THE CARBON DIOXIDE CONTENT AND THE pH OF THE PLASMA OF CAPILLARY AND VENOUS ASSOCIATED WITH DISTURBANCES OF RESPIRATION

by

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Part II

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THE CARBON DIOXIDE CONTENT AND THE pH OF THE PLASMA OF CATTLE AND THE CHANGES ASSOCIATED WITH DISTURBANCES OF RESPIRATION

GENERAL INTRODUCTION

The most important factors regulating respiration are, in all probability, the oxygen tension and the carbon dioxide tension in the blood, the hydrogen ion concentration of the blood, the nervous centres within the central nervous system and the array of impulses impinging on these centres. These numerous factors form also the substratum out of which respiration develops. Changes are continually taking place in these factors so that the respiratory pattern at any one time is the resultant of the interplay of all these factors.

The relationship between the partial pressure of carbon dioxide (pCO_2), the carbon dioxide concentration and the plasma pH is expressed by one form of the Henderson-Hasselbalch equation

$$pH = pK + \log \frac{\text{Total } CO_2 - \alpha\, pCO_2}{\alpha\, pCO_2}$$

where pK = constant = 6.1

α = constant = 0.0301 at 37° C.

The effects of carbon dioxide were amply demonstrated many years ago by Haldane who showed that inhalation of carbon dioxide caused a marked hyperventilation. The effect of inhalation of carbon dioxide is to raise the alveolar pCO_2 and hence the arterial pCO_2. The increase in the arterial partial pressure of carbon dioxide is stimulant to respiration both centrally and to a lesser extent at the chemo-receptors of the carotid and aortic glomi. As the pCO_2 within the

blood is increased, a greater hydration of CO_2 within the plasma will occur forming more carbonic acid.

$$H_2O + CO_2 \rightleftharpoons H_2CO_3$$

The increased quantity of carbonic acid will ionize according to the equation

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

These hydrogen ions produced by the ionization of carbonic acid are buffered by the buffering systems in the plasma with a slight fall in pH and the bicarbonate ions remain in the plasma. At the same time due to the increase in arterial pCO_2 a greater quantity of carbon dioxide diffuses into the erythrocytes where buffering mechanisms are available to deal with it. The concentration of bicarbonate increases within the erythrocytes and because of the increase in concentration within the erythrocytes diffusion of bicarbonate ions takes place out into the plasma while chloride ions from the plasma replace the bicarbonate ions in the erythrocytes. Thus as a result of the increase in the pCO_2 there is an increase in the bicarbonate present in plasma and thus an increase in the total CO_2 content of the plasma while the plasma pH is lowered.

It can also be shown that by decreasing arterial pCO_2 below the normal value the total CO_2 is lowered and the plasma pH is raised and ventilation is diminished.

It has been demonstrated above that changes in the pCO_2 lead to changes in plasma pH but it has been shown by Gray (1950) that alterations in plasma pH by themselves affect respiration. A lowering of plasma pH increases ventilation while raising of the plasma pH decreases ventilation.

the site of action is uncertain, the preponderant effect is thought to be central (Hoff and Breckenridge, 1955).

Changes in the partial pressure of oxygen within the blood have also been shown to affect respiration. A lowering of the oxygen tension stimulates respiration by increasing chemo-receptor activity (Heinz, Liljestrand and Robinson, 1939; Whitelaw, Bertels and Leichnetz, 1955) but hypoxia is depressant centrally (Watt, Dunlap and Comroe, 1943). Breathing pure oxygen has been shown to decrease chemo-receptor activity by Heinz et al. (1939) and to cause a decrease in ventilation of trained dogs by Watt et al. (1943). This decrease in ventilation disappeared after de-afferentation of the chemo-receptors. In man an initial decrease in ventilation following inhalation of 100% oxygen has been observed by Bojesen, Lehmann, Reynaud and Veillon (1957), Dripps and Comroe (1947), and Asmussen and Nielsen (1955). Dripps and Comroe (1947) noted also a secondary increase in ventilation following the initial decrease. They ascribed this increase to an irritant effect of pure oxygen.

By analysis of the blood it is possible to determine the concentration in the blood of some or all of the major components affected by or affecting respiration, e.g. carbon dioxide, hydrogen ions and oxygen. This type of analysis can also be used to study alterations caused by disturbances of respiration due to disease, drugs or the administration of gas mixtures different in composition from the air normally breathed. There are many advantages in such a study in cattle provided a method of obtaining blood samples can be evolved which

will give a true picture of the concentrations of carbon dioxide and oxygen and hydrogen ion actually affecting the central nervous system and the chemo-receptors. If arterial blood samples can be obtained, these will give a true indication of the plasma concentrations of carbon dioxide and hydrogen ion concentration of the blood impinging on the respiratory centre and chemo-receptors. In certain analysis of alveolar air and expired air may not immediately reflect changes in the blood chemistry, changes liable to cause disturbances of respiration particularly where a greater than normal barrier exists in the lung across which diffusion takes place, e.g. when alveolar hyalinisation and epithelialisation have occurred. In addition although alveolar gas analysis and analysis of expired air are possible, the gas samples obtained may not be true samples due to disturbance of respiration caused by fear, handling and excitement when collecting these samples and also in the sealing by the addition of saturated mucus gas.

In the present study suitable methods of obtaining blood samples have been evolved. Using these methods of sampling, the carbon dioxide concentration of the plasma and the pH of the plasma of healthy adult cattle and calves have been determined so have the variations in those taking place in healthy cattle than at rest during the day. Studies have been made also of the alterations in these two concentrations, as a result of lung damage due to pneumonia, as a result of the central depression of respiration caused by general anaesthetics and as a result of the inhalation of mixtures of nitrogen, oxygen and carbon dioxide different in amount to those present in the normal atmosphere breathed.

For comparative purposes a study has been made of the concentrations of carbon dioxide and the pH of the plasma of normal dogs and also of the effects of anaesthesia on the plasma pH and plasma carbon dioxide content of species other than rabbits, e.g. horses, dogs and sheep.

In order to describe the disturbances of respiration observed with pentobarbitone, anaesthesia and inhalation of foreign gas mixtures measurements have been made of the ventilation and respiratory rates of cats and rabbits.

PART I

THE CARBON DIOXIDE CONTENT AND THE pH OF THE PLASMA OF NORMAL CATTLE AND THEIR PHYSIOLOGICAL VARIATIONS. COMPARATIVE DETERMINATIONS IN THE DOG AND THE VENTILATION RATE AND THE RESPIRATORY RATE OF NORMAL CATTLE.

REVIEW OF THE LITERATURE

The carbon dioxide content of the plasma or serum obtained from venous blood of cattle has been determined by a number of workers. Some of these workers have also determined the blood or plasma pH.

Anderson, Gayley and Pratt (1930) in an analysis of the blood of 53 cattle gave a mean value of 58.9 volumes per cent (25 m.moles/litre) as the plasma bicarbonate concentration. Of these 53 animals only five were over one year of age and for these five a mean value of 43.4 volumes per cent (19.2 m.moles/litre) was found, which value is low in comparison to other workers' findings. Anderson et al. stated that the samples were collected without stasis from the jugular vein in such a manner that the blood was protected from exposure to the atmosphere by a layer of liquid paraffin. They gave the method of determination of the carbon dioxide content but did not explain how the values of carbon dioxide content were converted to plasma bicarbonate since plasma pH was not determined at the same time.

Blenco (1952) in a study of the effects of thermal stress on the venous plasma carbon dioxide content and the plasma pH of two Ayrshire calves found a total carbon dioxide concentration of 69 volumes per cent (30.6 m.mole/litre) and 62 volumes per cent (27 m.moles/litre) during

the control period. He also found a plasma pH of the two calves to be 7.51 and 7.52 during the control period. For each determination a sample was taken under oil into heparinized centrifuge tubes and centrifuged immediately and the determinations then carried out. Although only two animals were sampled the values for total carbon dioxide concentration found were within the range of other authors. The pH determinations were less valid since readings were taken at room temperature and corrected to the temperature of the animal using a temperature coefficient found by Henson (1953) whose paper on temperature coefficients was concerned with the determination of temperature coefficients for the pH of human plasma and urine.

Bunge (1948) in another study of the effects of thermal stress on the blood composition of dairy cattle found a mean value of 55.3 volumes per cent (25 m.moles/litre) for the carbon dioxide capacity of the plasma of six bovine subjects when in an ambient temperature of 56-60° F. prior to exposure to thermal stress. The method of taking the blood samples was not mentioned but since carbon dioxide capacity and not content was measured sampling methods were less critical.

Craige (1947) hypothesised that the cause of hypocalcaemia in cattle was an alkalosis. He made 18 determinations on the plasma of five cows and found a mean value for the carbon dioxide concentration of plasma of these cows to be 25 m.moles/litre. He did not state how the blood

samples were handled or from what blood vessels they were obtained. On another occasion Craige, Johnson, Blackburn and Coffin (1949) made a more complete study of three cows over a period of one month. Maximum and minimum values were given for the carbon dioxide content of each cow and these were between 23 and 22 m.moles/litre. Similarly maximum and minimum values of 7.45 and 7.29 were given for plasma pH. It was not stated from which vessel the blood samples were obtained but it was stated that the samples were withdrawn without exposure to the atmosphere.

Dale and Brody (1953) in studying the effects of thermal stress on the acid base balance of four cows gave mean control values of 54.6 volumes per cent (25 m.moles/litre) for the carbon dioxide concentration and 7.32 for the blood pH. They took the blood samples from the jugular vein with minimal stasis and minimal exposure to air into oiled heparinised syringes.

Dale, Gebesdian and Brody (1954) carried out a similar study on the effects of starvation on acid base balance and gave a mean value for the carbon dioxide capacity of the plasma of four cows to be 25.4 meq./litre. This plasma was obtained from jugular venous blood. Blood pH was determined on the same four cows and a range was given of 7.29 - 7.40. They took blood samples from the jugular vein into oiled heparinised syringes with minimal stasis and minimal exposure to air.

Krapf (1941) found the carbon dioxide capacity of the venous plasma

of 119 cattle to be between 55 volumes per cent (23 m.moles/litre) and 75 volumes per cent (33 m.moles/litre) while the plasma pH was from 7.35 - 7.50. No breed, sex or age differences were noticed. His samples were withdrawn with anaerobic precautions from the jugular vein.

McSherry and Grinyer (1954) measured the carbon dioxide content and the pH of bovine serum. The samples were collected with the minimal shock of the blood from the jugular vein into test tubes in such a manner that collection was under a layer of liquid paraffin. The samples were then allowed to clot at room temperature for two hours but despite this pH values were found similar to the values found by other authors. This is not that one would expect to find according to Rosenthal (1948) who showed that the autolysis taking place in blood samples which were allowed to stand at room temperature for one hour would cause a lowering of pH by as much as 0.1 pH units. McSherry and Grinyer found a mean pH value for 86 adult cattle of 7.42 ± 0.05 and 7.44 ± 0.14 for the pH value of 20 calves. A serum bicarbonate concentration for the adults of 29.5 ± 1.96 meq./litre and for the calves of 31.03 ± 1.9 was found. The bicarbonate concentration was calculated from the total carbon dioxide content and the serum pH.

Simpson and Hayden (1935) measured the carbon dioxide content of the plasma of 25 normal cows. A mean value was determined of 63.3

volumes per cent (23 m.moles/litre) with a maximum value of 79 volumes per cent (35 m.moles/litre) and a minimum value of 54 volumes per cent (25 m.moles/litre). No description was given of the methods of collection or handling of the blood samples.

The most extensive study so far of the respiratory performance of cattle was made by Brody (1945). He conducted numerous experiments and constructed many types of spirometer for respiratory measurements. From the hundreds of results obtained he plotted a graph of the logarithm of the ventilation rate in litres per minute against the logarithm of the body weight in kilograms. From this graph he obtained a regression for all the determinations. From this regression he predicted that a calf of 50 kg. body weight would have a ventilation rate of 25 litres per minute while an adult cow of 500 kg. body weight would have a ventilation rate of 87 litres per minute.

Doyle, Patterson, Warren, Botnaller and Reynolds (1958) have published some observations on respiratory function of adult cattle but weights of animals were not given. A mean value of 82 litres per minute was found for the ventilation rate of six cows while the respiratory rate averaged 26 respirations per minute.

Many authors in studying the disturbances of respiration produced experimentally have included control measurements made prior to the experimental alteration. Mellowall, Lee, Fohrman and Anderson (1953)

when studying the effects of thermal stress on respiration gave a mean value of 83 litres per minute for the ventilation rate, 43 respirations per minute for the respiratory rate of 10 adult cows whose weight averaged 450 kg., the ambient temperature varying from 57-84° F.

Findlay (1955) carrying out similar studies on calves in an environmental temperature of 20° C. gave an initial ventilation rate of 30 litres per minute and a respiratory rate of 34 respirations per minute but did not state the weights of the calves. In a full paper Findlay (1956) stated that he studied 10 four month old calves which had a mean control ventilation rate of 44 litres per minute and a respiratory rate of 70 respirations per minute in an environmental temperature of 20° C. This respiratory rate is much above that found by other workers. Such a value for respiratory rate indicates that his calves were excited, under a heat stress or affected with pneumonia. Again the body weights of the animals were not given.

Hansson and Johannisson (1958) studying the effects of anaesthesia gave ventilation rates of 38, 56, 49, 78 and 51 litres per minute and respiratory rates of 14, 18, 17, 23 and 16 respirations per minute for the control values of five Swedish Red cattle prior to anaesthesia. These values were somewhat lower than values found by other authors which may indicate a breed variation of Swedish Red cattle.

Summarising the values of the pH and the carbon dioxide content of

the plasma of cattle obtained by other workers it appeared that the pH of the venous plasma of cattle was between 7.30 and 7.50 pH units and that the venous plasma carbon dioxide concentration was between 20 and 33 m.moles/litre. From the values obtained for the respiratory rate of normal cattle it appeared that the normal respiratory rate of cattle was between 20 and 45 respirations per minute, probably depending on the prevailing ambient temperature. It was observed that the ventilation rate varied with the size of the animal and that a 50 kg. bovine subject had a ventilation rate of about 27 litres per minute while a 500 kg. bovine subject had a ventilation rate of about 90 litres per minute.

METHODS

(1) Blood sampling

(2) Plasma carbon dioxide content

(3) Plasma pH

(4) Ventilation rate

(5) Respiratory rate

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(1) Blood sampling

Venous blood samples were obtained from both the jugular and mammary veins. The jugular veins of cattle were not obvious on inspection or palpation unless some cardiac defect led to a rise in venous pressure. It was necessary to raise the vein by digital compression below the point of puncture. With the vein raised it was entered by means of a rapid stab puncture using a one inch 16 Swedish Wire Gauge needle. Once the vein was penetrated the digital compression was removed and the blood allowed to flow out of the needle for at least 30 seconds prior to taking a sample. The other vein from which venous samples were removed was the mammary or subcutaneous

abdominal vein. This vessel was not suitable in young animals but provided a readily accessible source of blood in adult cows. Compression of this vein was not necessary. The vein was entered at the so-called "milk well" where the vein entered the abdomen one hand-breadth lateral to the xiphoid cartilage.

Blood samples approximating mixed venous samples were obtained by catheterisation of the jugular vein. An area over the upper third of the jugular vein was clipped and washed with a solution of trimethyl alkyl ammonium bromides*. Five millilitres of a sterile 5% solution of procaine hydrochloride was infiltrated beneath the skin in this area. After raising the jugular vein by means of a rope around the neck the vein was entered with a two inch number 9 British Wire Gauge needle and the rope was removed. A 40 cm. length of 2 mm. diameter sterile nylon tube was inserted into the jugular vein through the needle and the needle was removed. A small piece of rubber tubing was attached to the nylon catheter and the catheter was filled with heparinised saline to keep it patent. The rubber tube was then closed with a Kehr's clip. The catheter was palpable within the jugular vein to the root of the neck but it was not possible to ascertain the exact position of the tip of the catheter. Figure 1 illustrates the jugular venous catheter in situ.

* Cetavlon: Imperial Chemical (Pharmaceuticals) Ltd.

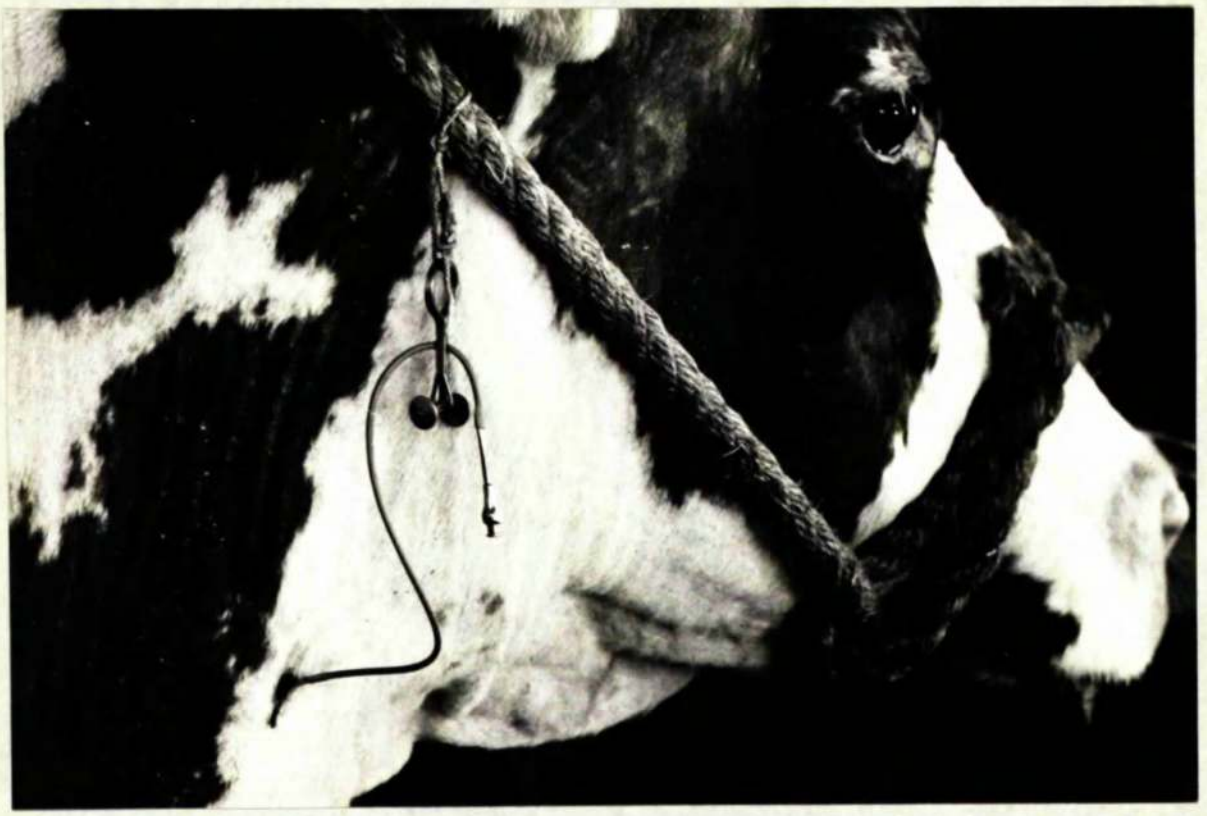

Fig. 1

Jugular catheter in situ.

To obtain mixed venous samples from the right ventricle of the human subject Cournand (1941) determined the exact position of the catheter tip radiographically. While it may have been possible to determine the position of the catheter in this manner in adult cattle the high dosage of X-rays necessary to penetrate the bovine chest was considered to constitute too great a hazard to handlers of the animals to adopt the practice.

Arterial blood samples have been obtained from cattle in many ways. Van Leersum (1923) described how in dogs it was possible, by surgical intervention, to bring the carotid arteries to a superficial position enclosed in a roll of skin. This procedure has been carried out in cattle (Jennings, 1956) but suffered the disadvantage that the loops broke down after three months. Moreover this could be carried out on experimental animals only.

Arterial blood has also been obtained from cattle by direct puncture of the carotid artery in the middle third of the neck (Sellers and Hemingway, 1951). They infiltrated the subcutaneous tissue with procaine hydrochloride and inserted the needle above the jugular vein and into the artery. However, when this method was repeated it was found that considerable restraint was necessary, and that slight movements of the neck withdrew the needle from the artery.

Swedish workers Hansson and Obel (1958) have described how they effected entrance into the abdominal aorta of cattle. They used a special needle 30 cm. in length, the last 8 cm. of which was curved and of 1.6 mm. external diameter for actual entrance into the artery, while the remainder of the shaft was of 10 mm. diameter. With the tip of the needle protected by a rubber finger shield the needle was inserted into the scrotum, through the rectal wall into the abdominal aorta. This method was not used in the present study.

In the present study it was found possible to obtain arterial blood samples from cattle of all ages by puncture of the brachial artery at the root of the neck (Fisher, 1956). The animal to be sampled was tethered by means of a halter so that its head was held slightly upward and to the right. The hand of the operator was placed under the point of the shoulder as shown in figure 2 and the brachial artery was palpated at the point where it crossed the first rib.

Fig. 2

Palpation of the brachial artery

In thin cows the brachial artery was easily found and rolled between the fingers and the first rib but in fat, thick-necked animals it was not possible even to feel the pulse at this point.

The proposed puncture site was infiltrated with a sterile 5% solution of procaine hydrochloride. A four inch 15 British Wire Gauge, long pointed needle was directed into the cow at a point just medial to the second finger as shown in figure 2, parallel to the long axis of the cow and at about 15° to the horizontal. The needle was inserted to a depth of one to three inches in order to effect entrance to the artery. Figure 3 illustrates this.

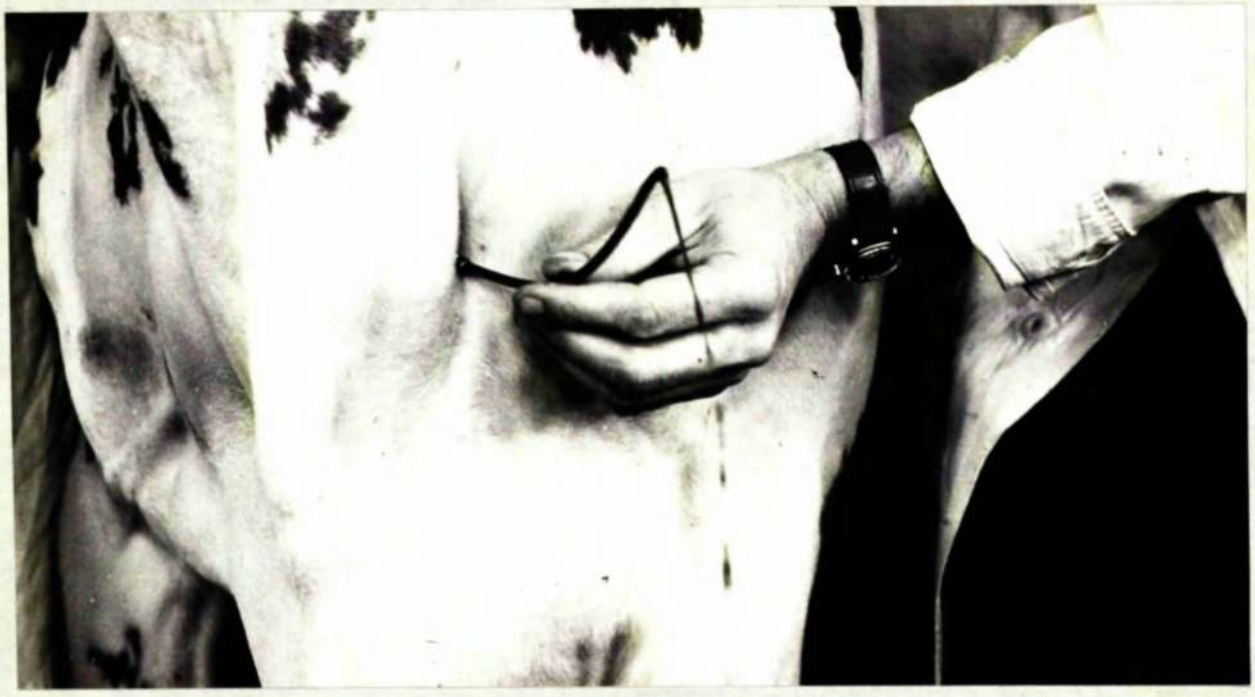

Fig. 3

Needle inserted into brachial artery

No fatalities have occurred in healthy animals using this method. Post mortem examinations of animals slaughtered for other reasons and on which arterial punctures had been carried out revealed only slight haematomata at the site of puncture in a few cases. The wall of the artery showed small puncture wounds but no significant damage had occurred.

In obtaining blood samples from cattle the following measures were adopted to ensure that the plasma samples provided an accurate estimate of the plasma carbon dioxide content and that contamination of these samples did not occur.

All blood samples were collected into clean Pyrex centrifuge tubes (fig. 4).

Fig. 4

Collection of blood into Pyrex centrifuge tube

these centrifuge tubes were thoroughly washed with soap and water to remove any trace of former samples. They were then rinsed twice in distilled water, soaked in concentrated hydrochloric acid and then rinsed again with two separate quantities of distilled water before drying in a hot air oven. Duplicate blood samples were taken into two separate tubes and duplicate estimations were carried out on each.

Blood on which an analysis was to be made was allowed to fill the soft plastic connecting tube and was then collected into heparinised centrifuge tubes under a layer of liquid paraffin so that no exposure to the atmosphere took place. In order to prevent a loss of carbon dioxide from the blood to the liquid paraffin, the quantity of liquid paraffin allowed to remain in contact with the blood was kept to a minimum. During collection blood was allowed to overfill the centrifuge tube so that most of the liquid paraffin was displaced from above the blood and a small volume only was left over the blood. The tube was then sealed with a soft rubber bung so that no air was included between the blood and the bung and more paraffin was extruded out of the centrifuge tube (fig. 5). The centrifuge tube was kept sealed until analysis of the plasma was undertaken.

Van Slyke showed many years ago that if blood was allowed to stand at room temperature, changes of autolysis took place and the pH fell. This was confirmed by Rosenthal (1946) who also showed that 30 minutes

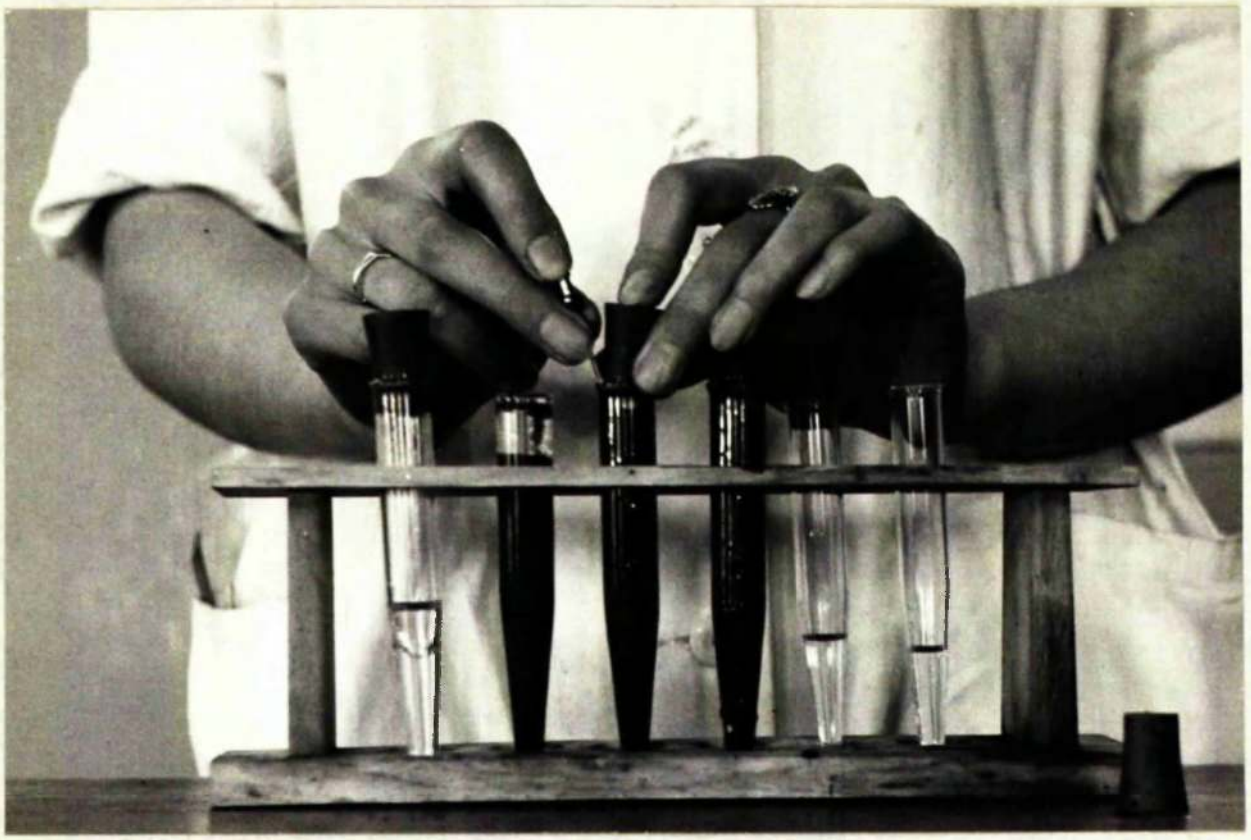

Fig. 5

Extrusion of liquid paraffin from above the blood

could elapse before changes took place and that these changes could be prevented by refrigeration. Blood samples in the present study were collected and the analysis for the carbon dioxide content of the plasma and the plasma pH was started within 30 minutes of collection. On occasion this was not possible and the samples were refrigerated for as long as necessary and before analysis were brought up to room temperature.

(2) Plasma carbon dioxide content

Two methods were used to determine the carbon dioxide content of plasma. The Manometric Method of Van Slyke (1924) was used for most of the estimations on normal animals. In the second half of the study the method of Conway (1950) was used when it was necessary to carry out large numbers of estimations in a short space of time.

In the Manometric Method of Van Slyke (1924) the gases present in the plasma are released by means of lactic acid and exposure to a vacuum within the special apparatus. The pressure (p_1) exerted by the gases when held at constant volume and constant temperature is measured. The carbon dioxide is absorbed by sodium hydroxide and the new pressure (p_2) is measured. From these two values, p_1 and p_2, it is possible, knowing the temperature, to calculate the carbon dioxide content of the plasma by reference to a special table produced by Van Slyke (Fig. 6). The method is described below.

Reagents

(a) 1.0 normal lactic acid.

(b) 1.0 normal sodium hydroxide solution.

(c) Distilled water.

These three reagents were made air free by extraction in the manometric apparatus. After they were made air free they were collected into special containers without contact with air.

(d) Caprylic alcohol.

Factors for Calculation of CO_2 Content of Blood (from Van Slyke and Sendroy (62))

Temperature	Factors by which millimeters P_{CO_2} are multiplied to give:									
	Millimoles CO_2 per liter of blood					Vol. per cent CO_2 in blood				
	Sample = 0.2 cc.	Sample = 1.0 cc.				Sample = 0.2 cc	Sample = 1.0 cc.			
		S = 3.5 cc.		S = 7.0 cc.			S = 3.5 cc.		S = 7.0 cc.	
	S = 2.0 cc. a = 0.5 cc. i = 1.037	a = 0.5 cc. i = 1.037	a = 2.0 cc. i = 1.017	a = 0.5 cc. i = 1.037	a = 2.0 cc. i = 1.017	S = 2.0 cc. a = 0.5 cc. i = 1.037	a = 0.5 cc. i = 1.037	a = 2.0 cc. i = 1.017	a = 0.5 cc. i = 1.037	a = 2.0 cc. i = 1.017
°C.										
15	0.1514	0.0313	0.1229	0.0341	0.1335	0.3370	0.0697	0.2735	0.0758	0.2974
16	07	11	22	38	25	54	93	19	52	50
17	0.1499	10	15	35	15	38	89	04	46	28
18	92	08	08	33	06	22	86	0.2690	41	06
19	86	06	02	31	0.1297	07	82	75	36	0.2886
20	79	05	0.1196	28	88	0.3292	78	62	31	66
21	72	03	90	26	79	78	75	48	26	48
22	66	02	83	24	70	63	71	34	21	28
23	59	00	77	22	62	48	68	20	16	08
24	53	0.0299	71	19	53	34	65	07	11	0.2790
25	46	97	65	17	45	20	61	0.2594	07	72
26	40	96	60	15	37	06	58	81	02	53
27	34	94	54	13	29	0.3193	55	69	0.0698	36
28	28	93	49	11	22	79	52	57	93	20
29	22	91	43	10	15	66	49	45	89	04
30	16	90	38	08	08	53	46	33	85	0.2688
31	11	89	33	06	01	40	43	22	82	74
32	05	88	28	05	0.1195	28	40	11	78	59
33	00	86	23	03	88	15	37	00	74	44
34	0.1394	85	18	01	82	03	34	0.2489	71	30

To obtain factors for a sample other than 1 cc., divide the above factors for 1 cc. by the cubic centimeters of sample analyzed: e.g., for a 2-cc. sample, S, A, and a being the same, the factors are one-half of those for a 1-cc. sample.

Fig. 6

Method

After cleaning the extraction chamber a drop of caprylic alcohol was drawn into the capillary above the cock at the top. A small amount of this caprylic alcohol was drawn into the extraction chamber. The stopcock was closed and any caprylic alcohol left in the cup was removed by suction. 2.5 ml. of carbon dioxide free water were placed in the cup. The sample of plasma in the special pipette with a rubber tip was run into the chamber under the water already in the cup. The stopcock was closed and the pipette removed. Any small amount of plasma left in the cup was washed in with some of the water. In a similar manner 0.2 ml. of 1 Normal lactic acid was run into the chamber and washed in with the remainder of the water until the mark on the lower end of the cup was reached. A small amount of mercury was placed in the cup and this was run into the stopcock to form the mercury seal.

The apparatus was evacuated by lowering the levelling bulb until the mercury in the chamber had fallen to the 50 ml. mark. The cock leading to the extraction chamber was closed and the reaction mixture was shaken for three minutes. When extraction was completed the mercury was re-admitted slowly until the gas volume in the chamber had been reduced to 2 ml. The reading on the manometer was then taken; this gives the value for p_1 in millimetres of mercury. The mercury in the levelling bulb was lowered once more to increase the gas volume to

about 10 ml. 1.2 ml. of 1 Normal air-free sodium hydroxide were placed in the top cup and 1 ml. of this drawn slowly into the reaction chamber. About 30 seconds were taken for running in the sodium hydroxide and during this time absorption of the carbon dioxide was completed. As previously the stopcock was sealed with a mercury seal. The volume of gas was once more reduced to 2 ml. The manometer reading now gave a value for p_2 in millimeters of mercury. The pressure of carbon dioxide (pCO_2) was obtained from the equation $pCO_2 = p_1 - p_2 - c$, 'c' being the correction previously determined. To obtain the carbon dioxide content of the plasma, the pCO_2 value was multiplied by the proper factor from the table given in Figure 6.

The 'c' correction was obtained by performing blank analyses, in the manner directed excluding the plasma. The $p_1 - p_2$ value found in the blank constituted the 'c' correction.

The 'c' correction may be the sum of two components. One of them is a slight amount of gas which may be yielded by the reagents themselves. The other is the fall in pressure reading caused even when no gas is removed between the p_1 and the p_2 readings, by the introduction of a given volume of absorbent solution such as the 1.0 ml. of 1 Normal sodium hydroxide used to absorb carbon dioxide in blood gas determinations. The introduction of solution between the two manometer readings, p_1 and p_2, lowers the p_2 value merely by increasing the volume of fluid between

the mercury surface and the water meniscus at the moment of reading. Thereby the level of the mercury surface in the chamber and hence also in the manometer is lowered. The extent of this effect can be determined by means of blank analyses in which, with the same amount of reagents but no gas in the chamber, the usual amount of absorbent solution is admitted. The manometer is read with water meniscus in the chamber at the same mark before and after the admission of the absorbent solution. The precise shape of the apparatus causes the area of the meniscus in the chamber to vary according to the volume of solution present and according to the location of the water meniscus, causes the mercury meniscus to be located at points of slightly different cross section in the conical upper portion of the chamber. Table 1 below gives the results of determination of the 'c' correction factor for the particular Van Slyke manometric apparatus used.

Table 1

Determination of the 'c' Correction Factor

P_1	P_2	$P_1 - P_2 = {}'c'$
90.8	89.4	1.4
90.0	88.8	1.2
91.5	90.1	1.4
91.6	90.2	1.4
92.4	91.0	1.4
93.1	91.9	1.2
93.9	92.6	1.3
93.0	91.7	1.3
94.8	93.5	1.3
93.5	92.0	1.5

Mean 'c' = 1.3 millimetres of mercury

Determinations were carried out of the carbon dioxide content of a standard solution of sodium bicarbonate containing a calculated 20 n.moles of carbon dioxide per litre. The results are presented below.

Table 2

Recovery of Carbon Dioxide from Bicarbonate Standard

Temp. °C.	p_1 mHg	p_2 mHg	$p_1-p_2-'c'$ mHg	Factor	n.moles CO_2 per litre
19	261.2	101.8	158.1	0.1202	19.16
19	267.2	103.0	162.9	0.1202	19.73
18	268.9	96.5	170.7	0.1208	20.78
18	267.0	95.8	170.7	0.1208	20.78
18	266.8	95.5	171.2	0.1208	20.83
18	269.5	96.5	171.7	0.1208	20.89
19	264.5	104.0	157.9	0.1202	19.13

Mean = 20.2 ± 0.6 n.moles/litre

It was concluded that the method as being used was suitable for carrying out a number of determinations.

In the method of Conway, within a gas-tight Conway unit the carbon dioxide is released from the plasma by means of a strong mineral acid. The carbon dioxide diffuses into the centre well of the Conway unit

there it is fixed by means of a solution of barium hydroxide so that a precipitate of barium carbonate is formed. The excess barium hydroxide is determined by titration with hydrochloric acid. Since the strength and volume of barium hydroxide originally present in the unit are known, the amount of carbon dioxide released from a known volume of plasma can be determined.

Certain modifications were made to the method as published by Conway (1950). Micro-Conway units were used at first but it was not found possible to obtain either accuracy or repeatability with the volumes used in these small units. It was decided therefore to use large size Conway units since there was no problem of volumes of plasma available from cattle.

Vaseline was found unsatisfactory as a seal for the Conway units used in the determination of carbon dioxide since diffusion of carbon dioxide appeared to take place through the vaseline. The gum tragacanth-glycerin-acid mixture mentioned by Conway was found to be satisfactory in this respect but if made according to the directions of Conway was far too mucous to spread and form an efficient seal. Water was therefore added to the mixture.

Each batch of barium hydroxide solution used for determinations was adjusted in strength so that 1 ml. (the volume placed in a Conway unit) required 4 to 4.5 ml. of N/50 hydrochloric acid. In this manner

the maximum accuracy was obtained. It was found that 1 ml. of barium hydroxide solution gave the optimal area for absorption of carbon dioxide. It was also found that the total volume which could be contained in the centre well of the Conway unit without overflow was 5.5 ml. so that 4 to 4.5 ml. of acid could be added.

The barium hydroxide with the indicator added was kept in a carbon dioxide free atmosphere. In this manner large volumes (500 ml.) of working solution could be made up, standardised and stored. The time for diffusion of the carbon dioxide from plasma was found to be in excess of the three to four hours given by Conway. The best results were obtained if the units were left overnight. To overcome the buffering capacity of plasma, it was found necessary to use a strong mineral acid (0.5 ml. of N/2 sulphuric acid) to release the carbon dioxide.

Titration of the Conway units was not accurate when performed open to the atmosphere, but titration in a carbon dioxide free atmosphere gave good results. Atmospheric carbon dioxide did not appear to interfere with the results but a sudden increase arising from the operator expiring over the units led to errors. A shield was designed to eradicate this error.

Apparatus

(a) A number of no. 1 size Conway units.

(b) Syringe pipettes of the "walking stick" pattern. Two sizes were used, 0.5 ml. and 1.0 ml.

(c) One 5.0 ml. Green Line* burette with automatic refill.

(d) A magnetic stirrer.

(e) A transparent shield behind which the Conway units were titrated.

The burette, magnetic stirrer and transparent shield were combined to form the titrating unit illustrated in figure 7.

Fig. 7

* H.J. Elliott Ltd., E-Mil Works, Pontypridd.

Reagents

(a) A saturated solution of barium hydroxide which was kept in a sealed bottle.

(b) From this stock solution, a solution of barium hydroxide was made up which was used in the determination. This solution consisted of a 1 in 4 dilution of the stock solution of barium hydroxide in distilled water together with about 5 ml. of the mixed indicator solution described below. The strength of the solution was adjusted so that 1 ml. required 4 to 4.5 ml. of N/50 hydrochloric acid for neutralisation. Once made up this solution was stored in a carbon dioxide free atmosphere. The carbon dioxide free storage system illustrated in figure 8 consisted of a storage bottle connected to a U tube full of soda lime so that air entering the bottle passed through the soda lime and became carbon dioxide free. The storage bottle fed by gravity the barium hydroxide solution to a two-way stopcock. This stopcock was normally kept closed but when estimations were carried out a quantity of the carbon dioxide free barium hydroxide could be fed into a burette tube from which fixed volumes could be removed to fill the centre wells of the Conway units.

(c) A solution of N/2 sulphuric acid.

(d) Indicator solution which consisted of a mixture of phenolphthalein

and thymolpthalein.

(e) A solution of hydrochloric acid made up accurately to be of N/80 strength.

(f) A seal for the lids of the Conway units which consisted of a mixture of tragacanth gum, glycerin, dilute sulphuric acid and water. The inclusion of acid in this mixture prevented a diffusion of carbon dioxide from the atmosphere into the sealed Conway units.

Fig. 8

Carbon dioxide free storage system.

Method

The glass rims and lids of the Conway units were smeared with the sealing compound. The 0.5 ml. N/2 sulphuric acid was then delivered into the outer well of each Conway unit diametrically opposite to the point where the plasma was added later. The lids were then placed on the Conway units leaving about one-half of the centre well uncovered. By means of a syringe pipette 1.0 ml. of the barium hydroxide indicator solution was delivered to the centre well of each unit and the units were immediately sealed. Some of these units set up in this manner were left like this to act as blanks to allow for the slow deterioration of barium hydroxide which occurred in the carbon dioxide free storage system.

The plasma in 0.5 ml. volume was removed from the sealed centrifuge tubes so that minimal exposure to the atmosphere took place. The lid of a Conway unit was moved over slightly and the plasma was delivered into the outer well of a Conway unit after which the unit was immediately sealed. The Conway unit was then rotated slightly to bring the sulphuric acid into contact with the plasma. The units were then left on the bench until next morning when the barium hydroxide left was titrated against the N/60 hydrochloric acid in the titration stand illustrated in figure 7. The colour changes of the indicator were blue to pink to colourless. Blank units were titrated first to determine

the amount of acid (B ml.) required to neutralise the barium hydroxide present in each unit before the addition of carbon dioxide. The units containing the plasma were then titrated to determine the amount of acid (U ml.) required to neutralise the barium hydroxide left after the absorption of the carbon dioxide released from 0.5 ml. of plasma. The calculations given below demonstrate how the concentration of carbon dioxide present in the plasma samples was obtained from the two titration values B and U.

Equation (a) $Ba(OH)_2 + CO_2 \longrightarrow BaCO_3 \downarrow + H_2O$

Equation (b) $Ba(OH)_2 + 2HCl \longrightarrow BaCl_2 + 2H_2O$

Equation (a) $Ba(OH)_2 + CO_2 \longrightarrow BaCO_3 \downarrow + H_2O$

Equation (b) $Ba(OH)_2 + 2HCl \longrightarrow BaCl_2 + 2H_2O$

Blank reading (B) gives amount $Ba(OH)_2$ present in solution
Unknown reading (U) gives amount $Ba(OH)_2$ left over after

absorbing the CO_2.

Therefore (B - U) gives the amount of $Ba(OH)_2$ required by CO_2.

But from equation (a) 1 m.mole $Ba(OH)_2$ requires 1 m.mole CO_2

and from equation (b) 1 m.mole $Ba(OH)_2$ requires 2 m.moles HCl.

Therefore 1 m.mole CO_2 = 2 m.moles HCl.

But strength of acid used was N/60 and the volume of plasma used 0.5 ml.

Therefore the number of m.moles CO_2 in 0.5 ml. plasma =

$$\frac{(B-U)}{2} \times \frac{1,000}{60} \times \frac{1}{1,000}$$

Therefore the number of m.moles CO_2 per litre of plasma =

$$\frac{(B-A)}{2} \times \frac{1,000}{30} \times \frac{2,000}{1,000} =$$

$$(B-A)\ \frac{100}{6}$$

The method of Conway as used contained several innovations and therefore a new complete analysis was made also was made for the Van Slyke method. A series of standard solutions of sodium carbonate was made up from Analar reagent grade anhydrous sodium carbonate. These solutions were fairly stable at room temperature but tended to pick up carbon dioxide from the atmosphere to form sodium bicarbonate as shown in the following equation.

$$Na_2CO_3 + H_2O + CO_2 \longrightarrow 2NaHCO_3$$

The solutions were freshly prepared and used on the same day that they were made. Solutions were made up which contained 10, 15, 20, 25, 30 and 35 millimoles of carbon dioxide per litre. Table 3 below gives the weights of sodium carbonate which dissolved in 1 litre of distilled water gave the required concentration of carbon dioxide.

Table 3

Standard Solutions of Sodium Carbonate

10 m.moles/litre required	1.06	gm. Na_2CO_3 per litre of water
15 " "	1.59	" " " " " " " "
20 " "	2.12	" " " " " " " "
25 " "	2.65	" " " " " " " "
30 " "	3.18	" " " " " " " "
35 " "	3.75	" " " " " " " "

Eight blank Conway units were set up together with seven Conway units for each standard solution. These were left overnight and titrated in the morning. Then there were eight values determined for the blank and seven values determined for each standard. By considering each blank titration value against each standard titration value a total of 56 results were obtained for each standard solution. These results are shown in tables 4 to 9. A mean result has been calculated for each standard solution together with the standard deviation. Frequency distribution curves were plotted for each standard and these are reproduced in figures 9 and 10.

Table A

Recovery of 10 m.moles/litre of Carbon Dioxide

Blanks (B) (ml.)	Number of blanks with this reading	Standard (S) (ml.)	Number of standards with this reading	B - S (ml.)	m.moles/litre
3.62	1	2.79	1	0.83	10.38
		2.81	1	0.81	10.13
		2.82	2	0.80	10.0
		2.83	3	0.79	9.88
3.63	3	2.79	1	0.84	10.5
		2.81	1	0.82	10.25
		2.82	2	0.81	10.13
		2.83	3	0.80	10.0
3.64	3	2.79	1	0.85	10.63
		2.81	1	0.83	10.38
		2.82	2	0.82	10.25
		2.83	3	0.81	10.13
3.65	1	2.79	1	0.86	10.75
		2.81	1	0.84	10.5
		2.82	2	0.83	10.38
		2.83	3	0.82	10.75

Mean value = 10.2 ± 0.19

Table 5

Recovery of 14 n.moles/litre of Carbon Dioxide

Blanks (B) (ml.)	Number of blanks with this reading	Standard (U) (ml.)	Number of standards with this reading	B − U (ml.)	n.moles/litre
3.62	1	2.44	6	1.18	14.75
		2.45	1	1.17	14.63
3.63	3	2.44	6	1.19	14.87
		2.45	1	1.18	14.75
3.64	3	2.44	6	1.20	15
		2.45	1	1.21	15.125
3.65	1	2.44	6	1.21	15.13
		2.45	1	1.22	15.25
				Mean value = 14.96 ± 0.19	

Table 6

Recovery of 20 n.molen/litre of Lactic Acid

Blanks (B) (ml.)	Number of blanks with this reading	Standard (S) (ml.)	Number of standards with this reading	B - S (ml.)	n.molen/litre
3.62	1	2.02	1	1.6	20.0
		2.04	1	1.58	19.75
		2.05	1	1.57	19.63
		2.07	1	1.55	19.38
3.63	3	2.02	1	1.61	20.13
		2.04	4	1.59	19.88
		2.05	1	1.58	19.75
		2.07	1	1.56	19.5
3.64	3	2.02	1	1.62	20.25
		2.04	4	1.60	20.0
		2.05	1	1.59	19.88
		2.07	1	1.57	19.63
3.65	1	2.02	1	1.63	20.38
		2.04	4	1.61	20.13
		2.05	1	1.60	20.0
		2.07	1	1.58	19.88
				Mean value = 19.92 ± 0.27	

Table 7

Recovery of 25 n.moles/litre of Carbon Dioxide

Blanks (B) (ml.)	Number of blanks with this reading	Standard (U) (ml.)	Number of standards with this reading	B - U (ml.)	n.moles/litre
3.62	1	1.62	2	2.00	25.0
		1.64	2	1.98	24.75
		1.66	3	1.96	24.5
3.63	3	1.62	2	2.01	25.13
		1.64	2	1.99	24.88
		1.66	3	1.97	24.65
3.64	3	1.62	2	2.02	25.25
		1.64	2	2.00	25.00
		1.66	3	1.98	24.75
3.65	1	1.62	2	2.03	25.5
		1.64	2	2.01	25.13
		1.66	3	1.98	24.75
				Mean value = 24.2 ± 0.27	

Table 3

Recovery of 30 n.moles/litre of Carbon Dioxide

Blanks (B) (ml.)	Number of blanks with this reading	Standard (U) (ml.)	Number of standards with this reading	B - U (ml.)	n.moles/litre
3.62	1	1.26	5	2.35	29.5
		1.27	1	2.35	29.38
		1.28	1	2.34	29.25
3.63	3	1.26	5	2.37	29.75
		1.27	1	2.36	29.5
		1.28	1	2.35	29.38
3.64	3	1.26	5	2.38	29.75
		1.27	1	2.37	29.63
		1.28	1	2.36	29.5
3.65	1	1.26	5	2.39	29.88
		1.27	1	2.38	29.75
		1.28	1	2.37	29.63

Mean value = 29.67 ± 0.15

Table 9

Recovery of 35 n.moles/litre of Carbon Dioxide

Blanks (B) (ml.)	Number of blanks with this reading	Standard (S) (ml.)	Number of standards with this reading	B - S (ml.)	n.moles/litre
3.62	1	0.84	1	2.74	34.25
		0.86	4	2.76	34.5
		0.88	2	2.78	34.75
3.63	3	0.84	1	2.79	34.88
		0.86	4	2.77	34.63
		0.88	2	2.75	34.38
3.64	3	0.84	1	2.80	35.0
		0.86	4	2.78	34.75
		0.88	2	2.76	34.5
3.65	1	0.84	1	2.81	35.13
		0.86	4	2.79	35.0
		0.88	2	2.77	34.63
				Mean value = 34.76 ± 0.21	

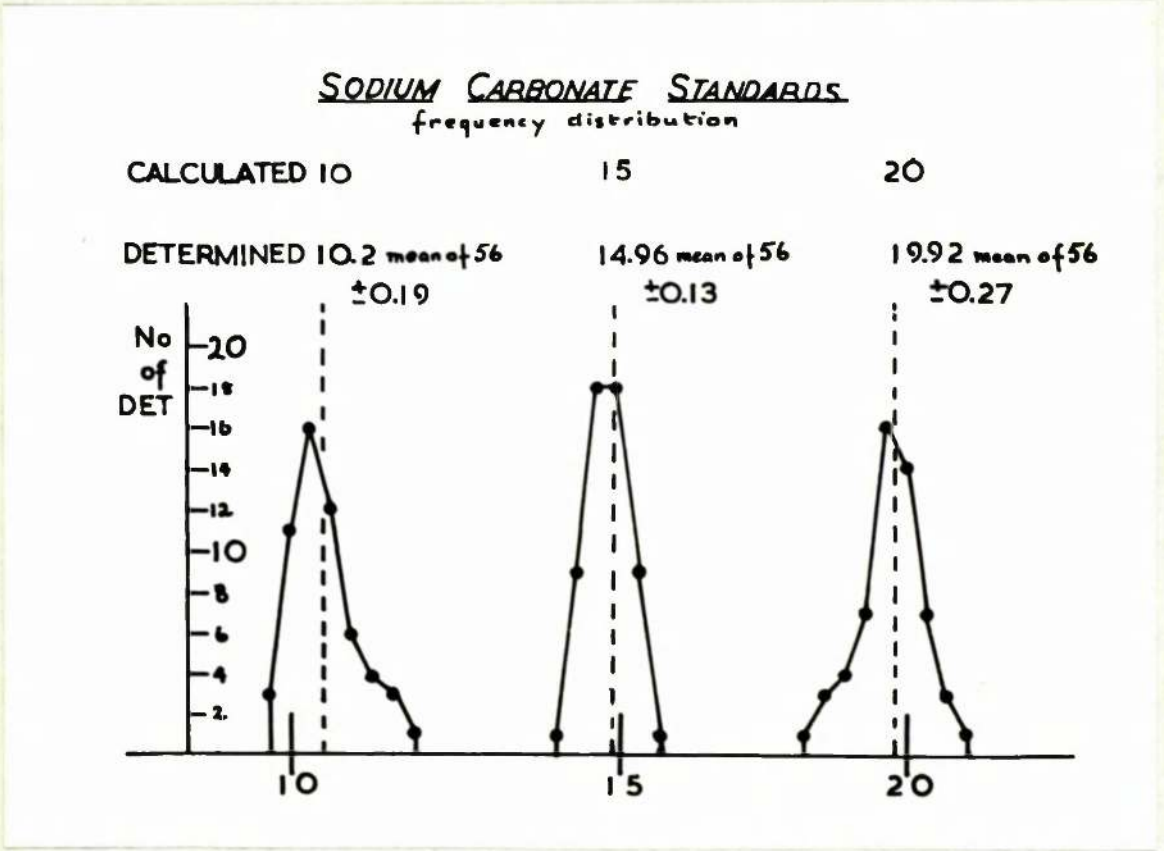

Fig. 2

Frequency distribution curves of sodium carbonate standards containing 10, 15 and 20 m.moles/litre of carbon dioxide

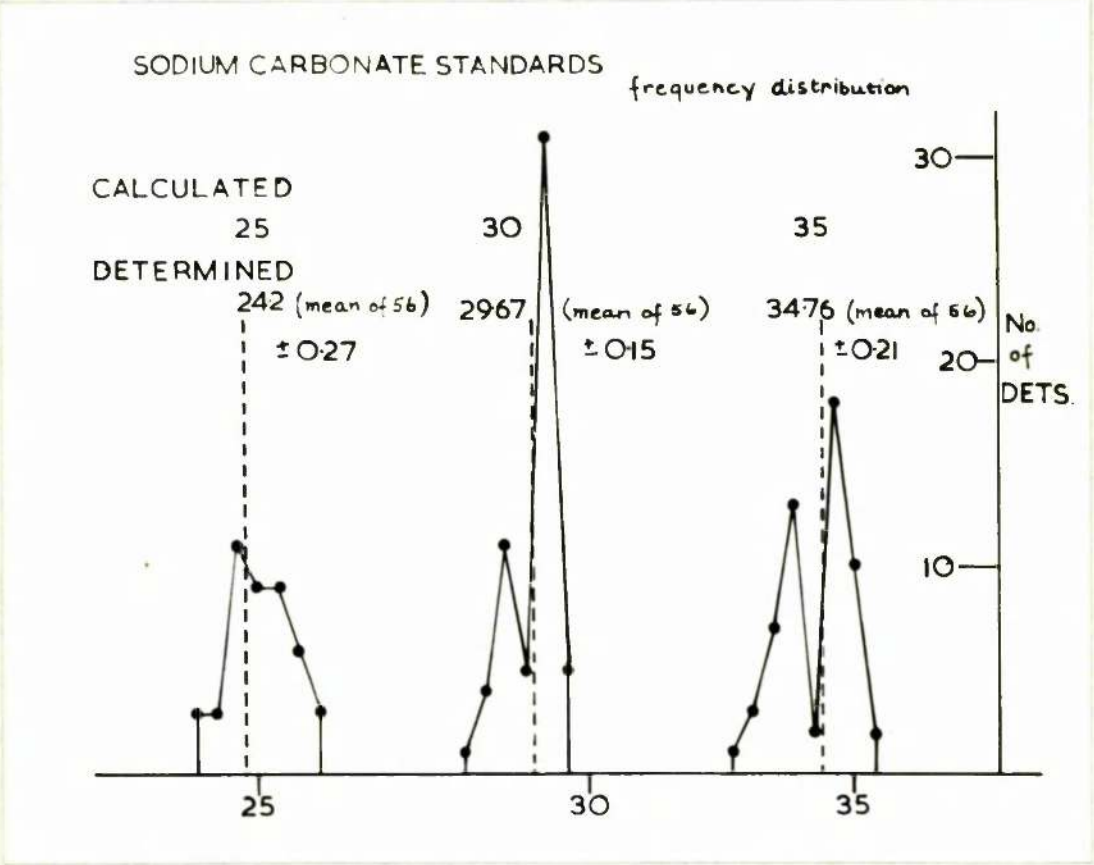

Fig. 10

Frequency distribution curves of sodium carbonate standards containing 25, 30 and 35 n.moles/litre of carbon dioxide

A number of plasma samples were also set up to test repeatability and the results are given in table 10.

Table II

Reproducibility of Plasma Samples

Blanks (B) (ml.)	Number of blanks with this reading	Unknown (U) (ml.)	Number of unknowns with this reading	B − U (ml.)	CO_2 conc. m.moles/litre
Plasma 1 4.37	1	2.35	3	1.91	23.88
		2.27	2	1.90	23.75
4.38	4	2.35	3	1.92	24.00
		2.27	2	1.91	23.88
				Mean value = 23.92 ± 0.06	
Plasma 2 4.37	1	2.14	4	2.03	25.38
		2.16	1	2.01	25.13
4.38	4	2.14	4	2.04	25.50
		2.16	1	2.02	25.25
				Mean value = 25.42 ± 0.1	
Plasma 3 4.37	1	1.66	1	2.51	31.38
		1.68	3	2.49	31.13
4.38	4	1.66	1	2.52	31.50
		1.68	3	2.50	31.25
				Mean value = 31.25 ± 0.12	
Plasma 4 4.37	1	2.08	2	2.09	26.13
		2.09	2	2.08	26.00
		2.10	1	2.07	25.88
4.38	4	2.08	2	2.10	26.25
		2.09	2	2.09	26.13
		2.10	1	2.08	26.00
				Mean value = 26.12 ± 0.115	

(3) Plasma pH

Since pH varies inversely with temperature the plasma pH had to be measured either at body temperature or at a lower temperature and temperature coefficients applied to the values obtained. Several colorimetric methods of determination of plasma pH have been described, many using phenol red as the indicator (Hastings and Sendroy, 1924; Stock and Hastings, 1934; Rutledge, 1948, and Nadeau, 1953). The following method described by Van Slyke, Weisinger and Van Slyke (1949) also using phenol red has been adapted to the "Vit" colorimeter* and has been used in the present study.

Reagents

(a) A stock solution of disodium hydrogen phosphate containing 142 gm. of the anhydrous salt per litre.

(b) A stock solution of potassium dihydrogen phosphate containing 136.1 gm. of the anhydrous salt per litre.

Keep the solutions were kept in stoppered Pyrex flasks in the refrigerator at 4° C.

(c) Phenol red. A stock solution of 0.1% phenol red was made up by dissolving 100 mg. of phenol red in 28.2 ml. of 0.01 N NaOH and diluting to 100 ml. This was prepared several days before required.

(d) A 0.002% solution of phenol red was made by dilution of 20 ml. of

* Evans Electroselenium Ltd., Harlow, Essex.

the 0.1% stock to 250 ml. with water. This solution was also stored in a refrigerator at 4° C.

(e) A 22.5% solution of sodium chloride.

(f) A 0.9% solution of sodium chloride.

(g) Neutralized liquid paraffin. This was made by mixing about 200 ml. of liquid paraffin with 200 ml. of distilled water to which a few drops of 0.1% phenol red solution had been added. The mixture was shaken vigorously and 0.02 N NaOH was added drop by drop until the watery solution of indicator became permanently pink. The oil was separated, centrifuged, re-separated and stored in a stoppered flask.

(h) Neutralized saline-dye solution with 8 mg. of phenol red per litre. This solution was made immediately before use since the pH fell if it stood for more than an hour or two. Into a 100 ml. volumetric flask were placed 4 ml. of the 22.5% solution of sodium chloride and about 80 ml. of water. 10 ml. of the 80 mg. per litre phenol red solution were measured accurately and added. Drop by drop N NaOH was added, stirring by rotation after each addition until the pH was about 7.4 (a colorimeter reading of 34). The flask was then filled with water until about 0.2 ml. of the mark and more 0.02 N NaOH added a drop at a time until the pH was between 7.4 and 7.6 (colorimeter readings 34 - 38). This flask was kept stoppered to prevent absorption of atmospheric carbon dioxide.

Preparation of standard phosphate-dye solutions of known pH

From the 0.5 M stock solutions previously prepared mixed solutions of disodium hydrogen phosphate and potassium dihydrogen phosphate were prepared. These solutions mixed in the proportions shown in table 11 gave the pH values indicated when they were diluted to M/15.

Table 11

Volumes of Phosphate Solutions

to be Mixed to Produce the pH Values Required

0.5 M stock solution		pH obtained at temperatures indicated when diluted to M/15	
Na_2HPO_4 ml.	KH_2PO_4 ml.	20° C.	25° C.
50	22.65	7.13	7.20
50	11.48	7.43	7.40
50	5.57	7.73	7.70

To obtain the correct M/15 dilution and correct pH, into three separate cuvettes were measured 8.35 ml. of water, 1.25 ml. of the appropriate 0.5 M mixed phosphate buffer and 1.0 ml. of the 20 mg. per liter phenol red solution. After addition of the dye, the mixtures were each mixed with a footed rod and immediately stoppered to protect from atmospheric carbon dioxide.

During the development of the method standard phosphate-dye solutions were prepared and readings of optical density were made in a Unicam S.P. 600 spectrophotometer[x]. A non-linear calibration curve was obtained as described by Van Slyke et al. (1949) with specially selected test tubes in the test tube carrier. This is illustrated in Figure 11.

It was possible to obtain repeatability at 20° C. but not at 37° C. Unpredictable errors occurred and the source of these errors was found to lie in the heavy metal construction of the cuvette carriers. A cuvette containing a phosphate buffer solution warmed to 37° C. and placed in the spectrophotometer, rapidly lost heat to the metal carrier so that the optical density was read at an unknown and random temperature somewhere between 37° C. and room temperature. The rapidity of heat loss from such a solution when placed in a test tube cuvette carrier in the Unicam S.P. 600 spectrophotometer is shown in table 12.

This source of error was overcome with the special cuvettes by heating the metal cuvette carrier prior to placing in the spectrophotometer and thus slowing the heat loss from the cuvettes enabling a reading to be taken.

A calibration curve was then attempted using an "EEL" colorimeter with a no. 625 Ilford gelatine filter whose absorption peak is in the region of 545 mμ. The phosphate-phenol red buffer mixtures were made

x Unicam Instrument Co. Ltd., Cambridge.

Table 12

Heat Loss from Test Tubes in the

Test Tube Cuvette Holder in the Spectrophotometer

Time (secs.)	Sample 1 Temp. °C.	Sample 2 Temp. °C.	Sample 3 Temp. °C.
0	39	39	39
5	38.5	38	38.5
10	38	37.5	38
15	37.5	37	37.5
20	37	36	36.7
25	36	35.4	35.8
30	34.6	34	35
45	33.5	33	34

up in the colorimeter tubes. Prior to reading at 20° C. the colorimeter tubes were heated in a water-bath to 21° C. The calibration curve obtained at 20° C. using a number 625 filter is illustrated in Figure 11 together with a calibration curve obtained on the spectrophotometer.

From the experience acquired in attempting to obtain a calibration curve of the phosphate buffer-dye solution in the spectrophotometer at 36° C., a check was made of the temperature loss from the colorimeter tubes containing 10.5 ml. of fluid when placed in the "EEL" colorimeter.

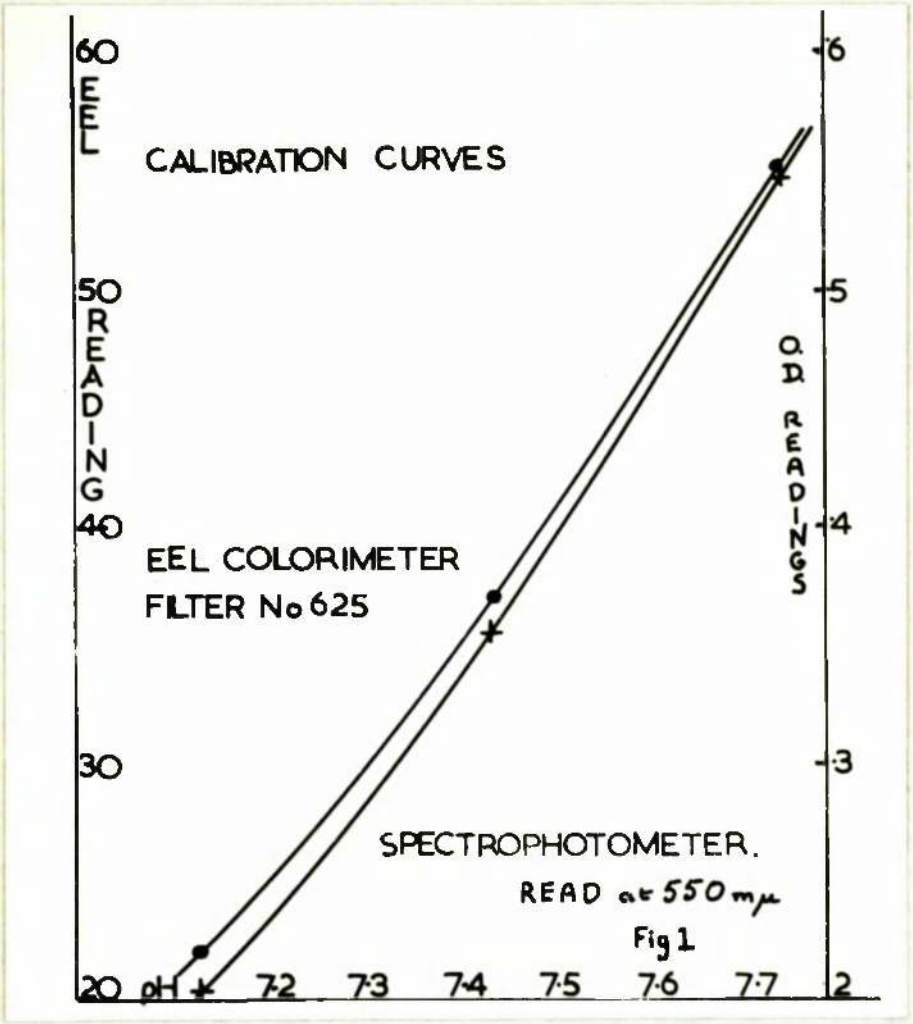

Fig. 11

Comparison of calibration curves obtained from the spectrophotometer and the colorimeter

A slower loss of heat was observed than occurred in the spectrophotometer and this was thought to be due to the plastic holder for the colorimeter tubes within the colorimeter. The results obtained in the colorimeter compared with heat loss in the spectrophotometer are given

in table 13.

Table 13

Comparison of Heat Loss Curves Obtained
With the Colorimeter and the Spectrophotometer

Time (secs.)	Temperature in degrees centigrade "E.I." colorimeter			Spectrophotometer		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
0	39	39	39	39	39	39
5				38.5	38	38.5
10	39	38	38	38	37.5	38
15				37.5	37	37.5
20	38	38	38	37	36	36.7
25				36	35.4	35.8
30	37	38	37.5	34.5	34	35
40	35.5	37.5	37.5			
45				33.5	33	34
50	36	36.5	37			

Calibration curves were then carried out using an O.S.R.I simple glass filter. A straight line calibration curve was obtained at 39° C. and at 38° C. Since reproducible, straight line calibration curves were obtained with the O.S.R.I filter, the method was then used to

determine plasma pH and the temperature coefficients of blood and plasma. These calibration curves are shown in figure 12.

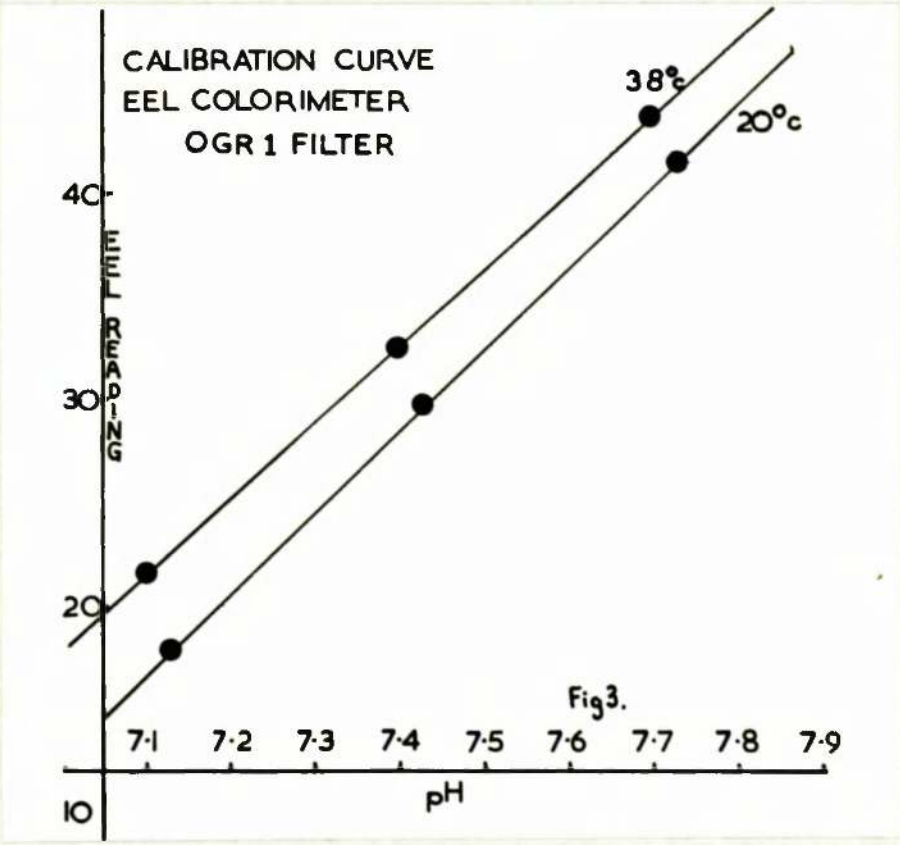

Fig. 12

Calibration curves phosphate buffers

For each determination three colorimeter tubes were used. Into two of these were pipettes 10 ml. of the neutralised saline dye solution for duplicate determinations and these solutions were immediately covered with a layer of neutralised liquid paraffin. Into the third

colorimeter tube 10 ml. of 0.9% saline were pipetted to receive a plasma blank. Into each of the two colorimeter tubes containing the 10 ml. of saline-dye solution was placed 0.5 ml. of plasma.

To obtain the 0.5 ml. of plasma from the centrifuge tube in which it had been collected, the rubber bung was removed and the plasma was withdrawn using a long tipped 0.5 ml. syringe pipette. A third 0.5 ml. of plasma was run into the colorimeter tube containing the saline. The contents of each colorimeter tube were mixed with a clean footed rod and the two tubes were placed in a water-bath at 21° C. for five minutes.

The zero of the colorimeter was set with the plasma-saline blank and the readings of the colorimeter tubes taken. By reference to a previously determined calibration curve the plasma pH was found.

It was necessary to check first the repeatability of the method with reference to plasma pH determinations and to do this pH determinations were made on 10 plasma samples. The results of the pH determinations at 20° C. are given in table 14. The results are not corrected to the body temperature of the animal. From these results it appeared that this method could be used for the determination of plasma pH of cattle.

Table 14

Error of Consecutive Determinations on the Same Sample

Sample	a	b	c	d	e	f
1	7.695	7.705	7.695	7.695	7.695	7.695
2	7.670	7.670	7.655	7.655	7.655	7.655
3	7.680	7.675	7.680	7.675	7.680	7.680
4	7.490	7.485	7.485	7.485	7.485	7.485
5	7.635	7.625	7.625	7.625	7.630	7.630
6	7.430	7.430	7.430	7.430	7.425	7.425
7	7.780	7.770	7.755	7.770	7.770	
8	7.755	7.755	7.780	7.760	7.770	
9	7.740	7.725	7.740	7.740		
10	7.770	7.780	7.780	7.780		

Since it was possible neither to find the blood pH directly at 38° C. nor to separate the plasma at 38° C., it was necessary to determine temperature coefficients for both blood and plasma. Rosenthal (1943) found that in order to obtain pH values for plasma, exactly equal to those of plasma as drawn, it was necessary to centrifuge at 38° C. If blood were cooled to 20° C. and centrifuged at that temperature and the separated plasma was then warmed to 38° C.

for pH measurements, the pH obtained was about 0.03 units greater than that observed when the blood was centrifuged at 38° C. It showed that the reason for this was the difference in temperature coefficients for blood pH and plasma pH, the blood temperature coefficient being greater. Van Slyke et al. (1949) considered that since practically all the data in the literature concerning the pH of human plasma were derived from blood centrifuged at room temperature, for comparison it was justifiable to continue to centrifuge at room temperature.

When blood samples were drawn from cattle they were not drawn in the relatively warm atmosphere of a hospital ward and immediately transported to a warm laboratory. Blood samples were withdrawn into a relatively cold atmosphere of a byre and as a consequence were subjected to considerable cooling prior to centrifugation. Although it was found that the temperature of the centrifuge used was in the region of 20° to 25° C. while in use, it was considered that the cooling, prior to centrifugation, may have affected the results obtained. A standard procedure of bringing blood samples to 20° C. prior to centrifugation was therefore adopted and temperature coefficients were found for both blood pH and plasma pH.

The temperature coefficient of the pH of bovine plasma was found by taking plasma samples and finding the pH difference when read on the "EEL" colorimeter at two known temperatures, 20° C. and 38° C., for which calibration curves had been prepared using the phosphate buffer-dye solutions. Results are given in table 25.

Table 15

Temperature Coefficient of Bovine Plasma (33 normal)

Sample	pH 20° C.	pH 38° C.	Difference pH units
1	7.605	7.465	.140
2	7.690	7.485	.145
3	7.665	7.510	.155
4	7.680	7.520	.160
5	7.720	7.570	.150
6	7.670	7.455	.115
7	7.695	7.490	.205
8	7.740	7.555	.185
9	7.745	7.560	.185
10	7.635	7.495	.140
11	7.645	7.510	.135
12	7.705	7.545	.160
13	7.660	7.535	.125
14	7.615	7.465	.150
15	7.645	7.490	.155
16	7.680	7.530	.150
17	7.690	7.495	.195
18	7.640	7.470	.170
19	7.650	7.495	.155
20	7.705	7.555	.150
21	7.655	7.570	.145
22	7.690	7.530	.160
23	7.695	7.490	.145
24	7.775	7.595	.180
25	7.740	7.580	.160
26	7.640	7.450	.190
27	7.660	7.575	.145
28	7.660	7.495	.165
29	7.655	7.525	.130
30	7.670	7.520	.150
31	7.690	7.530	.160
32	7.700	7.530	.170
33	7.650	7.505	.145
			Mean ± .155 ± 0.018

A mean difference in pH of 0.155 pH units was found for 38° C. A temperature coefficient for the pH of bovine plasma of 0.009 pH units per degree centigrade was calculated; in a similar manner a temperature coefficient was found for canine plasma. The results obtained are given in table 36.

Table 36

Temperature Coefficient of Canine Plasma (20 samples)

Sample	pH 25° C.	pH 38° C.	Difference pH units
1	7.66	7.43	.23
2	7.58	7.38	.20
3	7.59	7.38	.21
4	7.61	7.40	.21
5	7.67	7.46	.21
6	7.74	7.50	.24
7	7.69	7.48	.21
8	7.72	7.48	.24
9	7.70	7.47	.23
10	7.73	7.49	.24
			Mean = .222 ± 0.013

A mean difference of 0.222 pH units was found for 15° C. A temperature coefficient for the pH of canine plasma of .012 pH units per degree centigrade was calculated.

The temperature coefficient of bovine blood was determined in an indirect manner since a method for the determination of blood pH had not yet been developed. Rosenthal (1940) pointed out that blood and plasma had the same pH at the temperature of separation. Therefore if the plasma could be separated at two different temperatures and the plasma pH found at these different temperatures, then the blood pH could be ascertained at these temperatures; if the blood pH was known at two temperatures it was possible to find the blood pH temperature coefficient.

Two blood samples were taken from the same animal at the same time and separated at 0° C. and 25° C., respectively, and the plasma pH was found for each at 20° C. The plasma pH values were corrected to the respective temperatures of separation to obtain blood pH at 0° C. and 25° C. and from these two values the temperature coefficient of the pH of this bovine blood could have been determined. However, in order to deal with a number of blood samples from different cattle a slightly different procedure was adopted. Two samples were obtained from each animal and after separation at 0° C. and 25° C. the pH values were found for the plasma at 20° C. The results obtained are given in table 17.

Table IV

The Difference in Plasma pH Caused by Centrifugation of Blood at 25° C. and 0° C.

Sample	Plasma pH at 25° C. Separation at 25° C.	Separation at 0° C.	Difference pH units
	pH	pH	
1	7.620	7.565	.055
2	7.635	7.585	.050
3	7.595	7.670	.075
4	7.620	7.675	.055
5	7.555	7.630	.075
6	7.535	7.600	.065
7	7.560	7.630	.070
8	7.635	7.705	.070
9	7.595	7.605	.050
10	7.600	7.650	.050
11	7.545	7.630	.085
12	7.450	7.530	.080
13	7.550	7.595	.045
14	7.605	7.660	.055
15	7.635	7.720	.085
16	7.620	7.690	.070
17	7.610	7.685	.075
18	7.565	7.635	.070
19	7.635	7.720	.085
20	7.565	7.660	.095
			Mean = .070 ±0.015

From this table the mean difference in plasma pH due to difference in temperature of centrifugation of the blood was found to be 0.07 pH

units. A graph was constructed, as illustrated in figure 13, of temperature against pH and on this graph two parallel lines were drawn, of slope, the temperature coefficient of plasma and these two lines were separated by the mean difference of pH due to difference of temperature of centrifugation.

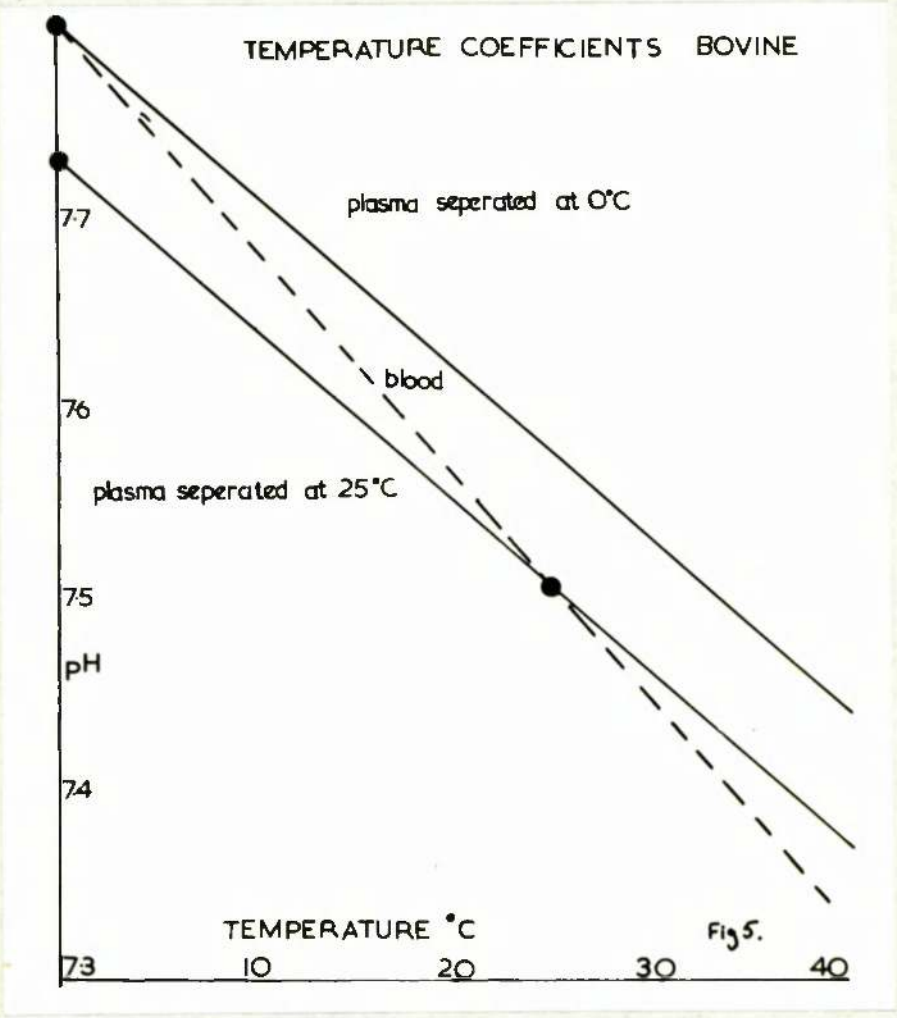

Fig. 13

Temperature coefficients bovine blood and plasma

Two points entered on this graph where blood and plasma had the same pH. These were the points on each plasma line at the temperature of centrifugation. By joining these two points a line of slope, the temperature coefficient of bovine blood, was obtained. The temperature coefficient of bovine blood found in this way was 0.012 pH units per degree centigrade.

Similarly the temperature coefficient of canine blood was also found. Duplicate samples were taken, spun at 0° C. and 25° C. and the plasma pH found at 20° C. The values obtained are given in table 13.

Table 13

The Difference in Canine Plasma pH Caused by Centrifugation of Blood at 25° C. and 0° C.

Sample	Plasma pH at 20° C. Separation at 25° C.	Separation at 0° C.	Difference pH units
	pH	pH	
1	7.66	7.74	.080
2	7.58	7.69	.090
3	7.61	7.70	.090
4	7.66	7.79	.080
5	7.49	7.51	.080
6	7.38	7.42	.040
7	7.40	7.47	.070
8	7.73	7.80	.070
9	7.73	7.77	.040
10	7.51	7.55	.040
			Mean = .060 ±0.018

The mean difference due to difference in temperature of centrifugation was found to be 0.06 pH units. A graph was drawn as illustrated in figure 14 with the two plasma lines separated by this mean difference.

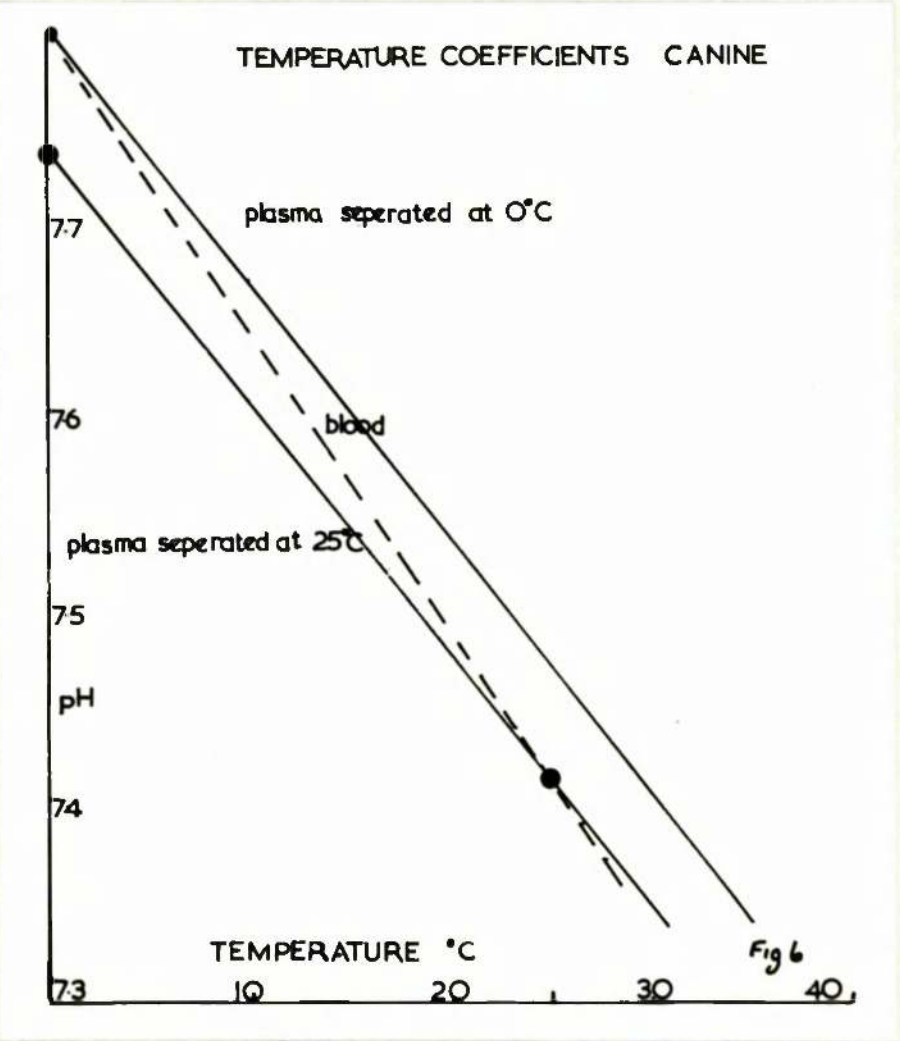

Fig. 14

Temperature coefficients canine blood and plasma

The pH of plasma and hence of blood at the temperature of separation was found and the line joining these was drawn, the slope of which was the temperature coefficient of the pH of canine blood. The temperature coefficient of the pH of canine blood found in this way was 0.0155 pH units per degree centigrade.

When temperature coefficients had been found for bovine and canine blood and plasma, respectively, the following method was adopted to determine the pH of cattle and dog plasma at 38° C. Blood samples were taken with special anaerobic precautions as previously described, brought to the laboratory and placed in a water-bath at 20° C. for five minutes to ensure a constant temperature before being placed in the centrifuge. The blood samples were centrifuged at a known temperature (usually 23° C.) and then the plasma pH was found using the "EEL" colorimeter as previously described. To the plasma pH found at 20° C. it was necessary to apply temperature coefficients to obtain the plasma pH at 38° C. The plasma pH at 20° C. was first corrected to the plasma pH at the temperature of separation by application of the temperature coefficient of bovine plasma and then the blood temperature coefficient was applied to correct the pH to 38° C. in the following manner.

For bovine blood and plasma if X be the temperature of separation and Y the temperature of reading then the plasma pH at 38° C. = pH at Y° C. + (X - Y)(0.009) - (38 - X)(0.012) where 0.009 is the calculated temperature coefficient of plasma and 0.012 is the calculated temperature coefficient of blood.

(4) Ventilation rate

Ventilation rate may be defined as the volume of gas expired in one minute. It is affected by the rate and depth of respiration, both of which may undergo alteration.

The extensive study reported by Brody (1950) was conducted using a variety of spirometers. In the present study the ventilation rate was measured by means of a face mask connected to unidirectional valves in such a manner that the bovine subject inspired from the atmosphere and expired to the atmosphere through a gas meter.

The face masks (Orr's mask and Haldane's mask) used for the anaesthetization of adult cattle were examined for their suitability for the measurement of the ventilation rate of cattle and were rejected since they were unwieldy, not gas tight and also the dead space within the masks was too large. A mask was therefore fabricated to overcome these disadvantages. For the main body of the mask a open aluminium cone as was used for shop lighting was required. This had a diameter of six inches at its base and was 10 inches long. A one and a half inch brass nipple was forced through the apex of the cone and secured in this position by means of jam-rings with rubber washers acting as gas seals. Attached to the open base of the cone, by means of adhesive Bostick[x], was a six inch width of flexible rubber tubing of five inches

x B.B. Chemical Co. Ltd., Leicester.

diameter. The rubber tubing enabled a gas tight seal to be made over the animal's face and the spun aluminium cone over the animal's nostrils could be moulded to the particular animal's facial contour. This is illustrated in figure 15.

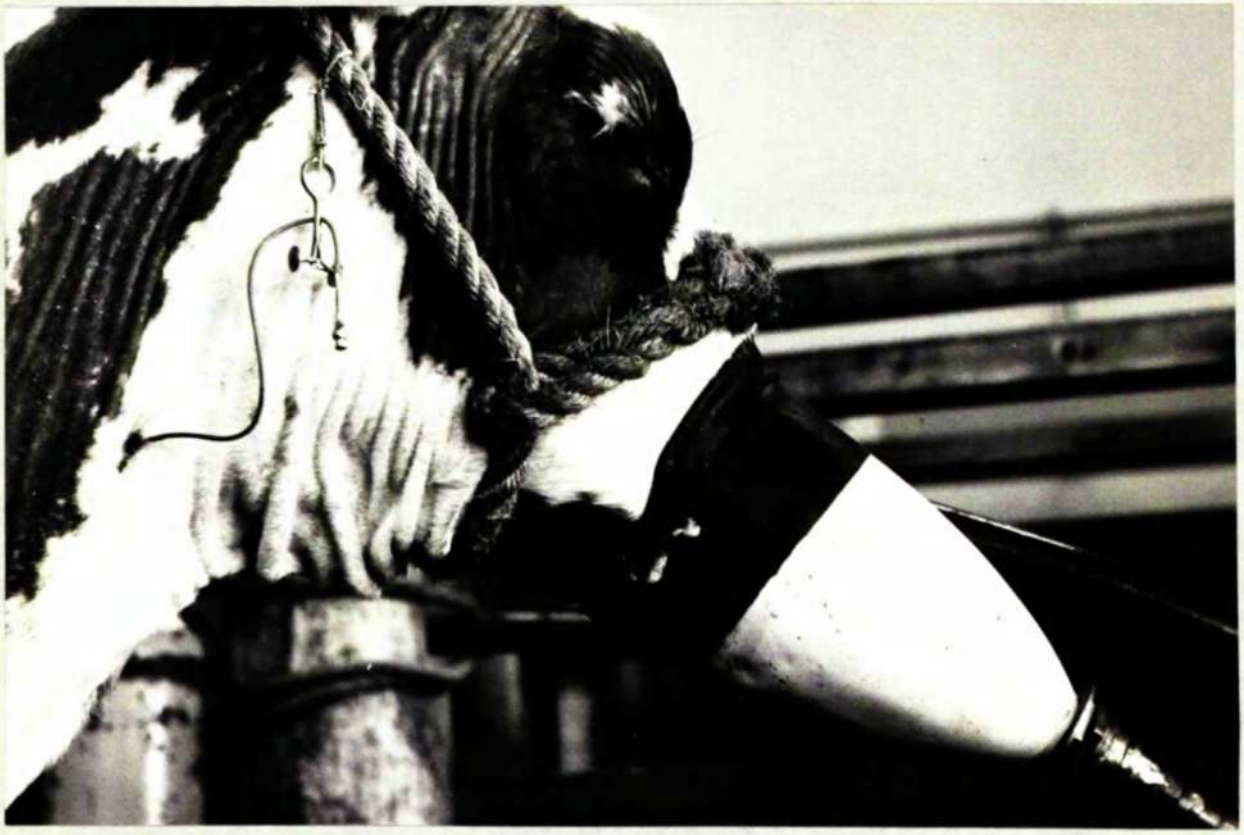

Fig. 15

Face mask in position on a cow.

For calves a similar mask was fabricated. A smaller spun aluminium cone of length five inches and diameter of base four inches

was selected. A three-quarter inch nipple was forced through the apex and secured with the appropriate size jam rings and rubber washers. The air seal of the base of the mask to the face of the calf was made in one of two ways. On some occasions a rubber ring obtained from the cuff of a surgical rubber glove was used to make the seal while on other occasions an inflatable cuff, as used for a human type face mask, was attached.

The mask for adult cattle is illustrated in figure 16.

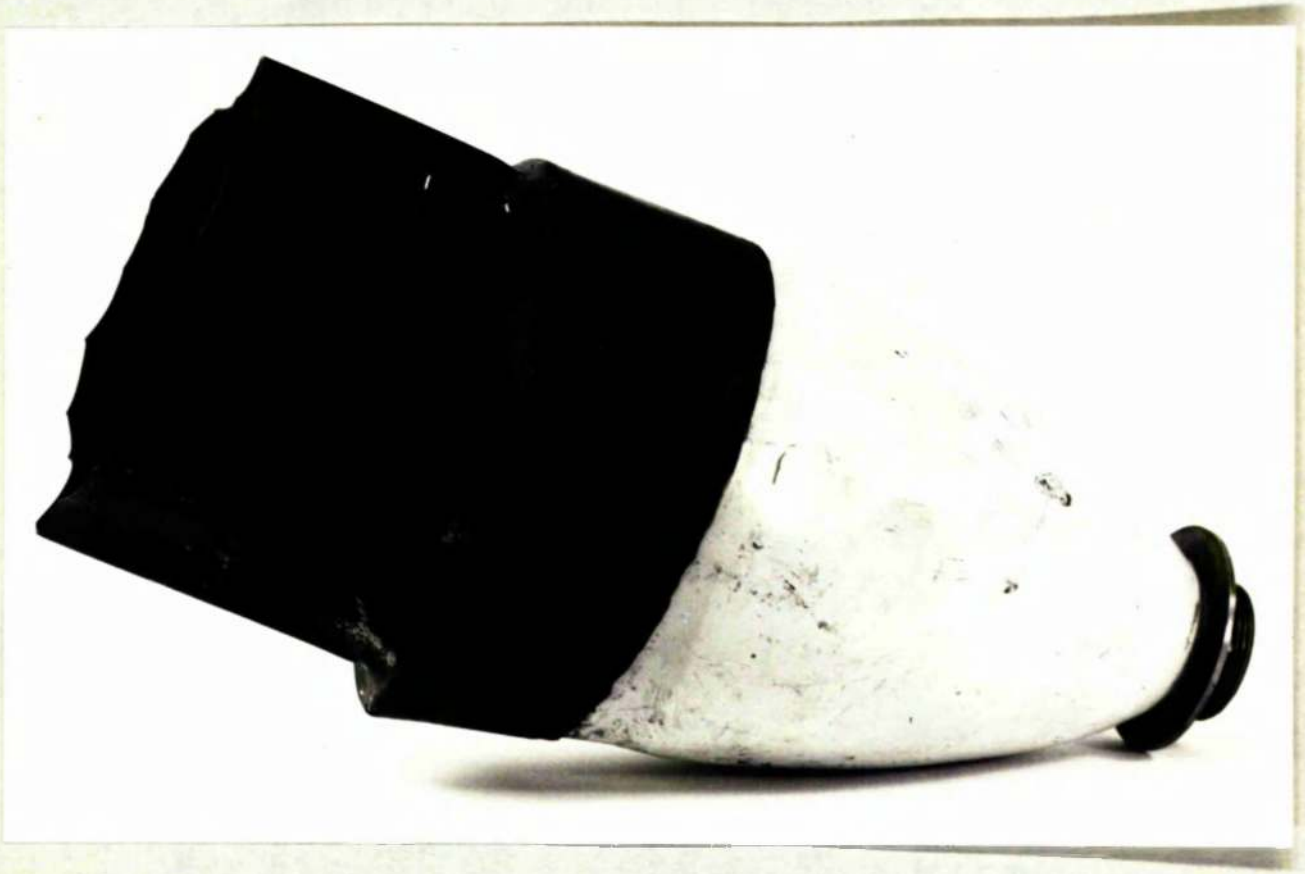

Fig. 16

Mask for adult cattle.

The masks used for calves are illustrated in figures 17 and 18.

Fig. 17

Calf mask with inflatable cuff seal.

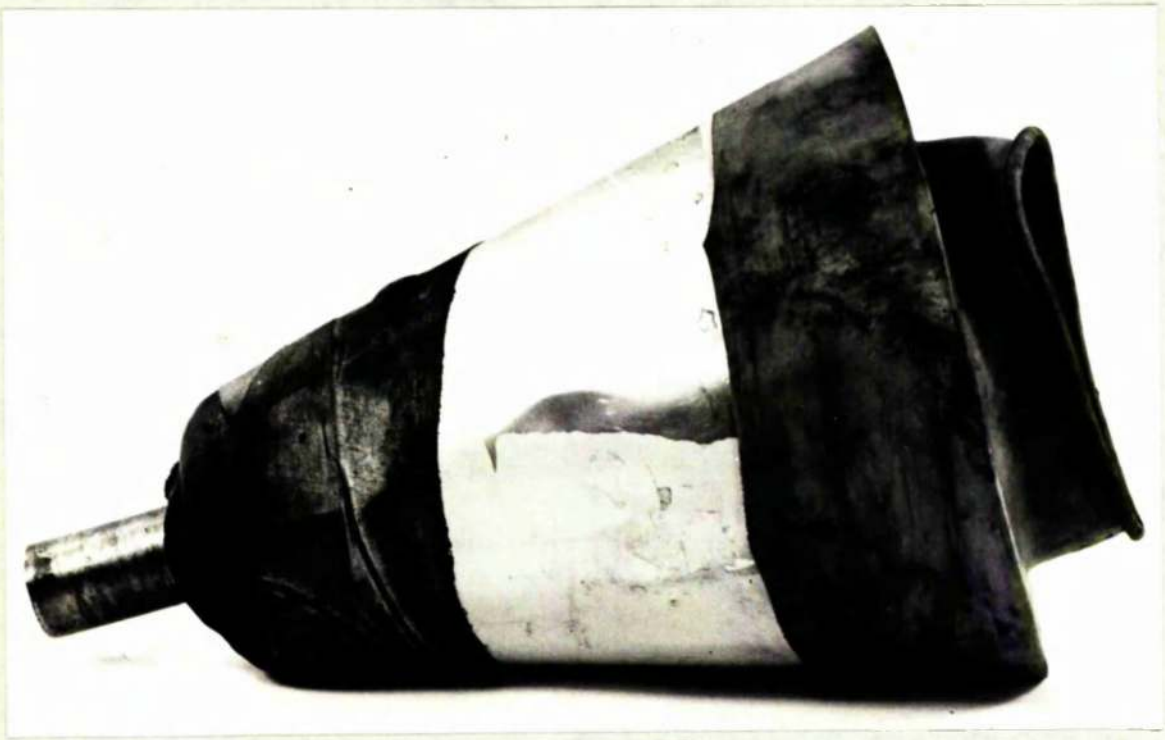

Fig. 18

Calf mask with rubber ring seal.

Each mask was connected by means of the brass nipple at the apex to a 'Y' piece of appropriate size. For the adult mask the nipple screwed into the one inch stem of a 'Y' piece made from one and a half inch diameter copper tube. The other two arms of the 'Y' piece were connected to an inspiratory and expiratory valve, respectively. These connections were made by means of 18 inch lengths of flexible plastic tube of one and a quarter inch internal diameter ("Hoovertex" [x]). For the calf a 'Y' piece made of three-quarter inch diameter copper tube was used and the connections to the unidirectional valves were of six inch lengths non-kink flexible rubber tubing of the kind used in an anaesthetic apparatus.

The unidirectional valves used were of the diaphragm type. For the adult cow all airways of the valves were of a minimum of one and a quarter inch internal diameter while the rest of the valve was of appropriate size. For the calf the airways were of three-quarter inch internal diameter. Drawings of the valves giving the dimensions are shown in figures 19 and 20.

The expiratory side of the unidirectional valve was connected directly to the inlet of the gas meter. The gas meter used was the Parkinson Cowan no. 004 (2½" outlet)[y] which measured a gas flow of up to 700 litres per minute with a minimal resistance. This gas meter is

x Hoover Ltd., Perivale, Middlesex.
y Parkinson Cowan Ltd., City Road, London.

shown in figure 21.

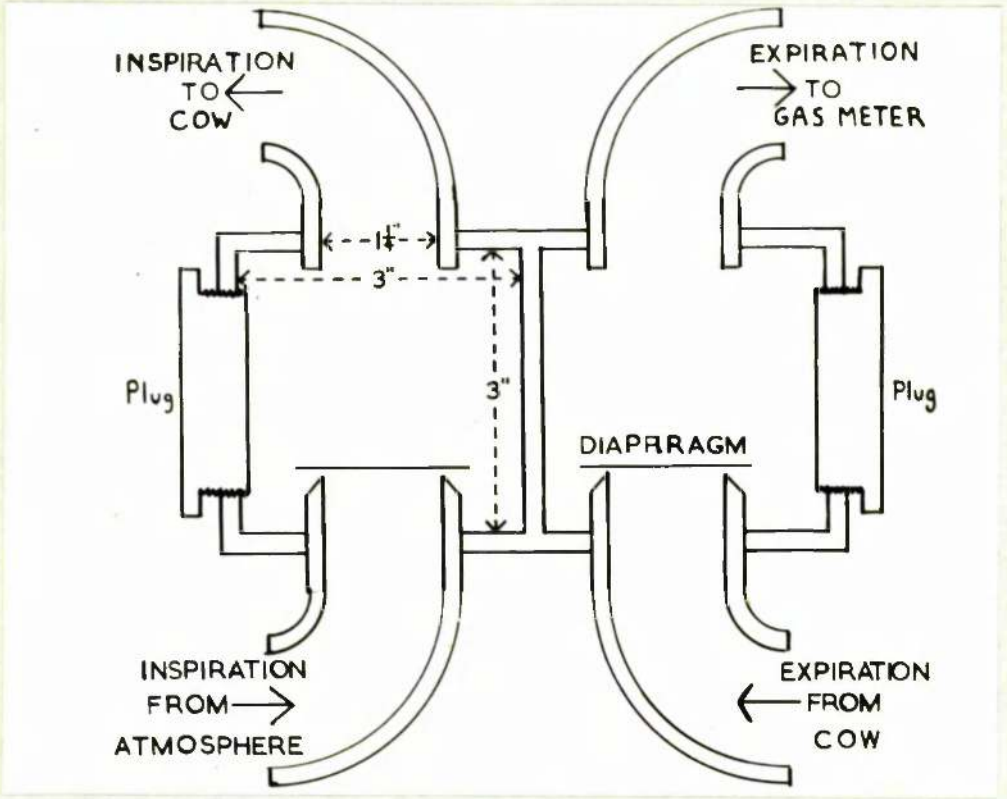

Fig. 19

Unidirectional valves for adult cattle.

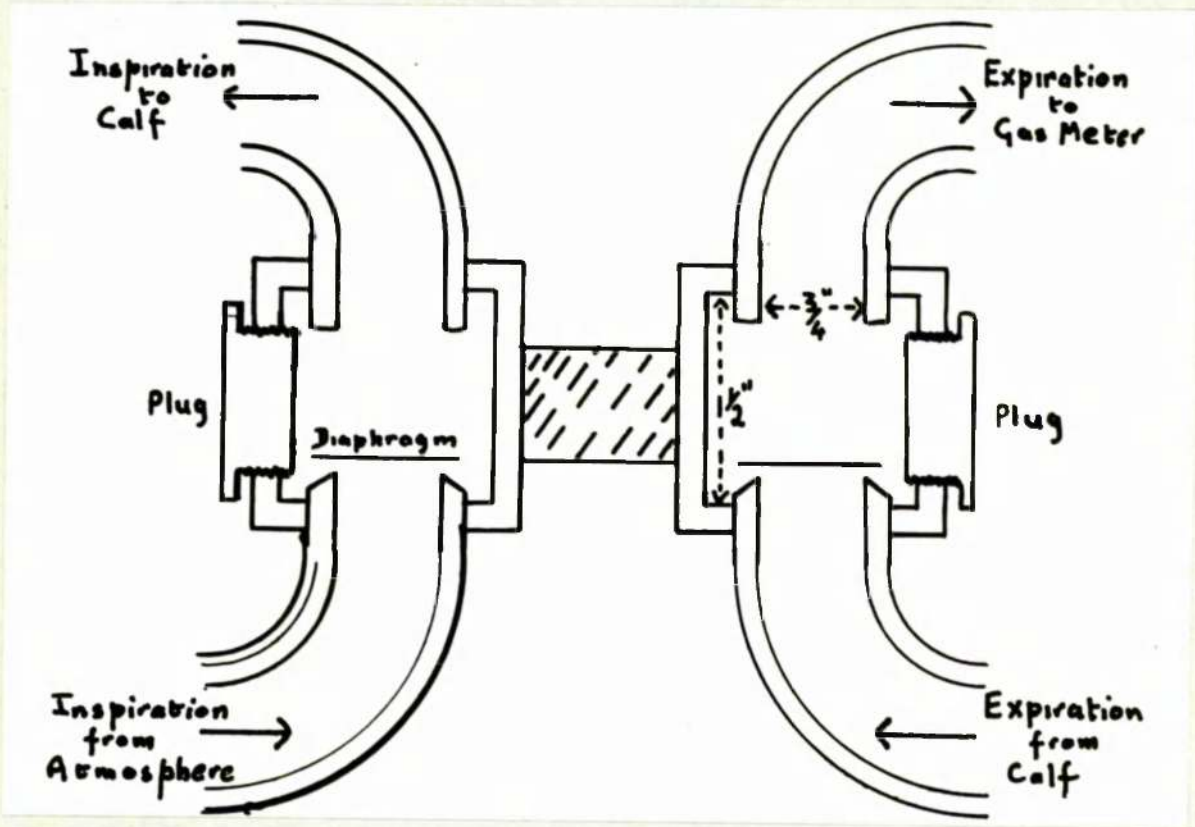

Fig. 20

Unidirectional valves for calves.

This particular meter was obtained after consultation with Dr. J. V. A. Dando who was using two such meters in the Scottish Hospitals Endowment Research Trust unit in Glasgow.

Measurements were made of the resistance to gas flow of the gas meter together with the multidirectional valves and connecting tubes as used when pulmonary ventilations were recorded. The results are given below.

	Rate of flow in litres per min.	Resistance in centimetres of water
(a) Small valves and gas meter	50	2
	100	5
	150	8
	180	14

Suitable for the measurement of pulmonary ventilations up to 50 litres/minute.

(b) Large valves and gas meter	130	2.5
	240	3.5
	320	4.5
	400	7.0

Suitable for the measurement of pulmonary ventilations over 50 litres/minute and less than 130 litres/minute.

Ability of the Eastman-Servo IIA Gas Meter to Record Accurately Pulsatile Air Flows

The ability of the gas meter to record accurately pulsatile air flows was tested by the use of an apparatus which had been developed by Dumin and Rooksby (1959) to calibrate the Ken Plank respirometer. This apparatus gave a pulsatile gas flow at physiological respiration frequencies (12 to 60 per minute) and covered a range of pulsatile flows up to 200 litres per minute.

The volume control on the apparatus was adjusted first to give a steady flow of 200 litres per minute and then the interrupter was started so that a pulsatile flow corresponding to a respiratory rate of 35 per minute was produced. Reduction of total flow took place to 60 litres per minute.

The output from the apparatus was put through the gas meter and the volume as recorded by the gas meter was noted. The volume was collected into a Douglas bag and afterwards measured.

The same experiment was repeated with a pulsatile flow corresponding to that obtained with a respiratory rate of 60 per minute and a ventilation of 30 litres per minute.

The steady flow was then increased to 400 litres per minute and measurements were made with the interrupter adjusted first to correspond

to a respiratory rate of 36 per minute and a ventilation of about 146 litres per minute when measurements were again made and also after adjustments had been made to the interrupter to correspond to a respiratory rate of 60 per minute and a ventilation of 168 litres per minute. Table 18(a) gives the results obtained.

Table 18(a)

Accuracy of Gas Meter in Recording Pulsatile Flow

Experiment	Steady Flow litres/min.	Respiratory Rate	Ventilation litres/min.	
			Gas Meter	Douglas Bag
1	200	36	68	68
2	200	36	67	67
3	200	60	79	81
4	200	60	80	82
5	400	36	146	142
6	400	36	144	140
7	400	60	168	168
8	400	60	164	168

The average error was 1.8% which would not interfere with the results.

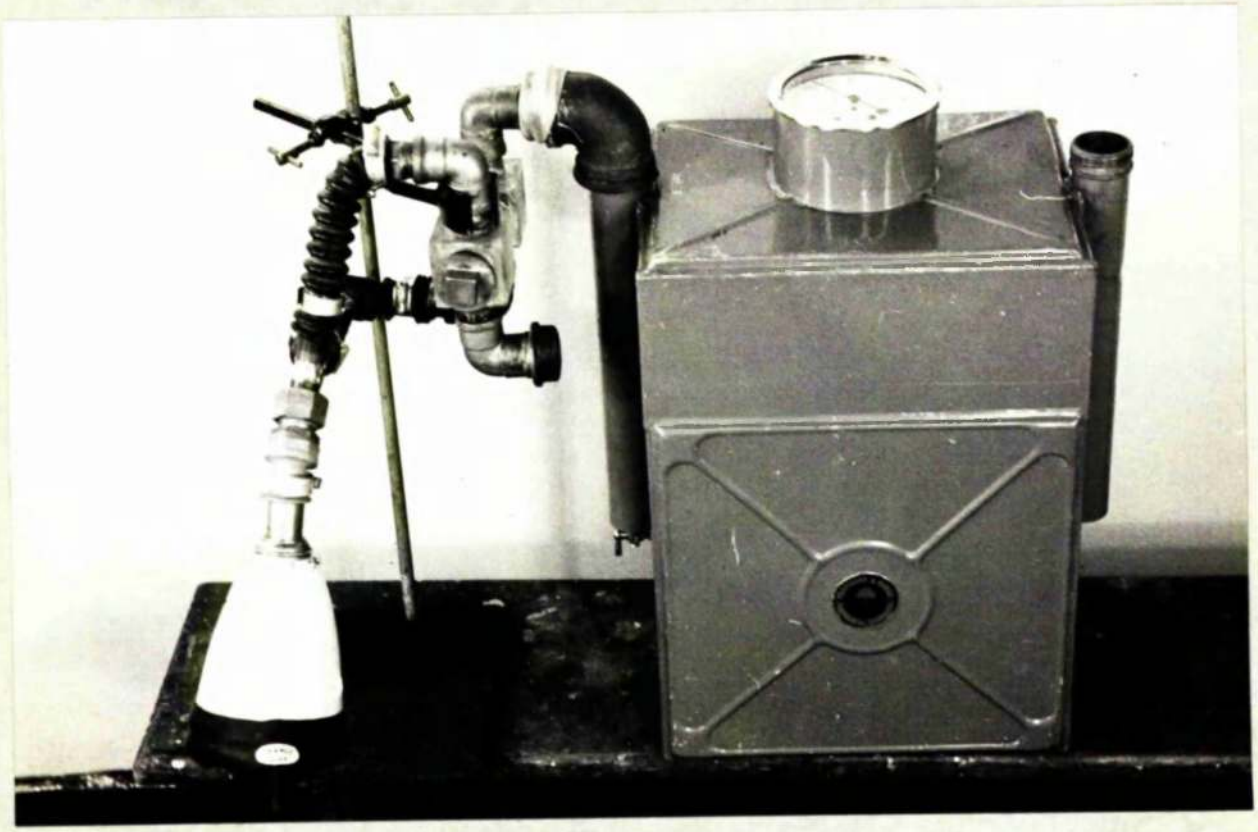

Fig. 21

Parkinson Cowan CD4 gas meter

with calf mask and unidirectional valves attached.

Having constructed the apparatus, the method of use was then developed. The respiratory efforts of a bovine subject were observed when the animal was at rest and not connected to the apparatus. Observation was made of both the rate and depth of respiration. The animal was then fitted with the face mask alone and observation was

again made of the depth and rate of respiration. It was noted that the majority of cattle resented the fitting of the face mask and required slight restraint to maintain the mask in place. Most animals, after the initial resentment to fitting the mask, required only their heads to be held gently with the mask in position so that within two minutes of fitting the mask the rate of respiration had returned to normal. With the face mask on the animal, connection was made to the unidirectional valves by means of the 'Y' piece and the flexible tubes. The ventilation rate was measured from the gas meter for periods of five or 10 minutes.

Calves were found to be more difficult subjects than adult cattle since on occasion, after they had apparently settled down, they suddenly decided to struggle violently, settled down and then struggled violently again. When this occurred studies had to be abandoned in some calves.

The measurement of the ventilation rate of a calf is illustrated in figure 22.

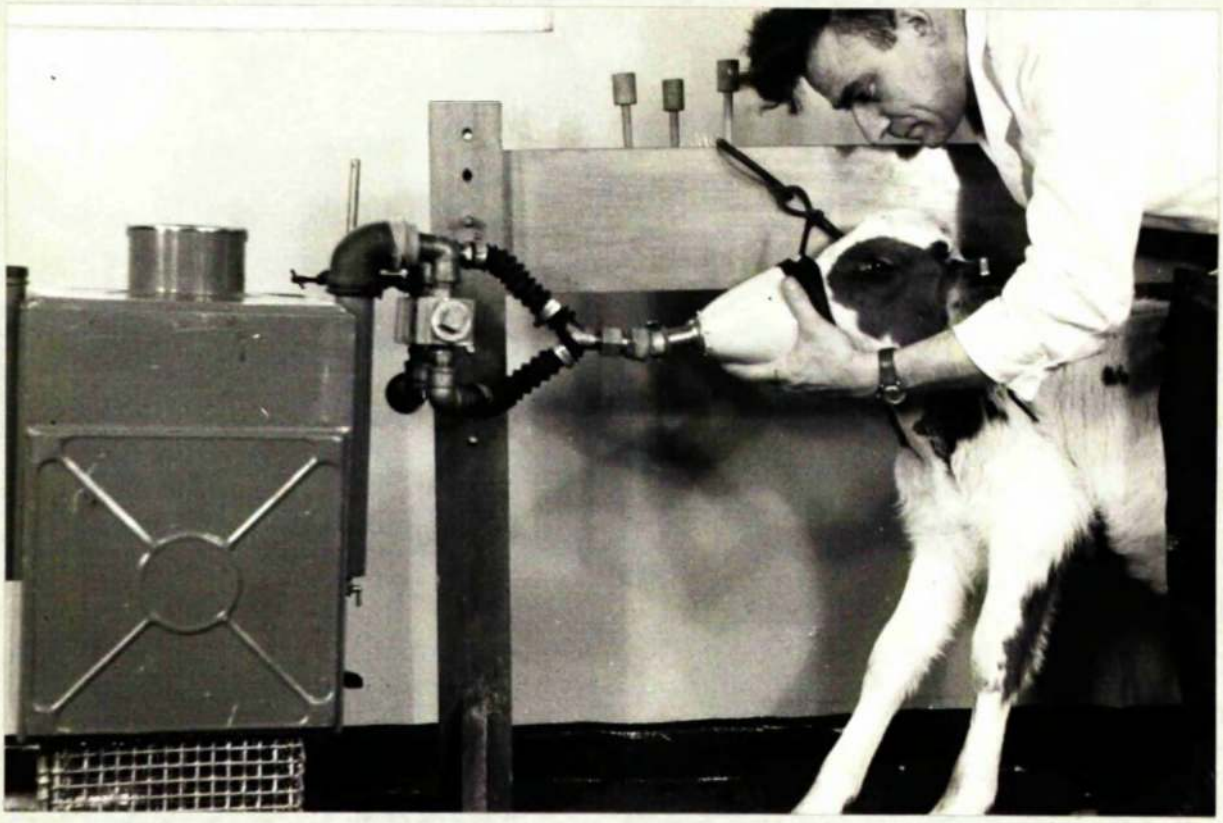

Fig. 22

Measurement of the ventilation rate of a calf.

(5) Respiratory rate

The respiratory rate was counted while the ventilation rate was being measured. The clicks of the diaphragm of the expiratory valve were counted and the total for each minute recorded.

RESULTS

(1) A comparison of the effects of errors of sampling on the carbon dioxide content and the pH of the plasma.

(2) The carbon dioxide content of the plasma of (a) normal Ayrshire cattle
(b) normal dogs.

(3) The pH of the plasma of (a) normal Ayrshire cattle
(b) normal dogs.

(4) The physiological variations of the carbon dioxide content and the pH of the plasma of normal Ayrshire cattle.

(5) The ventilation and respiratory rates of Ayrshire cattle.

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Statistical Analysis

Throughout this thesis where mean values are given they are followed by the standard deviation.

Differences stated as significant indicate that the probability of the given results having occurred by chance is less than 5%. Student's "t test" was used throughout.

(1) A comparison of the effects of errors of sampling on the carbon dioxide content and the pH of the plasma.

(a) Contamination of tubes

The possibility of errors arising from the contamination of blood sampling tubes was considered as follows. Duplicate blood samples were taken and duplicate examinations were carried out on the plasma of each sample. The results are given in table 19.

Table 19

Variations in the CO_2 content and the pH of Plasma

Due to Contamination of Sampling Tubes

Cow	CO_2 m.moles/litre Tube 1		Tube 2		pH Tube 1		Tube 2	
1	30.5	30.6	30.5	30.5	7.435	7.43	7.44	7.43
2	29.9	29.5	29.5	29.3	7.44	7.44	7.47	7.47
3	30.6	31	30.6	31	7.47	7.47	7.465	7.485
4	27.6	27.8	27.5	28	7.42	7.42	7.43	7.43

From these results it will be seen that with the precautions adopted for the cleaning of glassware, variations from this source were not more than 0.3 m.moles/litre in the carbon dioxide content and not

more than 0.03 pH units in the determination of the plasma pH. Nevertheless the variations in the determination of the plasma pH were of sufficient magnitude to justify the practice of taking duplicate samples and doing single pH determinations on these.

(b) Exposure to atmosphere

It has been emphasized repeatedly that blood or plasma samples examined for their carbon dioxide content should not be exposed to the air because of the loss of carbon dioxide to the atmosphere (Davenport, 1950). To study this source of error blood samples were obtained from the brachial artories of five cows. Two samples were taken at the same time from each cow and one sample was collected with exposure to air while the other was collected under liquid paraffin and sealed with a rubber bung. The time between taking the first sample from the first cow and analysis of the plasma was 25 minutes while the interval between taking the samples from the last cow and analysis of the plasma was approximately 35 minutes. The results are given in table 20.

Table 20

Effect of Exposure of Blood to the Atmosphere on the CO_2 Content and the pH of Plasma

Cow	CO_2 content in m.moles/litre		CO_2 loss in m.moles /litre	Plasma pH		pH Difference
	Aerobic	Anaerobic		Aerobic	Anaerobic	
1	25.9	27.1	1.2	7.50	7.44	.06
2	27	27.8	0.8	7.50	7.41	.09
3	29.9	30.4	0.5	7.35	7.30	.05
4	30	30.4	0.4	7.37	7.35	.02
5	26.8	27.5	0.7	7.37	7.37	.02

The results showed once more that blood samples must be removed without exposure to the atmosphere otherwise a loss of carbon dioxide of up to 1.2 m.moles/litre might occur with a pH difference as great as 0.09 pH units.

(c) Liquid paraffin seal

Van Slyke (1949) stated that absorption of carbon dioxide by a protective layer of liquid paraffin could lead to errors due to absorption of the carbon dioxide by the paraffin. This source of error was studied by collecting blood in 50 ml. centrifuge tubes on one occasion, and on another occasion by collecting in 25 ml. centrifuge

tubes. In the 50 ml. tubes the blood was covered by two inches, one inch, half an inch and minimal layers of liquid paraffin, while in the 15 ml. tubes the layers of liquid paraffin were half inch, quarter inch and minimal layers of liquid paraffin. The results are shown in table 21.

Table 21.

Effect of the loss of Carbon Dioxide to the Protective Layer of Paraffin on the CO_2 Content and the pH of Plasma

50 ml. centrifuge tubes	CO_2 content	Plasma pH
2" liquid paraffin above blood	29.25	7.36
1" liquid paraffin above blood	30.9	7.34
½" liquid paraffin above blood	31.25	7.33
Minimal liquid paraffin above blood	31.5	7.32
15 ml. centrifuge tubes		
½" liquid paraffin above blood	28.23	7.43
¼" liquid paraffin above blood	28.625	7.46
Minimal liquid paraffin above blood	29.75	7.44

It can be seen that an error as great as 1.2 m.moles/litre in the plasma carbon dioxide content and as great as 0.05 pH units can occur

due to absorption of carbon dioxide by liquid paraffin when the layer was greater than minimal. Accordingly minimal layers of liquid paraffin have been used in this study.

(d) Autolysis on plasma pH

Hathaway and Sawyer (1954) determined colorimetrically the pH of serum samples obtained by taking blood samples with anaerobic precautions and allowing them to remain on the bench for a period of two hours in order that the serum might separate out. It is difficult to see how they obtained serum samples from bovine blood after two hours when bovine serum usually takes much longer to separate out unless the samples were stored. Using this method they obtained apparently normal values in spite of the fact that Rosenthal (1948) had demonstrated that the effects of autolysis in blood samples standing for this length of time at 20° C. would cause a very marked fall in plasma pH.

An experiment was designed to check the effects of autolysis on plasma pH and to find out if immediate centrifugation would limit autolysis. Eight blood samples each in a 50 ml. centrifuge tube were taken from the brachial artery of a cow. All the tubes were placed in ice for 15 minutes to reduce to a constant temperature, after which they were exposed to room temperature (15° C.). Tubes 1 to 5 were immediately centrifuged for 15 minutes and plasma pH of tubes 1 and 5 determined. After one and a half hours exposure to 15° C., tube 6 was

centrifuged and the pH determined on tubes 2 and 6. This procedure was repeated at half hour intervals on tubes 3 and 7, and 4 and 8, respectively. The results obtained are given in table 22.

Table 22

Effect of Autolysis on Plasma pH

	Centrifugation at start Tube no.	pH	pH	Centrifugation at intervals Tube no.	pH	pH
Initial	1	7.50	7.50	5	7.48	7.475
½ hour	2	7.505	7.505	6	7.48	7.485
1 hour	3	7.456	7.465	7	7.38	7.39
1½ hours	4	7.41	7.41	8	7.38	7.39

The results confirmed Rosenthal's findings that autolysis caused a considerable drop of plasma pH (0.1 pH units) in one hour but that separation of the cells to the bottom of the centrifuge delayed the autolytic effect. As a result of these observations all blood samples were centrifuged and the pH determined within half an hour of collection. Where this was not possible, samples were placed in ice until the determinations were carried out.

It is difficult to draw any conclusion as to how McHenry and Grayer (1954) obtained their normal results by determination of the

serum pH. The only possible explanation is that by reading at room temperature they obtained a pH higher than the true pH of the serum samples since the effect of not taking into account a temperature coefficient for plasma or serum would counteract the lowering of serum pH caused by autolysis.

(2) The carbon dioxide content of the plasma of (a) normal Ayrshire cattle
(b) normal dogs

(a) Normal Ayrshire cattle

Blood samples by puncture were taken from the jugular veins of 29 healthy Ayrshire cows and the carbon dioxide content of the plasma was determined. The results are given in Appendix I. A mean value of 27.4 ± 1.9 m.moles/litre was obtained. Blood samples were also taken from the jugular veins of 10 cows by means of a jugular venous catheter as already described. It was assumed that these samples approximated to mixed venous samples of cattle. The mean value of 29.2 ± 2.7 m.moles/litre obtained for the carbon dioxide content of the plasma derived from mixed venous samples was not significantly higher than the mean value obtained for the carbon dioxide content of venous plasma derived from jugular venous blood obtained by puncture. The individual results are given in Appendix II.

Over a period of three years 159 arterial blood samples were taken from healthy cows in the University herd. This herd consisted of non-pedigree Ayrshire cows and was run on a commercial basis so that unproductive cows were quickly removed from the herd. This factor, combined with culling because of age, meant that there was a constant turnover of animals in the herd over the three years.

These cows were in varying stages of pregnancy and lactation. During the winter they were housed for most of the day but allowed out for a short time for exercise. In spring and autumn they were housed at night while in summer they were out day and night except for milking. They were fed a production mixture of cattle cake, beans and oats throughout the year and in addition, when grass was not available, they received hay, silage, roots and kale.

The arterial samples were all taken from these cows at approximately the same time each day (8 a.m.), one hour after morning milking. Note was made of the condition of the animal; whether dry or lactating, pregnant or non-pregnant. Samples were taken on nine different occasions during these three years as shown in table 23. The number of cows sampled on each occasion varied from eight to 23 and duplicate samples were taken from each cow on each of which a single estimation was made. Detailed results are given in Appendix III, while the mean values together with the standard deviations of the carbon dioxide content of the plasma obtained on each occasion are given in table 23.

The mean value obtained for the carbon dioxide content of the arterial plasma of 159 dairy cows was not significantly different from the mean values obtained for the carbon dioxide content of jugular venous plasma and mixed venous plasma and was identical with that given by Davenport (1950) for the arterial plasma carbon dioxide content of the

Table 22

Mean Values of the CO_2 Contents of the Plasma of Arterial Cows Together with the Dates of Sampling

Year	Month	No. of cows	Mean value CO_2 content m.moles/Litre
1955	April	20	27.6 ± 2.1
1955	June	39	25.4 ± 1.9
1955	October	10	27.5 ± 2.2
1956	January	23	27.3 ± 2.5
1956	May	20	27.0 ± 2.1
1957	June	20	25.2 ± 1.3
1958	January	19	28.2 ± 1.4
1958	March	6	27.2 ± 2.7
		Total 159	Mean 27.2 ± 2.6

human subject.

From this series of observations no significant difference was found between any of the mean values obtained so that it can be inferred that no significant variation occurred in the arterial plasma carbon dioxide contents of these cows due to seasonal variation.

The results obtained from pregnant and non-pregnant cows are compared in table 24.

Table 24

Mean Values of the CO_2 Contents of the Plasma of Pregnant and Non-Pregnant Cows

Condition of cows	No. of cows	Mean CO_2 content m.mole/litre
Pregnant	65	26.5 ± 1.73
Non-pregnant	76	27.3 ± 2.26

It will be observed that there was no significant difference.

Similarly the results obtained were analysed on the basis of the lactational status of the cows. The results are given in table 25 and again there was no significant difference.

Table 25

Mean Values of the CO_2 Contents of the Plasma of Dry and Lactating Cows

Condition of Cows	No. of cows	Mean CO_2 content m.mole/litre
Dry cows	15	27.7 ± 1.71
Lactating	126	27.0 ± 2.12

Arterial blood samples were also collected from 61 Ayrshire calves and the carbon dioxide content of the plasma was determined. These calves were purchased in a local market and brought to the Veterinary Hospital where they were housed in individual huts. For the first month of their lives they were fed "Osteomilk" no. 2*, a dried whole milk product, at a strength of 1 lb. to the gallon of water and they received six to eight pint daily in two feeds. At three weeks of age they were introduced to hay and calf manger nuts, and at four weeks the "Osteomilk" stopped. A variety of antibiotic supplements was given during the first two weeks of life to prevent diarrhoea.

Blood samples were removed from the brachial arteries of the calves the ages of which varied from one to eight weeks of age. The results obtained are presented in table 25.

The mean value obtained, 29.8 ± 2.5 m.moles/litre was significantly higher than that found for adult cows.

* Glaxo Laboratories, Ltd., Greenford, Middlesex.

Table 26

Mean Values for the CO_2 Contents of the Arterial Plasma of Calves Divided on the Basis of Age

Age	Number	Mean CO_2 content m.mole$\frac{s}{l}$/litre
7 days	18	30.53 ± 1.7
14 days	20	29.8 ± 2.5
4 weeks	8	30.6 ± 3.0
6 weeks	21	29.3 ± 2.2
8 weeks	14	28.9 ± 1.8
	Total 81	Mean 29.8 ± 2.5

(b) Normal dogs

The carbon dioxide content of the arterial plasma of dogs has been reported to be lower than in most species (Spector, 1956). The carbon dioxide content of the arterial plasma of 15 dogs was determined to confirm the reported results. The dogs from which the samples were taken were healthy normal dogs which were being maintained in the Veterinary Hospital. Blood samples were removed from the femoral artery. The mean value obtained, 21.33 ± 2.9 m.moles/litre, was similar to the value of 21.4 m.moles/litre reported by Spector (1956). These values for the carbon dioxide content of the arterial plasma of dogs are significantly lower than the values found for the arterial plasma of cattle.

(2) The pH of the plasma of (a) normal Ayrshire cattle

(b) normal dogs

(a) Normal Ayrshire cattle

As described in the section on methods, blood samples were brought to a constant temperature in a water-bath, centrifuged at a known temperature, 23-25° C., and the plasma pH was found at 20° C. Using the temperature coefficients previously determined the pH values were corrected to 38° C.

Duplicate determinations of the arterial plasma pH of 169 and of the venous plasma pH of 55 healthy Ayrshire cows were carried out. Some of these animals were housed in the Veterinary Hospital while others were cows in the University herd. The mean value obtained for the arterial plasma pH at 38° C. was 7.43 ± 0.025 pH units, and the mean value for the venous plasma pH at 38° C. was 7.39 ± 0.05 pH units.

Duplicate determinations were also made on arterial plasma samples from 33 normal calves housed in the Veterinary Hospital and a mean value of 7.42 ± 0.035 pH units was obtained. Venous samples were also obtained from 19 calves and the mean value found for the plasma pH was 7.38 ± 0.02 pH units. These values for plasma pH were similar to those found in adult cattle.

(b) Normal dogs

Determinations were made of the plasma pH of 10 dogs. Temperature coefficients had already been found for dog blood and plasma as described in the section on methods. Using these temperature coefficients the pH at 38° C. of the plasma obtained from femoral arterial blood of 10 dogs was determined. A mean value of 7.41 pH units with a standard deviation of ± 0.05 pH units was obtained.

These values are very similar to the values obtained for cattle in this study (pH = 7.43 ± 0.045) but higher than the value given by Spector (1958) for dogs (pH = 7.36).

(4) The physiological variations of the carbon dioxide content and the pH of the plasma of normal Ayrshire cattle

Davenport (1950) gave 1.1 m.moles/litre as the difference in the carbon dioxide content of arterial and venous plasma obtained at the same time from the same human subject. He also stated that some variation existed between venous plasma samples taken from different vessels in the same subject as did Holman and Kelentley (1935). In the literature there is no record of the extent of the arterio-venous difference in the carbon dioxide content of the plasma of cattle or of differences in the carbon dioxide contents of the plasma obtained from blood taken from different veins of cattle. The differences in the carbon dioxide content and pH of plasma obtained from different vessels were studied in five cows. Blood samples were obtained at the same time from the brachial artery, the jugular vein and the mammary vein of each cow. The results obtained for the plasma carbon dioxide contents are given in table XV.

The results demonstrate that an arterio-venous difference in the carbon dioxide content of plasma could be detected but that its magnitude depended on the vein selected. The mean arterio-venous difference between the carbon dioxide content of the plasma from the brachial artery and that from the jugular vein was 1.2 m.moles/litre while the mean arterio-venous difference between the carbon dioxide content of the plasma from the brachial artery and mammary vein was 2.9 m.moles/litre. In the five cows sampled, the mammary venous plasma carbon dioxide content was greater than the jugular venous carbon dioxide content, the mean jugular venous difference being 1.7 m.moles/litre.

Table 22

Arterio-venous Differences in the Plasma CO_2 content

Cow	Vessel	Plasma CO_2 m.mols/ litre	Brachial-jugular difference	Brachial-mammary difference	Mammary-jugular difference
12549	Brachial artery	29			
	Jugular vein	30.5	1.5	3.4	1.9
	Mammary vein	32.4			
12552	Brachial artery	33.1			
	Jugular vein	34.25	1.15	2.8	1.65
	Mammary vein	35.9			
12553	Brachial artery	28.75			
	Jugular vein	30.1	1.35	3.75	1.4
	Mammary vein	31.5			
12556	Brachial artery	28.0			
	Jugular vein	28.25	0.25	3.0	2.75
	Mammary vein	29.0			
12557	Brachial artery	25.0			
	Jugular vein	26.63	1.63	2.63	1.0
	Mammary vein	27.63			
		Mean	1.2	2.9	1.7

The results for the differences in plasma pH are given in table 23.

Table 23

Arterio-venous Difference in the Plasma pH

Cow	Vessel	Plasma pH	Brachial-Jugular difference	Brachial-Mammary difference
12649	Brachial artery Jugular vein Mammary vein	7.50 7.45 7.44	0.05	0.06
12652	Brachial artery Jugular vein Mammary vein	7.45 7.36 7.43	0.1	0.05
12653	Brachial artery Jugular vein Mammary vein	7.54 7.47 7.44	0.07	0.1
12656	Brachial artery Jugular vein Mammary vein	7.50 7.44 7.44	0.06	0.06
12657	Brachial artery Jugular vein Mammary vein	7.50 7.44 7.48	0.06	0.02
		Mean	0.07	0.06

It will be observed that there is an arterio-venous difference in plasma pH between the brachial artery and both the mammary and jugular veins. The pH of plasma from the mammary vein was not consistently different from the pH of the plasma from the jugular vein.

Variations have also been observed in the plasma carbon dioxide

content and the plasma pH of the healthy human subject throughout the course of a day which were unconnected to any specific activity (Cullen and Keele, 1929). A study was made of the variations of plasma pH and plasma carbon dioxide occurring in seven healthy adult cattle during the course of a day or part of the day when they were standing in the byre. These animals were given their normal feed of cattle cake at 7 a.m. and thereafter had hay and water ad libitum through the day. They were noticed to eat, drink, urinate, defaecate and urinate and behaved in every way as normal cattle, throughout the period of study.

Blood samples were withdrawn at intervals during the day from the same point in the same vessel.

The results obtained for those cows which have been numbered 1 to 7 are given in tables 29 to 32. Graphic representation of the results obtained from cows 1 and 2 are given in figures 23 and 24.

Table 22

Variation in the CO_2 content and the pH of the plasma obtained from brachial arterial blood of cow 3

Time of sampling	Plasma pH	Plasma CO_2 content m.moles/litre
9.35	7.40	27.6
10.35	7.36	27.8
11.35	7.43	27.0
12.35	7.38	27.2
13.35	7.41	27.8
14.35	7.41	27.8
15.35	7.41	27.1
16.35	7.39	27.0

The variations observed in the plasma carbon dioxide content were small, being less than 1 m.mole/litre. The variations in plasma pH were likewise small. No correlation was apparent over the day between changes in carbon dioxide content and pH.

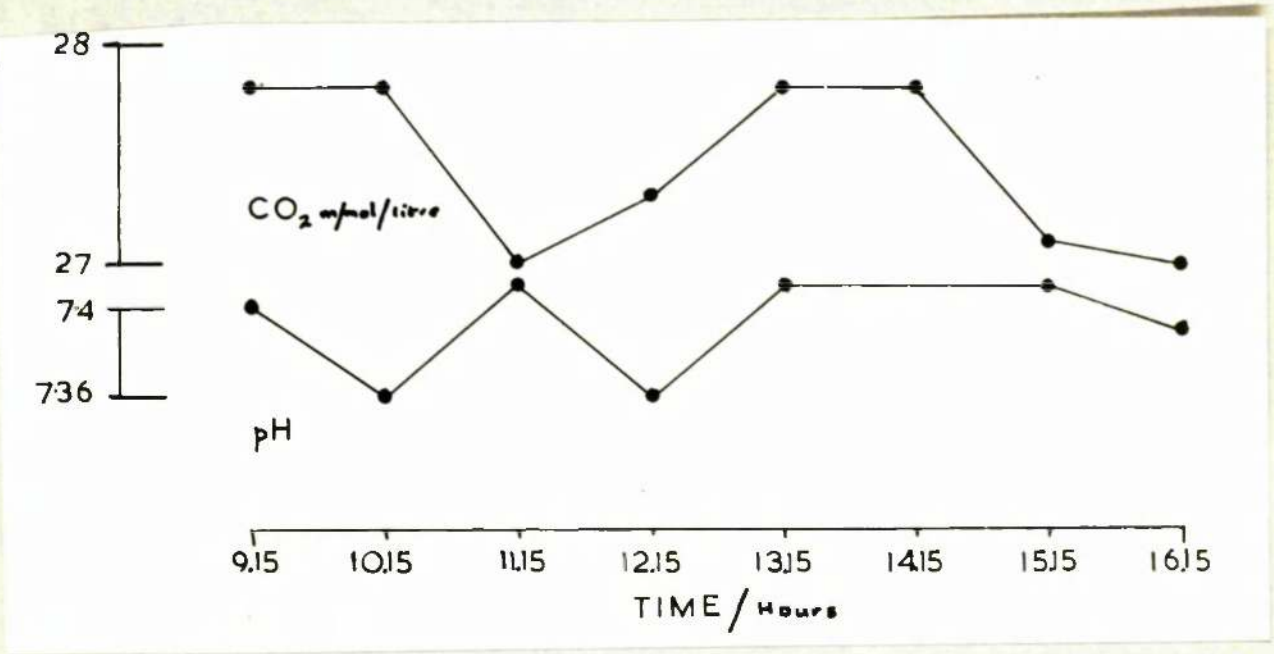

Fig. 23

Graphic representation of the results in table 29

Table 30

Variations in the CO_2 Content and the pH of the Plasma Obtained from Jugular Venous Blood of Cow no. 2 During the Course of an Afternoon

Time of Sampling	Plasma pH	Plasma CO_2 content m.moles/litre
14.00	7.43	29.5
14.35	7.42	32.5
15.10	7.41	29.5
15.45	7.32	30.3
16.00	7.31	29.9
16.10	7.36	28.8

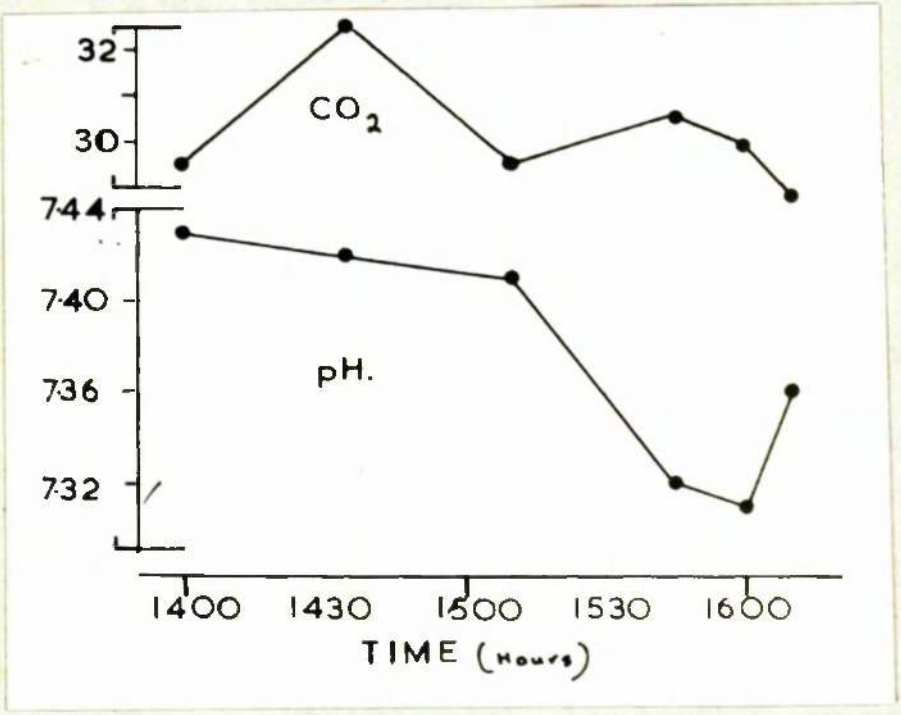

Fig. 24

Graphic representation of the results in table 30

This cow which was sampled six times in just over two hours showed much more marked changes in both the pH and the carbon dioxide content of the plasma.

Table 27

Variations in the pH and the CO_2 Content of Plasma Obtained from Brachial Arterial Blood of Dog no. 3 During the Course of an Afternoon

Time of sampling	Plasma pH	Plasma CO_2 content m.mole/litre
13.00	7.42	26.5
14.00	7.53	26.3
15.00	7.53	27.3
16.00	7.51	25.5

The variations in the plasma carbon dioxide content were small but there was a greater change in plasma pH.

Table 32

Variations in the CO_2 Content and the pH of Plasma Derived from Brachial Arterial Blood of Cat no. 4 Taken at Short Intervals Over 60 Minutes

Time of sampling	Plasma pH	Plasma CO_2 content m.moles/litre
13.25	7.42	27.3
13.32	7.42	27.3
13.35	7.42	27.9
13.37	7.42	27.9
13.40	7.43	27.9
14.45	7.42	30.0

Six arterial samples were taken from this cat in 60 minutes during which time no change in plasma pH and only very small changes in plasma carbon dioxide content occurred except at the last sample where a rise of 2 m.moles/litre took place unconnected with a change in plasma pH.

Table 33

Variations in the CO_2 Content and the pH of Plasma Obtained from Jugular Venous Blood of Cow Nos. 5, 6 and 7

Time of sampling	Cow 5 Plasma pH	Cow 5 Plasma CO_2 m.moles/ litre	Cow 6 Plasma pH	Cow 6 Plasma CO_2 m.moles/ litre	Cow 7 Plasma pH	Cow 7 Plasma CO_2 m.moles/ litre
11.15	7.40	28.6	7.39	28.3	7.38	28.5
12.30	7.40	28.6	7.41	30.9	7.40	26.6
13.45	7.39	29	7.43	29.3	7.40	27.0
15.15	7.37	29.6	7.42	29.5	7.40	25.6
16.30	7.34	30.0			7.34	25.1

These three cows which were studied at the same time showed slight variations in both the carbon dioxide content and the pH of their plasma.

Studies on these seven cows indicated that changes in the pH and carbon dioxide content of the plasma which occurred during the day unconnected with any activity, were small and within the normal values already determined for adult Ayrshire cattle.

(5) The ventilation and respiratory rate of Ayrshire cattle

The main purpose of this study was to determine the normal values for the plasma carbon dioxide and plasma pH of Ayrshire calves and cows to determine the changes associated with disturbances of respiration.

To study these disturbances of respiration it was necessary to measure both respiration rates and pulmonary ventilation. The methods of so doing have already been described and it is necessary only to state that each measurement in table 24 represents the mean of at least five and often 20 measurements taken during a period of five to 20 minutes.

The respiratory minute volumes in table 24 were corrected to B.T.P.S. It was found however that when this was done, due to the fact that the temperature of the two rooms in which these measurements were made was relatively constant between 13 and 15° C., although atmospheric pressure varied from 747 - 770 m.Hg., the conversion factor to correct to B.T.P.S. volumes was found to be a constant 1.112. This factor can be used to convert any other measured ventilation in this thesis to B.T.P.S.

The results show that the respiratory rate does not increase with body weight but the pulmonary ventilation does and as a consequence so must the tidal volume. The relationship between body weight and pulmonary ventilation is not linear according to Brody (1950) but logarithmic. Our results are in agreement with those of Brody.

Table 24

Respiratory Rate, Ventilation Rate and the Tidal Air of Normal Cattle Accused According to Body Weight

Body weight (kg.)	Ventilation rate Litres/min. (B.T.P.S.)	Respiratory rate /minute	Tidal air mL.
27	7	10	370
28	6	19	334
28	7	24	276
30	7	22	305
36	9	28	337
37	7	20	246
40	16	31	502
49	24	35	698
50	29	30	963
51	23	32	729
52	27	30	890
99	31	30	1097
123	48	22	2073
255	72	20	3558
364	79	25	3095
374	86	22	3592
386	87	28	3719
432	110	24	4533
445	322	30	5705
450	100	24	4270

DISCUSSION

The development of a method of obtaining arterial blood from the brachial artery of unanaesthetised cattle of all ages has made it possible for the determination to be made of the carbon dioxide content of arterial plasma of cattle. It is interesting to note that the mean value obtained for the arterial plasma of adult cattle was identical to that given by Davenport (1950) for the arterial plasma carbon dioxide of the human subject but both cattle and humans have a significantly higher arterial plasma carbon dioxide than does the dog.

The mean values obtained in this study for carbon dioxide content of venous plasma of both cows and calves are similar to the values given by the majority of other workers whose results are given in the review of the literature at the beginning of this section.

The mean value for the carbon dioxide content of the arterial plasma of dogs found in this study was almost identical to the value given by Spector (1953).

Similarly the mean plasma pH values found in this study were identical with the venous and arterial plasma pH values given by Davenport (1950) for the human subject and the venous plasma pH values were similar to those found by other workers.

Variations in the carbon dioxide content and the pH of the plasma of normal cattle occurring during the course of a day were similar to changes observed by Cullen and Eagle (1939) in the human subject.

The observations made in this study of the respiratory rate of cattle of all ages agreed with those of all the other workers with the exception of Findlay (1956) whose control values given for the respiratory rates of calves indicated the presence of exhibaent, thoracic stress or pneumonia in his calves.

The values found for the ventilation rate of cattle were in agreement with the far more extensive study of Brody (1950) and with results of others where a comparison on a body weight basis could be made.

CONCLUSIONS

From the various estimations made in Part I of this study certain conclusions were drawn.

(1) The following precautions were necessary in the collection and handling of blood samples.

(a) When duplicate blood samples were taken and single estimations were carried out on these samples, the variations obtained by this method were not more than 0.3 m.moles/litre in the carbon dioxide content and not more than 0.03 pH units in the plasma pH.

(b) Unless the collection of blood was made under a layer of liquid paraffin, carbon dioxide lost to the atmosphere caused errors as great as 1.2 m.moles/litre in the carbon dioxide content and 0.07 pH units in the plasma pH.

(c) Unless this layer of liquid paraffin left in contact with the blood was kept to a minimum, errors arose due to the absorption of carbon dioxide by the liquid paraffin of as much as 1.25 m.moles/litre carbon dioxide content and 0.05 pH units when this layer in a 50 ml. centrifuge tube was two inches deep.

(d) Unless determinations were carried out on plasma samples within

half an hour of collection, autolysis caused a lowering of plasma pH of as much as 0.1 pH units in one hour.

(2) Both the Conway method and the Manometric method of Van Slyke were found to be suitable for the determination of the carbon dioxide content of plasma, the Conway method being the more suitable for large numbers of determinations carried out in a short time. Using these methods the following results were obtained.

(a) The mean value for the arterial plasma carbon dioxide content of 159 adult Ayrshire cows was found to be 27.2 ± 2.7 m.moles/litre. No significant differences were found due to seasonal variations, lactational stains or pregnancy.

(b) The mean values for the carbon dioxide content of venous and mixed venous blood were found to be 27.4 ± 2.9 m.moles/litre and 29.2 ± 2.7 m.moles/litre, respectively.

(c) It was found that a mean difference of 1.7 m.moles/litre existed in the carbon dioxide content of jugular and mammary venous plasma of five cows, the mammary venous plasma carbon dioxide contents being greater.

(d) It was found that the mean value of arterial plasma carbon dioxide in 81 calves was 29.8 ± 2.5 m.moles/litre, a figure significantly lower than the mean for adult Ayrshire cows.

(3) The colorimetric method of determination of plasma pH using phenol

sed on the indicator was examined and shown to be reliable and reponsible.

(c) Using this method, temperature coefficients were found for bovine plasma of 0.009 and for bovine blood of 0.012 pH units per degree centigrade.

(b) Using these temperature coefficients and correcting all results to 38° C. mean values of 7.43 ± 0.045 pH units for the arterial plasma pH of 169 Ayrshire cows, of 7.39 ± 0.05 pH units for the venous plasma pH of 55 Ayrshire cows, and of 7.42 ± 0.036 pH units for the arterial plasma pH of 18 calves were found.

(c) Temperature coefficients of 0.0155 pH units and 0.012 pH units were found for canine blood and plasma, respectively.

(d) Using these temperature coefficients and correcting all results to 38° C., a mean value of 7.43 ± 0.045 pH units was found for the arterial plasma pH of 10 dogs.

(4) No significant variations in the values of plasma carbon dioxide content and plasma pH were observed when seven cows were sampled repeatedly during periods of up to six hours.

(5) It was shown that cattle of all ages had respiratory rates between 16 and 36 per minute and that the ventilation rate varied with the body weight, not in a linear manner but logarithmically so that a calf of body weight of 50 kg. had a ventilation rate of 25 litres per minute while a cow of 500 kg. body weight had a ventilation rate of about 100 litres per minute.

PART IX

THE CHANGES IN THE CARBON DIOXIDE CONTENT AND THE pH OF THE PLASMA

OF CATTLE CAUSED BY DISTURBANCES OF RESPIRATION

INTRODUCTION

Disturbances of respiration may be induced in a number of ways. It is possible to alter the central respiratory drive by factors affecting the respiratory centre either directly or reflexly as the result of stimuli elsewhere. It is possible also to produce changes in respiration by quantitative alterations in the blood, carbon dioxide or H ion concentration.

Little is known of the central respiratory mechanism of cattle or of the factors affecting it. It is assumed that cattle are the same as all other species studied with a respiratory centre which may be affected by other centres in the brain, by changes in its blood chemistry, by impulses arising from peripheral stimuli such as pain or limb movements and by impulses from chemo-receptors which respond to changes in blood chemistry.

In the present study certain disturbances of respiration of diverse origin were investigated in order to ascertain the changes in plasma pH and plasma carbon dioxide content as a result of these disturbances. Ventilation and respiratory rates have also been measured to define these disturbances of respiration.

The disturbances of respiration on which studies were made were

chosen because of availability of material. An intensive investigation had been started on the suitability of volatile and gaseous anaesthetics for cattle (Slater and Jennings, 1956(a), 1956(b)). This investigation was extended to determine the effects of certain anaesthetic agents, in particular Fluothane[1], on the plasma pH and the plasma carbon dioxide content of cattle together with the changes in the ventilation and respiratory rates.

The studies of Jarrett, Jennings, McIntyre, Milligan and Urquhart (1957) on the parasite Metastrongylus vivivorum made available many calves in different stages of parasitic pneumonia. Investigations were therefore made of the changes in plasma pH and the plasma carbon dioxide content of calves affected with parasitic pneumonia. Measurements were made at the same time of the ventilation and respiratory rates of calves with this disease.

In an attempt to explain the response of pneumonic and anaesthetised cattle, studies were carried out of the effects on respiration of gas mixtures different in composition to the atmosphere.

Since these three types of disturbances of respiration are widely different they are each presented in a separate section in this part of the thesis.

1 Imperial Chemical (Pharmaceuticals) Ltd., Wilmslow, Cheshire.

SECTION I

THE EFFECTS OF SOME GENERAL ANAESTHETIC AGENTS, HALOTHANE IN PARTICULAR, ON THE PLASMA pH AND THE PLASMA CARBON DIOXIDE CONTENT OF CATTLE.

REVIEW OF THE LITERATURE

General anaesthetic agents, because of their effects on the central nervous system have been shown to cause changes in respiration with changes in plasma pH and plasma carbon dioxide content of man and many other animals. A very extensive literature has been accumulated on the clinical effects of general anaesthetic agents in man, the advent of each new anaesthetic agent leading to the production of numerous articles. For example, between the introduction of Fluothane in 1956 and the middle of 1958 at least 30 articles appeared on its clinical use in man. Fewer reports have appeared on the biochemical effects of general anaesthetic agents on the human subject but the amount of published material is still extensive. Similarly a voluminous literature has been accumulated on the effects of general anaesthetic agents on dogs,

cats and monkeys when these animals were used as experimental subjects.

On the other hand the effects of general anaesthetics on the respiration of cattle have not been studied very fully. Until recently volatile and gaseous anaesthetics have been considered unsuitable for cattle because of the copious salivation and outpouring of bronchial secretions with consequent risk of post-anaesthetic pneumonia (Wright, 1952). Ether was stated to be ineffective as a general anaesthetic for large animals (U. Vet. Coder., 1952) although Hamlin (1935) described its use as a general anaesthetic for horses. However, the successful use of volatile and gaseous anaesthetics in cattle has been reported by Hall (1957), Hannon and Johannson (1958), and Fisher and Jennings (1958).

Hall (1957) in a clinical evaluation of the effects of Fluothane on four cows stated that respiratory depression occurred together with a slowing of the pulse rate. Hannon and Johannson (1958) reported the successful use of a mixture of thiopentone sodium, succinylcholine and nitrous oxide as a general anaesthetic for cattle. They observed slight changes in the partial pressure of carbon dioxide in the blood and slight changes in the blood pH. Some of the changes they reported may not have been of respiratory origin since on occasion a rise in the partial pressure of carbon dioxide occurred with a rise in the plasma pH. As they also assisted ventilation by means of a pump it was not possible to draw conclusions about the effects of the anaesthetic mixture used on

respiration. In another experiment they reported a slight decrease in pulmonary ventilation together with a decrease in carbon dioxide production and a slight decrease in oxygen consumption when thiopentone sodium alone was used as the anaesthetic agent. Haight (1952) reported an increase of pulse rate and respiratory rate during maintenance of general anaesthesia in cattle with chloral hydrate. Henderson (1944) has reported that the use of thiopentone sodium in young cattle caused a decrease in respiratory rate and an acceleration of the pulse. Furey (1950) reported favourably on the use of *Avertin*[x] in cattle but Haight (1957) reported tachypnoea and dyspnoea in similar circumstances.

x Imperial Chemical (Pharmaceuticals) Ltd., Wilmslow, Cheshire.

METHODS

(1) Fluothane

The closed circuit method of administration of Fluothane was used. A circle absorber apparatus was constructed of a size considered suitable for adult cattle and horses. The airways were of one and a half inch internal diameter, and an eight gallon reservoir bag was used. The outer cylinder of the soda-lime absorber was 10 inches in diameter and 10 inches in height while the inner cylinder holding the soda-lime was eight inches in diameter and eight inches in height and had a capacity of six litres.

This apparatus is illustrated in figures 25 and 26.

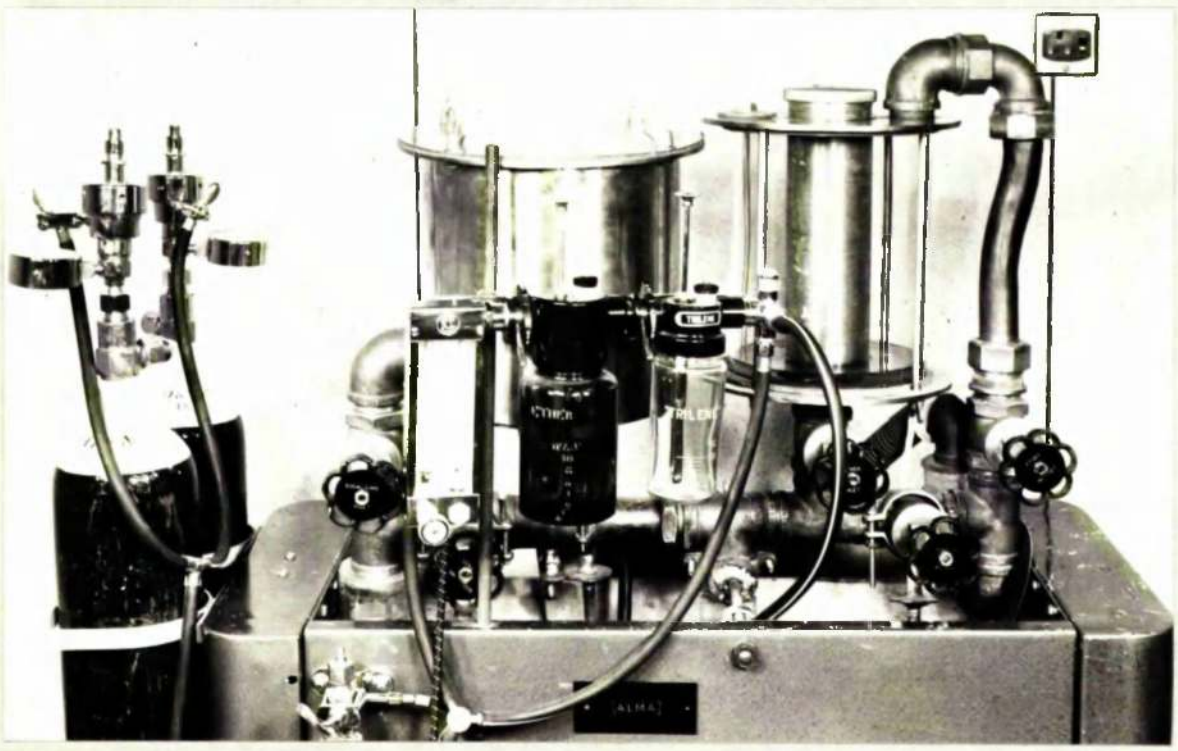

Fig. 25

Photograph of circle absorber

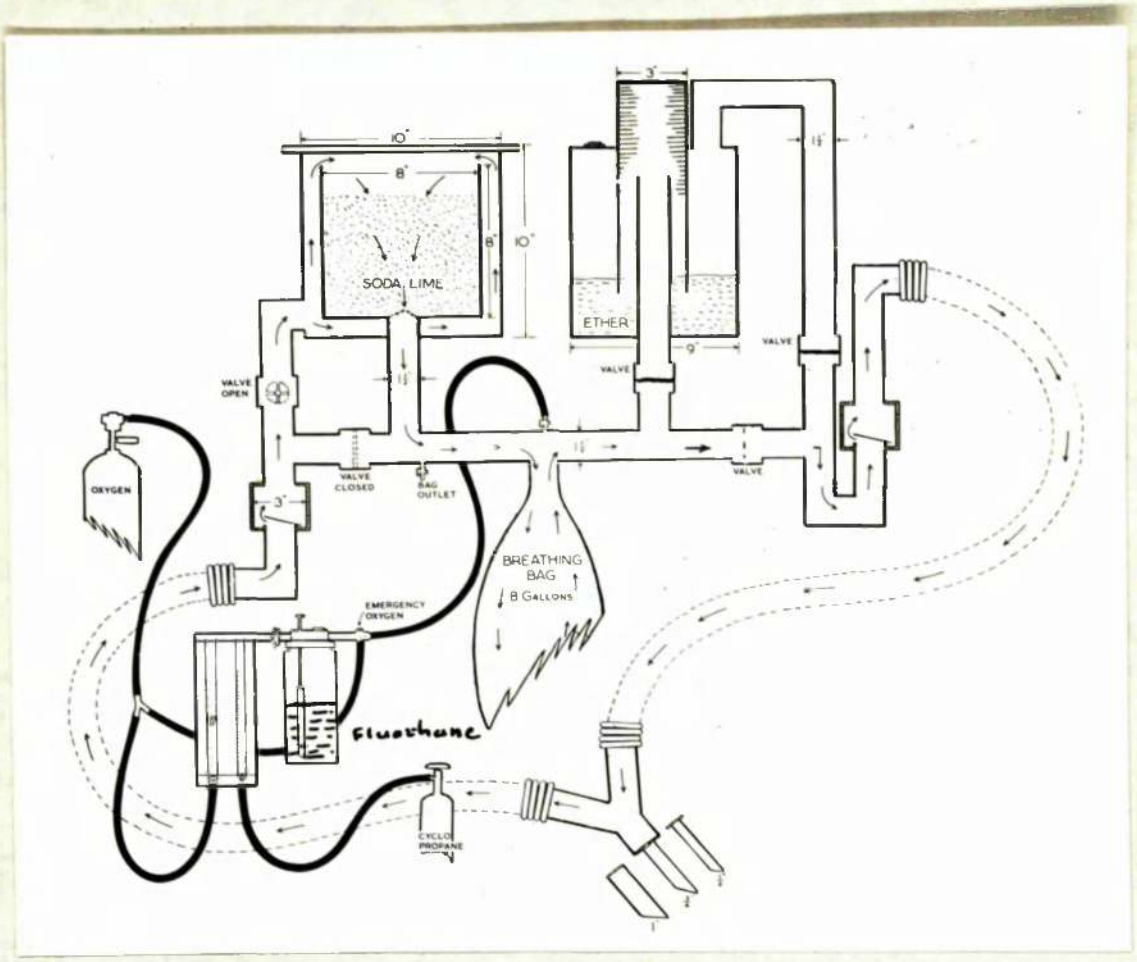

Fig. 26

Diagram of circle absorber

Animals were not starved before anaesthetisation and they were sometimes anaesthetised shortly after normal feeding. No pre-anaesthetic drugs were used. A catheter was introduced into the jugular vein about one hour before anaesthetisation to enable blood samples to be withdrawn easily from the site before, during and after an operation. Just before an operation, the ventilation rate was

was recorded for a period of 10 minutes using the face mask and gas meter with the unidirectional valves previously described. At the end of this period a blood sample was taken, the plasma was separated and the pH and carbon dioxide content determined. On some occasions the subject was made to breathe pure oxygen for a further period of 10 minutes during which time ventilation was recorded and afterwards blood samples were again taken, the plasma separated and the pH and carbon dioxide content of the plasma determined.

Induction of anaesthesia was commenced in every case with the animal in the standing position. As anaesthesia advanced the animals were either allowed to sink to the ground or, when a mechanically operated table was used, the table was brought from the vertical to the horizontal position so that the animal was pulled into lateral recumbency. Two methods of induction were used. In the first, anaesthesia was induced by means of a 10% solution of thiopentone sodium in normal saline. When thiopentone sodium was used for the induction of anaesthesia it was not usual to record the ventilation rate during induction. In the second method Fluothane was used as the inducing agent and in some of these cases ventilation was recorded during induction by including the gas meter in the anaesthetic circuit. When Fluothane was used for induction, the close fitting face mask was applied to the animal and the circle absorber was connected to the face

mask. Fluothane was administered to the animal from a Trilene[1] vaporizer bottle by passage of oxygen through the Fluothane. Once general anaesthesia had been induced a cuffed endotracheal tube was introduced into the trachea. The circle absorber was connected to the endotracheal tube and anaesthetization was continued. Ventilation rates were measured during anaesthesia by including the gas meter in the circuit between the expiratory valve and the circle absorber. In those experiments where a study was made of changes in plasma pH and plasma carbon dioxide, a blood sample was taken for analysis as soon as possible after induction.

Once surgical anaesthesia had been attained after connection to the circle absorber, the oxygen input was altered to by-pass the Fluothane in the Trilene vaporizer but increments of Fluothane were added at intervals to make up for losses due to leakage from the circuit. During the studies of Fluothane on ventilation and plasma carbon dioxide content and plasma pH, ventilations were recorded continuously and blood samples were taken at half-hourly intervals for analysis of the plasma pH and plasma carbon dioxide content.

After the period of anaesthesia the anaesthetic apparatus was disconnected but the endotracheal tube was left in situ until a stage of recovery was reached where the subject would no longer tolerate it.

[1] Imperial Chemical (Pharmaceuticals) Ltd., Wilmslow, Cheshire.

Ventilation rates were recorded to this point and only occasionally afterwards. Blood samples were usually taken until the plasma pH had returned to the control value.

In the comparative studies on Fluothane anaesthesia carried out in dogs, sheep and horses, the same general procedure was adopted. For the studies in dogs and sheep the same anaesthetic apparatus was employed but the large unidirectional valves were replaced by the three-quarter inch diameter unidirectional valves described in the section on the measurement of the ventilation rate of calves, and the tubes connecting these valves to the animal were also of three-quarter inch internal diameter. Control ventilations were not usually attempted in sheep or horses as both species became excited when they were fitted with a mask. For this reason it was possible to carry out induction of the horse with Fluothane on only one occasion.

(2) Thiopentone sodium

Thiopentone sodium was administered as a 10% solution in normal saline. On one occasion the calculated dose was injected rapidly into the jugular vein and changes in plasma pH and plasma carbon dioxide content followed. In two other instances was carried out more slowly until the stage of surgical anaesthesia was reached. Further increments of thiopentone sodium were added as required to maintain the animal in this plane of anaesthesia.

(3) Chloral hydrate

Chloral hydrate was administered to one cow and the changes in ventilation, plasma pH and plasma carbon dioxide content followed.

A catheter was introduced into the jugular vein and a 10% solution of chloral hydrate administered slowly. Pulmonary ventilation rate was followed before and during the administration of the chloral hydrate and during the recovery period. The cow was maintained at the stage of surgical anaesthesia for approximately one hour by the addition of small increments of chloral hydrate intravenously. Blood samples were taken at approximately half hour intervals and the plasma pH and plasma carbon dioxide content determined.

RESULTS

The results of the studies in this section are arranged in the following order.

(1) Fluothane (a) Cattle

(b) Horses

(c) Dogs

(d) Sheep

(2) Thiopentone sodium in cattle.

(3) Chloral hydrate in one cow.

(1) Fluothane

(a) Cattle

Experiments were carried out on eight cattle. In two of these experiments the effects of Fluothane on the ventilation rate, the plasma pH and the plasma carbon dioxide content were followed. In six experiments the effects of breathing pure oxygen for 10 minutes on the ventilation rate, the carbon dioxide content and the plasma pH before the animal was anaesthetized were also examined. Each experiment is described separately and each set of results is illustrated by a graph and given in a table.

Experiment I. Cow 850. In this experiment pre-anaesthesia control values of ventilation rate, carbon dioxide content and pH of plasma were determined and similar determinations were made over the period of anaesthesia. An hour after the cow had been removed from the anaesthetic circuit, a final blood sample was taken. The changes taking place are demonstrated in figure 27, and summarised in table 35.

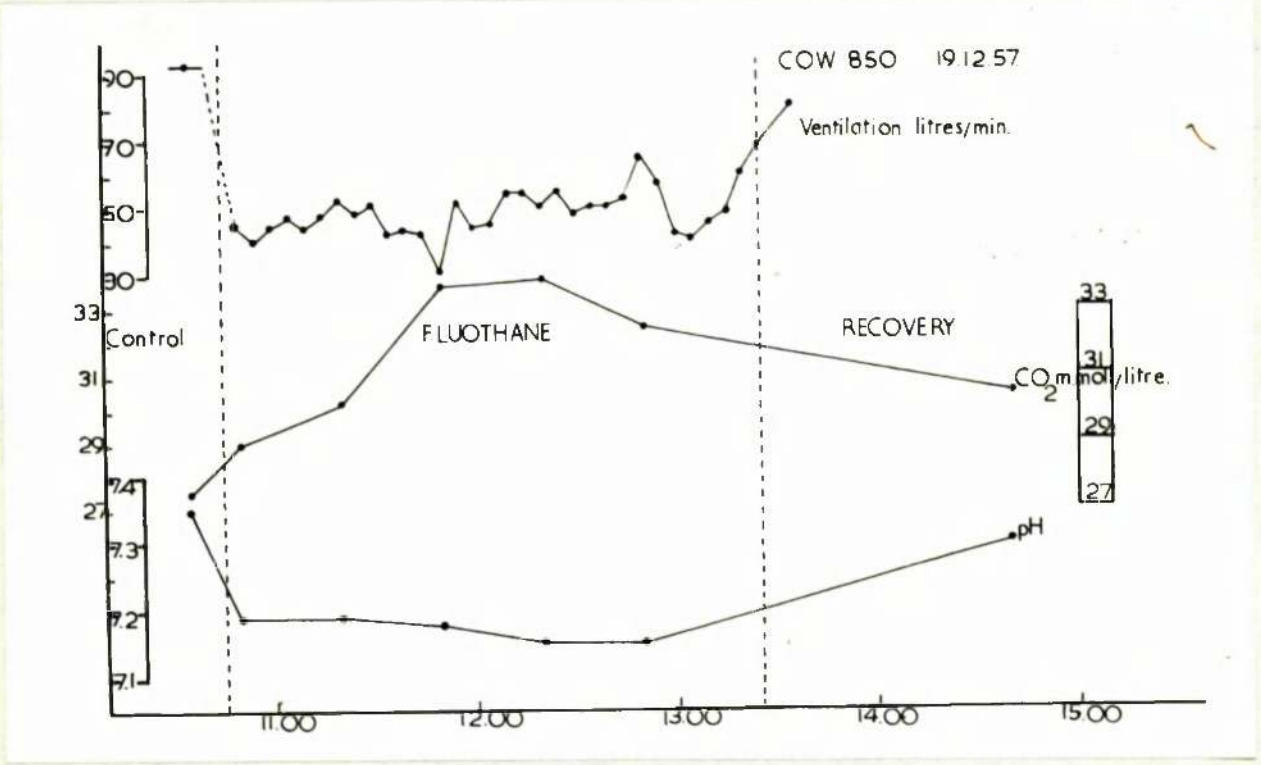

Fig. 27

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia.

Table 35

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before anaesthesia	During Anaesthesia (minutes) 5	30	60	90	120	During Recovery (minutes) 5	30	60
pH [a]	7.35	7.19	7.39	7.38	7.35	7.35			7.30
CO_2	27.5	27.9	20.7	33.5	33.9	32.5			20.5
Vent	93	44	47	42	54	53	76		

From these results it will be observed that depression of pulmonary ventilation occurred and that at the same time there was a fall in plasma pH and a rise in plasma carbon dioxide. The changes in pH and carbon dioxide were greater than those observed when following hourly variations in the normal unanaesthetized cow. There was evidence of some carbon dioxide retention and a lowering of plasma pH as long as one hour after removal from the anaesthetic atmosphere.

a Throughout these pH = Plasma pH

CO_2 = Plasma carbon dioxide content in m.moles/litre

Vent = Ventilation rate in litres/minute.

Experiment II. Bull 400. This experiment is similar to the previous one. The changes taking place during the whole period of anaesthesia and recovery are illustrated in figure 28, and summarised in table 36.

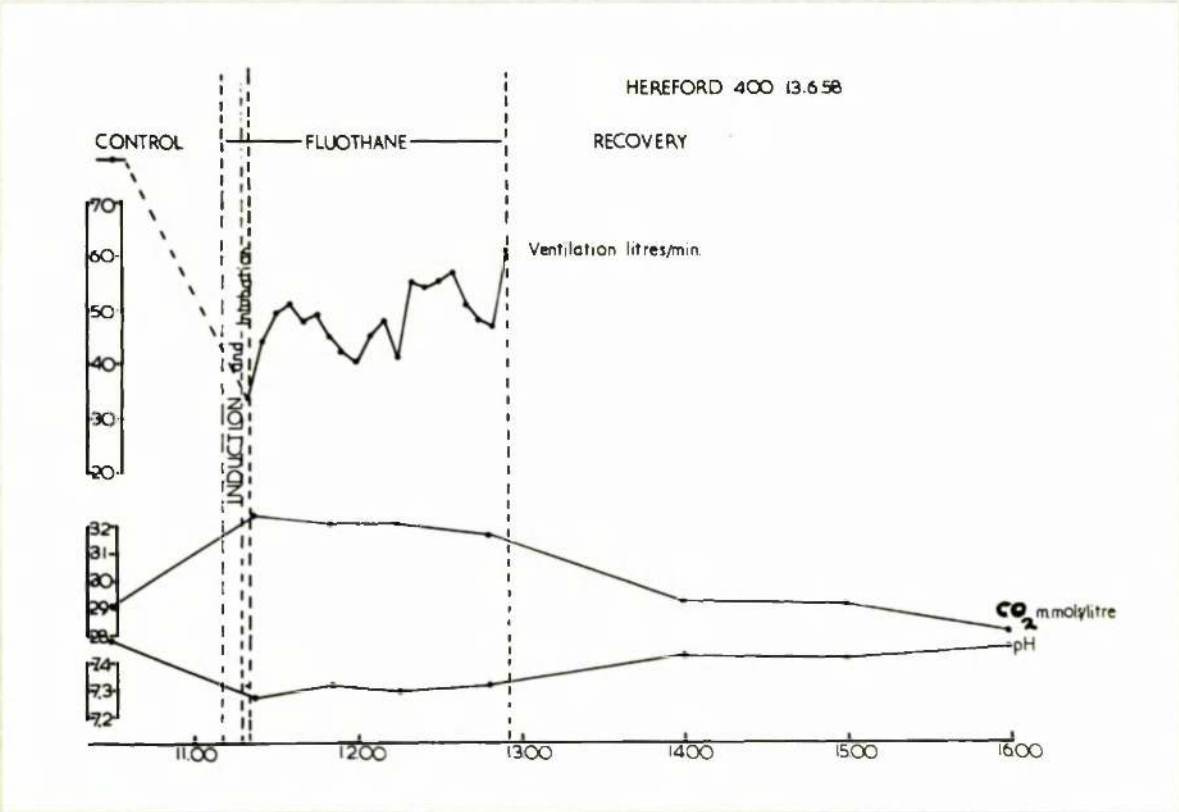

Fig. 28

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 26

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Ethethane Anaesthesia

Time	Before anaesthesia	During Anaesthesia (minutes) 5	30	60	90	During Recovery (minutes) 5	30	60	120	180
pH	7.47	7.27	7.31	7.29	7.31			7.42	7.43	7.45
CO_2	29.2	32.4	32.3	32.1	31.7			29.3	29.1	28.1
Vent	78	34	45	48	48	61				

In this experiment a depression of ventilation again occurred with fall in plasma pH and rise in plasma carbon dioxide content. One hour after anaesthesia the carbon dioxide content had returned to near the pre-anaesthetic value but the plasma pH took a further two hours to return to pre-anaesthetic values.

Experiment III. Bull 400. The changes taking place during anaesthesia and recovery are illustrated in figure 29 and summarised in table 37.

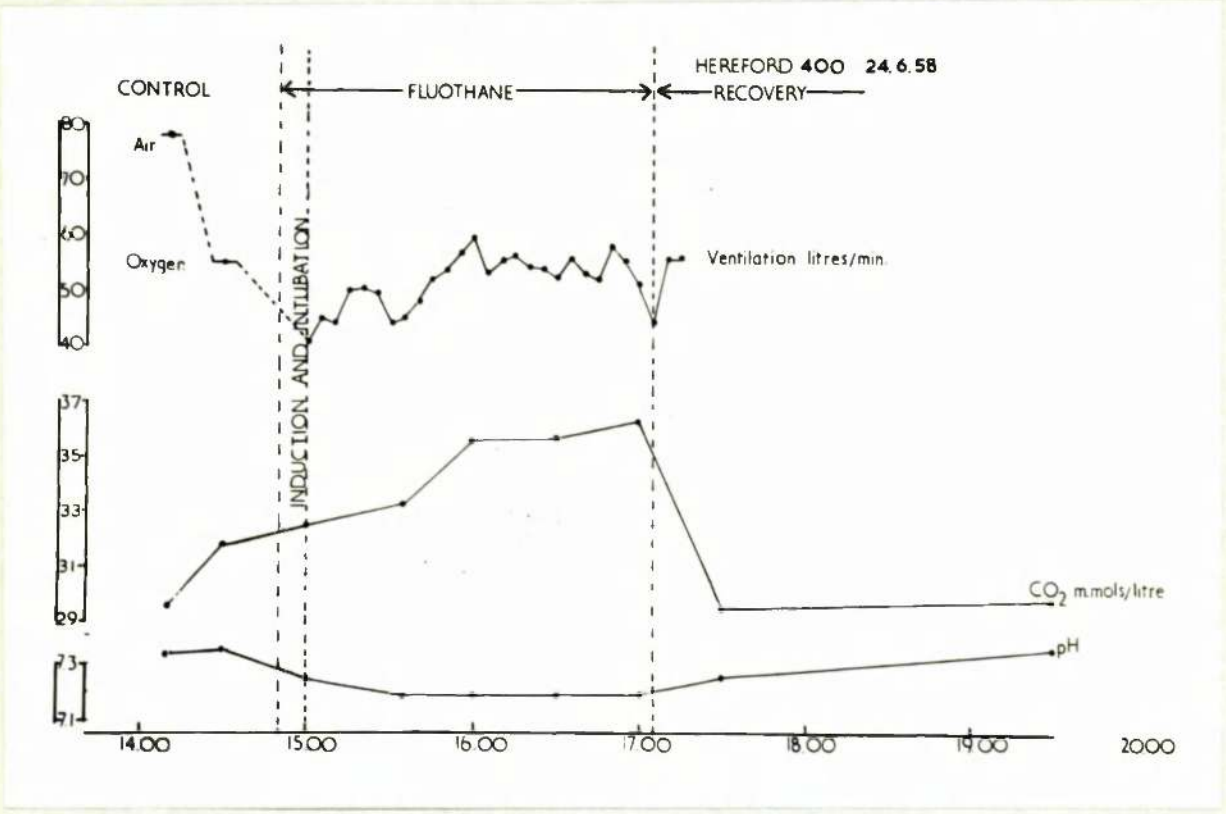

Fig. 29

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 37

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anesthesia

Time	Before Anesthesia Air	Before Anesthesia Oxygen	During Anesthesia (minutes) 5	20	60	90	120	During Recovery (minutes) 5	30	120
pH	7.34	7.35	7.25	7.39	7.39	7.39	7.39		7.25	7.35
CO_2	29.6	31.9	32.5	33.4	35.6	35.9	35.9		29.5	29.29
Vent	76	55	40	52	53	54.5	47	56		

When the animal was unanesthetized and breathing pure oxygen there was some reduction of pulmonary ventilation and a rise in plasma carbon dioxide content but the plasma pH was not significantly different. During anesthesia a depression of ventilation occurred but throughout the main part of anesthesia ventilation was not markedly different from that observed when the animal breathed pure oxygen. There was the expected carbon dioxide retention with a fall in pH and a rise in plasma carbon dioxide content above values observed in normal animals. Two hours after recovery from anesthesia the plasma pH and plasma carbon dioxide content were at their pre-anesthetic control values.

Experiment IV. Cow 900. This experiment was similar to the previous one. The changes which took place during the period of anaesthesia and recovery are illustrated in figure 30 and summarised in table 38.

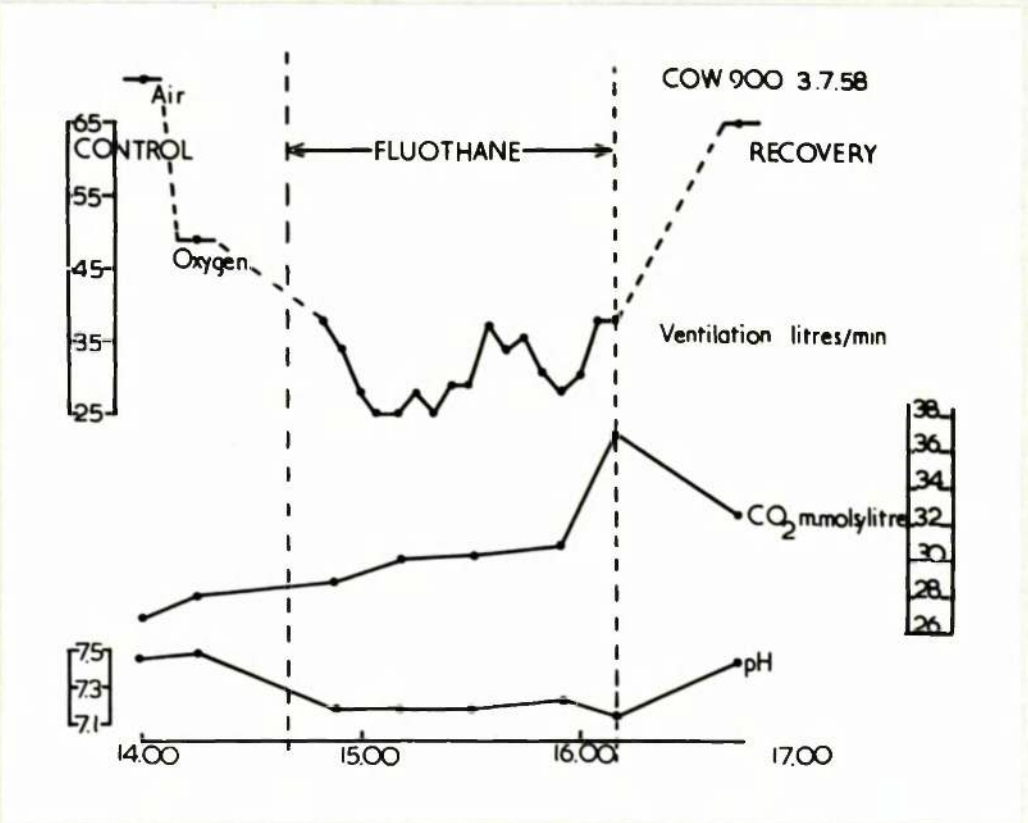

Fig. 30

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 24

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

	Before Anaesthesia		During Anaesthesia (minutes)					During Recovery (minutes)	
Time	Air	Oxygen	5	30	60	90	120	5	30
pH	7.45	7.45	7.37	7.35	7.33	7.29	7.30		7.44
CO_2	25.3	23	23.3	30.0	30.2	30.8	37		32.5
Vent	71	49	38	25	29	30	37	50	65

In this animal there was pre-anaesthetic reduction of ventilation with pure oxygen and during anaesthesia a depression of ventilation occurred. The plasma pH and plasma carbon dioxide content both rose during inhalation of pure oxygen. During the anaesthetic period retention of carbon dioxide occurred with a fall in plasma pH. Plasma pH had returned to the pre-anaesthetic control value but there was still some retention of carbon dioxide 30 minutes after the administration of anaesthetic had ceased.

Experiment V. Heifer 822. In this experiment which was similar to experiments III and IV the endotracheal tube was ejected in the middle of the experiment. The changes taking place during the period of anaesthesia and recovery are illustrated in fig. 31 and summarised in table 39.

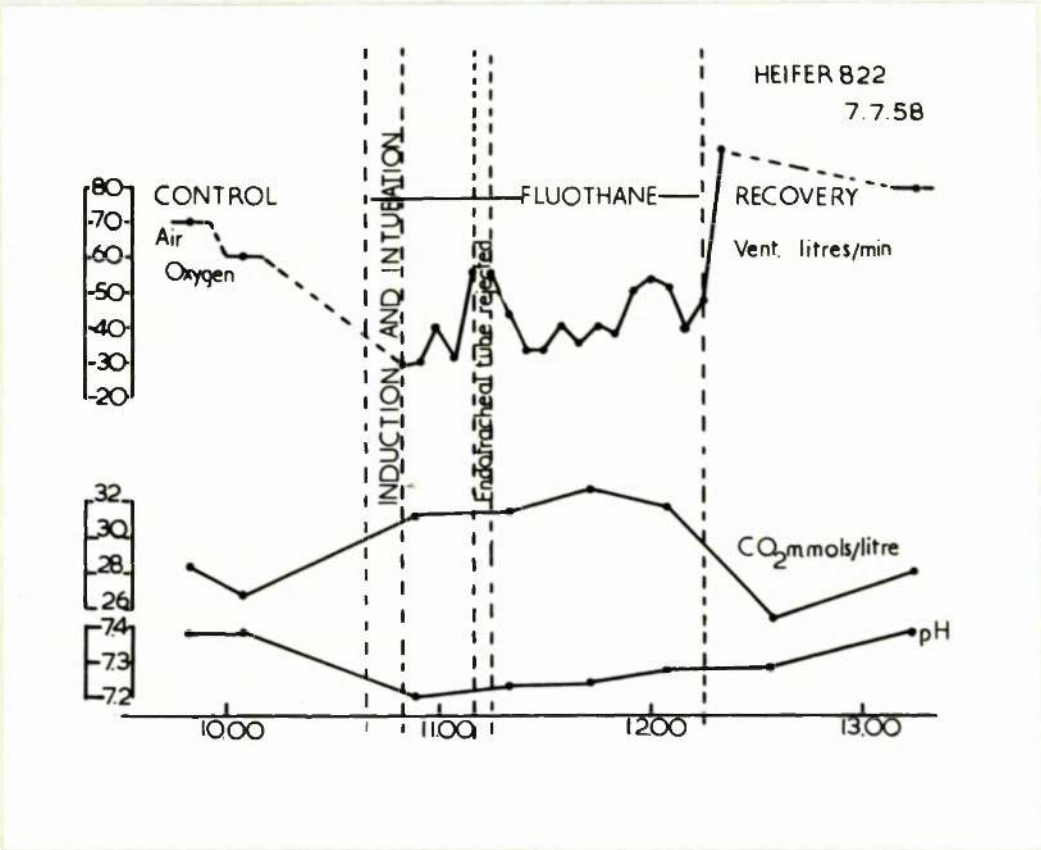

Fig. 31

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia.

Table 32

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before Anaesthesia		During Anaesthesia (minutes)				During Recovery (minutes)		
	Air	Oxygen	5	30	60	90	5	15	60
pH	7.38	7.38	7.20	7.23	7.24	7.28		7.39	7.39
CO_2	28.2	25.7	31.2	31.5	32.8	31.8		27.5	28.2
Vent	70	60	30	55	40.6	52	91	90	80

This animal also showed some reduction of ventilation during the administration of pure oxygen but struggled violently so that the reduction was less marked. The plasma carbon dioxide content gave no evidence of retention during the administration of pure oxygen. During anaesthesia depression of ventilation occurred with carbon dioxide retention and a fall in plasma pH. During recovery some hyperventilation occurred. At the end of an hour the plasma pH and plasma carbon dioxide content had returned to their pre-anaesthetic control values.

Experiment VI. Cow 940. This experiment was similar to the previous one. The changes which took place during the period of anaesthesia and recovery are illustrated in fig. 32 and summarised in table 40.

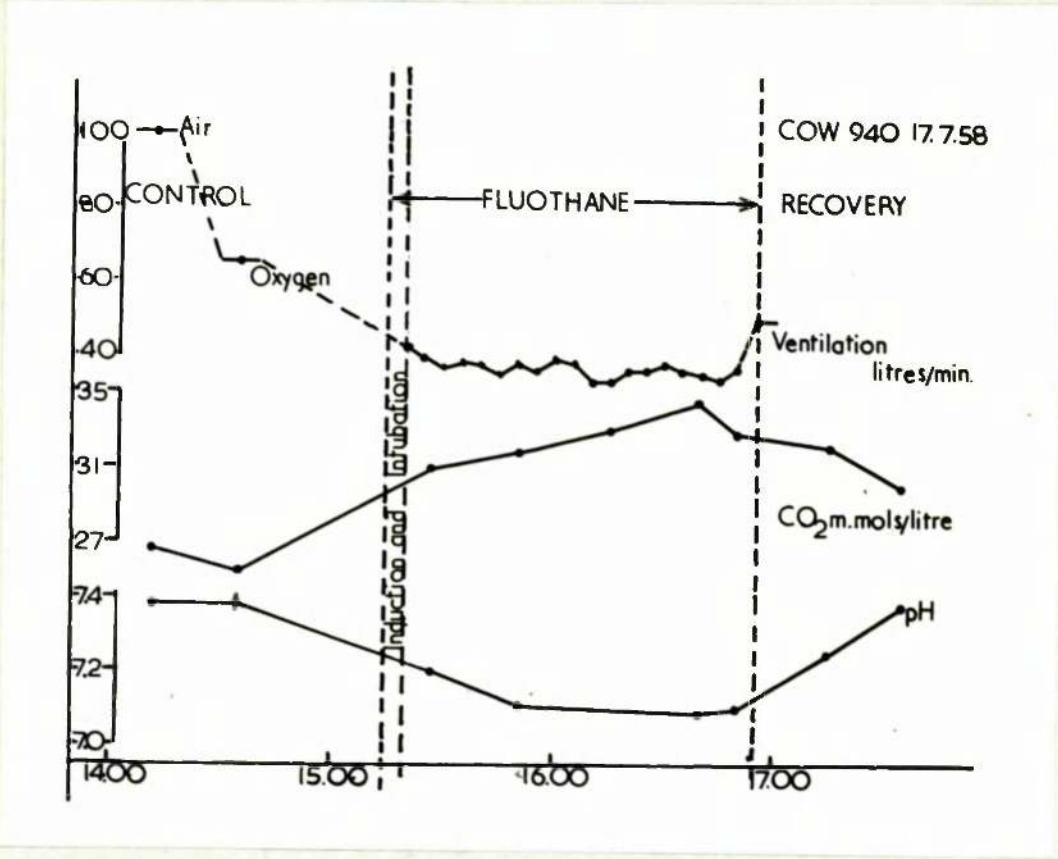

Fig. 32

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia.

Table 40

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before Anaesthesia Air	Oxygen	During Anaesthesia (minutes) 5	30	60	90	120	During Recovery (minutes) 5	15	60
pH	7.38	7.38	7.20	7.11	7.10	7.09	7.19		7.25	7.38
CO_2	26.5	25.25	30.9	31.9	33	34	32.9		32.5	30.1
Vent	99	65	39	35	33	35	35.6	50		

Inhalation of pure oxygen caused a decrease in ventilation but no marked changes were detected in the plasma pH and the plasma carbon dioxide content. The depression of ventilation occurred during Fluothane anaesthesia with carbon dioxide retention and a fall in plasma pH. The plasma pH returned to the control value one hour after the cat had been removed from the Fluothane atmosphere but there was still some carbon dioxide retention.

Experiment VII. Calf 272. This, too, was similar to the previous experiments. The changes which took place during the period of anaesthesia and recovery are illustrated in figure 33 and summarised in table 41.

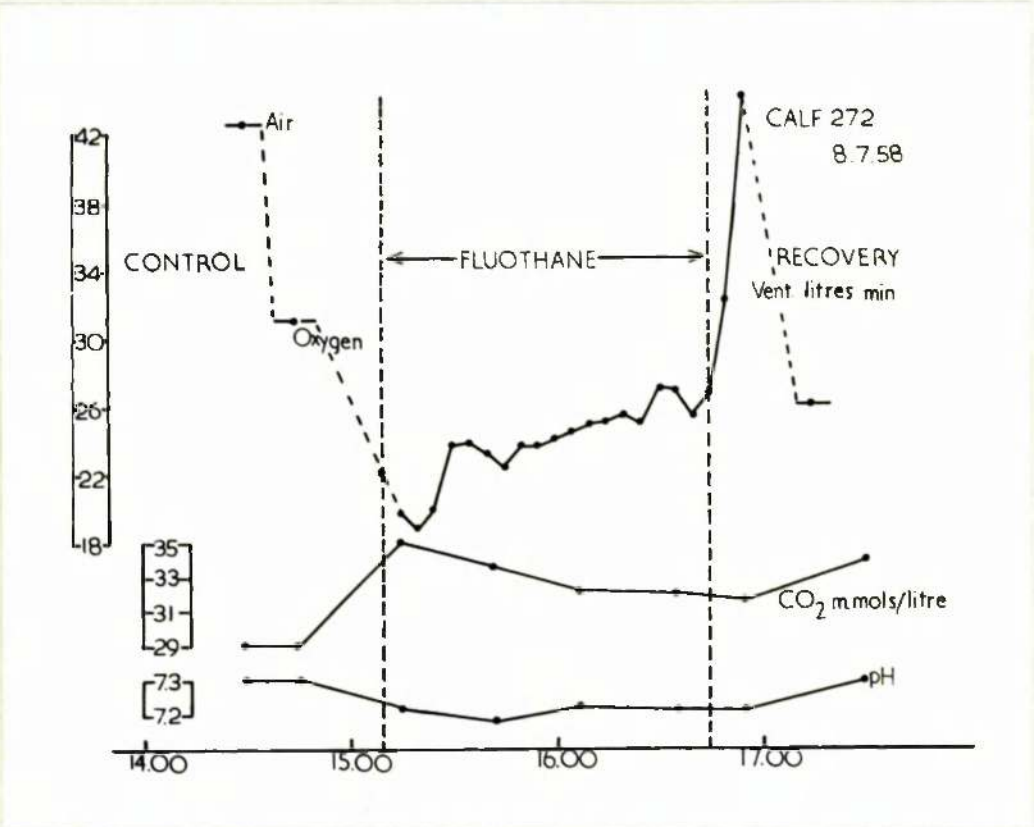

Fig. 33

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 41

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before Anaesthesia		During Anaesthesia (minutes)				During Recovery (minutes)	
	Air	Oxygen	5	30	60	90	5	30
pH	7.30	7.30	7.21	7.18	7.22	7.24	7.24	7.30
CO_2	29	29	35	34	32	32	31.6	34
Vent	42.5	31.0	29	23	25	26	33	26

In this calf similar changes to these in the adult cows took place. There was pre-anaesthetic lowering of pulmonary ventilation during inhalation of pure oxygen but changes were not detected in the plasma pH and the plasma carbon dioxide content. There A depression of ventilation occurred during Fluothane anaesthesia and although this was marked immediately after intubation, over the whole period of anaesthesia the depression was not great. Changes of plasma carbon dioxide and the changes of plasma pH reflected the ventilation changes. During recovery the plasma pH returned to the control value after half an hour although ventilation was still depressed and there was still more carbon dioxide retention.

Experiment VIII. Calf 270. This experiment was similar to the previous experiments. The changes which took place during the period of anaesthesia and recovery are illustrated in figure 34 and summarised in table 42.

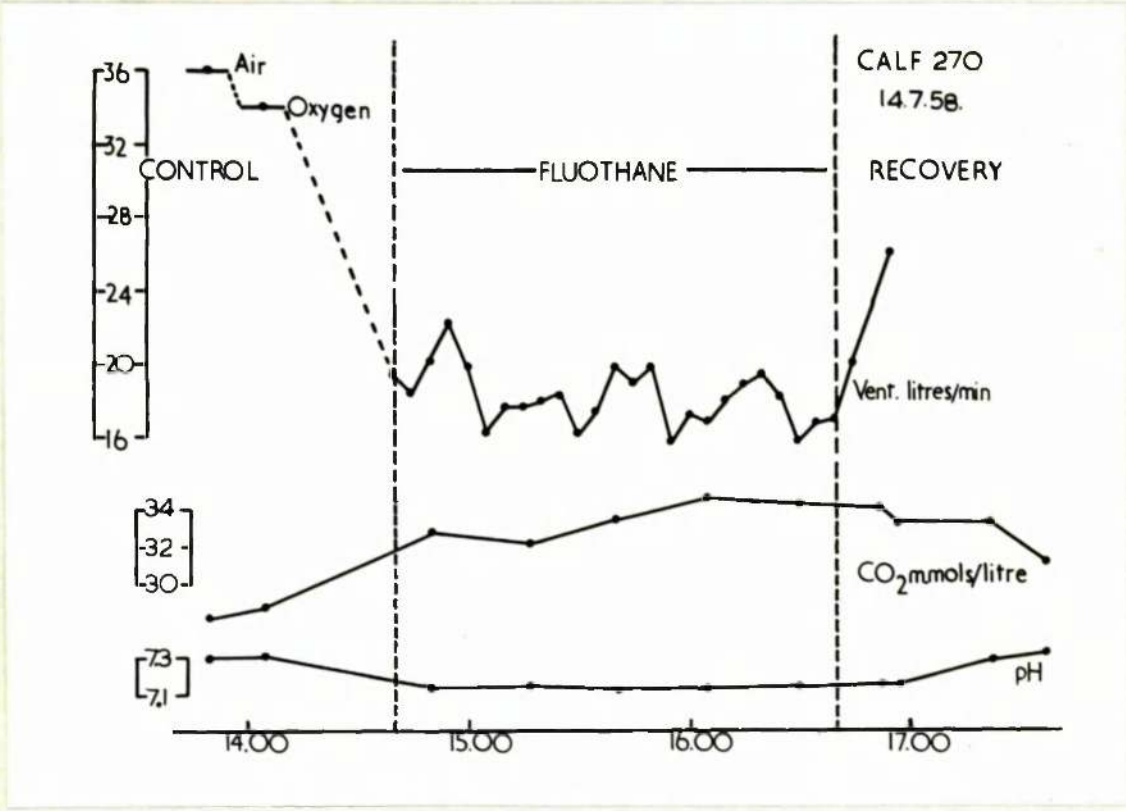

Fig. 34

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia.

Table 42

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before Anaesthesia		During Anaesthesia (minutes)					During Recovery (minutes)		
	Air	Oxygen	5	30	60	90	120	5	30	45
pH	7.30	7.31	7.34	7.35	7.34	7.34	7.35	7.36	7.39	7.38
CO_2	28	31.0	32.0	32.2	32.6	34.7		32.2	33.4	33.1
Vent	35	34	19	16	15	17	17	22		

This calf showed only slight pre-anaesthetic hypoventilation when inspiring pure oxygen and a slight rise in plasma carbon dioxide content. During anaesthesia a marked depression of ventilation occurred with carbon dioxide retention and a fall in plasma pH. During recovery, after 30 minutes, pH had returned to near the control value but after 45 minutes there was still evidence of carbon dioxide retention.

(b) Horses

Experiments were carried out on three horses. In the first experiment induction was by means of Fluothane while in the other two experiments thiopentone sodium was the inducing agent. In one experiment only was it possible to follow ventilation throughout. In all three experiments blood samples were taken and changes in the carbon dioxide content and the pH of the plasma followed.

Experiment I II. Hunter 1006. Induction of anaesthesia in this animal was by means of Fluothane. The results obtained during the period of anaesthesia and recovery are illustrated in figure 35 and summarised in table 43.

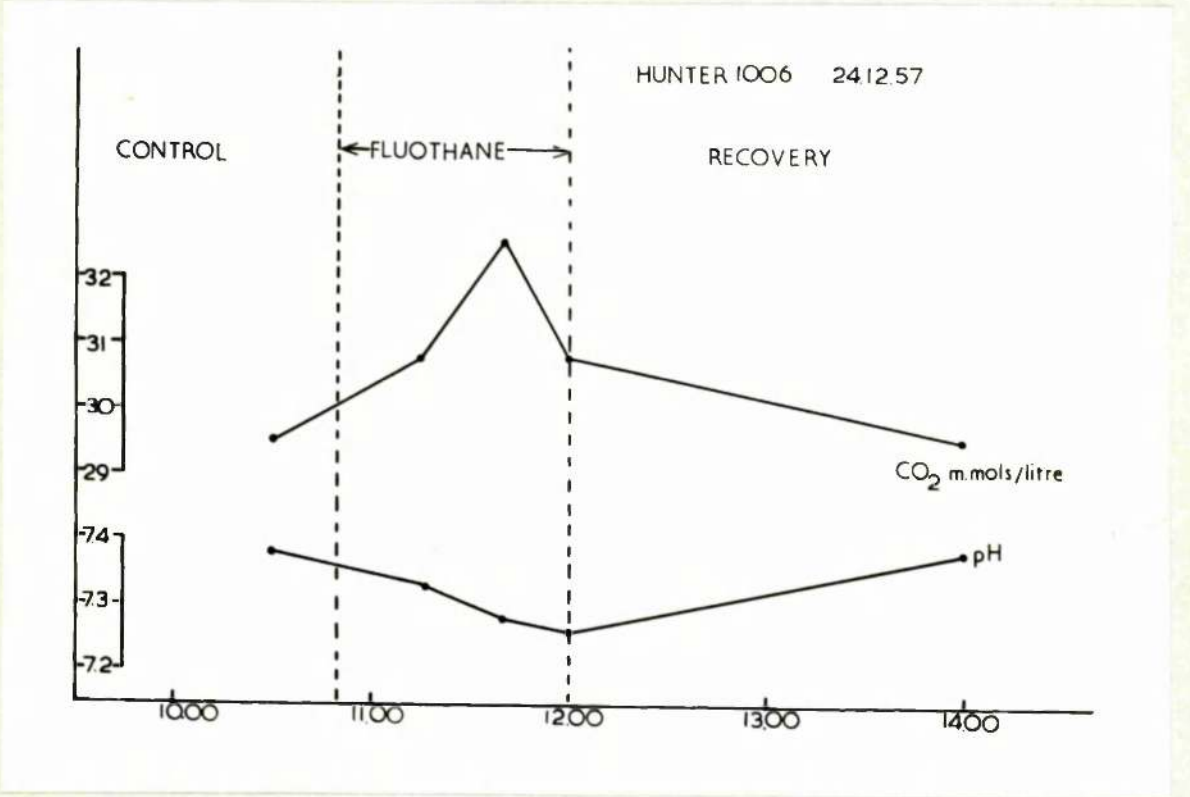

Fig. 35

Changes in plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table A3

Changes in Plasma Carbon Dioxide Content and Plasma pH

During Fluothane Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 5	30	50	70	During Recovery (minutes) 5	30	60
pH	7.38		7.33	7.23	7.25			7.38
CO_2	29.5		30.75	32.5	30.75			29.5

During anaesthesia the rise in plasma carbon dioxide and fall in plasma pH indicated a depression of ventilation. One hour after the animal had been removed from the anaesthetic atmosphere, plasma pH and plasma carbon dioxide had returned to the control values.

Experiment II H. Horse 1071. Induction and intubation were carried out using thiopentone sodium as the anaesthetic agent. Ventilation was recorded after induction. The results obtained during the period of anaesthesia and recovery are illustrated in figure 36 and summarised in table 44.

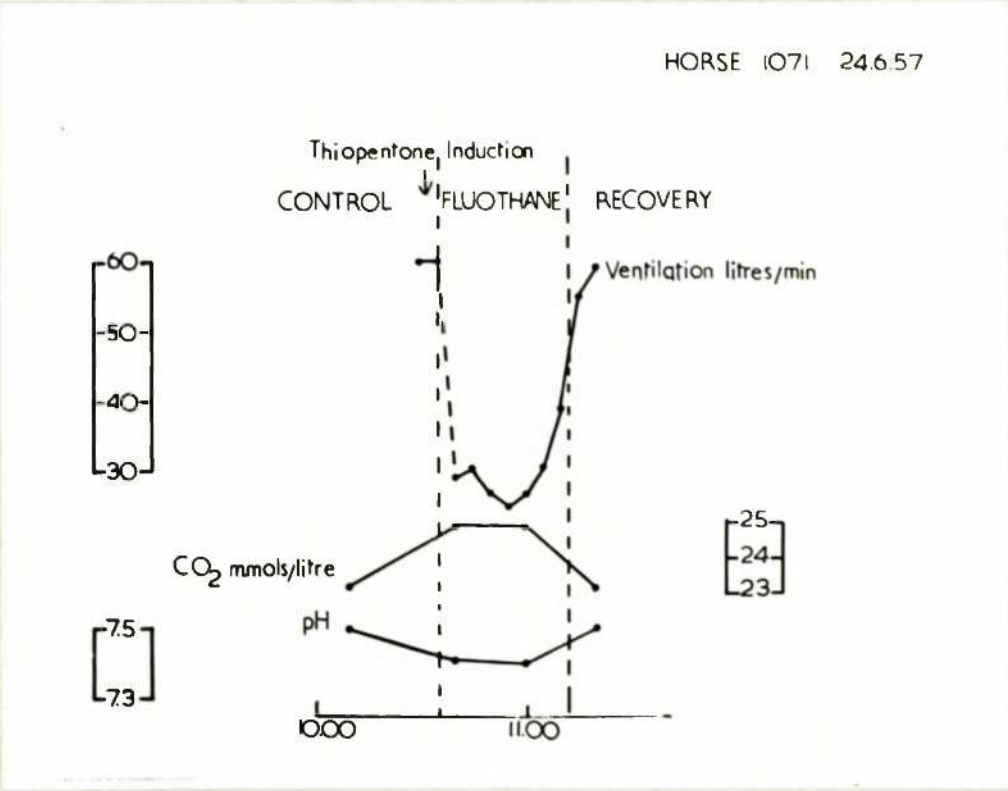

Fig. 36

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia.

Table 44

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 5	30	50	70	During Recovery (minutes) 5	20	60
pH	7.40	7.31	7.39			7.40		
CO_2	23.23	24.9	24.9			23.123		
Vent		30	27			57		

Depression of ventilation occurred but was not so marked as it appears from the graph since under chloroform and after intubation some respiratory stimulation occurred. The changes in plasma carbon dioxide content and plasma pH were small since the period of anaesthesia was short. Five minutes after removal from the anaesthetic atmosphere the plasma pH and plasma carbon dioxide content had returned to the pre-anaesthetic control values.

Experiment III H. Pony 792. It was possible in this animal to follow ventilation rate in addition. The results obtained during the period of anaesthesia and recovery are illustrated in figure 37 and summarised in table 45.

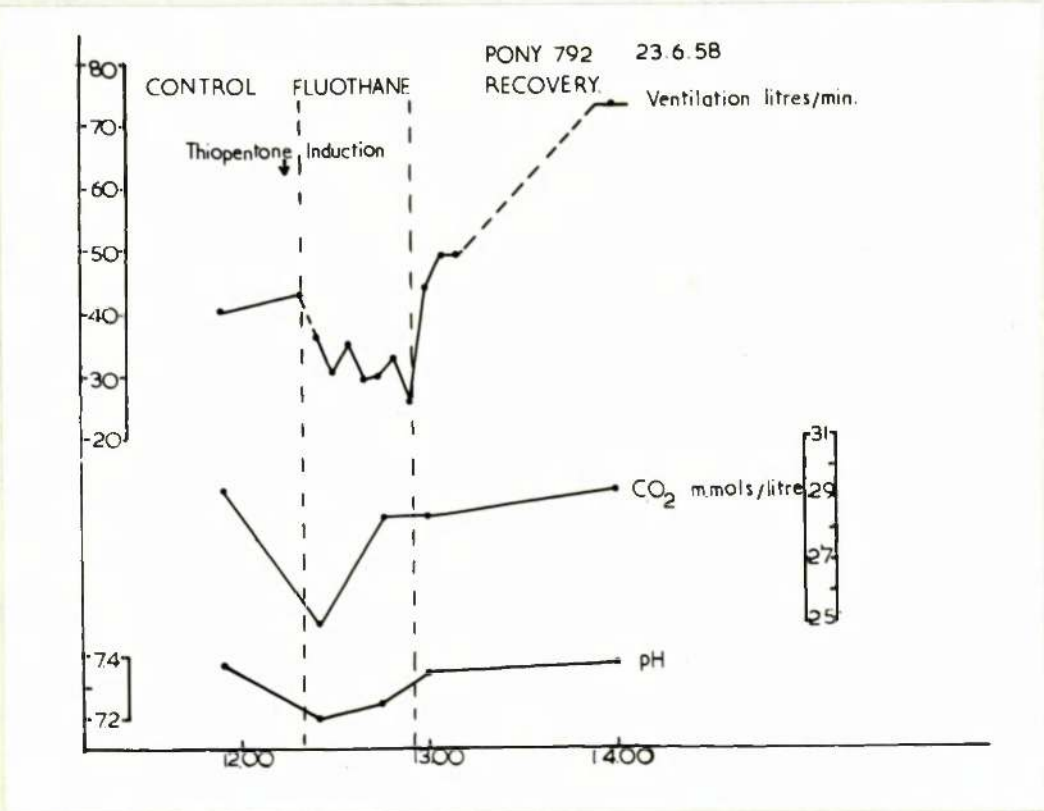

Fig. 37

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 45

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Thiopental Anesthesia

Time	Before Anesthesia	During Anesthesia (minutes) 5	30	60	70	During Recovery (minutes) 5	30	60
pH	7.37	7.19	7.23			7.34		7.37
CO_2	29.25	25	28.5			28.5		29.25
Vent	40.5	40	33			45		74

Following induction and intubation with thiopentone sodium some respiratory stimulation occurred. The plasma carbon dioxide at this point indicated hyperventilation but the plasma pH had fallen and may have indicated some degree of a metabolic acidosis as reported by Benbow, Boechez, Briggs, Brewster and Barnes (1951) during other anaesthesia in dogs. Beyond this point some respiratory depression occurred with a rise in plasma carbon dioxide content. During the recovery period, after five minutes the pH and carbon dioxide content were nearly back at the control values and after one hour they had returned to the control value. One hour after anaesthesia when the ventilation rate was measured, some hyperventilation was observed.

(c) Dogs

Experiments were conducted on three greyhounds. In each experiment it was possible to follow ventilation before, during and after anaesthesia. Blood samples were taken and the changes in the carbon dioxide content and the pH of plasma followed throughout each experiment.

Experiment I D. Greyhound 71. Induction of this animal was by means of Fluothane. The results obtained during the period of anaesthesia and recovery are illustrated in figure 38 and summarised in table 46.

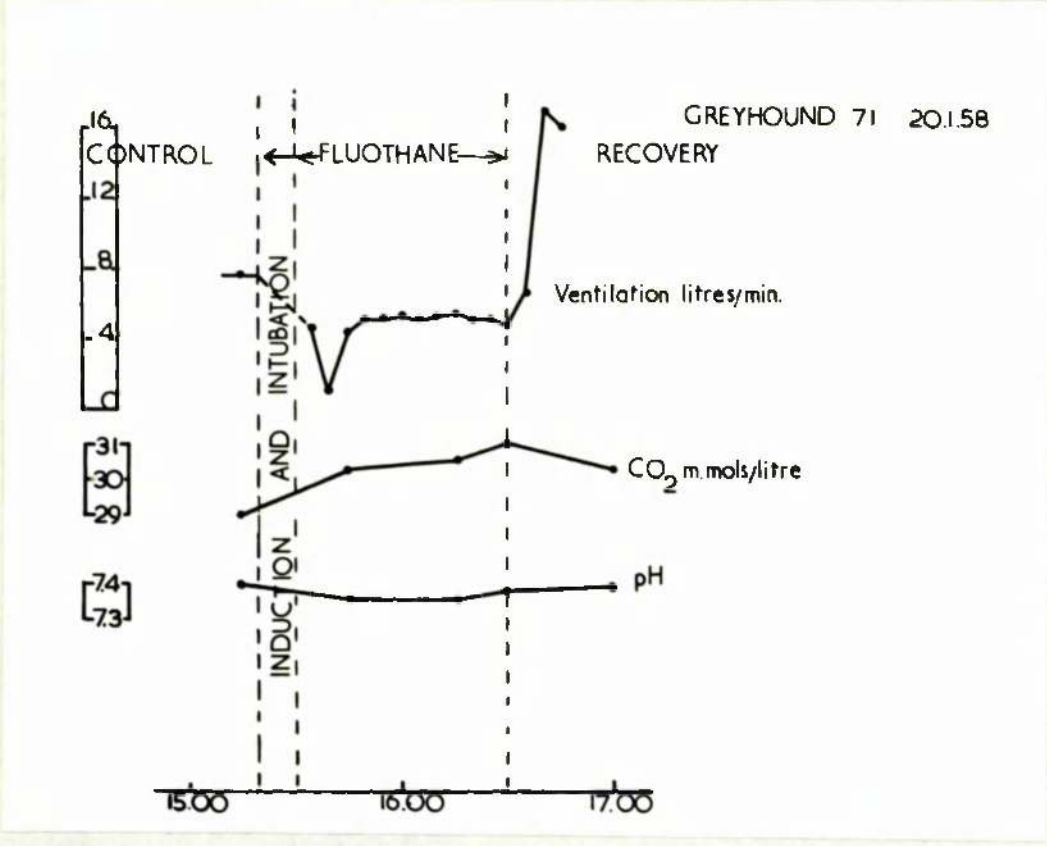

Fig. 38

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 46

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 5	30	60	90	During Recovery (minutes) 5	25	55
pH	7.40	7.36	7.35		7.38	7.39		
CO_2	29	30.2	30.5		30.3	30.3		
Vent	7.6	4.6	5.0		5.0	12.5		

The passage of the endotracheal tube after Fluothane induction caused a marked stimulation of respiration which was not recorded. Once the respiratory stimulation had ceased the ventilation rate was again recorded. A depression of ventilation occurred during Fluothane anaesthesia but marked changes were not detected in plasma pH and plasma carbon dioxide content. During the recovery from Fluothane anaesthesia considerable hyperventilation occurred.

Experiment II D. Greyhound 59. This dog also received Fluothane for induction. The results obtained during the period of anaesthesia and recovery are illustrated in figure 39 and summarised in table 47.

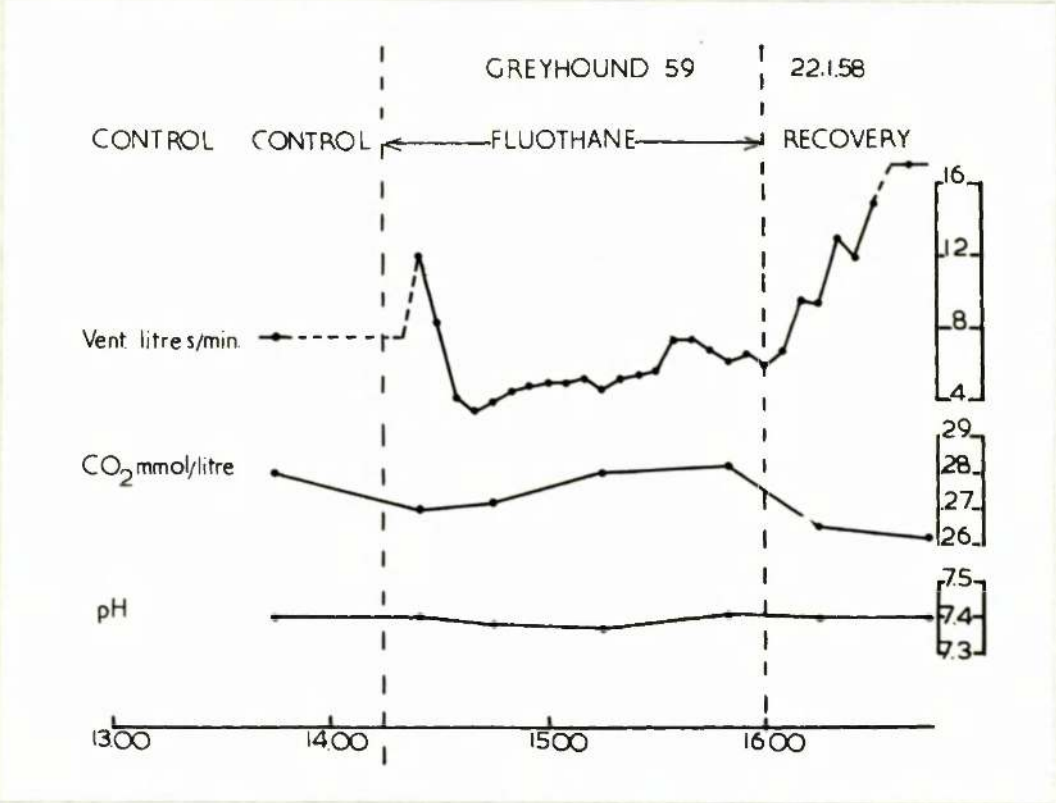

Fig. 39

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 42

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 5	30	60	90	During Recovery (minutes) 5	25	55
pH	7.40	7.40	7.39	7.38	7.41		7.40	7.40
CO_2	28	27	27.2	28	28.2		26.5	26.2
Vent	7.7	11	4	5	6		11	37

As in greyhound 71 intubation caused stimulation of respiration and hyperventilation. Following this hyperventilation respiratory depression occurred. There were very small changes of plasma pH during the experiment. The plasma carbon dioxide content fell as a consequence of the hyperventilation and then gradually rose again. During the recovery period the plasma pH remained satisfactory but the plasma carbon dioxide content fell as the animal was hyperventilating above the determined pre-anaesthetic control ventilation.

Experiment III D. Greyhound 70. Induction was by means of Fluothane. The results obtained during the period of anaesthesia and recovery are illustrated in figure 40 and summarised in table 48.

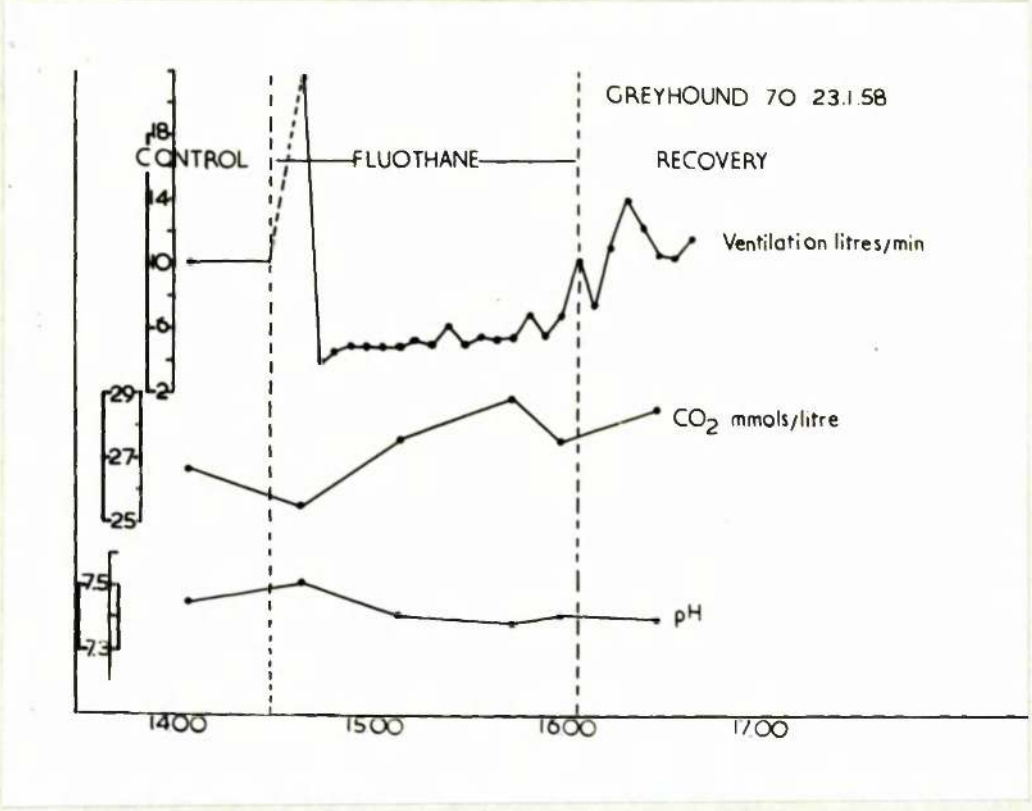

Fig. 40

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 48

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 5	30	60	90	During Recovery (minutes) 5	25	55
pH	7.45	7.51	7.41	7.39	7.41		7.40	
CO_2	26.7	25.5	27.5	26.8	27.5		28.5	
Vent	10.0	21.6	5.0	5.3	10.0		10.5	

Intubation caused a marked hyperventilation which lowered the plasma carbon dioxide content and caused the plasma pH to rise. Following the hyperventilation respiratory depression took place causing slight carbon dioxide retention and a fall in plasma pH. Twenty-five minutes after the animal had been removed from the Fluothane atmosphere the pulmonary ventilation was similar to the control value but the plasma pH was below the control value while the plasma carbon dioxide content was above the plasma control value.

(d) Sheep

Experiments were carried out on three sheep. Changes in the carbon dioxide content and the pH of plasma were followed in all three experiments but ventilation rate was measured in the first experiment only. Control measurements of ventilation rate in this experiment indicated the effect of anesthesia on ventilation rate of the unanesthetized sheep and therefore this measurement was abandoned in sheep.

Experiment I S. Sheep 805. Induction of anaesthesia was by means of Fluothane. The results obtained during the period of anaesthesia and recovery are illustrated in figure 41 and summarised in table 49.

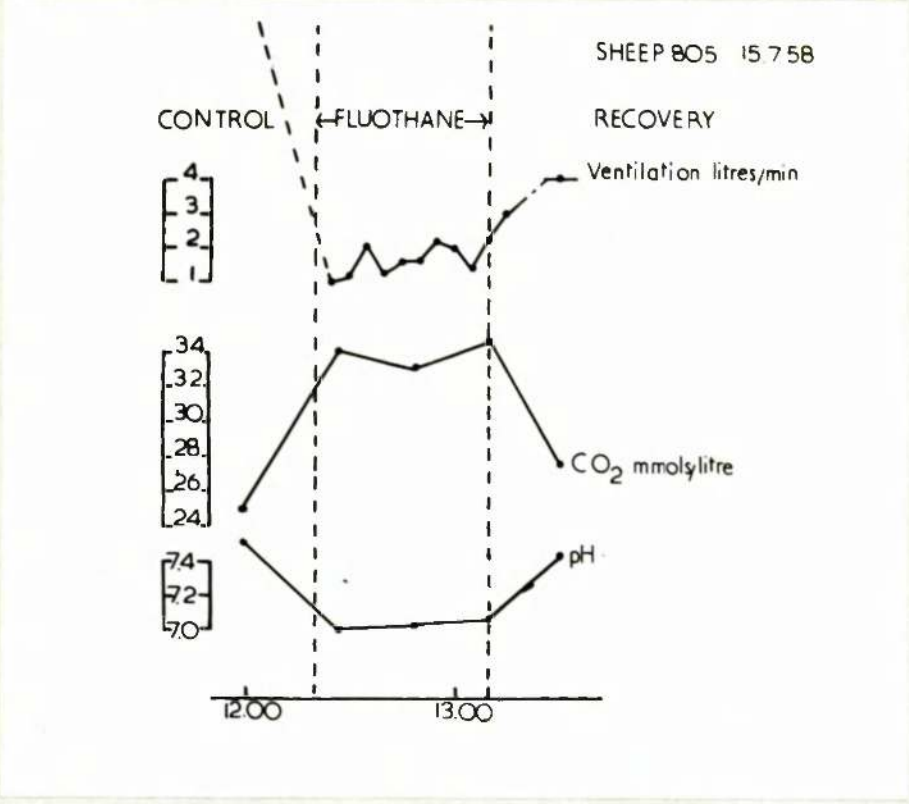

Fig. 41.

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 49

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluothane Anesthesia

Time	Before Anesthesia	During Anesthesia (minutes) 5	30	60	80	During Recovery (minutes) 20	30	40
pH	7.50	7.00	7.02	7.05		7.41		
CO_2	25	24	33	34.5		27.5		
Vent	35	1.0	1.5	2.2		4.0		

Marked respiratory depression occurred with startling changes in both the plasma pH and the plasma carbon dioxide content. Once the animal was removed from the Fluothane atmosphere both the plasma pH and the plasma carbon dioxide returned to near the control value.

Experiment II S. Sheep 813. In this animal ventilation was not recorded. Induction was by means of Fluothane. The results obtained during the period of anaesthesia and recovery are illustrated in figure 42 and summarised in table 50.

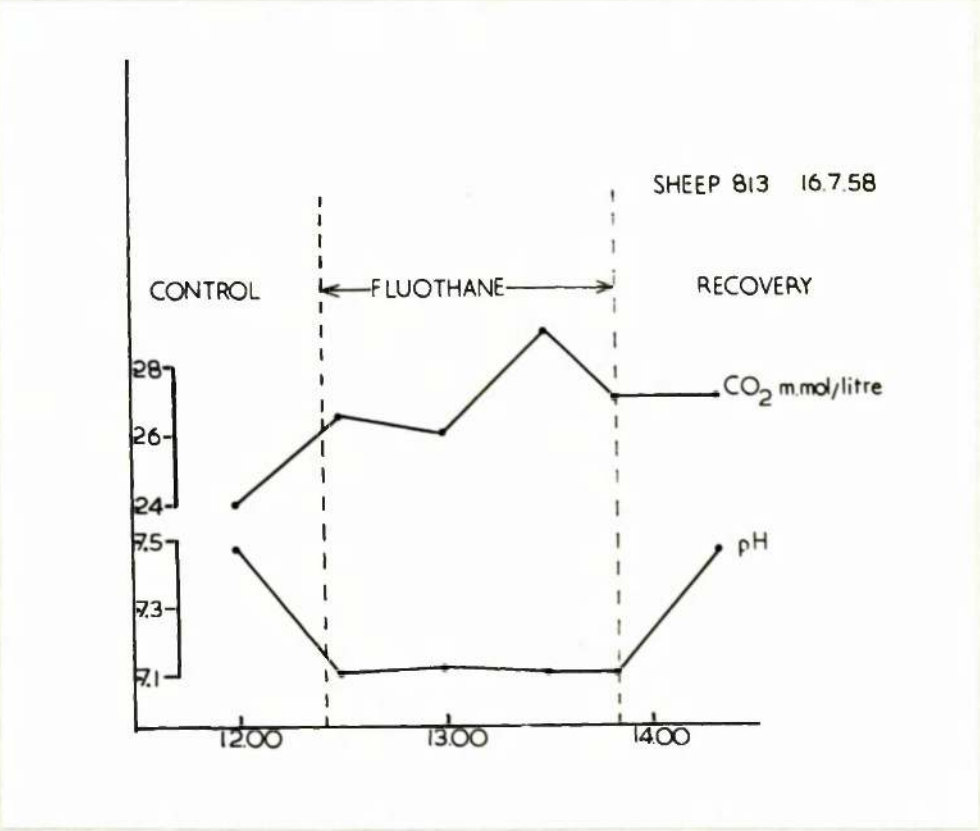

Fig. 42

Changes in plasma carbon dioxide content and plasma pH during Fluothane anaesthesia

Table 50

Changes in Plasma Carbon Dioxide Content and Plasma pH

During Fluothane Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 5	30	60	60	During Recovery (minutes) 20	30	40
pH	7.47	7.10	7.11	7.10	7.10	7.46		
CO_2	24	26.5	25	29	27	27		

Startling changes both in plasma pH and plasma carbon dioxide content were observed during Fluothane anaesthesia. During the recovery the plasma pH returned to the control pre-anaesthetic value before the plasma carbon dioxide content.

Experiment III S. Sheep 823. In this animal ventilation was not recorded. Induction was by means of Fluothane. The results obtained during the period of anaesthesia and recovery are illustrated in figure 43 and summarised in table 51.

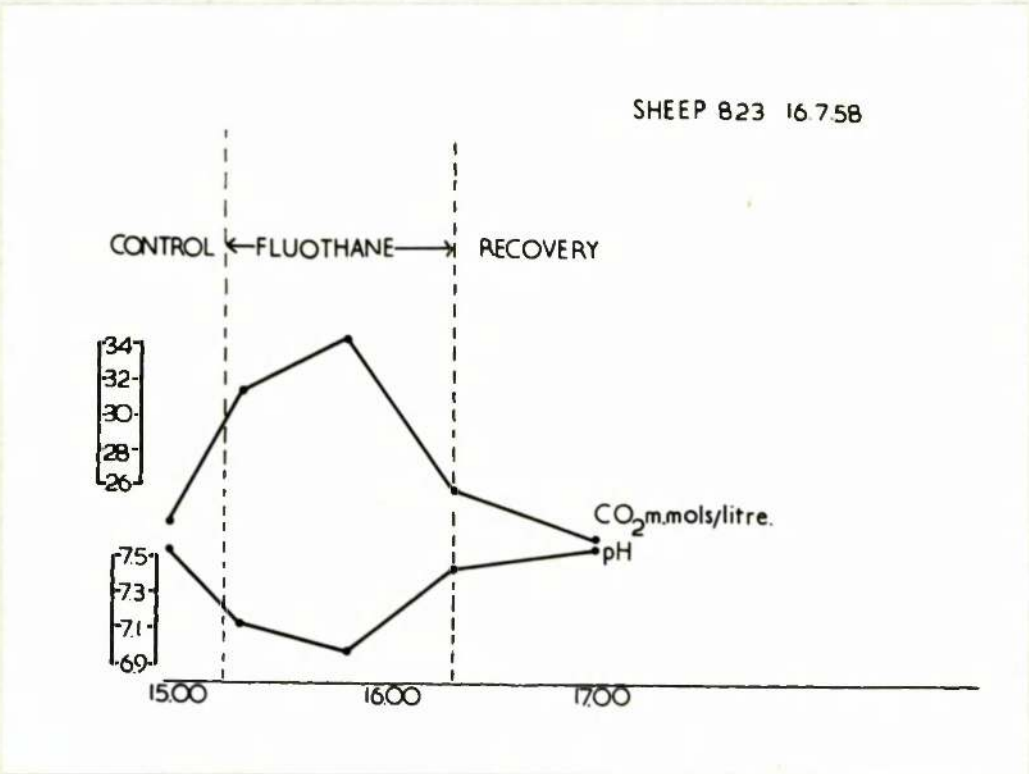

Fig. 43

Changes in plasma carbon dioxide content and plasma pH during Fluothane anaesthesia.

Table 52

Changes in Plasma Carbon Dioxide Content and Plasma pH

During Fluothane Anesthesia

Plas	Before Anes-thesia	During Anesthesia (minutes) 5	30	60	80	During Recovery (minutes) 20	30	40
pH	7.34	7.23	6.98	7.44				7.39
CO_2	34	31.4	34.3	25.4				29

Marked change in both plasma pH and plasma carbon dioxide occurred during Fluothane anesthesia but both plasma pH and plasma carbon dioxide returned to the control values 40 minutes after removal from the Fluothane atmosphere.

(2) Thiopentone sodium in cattle

Three experiments were carried out. In the first experiment thiopentone sodium was used as a single dose of 6 gm. while in the other two experiments thiopentone sodium was used not only to induce but also to maintain anaesthesia by the addition of increments of thiopentone sodium intravenously throughout the experiments.

Experiment TB I. This adult cow received an inducing dose of 6 gm. of thiopentone sodium. It collapsed in an anaesthetised state 17 seconds later. The results obtained during the period of anaesthesia and recovery are illustrated in figure 44 and summarised in table 52.

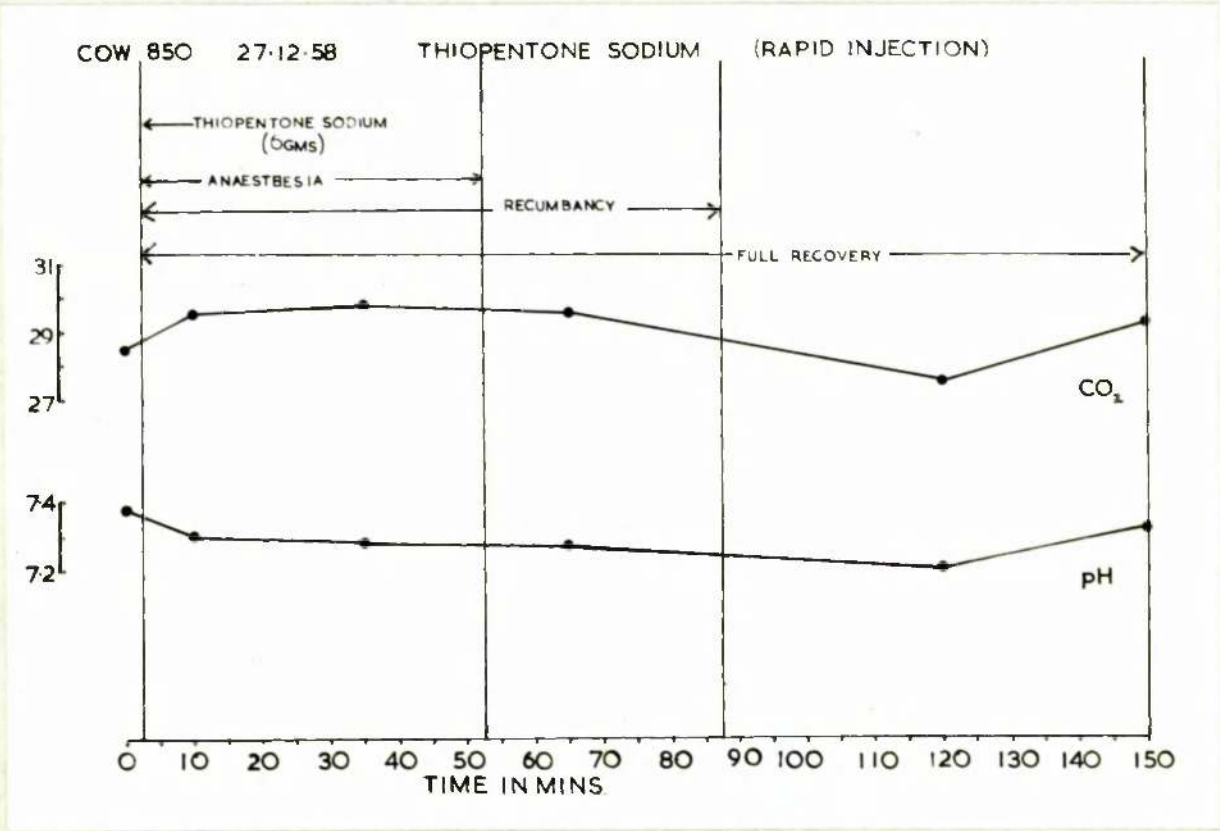

Fig. 44

Changes in plasma carbon dioxide content and plasma pH during thiopentone sodium anaesthesia

Table 52

Changes in Plasma Carbon Dioxide Content and Plasma pH

During Thiopentone Sodium Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 30	30	During Recumbency (minutes) 35	During Recovery (minutes) 40	70
pH	7.38	7.30	7.27	7.26	7.30	7.32
CO_2	28.5	29.5	29.75	28.0	27.5	29.25

The changes in plasma pH and plasma carbon dioxide content were not marked. It was noted that the period of surgical anaesthesia was about 30 minutes, and was followed by a period when the animal was at a higher level of anaesthesia when no operative interference would have been possible. After 50 minutes the cow could not be considered anaesthetized in any degree but she remained on her side and was not able to sit up for a further 40 minutes. The cow was then able to maintain breast recumbency but did not get on to her feet for a further hour.

Experiment TB II. Four week old calf. Blood samples were removed from the brachial artery and changes in blood carbon dioxide content were followed. Anaesthesia was maintained with increments of thiopentone sodium. The results obtained during the initial period of anaesthesia are illustrated in figure 45 and given in table 53.

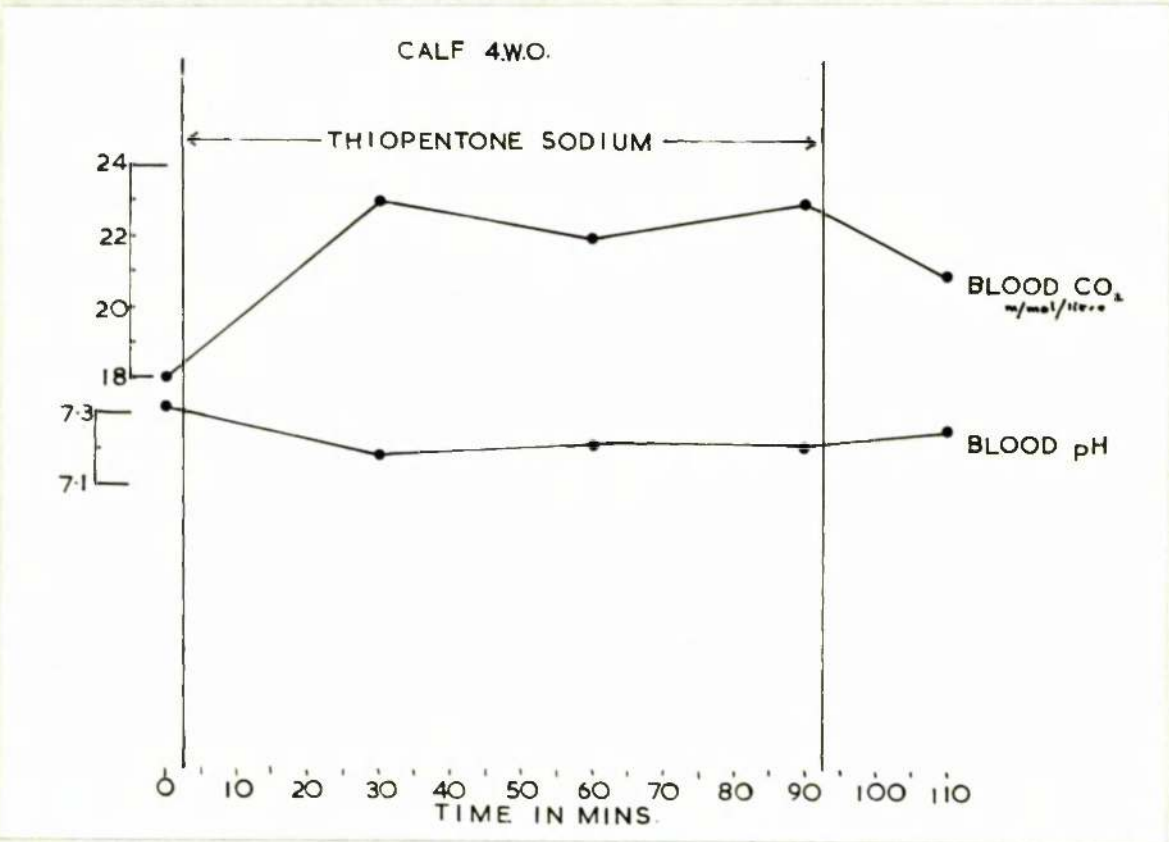

Fig. 45

Changes in blood carbon dioxide and pH during thiopentone anaesthesia

Table 53

Changes in Blood Carbon Dioxide and pH

During Thiamylene Anesthesia

Time	Before Anesthesia	During Anesthesia (minutes) 30	60	90	110
pH	7.32	7.19	7.21	7.21	7.25
Blood CO_2	38	28	22	23	22

Retention of carbon dioxide occurred and there was the usual fall in blood pH. The calf did not recover from anesthesia for a period of 15 hours.

Experiment TB III. Six week old calf. Blood samples were removed from the jugular vein by means of a catheter. The results obtained during anaesthesia are given in figure 46 and summarised in table 54.

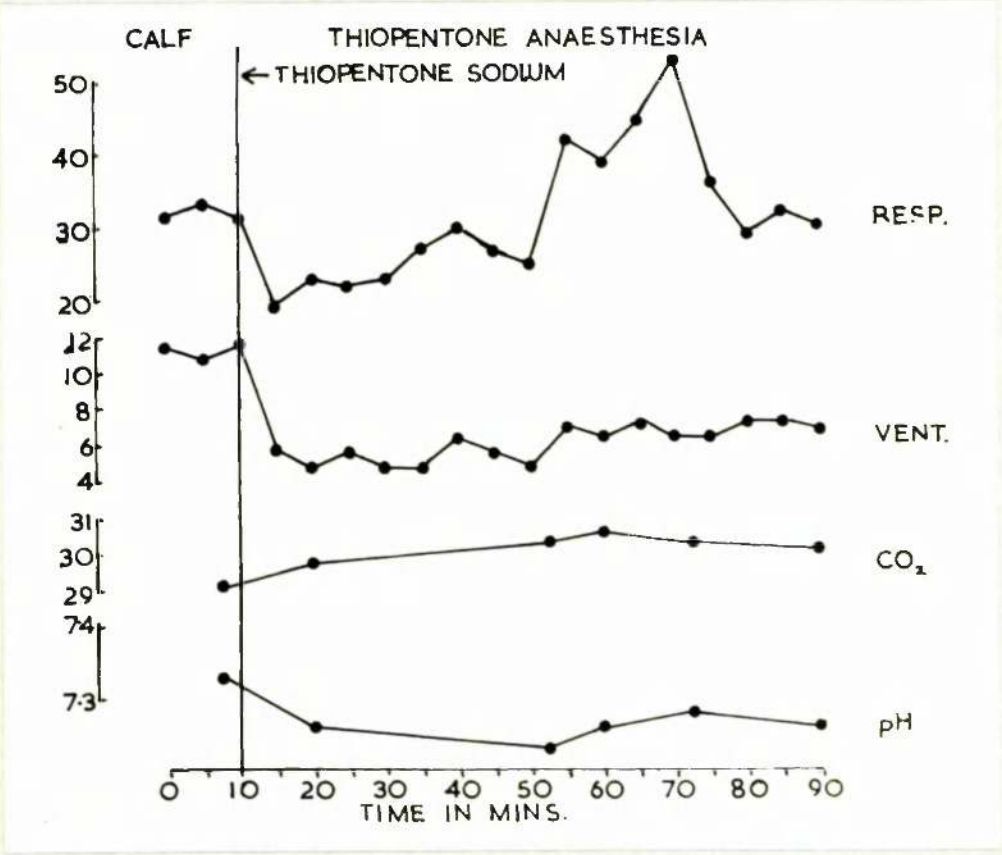

Fig. 46

Changes in ventilation and respiratory rates, plasma carbon dioxide content and plasma pH during thiopentone anaesthesia

Table 54

Changes in Ventilation and Respiratory Rate, Plasma Carbon Dioxide Content and Plasma pH During Fluroxene Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 30	40	50	60	80
* Resp	32	29	25	45	25	30
Vent	11	4.8	6	7.2	6.4	5.8
pH	7.33	7.35	7.29	7.26	7.29	7.26
CO_2	29.125	29.75	30.375	30.625	30.375	30.125

A marked depression of ventilation occurred but the changes in the plasma carbon dioxide content were not great. The calf was still anaesthetized at the end of the experiment and remained so for a further 12 hours.

* Throughout these Resp = Respirations per minute.

(3) Chloral hydrate in cattle

One experiment only was carried out the results of which are given in figure 47 and table 55.

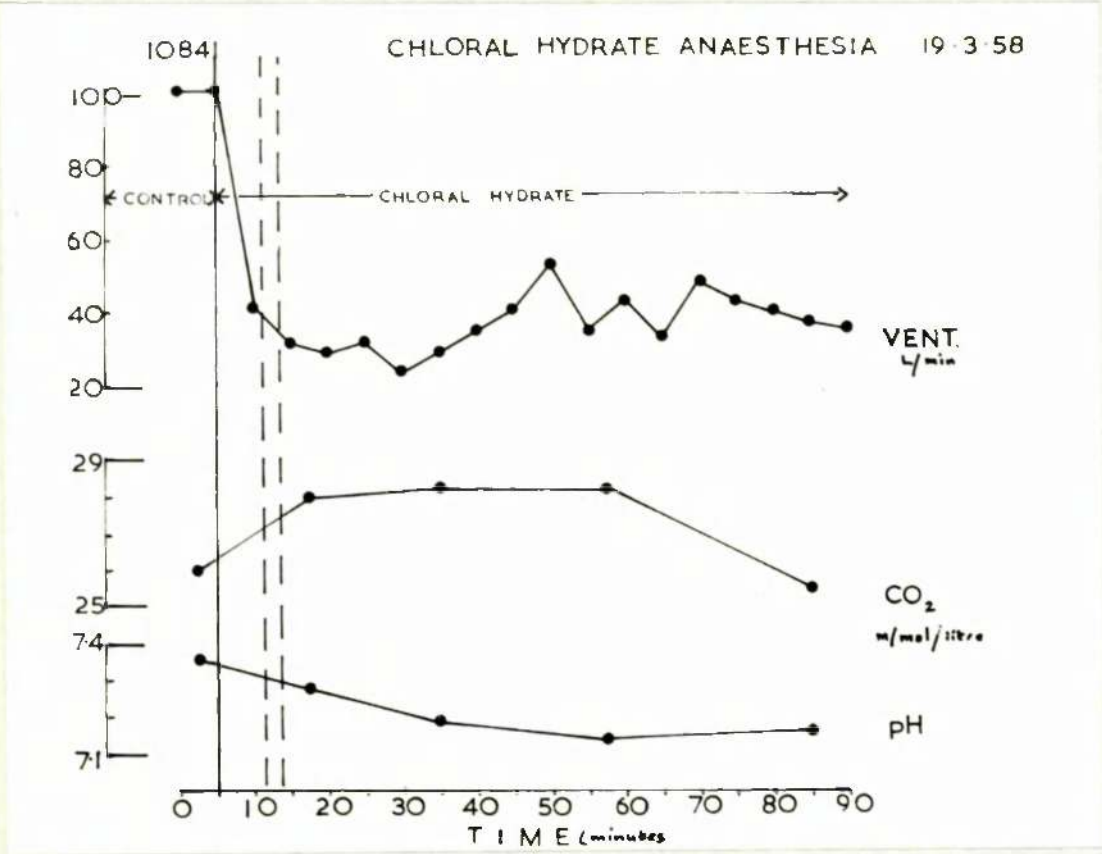

Fig. 47

Changes in ventilation rate, plasma carbon dioxide content and plasma pH during chloral hydrate anaesthesia

Table 55

Changes in Ventilation Rate, Plasma Carbon Dioxide Content and Plasma pH During Chloral Hydrate Anaesthesia

Time	Before Anaesthesia	During Anaesthesia (minutes) 5	20	50	80
Vent	100	30	31	35	57
pH	7.35	7.23	7.19	7.14	7.36
CO_2	29	28	28.25	28.25	29.5

Respiratory depression occurred and as a consequence carbon dioxide retention and a fall in plasma pH. After chloral hydrate administration had been discontinued respiration still remained depressed but the plasma carbon dioxide content returned to the control pre-anaesthetic level although the pH was still below the control value.

DISCUSSION
(Part II section 1)

The changes in pulmonary ventilation, plasma pH and plasma carbon dioxide concentration of cattle during Fluothane anaesthesia were contrasted with expected changes in general anaesthesia. There was a greater depression of ventilation than with the other anaesthetics, thiopentone sodium and chloral hydrate, which were studied. Fluothane was administered together with pure oxygen and it is possible that the increased oxygen tension eliminated any chemo-reflex stimulation of ventilation that might have arisen due to hypoxia caused by central depression of ventilation due to anaesthesia (Comroe, Forster, DuBois, Briscoe and Carlsen, 1955). It is possible that the cardiovascular effects of Fluothane in producing a fall in cardiac output and a fall in blood pressure (Dunn, Epstein, Folgan and Paton, 1957) may have produced interference with gaseous exchange by alterations of pulmonary blood flow. It has been shown that if Fluothane is administered with air to the human subject cyanosis tends to occur (Dunn, Fuchin, Gygax and Robertson, 1957). The rapid excretion of Fluothane through the lung once the animal has been removed from the anaesthetic atmosphere causes a rapid increase in pulmonary ventilation during recovery. The changes observed in cattle were similar to those reported by

Bovine, Hamilton and Fiddinger (1938) in the human subject and qualitatively similar to the effects on the ventilation of horses, sheep and dogs. Accompanying the depression of ventilation there was a rise in venous plasma carbon dioxide content during Fluothane anaesthesia to a value greater than that observed in the venous plasma of normal cattle. In prolonged periods of anaesthesia there was usually stabilization at a higher concentration of CO_2 than normal and towards the end of experiments a tendency for the carbon dioxide concentration of the plasma to fall. These observations of stabilization and tendency for the carbon dioxide content of the plasma to fall may have been explicable in the light of Quastel's (1952) work when he showed that oxidative metabolism was decreased during general anaesthesia in which case there would be a decreased carbon dioxide production. This decreased carbon dioxide production could possibly be handled by the depressed ventilation.

The changes of carbon dioxide content of the plasma during general anaesthesia were qualitatively similar in all species studied but quantitatively much greater in the sheep. Although ventilation was not generally recorded it was observed that many sheep became apnoic during Fluothane anaesthesia which might explain the high values obtained. The increases in the carbon dioxide content of cattle plasma during Fluothane anaesthesia were more marked than those observed with chloral hydrate or thiopentone sodium.

Plasma pH fell quite sharply after induction and remained at a low level during anesthesia. The changes observed were correlated with carbon dioxide retention and a respiratory acidosis. The changes observed in all species were qualitatively similar but the sheep showed greater changes. The decreases which took place in plasma pH during Fluothane anesthesia were greater than those observed with chloral hydrate or thiopentone sodium. It was noted that towards the end of anesthesia and during recovery that the plasma pH returned to the pre-anesthetic control value before the plasma carbon dioxide content returned to the control value. This may have been due to some other buffering capacity or buffering regulating mechanism coming into play during recovery. This could indicate interference with renal regulation during Fluothane anesthesia as has been shown to occur with barbiturate anesthesia in dogs (Blake, 1957).

CONCLUSIONS

From the results obtained in these experiments the following conclusions were drawn.

(1) A depression of pulmonary ventilation occurred during the administration of Fluothane to cattle, horses, sheep and dogs.

(2) Of the species studied, the greatest respiratory depression occurred in sheep, these animals often becoming apnoic during the administration of Fluothane.

(3) Accompanying the depression of ventilation there was a rise in the plasma carbon dioxide content, the extent of which depended on the depression of ventilation.

(4) The retention of carbon dioxide caused a lowering of plasma pH and hence the production of a respiratory acidosis.

(5) Recovery from the anaesthetic effects of Fluothane was rapid, the plasma pH in most cases returning to the pre-anaesthetic value one hour after removal from the Fluothane atmosphere, while at this point the carbon dioxide content was still elevated.

SECTION 2

THE DISTURBANCE OF RESPIRATION CAUSED BY PNEUMONIA.

REVIEW OF THE LITERATURE

The pathological changes taking place in the lungs of cattle as a result of pneumonia have been extensively studied by Jarrett (1956) and Jarrett, Jennings, McIntyre, Mulligan and Urquhart (1957). The disturbance of the respiratory rate in pneumonia caused by Dictyocaulus viviparus has been followed by Jarrett, McIntyre and Urquhart (1954) and has been used by them to assess the severity of the pneumonic process. Simpson and Hayden (1935) reported no change had taken place in the carbon dioxide capacity of the plasma of cattle with pneumonia but did not indicate the severity of the disease in the animals studied.

METHODS

The calves used in this series of experiments were reared in a similar manner to that described in Part I.

The pneumonia was produced by giving 4,000 Dictyocaulus viviparus larvae to each calf when it was about eight weeks of age. Three weeks after infection a severe clinical pneumonia was invariably present in these calves.

The calves described as normal in these experiments were not infected with Dictyocaulus viviparus larvae but nevertheless had a slight disturbance of respiratory rate due to residual lesions of curling pneumonia, a ubiquitous disease of calves. The ventilation and respiratory rates were measured as described in Part I. Blood samples were taken and estimations carried out on the plasma as described in Part I.

RESULTS

The results of five experiments are given and the variations in design are described separately.

Experiment I

The plasma carbon dioxide content of arterial blood was determined in two groups of calves of 12 weeks of age. In this experiment one group of 10 pneumonic calves was studied together with another group 10 normal calves. Over a period of nine days 19 blood samples were taken from the pneumonic group, all but one of the calves being sampled on two occasions. From the normal group of 10 calves 16 blood samples were taken, only six of the calves being sampled on two occasions. The results of the estimations are given in table 56.

The mean value for the determinations made on the pneumonic calves was significantly higher than the mean value obtained for the normal calves indicating that some carbon dioxide retention had occurred in these pneumonic calves.

Table 56

Carbon Dioxide Content of the Arterial Blood of Normal and Pneumonic Calves

Date	Normal Calves		Pneumonic Calves	
	Calf no.	Total CO_2 m.moles/litre	Calf no.	Total CO_2 m.moles/litre
4/6	18	30.2	33	32.9
	19	23.4	34	32.4
			35	32.6
			36	32.4
			37	34.6
7/6	15	31.1	30	33.7
	16	31.9	31	33.7
	17	31.0	32	30.5
	18	31.4	33	35.6
	19	31.0	34	31.5
11/6	40	29.5	25	32.9
	41	29.6	36	31.7
	42	27.4	37	31.4
	43	23.8	28	33.7
	44	25.2	29	31.9
12/6	16	28.6	30	31.9
	17	25.6	31	28.7
	18	23.8	33	30.5
	19	24.3	34	29.2
	Mean values		25.9 ± 2.3	32.6 ± 1.6

Experiment II

In this experiment the arterial plasma pH values of a group of 19 pneumonic calves were compared with the plasma pH values of 13 normal calves. Both groups of calves were about 12 weeks of age. The pneumonic group had been suffering from pneumonia for about three weeks. The results obtained are given in table 37.

There were no significant differences for the values for the plasma pH obtained in the two groups of calves and all values found were within the range of values determined for calves in Part I of this thesis.

Table 37

The Arterial Plasma pH of Normal and Pneumonic Calves

Normal Calves		Pneumonic Calves	
Calf no.	Plasma pH	Calf no.	Plasma pH
9732	7.47	9836	7.38
9729	7.44	9831	7.44
9718	7.42	9842	7.42
9725	7.44	9834	7.44
9735	7.45	9833	7.40
9727	7.47	9818	7.39
9730	7.44	9823	7.38
9731	7.40	9821	7.44
9740	7.47	9819	7.38
9502	7.42	9816	7.42
9739	7.38	9814	7.36
9726	7.42	9827	7.40
9512	7.38	9817	7.39
Mean values	7.43 ± 0.04		7.40 ± 0.04

Experiment III

In this experiment determinations of the carbon dioxide content and the pH of plasma were made simultaneously in two groups of calves, one consisting of five normal calves and the other of 19 pneumonic calves. The results obtained are given in table 58.

These results confirmed those of the previous two experiments that parasitic pneumonia in calves caused a carbon dioxide retention with a significantly higher plasma carbon dioxide content and not a significantly lower plasma pH than normal calves exposed to similar conditions.

Table 34

The Arterial Plasma pH and Carbon Dioxide Content of the Plasma of Normal and Pneumonic Calves

Normal Calves			Pneumonic Calves		
No.	pH	CO_2 m.mols/litre	No.	pH	CO_2 m.mols/litre
31	7.38	27.4	4	7.42	27.8
32	7.47	27.8	5	7.44	29.1
33	7.44	27.9	GH17	7.42	31.2
34	7.36	28.8	GH19	7.38	26.4
931	7.42	27.6	62	7.40	30.4
			61	7.42	30.1
			55	7.39	32.0
			56	7.34	32.6
			57	7.36	32.4
			58	7.40	29.1
			59	7.38	33.5
			60	7.35	30.5
			37	7.33	29.8
			38	7.40	28.1
			39	7.36	28.8
			49	7.36	26.8
			932	7.38	29.0
			933	7.46	29.9
			768	7.38	28.3
Mean values	7.41 ± 0.04	27.9 ± 0.5%	Mean values	7.38 ± 0.04	29.8 ± 2.0

Experiment IV

The pneumonic group in this experiment was different from that in all other experiments in that the calves had picked up their parasitic infection on a heavily contaminated pasture. These calves were therefore much more severely infected than the calves in the other pneumonic groups and in fact all the calves died a few days after the measurements were made. The results obtained are given in table 39.

There was a considerable difference in the plasma carbon dioxide contents of the two groups of calves but still no significant difference between the plasma pH values. The severity of the pneumonia is indicated by the great increase in respiratory rate. Although the ventilation rates in the pneumonic group had increased by nearly 50%, the tidal air had decreased by about one half.

Table 29

The Plasma pH and the Carbon Dioxide Content Together with the respiratory and ventilation rates of normal and pneumonic calves

Calf	CO_2 m.moles/litre	pH	Ventilation Litres/min.	Respiratory rate/min.	Tidal air
Normal group					
a	28.6	7.40	28	42	680
b	31.3	7.38	22	35	620
c	29.4	7.35	25	30	860
d	29.1	7.38	24	30	800
Mean	29.6	7.38	25	34	740
Pneumonic group					
e	37.6	7.25	39	75	490
f	31.8	7.30	30	62	480
g	34.9	7.35	40	80	500
h	35.1	7.40	35	67	430
i	32.3	7.35	45	82	540
Mean	34.3	7.33	37	77	470

Experiment I

In this experiment a group of five calves was studied over a period of 40 days during which time six examinations were carried out approximately at weekly intervals. The first examination was carried out 10 days after an infecting dose of 4,000 Dictyocaulus viviparus larvae had been given, i.e. at the early pneumonic stage.

The results for each calf are given in tables 60 (a) - (f). In table 60 (f) the mean values for each measurement are given.

In this experiment it was possible to follow the changes from the early to the severe pneumonic state. The plasma carbon dioxide contents increased until the fourth week from infection after which they tended to stabilise at a high value. This was also true of respiratory rate. On the other hand the pH did not alter significantly.

Table 60

Changes in Plasma pH, Plasma Carbon Dioxide Content,

Ventilation and Respiratory Rates of 6 Calves

Following R. vivipara Infection

Table 60 (a)

Calf 55

Date	CO_2 m.moles/litre	pH	Ventilation litres/min.	Respiratory rate/min.	Tidal air
12/9	29.1	7.38	24	30	800
19/9	27	7.35	25	47	530
25/9	26.7	7.44	30	55	545
3/10	31	7.39	32	52	615
10/10	34.3	7.38	32	50	640
23/10	26.3	7.28	31	55	563

Table 60 (b) Page 97

Date	CO_2 m.moles/litre	pH	Ventilation litres/min.	Respiratory rate/min.	Tidal c/s
12/9	29.4	7.35	26	30	670
19/9	25.9	7.39	18	32	560
26/9	24.0	7.44	23	52	540
3/10	29.4	7.40	25	57	460
10/10	24.6	7.38	18	43	420
21/10	26.3	7.36	36	73	490

Table 60 (c) Page 98

Date	CO_2 m.moles/litre	pH	Ventilation litres/min.	Respiratory rate/min.	Tidal c/s
12/9	30.6	7.35	12	44	272
19/9	28.4	7.39	15	60	250
26/9	30.5	7.41	18	69	260
3/10	32.5	7.46	18	66	272
10/10	33.5	7.39	16	78	205
22/10	35.6	7.38	29	115	255

Table 60 (d) Gulf 52

Date	CO_2 m.moles/litre	pH	Ventilation litres/min.	Respiratory rate/min.	Tidal air
12/9	28.8	7.40	23	41	680
19/9	28.6	7.38	24	50	480
26/9	31.5	7.44	27	75	360
3/10	30.6	7.38	23	57	560
10/10	34.6	7.37	29	50	580
21/10	31.6	7.33	37	69	540

Table 60 (e) Gulf 100

Date	CO_2 m.moles/litre	pH	Ventilation litres/min.	Respiratory rate/min.	Tidal air
12/9	31.3	7.38	22	35	630
19/9	31.3	7.38	31	99	330
26/9	36.2	7.30	24	78	308
3/10	25.8	7.28	29	97	300
10/10	37.9	7.34	30	89	337
21/10	35.1	7.32	37	80	462

Table 60 (3)

Mean Values all 5 Calves

Date	CO_2 m.moles/litre	pH	Ventilation litres/min.	Respiratory rate/min.	Tidal air
12/9	29.8	7.37	20	36	555
19/9	33.4	7.38	29	56	510
25/9	33.7	7.42	25	66	378
3/10	32.6	7.39	25	66	393
10/10	34.9	7.37	25	62	403
21/10	32.9	7.39	29	79	436

DISCUSSION
(Part II section 2)

Calves affected with a parasitic pneumonia showed an increase in ventilation and respiratory rates, the increase in the respiratory rate being the greater so that the tidal air fell indicating shallower and less efficient respiration. The carbon dioxide content of the plasma of these calves was significantly higher than that of normal calves. It was noteworthy that, with the exception of one anoxaemic severely ill calf which died the next day, the plasma pH of these pneumonic calves was never below normal. A compensated respiratory acidosis therefore existed in these calves.

The pneumonic animals showed a continuing increased respiratory response to pneumonia so that there must have been a continuous stimulation to respiration. The increased carbon dioxide content of the plasma would also act as a stimulus, but since the plasma pH was within the normal range, the pH would not act as a stimulus.

It was observed that the arterial blood of these pneumonic calves was darker in colour than arterial blood from normal calves. The lowered oxygen content which this indicated could also have acted as a stimulus to respiration by its effect at the chemo-receptors although this might be counteracted by the fact that anoxia centrally has a

department offset on negligible.

CONCLUSIONS

(1) Calves affected with a parenteric pneumonia showed an increase in both the respiratory and ventilation rates compared to normal calves.

(2) The increase in the respiratory rate was greater than the increase in ventilation rate so that respiration became shallower and less efficient.

(3) These pneumonic calves showed an increase in the plasma carbon dioxide content but no marked change in the plasma pH.

SECTION 3

THE EFFECTS OF ALTERATIONS IN THE ATMOSPHERE BREATHED TO GIVEN TO BEHAVIOUR ON THE VENTILATION RATE, THE PLASMA pH AND PLASMA CARBON DIOXIDE CONTENT OF CATTLE

REVIEW OF THE LITERATURE

Alterations in the atmosphere on animal breathing affect respiration and the changes in plasma carbon dioxide tension, plasma carbon dioxide content and plasma pH have been extensively studied in man and experimental animals.

The preponderant role that carbon dioxide plays in the day by day regulation of respiration emerged from the studies of Haldane many years ago whose results are still quoted in most textbooks of physiology.

A good summary of the effects on respiration of the tension of oxygen and carbon dioxide in the blood and the plasma pH was given by Grey (1950). He stated that an increase in the arterial carbon dioxide tension (pCO_2) caused hyperventilation and a lowering caused hypo-ventilation. He described the stimulant effect of hypoxia on respiration

as slight, no appreciable change in respiration taking place until the content of oxygen in inspired air fell below 14%. Gray discussed the reasons for this and suggested that the hyperventilation caused by the lowered arterial pO_2 resulted in acapnia the depressant effect of which counteracted the stimulation of hypoxia so that the resultant respiratory stimulation was slight. He also stated that the effects of increasing the oxygen content of inspired air were negligible in the normal human subject. However, there is some evidence for the normal arterial pO_2 of the unanaesthetized human subject acting as a tonic stimulus to respiration. The measurements of arterial pO_2 in resting human subjects breathing room air found by Asmussen and Nielsen (1950) indicated values below the threshold found in animal experiments by Mitchell et al. (1955). Similarly Nett et al. (1943) found evidence for the presence of tonic chemo-receptor activity at the normal arterial pO_2 of dogs. They produced experimentally a decrease in ventilation if dogs were given pure oxygen to breathe. This decrease no longer took place when the chemo-receptors were denervated.

Examination of the literature revealed no evidence of studies of the respiratory behaviour of cattle when exposed to gas mixtures of different composition to atmospheric air.

METHODS

Studies were carried out on Ayrshire calves between six and 12 weeks of age. The calves were lightly restrained in a dog stand and the close fitting mask previously described was fitted. This procedure seldom disturbed the calf provided their heads were held gently. The mask was connected by means of a brass 'Y' tube and neoprene rubber tubing to a pair of unidirectional valves. The expiratory portion of these valves led to the gas meter so that the ventilation could be recorded. The inspiratory side of the unidirectional valves was connected to a length of flexible tube (Hoovertube). At the end of this flexible tube was a 'T' junction to the end of which was attached a one and a half inch stove release valve which could be opened to the atmosphere. To the third arm of the 'T' an eight gallon reservoir bag was fitted. The other end of the reservoir bag had a small diameter rubber connection through which measured gas mixtures were fed into the reservoir bag.

The calves were allowed to inspire room air through the open stove valve for a period of 10 minutes during which time ventilation rates and respiratory rates were recorded. At the end of this control period, if blood changes were being followed, control blood samples were taken from

the jugular vein or brachial artery. The stop valve was then closed and the gas mixtures, the effects of which were being studied, was fed into the reservoir bag. Gas mixtures were fed in at such a rate as to keep the reservoir bag about half full, the animal's inspiratory efforts being plainly visible in the extraction of the gas mixture from the bag. Gas mixtures were either made up as required or purchased ready made from British Oxygen Gases, Ltd., Ronaldsway Works, Ballasalla, Glasgow. The following mixtures were purchased: (a) 90% nitrogen and 10% oxygen, (b) 95% oxygen and 5% carbon dioxide, (c) 100% carbon dioxide, and (d) 100% oxygen. Accurate analysis of the gas mixture presented to the animals was not attempted.

The calves were allowed to breathe the gas mixtures for varying periods of time during which respiratory and ventilation rates were recorded. Blood samples were taken at intervals, centrifuged and the plasma pH and plasma carbon dioxide content were determined. In a number of cases, at the end of an experiment, the calf was allowed to inspire room air for a period while ventilation rates and respiratory rates were again recorded and blood samples taken for the determination of plasma pH and plasma carbon dioxide content.

RESULTS

The results of the studies in this section are arranged in the following order.

(1) Hypoxemia

(2) Hypercapnia

(3) Anemia

(2) Hyperoxygenation

In this section a variety of experiments have been carried out. In experiments I and II the effects of hyperoxygenation on the ventilation rates of three calves and three dogs, respectively, were studied.

In experiments III and IV more extensive studies were carried out in that the effects of the gradual increase in the concentration of oxygen inspired on the carbon dioxide content and the pH of plasma, the ventilation and respiratory rates were studied.

In experiment V, similar determinations were made on a normal calf breathing pure oxygen for 35 minutes.

In experiments VI and VII the studies on the effects of pure oxygen were continued in two pneumonic calves.

Experiment I

Each calf was allowed to breathe room air for a period of 10 minutes during which time the ventilation rate was recorded. At the end of this time the release valve was closed and each calf was made to inspire pure oxygen from the reservoir bag for a further 10 minutes and the ventilation rate was again recorded. The results obtained are given in figures 48 and 49.

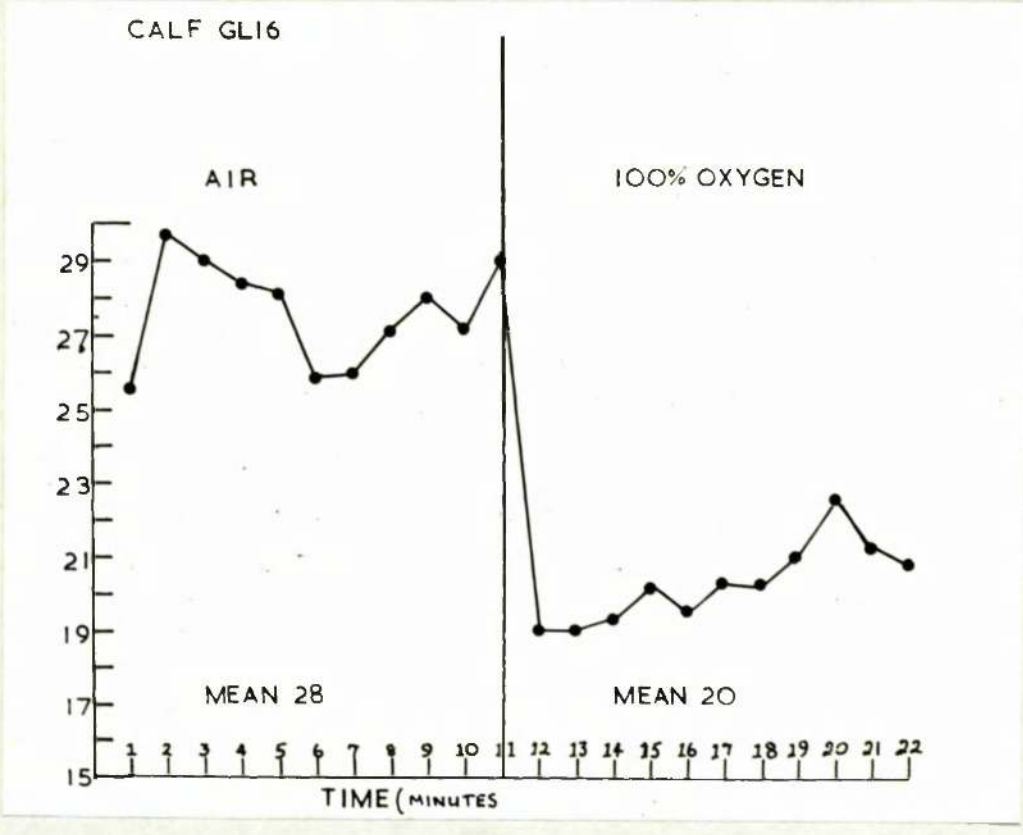

Fig. 48

Depression of ventilation rate caused by inhalation of pure oxygen

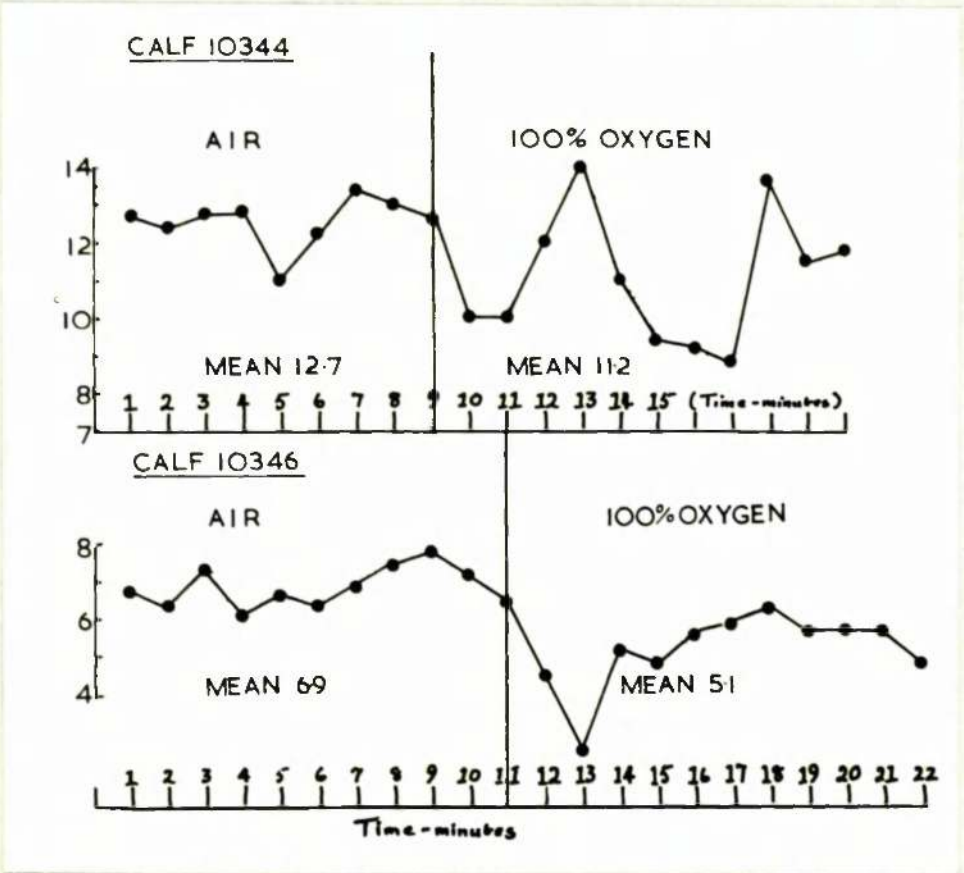

Fig. 19

Reduction of ventilation rate caused by inhalation of pure oxygen

A reduction of ventilation of varying degree occurred in each calf.

Experiment II

Each of three greyhounds was allowed to inspire room air for a period of 10 minutes during which time its ventilation rate was recorded. Each greyhound was then made to inspire pure oxygen for 10 minutes when the ventilation rate was again recorded. The results obtained are given in figures 50 and 51.

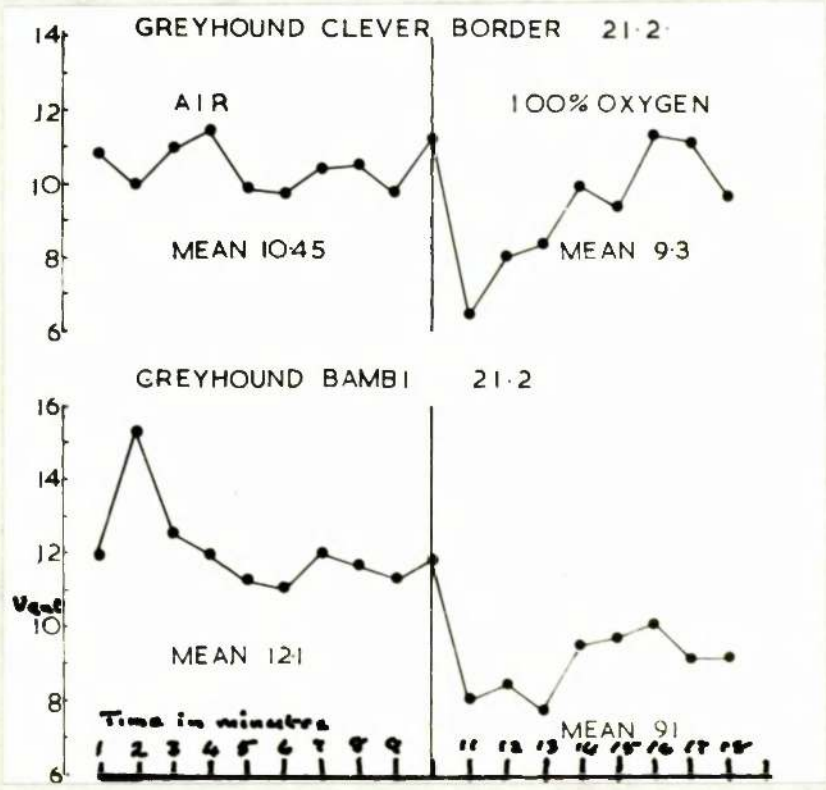

Fig. 50

Reduction of ventilation rate caused by inhalation of pure oxygen

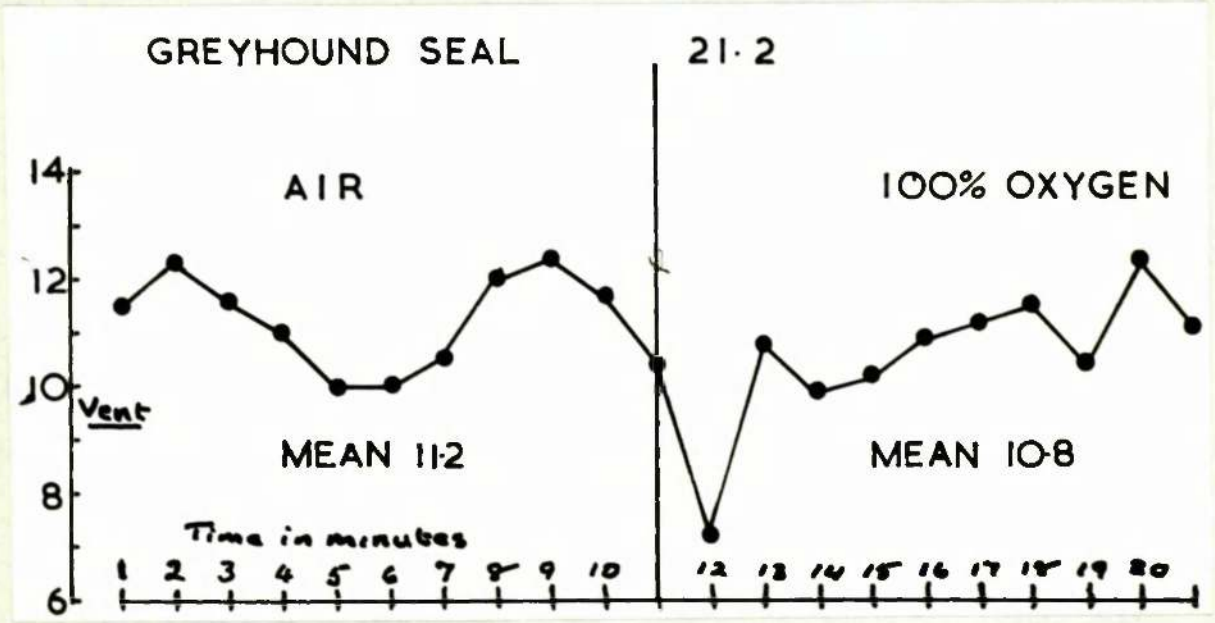

Fig. 51

Reduction of ventilation rate caused by inhalation of pure oxygen

As in the calves in experiment I these dogs showed a varying reduction of ventilation rate.

Experiment III

In this experiment one calf was subjected to alternate periods of 10 minutes of air and air/oxygen mixtures. On each of the three periods of oxygenation the concentration of oxygen was increased, terminating in pure oxygen on the third occasion. Determinations carried out are given in table 62 and illustrated graphically in figure 52.

Table 61

Effects of Inhalation of Air Enriched with Different Proportions of Oxygen

Mixture inhaled	Air	Air + 1 litre O_2 per min.	Air	Air + 4 litres O_2 per min.	Air	Pure O_2	Air
pH	7.40	7.32	7.41	7.36	7.38	7.23	
CO_2	31	33	32.9	33	31.9	33.3	
Resp	57	62		63		65	59
Vent	41	39		35		34	39

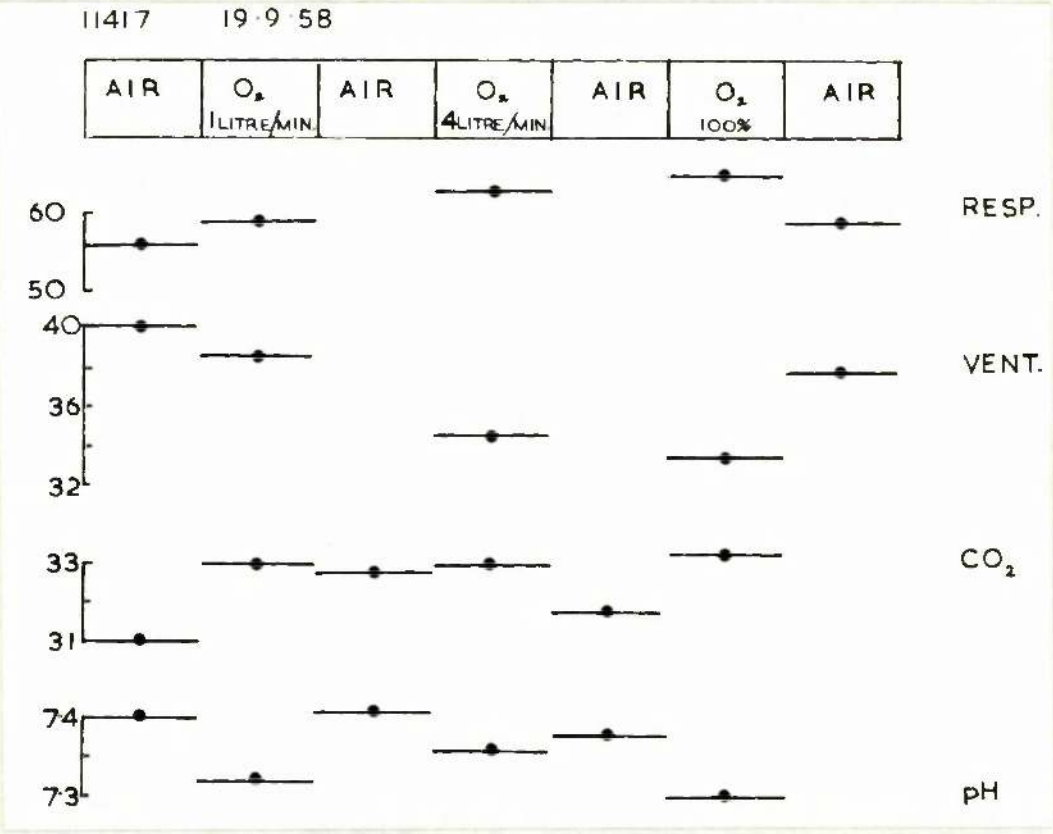

Fig. 52

Effects of inhalation of air enriched with different proportions of oxygen

It was noted that a slight increase in the respiratory rate and a decrease in the ventilation rate occurred, the changes being greater the higher the concentration of oxygen in the inspired air. The changes in plasma pH and plasma carbon dioxide content were less obvious but each time enriched air was given the pH fell and the plasma carbon dioxide content rose but in the control periods between enriched mixtures neither pH nor carbon dioxide content returned to the initial values.

Experiment IV

A similar experiment was conducted on a calf with a more severe parasitic pneumonia. The periods between the administration of different levels of oxygen were lengthened to half an hour. The results obtained are given in table 62.

Table 62

Effects of Inhalation of Air Enriched with Different Percentages of Oxygen

Mixture Inhaled	Air	Air + 1 Litre O_2 per min.	Air	Air + 4 Litres O_2 per min.	Air	Pure O_2
pH	7.39	7.35	7.40	7.35	7.39	7.27
CO_2	39	38.4	38.1	38.3	39.1	39.4
Resp	94	95		87		92
Vent	36	36		32		31

It will be seen that a reduction of ventilation occurred with the high concentrations of oxygen but that no reduction occurred when 1 litre of oxygen per minute was added to a ventilation rate of 36 litres per minute. The respiratory rate was lowered slightly by high concentration of oxygen. The plasma pH showed the expected trends but apart from the marked fall during the administration of pure oxygen the

changes were not significant. The plasma carbon dioxide content, like the plasma pH, showed the expected trend in that it rose when the ventilation rate fell but the changes were again not significant.

Experiment V

In this experiment the effects of more prolonged administrations of pure oxygen were studied in a two week old calf. The determinations made are given in table 69 and illustrated in figure 53.

Table 69

Effects of Inhalation of Pure Oxygen

	Air 5 min.	100% O_2 5 min.	25 min.	35 min.	Air 7 min.
Resp	18	37.6	18.5	19	22
Vent	6	4.5	4.7	5.5	6.0
CO_2	35.5	36	35.4	36.6	35.6
pH	7.40	7.35	7.35	7.33	7.43

It was observed that a slight increase in the respiratory rate occurred during the administration but a noticeable increase occurred afterwards. Ventilation fell during the administration of pure oxygen but rose again before the cessation of the administration. This animal was not premonic but initial high values of unknown origin were observed for the plasma carbon dioxide content. Concomitant with the changes in ventilation the plasma carbon dioxide content rose and the plasma pH fell but the changes were small. There was a rapid return

to normal values once the calf again breathed air.

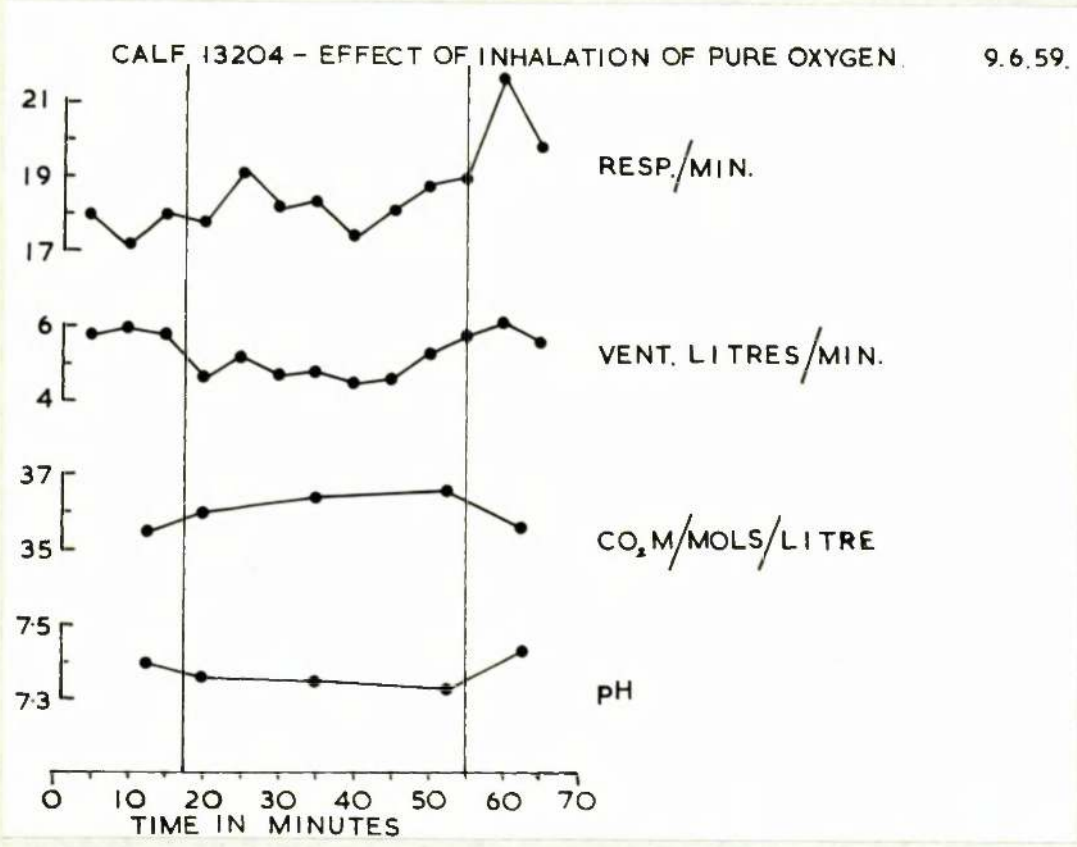

Fig. 53

Effects of inhalation of pure oxygen

Experiment VI

In this experiment the prolonged administration of pure oxygen to a severely pneumonic calf was studied. The results of the determinations are given in table 64.

Table 64

Effects of Inhalation of Pure Oxygen

	Air	Inhalation of pure O_2 (minutes) 10	20	30	45	Recovery (minutes) 5	10
pH	7.38	7.29	7.22	7.21	7.29		7.39
CO_2	37.5	38	38.8	39.4	38.9		38.6
Resp	96	94	97	93	94	95	94
Vent	34	29	31	30	32	30	37

It was observed that the respiratory rate was little affected by inhalation of pure oxygen but that the ventilation rate fell. After the calf had ceased breathing pure oxygen the ventilation rate rose above the initial level probably because of the higher than normal plasma carbon dioxide content. The plasma pH fell quite sharply and remained low. The plasma carbon dioxide content which was initially high rose to even higher levels during the administration of pure oxygen and had not returned to the initial level after 10 minutes.

Experiment VII

The effects of the inhalation of pure oxygen on a pneumonic calf were again studied. This Ayrshire calf was as severely affected as the previous calf. The results obtained are given in table 65 and illustrated in figure 54.

Table 65

Effects of Inhalation of Pure Oxygen

	Air	Inhalation of pure O_2 (minutes)					Recovery (mins.)		
		5	15	25	35	45	5	10	20
pH	7.42	7.31	7.27	7.25	7.23	7.22	7.33	7.35	
CO_2	39.4	39.6	41.3	42.1	41.6	43.1	41.3	41.3	
Resp	69	76	60	75	69	78	84	85	73
Vent	32	36	25	26	23	29	35	34	32

The ventilation rate was observed to fall as did the respiratory rate during the administration of pure oxygen. When the administration of pure oxygen had ceased respiratory rate rose as did the ventilation rate to values above the initial values. There was in this calf a marked rise in the plasma carbon dioxide content and a fall in plasma pH and even 10 minutes after the administration of pure oxygen had

ceased the pH and plasma carbon dioxide content had not returned to the initial values.

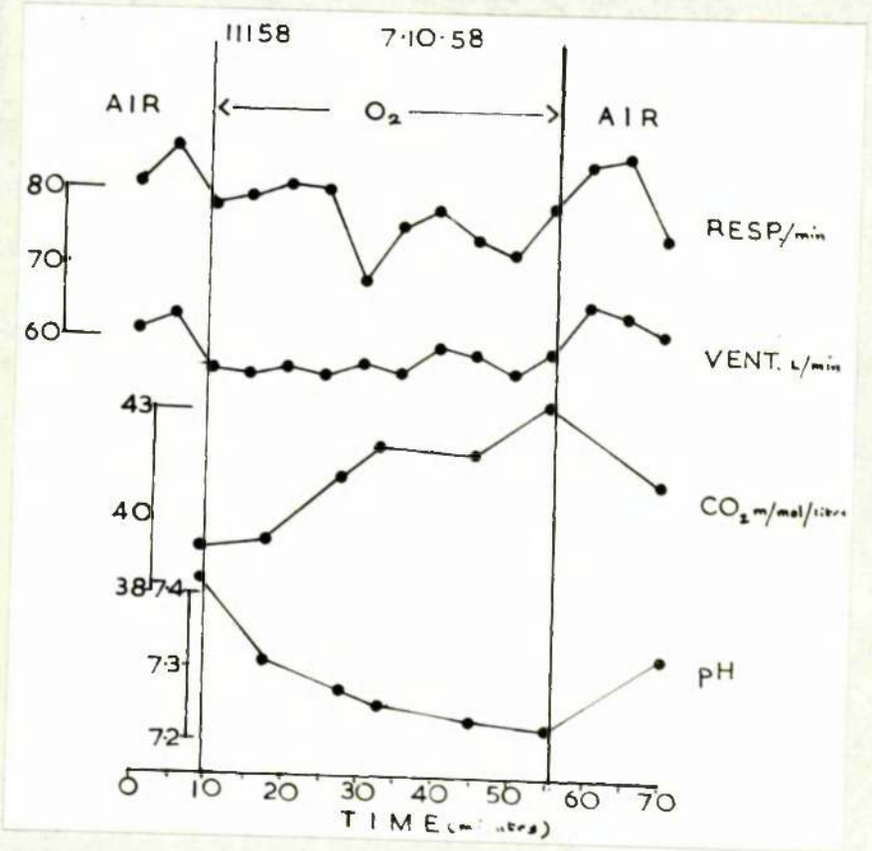

Fig. 54

Effects of inhalation of pure oxygen

(2) Hypercapnia

In this section a variety of experiments have been carried out on the effects of hypercapnia. In experiments I to IV the plasma pH and plasma carbon dioxide changes due to inhalation of 95% oxygen and 5% carbon dioxide were followed in normal two-month old calves. In experiments II, III and IV the ventilation rates were also measured and in experiments III and IV respiratory rates were measured.

The effects of hypercapnia on calves with mild and severe pneumonia were studied in experiments V and VI, respectively.

Experiments I, II, III and IV

The results obtained from experiments I to IV are given in tables 66, 67, 68 and 69, respectively, and in addition the results from experiment IV are illustrated graphically in figure 55.

Table 66

Experiment I
Venous plasma

Effects of Inhalation of 5% Carbon Dioxide

	Mix	5% CO_2 inhalation (minutes)			Recovery (minutes)	
		10	20	30	5	15
pH	7.35	7.24	7.24	7.21	7.27	7.32
CO_2	30.1	31.5	31.5	32.5	29.6	30.1

Table 67

Experiment II
Venous plasma

Effects of Inhalation of 5% Carbon Dioxide

	Mix	5% CO_2 inhalation (minutes)		Recovery (minutes)
		10	20	30
pH	7.37	7.25	7.24	7.37
CO_2	29.4	31.4	29.1	32.5
Vent	29	30	32	25

Table 68

Experiment III
Venous plasma

Effects of Inhalation of 5% Carbon Dioxide

	Air	5% CO_2 inhalation (minutes)			Recovery (minutes)		
		5	15	30	5	10	30
pH	7.37	7.34	7.29	7.21	7.25	7.32	7.37
CO_2	32.0	32.75	34.4	34.5	33.75	33.0	32.0
Resp	32	40	55	58	40	35	

Table 69

Experiment IV
Venous plasma

Effects of Inhalation of 5% Carbon Dioxide

	Air	5% CO_2 inhalation (minutes)			Recovery (minutes)	
		5	15	30	5	15
pH	7.36	7.27	7.27	7.25	7.35	7.35
CO_2	27.9	29.5	29.6	30.5	29	27.5
Resp	25	29	36	29	27	25
Vent	36	33	37	33	30	30

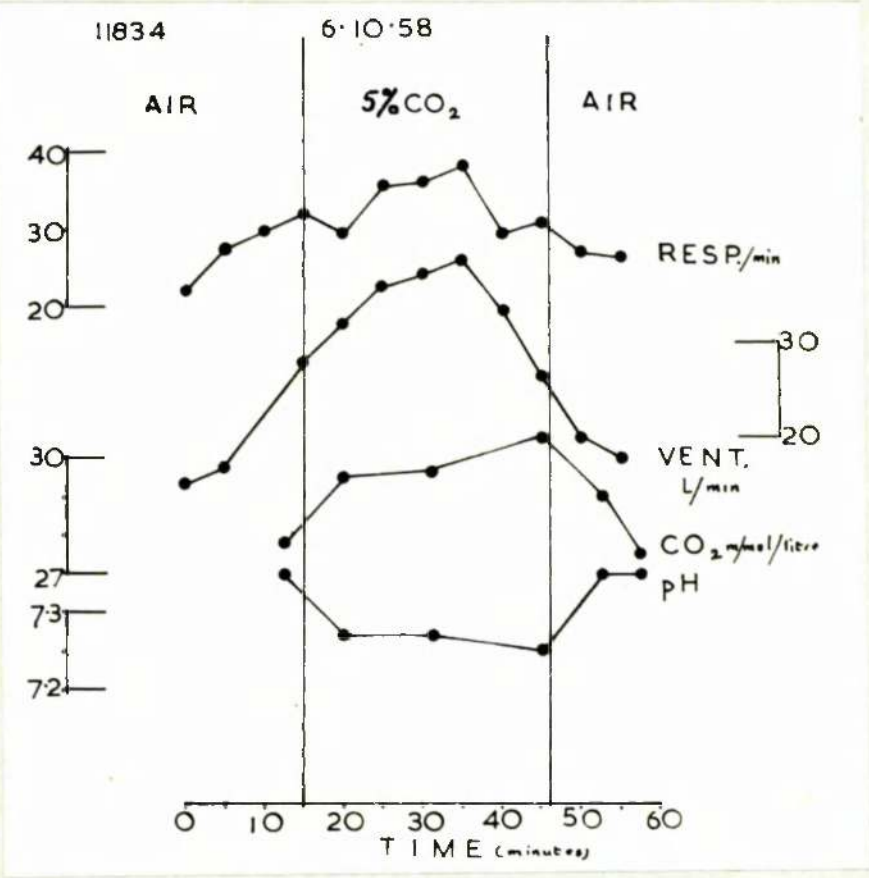

Fig. 55

Effects of inhalation of 5% carbon dioxide

These four calves showed an increased plasma carbon dioxide content and a lower plasma pH. Where ventilation rate was measured this showed an increase as did respiratory rate when this was recorded. In all cases there was a rapid return to the initial values when carbon dioxide administration ceased.

Experiment V

The results obtained from studying the effects of hypercapnia in a mildly pneumonic calf are given in table 70 and figure 56.

Table 70

Effects of Inhalation of 5% Carbon Dioxide

	Air	5% CO_2 inhalation (minutes) 5	15	Recovery (minutes) 1	15	30
pH	7.37	7.23	7.18	7.26	7.29	7.35
CO_2	33.9	35.8	35.0	34.3	33.0	33.5
Resp	44	56	54	65	75	60
Vent	25	38	45	40	35	28

The respiratory rate of this calf continued to increase throughout the period of administration of the 95% oxygen and 5% carbon dioxide and this increase was maintained through about half of the recovery period. The ventilation rate rose and started to fall while the calf continued to breathe the gas mixture. The plasma carbon dioxide content rose upon administration of the carbon dioxide but started to fall before this administration had ceased. The plasma pH fell quite sharply and continued to fall throughout the administration of carbon dioxide.

During recovery which was slower than in the non-pneumonic calf, return to the initial values of carbon dioxide and pH took 30 minutes but at this point both respiratory rate and ventilation rate were still above the initial values.

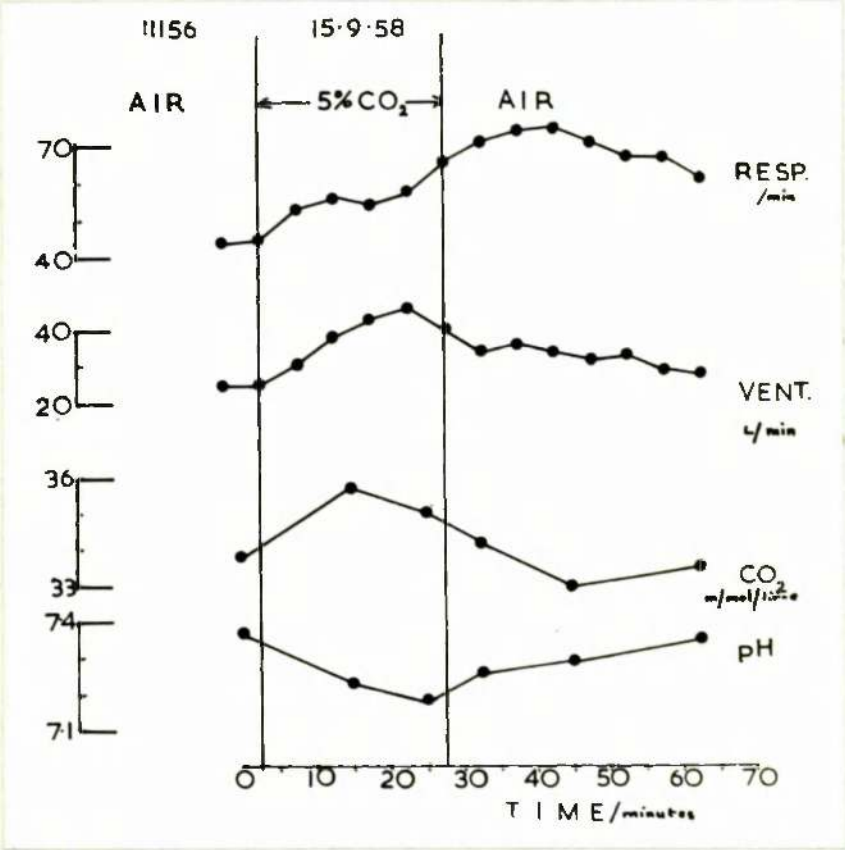

Fig. 56

Effects of inhalation of 5% carbon dioxide

Experiment VI

The results obtained from studying the effects of hyperpnoea in a normally pneumonic calf are given in table 71 and figure 57.

Table 71.

Effects of Inhalation of 5% Carbon Dioxide

	Air	Inhalation of 5% CO_2 (minutes) 14	20	25	32	37	46	Recovery (minutes) 5	10	20
pH	7.34	7.24	7.36	7.37	7.37	7.37	7.36		7.28	7.31
CO_2	34.9	36.6	40.3	41.9	41.8	40.4	40.5		39.4	37
Vent	30	27	30	26	30	32	33	35	33	32
Resp	96	70	63	62	66	66	72	61	65	85

This calf differed from the other calves in that during the administration of carbon dioxide the respiratory rate underwent a marked decrease but began to rise again towards the end of the administration. The ventilation rate showed some decrease initially but a rise occurred towards the end of the administration of the gas mixture to above the initial value. The plasma carbon dioxide content rose and the plasma pH fell initially and then the carbon dioxide content fell and the plasma pH started to rise when the ventilation increased during the administration

of the gas mixture. Changes in plasma pH and carbon dioxide content were quite marked. During the first 20 minutes of the recovery period there was a slight return towards the initial values of plasma pH and plasma carbon dioxide content.

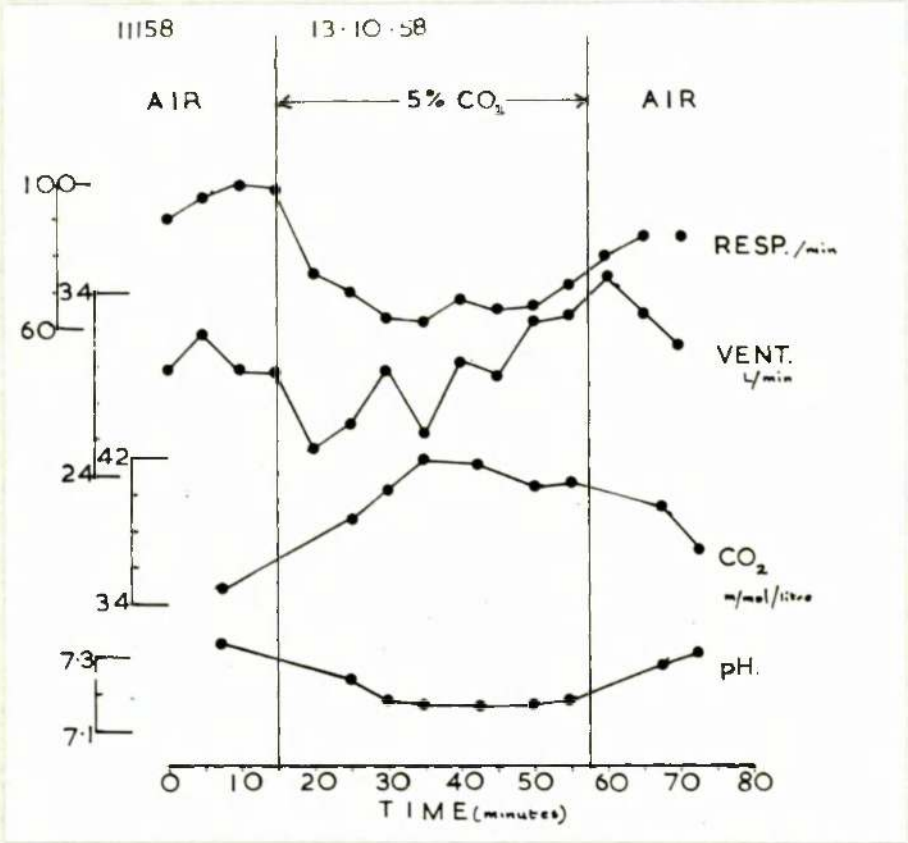

Fig. 57

Effects of inhalation of 5% carbon dioxide

(2) Anoxia

Two experiments were carried out to study the effects of anoxia in calves by the administration of a gas mixture deficient in oxygen. These calves were made to inspire a gas mixture containing 90% nitrogen and 10% oxygen.

Experiments I and II

The results obtained in experiments I and II are given in tables 72 and 73 and figures 58 and 59.

Table 72

Effects of Anemia on the Ventilation and Respiratory Rate, the Carbon Dioxide Content and the pH of the Plasma of a Calf

	Air	Inhalation of N_2 mixture (minutes)				Recovery (minutes)		
		10	22	35	47	5	19	27
pH	7.36	7.49	7.47	7.48	7.43		7.35	7.32
CO_2	26.6	26.6	25.3	25.8	26.6		25.5	25.1
Resp	29	33	24	33	34	35	33	31
Vent	16.4	21	25	22	21	15	14	13

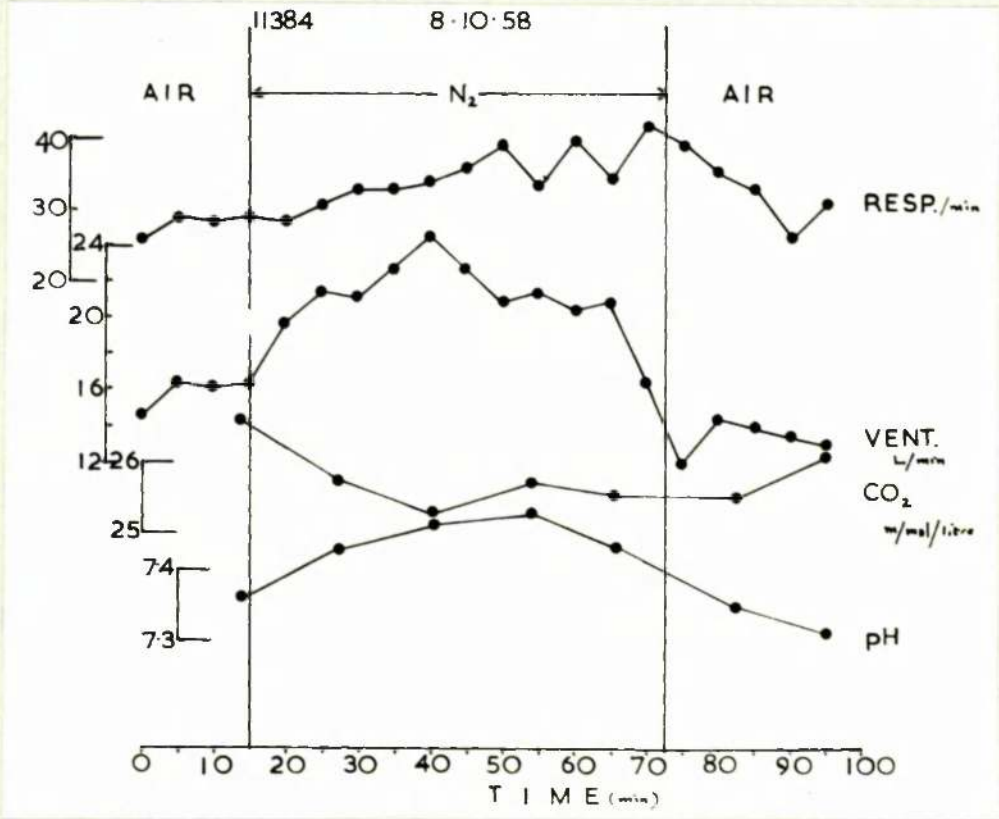

Fig. 58

Effects of anoxia on the ventilation and respiratory rates, the carbon dioxide content and the pH of the plasma of a calf

Table 73

Effects of Anoxia on the Ventilation and Respiratory Rates, the Carbon Dioxide Content and the pH of the Plasma of a Calf

	Air	Inhalation of N_2 mixture (minutes)				Recovery (minutes)	
		12	30	45	55	10	20
pH	7.43	7.52	7.53	7.58	7.57	7.44	7.44
CO_2	30	30.8	30	29.1	28.6	29.1	29.8
Resp	18	24	25	27	27	20	20
Vent	4	7	5	9	10	5	5

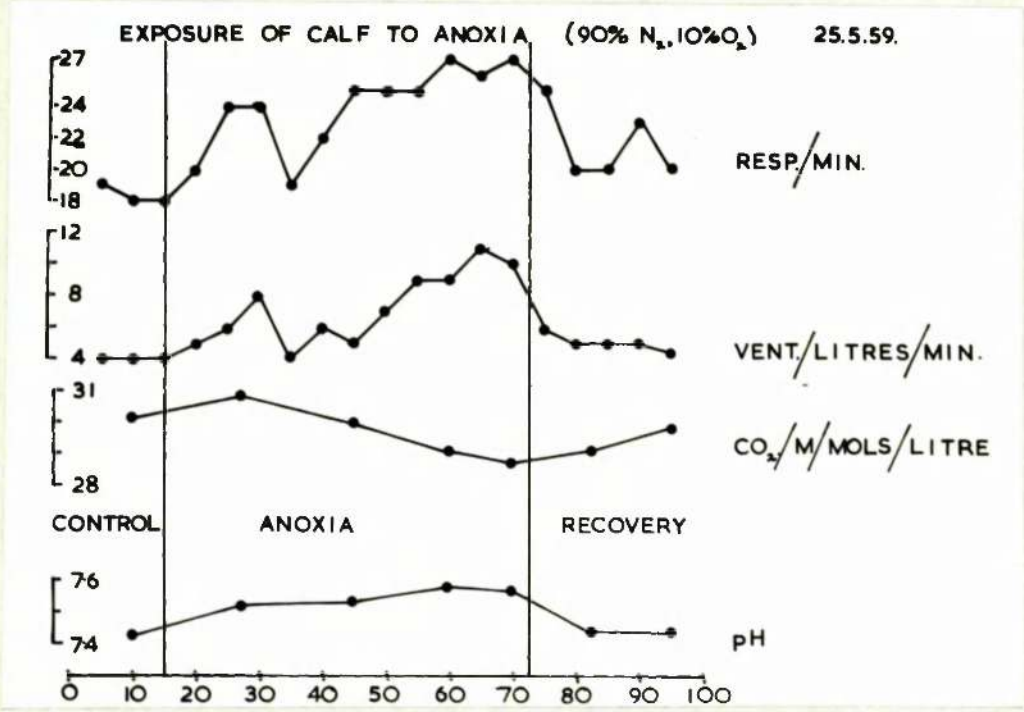

Fig. 52

Effects of anoxia on the ventilation and respiratory rates, the carbon dioxide content and the pH of the plasma of a calf

Both calves responded to the anoxic stimulus with an increase in the respiratory and the ventilation rates. As a result of the increased ventilation additional carbon dioxide was excreted so that the plasma carbon dioxide content fell and the plasma pH rose. It seemed that the lowest plasma carbon dioxide content and the high pH had a less stimulating effect on respiration because the ventilation rate then fell while the

respiratory rate showed greater variation although the oxygen mixture was still being administered. Plasma pH then fell and plasma carbon dioxide content rose. After the administration of the oxygen mixture had ceased the ventilation rate and the respiratory rate continued to fall while the plasma pH also fell and the plasma carbon dioxide content rose.

DISCUSSION
(Part II section 3)

The administration of oxygen at one atmosphere caused a reduction of the ventilation of calves and the greyhounds studied but had little effect on the respiratory rate of the calves. The effect of the hyperventilation of the calves was to cause a rise in the carbon dioxide content and a fall in the pH of the plasma. In normal calves and calves not severely affected with pneumonia the changes produced by oxygen administration were small and when the subject was again made to breathe atmospheric air, a rapid return to the initial values took place.

Experiments by Euler et al. (1939) on cats showed that the chemoreceptors of the carotid glomus of this species discharged at tensions of oxygen found in normal circumstances and that as the oxygen tension was increased the frequency of the impulses decreased. These results suggested that in cats normal breathing reflects a certain amount of chemoreceptor drive due to oxygen lack. Using trained dogs Watt et al. (1943) found that a reduction of ventilation occurred immediately following the inhalation of pure oxygen. This effect was abolished by denervation of the carotid and aortic bodies.

Experiments conducted in man by Dripps and Comroe (1947), Dejours et al. (1957) and by Asmussen and Nielsen (1955) have shown a decrease

in ventilation immediately following the inhalation of pure oxygen. Dalgas and Gemzo (1947), however, noted a secondary rise in ventilation after the first one or two minutes. They attribute this secondary rise to the irritant effect of oxygen. From their determination of arterial pO_2 in man, at rest, breathing room air, and a comparison with the threshold value for chemo-receptor drive found by Mitchell et al. (1955), Asmussen and Nielsen (1956) concluded that with oxygen at the tensions present in blood in man breathing normal air there was stimulation of the chemo-receptors.

Findings in the present series of experiments on cattle of a decrease in ventilation with a fall in plasma pH and a rise in the plasma carbon dioxide content during inhalation of pure oxygen require more extensive investigation. It would not be surprising if cattle showed species differences in their respiratory behaviour compared to man since in addition to these results obtained with hyperoxygenation it has been shown that on a weight basis their pulmonary ventilation is high (Brody, 1960), the packed cell volume of bovine blood is low as is the haemoglobin concentration compared to the human subject (Holman, 1955).

When 100% oxygen was administered to severely pneumonic calves a reduction of ventilation again occurred with greater changes in plasma pH and plasma carbon dioxide content. These calves took longer to return to the initial values when they were again breathing room air. However, the pneumonic calves studied increased their respiratory

efforts in order to remove the increased plasma carbon dioxide content produced by the hypoventilation due to the administration of pure oxygen. It is possible that the administration of pure oxygen for prolonged periods to a severely pneumonic calf making its maximum respiratory efforts might prove deleterious. It has been shown that continual inhalation of pure oxygen at one atmosphere may produce pulmonary edema (Lilly, 1955). The reduction of ventilation might raise the plasma carbon dioxide content to acute levels which the calf could not then get rid of.

The administration of a 95% oxygen and 5% carbon dioxide mixture to normal calves caused an immediate increase in the respiratory rates and pulmonary ventilation. At the same time there was a marked fall in plasma pH and a rise in the plasma carbon dioxide content. The calf mildly affected with pneumonia responded slightly differently in that the increase in ventilation was continued for a longer period during recovery from the administration of 95% oxygen and 5% carbon dioxide. The severely pneumonic calf responded to 95% oxygen and 5% carbon dioxide with a reduction of both pulmonary ventilation and respiratory rate although the plasma pH fell to very low values and the carbon dioxide content rose to very high values. This calf apparently responded to the 95% oxygen content of the gas mixture and was not responsive to the increased carbon dioxide intake until concentrations within the plasma above those normally found in pneumonic had arisen.

The findings in this calf are in agreement with the findings by Boutourline-Young and Whittenberger (1951) that the respiratory centre may in certain circumstances become adapted to chronically high carbon dioxide by losing its responsiveness.

The respiratory rates of normal calves when made hypercapnic were not as high as those observed in pneumonic calves although the plasma carbon dioxide content rose to values observed in pneumonic animals and the plasma pH fell below the values observed in pneumonic animals. An increased carbon dioxide content of the plasma and the rise in pCO_2 could not therefore be the only stimulus to the increased respiratory rate of pneumonic calves.

When calves were exposed to hypoxia by the administration of gas mixtures containing less than 20% oxygen there was at first an increase in both respiratory rates and pulmonary ventilation while the plasma carbon dioxide content fell and the plasma pH rose. Although the hypoxic gas mixture was still being administered the pulmonary ventilation fell. These observations might be explained on the basis of Gray's hypothesis (1950) that the final drive to respiration due to changes in the blood gases is the resultant of a number of factors some stimulant, some depressant. In these calves the fall in ventilation which occurred while the animals were still being rendered anoxic was no doubt due to the resultant depression from the sum of central anoxic depression, central hypocapnic depression, central alkalotic depression, reduction in stimulation of the chemo-receptors from hypocapnic and alkalosis and chemo-receptor stimulation due to hypoxia.

CONCLUSIONS

From the results obtained in these experiments the following conclusions were drawn.

(1) Hyperoxygenation of calves by the administration of 100% oxygen at one atmosphere caused a depression of pulmonary ventilation leading to an elevation of plasma carbon dioxide content and a fall in plasma pH.

(2) These changes were much greater in pneumonic calves than in normal calves.

(3) Hypercapnia caused an immediate increase in respiratory and ventilation rates, a rise in the carbon dioxide content and a fall in the pH of plasma.

(4) Pneumonic animals took longer to recover from the effects of hypercapnia than normal animals.

(5) Acidn caused stimulation of respiratory and ventilation rates of calves which led to a respiratory alkalosis.

DISCUSSION
(Part II)

The study of the disturbance of respiration of cattle caused by general anaesthesia with Fluothane, thiopentone sodium and chloral hydrate showed that there was a depression of pulmonary ventilation which was greatest when Fluothane was the anaesthetic agent. A part of the marked hypoventilation may be due to the concomitant administration of oxygen, which was shown to be responsible for some reduction of ventilation when administered to unanaesthetised cattle, but there is no proof of this. Horses, dogs and sheep also showed a depression of ventilation when anaesthetised with Fluothane, sheep often becoming apneic.

Because of the depression of ventilation, carbon dioxide retention took place so that the plasma carbon dioxide content rose to higher concentrations than those found in normal unanaesthetised animals. As a consequence of the carbon dioxide retention, the increased pCO_2 caused an increase in the H ion concentration, a fall in plasma pH, and a respiratory acidosis was produced. The changes in plasma pH which occurred in general anaesthesia were greater than the variations observed in normal unanaesthetised cattle when the latter were studied over periods from one to six hours. It was noticed that in prolonged general anaesthesia of two to three hours duration the plasma carbon dioxide content did not always continue to rise but stabilised at a high

concentration or fall while the animal was still being maintained in a state of surgical anaesthesia. It was considered that this may have been as a result of a general depression of metabolism due to anaesthesia and hence a decreased carbon dioxide production.

During the anaesthetization of animals with Fluothane and oxygen it was noticed that venous blood became arterial in colour so that muscle was not a feature of this method of anaesthesia provided the circulation was maintained. The rapid recovery from Fluothane anaesthesia meant that the ventilation returned to normal or somewhat above normal quite rapidly and the carbon dioxide content of the plasma fell while the plasma pH rose. The return of plasma pH to the pre-anaesthetic control value before the return of the plasma carbon dioxide content to the pre-anaesthetic value may have been due to renal compensation which was previously inhibited by the anaesthetic agent. It has been shown that general anaesthesia can interfere with renal regulation (Blake, 1957).

Thiopentone sodium and chloral hydrate anaesthesia in cattle produced changes that resembled those produced by Fluothane anaesthesia.

Neonatal calves showed an increase in respiratory rates and pulmonary ventilation, the greater increase occurring in the respiratory rate, so that these calves had a smaller tidal volume and smaller respiration than normal calves. The plasma carbon dioxide concentrations of neonatal calves were higher than those observed in normal calves but lower than the concentration found in anaesthesia and the concentrations

obtained by the administration of 95% oxygen and 5% carbon dioxide to calves. It was noteworthy that with the exception of one muscle, severely ill calf the plasma pH was never below normal. This contrasted with unanesthetized animals. A compensated respiratory acidosis therefore existed in these pneumonic calves.

Hyperoxygenation by the inhalation of pure oxygen at one atmosphere pressure caused a reduction of the ventilation of calves and dogs. In calves it was shown that this reduction of ventilation caused a rise in the plasma carbon dioxide concentration and a fall in the plasma pH. The changes were small in normal calves and more marked in pneumonic calves. There was little change in the respiratory rate of these calves. After removal from the pure oxygen normal calves quickly returned to the pre-administration values of plasma carbon dioxide content and plasma pH, while the pneumonic calves studied were able to increase their respiratory efforts to get rid of the extra carbon dioxide which had built up in their circulation as a result of the hypoventilation caused by pure oxygen inhalation. It was thought that some pneumonic animals may not have been able to do this so that the administration of pure oxygen to them might prove deleterious.

When the effects of the administration of 5% carbon dioxide and 95% oxygen were studied the results obtained in normal animals were as expected in that there was an increase in respiratory rate and pulmonary ventilation and plasma carbon dioxide content, and a fall in plasma pH.

Although the plasma carbon dioxide content rose to values observed in pneumonia, the respiratory rate did not increase to the values observed by Jennett et al. (1957) in pneumonia.

Acute anemia in calves produced by the inhalation of 90% nitrogen and 10% oxygen caused initially an increased respiratory rate and pulmonary ventilation so that a respiratory alkalosis was produced. Ventilation then decreased while this gas mixture was still being administered and as a consequence plasma pH fell and plasma carbon dioxide content rose again. Hypoxia by itself did not act as a marked stimulus to respiration.

The results of these studies on respiratory disturbances in cattle have raised some interesting problems on certain aspects and shed light on other aspects of clinical work. The fact that calves suffering from a severe parasitic pneumonia did not become acidotic is probably the most surprising result obtained. In this connection, too, it is interesting to note that it was not possible to produce as great a degree of tachypnoea by means of artificial hypercapnia or hypoxia as did pneumonia. Further investigation is necessary into the locus for the stimulus for the tachypnoea of parasitic pneumonia.

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THE CARBON DIOXIDE CONTENT AND THE pH OF THE PLASMA OF CATTLE AND THE CHANGES ASSOCIATED WITH DETERMINATIONS OF RESPIRATION

By E. V. Mather

Summary

A study was made of various methods of obtaining blood samples from cattle and a technique evolved whereby arterial blood samples could be obtained from the brachial arteries of cattle of all ages.

Examination was made of the errors liable to arise when determinations were made of the plasma carbon dioxide content and the plasma pH if certain precautions were not taken in the handling of blood samples. It was shown that it was necessary to collect blood samples under a layer of liquid paraffin but this layer must be kept to a minimum thickness to prevent the absorption by it of the carbon dioxide from plasma. Determinations had to be carried out within half an hour of collection of samples in order to prevent enzymic lowering of plasma pH.

Both the Conway method and the manometric method of Van Slyke were

found suitable for the determination of the carbon dioxide content of the plasma of cattle. The mean value for the arterial carbon dioxide content of 159 adult Ayrshire cows was found to be 27.2 ± 2.7 m.moles/litre. No significant differences were found due to seasonal variations, lactational status or pregnancy. The mean values found for the carbon dioxide content of venous and mixed venous plasma were found to be 27.4 ± 1.9 m.moles/litre and 29.2 ± 2.7 m.moles/litre, respectively, and the mean value of the carbon dioxide content of the arterial plasma of 61 calves was found to be 29.8 ± 2.5 m.moles/litre which value is significantly higher than similar determinations in adult cattle. The mean value of 21.12 ± 2.9 m.moles/litre found for the arterial plasma carbon dioxide content of 15 dogs was significantly lower than the mean value found for the arterial plasma carbon dioxide content of cattle.

A colorimetric method for the determination of plasma pH was evolved and with this method, temperature coefficients for pH of blood and plasma of cattle were found to be 0.012 pH units per degree centigrade and 0.009 pH units per degree centigrade. Using these temperature coefficients a mean value of 7.43 ± 0.025 pH units was found for the arterial plasma pH at 38° C. of 159 Ayrshire cows, and 7.39 ± 0.05 pH units for the venous plasma pH of 55 Ayrshire cows. Temperature coefficients were also found of 0.0155 pH units and 0.012 pH units for canine blood and plasma, respectively, and with these temperature coefficients the mean value of 7.43 ± 0.045 pH units was found for the arterial plasma pH at 38° C. of 30 dogs.

Studies were made of diurnal variations in the carbon dioxide content and the pH of the plasma of five cows. No significant variations were found over periods of up to six hours.

Respiratory and ventilation rates were measured in cattle of all ages. It was found that all cattle had respiratory rates between 15 and 35 per minute but the ventilation rate varied with body weight in a logarithmic manner so that a calf of 50 kg. body weight had a ventilation rate of about 25 litres/minute while a cow of 500 kg. body weight had a ventilation rate of 100 litres/minute.

With methods developed and normality defined, studies were made on three types of disturbance of respiration of cattle.

General anaesthetics caused a depression of the pulmonary ventilation of cattle which was greatest when Fluothane was the anaesthetic agent. Horses, dogs and sheep also showed a depression of ventilation when anaesthetized with Fluothane, sheep sometimes becoming apnoic. The plasma carbon dioxide content of anaesthetized animals rose above the levels found in unanaesthetized animals and the plasma pH fell below the normal values so that a respiratory acidosis was produced. Recovery from Fluothane anaesthesia was rapid; the ventilation rate quickly returned to the pre-anaesthetic control values, the plasma carbon dioxide content fell and the plasma pH rose. Plasma pH usually returned to the pre-anaesthetic control value before the plasma carbon dioxide content.

Anaemic calves showed an increase in both respiratory and ventilation rates, the increase in the respiratory rate being greater so that the tidal air fell. The plasma carbon dioxide content of these calves was greater than the plasma carbon dioxide content of normal calves but generally lower than the levels found in anaesthetic. It was noteworthy that with the exception of one anaemic, severely ill calf the plasma pH was never below normal which contrasted with the effects of general anaesthesia. A compensated respiratory acidosis therefore existed in these calves.

Hyperoxygenation caused a reduction of ventilation of calves and dogs. Calves showed an increase in the plasma carbon dioxide content and a fall in plasma pH but these changes rapidly reversed when the calves were again breathing room air. Anaemic calves showed greater changes and took longer to recover.

Hypercapnia by the administration of 95% oxygen and 5% carbon dioxide caused an increase in respiratory and ventilation rates and in plasma carbon dioxide content, and a fall in plasma pH. The plasma carbon dioxide content rose to the values observed in anaemia but the respiratory rate did not increase to that of anaemic animals.

Hypoxia in calves produced by the administration of 90% nitrogen and 10% oxygen caused initially an increase in respiratory and ventilation rates as a consequence of hyperventilation, hypocapnia and alkalosis. Ventilation decreased while the hypoxic gas mixture was still being administered so that the plasma pH fell and the plasma carbon dioxide content rose.

Appendix I

Carbon Dioxide Contents of Venous Plasma Samples
Obtained by Puncture of the Jugular Vein
of Healthy Ambulant Cows in the University Herd

Date of Sampling: April 1954

Sample No.	CO_2 content n.moles/litre	Sample No.	CO_2 content n.moles/litre
1	26.94	15	28.75
2	27.40	16	28.22
3	27.50	17	26.75
4	30.09	18	25.81
5	25.86	19	26.99
6	29.24	20	26.72
7	28.24	21	25.72
8	25.34	22	27.45
9	31.08	23	26.20
10	28.53	24	29.95
11	27.71	25	29.69
12	27.92	26	31.32
13	29.29	27	29.19
14	25.09	28	25.36
		29	28.19

Mean = 27.42 $\pm$ 1.9 n.moles/litre

Appendix IV

Carbon Dioxide Contents of Wood Versus Moisture

Sample No.	CO_2 content m.moles/litre
1	24.5
2	29.1
3	29.6
4	25.5
5	30.1
6	25.0
7	29.4
8	32.0
9	25.0
10	28.5

Mean = 29.2 ± 2.7 m.moles/litre

Appendix III

Arterial Plasma Carbon Dioxide Contents of Healthy Anesthetized Cows in the University Herd

Sampling Date April 1956

Cow	CO_2 content m.moles/litre
Benton	22.3
Brownie	27.1
Poppy	25.1
Nell	26.8
Elsie	27.3
April	27.0
Ina	25.1
Gleize	25.4
Sylvia	27.8
Marie	30.3
Eva	29.9
Queen	27.7
Blanch	30.6
Peggy	26.6
Crumple	30.9
Ruby	28.6
Juno	25.7
Stetson	27.6
Sonn	29.8
Granny	29.3

Mean = 27.6 ± 2.135 m.moles/litre

Sprotline late June 1965

Key	CO_2 content m.moles/litre
Eva	25.5
Keto	25.2
Elsie	25.1
Iggy	24.5
Gwenny	26.9
Diesle	25.9
Violet	25.1
Bobby	25.6
Ruby	27.0
Anne	23.7
Juno	25.9
Dinah	23.6
Quiff	25.7
Queen	23.7
Benton	23.5
Aston	24.3
Ivy	24.7
Poppy	24.6
Susie	23.1

Mean = 25.4 ± 1.9 m.moles/litre

Sampling Date October 1955

Car	HCO_3 content m.moles/litre
Bowden	29.3
Astor	26.5
Granny	28.5
Poppy	28.5
Avon	22.8
Apple	25.3
Eva	30.8
Miele	27.4
Kate	28.5
April	25.0

Mean = 27.5 ± 2.24 m.moles/litre

Sampling Date January 1956

Cow	SO_2 content m.moles/litre
Bobby	25.2
Aston	23.3
Susie	27.1
Avril	25.3
Granny	29.8
Blaire	29.0
Poppy	27.8
Minah	29.3
Dunbar	29.0
Eve	29.7
Arter	23.6
Foggy	25.2
Ivy	31.9
Sylvia	29.0
Miss	22.6
Ina	23.8
Alla	30.2
Jock	29.9
Steven	25.3

Sampling Date January 1956
(contd.)

Day	CO_2 content n.moles/litre
June	24.6
Rory	24.7
Bunny	25.6
Violet	29.0

Mean = 27.5 ± 2.53 n.moles/litre

Sampling Date May 1995

Girl	CO_2 content n.moles/litre
Avril	27.3
Granny	31.2
Hanbon	23.5
Anton	27.7
Ivy	23.6
Poppy	30.3
Aedon	27.6
Rita	24.3
Keko	27.8
Sylvia	30.1
Posy	27.0
Alia	24.0
Strung	24.1
Ruth	23.6
Violet	23.4
June	24.6
Ruby	29.1
Poppy	23.8
Dinah	23.8
Guest	23.6

Mean = 27.0 ± 2.165 n.moles/litre

Sampling Date June 1957

Boy	CO_2 content m.moles/litre
Dawson	27.0
Arter	27.6
Ivy	26.7
Sarah	26.3
Aston	25.2
Miss	25.8
Poole	24.9
Syfell	26.4
Avell	23.1
Clarke	24.0
Stephens	29.0
Eva	26.6
Scribb	24.9
Saddle	24.5
Rushol	26.7
Rom	26.0
Sorrel	25.1
Foxy	23.6
Sandra	27.6
Sloppy	25.9

Mean = 26.2 ± 1.257 m.moles/litre

Reading Date January 1951

Girl	^{40}Ca content m.mol/litre
Megan	27.5
Helena	29.0
Beatan	25.3
Aster	28.2
Sandi	27.2
Avon	25.8
Sanjo	28.0
Rita	26.8
Ann	27.1
Gladys	25.3
Thora	29.2
Tina	30.2
Jane	28.2
Sally	30.7
Jack	29.6
Ruth	30.0
Strang	25.2
Alla	27.7
Sophia	30.5

Mean = 28.2 ± 1.47 m.mol/litre

Examples Data March 2018

Day	CO_2 content n.moles/litre
Denise	25.0
Granny	30.1
Peter	25.7
Susie	27.0
Terry	31.2
Gloria	27.1
Jane	24.0
Sally	26.5

Mean = 27.2 ± 2.725 n.moles/litre

Spreading Data May 1958

Day	CO_2 content m.moles/litre
Martin	23.62
Granado	27.50
Astor	23.75
Sarah	23.62
Arden	24.50
Min	25.87
Terry	30.12
Ina	28.00
Eva	25.87
Sorbic	29.75
Resin	29.75
Sonden	26.25
Shoppy	27.50
June	27.00
Sally	27.75
Jack	26.25
Violet	29.75
Ruth	25.50
Sonny	26.00
Ella	25.50

Mean = 26.9 ± 1.8 m.moles/litre