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OXYGEN UPTAKE IN RELATION TO WATER BALANCE
OF A MITE IN UNSATURATED AIR

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
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* * * * * *

The Ohio State University
1963

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INTRODUCTION

Conservation of body-water reserve against the drying forces of the terrestrial environment is a major problem to be solved if life is to survive on land. Organisms must maintain a fairly constant internal medium of their own to gain independence from an aquatic environment. Different groups of land animals have solved this problem in different ways. Among invertebrates the arthropods are remarkable in their power of adaptation to land habitats. Referring to the Arthropoda, Cloudsley-Thompson (1958) has said,

Each aspect of their adaptation to life on land affects and is affected by other aspects. For example, it might appear a simple matter for an organism to eliminate water loss by the evolution of an integument completely impervious to water vapor, but such an integument would also be impervious to oxygen and carbon dioxide. A respiratory mechanism has therefore had to be evolved which permits gaseous exchange without excessive water loss. If the integument is rigid and provides support, then growth becomes impossible except by moultng and this limits size. The physiology of nutrition and excretion too, are closely concerned with water conservation and superimposed upon this basic physiological requirement are the innumerable concomitants of behaviour and ecology.

Besides the structural and physiological modifications, the over-all size of an animal has a great bearing on the regulation of its body water. This was recognized by
Kennedy (1927) who pointed out that all other parameters remaining the same, the water content is a function of the volume of the animal whereas evaporation is a function of the surface area. Small animals have very large surfaces in comparison to their volume. Consequently, the evaporative loss of water from their body surfaces becomes greater. Hence the greater the ratio of surface area to volume the more acute is the problem of water regulation in animals.

Homeostatic biological systems are always in dynamic equilibrium with their immediate environment: losses being counterbalanced by gains or vice versa. From this standpoint water balance in the Arthropoda can be analyzed into its components: viz., the loss and the gain of water. The factors that are concerned with the regulation of water in an organism can be empirically grouped under two broad headings: physical and physiological. However, it should be pointed out that a sharp line of demarcation between these two sometimes becomes obscure. Both the loss and the gain of water by an organism are influenced by these two classes of factors. Among the physical factors, the influence of temperature and humidity on water relations of arthropods as living systems has been a subject of numerous investigations in the past. This aspect of the subject has been discussed and reviewed by Buxton (1924), Uvarov (1931), Mellanby (1935), Johnson (1942), Ludwig (1945), Wigglesworth (1945,
1948), Richards (1951), Andewartha and Birch (1954), Edney (1957), and Beament (1954, 1961).

Aside from the physical agents of the environment, the loss and the gain of water by the organism also occurs as a product of its physiological processes. Thus, loss of water can occur through egestion, excretion, and respiration (Wigglesworth 1953). On the other hand the gain of water may result from taking it in with food, oral and anal drinking (Spencer and Edney 1954), production of metabolic water (Buxton 1930; Mellanby 1932), and in some cases by the absorption of water vapor from unsaturated air (Ludwig 1937; Lees 1946, 1947; Edney 1947; Browning 1954; Winston 1959; and Wharton and Kanungo 1962).

Mites, as a group, are the smallest of all terrestrial animals. As a result, the conservation of body water becomes of enormous importance for survival of those that inhabit comparatively dry environments. Frequent feeding and therefore close association with the source of food is a common mechanism used by terrestrial acari for replenishment of their body water. However, in many ectoparasitic forms such as the ticks, engorgement followed by fasting is a frequent pattern. Similar situations are also met in the case of the nest-dwelling ectoparasitic mesostigmatid mites. Acarines that face such odd situations must possess special mechanisms to withstand desiccation and to replenish their depleted body-water reserve for operation of their basic
physiological processes. Not all of the means alluded to above by which the arthropods can gain water are available under unfavorable circumstances. The only methods that can be exploited by the organism under most conditions are production of metabolic water and/or absorption of water vapor from the surrounding air.

Buxton (1930) showed that Tenebrio larvae, starved at different relative humidities below 60 percent, lost more weight in lower humidities than in high, but that their proportion of dry weight to wet weight remained constant. This he explained by supposing that, in dry air, additional dry material was oxidized and the water of metabolism retained to compensate for that lost by evaporation. A similar constancy in wet/dry weight ratio has been reported for the larvae of Tenebrio starved at different temperatures and humidities (Mellanby 1932). Oxidation of additional fat in dry air, presumably to produce water of metabolism to compensate for the loss by evaporation, has been shown to occur in starving Glossina (Buxton and Lewis 1934). That the insects metabolize more dry material in dry air than in moist air is apparent from the above work. Similar results have also been obtained by Fraenkel and Blewett (1944) for the larvae and pupae of Tribolium, Ephelia, and Dermetes.

Absorption of water vapor from humid air to compensate for the losses has also been reported in some arthropods. For example, Buxton (1930) reported that the starved larvae
of *Tenebrio* could obtain water from air at 90% relative humidity (r.h.) or above and thus gain weight. This gain in weight was found to be significantly higher than could be accounted for by gain in water resulting from the metabolism of food. Buxton's result has been confirmed by Mellanby (1932). Similar results for starved *Tenebrio* larvae have been obtained by Lafon and Teissier (1939) and for the nymphs of the grasshopper *Chortophaga* by Ludwig (1937) and for the prepupae of *Xenopsylla brasiliensis* by Edney (1947).

Although few acarines have been studied from this point of view, nevertheless the ability to absorb water vapor from unsaturated air has been found to exist in the ones in which it has been studied. The classical studies by Lees (1946, 1947) on *Ixodes ricinus* and various other species of ticks showed that this phenomenon exists in this group of arthropods. Similar results have been obtained for the tick *Ornithodoros moubata* (Browning 1954) and for *Hyalomma asiaticum* (Balashov 1960). Corroborative evidence to this effect has also been reported for the mites. Thus, Winston (1959) reported that the common clover mite, *Bryobia praetiosa*, could extract water vapor from air at a r.h. of 52%. *Acarus siro*, when transferred from lower r.h. to a higher one, i.e., from 70% r.h. to 80%, 90% or above, was also found to gain weight (Solomon 1962; Knüll 1962). Starved and predesiccated females of the spiny rat mite,
Echinolaelaps echidninus, could also gain weight at 90% r.h. or above (Wharton and Kanungo 1962). That this gain in weight is due to the movement of water from the surrounding humid air into the mite has been shown by the above authors.

That absorption of water vapor in humid air is a physiological process and is inhibited by a high concentration of nitrogen (more than 90%) or carbon dioxide (30% to 45%) in air has been demonstrated for the tick O. moubata (Browning 1954). Loss of weight by starved and predesiccated females of E. echidninus in an atmosphere of pure nitrogen while the relative humidity was 95% or above also supports the above conclusion (Kanungo, in press). Furthermore, the absorption of water vapor from unsaturated air by the ticks (Lees 1946) and by the spiny rat-mite (Wharton and Kanungo 1962) is reported to occur against a vapor pressure gradient which, for its operation, needs expenditure of energy on the part of the organism.

The above authors have also pointed out that the amount of energy needed to absorb water against an osmotic gradient of 140 atmospheres in the case of the females of the spiny rat-mite E. echidninus is not great. A change in temperature of less than 2°C will achieve it so that less than two calories per gram of water is required. The present investigation is an attempt to get an approximation of the magnitude of the efficiency of the active process.
involved in overcoming the above osmotic gradient in female
E. echidninus and to draw an over-all balance sheet for the
exchange of water in this mite.

A knowledge of the rate of metabolism of a living
system is a prerequisite for the assessment of the energetic
efficiency of any of its physiological processes. Usually
the rate of consumption of oxygen is taken as an index of
the rate of metabolism of aerobes. While a great deal of
information exists on the rate of consumption of oxygen and
respiratory metabolism of different arthropods, particularly
the insects (Krogh 1941; Edwards 1953; Buck 1962), the
physiology of respiration in acarines is scarcely known.
Mites occur in places where the oxygen tension in the
environment may be low as in bulk stored food materials in
the case of many acarids (Hughes 1959). Acarus siro can live
normally in an atmosphere with oxygen concentration as low
as 2% and can survive for a time at 1.2% (Hughes 1943).
Similarly, the females of E. echidninus can function normally
in an atmosphere containing 2.5% O₂ but are killed in a pure
nitrogen atmosphere. Thus, these mites also have the
ability to survive under conditions of low O₂ tension
(Kanungo, in press).

The methods that have been used to measure the rate
of oxygen uptake and carbon dioxide output by living systems
are diverse (Dixon 1952; Umbriet et al. 1959; Glick 1961).
Of all the gasometric techniques, the Cartesian diver manometry is of comparatively recent origin. Since its first application by Linderstrom-Lang (1937), the principle of the Cartesian diver to the manometric determination of changes in small quantities of gas has been used for the study of a variety of biological reactions. Linderstrom-Lang and Glick (1938) used this for the determination of cholinesterase. From that time on many modifications of the Cartesian diver technique have been introduced to meet the needs of the various investigators. Boell, Needham, and Rogers (1939), Boell and Needham (1939), and Boell, Koch, and Needham (1939) introduced some useful modifications of this technique in their investigations on the respiration and anaerobic glycolysis of regions of the amphibian gastrula. A recent review of Cartesian diver respirometry is given by Glick (1961). The size of the Cartesian diver which normally contains a gas volume of 1 to 10 μl has been altered by Zeuthen (1943) to give a total gas volume of 0.1 μl. On the other hand, Smith (1958) employed a diver of gas volume 188.6 mm³ to study the respiration of embryonic chick heart. Similarly, the shape of the diver has been modified to meet specific needs. Among these the cylindrical and the flask-shaped diver are noteworthy (Holter 1943).

Various refinements and alterations introduced into the diver technique are due to a need for an increase in the accuracy of measurements and to make the diver system
suitable for specific investigation. Thus, the loss of
gases from the charged divers was minimized by the use of
solid glass stoppers in the neck (Holter 1943). Likewise,
a coating of paraffin in the inside of the neck of the
diver has been used to prevent aqueous neck seals from
spreading (Anfinsen and Claff 1947). Silicone coating was
found to be superior and has been used in place of paraffin
to make the inside of the diver hydrophobic (Schwartz 1949;
Waterlow and Burrow 1949). The sensitivity of the Cartesian
diver system as a gasometric apparatus has also been
increased by the use of refined divers ("ampulla divers")
in conjunction with a sensitive manometer (Zeuthen 1953)
and also minimizing the fluctuations in the temperature of
the diver bath to about 2 to 3 x 10^{-4} C. (Lovlie and
Zeuthen 1962).

The spectrum of biological materials that has been
studied with the Cartesian diver method includes the measure-
ment of: the activity of enzyme systems; e.g., cholinesterase
(linderstrom-Lang and Glick 1938; Zajicek and Zeuthen 1961);
succinoxidase (Waterlow and Borrow 1949); (Borrow and Penney
1951) (an elaborate list of biochemical systems studied in
this way has been given by Holter 1961); the respiration of
the developmental stages of various organism, e.g., eggs of
rat (Boell and Nicholas 1948), eggs and embryos of Ciona
intestinalis (Holter and Zeuthen 1944), eggs of Rana
platyrrhina (Zeuthen 1946), sea urchin eggs (Zeuthen 1950a and b; Frydenberg and Zeuthen 1960), eggs of surf clam (Sculifer 1955); regions of amphibian gastrula (Boell et al. 1939), vegetative halves of echinoderm embryos (Lindahl and Holter 1940); cells from the following tissue cultures: embryonic chick heart fibroblast (Danes 1955; Smith 1958), mouse subcutaneous fibroblast (Danes and Kieler 1958; Paul and Danes 1961), megakaryocytes of rat bone marrow (Zajicek and Zeuthen 1961); live protozoa, e.g., amoebae (Holter 1943; Zeuthen 1943; Holter and Zeuthen 1948) and larval forms such as veliger and trochophore (Zeuthen 1947).

In addition to the above, the Cartesian diver has been used as a gasometric tool in the analysis of certain inorganic reactions (see Holter 1961 for references) and also as a balance to measure the underwater or reduced weights of small samples of living cells and single amoebae (see Zeuthen 1961 for review).

Although the versatility and great sensitivity of the diver technique has been used extensively in biological investigations, its use to measure the respiratory rates of mobile stages of terrestrial arthropods has not been previously reported. The present study on the respiration of an active mite by the methods of the Cartesian diver is new. The inherent sensitivity of diver manometry to detect
changes in gas volume of the order of one μl rendered its use most appropriate for the present investigation on the rate of uptake of oxygen by individual females of E. echidninus.
MATERIALS AND METHODS

A. The Organism

The organism used in the present investigation was *Echinolaelaps echidinus* (Berlese, 1887) (Acarina:Laelaptidae), an ectoparasite of rats. In order to reduce the variability, only adult females were selected for study. A supply of mites was obtained from mass cultures maintained in the Institute of Acarology. The mass cultures were maintained in the way described by Strandtmann and Wharton (1958). The method for collection of mites has been described by Wharton and Kanungo (1962).

B. Standardization of Mites

Mites were collected in glass vials having the following dimensions: outside diameter 8 mm, inside diameter 7 mm, and length 40 mm. Both ends of the vial were closed with bolting silk. Dehydration of mites prior to feeding was affected by placing the vial containing the mites over drierite (anhydrous calcium sulfate) for 5 to 60 hours. The mites were then supplied with heparinized blood collected from the heart of a rat and were allowed to feed for about 30 minutes through bolting silk, following the technique developed by Cross (1954), after which a selection was made.
Only recently fed females were selected by placing all the mites in an enameled iron tray, the sides of which were electrically heated by nichrome wire. The amount of current and hence the heating of the wire was controlled by a rheostat. Heating prevented the mites from leaving the tray and thus facilitated the collection. Selected mites were kept in another glass vial similar to one described above and were used for further standardization. It was possible to see the blood meal in gut diverticula with the aid of a dissecting microscope.

Standardization of mites was carried out qualitatively. The method used is as follows: Recently fed mites were confined individually in numbered glass vials of the same dimensions as described above. The ends of the vials were closed with bolting silk and a small piece of rubber tubing was fitted exactly to the inside diameter of the tubes. Fifty mites and five different relative humidities (r.h.) at 80°F. were used for standardization. The mites were observed every day under a binocular microscope with 30X magnification to ascertain the state of the blood meal in the gut. During observation the mites were anesthetized with carbon dioxide. Arbitrary values were assigned to various stages of digestion of the meal in the gut. These values had 5 and 0 as upper and lower limits, respectively. A fully fed mite was ranked as 5 whereas a mite with no
visible blood in the gut was ranked as 0. The relative
humidities used were 70, 80, 85, 90, and 95% ± 1% of 80°F.
Temperature and humidities were regulated by a "Humidity-
Gradient" machine (see below for description).

From the results obtained from this standardization
experiment (see Results), it was decided to starve the
recently fed mites for 6 to 7 days in a wet plaster-charcoal
jar at room temperature. The humidity of the jar was over
90% as shown by the color of the plaster-charcoal mat
(Huber 1958). After this starvation period, the mites were
called the "standardized" mites.

C. Weighing

The weight of each individual mite was taken on a
Cahn electrobalance, Model M-10, using the 10 mg. range.
At this range, the weight is read directly in micrograms.
The last digit in the weight was read from a vernier attached
to the dial of the balance. The mites were anesthetized by
CO₂ (see below for description of anesthetizing chamber) and
were handled with a camel's hair brush.

D. Regulation and Determination of
Temperature, Relative Humidity,
and Barometric Pressure

Moving air at desired temperature and relative humid-
ities was obtained by using a "Humidity-Gradient" machine
(Figure 1). The latter was made by joining two Aminco-aires
Figure 1. Diagramatic representation of the "Humidity-Gradient" machine

A. Front view of the "Humidity-Gradient" machine.
   RTC = rubber tubings connecting exposure chambers to the metal pipes

B. Schematic drawing of the "Humidity-Gradient" machine.
   EC = exposure chamber, MP = metal pipe

C. Sectional view of one of the exposure chambers with the sensing element in place.
   EC = exposure chamber, AI = air outlet
   SE = sensing element, MT = vertical metal tube
Figure 1.
(air conditioners manufactured by the American Instrument Company, Silver Spring, Maryland) with two insulated metal pipes. The two Aminco-aikes were maintained at the same temperature (80° F.), but the relative humidity of one was kept at 70% and the other at 95%. The flow of air in the pipes was controlled by two dampers, one placed on each pipe. Each exposure chamber consisted of a vertical metal tube 60 mm in diameter. The circulating air in the main pipes was allowed to pass through these vertical tubes by cross connections. There were two cross connections for each exposure chamber, one for each main pipe. Thus, the actual mixing of air took place in these vertical tubes. Each cross connection was guarded by dampers to control the amount of air passing into the tubes. By adjusting the dampers, different proportions of air at low and high relative humidities were mixed, and a desired gradient of relative humidities between the two extremes was obtained. Two threaded Mason jar caps were soldered end to end. One end of this soldered cap was fitted tight at the top of each vertical tube while the other end was fitted with a jar (diameter 60 mm). The latter had a circular hole at its base (Figure 1). Thus, when the jar was fitted to the vertical tube, the hole came at the top. A wire net was placed at the top of each vertical tube to serve as a base on which the tubes containing the mites could be placed.
An appropriate sensing element (purchased from Hydrodynamics Inc., Silver Spring, Maryland), both for the temperature and the relative humidity, was placed inside each vertical tube (Figure 1) and was connected to a Dynamaster Recorder (a wide-strip recording system made by the Bristol Company, Waterbury, Connecticut). This recording system provided a continuous record of the temperature and the relative humidity in each of the exposure chambers.

There were six exposure chambers, and the gradients of the relative humidities regulated were 70, 75, 80, 85, 90, and 95% at 80°F. The exposure chambers were located inside a wooden box (Figure 1), and the air passing through these chambers were returned to the main pipes by means of rubber tubing (Figure 1). Thus, the whole "Humidity-Gradient" machine was a closed unit in itself. The variation in the relative humidities was ±1% and it was ±0.5°F. for the temperatures.

The room was provided with an air-conditioning unit. The temperature and the relative humidity of the room were measured by an electric hygrometer (purchased from Hydrodynamics, Inc., Silver Spring, Maryland). The air pressure in the room was measured by a Fortin-type barometer (manufactured by the Precision Thermometer & Instrument Co., Philadelphia, Pennsylvania).
The consumption of oxygen by individual mites was measured using the technique of the Cartesian diver. The set-up for this purpose is illustrated in Figure 2. In addition to the divers, the assembly consisted of the following: the flotation vessels in which the divers were placed during the experiment, the connecting manifold, the manometer, the pressure regulator, the water jacket, and the water bath. The water jacket, the connecting manifold, the manometer, and the pressure regulator were mounted on a wooden frame.

1. The flotation vessel (Figure 3)

This was a glass tube with an outside diameter of 2.6 cm and an inside diameter of 2.5 cm. The total length of the vessel was 18.4 cm. The bottom was hemispherical in shape. This helped to bring the diver to the center of the tube when the former was at rest at the bottom. The open end of the vessel was a ground glass mouth of wider diameter than the rest of the tube. Two fine circular marks were drawn on the outside of the vessel. The lower mark served as the fiduciary line, while the upper one was used for the upper level of the flotation medium. Two of the vessels were used for experimental divers and a third one contained the control diver called the "Thermobarometer."
Figure 2. A schematic diagram of the Cartesian diver assembly

CM = connecting manifold
FV = flotation vessel
IB = iron bar
M = manometer
PR = pressure regulator
SC = meter scale
Th = thermometer
WF = wooden frame
WI = water inlet
WJ = water jacket
WO = water outlet
Figure 3. Flotation vessel

D = diver
FL = fiduciary line
S = ground glass stopper
UM = upper level of the flotation medium
Figure 3.
During the experiment the flotation vessels were connected to the manometer through a connecting manifold by means of rubber tubing and hollow ground glass stoppers (Figure 2).

The flotation medium. Lithium chloride (LiCl) solution of the same concentration (≈11N) used by Boell, Needham, and Rogers (1939) was used as the flotation medium for the divers. Four hundred and seventy-six grams of LiCl were weighed in a Harvard trip balance and the solution was made with distilled water. LiCl was first dissolved in 800 ml of distilled water and was then vacuum filtered. Since the reaction between LiCl and water is an exothermic one, the filtered solution was allowed to cool down to room temperature, after which the volume of the total solution was made up to a liter. The solution was stored in containers which were only half filled so that enough air remained in the containers. Thus, the solution remained nearly saturated with air.

The medium was filled up to the upper mark on the vessel (Figure 3). The height of the medium in all three vessels was 5.5 cm and the distance between the fiduciary line and the top of the medium was 2.0 cm.
2. The connecting manifold (Figure 2)

The ground glass mouths of the flotation vessels were fitted separately to hollow ground glass stoppers. These stoppers passed through a rubber stopper which was used to close the mouth of the water jacket (see below). The glass tubes of the joints sticking out through the rubber stopper were connected to the connecting tube by means of thick-walled rubber tubing.

The connecting tube was a thick-walled glass tube shaped like a rake (T T T T) and it ran over the water jacket. The distance between the connecting tube and the top of the rubber stopper was 62 cm. The connecting tube was then connected to one arm of the manometer by a ground glass joint. This rake had three three-way stopcocks and thus every flotation vessel could be made to communicate separately with the manometer. The connecting tube was open to the air at the end opposite the manometer. However, during the experiment the direct connection between the outside and the flotation vessel was cut off by the three-way stopcock. All ground glass joints were greased with stopcock grease.

3. The manometer (Figure 2)

This consisted of a U-shaped glass tube, one arm of which was open to the air while the other arm was connected at the top to the rake-shaped connecting tube described above.
The length of the open and the closed arm was 124 and 105 cm, respectively. The outside and the inside diameter of the arms of the manometer were 1 cm and 0.8 cm, respectively. A short glass tube was fused at the bottom of this U-shaped manometer to which a rubber tubing was sealed with a glass rod. The length, the outside and the inside diameter of this rubber tubing were 125.0, 1.5 and 1.0 cm, respectively. The manometer was mounted vertically on a wooden board carrying between the arms a 100 cm long scale graduated in millimeters.

4. The pressure regulator (Figure 2)

An iron bar 50 cm in length, 3 cm in width, and 1.0 cm in thickness was mounted horizontally on the baseboard supporting the vertical wooden frame. One end of this iron plate was tightly hinged to the board while the other end was left free. A portion of the rubber tubing connecting the manometer was placed underneath this iron bar and was kept in place by passing its closed end through a hole at the fixed end of the bar. At a distance of 32 cm from the fixed end of the bar a screw clamp was tightly fixed to the baseboard. The diameter and the length of the screw were 0.5 and 14.0 cm, respectively. A downward movement of the screw pressed the iron bar which in turn squeezed the rubber tubing underneath. The reverse process occurred when the screw was loosened and moved upwards.
The manometer fluid consisted of distilled water slightly tinged blue with a very dilute solution of sodium taurocholate and bromocresol purple. The fluid was infused into the rubber tubing with a syringe until it reached a height of 50 cm on the manometer. Any air bubble present in the fluid in the rubber tubing and in the manometer was removed with a quick upward and downward movement of the iron bar. The menisci of the fluid in the manometer were slightly concave and the readings were taken from the lower tips of the curvatures.

5. The water jacket and the water bath (Figure 2)

During the experiments the flotation vessels were enclosed in a water jacket. The water jacket consisted of a thick-walled glass cylinder whose inside diameter and length were 9.0 cm and 32.0 cm, respectively. It was mounted vertically in a wooden frame. The water jacket was connected to the water bath by an inlet at the bottom and an outlet at the top. These two connections, for the most part, consisted of pressure tubing. A screw-type pinchcock was placed on the inlet tubing to regulate the rate of flow of water from the bath to the jacket. It was so regulated that about a liter of water could circulate through the jacket in 40 seconds. The water jacket was closed at the top with a rubber stopper. There were five holes in the stopper: one for the thermometer, one for the outlet of water, and the
other three for the glass tubes connecting the hollow stoppers of the three flotation vessels. The water bath was a Blue M Magni-Whirl utility water bath (manufactured by the Blue M Electric Company, Blue Island, Illinois). The temperatures of the bath and the jacket were recorded by Fahrenheit thermometers calibrated to $0.05^\circ$. The fluctuation of temperature during the experiment was $\pm 0.5^\circ$ F.

6. The diver (Figure 4)

Making the diver.—Divers were made from Kimax melting point glass capillaries with an approximate 1.4 mm outside diameter and a 0.8-1.2 mm inside diameter. The capillary was bent at right angles in the middle. A piece of polyethylene tubing 1.7 mm outside diameter and 1.2 mm inside diameter was fitted to one end of this bent capillary. This polyethylene tubing served as a mouth blower with which the size of the bulb of the diver could be regulated. The other end of this capillary was then heated over the micro-flame. The opening of the capillary at this end was closed by sucking in air through the mouth blower. This produced a flattened solid bulge ("Lining cone") which, when the diver was completed, was situated at the top of the bulb opposite the neck and served to line up the diver with the fiduciary line (Figure 3) drawn on the flotation vessel. The size of the bulb of the final diver was judged from a "Reference diver." The latter was prepared in the same way.
Figure 4. A diver with various seals

AS = sodium hydroxide seal
LC = lining cone
OS = oil seal
MS = medium seal
PP = polyethylene plug
as described above and was tested in the flotation medium for desired buoyancy. The total length of the diver was kept constant by cutting it off from the capillary over a premeasured line. Three divers were used at a time.

**Calibration of the diver.**—The total gas volume of each diver was measured by filling the divers with distilled water from a Rehburg Micro burette calibrated to deliver 1 μl per division. The diver was cleaned and dried before calibration. It was filled with distilled water, and the volume delivered was noted. The diver was then dried thoroughly and the measurement repeated. In all cases two measurements gave exactly the same value and therefore repetitions of calibration were thought to be unnecessary.

**Dimensions of the diver.**—Six divers were used in the present experiment. The volume (total gas volume of empty diver) of the divers ranged from 25.0 μl to 27.5 μl (Table 1). Besides the volume, the diameter of the neck and the length of the divers varied also. However, in all cases except one the inside diameter of the neck was 1.0 mm while the length of the neck and the total length of the divers varied from 17.0 to 19.5 mm and 21.0 to 23.0 mm, respectively (Table 1).
TABLE 1

DIMENSIONS OF THE DIVERS

<table>
<thead>
<tr>
<th>Diver No.</th>
<th>Average volume (µl)</th>
<th>Diameter of neck</th>
<th>Length of neck (mm)</th>
<th>Total length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inside (mm)</td>
<td>Outside (mm)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>27.0</td>
<td>1.0</td>
<td>1.38</td>
<td>19.0</td>
</tr>
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<td>2</td>
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<td>1.0</td>
<td>1.48</td>
<td>19.0</td>
</tr>
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<td>3</td>
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<td>0.93</td>
<td>1.38</td>
<td>19.0</td>
</tr>
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<td>4</td>
<td>26.0</td>
<td>1.0</td>
<td>1.38</td>
<td>17.0</td>
</tr>
<tr>
<td>5</td>
<td>25.0</td>
<td>1.0</td>
<td>1.31</td>
<td>18.0</td>
</tr>
<tr>
<td>6</td>
<td>27.5</td>
<td>1.0</td>
<td>1.38</td>
<td>19.5</td>
</tr>
</tbody>
</table>

Handling, storing, and cleaning the diver.—The calibrated divers were always handled with a pair of jeweler's forceps, which were always cleaned and dried beforehand. Very little pressure was exerted on the prongs of the forceps to avoid scratching the divers.

When not in use, the cleaned and dried divers were stored in plastic boxes 2.8 cm x 2.8 cm. The floor of the boxes was matted with tissue paper to avoid unnecessary damage to the divers. These boxes were then placed in large plastic boxes 15.5 cm x 6.0 cm. In order to keep the divers thoroughly dry, some drierite crystals were placed in the large box. The drierite was changed every 4 or 5 days.

The procedure of cleaning the divers was as follows: At the end of each experiment the diver was quickly removed
from the flotation vessel and was rolled over a filter paper to wipe away the medium sticking to the outside of the diver. The medium seal, the oil seal, and the sodium hydroxide were then removed one after the other with balls of filter paper. These were made from narrow strips of filter paper which were rolled between the fingers and made as compact as possible. Care was taken to remove all the liquids from the neck of the diver as completely as possible. The polyethylene plug (see below) was removed with a syringe needle. The confined mite usually crawled out as soon as the polyethylene plug was removed. However, in some cases the mite stopped at some point in the neck of the diver. A thin wire bent to form a hook at one end was then used to pull the mite out of the diver.

The empty diver was then filled with xylene with a capillary pipette. After about a minute the xylene was pipetted from the diver which was again refilled with fresh xylene. This procedure was repeated three times for xylene, isopropyl alcohol, and distilled water which followed one after the other in sequence. After removal of the distilled water, the diver was placed in a cleaning solution. This solution was made of the following ingredients: sixty-three grams of potassium dichromate were dissolved by heating with 35 ml of water and then concentrated sulfuric acid was added to make up to a liter (Umbreit et al. 1959). The diver was
filled with the cleaning solution by suction through a sink aspirator and then was left over night in the cleaning solution.

The diver was removed from the cleaning solution the next day and was placed in tap water in a small glass jar. The jar was fitted with a two-hole rubber stopper through which glass tubing was passed. The jar was then connected to the sink aspirator through one glass tube while the other tube served to control the evacuation process. Usually a small air bubble was introduced into the diver filled with the cleaning solution. Then, by controlled suction and subsequent release of pressure in the jar the air bubble was moved to the extreme end of the bulb of the diver. During this process some of the cleaning solution was forced out and was replaced by water. This resulted in the dilution of the cleaning solution in the diver which could be expelled with a little suction. When the pressure inside the jar was reduced, the air bubble expanded and forced the diluted cleaning solution out. However, the cleaning solution was not removed from the diver in one suction. With alternate application and release of suction to a desired degree, the diluted cleaning solution was moved back and forth four or five times inside the neck of the diver and was finally expelled. The diver was then filled with tap water in a similar way and the process was repeated until no yellow tinge of dichromate was visible.
The diver was placed over a washing set-up (Figure 5A) for continuous cleaning with tap water. The washing set-up was attached to the sink. It consisted of a metal pipe with six vertical outlets and a horizontal outlet at the end. The inlet at the sink and all the outlets were provided with needle valves so that the rate of the flow of water could be regulated. Each of the six vertical outlets was fitted with a rubber tubing at the top of which was placed a syringe needle pierced through a rubber stopper of an appropriate size to fit the inside diameter of the rubber tubing. When properly regulated, the water flowed through the needle forming a vertical jet. The length of the needle was so adjusted that when the diver was placed on it, the tip of the needle could touch the end of the bulb of the diver. In this way a continuous flow of water was maintained through the diver when it was washed with tap water, a procedure which lasted for about an hour. During this washing with tap water the end outlet of the set-up was closed.

After washing with tap water, the diver was cleaned with distilled water. At this time the inlet of the washing set-up was closed and the end outlet was opened. The tap water from the metal tube was aspirated out by connecting a sink aspirator to the end outlet. The jar containing the distilled water was connected to the set-up through the end
outlet. Distilled water was then pumped into the system and the flow was maintained by air pressure inside the jar. The water jet was similarly regulated by the needle valves. Washing with distilled water was continued for half an hour to an hour after which the diver was dried.

The drying of the diver was effected by a "suction drier" (Figure 5B). This was made up of a glass Y-tube, the two arms of which were fitted to two glass tubes that were closed by rubber stoppers. A syringe needle was pierced through each of these stoppers. This set-up was then connected to the sink aspirator. The length of the syringe needle, as in the washing set-up, was adjusted to reach the end of the diver bulb when the diver was inverted over the needle. With this set-up, complete drying of the diver was accomplished in 10 to 15 minutes.

**Charging the diver.**—The loading of the diver for the experiment comprised the following steps: (a) placing and confining the mite in the diver; (b) placing of the various neck seals, _viz._, the sodium hydroxide (NaOH) seal, the oil seal, and the medium seal; and (c) adjusting the loaded diver to proper buoyancy in the flotation medium.

Immediately after it was weighed, the mite was introduced into the diver. The anesthetized mite was picked up by holding its legs with a pair of forceps. The anterior end of the mite was introduced through the mouth of the
Figure 5. Contrivances for washing and drying the divers

A. Contrivance for washing the divers

D = diver, MP = metal pipe, N = syringe needle
NV = needle valve, OL = outlet, RT = rubber tubing
SAP = sink aspirator

B. Contrivance for drying the divers

CON = connection to the sink aspirator
ST = stand
T = Y-tube
Figure 5.
diver and then it was pushed into the bulb with a long polyethylene tube. This process was carried out in an atmosphere of carbon dioxide maintained in an anesthetizing chamber (Figure 6). This chamber consisted of a plastic box 12.0 cm x 10.5 cm covered by a cardboard at the top. Rubber tubing was passed through a hole in one of the sides. A petri dish was placed inside the box where all the operations were carried out. The plastic box was connected to a CO₂-cylinder and the flow of gas was regulated by a needle valve (Figure 6). In order to prevent excessive dehydration of the mite during anesthetization, a water bottle was introduced between the anesthetizing chamber and the CO₂ cylinder (Figure 6).

After the mite was secured in the diver, the diver was taken out of the anesthetizing chamber and was flushed with 10 cc of air delivered through a syringe. In most cases the mite came out of the anesthesia before the flushing.

The groups of standardized mites for the investigation of consumption of oxygen were of two different kinds as regards their water content. One group, the dehydrated mites, after the period of standardization, was exposed to 80% r.h. at 80° F. for 24 hours prior to the experiment while the second group, the hydrated mites, after being treated in a similar way as the first group, was exposed to 95% r.h. at 80° F. for about 24 hours before the start of the experiment. In order to reduce the random experimental
Figure 6. The chamber for anesthetizing the mites

AC = anesthetizing chamber
CC = carbon dioxide cylinder
P = petri dish
PB = plastic box
WB = water bottle
errors while comparing the consumption of O₂ between the two kinds of mites, one of each type was used side by side in all experiments.

The divers used for each kind of mite were selected by rolling a die. Polyethylene tubing with 1.27 mm outside diameter, 0.86 mm inside diameter, and 5 mm in length was used as a plug to keep the mite confined in the diver bulb (Figure 4). A small amount of 1% NaOH solution, used as the neck seal, was placed in the polyethylene tubing.

The sodium hydroxide (NaOH) seal consisted of 1% NaOH. It was delivered to the neck of the diver with a fine capillary attached to a Rehburg micro burette. The diver with the confined mite was placed over a stand which could be moved up and down by rack and pinion. The latter was made from an old microscope from which the lens assembly had been removed (Figure 7). A rubber tube of an appropriate inside diameter to hold the bulb of the diver was fastened to the stand and the diver was placed on this rubber tube. The stand with the diver was brought under the burette and the delivery capillary was introduced into the diver by raising the stand slowly. The NaOH seal was placed according to the method described by Holter (1943). The seal was placed a short distance from the tip of the polyethylene plug so that it would not come in contact with the plug due to the variation in pressure during measurements.
Figure 7. The diver stand for charging the divers

D = diver
MB = microburette
RP = rack and pinion
ST = stand
Figure 7.
White mineral oil (paraffin oil) was used for the oil seal. It was delivered to the diver in the same manner as the delivery of the NaOH seal. Due to the high viscosity of the oil, it was difficult to deliver less than 1 µl at one time. When more than 1 µl was placed in the diver, the excess was sucked out until the length of the seal gave an approximate volume of 1 µl or less.

The medium seal consisted of the flotation medium (~11.2 N LiCl). In order to make the medium seal slide easily in the neck of the diver, 0.1 gram of sodium taurocholate was added to 100 ml of the flotation medium used for the seal. The delivery of the medium seal to the neck of the diver was effected by a capillary pipette. This pipette was attached to polyethylene tubing which served as a mouth blower to regulate the amount of the medium delivered.

Adjustment of the diver to proper buoyancy.—One of the flotation vessels filled with the flotation medium to the desired height was used for testing the buoyancy of the charged diver. The medium was brought to the required temperature (80° F.) by placing the flotation vessel into a pre-set water bath and in which it was kept during the test period. Since the room temperature did not vary too much from 80° F., the temperature gradient between the air and the medium was neglected. The barometric pressure and the
temperature of the room were recorded during the adjustment of the divers.

The adjustment was slightly different in the thermobarometer diver from that of the divers containing the mites. All of the divers were adjusted by varying the length of the medium seal. The thermobarometer was adjusted so that no more than 100 mm of pressure would be required to bring it to the level of the fiduciary line. However, the experimental divers, if adjusted the same as the thermobarometer, got heavy by the time the actual measurements started. In some preliminary experiments the experimental divers adjusted the same as the thermobarometer got so heavy that with the pressure-difference that could be created in the present set-up, it was impossible to bring the divers to the level of the fiduciary line. For this reason the experimental divers were always made lighter at the time of testing. This initial lightness of the charged experimental divers was judged visually by observing the speed of the rise of the divers in the flotation medium. It took 5 to 10 minutes to adjust each diver to the desired buoyancy. After adjustment each diver was kept in the medium in its respective flotation vessel until the lengths of the various seals were determined.

The lengths of the seals and the NaOH in the polystyrene plug were determined with a binocular dissecting
microscope fitted with an ocular micrometer. The smallest distance between the menisci of each seal was measured for the above purpose.

Transfer of the diver to the flotation vessel and equilibration of temperature.---Immediately following the measurements, the divers were returned to their respective vessels. The air bubbles sticking to the outside of the divers were carefully removed with a fine glass rod. A glass rod bent to form a circular loop of about the same diameter as the bulb of the diver was used to place and to remove the divers from the flotation medium. As these divers were without tails, they floated with their "lining cones" up and necks down in the medium.

The flotation vessels containing the charged divers were then placed in the water jacket and were connected to the manifold. While fitting the ground joints of the vessels, the manifold was open to the air by means of the three-way stopcocks so that no sudden change in pressure would occur in the vessels. The pump of the main water bath was turned on and thus the circulation of water from the bath to the jacket was regulated. The system was left in that way for about an hour for the equilibration of temperature.
F. **Measurements**

At the end of the one-hour period of temperature equilibration the flotation vessels were cut off from air. The barometric pressure in the room was recorded. In all cases the measurements were started and ended with the thermobarometer. In each case the diver was brought to the level of the fiduciary line until a black circle appeared in the "lining cone." The circle was gradually made smaller and smaller until it was reduced to its smallest size (see evaluation of measurement below) at which time the heights of water in both the arms of the manometer were recorded. At least three consecutive readings for each diver were taken each time. The duration of each experiment, i.e., the time the readings were started and ended, was 60 ± 5 minutes. The interval for each cycle of reading was timed in such a way as to permit at least six cycles of readings during a one-hour period of experimental duration. The temperature of the water bath was intermittently recorded during the period of the actual experiment.

G. **End of the Experiment**

At the conclusion of the experiment, the divers were removed from the vessel by the glass loop and were rinsed with distilled water. The various neck seals were carefully removed one by one. The polyethylene plug was removed with a syringe needle. The mite confined in each of the
experimental divers was taken out immediately and weighed. The divers were then cleaned and stored as described above.

Immediately after the final weights were taken, the mites were put individually in numbered vials and were placed over drierite (anhydrous CaSO₄) in a desiccator at room temperature. The mites were weighed again after 3 days of drying over drierite. The weights so obtained are called "Dry weights" (Wd).

H. Calculations

1. Volumes of various liquid columns in the diver

   The volume of each column was calculated assuming that each column was a cylinder.

   Hence, \( V = \pi r^2 L_D \)
   
   where, \( V = \) volume in \( \mu l \)
   \( r = \) inner radius of the diver capillary or of the polyethylene plug (mm)
   \( L_D = \) the effective length of column in mm

   Since the measurements of lengths were made at the smallest distance between the menisci, the effective length (including two menisci for each column) of each column was calculated from the formula:

   \( L_D = L + \frac{2r}{3} \) (Linderstrom-Lang 1943)

   where, \( L = \) length at the smallest distance between the menisci (in mm)
2. Change in equilibrium pressure

As mentioned above, the heights of water in both arms of the manometer were recorded every time the diver was brought into line with the fiduciary line. The reading of the closed arm of the manometer was subtracted from that of the open arm to get the difference in height of water in the manometer (see sample experiment below). Such initial difference taken at interval 0 is called "equilibrium pressure" and was subtracted from each of the successive differences to get the net change in pressure corresponding to the interval. This net change of pressure for the experimental divers is termed $\Delta P_e$ and for the thermobarometer is $\Delta P_c$. These were always recorded in millimeters of water.

3. The diver constant

The volume of gas in a charged diver (the diver constant) was calculated by subtracting the volume of the various liquid columns, the volume occupied by the polyethylene plug and the volume of the mite (in experimental divers only) from the total volume of the diver. All measurements expressed in mm$^3 = \mu l$.

$$V_e = V_g - (V_t + V_{tn} + V_n + V_o + V_m + V_a)$$

where, $V_e =$ the diver constant for experimental divers

$V_g =$ total volume of the diver

$V_t =$ volume occupied by polyethylene plug
\( V_{tn} \) = volume occupied by NaOH in polyethylene plug

\( V_n \) = volume occupied by NaOH seal

\( V_o \) = volume occupied by oil seal

\( V_m \) = volume occupied by medium seal

\( V_a \) = volume occupied by the mite

The average volume of the hydrated and dehydrated mite was calculated from the average weights of each kind of mite, assuming the density of the mite is 1.0. Thus, the average volume of a dehydrated mite was found to be 0.16 mm\(^3\) while that of a hydrated one was 0.19 mm\(^3\).

4. The diver equation

The volume of oxygen used in a given interval of time was calculated from the change of pressure for that interval. The equation used for the purpose was basically the same as the one recommended by Glick (1961) and Holter (1943). However, in view of the fact that the diver constants for the experimental divers (\( V_e \)) and for the thermobarometer (\( V_c \)) were not always equal, this difference in volumes was taken into consideration while correcting the pressure changes for the experimental divers against those of the thermobarometer. Hence, the pressure changes were made proportionate to the volumes using the equation:

\[ \Delta P_e \times \frac{V_e}{V_c} = \Delta P'_e \]
where, $\Delta P_e$ = pressure change for experimental diver (mm)
$V_e$ = diver constant for experimental diver (\mu{l})
$V_c$ = diver constant for thermobarometer (\mu{l})
$\Delta P'_e = \Delta P_e$ corrected for differences in $V_e$ and $V_c$

Since the factors affecting the measurements of changes of pressure for the thermobarometer ($\Delta P_c$) must have affected the pressure changes for the experimental divers ($\Delta P_e$), the former has to be subtracted from the latter to get the correct $\Delta P_e$. As $\Delta P_c$ could not be measured at the same time as $\Delta P_e$, the former was plotted against time. $\Delta P_c$ for the corresponding time of $\Delta P_e$ was read from such a curve (see sample experiment and Figure 8) and the former was subtracted from the $\Delta P'_e$. This corrected change of pressure for the experimental divers (i.e., $\Delta P'_e - \Delta P_c$) is called $\Delta P$ and was used in the following diver equation for the calculation of the change in volume of gas in the diver ($\Delta V$).

This change in volume of gas in the diver was due to the consumption of oxygen by the mite and hence oxygen was continuously being lost from the gas phase in the diver. Hence, $\Delta V = \Delta V_{O2}$ where $\Delta V_{O2}$ is the volume of oxygen consumed
Figure 8. A sample curve showing the fluctuations of the equilibrium pressure for the thermobarometer during the experiment.
Figure 8.
by the mite and is expressed, in the present case as μl per hours. This rate has been computed in three different ways, viz., on the basis of unit of dry weight of the mite, on the basis of unit of live initial weight, and on a per mite basis (see Results).

Thus, the equation for the calculation of the volume of $O_2$ consumed is

$$\Delta V_{O_2} = \frac{V_e (\Delta P_e \times \frac{V_e}{V_o} - \Delta P_o) T_0}{P_o}$$

$$= \frac{V_e (\Delta P_e - \Delta P_o) T_0}{T}$$

$$= \frac{(V_e \times \Delta P) T_0}{P_o}$$

where, $\Delta V_{O_2}$ = the rate of oxygen consumption expressed as the volume (μl) of oxygen used by the mite per hour

$T_0$ = freezing point of water (Abs.) = 273°

$T$ = temperature of the diver system (Abs.) = 299.7°

$P_o$ = normal pressure of manometer fluid (in this case water) = 10367 mm

In all cases the right hand term in the above expression is negative because of the negative sign of $\Delta P$ (see sample experiment). This minus sign indicates that gas was lost from the diver and hence the pressure was decreased to bring the diver to the equilibrium position.
SAMPLE EXPERIMENT

As an example of the actual performance in determining the respiration intensities of the starved dehydrated and hydrated females of *E. echidninus*, the following observations covering one complete experiment in one day are given so that one can get an over-all picture of the procedure from such a sample experiment (Tables 2 to 6). Furthermore, it also shows the reproducibility of the individual measurements and gives the magnitude of the random variations introduced into the measurements as they influenced the "thermobarometer" in the diver system. All in all, the aim of the sample experiment is to aid in understanding the procedures of actual measurements with the Cartesian diver as it was used in the present investigation. However, it should be pointed out that the actual observations reported in the sample experiment in Tables 2 to 6 are just the observations made in one experiment on that particular day and do not necessarily reflect the magnitude of respective observations for all the experiments on which the present discussion is based.
TABLE 2

SAMPLE EXPERIMENT: CONSUMPTION OF OXYGEN BY
FEMALE ECHINOLAECLAPS ECHIDNINUS

Date - September 15, 1962
Diver No. 4
Dehydrated mite

<table>
<thead>
<tr>
<th>Time (P.M.)</th>
<th>Interval (minutes)</th>
<th>Open arm (O.A.)</th>
<th>Closed arm (C.A.)</th>
<th>Difference (mm) (O.A.-C.A.)</th>
<th>Net change (mm) (ΔP&lt;sub&gt;e&lt;/sub&gt;)</th>
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</thead>
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<td>-440</td>
<td>0</td>
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<td>879</td>
<td>-440</td>
<td>0</td>
</tr>
<tr>
<td>3:22</td>
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<td>438</td>
<td>878</td>
<td>-440</td>
<td>0</td>
</tr>
<tr>
<td>3:29</td>
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<td>885</td>
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<tr>
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<td>436</td>
<td>885</td>
<td>-449</td>
<td>-9</td>
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<td>436</td>
<td>885</td>
<td>-449</td>
<td>-9</td>
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<tr>
<td>3:42</td>
<td>21</td>
<td>390</td>
<td>865</td>
<td>-475</td>
<td>-35</td>
</tr>
<tr>
<td>3:42</td>
<td>21</td>
<td>390</td>
<td>865</td>
<td>-475</td>
<td>-35</td>
</tr>
<tr>
<td>3:43</td>
<td>22</td>
<td>388</td>
<td>864</td>
<td>-476</td>
<td>-36</td>
</tr>
<tr>
<td>3:58</td>
<td>37</td>
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<td>876</td>
<td>-503</td>
<td>-63</td>
</tr>
<tr>
<td>3:59</td>
<td>38</td>
<td>373</td>
<td>876</td>
<td>-503</td>
<td>-63</td>
</tr>
<tr>
<td>3:59</td>
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<td>372</td>
<td>876</td>
<td>-504</td>
<td>-64</td>
</tr>
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<td>47</td>
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<td>878</td>
<td>-525</td>
<td>-85</td>
</tr>
<tr>
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<td>878</td>
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<tr>
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<td>48</td>
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<td>878</td>
<td>-525</td>
<td>-85</td>
</tr>
<tr>
<td>4:20</td>
<td>59</td>
<td>340</td>
<td>884</td>
<td>-544</td>
<td>+104</td>
</tr>
<tr>
<td>4:20</td>
<td>59</td>
<td>339</td>
<td>884</td>
<td>-545</td>
<td>-105</td>
</tr>
<tr>
<td>4:21</td>
<td>60</td>
<td>339</td>
<td>884</td>
<td>-545</td>
<td>-105</td>
</tr>
<tr>
<td>4:22</td>
<td>61</td>
<td>339</td>
<td>884</td>
<td>-545</td>
<td>-105</td>
</tr>
</tbody>
</table>
TABLE 3
SAMPLE EXPERIMENT: CONSUMPTION OF OXYGEN BY FEMALE ECHINOLAEELS ECHIDNINUS
Date - September 15, 1962
Diver No. 5
Hydrated mite

<table>
<thead>
<tr>
<th>Time (P.M.)</th>
<th>Interval (minutes)</th>
<th>Manometer reading (mm)</th>
<th>Open arm (O.A.)</th>
<th>Closed arm (C.A.)</th>
<th>Difference (mm) (O.A.-C.A.)</th>
<th>Net change (mm) (ΔP_e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:24</td>
<td>0</td>
<td>471</td>
<td>725</td>
<td>-254</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3:25</td>
<td>1</td>
<td>472</td>
<td>725</td>
<td>-253</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>3:25</td>
<td>1</td>
<td>474</td>
<td>726</td>
<td>-252</td>
<td>+2</td>
<td></td>
</tr>
<tr>
<td>3:31</td>
<td>7</td>
<td>597</td>
<td>892</td>
<td>-295</td>
<td>-41</td>
<td></td>
</tr>
<tr>
<td>3:32</td>
<td>8</td>
<td>599</td>
<td>893</td>
<td>-294</td>
<td>-40</td>
<td></td>
</tr>
<tr>
<td>3:38</td>
<td>14</td>
<td>577</td>
<td>880</td>
<td>-303</td>
<td>-49</td>
<td></td>
</tr>
<tr>
<td>3:39</td>
<td>15</td>
<td>574</td>
<td>880</td>
<td>-306</td>
<td>-52</td>
<td></td>
</tr>
<tr>
<td>3:44</td>
<td>20</td>
<td>436</td>
<td>751</td>
<td>-315</td>
<td>-61</td>
<td></td>
</tr>
<tr>
<td>3:45</td>
<td>21</td>
<td>435</td>
<td>751</td>
<td>-316</td>
<td>-62</td>
<td></td>
</tr>
<tr>
<td>4:02</td>
<td>38</td>
<td>409</td>
<td>751</td>
<td>-342</td>
<td>-88</td>
<td></td>
</tr>
<tr>
<td>4:02</td>
<td>38</td>
<td>408</td>
<td>751</td>
<td>-343</td>
<td>-89</td>
<td></td>
</tr>
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<td>4:03</td>
<td>39</td>
<td>410</td>
<td>752</td>
<td>-342</td>
<td>-88</td>
<td></td>
</tr>
<tr>
<td>4:24</td>
<td>60</td>
<td>360</td>
<td>743</td>
<td>-383</td>
<td>-129</td>
<td></td>
</tr>
<tr>
<td>4:24</td>
<td>60</td>
<td>359</td>
<td>743</td>
<td>-384</td>
<td>-130</td>
<td></td>
</tr>
<tr>
<td>4:25</td>
<td>61</td>
<td>359</td>
<td>743</td>
<td>-384</td>
<td>-130</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4

SAMPLE EXPERIMENT: CONSUMPTION OF OXYGEN BY FEMALE ECHINOLAEELAPS ECHIDNINUS

Date - September 15, 1962
Diver No. 6

Thermobarometer

<table>
<thead>
<tr>
<th>Time (P.M.)</th>
<th>Interval (minutes)</th>
<th>Manometer reading(mm)</th>
<th>Net change(mm)</th>
<th>Net change(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Open arm (O.A.)</td>
<td>Closed arm (C.A.)</td>
<td>Difference (O.A.-C.A.)</td>
</tr>
<tr>
<td>3:15</td>
<td>0</td>
<td>542</td>
<td>668</td>
<td>-126</td>
</tr>
<tr>
<td>3:16</td>
<td>1</td>
<td>542</td>
<td>668</td>
<td>-126</td>
</tr>
<tr>
<td>3:16</td>
<td>1</td>
<td>541</td>
<td>668</td>
<td>-127</td>
</tr>
<tr>
<td>3:26</td>
<td>11</td>
<td>575</td>
<td>695</td>
<td>-120</td>
</tr>
<tr>
<td>3:27</td>
<td>12</td>
<td>577</td>
<td>696</td>
<td>-119</td>
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<tr>
<td>3:27</td>
<td>12</td>
<td>576</td>
<td>695</td>
<td>-119</td>
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<td>3:35</td>
<td>20</td>
<td>578</td>
<td>697</td>
<td>-119</td>
</tr>
<tr>
<td>3:36</td>
<td>21</td>
<td>578</td>
<td>697</td>
<td>-119</td>
</tr>
<tr>
<td>3:36</td>
<td>21</td>
<td>578</td>
<td>697</td>
<td>-119</td>
</tr>
<tr>
<td>3:47</td>
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</tr>
<tr>
<td>4:05</td>
<td>50</td>
<td>573</td>
<td>695</td>
<td>-122</td>
</tr>
<tr>
<td>4:06</td>
<td>51</td>
<td>573</td>
<td>695</td>
<td>-122</td>
</tr>
<tr>
<td>4:16</td>
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<td>-129</td>
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</tr>
<tr>
<td>4:26</td>
<td>71</td>
<td>571</td>
<td>696</td>
<td>-125</td>
</tr>
<tr>
<td>4:26</td>
<td>71</td>
<td>572</td>
<td>696</td>
<td>-124</td>
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<tr>
<td>4:27</td>
<td>72</td>
<td>572</td>
<td>696</td>
<td>-124</td>
</tr>
</tbody>
</table>
## TABLE 5

SAMPLE EXPERIMENT: TEMPERATURE, RELATIVE HUMIDITY, AND BAROMETRIC PRESSURE DATA

**Date - September 15, 1962**

<table>
<thead>
<tr>
<th>Time (P.M.)</th>
<th>Room Temperature °F</th>
<th>Room r.h. %</th>
<th>Barometric pressure (mm.Hg)</th>
<th>Temperature of Water bath °F</th>
<th>Water jacket °F</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:14</td>
<td>80</td>
<td>27.5</td>
<td>741.1</td>
<td>80.0</td>
<td>80.2</td>
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<td>-</td>
<td>80.0</td>
<td>80.2</td>
</tr>
<tr>
<td>3:36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>80.1</td>
<td>80.2</td>
</tr>
<tr>
<td>3:46</td>
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<tr>
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<td>-</td>
<td>740.5</td>
<td>79.95</td>
<td>80.3</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>80.0</td>
<td>80.3</td>
</tr>
<tr>
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<td>79.9</td>
<td>80.1</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>80.0</td>
<td>80.1</td>
</tr>
<tr>
<td>4:28</td>
<td>80</td>
<td>27.5</td>
<td>741.0</td>
<td>80.0</td>
<td>80.1</td>
</tr>
</tbody>
</table>
### TABLE 6

**SAMPLE EXPERIMENT: DATA ON WEIGHTS OF MITES AND VARIOUS DIVER SEALS**

Date - September 15, 1962

<table>
<thead>
<tr>
<th>Diver No.</th>
<th>Type of mite</th>
<th>Time (P.M.)</th>
<th>Weight (µg) in diver (observed) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$W_i$</td>
<td>$W_f$</td>
</tr>
<tr>
<td>4</td>
<td>Dehydrated</td>
<td>1:40</td>
<td>4:44</td>
</tr>
<tr>
<td>5</td>
<td>Hydrated</td>
<td>1:47</td>
<td>4:47</td>
</tr>
<tr>
<td>6</td>
<td>No mite</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(Thermobarometer)
EVALUATION OF THE EXPERIMENTAL METHODS

A. Standardization of Mites

The aim of standardization was to obtain starving mites in which the loss of weight due to egestion and excretion would be minimum. This was achieved by following the method described before. The guts of the fully fed mites became empty after 6 to 7 days in 90% and 95% r.h. at 80°F. The amount of blood in various diverticula of the gut just after feeding was variable from individual to individual (Figure 9). A mite with all the gut diverticula and the fore- and mid-guts filled with blood was assigned an arbitrary number 5. Since all the mites did not feed to a state to be rated as 5, minus signs were used for the incompletely fed ones. The degree of incomplete feeding could therefore be judged from the number of minuses a particular mite was assigned just after feeding. As the days of starvation progress, the amount of blood in the gut decreased due to continued digestion. The status of blood meal during successive days of starvation (Figures 10, 11) was again assigned arbitrary numbers, viz., 4, 3, 2, and 1, while a mite with a visibly empty gut was rated as 0 (Figure 11).
Figure 9. Diagrammatic representation of various stages of engorgement during feeding by the females of *E. echidninus*. Circles indicate the blood. See Figure 11 for legend.
Figure 9.
Figure 10. Diagrammatic representation of the different stages of digestion of blood in different parts of the gut of the females of E. echidninus during the period of starvation. Circles indicate blood. Cross-hatches indicate the digested blood meal. See Figure 11 for legend.
Figure 10.
Figure 11. The stage of starvation of female *E. echidninus* when no blood is visibly present in the gut

A = oesophagus
B = anterior caecum
C = anterior chamber of ventriculus
D = posterior dorsal caecum
E = posterior chamber of ventriculus
F = posterior ventral caecum
G = rectum
Figure 11.
The number of excretory pellets was found to be maximum during the first 24 hours but these gradually declined in successive days of starvation. The average number of days needed to attain 0 status at different r.h. varied from 3 to 7 days depending on the status of feeding (Table 7). In most cases it was between 5 to 7 days.

**TABLE 7**

**EFFECT OF RELATIVE HUMIDITY ON DIGESTION IN FEMALE E. ECHIDNINUS**

<table>
<thead>
<tr>
<th>State of gut</th>
<th>Average days taken to reach 0-stage in % r.h.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% r.h. 70 80 85 90 95</td>
</tr>
<tr>
<td>5 ---</td>
<td>Dead on 2nd day</td>
</tr>
<tr>
<td>5 --</td>
<td>Most of these died on 2nd day</td>
</tr>
<tr>
<td>5 -</td>
<td>Most of these died before 0</td>
</tr>
<tr>
<td></td>
<td>7 to 8 5 to 6 6 to 7 5 to 7 6 to 7</td>
</tr>
</tbody>
</table>

*Ten mites were used for each determination.*
B. Measurements with the Diver Set-up

In experimental science the significance and interpretation of any numerical data depends on the accuracy of the method used to obtain such data. The results are of little value unless the magnitude of error is pointed out for every step. As such the following evaluations are of importance insofar as they bring out the limits of accuracy of the methods employed in the present investigation.

The measurements with the Cartesian diver can be influenced by errors from two sources, viz., (1) errors brought about by factors other than the ones under investigation in the measurement of the change in equilibrium pressure, and (2) errors in determination of the true diver constant.

The former category includes the factors such as the escape of gas from the diver under a diffusion gradient, the capillary forces, the fluctuation of temperature in the diver system, and the changes in atmospheric pressure. Carlsberg workers have estimated the magnitude of error that could enter due to the operation of the above factors (Linderstrom-Lang and Holter 1942; Linderstrom-Lang 1943; Holter 1961). From their exhaustive and theoretical analysis of various diver systems based on practical measurements, it is evident that the errors introduced by the above factors, except the variation in temperature, can be disregarded.
1. Loss of Gas Other than Water Vapor

Diffusive loss of gas, except water vapor, from the charged diver is very small. For example, the loss of oxygen from a diver with a neck area $0.4 \text{ mm}^2$ and a $4 \text{ mm}$ mouth seal (Holter's medium) without a glass stopper is $0.0008 \mu l$ per hour (Linderstrom-Lang 1943). The consumption of oxygen by an individual mite is in the order of magnitude of $0.1 \mu l$ per hour. The neck area of the present divers was about $0.8 \text{ mm}^2$ and the mouth seal (the medium that entered into the neck of the diver due to capillarity) was about 1 millimeter. However, in view of the above rate of diffusive loss of oxygen from that particular diver system, the error introduced in the measurement of the change in equilibrium pressure by such a loss of oxygen in the present investigation is very slight and is therefore neglected.

2. Loss of Water Vapor

The loss of water vapor from the charged divers is of importance not only from the standpoint of the measurement of change in equilibrium pressure, but also in the maintenance of a constant high humidity atmosphere for the mites. Considerable quantities of water vapor are transported through the oil seal by the pressure gradient formed because of the difference in vapor pressure of the NaOH seal and that of the medium. ($\Pi_n - \Pi_m$, where $\Pi_n$ and $\Pi_m$ are the vapor measures of NaOH in the diver and flotation medium, respectively.)
The following calculations give the magnitude of the amount of change in the diver constant \( (V) \) and its effect on equilibrium pressure \( (P) \) due to the loss of equivalent quantity of fluid water corresponding to the amount of vapor lost. This calculation is based on the equation given by Linderstrom-Lang (1943)

\[
\frac{dV}{dt} = \frac{\rho_m - \rho_n}{\rho_m} \times \frac{18.02}{22410} \times \frac{273}{273 + t^0} \times \frac{A' \varepsilon' H_2O}{L_D} (\Pi_n - \Pi_m)
\]

where, \( \frac{dV}{dt} \) is the change in diver constant per hour. \( \rho_m \) and \( \rho_n \) are the densities of flotation medium and NaOH solution in the diver, respectively; \( t^0 \) is the temperature of the diver system in °C, \( A' \) is the cross sectional area of the oil seal, \( L_D \) is the effective diffusive length of the oil seal, and \( \varepsilon' H_2O \) is the standard rate of passage of water vapor through the oil. (Standard rate of passage of any gas is defined as the gas volume in µl at 760 mm.Hg and the temperature of the system \( (t^0) \) which in 1 hour diffuses through a column with an area 1 mm² and length 1 mm, the pressure fall along the column being 1 atmosphere (Linderstrom-Lang and Holter 1942).)

Introducing \( \rho_m = 1.245, \rho_n = 1.0, t^0 = 26.7° \text{ C}. \), \( \varepsilon' H_2O = 3.0 \mu l/hr \), \( A' = 0.785 \text{ mm}^2 \), \( L_D = 1.0 \text{ mm} \) (average), \( \Pi_n - \Pi_m = 0.025 \text{ atmosphere} \) (\( \Pi_m \) is taken to be 6 mm Hg and \( \Pi_n, 25 \text{ mm Hg} \)), it was found that \( \frac{dV}{dt} = 0.85 \times 10^{-5} \mu l \text{ per hour} \).
The corresponding change in equilibrium pressure, \( P \), is

\[
\frac{dP}{dt} = \frac{P}{V} \frac{dV}{dt} \quad \text{(Linderstrom-Lang (1943))}
\]

for \( V = 16 \mu l \) (the minimum diver constant in this case)

and \( P = 10367 \text{ mm.H}_2\text{O} \)

\[
\frac{dP}{dt} = 0.07 \text{ mm H}_2\text{O per hour}
\]

The volume of fluid water removed (\( dV_w \)) is

\[
\frac{dV_w}{dt} = \frac{S_m}{S_m - S_n} \times \frac{dV}{dt} \quad \text{(Linderstrom-Lang 1943)}
\]

Substituting the above value for \( \frac{dV}{dt} \),

\[
\frac{dV_w}{dt} = 4.3 \times 10^{-5} \mu l \text{ per hour.}
\]

In 4 hours (the maximum period of confinement of mites in the diver) \( 17.2 \times 10^{-5} \mu l \) of liquid water will be lost from the diver, a quantity which is negligible in comparison to 2\( \mu l \) of NaOH used in the seal.

3. Effect of the Loss of Water on the Concentration of Sodium hydroxide solution

The concentration of NaOH solution in the diver plays two important roles: (a) maintaining a high humidity atmosphere in the diver for the mite, and (b) absorption of CO2. In order to fulfill the former criterion, the concentration of NaOH in the solution should be low. However, the concentration should not be so low as to fail to absorb CO2.
completely from the diver. Linderstrom-Lang (1943) has pointed out that the average concentration of NaOH in the seal should not fall below 0.1 N (=0.4%). Thus, the upper limit for the concentration of NaOH solution was fixed by the criterion of maintaining a high humid atmosphere while the lower limit was 0.4%. Preliminary experiments using a diver with a dehydrated mite confined in it showed that the mite gained weight when 1% NaOH solution was used as the seal.

**Maintaining high relative humidity.** A NaOH solution of 1% maintains about 98% r.h. at 25° C. (extrapolated from the data given by Lange 1961). As such, this concentration was used in the NaOH seal in all the experiments. However, the possibility still exists that the initial concentration of NaOH may go up as a result of loss of water from the charged diver and hence the r.h. in the air surrounding the mite might fall. As has been shown above, the rate of loss of water from the NaOH seal to the medium is $4.3 \times 10^{-5}$ μl per hour so that in 4 hours only 0.00017 μl will be lost from the diver. This amount in comparison to the average volume of NaOH solution used in the diver (2 μl) is too small to bring about a significant change in concentration of the NaOH solution. To bring the concentration up to 5.5%, more than 1.7 μl of water would have to be taken out from 2 μl of NaOH solution so that the r.h. would fall below 95%. Therefore, a fall in r.h. due to rise in concentration
of NaOH in the present case is not serious. Furthermore, the fact that the dehydrated mites on the average weighed more after the experiment is evidence that the r.h. in the air surrounding the mite in the diver was high.

In order to meet the requirement of providing an atmosphere of high relative humidity in the diver, the NaOH solution would have to give out water to the air space in the diver. Since this would occur only by evaporation and distribution of water vapor molecules in the air space, the factors limiting the rate of evaporation would be the controlling agents in determining the time required for the air space in the diver to come to a required humidity. The NaOH solution in the neck (neglecting the NaOH solution in the polyethylene plug) was assumed to have a cylindrical shape. The rate of evaporation of water from such a cylindrical column can be calculated from the following expression:

\[ V = \frac{A\Delta}{h} \log_e \left( \frac{P-P_0}{P-P_s} \right) \]  
(Egerton 1929)

where, 
\( V \) = rate of evaporation (volume per unit of time)  
\( A \) = area of the cross section of the tube  
\( h \) = distance from upper end to the surface  
(in this case it is taken as 1 mm)  
\( \Delta \) = diffusion coefficient (in the case of water vapor in air = 0.239 cm² per second at 80°C.)  
(Handbook of Chemistry & Physics, 1958-59)
\[ P = \text{total gas pressure} \]

\[ P_0 = \text{pressure of vapor in gas at distance from the surface} \]

\[ P_s = \text{saturation pressure at surface of the liquid} \]

Hence, the above expression becomes

\[ V = 18.76 \log_e \frac{P - P_0}{P - P_s} \]

The last term in the above expression is minor compared to the first term and if it is neglected then

\[ V = 18.76 \text{ mm}^3 \text{ per second}. \]

Assuming the air space of the diver had a relative humidity of 30% when the neck seals were put in place, it would take only 3.6 seconds to bring an air volume of 10 \( \mu l \) from 30% to 95% r.h. The corresponding amount of liquid water that has to be removed from the NaOH solution to bring 10 \( \mu l \) of air from 30% to 95% r.h. can also be calculated by taking into consideration the density of water vapor and liquid water at that temperature. From this calculation it was found that the amount of liquid water necessary to raise the r.h. from 30% to 95% at 80 F. is about \( 0.57 \times 10^{-8} \mu l \).

The above calculations, although approximate, show the magnitude of the quantities involved. In view of the short time and the corresponding amount of liquid water to be removed to provide the required r.h. in the air surrounding the mite in the diver, their effects have been disregarded in the present discussion.
Furthermore, when \( \text{CO}_2 \) is absorbed by the \( \text{NaOH} \) solution, it produces not only sodium bicarbonate (\( \text{NaHCO}_3 \)) but also \( \text{H}^+ \) and \( \text{HCO}_3^- \) ions in accordance with the following equation:

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-.
\]

These ions as particles affect the osmotic pressure of the \( \text{NaOH} \) solution. Since vapor pressure, like osmotic pressure, is a colligative property, a change in osmotic pressure will affect the vapor pressure and consequently the r.h. The following calculations give the magnitude of these ions to be encountered in the present system.

As \( \text{CO}_2 \) is absorbed by the \( \text{NaOH} \) solution, more and more bicarbonate is formed. In the presence of bicarbonate and \( \text{CO}_2 \), most of the \( \text{Na}^+ \) ions are associated with bicarbonate but some carbonate does always exist. The \( \text{CO}_2 \) or acid produced will combine with carbonate and convert it to bicarbonate. Since this reaction releases no gas, the \( \text{CO}_2 \) or acid involved will escape manometric estimation. The following equations given by Umbreit et al. (1959) show the reactions and dissociation constants (\( K_1 \) and \( K_2 \) involved):

\[
\frac{(\text{H}^+)(\text{HCO}_3^-)}{\text{H}_2\text{CO}_3} = \frac{(\text{H}^+)(\text{HCO}_3^-)}{\text{CO}_2} = K_1 = 3 \times 10^{-7}
\]

\[
\frac{(\text{H}^+)(\text{CO}_3^{2-})}{\text{HCO}_3^-} = K_2 = 6 \times 10^{-11}
\]

\[
K_1/K_2 = K = \frac{(\text{H}^+)(\text{HCO}_3^-)}{\text{CO}_2} / \frac{(\text{H}^+)(\text{CO}_3^{2-})}{\text{HCO}_3^-} = (\text{HCO}_3^-)^2/(\text{CO}_2)(\text{CO}_3^{2-})
\]
Thus \( K = \frac{3 \times 10^{-7}}{6 \times 10^{-11}} = 5000 \)

Therefore the concentration of carbonate is, \( \text{CO}^- = \frac{(\text{HCO}_3^-)^2}{\text{CO}_2 \times K} \)

Assuming an R. Q. of 1, a mite gives off approximately 0.17 \( \mu \)l of \( \text{CO}_2 \) per hour. In 2 hours the amount of \( \text{CO}_2 \) given off is 0.34 \( \mu \)l. This amount of \( \text{CO}_2 \) is 0.34/22.4 or 0.015 micromoles. If all of this is converted to sodium bicarbonate, then at the end of 2 hours 2 \( \mu \)l of NaOH solution in the diver will have a sodium bicarbonate concentration of 0.015 micromoles (0.15 \( \times \) 10^{-7} moles) or 0.75 \( \times \) 10^{-8} moles per \( \mu \)l of NaOH solution. Therefore, the concentration of sodium bicarbonate in the NaOH solution in the diver at the end of 2 hours is 0.75 \( \times \) 10^{-2} molar or 0.0075 molar.

Using the above expression, Umbreit \textit{et al.} (1959) have pointed out that 5% \( \text{CO}_2 \) with a bicarbonate concentration of 0.01 molar at 25°C, the concentration of carbonate (CO\(_3\^-\)) is 1.32 \( \times \) 10^{-5} moles per liter. If the initial concentration of sodium bicarbonate is taken as 0.0075 molar, then, using the above expression, the concentration of CO\(_3\^-\) is found to be \( \frac{(0.0075)^2}{5000} = \frac{0.05625}{5} = 0.01125 \)

\( = 1.125 \times 10^{-2} \) moles per liter = 1.125 \( \times \) 10^{-8} moles per \( \mu \)l or about 1.13 \( \times \) 10^{-8} moles per \( \mu \)l, which is a very small quantity. In the above calculation the amount of \( \text{CO}_2 \) in the gas phase of the diver at any time has been neglected because of the fact that the amount of \( \text{CO}_2 \) produced is very
small and it is absorbed by the NaOH solution as soon as it is released to the gas phase.

The increase in osmotic pressure due to the above number of particles is very small and therefore its effect on the decrease in vapor pressure and r.h. in the diver is also negligible.

The number of H⁺ ion in the NaOH solution, which may be figured from the equations for dissociation constants given above, is also small and its effect on r.h. in the diver is also minor.

Absorption of CO₂. The efficiency of the NaOH seal in absorbing carbon dioxide needs further scrutiny. If the carbon dioxide is not absorbed as soon as it is released to the gas phase or if the NaOH fails to absorb all the CO₂ given off by the mite, then the measurements based on the changes in equilibrium pressure will not reflect the pressure changes due to the consumption of oxygen by the mite. The rapidity with which the NaOH seal will take up CO₂ from the gas phase will depend upon the rate with which hydroxyl ions are carried to the surface and carbonate ions are removed from the surface of the seal. Since these processes are brought about by diffusion, the concentration of NaOH should be in excess of the amount needed for the absorption of CO₂ released. Following the method given by Linderstrom-Lang (1943) for calculating the minimum concentration of the NaOH solution required for the complete absorption of CO₂,
it was found that 0.4% of NaOH solution would be sufficient to absorb all of the CO₂ given off by a mite in 4 hours (assuming R. Q. of the mite is 1, about 0.17 μl of O₂ is used per hour). Hence, it is evident that there was more than two times the concentration of NaOH present in the seal than was required for all the CO₂ evolved.

4. Effect of Uptake or Release of Water by the Mite on the Concentration of NaOH solution

Aside from the loss by diffusion, water vapor taken up by the dehydrated mite is also ultimately removed from the NaOH solution in the diver. The following evaluation is aimed to give a magnitude of such a loss and vice versa for the dehydrated mite. The dehydrated mites gain on the average of 1.0 μg per hour. If this gain in weight is all due to the absorption of water from the surrounding air, then in 4 hours they absorb about 4.0 μg. The corresponding volume for liquid water is 0.004 μl. Since this removal of water from NaOH is not a loss from the charged diver, it will have no effect on the measurements of equilibrium pressure. However, from the point of view of change in concentration of NaOH solution and hence on r.h. in the diver, this loss of water due to uptake by the mite needs explanation. From the foregoing calculations it has been found that 0.00017 μl of liquid water is removed from the NaOH solution to the medium by diffusion. To this quantity should
be added the amount lost caused by the uptake of water by the dehydrated mite. Thus, the total amount of liquid water removed from the NaOH seal in 4 hours is 0.00417 μl. As mentioned before, more than 1.7 μl of water must be taken from about 2 μl of a 1% NaOH solution in the diver so that the resulting rise in concentration might bring the relative humidity down to 95%. The total amount of water lost in 4 hours is only about 400th of 1.7 μl and is therefore neglected.

Water given off by the hydrated mite will decrease the concentration of NaOH solution. Hence, it will increase the r.h. in the air surrounding the mite. Since the loss in weight of the hydrated mites is approximately equal to the gain in weight by the dehydrated mites, the amount of liquid water added to the NaOH is also of the same order of magnitude. Therefore, if 0.004 μl is added to 2 μl of NaOH solution in 4 hours, the concentration will decrease about 0.2%. As has been pointed out above, the minimum concentration of NaOH solution required to absorb all the CO₂ liberated by the mite in 4 hours is 0.4%. Hence, it is obvious that a decrease of 0.2% in concentration of 1% NaOH solution will not substantially affect the absorption of CO₂ from the diver.
5. Effect of Variations in Temperature

The significant factors that contribute to the error introduced into the determination of equilibrium pressure \( P \) by changes in the temperature of the diver system during the experiment are the change in gas volume and the change in the diver constant (Holter 1961). The upper limit of temperature variation permissible can be calculated from the expression: \( \delta P = 4.5 \delta t^0 \) (Linderstrom-Lang 1943; Holter 1961). In the present case, \( \delta P = 0.3 \, \text{cm H}_2\text{O} \)
\[ \delta t^0 = 0.3/4.5 = 0.07^\circ \text{C.} = 0.13^\circ \text{F}. \]
The average fluctuation in temperature noted was \( \pm 0.5^\circ \text{F} \). Hence, the error introduced in measurement of equilibrium pressure due to this fluctuation in \( t^0 \) seems to be significant and should, therefore, be accounted for.

According to Linderstrom-Lang (1943) and Holter (1961), \( \Delta V/\Delta t^0 \) is nearly proportional to \( V \) (because the weight of the diver is nearly proportional to the volume of gas in the diver \( V \)), \( \delta P \) is almost completely independent of \( V \). This makes it easy to correct for temperature changes by running control divers along with the experiment. As such the variations in \( \Delta P_e \) was corrected by subtracting \( \Delta P_c \) from \( \Delta P_e \) for each measurement (see calculations above).
6. Effect of Changes in Atmospheric Pressure

Since the manometer was open to the air, the variation in barometric pressure \( (P_B) \) would affect the measurement of \( P \). However, this effect can be neglected in view of the fact that \( P_B \) appears in small terms in the diver equations (Linderstrom-Lang 1943). Any change in \( P_B \) would affect the change in equilibrium pressure for the thermobarometer as well as the experimental divers. The magnitude of this effect will depend on \( V_c \) and \( V_e \). Thus, the correction of the change in equilibrium pressure for the experimental \( (\Delta P_e) \) from the corresponding pressure change for the thermobarometer \( (\Delta P_c) \) in proportion to their respective gas volumes \( V_e \) and \( V_c \) (see Calculations) would eliminate the error.

7. Effect of capillary forces

Capillary forces tend to deform the menisci and delay the movement of menisci to their new positions when the diver is subjected to changes in pressure. However, the influences of such forces are rather small and still less when a surface active substance (like sodium taurocholate) is added to the liquid (Holter 1961). The variation in equilibrium pressure due to the sluggish movement of menisci generally fades out after some minutes so that a couple of measurements are sufficient to obtain a fairly good value of equilibrium pressure (Linderstrom-Lang 1943).
The medium seal, in the present case, contained 0.1% sodium taurocholate so that moistening of the glass wall of the diver by the medium was increased. However, oil and NaOH moisten the wall so well that significant systematic errors of the type may be excluded (Linderstrom-Lang 1943). In view of the above considerations and the fact that the divers used in the present experiment were thoroughly cleaned and dried beforehand and hence no hydrophobic effect could bring about variations, the error introduced due to capillary forces is minor and negligible.

8. The Diver Constant

Since the diver constant has a great bearing on the measurement of equilibrium pressure (P) and contributes significantly to the calculation for the consumption of oxygen (see the diver equation above), the accuracy with which it is determined would reflect the over-all accuracy of the results. In the following evaluation, the diver constant for the experimental diver (Ve) and for the thermo-barometer (Vc) are combined under one term and is represented by "V."

V, in the present investigation, was determined in a way similar to that of Boell, Needham and Rogers (1939). Carlsberg workers have pointed out that the diver constant obtained in the above way (V') is different from the true diver constant (V) because the temperature (t') and
pressure \( (P') \) under which the diver is charged are different from that of the actual experiment. The two diver constants can be related by the following expression (Holter 1961)

\[
V = V' \frac{P'}{P} \times \frac{273 + t^0}{273 + t^1}
\]

where, \( P \) is the equilibrium pressure and \( t^0 \) is the temperature \((^\circ C.)\) of the diver system. In the present investigation \( t^0 \) and \( t^1 \) were kept almost identical (see Materials and Methods). Hence, the error introduced by the last term in the above equation is negligible. However, the difference between \( P \) and \( P' \) needs to be accounted for if \( V \) and \( V' \) are to be taken as identical.

The equilibrium pressure \( (P) \) of the diver is the sum of several pressures, viz., the barometric pressure \( (P_B) \), the pressure difference in the manometer \( (P_O) \), the hydrostatic pressure of the column of the medium from the top to the neck of the diver when the diver is in line with the fiduciary line \( (h_m \cdot \rho_m) \) and the pressure due to the medium entering into the neck of the diver by capillarity \( (\delta \cdot \rho_m) \).

Hence, \( P = P_B + h_m \cdot \rho_m + P_O + \delta \cdot \rho_m \) (Linderstrom-Lang 1943) where, \( h_m \) and \( \rho_m \) are the height and the density of the medium respectively and \( \delta \) is the height of rise of the medium in the neck of the diver due to capillary forces. \( P_B \), in the present case, was \( \sim 960 \) cm. \( H_2O \), \( h_m \) was \( \sim 4.3 \) cm; hence, \( h_m \cdot \rho_m = 5.6 \) cm. Putting \( P_O \) as \( -30 \) cm. \( H_2O \) on the
average and neglecting the last term in the above expression
\[(6 \cdot \mathfrak{f}_m) \frac{P_1}{P} = 1.025.\] For the smallest diver constant, which was 16 µl in the present case, the error introduced in the calculation of diver constant due to the above difference between \(P\) and \(P_1\) was 2.5%.

Since this was an error which entered into the divers used for dehydrated and hydrated mites all through the experiment and since the divers were used at random, it was cancelled out while comparing the oxygen consumption of the two groups of mites.

9. Error in the Measurement of Equilibrium Pressure (\(P\))

Aside from the errors introduced by other sources into the measurement of \(P\), there was a degree of variation in the readings for \(P\). As described above (Materials and Methods), the reading of \(P\) was taken at the point where the size of the elliptical reflection of the fiduciary line on the lining cone was at its minimum. Since this was an eye estimation, there was a variation in determining the minimum size of the reflection and hence in the determination of \(P\). All the readings for \(P\) were taken when the reflections were approximately of the minimum size shown in Table 8. The spread in the difference for the minimum sized reflection ranged from -203 to -206 mm. Hence, the accuracy of \(\Delta P\) in the present investigation was approximately
TABLE 8
VARIATION IN EQUILIBRIUM PRESSURE

<table>
<thead>
<tr>
<th>Schematic diameter of the reflection in lining cone (mm)</th>
<th>Height of H₂O in manometer (mm)</th>
<th>Difference (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open arm</td>
<td>Closed arm</td>
</tr>
<tr>
<td>20</td>
<td>353</td>
<td>559</td>
</tr>
<tr>
<td>10</td>
<td>352</td>
<td>555</td>
</tr>
<tr>
<td>5</td>
<td>349</td>
<td>553</td>
</tr>
<tr>
<td>1.5</td>
<td>346</td>
<td>550</td>
</tr>
<tr>
<td>0.5</td>
<td>343</td>
<td>547</td>
</tr>
<tr>
<td>20</td>
<td>334</td>
<td>537</td>
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<td>533</td>
</tr>
<tr>
<td>1.5</td>
<td>324</td>
<td>529</td>
</tr>
<tr>
<td>0.5</td>
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<td>528</td>
</tr>
<tr>
<td>20</td>
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<td>519</td>
</tr>
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<td>10</td>
<td>310</td>
<td>515</td>
</tr>
<tr>
<td>5</td>
<td>311</td>
<td>515</td>
</tr>
<tr>
<td>1.5</td>
<td>309</td>
<td>513</td>
</tr>
<tr>
<td>0.5</td>
<td>304</td>
<td>507</td>
</tr>
</tbody>
</table>
+ 3 mm H$_2$O. However, in view of the large ΔP at the end of 1 hour of experimental duration, the error introduced by this fluctuation of + 3 mm is a minor one and considered negligible in the present case.

10. The sensitivity of the present diver system

In view of the small rate of oxygen consumption of the mite, the ability of the present measuring system becomes of importance in evaluating the results. The sensitivity of the present system was calculated by dividing the mean rate of consumption of oxygen by the mean net pressure change during a one-hour period. The average sensitivity, thus determined, was found to be $1.5 \times 10^{-3}$ μl/mm·H$_2$O, the minimum pressure change was 44 mm H$_2$O while the maximum was 180 mm H$_2$O. The average rate of oxygen consumption per mite is $165 \times 10^{-3}$ μl/hr (Table 10).

As has been pointed out before, there was a variation of about 3 mm·H$_2$O in the readings of the change in pressure in the manometer (Table 8). Thus a change of about $5 \times 10^{-3}$ μl in gas volume in the diver system would escape from observations. However, the total oxygen consumption of a mite in an hour is of the order of magnitude of $150 \times 10^{-3}$ μl which is 30 times greater than the minimum significant change in gas volume that could be detected by the present system.
11. The reproducibility of measurements with the present diver system

The reproducibility of the present diver system can be judged from the rates of oxygen consumption of the individual mites (Table 10) and also from the change in pressures in consecutive readings made at about the same time (Tables 2,3). Aside from influences of variations in temperature, barometric pressure, etc., the reproducibility of the readings with a given diver system under identical conditions will depend on the correctness of the judgment of whether or not the diver is in "equilibrium" position. This judgment with the present system was based on eye-estimation and the variation noted in the readings of the change in pressure was \( \pm 3 \text{ mm H}_2\text{O} \) (see Sample Experiment above). However, this variation of \( \pm 3 \text{ mm H}_2\text{O} \) is a minor one in comparison to the total change in pressure observed during the experiment in a one-hour period.

C. Loss of Weight by the Mite During Exposure to Room Conditions

The relative humidity of the room was very low when the present series of experiments were carried out. As such, the mites lost weight during the time their weights were taken and the diver was flushed with air (see Materials and Methods). To get an estimate of such a weight-loss, a group of dehydrated mites and another group of hydrated mites were weighed individually and treated in the same way as they
would have been if used in the diver experiment. The initial weights of the mites were taken, they were put in the diver, the diver flushed with air, the mites were taken out, and the final weights taken. The interval for each step was noted. From such an experiment, it was found that the dehydrated mites lost about 0.3% of their initial weight per minute whereas the hydrated mites lost about 0.5% of their initial weight per minute (Table 9). The room temperature varied from 79° to 81° F. while the relative humidity fluctuated from 28 to 35% during the period when the experiments were run. Since one-half of the above loss in weight occurred after the initial weight was taken and the other half of the loss occurred before the final weight was determined, they cancel each other when change in weight was calculated according to the following

\[ \Delta W = \left( W_f + \frac{W^I}{2} \right) - \left( W_i + \frac{W^I}{2} \right) \]

\[ = W_f - W_i \]

where, \( \Delta W \) is the change in weight of the mite

\( W_f \) is the final weight after the experiment

\( W_i \) is the initial weight

\( W^I \) is the loss in weight while the mite was exposed to room conditions
<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>CO₂</th>
<th>Initial Weight (µg)</th>
<th>Final Weight (µg)</th>
<th>Change in Weight (%) Initial wt.</th>
<th>Change in Weight (µg)</th>
<th>Weight Change (%) Initial wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dehydrated mites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1</td>
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<td>172.0</td>
<td>+0.6</td>
<td>+0.35</td>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
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<td>177.8</td>
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<td>+2.14</td>
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</tr>
<tr>
<td><strong>Hydrated mites</strong></td>
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<td></td>
<td></td>
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<tr>
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<td>-3.0</td>
<td></td>
</tr>
</tbody>
</table>
D. Conclusions from the Above Evaluations

The procedure of standardization produced mites in which the egestion and excretion were minimal.

The errors introduced from various sources into the measurement of the change in equilibrium pressure in the Cartesian diver manometer and hence into the calculation of the consumption of oxygen by the mite are in most cases minor and negligible. The variations in temperature of the diver system, the effect of capillary forces, the changes in barometric pressure, and the variation in the readings of equilibrium pressure were random and, therefore, should not introduce any systematic errors that might give a cumulative effect. A similar argument will apply for the change in equilibrium pressure of the diver due to the deposit of any excretory pellet by the mite in the diver. No pellet was, however, noticed in the diver after the experimental duration. The escape of gases from the charged diver was a rather one-way loss until a steady state was reached and therefore might have introduced positive errors in diver measurements. These errors have been disregarded mostly on the basis of their magnitude in comparison to the quantities involved in the measurements.

Furthermore, the errors introduced by the above-mentioned sources had an equivalent effect on the measurements of equilibrium pressures for hydrated and dehydrated
mites. Therefore, the errors must have balanced each other or have been reduced to a negligible minimum when the comparison is made between the rate of oxygen consumption of the hydrated and the dehydrated mites.
RESULTS

**Oxygen Consumption**

The volume of oxygen consumed by an individual mite was calculated according to the formula given above (see Calculations in Materials and Methods). The rate of oxygen consumption was calculated for each mite in each of the two groups in three ways, viz., volume of oxygen per hour per unit of dry weight \((W_d)\), volume of oxygen per hour per unit of initial live weight \((W_1)\), and volume of oxygen per hour per mite. The average rate of consumption of oxygen in the dehydrated mite is \(3.03 \times 10^{-3} \pm 0.707 \times 10^{-3}\) \(\mu l/hr/\mu g\) of \(W_d\) or \(1.05 \times 10^{-3} \pm 0.279 \times 10^{-3}\) \(\mu l/hr/\mu g\) of \(W_1\) or \(167.72\times 10^{-3} \pm 47.958 \times 10^{-3}\) \(\mu l/hr/mite\) (Table 10). Similarly, the average rate for the hydrated mite is \(2.83 \times 10^{-3} \pm 0.413 \times 10^{-3}\) \(\mu l/hr/\mu g\) of \(W_d\) or \(0.89 \times 10^{-3} \pm 0.446 \mu l/hr/\mu g\) of \(W_1\) or \(172.08 \pm 28.78 \mu l/hr/mite\) (Table 10). Since one dehydrated mite and one hydrated mite were used simultaneously in the experiment, the difference in the rate of consumption of oxygen was determined for each pair. The rate of oxygen consumption of the hydrated mite was subtracted from that of the dehydrated mite to get the difference in each pair. The differences were also calculated in each of the three ways mentioned above and the mean difference in
rates with standard errors was $+0.19 \times 10^{-3} \pm 0.68 \times 10^{-3}$
$\mu l/hr/\mu g$ of $W_d$, $+0.16 \times 10^{-3} \pm 0.29 \times 10^{-3}$ $\mu l/hr/\mu g$ of $W_1$
and $-4.35 \times 10^{-3} \pm 52.56 \times 10^{-3}$ $\mu l/hr/mite$. Obviously the
differences were not significant since the mean differences
were less than their standard errors.

The rates of oxygen consumption of the mites that were
caught in NaOH solution in the diver were abnormally high.
The rate was more than three times higher than the rate of
the same type of mite which was not caught in NaOH solution.
In one such case the rate of consumption of oxygen as cal-
culated from the change in equilibrium pressure ($\Delta P$) by a
hydrated mite was $523 \times 10^{-3}$ $\mu l/hr/mite$ whereas the dehy-
drated one belonging to the pair had the rate $164.7 \times 10^{-3}$
$\mu l/hr/mite$ (Fig. 12). Such higher rates of oxygen consump-
tion under this abnormal condition have been observed for
both hydrated and dehydrated mites.

**Weight Change ($\Delta W$)**

The change in weight of the mites during the period
they were in the diver should be taken into account in draw-
ing a balance sheet of movement of water into or out of the
mites. The average rate of gain of water ($=\Delta W$) by the
dehydrated mites is $1.0 \pm 0.53 \mu g/hr/mite$ or $0.62 \pm 0.33\%$ of
initial weight ($W_1$) per hour or $1.3 \pm 0.84\%$ of their dry
weight ($W_d$) per hour (Table 11). Similarly the average rate
of loss of water by the hydrated mites is $1.0 \pm 0.59 \mu g/hr/$
mite or $0.54 \pm 0.32\%$ of their $W_1$ per hour or $1.8 \pm 0.89$ of $W_d$ per hour (Table 11). The changes in weights by dehydrated and hydrated mites are significantly different from zero and are thus true changes due to the movement of water into or out of the mite.

The rate of loss of weight by the mites that were caught in NaOH solution in the diver during the experiment was 6 to 7 times higher than that of the normal mites. In one such case the rate was $7 \mu g/hr/mites$. Likewise, the rate of loss of weight of a mite that died during the experiment went as high as $14 \mu g/hr/mite$. 
Figure 12. Curve showing the increased rate of oxygen uptake by a struggling mite caught in NaOH solution in the diver during the experiment.
TABLE 10
RATE OF CONSUMPTION OF OXYGEN ($\Delta V_O^2$) BY STANDARDIZED DEHYDRATED AND HYDRATED FEMALES OF E. ECHIDNINUS

<table>
<thead>
<tr>
<th>Trial</th>
<th>$\Delta V_O^2 \times 10^3$</th>
<th>$\mu l/hr/\mu g$ of $W_d$</th>
<th>$\mu l/hr/\mu g$ of $W_i$</th>
<th>$\mu l/hr$/mite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dehydrated mites</td>
<td></td>
<td>Hydrated mites</td>
</tr>
<tr>
<td>1</td>
<td>2.65</td>
<td>0.82</td>
<td>116.60</td>
<td>131.34</td>
</tr>
<tr>
<td>2</td>
<td>2.25</td>
<td>0.77</td>
<td>135.00</td>
<td>104.88</td>
</tr>
<tr>
<td>3</td>
<td>2.96</td>
<td>0.99</td>
<td>148.00</td>
<td>315.98</td>
</tr>
<tr>
<td>4</td>
<td>2.54</td>
<td>0.73</td>
<td>111.76</td>
<td>129.12</td>
</tr>
<tr>
<td>5</td>
<td>2.75</td>
<td>0.93</td>
<td>143.00</td>
<td>236.16</td>
</tr>
<tr>
<td>6</td>
<td>2.30</td>
<td>0.87</td>
<td>133.40</td>
<td>224.00</td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>0.36</td>
<td>58.00</td>
<td>68.12</td>
</tr>
<tr>
<td>8</td>
<td>7.75</td>
<td>2.95</td>
<td>496.00</td>
<td>167.00</td>
</tr>
<tr>
<td>Mean</td>
<td>3.03</td>
<td>1.05</td>
<td>167.72</td>
<td></td>
</tr>
<tr>
<td>S.E.</td>
<td>$\pm 0.707$</td>
<td>$\pm 0.279$</td>
<td>$\pm 47.95$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hydrated mites</td>
</tr>
<tr>
<td>1</td>
<td>1.99</td>
<td>0.68</td>
<td>131.34</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.38</td>
<td>0.53</td>
<td>104.88</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.27</td>
<td>1.58</td>
<td>315.98</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.69</td>
<td>0.70</td>
<td>129.12</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.69</td>
<td>1.13</td>
<td>236.16</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.00</td>
<td>1.19</td>
<td>224.00</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.31</td>
<td>0.39</td>
<td>68.12</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.34</td>
<td>0.90</td>
<td>167.00</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.83</td>
<td>0.89</td>
<td>172.08</td>
<td></td>
</tr>
<tr>
<td>S.E.</td>
<td>$\pm 0.413$</td>
<td>$\pm 0.446$</td>
<td>$\pm 28.78$</td>
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</tr>
</tbody>
</table>
### TABLE 11

WEIGHTS AND WEIGHT CHANGES OF STANDARDIZED INDIVIDUAL FEMALES OF *E. ECHIDNINUS*

<table>
<thead>
<tr>
<th>Trial</th>
<th>(W_1) ((\mu)g)</th>
<th>(W_d) ((\mu)g)</th>
<th>(\Delta W) per hour</th>
<th>% of (W_1)</th>
<th>% of (W_d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>142</td>
<td>44</td>
<td>+0.67</td>
<td>+0.40</td>
<td>+1.5</td>
</tr>
<tr>
<td>2</td>
<td>176</td>
<td>60</td>
<td>+2.00</td>
<td>+1.13</td>
<td>+3.3</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>50</td>
<td>+0.67</td>
<td>+0.43</td>
<td>+1.3</td>
</tr>
<tr>
<td>4</td>
<td>154</td>
<td>44</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>154</td>
<td>52</td>
<td>+0.67</td>
<td>+0.43</td>
<td>+1.3</td>
</tr>
<tr>
<td>6</td>
<td>154</td>
<td>58</td>
<td>+2.50</td>
<td>+1.63</td>
<td>+4.3</td>
</tr>
<tr>
<td>7</td>
<td>162</td>
<td>58</td>
<td>+2.00</td>
<td>+1.23</td>
<td>+3.4</td>
</tr>
<tr>
<td>8</td>
<td>168</td>
<td>64</td>
<td>-0.50</td>
<td>-0.30</td>
<td>-0.8</td>
</tr>
<tr>
<td>Mean</td>
<td>158</td>
<td>54</td>
<td>+1.00</td>
<td>+0.62</td>
<td>+1.8</td>
</tr>
<tr>
<td>S.E.</td>
<td>±3.8</td>
<td>±2.6</td>
<td>±0.53</td>
<td>±0.33</td>
<td>±0.84</td>
</tr>
</tbody>
</table>

**Hydrated mites**

<table>
<thead>
<tr>
<th>Trial</th>
<th>(W_1) ((\mu)g)</th>
<th>(W_d) ((\mu)g)</th>
<th>(\Delta W) per hour</th>
<th>% of (W_1)</th>
<th>% of (W_d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>192</td>
<td>66</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>198</td>
<td>76</td>
<td>-0.67</td>
<td>-0.33</td>
<td>-0.9</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>74</td>
<td>-2.00</td>
<td>-1.00</td>
<td>-2.7</td>
</tr>
<tr>
<td>4</td>
<td>184</td>
<td>48</td>
<td>-2.00</td>
<td>-1.10</td>
<td>-4.2</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>64</td>
<td>+0.67</td>
<td>+0.33</td>
<td>+1.1</td>
</tr>
<tr>
<td>6</td>
<td>188</td>
<td>56</td>
<td>+0.50</td>
<td>+0.28</td>
<td>+0.9</td>
</tr>
<tr>
<td>7</td>
<td>176</td>
<td>52</td>
<td>-2.00</td>
<td>-1.15</td>
<td>-3.8</td>
</tr>
<tr>
<td>8</td>
<td>186</td>
<td>50</td>
<td>-2.50</td>
<td>-1.35</td>
<td>-5.0</td>
</tr>
<tr>
<td>Mean</td>
<td>191</td>
<td>61</td>
<td>-1.00</td>
<td>-0.54</td>
<td>-1.8</td>
</tr>
<tr>
<td>S.E.</td>
<td>±3.1</td>
<td>±3.8</td>
<td>±0.59</td>
<td>±0.32</td>
<td>±0.89</td>
</tr>
</tbody>
</table>

**Symbols:**
- \(W_1\) = initial weight of the mite.
- \(W_d\) = dry weight of the mite.
- \(\Delta W\) = change in weight.
- + and - signs indicate gain and loss in weight respectively.

*Range of change in weight is calculated from the change in weight during the period of confinement in the diver.*
DISCUSSION

Previous work on the water balance of starved predesiccated females of *E. echidninus* has shown that the mites lose weight when exposed to 80% relative humidity (r.h.) but that they can maintain water balance at 90% r.h. or above (Wharton and Kanungo 1962). It has been shown that when these mites are exposed to a r.h. of over 90%, they gain weight for a period of about 24 hours, attain a peak and then drop down a little after which they stay at an equilibrium level called "equilibrium weight." That these changes in weight are the result of the movement of water into and out of the mite has also been indicated in the above work. From their study, Wharton and Kanungo (1962) have concluded that 90% r.h. is the "equilibrium humidity" for standardized females of *E. echidninus*. The equilibrium humidity is defined as the lowest relative humidity at which the organism achieves an equilibrium weight.

Any relative humidity below the equilibrium humidity is called a "desiccating humidity" whereas any above the equilibrium humidity is referred to as a "hydrating humidity." In the present discussion, the standardized mites that were dehydrated in a desiccating humidity but were not exposed to equilibrium or hydrating humidities are referred to as
"dehydrated mites." Likewise, the standardized, dehydrated mites that have been exposed to a hydrating humidity for a period sufficient to elevate their weights above their equilibrium weights are designated as "hydrated mites."

That the gain in weight of a dehydrated mite in a hydrating humidity is caused by the absorption of water vapor against a vapor pressure gradient has already been established for this mite (Wharton and Kanungo 1962; Kanungo, in press). The hypothesis that has been advanced by these authors is that the mechanism by which these mites absorb water from unsaturated air operates with greater intensity in dehydrated mites, gradually diminishes as the mite gets hydrated, and is minimal, or ceases completely, when the mite is at the peak of hydration. If this hypothesis be true, then the dehydrated mites would be expected to do more physiological work in a hydrating humidity than the hydrated ones in the hydrating atmosphere. Therefore, in a hydrating atmosphere the expected rate of consumption of oxygen for a hydrated mite would be greater than that of a hydrated mite. Dehydrated and hydrated mites were therefore chosen for the present investigation so that the dehydrated mite would be at the base of the time-weight curve whereas the hydrated mites would be at the peak. Furthermore, if the above hypothesis is true, then the dehydrated mites would gain weight while the hydrated ones would lose during the experiment. It is evident from the results that were obtained that
the changes in weight of each kind of experimental mite followed the expected pattern: The dehydrated mites, on the average, gained 0.62% of their initial weight per hour, whereas the hydrated ones, on the average, lost 0.54% of their initial weight per hour during the experiment.

Absorption of water vapor by dehydrated females of *E. echidninus* in a hydrating air is known to occur against a vapor pressure gradient. Dehydrated mites, therefore, need extra energy to perform this osmotic work. The energy required can be calculated thermodynamically from the expression:

\[ -G = nRT \ln \frac{P_0}{P_1} \]

where, \(-G\) = the change in free energy

\(n\) = the amount of material (in this case water) transported in moles

\(R\) = the gas constant

\(T\) = absolute temperature

\(P_1\) and \(P_0\) are the vapor pressures of inside (haemolymph of the mite) and outside (air with 98% r.h.) respectively

The vapor pressure of the haemolymph of the mite is very close to the vapor pressure of the saturated air at 80°F. Thus, putting \(P_1 = 0.033\) atmospheres, \(P_0 = 0.034\) atmospheres and \(T = 299.7^\circ\), it is found that \(-G\) in the above expression is 17.56 calories per mole of water or \(0.98 \times 10^{-6}\) calories per \(\mu g\) of water transported. A
dehydrated mite, takes up, on the average, \(19 \times 10^{-3} \mu g\) \(H_2O/hr/\mu g\) of dry weight (Table 11). The energy necessary to transport the above amount of water is thus \(17.64 \times 10^{-9}\) \((0.98 \times 10^{-6} \times 18 \times 10^{-3})\) calories. One milliliter of oxygen consumed corresponds approximately to five calories (Bayliss 1960). Hence, the rate of oxygen consumption that will produce \(17.64 \times 10^{-9}\) calories per hour is about \(3.53 \times 10^{-6} \mu l/hr\). The rate of oxygen consumption of a dehydrated mite is about \(3 \times 10^{-3} \mu l/hr/\mu g\) of dry weight. Thus, the amount of oxygen needed to supply energy for the active absorption of water is very small compared to the amount of oxygen taken in by the mite. Any difference that might have existed in the rates of oxygen consumption between hydrated and dehydrated groups of mites is masked by large variations in the above rates within the groups and between the groups (see Results).

The higher rate of uptake of oxygen by the mites that were caught in NaOH solution in the diver needs to be evaluated against the respiratory rates of the normal mites. The mites that were caught in NaOH were struggling, and as a result their motor activity was higher than the normal ones. The normal ones were either quiescent or with less activity. An increased rate of oxygen consumption with an increase in activity of the organism is a well known physiological principle.
The higher rate of oxygen uptake in struggling mites indicates that the normal experimental mites did not consume oxygen to their maximum ability. It is, however, difficult to say that the observed respiratory rate, in the case of normal mites, was an indicator of "basal" metabolism. The mites were found moving in the diver. This itself suggests that the "basal" conditions were not attained by the normal mites in the diver although the activity was less. The term "standard" metabolism has been referred to oxygen consumption measured with minimal motor activity (Prosser and Brown 1962). According to Cook (1949), "standard" metabolism envisages a condition wherein the animal shows physical activity, but the degree or level of activity is not excessive and is reproducible. The results obtained in the present case show that the measurements of oxygen uptake are reproducible provided the conditions are the same as they were in the present experiments. Furthermore, it is evident that the rate of oxygen consumption is at a lower level in normal mites when it is contrasted against the oxygen uptake of struggling mites. It can, therefore, be concluded that the oxygen consumption observed in the present case was an index of "standard" metabolism for the normal mites.

As has been pointed out, the changes in weight followed an expected pattern in the two kinds of experimental mites: The dehydrated ones, on the average, gained weight while the hydrated ones lost weight during the experiment.
The validity of equating the observed weight changes with the changes in water content of standardized females of *E. echidninus* has been stressed by Wharton and Kanungo (1962). The death of a mite in hydrating air results in a comparatively high loss of weight has also been indicated by the above workers. Aside from the loss of weight by the hydrated mites and by the mites that died during the experiment, the excessive loss of weight by those that were caught in NaOH solution in the diver needs to be explained here. This loss could be due to the increased loss of water by transpiration resulting from the increased activity of the mite, or the concentration of NaOH solution might be hypertonic with respect to the haemolymph of the mite in which case the water might be drawn out of the mite when the latter was caught in NaOH solution. The osmotic pressure of 1% NaOH solution is about 8 atmospheres. The osmotic pressure of the blood of the mite, although unknown, is probably not greater than 10 atmospheres (Wharton and Kanungo 1962). Thus, there is not a great difference between the osmotic pressure of NaOH solution in the diver and the osmotic pressure of the blood of a mite. The increased transpirational loss of water is responsible for the excessive loss of weight by the struggling mites is therefore indicated.

It should be pointed out that the higher rate of consumption of oxygen and the higher rate of loss of weight
by the struggling mites could be determined because individual mites were used for such determinations. This would have been otherwise obscured if groups of mites had been used as was done in the case of spider mites (McEnroe 1961) and flour mites (Hughes 1943).
SUMMARY

The rate of consumption of oxygen by the standardized females of *Echinolaelaps echidninus* was studied using the techniques of the Cartesian diver. The procedure of standardization yielded mites that had no visible food materials in the gut. The mites used in the present investigation were of two different types as regards to their body water content. One group, the "dehydrated" mites, was exposed to a desiccating atmosphere with 80\% relative humidity at 80\° F. for 24 hours while the others, the "hydrated" mites, after the above period of desiccation, was exposed to hydrating atmosphere of 95\% r.h. at 80\° F. for about 24 hours. The dehydrated group was on the negative side of their normal water content while the hydrated group was on the positive side.

It is known that the dehydrated mites when exposed to hydrating air absorb water vapor from ambient air against a gradient of vapor pressure. On the other hand in hydrated mites this active absorption is minimal or ceases completely, and the mites lose water to the surrounding hydrating air. Thus, dehydrated mites gain weight while hydrated ones lose weight when placed in a hydrating atmosphere. Therefore, if
the absorption of water vapor from ambient air by dehydrated mites is an active process, the rate of oxygen consumption in these mites would be higher than in hydrated ones. The aim of the present investigation was to test the above hypothesis and the two types of mites were therefore used for the purpose.

Although the usual techniques of the Cartesian diver were followed, certain modifications were introduced in order to make the technique suitable for the present study. A polyethylene tubing of appropriate diameter was placed inside the diver to prevent the mite from crawling into the neck of the diver. The divers used were without tails and with a "lining cone" situated at the top of the diver bulb. This served to bring out the reflection of the "fiduciary" line marked on the flotation vessel. This reflection in turn was used to bring the diver to the equilibrium level when the readings of the equilibrium pressure were taken. One percent solution of NaOH was used as alkali to absorb CO₂ and to provide a high humid atmosphere (about 98% r.h.) for the mite in the diver. The temperature of the system was 80° ± 0.5° F. White mineral oil served as the oil seal and the flotation medium (~LiN LiCl) with 0.1% sodium taurocholate was used as the neck seal. There were six divers; three were used at each time and were selected at random. Two of these divers contained experimental mites, one with a hydrated mite and the other with a dehydrated one,
while the third was charged exactly as the experimental divers except it did not contain a mite. This third diver served as a control and was designated as the "thermo-barometer." The readings of equilibrium pressures were taken for about an hour. The initial and the final weights of the mites were taken on a Cahn electrobalance.

The various sources of error that might have influenced the readings of the equilibrium pressure have been discussed and the magnitude of error has been pointed out in each case. In most cases, the errors are minor and therefore have been ignored. However, appropriate corrections have been made for the discrepancies that could have introduced significant errors in the measurements.

No significant difference in the rates of oxygen consumption between the two groups of mites was detected, the average rates being $3.03 \times 10^{-3} \pm 0.707 \times 10^{-3}$ $\mu l/hr/\mu g$ of dry weight and $2.83 \times 10^{-3} \pm 0.413 \times 10^{-3}$ $\mu l/hr/\mu g$ of dry weight for the dehydrated and hydrated mites, respectively. Any difference that might have existed in the rates of oxygen consumption between the two groups of mites has been masked by large variations within the groups.

The changes in weights of the two groups of mites during the period of the experiment followed an expected pattern: the dehydrated ones gained while the hydrated ones lost weight. The dehydrated group, on the average, gained 0.62 percent of their initial weight per hour while the
hydrated ones, on the average, lost 0.54 percent of their initial weight per hour.

Calculations based on change in free energy revealed that the amount of oxygen required by a dehydrated mite to supply energy for the absorption of water vapor against an osmotic gradient is very small in comparison to the amount of oxygen taken in.

In the course of the present experiment, some mites were found trapped in the NaOH solution in the diver. These cases have been dealt with separately, and have not been included in the computation of the above results. Once caught in NaOH, the mites struggled and as a result their activity was at a higher level than the normal mites for which the rate of O$_2$ consumption was measured. This increased activity demanded a faster rate of O$_2$ consumption and consequently the rate was more than thrice the usual rate of oxygen consumption. The increased rate of O$_2$ uptake by the struggling mites indicates that the normal mites were not taking O$_2$ to their maximum capacity, and actually were at a low level of activity during the period of the experiment. Since there was some activity shown by the normal mites, the metabolism of the mites under the present experimental conditions has been referred to as "standard" metabolism.

The changes in weight by these struggling mites were also greater than those of the normal ones. One such mite
lost weight at the rate of 7 μg/hr/mite. It has been indicated that this increased loss of weight by these mites could be caused by an increased loss of water by raised transpiration. The rate of loss of weight due to the death of a mite during the experiment went as high as 14 μg/hr/mite.

The higher rate of oxygen consumption and the increased loss of weight by the struggling mites could be determined because individual mites were used in the experiment. Such results would have been obscured if groups of mites had been used.
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I, Kalpataru Kanungo, was born in the Village of Kuradha, District Cuttack, Orissa, India, February 12, 1931. I received my secondary school education in the public schools of Cuttack, Orissa and my undergraduate training at Utkal University, Orissa, India, which granted me the Bachelor of Science degree in 1952. From the University of Allahabad, India, I received my Master of Science degree in 1955. I joined the staff of the Orissa College of Veterinary Science and Animal Husbandry, Orissa as an Assistant Professor of Zoology and taught there until December 1958.

In February 1959, I was appointed as Graduate Assistant in the Department of Zoology, University of Maryland, U.S.A. and worked there towards my Doctor of Philosophy degree. In September 1961, I moved to The Ohio State University and the Ohio Agricultural Experiment Station where I completed my work for the degree of Doctor of Philosophy.

I have accepted a position as Research Associate in Entomology at Cornell University.