequency in the above experiments, in some cases it changed the frequency from the resting level and the effect was found to be inconsistent. Fig. 8 shows the effect of breathing different concentrations of CO_2 for 30 minutes in 15 experiments. In 7 of these experiments the frequency was increased, in 6 it was decreased and in 2 there was no change; and the change seemed to bear no relation to the concentration of CO_2.

This differs from the observations of Andersen & Lövö (1964) who found a tendency for the frequency to decrease when the percentage of CO_2 in the inspired air increased. On the whole the effect of CO_2 on the respiratory frequency seems insignificant compared to the effect of temperature from observations on hens: a definite increase in frequency occurring at an air temperature of 35°C and as the temperature increases further, the respiratory rate rises rapidly to a maximum of 160 /min at 43°C (Lee, Robinson, Yeates & Scott, 1945).

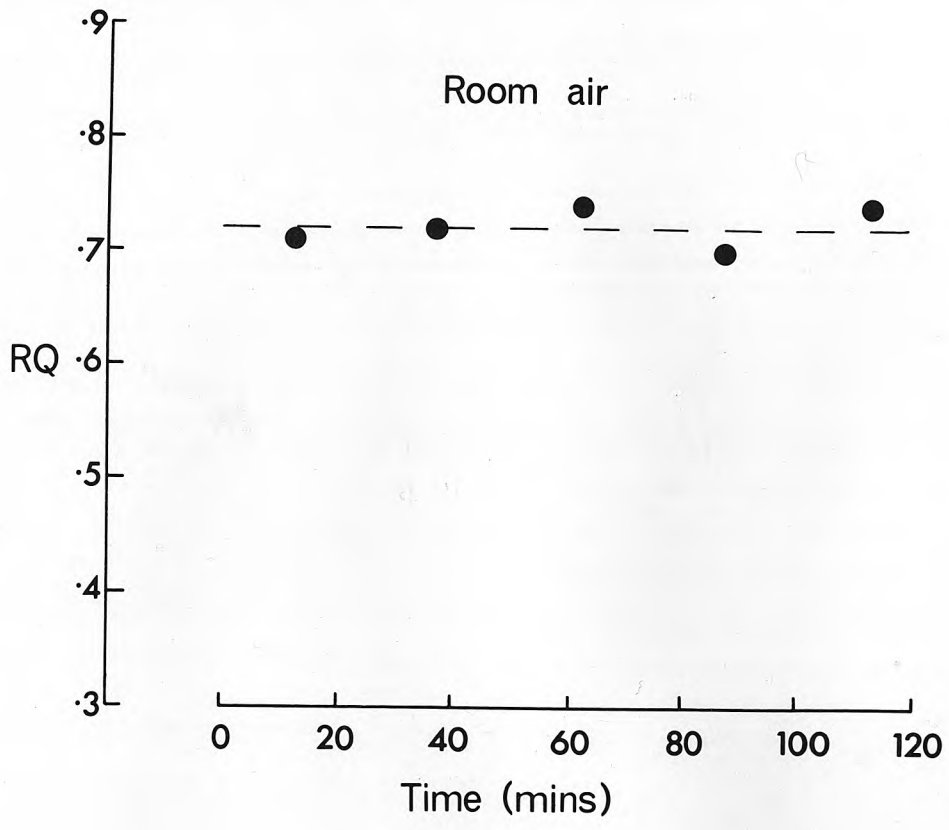


Fig. 9. The extent of variation of resting R.Q. breathing room air. (Experiment 7)

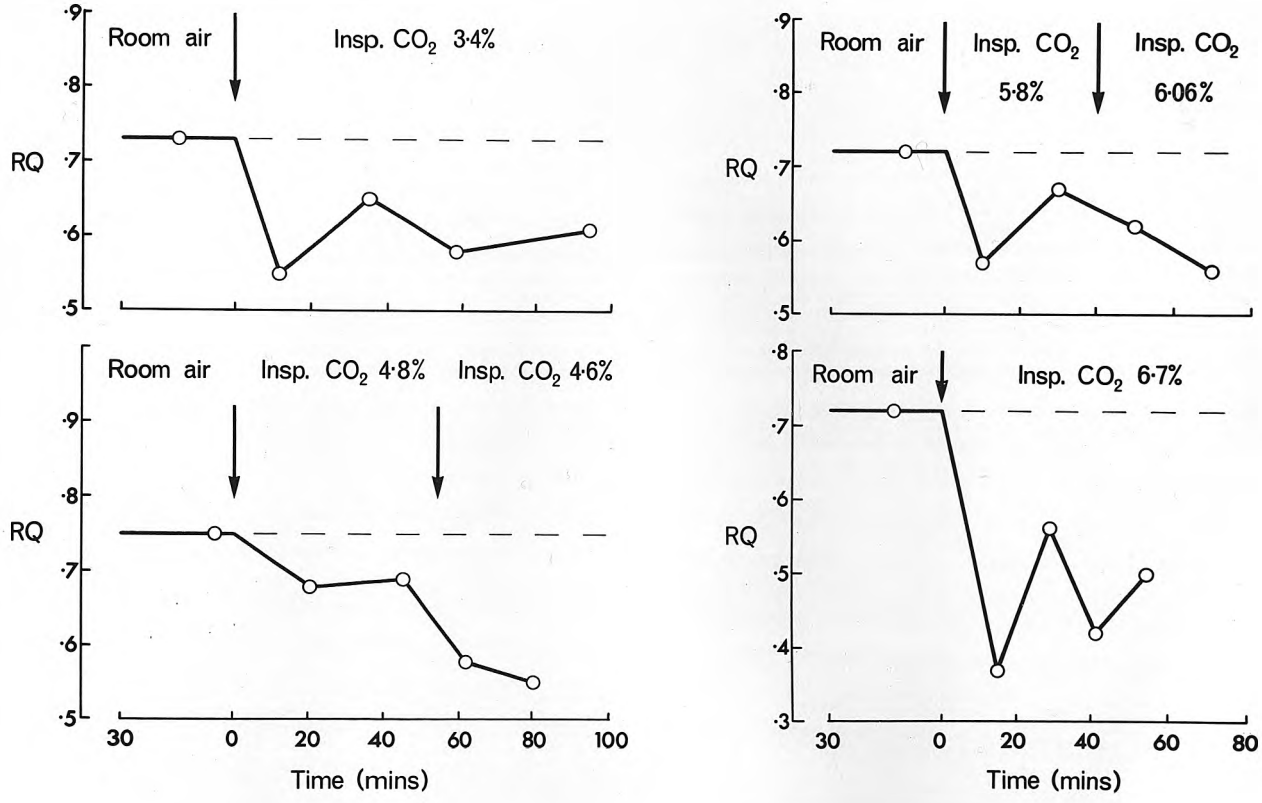


Fig. 10. R.Q. changes on CO_2 inhalation in 4 experiments on ducks with different inspired CO_2 concentrations (Experiments 2, 3, 5 and 9)

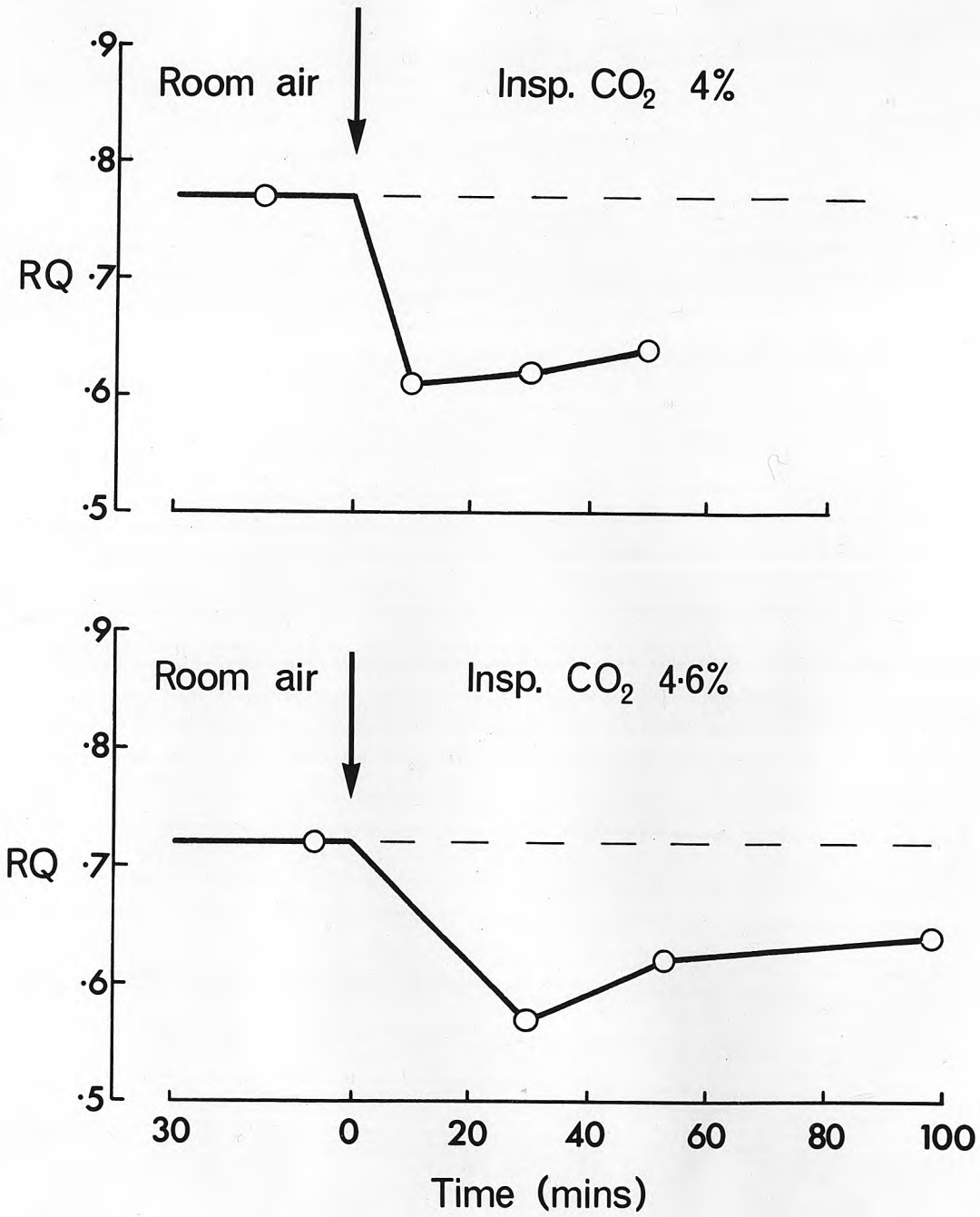


Fig. 11. Pattern of R.Q. changes on CO_2 inhalation in 2 hens.

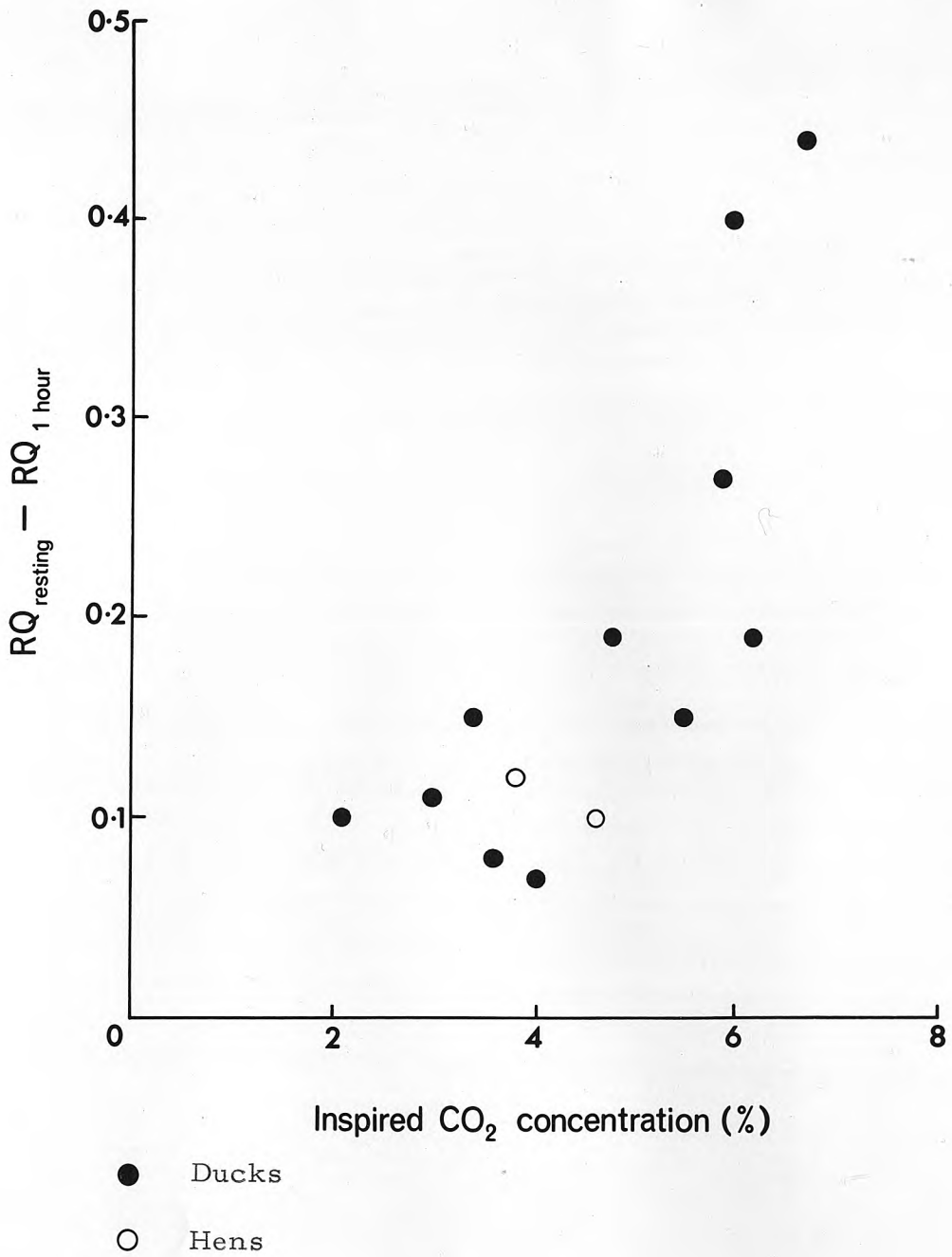


Fig. 12. The change of R.Q. from the resting level at about one hour during inhalation of different CO_2-air mixtures in ducks and hens.

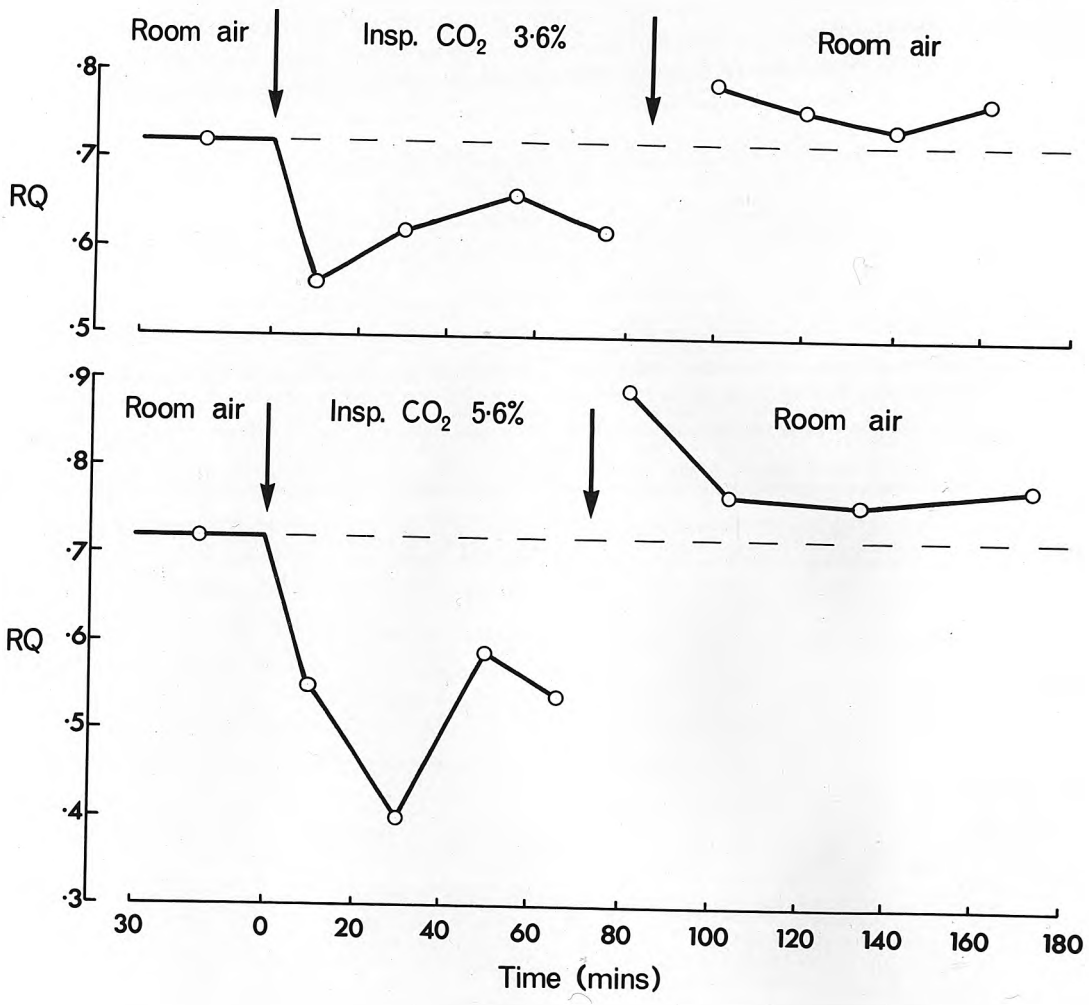


Fig. 13. Pattern of R.Q. changes during and after CO_2 inhalation for 2 ducks. (Experiments 4 and 6)

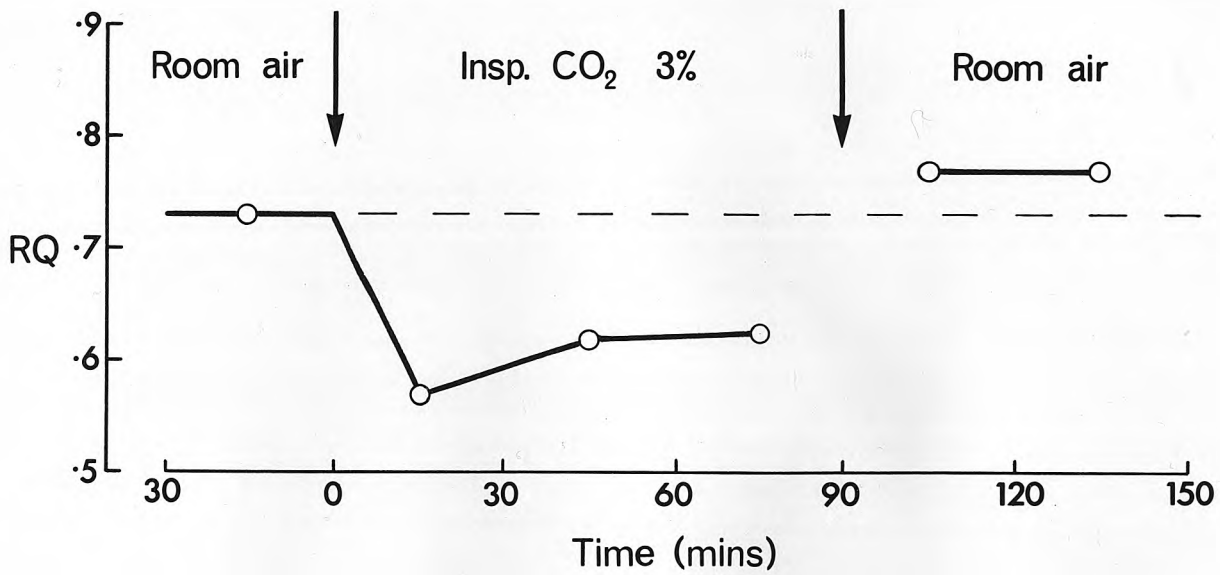


Fig. 14. An experiment in which respiratory gas exchange was measured during and after inhalation of CO_2.
139 cc of CO_2 was taken up during CO_2 inhalation
29 cc of CO_2 was recovered in the subsequent period breathing room air. (Experiment 15)

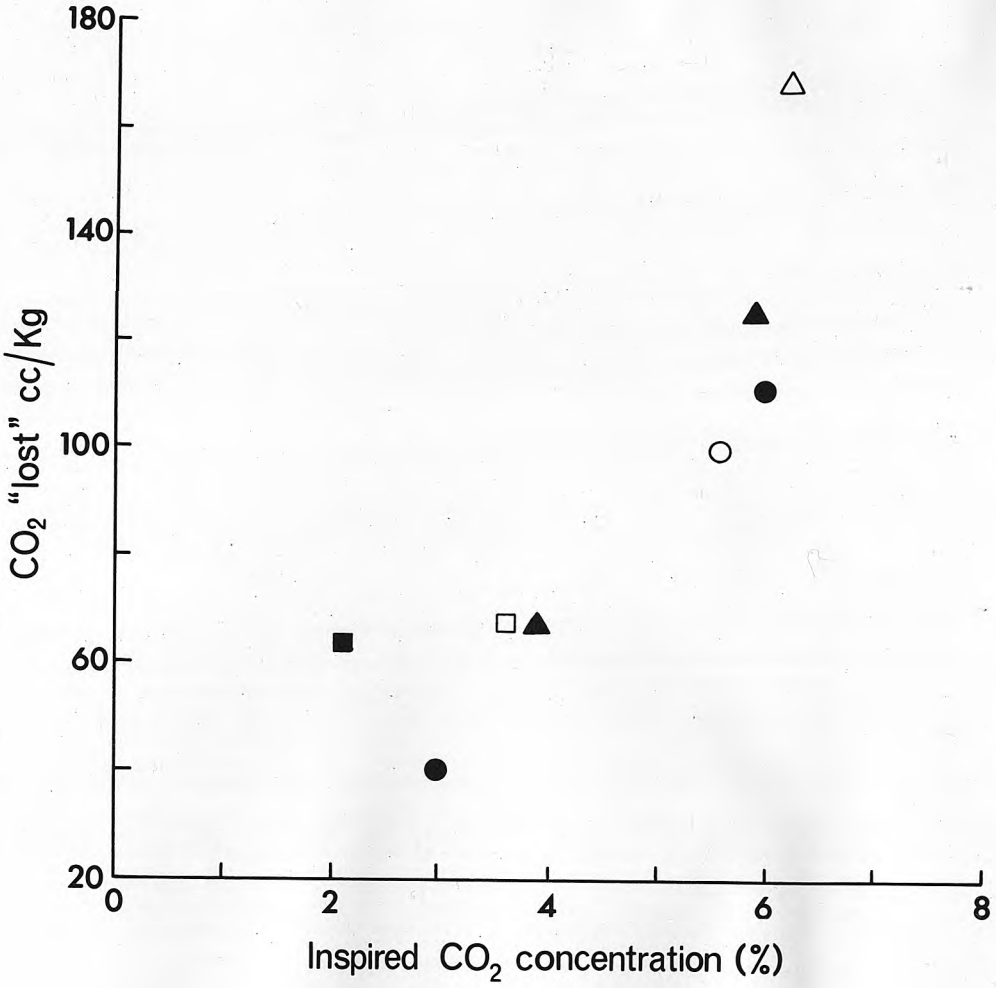


Fig. 15. The relation between inspired CO_2 concentration (%) and CO_2 accumulation by the ducks.

N.B. - The values for experiments 4 and 6 are based on assumed oxygen uptakes: see text.

Table II. The changes of body CO_2 stores during and after inhalation of CO_2 in decerebrate ducks.

Expt. No.	Wt (kg)	ICO_2 %	Duration of CO_2 inhalation.	CO_2 taken up (cc)	CO_2 recovered (cc)	CO_2 "lost" (cc per kg)
12	2.75	2.13	90 min	198	13	64
13	2.50	6.16	90 "	497	78	168
15	2.65	2.96	90 "	132	29	39
16	2.65	5.95	90 "	330	36	111
17	3.25	3.93	90 "	219	-	67
18	3.25	5.88	45 "	486	81	125
4	2.5	3.6	90 "	258	91	67
6	2.75	5.6	73 "	497	224	99

N.B. In experiments 4 and 6 respiratory gas exchange was not measured. The CO_2 store changes were calculated with values of O_2 consumptions based on the average oxygen consumption found in ducks.

II. The metabolic effects of CO_2 inhalation

A. The resting respiratory quotient.

The resting respiratory quotients measured on the morning after decerebration and in some cases on subsequent days, varied very little from one to another. All the values for the respiratory quotients are listed with the other experimental data in Appendix 1.

In 17 experiments on these 10 ducks the range was 0.67-0.74 with a mean of 0.72 and a S.D. of 0.02.

In the 2 hens studied the respiratory quotients at four and six hours after decerebration were 0.77 and 0.72.

These figures of the resting respiratory quotient are similar to that obtained by Benedict & Riddle, 1929; the fasting respiratory quotient for the chicken was 0.70 after 48 hours and for the pigeon it was 0.71-0.72 after 28 hours. According to Barott & Pringle (1946) the fasting respiratory quotient of chickens of all ages is approximately the same (0.715).

Two ducks were used (Experiments 7 and 10) to study the extent of variation in the respiratory quotient while breathing room air for a period of 120 and 100 minutes. The average respiratory quotient for experiment 7 (Fig. 9) was 0.72 and the maximal variation from this value was 0.02. In experiment 10 the average respiratory quotient was 0.73 with a similar variation. These 2 control experiments indicated that there is very little basal metabolism change over the duration of the experiments.

B. The effect of CO_2 inhalation on the R.Q.

1. The pattern of R.Q. changes during CO_2 inhalation.

During the first 20-30 minutes of CO_2 inhalation the R.Q. fell below the resting level to an extent depending on the concentration of inspired CO_2. In most cases the 60-90 minute R.Q.s rose again but always remained well below the resting level. Fig. 10 depicts the R.Q. changes during CO_2 inhalation in 4 experiments with different inspired CO_2 concentrations. In the 2 hens studied similar changes in R.Q. followed on inhalation of CO_2 (Fig. 11). In man inhalation of similar concentrations of CO_2 does not change the respiratory gas ex-:change to such an extent. Campbell, Douglas & Hobson (1914) demonstra-:ted in 3 experiments with inspired CO_2 concentrations of 4.5% that during the course of one hour the respiratory quotient did not change significantly from the resting level. In 2 other experiments breathing 3.5% CO_2 they found that although the R.Q. decreased in the first 30 minutes the R.Q. returned close to the resting level at 60 minutes.

As the R.Q. fell to an extent depending on the inspired CO_2 concentra-:tion, the difference between the resting and the one-hour R.Q. increased with increasing inspired CO_2 concentration. Fig. 12 shows the change in R.Q. from the resting value at an hour during inhalation of different CO_2-air mixtures in 11 experiments on ducks and in 2 hens.

2. Pattern of R.Q. changes during CO_2 inhalation and in the recovery period.

In all the experiments the R.Q. remained below the resting level during CO_2 inhalation, but during the first 30 minutes of the recovery period on subsequent breathing of room air the R.Q. rose above the resting value. Then the R.Q. returned to near the resting value within the next 30-60 minutes. The extent of this rise was always found to be much less than the extent of the fall in R.Q. during the period of CO_2 inhalation. Fig. 13 shows the changes in the R.Q. during and after inhalation of CO_2 for 2 experiments (Experiments 4 and 6) on the ducks breathing different concentration of CO_2.

This response in the duck is probably different from that occurring in man: results obtained by different workers (Campbell, Douglas & Hobson, 1914; Adolph, Nance & Shiling, 1929) are somewhat contradictory.

C. Changes in body CO_2 stores.

The amount of CO_2 accumulated by the body during CO_2 inhalation and the amount eliminated on subsequent breathing of room air.

Carbon dioxide lost from the inspired air on breathing CO_2-air mixtures was calculated from the difference between the amount of CO_2 accumulated by the body during CO_2 inhalation and the amount eliminated in the recovery period on subsequent breathing of room air. The CO_2 accumulated was calculated as follows:

$$CO_2 \text{ accumulated} = \text{observed } \dot{V}_{O2} \text{ (Resting - observed R.Q.)}$$

Experiment 15 (Fig. 14) is a typical experiment where the gas exchange was measured and the total amount of CO_2 lost on inhalation of 3% CO_2 for 90 minutes was calculated. 132 cc of CO_2 were accumulated during the CO_2 inhalation and 29 cc were eliminated in one hour of the recovery period. The net CO_2 'lost' being 103 cc.

The body CO_2 store changes on inhalation of varying CO_2 - air mixtures for 8 experiments are given in Table II. In experiments 4 and 6 listed in this table the gas exchange was not directly measured. The CO_2 store changes were calculated from assumed oxygen consumption based on the average value of resting oxygen consumption found in the duck and the observed difference in the R.Q. The figures used for oxygen consumption in these experiments are listed in Appendix 2 together with calculation of the changes of CO_2 stores for the other experiments.

In keeping with the relationship between the change in R.Q. and the inspired CO_2 concentration the calculated amount of CO_2 'lost' increased with the increasing inspiration CO_2 concentration (Fig. 15).

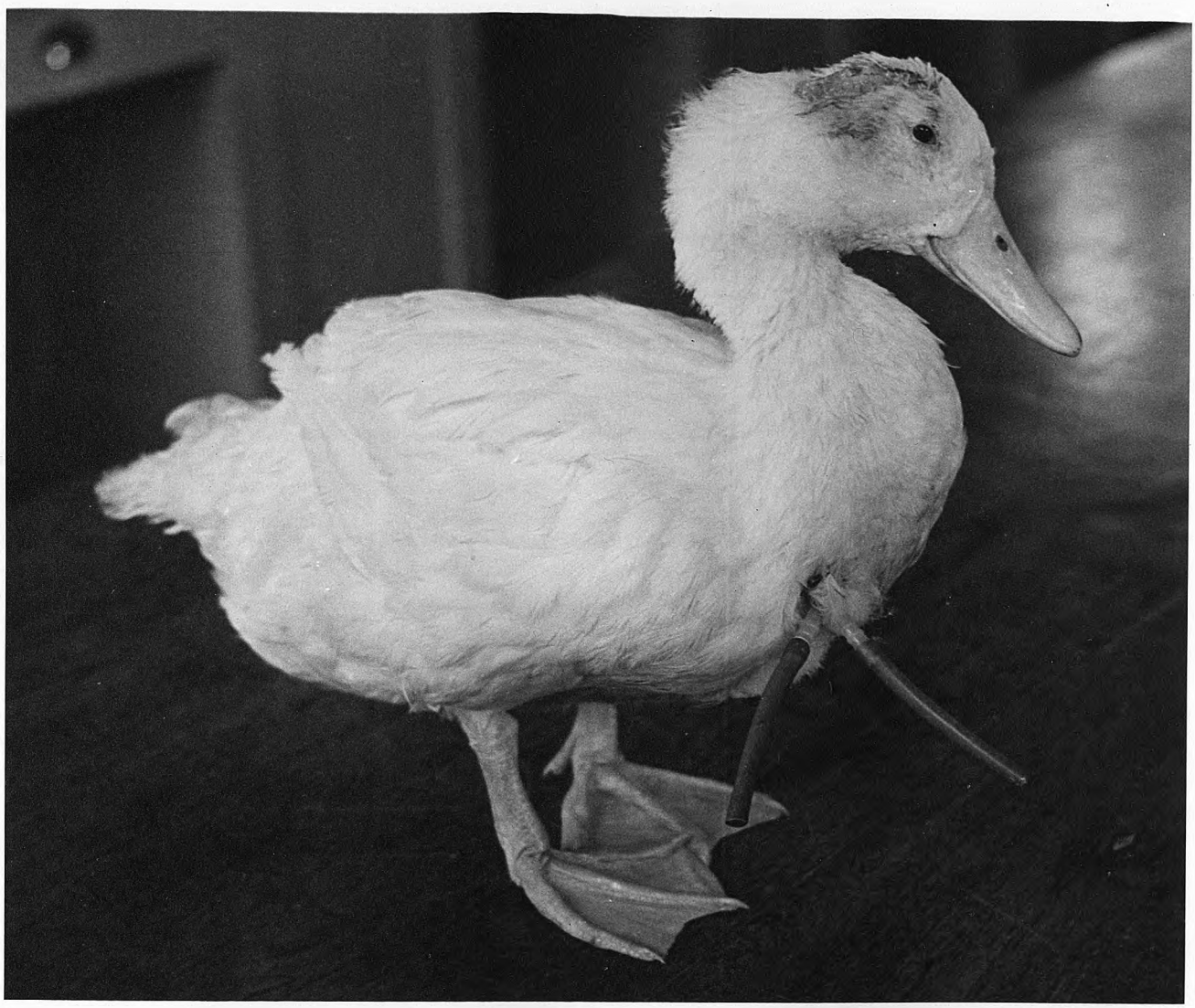


Plate 1. Duck - 4 days after decerebration

DISCUSSION

METHODS

For the purpose of studying the effect of CO_2 inhalation on pulmonary ventilation and respiratory gas exchange in the ducks, the use of conscious birds is not very satisfactory as they tend to be disturbed very easily. When conscious birds are used for such studies some physical restraint is required (King & Payne, 1964) which might increase the resting pulmonary ventilation.

Ducks were found to do very well after decerebration. Some of the ducks were in good condition even up to 6-7 days. Their postural and righting reflexes remain normal and they even walk about without running into objects. Their temperature regulation too, is not affected by decerebration. Plate 1. Picture of a duck on the 4th day after decerebration.

It is well known that decerebrate animals are able to maintain rhythmic breathing. It was observed by Lumsden (1923) that section of the cat's brain behind the posterior corpora quadrigemina, but above the pons caused no change in either rate or depth of respiration. On the other hand, when general anaesthesia is employed, it is known that sensitivity of the respiratory centre is modified. Decerebration eliminates the need for the use of anaesthesia.

Kao, Schlig & McC. Brooks (1955) have demonstrated that the slopes of the relation between ventilation and oxygen consumption at all levels of ventilation during induced exercise in conscious and decerebrate dogs were not statistically different from each other, whereas the slope for the same

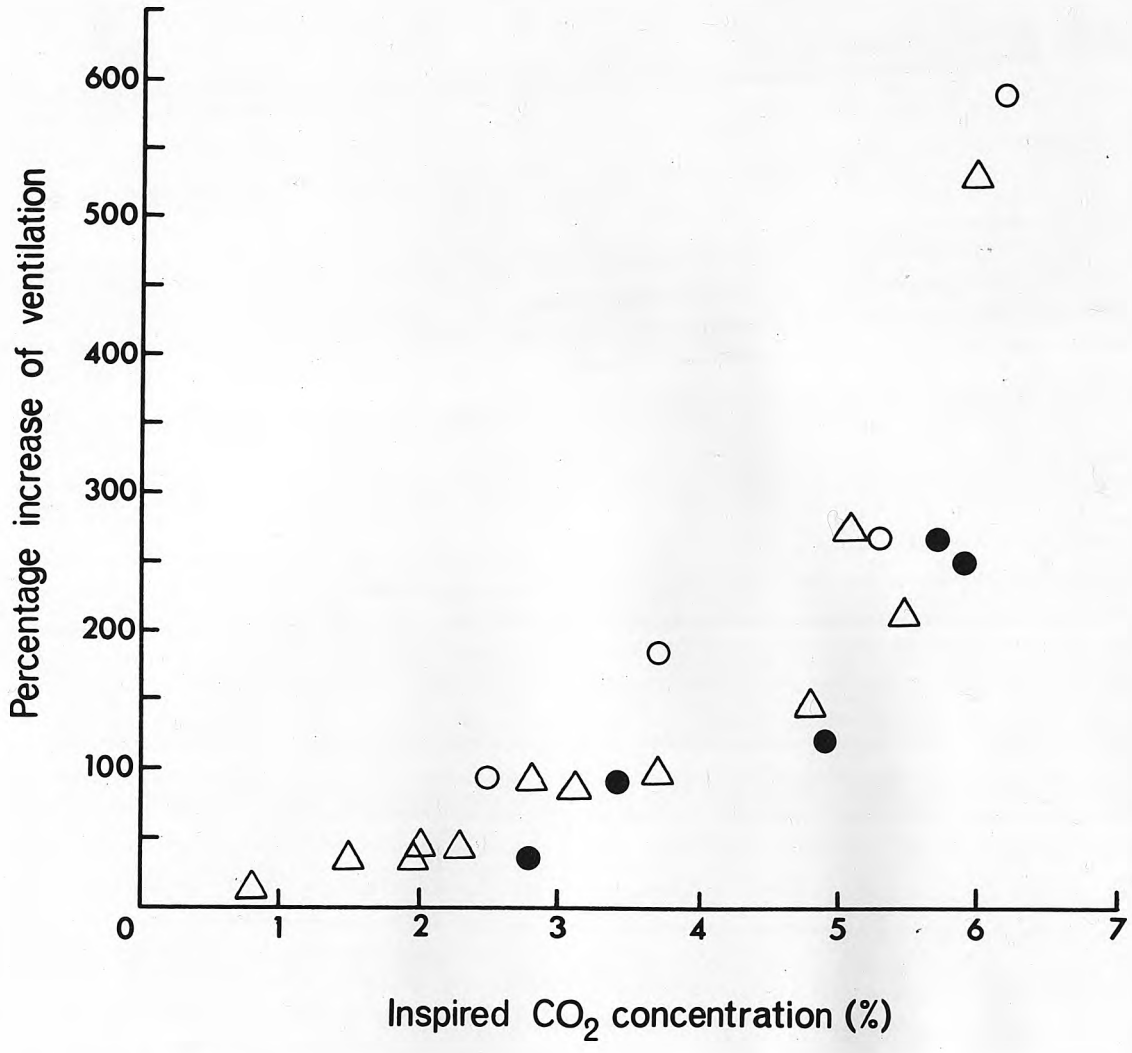
relationship in the dog under general anaesthesia was found to be variable. They also gave evidence that this variation was due to the effect of anaesthesia on the respiratory centre and not due to abnormal sensory stimuli generated in the anaesthetized dogs by the induced exercise.

It seems therefore, that to evaluate the effect of any one stimulus on pulmonary ventilation the use of a decerebrate animal preparation reduces the effect of additive sensory stimuli on the respiratory centre. As can be seen from the results of Andersen & Lövö (1964) the resting ventilatory minute volumes measured in conscious ducks are rather higher than the values for the decerebrate ducks of this study.

The range of inspired CO_2 concentration used was between 2-9%. In man when more than 10% CO_2 was breathed it produced stupefaction (Haldane, 1935) and CO_2 in high concentration (20-30%) was used as an anaesthetic before chloroform was adopted. To avoid this narcotic effect of CO_2, concentrations below 10% were used. This narcotic effect of CO_2 may well explain the apnoeic response to inhalations of 10-20% CO_2 in ducks observed by Orr & Watson (1913). To use an inspired CO_2 concentra-:tion of over 10% would seem abnormally high considering that even during 2 successive dives lasting for 30 minutes the alveolar pCO_2 of the ducks rarely rose to 15% (Andersen, 1959).

In assessing the ventilatory response to CO_2 inhalation it is important to measure the ventilatory volumes only when a new steady state is reached on inhalation of a new CO_2-air mixture. In man experiments of Douglas & Haldane (1909) had shown that some time must elapse before the respiratory centre reacted completely and steadily to any given change in alveolar CO_2 percentage. Later it was shown by Campbell, Douglas, Haldane & Hodson

(1913) that breathing becomes quite steady 10 minutes after the beginning of CO_2 inhalation. In this study it was found that during the first 10 minutes of inhalation of a new CO_2-air mixture the ventilation rate was somewhat lower than the steady state value obtained from the second 10 minutes onwards. Although quantitative measurements of ventilatory response to CO_2 inhalation were made in the ducks by Andersen & Lövö (1964) their measurements were made during the second half of 5 minutes' inhalation of each concentration of CO_2 and it seems most unlikely that their ducks ever reached a steady respiratory state.



● Duck (Experiment 1)

○ Hen (Johnston & Jukes, 1966)

△ Man (Haldane & Priestley, 1905)

Fig. 16. Comparison of respiratory response to inhalation of CO_2 in decerebrate duck, a decerebrate hen and conscious man.

RESULTS I

A.

The ventilatory response curve for inspired CO_2 of 1-6% in the decerebrate duck was found to be similar to that curve found by Johnston & Jukes (1966) in decerebrate hens breathing the same range of CO_2 concentra-:tion. It was also found that the ventilatory response to CO_2 in this diving bird was not only similar to a non-diving bird but was comparable to the response curve in man in terms of percentage increase of ventilation. Fig. 16 shows the relation of percentage increase of ventilation and the inspired CO_2 concentration in a duck, a hen and a man. The figures for the hen are from Johnston & Jukes (1966) and that of man for Haldane & Priestley (1905).

It is seen that the CO_2 response curves agree fairly well with one another up to inspired CO_2 concentration of 6%, considering that CO_2 response in the same individual may vary from time to time (Lloyd, Jukes & Cunningham, 1958), and that there is a much wider variation among individuals (Lambertsen, 1960). In fact, Schaefer (1958) observed 2 distinct groups in man as regards their ventilatory response to CO_2 inhalations. He classified subjects who responded to inhalation of 5.4% CO_2 with an increase in minute volume less than four times the basic volumes on air as belonging to the low ventilation group.

The ventilation of the ducks in this study was found to be doubled by an inspired CO_2 concentration of 3% in almost every case. This concentra-:tion of CO_2 for doubling the ventilation is much lower than that in the experiments of Andersen & Lövö (1964) on ducks. In their observations,

on the average ventilation was doubled at 7.5% inspired CO_2 concentration. Their values for resting ventilation were higher and the ventilatory response to CO_2 inhalation was much lower, because the ducks were conscious and equilibration period for each inspired CO_2 concentration was short.

Although the ventilatory response was of the same order in ducks of this series as that of hen and man for inspired CO_2 concentrations of up to 6%, raising the inspired CO_2 concentration above this was found to depress the ventilation in ducks. This depression of ventilation is quite different from man, as it is known that much higher concentrations of CO_2 are re-:quired to depress the respiration in man. In the experiments which Haldane & Priestley (1905) carried out on themselves, increasing ventila-:tion was seen with inspired CO_2 concentrations of up to 7.66% studied. Dripps & Comroe (1947) have shown that although inhalation of 7.6 and 10.4% CO_2 in man raises the average minute volumes to 51 l. and 76 l. respectively there are wide individual differences. Only 2 of their 31 subjects could tolerate 10.4% CO_2 for five minutes. Definite depression of respiration in man to inhalation of 12.4% CO_2 was observed by Brown (1930).

It is thus obvious that inhalation of high concentrations of CO_2 in man brings about respiratory depression but that much lower concentrations bring about such depression in the decerebrate duck. But Andersen & Lövö (1964) found that respiration in ducks was increased until the CO_2 concentration was raised above 10%. This finding again may be due to their very short exposure to each new concentration of CO_2 and thus lack of equilibration.

Depression of respiration on inhalation of CO_2 in some birds and

ducks has been known for a long time (Orr & Watson, 1913; Windle & Nelson, 1938; Hiestand & Randall, 1941). But a direct depressant action of CO_2 on the respiratory centre of these birds was called into question when CO_2 sensitivity in the nasopharyngeal region in the duck was demonstrated by Hiestand & Randall (1941) and CO_2 stimulated respiration when it by-passed the upper respiratory tract (Dooley & Koppányi, 1929; Andersen & Lövö, 1964). Although the CO_2 sensitive endings are believed to exist, so far neither their structure nor the afferent pathways are known.

It can be concluded from this study that although CO_2 administered via a low tracheostomy tube stimulated respiration in ducks as in the hen and mammals, the higher limit of CO_2 concentration causing pure stimula-:tion was much lower in the duck. Whether this phenomenon in some ways helps in the adjustment of respiration during diving is not known. Two other facts also need clarification (1) whether there is a narcotic effect on the respiratory centre of the duck at this level of 6% inspired CO_2 and (2) whether it is an inhibitory effect via the CO_2 sensitive receptors at a high blood pCO_2.

B.

It was found that the increase in minute volume on CO_2 inhalation in the decerebrate duck was brought about mainly by an increase in the tidal volume up to the observed increases in ventilation of 300%. This level of ventilation is also inside the limit observed in man within which there is a linear relationship between the ventilation and the tidal volume (Hey, Lloyd, Cunningham, Jukes & Bolton, 1966). Earlier, Haldane & Priestley (1905) had demonstrated that the respiratory frequency does not

rise significantly in man until the inspired CO_2 concentration is increased up to 5-6%. Johnston & Jukes (1966) found that in some hens no increase in respiratory frequency occurred as pulmonary ventilation increased on CO_2 inhalation and in others very little change was observed as compared to the effect of temperature on respiratory frequency.

Andersen & Lövö (1964) observed in their experiments on ducks that the respiratory frequency tended to fall with increasing inspired CO_2 concentrations but that the increase in the tidal volume well compensated this effect to bring about an increase in the minute volume. But in the present study it was found that CO_2 did not have such a consistent depressive effect on the respiratory frequency of the duck. In fact, in most of the experiments the frequency was not affected by the increased inspired CO_2.

It is known from direct electrical stimulation of the respiratory centre (Comroe, 1943) with solutions containing bicarbonate buffers and CO_2 that these chemicals bring about a prompt increase in depth or in both depth and rate of respiration. These chemical solutions used had a pCO_2 of 250 mm Hg. Perhaps the direct application of such high concentrations of CO_2 brings about an increase in the rate of respiration. Occasionally following chemical stimuli expiratory apnoeas were observed which might help to explain periods of apnoea on administration of 10-20% CO_2 by tracheal tube in the experiments of Orr & Watson.

It seems that for the hyperventilation produced in the present study using inspired CO_2 concentrations of up to 9% the increase in ventilation was well within the region where it could be affected by increasing the tidal volume. On the other hand, no consistent depression of the respiratory frequency by inhaled CO_2 was seen.

In view of the fact that CO_2 has similar effects on the basic pattern of the relation between the depth and frequency of respiration in duck, hen and man, it suggests that there is no difference in the mechanism of action of CO_2 on this aspect of respiration in the diving and the non-diving animals.

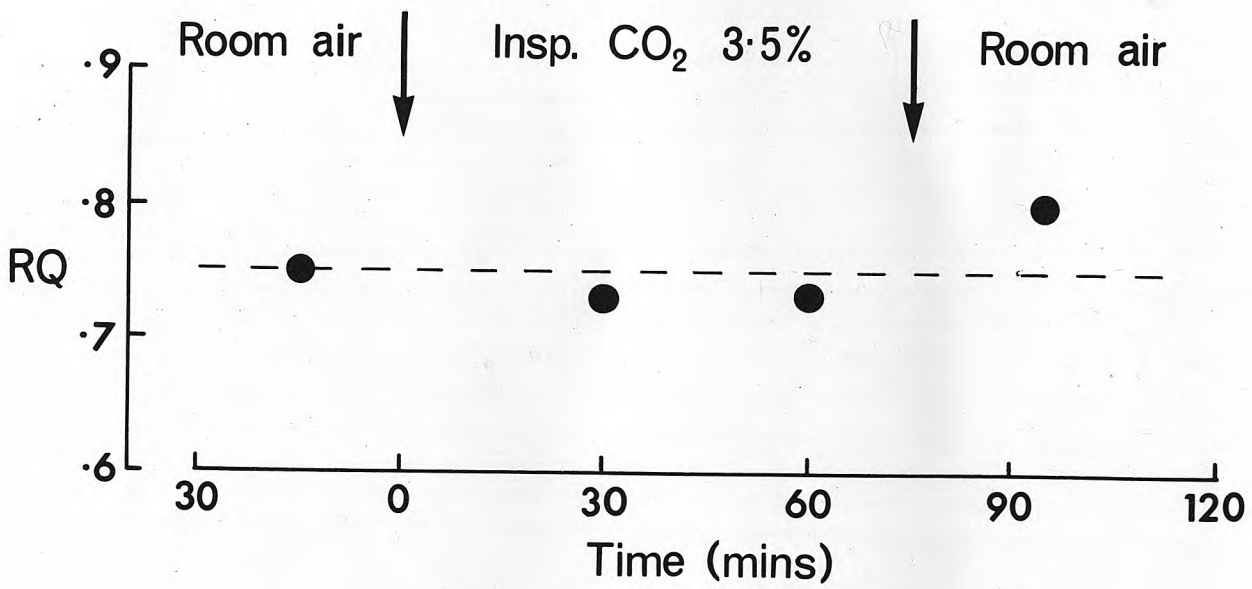


Fig. 17. Time course of R.Q. changes in man during and after inhalation of CO_2 (From Campbell, Douglas & Hobson, 1914).

Table III. The slopes of CO_2 uptake and elimination in decerebrate ducks.

Expt. No.	Body weight (kg)	Δ mm pCO_2	CO_2 uptake (cc/kg)	CO_2 recovered (cc/kg)	Slope of CO_2 uptake cc/kg/mmpCO_2	Slope of CO_2 elimination cc/kg/mmpCO_2
12	2.75	1.5	72	4.7	48	3.1
13	2.50	14	199	31.2	14	2.2
15	2.65	2	50	10.9	25	5.4
16	2.65	13	124	13.6	10	1.0
17	3.25	3	67	-	22	-
18	3.25	12	150	24.9	13	2.1
					Average 14.5	2.0

RESULTS II

The mean fasting R.Q. at rest of 0.72 in the decerebrate ducks observed in this study is the same as the fasting R.Q. of chickens of all ages (0.71-0.72) found in most studies. And, as this figure is not much lower than the fasting R.Q. of 0.75 found in man, this suggests that the ventilation in these decerebrate ducks was not depressed. From the control experiments where the resting R.Q.s did not vary from the average value by more than 0.02 at various times during 2 hours it is assumed that no true changes in metabolism occurs during the course of the experiments of CO_2 inhalation.

In the decerebrate ducks and hens on inhalation of CO_2 the R.Q. fell initially in direct proportion to the inspired CO_2 concentration. The lowest R.Q. was found in the first 30 minutes, the R.Q. rising slightly towards the resting level but remaining much below it in the next 60 minutes of CO_2 inhalation. As this response of R.Q. to CO_2 inhalation was the same in the duck and the hen, it is presumably not a peculiarity of the diving animal. This response is quite different to the observations made in man. In most of the experiments of Campbell, Douglas & Hobson (1914) breathing 3.5 and 4.5% CO_2 the R.Q. fell to a much smaller extent in the ducks or the hens in 30 minutes and the R.Q. returned to nearly the resting level by 60 minutes.

The results of R.Q. changes on CO_2 inhalation in man are varied and contradictory, not only from one study to another but, from experiment to experiment in the same study. In one of the experiments of Adolph et al. (1929) during 30 minutes inhalation of 4.3% CO_2 the R.Q. fell but it returned to a value close to the resting level within 20 minutes: in another experiment

however, in the same study the R.Q. stayed down below the resting level throughout the 30 minutes inhalation of the same concentration of CO_2. The duration of CO_2 inhalation in these experiments was short and is comparable only to the first 30 minutes in the present study and of the experiments of Campbell et al. (1914).

By comparing the extent of R.Q. change in CO_2 inhalation in the duck and that of man found in the experiments of Campbell et al. (1914) which have similar time course and equilibrations with CO_2 as this study, it is apparent that much greater quantities of CO_2 are accumulated by the duck for similar concentrations of inspired CO_2. Fig. 17 is an experiment in man showing the time course of R.Q. changes during and after inhalation of 3.5% CO_2 (from Campbell et al., 1914). By using the figures from this experiment the slope for the whole body dissociation curve at one hour was calculated and was found to be 2.3 cc/kg/mm pCO_2. The value lies within range of whole body CO_2 dissociation curve in most studies.

Farhi & Rahn (1955) obtained identical results for slopes of whole body dissociation curves from hyperventilation experiments and from CO_2 inhalation experiments in dogs. The average slope of 1.5 cc/kg/mm pCO_2 in their study agrees well with the slope of 1.78 cc/kg/mm pCO_2 observed in cats by Shaw & Messer (1930). The duration of experiments in these two studies were 40-50 minutes and 45-140 minutes respectively. Vance & Fowler (1960) in their hour long hyperventilation experiments on man obtained similar results (2 cc/kg/mm pCO_2) although much larger slopes of 11.6 cc/kg/mm pCO_2 were obtained in rats for periods of equilibrium with CO_2 lasting for 6.28 days (Freeman & Fenn, 1963). The value of the CO_2 dissociation slopes for mammals, in experiments lasting 1-2 hours lies within 1.5-3.8 cc/kg/mm pCO_2 (Rahn & Farhi, 1963).

The whole body dissociation curves for the ducks were calculated from the amounts of CO_2 retained during CO_2 inhalation and also the amounts eliminated during the recovery period. The difference in CO_2 tension in the alveolar air for the particular inspired CO_2 concentration was derived from the relation of inspired pCO_2 to alveolar pCO_2, observed in the experiments of Jukes (1958), which is similar to that given by Haldane & Priestley (1905).

Table III, columns 6 and 7 show the slopes of CO_2 uptake on CO_2 inhalation and the slopes of CO_2 elimination in the recovery period on subsequent breathing of room air in 6 experiments. The average slopes were 22 cc/kg/mm pCO_2 and 2.3 cc/kg/mm pCO_2 respectively; the dura-:tion of CO_2 inhalation varied from 45-90 minutes.

The slope of the whole body CO_2 dissociation curve calculated from the amount of CO_2 eliminated during the recovery period in the duck is of the same order as that found in most studies on mammals for experiments of similar duration. But the slope of CO_2 uptake is very much larger than the slope elimination of this study, unlike the experiments of Farhi & Rahn, (1955) on dogs when the slopes of hyperventilation experiments (1.5 cc/kg/mm pCO_2) were the same as the slope for the CO_2 inhalation experiments. From the experimental data of Irving, Ferguson & Plewes (1930) on cats, the whole body CO_2 dissociation slopes of over 10 cc/kg/mm pCO_2 can be obtained for hyperventilation or CO_2 elimination experiments, but the duration of hyperventilation or CO_2 inhalation was much longer than in this study (2-5 hours).

From the difference between the slope of CO_2 uptake and the slope of CO_2 elimination in the ducks it can be concluded that 19.7 cc/kg/mm pCO_2 of CO_2 was retained by the body; 14% of the CO_2 uptake during CO_2

inhalation was eliminated within 30-60 minutes of breathing room air. As the extra CO_2 elimination above the metabolic CO_2 production ceases within 60 minutes, a larger proportion of the retained CO_2 is not recovered. Irving et al. (1930) had shown that in cats the large amount of CO_2 retention cannot be accounted for by the increases of CO_2 in the blood, muscle and bones.

Ducks are known to possess large air sacs having total capacities of 280 cc (Sturkie, 1954) and pneumatic bones which communicate with the air sacs and the lungs (Dooley & Koppányi, 1929). But even the volume of these could far from account for the large volumes of CO_2 'lost' from the inspired air when high concentration of CO_2 are inhaled. In experiment 13 with an inspired CO_2 concentration of 6% about 170 cc of CO_2 was 'lost' during inhalation of the CO_2-air mixture for 90 minutes; 170 cc of CO_2 in the form of a 6% mixture would occupy a volume of about 2800 cc .

It is not known how the duck deals with this large volume of retained CO_2 which cannot be accounted for by the capacities of the body tissues. The extent to which the kidney helps in eliminating the extra CO_2 is not certain. Normally, the intracellular and interstitial pCO_2 of kidney is found to be the same as the pCO_2 of plasma (Kennedy, 1960). During in-:duced respiratory acidosis by CO_2 inhalation of pCO2 of the renal tissue as well as the filtrate is likely to rise along with the raised blood pCO_2 and thus eliminating some amount of retained CO_2. However, the quantity of CO_2 which might be eliminated by this means is not known. An investiga-:tion measuring the rise of urinary pCO_2 during CO_2 inhalation would help to shed some light on the role of the kidney in dealing with the retained CO_2.

The results of the present investigation suggest that inhalation of CO_2 has a much greater effect on the body stores of CO_2 in the duck than in mammals according to the results of most studies. It is believed that measurement of the urinary pCO_2 during CO_2 inhalation would indicate whether the loss of CO_2 via the kidney could account for the larger quantity of CO_2 retained by the duck.

SUMMARY

1. The effect of CO_2 inhalation on respiration of decerebrate ducks has been investigated.

2. The respiratory centre took about 10 minutes to achieve steady-state on inhalation of a new CO_2-air mixture when the change in inspired CO_2 was small.

3. Inhalation of CO_2 stimulated the pulmonary minute volume with inspired CO_2 concentrations of up to 6%. Pulmonary ventilation was doubled at inspired CO_2 concentration of 3%.

4. The pulmonary minute volume was depressed on increasing the inspired CO_2 concentration above 6%.

5. Inhalation of CO_2 had little effect on the respiratory frequency.

6. Pulmonary ventilation increased mainly by the increase of the tidal volume. A linear relationship of the ventilation of tidal volume was obtained for increase of ventilation up to 300%.

7. On CO_2 inhalation the R.Q. fell in [redacted] proportion to the concentra-:tion of inspired CO_2.

8. The extent of changes of R.Q. was similar to that observed in hen but much greater changes than that found in man.

9. The CO_2 uptake curve of 4.5 cc/kg/mm pCO_2 and CO_2 elimination curve of 2.0 cc/kg/mm pCO_2 was obtained.

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APPENDIX 1

TABLES OF EXPERIMENTAL DATA

Explanation of column headings

t = Time in minutes, from the start of inhalation of each concentration of CO_2.

$ICO_2\%$ = Percentage by volume of inspired CO_2.

f = Respiratory frequency per minute

$\dot{V}_E$ = Ventilation per minute, cc/min, BTPS.

TV = Tidal volume, cc [redacted], BTPS.

$\dot{V}_E$, % incr = Percentage increase of ventilation above resting level.

$\dot{V}_{CO_2}$ = Difference between expired and inspired CO_2, cc/min.

$\dot{V}_{O_2}$ = Difference between inspired and expired O_2, cc/min.

R.Q. = Respiratory quotient.

Duck No. 1 Weight 1.9 kg

Experiment No. 1 Post-operative day 0

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_E$ % incr.	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-31 - -12	0.03	33	468	14		12.3	16.0	0.77
2	40 - 49	2.8	15	621	41	33	7.9	16.0	0.50
3	45 - 60	3.4	32	878	27	88	8.9	14.3	0.61
4	38 - 50	4.92	37	1042	28	123	5.8	12.9	0.45
5	9 - 17	5.74	45	1792	40	283	6.8	12.8	0.50
6	10 - 16	5.92	41	1635	40	249	11.2	16.8	0.66

Duck No. 2 Weight 2.75 kg

Experiment No. 2 Post-operative day 1

Sample	t	$ICO_2\%$	f	R.Q.
1	-31 - -1	0.03	15	0.73
2	1 - 22	3.4	13	0.55
3	28 - 43	3.4	14	0.65
4	50 - 65	3.4	15	0.58
5	86 - 107	3.4	14	0.61

Duck No. 3 Weight 3.0 kg

Experiment No. 3 Post-operative day 1

Sample	t	ICO_2%	f	R.Q.
1	-33 - -17	0.03	17	0.74
2	-5 - 20	4.93	17	0.59
3	28 - 43	4.93	17	0.66

Duck No. 4 Weight 2.5 kg

Experiment No. 4 Post-operative day 1

Sample	t	ICO_2%	f	R.Q.
1	-68 - -38	0.03	15	0.72
2	1 - 21	3.59	18	0.56
3	21 - 41	3.59	18	0.62
4	45 - 65	3.59	18	0.66
5	66 - 86	3.59	-	0.62
6	91 - 111	0.03	-	0.79
7	111 - 131	0.03	-	0.76
8	132 - 152	0.03	-	0.74
9	153 - 173	0.03	16	0.77

Duck No. 5 Weight 2.25 kg

Experiment No. 5 Post-operative day 1

Sample	t	ICO_2%	f	R.Q.
1	-24 - -9	0.03	16	0.72
2	0 - 11	6.73	20	0.37
3	12 - 27	6.73	-	0.56
4	27 - 37	6.73	-	0.42
5	40 - 50	6.73	-	0.50
6	55 - 60	6.73	26	0.28

Duck No. 6 Weight 2.75 kg

Experiment No. 6 Post-operative day 1

Sample	t	ICO_2%	f	R.Q.
1	-30 - -0	0.03	12	0.72
2	1 - 21	5.55	17	0.55
3	21 - 39	5.55	-	0.39
4	39 - 59	5.50	18	0.59
5	59 - 74	5.50	-	0.54
6	74 - 95	0.03	-	0.89
7	95 - 125	0.03	11	0.77
8	125 - 155	0.03	-	0.76
9	165 - 190	0.03	-	0.78

Experiment No. 7 Post-operative day 3

Sample	t	ICO_2%	f	R.Q.
1	0 - 20	0.03	15	0.71
2	20 - 40	0.03	15	0.72
3	40 - 60	0.03	15	0.74
4	60 - 80	0.03	-	0.70
5	80 - 100	0.03	12	0.74

Duck No. 7 Weight 3.5 kg

Experiment No. 8 Post-operative day 1

Sample	t	ICO_2%	f	R.Q.
1	-35 - -11	0.04	17	0.71
2	1 - 20	5.26	12	0.57
3	20 - 40	5.26	10	0.53
4	40 - 60	5.28	9	0.27
5	60 - 80	5.28	10	0.65
6	80 - 100	5.27	10	0.67

Experiment No. 9 Post-operative day 1

Sample	t	ICO_2%	f	R.Q.
1	-20 - 0	0.03	10	0.72
2	1 - 21	5.8	10	0.57
3	21 - 41	5.8	11	0.67
4	42 - 62	6.06	11	0.62
5	62 - 82	6.06	10	0.56

Experiment No. 10 Post-operative day 2

Sample	t	ICO_2%	f	R.Q.
1	0 - 20	0.03	16	0.71
2	20 - 40	0.03	16	0.75
3	40 - 60	0.03	16	0.73
4	60 - 80	0.03	16	0.73
5	80 - 100	0.03	13	0.72

Duck No. 8 Weight 2.75 kg

Experiment No. 11 Post-operative day 1

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-30 - 0	0.03	14	632	45	24.2	36.0	0.67

Experiment No. 12 Post-operative day 2

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-31 - -1	0.03	10	691	69	26.4	35.7	0.74
2	1 - 31	2.13	12	762	64	21.6	33.2	0.65
3	31 - 61	2.13	8	677	85	19.4	30.1	0.64
4	62 - 92	2.13	8	813	102	25.0	38.0	0.67
5	93 - 123	0.03	7	363	53	16.0	21.8	0.76
6	124 - 154	0.03	7	447	63	17.5	25.4	0.69
7	154 - 184	0.03	10	379	38	16.0	23.2	0.68

Duck No. 9 Weight 2.5 kg

Experiment No. 13 Post-operative day 1

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_E$ % incr.	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-30 - 0	0.09	17	642	39		23.6	33.4	0.71
2	1.5 - 31.5	6.16	9	1360	151	112	18.2	35.4	0.52
3	31.5 - 61.5	6.16	14	1712	122	167	18.5	31.8	0.58
4	62 - 92	6.14	15	1633	109	154	13.7	27.6	0.50
5	93 - 125	0.09	20	717	36	12	23.5	29.5	0.80
6	125 - 155	0.09	21	616	29	-4	18.9	26.8	0.70
7	155 - 185	0.09	24	232	10	-	6.9	10.5	0.65

Experiment No. 14 Post-operative day 3

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_E$ % incr.	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-30 - -10	0.09	10	339			15.3	22.1	0.69
2	11 - 31	3.01	14	1002	72	151	19.1	30.5	0.682
3	3 - 23	4.52	10	854	85	114	11.7	22.2	0.53
4	1 - 21	4.99	10	1066	107	167	13.2	31.9	0.42

Duck No. 10 Weight 2.65 kg

Experiment No. 15 Post-operative day 1

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_E$ % incr.	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-30 - 0	0.10	8	343	43		8.5	11.6	0.73
2	1 - 31	2.96	9	857	95	150	7.5	13.0	0.57
3	31 - 61	2.96	10	667	67	94	5.9	9.5	0.62
4	61 - 91	2.96	9	869	96.5	153	8.0	13.0	0.62
5	91 - 121	0.10	6	264	44	-23	6.7	8.7	0.77
6	121 - 151	0.10	8	528	66	54	11.9	15.4	0.77

Experiment No. 16 Post-operative day 3

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_E$ % incr.	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-30 - 0	0.10	5	133	26		3.8	5.5	0.68
2	1 - 31	5.95	6	413	69	210	1.1	10.5	0.10
3	31 - 61	5.59	4	287	72	116	1.5	5.1	0.29
4	61 - 91	5.95	4	455	114	242	2.8	8.3	0.33
5	91 - 121	0.10	5	170	54	28	5.8	6.9	0.84
6	121 - 151	0.10	5	146	29	10	4.4	6.3	0.70

Duck No. 11 Weight 3.25 kg

Experiment No. 17 Post-operative day 1

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_E$, % incr.	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-30 - 0	0.04	10	604	60		22.7	30.6	0.74
2	1 - 31	3.93	12	1338	112	122	24.0	35.2	0.68
3	31 - 61	3.93	14	1476	105	144	23.9	35.6	0.67
4	61 - 91	4.04	14	1537	110	154	22.0	33.2	0.66
5	91 - 121	0.04	10	593	59	-2	21.2	29.8	0.71
6	121 - 161	0.04	10	390	39	-35	15.2	21.5	0.70

Experiment No. 18 Post-operative day 2

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_{CO_2}$	$\dot{V}_{O_2}$	R.Q.
1	-30 - 0	0.04	9	338	38	14.0	19.8	0.71
2	1 - 16	5.88	7	784	112	15.4	34.0	0.45
3	16 - 31	5.88	10	1108	111	13.0	35.7	0.36
4	31 - 46	5.88	10	1077	108	18.0	41.2	0.44
5	46 - 76	0.04	6	490	82	23.3	29.0	0.80
6	76 - 106	0.04	8	320	40	13.5	19.6	0.69

Duck No. 12 Weight 3.0 kg

Experiment No. 19 Post-operative day 1

Sample	t	ICO_2%	f	$\dot{V}_E$	TV	$\dot{V}_E$, % incr.
1	- 29 - 0	0.03	12	644	54	
2	1 - 31	2.1	12	754	63	17
3	1 - 31	3.0	12	1277	106	98
4	1 - 31	4.0	13	1628	125	153
5	1 - 31	5.9	14	2254	161	250
6	1 - 31	9.1	14	1585	113	146

Duck No. 13 Weight 2.75 kg

Experiment No. 20 Post-operative day 1

Sample	t	ICO_2%	f	$\dot{V}_E$		TV	$\dot{V}_E$ % incr.
1	-30 - 0	0.03	10	450		45	
2	1 - 11	2.4	12	527	643	58	56
3	11 - 31	2.4	11	702			
4	1 - 11	3.36	15	896	861	66	87
5	11 - 31	3.36	13	843			
6	1 - 11	4.7	16	1160	1395	107	236
7	11 - 31	4.7	13	1513			
8	1 - 11	6.1	12	1565	1793	128	324
9	11 - 31	6.1	14	1906			
10	1 - 11	7.35	13	1513	1541	118	246
11	11 - 31	7.35	13	1555			

Duck No. 14 Weight 3.2 kg

Experiment No. 21 Post-operative day 1

Sample	ICO_2%	t	$\dot{V}_E$
1	0.04	0 - 10	533
2	0.04	10 - 20	746
3	0.04	20 - 30	646
4	0.04	30 - 40	606
5	2.98	0 - 10	623
6	2.98	10 - 20	775
7	2.98	20 - 30	904
8	2.98	30 - 40	932
9	4.04	0 - 10	1028
10	4.04	10 - 20	1244
11	4.04	20 - 30	1283
12	4.04	30 - 40	1163
13	4.90	0 - 10	1225
14	4.90	10 - 20	1373
15	4.90	20 - 30	1266
16	4.90	30 - 40	1348
17	5.82	0 - 10	852
18	5.82	10 - 20	1311
19	5.82	20 - 30	1334
20	5.82	30 - 40	1212

Hen No. 1 Weight 3.6 kg

Experiment No. 1 Post-operative day 0

Sample	t	ICO_2%	f	$\dot{V}_E$	R.Q.
1	-35 - -28 + -18 - -12	0.04	52	78	0.72
2	10 - 25	4.64	-	263	0.57
3	31 - 51	4.64	-	686	0.62
4	76 - 96	4.64	60	592	0.64

Hen No. 2 Weight 2.0 kg

Experiment No. 2 Post-operative day 0

Sample	t	ICO_2%	f	R.Q.
1	30 - 0	0.04	16	0.77
2	1 - 20	3.98	21	0.61
3	20 - 40	3.98	24	0.62
4	40 - 60	3.98	30	0.65

APPENDIX 2

TABLES FOR CALCULATION OF CO_2 'LOST'

Explanation of column headings

$I_{CO_2}\%$ = Inspired CO_2 concentration (%).

R.Q.$_R$ = Resting respiratory quotient.

R.Q. (obs) = Observed R.Q. during CO_2 concentration.

Δ R.Q. = Difference between R.Q.$_R$ and R.Q. (obs).

$\dot{V}_{O_2}$ (obs) = Observed oxygen consumption.

ΔCO_2 = Difference between observed $\dot{V}_{CO_2}$ and calculated $\dot{V}_{CO_2}$.

t = Duration in minutes of each successive samples.

Total Δ CO_2 = Difference between $\dot{V}_{CO_2}$ (obs) and $\dot{V}_{CO_2}$ calculated for the duration of the experiment.

Duck No. 8 Weight 2.75 kg

Experiment No. 12

$R.Q._R = 0.72$

$ICO_2\%$	R.Q.(obs)	ΔR.Q.	$\dot{V}_{O_2}$(obs)	ΔCO_2	t	Total ΔCO_2
2.13	0.65	0.07	33.2	2.32	30	- 69.7
2.13	0.64	0.08	30.1	2.41	30	- 72.2
2.13	0.67	0.05	37.6	1.88	30	- 56.4
0.04	0.74	0.02	21.8	0.44	30	+ 13.1
0.04	0.69	0.03	25.4	0.76	30	- 23
0.04	0.68	0.04	23.2	0.93	30	- 28

Duck No. 9 Weight 2.5 kg

Experiment No. 13

$R.Q._R = 0.71$

$ICO_2\%$	R.Q.(obs)	ΔR.Q.	$\dot{V}_{O_2}$(obs)	ΔCO_2	t	Total ΔCO_2
6.16	0.52	0.19	35.4	6.7	30	- 200
6.16	0.58	0.13	31.8	4.1	30	- 123
6.16	0.50	0.21	27.6	5.8	30	- 174
0.04	0.80	0.09	29.5	2.6	30	+ 78
0.04	0.70	0.01	26.8	0.3	30	- 9

Duck No. 10 Weight 2.65 kg

Experiment No. 15

$R.Q._R = 0.73$

$ICO_2\%$	R.Q.(obs)	ΔR.Q.	$\dot{V}_{O_2}$(obs)	ΔCO_2	t	Total ΔCO_2
2.96	0.57	0.16	13.03	2.1	30	- 63
2.96	0.62	0.11	9.47	1.0	30	- 30
2.96	0.63	0.10	12.95	1.3	30	- 39
0.04	0.77	0.04	8.66	0.35	30	+ 10
0.04	0.77	0.04	15.4	0.62	30	+ 19

Experiment No. 16 $R.Q._R = 0.68$

$ICO_2\%$	R.Q.(obs)	R.Q.	$\dot{V}_{O_2}$(obs)	CO_2	t	Total CO_2
5.95	0.10	0.58	10.53	6.1	30	- 183
5.95	0.28	0.40	5.14	2.0	30	- 60
5.95	0.33	0.35	8.33	2.9	30	- 87
0.04	0.84	0.16	6.88	1.1	30	+ 33
0.04	0.70	0.02	6.26	0.1	30	+ 3

Duck No. 11 Weight 3.25 kg

Experiment No. 17

$R.Q._R = 0.74$

$ICO_2\%$	R.Q.(obs)	ΔR.Q.	$\dot{V}_{O_2}$(obs)	ΔCO_2	t	Total ΔCO_2
3.93	0.68	0.06	35.20	2.1	30	- 63
3.93	0.67	0.07	35.58	2.5	30	- 75
3.93	0.66	0.08	33.20	2.7	30	- 81
0.04	0.71	0.03	29.84	0.89	30	- 27
0.04	0.70	0.04	21.52	0.86	30	- 25

Experiment No. 18 $R.Q._R = 0.71$

$ICO_2\%$	R.Q.(obs)	R.Q.	$\dot{V}_{O_2}$(obs)	CO_2	t	Total CO_2
5.88	0.45	0.26	33.96	8.8	15	- 132
5.88	0.36	0.35	35.68	12.5	15	- 188
5.88	0.44	0.27	41.16	11.1	15	- 166
0.04	0.80	0.09	29.00	2.69	30	+ 81
0.04	0.69	0.02	19.64	0.39	30	- 12

Duck No. 4 Weight 2.5 kg

Experiment No. 4

$R.Q._R = 0.72$

Calculation of missing CO_2

assuming $\dot{V}_{O_2}$ at rest = 25 cc/min

" during CO_2 inhalation = 28 cc/min

" during recovery = 25 cc/min

$ICO_2\%$	Δ R.Q.	$\dot{V}_{O_2}$	t	Total ΔCO_2
3.59	0.16	28	20	- 90
3.59	0.10	28	22	- 62
3.59	0.06	28	23	- 39
3.59	0.10	28	24	- 67
0.04	0.07	25	20	+ 35
0.04	0.04	25	21	+ 21
0.04	0.02	25	21	+ 10
0.04	0.05	25	20	+ 25

Duck No. 6 Weight 2.75 kg

Experiment No. 6

$R.Q._R$ = 0.72

Calculation of missing CO_2

assuming $\dot{V}_{O_2}$ at rest = 25 cc/min

" during CO_2 inhalation = 33 cc/min

" during 1st recovery = 28 cc/min

" during 2nd recovery = 25 cc/min

$ICO_2\%$	$\Delta R.Q.$	$\dot{V}_{O_2}$	t	Total ΔCO_2
5.55	0.17	33	20	- 112
5.55	0.33	33	18	- 207
5.55	0.13	33	20	- 86
5.55	0.18	33	15	- 92
0.04	0.17	28	21	+ 106
0.04	0.05	25	30	+ 38
0.04	0.04	25	35	+ 35
0.04	0.06	25	30	+ 45

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