STUDIES ON THE

CONTROL OF RESPIRATION

A Thesis submitted to the University

of Glasgow in candidature for the degree of

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in the

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BY

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CONTENTS.

Studies on the Control of Respiration.

January 22nd., 1665-66.

"I back presently to the Crowne taverne behind the Exchange, by appointment, and there met the first meeting of Gresham College* since the plague..... But what, among other fine discourse pleased me most, was Sir G. Ent about Respiration; that it is not to this day known, or concluded on among physicians, nor to be done either, how the action is managed by nature, or for what use it is".

Samuel Pepys.

* The Royal Society.

Definitions of Symbols used in the Text.

$\dot{V}$ - Pulmonary ventilation. Expressed in litres per minute, at body temperature, the prevailing atmospheric pressure, and saturated with water vapour. (l/min., BTPS)

pO_2 - Partial pressure or tension of oxygen. Expressed in millimeters of mercury. (mm.Hg.)

pCO_2 - Partial pressure or tension of carbon dioxide. Expressed in millimeters of mercury. (mm.Hg.)

$[H^+]$ - Hydrogen ion concentration. Expressed in millimicromoles per litre. ($m\mu M/l.$)

pCO_2^s - "Setting" value of the partial pressure of carbon dioxide for the controlling system.

MR_{O_2} - Oxygen consumption or metabolic rate as measured by pulmonary gas exchange. Expressed in cubic centimetres per minute, at standard temperature and pressure, dry. (cc./min., STPD)

MR'_{O_2} - Actual oxygen consumption or energy output of the tissues at a given moment.

MR_{CO_2} - Carbon dioxide production as measured by pulmonary gas exchange. Expressed in cubic centimetres per minute, at standard temperature and pressure, dry. (cc./min., STPD)

RQ - Respiratory Quotient $= \dfrac{MR_{CO_2}}{MR_{O_2}}$

X - The exercise stimulus to ventilation.

VE_{O_2} - Ventilation Equivalent for Oxygen

$$= \frac{\text{Ventilation (l/min) x 100}}{\text{Oxygen Consumption (cc./min.)}}$$

VR - Ventilation Ratio $= \frac{\text{Ventilation under treatment}}{\text{Resting ventilation}}$

MRR - Metabolic Rate Ratio $= \frac{MR_{O_2} \text{ under treatment}}{\text{Resting } MR_{O_2}}$

DNP - 2: 4 - dinitrophenol.

P - Probability

r - Correlation coefficient

s - Standard deviation.

BOOK I.

THE CONTROL OF RESPIRATION IN MODERATE EXERCISE.

PART I.

GENERAL INTRODUCTION AND REVIEW OF THE LITERATURE.

Introduction.

Regulation is the essence of physiology. The body has many interconnected and interdependent systems and each must function in an orderly manner to maintain the "constant internal environment". It is the physiologist's role to study the action of the various systems and the study of the regulation of the systems has been an important adjunct to that aim. The regulation of breathing, the circulation, the kidney, water balance, reproduction, to mention but a few, have been, and still are, under examination by the physiologist. In recent years new tools and ideas have enabled the experimental worker to concentrate more and more on the quantitative aspects of physiological regulation, and none more than the respiratory system. The respiratory system is probably the simplest system in the body for quantitative study. The measurement of gas volumes, their composition, and the changes produced by different experimental conditions can be fairly easily and accurately measured. The question of control, however, is not so easily determined, in common with most other systems of the body.

The output of the respiratory pump, the volume of air breathed by the organism in unit time, in other words, the pulmonary ventilation in litres per minute ($\dot{V}$), is the measurable factor, the control of which interests the respiratory physiologist.

Many years before the development of the theory and design of regulator systems and the science of control, it was recognised that a system is usually controlled, at least in some measure, by the level or quality of what it itself produces. Thus, since breathing was considered to be for the provision of oxygen to the body, the earliest theory of respiratory control was that the oxygen level in the arterial blood controls the ventilation in an inverse fashion, (Rosenthal, 1880). This theory holds to a certain extent in a qualitative fashion for the conditions prevailing in anoxia but not for the responses of the respiratory system to other conditions, such as metabolic acidosis, or the inhalation of carbon dioxide.

Haldane & Priestley (1905) suggested that the ventilation is controlled by the carbon dioxide tension (pCO_2) of the arterial blood. They considered that the pCO_2 of the alveolar air was equivalent to that of the arterial blood and found that the pCO_2 of the alveolar air was almost constant at rest at sea level, down a coal mine, and on top of a mountain. They investigated the effects of breathing CO_2 and of work and concluded that under normal conditions the regulation of ventilation depends on the pCO_2 of the arterial blood acting on the respiratory centre. This theory, however, is untenable since although it explains qualitatively the respiratory response to inhalation of

CO_2, it does not hold for any other condition. Haldane & Priestley believed the theory to hold for the ventilatory rise in exercise but since this view was based on analyses of alveolar air samples obtained by faulty methods it was soon discarded.

The factor next to be considered as the factor controlling respiration was the hydrogen ion concentration of the arterial blood. (Winterstein, 1911). Ventilation was considered to be directly related to $[H+]$. Gesell (1925) modified the original conception of this theory and presented it as three precepts;

1). The respiratory centre possesses an acid metabolism of its own.

2). The rate of formation of acid in the centre and the rate of transport of acid from the centre determine the acidity of the centre.

3). Changes in the hydrogen ion concentration of the respiratory centre, rather than that of the blood, constitute the prime factor in respiratory control.

Winterstein's original theory could only hold qualitatively for inhalation of carbon dioxide, metabolic acidosis, or severe exercise but not for anoxia.

Of more importance is the fact that such a theory only holds in a quantitative manner for the response to metabolic acidosis.

The great drawback of Gessell's modification of Winterstein's theory is that it cannot be experimentally tested. More recently (1953) Winterstein has suggested that the hydrogen ion concentration of the cerebro-spinal fluid is the controlling factor for respiration. It seems probable that the $[H^+]$ of the c.s.f. is connected with the control of respiration but quantitative correlation between it and pulmonary ventilation has not been forth-coming.

Lindhard (1915) and Nielsen (1936) proposed that the pCO_2 of the arterial blood passing to the respiratory centre is the "real" stimulus to ventilation and that all other stimuli merely altered the sensitivity of the respiratory centre to CO_2.

Experimental data, however, is not compatable with this Sensitivity Theory and Grodins (1950) has shown theoretically that it does not hold.

Thus over some 60 years it has been shown that the concept of any one chemical stimulus acting alone on the respiratory centre is not able to account for the response of the respiratory system under the various conditions with which it has to deal.

Gray, (1946,1950) has advanced a theory of the control of respiration which deals with the problem in a much more satisfactory manner. His "Multiple Factor Theory" states that the pO_2, pCO_2, and $[H^+]$ in the arterial blood act

together to control the level of pulmonary ventilation ($\dot{V}$). Each agent exerts an independent effect on $\dot{V}$ and the actual level of $\dot{V}$ at any instant is determined by the algebraic sum of the partial effects of all stimuli.

Some modifications of the original theory have been found to be necessary as experimental methods have improved and new data has been forthcoming. It is probable for example, that it is "tissue" pCO_2 or venous pCO_2 and not the arterial blood level which is important. The quantitative aspect of pO_2 is probably the weakest point of the original theory but new experimental data is becoming available which will enable this to be determined. The basic principles of the theory as stated above, however, have only been further supported in recent years. The steady state responses of pulmonary ventilation to CO_2 inhalation, arterial anoxaemia, and disturbances in acid-base balance are successfully accounted for by the multiple factor theory, not only qualitatively but quantitatively.

This modern theory of respiratory control will be used to outline a possible respiratory regulator. The theoretical behaviour of this model will then be discussed and used as a basis for a critical analysis of the literature in order to ascertain the present state of knowledge on the subject of the control of respiration in exercise.

Theoretical Considerations and Review of the Literature.

The Respiratory Chemostat.

I. The Basic System.

The Multiple Factor Theory states that the chemical stimuli pCO_2, pO_2, and $[H^+]$ exert independent effects on $\dot{V}$, the net effect resulting from an algebraic summation of their partial effects. Although each agent has an independent effect on $\dot{V}$, their arterial levels are interdependent and not only do the levels of the chemical agents regulate ventilation, but the ventilation in turn regulates the levels of the agents in the arterial blood. Thus the theory visualises the respiratory control system as a "chemostat" which, in form, is an example of a feedback regulator.

At this point it is necessary to specify certain conditions before outlining the system further. viz.

1). In the range of moderate exercise to be considered changes in pO_2 may be neglected.

2). No metabolic disturbances in acid-base balance occur in the range of exercise considered.

3). The subjects work at sea-level, breathing air.

4). Only steady state conditions are considered.

These conditions enable a simple chemostat to be outlined. This is shown in figure 1.

The system may be described in terms of its two components,

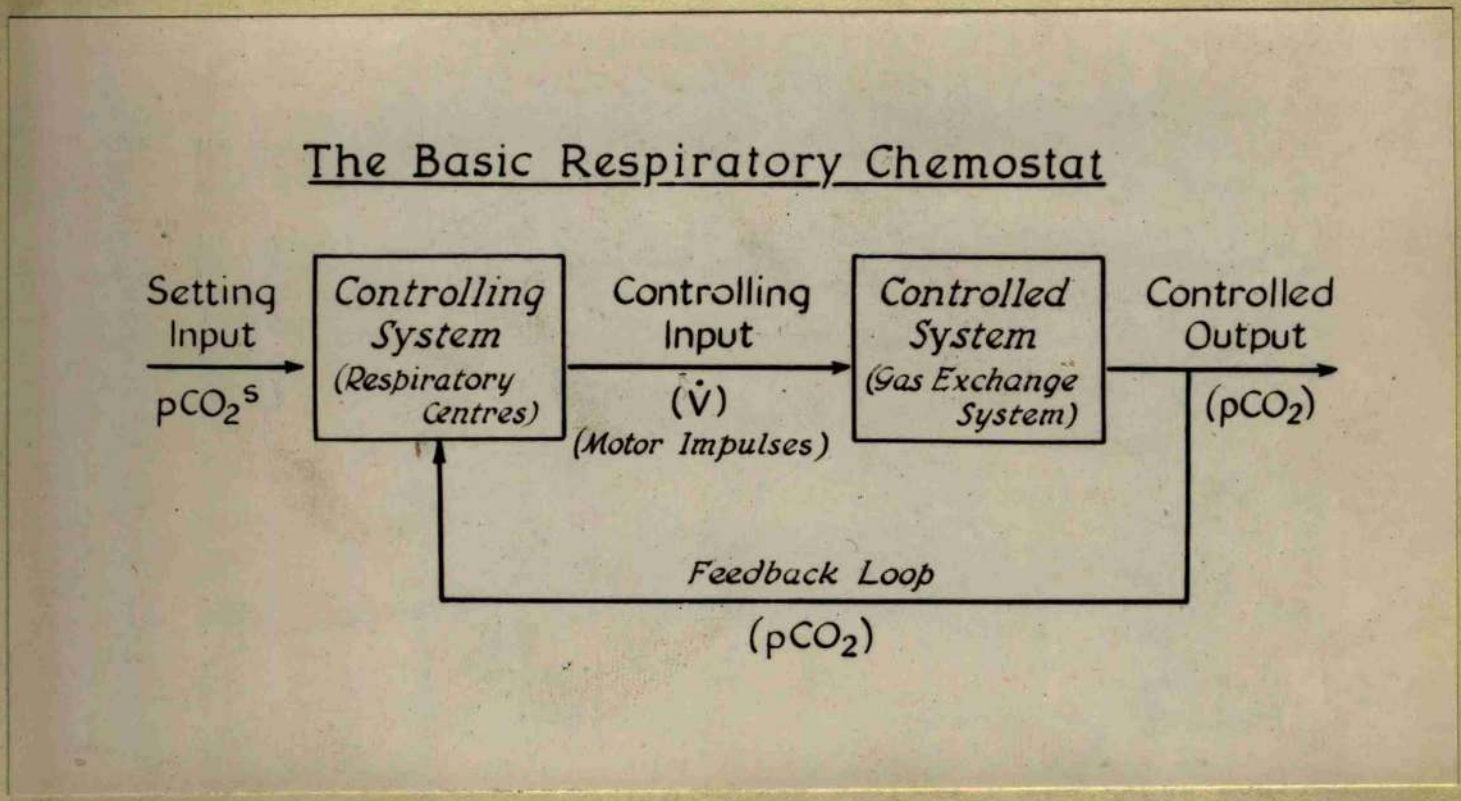

The Basic Respiratory Chemostat
Setting Input
pCO_2^s
Controlling System (Respiratory Centres)
Controlling Input
$(\dot{V})$
(Motor Impulses)
Controlled System (Gas Exchange System)
Controlled Output
(pCO_2)
Feedback Loop
(pCO_2)

Figure 1.

the Controlling and Controlled Systems. Each component system has an input and an output, thus an equation may be assigned to each system which will define its operation by expressing the output as a function of the input.

The controlled system (the gas exchange system) has $\dot{V}$ as its input, in terms of motor impulses from the respiratory centre, and its output consists of the arterial pCO_2, pO_2, and $[H^+]$ levels. The simplifying conditions made above enable the pO_2 to be neglected and the $[H^+]$ to be expressed in terms of pCO_2. Thus the output of the Controlled System may be considered to be pCO_2, and the equation of the system is:

$$pCO_2 = \frac{K.MR_{O_2}.RQ}{\dot{V}} \qquad \ldots\ldots\ldots (1).$$

since pCO_2 is inversely proportional to $\dot{V}$ and $MR_{O_2}.RQ = MR_{CO_2}$.

The controlling system (the respiratory centre) has pulmonary ventilation ($\dot{V}$) as its output. The input consists of the actual level of arterial pCO_2 reaching it via the feed-back loop and also of pCO_2^s, the "setting" of the regulator. Thus the operating input is the difference between the desired level of arterial pCO_2 (pCO_2^s) and the actual level of pCO_2. This difference may be called the "error". The controlling system equation may then be written:

$$\dot{V} = a(pCO_2 - pCO_2^s) + b \qquad \ldots\ldots\ldots (2).$$

If equations (1) and (2) be combined the result is an equation describing the operation of the complete, closed

loop regulator,

$$\dot{V} = a\left(\frac{K.MR_{O_2}.RQ}{\dot{V}} - pCO_2^s\right) + b \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3).$$

which is a quadratic equation in $\dot{V}$.

Before discussing the operation of this system in exercise it is necessary to distinguish between two methods by which $\dot{V}$ may be altered or "forced" in the operation of the system. If MR_{O_2} were to be increased then pCO_2 would rise in accordance with equation (1). This pCO_2 would travel via the feedback loop to the controlling system to increase $\dot{V}$ as described by equation (2). The increase in MR_{O_2} thus causes a rise in $\dot{V}$ in an indirect manner, in that it acts first via the controlled system on pCO_2, and the latter in turn increases $\dot{V}$ via the feedback loop. This type of forcing depending on an initial operation on pCO_2 through the controlled system will be referred to as <u>secondary forcing</u>. The term <u>primary forcing</u> will be used to designate a change in $\dot{V}$ brought about by a method not involving a preliminary effect on pCO_2 through the controlled system. As the system is set up at present, there are only two ways in which primary forcing of $\dot{V}$ may be brought about:

1). By injecting H_2CO_3 into the arterial blood entering the respiratory centre.

2). By changing pCO_2, the chemostat setting.

The response of this system in exercise, i.e. an increase in MR_{O_2} (gas exchange) would be as follows.

1). The increase of MR_{O_2} forces pCO_2 through equation (1). This pCO_2 operating via the feedback loop.

2). Forces $\dot{V}$ through equation (2). In other words $\dot{V}$ undergoes secondary forcing by the increase in MR_{O_2}.

The net result is an increase in $\dot{V}$ accompanied by a rise in pCO_2, i.e. a steady state error in pCO_2, and a fall in VE_{O_2} since $\dot{V}$ rises relatively more than does MR_{O_2}. Such behaviour is not in accordance with observed results in exercise. (Nielsen, 1936; Asmussen & Nielsen, 1946.)

Since the simple system described does not function as the real system has been observed to function, it is necessary to introduce a new quantity to operate on $\dot{V}$. This new quantity is termed the "exercise stimulus". (Grodins, 1950.)

II. The "Exercise Stimulus".

Certain postulates are made about the exercise stimulus, X, before discussing the respiratory control system as modified by the introduction of X.

1). The exercise stimulus has a primary forcing action on $\dot{V}$ through the controlling system and does not operate via the feedback loop.

2). The partial effect of X on ventilation is linear

$$\text{i.e.} \quad \dot{V}_x = kX \qquad \ldots\ldots\ldots\ldots (4)$$

3). The magnitude of X is normally directly proportional to the metabolic rate, as measured by pulmonary gas exchange (MR_{O_2}). Pulmonary gas exchange, in the steady state, serves

as a convenient measure of the total energy output of the working tissues ($MR^{1}_{O_2}$). Although normally MR_{O_2} is equal to $MR^{1}_{O_2}$, under certain experimental or abnormal physiological conditions this equality does not necessarily hold true.

Therefore $X = k^{1}MR^{1}_{O_2}$ . . . . . (5)

or, from eqn.(4) $V_x = K\,MR^{1}_{O_2}$ . . . . . (6)

4). The exercise stimulus acts in an <u>additive</u> manner with pCO_2 and not by altering the sensitivity of the respiratory centre to one or all of the chemical stimuli. (Nielsen, 1936.)

Grodins (1950) shows that an exercise stimulus of the magnitude postulated can account for the direct proportionality of ventilation and oxygen consumption observed (Graph 1) whether acting as an additive stimulus or as a stimulus altering sensitivity. Existing data on the effects on ventilation of CO_2 inhalation and of metabolic acidosis during exercise can, however, only be explained by the additive hypothesis.

It is not known by what pathway the exercise stimulus reaches the controlling system (the respiratory centre) nor where it originates. The possible pathways may be

(i) Humoral

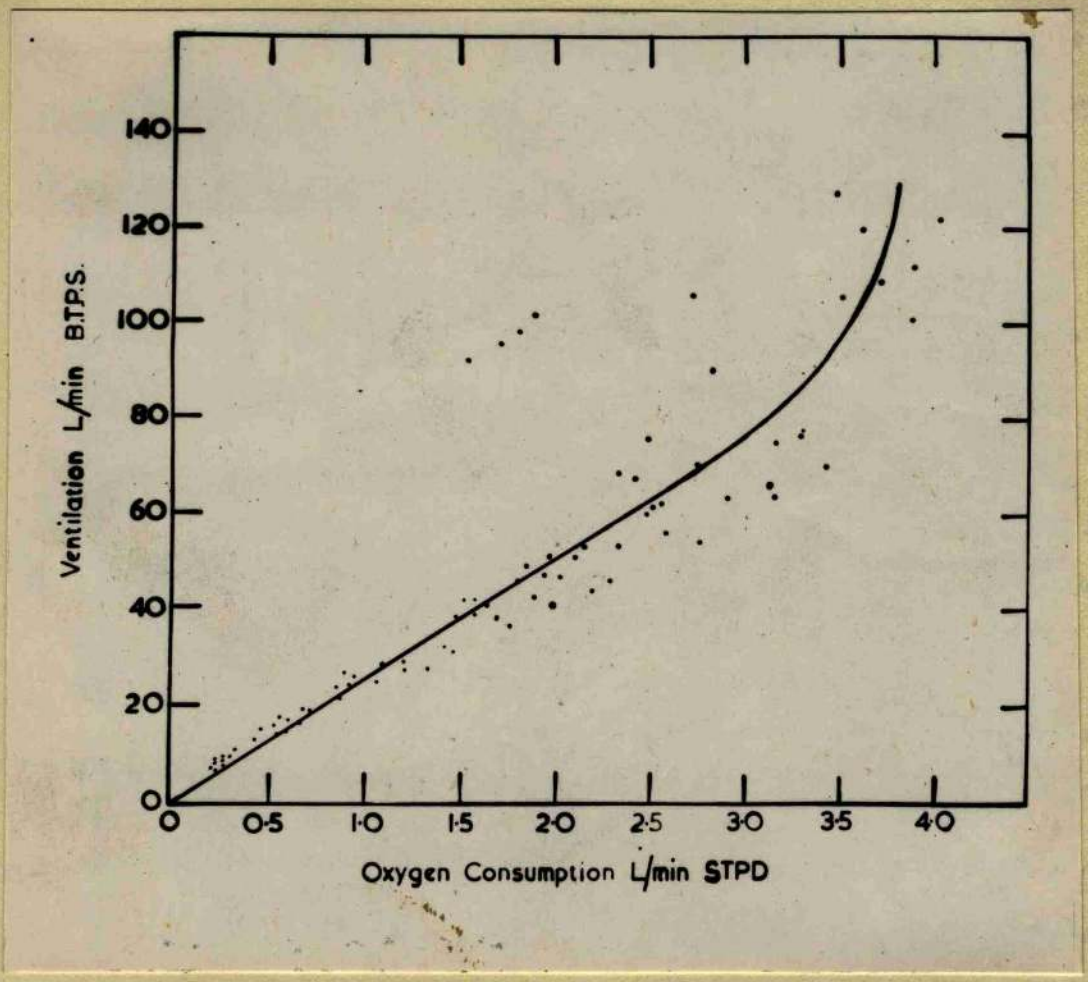
(ii) Neural

and the possible sites of origin are

(i) Central (brain)

(ii) Peripheral (working muscles)

(iii) Other peripheral sites


Ventilation L/min BTPS.
140
120
100
80
60
40
20
0
0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
4·0
Oxygen Consumption L/min STPD

Various experimental procedures have been used to attempt a differentiation of the above possibilities. Before discussing these various methods it is necessary to examine the responses of the model system as modified by the addition of the exercise stimulus X.

Figure 2 represents the modified ventilatory control system, now including the exercise stimulus, X. The following equations describe its component parts.

The controlling system:

$$\dot{V} = a\,(pCO_2 - pCO_2^s) + bX - c \quad \ldots\ldots\ldots \quad (7)$$

The controlled system:

$$pCO_2 = \frac{K\,MR_{O_2}\,RQ}{\dot{V}} \quad \ldots\ldots\ldots\ldots \quad (1)$$

The operation of the modified system in exercise would be as follows.

$\dot{V}$ may be forced in two ways (i) Primary forcing by $X = MR^1_{O_2}$ as described by equation (7)

(ii) Secondary forcing by MR_{O_2} via the feedback loop as described by equation (1).

Normally $MR^1_{O_2} = MR_{O_2}$ and then $\dot{V}$ is directly proportional to MR_{O_2} ($\dot{V}_x = kMR_{O_2}$). Thus the result is a rise in ventilation directly proportional to the rise in MR_{O_2}, pCO_2 and VE_{O_2} remaining constant. This behaviour of the system agrees with observations on the response of the real system in exercise.

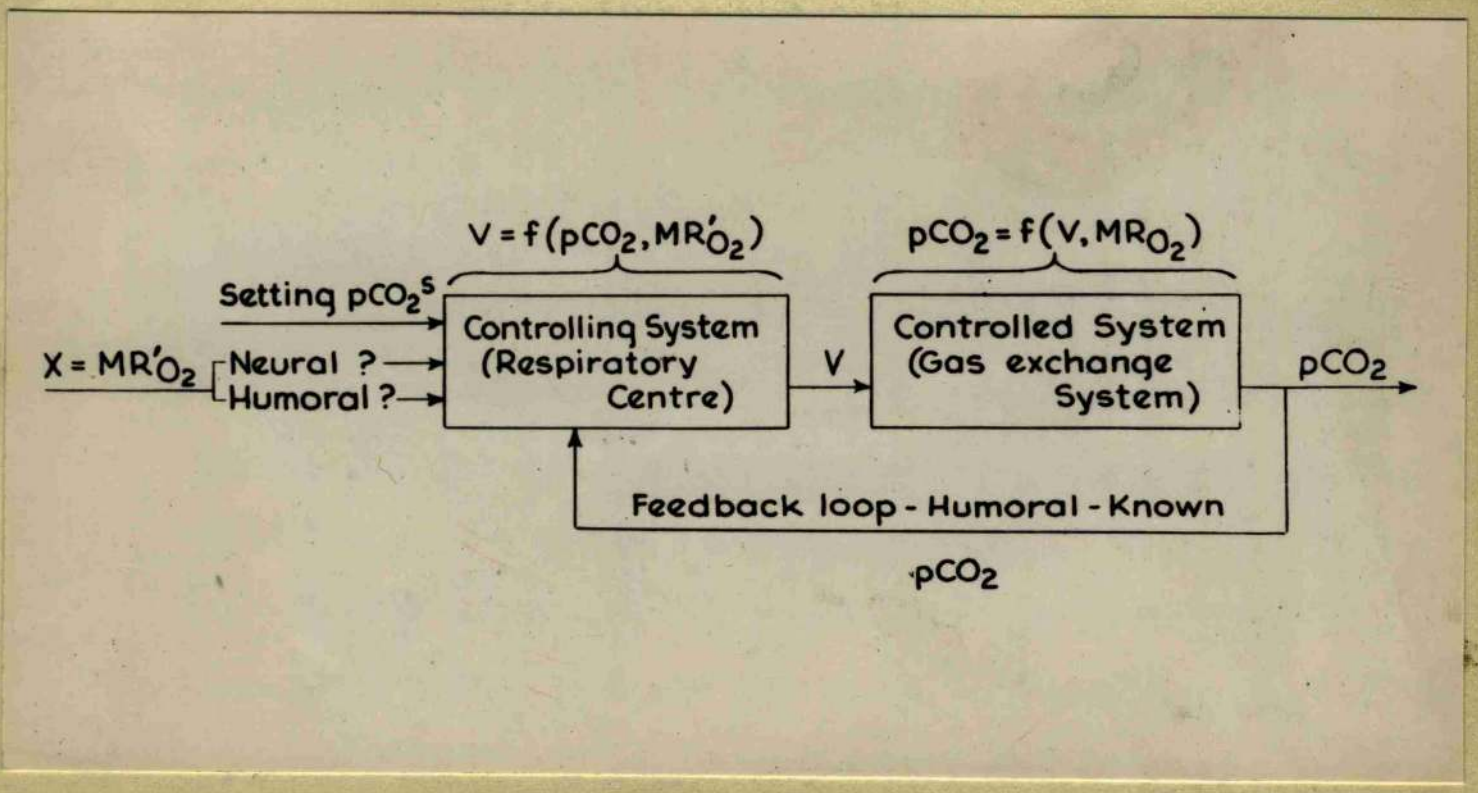


Figure 2. The Modified Respiratory Chemostat.

Site of Origin and Pathway of the Exercise Stimulus.

The concept of an exercise stimulus acting as an additional stimulus to ventilation impinging on the respiratory centre could be satisfied by three means:

1). "Spread" of excitation from the cortical motor areas acting on the respiratory centre.

2). Stimulation of the centre by neural stimuli originating peripherally.

3). Stimulation of the centre by a chemical substance, released from the working muscles, transmitted humorally.

Krogh & Lindhard (1913; 1920) claimed that cortical irradiation is the mechanism involved and concluded that such irradiation acts by altering the excitability of the respiratory centre to one or all of the chemical stimuli. As has already been noted, analysis of existing data has shown that the exercise stimulus probably acts in an additive fashion and not by alteration of the respiratory centre excitability. An experimental procedure for investigating the theory of irradiation of the respiratory centre by the motor cortex would be to compare the ventilatory response to voluntary exercise with that observed during electrically induced exercise. If the assumption is made that there is no abnormal sensory stimulation produced by the electrical stimulation then the system (Fig. 2) should act as follows:

1). If cortical irradiation is the exercise stimulus

a) In induced exercise: $\dot{V}$ is secondarily forced by an increase in MR_{O_2} via pCO_2 and the feedback loop only, since X has been eliminated.

b) A lag in time between the start of exercise and an increase in $\dot{V}$ will be found.

c) $\dot{V}$ will rise less than MR_{O_2}, proportionately, giving a rise in pCO_2 i.e. a steady state error in pCO_2 will be produced.

2). If cortical irradiation is <u>not</u> the exercise stimulus.

a) The ventilatory response in electrically induced exercise will be the same as that in voluntary exercise.

b) $\dot{V}$ and MR_{O_2} will rise in direct proportion and there will be no steady state error in pCO_2.

Krogh & Lindhard (1917) found an immediate increase in ventilation both in voluntary and in electrically induced work. They noted, however, during the induced work, a much higher ventilation than the increase in oxygen consumption would warrant. This finding, together with very irregular results and reported subjective sensations with the induced work, indicates that abnormal sensory stimulation was being produced. No conclusions can therefore be drawn from the results reported by these authors as to the identification or otherwise of cortical irradiation with the postulated exercise stimulus.

Assmusson, Nielsen & Wieth-Pedersen (1943) performed similar

experiments and reported 1) That the ventilatory response to electrically induced exercise was identical to that in voluntary exercise.

2) That the alveolar pCO_2 remained constant, i.e. there was no steady state error in pCO_2.

Kao and Ray (1954) reported the respiratory and circulatory responses of anaesthetised dogs to electrically induced exercise. They found that electrical stimulation of the hind limbs for 15 minute periods resulted in an increase of ventilation and also oxygen consumption. Ventilation remained directly proportional to oxygen consumption, VE_{O_2} remaining constant.

Kao, Schlig and Brooks (1955) give results of experiments on decerebrate dogs. Electrically induced work once again produced a rise in ventilation directly proportional to the increase in oxygen consumption. They also report that the sensitivity of the respiratory centres to exercise in decerebrate dogs is not significantly different to that in unanaesthatized, trained dogs as reported by Slowtzoff (1903) and Zuntz (1897).

The results of all these workers are in accordance with the predicted response of the system if cortical irradiation were <u>not</u> the exercise stimulus. The reports of Kao and his co-workers give strong evidence for the view that the exercise stimulus does not originate in the cortex but in the periphery.

If, on the basis of the forementioned evidence, it is assumed that the exercise stimulus originates peripherally in, or close to, the working muscles then the problem is to identify

the pathway followed by the exercise stimulus from the periphery to the respiratory centre.

Several types of experimental procedure have been used in attempts to identify the pathway of the exercise stimulus. Vascular occlusion of an exercising extremity is one such type. Nerve section, either of the spinal cord or portions thereof, is a second. Passive movement of the extremities is still a third type of procedure which has been used. These three types have also been used in conjunction, as well as separately, and the following outline will be used for their review:

I. Vascular occlusion experiments.
   A. With voluntary work.
   B. With electrically induced work.
II. Spinal cord section experiments.
III. Passive movement experiments.
IV. Other experimental work.

Before reviewing the literature on these experiments the operation of the modified control system (Fig. 2) under the above conditions will be considered. It is assumed that X, the exercise stimulus, originates peripherally.

I. Vascular occlusion

A. Voluntary work

1. On exercise with the circulation intact.

$\dot{V}$ will undergo primary forcing by X and secondary forcing by MR_{O_2} via the feedback loop. The steady state result will be a rise in $\dot{V}$ directly proportional to MR_{O_2} with

pCO_2 and VE_{O_2} remaining constant.

2. Vascular occlusion of the extremity at rest.

Assuming that no abnormal stimuli are produced by the process of occlusion then a fall in MR_{O_2} by secondary forcing causes a fall in $\dot{V}$.

If X travels by neural pathway and is in operation at rest then the reduction in $\dot{V}$ will not be proportional to the fall in MR, therefore VE_{O_2} will rise.

If X travels by humoral pathway then $\dot{V}$ and MR_{O_2} fall in proportion and VE_{O_2} remains constant.

3. Vascular occlusion of the extremity with exercise.

Assuming 1) the exercise is begun after occlusion of the circulation.

2) no abnormal stimuli are produced by the occlusion.

If X travels by neural pathway then $\dot{V}$ undergoes primary forcing. The rise in $\dot{V}$ reduces pCO_2 which in turn tends to reduce $\dot{V}$. The net result is a rise in $\dot{V}$ and VE_{O_2} and a steady state reduction in pCO_2.

If X travels by humoral pathway no change in $\dot{V}$, VE_{O_2}, or pCO_2 will occur.

B. Electrically induced work with vascular occlusion.

1. With the nerves to the extremity intact.

Assuming that neither the electrical stimulation nor the vascular occlusion produces any abnormal stimuli then the response of the system will be the same as

in voluntary work (A above).

2. With the nerves to the extremity cut.

Assuming no abnormal stimuli are produced by the procedure. If X travels by neural pathway then $\dot{V}$ will undergo secondary forcing giving a net result of an increase in $\dot{V}$, fall in VE_{O_2} and a steady state increase in pCO_2 (the basic system response).

If X travels by humoral means the response will be as in the normal subject (A above).

If vascular occlusion is instituted before inducing exercise then, whether X travels by neural or humoral means, no change in MR_{O_2}, pCO_2, $\dot{V}$, or VE_{O_2} will occur.

II. Spinal section.

The responses of the system in this condition would be essentially the same as those described above for electrically induced work with the nerves from the extremities cut.

III. Passive movement.

In passive movement experiments it is assumed that there is no increase in MR_{O_2} in response to the movement.

If X is elicited by passive movement of an extremity then $\dot{V}$ undergoes primary forcing, pCO_2 is thereby reduced which tends to reduce $\dot{V}$. The net result in the steady state is an increase in $\dot{V}$ and VE_{O_2}, since MR_{O_2} is assumed not to change, and a fall in pCO_2.

If X is <u>not</u> elicited by passive movement then no change in $\dot{V}$, VE_{O_2}, or pCO_2 will result.

Having examined the theoretical responses of the respiratory control system under various conditions it is now possible to review and evaluate the experimental work done by other workers in attempts to elucidate the pathway of the exercise stimulus.

I. Vascular Occlusion Experiments.

A. With Voluntary Work.

Harrison, Harrison, Calhoun & Marsh (1932) did a series of experiments in which three cardiac patients performed voluntary exercise, viz. rapidly opening and closing the hands or rotating the feet. Respiration was recorded by means of a Benedict-Roth Spirometer before, during, and after such exercise. The exercise was then repeated with sphygmomanometer cuffs, inflated to a pressure of 200 mm. of mercury, placed around the proximal portions of the extremities. The results of these experiments are reproduced in Table 1.

Table 1.

Ventilatory Response in Cardiac Patients to Mild Exercise (Movement of Feet and Hands).

Ventilation in litres per minute. Harrison et al. (1932).

Subject	Control	Exercise with Circulation Intact	Control	Exercise with Vascular Occlusion
1	8.05	9.21	8.29	10.15
2	6.66	9.16	8.12	8.74
3	7.90	10.80	7.48	10.80
Mean	7.54	9.72	7.96	9.83
		t = 4.18		t = 2.48
		P lies between 0.1 and 0.2		P lies between 0.2 and 0.3

Statistical analysis of these results was not done by the authors and on performing such an analysis it was found that

the mean rise in ventilation produced by the exercise, either with or without occlusion of the blood supply, is not significant. The experiments were also carried out on four normal subjects. The results of this series are given in Table 2.

Table 2.

Ventilatory Response in Normal Subjects to Mild Exercise (Movement of Feet and Hands).

Ventilation in litres per minute. Harrison et al. (1932).

Subject	Control	Exercise with Circulation Intact	Control	Exercise with Vascular Occlusion
1	7.70	12.06	7.70	13.20
2	6.85	18.40	6.49	15.32
3	7.24	8.73	8.32	8.52
4	7.56	12.30	8.36	14.10
Mean	7.34	12.87	7.71	12.79
		t = 2.60		t = 2.83
		P lies between 0.05 and 0.1		P lies between 0.05 and 0.1

Statistical analysis of these results also shows an insignificant elevation of ventilation. It would seem unlikely that there would be any difference in a neural pathway from the exercising muscles between cardiac patients and normal subjects. If both the cardiac patients and the normal subjects are amassed as one group and a paired comparison made of the resulting set of data, then it is found that the pulmonary ventilation is significantly raised by the exercise, both when the circulation is intact and when vascular occlusion has been effected. (Table

3 and Graph 2).

Table 3.

Ventilatory Response to Mild Exercise.(Movements of Feet & Hands).

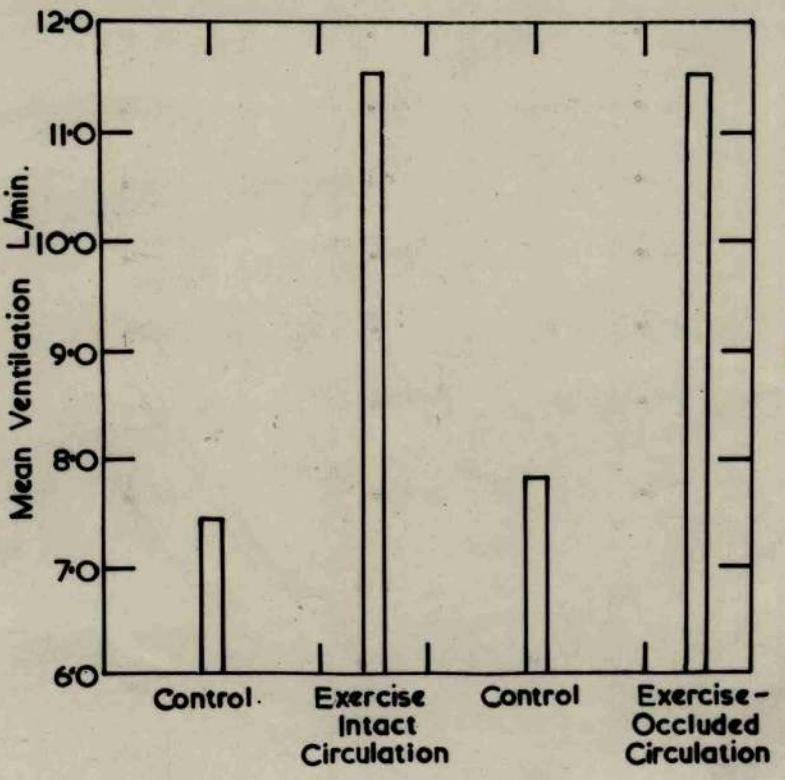
Ventilation in litres per minute. Harrison et al. (1932).

Subject	Control	Exercise with Circulation Intact	Control	Exercise with Vascular Occlusion
1	8.05	9.21	8.29	10.15
2	6.66	9.16	8.12	8.74
3	7.90	10.80	7.48	10.80
4	7.70	12.06	7.70	13.20
5	6.85	18.40	6.49	15.32
6	7.24	8.73	8.32	8.52
7	7.56	12.30	8.36	14.10
Mean	7.42	11.52	7.82	11.55
		t = 3.06		t = 3.14
		P lies between 0.02 and 0.05		P lies between 0.02 and 0.05

The difference in elevation of the ventilation between one procedure and the other is not significantly different, indicating that under the experimental conditions the stimulus to ventilation arising from the limbs was of the same order of magnitude whether vascular occlusion had been effected or not. Such an indication only partially fulfils the previously described theoretical actions of the control system. Since the experimental conditions were not fully described, e.g. duration of exercise, span of recording time, oxygen

Graph 2 .

Ventilation at Rest and during Exercise of the Extremities with and without Vascular Occlusion. (Harrison et al, 1932)



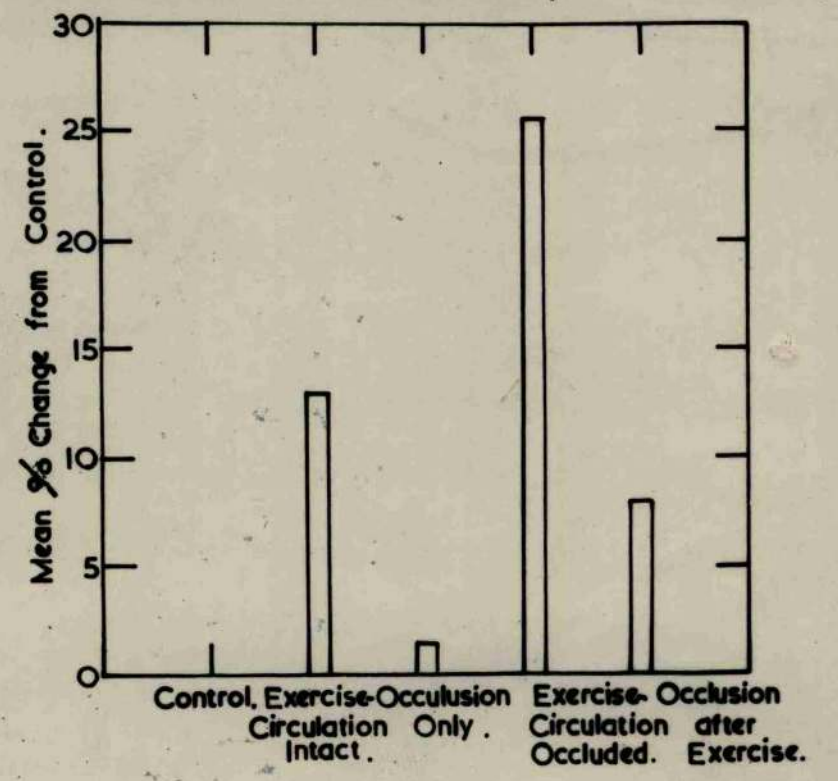
consumption, or alveolar pCO_2, it is impossible to compare the reported results directly with the theoretical response. There is some indication that the experimental procedures produced a stimulus to ventilation but whether this stimulus arose in the working limb, was identical in ischemia and with normal circulation, or was physiological or abnormally produced cannot be said.

Comroe and Schmidt (1943) also carried out some experimental work on pulmonary ventilation changes with and without vascular occlusion of an exercising limb. The right forearm and hand were exercised by flexing the fingers once per second, raising 1360 gm. 5 - 7 cm. The blood flow to the arm could be obstructed by a sphygmomanometer cuff on the upper arm. The experiment was divided into five two minute intervals

1) Resting control
2) Exercise with the circulation intact
3) Occlusion of the circulation in the right arm
4) Exercise with the circulation occluded
5) Cessation of exercise with the circulation occluded.

The ventilation was recorded by means of a spirometer throughout the experimental periods and the subjective sensations of the subjects were also noted. In the experiments where vascular occlusion was instituted the cuff was inflated before the recording of ventilation was begun. It is also noteworthy that each procedure noted above was a separate procedure, the subject returning to the normal resting state after each, with

Graph 3
Changes in Ventilation on Exercise
with and without Vascular Occlusion (Comroe et al., 1943.)



the exception of parts 4 and 5 which were concurrent. The changes in ventilation were all referred to the control level and the results are presented as percentage changes from the control which makes interpretation of the data difficult. Statistical analysis of the data, shown in Table 4 and Graph 3, has been carried out using the paired comparison method. Since absolute values for the ventilation are not given the relative changes are treated as absolute differences from the control level. As may be seen in Table 4, Comroe et al. found a significant rise in ventilation on mild exercise with or without occlusion and also if the occlusion of the blood supply to the exercising limb was continued after cessation of exercise. On the circulation to an arm being blocked, ventilation was reduced in some cases, raised in others, and showed no change in the remainder. The mean result was not significantly different from zero change but the numerical result was positive. In recording subjective sensations there was noted, in the majority of cases, tingling or numbness in the ischemic arm, raising the possibility that there was stimulation of ventilation via pain or kinesthetic stimuli. It would appear, therefore that some of the increase in ventilation which occurred on exercising the ischemic limb was due to stimuli other than any possible "exercise stimulus", probably similar to and including those stimuli giving rise to the subjective sensatinns noted. That this is almost certainly true is borne out by the finding that the ventilation was still

Table 4.

Changes in ventilation on exercise with and without vascular occlusion. (Comroe et al., 1943)

Ventilation expressed as percentage change from control level.

Experimental Procedures.

1 - Change from control to exercise

2 - " " " " ischemia

3 - " " " " exercise with ischemia

4 - " " " " occlusion after exercise

Subject	Ventilation Experimental Procedures			
	1	2	3	4
1. Mean of 3	+ 4	- 4	+13	+17
2. Mean of 6	+28.3	-16	+36.6	+27.2
3. Mean of 3	- 3.3	- 4.6	+24.6	+18.3
4. Mean of 2	+38	+13	+58	+13
5. Mean of 2	+15	+12.5	+ 3.5	+ 4.5
6.	0	0	+33	+ 7
7.	- 8	0	+13	+ 3
8.	+10	+12	+31	0
9.	+20	0	+28	0
10.	+11	0	+ 7	+ 3
11.	+27	+ 2	+35	- 4
Mean	+12.9	+ 1.35	+25.7	+ 8.0
t	2.97	0.52	5.34	2.80
P	0.01-0.02	0.6-0.7	0.001	0.01-0.02

significantly higher than the control level on maintaining the vascular occlusion after the exercise had ceased. Although the mean elevation of ventilation was only 8% over the control, it should be noted that the net ventilation in this condition is the result of the abnormal stimuli tending to raise ventilation and a respiratory alkalosis, produced by hyperventilation during the exercise, which would tend to depress ventilation. The authors themselves comment that the greater ventilatory response on exercising the ischemic limb might be due to either a specific reflex initiated by chemical substances, whose escape was prevented by the cuff, acting locally in the muscles, or to a non-specific pain-type stimulus. They add that they consider the latter explanation to be the case.

The level of work actually performed by the subjects was really quite low, amounting to only about 5 kg. metres/min. Such a low level of work does not produce big enough changes in the ventilation for any adequate conclusions to be drawn. Another point of importance is that the length of the experimental periods (two minutes) was too short to allow the subjects to reach a steady state of ventilation. Thus all the results have really been obtained in the transient, which makes interpretation almost impossible. Taking all these factors into consideration this series of experiments can hardly be said to either confirm or deny any postulate as to the pathway of the exercise stimulus.

Assmussen and Nielsen (1948) report some observations on young medical students working on a modified bicycle ergometer. Ventilation was recorded using a dry gas meter and the ventilation per minute was calculated every 0.3 minutes. They found that ventilation increases immediately, on commencing work, as did Krogh et al. (1913). The subjects did not know beforehand what grade of work they were to perform and it was found that ventilation increased more rapidly in heavy work than light work. The authors use a slightly different method than before described for their experiments on work with occlusion of the circulation. The subjects started exercising and reached a steady state of work before the pressure cuffs were applied. This means that the ventilatory response to exercise with the circulation occluded must be compared to exercise with the circulation intact and not to the resting control level as heretofore. Before examining the results obtained by Asmussen et al., it is necessary to consider the response of our modified model respiratory control system under these conditions.

If X is assumed to travel neurally

1) MR_{O_2} will fall

2) pCO_2 will fall

3) $\dot{V}$ (by secondary forcing) will fall, but not as much as MR_{O_2} since X is still operating, therefore VE_{O_2} will rise.

If X is assumed to travel humorally

MR_{O_2} and $\dot{V}$ will fall in proportion, VE_{O_2} and pCO_2 remaining constant.

In the work being reviewed when, during the steady state of work, the circulation to the legs was cut off, oxygen uptake and CO_2 production declined sharply but the ventilation remained at the same level. This latter finding is not in agreement with the predicted response of the system outlined above, assuming either humoral or neural transmission of the exercise stimulus.

Assmussen, Christensen & Nielsen (1943) also conducted a series of experiments where the subjects exercised on a bicycle ergometer. Metabolism and ventilation were determined using a Douglas bag. All determinations were done after 15 minutes of work, in the steady state. Immediately after taking readings while exercising with the circulation intact the pressure cuffs on the upper thighs were inflated, occluding the circulation to the working muscles. Ventilation was recorded for from five minutes for light work to one minute for heavy work. The same outline of experiment was also used with the subject at rest in an attempt to differentiate between the effects of the occlusion and occlusion plus exercise. The results of experiments at rest are shown in Table 5. It may be noted that on occlusion of the circulation to the legs both $\dot{V}$ and MR_{O_2} fell and the VE_{O_2} remained constant. Such a result is in accordance with the predicted response of the modified

control system, assuming that X is acting at rest and that it travels by a humoral pathway. If X travels neurally but is not acting at rest these results would also fit in with the response expected of the system.

Table 5.

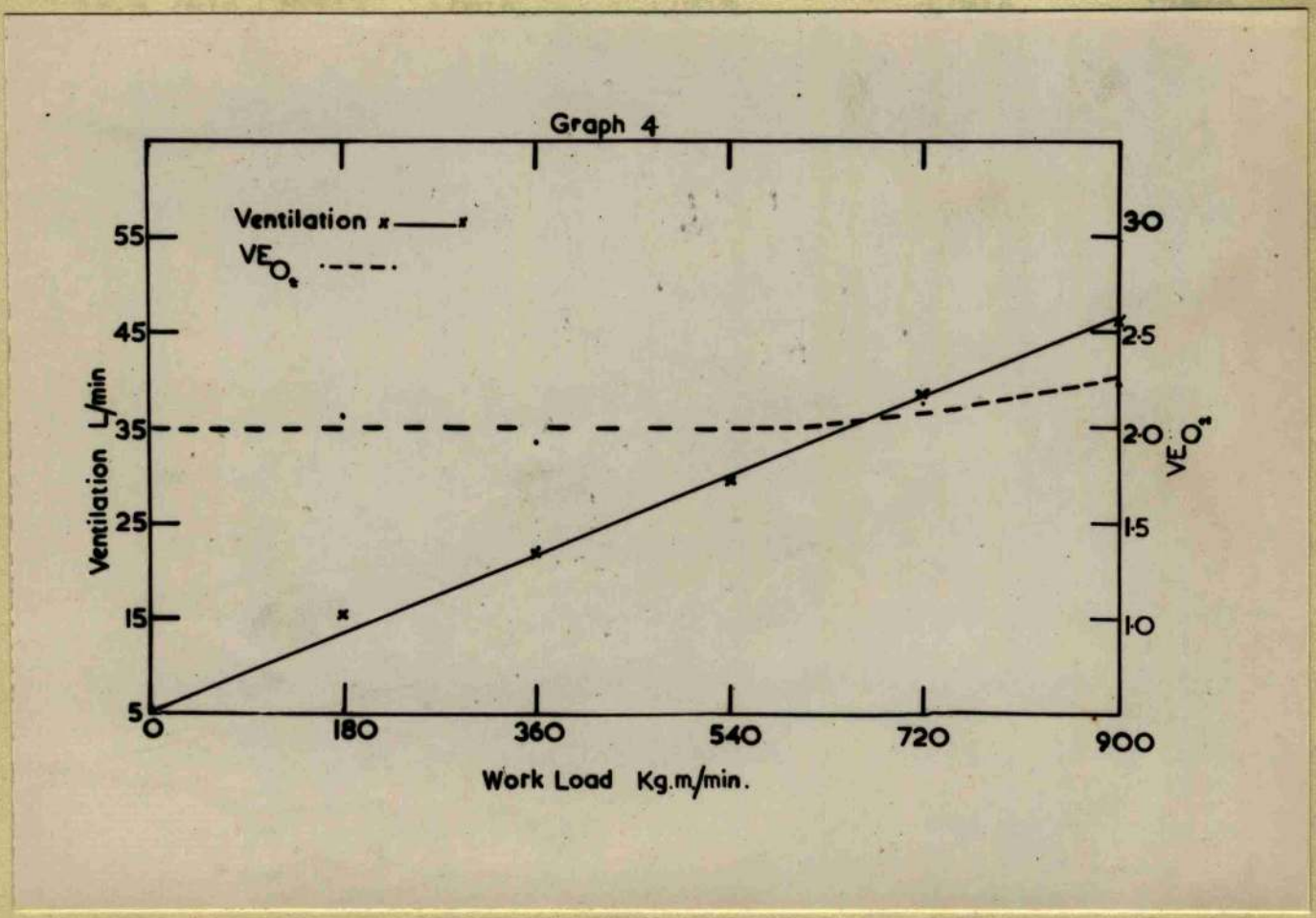
Normal subjects at rest.

Circulation to legs intact and occluded. (Assmussen et al., 1943).

Subject	Number of Expts.	Ventilation L/min.		Oxygen Uptake cc/min.		VE_{O_2}	
		Normal	Circulation Occluded	Normal	Circulation Occluded	Normal	Circulation Occluded
E.A.	15	5.66	5.25	256	254	2.20	2.21
		±0.06	±0.09	± 2.1	± 2.8	-	-
M.M.	21	6.93	6.26	237	231	2.72	2.70
		±0.12	±1.2	± 2.1	± 1.4	-	-

	Subject	Ventilation	O_2 Consumption
% change	E.A.	-7.3	-7.4
	M.M.	-9.7	-9.1

The results of experiments at work are given in Table 6. Here it is seen that with intact circulation the ventilation is directly proportional to the degree of work being performed (Graph 4) and the VE_{O_2} remains constant, only showing a slight rise at the higher work levels which may be attributable to the steady state having not been completely attained when readings were taken. When the circulation to the working muscles was blocked there was a sharp drop in oxygen consumption but little change in


Graph 4
Ventilation x——x
VEO_2 ·----·
55
45
35
25
15
5
Ventilation L/min
30
2·5
2·0
1·5
1·0
VEO_2
0
180
360
540
720
900
Work Load Kg.m/min.

ventilation, thus giving a rise in the VE_{O_2}.

Table 6.

Normal subjects working. (Assmussen et al., 1943)

Rate of Work	Number of	Normal Work			Circulation Occluded		
		O_2 intake	Ventilation	VE_{O_2}	O_2 intake	Ventilation	VE_{O_2}
Kg.m./min.	Expts.	L/min.	L/min.		L/min.	L/min.	
180	4	0.735	15.1	2.05	0.575	15.7	2.74
360	22	1.12	21.5	1.92	0.780	21.3	2.74
540	2	1.44	29.1	2.02	0.740	28.1	3.80
720	2	1.78	38.2	2.14	1.08	37.4	3.49
900	2	2.08	46.3	2.23	1.09	47.5	4.35

On comparison with the predicted response it may be seen that these results only partially fulfil the response expected assuming X to travel by a neural pathway and do not come close to that expected if X were humorally transmitted. The authors conclude, however, that the exercise stimulus travels by a neural pathway from the working muscles. Such a conclusion is not really valid since the whole of the stimulus transmitted neurally from the limbs could have been produced by the ischemia or by a combination of ischemia and work. There is no way of differentiating between the possible abnormal stimuli and a true "exercise stimulus". Nevertheless, this data is probably the best available using vascular occlusion methods in an attempt to identify the pathway of the exercise stimulus. There are

fairly adequate controls and the data includes metabolic and work level determinations enablingadequate analyses to be made.

Barman, Moreira & Consolazio (1943) carried out some experiments similar to those reported by Assmussen et al. In their first series, recumbent subjects exercised the arm muscles by squeezing inflated rubber bulbs sixty times per minute to perform work at a rate of 12 kg.m./min. Cuffs were placed about the upper arm in the usual manner. The pulmonary ventilation was measured

a) at rest

b) at rest with the circulation blocked

c) during hand and arm exercise

d) during the same exercise with circulation occluded.

The results of this series are shown in Table 7. Analyses of variance were performed on this data and the results of these tests (summarised in Table 8) show the following:

1) Vascular occlusion alone did not produce any significant change in ventilation.

2) Exercise alone and with ischemia significantly raised the ventilation.

3) There is no significant difference in the level of ventilation produced by exercise alone and that produced by exercise with the circulation blocked.

In the second group of experiments done by Barman etal. the subjects walked on a treadmill on a gradient of 8.6 in 100 at a speed of 3.5 m.p.h. until a steady state of ventilation was

Table 7.

Ventilation (l/min.). Exercise by hands. (Barman et al., 1943).

Subject	Experiment	Experimental Conditions 1. Rest	2. Rest & Ischemia	3. Exercise	4. Exercise & Ischemia
Bar.	1	6.90	7.00	8.10	7.30
	2	7.50	8.20	8.90	8.90
	3	7.20	8.00	8.50	8.50
	4	6.40	7.60	7.60	7.00
	5	6.80	7.80	8.10	7.80
	6	6.80	7.50	9.00	8.10
	7	6.70	6.90	6.80	7.10
	8	6.70	6.70	7.30	7.30
	9	6.72	7.20	7.20	7.20
	10	7.00	7.80	7.20	7.0
Mor.	1	6.70	9.00	8.90	8.90
	2	7.74	7.74	8.40	7.90
	3	7.80	8.20	7.90	9.10
	4	6.80	7.60	7.70	7.80
	5	6.80	6.90	8.60	9.10
	6	7.95	8.20	8.45	8.00
	7	6.75	6.80	7.43	6.75
	8	7.80	7.85	7.85	7.20
	9	8.10	8.00	9.90	9.00
	10	7.80	8.00	9.50	10.00
	11	6.90	8.10	9.10	8.00
Hol	1	6.80	8.10	8.50	8.20
	2	6.50	7.10	8.80	8.20

reached. The ventilation was then determined under the following conditions:

a) at work - steady state

b) at work - circulation to the legs blocked

c) at work - one minute after releasing occlusion

d) at work - ten minutes after releasing occlusion.

Oxygen consumption was also recorded under these varying conditions. A summary of the results of this group of experiments is to be seen in Graph 5 in which VE_{O_2} is also included. The mean results for each of the three subjects are plotted together with the overall means.

Consideration of the results of the first group of experiments reported reveals that the ventilatory responses do not approach the predicted responses, assuming either humoral or neural transmission of the exercise stimulus. Since oxygen consumption figures are not available for this series comparison must be restricted to ventilation responses alone and in this respect the results most closely resemble those expected if a neural exercise stimulus were in action. The level of exercise was, however, rather low and for this reason alone criticism attaches to the data. Certainly humoral transmission cannot be said to have been substantiated.

In the second group of experiments once again the results do not fulfil the predicted response for either neural or humoral propagation of the exercise stimulus. The data is closer to that expected if the neural pathway were the case and

Table 8.

Analyses of Variance on Data in Table 7. (Barman et al., 1943).

Experimental conditions	1 and 2	1 and 3	1 and 4	3 and 4
Corrections	2504.91	2707.20	2625.28	3042.08
Totals	16.95	161.66	158.22	30.68
Subclasses	6.34	19.72	14.37	5.99
Within subclasses	10.61	141.94	143.85	24.69
Subject variance	0.74	8.10	4.82	3.65
Condition variance	3.50	16.05	10.47	0.63

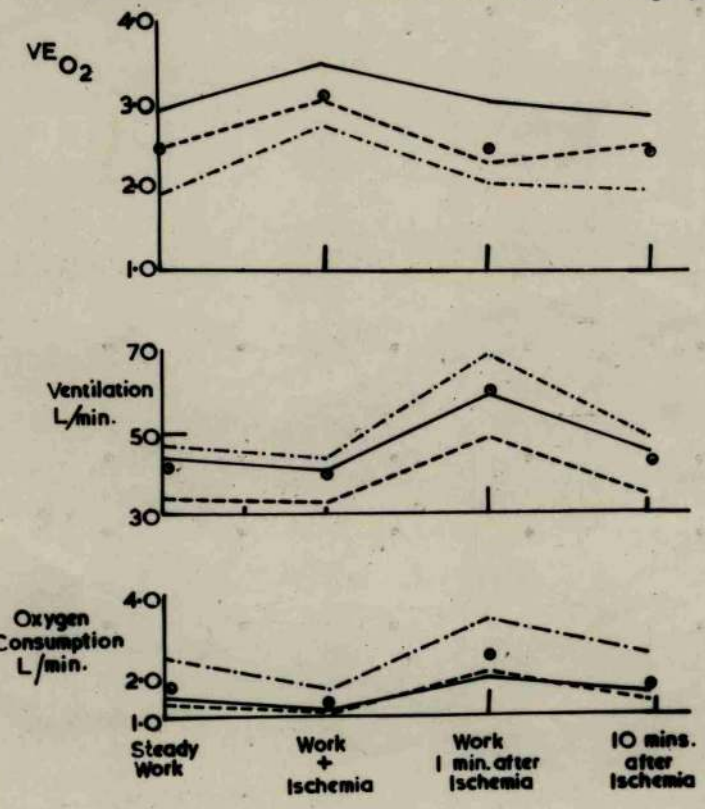
Source of Variation	Degrees of Freedom	1 and 2		1 and 3		1 and 4		3 and 4	
		Sum of Squares	Mean Square	Sum of Squares	Mean Square	Sum of Squares	Mean Square	Sum of Squares	Mean Square
Subjects	2	0.74	0.37	8.10	4.05*	4.82	2.41	3.65	1.82
Exp. Conditions	1	3.50	3.50	16.05	16.05**	10.47	10.47**	0.63	0.63
Error	40	10.61	0.265	141.94	3.55	143.85	4.59	24.69	0.617
Total	43	14.85	4.135	166.09	23.65	159.14	17.47	28.97	3.067

* Significant at 5% level

** Significant at 1% level

Graph 5.

VE_{O_2} Ventilation and Oxygen Consumption in Subjects working on a Treadmill with and without Vascular Occlusion of the Legs. (Barman et al; 1943.



⊙ – mean points.

humoral transmission can be given no support from this work.

It is difficult to conceive, therefore, how the authors of this paper came to the conclusion that their experimental results support the theory of chemical stimulation of the respiratory centre via a blood-borne agent from the working muscles. Such a conclusion is incompatable with the data published. Actually neither theory of propagation of the exercise stimulus can be said to receive support from this work. It is felt that the published results do not represent the normal respiratory response to exercise and their application to explaining the mechanisms of the normal response cannot be valid.

The problem of tight boots, as used in aviation, may seem rather removed from the problem at hand. Delucchi (1943) however, has investigated the question of obstruction of blood flow to the exercising legs by tight fitting boots and his results are relevant to this discussion. His subjects were seated with pneumatic cuffs on both legs and their feet resting on imitation rudder pedals. The ventilation was recorded under three conditions

1) With circulation intact.
2) Vascular obstruction by a pressure equal to one half systolic blood pressure.
3) Vascular obstruction by a pressure equal to two thirds systolic blood pressure.

Each of these conditions was instituted at three levels of static work.

a) at rest

b) with steady push of 10 lbs.

c) with steady push of 20 lbs.

The results are summarised in Table 9.

Table 9.

Ventilation (L/min.) with steady work with and without vascular obstruction. (Delucchi, 1943).

Condition	Rest	10 lbs. push	20 lbs. push
No obstruction	6.67 ± 1.17	7.39 ± 1.36	8.13 ± 0.78
½ Systolic pressure	6.78 ± 1.17	7.56 ± 1.27	8.26 ± 0.74
2/3 Systolic pressure	5.99 ± 1.19	8.48 ± 0.91	8.88 ± 0.72

None of the changes in ventilation are really statistically significant but there are some indicative points worthy of comment. Firstly, the ventilation increases in a linear fashion from rest to work of 20 lbs. pushing, either with no vascular obstruction or with obstruction equal to one half systolic pressure. The greater increase of ventilation at the higher level of steady work might possibly be due to subjective sensations affecting the respiration. Since only ventilation was recorded it is not possible to determine this point. Secondly, little change in ventilation is produced by obstructing the circulation to the legs by applying a pressure equal to one half systolic blood pressure. Such a pressure would be adequate to prevent venous return from the legs and yet ventilation is maintained at a

raised level during work. Thirdly, no motion of the legs occurs during the work, eliminating the possibility of stimuli arising from joint movements causing the increase in ventilation, although not the possibility of the initiation of stimuli from tension in the muscles or tendons.

The reported results thus, although not at all conclusive in themselves, support the possibility of the exercise stimulus being transmitted neurally from the working muscles.

The experiments using vascular occlusion only really show that ischemia of working muscles can produce a stimulus to ventilation which is transmitted neurally. Whether or not this is, in whole or part, the physiological exercise stimulus is an open question. That the stimulus is neurally transmitted has been neither excluded nor proven and any conclusions, based on these experiments, that such is true cannot stand up under critical analysis.

B. With Electrically Induced Work.

Krogh & Lindhard (1917) used an adaptation of a Bergonié stimulator to compare the response to voluntary leg work and to electrically induced leg work in man. They found that, under their experimental conditions, there was an immediate increase in ventilation both in voluntary and induced work. There was, however, a higher level of ventilation, compared to the oxygen consumption, in induced work than in voluntary work and also a close relation between ventilation, the

strength of stimulation, and the sensations produced by the process. There is thus adequate evidence that there was stimulation, not only of the motor nerves and the muscles themselves, but also sensory nerves from the exercising limbs. The experiments described are therefore of little value in determining whether or not the increase in ventilation with work is via peripheral "reflexes" or by a central mechanism. The authors mention that in their opinion there is no indication of an initial chemical regulation of respiration in work.

Comroe & Schmidt (1943) report some experimental observations on cats and dogs anaesthetised with barbitone or chloralose. The ventral lumbar spinal roots were exposed, cut free, and placed on raised, insulated electrodes. The roots were then stimulated from a thyrotron stimulator four times per second at a strength of stimulation to give a maximum response. No quantitative data was published for this series but the authors note the following:

With cats: 1) In every case some increase occurred in ventilation.

2) A latent period of at least 15 seconds was seen from the start of stimulation to the start of the ventilatory response.

3) On stopping stimulation, ventilation returned gradually and slowly towards the resting level.

4) Clamping the blood vessels to the legs produced no change in ventilation.

5) Exercise induced while the blood vessels were clamped had less effect on breathing than when the circulation was intact and when the vessels were released, hyperpnea promptly appeared.

6) When the spinal cord was transected in the lower dorsal region, induced exercise still resulted in an increase in ventilation.

When dogs were used the following observations resulted:

1) In 35 out of 39 cases an increase in ventilation was noted.

2) The ventilatory response to induced exercise was immediate on starting stimulation.

3) Ventilation returned to normal almost immediately after stopping stimulation.

4) Stimulation of ventilation was principally evoked by an increased rate of breathing rather than depth.

5) Transection of the spinal cord completely abolished any ventilatory response to induced exercise.

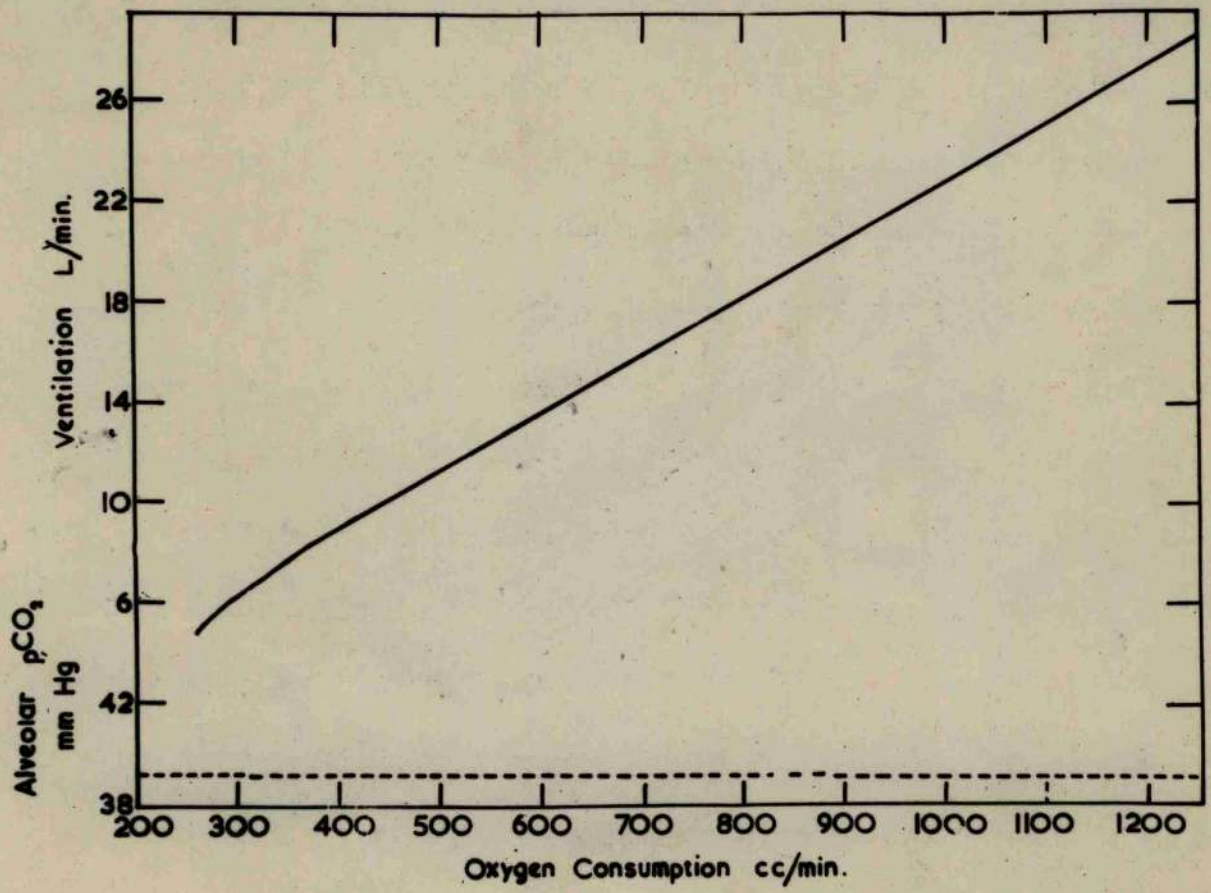
Several points in these observations deserve comment. Firstly, the difference in response between the cat and dog. The

authors suggest that a species difference exists in regard to the control of ventilation in exercise. This is certainly possible but is not considered probable. It would seem more likely that differences in current spread at the point of stimulation and of surgical trauma were the causative factors. Secondly in the cat experiments, clamping of the blood vessels to the legs produced no change in ventilation. This result is not compatable with the other findings nor with the authors' conclusions that, in the cat, the exercise stimulus is humorally transmitted. Thirdly, in the dog experiments, transection of the cord completely abolished any ventilatory response to induced exercise. Although certainly any neural response would be abolished a secondary, humorally transmitted increase in ventilation should have been noted. It is felt that this series of experiments is of questionable value, certainly an adequate evaluation is impossible since no experimental data is presented.

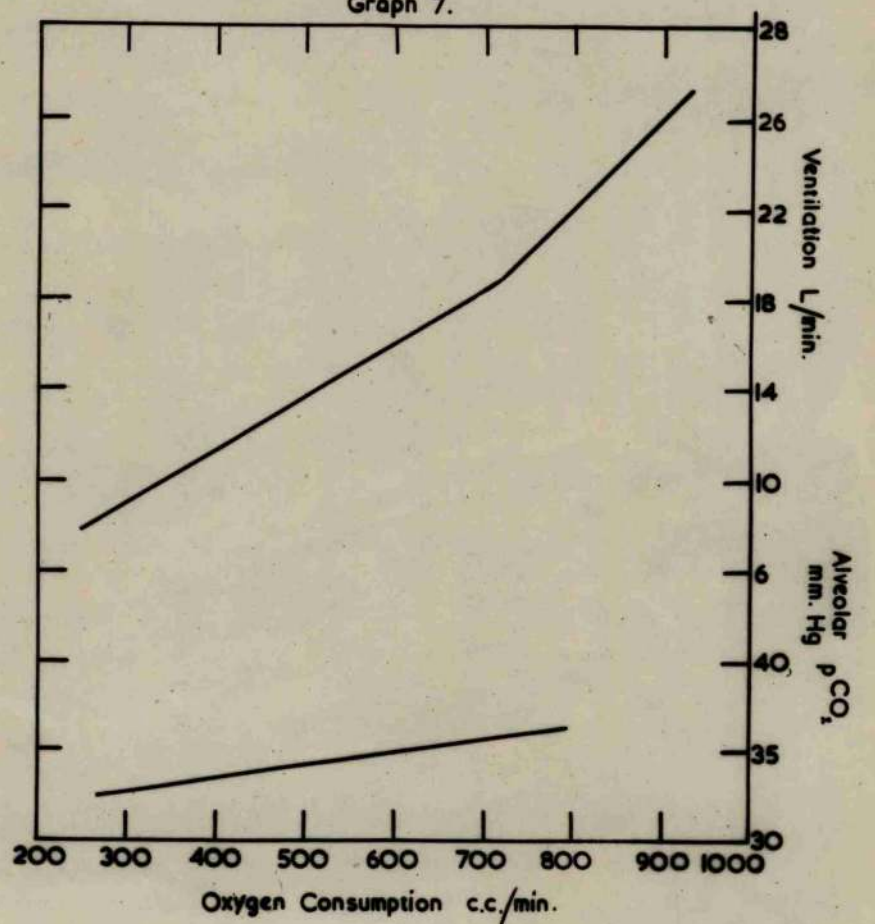
Assmuss en, Nielsen & Wieth-Pedersen (1943) attempted to differentiate between possible "reflex" or "cortical irradiation" control of respiration during muscular work. These investigators used human subjects seated with their feet on a bar which moved in a horizontal path. The movement of the bar was resisted by springs. An indifferent electrode was placed on the lower back of the subject and stimulating electrodes placed on the thighs and calves. Electrical stimulation thus produced stretching movements of the legs

against the resistance of the spring-loaded bar. After 10 - 15 minutes of induced work the oxygen consumption and ventilation were determined using a Douglas bag, and the alveolar pCO_2 calculated. During work induced as described in a normal subject it was found that the increase in ventilation with work corresponded with the increase in oxygen consumption exactly as in voluntary work (Graph 6). The alveolar pCO_2 also remained steady at the control value of 39 mm. Hg. From these results the authors concluded that the exercise stimulus is not irradiation from the motor cortex but by "reflexes" from the working muscles. They considered that the reflex impulses stimulating ventilation passed up to the higher centres as "kinesthetic sensations" in the posterior fascicles of the spinal cord. The experiments were therefore repeated on a subject who had had tabes dorsalis for a period of several years. As is seen in Graph 7, in the subject ventilation increased in direct proportion to oxygen consumption as with the normal subject. There are, however, some differences from the graph of the normal subject. Firstly the alveolar pCO_2 remains steady at 39 mm. in the normal whereas there is a linear rise from the low level of 32.5 mm., at 270 cc/min. oxygen consumption, to 36 mm., at 780 cc/min. oxygen consumption, in the subject with tabes dorsalis. This difference is not accounted for and there is no basis in the paper for speculation as to its cause. Secondly VE_{O_2} falls, in the range between 400 cc and 250cc/

Graph 6.


Ventilation L/min.
26
22
18
14
10
6
Alveolar pCO_2 mm Hg
42
38
200
300
400
500
600
700
800
900
1000
1100
1200
Oxygen Consumption cc/min.

Graph 7.


28
26
22
18
14
10
6
40
35
30
Ventilation L/min.
Alveolar mm. Hg p_{CO_2}
200
300
400
500
600
700
800
900
1000
Oxygen Consumption c.c./min.

min. oxygen consumption, in the normal subject and, thirdly, VE_{O_2} rises, in the range between 700 cc and 930 cc/min. oxygen consumption, in the tabetic subject. The first of these findings on VE_{O_2} is unaccountable, the second may be due to the stimulation, but there remains the fact that the two sets of results are not fully compatable. The authors do not discuss these differences but only conclude that the exercise stimulus must be carried by sensory nerves outside the posterior fascicles. This conclusion does not seem to be fully justified from the results since the electrical stimulation used could hardly be said to approximate physiological stimulation of the muscles and also from the unexplained differences in results between the two subjects. It should be noted too that the patient with tabes dorsalis was at least pathological as far as his spinal cord was concerned and there is no means of ascertaining if his ventilatory regulation system was not also pathological and therefore providing spurious results for application to physiological functions. The general tenor of the conclusions has, however, received recent support in work which will be reviewed later (Kao & Ray, 1953).

II. Spinal Cord Section Experiments.

Geppert & Zuntz (1888) were the first to study the hyperpnea of muscular exercise. They transected the spinal cord and noted the changes in ventilation produced by electrical tetanization of the muscles of the hind limbs. These authors noted that an increase in ventilation, though but slight, was produced and concluded that the exercise stimulus was humorally transmitted. The small amount of work induced and the extreme abnormality of tetanic stimulation makes such an unequivocal conclusion impossible.

Von Euler and Liljestrand (1946) attempted to show the effects of spinal cord transection and sinus denervation on the changes in ventilation produced by induced work. In all cases where the oxygen consumption was also noted they found a fall in VE_{O_2} with induced work, even in the intact animal. The values for Ventilation Equivalent for their animals were high to begin with, indicating an abnormal regulatory state. The fall in VE_{O_2} with work indicates the possibility of the stimulation producing some inhibition of respiration. Such a possibility is considerably strengthened by the authors' note that on the inception of electrical stimulation the lung resting volume increased, thus increasing dead space and residual volume. This note points to the probability that the inspiratory musclature was being stimulated either directly or via a spread of current or impulses to the higher centres. If this be true then the results reported by these workers are invalid. It is

interesting to note that Harrison et al. (1932) report that section of the cord at the level of the 8th dorsal root did not diminish the ventilatory response to movement of the hind leg whereas section of the spinal cord at the level of the 4th dorsal root abolished any effect. The level of transection carried out by von Euler et al. was the 12th dorsal root so that it would appear that impulses from the exercising limbs might not be wholly eliminated in his experiments.

Grodins & Morgan (1950) did a series of experiments in which they compared the effects of exercise, electrically induced, of the hind limbs in dogs both intact and with spinal transection at T 10. They found a relationship between oxygen consumption and ventilation which was qualitatively compatable with the theory that section of the cord blocked the exercise stimulus. The chemical changes in the blood of the dogs with spinal transection could not, however, explain all the increase of ventilation and spinal section in itself produced a significant rise in the resting level of ventilation. The mean resting values for VE_{O_2} for both their intact (Morgan & Grodins, 1950) and cord sectioned groups are quite high, indicating that the respiratory regulating systems of the animals were not operating at a normal level and possibly not in a normal fashion. A further criticism of this work is that the sensory stimulation of the electric current used to produce exercise could well be

resulting in indeterminable artefacts in the data.

It is felt that no justifiable conclusions can be drawn from the experiments using section of the spinal cord. The published results in themselves neither substantiate nor deny the theory of either humoral or neural transmission of the exercise stimulus.

III. Passive Movement Experiments.

The study of the ventilatory response to passive exercise of the extremities has as its basis the assumption that by passive movement the metabolism of the muscles would not be changed and any effect on ventilation would be from movement, tension on tendons, or from other proprioceptive stimuli. Several reports have appeared in the literature of experiments of this nature on both animals and man. Harrison et al. (1932) report a 20% increase in ventilation on passive movement of the hands and feet in man. With dogs, shaking of the hind limb 300 times per minute produced an 8% rise in ventilation which was not affected by ischemia of the muscles but was eliminated by section of the sciatic nerve. Comroe & Schmidt (1943) repeated Harrison's work and also found a slight but very variable increase in ventilation on moving the disarticulated hind limb of a cat 200 times per minute. The increase was maintained during vascular occlusion and with the tendons cut but was abolished by spinal section and by section or anaesthetisation of the nerves to the knee joint. These workers concluded that proprioceptive reflexes from the extremities together with other factors such as cortical irradiation and impulses from the lungs act as the stimulus to ventilation in exercise.

Gardner & Jacobs (1948) used various stimuli such as

passive flexion 150 - 300 times per minute, trunk movements, rubbing wound edges, tracheal cannula movements and electrical stimulation of joint nerves. They report variable but slight changes in ventilation from all their experiments and conclude that any neural stimuli are unimportant in the regulation of respiration in exercise. Grandpierre, Franck & Violette (1951) report some experiments on passive movement of the extremities in dogs and man. These workers too get variable results but an overall slight increase in ventilation resulted from movement of the hind leg in the dog or forearm in man 30 times per minute. They found that, in man, subjects who showed hyperventilation with low concentration (2 - 4%) of CO_2 in the inspired air showed a more marked response to passive movement than those who required a higher (5 - 7%) concentration of CO_2 in the inspired air to produce hyperventilation. Grandpierre concluded that the passive movement produces, via reflexes from the muscles and tendons, an increase in ventilation whose intensity depends on the excitability of the respiratory centre. These same workers recently report that if a subject ingests a quantity of glucose one hour before a passive motion experiment is performed on him a greater increase in ventilation is produced than when glucose is not previously taken. The opposite result is reported when a barbiturate is taken (Grandpierre, Franck, Violette & Arnould, 1952). Taking

these latter results into consideration, Granpierre concluded that the response of the respiratory centre to passive movement is a function of the momentary excitability of the centre.

As noted before, the rationale of passive movement experiments is partly that no increase in metabolism will result from the movement. That this premise is incorrect has been shown by Bahnson, Horvath and Comroe (1949) and also by Liljestrand and Stenstrom (1922) who agree that passive movement of the types used produces an increase in oxygen consumption of 32 - 40%. Otis (1949) reports a series of experiments where oxygen consumption and alveolar pCO_2 were measured during passive exercise. He confirmed the finding that limb movements cause a considerable increase in ventilation but also in oxygen consumption and carbon dioxide production. Otis also noted that such movements may also introduce previously pooled blood into general circulation. Gardner et al. (1948) noted an increase in tonus of the leg muscles on passive movement, an indication that work was being done by the muscles against the movement. Such findings are contrary to the basic precept of passive movement experiments and when it is also considered that the movement concerned in many experiments was a violent shaking of radically traumatized limbs it is only prudent to conclude that this type of investigation has added little to the knowledge of the control of respiration in exercise.

IV. Other Experimental Work.

Other experimental work reported in the literature pertinent to the discussion at hand consists mainly in various types of cross circulation experiments. Heymans, Jacob & Liljestrand (1947) noted the laryngeal movements of an isolated head perfused, via carotid-jugular connections, by a donor dog. The effects of electrically induced work, hyperventilation, and oxygen inhalation by the donor dog were investigated. Making the questionable assumption that the laryngeal movements in the isolated head are a measure of what the respiration would be in the intact animal this procedure would provide data on the purely humoral control of respiration. Increase in rate and amplitude of the laryngeal movements was produced by electrically induced work in the donor dog, mainly in the later stages and after the work. A decrease in the movements was produced by hyperventilation and also by oxygen inhalation by the donor dog. These experiments only point out that chemical factors have a role in the regulation of respiration in exercise. That chemical factors do indeed play a part in the fine regulation of ventilation has already been described in the discussion of the modified regulatory system. Experimental evidence for this view was provided by Kao & Grodins (1952). By cross circulation techniques these workers obtained results which were qualitatively compatable with the assumption that

the exercise stimulus, which initiates the increase of ventilation on exercise, is neurally transmitted from the working muscles.

Kao & Ray (1953) recently reported some experiments in which the hind limbs of a recipient dog were perfused via the aorta and vena cava with blood from a donor dog. The recipient dog's limbs were then exercised and the ventilation and oxygen consumption noted with

a) spinal cord intact

b) section of the dorsal columns at T 11

c) section of the lateral columns at T.11

d) total cord section.

They found that when the cord was intact or when the dorsal columns were cut there was a significant increase in ventilation and VE_{O_2}. When the lateral columns were sectioned, and with total cordotomy, no significant increase of ventilation was recorded. Kao concluded from these results that the exercise stimulus is neurally transmitted via the lateral columns of the spinal cord. It is interesting to note that this work supports the findings of Assmussen et al. (1943) on a subject with tabes dorsalis which was reviewed earlier. Kao's results are more satisfactory from the point of view that humoral transmission is definitely ruled out, it is known exactly what parts of the spinal tracts are interrupted, and enough experiments were done to enable statistical

analysis of the results to be carried out.

To summarise the discussion in this section on the site of origin and pathway of the exercise stimulus it must be said that there is insufficient evidence in the literature to decide conclusively the modus operandi of the stimulus. One thing is clear, however, which is that the beliefs of Volkmann (1841) and Vierordt (1844) that respiration in exercise is regulated chiefly by "reflexes" now has much firmer experimental support. That the control of ventilation in exercise is either wholly or principally by the common chemical agents, pCO_2, pO_2 and $[H^+]$, is disproved and the weight of evidence points to the conclusion, as yet unconfirmed, that the exercise stimulus originates in the working muscles and is transmitted neurally via the lateral columns of the spinal cord to the respiratory centres.

PART II.

THE SPECIAL ROLE OF MUSCLE ACTION IN THE CONTROL OF RESPIRATION IN MODERATE EXERCISE.

Nature of the Exercise Stimulus.

An attempt has been made in the foregoing pages to show that the weight of evidence in the literature gives basis to the conception of the exercise stimulus originating in the working muscles and being transmitted thence to the respiratory centre by neural means. If such a working assumption is made the question now arises as to the nature of the exercise stimulus and the type of receptor upon which it may act. One of several types of receptors known to be present in the body, or a combination thereof, may be responsible. Possible receptor systems are:

1) Thermoreceptors sensitive to temperature changes in the muscles.

2) Proprioceptors sensitive to changes in tension of the muscles or tendons or to movement or the position of the working parts.

3) Chemoreceptors sensitive to changes in $[H^+]$, pCO_2 or pO_2, or other tissue metabolites.

With the exercise stimulus in mind each of these possible receptor systems will be discussed.

1. Thermoreceptors.

The ventilation and oxygen consumption during exercise are directly related, as has already been noted, and therefore magnitude of the exercise stimulus must also be directly related to oxygen consumption. It is suggested by Gray (1950) and Grodins (1950) that temperature changes in the working

muscles may provide the exercise stimulus. Kao and Grodins (1951) found a high correlation between muscle temperature, oxygen consumption, and ventilation. Such a finding would seem to lead to the conclusion that it is the heat produced by muscular work which stimulates the ventilation. If the temperature changes were the factor involved, and not just a correlated but inactive by-product of work, it would be expected that heating of the muscles by hot baths, diathermy, heating perfusing blood, or other means of producing the same order of muscle temperature rise as in exercise, would give a concomitant rise in ventilation. That such is not the case has been shown by Lim (1951), Lim & Grodins (1951), Morgan (1953) and Landis, Long, Dunn, Jackson & Meyer (1926).

Quite recently Barltrop (1954) described work on the relation between body temperature and respiration. He measured the steady state ventilatory response to raising the body temperature by means of hot baths. The author concludes "that the rise of body temperature in exercise constitutes a small part only of the 'exercise stimulus' for respiration".

Cotes (1955) reports ventilation, pCO_2, and body temperature (rectal temperature) during exercise in a single subject. As did Kao et al. (1951), he found a high correlation between oxygen consumption, ventilation, and body temperature. Cotes concluded that ventilation is

controlled by pCO_2 and body temperature acting additively. Such a conclusion does not seem valid from the results reported. There is no justification given for the assumption that the temperature is the independent variable and ventilation the dependent variable. It is perhaps pedantic to say that if two things show correlation then it is not necessarily the case that one is dependent on the other. Also this author does not investigate the time relationships of changes in temperature and ventilation - which would be important in justifying his conclusion.

These last two papers appeared after the experimental work to be described was completed and their implications in the light of the experimental results obtained will be discussed later.

It is considered that the weight of evidence from the papers cited above make it necessary to discard muscle temperature as the exercise stimulus and thermoreceptors as the receptor system. It should be noted, however, that it is recognised that in any experimental work it is important to take data on muscle temperature changes and to consider the possibility of any such changes having an effect on ventilation.

2. Proprioceptors.

Passive movement experiments have been reviewed in an earlier section. These experiments provide the basis for the postulate that proprioceptive impulses may initiate the

exercise stimulus. The main argument against this idea is that the exercise stimulus must be directly related to the metabolic rate. The same movement of an arm or leg can be used to do 1 ft. lb. or 10 ft. lbs. of work, although tendon tension may have some relation to the work done. Movement is in no way a constant function of metabolic activity. In "static work", for example, there is no motion at all, although the oxygen consumption and ventilation rise as in active work. It is considered that the proprioceptors can be discarded as a possible receptor system for the exercise stimulus.

3. Chemoreceptors.

Experiments where chemical products of muscle metabolism have been injected intra-arterially, (Moore, Moore & Singleton, 1934; Comroe & Schmidt, 1943) intra-muscularly (Comroe et al., 1943) or intravenously (Morgan, 1953) have not resulted in a rise in ventilation in any degree similar to that produced by exercise. Kao (1951) produced an eightfold increase in the ventilation of a dog by allowing it to rebreathe its own expired air. In an animal whose hind limb was perfused with blood from the first animal, however, no change in ventilation was noted. He concluded that there was no evidence that chemoreceptors were responsible for transmission of the exercise stimulus.

It has been suggested that there may be venous chemoreceptors involved in the control of ventilation in exercise.

Recently Dejours, Mithoefer and Teillac (1955) report experiments on human subjects, using a vascular occlusion method, from the results of which they discard the possibility of such venous chemoreceptors. On the other hand Hortolemei, Proca, Busu, Enescu & Litarczek (1954), in a paper which will be discussed in greater detail later, suggest that there must be chemoreceptors sensitive to an increase in pCO_2 and O_2 lack and to other chemical substances and that these receptors are located in the muscles or the blood vessel walls. The suggestion by these workers that there may be receptors sensitive "to other chemical substances" is the premise on which the experimental work to be described was based. Bradley, Gaskall, Holland, Lee & Young (1954) also give results which suggest the existence of receptors sensitive to something other than changes in pCO_2 or pO_2. As has been emphasised previously the increase in ventilation on exercise occurs rapidly and in direct relation to the increase in metabolism of the muscles. As Dejours, Raynaud, Cuenod, & Labrousse (1955) state, there must be a factor .... "en rapport avec l'activite motrice, apparaissant et disparaissant avec elle, résponsible des variations instantanées du debut et de la fin de l'exercise".

It does not seem to have been considered that the actual metabolic activity of the working muscle cells might be the initiating stimulus rather than some "by-product" of such activity like metabolites, heat or movement. By 'metabolic

activity' is meant the actual chemical activity of the muscle cell upon which energy output of any form, be it heat, tension, or movement, depends.

It is thus postulated that there are receptors in muscle which are sensitive to some phase or phases of the ATP-glycogen-phosphocreatine cycle. In order to differentiate these postulated receptors from recognised chemoreceptors they are designated metaboreceptors. Anything which would increase the rate of operation of the energy cycle in the muscle, such as work, hyperthyroidism, or chemical agents, under either aerobic or anaerobic conditions would cause impulses to be initiated in these metaboreceptors and then travel, by neural pathway via the lateral columns of the spinal cord, to the respiratory centre. These neural impulses would stimulate ventilation in a manner directly proportional to the energy output, and hence oxygen consumption, of the muscles.

Method for Examination of the Metaboreceptor Theory.

In order to test the postulate of receptors sensitive to changes in muscle metabolic rate it is necessary to change the metabolism, without directly causing any change in muscle temperature, without movement of the limb or change in tension of the muscle, and measure any alteration in ventilation produced.

It is also important to avoid production of any abnormal chemical changes in the muscle within the time

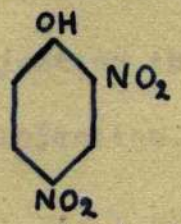
course in which the ventilation is being measured. One possible method of doing this is by using a drug whose sole effect is to increase the metabolism of the muscle cells. The ideal drug would not alter the normal chemistry of the cells, have no irritating effects on the tissues, no direct stimulating action on nerve endings, and no central effect on the respiratory centre. Such an ideal drug is not known but one chemical which approaches the ideal is 2 - 4, dinitrophenol.

Review of the literature on the pharmacology of this drug follows.

The Pharmacology of 2:4 - dinitrophenol (DNP).

Physical Properties.

2:4 - dinitrophenol (DNP) is a pale yellow crystalline powder. It has the chemical structure



DNP is sparingly soluble in water but may be used as a 2% solution in ethylene or propylene glycol or a 3% solution in 1.5% soldium bicarbonate. The latter preparation is more commonly used. It is a deep orange solution, quite stable and with a pH of about 8.1 (Tainter & Cutting, 1933).

Dosage.

The dosage of DNP is given in terms of milligrams of the acid, although the solution of the sodium salt is administered.

The following dosage levels have been established as applicable to most species of experimental animals (Magne, Mayer & Plantefol, 1932a; Tainter, 1934).

Minimum Lethal Dose 10mg/kg. body weight

LD_{50} 25mg/kg.

LD_{100} 50mg/kg.

G eneral Response.

The response of an animal to injection of DNP is characterized by a rise in oxygen consumption of the animal and an increase in the rate and depth of breathing which, with high dosages, progresses to a marked hyperpnea. Body

temperature rises secondarily and a marked pyrexia may be produced.

The duration and intensity of the response depends on the route of injection and dosage level. With subcutaneous and intramuscular injection there is a slight latent period whereas intravenous injection results in an almost immediate response.

If a fatal dose is administered respiration eventually becomes irregular, oxygen consumption falls, and the animal dies in rigor.

Causes of Death from Fatal Doses of DNP.

The immediate cause of death resulting from injection of DNP may be one of three (Tainter et al., 1933).

1) Large doses of DNP intravenously may cause cardiac depression and failure before either body temperature or respiration are affected.
2) Smaller doses of DNP may lead to death from hyperpyrexia. The circulating blood remains well oxygenated up to the point of death.
3) Small or medium doses of DNP may lead to a great increase in oxygen consumption and ventilation until the respiratory system can no longer adequately oxygenate the blood. A progressively more severe anoxemia and metabolic acidosis rapidly develops and cessation of respiration and cardiac arrest occur almost together. The animal develops rigor almost

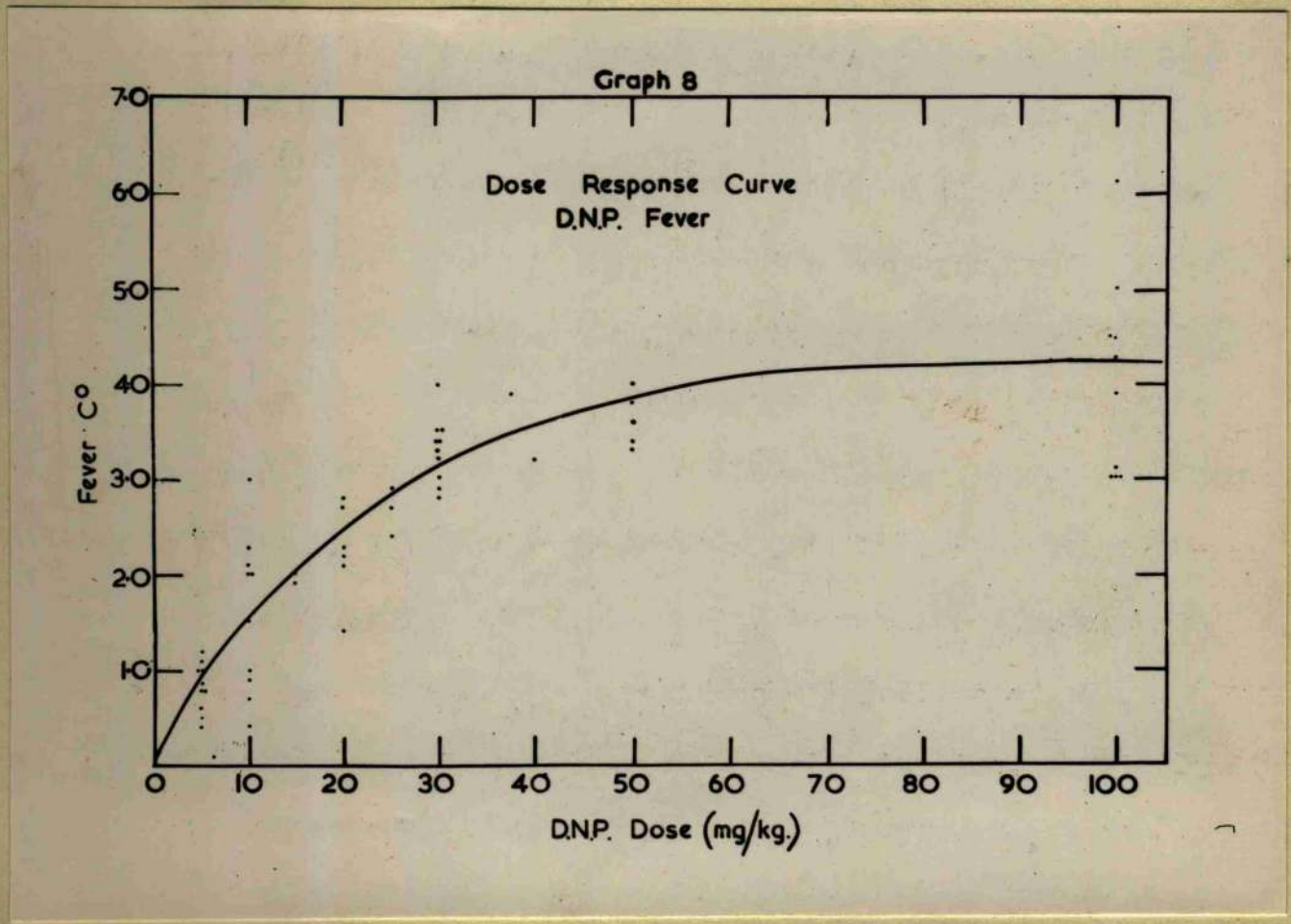
immediately after death.

Specific Effects of DNP Administration.

Body Temperature.

In Graph 8 is shown the relationship between DNP dosage level and the degree of fever produced in dogs, cats, and rabbits, (Tainter et al., 1933; Magne et al., 1932a, b, c; Hall, Field, Sahyun, Cutting & Tainter, 1933). Tainter et al. (1933) state that the degree of fever is also dependent on the route of administration of the drug but examination of the data available shows that the pyretic response produced by a particular dose is the same, no matter by what parenteral route it is administered, although the time to attain that response may be different.

Data published by Magne et al. (1932a) shows that there is a tendency for a drop in rectal temperature for a period of 5 - 10 minutes after subcutaneous injection of DNP. After this period the temperature then begins to rise. This finding is probably the result of two factors. Firstly, a time lag during which the drug is being absorbed and, secondly, increased heat loss in the initial stages of increased ventilation. This latter factor is shown by Hall et al. (1933) who found that if the temperature of the inspired air was kept low, the degree of fever produced by a given dose was less. These workers also noted the time lag between injection and rise in rectal temperature.


Graph 8
Dose Response Curve
D.N.P. Fever
Fever · C°
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
10
20
30
40
50
60
70
80
90
100
D.N.P. Dose (mg/kg.)

That the rise in body temperature is not produced by central effects was shown by van Uytvaneck (1931), Magne et al. (1932a) and Tainter et al. (1933). Extirpation of the thermoregulatory centre, brain section below the medulla, complete brain destruction and also destruction of the spinal cord do not prevent the production of fever by the injection of DNP. Magne and Tainter also report that ablation of the viscera does not eliminate the temperature rise. Tainter concluded that the drug acts peripherally on the muscles. Before coming to the same conclusion Magne placed thermocouples in various regions of the body, both peripheral and central, and followed the temperature changes after DNP injection. He found that skeletal muscle and arterial blood temperatures rose first and to a higher degree than liver or intestine temperatures.

Magne also showed that the temperature rise is not caused by increased muscular activity, such as shivering, by recording the characteristic rise in temperature in curarised animals. An interesting point in this connection is that Hall, Crisman & Chamberlin (1937) found that DNP depresses shivering. This is not explained by the rise in body temperature since shivering did not occur in animals, injected with DNP, whose body temperatures were low enough to normally elicit shivering.

That the rise in body temperature is secondary to the

increased metabolism of the tissues was shown by Hall et al. (1933) who report that the oxygen consumption of an animal injected with DNP may be doubled before any rise in temperature occurs. These authors also point out that the increase in oxygen consumption is only accounted for to a very small degree by the rise in body temperature.

Circulation.

It is repeatedly noted in the literature that no consistent change in arterial blood pressure or in pulse rate occurs after injection of DNP (Cutting, Mehrtens & Tainter, 1933; Tainter, 1934; Freeman, 1934; Rosenblum, 1934; Rabinowitch & Fowler, 1934). Tainter et al. (1933) do, however, note that the pulse rate in their animals increased 20 - 40 beats per minute but this change in pulse rate occurred only after considerable fever had been produced.

It is to be expected that a large increase in metabolism would result in an increased cardiac output. Hall et al. (1933) calculated cardiac output by the Fick method and found after DNP injection a mean increase of two and one half times the control value.

Rabinowitch & Fowler (1934), in two determinations on human subjects receiving low oral dosages of DNP (1.5 - 3mg/kg), noted an increase in cardiac output in one subject and a decrease in the second.

Freeman (1934) noted in human subjects receiving DNP

(3 - 5 mg/kg per day) that after seven days there was a 22% reduction in the mean arm-carotid circulation time. On the other hand, Rosenblum (1934) reports no significant change in arm-tongue circulation time in obese patients receiving DNP. This latter author does not report the dosage used nor the time of DNP administration or circulation time determinations.

Metabolism.

Injection of DNP can increase oxygen consumption to a high degree, up to 14 times the normal resting metabolism, before death ensues. There is little quantitative evidence in the literature on which to base a dose-response curve, and of the data available, there are only three or four experiments where anything approaching a steady state of oxygen consumption has been recorded.

The mode of action of DNP in increasing the metabolism is generally accepted to be by direct action on the cell oxidation processes. Magne et al. (1932a) concluding as described above that DNP acts peripherally on the muscles found that the oxygen consumption of an isolated limb was increased when perfused with blood containing DNP. From the finding they concluded that DNP acts on the muscle cells directly. Such a conclusion was put on a firmer basis by Plantefol (1932) who reported the increased oxygen consumption of vegetable and animal cells in vitro when treated with DNP.

Tainter (1934) postulated that DNP might increase the efficiency of oxygen utilization by the tissues and compared the

resistance to anoxemia of normal rats, thyroid treated rats, and DNP treated rats. He found that there was no difference in sensitivity to oxygen lack between the thyroid treated and DNP treated animals, provided the degree of metabolic stimulation was the same. From these results the author concluded that DNP does not promote more efficient utilization of oxygen by the tissues.

Hall and Chamberlin (1937) report a synergistic action of adrenaline and DNP. On anaesthetised cats adrenaline infusion produced only a slight rise in oxygen consumption after one hour; DNP (10 mg./kg. intramuscularly) resulted in a rise to approximately twice the resting level after one hour. When the two drugs were administered together the metabolic rate after one hour was almost three times the resting level. The authors do not comment on the possible significance of their findings and it cannot be said where these results may fit into an explanation of the action of DNP in increasing the oxygen consumption of the cells.

Injection of DNP produces a marked hyperglycemia and depletion of liver and muscle glycogen stores (Magne et al., 1932b; Hall et al., 1933). No significant changes are found in the blood levels of fatty acid, cholesterol or non-protein nitrogen. Hall et al (1933) carried out chemical studies on the blood and tissues of dogs injected with DNP. The important point of their findings with regard to the present review is that no significant changes were found in blood lactate, blood

pH, or plasma CO_2 combining power unless the oxygenation of the blood was impaired. This latter condition only occurs just before death.

Hall, Brown, & Sahyun (1933) attempted to explain the mechanism of the hyperglycemia produced by DNP injection. They show that it is not produced secondary to general asphyxia in anaesthetised cats. They actually found an increase in arterial pO_2 and a decrease in pCO_2 and $[H^+]$ in following for $1\frac{1}{2}$ hours the response to a dose of 20 mg./kg. of DNP.

DNP is excreted by the kidney partially unchanged and partially as 2 amino - 4 nitrophenol (Goodman & Gilman, 1941; Guerbet & Meyer, 1932).

Respiration.

Although marked increases in ventilation after DNP injection have been noted by all investigators who have studied the actions of the drug only two papers give any adequate quantitative data. Hall et al. (1933) noted the respiratory responses of anaesthetised dogs to doses of 10 mg. and 20 mg./kg. but only publish "before" and "after" readings. Magne et al. (1932) publish data recorded throughout the experimental period. The latter workers used mainly unanaesthetised dogs but also unanaesthetised and anaesthetised rabbits and one anaesthetised dog. The DNP dosages used were 100 mg./kg. and 50 mg./kg. which were fatal in all cases. The animals, although unanaesthetised, had tracheal cannulae inserted and, in every case but one, the resting VE_{O_2} was high (over 3.5). In these animals the VE_{O_2}

rose after DNP injection and continued to rise indicating the presence of respiratory stimuli other than the increase in metabolism. The origin of these stimuli - whether traumatic, subjective or other - cannot be ascertained from the data reported. In the one anaesthetised dog the resting VE_{O_2} was near normal (2.6), after injection it fell slightly (2.17), and then rose after approximately 14 minutes, continuing to rise until respiratory failure and death ensued. Thus, in this case, the ventilation followed quite closely the increase in metabolic rate up to approximately five times the resting level, as in normal exercise. It is not considered, however, that valid conclusions as to the action of DNP compared to normal exercise may be drawn from the data presented in this paper.

Hall et al. (1933) do not report the time course of their experiments, nor the time elapsed from injection of the drug to the point at which they took their "After injection" readings. Once again, therefore, it is impossible to compare DNP with exercise as to the effects on metabolism and respiration. The authors do, however, make some general comments on their experimental findings. They state that the increase in ventilation occurring after DNP injection is brought about by an increase in both tidal volume and respiratory rate but that the increase in rate is the more important. They further add that the respiratory stimulation is proportionately greater than the metabolic stimulation although whether this is found

throughout the experimental period, or only after a metabolic acidosis is produced, is not noted.

It is therefore not possible to determine from the existing data whether an increased oxygen consumption as produced by DNP injection gives a respiratory response in any way similar to that engendered by a similar rise in metabolic rate as brought about by exercise. It is considered that if DNP can produce metabolic and respiratory responses similar to those found in exercise, they would be more likely to be evinced at lower dosage levels than those used in the experiments reviewed above. Where dosages of 50-100 mg/kg. were used, giving increases in oxygen consumption of up to 1400%, doses of 3 - 10 mg./kg., giving increases of perhaps up to a maximum of 400%, would probably result in responses closer to those found in normal exercise.

Mode of Action of DNP.

In recent years considerable interest has been shown in the mode and site of action of DNP and other similar drugs. Most of the experimental work has been done on tissue cell preparations and cell free mitochondria preparations in vitro.

Loomis & Lipmann (1948) were the first to note that mitochondria in vitro when treated with DNP failed to combine with phosphate in the usual manner. This finding was extended further and Parker, Barnes & Denz (1951) presented a theory of the possible mode of action of the dinitrophenols. The main points of their postulate are as follows:

1. Carbohydrate is the main energy source for the body and the energy is tapped off by formation of compounds with high energy phosphate bands e.g. Adenosine triphosphate (ATP).
2. ATP is the immediate source of available energy.
3. With DNP, ATP formation is inhibited.
4. Oxidative processes speed up and the excess energy usually used to synthesise ATP is dissipated as heat.

In recent years this theory has been changed only slightly as further work has elucidated the site of action of DNP more clearly. It is now generally accepted that the metabolic rate of intact cells is controlled by the rate at which energy rich phosphate is consumed or broken down by the enzyme ATPase, (Simon, 1953). DNP acts as such an enzyme and allows metabolism to proceed at a faster rate since less "Phosphate acceptor" is required. That is to say, DNP acts as an inhibitor of oxidative phosphorylation and thus stimulates glycolysis rather than oxidative metabolism as such.

Whether the actions of DNP noted above actually occur or represent the mechanisms in the intact cell and the intact animal is a matter for speculation. The reactions postulated can explain some of the results obtained from DNP injection in the intact animal but whether they tell the whole story or not is not known.

One interesting inference to be drawn from the theory of DNP action as presented above has to do with the increase in

body temperature noted after injection of the drug. This inference is that the rise in body temperature is not due to any central effect on a heat dissipating centre but that more heat is being produced by the oxidation processes than normally.

Original Observations.

Part of the work on the following pages was carried out in the Physiology Department, Northwestern University Medical School, Chicago, Illinois, U.S.A.

A.

The Effects of Increased Metabolism on the Pulmonary Ventilation in Cross-circulated Dogs.

Experimental Design and Objectives.

Having a drug, DNP, available which will increase the metabolic activity of the tissues it is possible to ascertain if an increase in metabolism, as produced by the drug, will evoke a neural stimulus to ventilation.

If a limb of an animal is prepared in such a way that it is functionally connected to the parent body only by nerves and is perfused with blood from a donor animal then the effect on ventilation of neural stimuli arising from that limb alone may be examined. DNP may be introduced into the blood perfusing the limb and the effect of the increased metabolic activity of the leg on the ventilation of the recipient animal noted.

Using such a cross-circulation technique the experimental objectives would be as follows:

1) Does an increase in metabolism of the perfused limb, as produced by DNP, cause an increase in ventilation in the recipient animal?
2) If the nerves connecting the perfused limb and the recipient animal be cut does a rise in metabolism of the leg produce a rise in ventilation?
3) Is any rise in ventilation noted due to the rise in metabolism of the leg produced or is it the result of a direct stimulating effect of DNP on nerve endings?

Experimental Methods.

Three groups of mongrel dogs weighing 9 - 18 kg. were used. In the first group, the "nerve intact" group, consisting of ten pairs of animals, the sciatic and femoral nerves were left intact as described below. The second group, the "nerve cut" group, also consisting of ten pairs of animals, differed from the first group in that the sciatic and femoral nerves were sectioned before instituting the cross perfusion. The procedure followed is detailed below. The third group, the "control" group contained eight pairs of animals and treatment of these animals only differed from that of the "nerve intact" group in that saline instead of DNP was injected.

The dogs were under morphine and barbitone sodium (sodium diethylbarbiturate) anaesthesia during the surgical and experimental procedures. Morphine (40 mg./kg. body weight) was injected subcutaneously and, after 30 minutes, anaesthetisation was completed with barbitone (200 mg./kg. body weight).

After placing the animals in a supine position tracheal cannulae were inserted. In the animal arbitrarily chosen to be the recipient the sciatic nerve in the right hind limb was exposed via an incision in the lateral part of the thigh. In the "nerve cut" group 1% procaine hydrochloride was injected into the nerve sheath and the nerve sectioned using an electric cantery. The wound was then closed with wound clips. In the "control" and "nerve intact" groups the nerve was gently elevated

and a waxed rope, to be used as a tournequet on the leg, passed under it. The nerve and exposed tissues were covered with moist gauze packs.

The femoral artery and vein in the right hind limb were then exposed for cannulation and the femoral vein of the left hind limb exposed for any necessary injections.

In the donor dog the femoral artery and vein of both hind limbs were exposed for cannulation.

The animals were then left for one hour to allow haemostasis to be established. After this interval 2000 units of heparin was injected intravenously into each dog and the previously exposed blood vessels cannulated. The cannulae and tubing system were completely filled with physiological saline before use. The cross circulation system is shown in Figure 3. The cannulae, T-tubes and Y tubes were made of glass, thoroughly cleaned and silicone coated. The connecting tubing was polyethylene transfusion type tubing. The heparin drip system was regulated so that a flow of 1000 units of heparin in 60 cc of saline passed into the venous cannula system per hour. Once flow through the system had been established the rope tournequet was put in position firmly as high on the limb as possible. The measuring and recording apparatus was then put in place.

The muscle temperatures of the hind limbs of the recipient animal, both perfused and normal, were measured through two copper-constantin thermocouples, shielded by No. 13 needles 3

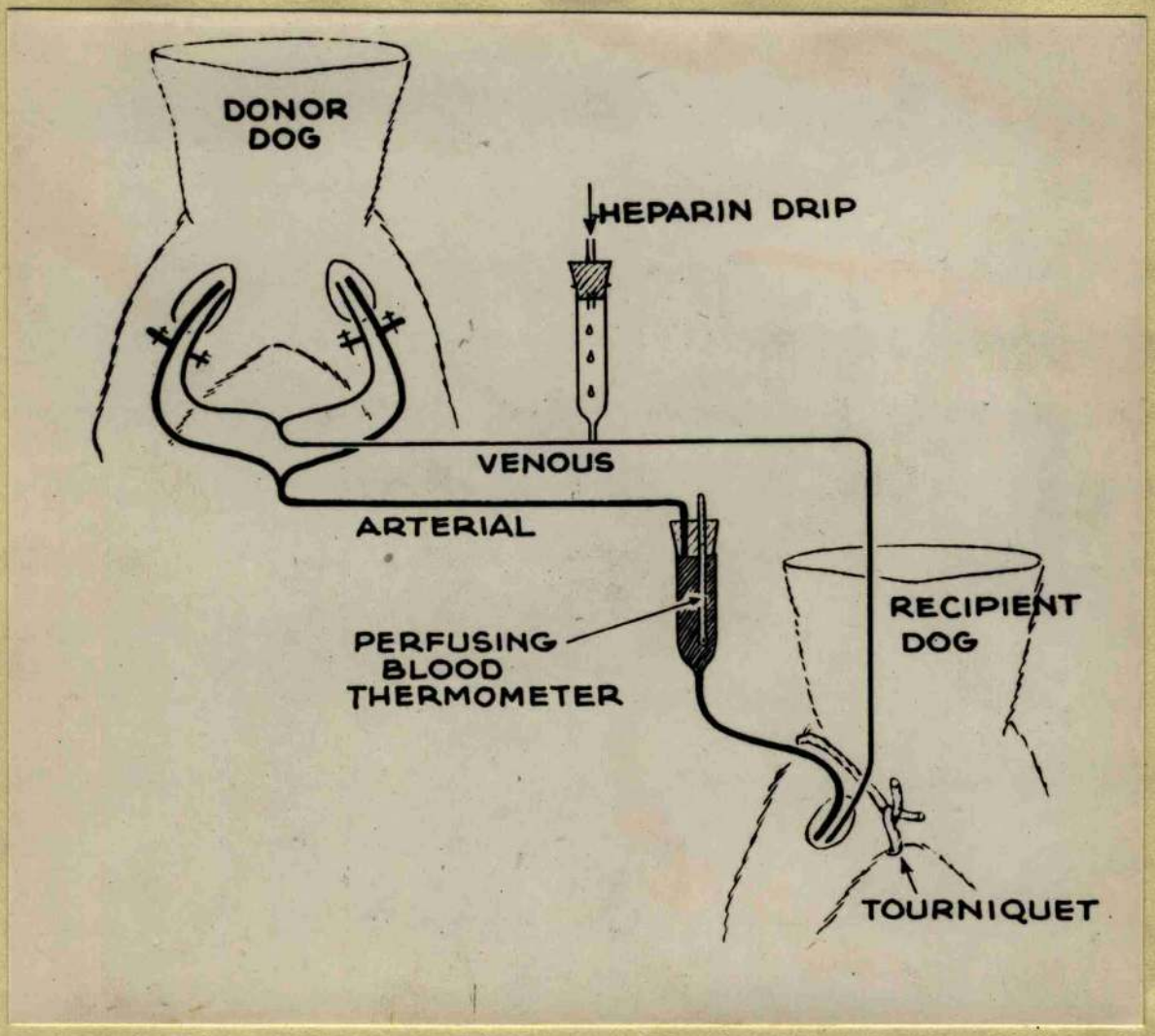


Figure 3. Cross circulation arrangement (diagrammatic).

inches long. These needles were thrust deep into the muscles of the limb. The temperatures were recorded automatically by a two channel Honeywell recording instrument.

Rectal temperatures were measured by mercury thermometers and the perfusing blood temperature by a mercury thermometer situated at the distal end of the arterial cannula system as shown in Figure 3.

Benedict-Roth Spirometers, one to each animal, were connected to to the tracheal cannulae and used to measure and record the respiratory movements. Records were taken for five minute periods every ten minutes and from the graphics thus obtained respiratory rate, tidal volume, pulmonary ventilation and oxygen consumption were calculated.

After the measuring and recording apparatus was all in place readings were taken for a period of 35 minutes after which time the injection of DNP was made. The preparation used was a 3% solution of DNP in 1.5% sodium bicarbonate. The dosage was 5 mg. DNP/kg. body weight of the donor dog and was administered by injecting into the arterial cannula system to the perfused leg. Readings were taken for the succeeding 35 minutes and the dogs then sacrificed by intravenous injection of an overdose of Nembutal.

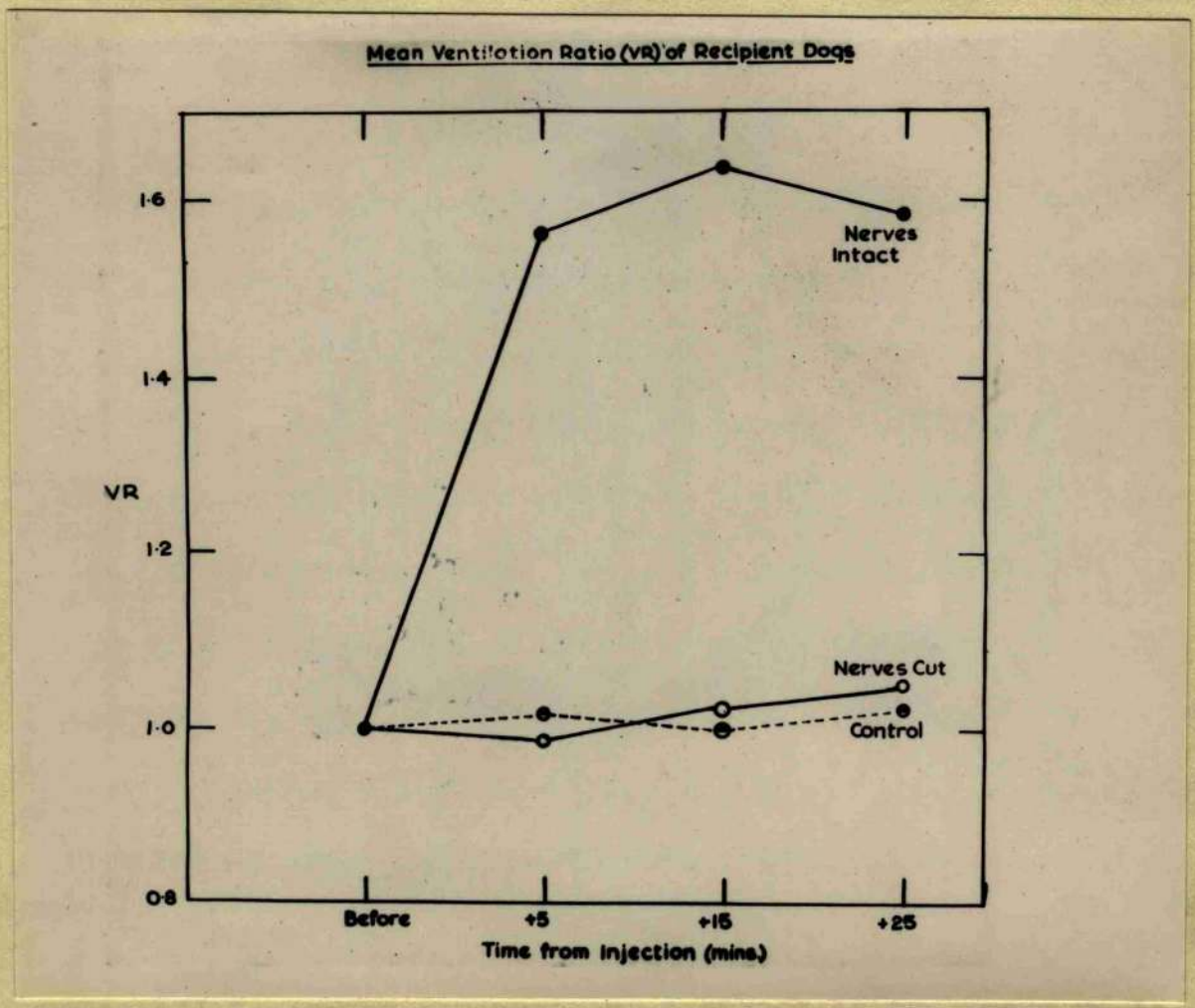
Results.

I. Respiratory and metabolic responses of animals with perfused hind limbs.

The object of the experiments here reported was to determine any change in ventilation of the recipient animal when the oxygen consumption of the perfused limb was raised by DNP.

The results are presented as graphs of mean values, from which may be noted the overall responses of the groups.

Since the absolute values of ventilation vary considerably between animals in each group, these measurements were converted to ratios. The mean ventilation of each animal, in litres/minute, during each five minute interval after the injection of DNP was compared to the corresponding mean ventilation during the 35 minute control period before injection. The mean ventilation ratio (VR) for each group of animals was then calculated and these values are shown in Graph 9. It may be seen here that five minutes after the injection of DNP into the perfusing blood the ventilation of the recipient animal, when the nerves from the leg were intact, increased to a mean level of 1.57 ($\pm$0.63) times the resting control level. In the group of animals in which the sciatic and femoral nerves supplying the perfused leg were cut the mean VR was 0.99 ($\pm$0.1) five minutes after injection, and, in the control group, where saline instead of DNP was injected, the mean VR was 1.01 ($\pm$0.05) five minutes after injection. The mean VR of the

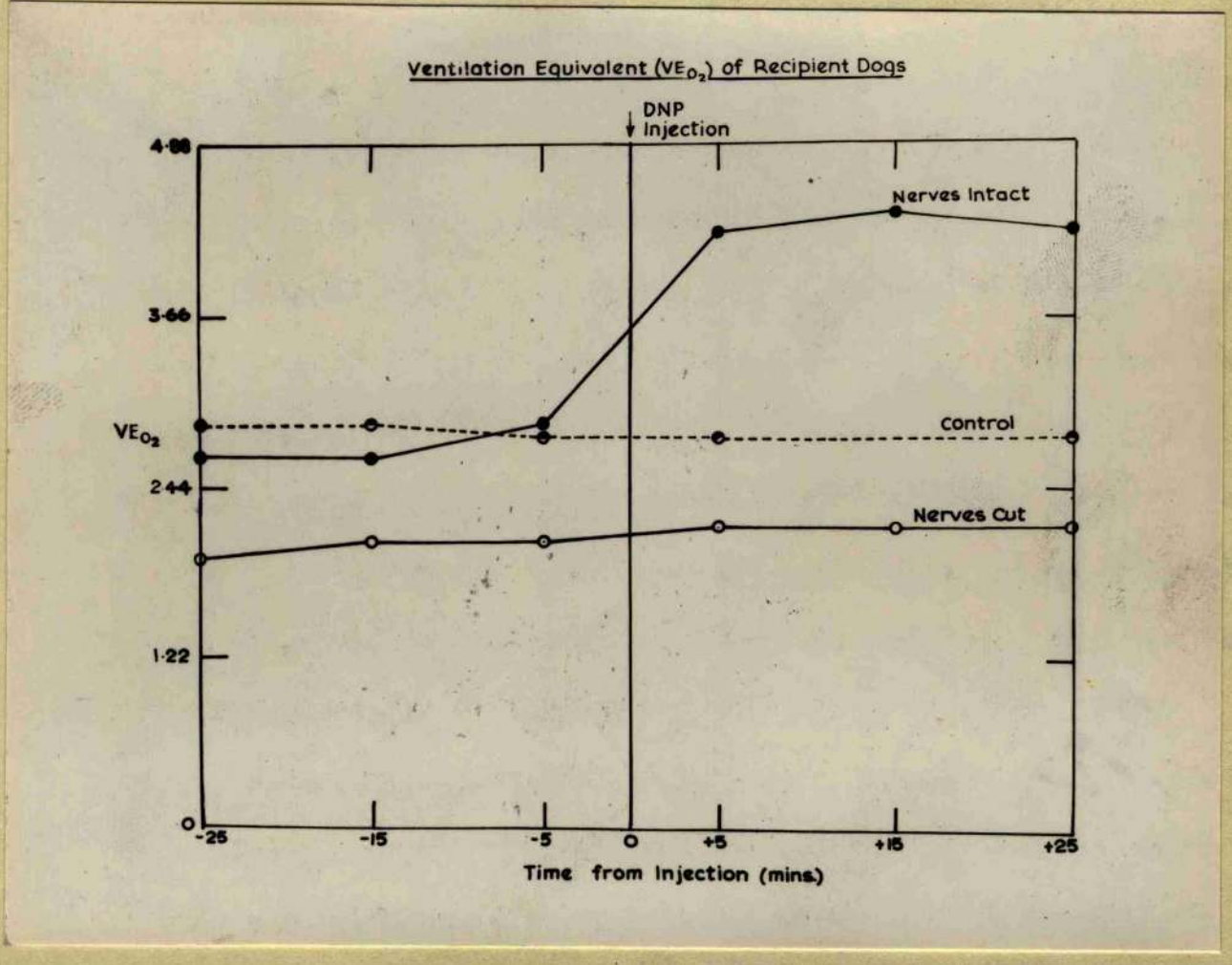

Mean Ventilation Ratio (VR) of Recipient Dogs
1·6
1·4
1·2
1·0
0·8
VR
Nerves Intact
Nerves Cut
Control
Before
+5
+15
+25
Time from injection (mins.)

nerve intact group is significantly raised from both the nerve cut group (P=0.01) and the control group (P=0.024). It is apparent from these results that an increase in metabolism, as produced by DNP, of the perfused limb produces, via neural stimuli, an increase in the ventilation of the recipient animal, to a value approximately 1.6 times the resting value, in the steady state.

The increase in ventilation shown in Graph 9 is not because of any increase in metabolic rate of the recipient animals. No significant change in the oxygen consumption of the recipient animals occurred in any group throughout the experimental procedure. From these findings it is also inferred that no "leakage" of DNP from the perfused leg into the body of the recipient animal occurred. In one experiment, which was discarded, some leakage of DNP did occur, resulting in an immediate increase in oxygen consumption of the recipient animal.

The derived measurement VE_{O_2} $\left(\frac{\text{Ventilation (l/min. BTPS) x 100}}{O_2 \text{ consumption (cc/min. STPD)}}\right)$ is useful in considering the relationship between ventilation and oxygen consumption. Since ventilation is normally directly proportional to oxygen consumption, a change in the value of VE_{O_2} from the normal (approximately 2.5) may be used as a measure of additional stimuli affecting the respiratory system. The values for VE_{O_2} are presented in Graph 10.

In the present series of experiments it would be expected that the mean resting control value of the VE_{O_2} would be the same, or nearly so, for both the "nerve intact" and the "control"


Ventilation Equivalent (VE_{O_2}) of Recipient Dogs
DNP Injection
4·88
3·66
2·44
1·22
0
VE_{O_2}
Nerves Intact
Control
Nerves Cut
-25
-15
-5
0
+5
+15
+25
Time from Injection (mins.)

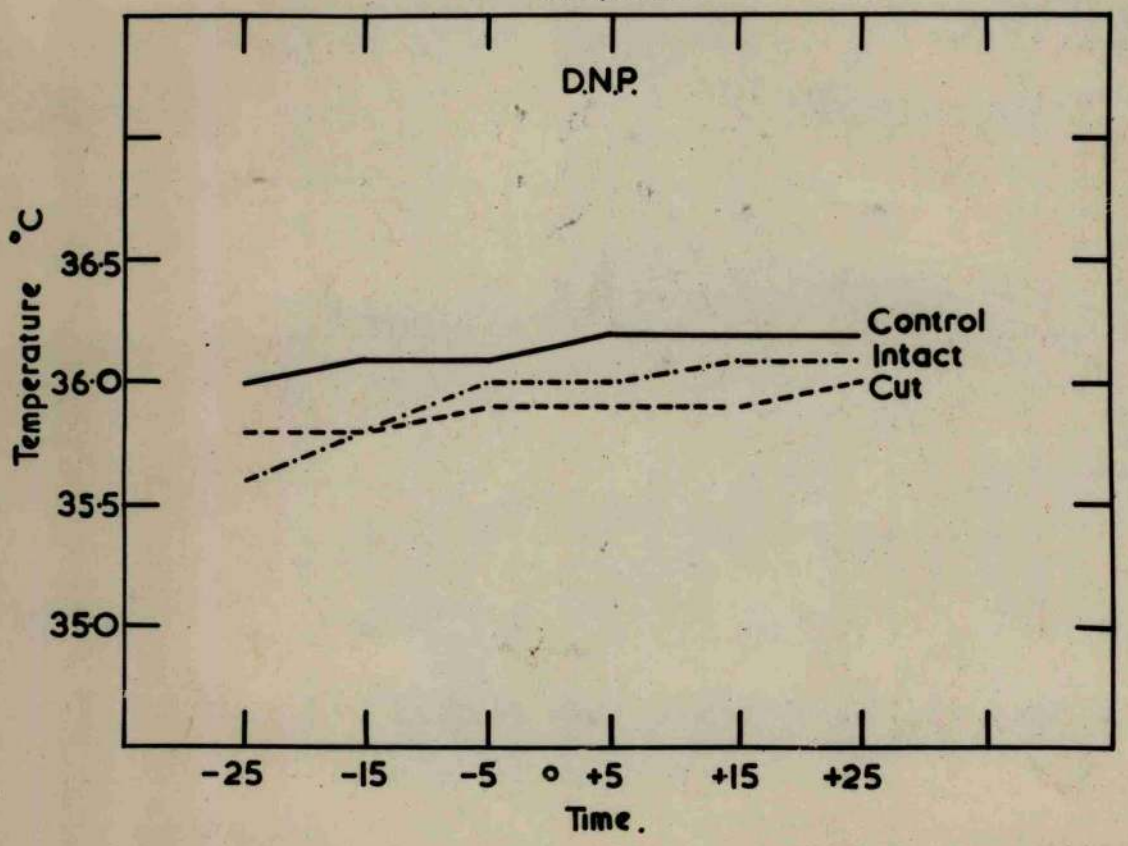
groups, since the animals in these two groups are under identical conditions. It would also be expected that the resting VE_{O_2} of the "nerve cut" group would be lower than either of the other two groups, since any stimuli to ventilation arising in the perfused limb, and travelling by nerural pathway, are eliminated. Graph 10 illustrates that these predictions are fulfilled. From this graph it may also be seen that 5 minutes after injection of DNP the mean value for the VE_{O_2} of the nerve intact group rose to 4.17 ($\pm$1.5) whereas the value for the control group remained steady at 2.82 ($\pm$0.5) as did the nerve cut group at 2.10 ($\pm$0.4). Because of considerable variation between the individual animals, the mean value of VE_{O_2} for the nerve intact group 5 minutes after DNP injection is not significantly different from that of the control group ($P=0.06$), but it is significantly different from the mean value for the nerve cut group ($P= 0.01$).

There is therefore adequate evidence that the addition of DNP to the blood perfusing the vascularly isolated limb initiates a stimulus to ventilation which is transmitted to the respiratory centre by a nervous pathway.

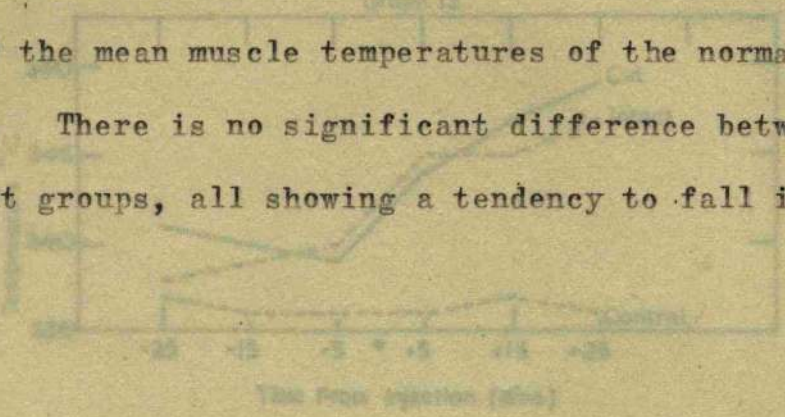
The mean values for the rectal temperatures throughout the experimental procedures are plotted in Graph 11. It may be seen that no significant difference in rectal temperature existed between any of the groups.

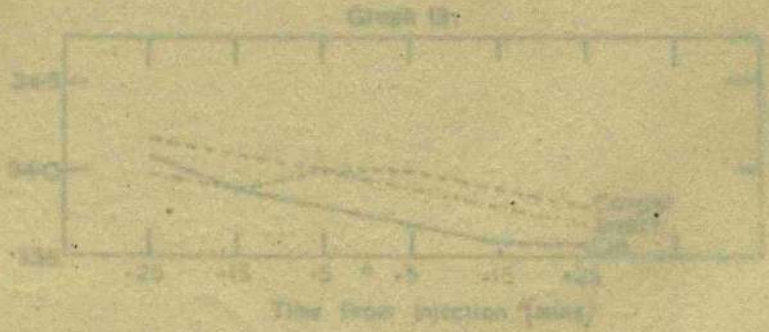
The mean muscle temperatures of the perfused right hind limb and normal left hind limb of the recipient animals are

Graph 11.

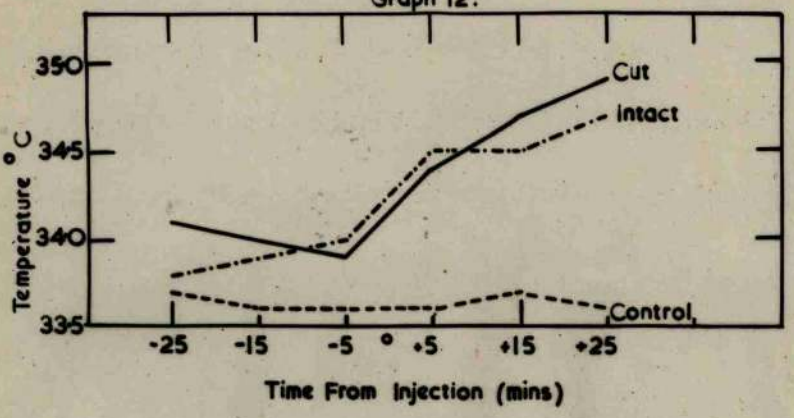

D.N.P.
Temperature °C
36·5
36·0
35·5
35·0
Control
Intact
Cut
-25
-15
-5
o
+5
+15
+25
Time.

shown in Graphs 12 and 13. It may be seen in Graph 12 that injection of DNP into the perfusing blood produced a fairly steep rise in temperature. The mean rise in muscle temperature only amounted to approximately 0.85 C^{o} in 25 minutes, however, which does not constitute a very great increase in muscle temperature, and also, since it is in the mean range of 34-35^{o}C cannot account for the rise in ventilation noted. In Graph 13 the mean muscle temperatures of the normal legs are plotted. There is no significant difference between the different groups, all showing a tendency to fall in temperature.

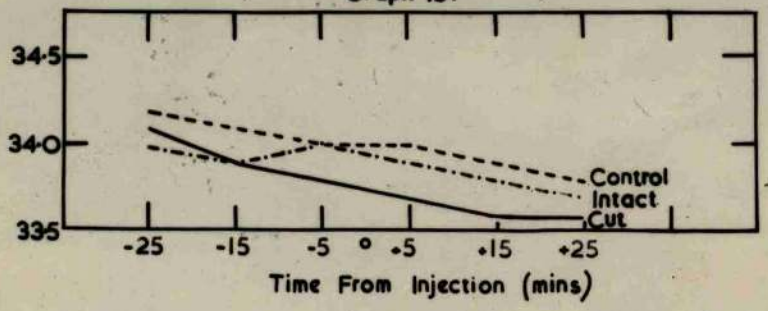




Graph 12.



Graph 13.



II. Respiratory and metabolic responses of the donor animals after DNP injection.

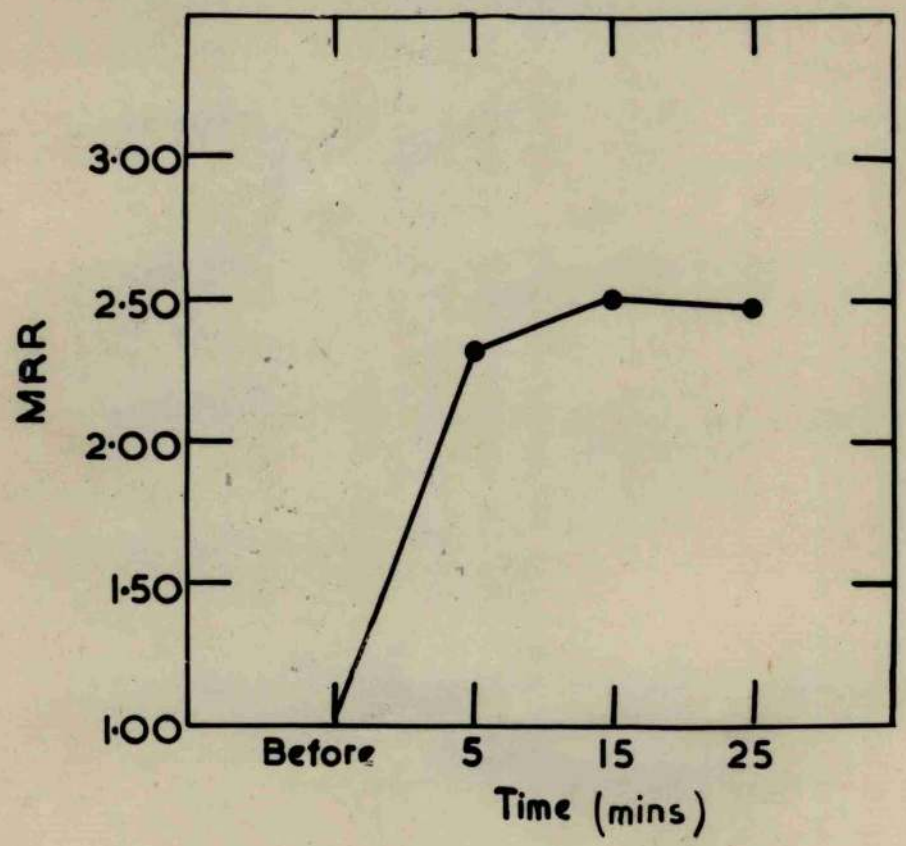
It was felt that the data collected on the oxygen consumption, respiration and rectal temperature changes of the animals injected with DNP would be a useful contribution to the knowledge of the pharmacology of the drug. For this reason the measurements recorded from the donor animals are presented.

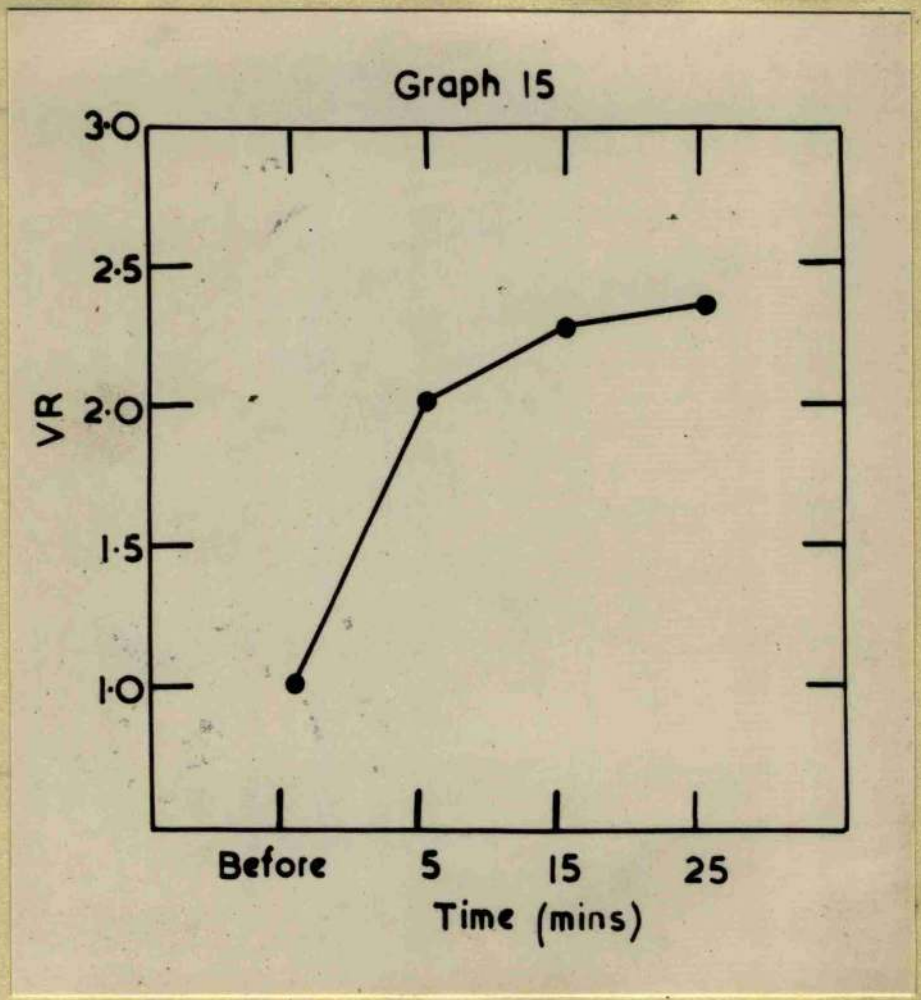
Graph 14 shows the mean metabolic rate ratio (MRR) values. It may be seen from the graph that 5 minutes after injection of 5 mg./kg. body weight of DNP the mean oxygen consumption is raised to 2.33 ($\pm$0.44) times the mean resting level. The steady state is reached in from 10 to 20 minutes with a mean increase to approximately 2.5 ($\pm$0.4) times the mean resting level. That is to say that a dosage of DNP of 5 mg./kg. increases the metabolic rate to two to three times the resting level.

Corresponding data on the ventilation ratio are given in Graph 15. Five minutes after injection of DNP the ventilation had risen to a mean value of 2.03 ($\pm$0.56) times the mean resting level. The steady state was reached in 15–25 minutes at a level of ventilation approximately 2.4 ($\pm$0.7) times the resting level. This range of increase of ventilation is not significantly different from that noted above for the increase in metabolism.

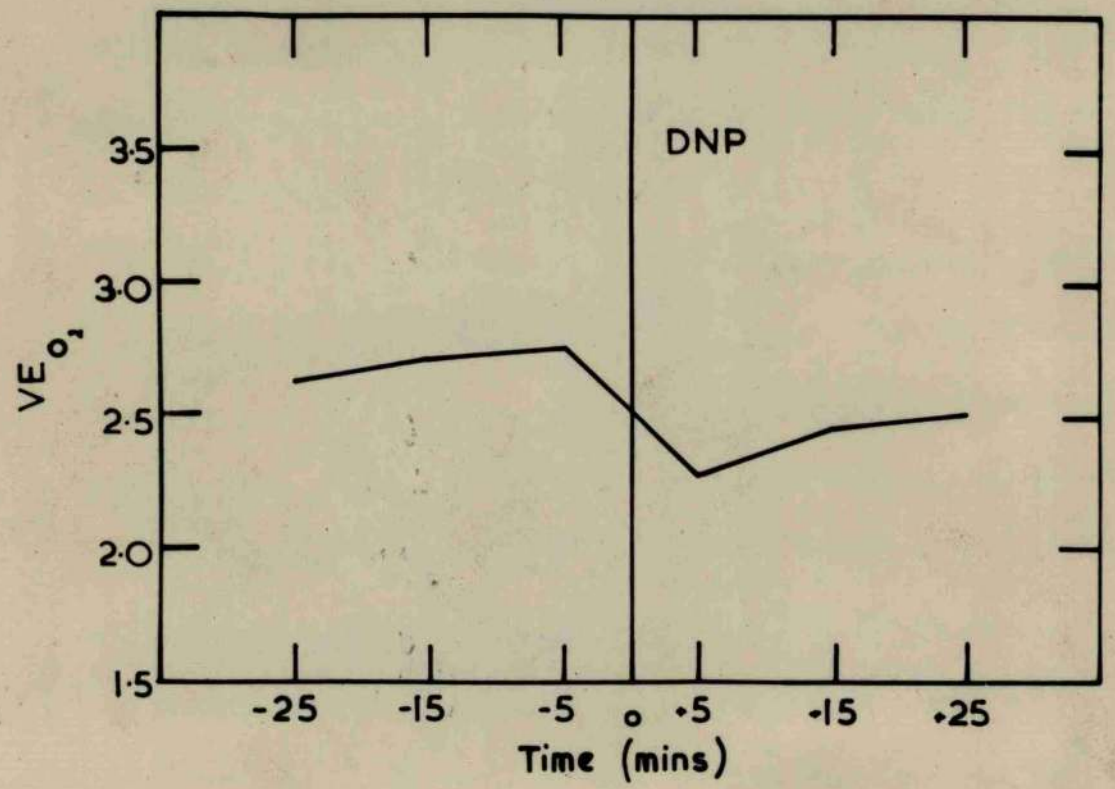
Data on VE_{O_2} are presented in Graph 16. The most striking feature of this data is the sharp drop in VE_{O_2} after the injection of DNP. When the experimental conditions are considered, however, it is seen that such behaviour of the VE_{O_2}

Graph 14.




Graph 15
3·0
2·5
2·0
1·5
1·0
VR
Before
5
15
25
Time (mins)

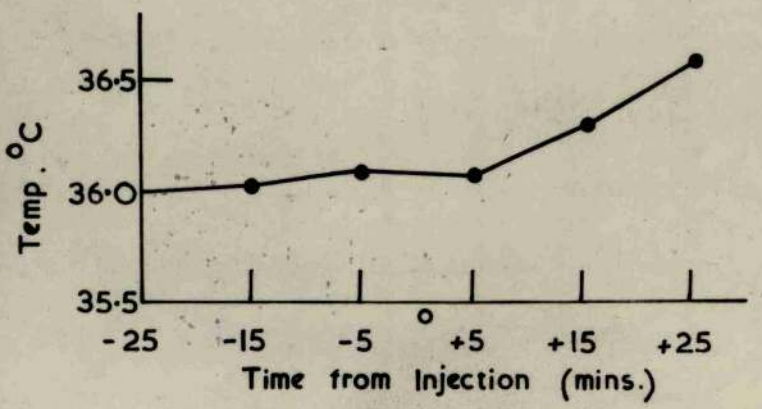
Graph 16.



is to be expected. The drug is injected into the arterial perfusing blood of the perfused limb and the initial stimulus to ventilation in the donor animal will be almost wholly humoral, i.e., the oxygen consumption will increase to an extent relatively greater than the ventilation, giving a fall in VE_{O_2}. In the steady state humoral stimulation will still be greater than neural stimulation, proportionately, since there is no neural connection with the perfused leg, and the VE_{O_2} will be lower than the resting control level. Graph 16 shows that the mean values do indeed follow this pattern.

The rectal temperatures of the donor animals were recorded throughout the experimental procedures. The measurements are given in Graph 17. The graph shows the rise in rectal temperature which is one of the characteristic responses to administration of DNP. It should be noted that the mean rectal temperature does not begin to rise until 10-15 minutes after the injection is made, and that 25 minutes after injection it is still rising. It was not determined how long a time would be required for the rectal temperature to reach a steady state.

Graph 17.
Rectal Temperatures.
Donor Dogs.



Discussion.

The results of the experiments reported in the previous section show that an increase in metabolism of the leg muscles, as produced by the drug DNP, initiates a stimulus to ventilation which is transmitted neurally to the respiratory centre. Before such a result can be said to support the theory of the existence of receptors sensitive to some part of the energy producing cycle in muscle it must be shown that

a) DNP acts in the energy producing cycle.

b) The ventilatory response to an increased metabolism produced by DNP is the same as that to normal exercise.

c) DNP does not itself directly stimulate nerve endings.

The first requirement has been shown as is noted in the review of the literature on DNP earlier in this paper.

The second requirement has not been investigated at present. The literature available, as earlier noted, is scant with regard to accurate quantitative data on this matter. The data presented in this section is a beginning towards establishing a dose-response curve for the drug, which is one of the first requisites to supporting the requirement being discussed. The data obtained from the donor dogs agrees closely with the ventilatory response to normal exercise. Further investigation is necessary before it can definitely be said that the ventilatory response to increased metabolism, as produced by DNP is the same as that to normal exercise.

The third requirement has, as yet, no experimental verification,

There are, however, some points of evidence which suggest that the DNP solution used does not directly stimulate nerve endings. Moore et al. (1934) investigated the ability of acids, alkalis, and certain ions to stimulate nerve endings in muscle directly. It was found that solutions of concentration 0.1$\bar{N}$ or higher or with a pH lower than 6.3 or higher than 9.2 stimulate nerve endings. Solutions of less concentration and within the pH range 6.4 - 9.0 do not stimulate nerve endings. From these findings it can at least be said that stimulation of nerve endings did not occur from injection of DNP by virtue of the pH or concentration of the solution. Another point to be taken into consideration is that direct stimulation of nerve endings by chemicals injected into the circulation of a limb causes muscle twitching, extension of the limb, gasping respiration and other such phenomena. No phenomena of this type occur from injection of DNP.

The experiments reported here have shown that a neural stimulus to ventilation arises in muscle whose metabolism has been raised, thus giving support to one part of the metaboreceptor theory. It is considered that the experimental data presented provides a basis for the inference that the mechanisms of the increase of ventilation in normal exercise and after injection of DNP are at least similar if not identical. Further experimental data on the actions of DNP on the metabolism - ventilation relationship and on nerve are necessary before the theory of metaboreceptors and their part in the control of respiration can

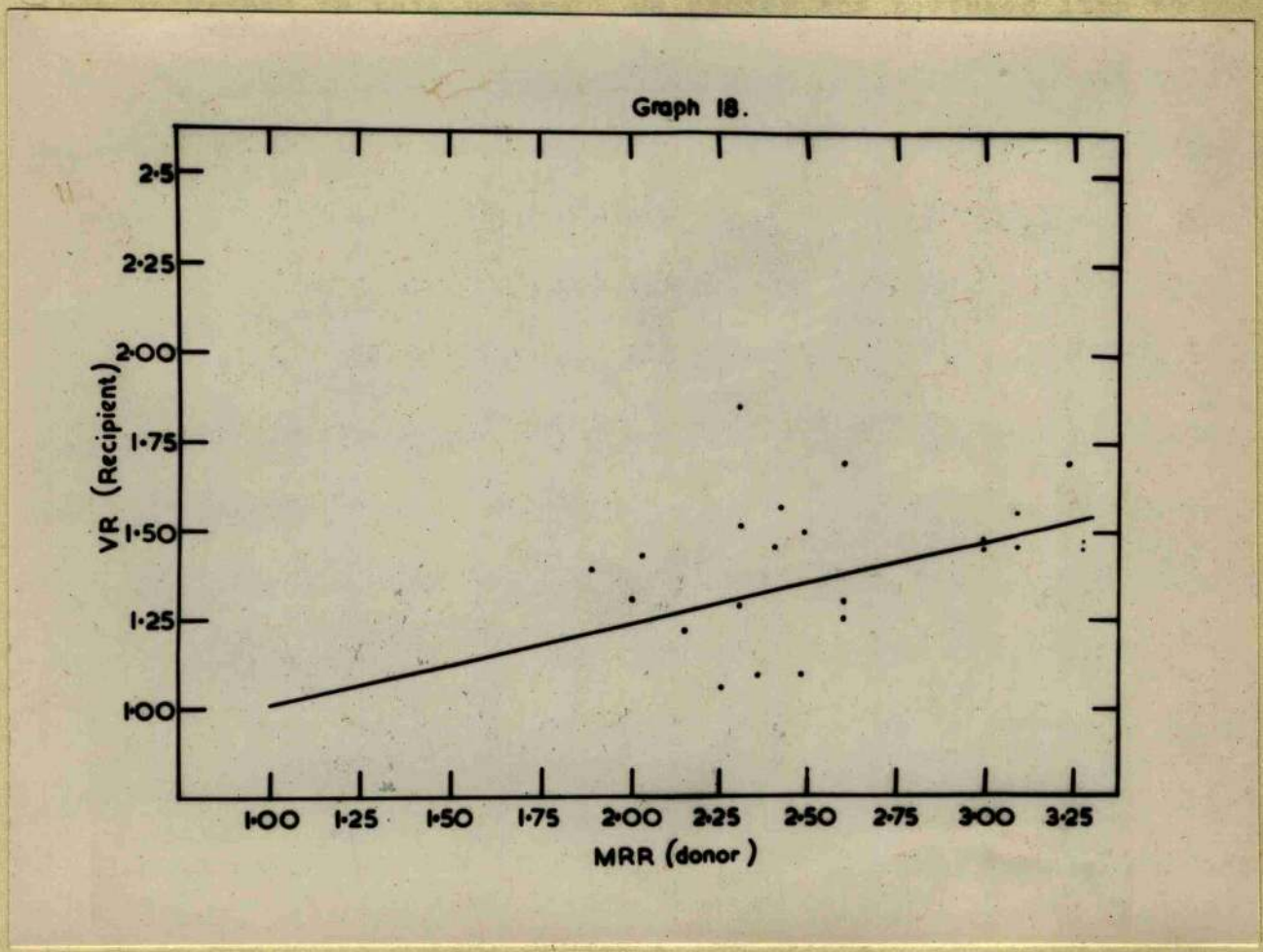
be fully supported.

As mentioned earlier, since this work was completed, some Roumanian workers (Hortolemei et al., 1954) have reported the results of experiments using a preparation similar to that described in this paper. These workers changed the conditions of the blood perfusing the isolated limb and recorded resulting changes in the ventilation of the recipient animal. By adding various drugs such as acetylcholine and adrenaline and various buffer solutions to the perfusing blood and also by giving the donor animal various gas mixtures to breathe, they produced changes in the ventilation of the recipient dog. Hortolemei claims that the results obtained prove the existence of nerve receptors in muscle which are sensitive to chemical changes in the muscles and their blood supply. This work gives considerable support to the theory advanced earlier in this paper and the conclusions, independently arrived at by Hortolemei and his co-workers, show close agreement to those of this paper.

The correlation of the results with previous experimental data.

In Graph 18 the ventilation ratio (VR) of the recipient animals is plotted against the metabolic rate ratio (MRR) of the donor animals. The values used are the steady state values obtained 15 and 25 minutes after injection of DNP.

It was found that there was a significant linear correlation between the two sets of measurements ($r = 0.46$. $P = 0.05$). It was further calculated that a line with the equation $Y = 0.23X + 0.78$ represents the relationship between MRR (Donors)


Graph 18.
2·5
2·25
2·00
1·75
1·50
1·25
1·00
VR (Recipient)
1·00
1·25
1·50
1·75
2·00
2·25
2·50
2·75
3·00
3·25
MRR (donor)

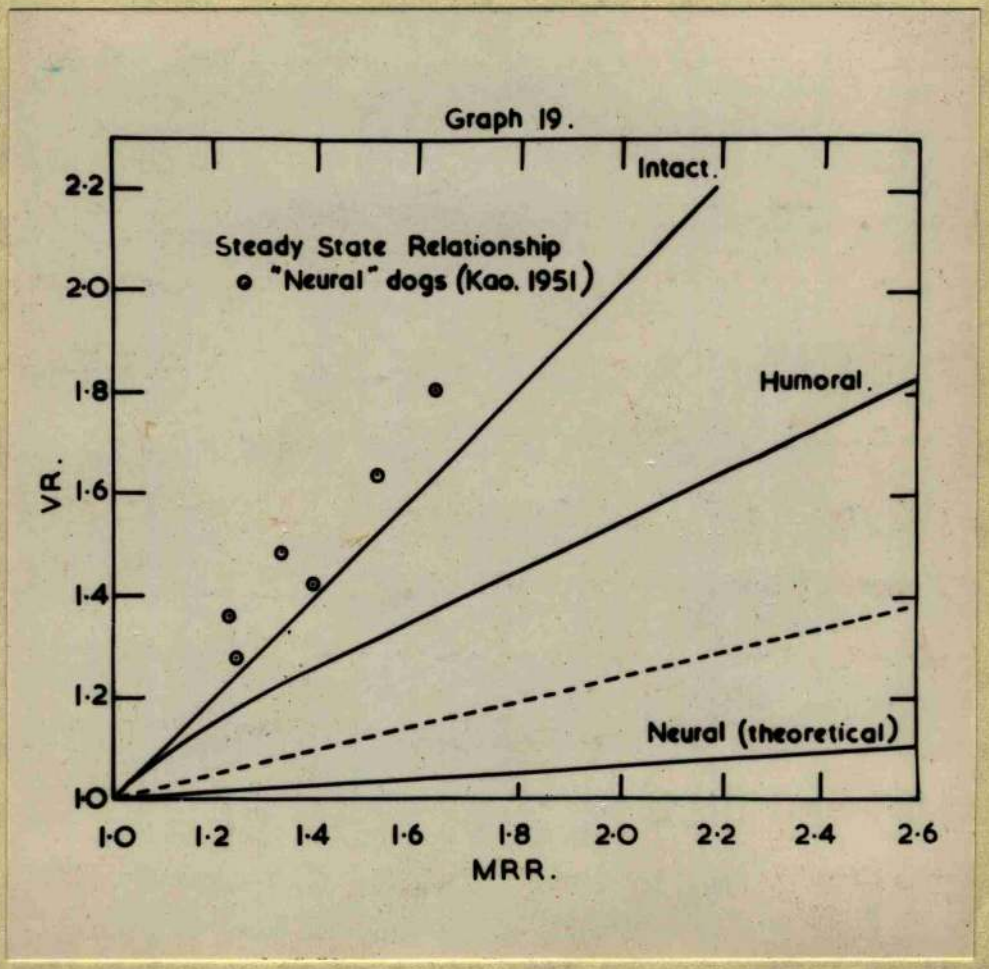
and VR (Recipients) under the experimental conditions.

With such a relationship established it is possible to compare the data with previous findings obtained from similar experimental preparations.

Kao (1951) used a similar cross circulation technique to that described in this paper. He caused the perfused limb to exercise by electrical stimulation and attempted to differentiate between neural and humoral components of the stimulus to ventilation produced.

In Graph 19 are shown the various relationships. The line marked "Intact" represents the direct relationship between VR and MRR found in intact man. Induced exercise in the intact anaesthetised dog has been shown to give a relationship not significantly different from this (Morgan & Grodins, 1950; Kao & Ray, 1954a; Kao, Schlig, & Brooks, 1955). The curve marked "Humoral" represents the ventilatory response in exercise to chemical stimuli alone as noted in Kao's "Humoral dogs".

The problem at this point is to establish the relationship of VR to MRR under purely neural stimulation. The theoretical relationship is represented by the line marked "Neural" in Graph 19 (Grodins, 1950). Before data from the recipient animals may be compared with this theoretical line the metabolic rate of the perfused limb must be established. With electrically induced exercise of the cross perfused legs it may be assumed that all of the increase of metabolism recorded in the donor dog is due


Graph 19.
Intact.
Steady State Relationship
"Neural" dogs (Kao. 1951)
Humoral.
Neural (theoretical)
VR.
MRR.
2·2
2·0
1·8
1·6
1·4
1·2
1·0
1·0
1·2
1·4
1·6
1·8
2·0
2·2
2·4
2·6

to the leg exercise. The experimental points obtained by Kao, noted on the graph, are much too high and also the increase in MRR is of a low order of magnitude. The artefacts and abnormal stimulation caused by the electrical stimulation probably are the reasons for these findings. With a rise in metabolism of the perfused limb being produced by DNP in the present series of experiments it is necessary to know what fraction of the increase in MR_{O_2} of the donor dog may be attributed to the perfused limb. This figure could be found by analysis of the perfusing blood and recording the rate of flow of the perfusing blood or by clamping off the perfused leg and noting any change in the MR_{O_2} of the donor dog. The points on Graph 19 and the line fit to these points, as noted in Graph 18, represent the steady state data of the present experiments. They are entered on the graph assuming that 100% of the increase of MR_{O_2} of the donor dog is due to the perfused limb. It may be seen that the points are not as high as those obtained by Kao but their position on the horizontal scale cannot be determined without knowledge of the MR'_{O_2} of the perfused limb.

Another factor to be taken into consideration is that under anaesthetic, the sensitivity of the respiratory centres to humoral stimuli is almost certainly lowered. The effect of this would be to lower the "Humoral" and raise the "Neural" curves in Graph 19. Thus not only can the experimental points not be accurately placed on the graph with the available data but the theoretical

lines may not hold under the prevailing experimental conditions.

In order to fully evaluate the implication of the results obtained in the present study, and their relationships to previous work it is necessary to determine

a) the fraction of the overall increase in oxygen consumption measured in the donor animals which is attributable to the perfused limb.

b) the effect of DNP in the intact animal and the relation of this effect to that produced by exercise.

c) the effect of anaesthesia on the respiratory response to DNP.

All of the work described on succeeding pages was carried out in the Institute of Physiology, University of Glasgow.

B.

The Measurement of the Oxygen Consumption of the Isolated, Perfused Hind Limb of the Dog.

Examination of the data from the experimental work described earlier showed the necessity for further investigation of certain points before full evaluation of the data could be made.

The first consideration was the determination of the changes in oxygen consumption of the perfused limb.

METHODS.

Respiratory Measurements.

It was considered that if accurate measurements of oxygen consumption and ventilation in dogs, and as later described, in cats, were to be made, then the standard Benedict-Roth spirometer was not adequate. Trouble had also been experienced, on occasion, with the recording of rapid rates of breathing on the standard Benedict-Roth. Bernstein and Mendel (1953) described a new type of spirometer designed for taking Maximum Breathing Capacity measurements in human subjects and it seemed that their design would form a good basis for an accurate animal spirometer. A spirometer half the size of that described by Bernstein and Mendel, but of the same design, was constructed. A scale diagram is shown in Figure 4. A photograph of the spirometer is shown in Figure 5. The perspex bell has an effective volume of 1400 ml. which has been found to be quite large enough for use with dogs, and small enough to record tidal volumes accurately to 2 ml. In order to achieve such accuracy a special frontal-writing lever was attached to the counter weight of the spirometer bell so that the movements of the bell could be subjected to

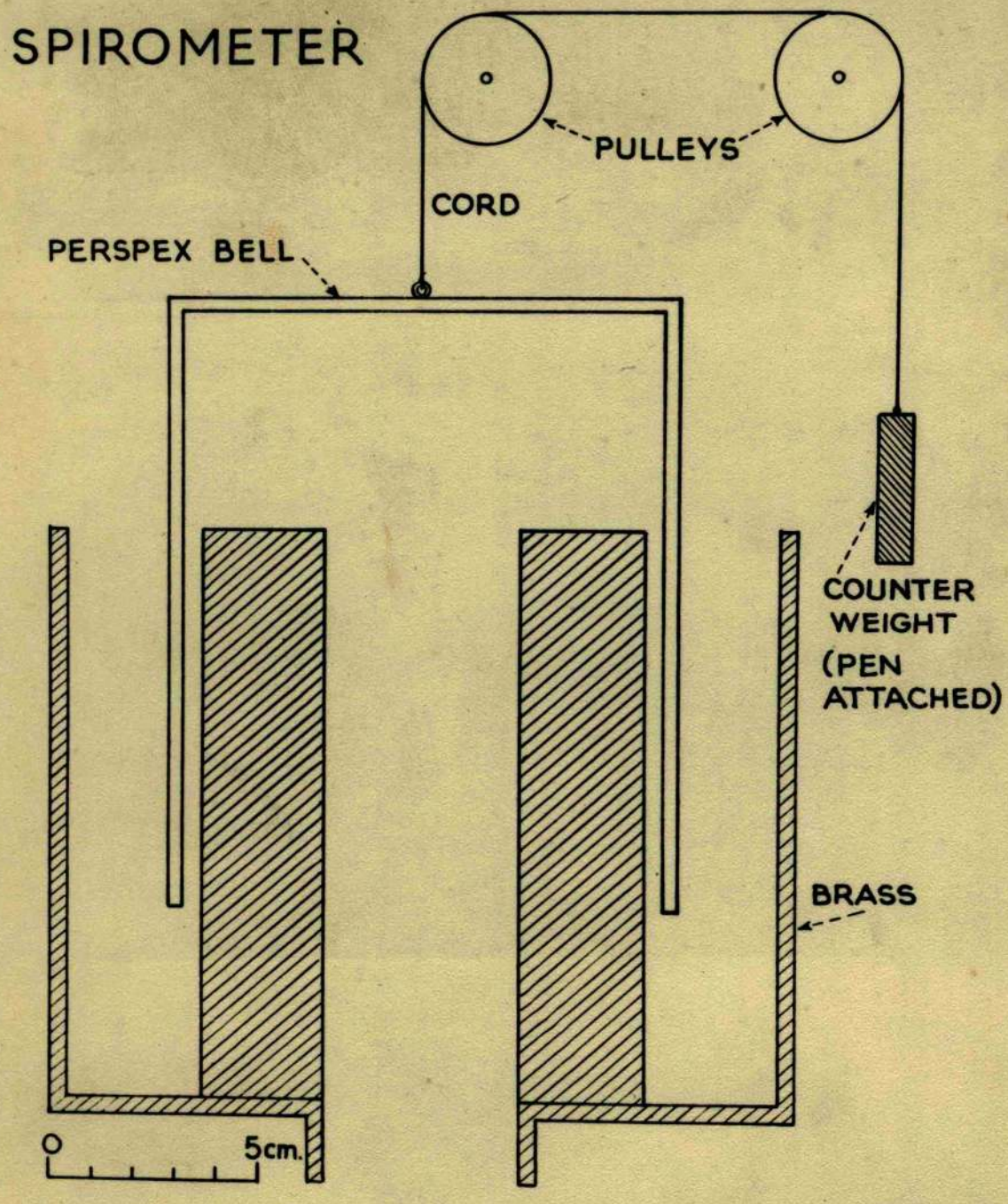


Figure 4. Scale diagram of spirometer.

Figure 5. View of spirometer. The brass upright to the immediate right of the spirometer is the support for the pulleys and counterweight.

varying degrees of lever-magnification. The arrangement is shown in Figures 6 and 7. The type of trace recorded from a dog is seen in Figure 8. This photograph was taken during an actual experiment, movement of the lever causing it to appear somewhat blurred.

In order to measure oxygen consumption in the same manner as with a Benedict-Roth Spirometer it was necessary to have some means of absorbing expired CO_2 and also some valve mechanism to ensure one-way gas flow in the system. In order to provide adequate means for the absorption of CO_2 special gas absorbing tubes were devised. Soda Asbestos ("Carbosorb") 3 - 6 mesh, was used as the absorbing medium. As the respirometer system was first set up, water valves were used to provide for one-way gas flow. A schematic diagram which shows the construction of both the CO_2 absorbing tubes and the water valves and their positions in the system is shown in Figure 9. Two absorbing tubes were used connected in parallel to ensure as low a resistance to gas flow as possible and to minimise the danger of blockage in the absorbing tubes. Figure 10 is a photograph of the apparatus ready for use. Figure 11 shows another view of the CO_2 absorbing tubes. The water valves were found to be quite successful when initial testing of the apparatus was carried out with cats. When, however, dogs were used it was found that the water valves were not fully satisfactory at the higher volume and flow rates involved. For this reason, low

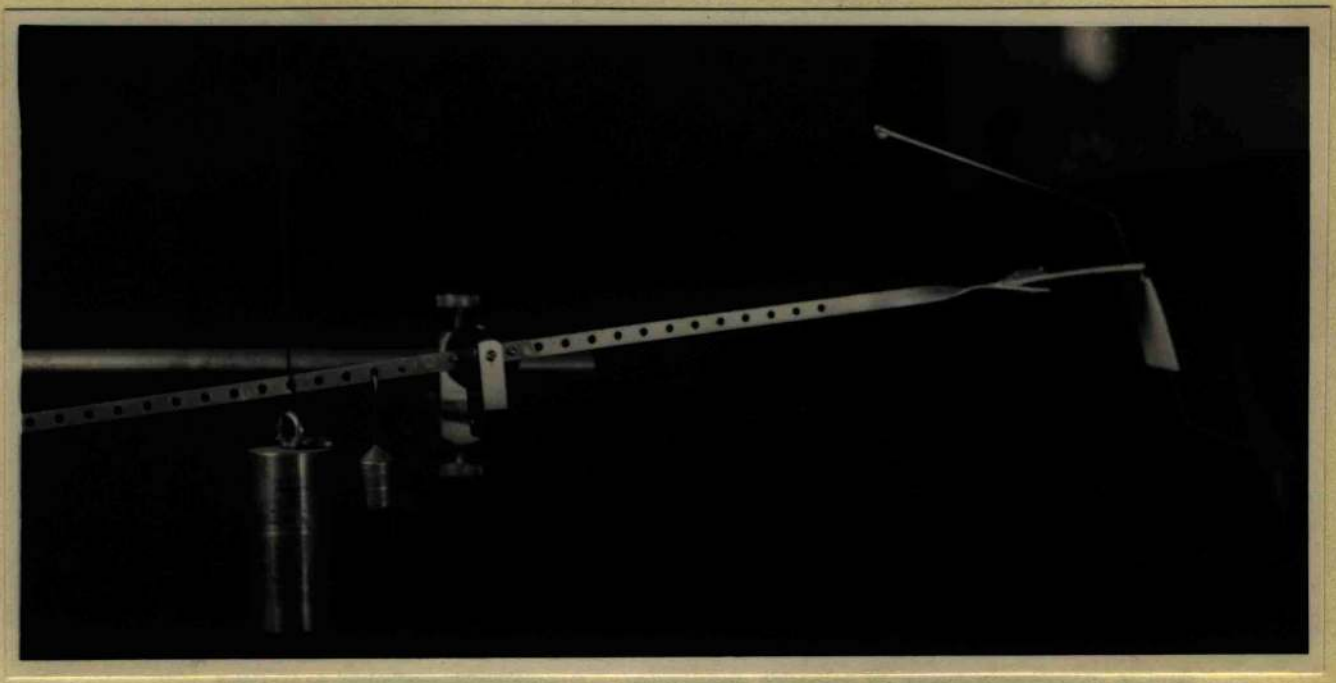


Figure 6. Lever and frontal writing point and attachment to counter weight of spirometer.

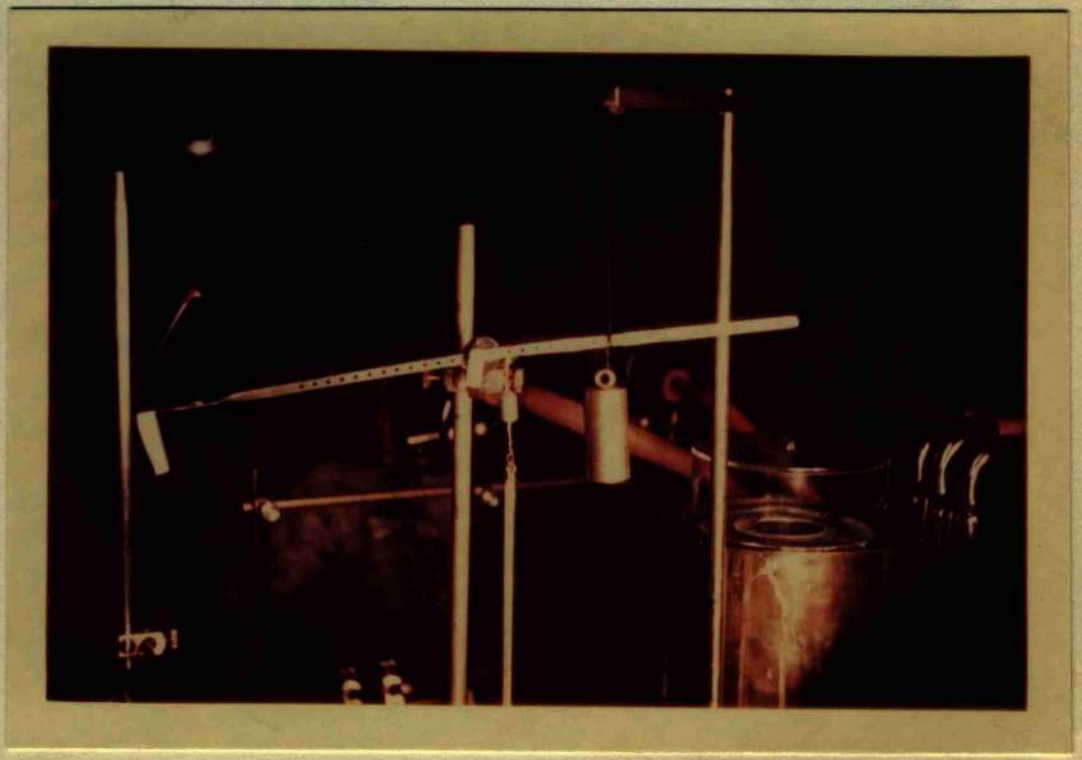


Figure 7. Another view of method of attachment of counter weight to lever.

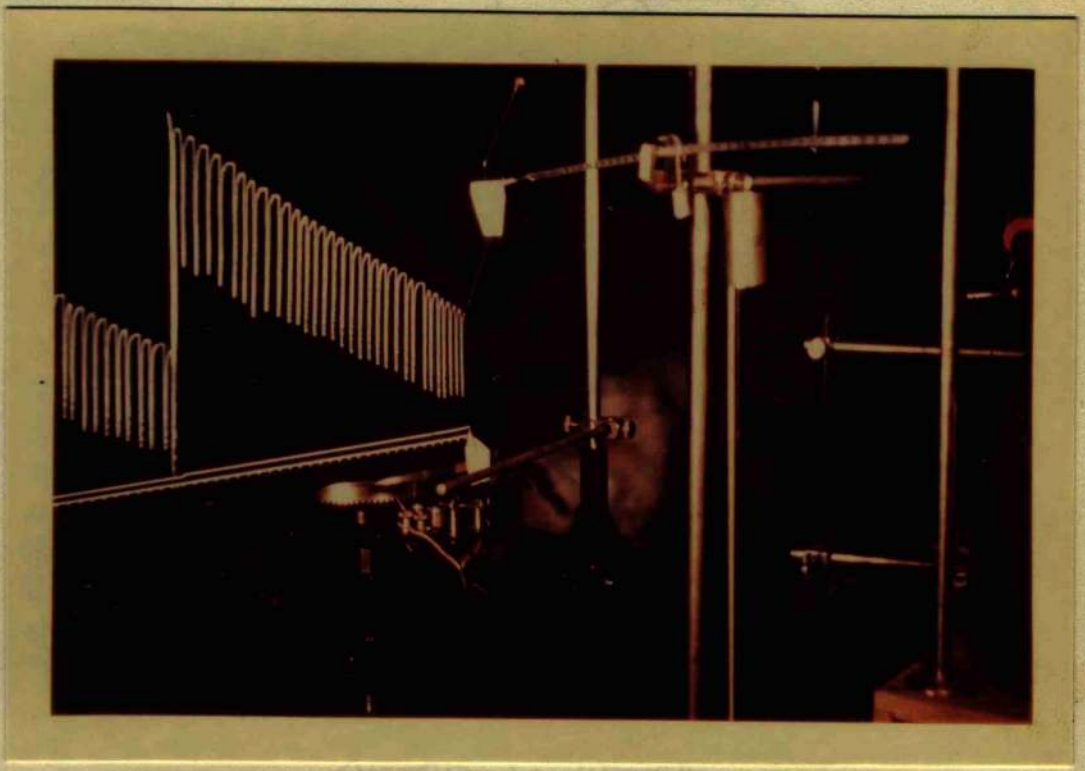


Figure 8. Respiratory tracing as obtained from a dog.

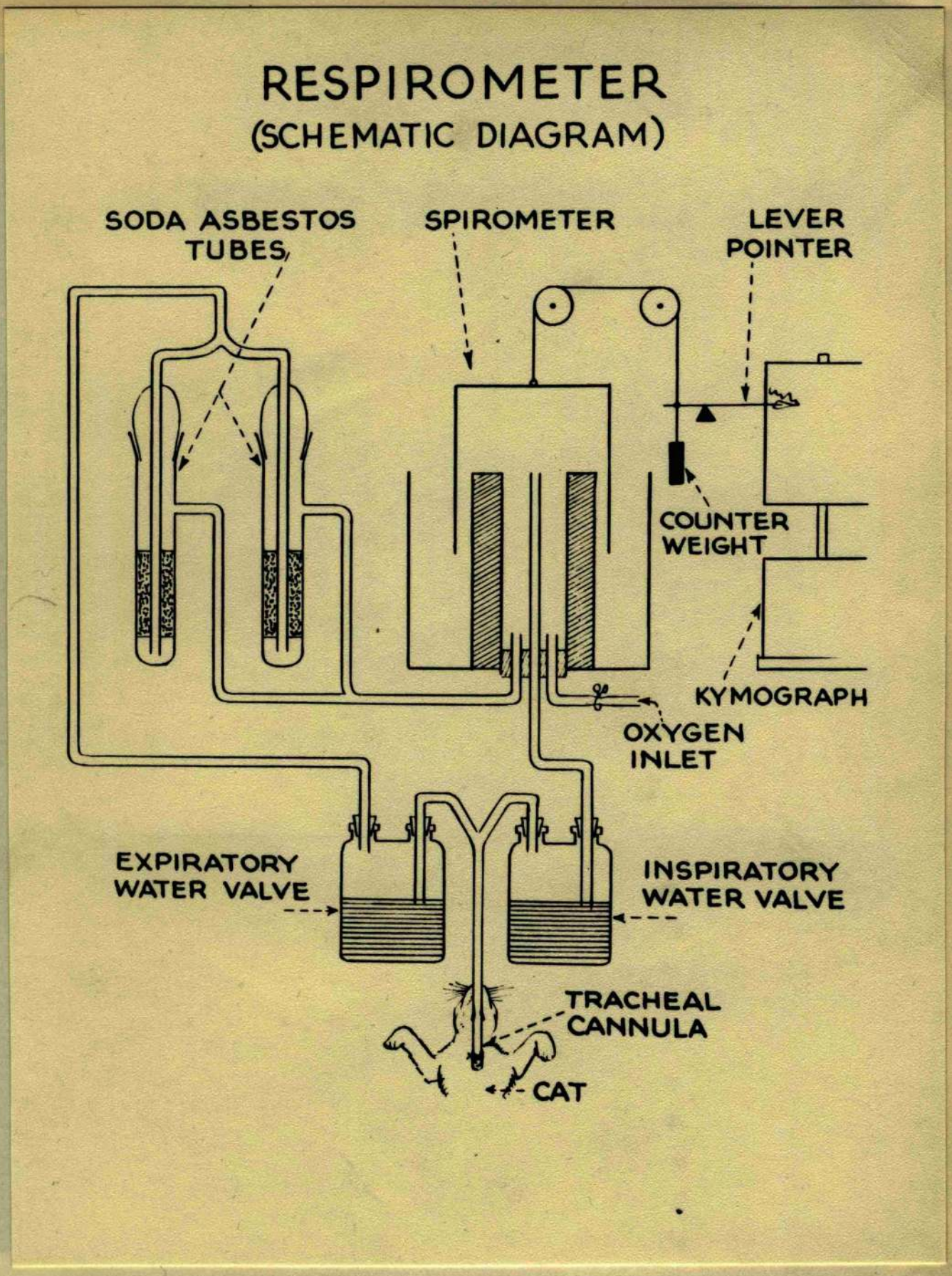

RESPIROMETER
(SCHEMATIC DIAGRAM)
SODA ASBESTOS TUBES
SPIROMETER
LEVER POINTER
COUNTER WEIGHT
KYMOGRAPH
OXYGEN INLET
EXPIRATORY WATER VALVE
INSPIRATORY WATER VALVE
TRACHEAL CANNULA
CAT

Figure 9.

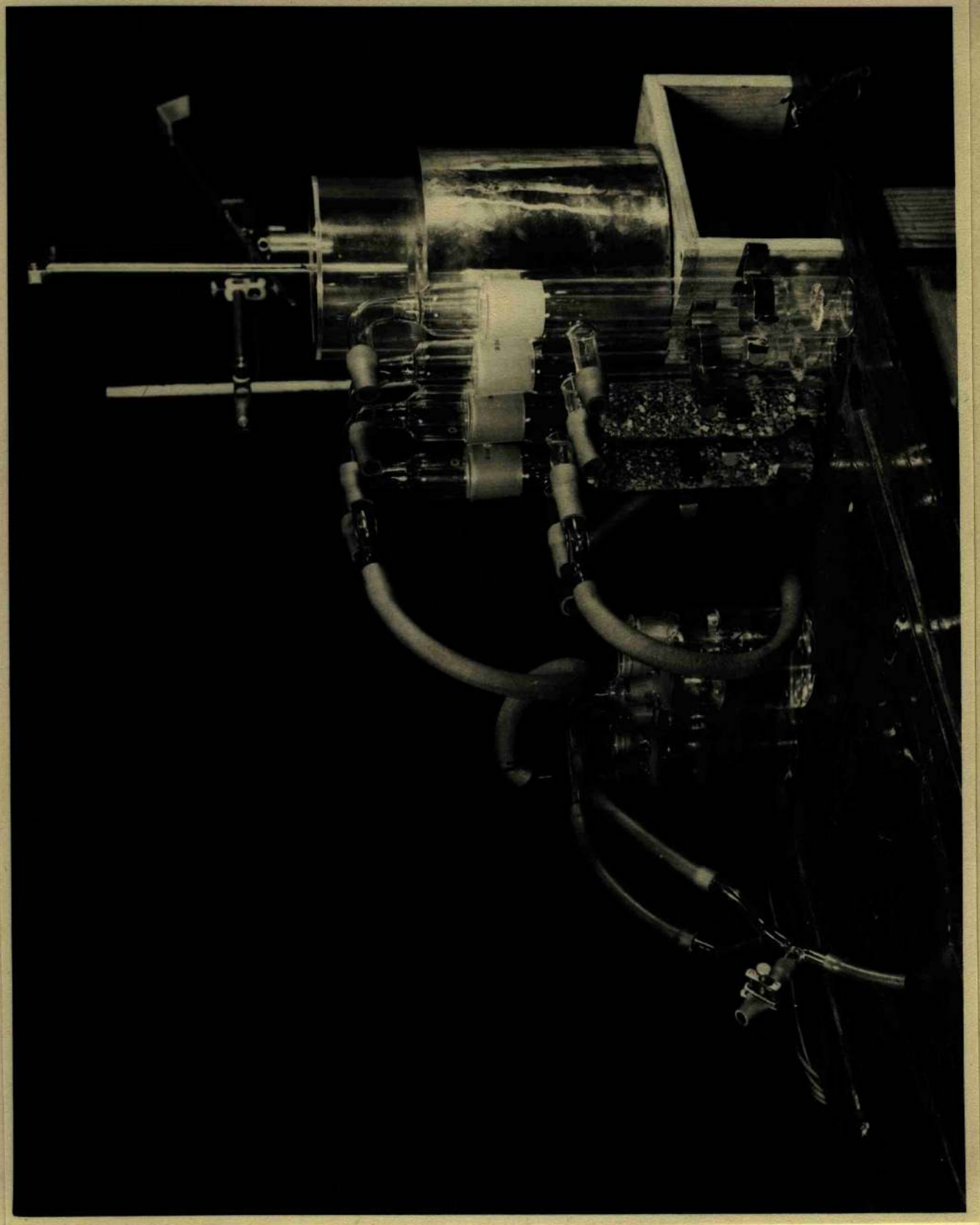


Figure 10. View of respirometer with water valves (extreme left) and CO_2 absorbing tubes in position.

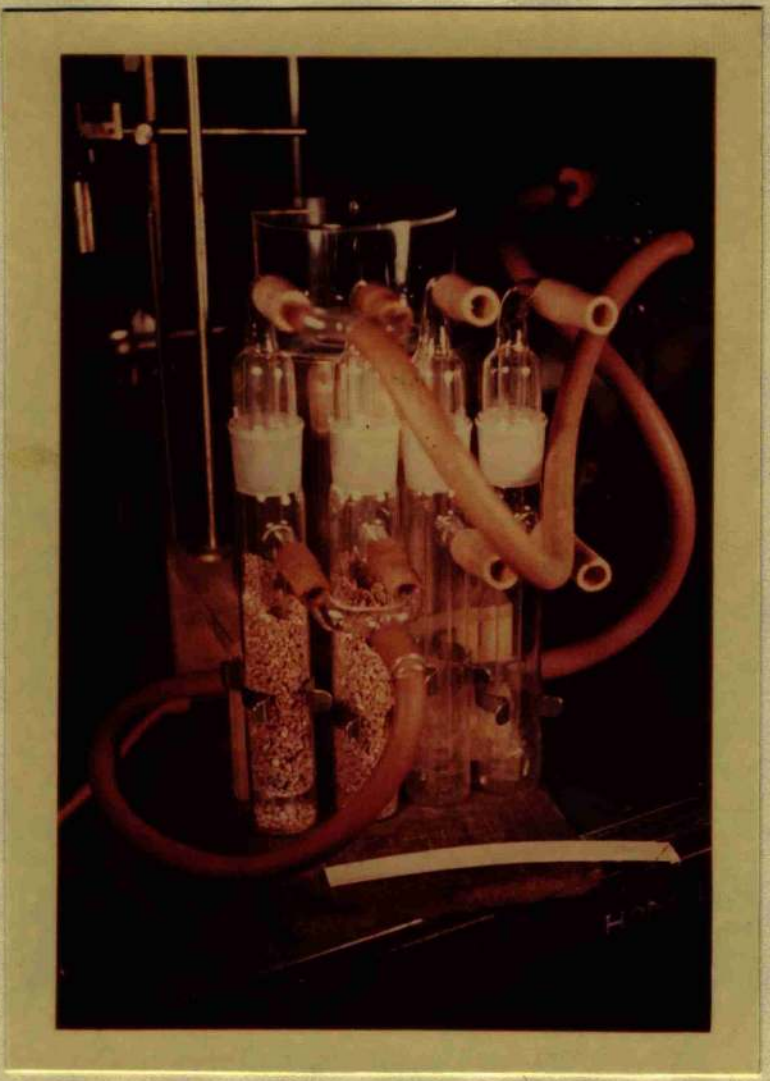


Figure 11. Carbon dioxide absorbing tubes.

resistance rubber flap valves were devised. A sectional drawing of the type of valve used is seen in Figure 12. Figure 13 is a photograph of the valves and their connections to the tracheal cannula in a dog. Their mounting and connections to the spirometer and CO_2 absorbing tubes are shown in Figure 14.

Experimental Methods.

Two techniques were used for determination of the rate of metabolism of the perfused hind limb in the recipient animal. The first method was as follows.

The respiration and oxygen consumption of the donor animals was recorded by means of the respirometer described above. After a series of control recordings, 5 mg./kg. body weight DNP was injected into the perfusion system. When a steady state had been attained (25 - 35 minutes) the perfusion system was clamped and the recording of respiration and oxygen consumption continued. Any fall in oxygen consumption recorded would be caused by the cutting off of the blood flow to the perfused limb. This method produced results which will be presented in the following section. The data indicated the order of magnitude of the oxygen consumption of the perfused limb but the method was not considered to be satisfactory since no repetitive checks could be carried out and the clamping off of the perfusion system might have some effect on the recipient animal. For these reasons a second method was used. In

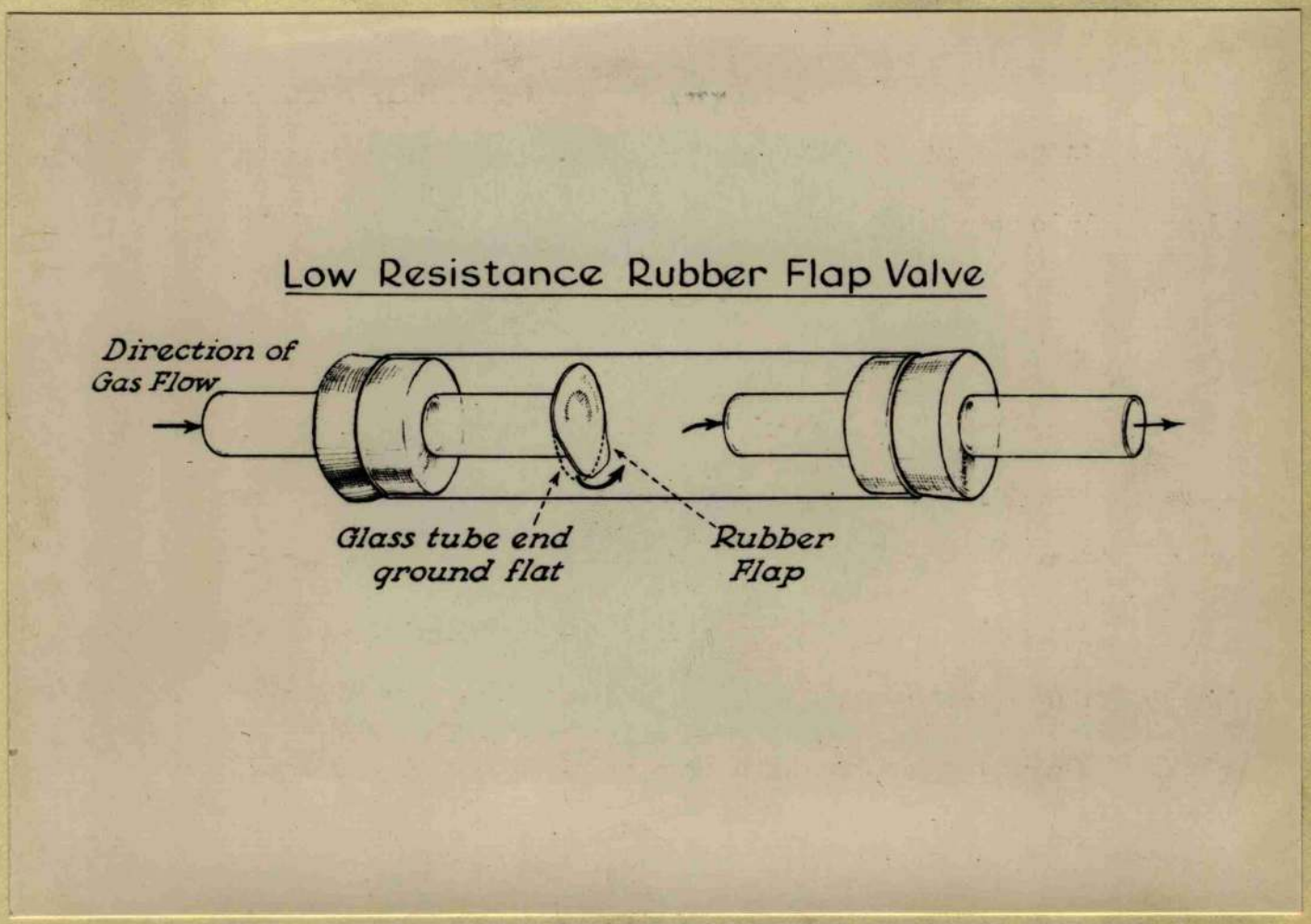

Low Resistance Rubber Flap Valve
Direction of Gas Flow
Glass tube end ground flat
Rubber Flap

Figure 12.

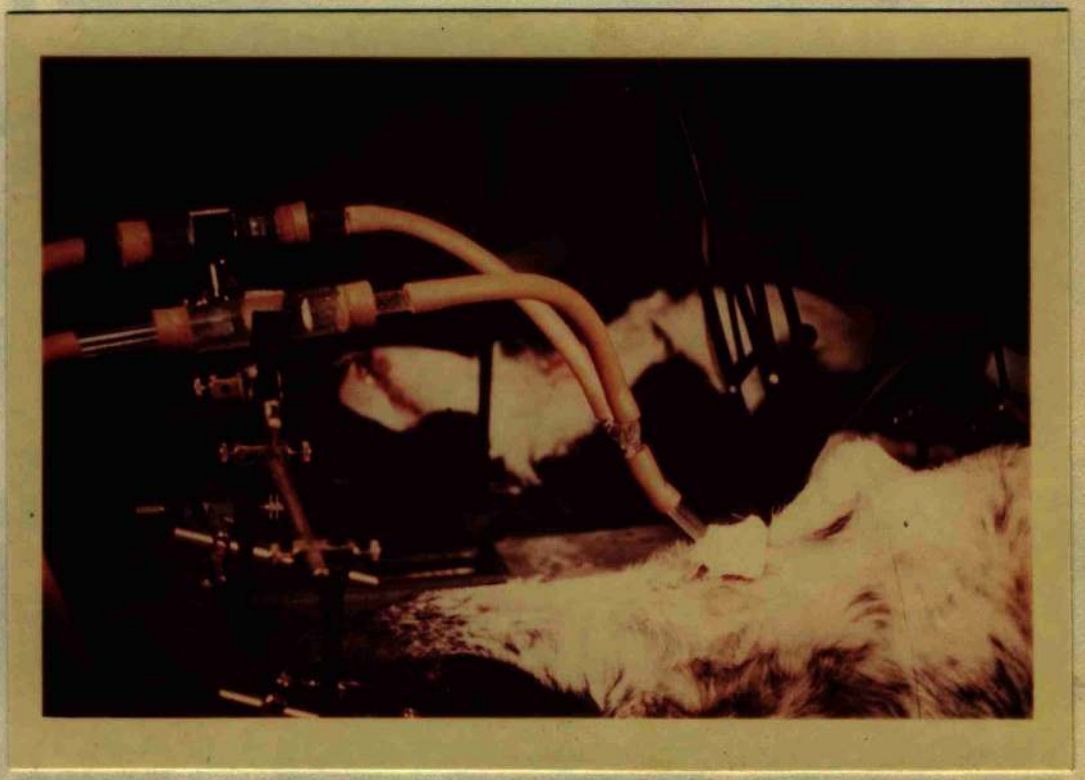


Figure 13. Low resistance rubber flap valves (upper left) connected to tracheal cannula in dog (centre).

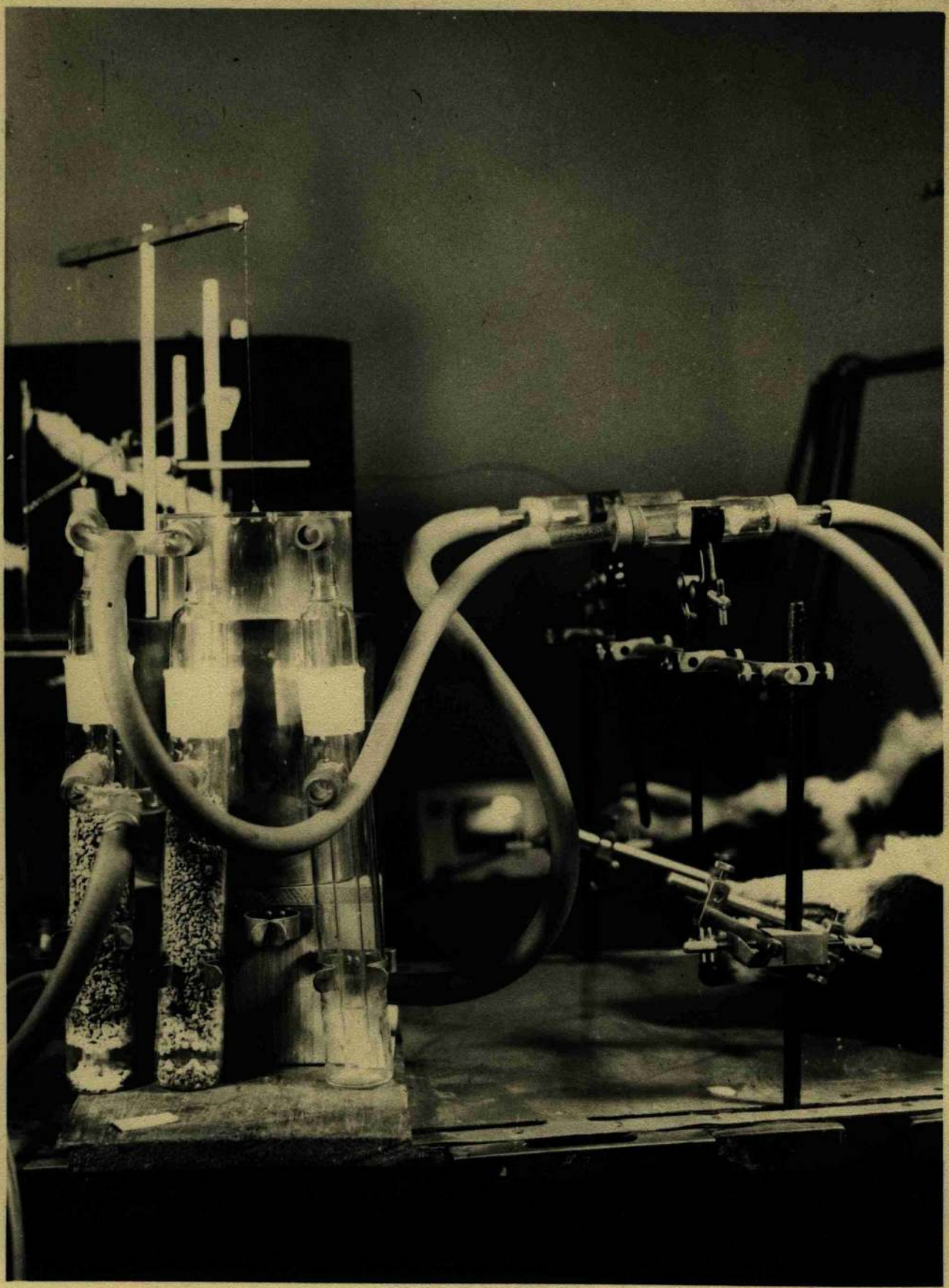


Figure 14. Flap valves (upper right) connected to spirometer and CO_2 absorbing tubes (left).

this method the Fick Principle was used to determine the oxygen consumption of the perfused limb. A Rotameter was inserted into the venous side of the perfusion system so as to provide continuous indication of the blood flow through the limb. The cross circulation system is shown diagrammatically in Figure 15. Figure 16 is a photograph showing the complete experimental arrangement. The Rotameter may be seen in the centre of the picture behind which is the heparin drip system. A clearer picture of the Rotameter and drip feed is shown in Figure 17. Figure 18 is a view showing the cannulae inserted into the femoral vessels of the donor animal.

Blood samples were taken from the arterial and venous sides of the perfusion system close to the perfused limb. These samples were drawn into oiled tuberculin syringes and the oxygen content determined using a Roughton-Scholander Syringe (Consolazio, Johnson & Marek, 1951). Samples were taken at the resting steady state level and also the steady state after injection of DNP.

Temperature Measurement.

As noted previously it was considered advisable in any further experimental work on the effect of artificial increase in metabolism on respiration to take careful note of temperature changes in the animals. It was decided that if temperature measurements were to be taken at all then they should include not only rectal temperatures but also normal and perfused muscle

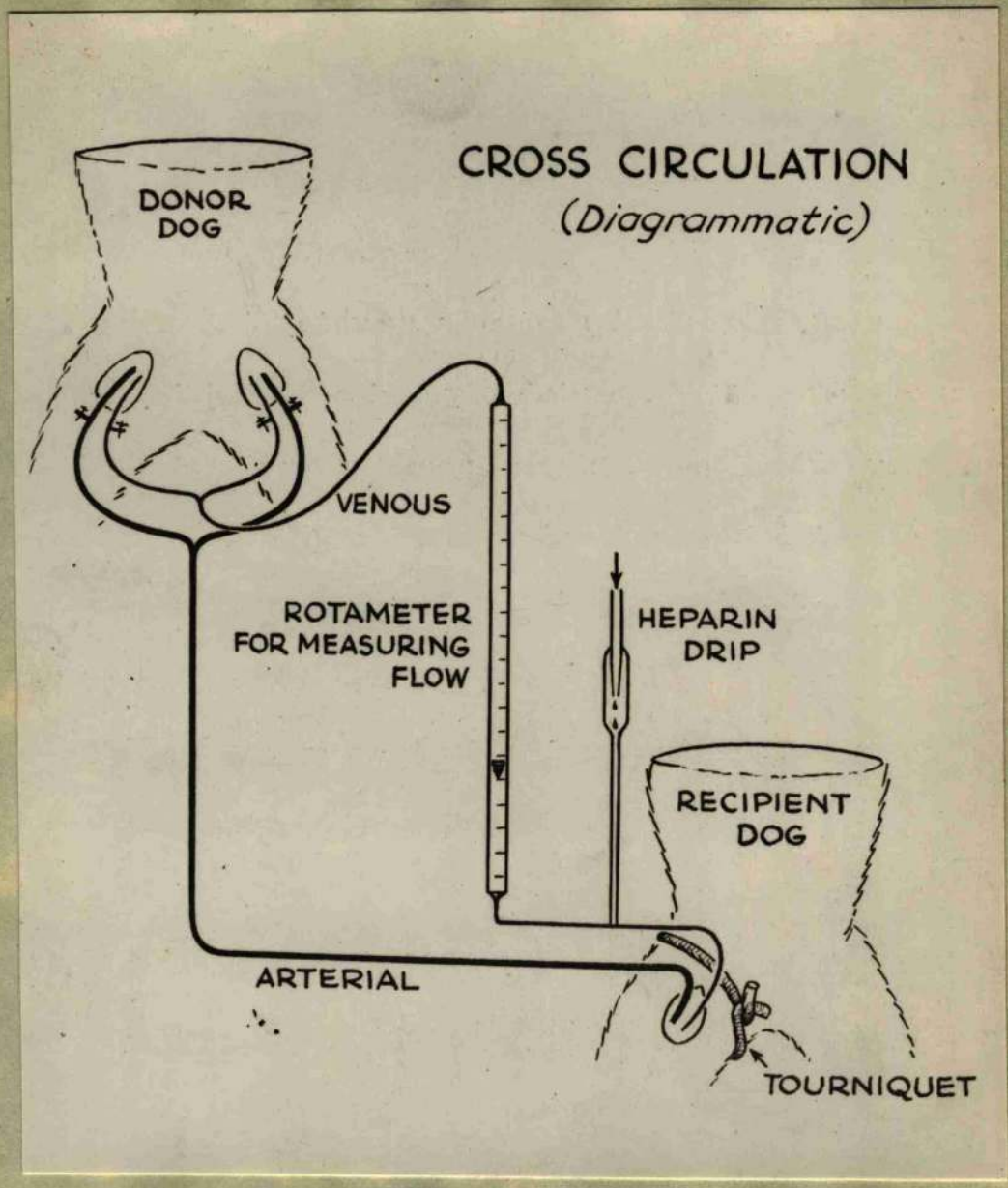


Figure 15. Arrangement for cross circulation experiments using a Rotameter for measuring the blood flow.

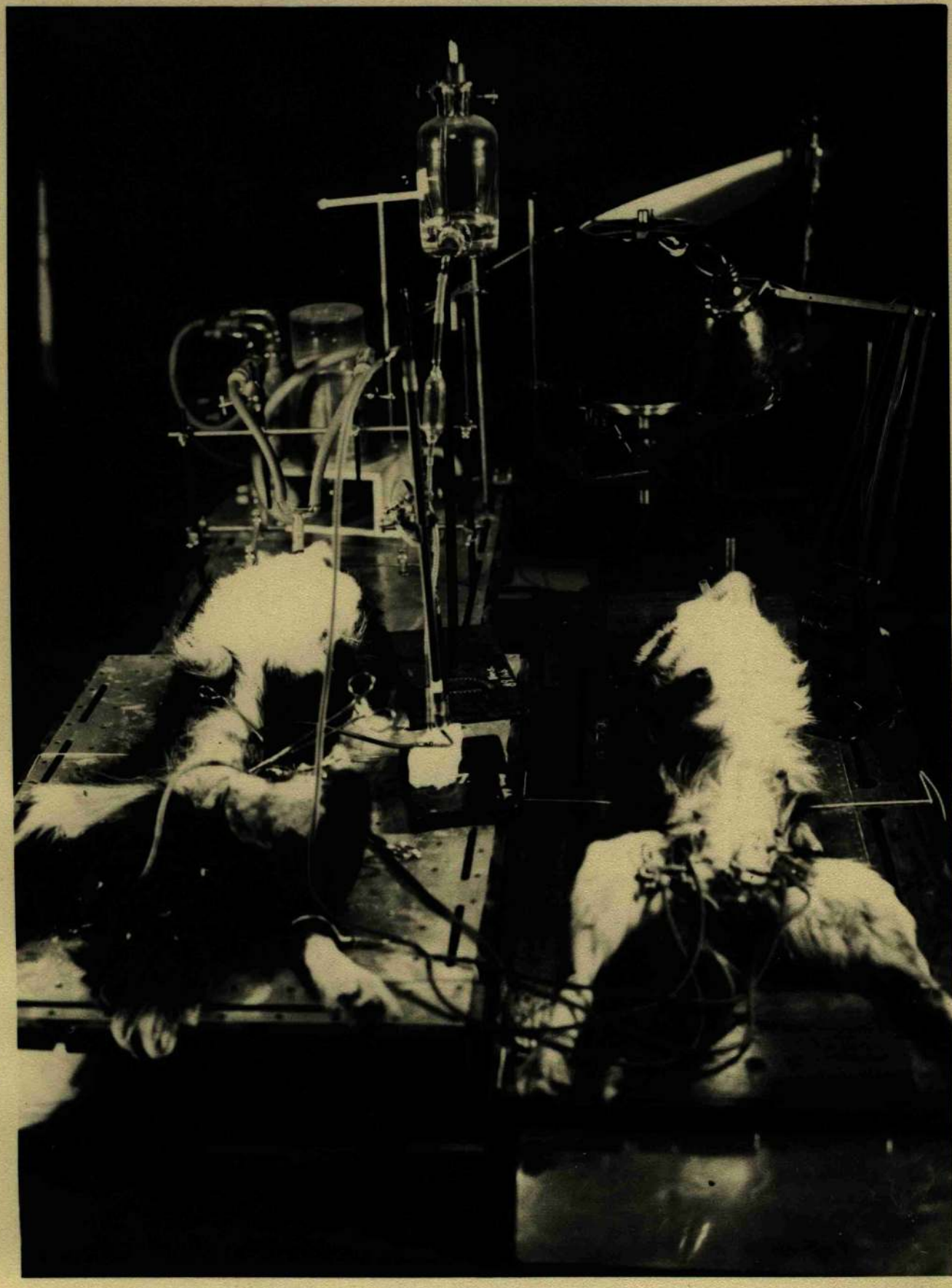


Figure 16. View of cross circulation experiment.

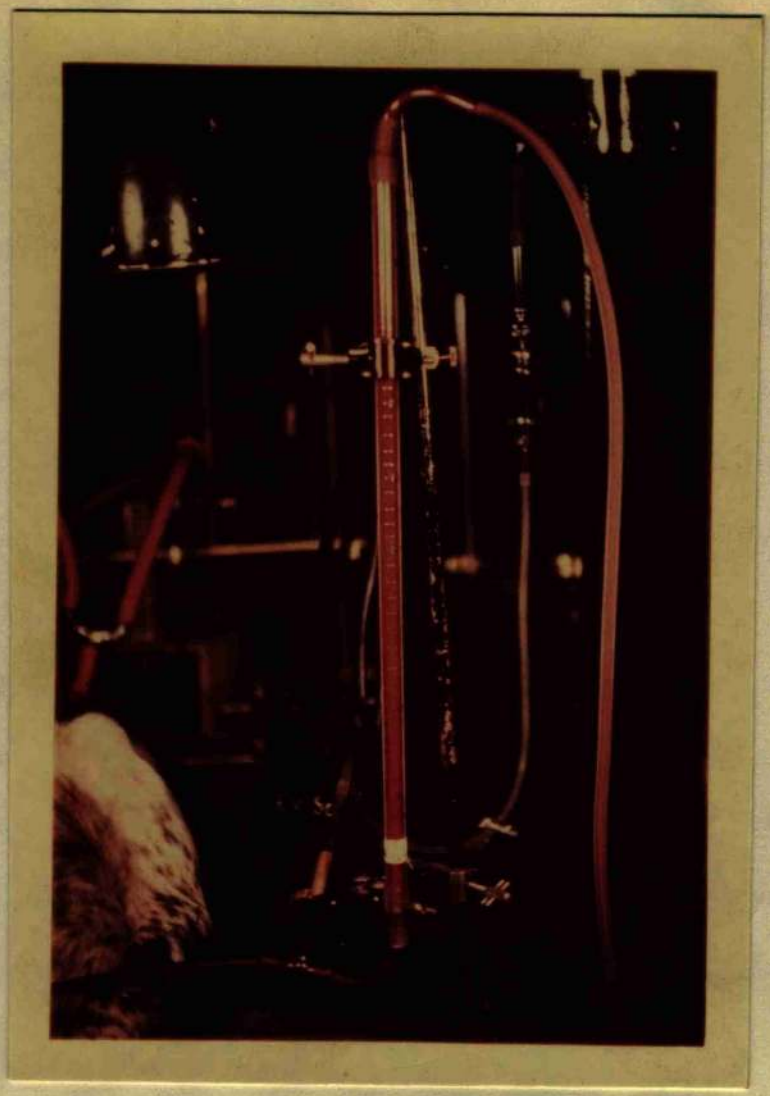


Figure 17. Rotameter for measuring blood flow.

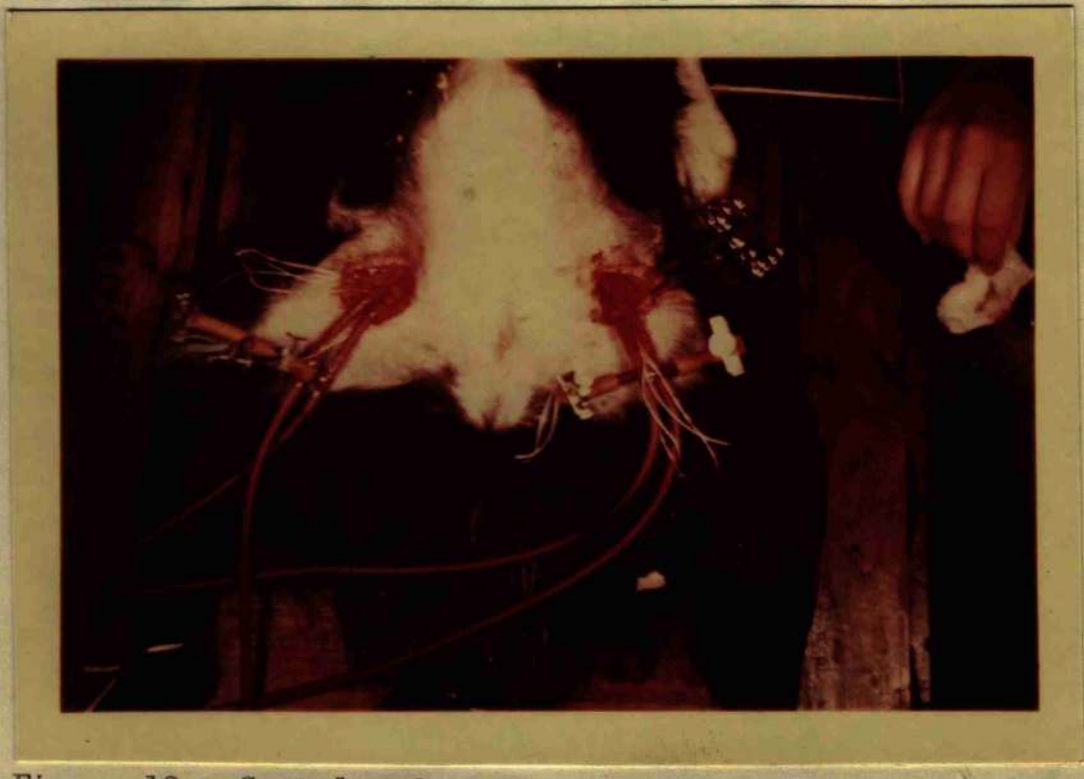


Figure 18. Cannulae for cross circulation in femoral vessels of dog.

temperatures and, if possible, central temperatures. Mercury-in-glass thermometers were obviously inadequate for such measurements because of size, slow response, and inaccuracy. It was therefore decided to construct a multi-thermocouple system giving a high degree of accuracy and sensitivity, quick response, and ease of working. Six copper-constantin thermocouples were made and carefully matched to produce the same response to a unit increase in temperature. The current output of these thermocouples was of the order of 40 μA. The copper leads from the thermocouples were connected through a selector switch to one side of a Pye Spot Galvanometer. The constantin leads were bonded together and connected to the constantin lead of a reference thermocouple. This reference thermocouple was kept in a constant temperature unit. The copper lead from the reference thermocouple was connected through a series potentiometer to the other side of the galvanometer. The potentiometer was used to control the sensitivity of the galvanometer. The circuit is shown in Figure 19. Figure 20 is a photograph of the apparatus. At the lower left and right are shown the selector switch and the thermocouples in their hypodermic needle sheaths. On the table may be seen the Spot Galvanometer and constant temperature unit. It was found in practice that the slow response of the Spot Galvanometer limited the usefulness of the apparatus. A Pye Microvoltmeter was obtained and connected in place of the

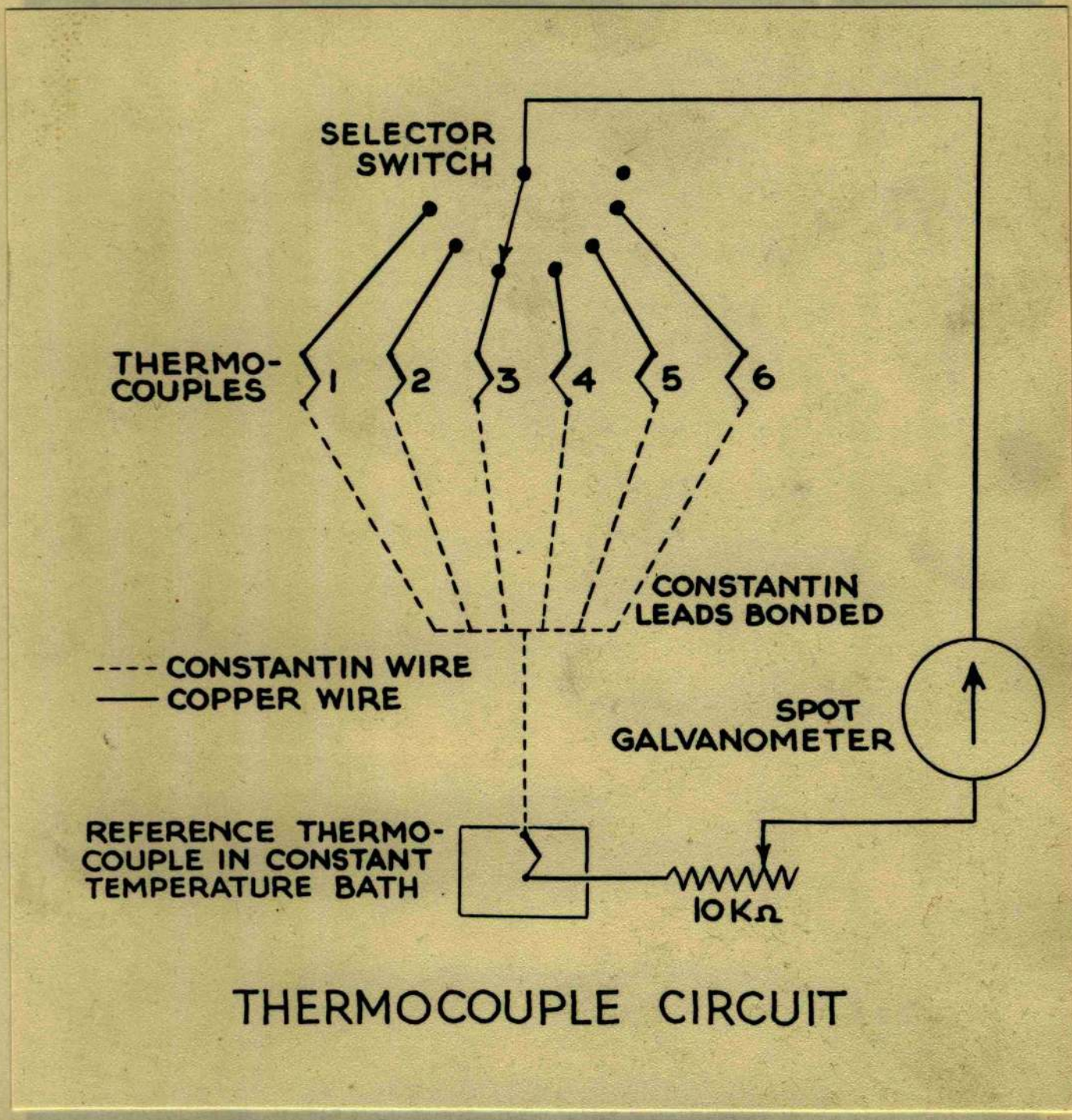

SELECTOR SWITCH
THERMO-COUPLES
1
2
3
4
5
6
CONSTANTIN LEADS BONDED
---- CONSTANTIN WIRE
—— COPPER WIRE
SPOT GALVANOMETER
REFERENCE THERMO-COUPLE IN CONSTANT TEMPERATURE BATH
10 KΩ
THERMOCOUPLE CIRCUIT

Figure 19.

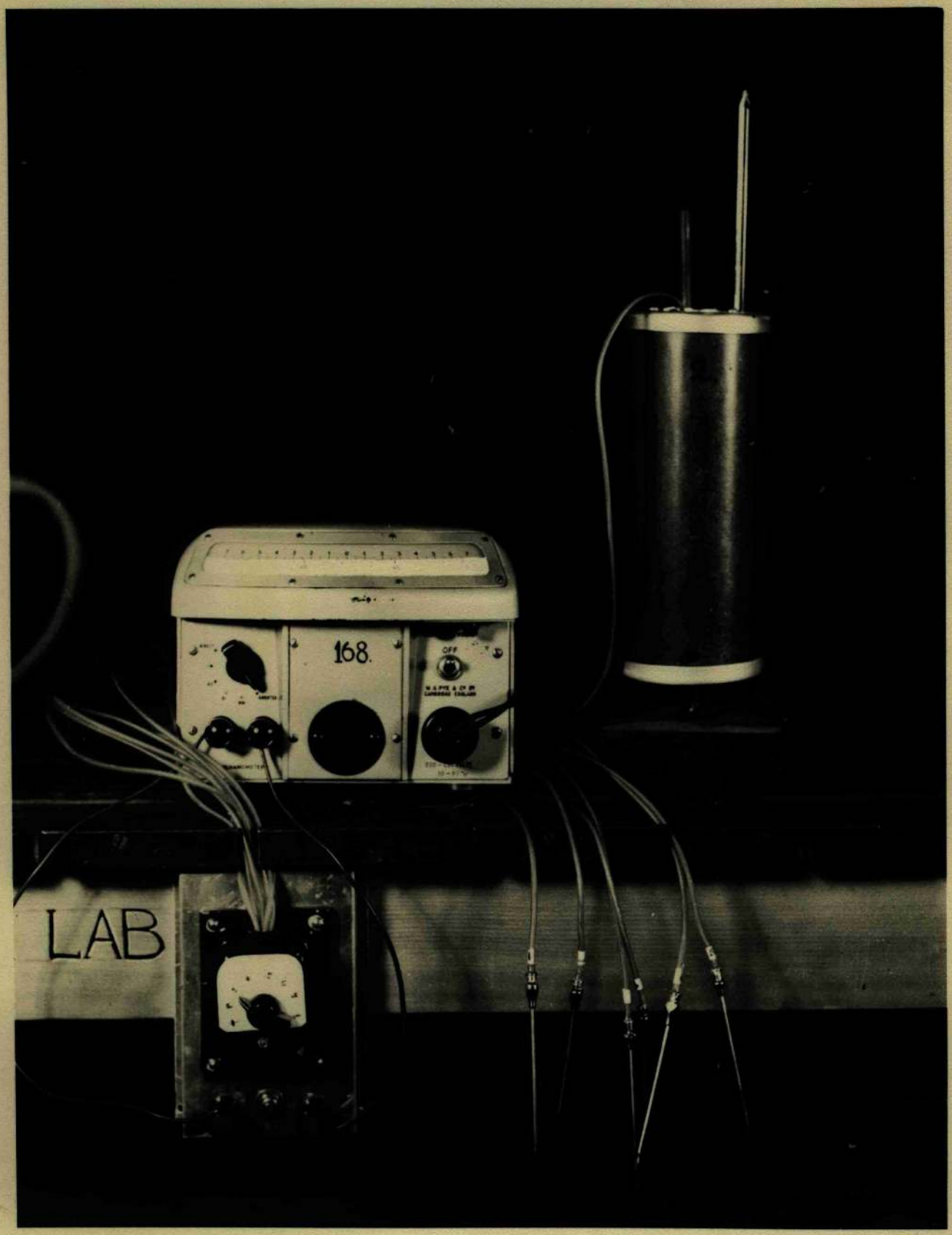


Figure 20. Temperature measuring apparatus. Upper left, Pye Galvanometer; Upper right, constant temperature unit; Lower left, selector switch; lower right, shielded thermocouples.

galvanometer. The only change in the circuit of Figure 19 required was the substitution of a 2.5 KΩ potentiometer for the 10 KΩ component originally used. The speed of response of the Microvoltmeter meant that it was possible to take six temperature readings as fast as the switching and reading of the values permitted.

Temperature readings were taken with the thermocouples in the following positions. In the donor animal:- rectum, shoulder muscle, and in the muscles of the back of the neck close to the spine. In the recipient animal:- rectum, in the gastrocnemius of the perfused limb and the normal hind limb.

Animals.

The animals used were mongrel dogs of 10-15 kg. body weight. The anaesthesia was a s previously described - 4 mg./kg. morphine sulphate subcutaneously followed, after 30 mins., by 200 mg./kg. barbitone sodium 15%, intravenously. DNP was administered in the form of a 3% solution in 1.5% sodium bicarbonate, the dose being 5 mg./kg. intravenously.

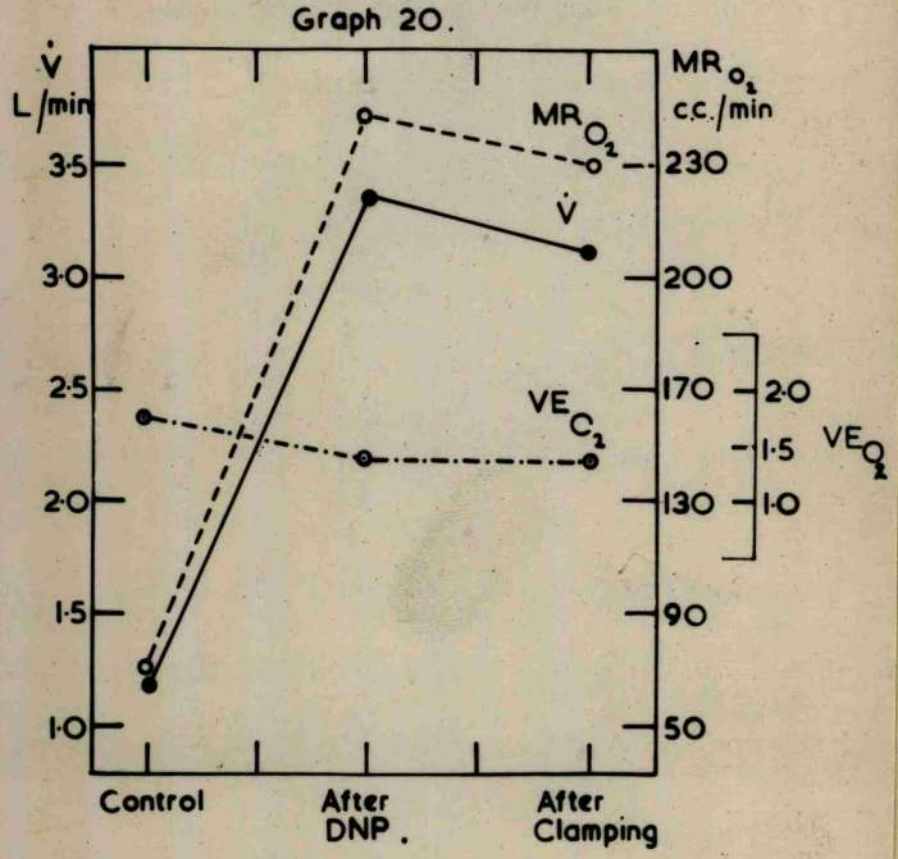
RESULTS.

Graph 20 shows the results obtained from the first method used. It will be recalled that in this method the cross circulation is set up and DNP given to the donor animal. After a steady state has been reached (25-35 mins.) the perfusion system is clamped. Any fall in oxygen consumption measured in the donor animal is then assumed to be due to the isolation of the perfused limb. As may be seen in Graph 20, the mean MR_{O_2} of the donor animals rose from 66.6 cc/min. to 247.2 cc/min. after injection of DNP. After clamping the perfusion system the mean MR_{O_2} fell to 230 cc/min., a fall of 17.2 cc/min. ($\pm$2.3). The VE_{O_2} of the donor animals showed the same type of response as in the previous experiments and remained steady after the clamping procedure.

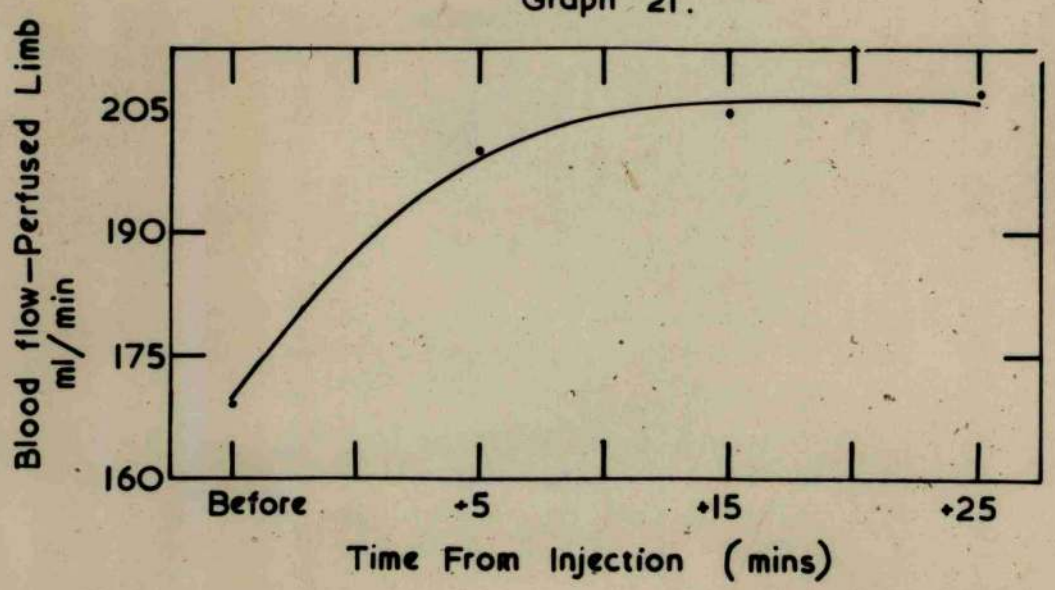
For reasons mentioned earlier the above method was not considered entirely satisfactory. The second method described, using a Rotameter in the perfusion system, was then instituted.

It was found that the average rate of blood flow through the perfused limb at rest was 169 ml./min. When DNP was then injected into the donor animals the blood flow rose to a steady state average value of 206 ml./min. This is shown in Graph 21. From analyses of blood samples drawn from the arterial and venous sides of the perfusion system it was found that, at rest, there was an average A-V oxygen difference of 5.31 vols.%. After DNP was injected the average A-V difference for oxygen across

Graph 20.


V̇ L/min
3·5
3·0
2·5
2·0
1·5
1·0
MR O2 c.c./min
230
200
170
130
90
50
MR O2
V̇
VE C2
2·0
1·5
1·0
VE O2
Control
After DNP.
After Clamping

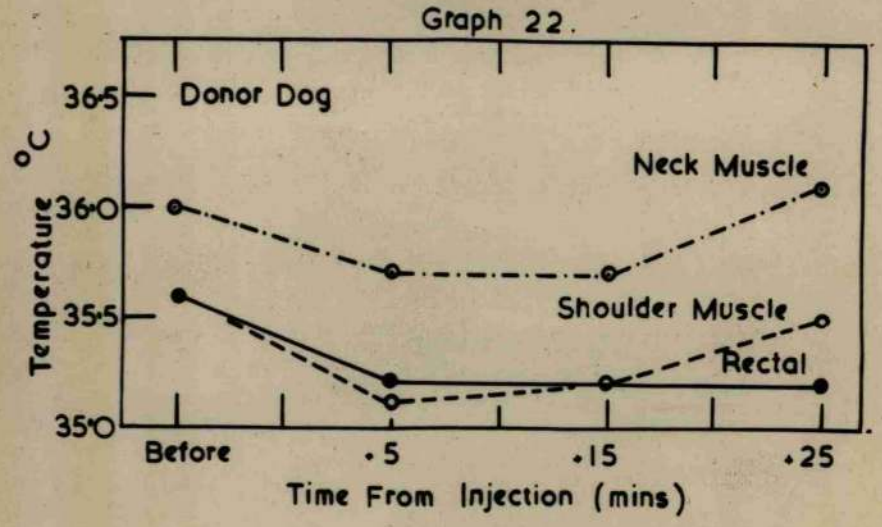
Graph 21.


Blood flow—Perfused Limb
ml/min
205
190
175
160
Before
+5
+15
+25
Time From Injection (mins)

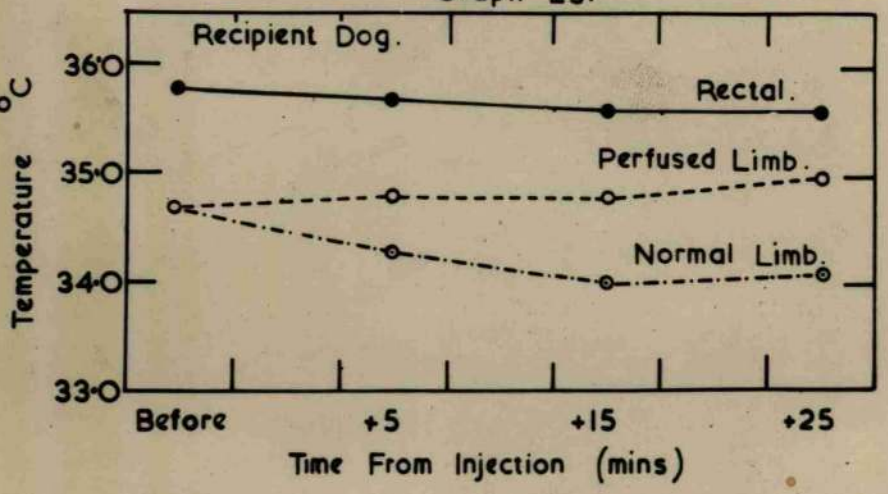
the perfused limb was found to be 11.26 vols. %. These figures mean that the average oxygen consumption of the perfused limb at rest was 8.97 cc/min. and, after DNP injection, 23.19 cc/min., a mean increase of 2.54 times the resting level. This increase of MR_{O_2} of 2.54 agrees closely with the increase in MR_{O_2} of 2.49 ($\pm$0.46) recorded from the donor animals in the previous experiments.

In Graphs 22 and 23 are plotted the average temperature recordings. Graph 22 shows those from the donor animals. It may be noted that the temperature in all three sites of measurement fell in the 5 minutes following injection of DNP. Graph 23 shows the results obtained from the recipient animals. In this case the temperature recorded from the perfused limb shows a mean rise of 0.1C° five minutes after the injection of DNP but the other two sites show a fall in temperature.

Graph 22.



Graph 23.



DISCUSSION.

The average oxygen consumption of the donor animals in all the cross circulation experiments (22 animals) is 101.4 cc/min. Thus the oxygen consumption of the perfused limb (23.2 ml./min.) represents approximately 23% of the metabolism of the donor. This figure may be used to make the regression line fitted to the data of the previous experiments, shown in Graph 19, more comparable to the data of Kao (1951). The result is shown in Graph 24. The line is of steeper slope and slightly higher than that for the "intact" animal. The points obtained from Kao's "neural" dogs, however, fit fairly closely to the new, calculated line.

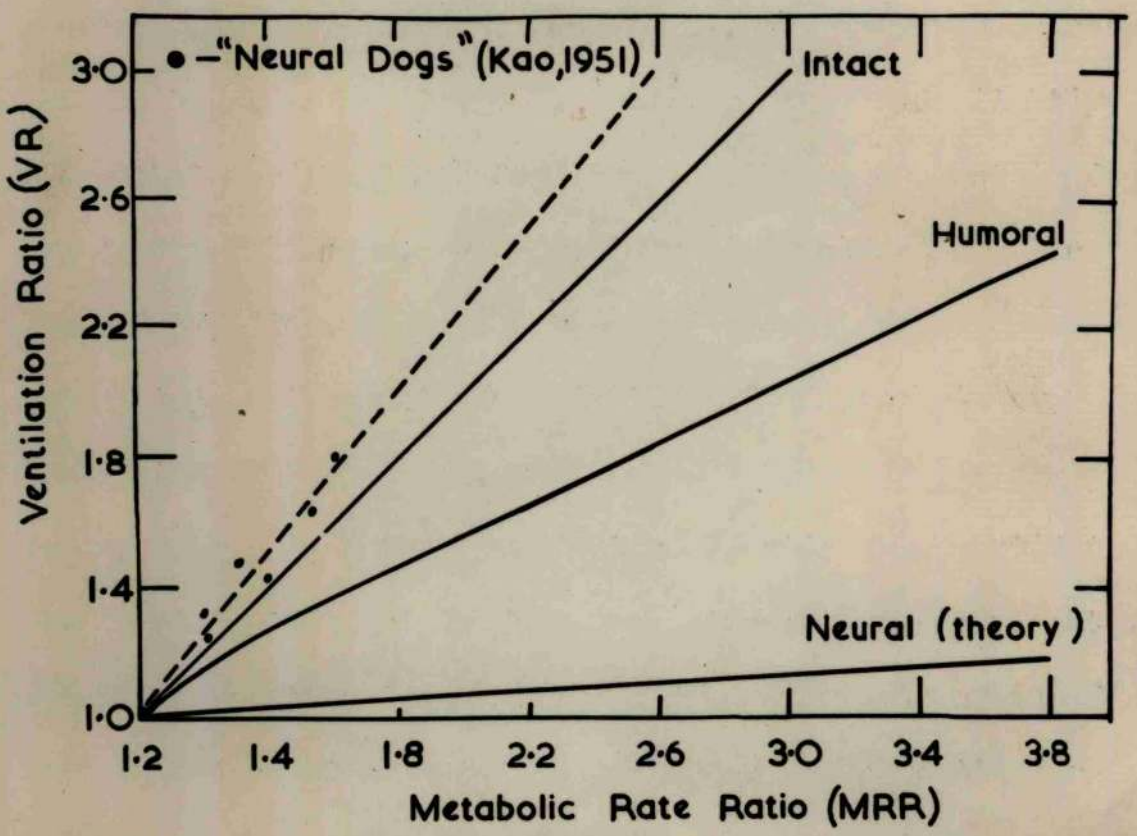
It is not easy to explain these findings but several suggestions may be made.

1. There is some other factor affecting the ventilation of the recipient animal in the cross circulation experiments. Such a suggestion is possibly an "easy way out" of the difficulty but is not considered to be plausible. The ventilatory control of the recipient animals seems to be working in a normal manner and any stimuli from trauma etc., would be expected to operate during the control period as well as after injection of DNP.

2. DNP may stimulate nerve endings and so stimulate ventilation directly as well as via its effect on metabolism.

Experiments on the respiratory response to DNP in the intact animal and comparison with exercise are necessary before

Graph 24.


● —"Neural Dogs" (Kao, 1951)
Intact
Humoral
Neural (theory)
Ventilation Ratio (VR)
3·0
2·6
2·2
1·8
1·4
1·0
1·2
1·4
1·8
2·2
2·6
3·0
3·4
3·8
Metabolic Rate Ratio (MRR)

this possibility can be either substantiated or ruled out.

3. The anaesthesia employed may radically alter the response of the respiratory system to an increase in metabolism.

This also requires further experimental work before an opinion can be given.

4. The data obtained by Kao and plotted on Graph 24 and the calculated line drawn on the same graph may differ from the "intact animal" response only by experimental error. The implications of this suggestion are far reaching. It would mean that the respiratory response to moderate increases in metabolism was wholly controlled by neural impulses from the periphery. The humoral control would only be of importance in severe exercise or for rough control. If such were the case then many concepts of the control of respiration would have to be rearranged. Some experimental evidence that this is actually the case is available (Kao, 1955, personal communication). Further information is, however, necessary on the actions of DNP in the intact animal and the effects of anaesthesia on the respiration.

C.

The Respiratory and Metabolic Responses of the Intact Animal to Intravenous Injection of DNP and the Effects of Barbiturate Anaesthesia.

In general, previous considerations of the relationships between anaesthesia and respiration have been concerned with either the mode of action of anaesthetics or on how observation of respiration can give information on the functional state of the anaesthetised individual. For example, Morton (1950) wrote: "Since the earliest days in anaesthesia, respiration has provided helpful signs for those who conduct fellow human beings on journeys through unconsciousness. We have no reason to suspect that the last secret has been revealed; that no more useful information is forthcoming. Let us then apply ourselves with renewed vigour to the study of respiration, and progress in anaesthesia will surely result." On the other hand Proctor (1953) wrote: "Alterations in respiration provide the most reliable signs of depth of narcosis during anaesthesia."

Christensen (1955) said: "One has to remember that results on anaesthetised animals may have to be obtained under experimental conditions that are far from the normal physiological ones. It is particularly important to know the effect of the anaesthetics used."

As far as can be ascertained no previous work has been done on the effects of anaesthesia on the control mechanism of the respiratory system. The experiments to be described had a two-fold purpose. Firstly to determine the effect on respiration of DNP in the intact animal and, secondly, to determine the effects, if any, of anaesthesia on such responses.

Methods.

Animals.

Adult cats of both sexes were used with a range in body weight of 1.9 to 4.2 kg.

Experimental.

Three groups of animals were used. In the first group, designated Group AD, the cats were anaesthetised in an ether box, a tracheal cannula inserted (See Figure 21) and a femoral vein exposed. 2 mg./kg. morphine sulphate was then injected subcutaneously. Ether anaesthesia was discontinued and anaesthesia by barbitone sodium (200 mg./kg.) induced. Half of the calculated dose of barbiturate was injected immediately into the exposed femoral vein. The remaining amount was given in small portions over 15-20 mins. By this means a smooth change over from ether to morphia-barbiturate anaesthesia was obtained with the animal never getting too deep to halt respiration nor too light to allow reflex movements to obtrude. After 30-60 minutes, to allow for stabilisation of anaesthesia, control recordings of ventilation and oxygen consumption were made using the respirometer previously described. DNP, in amounts ranging from 2 to 10 mg./kg., was then injected intravenously and steady state levels of ventilation and oxygen consumption recorded.

In the second group of cats, designated Group DD, the animals were anaesthetised in an ether box and then decerebrated.

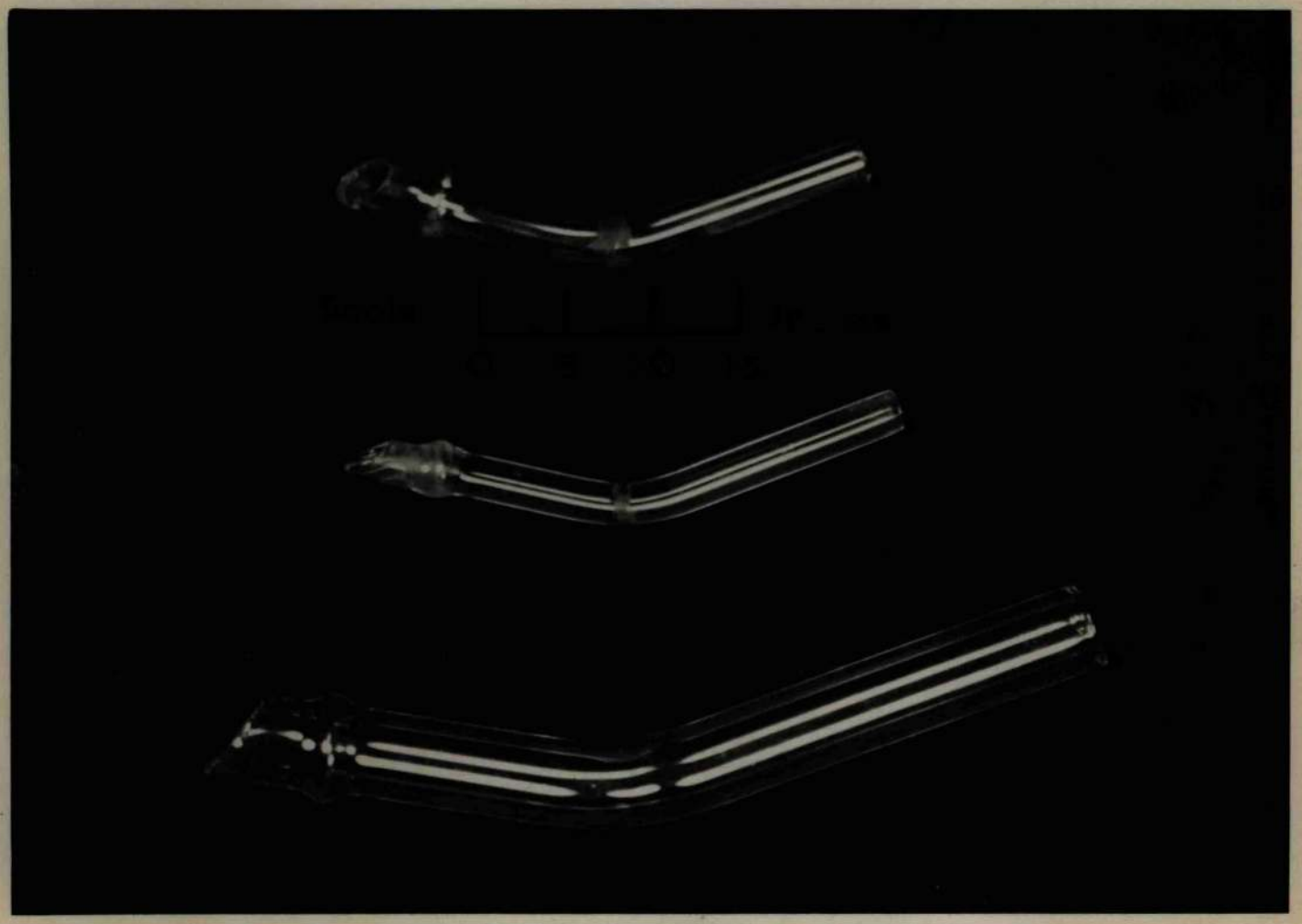


Figure 21. Glass tracheal cannulae as used in cats (top and centre) and dogs (bottom).

The routine method for decerebration used in this department was used. In this method, after insertion of a tracheal cannula and both carotid arteries have been tied off, a 1 cm. hole is made on the right side of the skull. After the hole is enlarged with bone-nibbling forceps the dura is slit and the brain stem sectioned just forward of the bony tentorium. The brain anterior to the section is then scooped out. Care is taken to procure haemostasis inside the skull, the simplest and most reliable method being by the use of pledgets of G el-Foam on cotton wool swabs applied with pressure to bleeding points.

After decerebration the animals were allowed to stabilise for 30-60 minutes and then recordings of ventilation and oxygen consumption taken both before and after DNP injection as with Group AD.

The third group of animals, group DDA, were decerebrated in an identical manner to those of Group DD. After the period of stabilisation, however, they were anaesthetised with morphia-barbitone anaesthesia exactly as Group AD. Recordings were then made of the respiratory responses in these animals as in the other two groups.

Temperature.

Readings were taken of the temperature changes in various parts of the body throughout the experimental period. The thermocouple system described previously was used and the sites

where the thermocouples were placedwere: in the muscles of all four limbs, the rectum, and the back of the neck close to the spine.

The animals lay supine on a heated operating table maintained at 40°C by thermostatic control.

RESULTS.

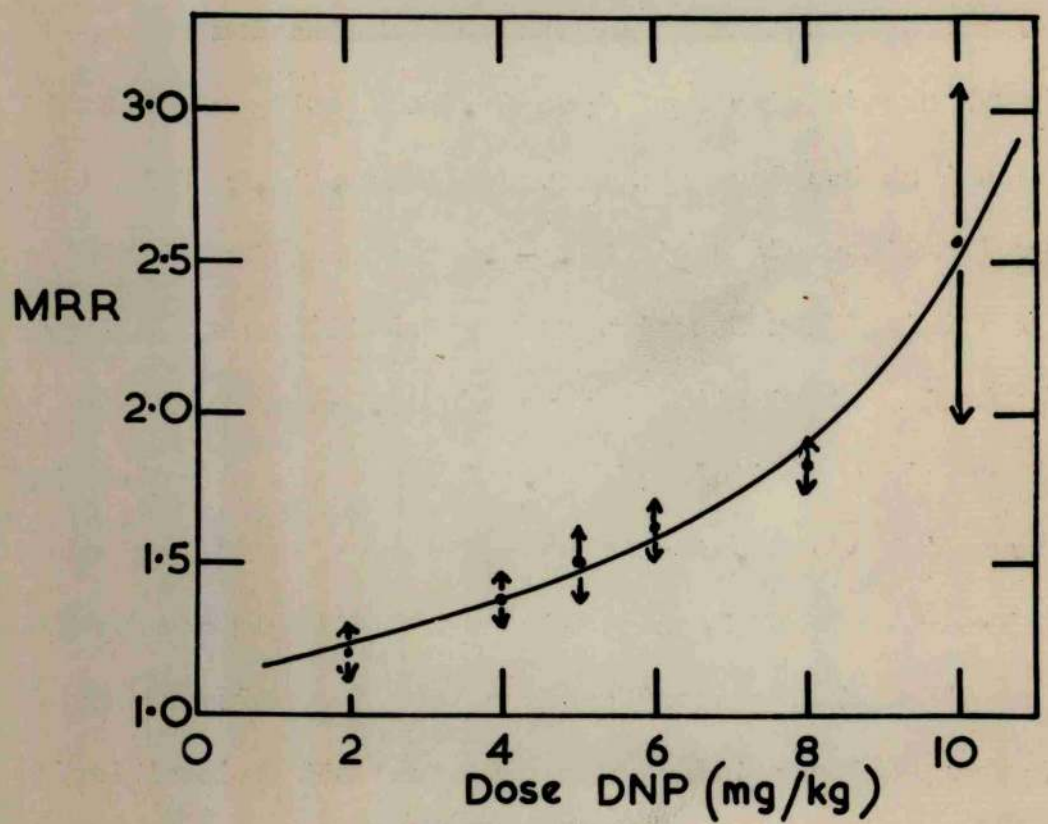
Metabolism.

One of the first things which became apparent when this series of experiments was begun was that the dose of DNP used for dogs (5 mg./kg.) was not producing the same order of stimulation of oxygen consumption in cats. In the donor dogs it had been found that a dose of 5 mg./kg. DNP intravenously produced an increase in metabolic rate of 2.5 ± 0.4 times the resting level. In intact cats it was found that a dose of 5 mg./kg. DNP intravenously produced an increase in metabolic rate of only 1.5 ± 0.08 times the resting level. In Graph 25 are plotted the mean values for MRR at the various dosage levels used. The arrows indicate the standard deviation of each plotted mean value. The plotted points on this graph represent 212 observations on 24 cats. The data on metabolic rate increase with different doses of DNP is amassed from all three experimental groups since there was found to be no significant difference in response between the groups.

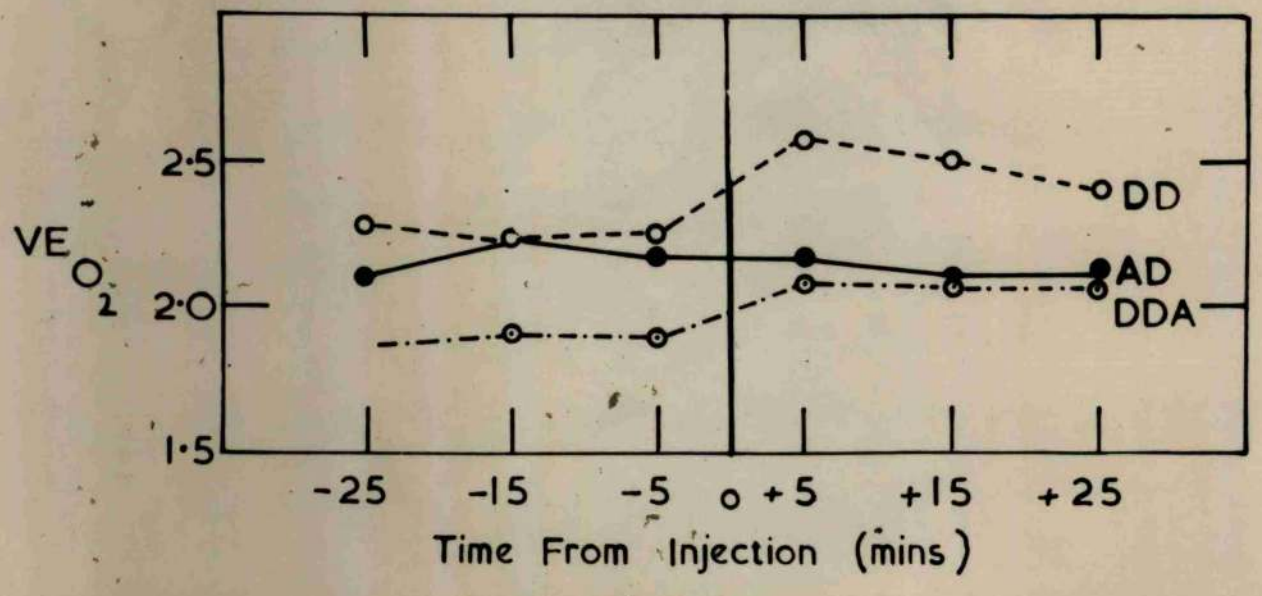
Respiratory Responses.

Ventilation Equivalent. In G raph 26 are plotted the mean values for VE_{O_2} for all three groups separately. Although the mean value for the VE_{O_2} shows a tendency to rise in groups DD and DDA, statistical analysis of the data indicates that this is not significant. In the group of animals which were decerebrated only (Group DD) the mean VE_{O_2} before injection of DNP was 2.31 and that after DNP 2.43. The standard error of

Graph 25.



Graph 26.



the difference between these values is $\pm$0.12. The difference is not significant. In Group DDA, where the animals were decerebrated and also anaesthetised, the mean values for VE_{O_2} before and after injection of DNP were 1.89 and 2.09 respectively. Once again, the difference between these values is not significant. The animals of Group AD, which were anaesthetised only, had a mean value for VE_{O_2} before DNP of 2.17. After DNP injection the VE_{O_2} recorded was 2.12, which is not significantly different from the "before" value. The differences were tested by a paired comparison analysis.

Relationship of Ventilation and Oxygen Consumption. In reporting and discussing the results of the initial experimental work (Part II, Section A of this thesis) it was more convenient to deal with non-dimensionalised values for metabolism and ventilation. Similarly with the effect of DNP on oxygen consumption as reported above. The results to be reported here, however, are better dealt with as absolute and not relative values. Pulmonary ventilation ($\dot{V}$) is therefore dealt with in litres per minute, BTPS, and oxygen consumption (MR_{O_2}) in cubic centimeters per minute, STPD.

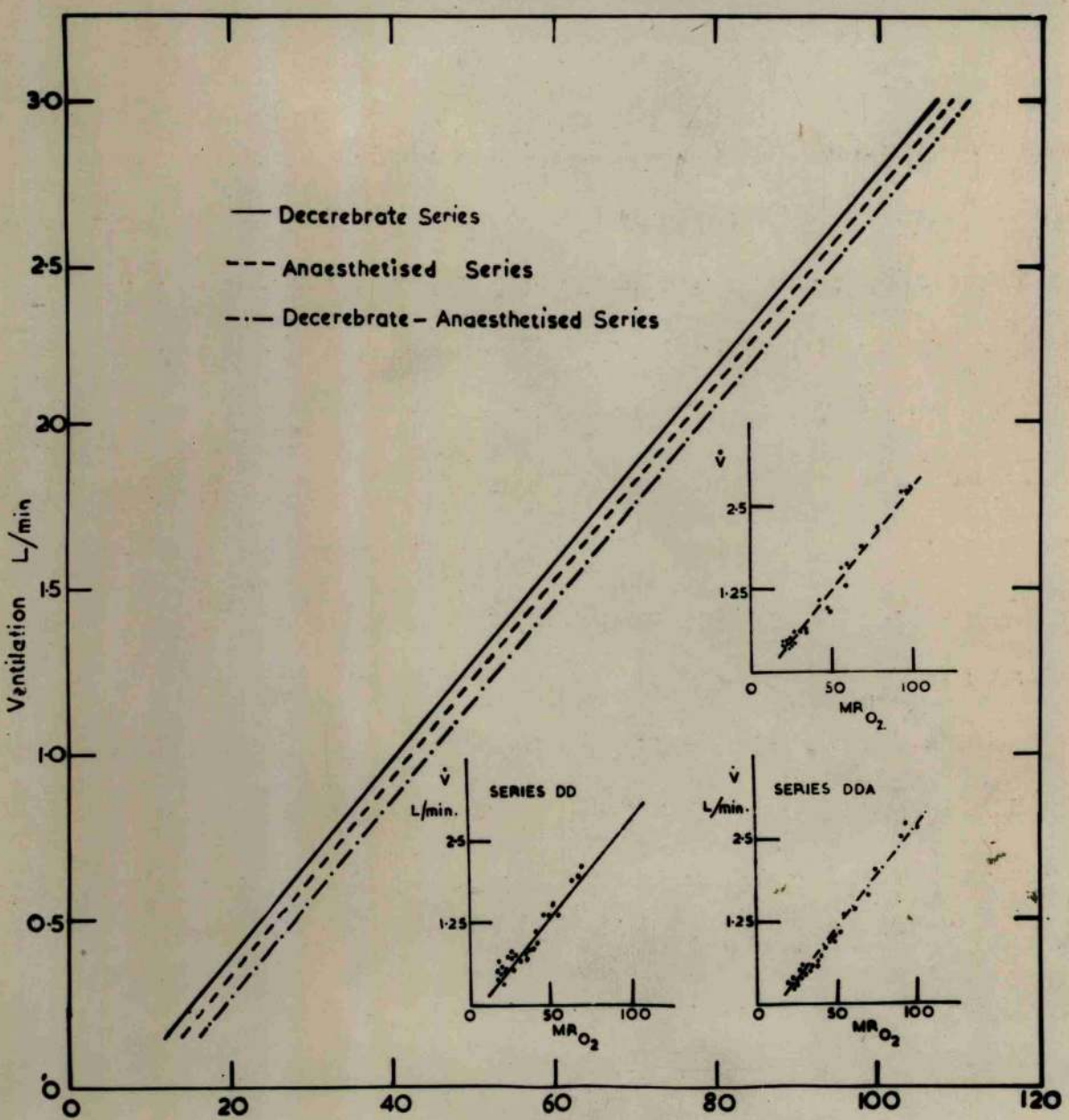
Regression analyses were carried out on the data for $\dot{V}$ and MR_{O_2} for each group, AD, DD, and DDA, individually. The results are seen in Table 9 below.

Table 9.

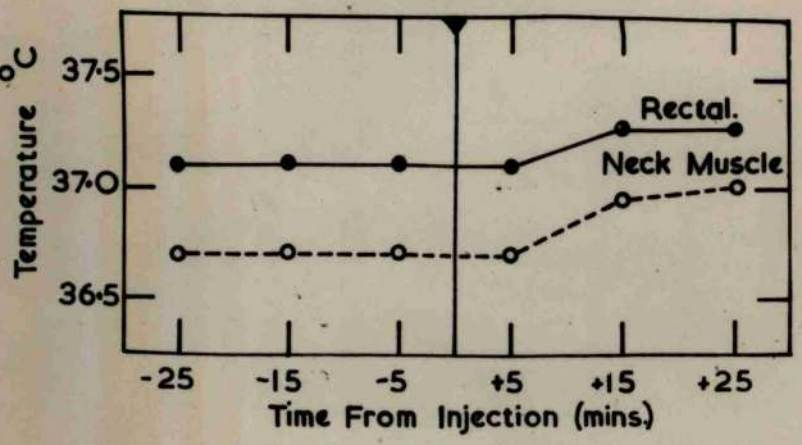
Group	Regression Equation	b.	r.
AD	Y = 0.03X-0.264	0.029	0.985
DD	Y = 0.03X-0.205	0.030	0.935
DDA	Y = 0.03X-0.35	0.030	0.975

As may be noted from the Table, a high correlation was found between $\dot{V}$ and MR_{O_2} in all groups; the regression coefficient (b) is almost identical for all three groups; the regression equations for each group are not significantly different one from another. In the main part of Graph 27 are shown the three regression lines. The smaller graphs show the regression lines for each group and the points from which the lines are derived. These graphs are derived from 84 observations on 25 animals.

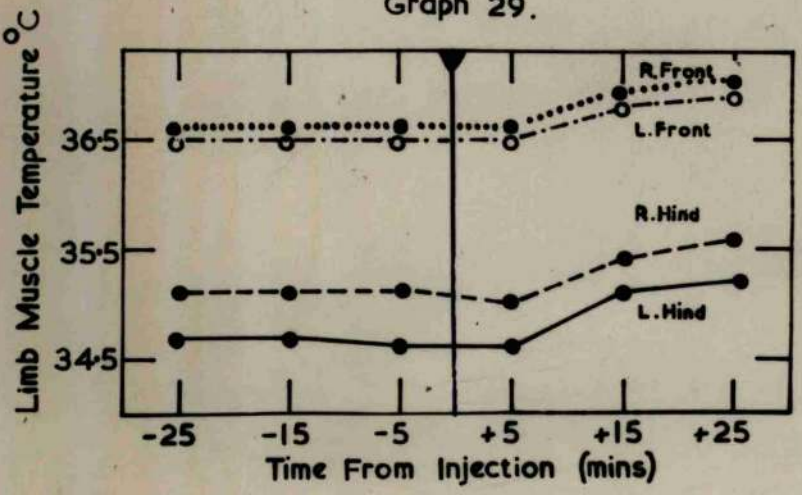
Temperature. In Graphs 28 and 29 are plotted the mean temperatures recorded in the various sites noted. It can be seen that no rise in temperature is recorded in any site until 15 minutes after injection of DNP. The maximum rise in temperature noted was seen in the left hind limb, that of 0.6°C 25 minutes after the DNP injection. None of the temperatures recorded was as high as the normal temperature in an intact unanaesthetised cat which is approximately 38°C.


Decerebrate Series
Anaesthetised Series
Decerebrate – Anaesthetised Series
Ventilation L/min
Oxygen Consumption cc/min.
SERIES DD
SERIES DDA
L/min.
MR O2

Graph 28.



Graph 29.



Discussion.

The data obtained from the experiments just described enable some of the questions arising from previous sections to be answered.

From the data presented in Graph 27 it is obvious that anaesthesia by barbitone-morphia does not alter the respiratory response to an increase in oxygen consumption as produced by DNP. This res ult means that an alteration of the normal respiratory control by anaesthesia cannot be held to account for the position of the calculated line in Graph 24. (See discussion of previous section.) Decerebration does not alter the respiratory response since the data from Group AD (anaesthetised) is not different from that from Group DD (decerebrated). There is still the previously mentioned possibility that the respiratory response to DNP injection is not the same as that produced by exercise.

Slowtzoff (1903) and Zuntz (1897) reported that in trained, normal, intact dogs, ventilation increases in direct proportion to oxygen consumption during voluntary work. A regression analysis of their data gives a regression line with the equation:

$$\dot{V} = 0.030\ MR_{O_2} - 1.142$$

Asmussen et al. (1943) reported the ventilation and oxygen consumption during electrically induced exercise in normal and tabetic men. The equation of the line drawn for the normal subjects is approximately

$$\dot{V} = 0.022\ MR_{O_2} + b.$$

The equation for the data on the tabetic subject is

$$\dot{V} = 0.024\ MR_{O_2} + b.$$

Gray (1950) states that analyses of data for normal men exercising voluntarily shows that the equation $\dot{V} = f(MR_{O_2})$ is a rectilinear function with a direct proportionate relationship. The equation of a line expressing this relationship is

$$\dot{V} = 0.032\ MR_{O_2}$$

Kao et al. (1951) report that in dogs anaesthetised with barbitone, exercise induced by electrical stimulation of the leg muscles produced relative changes in respiration and metabolism described by the equation

$$\dot{V} = 0.031\ MR_{O_2} - 0.139$$

Kao et al. (1952) published data on the ventilation and oxygen consumption of dogs anaesthetised with Nembutal. Exercise was induced in the hind limbs by electrical stimulation. The equation of the line fitted to their data is

$$\dot{V} = 0.034\ MR_{O_2} + 0.123$$

Kao (1952) describes how he exercised the hind limbs of dogs, anaesthetised with Nembutal, by stimulation of motor roots. The regression line fitted to his data on ventilation and metabolic rate is

$$\dot{V} = 0.031\ MR_{O_2} + 0.158$$

Kao et al. (1953) recorded ventilation and oxygen consumption in dogs anaesthetised with chloralose-urethane. Exercise was induced by electrical stimulation of the hind limbs.

The relationship of ventilation to oxygen consumption is expressed by the equation

$$\dot{V} = 0.025\ MR_{O_2} + 0.001$$

In 1955 Kao et al. published work on the regulation of respiration during induced muscular work in decerebrate dogs. The relationship of ventilation to oxygen consumption in the animals is expressed by the equation

$$\dot{V} = 0.033\ MR_{O_2} + 0.154$$

In the experiments reported in this thesis the oxygen consumption of cats was increased by the drug DNP. In three groups of cats, anaesthetised, decerebrate, and decerebrated and anaesthetised, the respiratory response was identical. The relationship of ventilation to oxygen consumption is expressed as

$$\dot{V} = 0.030\ MR_{O_2} - 0.264.$$

In no case does the intercept value of any of the above regression equations differ significantly from zero. The slopes of the lines are not significantly different one from another. It can therefore be said, with reasonable assurance, that the respiratory response to injection of DNP is identical to that produced by exercise.

The data from previous work, as quoted above, combined with the present experiments, also shows that an increased oxygen consumption, whether produced by voluntary exercise, induced exercise (peripherally or via motor roots) or by DNP in man, dogs, or cats, whether anaesthetised or decerebrated or intact

and unanaesthetised, in short, by any means and in any state, will produce exactly the same response in pulmonary ventilation. This fact is strong evidence in favour of the postulate made in Part II of this Book that there are receptors - metaboreceptors - in the periphery which are sensitive to changes in tissue metabolism. Peripheral receptors which are concerned in the control of respiration are not depressed by anaesthetics, (von Euler & Soderberg, 1952; Dripps & Dumke, 1943). This is in agreement with the finding that anaesthesia does not alter the respiratory response to increased oxygen consumption as described above.

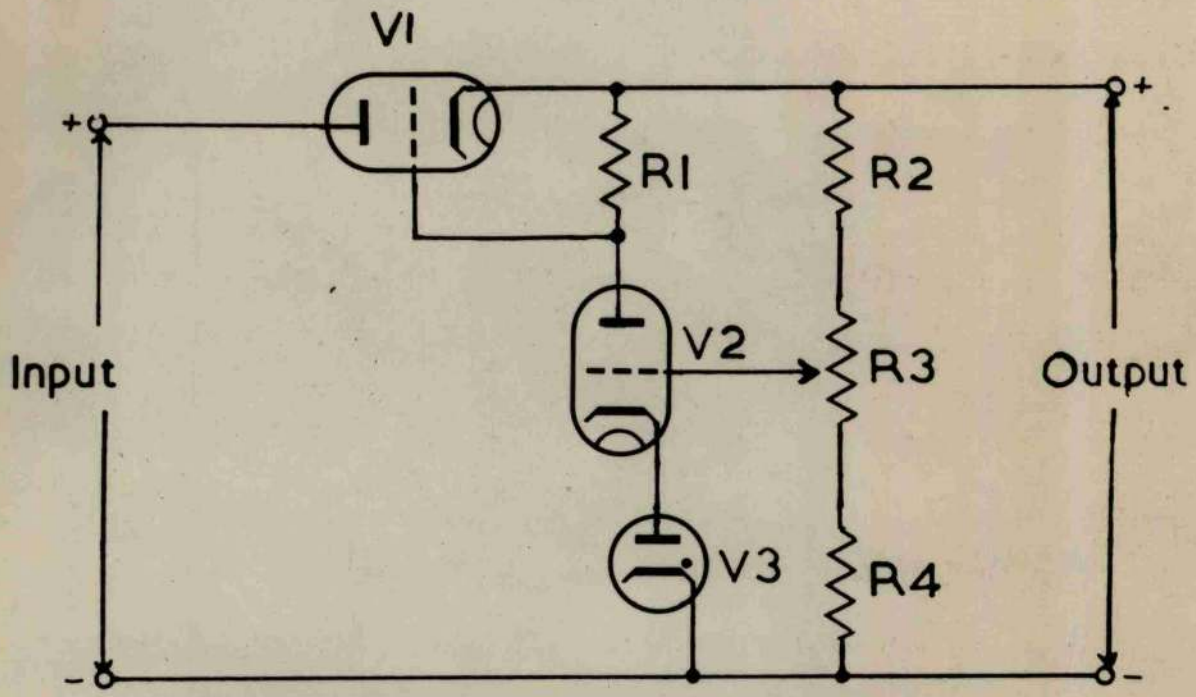
The results of the experiments described in this section also mean that only one explanation is possible for the respiratory responses of Kao's "neural" dogs and the "recipient" animals in the initial experiments (Graph 24). That explanation is that the respiratory response to moderate increases in metabolism is wholly controlled by neural impulses from the periphery. It is considered that there is sufficient evidence, both from the experimental work described in this thesis and from the literature, to put forward the following theory:

The pulmonary ventilation is controlled normally not purely by a humoral feedback mechanism as outlined in Figure 2. For moment to moment control of ventilation, to keep in step with changes in oxygen requirements of the tissues, there is a second feedback control, with neural connections to the controlling system. The input of this feedback is represented by X,

the exercise stimulus, in Figure 2. The humoral control will always be acting but is slow in action and of minor importance except in cases where major changes in blood chemistry occur, e.g. disturbances in acid base balance or severe exercise. For all ranges of normal activity, including moderate exercise, the neural control mechanism is "in charge" and controls the ventilation so that it is directly proportional to the requirements of the tissues for oxygen. The receptors for this control system are in the periphery.

It is not easy to outline a control system, such as is required by this theory, in the same way as has already been done in Figures 1 and 2. There are, however, analogies in electronic circuits which describe such a control system. In Figure 22 is shown the circuit of a series-parallel voltage stabilizer. The action of this stabilizer is to keep the output (blood chemical agents) steady. The reference for the feedback control in this circuit in the output itself. This circuit is then analogous to the Basic Chemostat outlined in Figure 1. This stabilizer, however, is only adequate if the load on the output, which could be compared to tissue oxygen consumption or CO_2 production, remains steady. Figure 23 shows the circuit of a stabilizer with improved stabilization of output. In this circuit a fine control for keeping the output steady under changes in load is obtained by inserting R_6. The previous feedback from the output - analogous to the humoral

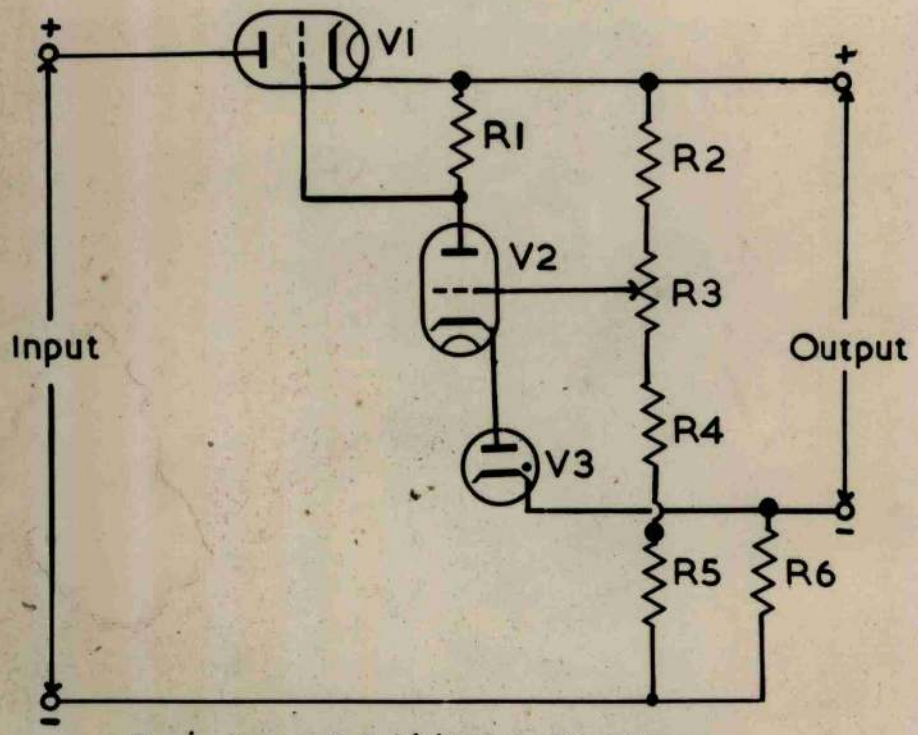
Figure 22.



Series–parallel Voltage Stabilizer

Figure 22.

Figure 23.



Series-parallel Voltage Stabilizer
With Improved Stabilization.

Figure 23.

feedback in Figure22 - is still present but a second feedback from the output load (MR_{O_2}) is brought in. This second feedback is analogous to the signals from the peripheral "metaboreceptors".

Thus the theory of the control of pulmonary ventilation put forward above allows the explanation of available experimental data. The type of control system required by the theory is also not only readily available in a simple, and even bas ic, electronic circuit but actually provides a more stable and flexible control than the previous idea of the control system could do.

Summary of Book I.

1. A general introduction dealing with theories of control of respiration is given.
2. Theoretical considerations of the respiratory control system and a critical review of the literature on the control of ventilation in exercise is presented.
3. The possible role of muscle action in the stimulation of ventilation in exercise is discussed. A theory of special receptors situated peripherally and sensitive to changes in metabolism is advanced.
4. The literature on the use of the drug 2: 4 - dinitrophenol as a metabolic stimulant is reviewed.
5. Experimental work, using cross circulated dogs, was described. This work showed that an increase of muscle metabolism, as produced by DNP, initiates a neurally transmitted stimulus to ventilation.
6. The metabolism of the vascularly isolated perfused limb of a dog was determined. This data made possible the evaluation of the results from the previous work with reference to earlier, similar experiments.
7. A dose-response curve for the stimulation of metabolism by intravenous DNP in cats was determined.
8. It was found that the respiratory response to an increase in metabolism as produced by DNP in the cat was identical to that produced by exercise. It was further shown that

anaesthesia did not quantitatively alter this response in any way.

9. By reference to past literature it was shown that an increase in metabolism, whether produced by voluntary or electrically induced exercise, or DNP in man, dogs, or cats, anaesthetised, decerebrate, or unanaesthetised, produced an identical response in pulmonary ventilation.

10. A new theory of the general control of respiration was advanced. It was shown that the form of the respiratory control system required by this theory has a physical counterpart in a simple electronic stabilizer circuit.

BOOK II.

The Effect of Intravenous Injection of Salicylates and their Chemical Isomers on the Respiration.

Part I.

Introduction and Review of the Literature.

INTRODUCTION AND REVIEW OF THE LITERATURE.

The various derivatives of salicylic acid are probably the most widely used drugs in medicine. Almost every affection or affliction of the human organism is, or has been, treated with sodium salicylate or one of its close chemical relatives. It would be expected that such a commonly used drug would be thoroughly investigated and its pharmacological and physiological actions known and understood. It is true that much experimental work has been published on the action of salicylates in the body. Many of these reports, however, have been contradictory or conflicting in substance, and some of the work done has been frankly misleading. The result is that most salicylate therapy is empirical in basis and many of the effects of the salicylates on body actions, although recognised and accepted, have not been adequately examined or understood.

Very shortly after sodium salicylate was introduced as an antipyretic by Buss in 1875 it was noted that during salicylate therapy there was an increase in "breathing". The mechanism of stimulation of the respiration by salicylates has proved to be of interest to many workers over the past 60 years. The numbers of explanations put forward were almost as numerous as the investigators - and many of them seem rather ingenuous today. It is probable that the

confused state of the existing knowledge on the physiology of respiration itself partially accounts for this.

Johnson (1930), in one of a long series of papers on the salicylates, discusses the question of acidosis following the administration of salicylates. He reports that on parenteral or oral administration of sodium salicylate and aspirin there is a marked respiratory stimulation with a "depletion of alkali reserve", but that the pH of the blood is unchanged. Johnson considered that salicylates produced a "fixed acid acidosis" which was compensated for by a loss of CO_2. This idea of an acidosis being produced by salicylates persisted, as will be seen, for some years. It is now generally recognised that the "alkali reserve" as measured by Johnson and many other workers, is of little value in itself- and only of limited significance - for the determination of changes in blood acid-base balance. Limitations of technique and inadequate data led Johnson - and others - to unwarranted conclusions.

In 1932 Odin noted that at that time salicyl poisoning was almost universally considered to be an acidosis. He sets out to consider the possibility that symptoms of intoxication appearing during therapy with salicylates may be due to poisoning with acid, either from the salicylates or from some other acid secondarily formed in the body. Odin also suggested that the result of salicylate administrations

might not be an acidosis but a respiratory alkalosis and further suggests that the data given by van Slyke & Cullen (1917) would support this contention. Odin reports on determinations made on 27 patients treated with salicylates. He first rules out any possiblity of the response being an allergic one. He then compares the findings in severe diabetic acidosis with those obtained from cases of salicyl poisoning and shows that it is not justifiable to consider salicyl poisoning as a pure and simple acid poisoning. This careful worker concludes that the "results speak strongly for the view that deep respiration (in salicylate therapy) is not due to poisoning with acid but to a toxic irritation of the respiratory centre by the salicyl". With this suggestion, Odin was following the same line of explanation as offered for the action of salicylate as an antipyretic. That it is not true does not detract from the value of Odin's observations.

In the first of a series of papers on the electrolyte structure of the blood after administration of salicylates, Rapoport and Guest (1945) produce evidence that a respiratory alkalosis is produced. From their experiments on monkeys and dogs they conclude that salicylates cause a primary hyperventilation. Guest, Rapoport, & Roscoe (1945) note that Hanzlik (1926) had the impression that non-toxic,

therapeutic doses of salicylate in man do not affect the respiration. Guest and his coworkers show, however, from their observations on 21 patients that an increase in respiration is seen with therapeutic doses of salicylate. They also confirm the findings of Veil & Grauber (1926) and Gebert (1931) that there is a fall in arterial pCO_2 even after a single dose of sodium salicylate in man.

Farber, Yiengst, & Shock (1949) report their experiments on 10 healthy adult males. The subjects were given therapeutic doses of aspirin and measurements were made of ventilation, serum bicarbonate, pH, and total CO_2 of the blood. These workers show without doubt that salicylate therapy produces a respiratory alkalosis and not an acidosis. Farber et al. stress the importance of measuring both the CO_2 content of the blood and the pH before it can be said whether an alkalosis or acidosis of respiratory or metabolic origin is present. They do not, however, offer any explanation of the mechanism of production of the respiratory alkalosis.

In a rather confused paper which, unfortunately, has been rather widely quoted, Graham & Parker (1948) report some observations on rheumatic patients and on rabbits and cats after salicylate administration. By the rather inadequate method of measuring the CO_2 combining power of the blood they claim to show that salicylates cause a primary

stimulation of respiration and hence a respiratory alkalosis. In their animal experiments Graham & Parker recorded respiratory movements by means of a stethograph. This method is qualitative at best and may actually give spurious recordings at worse. They claim to show, however, that removal of the carotid and aortic chemoreceptors, or injection of atropine or acetylcholine do not abolish the stimulation of respiration by intravenous sodium salicylate. They do claim to abolish this stimulation, however, by bilateral vagotomy in the neck. Graham and Parker then conclude that the site of stimulation of respiration by salicylates is peripheral, acting through vagal fibres.

A further confusing point arises from a report by Reid, Watson & Sproull (1955). They investigated the mode of action of salicylate in acute rheumatic fever and from this data it appears that their patients actually had a respiratory alkalosis present before salicylate therapy, the alkalosis being made more severe after therapy. In this respect it is probably best to consider this report as confirmation of the findings of Farber et al. (1949) on normal men. The paper by Reid and his coworkers does, however, touch on certain metabolic effects of therapy with salicylate and the literature on this aspect of the action of salicylates will now be considered.

As early as 1901 Singer reported an increase in oxygen

consumption in rabbits after being given toxic doses of aspirin.

Denis & Means (1916) report the results of their observations of the influence of salicylate on metabolism in man. Three adult males were given therapeutic doses of sodium salicylate. There was a rise in the urinary excretion of nitrogen, phosphate, and uric acid and a fall in ammonia excretion. There was also a slight drop in the Respiratory Quotient. The authors also noted an increase in oxygen consumption in their patients during salicylate therapy.

Sylla (1935) reported an increase in metabolic rate of a woman with salicylate poisoning. He attributed the increase largely to the muscular exertion of the deep and rapid respiration also seen in this woman.

Dodd, Minot & Arena (1937) attempted to explain some of the more serious manifestations of salicylate poisoning. They recorded the oxygen consumption, by means of a Benedict-Roth Spirometer, rectal temperature, pulse, and blood pressure in anaesthetised dogs. On intravenous administration of sodium salicylate there was an abrupt rise in oxygen consumption. The rectal temperature rose after one hour but although the pulse rate often was increased no significant alteration in blood pressure was noted. The only data on

respiration given by these workers is on respiratory rate. No information on ventilation and its relation to the increased oxygen consumption is therefore available from this paper.

Cochran (1952) gave data on the respiratory effects of salicylate in man. His subjects were both normal adults and patients with rheumatic disease. The respiratory measurements were made with a Knipping Spirometer and these measurements included oxygen consumption and CO_2 production, tidal volumes and respiratory rates. It is not noted if the gas volumes are corrected to any standard temperature or not and it is assumed that they were not. The actual pulmonary ventilation was not calculated but enough data is published for this to be done. In normal subjects it was found that intravenous and oral salicylate produced a marked rise in oxygen consumption and CO_2 production. There was a coincident rise in ventilation. Observations were not made on a sufficiently large number of subjects for statistical analyses to be carried out on the data but it would appear that the VE_{O_2} is not greatly changed after salicylate administration although the ventilation equivalent for carbon dioxide may rise slightly. This paper is the first in which sufficient data is given for the relationship between metabolism and ventilation to be examined.

Reid (1952, unpublished data) also noted a marked increase in the oxygen consumption of rabbits after injection of salicylate. Sproull (1954) demonstrated that sodium salicylate is a powerful metabolic stimulant by showing that the Q_{O_2} of mouse liver slices in vitro was markedly increased by treatment with 3.5 to 7.5 x 10^{-4} M sodium salicylate.

There is thus adequate evidence that salicylates produce an increase in pulmonary ventilation and also an increase in oxygen consumption. Salicylates are capable of producing an increase in oxygen consumption invitro and it is possible that their action on ventilation and metabolism in the intact animal are not unlike those of exercise or DNP injection. There is, however, very little quantitative data on these possible actions of salicylate and it was considered that it would be of value to obtain some such measurements.

Part II.

Original Observations.

METHODS.

Animals. The experimental animals used were cats. In cross-circulation experiments dogs were used. The cats were anaesthetised with ether and then decerebrated in the routine manner as previously described. The dogs were anaesthetised with morphine-barbitone as described earlier.

Respiratory Measurements. Records of respiratory rate, tidal volume, and oxygen consumption were obtained by means of the special animal respirometer described in detail in Section B of Book I.

Drugs and Doses. The drugs used were sodium salicylate and stabilized calcium aspirin (calcium acetylsalicylate) 15% solution. 7.5% solutions of sodium meta - and para - OH - benzoate, the chemical isomers of sodium salicylate, were also used. The drugs were injected intravenously in doses varying from 0.05 to 0.1 g./kg. body weight.

RESULTS.

Intact decerebrate cats. Figure 24 shows the type of record obtained when a dose of 0.1 g./kg. sodium salicylate was injected intravenously in an intact decerebrate cat. In this experiment the resting ventilation was 1.10 l./min. Immediately after injection the ventilation increased to 3.61 l./min. After approximately 1½ mins. the ventilation had fallen to 2.21 l./min. Oxygen consumption also rose, reaching a steady state level, in this experiment, of 2.3 times the pre-injection level. Figure 25 shows the response after an injection of 0.05 g./kg. sodium salicylate. In this experiment the relavent values for ventilation are:

Steady State pre-injection - 0.77 l./min.

Immediately post-injection - 1.06 l./min.

Steady State post-injection - 0.91 l./min.

This picture of an immediate increase in ventilation on injection of sodium salicylate followed by a fall to a steady state value higher than the pre-injection level was seen in all experiments where the drug was injected into intact decerebrate animals.

The ventilation equivalent for oxygen (VE_{O_2}) is the most useful measurement for examining the relationship between ventilation and oxygen consumption. Comparison of the mean VE_{O_2} of these animals during the steady state pre-injection period and that of the steady state post-injection period

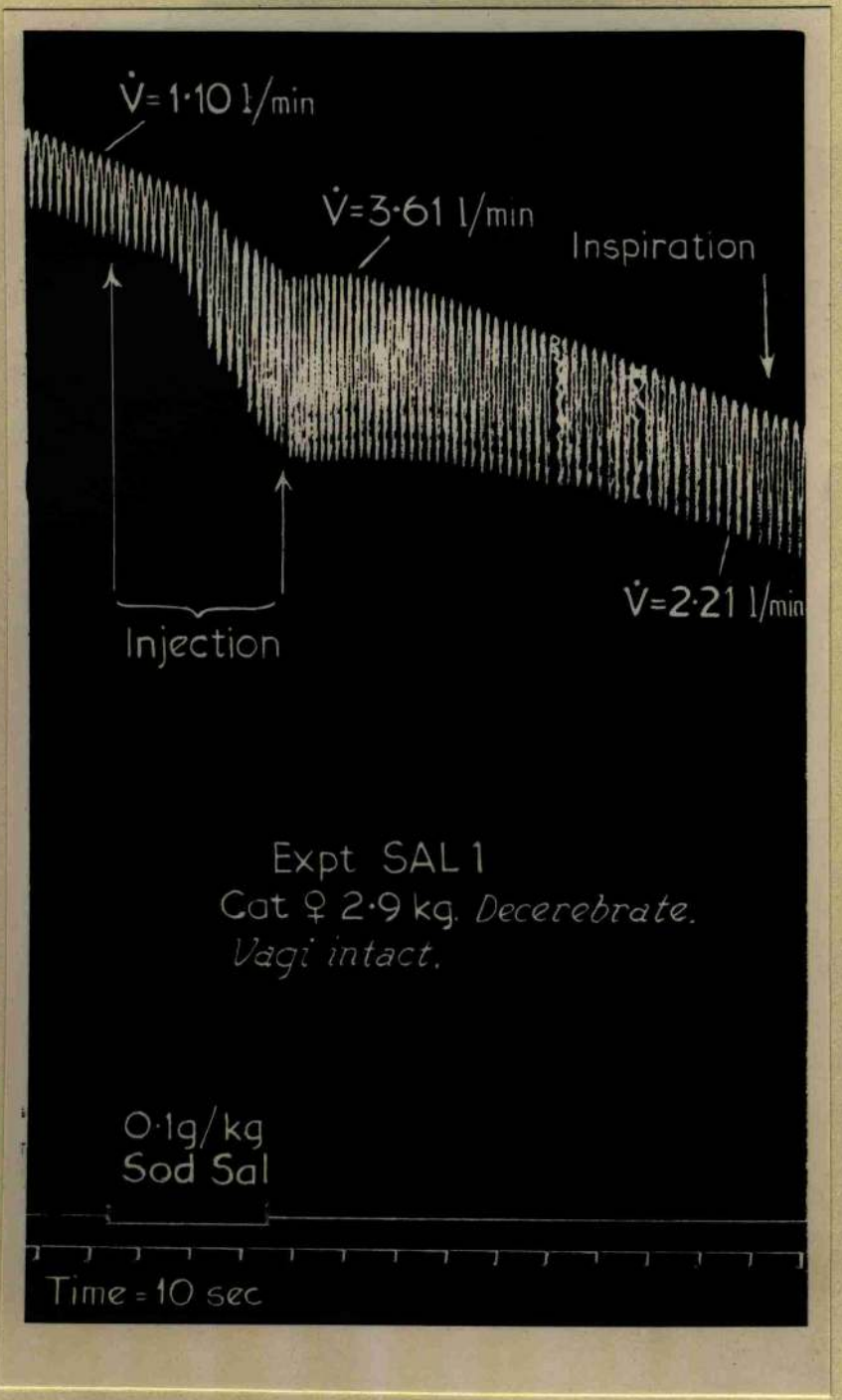

V̇= 1·10 l/min
V̇=3·61 l/min
Inspiration
V̇=2·21 l/min
Injection
Expt SAL 1
Cat ♀ 2·9 kg. Decerebrate.
Vagi intact.
0·1g/kg
Sod Sal
Time = 10 sec

Figure 24.


Inspiration
Injection
Expt SAL 2
Cat ♂ 2·8 kg
Decerebrate
0·05g/kg
Sodium Salicylate
Time 10 secs.

Figure 25.

shows that there was no change in these values. The relevent figures are:

Pre-injection - 2.70

Post-injection - 2.69

The significance of this finding will be discussed in the next section.

Venous blood samples were taken from two of the animals 35 mins. after injection of 0.1 g./kg. sodium salicylate. The plasma salicylate level was found to be 34 mg.% and 36 mg.% in these samples. These levels are of the same order of magnitude as is attained in salicylate therapy in acute rheumatic conditions.

Figures 26 and 27 show that intravenous injection of 0.1 g./kg. Calcium Aspirin does not produce the initial marked increase in ventilation seen on injection of sodium salicylate. The secondary increase in ventilation coincident with a rise in oxygen consumption is still seen, however. With injection of calcium aspirin, furthermore, there is a longer time interval after the injection before a steady state is reached - 20-30 mins. instead of 10-20 mins. after sodium salicylate. A further point of difference in the response to injection of calcium aspirin from that of sodium salicylate is that, weight for weight, calcium aspirin is less effective. For example, 0.1 g./kg. Ca. aspirin will increase the oxygen consumption to approx-

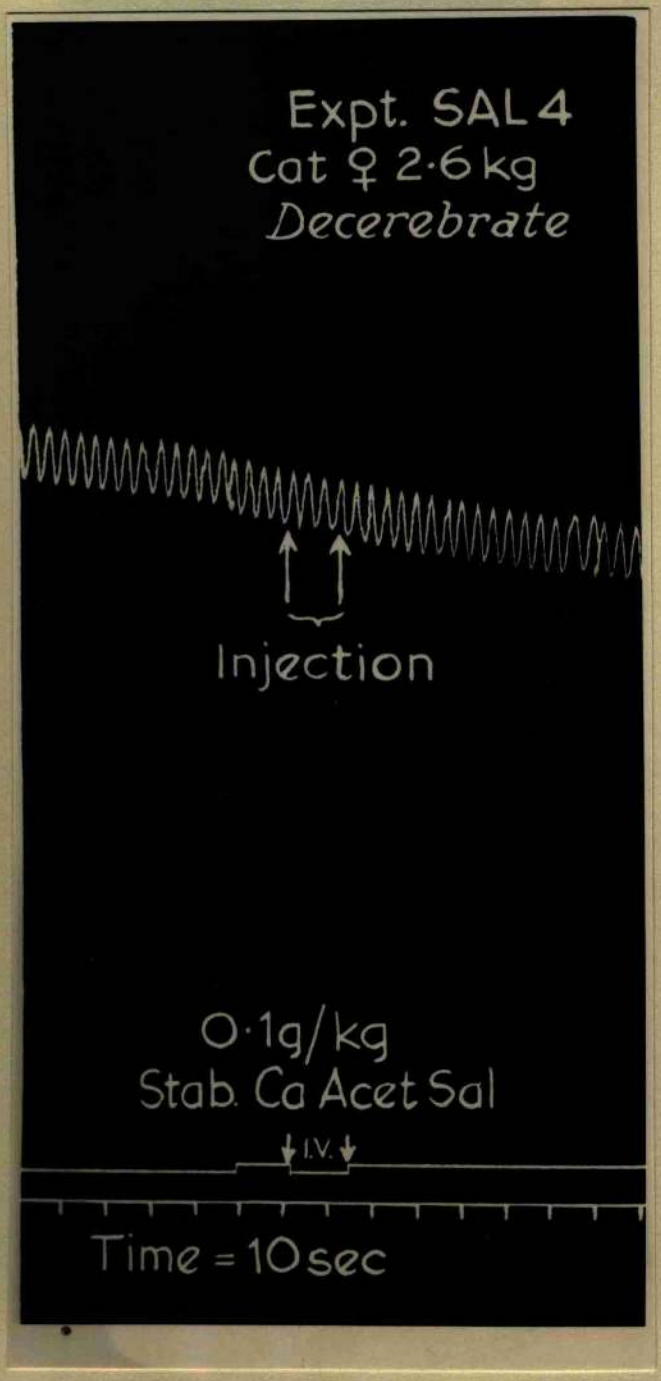

Expt. SAL 4
Cat ♀ 2·6 kg
Decerebrate
Injection
0·1g/kg
Stab. Ca Acet Sal
I.V.
Time = 10 sec

Figure 26.

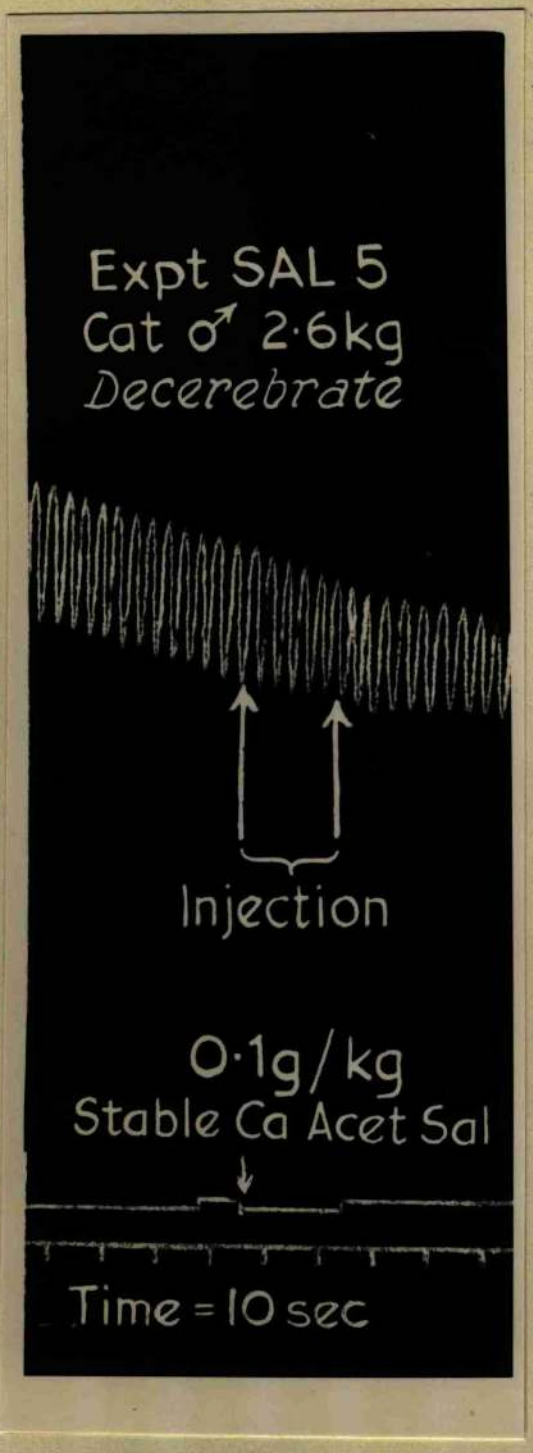

Expt SAL 5
Cat ♂ 2·6kg
Decerebrate
Injection
0·1g/kg
Stable Ca Acet Sal
Time = 10 sec

Figure 27.

imately 1.4 times the resting level. 0.1 g./kg. sodium salicylate will double the resting metabolic rate.

Blood samples taken 35 minutes after injection of 0.2 g./kg. calcium aspirin showed plasma salicylate levels of 39 mg.% and 41 mg.% respectively.

The Stabilized Calcium Aspirin from which the solution injected was prepared has the following composition:

Calcium Acetylsalicylate	96.75%
Sodium Chloride	0.5 %
Calcium Chloride	2.5 %
Calcium Carbonate	0.25%

When solutions of the isomers of sodium salicylate are injected intravenously the results, as recorded by the respirometer, are as shown in Figures 28 and 29. As can be seen, intravenous injection of 0.1 g./kg. of the isomers produces no immediate change in either ventilation or oxygen consumption. There may be a slight decrease in tidal volume for about 20 seconds following injection of sodium - p - OH - benzoate, but it does not appear to be significant. No change in oxygen consumption resulted from administration of the isomers of sodium salicylate.

Decerebrate vagotomised cats: As noted previously Graham et al. (1948) suggested that salicylates exert a primary stimulatory action on respiration and claimed to have localised the action at peripheral vagal nerve endings. Since other work (Cochran, 1952, 1954; Sproull, 1954)


Inspiration
Injection
Expt SAL 7
Cat ♂ 3·6 kg
Decerebrate
0·1 g/kg
Na-p-OH-benzoate
Time 10 secs

Figure 28.

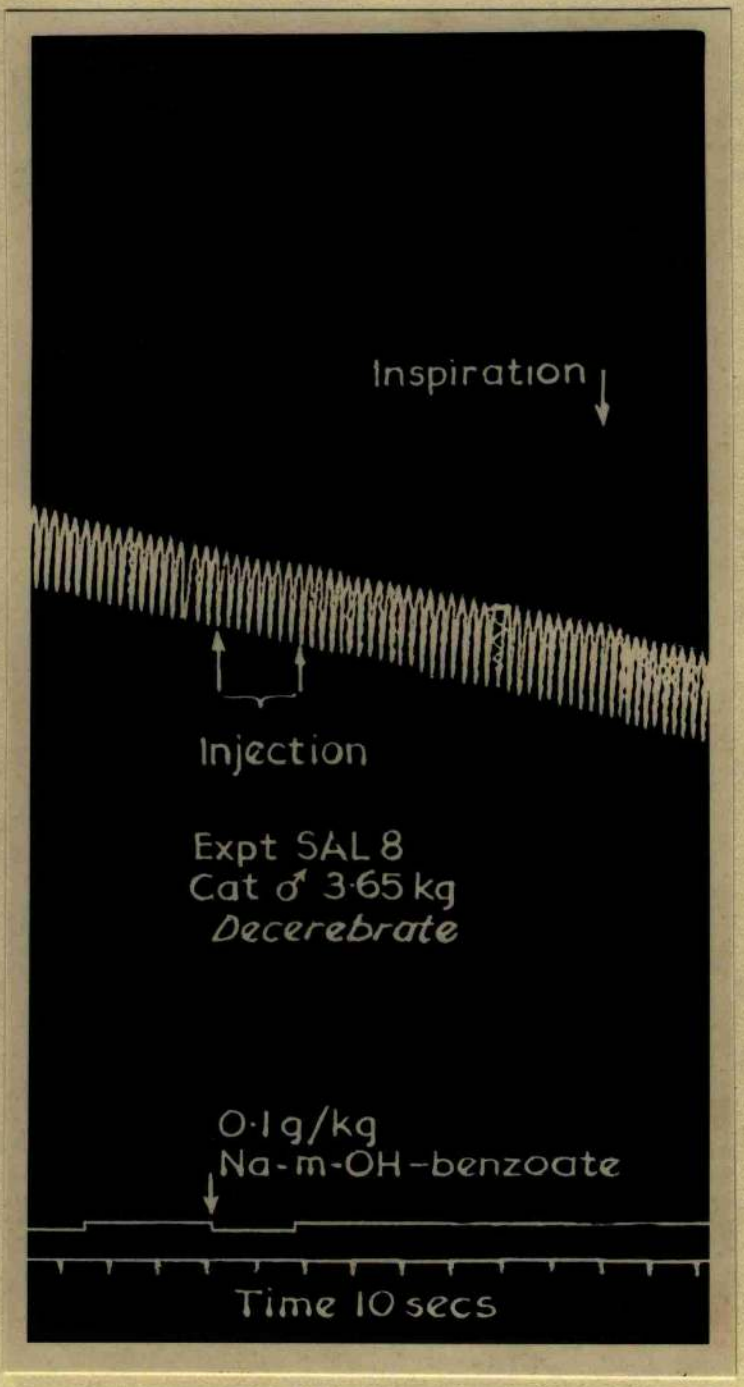

Inspiration
Injection
Expt SAL 8
Cat ♂ 3·65 kg
Decerebrate
0·1 g/kg
Na-m-OH-benzoate
Time 10 secs

Figure 29.

had indicated that the primary action of salicylates may be metabolic and not respiratory, it was considered that a re-examination of Graham and Parker's claim should be undertaken.

The vagi of cats, decerebrated in the routine manner, were sectioned in the neck. After a period of stabilization control records were taken of the respiration. Sodium salicylate (0.05 g./kg.) was then injected intravenously. The response obtained is shown in Figure 30. As may be seen the response was qualitatively similar to that already noted in the intact decerebrate cat. There was an immediate and marked increase in ventilation followed by a fall to a steady state level higher than the resting level, coincident with a rise in oxygen consumption. In the experiment illustrated the ventilation was increased from 0.94 l./min. at rest to 1.2 l./min. at steady state post-injection. As with the intact decerebrate animals, no change in VE_{O_2} was noted.

Cross circulation experiments. In an attempt to ascertain whether or not the stimulatory effects of salicylates on respiration are central or peripheral in origin some cross circulation experiments were done. The experimental set-up was as already illustrated in Figure 15. Records of respiration were obtained from the recipient dog.

Figure 31 shows the response obtained when 0.1 g./kg. donor dog body weight sodium salicylate was injected into the arterial side of the perfusion system. As illustrated,


Inspiration
Injection
Expt SAL 3
Cat ♂ 3·98 kg
Decerebrate:
Vagi cut
0·05g/kg
Sodium Salicylate
Time 10 secs.

Figure 30.

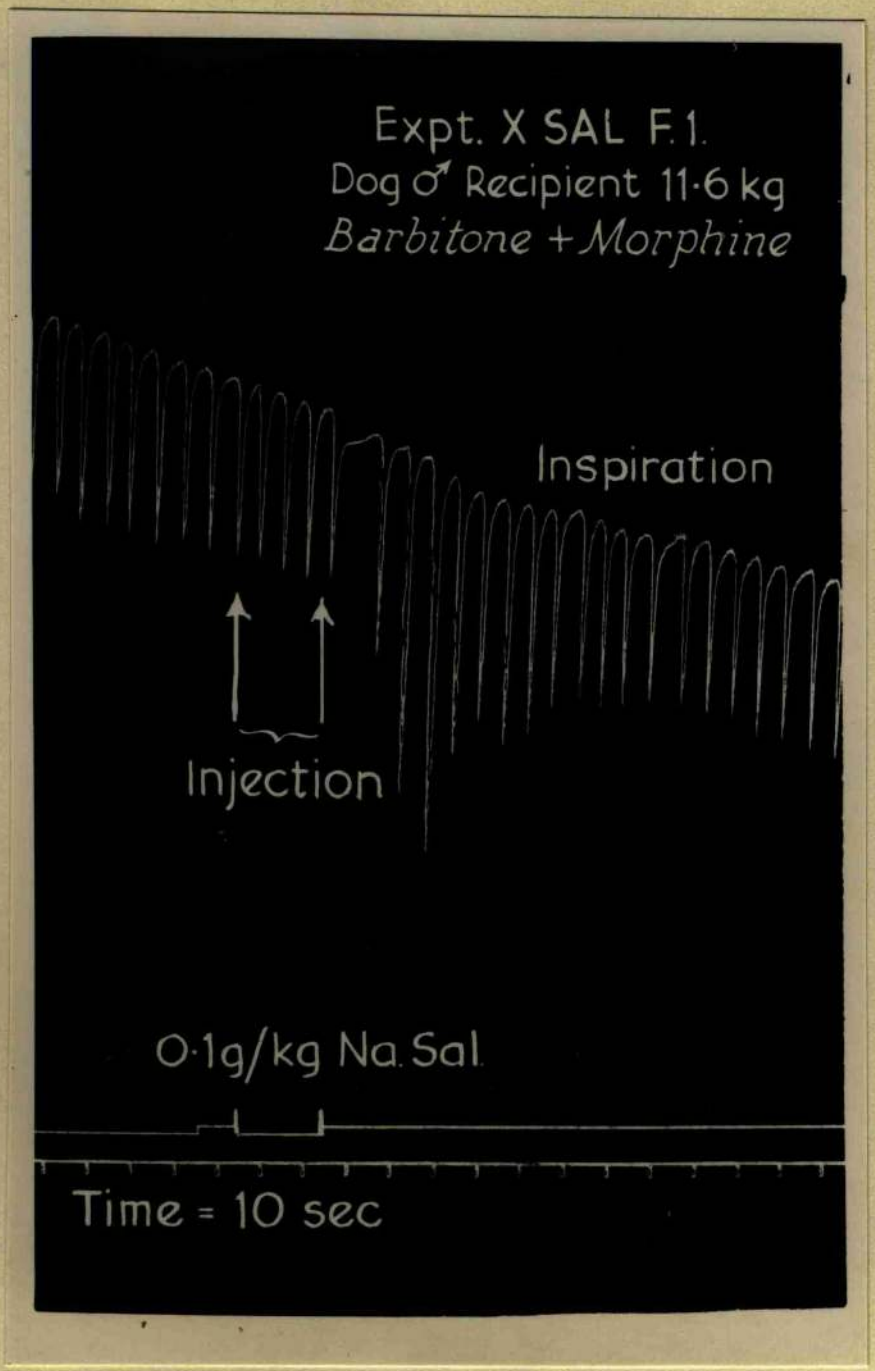

Expt. X SAL F.1.
Dog ♂ Recipient 11·6 kg
Barbitone + Morphine
Inspiration
Injection
0·1g/kg Na. Sal.
Time = 10 sec

Figure 31.

there was an immediate, transient, marked increase in the ventilation of the recipient dog. As shown in Figure 32, when the sciatic and femoral nerves connecting the cross-circulated limb from its parent body were cut, no such response was seen.


Injection
Expt. X SAL F.4.
Dog ♂ Recipient 19 kg
Sciatic and Femoral nn. cut.
Na Sal
IV-cannula
Time = 10 sec

Figure 32.

DISCUSSION.

It is obvious from the results of the experiments on intact decerebrate cats that the respiratory response to intravenous injection of salicylates is not a simple one. There are evidently two different types of response produced. (i) A primary, immediate response on injection - a rapidly appearing but transitory increase in pulmonary ventilation - and (ii) a secondary response - an increase in ventilation developing over 10-20 mins. after injection coincident with a rise in oxygen consumption. The secondary response is that referred to in the literature as "salicylate hyperpnea" and is of most physiological interest. The primary response is that dealt with by Graham et al. (1948).

In the experiments on vagotomised animals by Graham and Parker it would seem that the slowing and deepening of respiration produced by vagotomy, (c.f. Figures 24 and 30) combined with the insensitive stethographic recording method used, masked the immediate respiratory response to intravenous injection of sodium salicylate. Their method of recording respiration was not sensitive enough, neither did they take records for a long enough period after injection of the drug, to note the secondary and prolonged stimulation of respiration. The use of more accurate methods of recording respiration in the experiments here reported shows

that vagotomy does not, in fact, abolish the immediate stimulatory effect on the respiration of the intravenous injection of sodium salicylate. The cross circulation experiments show quite clearly that this immediate effect - and probably the secondary respiratory stimulation also - arises peripherally. The finding that stabilized calcium aspirin solution ("neutral" or "soluble" aspirin) when injected intravenously will not elicit the primary response indicates that the drug itself may have some stimulatory action on nerve endings at, or close to, the site of injection. Sodium salicylate solution is used as a sclerosing agent and is not, by any means, non-irritant to tissues. Sharp pain may give rise to respiratory gasps or short transient periods of increased ventilation. It is probable that the injection of sodium salicylate stimulates pain endings at, or close to, the site of injection and s o produces the initial effect on respiration noted.

The secondary, prolonged, increase in ventilation is coincident with a rise in oxygen consumption. The increase in ventilation is in direct proportion to the increase in oxygen consumption, in other words, VE_{O_2} remains steady. This response is the same as that seen in exercise or after metabolism has been increased by injection of DNP, as

described in Book I. Salicylate hyperpnea, therefore, can be adequately explained, physiologically, by the action of salicylate as a metabolic stimulant. This metabolic stimulation probably occurs at the cellular level and the stimulus to respiration is probably akin to, if not identical with, the exercise stimulus.

The discussion above leaves unexplained the mode of production of the respiratory alkalosis found during therapy with salicylates. In subjects and animals where salicylate is administered by intravenous injection the initial transitory hyperpnea will certainly produce some measure of acapnia. There is, however, no reason for this condition to remain for any time after the hyperventilation has diminished and the respiration is a true metabolic hyperpnea. In normal circumstances it would be expected that such an acapnia would be eliminated, by the normal respiratory control system, within a few minutes. Oral administration of salicylate would also not be expected to produce the initial transient hyperventilation produced by intravenous injection. Since this work was completed two publications have come to hand in which an attempt is made to deal with this problem.

Alexander, Spalter and West (1955) report on modifications of the respiratory response to CO_2 inhalation by administration of salicylate. They claim that after administration of aspirin orally, their subject showed an increase in the

sensitivity of the respiratory centre to the CO_2 stimulus. Apart altogether from the fact that their conclusions are based on very scant data there are several reasons why this is not considered to be the solution to the problem.

If the subject already had developed a respiratory alkalosis when the "sensitivity test" was carried out, then the gradient of the line expressing sensitivity, as these workers expressed it, would be greater than the "control" line. This would occur since the increase in ventilation from a unit increase in pCO_2 would be the result of stimulation by a rise in pCO_2 and also a removal of some of the inhibition of respiration exerted by the acapnia and alkalosis.

The suggestions of Alexander et al. would mean that salicylates exert an action centrally on the respiratory centre. All the actions of salicylates were at one time considered to result from central effects by the drug. Antipyresis, metabolic stimulation, stimulation of ventilation; - all have been shown to be peripheral in basis. It is difficult to believe that salicylates will produce an alkalosis by an action centrally whereas all the other actions originate peripherally. Grodins (1950) showed that it was not necessary, nor true, to postulate an increase in sensitivity of the respiratory centre to explain the hyperpnea of exercise. It is considered that this is also true for

salicylate hyperpnea.

Tenney and Miller (1955) give a fairly extensive report on the respiratory and circulatory actions of salicylate. These workers give data which confirms that given in the present report. Their data on intravenous injection of sodium salicylate in dogs show the same type of responses as described above. They did not, however, recognise that there is a dual respiratory response. Tenney and Miller also found that 0.1 g./kg. sodium salicylate will approximately double the oxygen consumption in anaesthetised dogs as was found with intact decerebrate cats. The above workers found that after denervation of the carotid and aortic chemoreceptors and bilateral vagotomy injection of sodium salicylate still produced an increase in ventilation. They conclude that the locus of action of salicylate is the respiratory centre in the medulla. Such a conclusion is, of course, unwarranted. Tenney et al. further note that after administration of salicylate, the cardiac output rises, but not so quickly as the rise in ventilation. Thus, they suggest, the ratio of ventilation to blood flow through the lungs rises, producing the equivalent of a hyperventilation and so a respiratory alkalosis. Such an explanation would be compatible with the data and conclusions already presented in this thesis. Its validity is not, however, confirmed.

The knowledge of the control of cardiac output and circulation is not as yet adequate for this to be done.

It would appear, therefore, that there are two possible explanations for the production of a respiratory alkalosis with salicylate therapy. Firstly, there is the possibility of an increase in sensitivity of the respiratory centre. This possibility, although considered not probable, is not disproved, and there is some evidence that may be held to support it. Examination of various theoretical aspects of respiratory control as developed by Grodins, Gray, Schroeder, Norins and Jones (1954) as well as the action of the control system as necessitated by the theory presented in Book I, show that an increase in sensitivity of the centres could explain the respiratory alkalosis produced by salicylates. Experimental work is necessary to prove or disprove such a contention. An experiment which might settle whether or not salicylate has a central action would be as follows. The head of one animal (the recipient) could be cross-circulated with arterial blood from a donor animal. Salicylate would then be given to the donor. If there is a hyperventilation produced in the recipient animal then salicylate could be said to have some central action. Quantitative measurements could not, however, be made in the recipient animal since the humoral feedback loop in the respiratory control system would

be broken in that animal. The second possible explanation of the respiratory alkalosis produced by salicylate is that the lung ventilation/perfusion ratio rises giving the equivalent of a hyperventilation. Confirmation or refutation of this suggestion must await further information and quantitative data on the mechanism of control of the circulation.

Summary of Book II.

1. A review of the literature on the effects of salicylate administration on respiration is presented.
2. Experiments on dogs and cats in which quantitative measurements of ventilation and oxygen consumption were made both before and after injection of salicylates are described.
3. It is shown that intravenous injection of sodium salicylate produces a respiratory response having two components - an immediate, marked, but transitory increase in ventilation and a long lasting increasee in ventilation coincident with a rise in oxygen consumption.
4. It is shown that vagotomy does not abolish the immediate respiratory stimulation by intravenous sodium salicylate and, with the help of cross-circulation experiments, it is demonstrated that this effect, and also the secondary respiratory stimulation, is peripheral and not central in origin.
5. The implication of the experimental results are discussed and it is shown that salicylate hyperpnea is similar to, if not identical with, exercise hyperpnea.
6. The possible modes of production of the respiratory alkalosis found during salicylate therapy are discussed. It is shown that further experimental work is necessary for the elucidation of this problem.

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