THE RESPIRATORY RATE AND VOLUME OF THE GUINEA PIG

by

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A thesis submitted in Partial fulfilment of
the requirements for the degree of
Master of Science in Agriculture
in the Department

of

Animal Husbandry

We accept this thesis as conforming to the standard required from candidates for the degree of MASTER OF SCIENCE IN AGRICULTURE.

Members of the Department of Animal Husbandry

THE UNIVERSITY OF BRITISH COLUMBIA

May, 1954

ABSTRACT

The present methods for the measurement of respiratory rate and volume of laboratory animals have been reviewed. The review suggests that these methods leave much to be desired. For this reason an apparatus has been developed in the hope that it may overcome the difficulties associated with those described in the literature. The apparatus consists of a mechanism to interrupt a beam of light in time with the animal's breathing, and the signallso produced is amplified to close a double pole double throw relay. state of the relay, open or closed, selects an arrester on either the air supply spirometer or the air receiver spirometer. When the animal breathes in the air supply spirometer is free to move and the air receiver is locked allowing air to be taken from the air supply spirometer only. On expiration the reverse situation is in effect. A stylus attached to each spirometer records the downward or upward travel and hence the volume on a kymograph drum. An electro-magnetic stylus records each inspiration on the kymograph and a five second timer writes a time base.

A limited amount of data on female and male guinea pigs is presented to indicate the validity of the measurements made with the apparatus. Equations based on these data relating respiratory rate and volume of the guinea pigs to body weight have been derived. These equations are compared with those of other workers and suggest that the apparatus devised may be superior to those described in the literature.

Acknowledgments

The writer wishes to thank Professor H. M. King, Head of the Department of Animal Husbandry for his kind permission to carry out this work and for his provision of the laboratory facilities used.

To Dr. A. J. Wood, Associate Professor in the Department of Animal Husbandry, sincere gratitude is expressed, for his inspiration, enthusiastic interest and constructive criticism throughout the course of this work.

The writer also wishes to thank Defence Research Board of Canada for making this problem available.

Special thanks are tendered my many associates at the Animal Nutrition Laboratory for their encouragement and helpful criticisms throughout the duration of this work.

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Ι

Introduction

Since 1777 when Lavoisier and Laplace did their classical experiment on animal calorimetry and demonstrated a definite relation-ship between the heat produced by an animal and the carbon dioxide exhaled, interest in the quantitative aspects of respiration has slowly but surely increased to its present day position as a major field of investigation.

Present day interest in respiratory rate and volume revolves around such basic fields of investigation as animal energetics, pathology, toxicology, and homeostasis. These several branches of research may be considered to have two important aspects, namely, the fundamental and the practical, both of which are of major importance to the agricultural researcher.

Bio-energetics or animal calorimetry falls into two major categories, direct and indirect calorimetry. In practice, since the direct method is much more expensive and complicated it is rarely used, except when the caloric values of O_2 and CO_2 are in doubt. Indirect calorimetry was first used by Lavoisier and is based on the fact that, normally, O_2 consumption and CO_2 production are closely correlated with heat production. These studies therefore require a means of measuring respiratory volume or more important a means of measuring O_2 consumption, CO_2 production and hence Respiratory Quotient.

Animal pathology, particularly with respect to infections gaining admittance to the host via the respiratory route, e.g. Newcastle Disease, necessitate an accurate measure of respiratory volume, and also a means to partition or separate the inspired air carrying a known concentration of organisms and the expired air in which the concentration of organisms can be determined. From this data can be determined the bacterial dosage and subsequently the L.D.50 for a particular agent by the respiratory route.

With the increasing use of chemical insecticides, weed killers and the ever existent possibility of a conflict involving chemical warfare, the importance of knowing the tolerance of our farm animals to these agricultural chemicals on the one hand, and having a means of assessing, by animal tests, the lethality of our chemical warfare agents on the other, adds greatly to the necessity of having at our disposal a means of measuring tidal air volume and what is more important separating inspired and expired air.

The principle that all homeothermic organisms react to changing conditions in such a manner as to maintain constant their internal environment has long been known. However, the extent to which animals may adjust to changes in their environment, e.g. Temperature, humidity, and the role played by respiration in these changes is a subject of much research. The air conditioning of a dairy barn or a movie house is closely related to data obtained on the respiratory exchange and its role in thermal regulation of the inhabitants.

The many avenues of investigation cited or implied, fundamental

Iaboratory investigations are restricted by financial and physical limitations to workings mainly on small animals, namely the guinea pig, rabbit, white rat, white mouse and hamster. These requirements and limitations then would surely make any apparatus for directly measuring respiratory rate, respiratory volume, inspired volume and expired volume on small laboratory animals extremely useful. The fact is that much work is at present in a state of "postulative uncertainty" through lack of a suitable method of obtaining respiratory data, and by the same limitation much work has yet to be done.

II

Review of Literature

The few methods presently used for the determination of respiratory volume may be considered under several headings. One of the earliest methods calculated the volume indirectly from the oxygen consumption. Various methods for the determination of O2 consumption and CO2 production have been developed as a tool in the study of animal energetics. A closed-circuit-spirographic mask method originated by Frederica (1887) and perfected by Benedict (1920) in the U. S. A. and Keogh (1923) in Europe is now the standard Benedict-Roth-Collins clinical metabolism apparatus. The apparatus functions by connecting the pulmonary system of the subject to an oxygen spirometer, and thus measuring the rate of oxygen consumption by the rate of decline of the oxygen bell. The air is circulated freely through porous soda-lime in one direction by valves thus removing the CO₂ from the expired air. The oxygen bell, which floats freely in a water seal, is counterbalanced by a weight, and so will not rise or fall except when acted upon by the circulating air. The decline of the bell is recorded graphically on a clock kymograph drum. rate of oxygen consumption is computed from the slope of this graphic This method works well for large farm animals and human beings and has been used extensively by Brody (1945) in his work on metabolic rates of steers, dairy cattle, horses, sheep and goats.

A closed-circuit-chamber system for small animals was originated by Regnault and Reiset (1849). This method involves rebreathing the same air after removing the CO2 by circulating through soda lime, and replacing the consumed O2 by fresh O2. In the United States Kleiber (1940) used a modification of this apparatus for measuring the metabolism of rats, and Winchester (1940) has adapted it for use with chickens. This apparatus consists of four (1) constant-temperature cabinet, (2) burette system consisting of three tubes, two large and one ordinary titration burette, all interconnected so that they have the same water level, (3) Mariotte bottle, (4) CO2 absorbers. There are also auxiliary items, including pressure gauge; equilibrator, which adjusts temperature of water in Mariotte bottle to that of chamber, and 02 concentration of the water to that prevailing at chamber temperature; O2 spirometer, which keeps air out of the top of the Mariotte bottle by connection with pure 02; rocking mechanisms and fans.

The rate of O_2 consumption is measured by the rise of the water in the burette. As the O_2 from the burette system is consumed, it is replaced by water which automatically flows from the Mariotte bottle whenever the pressure at the siphon tube outlet falls below that at the inlet.

The CO_2 is absorbed in the absorber battery by a saturated $Ba(OH)_2$ solution in two sets of flat-bottomed flasks joined near the bottom by a glass tube. The battery is rocked at the rate of 40 oscillations per minute and the alkaline solutions flow from one set

of flasks to the other, alternately drawing air from and returning it to the chamber at the rate of 12 liters per minute.

The O_2 consumption measurements do not begin until after the animal has been in the chamber for 1/2 hour, in order to (1) accustom the animal to the chamber, (2) bring the system to a standard temperature and (3) establish an equilibrium between the absorbing rate of the battery and the OO_2 production rate.

Possible objections to these methods are: (1) the necessity of using O_2 , (2) the accumulation of water vapour which tends to depress the heat regulation by water vaporization at higher temperatures, (3) in the case of Winchester's apparatus it does not permit measurement of the water vaporized.

The open-circuit gravimetric method or air flow method was devised by Haldane (1892). This consists of a respiration chamber, in which the animal is kept, with several H₂O and CO₂ absorbers. The H₂O is usually absorbed by concentrated H₂SO₄ (in which lumps of pumice stone may be placed for increased area) or by such dry H₂O absorbers as magnesium perchlorate. The CO₂ is absorbed by alkali, such as a concentrated solution of NaOH or Ba(OH)₂, or more conveniently by "shell caustic." Air is drawn through the chamber and absorbers by a pump and a record of the rate of air passage is recorded by a wet test gas meter or similar meter. The air is freed of its CO₂ and H₂O by absorbers before it enters the chamber and also as it leaves the chamber by another set of absorbers. Thus some atmospheric O₂ is retained by the system in the form of CO₂, but nothing leaves the system. Hence, while the animal loses weight during the trial, it

loses CO_2 and H_2O , the system as a whole, the chamber and effluent absorbers gain in weight. The gain in weight represents O_2 consumed. From a trial with an apparatus such as this the H_2O vaporized, the CO_2 produced, and the O_2 consumed may be determined.

Modifications of the Haldane type apparatus can be used for large farm animals but as the chamber is of necessity too large for weighing, and the CO_2 production too much for absorption, air flow metering, aliquoting and gas analysis are employed. The air coming into the chamber is assumed to contain 0.031 per cent CO_2 and 20.939 per cent O_2 , the outgoing air is analyzed for its CO_2 and O_2 content. The rate of circulation is measured. The percentages of O_2 decrement and CO_2 increment in the outgoing air are computed, the product of these and the ventilation rate is the rate of O_2 consumption and CO_2 production. The measurement of the ventilation rate may be done with large commercial gas meters but the aliquoting is a complex matter and the analysis of chamber air, which is outdoor air only slightly contaminated, one per cent, with expired air is tedious as it must be done most accurately.

An open circuit mask method for large animals involving gas analysis has long been used for measuring human metabolism, it involves collection of all the expired air into a Douglas bag or into a Tissot spirometer over a short period. The analysis of directly expired air, containing several per cent $\rm CO_2$ increment and $\rm O_2$ decrement, is very much simpler than that of chamber air containing a fraction of a per cent of $\rm CO_2$.

Kleiber (1944) motivated by an increasing need for information concerning tidal air of laboratory animals presented a general formula for such a determination on resting and fasting animals. In determining the tidal air by the formula presented it must be assumed that animals use up the same proportion of O2 from the air as man, i.e. approximately 5 per cent. Thus animals under basal conditions inspire 20 liters of air for each liter of 02 consumed. The O2 consumption in 24 hours in animals and man can be estimated from the basal heat production which for mature homeothermic animals from rats to steers averages 72W. Calories, where W is the body weight in kilograms. Since one liter of 02 consumed by fasting animals represents 4.7 Calories of heat, the basal rate of 02 consumption amounts to $\frac{72}{4.7}$ W^{.75} = 15.3 W^{.75} liters O₂ per day, or 306 W^{.75} air per day or $\frac{306 \times 1000}{1000}$ W^{.75} = 212 W^{.75} c.c. of air per minute. Kleiber calculated the tidal air of 27 day old albino Swiss mice from the metabolism rate. The metabolism rate was determined by using a closed-circuit-chamber method previously described. Unfortunately Kleiber was forced to assume a decrement of 5 per cent on the one hand and had no means at his disposal to test the validity of his general formula by actual respiratory volume data.

Loosli (1943) while making an estimation of the amount of air-borne virus necessary to produce infection found it necessary to determine the tidal air of the mouse. The apparatus used consisted of two thistle tubes joined by a length of glass tubing having a side arm equidistant from each end. Over the open end of one thistle tube was stretched a latex diaphragm in which there was a hole to take the

animal's nose. Over the other thistle tube there was also a rubber diaphragm to which there was cemented a small mirror. The system was filled with O_2 and some soda lime placed in the bottom of one thistle tube to take up the CO2. In operation the mouse was lightly anesthetized, tied on a board and after greasing the face piece with vaseline its nose was pushed through the hole in the rubber diaphragm covering the open end of the thistle tube. The side arm in the connecting tube was opened to allow the air pressure in the system to return to normal. A light beam was directed on to the mirror cemented to the diaphragm on the opposite thistle tube and the excursions of the reflected light beam corresponding to the breathing rhythm of the amimal were recorded on a moving photographic film. After several observations the aperture for the animal s nose was plugged and the light beam excursion calibrated by injecting and withdrawing known amounts of air with a syringe via the side arm in the tube connecting the thistle tubes. This method suffers from the disadvantages of having to use 0, and having to anesthetize the animal and then restrain it in a somewhat unnatural position.

Chapman (1944) in a modified closed circuit apparatus for measuring respiratory metabolism, attached a concave mirror to the axis of the float of a Krogh spirometer. Any change in the volume of the spirometer changed the position of the float and hence caused a rotation of the mirror. The image from a single filament bulb was focused by the concave mirror on an arc equipped with a centimeter scale, the radius of which was such that a 1 cm. movement of the light beam was equal to a volume change of 1 c.c. in the spirometer.

Guyton (1947), because data in the literature on respiratory volume of laboratory animals was very scanty, and a tremendous amount of study was being directed toward the etiology and pathogenesis of respiratory disease, did a comprehensive study of the subject. Guyton implies that there exists a need for a more satisfactory method of obtaining data on respiratory volume of laboratory animals and offers two methods he used to gather data and thereby augment the scanty literature.

(1) Valve method: The valves used were of delicate construction utilizing very minute thin rubber discs, which were hinged loosely over the tips of polished glass inlet and outlet tubes. These valves were then connected directly by means of a glass seal either to a tight-fitting headpiece or to a tracheal cannula. Collection of air from the outlet valve was accomplished by two methods. The expired air enters a collecting chamber via a rubber tube attached to the top of the mercury filled collecting chamber, which may be connected to a water or mercury manometer on either side of the collecting column. By manipulating the stopcock at the bottom of the collecting column, mercury is allowed to fall at a rate which will equalize that of expired air and maintain pressure within the chamber at exactly zero. By measuring the fall of the mercury column for one minute while the repressure within the chamber is maintained at atmospheric pressure, the respiratory volume per minute may be measured directly.

The second device for collecting the air from the outlet valve was constructed to that no water can flow from an upper siphon

jar into a lower jar until an equal volume of air is introduced into the air space above the water in the upper jar. Special precautions were taken so that the pressure against which the animal must breathe is small and always constant, approximately 1/2 cm. of water pressure, this value being considered negligible as an obstruction to respiration. The respiratory volume is read directly from the calibration of the lower bottle.

(2) Oscilloscope respirograph method: In this method water flows from an upper bottle to a lower bottle forcing air from the lower bottle past the head of the animal into the upper bottle, thereby completing a closed circuit. The rate of air flow is adjusted so that the volume of air is at least five times as great as that required for normal respiration of the animal. A third tube leads from the head piece to an airtight bellows on the top of which is an electrical condenser made of alternate layers of insulating paper and tinfoil. As the animal breathes in and out, pressure within the bellows alternately increases and decreases by a minute amount. of the condenser likewise alternately become closer and farther apart. The changes in capacity of the condenser by the changes in distance between the plates of the condenser are measured on an oscilloscope. A syringe connected to the bellows is used to calibrate the apparatus. All readings may be made directly from the screen of the oscilloscope, or the respiratory pattern may be accurately recorded with a continuous camera. Animals tested by this modified respirograph method were under as nearly normal physiological conditions as possible except for the matter of fear. This was overcome by allowing the animals to

remain in the head piece for a long period of time before actual measurements were made. The volume of the bottles used was adjusted for different animals so that enough air was present to last usually ten minutes and so that the operating pressure within the system varied from atmospheric pressure by approximately 1/5000 of an atmosphere with each respiratory cycle.

None of the methods presently reported in the literature for measuring respiratory volume adequately meet all the requirements of a respiratory apparatus. Early methods are based on a calculation from O2 consumption assuming a certain O2 decrement per inspiration. The method of Loosli requires the use of O2 and also restrains the animal in a somewhat unnatural position. The use of valves introduces an appreciable time lag and also presents surfaces upon which materials presented to the animal may impinge. Although the oscilloscopic respirograph of Guyton appears to come very close to requirements for an apparatus it does not allow for the separation of inspired and expired air with the option of presenting anything to the animal independently of the expired air, and as is the case with many indirect methods it is not a simple apparatus.

III

Experimental

A. The Animal Nutrition Laboratory at the University of British Columbia has sought for the past six years to arrive at some suitable method for measuring the respiratory rate and volume of laboratory animals, particularly the guinea pig.

The problem, as defined, was to devise a method of measuring respiratory rate and volume within the following limitations:

- 1. The method must be accurate and allow a high degree of reproducability of results.
- 2. The apparatus must be basically of closed-circuit design to allow for the use of toxic or pathogenic material.
- 3. There must be provision to separate the expired air from the air to be inspired and a means to measure both independently of each other.
- 4. Remembering that in the case of small animals the force that can be applied against respiratory activity is very small before and effect is obvious, the animal must be asked to do very little work, and the work it is asked to do must be harnessed in a subtle manner.
- 5. The several functions measured by the apparatus should be suitable and permanently recorded.
 - 61 The use of valves is undesirable due to their time lag and

the fact that they offer points upon which air borne particulates can impinge.

- 7. The apparatus must be capable of measuring events occurring at a relatively high rate of speed, i.e. two respirations per second.
- 8. The apparatus and method must be simple in design and operation.

McQuarrie (1948) sought to solve the problem by using a Haldane type apparatus. However, this approach did not satisfy the requirements for a closed system nor did it give a measure of rate or allow for separating expired and inspired air.

Patterson (1950) measured the respiratory rate by visual observation over a definite time period. This apparatus consisted of a glass tube containing a bubble of ink into which the animal breathed. The oscillations of the bubble were counted and this gave an estimate of the rate. The animal while under test was breathing the same air over and over again and as the volume of the tube and head piece was small, the trial period was short, and the animal was not altogether free from the effect of increasing CO₂ concentration.

The writer took up the problem in 1952 and has attempted to arrive at an apparatus fulfilling the previously stated requirements. Several methods were tried all of which had one or two points in common. They are the type of head piece used on the animal and using an interrupted beam of light to activate the apparatus.

B. The head piece for the animal was relatively simple, being a latex mask to go over the entire head and fit closely around the neck. Two rubber tubes led from the head piece, one bringing air to the animal, and one taking expired air away. The mask was made by applying seven successive layers of liquid latex to a 200 ml. volumetric flask which was used as a form. This gave a msk of suitable shape and size, the dead air space being very small. Figure 6.

While in the head piece the animal is placed in a restraint box. This is not absolutely necessary in most cases but some animals move around while the test is in progress. The restraint box is of plywood construction $6\frac{1}{2}$ " x 5" x 4". The box opens longitudinally through the centre and is hinged on the back. A round opening in the front of the box allows the animal's head to protrude, while fitting close enough around the neck to prevent the head being drawn back into the box.

C. It was quite obvious early in the preliminary investigations that the guinea pig was quite unable to do any useful work in the usual sense, without imposing a considerable force against respiration. However, there existed the necessity of having the animal activate the apparatus in time with its breathing pattern.

The first attempt to have the animal activate the system was by placing a "T" in the tube conducting air to the animal, and connecting to this "T" a 2 m.m. I.D. U-tube. Water was placed in the U tube to a height of 1 cm. in each leg. It was hoped that during inspiration there would be sufficient pressure drop in the leg of

the U connected to the air tube, and that the water level would rise in this leg by a distance sufficient to cut off a beam of light directed through the glass tube on to a photo-cell. Although the problem of water meniscus could be overcome by treating the glass tube with Desicote, the actual pressure drop was only sufficient to allow the water level to be raised 1 m.m. which was not a usable amount.

Although not enthusiastically pursued because in principle it disregarded one of the requirements of the apparatus, namely that of having no obstruction in the air tubes, an attempt was made to have the animal move a balsa wood flap suspended in an enlarged glass section of the inlet tube. The flap while being unable to move from the vertical position on expiration was able to travel through 45° on inspiration, and it was thought that this would allow a light beam to pass through the glass cell on inspiration and cut it off on expiration. This system did not prove to be sufficiently positive in action or consistent in distance travelled. It also had the aforementioned disadvantage of being an obstruction to air flowing in the tube and a surface upon which particles of material might impinge.

Finally a tambour was connected to the "T" in the tube to the animal. Although the usual rubber dam used on the tambour was under too much tension when held in place by the split ring to be moved by the animal, a satisfactory diaphragm was moulded to an air tight fit with liquid latex. The latex diaphragm actually moved only a fraction of a millimeter on inspiration but this movement was amplified considerably by a six inch 1/8" x 1/8" balsa wood arm

resting on the tambour diaphragm l" from the fulcrum. This scheme gave a movement of 0.5 cm. at the end of the arm. To this end of the balsa arm was cemented a l" x 1/4" flag which cut the light beam off in the normal position, expiration, and was depressed sufficiently to let light on to the photocell on inspiration. Fig. 1, 2. The pressure differential required for this movement was approximately 2 m.m. of water.

Having arrived at a suitable method for interrupting the light on to the photocell it then was necessary to amplify the signal from the photocell to a point where it could activate a relay.

D. The first amplifier built consisted of a 6SN7, 1/2 as a cathode follower, 1/2 as an amplifier, and a 6V6 as an output tube. The phototube was connected in the grid-cathode circuit of the first half of the 6SN7. The relay, a 10,000 ohm plate circuit relay, was connected in the plate circuit of the 6V6. This amplifier required a 250 volt, 90 mill power supply that was well regulated. Inasmuch as this made the apparatus very large and expensive, it was decided to find some other photo-electric relay amplifier circuit that would fulfill the requirements without the disadvantages mentioned.

The amplifier finally decided upon was a photo-relay circuit using a gas tetrode as an amplifier coupled to the phototube. Fig. 3. One-half of a 6SN7 was used as a diode rectifier, the other half as an amplifier. The phototube is connected in the grid-cathode circuit of the amplifier half of the 6SN7. The output of the amplifier is applied to the grid of the 2051 gas tetrode thereby controlling the



Fig. 1 Respiration Apparatus

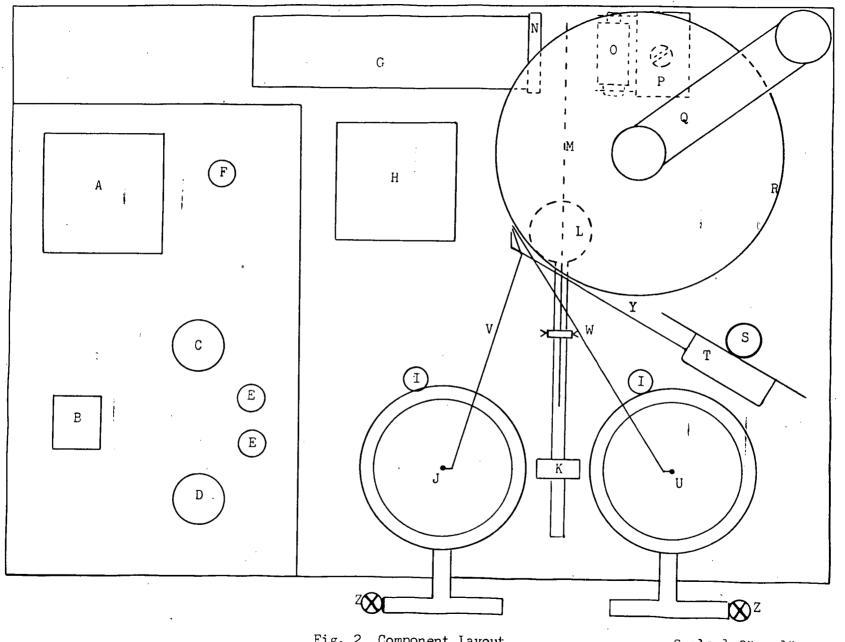


Fig. 2 Component Layout

Scale 1/2" = 1"

firing point by the light reaching the phototube. The gas tetrode was used as its conduction is on all or none action, and after firing takes place the grid has no more control. The tube ceases to conduct on the first negative half cycle of the applied voltage, after the negative grid bias reaches a cut-off valve. A 110 volt A.C. D.P.D.T. relay is connected in the plate circuit of the 2051 tube. This photo relay circuit appeared in the January issue of Electronics, 1941.

(1) The second phase of development was to arrive at a Ε. method of presenting air to be inspired and collecting the expired air in a closed system. It seemed impossible at the outset to accomplish this without the use of valves, and in the face of their disadvantages several types of valves were tried. Valves for such an application have certain requirements. First they must be fast in action, they must be positive and air tight, the valve orifice must not be critical and the valve must be compact. General Electric A.30 solenoid valves were tried first. This valve has a 3/32 inch orifice, works up to 150 pounds pressure and requires 7 watts on 110 volts for operation. These valves open when the solenoid coil is energized and close by gravity when de-energized. The distance travelled by the valve-seat piston is approximately 3/4 inch to open, and in falling the same distance to close the piston has a tendency to bounce in the seat which does not allow positive cut off. Another limitation to the use of this valve is the size of the orifice, 3/32, which was considered borderline with respect of limiting sir flow.

To overcome the difficulties encountered with the G. E. solenoid valve, a valve was designed at the Nutrition Laboratory. This

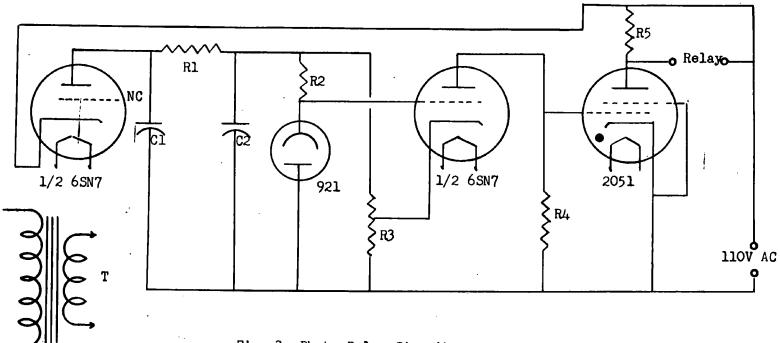


Fig. 3 Photo-Relay Circuit

C1, C2	8 mfd 450 W.V.	R5	1,000 ohm 20 watt
Rl	10,000 ohms 1/2 watt	T	6.3 Volt Transformer
R2	10 meg. 1/2 watt	Relay	115 Volt AC Coil
R3	10,000 ohm		Contacts 1.5 amp 115 Volts
R4	1 meg. 1/2 watt		

valve consisted of two concentric chambers, a central chamber 1/4 inch in diameter with a 1/8 inch wall, and an outer chamber 1/4 inch in width around the inner one, The bottom was closed in each case but the top was open. A tube 1/4 inch I.D. led into the outer chamber and a similar size tube into the inner chamber. A latex cap was fastened over the open ends of the chambers, and on to this was cemented an iron disc 1/2 inch in diameter and 3/16 inch thick. The disc held the diaphragm tightly to the top edge of the inner chamber and air entering the outer chamber could not leave unless the iron disc, and consequently the diaphragm, was lifted, allowing the air to spill over the edge into the inner chamber and exit by the tube running into it. The disc was lifted by an electro-magnet mounted above it. This valve satisfied most requirements but had one serious disadvantage, that of presenting a surface upon which material might collect and prevent an airtight seal.

(2) Various combinations of the methods of light cut off and valves mentioned above were tried with the following two devices for separating and recording inspired and expired air volumes.

A modified version of a micro-respirometer reported by

Tyler (1941) was tried. This consisted of a three foot five m.m.

I.D. glass tube connected to the inlet valve and a similar one connected to the outlet valve. The valves had a short 1/4 inch I.D. copper "T" connecting them. A bubble of water was placed at the 30-inch mark on the inlet tube and at the zero mark on the outlet tube. The animal and tambour were connected to one leg of the "T" joining the two valves.

As the animal inspired the inlet valve was opened allowing air to be

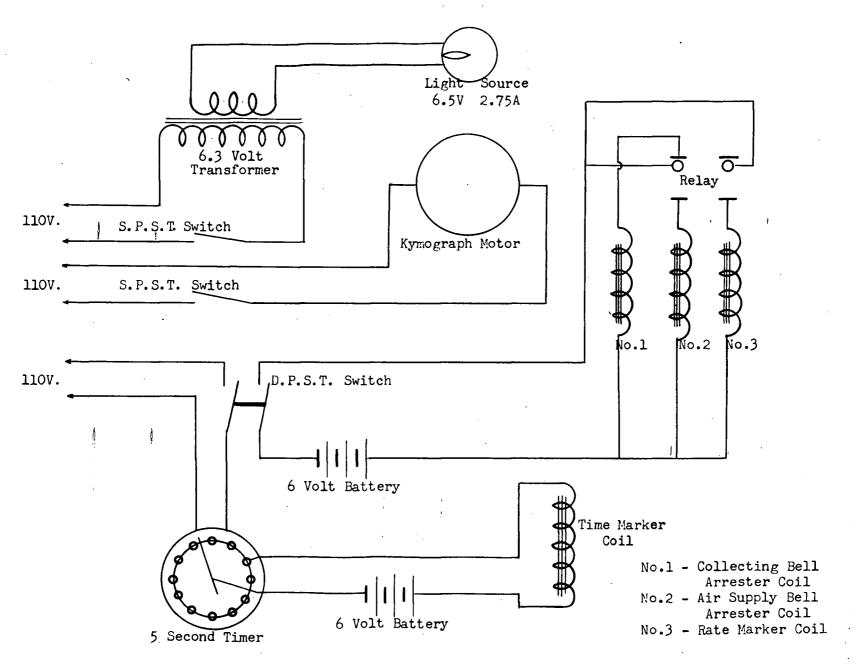
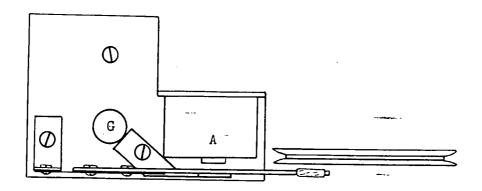


Fig. 4 Respirometer Wiring Diagram

taken from the air supply tube. This would cause the water bubble, which acted as a seal, to be drawn down the tube by a distance, which multiplied by the cross sectional area of the tube, gave the volume inspired. On expiration the inspiration valve would be closed and the expiration valve open, allowing air into the collecting tube and moving the bubble a distance indicating the expired volume. Aside from the undesirable feature of being valve operated, great difficulty was experienced in holding the water bubble. The bubble tended to decrease in size, as the inside of the tube was wet, to a point where it broke as a seal. If a non-wettable surface was put on the glass the bubble would not form at all.

A second attempt somewhat along the lines of Guyton's method of providing air flow in his oscillographic respirometer was tried. This scheme employs two air-water reservoirs. One reservoir contained air, and when the inspiration valve was open water entered to equal the air removed and thereby keep the pressure equal to atmospheric. The other reservoir contained water, and as air was admitted, on expiration, water was allowed to run out equalizing the pressure in the reservoir with that outside. Volume was measured by the amount of water entering one reservoir or leaving the other. This system showed promise but aside from using valves had the difficulty that the weight of water in a system large enough for trials of any time duration presented a positive pressure against the air supply on the one hand and a negative pressure on the receiving reservoir on the other.

The several methods so far described were endowed in some cases with obstacles unsurmountable, and in other cases with disadvantages



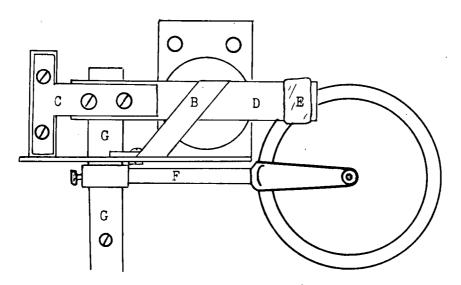


Fig. 5 Pulley Arrester Scale 1" = 1"

- 6 Volt Relay Coil
- Armature Stop
- Spring Steel
- Armature

- Rubber Tip
- F Pulley Mounting
- G Pulley Mounting and Arrester Standard

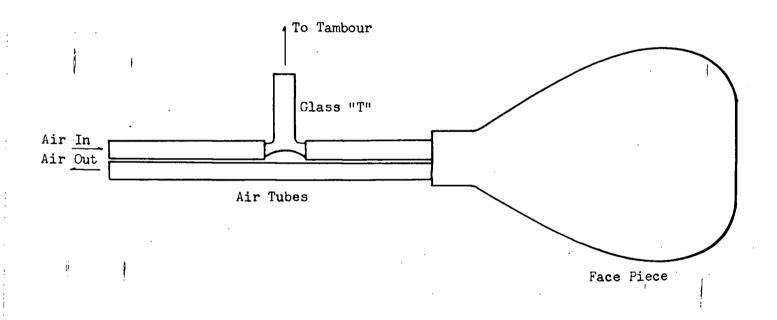


Fig. 6 Guinea Pig Face Mask for Respirometer

with respect of the limitations originally laid down as to make further investigations on them unwarranted.

- F. Finally it was decided to try two spirometers, one to be filled with air at the start of the trial and subsequently to be a source of air to the animal as and when required, the other spirometer to be empty at the start and be a receiver for expired air. The problem of valves again arose but it appeared that if at all possible they should not be used. After careful consideration the idea gradually developed that if the spirometers could be fixed or arrested when not required to deliver or receive air, and the animal did not have the capacity to compress the air in the receiver or withdraw air against a pressure in the supply bell, the spirometers themselves would act as valves. To this end electro-magnetic arresters were made and mounted so as to stop or lock the pulleys that carry the balance-weight chains in phase with the breathing pattern. Fig. 5. In other words when the animal is breathing in the air supply bell is free to move and the air receiver bell is arrested. This causes the animal to obtain its air from the air supply bell. On breathing out the reverse situation is in effect, and the expired air must go into the receiver bell. This proved to be a satisfactory method of providing air to the animal, and collecting expired air without the use of valves. The arrester coils operated on 6 volts D.C. in order to get away from vibration that often occurs in A.C. coils.
- G. The recording apparatus consisted of a kymograph drum mounted so that a writing stylus attached to the top of each spirometer would record the downward or upward travel, hence the volume

change in the spirometers. A stylus operated by an electro-magnet was mounted to record the respiratory rate on the kymograph. A five second contact timer was made and a five second time record drawn on the kymograph along with the volume and rate record. Fig. 7 shows a typical recording of rate, volume and time.

H. The whole apparatus was assembled on a 17" x 12" x 3" chassis. The amplifier was built on a 10" x6" x 2" chassis that was mounted on the larger chassis. Figs. 1 and 2 show the arrangement of the components on the chassis, and Fig. 4 shows the wiring exclusive of the amplifier.

As an accessory item two constant temperature cabinets were used, one in which to hold the animals over a period of time for acclimation to temperature and humidity if experiments on the influence of these factors were to be done, and the other cabinet kept under the same conditions in which the animal was to be placed for test. These cabinets were 24" x 18" x 24" and constructed of 3/8 inch plywood. The front of the cabinet was a glass door by which the animals could be observed. Each cabinet contained the following equipment:

- 1. A Cenco thermostat
- 2. 150 watt light bulb as a heat source
- 3. pilot light
- 4. Taylor humidiguide
- 5. 6 volt auto-type fan.

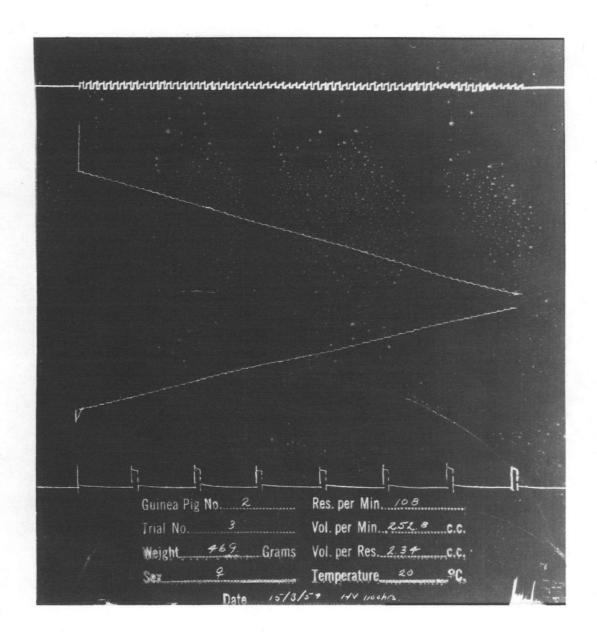


Fig. 7 Typical Record of Volume, Rate and Time

I. The operating cycle of the apparatus is as follows. The head piece is put on the animal and the animal placed in the restraint box. The restraint box is placed in the constant temperature cabinet and the air tubes pushed through the holes provided for them. While the animal is adjusting to the head piece the amplifier is turned on and allowed to warm up, at the same time the air supply spirometer is filled and the air receiver emptied. Now the air tubes from the head piece are connected to their respective spirometers, and the tube leading to the tambour is connected. The light source, rate counter, arresters and timer are turned on, and when all components are working smoothly the kymograph motor is started.

As the animal breathes in, light is allowed to strike the phototube, this extinguishes the 2051 tube and causes the relay to open. With the relay open the air supply spirometer is free and the receiver spirometer locked. Air is drawn from the supply bell by the animal and the bell descends by an amount indicating the volume of air breathed in. The stylus fastened to this spirometer bell draws a descending line on the kymograph. At the same time the rate counter coil is energized and a respiration mark is pat on the record. the animal exhales, the tambour returns to the normal position cutting the light off the phototube. This removes the bias on the 2051 tube allowing it to conduct and causing the relay to close. In the closed position the arrester on the supply bell is energized locking it in place and the receiver bell is freed allowing air to enter and raise the bell. The stylus on this spirometer draws an ascending line on the kymograph. The rate marker is returned to normal position. This

completes one respiratory cycle.

Throughout the trial period the timer is recording the time in five second intervals.

The female guinea pigs to be tested were kept in the constant temperature holding cabinet for a period of three weeks and the data were collected over a period of one week. The male guinea pigs to be tested were kept in the holding cabinet one week and the data were collected over a period of two days. It was found to be undesirable to house male guinea pigs of such a wide weight range together for too long a period as they fought almost continuously. Whether the effect of fighting or the short holding period or both would affect the data in the case of the male guinea pigs remained to be seen. J. The completed apparatus was calibrated with a Baltimore automatic pipette manufactured by Baltimore Biological Laboratory Incorporated, Baltimore, Maryland. This pipette is a reciprocating piston type that can be adjusted to deliver 0 to 10 c.c. per stroke at 0 to 200 strokes per minute. The inlet and outlet tubes of the pipette were attached to the head piece of the respiration apparatus and the apparatus operated by the same procedure as if an animal were harnessed to it. The pipette was adjusted to deliver various volumes at several delivery rates. The table for the calibration of the respiration apparatus is shown in Appendix II.

IV

Results

Sample data collected on five female guinea pigs and four male guinea pigs are presented in table form in Appendix 2. This table also gives the preparation of the data for fitting it to a satisfactory equation.

The animals tested were selected at random from the guinea pig colony at the Animal Nutrition Laboratory. The colony is kept at a temperature of 20°C. and fed a stock ration known as U.B.C. Ration No. 8. The constituents of this ration are shown in Appendix 1.

The arithmetic mean of the respiratory rate and volume of the female animals was calculated and these values plotted on an arithmetic grid, Fig. 8, and on a log-log grid, Fig. 9. A comparison of these plots revealed that a logarithmic relationship rather than an arithmetic relationship existed between weight and rate and weight and volume.

The equations for the relationship between body weight and respiratory rate and body weight and respiratory volume were calculated by the method shown in Appendix 3a and checked by the method shown in Appendix 3b.

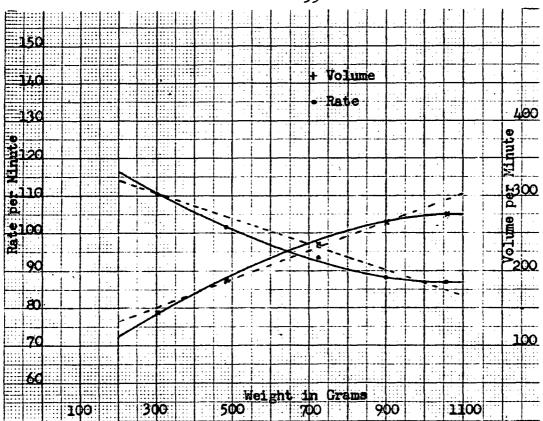


Fig. 8 Weight plotted against respiratory rate and respiratory volume. Points used are the arithmetic means of the data on female Guinea Pigs.

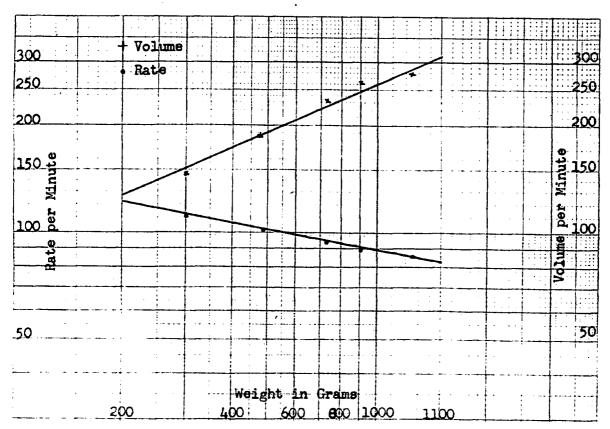


Fig. 9 Log of weight plotted against log of respiratory rate and respiratory volume. Points used are arithmetic means of the data on female Guinea Pigs.

Equation relating body weight to respiratory rate for the female guinea pig.

$$Y = 347.7W^{-.2}$$

S.E.E. = +11.8%
-10.5%
 $= 0.62$

Equation relating body weight to respiratory rate for the male guinea pig.

$$Y = 337W^{-.2}$$

S. E. E. = +10.5%
- 9.5%
 \sim = 0.59

Equation relating body weight to respiratory volume for the female guinea pig.

$$Y = 6.06W^{.55}$$

S. E. E. = +12.3%
-10.9%
 $= 0.90$

Equation relating body weight to respiratory volume for the male guinea pig.

$$Y = 13.2W^{-44}$$

S.E.E. = +14.00%
-12.28%
= 0.78

Respiratory volume as a function of body weight is shown plotted on a log-log grid in Figs. 10, 11 and respiratory rate as a function of body weight is shown plotted on a log-log grid in Figs. 12, 13.

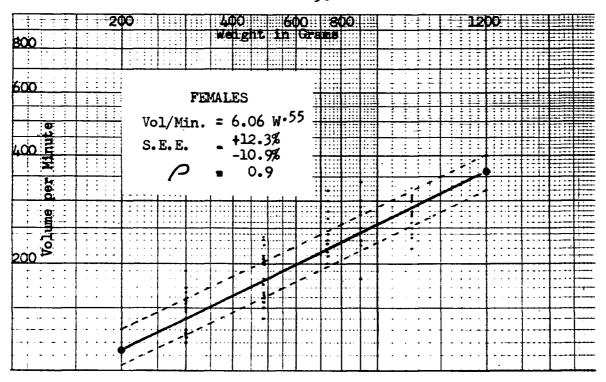


Fig. 10 The relation of respiratory volume to body weight. Plotted on a log-log grid. Female Guinea Pigs.

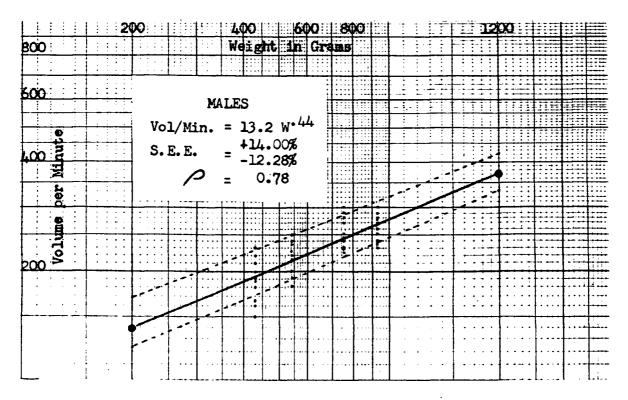


Fig. 11 The relation of respiratory volume to body weight. Plotted on a log-log grid. Male Guinea Pigs.

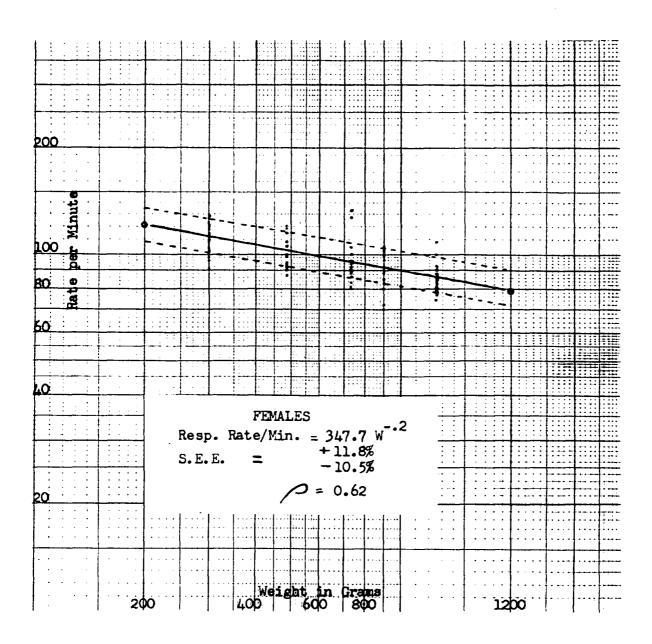


Fig. 12 The relation of respiratory rate to body weight. Plotted on a log-log grid. Female Guinea Pigs.

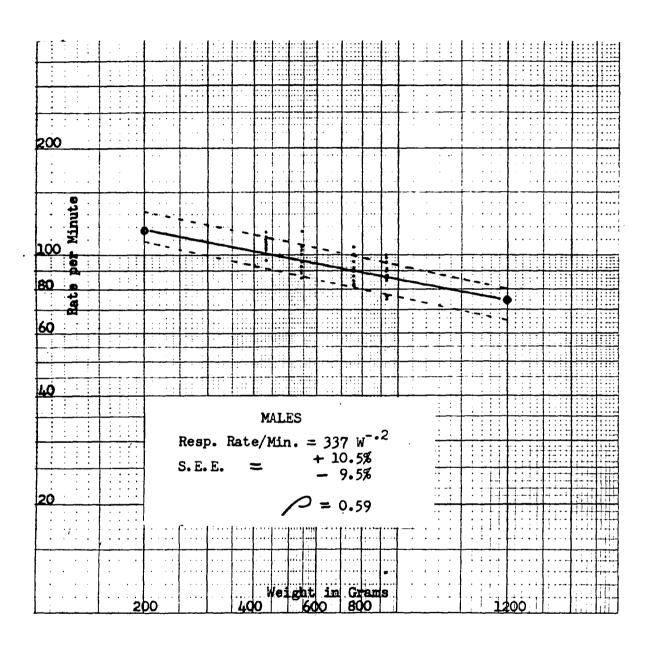


Fig. 13 The relation of respiratory rate of body weight. Plotted on a log-log grid. Male Guinea Pigs.

V

Discussion

It is intended that the limited data collected with the apparatus described in this thesis should provide some indication of the validity and reliability of this apparatus in measuring respiratory rate and volume, rather than establish a respiratory pattern for the experimental animals used.

Guyton (1940) proposed an equation for the respiratory volume of a number of species of small animals including the guinea pig, volume equals 2.1 W.75, where W is weight in grams.

Table 1 below shows a comparison between the average values for respiratory volume obtained on animals of different weight and sex, and the values calculated by the equation of Guyton and that suggested by the writer.

Table 1. Comparison of Respiratory Volume Obtained by Equations of Guyton and Nordan

Body Wt.	Sex	Actual	Guyton H	<i>i</i> quation	Nordan Equation		
Gms.		Ave.Vol. c.c./Min.	Females V=2.1W•75	Males V=2.1W ^{•75}	Females V=6.06W ⁵⁵	Males V=13.2W°44	
300	f	144.1	144.2		140.2		
485	f	186.8	206.7		184.2		
725	f	234.5	293.4		230.0		
900	f	264.2	345.0		259.0		
1052	f	275.2	387.7		282.4		
430	m	191.5		198.3		191.2	
541	m	210.0		235.6		211.5	
756	m	253.0		302.8		245.2	
922	, m	263.0		351.4		267.7	

Acclose examination of Table 1 shows the effect of the different exponents used in the equations. Both equations show similar values at the lower weights but the values calculated by the Guyton equation depart from the actual values to a greater and greater extent as body weight increases. The data reported here were collected on growing animals of one species only, Guyton on the other hand derived his equation from growing and mature animals of a number of species. This fact may account in part for the departure from actual values his equation gives when applied to growing animals.

Brody (1940) reports resting O_2 consumption for several large animals in the growing and mature state. The equations for the O_2 consumption of these large species appear in Table 2.

Table 2. Resting O₂ Consumption of Several Large Species.

Data from Brody 1945.

Animal	Weight lbs.	Weight K <i>g</i> ms.	Respiratory Volume Equation (W in Kgms.)
Percheron Female	200-1100	90-500	77.5 W.54
Percheron Female	1100-2000	500-900	6.22 W ^{•97}
Percheron Gelding	200-1100	90-500	84.7 W ^{.54}
Percheron Gelding	1100-2000	500-900	1.63 W ^{1.18}
Holstein	70-310	30-150	19.3 W.81
Holstein	310-1200	150-600	53.7 W.60
Jersey	60-220	28-100	18.3 W.84
Jersey	220-900	100-450	61.2 W.56
Shetland Ponies	90-800	40-300	90.7 W.52

Figures in Table 2 while admittedly not for ventilation volume as such indicate that a change in slope occurs in respiratory volume between growing and mature animals. Further they indicate that a slope for a group of animals representing the growing and mature phases would probably be in the order of .75.

This is consistent with Guyton's value which was derived from a group of growing and mature animals. The equation for resting 0_2 consumption of growing horses as reported by Brody is in the order of .55.

Although there appears to be some disagreement between the equations presented by the writer for female and male animals, it is feld that the value obtained for males may have been complicated by the short acclimation period and the fact that male guinea pigs when housed together tend to fight.

Guyton (1940) derived the following equation for the respiratory rate of small animals. Respiratory Rate equals 295 W where W is body weight in grams. This equation applies to all animals of both sexes and does not differentiate between growing and mature animals.

Patterson (1950) derived the following formulae for male and female guinea pigs:

Male $R = 447.6 \text{ W}^{-.22}$

Female $R = 304.1 \text{ W}^{-.19}$

W body weight in grams

Patterson used a large group of growing animals and only a small

number that could be considered mature. Table 3 shows a comparison of respiratory rate data calculated by the equations of Guyton,

Patterson and the author.

Guyton has noted that mice and guinea pigs breathe somewhat faster than would be calculated by the equation.

Allowing for this discrepancy as noted by Guyton, the results obtained from the equations derived by the three workers are in fairly good agreement.

From a comparison of the available data on respiratory rate and volume of the guinea pig it would appear that the apparatus described will give reasonable values for rate and volume.

One of the most serious difficulties with any apparatus or method for making such determinations is certainly the care with which the animals are prepared for the experimental work.

Constant temperature, humidity and acclimation to the new environment and apparatus are most important.

£3

Table 3. Comparison of Respiratory Rate Values Obtained by Equations of Guyton, Patterson and Nordan

Body Wt. in Grams	Sex	Actual Average Rate per Min.	Guyton Equation R=295 W-•25	Patterson Females R=304.1Wgm	Equation Males R=447.6Wgm	Nordan Females R=347.7Wgm	Equation Males R=337.0Wgm
300	f	110.3	70.88	102.9		111.1	
485	f	101.5	62.86	93.88		101.0	
725	f	93.5	56.85	86.98		93.14	
900	f	F88.5	53.86	83.48		89.20	
1052	f	86.3	51.80	81.04	,	86.46	
430	m	105.8	64.78		117.9		100.36
541	m	994•75	61.17		112.1		95.72
756	m	89.4	56.26		104.1		89.52
922	m	91.2	53.54		99.69		86.00

VI

SUMMARY

- 1. A review of the literature suggests that the present methods for measuring respiratory rate and volume leave much to be desired.
- 2. An apparatus has been developed to overcome the difficulties associated with those described in the literature.
- 3. The apparatus records on a kymograph drum volume inspired, volume expired, rate of breathing and a five second time base.
- 4. The air to be inspired is kept separate from that expired.
- 5. Simplicity of operation has been maintained.
- 6. The data obtained from the apparatus, while admittedly limited, suggest that the apparatus may be superior to those proposed by other workers.

VII

APPENDICES

APPENDIX I

Guinea Pig Ration

U.B.C. RATION No. 8

Rolled Oat Flour 450	
Flaked Wheat 140	
Flaked Barley 200	
Wheat Bran 350	
Dehydrated Grass 100	
Beet Pulp 80	
Cocoanut Meal 200	
Soyabean Meal 175	
Oil Cake Meal 250	
Mineral Pre-mix 50	
Salt 20	
Vitamin D ₂ Pre-mix25	5
2015	

APPENDIX II

Calibration Table for Respiration Apparatus

Vol./Min. Recorded	Strokes/Min. Recorded	Vol./Min. Delivered	Vol./Stroke	Strokes/Min.
c.c. 79	80	c.c. 80	c.c. 1.00	80
162	81	160	2.00	80
243	80	240	3.00	c 80
90	91	90	1.00	90
184	90	180	2.00	90
275	90	270	3.00	90
98	100	100	1.00	100
204	100	200	2.00	100
310	100	300	3.00	100
111	111	110	1.00	110
225	110	220	2.00	110
336	111	330	3.00	110
121	120	120	1100	120
245	120	240	2.00	120
367	122	360	3.00	120

•

APPENDIX III

Data on five female guinea pigs and preparation of data for fitting to a logarithmic equation.

- (a) Volume
- (b) Rate

						·	
No.	Body Wt.X	LogX	Vol. /Min.Y	LogY	LogX.LogY	Log ² X	Log ² Y
1	300	2.47712	149	2.17318	5.38324	6.13612	4.72273
2	300	2.47712	140	2.17609	5.39044	6.13612	4.73537
3	300	2.47712	165	2.21748	5.49397	6.13612	4.91723
	300	2.47713	150	2.17609	5.39044	6.13612	4.73537
4 5 6	300	2.47712	124	2.09342	5.18565	6.13612	4.38241
	300	2.47712	130	2.11384	5.23649	6.13612	4.46875
7	300	2.47712	140	2.14612	5.31621	6.13612	4.60586
8	300	2.47712	140	2.14612	5.31621	6.13612	4.60586
9	300	2.47712	150	2.17609	5.39044	6.13612	4.73537
10	300	2.47712	154	2.18752	5.41875	6.13612	4.78524
11	300	2.47712	120	2.07918	5.15038	6.13612	4.32299
12	300	2.47712	140	2.14612	5.31621	6.13612	4.60586
13	300	2.47712	120	2.07918	5.15038	6.13612	5.32299
14	300	2.47712	130	2.11394	5.23649	6.13612	4.46875
15	300	2.47712	129	2.11059	5.22818	6.13612	4.45459
16	300	2.47712	125	2.09691	5.19429	6.13612	4.39703
17	300	2.47712	170	2.23044	5.52509	6.13612	4.97490
18	300	2.47712	190	2.27875	5.64474	6.13612	5.19271
19	300	2.47712	150	2.17609	5.39044	6.13612	4.73537
20	300	2.47712	156	2.19312	5.43263	6.13612	4.80979
21	485	2.68574	225	2.35218	6.31735	6.21321	5.53276
22	485	2.68574	232	2.36548	6.35309	7.21321	5.59553
23	485	2.68574	225	2.35318	6.31735	7.21321	5.53276
24	485	2.68574	180	2.25527	6.05708	7.21321	5.08625
25	485	2.68574	150	2.17609	5.84441	7.21321	4.73537
26	485	2.68574	210	2.32221	6.23688	7.21321	5.39270
27	485	2.68574	209	2.32014	6.23131	7.21321	5 . 33 97 2
28	485	2.68574	156	2.19312	5.89016	7.21321	4.80979
29	485	2.68574		2.24551	6.03086	7.21321	5.04232
30	485	2.68574	180	2.25527	6.05708	7.21321	5.08625
31	485	2.68574	165	2.21748	5.95558	7.21321	4.91723
32	485	2.68574	150	2.17609	5.84441	7.21321	4.73537
33	485	2.68574	175	2.24303	6.02422	7.21321	5.03121
34	485	2.68574	140	2.14612	5.76394	7.21321	4.60586
35	485	2.68574	235	2.37106	6.36807	7.21321	5.62196
36	485	2.68574	225	2.35218	6.31735	7.21321	5.53276
37	485	2.68574	163	2.21218	5.94136	7.21321	4.89377
38	485	2.68574	163	2.21218	5.94136	7.21321	4.89377
39	485	2.68574	160	2.20412	5.91969	7.21321	4185814
40	485	2.68574	205	2.31175	6.20877	7.21321	5.34420
41	485	2.68574	200	2.30103	6.17997	7.21321	5.29473
42	725	2.86033	224	2.35024	6.72250	8.18153	5.52366
43	725	2.86033	200	2.30103	6.58172	8.18153	5.29473
44	725	2.86033	225	2.35218	6.72803	8.18153	5.53276
45	725	2.86033	320	2.50515	7.16557	8.18153	6.27577
46	725	2.86033	300	2.47712	7.08540	8.18153	6.13612
47	725	2.86033	240	2.38021	6.80820	8.18153	5.66540
48	725	2.86033	240	2.38021	6.80820	8.18153	5.66540
49	725	2.86033	244	2.38739	6.82874	8.18153	5.69963
50	725	2.86033	240	2.38021	6.80820	8.18153	5.66540
51	725	2.86033	200	2.30103	6.58172	8.18153	5 • 29473
52	725	2.86033	240	2.38021	6.80820	8.18153	5.66540
53	725	2.86033	200	2.30103	6.58172	8.18153	5.29473

No.	Body Wt.X	LogX	Vol. /Min.Y	LogY	LogX.LogY	Log ² X	Log ² Y
54	725	2.86033	200	2.30103	6.58172	8.18153	5.29473
55	725	2.86033	200	2.30103	6.58172	8.18153	5.29473
56	725	2.86033	280	2.44715	6.99969	8.18153	5.98858
57	725	2.86033	200	2.30193	6.58172	8.18153	5.29473
58	725	2.86033	244	2.38739	6.82874	8.18153	5.69963
59	725	2.86033	240	2.38021	6.80820	8.18153	
60		2.86033	240 268				5.66540
	725			2.42813	6.94528	. 8.18153	5.89583
61	725	2.86033	215	2.33243	6.67156	8.18153	5.44026
62	725	2.86033	230	2.36172	6.75534	8.18153	5.57775
63	725	2.86033	240	2.38021	6.80820	8.18153	5.66540
64	725	2.86033	225	2.35218	6.72803	8.18153	5.53276
65	725	2.86033	210	2.32221	6.64233	8.18153	5.39270
66	900	2.95424	300	2.47712	7.31801	8.72755	6.13612
67	900	2.95424	180	2.25527	6.66262	8.72755	5.08625
68	900	2.95424	.180	2.25527	6.66262	8.72755	5.08625
69	900	2.95424	300	2.47712	7.31801	8.72755	6.13612
70	900	2.95424	300	2.47712	7.31801	8.72755	6.13612
71	900	2.95424	300	2.47712	7.31801	8.72755	6.13612
72	900	2.95424	280	2.44715	7.22949	8.72755	5.98858
73	900	2.95424	300	2.47712	7.31801	8.72755	6.13612
74	900	2.95424	280	2.44715	7122949	8.72755	5.98858
75	900	2.95424	290	2.46239	7.27452	8.72755	6.06340
76	900	2.95424	250	2.39794	7 .0 8409	8.72755	5.75011
77	900	2.95424	250	2.39794	7.08409	8.72755	5.75011
78	900	2.95424	_ 340	2.53147	7.47860	8.72755	6.40838
7 9	900	2.95424	180	2.25527	6.66262	8.72755	5.08625
800	9:00	2.95424	230	2.36172	6.97711	8.72755	5 • 57775
81	900	2.95424	225	2.35218	6.94892	8.72755	5.53276
82	900	2.95424	300	2.47712	7.31801	8.72755	6.13612
83	900	2.95424	250	2.39794	7.08409	8.72755	5.75011
84	900	2.95424	250	2.39794	7.08409	8.72755	5.75011
85	900	2.95424	250	2.39794	7.08409	8.72755	5.75011
86	900	2.95424	278	2.44404	7.22030	8.72755	5 • 97335
87	900	2.95424	300	2.47712	7.31801	8.72755	6.13612
88	1052	3.02201	325	2.51188	7.59095	9.13258	6.30955
89	1052	3.02201	305	2.48430	7.50759	9.13258	6.17174
90	1052	3.02201	300 -	2.47712	7.48589	9.13258	6.13612
91	1052	3.02201	298	2.47421	7.47712	9.13250	6.12174
92	1052	3.02201	250	2.39794	7.24661	9.13258	5.75011
93	1052	3.02201	300	2.47712	7.48589	9.13258	6.13612
94	1052	3.02201	240	2.38021	7.19303	9.13258	5.66540
95	1052	3.02201	275	2.43933	7.37170	9.13258	5.95034
96	1052	3.02201	280	2.44715	7.39535	9.13258	5.98858
97	1052	3.02201	260	2.41497	7.29808	9.13258	5.83209
98	1052	3.02201	250	2.39794	7.24661	9.13258	5.75011
99	1052	3.02201	250	2.39794	7.24661	9.13258	5.75011
íóo	1052	3.02201	235	2.37106	7.16540	9.13258	5.62196
101	1052	3.02201	3 1 0	2.49136	7.52893	9.13258	6.20688
102	1052	3.02201	300	2.47712	7.48589	9.13258	6.13612
103	1052	3.02201	330	2.34242	7.07883	9.13258	5.48694
104	1052	3.02201	250	2.39794	7.24661	9.13258	5.75011
105	1052	3.02201	278	2.44404	7.38594	8.13258	5.97335
106	1052	3.02201	272	2.43456	7.35730	9.13258	5.92712
107	1052	3.02201	306	2.48572	7.51188	9.13258	6.17880
TO (10/2	J. UZZUI	700	~•40)(~	1 • 74400	/ • ± J & J O	0.11000

(b) Rate

						·	
No.	Body Wt.X	Logx	Rate /Min.Y	LogY	LogX.LogY	Log ² X	Log ² Y
1	300	2.47712	91	1.95904	4.85278	6.13612	3.83784
2	300	2.47712	100	2.00000	4.95424	6.13612	4.00000
3.	300	2.47712	106	2.02530	5.01692	6.13612	4.10186
4	300	2.47712	108	2.03342	5.03703	6.13612	4.13481
5 6	300	2.47712	123	2.08990	5.17694	6.13612	4.36770
	300	2.47712	122	2.08636	5.16816	6.13612	4.35289
7	300	2.47712	125	2.09691	5.19430	6.13612	4.39703
8	300	2.47712	118	2.07188	5.13230	6.13612	4.29269
9.	300	2.47712	95	1.97772	4.89906	6.13612	3.91139
10	300 -	2.47712	98	1.99122	4.93250	6.13612	3.96498
11	300	2.47712	120	2.07918	5.15038	6.13612	4.32299
12	300	2.47712	116	2.06445	5.11391	6.13612	4.26198
13	300	2.47712	101	2.00432	4.96494	6.13612	4.01730
14	300	2.47712	110	2.04139	5.05677	6.13612	4.16728
15	300	2.47712	111	2.04532	5.06651	6.12612	4.18334
16	300	2:47712	109	2.03742	5.04695	6.13612	4.15110
17	300	2.47712	103	2.01283	4.98604	6.13612	4.05151
18	300	2.47712	114	2.05690		6.13612	4.23085
19	300	2.47712	128	2.10721	5.21981	6.13612	4.44033
20	300	2.47712	109	2.03742	5.04695	6.13612	4.15110
21	485	2.68574	119	2.07554	5.57438	7.21321	4.30789
22	485	2.68574	116	2.06445	5.54460	7.21321	4.26198
23	485	2.68574	120	2.07918	5.58414	7.21321	4.32299
24	485	2.68574	120	2.07918	5.58414	7.21321	4.32299
25	485	2.68574	100	2.00000	5.37148	7.21321	4.00000
26	485	2.68574	105	2.02118	5.42839	7.21321	4.08520
27	485	2.68574	108	2.03342	5.46125	7.21321	4.13481
28	485	2.68574	105	2.02118	5.42839	7.21321	4.08520
29	485	2.68574	93	1:96848	5.28683	7.21321	3.87492
30	485	2.68574	94	1.97312	5.29931	7.21321	3.89323
31	485	2.68574	90	1.95424	5.24859	7.21321	3.81906
32	485	2.68574	93	1.96848		7.21321	3.87492
33	485	2.68574	87	1.93951	5.20904	7.21321	3.76173
34	485	2.68574	92	1.96378	5.27422	7.21321	2.85646
35	485	2.68574	93	1.96848	5.28683	7.21321 7.21321	3.87492
36	485	2.68574	108	2.03342	5.46125		4.13481 3.92939
37	485	2.68574	96 04	1.98227	5.32386	7.21321 7.21321	3.92 93 9
38	485	2.68574	96	1.98227	5,32386 5,37031	7.21321	3.99826
39	485 1.05	2.68574	99	1.99956 1.99956		7.21321	3.99826
40	485 1.85	2.68574	99		5.37031	7.21321	4.00000
41	485	2.68574 2.86033	100	2.00000 1.90309	5.37148		3.62175
42	725		80 80		5.44348	8.18153	
43	725	2.86033		1.90309	5.44348	8.18153	3.62175
44	725	2.86033	106 96	2.02530	5.79306 5.66996	8.18153 8.18153	4.10186 3.92939
45	725 725	2.86033 2.86033		1.98227	5.64381	8.18153	3.89323
46	725	2.86033	944 96	1.98000	5.66347	8.18153	3.92939
47	725	2.86033	96 100	2.00000	5.72067	8.18153	4.00000
48	725	2.86033	128	2.10721	6.02733	8.18153	4.44033
49	725 725	2.86033	96	1.98227		8.18153	3.92939
50 51	725	2.86033	90 80	1.90309	5.44348	8.18153	3.62175
5 2	725 725	2.86033	86	1.90309	5.53331	8.18153	3.74228
53		2.86033	83	1.91907	5.48921	8.18153	3.68286
22	725	2.000)	ره	⊥ • 7 ⊥ 7∪ (ノ・サロブルエ	し・エしエノノ	J. 00200

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55 725 2.86033 90 1.95424 5.58979 8.18153 3.64231 5.7725 2.86033 95 1.97072 5.65695 8.18153 3.64231 5.7725 2.86033 95 1.97072 5.65695 8.18153 3.91139 58 725 2.86033 84 1.92427 5.50408 8.18153 3.70285 60 725 2.86033 84 1.92427 5.50408 8.18153 3.70285 60 725 2.86033 84 1.92427 5.50408 8.18153 3.70285 60 725 2.86033 81 1.90848 5.45691 8.18153 3.64231 61 725 2.86033 96 1.98227 5.666996 8.18153 3.9239 62 725 2.86033 91 1.96378 5.61709 8.18153 3.85466 63 725 2.86033 91 1.95904 5.60351 8.18153 3.85466 63 725 2.86033 91 1.95904 5.60351 8.18153 3.85466 63 725 2.86033 133 2.12385 6.07493 8.18153 3.85466 64 725 2.86033 133 2.12385 6.07493 8.18153 3.85466 69 900 2.95424 70 1.84509 5.45086 8.72755 3.40488 69 900 2.95424 70 1.84509 5.45086 8.72755 3.40438 69 900 2.95424 70 1.84509 5.45086 8.72755 3.40438 69 900 2.95424 90 1.95422 5.85611 8.72755 3.81966 70 900 2.95424 90 1.95422 5.85611 8.72755 3.81966 71 900 2.95424 91 1.9504 5.78748 8.72755 3.80382 72 900 2.95424 83 1.92941 5.69997 8.72755 3.80387 72 900 2.95424 83 1.91907 5.66942 8.72755 3.803784 72 9.95424 83 1.91907 5.66942 8.72755 3.68286 77 900 2.95424 83 1.91907 5.66942 8.72755 3.68286 77 900 2.95424 83 1.91907 5.66942 8.72755 3.68286 77 900 2.95424 80 1.93909 5.62219 8.72755 3.68286 77 900 2.95424 83 1.91907 5.66942 8.72755 3.68286 77 900 2.95424 83 1.91907 5.66942 8.72755 3.68286 77 900 2.95424 80 1.90309 5.62219 8.72755 3.68286 77 900 2.95424 80 1.90309 5.62219 8.72755 3.68286 77 900 2.95424 80 1.90309 5.62219 8.72755 3.68286 90 2.95424 80 1.90309 5.62219 8.72755 3.68286 90 2.95424 90 1.95404 90 1.95		Wt.X		/Min.Y				
56 725 2.86033 81 1.90848 5.45891 8.18153 3.64231 57 725 2.86033 83 1.91907 5.48921 8.18153 3.70285 59 725 2.86033 84 1.92427 5.50408 8.18153 3.70285 60 725 2.86033 84 1.92427 5.66996 8.18153 3.62286 61 725 2.86033 96 1.9627 5.66996 8.18153 3.62281 61 725 2.86033 92 1.96378 5.61709 8.18153 3.69293 62 725 2.86033 105 2.02118 5.78128 8.18153 3.6924 64 725 2.86033 105 2.02118 5.78128 8.18153 3.6924 64 725 2.86033 105 2.02118 5.78128 8.18153 3.6924 67 705 2.95424 70 1.84509 5.45768 8.72755 3.9834 </td <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>								
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				79	1.89762	5.73465	9.13258	3.60098
	107	1052	3.02201	77	1.88649	5.70100	9.13258	3.55884

APPENDIX IV

Data on four male guinea pigs and preparation of data for fitting to a logarthmic equation

- (a) Volume
- (b) Rate

(a) Volume

No.	Body Wt.X	LogX	Vol. /Min.Y	LogY	LogX.LogY	Log ² X	Log ² Y
1	541	2.73320	225	2.35218	6.42898	7.47038	5.53571
2	541	2.73320	200	2.30103	6.28918	7.47038	5.29474
3	541	2173320	212	2.32634	6.35835	7.47038	5.41186
4	541	2.73320	250	2.38021	6.50559	7.47038	5.66540
5 6	541	2.73320	200	2.30103	6.28918	7.47038	5.29474
	541	2.73320	250	2.39794	6.55405	7.47038	5.75012
7 8	541	2.73320	180	2.25527	6.16410	7.47038	5.08624
8	541	2.73320	192	2.28330	6.24072	7.47038	5.21346
9	541	2.73320	236	2.37291	6.48564	7.47038	5.63070
10	541	2.73320	190	2.27875	6.22828	7.47038	5.19270
11	541	2.73320	200	2.30103	6 ,28 918	7.47038	5.29474
12	541	2.73320	190	2.27875	6.22828	7.47038	5.19270
13	541	2.73320	190	2.27875	6.22828	7.47038	5.19270
14	541	2.73320	180	2.25527	6.16410	7.47038	5.08624
15	541	2.73320	220	2.34242	6.40230	7.47038	5.48693
16	541	2.73320	220	2.34242	6.40230	7.47038	5.48693
17	541	2.73320	200	2.30103	6.28918	7.48038	5.29474
18	541	2.73320	250	2.39794	6.55405	7.46038	5.75012
19	541	2.73320	240	2.38021	6.50559	7.47038	5.66540
20	541	2.73320	240	2.38021	6.50559	7.47038	5.66540
21	756	2.87852	228	2.35793	6.78735	8.28588	5 • 55 983
22	756	2.87852	240	2.38021	6.85148	8.28588	5.66540
23	756	2.87852	230	2.36173	6.79829	8.28588	5 • 57777
24	756	2.87852	230	2.36173	6.79829	8.28588	5 • 57777
25	756	2.87852	228	2.35793	6.78735	8.28588	5.55983
26	756	2.87852	225	2.35218	6.77080	8.28588	5.53275
27	756	2.87852	244	2.38739	6.87215	8.28588	5.69963
28	756	2.87852	245	2.38917	6.87727	8.28588	5.70813
29	756	2.87852	248	2.39445	6.89247	8.28588	5 . 7 3 339
30	756	2.87852	250	2.39794	6.90252	8.28588	5.75012
31	756	2.87852	250	2.39794	6.90252	8.28588	5.75012
32	756	2.87852	250	2.39794	6.90252	8.28588	5.75012
33	756	2.87852	300	2.47712	7.13044	8.28588	6 11 3612
34	7 56	2.87852	250	2.39794	6.90252	8.28588	5.75012
35	756	2.87852	290	2.46240	7: 08807	8.28588	6.06341
36	756	2.87852	3: 0 0	2.47712	7.13044	8.28588	6.13612
37	756	2.87852	250	2.39794	6.90252	8.28588	5.75012
38	756	2.87852	250	2.39794	6.90252	8.28588	5.75012
39	756	2.87852	280	2.44716	7.044 2 0	8.28588	5.98859
40	756	2.87852	270	2.43136	6.99872	8.28588	5.91151

(a) Volume (contid)

No.	Do		- ;; -		····		
NO.	Body Wt.X	LogX	Vol. /Min.Y	LogY	LogX.LogY	Log ² X	Log ² Y
41	922	2.96473	273	2.43616	7.22256	8.78962	5.93488
42	922	2.96473	250	2.39794	7.10924	8.78962	5.75012
43	922	2.96473	240	2.38021	7.05668	8.78962	5.66540
44	922	2.96473	260	2.41497	7.15973	8.78962	5.83208
45	922	2.96473	236	2.37291	7.03504	8.78962	5.63070
46	922	2.96472	243	2.38561	7.07269	8.78962	5.69114
47	922	2.96473	230	2.36173	7.00189	8.78962	5.57777
48	922	2.96473	250	2.39794	7.10924	8.78962	5.75012
49	922	2.96473	290	2.46240	7.30035	8.78962	6.06341
50	922	2.96473	290	2446240	7.30035	8.78962	6.06341
51	922	2.96473	231	2.36361	7.00746	8.78962	5.58665
52	922	2.96473	271	2.43297	7.21310	8.78962	5.91934
53	922	2.96473		2.43136	7.20832	8.78962	5.91151
54	922	2.96473	280	2.44716	7.25517	8.78962	5.98859
56	922	2.96473	250	2.39794	7.10924	8.78962	5.75012
57	922	2.96473	230	2.36173	7.00189	8.78962	5.57777
58	922	2.96473	300	2.47712	7•34399	8.78962	6.13612
59	922	2.96473	290	2.46240	7.30035	8.78962	6.06341
60	922	2.96473	2 9 0	2.46240	7.30035	8.78962	6 .6 6341
61	430	2.63347	165	2.21748	5.83967	6.93516	4.91722
62	430	2.63347	180	2.25527	5.93918	6.93516	5.08624
63	430	2.63347	187	2.27184	5.98282	6.93516	5.16126
64	430	2.63347	160	2.20412	5.80448	6.93516	4.85814
65	430	2.63347	150	2.17609	5.73067	6.93516	4.73537
66	430	2.63347	200	2.30103	6.05969	6.93516	5.29474
67	430	2.63347	176	2.24551	5.91348	6.93516	5.04232
6 8	430	2.63347	180	2.25527	5.93918	6.93516	5.08624
69	430	2.63347	200	2.30103	6.05969	6.93516	5.29474
70	430	2.63347	200	2.30103	6.05969	6.93516	5.29474
71	430	2.63347	150	2.17609	5.73067	6.93516	4.73537
72	430	2.63347	210	2.32222	6.11550	6.93516	5.39270
73	430	2.63347	200	2.30103	6.05969	6.93516	5.29474
74	430	2.63347	190	2.27875	6.00102	6.93516	5.19270
75	430	2.63347	200	2.30103	6.05969	6.93516	5.29474
76	430	2.63347	220	2.34242	6.16869	6.93516	5.48693
77	430	2.63347	230	2.36173	6.21954	6.93516	5.57777
78	430	2.63347	220	2.34242	6.16869	6.93516	5.48693
79	430	2.63347	210	2.32222	6.11550	6.93516	5.39270
80	430	2.63347	200	2.30103	6.05969	6.93516	5.29474

NT-	Deale						
No.	Body Wt.X	LogX	Rate	LogY	LogX.LogY	Log2X	Log ² Y
-	WU.A		/Min.Y	- G-		6	
1	541	2.73320	93	1.96848	5.38025	7.47038	3.87491
2	541	2.73320	86	1.93450	5.28738	7.47038	3.74229
2 3	541	2.73320	82	1.91381	5.23083	7.47038	3.66265
4	541	2.73320	100	2.00000	5.46640	7.47038	4.00000
5 6	541	2.73320	90	1.95424	5.34133	7147038	3.81905
	541	2.73320	93	1.96848	5.38025	7.47038	3.87491
7	541	2.73320	95	1.977722	5.40550	7.47038	3.91138
8	541	2.73320	86	1.93450	5.28738	7.47038	3.74229
9	541	2.73320	101	2.00432	5.47821	7.47038	4.01730
10	541	2.73320	90	1.95424	5.34133	7.47038	3.81905
11	541	2.73320	96	1.98227	5.41794	7.47038	3.92939
12	541	2.73320	90	1.95424	5.34133	7.47038	3.81905
13	541	2.73320	89	1.94939	5.32807	7.47038	3.80012
14	541	2.73320	106	2.02531	5 • 53 557	7.47038	4.15816
15	541	2.73320	1188	2.07188	5.66286	7.47038	4.29269
16	541	2 .73 320	100	2.00000	5.46640	7.47038	4.00000
17	541	2.73320	98	1.99123	5.44243	7.47038	3.96500
18	541	2.73320	93	1.96848	5.38025	7.57038	3.87491
19	541	2.73320	96	1.98227	5.41794	7.47038	3.92939
20	541	2.73320	93	1.96848	5.38025	7.47038	3.87491
21	756	2.87852	95	1.97772	5.69291	8.28588	3.91138
22	756	2.87852	100	2.00000	5.75704	8.28588	4.00000
23	756	2.87852	87	1.93952	5.58295	8.28588	3.76174
24	756	2.87852	87	1.93952	5.58295	8.28588	3.76174
25	756	2.87852	85	1.92942	5.55387	8.28588	3.72266
26	756	2.87852	82	1.91381	5.50894	8.28588	3.66267
27	756	2.87852	83	1.91908	5.52411	8.28588	3.68287
28	756	2.87852	89	1.94939	5.61136	8.28588	3.80012
. 29	756	2.87852	81	1.90849	5.49363	8.28588	3.64233
30	756	2.87852	90	1.95424	5.62532	8.28588	3.81905
31	756	2.87852	90	1.95424	5.62532	8.28588	3.81905
32	756	2.87852	80	1.90309	5.47808	8.28588	3.62175
33	756	2.87852	96	1.98227	5.70600	8.28588	3.92939
34	756	2.87852	100	2.00000	5.75704	8.28588	4.00000
35	756	2.87852	84	1.92428	5.53908	8.28588	3.70285
36	756	2.87852	84 .	1.92428	5.53908	8.28588	3.70285
37	756	2.87852	105	2.02119	5.81804	8.28588	4.08521
38	756	2.87852	90	1.95424	5.62532	8.28588	3.81905
39	756	2.87852	90	1.95424	5.62532	8.28588	3.81905
40	756	2.87852	90	1.95424	5.62532	8.28588	3.81905

No.	Body Wt.X	LogX	Rate /Min.Y	LogY	LogX.LogY	Log ² X	Log ² Y
41	922	2.96473	87	1.93952	5.75015	8.78962	3.76174
42	922	2.96473	100	2.00000	5.92946	8.78962	4.00000
43	922	2.96473	100	2.00000	5.92946	8.78962	4.00000
44	922	2.96473	85	1.92942	5.72021	8.78962	3.72266
45	922	2.96473	100	2.00000	5.92946	8.78962	4.00000
46	922	2.96473	87	1.93952	5.75015	8.78962	3.76174
47	922	2.96473	91	1.95904	5.80802	8.78962	3.83784
48	922	2.96473	95	1.97772	5.86340	8.78962	3.91138
49	922	2.96473	90	1.95424	5.79379	8.78962	3.81905
50	922	2.96473	90	1.95424	5.79379	8.78962	3.81905
51	922	2.96473	74	1.86923	5.54176	8.78962	3-49402
52	922	2.96473	95	1.97772	5.86340	8.78962	3.91138
53	922	2.96473	98	1.99123	5.90346	8.78962	3.96500
54	922	2.96473	90	1.95424	5•79379	8.78962	3.81905
55	922	2.96473	75 -	1.87506	5.55905	8.78962	3.51585
56	922	2.96473	100	2.00000	5.92946	8.78962	4.00000
57	922	2.96473	98	1.99123	5.90346	8.78962	3.96500
58	922	2.96473	90	1.95424	5 •7937 9	8.78962	3.81905
59	922	2.96473	90	1.95424	5.79379	8.78962	3.81905
60	922	2.96473	90	1.95424	5.79379	8.78962	3.81905
61	430	2.63347	104	2.01703	5.31179	6.93516	4.06841
62	430	2.63347	101	2.00432	5.27832	6.93516	4.01730
63	430	2.63347	104	2.01703	5.31179	6.93516	4.06841
64	430	2.63347	101	2.00432	5.27832	6.93516	4.01730
65	430	2.63347	102	2.00860	5.28959	6.93516	4.03447
66	430	2.63347	105	2.02119	5.32274	6.93516	4.08521
67	430	2.63347	105	2.02119	5.32274	6.93516	4.08521
68	430	2.63347	109	2.03743	5.36551	6.93516	4.15112
69	430	2.63347	106	2.02531	5.33359	6.93516	4.10188
70	430	2.63347	111	2.04532	5.38629	6.93516	4.18333
71	430	2.63347	111	2.04532	5.38629	6.93516	4.18333
72	430	2.63346	115	2.06070	5.42679	6.93516	4.24648
73	430	2.63347	112	2.04922	5.39656	6.93516	4.19930
74	430	2.63347	110	2.04139	5.37594	6.93516	4.16727
75	430	2.63347	96	1.98227	5.22025	6.93516	3.92939
76	430	2.63347	108	2.03342	5.35495	6.93516	4.13480
77	430	2.63347	106	2.02531	5.33359	6.93516	4.10188
78	430	2.63347	104	2.01703	5.31179	6.93516	4.06841
79	430	2.63347	105	2.02119	5.32274	6.93516	4.08521
80	430	2.63347	102	2.00860	5.28959	6.93516	4.03447

APPENDIX V

Calculation of respiratory rate and volume equation and standard error of estimate, Method 1.

$$Y = aX^b$$

or
$$\log Y = \log a + b \log X$$

(2)
$$\leq (\log X \times \log Y) = \log a \leq (\log X) + b \leq (\log^2 X)$$

(1)
$$249.37155 = 107\log a + 300.02468b$$

(2)
$$701.41291 = 300.02468\log a + 845.21453b$$

Divide each equation by coefficient of log a

(1)
$$2.33057 = \log a + 2.80396b$$

$$(2) 2.33785 = \log a + 2.81715b$$

$$.00728 = .01319b$$

$$b = .55215$$

Substitute value "b" in equation (1) solve for "a"

$$2.33057 = \log a + 2.80396(.55)$$

$$2.33057 = \log a + 1.54758$$

$$log a = .78236$$

$$a = 6.06$$

equation
$$Y = 6.06X^{.55}$$

 $S_{\log y}^2 = \frac{(\log^2 Y) - \log a \xi(\log Y) - b \xi(\log X \times \log Y)}{N^1}$

$$= \frac{582.65608 - (.78236 \times 249.37155) - .55215 \times 701.41291}{105}$$

$$= \frac{582.65608 - 195.098325 - 387.285138}{105} = \frac{.272617}{105} = \frac{.00259}{105}$$

 $S_{log y} \cdot log x = .0502$

$$+ S_n = 2 + .0502 = 2.0502 = 112.3 = 12.3\%$$

$$-s_r = 2 - .0502 = 1.9498 = 89.9 = 10.91\%$$

N¹ is the "degrees of freedom". The "degrees of freedom" are the number of data points less the number of constants in the equation.

APPENDIX VI

Calculation of respiratory rate and volume equation, standard error of estimate and correlation coefficient, Method 2.

Method 2

$$\begin{array}{c} \underbrace{\text{slog X}}_{\text{N}} = 300.02468 & \leqslant \log Y = 249.37155 \\ \underbrace{\left(\frac{\log X}{N}\right)}_{\text{N}} = 2.80396 & \underbrace{\left(\frac{\log X}{N}\right)}_{\text{N}} = 2.330575 \\ \underbrace{\left(\frac{\log X}{N}\right)}_{\text{N}} \approx 10g X = 841.25720 & \log^2 Y = 582.65608 \\ \log^2 X = 845.21453 & \underbrace{\left(\frac{\log Y}{N}\right)}_{\text{N}} \approx \log^2 Y = 581.17910 \\ & \leqslant \log^2 X = 3.95733 & \underbrace{10g^2 Y}_{\text{N}} \approx 107 \\ & \leqslant \log X \times \log Y = 701.41291 \\ \underbrace{\left(\frac{\log X}{N}\right)}_{\text{N}} \approx \log X \times \log Y = 2.18506 \\ \log Y = \underbrace{\left(\frac{\log X}{N}\right)}_{\text{N}} + \underbrace{\frac{\log X}{N} \log Y}_{\text{N}} = \frac{\log X}{N} \\ \log Y = 2.330575 + \underbrace{\frac{2.18506}{2.18506}}_{\text{N}} \log X - (2.80396) \\ = 2.330575 + .55215\log X \\ Y = 6.06X.55 \\ \Rightarrow 2 = \underbrace{\frac{\log (X \times \log Y)}{8 \log^2 Y}}_{\text{N}} = \underbrace{\frac{.55(2.18506)}{1.47698}}_{\text{N}} = \underbrace{\frac{1.20178}{1.47698}}_{\text{N}} = \cdot .813 \\ \Rightarrow .901 \\ \text{Slog Y} \cdot \log X = \underbrace{\frac{8\log^2 Y}_{N} - \log(\frac{\log X}{N} \times \log Y)}_{\text{N}} = \underbrace{\frac{1.47698}_{1.47698}}_{\text{105}} = \frac{1.20648}{105} \\ = \frac{.2705}{105} = .002528 \\ \text{Slog Y} \cdot \log X = \underbrace{\left((\text{antilog }.0502) - 1\right]}_{\text{100}} = (1.123 - 1)100 = 12.3\% \\ \end{aligned}$$

 $-S_{y.x} = \frac{12.3}{1.123} = 10.95\%$

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