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**DETERMINATION OF MINIMUM CONCENTRATIONS OF ENVIRONMENTAL
WATER CAPABLE OF SUPPORTING LIFE**

**G. W. Wharton
Acarology Laboratory**

1 November 1968 - 30 April 1969

**NATIONAL AERONAUTICS & SPACE ADMINISTRATION
Washington, D.C. 20546**

Grant No. NGR 36-008-015

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Semiannual

REPORT

By

THE OHIO STATE UNIVERSITY
RESEARCH FOUNDATION

1314 KINNEAR RD.
COLUMBUS, OHIO 43212

To..... National Aeronautics & Space Administration.....

..... Washington, D.C. 20546.....

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On..... Determination of Minimum Concentrations of.....

..... Environmental Water Capable of Supporting Life.....

For the period..... 1 November 1968 - 30 April 1969.....

Submitted by..... G.W. Wharton.....

..... Director, Acarology Laboratory.....

Date..... 10 June 1969.....

SEMIANNUAL REPORT

1 November 1968 - 30 April 1969

TITLE: Determination of Minimum Concentrations of Environmental
Water Capable of Supporting Life

ABSTRACT

During the period of this report a Ph.D. dissertation was prepared by Mr. Terry Lee Devine. The thesis covers the work done on the non-equilibrium aspects of water balance done under the project. Mr. Devine has completed all the requirements for the degree which will be awarded in June 1969.

G.W. Wharton, Principal Investigator
Director - Acarology Laboratory
The Ohio State University

DETERMINATION OF MINIMUM CONCENTRATIONS OF ENVIRONMENTAL WATER CAPABLE OF SUPPORTING LIFE

Deserts

The paper on the evolution of desert faunas and floras was found to be unsuitable for Bioscience. It appears that the concepts presented are too revolutionary for a short presentation that can offer only suggestions rather than demonstration of the mechanism suggested. Support for a research program that will provide an experimental answer to the validity of the hypothesis presented in the paper is being sought.

Arthropods

The work on arthropods is discussed in the attached thesis. It covers work done over the last three years.

Future Work

This will be confined to preparing the thesis for publication and to preparing a final report of the project.

A SYSTEMATIC ANALYSIS OF THE EXCHANGE OF WATER
BETWEEN A MITE LAELAPS ECHIDNINA AND THE
SURROUNDING VAPOR

ABSTRACT OF
DISSERTATION

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

By

Terry Lee Devine, B.S., M.Sc.

* * * *

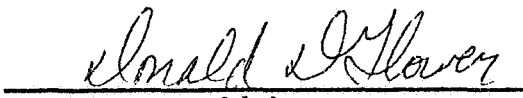
The Ohio State University

1969

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A SYSTEMATIC ANALYSIS OF THE EXCHANGE OF WATER
BETWEEN A MITE LAELAPS ECHIDNINA AND THE
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By

Terry Lee Devine, Ph.D.

The Ohio State University, 1969

Professor G.W. Wharton, Research Advisor

Professor Donald D. Glower, Academic Advisor

Tritiated water was utilized to determine the rates and mechanisms of the exchange of water between a living organism, the spiny rat mite, Laelaps echidnina Berlese, and the surrounding vapor. This mite is of special interest for its ability to actively transport water from unsaturated vapor into nearly saturated body fluids.

The water exchange rates were determined from observations of the water mass and tritium content of the mite following a step change in the environmental water balance parameters. The resulting change in water content was shown to be a first order rate process according to the theory of surface limited diffusion from solids. This model was further developed to relate the observed tritium content and specific activity to the total transpiration of water from the body fluids and the total sorption of water from vapor, respectively.

The first order rate constants for transpiration, sorption, and net mass loss were measured under several environmental conditions.

The rate constant for transpiration varies from three to five per cent per hour in air at 25°C, eight to ten per cent per hour in a high concentration of carbon dioxide, and one to three per cent per hour in air at 15°C. The rate constant for sorption varies significantly with the experimental conditions but tends to be proportional to the product of the transpiration rate constant and the water vapor activity.

The components of water exchange were hypothesized to include bulk flow, metabolic production and loss, diffusion, and the little understood active transport pump. Diffusion contributes to both transpiration and sorption through membranes, both of which were significant in the mite. The active and passive components of exchange through the cuticle and tracheal system, the major exchange surfaces, were examined with regard to the observed variation of transpiration and sorption with environmental parameters and with regard to certain other information on cuticular transport. The variations of permeability of the water exchange barrier were tentatively associated with the diffusion through the tracheal system. The cuticular exchange was tentatively associated with primarily active processes.

The application and theory of tracers utilized in this study are applicable to the determination of the individual transport components of any process which involves multiple exchange mechanisms or in which diffusion is significant.

A SYSTEMATIC ANALYSIS OF THE EXCHANGE OF WATER
BETWEEN A MITE LAELAPS ECHIDNINA AND THE
SURROUNDING VAPOR

DISSERTATION

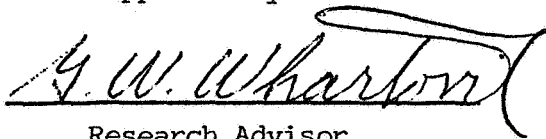
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* * * *

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The extent of this study and the consistency of the findings were made possible by the virtually unlimited supply of carefully prepared mites furnished by Dr. Willi Knülle and his staff at the Acarology Institute of the Ohio Agricultural Research and Development Center.

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J. Insect Physiology 11, 347-353.

Devine, T. L., and Peterle, T. J. (1968) Possible differentiation of natal
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Wharton, G. W., and Devine, T. L. (1968) Exchange of water between a mite,
Laelaps echidnina, and the surrounding air under equilibrium
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FIELDS OF STUDY

Major Field: Nuclear Engineering

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CHAPTER I

INTRODUCTION

Purpose and Scope of the Study

The availability of water is often a controlling factor in the ecology of terrestrial organisms, particularly for the smaller organisms that have a higher ratio of surface to volume with correspondingly more severe evaporative losses. Certain arthropods have adapted to desiccating environments by the evolution of special mechanisms for the regulation of water balance between the environment and their body fluids, which have a uniformly high activity of water. Among these mechanisms are limitation of respiratory gas exchange, minimization of soluble excreta, waterproofing of the cuticle, production of metabolic water, and an active transport pump for the extraction of water from vapor. The spiny rat mite, Laelaps echidnina Berlese, exhibits all of these mechanisms.

The study of L. echidnina began with the measurement of the exchange of tritiated water between a mite and the surrounding vapor under environmental conditions selected to allow constant water mass in the mite fluids. At that time the exchange of water was believed to be much less than one per cent per hour because of the waterproofing mechanism, and sorption was thought to result almost entirely from the active transport pump. This exchange of water, with constant water content, was observed and can be described as a first order rate

process having a rate constant of a few per cent per hour (Wharton and Devine, 1968). The net loss of water mass in a desiccating atmosphere was also found to be a first order rate process similar to surface limited evaporation from solids. In this study rate constants were determined for sorption, transpiration, and net mass loss at numerous combinations of water vapor activity and temperature both in air and in a high concentration of carbon dioxide. Permeabilities and asymmetries of the exchange system were calculated from the observed rate constants to produce an empirical model of the system. The magnitudes and variations of these three parameters were then used to compare certain hypothetical models to the empirical model in an effort to estimate the water exchange through the various anatomical structures and to estimate the active and passive components of the observed exchange.

Water Balance in Arthropods

The literature concerning the water relations of organisms is extensive. A resume of studies on transpiration and sorption of water by cells and the associated physical chemistry of water transport phenomena has been published by Dick (1966). Studies of evaporation and uptake of water by arthropods have been summarized by Edney (1957). The reviews of Bursell (1964), and Rapoport and Tschapek (1967) cover later reports and provide excellent bibliographies. A review of the environmental and anatomical factors affecting water exchange as reported for a variety of arthropods will serve as an introduction to the less extensive literature regarding water balance in Laelaps echidnina.

Studies of arthropod water balance are usually conducted under starving conditions, i.e. without access to food or liquid water. In most cases these arthropods can be induced to refrain from laying eggs or passing fecal material. When the arthropods can be further restrained to remain at rest the metabolic rate is low and the associated production of water is assumed to be insignificant. Under these conditions the observed changes in weight correspond to the changes in water content.

Under starving, resting conditions the principal water exchange surfaces are those of the integument and the tracheal system. Some arthropods, such as the larvae of the tick Dermacentor variabilis, have no tracheal system and so must exchange gas and water through the integument. This water transport is limited principally by a wax layer in the outer region of the integument, the effectiveness of which has been stressed by Edney (1957). For each species there is a critical temperature above which water balance cannot be maintained, generally around 35°C. These temperatures correspond to the observed melting points of wax derived from the wax layer of some insects studied.

Arthropods exhibiting active transport of water from vapor have a minimum or critical vapor activity below which water balance cannot be maintained. These critical equilibrium activities are characteristic of the species and the stage in the life cycle. Most of the organisms studied have critical equilibrium activities between 0.80 and 0.90 at 20°C to 25°C.

The net gain of water from unsaturated vapor by certain ticks was determined by Lees (1964) to take place through the cuticle. He

also found that the active uptake of water is less at 15°C and at 35°C than at 25°C. All of the arthropods which exhibit active transport of water are believed to have pore canals and dermal glands although some do not have trachea (Beament, 1964b). The dermal glands secrete the substance of the outermost layer of the cuticle, the cement layer, and the underlying wax layer is secreted through the pore canals. Extensions of the epidermal cells are extended into these pore canals. Certain insects lose their ability to extract water from vapor when the cytoplasmic processes of the epidermal cells are withdrawn from the pore canals prior to moulting (Beament, 1964b). The isolated cuticle of certain arthropods has been reported to show a difference in permeability with direction of net flow (Richards et al, 1953). This may result from a significant resistance to water transport in more than one layer of the cuticle as in the composite membranes studied by Hartley (1948). The permeability of the cuticle is known to vary with temperature and with the state of hydration (Ludwig, 1937).

The mechanism by which water is extracted from vapor has not been identified. Knulle (1967b) points out that the activity of water at the absorbing site must be lower than the activity of water in the air from which the water is extracted and that an active mechanism or pump is required to move the water from the site of sorption to the haemolymph. An electrostatic mechanism for active transport was considered by Beament (1964 a & b) who postulated a bi-molecular leaflet of organized lipid to produce an electrostatic field to force water through the membrane. In many cases water follows the active transport of other materials as in the transport of Na^+ ions in the

kidney of mammals. In such cases the actively transported substance is said to be a carrier molecule if a net transport of the second substance, such as water, results.

Few papers report the exchange of isotopically labelled water between arthropods and vapor. Govaerts and Lecercq (1964) determined the exchange of D_2O between insects and vapor in terms of equilibration time. Marcuzzi and Santoro (1959) reported the exchange of HTO between Tenebrio molitor and vapor in terms of the half time of equilibration. These studies indicate a continuous turnover of water in the insects with rate constants of a few per cent per hour but do not reveal whether the exchange occurs in all vapor conditions, as in the case of diffusion, or only as a result of an active transport pump.

The literature of water balance in organisms has described the net water loss, as measured by weight loss, in terms of the vapor pressure deficit, activity of water, and other dimensions that are discussed by Wharton and Devine (1968). In some studies the term transpiration has been applied to the observed net mass loss, and sorption has been used to describe those cases where a net mass gain was observed; this erroneously suggests a bulk flow under a pressure gradient rather than a random migration of molecules resulting from thermal motion. Diffusion can be described in terms of the total number of molecules which have left a system and the total number which have entered, the difference of which produces a net change of mass (Glasstone, 1941). Transpiration and sorption may be defined as these components of molecular flow.

Water Balance in Laelaps echidnina Berlese

Studies on the net movement of water between females of the spiny rat mite Laelaps echidnina and their surroundings indicate a mean critical equilibrium activity of 0.90 at 25°C (Wharton and Kanungo, 1962). This critical value was reported to be independent of temperature between 15°C and 25°C but near 30°C a critical temperature was detected. The activity of water in the haemolymph has not been precisely determined but it is known to be greater than 0.99 (Wharton and Kanungo, 1962). Thus Laelaps echidnina adult females can extract water from unsaturated air against an activity difference of 0.09 or an osmotic pressure difference of 129 atmospheres. The rate of net water movement was studied by Wharton and Kanungo (1962) who found that live mites exposed to water vapor activity of 0.99 could gain water at the rate of 0.8 ug/hr and that dead mites exposed to water vapor activity of 0.80 lost water at the rate of 1.2 ug/hr. These rates represent about 1 %/hr of the total body water. The energy requirements of the active transport mechanism are known to be only a small fraction of the total metabolic activity of the mite (Kanungo, 1965). Daily dehydration followed by daily rehydration of this mite was found to have little effect on its longevity under starving conditions (Knulle, 1967a). Kanungo (1963) determined that these mites could maintain water balance in unsaturated air in an atmosphere containing as little as 2.5% oxygen in nitrogen but were killed by an atmosphere of pure nitrogen in which they lost weight at about 0.4 %/hr. The oxygen uptake from air was determined by Kanungo (1965) to be approximately 0.17 ul/hr. The production of metabolic water associated

with this O_2 uptake rate is of the order of 0.1 ug/hr or, in terms of the total body water, about 0.1 %/hr.

Study of the water exchange of the mite has been simplified by design of the experimental conditions to minimize or eliminate some of the water exchange mechanisms. Mites are normally starved for a period of time before experimental use in order to minimize both excretion and the metabolism associated with digestion of food. Experimental procedures can be used that allow the mite to remain in a resting condition with low metabolic rate. The integument and tracheal system remain as the major water exchange surfaces. Of these the integument is the probable site of the active transport of water from vapor since active transport is known to occur in acari that have no tracheal system, and in L. echidnina excessive water loss is associated with any condition that results in elevated consumption of oxygen (Kanungo, 1965).

The integument of the mite Laelaps echidnina as described by Wharton et al (1968) is quite similar to that of insects described by Locke (1964). A thin layer of epidermal cells secrete the substance which forms an overlying cuticle of about ten microns thickness. The cuticle has distinct layers of which the innermost 0.025 to 0.05 μm is granular succeeded by a homogeneous layer 0.4 μm thick known as the endocuticle. The next layer, the exocuticle, is about 10 μm thick composed of lamellae of 0.1 μm separated by 0.01 μm . The outermost region, the epicuticle, is about 0.15 μm thick and composed of four layers. The innermost of these layers is similar to the exocuticle but with more compressed lamellae and is the thickest of the four layers.

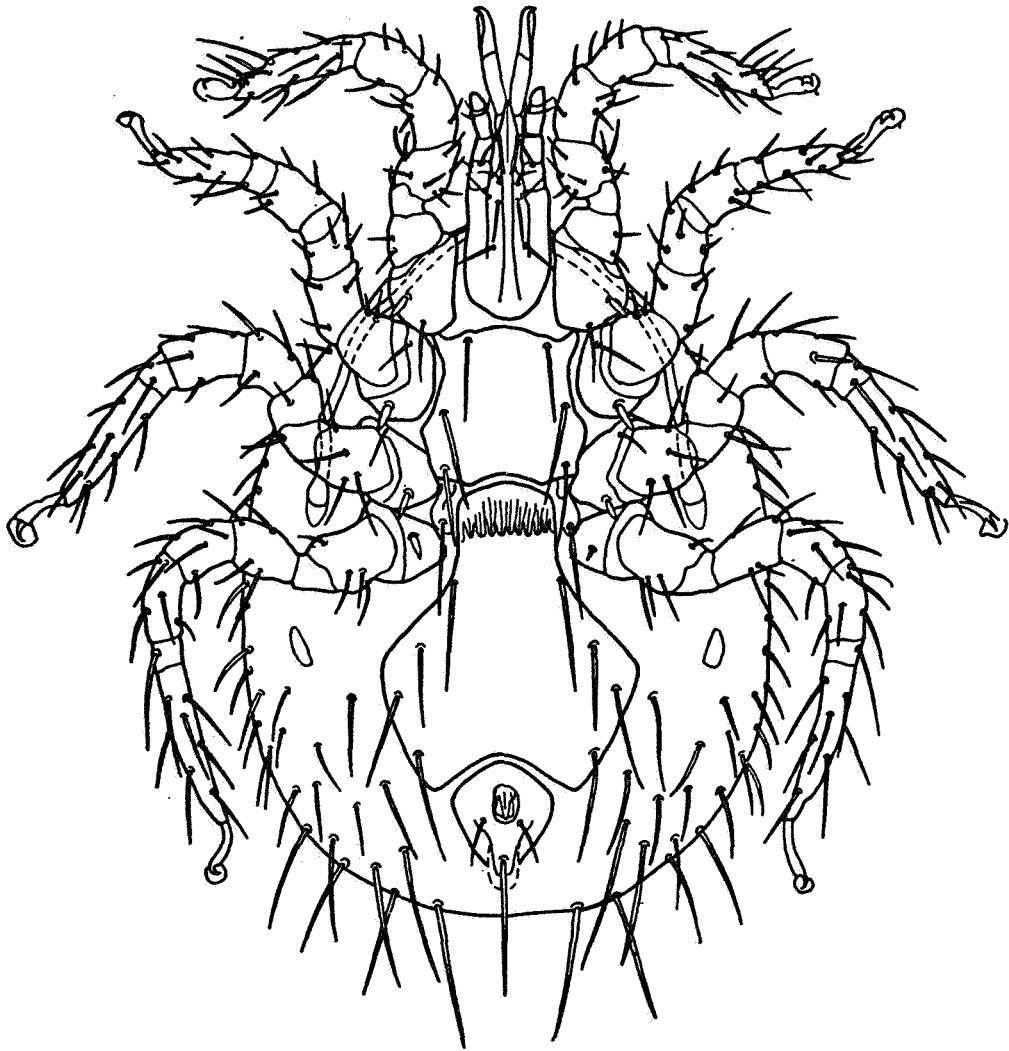


Figure 1.-- Venter of the female of Laelaps echidnina Berlese

The uppermost three layers are the cuticulin, the wax layer, and the cement layer.

Pore canals 0.1 μ m in diameter extend from the epidermal cell to, but not through, the cuticulin layer. The canals bifurcate about two microns from the surface of the cuticle. Wax filaments extend from the ends of the pore canals to the surface. The pore canals are thought to be filled with fluid, and cytoplasmic extensions of the epidermal cells extend into the base of the pore canals.

A number of larger pores appear over the surface of the cuticle. These are associated with the ducts from dermal glands. Forty-four such pores appear on the dorsal shield and a number have been identified on other surfaces. Some of these glands are believed to secrete the substance of the cement layer, although their physiology is not known.

The wax layer of the cuticle is believed to be a major barrier to the exchange of water through the cuticle. Although the melting temperature of this wax substance in L. echidnina has not been determined, the observed critical temperature corresponds to the melting point of such waxes in insects (Wigglesworth, 1945; Edney, 1957).

The tracheal system of L. echidnina opens through a spiracle on either side of the venter. Just within this spiracle is an atrium from which the major tracheal trunks originate. These repeatedly branch into successively smaller vessels, of which the final branches, referred to as tracheoles, permeate every tissue. The trachea are lined with epidermis and cuticle similar to that of the integument. At the spiracles a number of spines extend over the orifice and

presumably regulate the exchange of air. The extent to which the spiracles can be closed in the resting mite has not been determined but the water loss rate from active or anesthetized mites is much greater than that from normal resting individuals. Water exchange through the tracheal system probably occurs to some extent in all conditions and may be controlled by a spiracular valve as is the common situation in insects.

The transpiration of water from the mite may result from diffusion, hydrostatic flow under pressure of the integument, and from an efflux of the active transport pump. Sorption of water from vapor must have components of diffusion and active transport. Some of these components, such as diffusion, must occur through both the trachea and the cuticle. Measurements with isotopically labelled water can be designed to determine the total transpiration and the total sorption rates. The variation of these data with experimental conditions and the comparison of the variations in L. echidnina to acarines that have no trachea may suggest assumptions by which total transpiration and sorption may be resolved into the various components.

CHAPTER II

WATER EXCHANGE KINETICS

Tracers such as HTO have found diverse application in studies of rate processes. The exchange of tracer between compartments was discussed by Comar (1955) with emphasis on physiological systems. More general treatment of systems of competing and sequential processes has been given by Benedict and Pigford (1957) and by Rose (1961). These discussions are most helpful where the arrangement of compartments of the system and their exchange mechanisms are known.

The discussion of the water exchange in Laelaps echidnina given above suggests that the body water may be a single, well mixed compartment whose exchange with vapor is limited by the surface of the integument. Several processes may contribute to water exchange, and metabolism produces some water within the mite. The exchange processes can be identified by comparison of the observed response of the water mass and tritium content of the mite following a step change in the environmental water balance parameters to the various mathematical models associated with the possible exchange mechanisms.

If both sorption and transpiration rates were constant in time the water mass would be a linear function of time. This model does not agree with the response to an increase in water vapor activity observed by Wharton and Kanungo (1962).

If the sorption rate were constant and the transpiration rate were proportional to the activity of water in the body fluids the water mass and tritium content relations become rather complicated and of limited usefulness.

A suitable model results from the assumption that both transpiration and sorption are combinations of constant and differential rate processes. If the net mass change rate of the constant processes is $\dot{m}_c$ and that of the differential processes is k_m the resulting expression for water mass as a function of time is:

$$m = m_o e^{k_m t} - \frac{\dot{m}_c}{k_m} (1 - e^{k_m t})$$

where m is the water mass at time t and m_o is the initial water mass. This expression holds only if k_m is less than zero, i.e. if the differential processes of transpiration exceed those of sorption. In this case the equilibrium mass is $m_\infty = \dot{m}_c / k_m$ and the expression above becomes:

$$\frac{m - m_\infty}{m_o - m_\infty} = e^{-k_m t}$$

This expression accurately describes the observed behavior of the water mass of the live mite after a step change from one equilibrium condition to another if the linear term, $\dot{m}_c$, is proportional to the water vapor activity and greater than zero. In extreme environmental conditions where k_m is large and negative and $\dot{m}_c$ is small and positive the equilibrium mass is approximately zero and water mass is described by:

$$m = m_o e^{-k_m t}$$

The theory of diffusion in surface limited solids supports the validity and generality of first order kinetics for water mass as derived here for the body fluids of the mite.

Radial diffusion from a solid sphere of radius r_0 with a surface evaporation coefficient, c , that is small compared to $r_0 D$, where D is the diffusion coefficient, is discussed by Crank (1964). The expression for the total amount of substance, m , entering or leaving the sphere in time t is:

$$\frac{m - m_0}{m_\infty - m_0} = 1 - \sum_{n=1}^{\infty} \frac{6 L^2 e^{-B_n^2 D t / r_0}}{B_n^2 (B_n^2 + L(L-1))}$$

The B_n^2 are the roots of $B_n \cot B_n + L - 1 = 0$, $L = \frac{r_0 c}{D}$ and the other symbols are as in the previous example. Representative values for the mite are: $B_1^2 D / r_0 = 0.038 \text{ hr}^{-1}$, $L=0.01$, $r_0=4 \times 10^{-2} \text{ cm}$, $B_1 = 0.173$, and $B_2 = 4.496$. Substitution of these values into the equation above gives the rapidly converging series:

$$\frac{m - m_0}{m_\infty - m_0} = 1 - e^{-0.038 t/\text{hr}} + 3.7 \times 10^{-8} e^{-25 t/\text{hr}} + \dots$$

Similar results can be derived for the cases of the plane sheet and the cylinder in which the rate constant is of the form:

$$k = \frac{B^2 D}{r_0^2}$$

where B is a function of $r_0 c/D$ with r_0 the half thickness of the solid. These series converge rapidly for surface limited solids. Since the value of B decreases with r_0 the value of k does not change greatly as the shell collapses.

Crank (1964) gives a family of curves for each of many cases of diffusion from solids, as do Carslaw and Jaeger (1959) for analogous cases of heat transfer. The values from these graphs for surface limited solids can be shown to closely approximate the single exponential model.

Because the first order, or exponential, model satisfies both the experimental observations of the water mass of the mite and the theoretical arguments for water exchange from a surface limited solid it will be used in the interpretation of the data presented here.

The first order rate constant of the water mass, k_m , may be regarded as the difference of rate constants for sorption, k_s , and for transpiration, k_T , all of which are constant in time:

$$k_m = k_s - k_T$$

Multiplying this expression by the water mass, m , gives the mass balance relation:

$$\dot{m} = \dot{m}_s - \dot{m}_T$$

The rate constants are related to the mass flow rates by:

$$k_m = \frac{\dot{m}}{m}, \quad k_s = \frac{\dot{m}_s}{m}, \quad k_T = \frac{\dot{m}_T}{m}$$

In the experiments reported here, mites in equilibrium with HTO of high water vapor activity were subjected to a step change to a desiccating atmosphere of non-tritiated water. Their water mass is described by:

$$m = m_0 e^{-k_m t}$$

where m_0 is the initial water mass and m is the water mass at time t .

This relation may be written in logarithmic form as:

$$\ln \frac{m}{m_0} = k_m t$$

The rate constant can be evaluated as the slope of the graph of the logarithm of observed water mass plotted against time in the test environment.

An organism containing tritiated water of activity T^* and water mass m , with associated specific activity $S = T^*/m$, loses tritium to untritiated vapor at a rate dT^* / dt equal to the mass transpiration rate, $\dot{m}_T$, multiplied by the specific activity, S , or:

$$\frac{dT^*}{dt} = -\dot{m}_T S = -\dot{m}_T \frac{T^*}{m} = -k_T T^*$$

Integration of this expression with respect to time gives the tritium content:

$$T^* = T_0^* e^{-k_T t} \quad \text{OR} \quad \ln \frac{T^*}{T_0^*} = -k_T t$$

where T_0^* is the initial tritium content and k_T is the rate constant for both tritium content and transpiration.

$$k_T = \frac{1}{T^*} \frac{dT^*}{dt} = \frac{\dot{m}_T}{m}$$

The rate constant k_T may be evaluated as the slope of graph of the logarithm of observed tritium content or count rate plotted against time in the test environment.

The equation for specific activity as a function of time in the test environment is derived by differentiating the relation for specific activity to tritium content and water mass and substituting the rate

constant relations:

$$\frac{d}{dt} S = \frac{d}{dt} \frac{T^*}{m} = \frac{1}{m} \frac{dT^*}{dt} - \frac{T^*}{m} \frac{\dot{m}}{m} = - \frac{\dot{m}_T}{m} S - S \frac{\dot{m}_S - \dot{m}_T}{m} = - k_s S$$

Integration of this result with respect to time gives:

$$S = S_0 e^{-k_s t} \quad \text{OR} \quad \ln \frac{S}{S_0} = -k_s t$$

where S_0 is the initial specific activity and k_s is the slope of the graph of the logarithm of specific activity plotted against time.

These equations give one method for the evaluation of sorption and transpiration from data of water mass and tritium content during desiccation of the mite. The rate constants may be evaluated graphically or statistically with the rate constant for tritium content identical to the rate constant of transpiration and the rate constant for the specific activity identical to that of sorption. These rate constants may represent the sum of several water transport processes but according to the argument above only the differential processes contribute to the rate constants. Constant components such as metabolic production of water contribute only to the equilibrium mass term and if all sorption of water from vapor were constant in time the mass rate constant would be identically that of transpiration.

CHAPTER III

EXPERIMENTAL PROCEDURES

The mites used in this study were cultured in the Acarology Laboratory of the Ohio Agricultural Research and Development Center by the techniques of Strandtmann and Wharton (1958). Adult females were standardized by the method of Wharton and Kanungo (1962). After transport to the experimental facilities at The Ohio State University the mites were held for several days over tritiated water saturated with KNO_3 that maintained a water vapor activity of 0.925 at 25 C. Throughout the experiment the mites were held in cages of glass tubing ($\frac{1}{2}$ inch long, $\frac{1}{4}$ inch i.d.) covered at the ends with nylon cloth held by tygon washers. During the equilibration period test chambers were prepared with the desired test vapor. Two types of test chamber were used.

The "vapor chamber" (Figure 2) was a double walled polystyrene box with saturated salt solution in the bottom of the inner box, a fan to circulate air over the salt solution and cages, a cross-bored piston to introduce the cages of mites with a minimum of air exchange, and a balloon vented to the outer air to provide equilibration of pressure with the moving piston. Glass wool was packed between the walls for thermal insulation. Fixed joints were sealed with polystyrene cement and sliding surfaces were packed with stopcock grease.

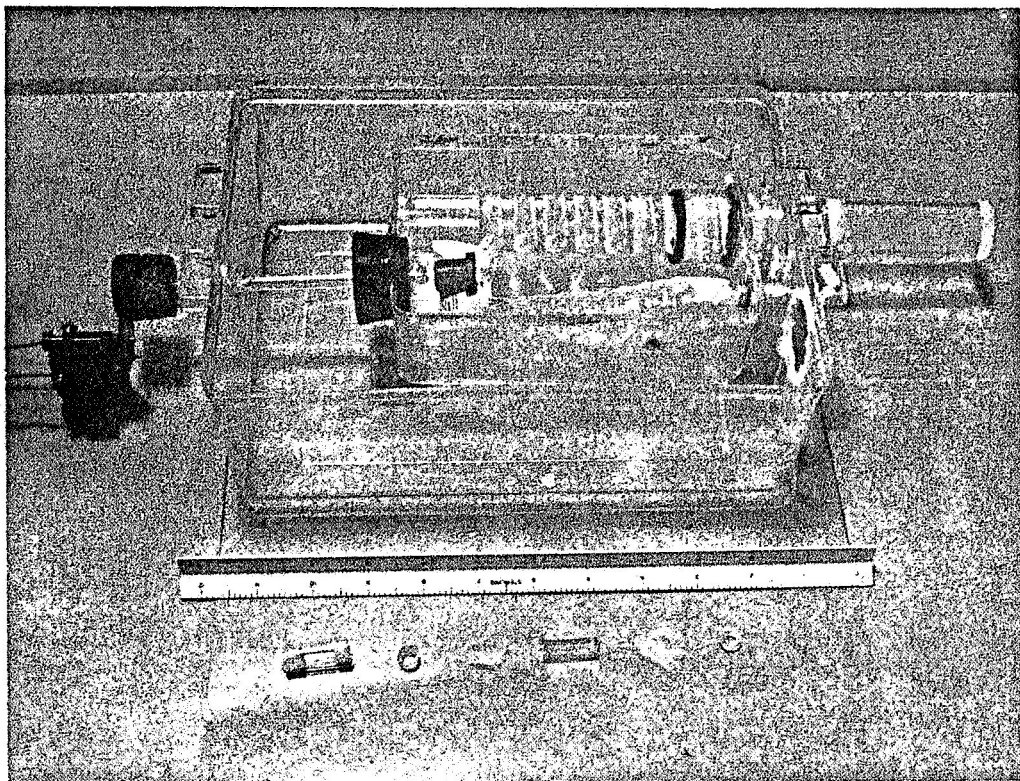


Figure 2 -- Vapor Chamber Components

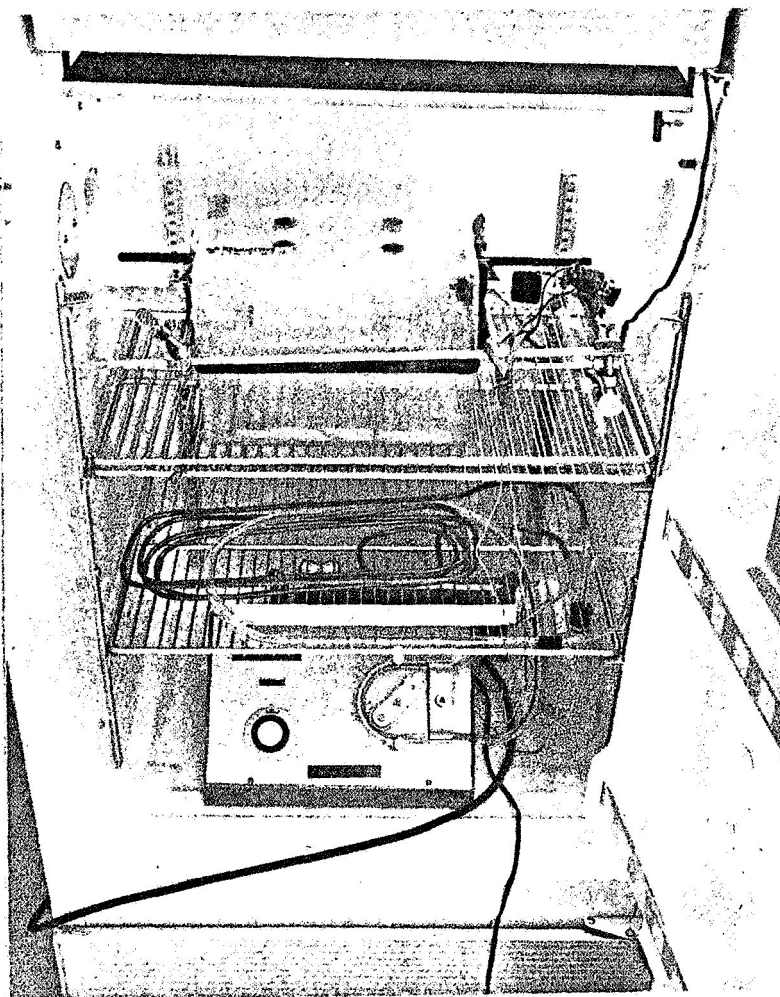


Figure 3 -- Vapor Chamber and Vapor Train in B.O.D. Incubator

In the "vapor train" air was continuously pumped through copper tubing, bubbled through salt solution, then forced through tygon tubing and mite cages back to the pump in a closed loop (Figure 3).

Saturated salt solutions were prepared with an excess of reagent grade salt stirred into distilled water heated to about 35°C then held at the temperature of the incubator for several days before use in the test chambers. These procedures tended to remove any volatile substances from the salt solutions which might affect the mites, although these mites are rather resistant to the salts and ammonia which normally occur in the nests of rats. The values of the water vapor activity over saturated salt solutions were taken from Winston and Bates (1960). The selected salt solution, at the temperature of the incubator, was placed in the test chamber one day before beginning a test in order to equilibrate the water vapor with the salt solution.

At the start of each experiment, cages containing a few dozen mites were removed from the equilibration chamber. Groups of about twenty live mites from these cages were anesthetized with carbon dioxide, transferred to test cages, and placed in the test chamber. This procedure required only a few minutes during which the exchange of water between mites and vapor was negligible. Several live mites were immediately weighed, individually, on a Cahn electrobalance to an accuracy of a few micrograms, and each was placed in a vial of scintillator solution. After several hours at room temperature these vials were assayed for tritium activity in a Packard liquid scintillation counter. At selected time intervals a cage of mites was removed from the test chamber and the surviving mites were individually weighed and assayed for tritium content.

The scintillation solution was prepared in bulk with eight grams of Packard BBOT phosphor in one liter of reagent methanol plus one liter of reagent toluene. This scintillator solution was found to be well suited for these experiments although more efficient scintillators were available. A number of glass scintillation counting vials sufficient for a series of experiments was prepared with ten milliliters of scintillator solution in each and the background count rate was determined by repeated counts on each vial to a total of a few thousand counts. After the rejection of an occasional vial the background of the remaining vials used was consistently 10 cpm. Untritiated mites had no significant effect on either the background or the efficiency of the counting system. Throughout the experiments the reproducibility of the counting data was well within the theoretical limits. The background count rate of 10 cpm was subtracted from all counting data to determine the net count rate which was then used as a measure of the HTO content without other adjustments.

The average dry mass of the mite was determined from that of a large number of mites which had been desiccated to constant mass over P_2O_5 . This value of 35 ug was subtracted from all mass data to obtain the water content of the mites.

The biological effects of the tritium on the mite are believed to be insignificant. At the specific activity used, the dose rate from the tritium beta radiation was of the order of 0.1 rad/hr and the ratio of tritium to hydrogen was of the order of a few parts per billion. More important to the interpretation of the data is the integrity of tritium as a tracer of water. The dissociation rate

is known to be high for water and tritium could conceivably be transported through the water exchange barrier as an ion or other non aqueous form. Also tritium may be exchanged with hydrogen in other compounds within the mite. However, tritiated mites that had been desiccated to constant mass over P_2O_5 and then fragmented into scintillator solution showed no radioactivity, indicating that the tritium remained volatile. The tritium was also determined to remain exchangeable with vapor in initially tritiated mites held for long periods over H_2O salt solutions, and the rate of loss of tritium from the mite is in agreement with the maximum mass loss rate. These observations indicate that non-aqueous exchange of tritium is insignificant.

Analysis of Data

The mean and standard deviation of the water mass, tritium content, and specific activity were calculated from the corresponding values of the several mites in each test group. These values of mean and standard deviation were then plotted on semi-logarithmic coordinates as functions of time in the test vapor. Two traces were then drawn through the extremes of these plotted data so as to produce lines of maximum and minimum slope for each set of data. The average value of these two slopes is the mean value of the rate constant and their difference is taken as two standard deviations of the rate constant. In extreme water vapor activity the smaller rate constant was calculated from the mass balance relation, $k_m = k_s - k_T$. Goodness of fit of the rate constants were not calculated for the reasons given by E. B. Wilson (1930). Confidence intervals determined by inspection are sufficient for the

present purpose of demonstrating the variation of the rate constants with environmental conditions. These variations can be demonstrated by graphs of the rate constants plotted against the activity of water vapor at constant temperature. If the variation of the rate constant with activity of water vapor had been determined in detail at several temperatures a three dimensional surface could be presented on axes of rate constant, temperature, and water vapor activity.

The effective permeability of the water exchange barrier can be determined from the ratio of the rate constant to the associated activity of water: k_s/a_v for permeability to sorption, k_T/a_m for transpiration permeability, and $k_m / (a_m - a_v)$ for the net mass loss permeability. The last term differs only by a constant factor from the commonly used definition of permeability, the ratio of net mass flow per unit area to the concentration difference across the membrane. The simplicity of the ratio of the rate constant to the activity of water and the direct reference to experimental data is preferred for describing the variations of permeabilities with environmental parameters. These variations will be demonstrated by graphs similar to those described for the rate constants. The confidence intervals of the permeabilities are the same as those of the corresponding rate constants since no information was available on the uncertainty of the water vapor activity.

The parameter $k_s a_m / k_T a_v$, referred to here as asymmetry, is the ratio of the permeability of sorption to the permeability of transpiration in the mite. The value of asymmetry exceeds one in water vapor activity above the critical equilibrium value and is less than unity in water vapor activity below the critical value.

The increase in the rate constants in a high concentration of carbon dioxide over those in air can be plotted against water vapor activity to illustrate the difference in the effect of carbon dioxide on sorption from that on transpiration as well as the variation in the rate constant increase with water vapor activity. These observations are important in the determination of the water exchange mechanisms.

The increase in the rate constants at 25°C over those at 15°C were calculated and their variations are demonstrated similar to those of the increases in carbon dioxide. The increase in the rate constant with temperature, or the temperature coefficient of the rate constant reflects on the mechanism of water transport. The ratio of the rate constant at 25°C to that at 15°C, referred to as the Q_{10} of the reaction, is usually less than one for passive physical processes such as diffusion and varies from one to four for active biological processes.

CHAPTER IV

RESULTS

The raw data from weighing and tritium assay of the individual mites, too voluminous to reproduce here, were converted to means and standard deviations of the net water content, tritium count rate, and specific activity of each test group (Appendix A). The fractional standard deviation of the water content was about ten per cent for mites from the equilibration chamber and increased during desiccation to twenty per cent in the more dehydrated mites. Repeated weighings of mass standards gave a fractional standard deviation of about three per cent. The fractional standard deviation of tritium content increased with desiccation from five per cent to forty per cent although the counting statistics were held to one per cent. In most groups of mites the specific activity showed less variation than the mass and tritium content.

The means and standard deviations were further reduced to rate constants by the methods given above (Table 1). Water mass, specific activity, and tritium content each exhibit a single first order time dependence (Figures 4, 5, and 6). The absence of an initially varying slope indicates that the water exchange mechanisms respond rapidly to the test environment, and the constant slope at larger values of time indicates a single effective compartment for water and tritium. These observations support the hypothesis of a single surface limited compartment

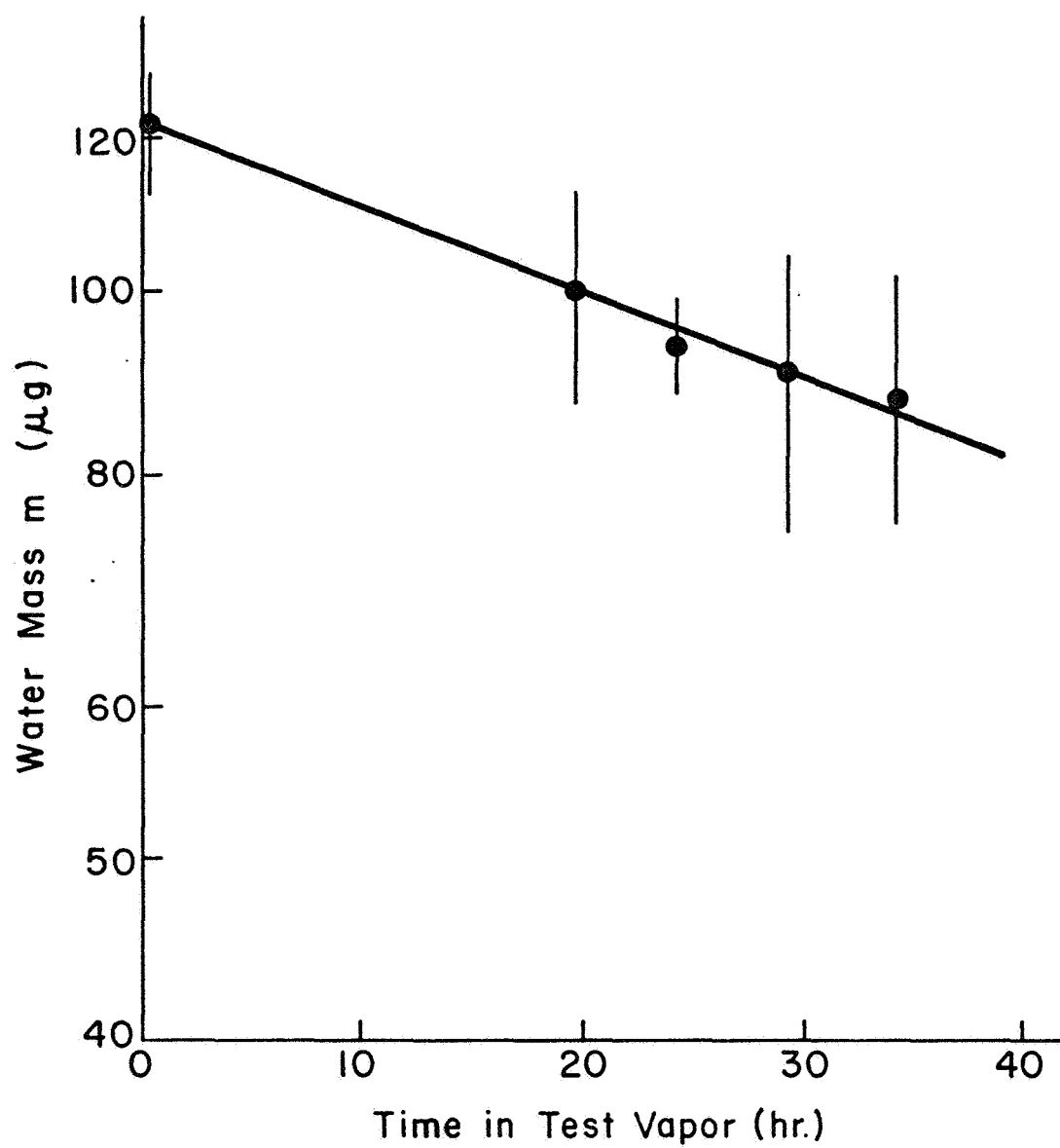


Figure 4. Mean and standard deviation of the water mass of mites held in water vapor activity 0.755 at 25°C for various time intervals.

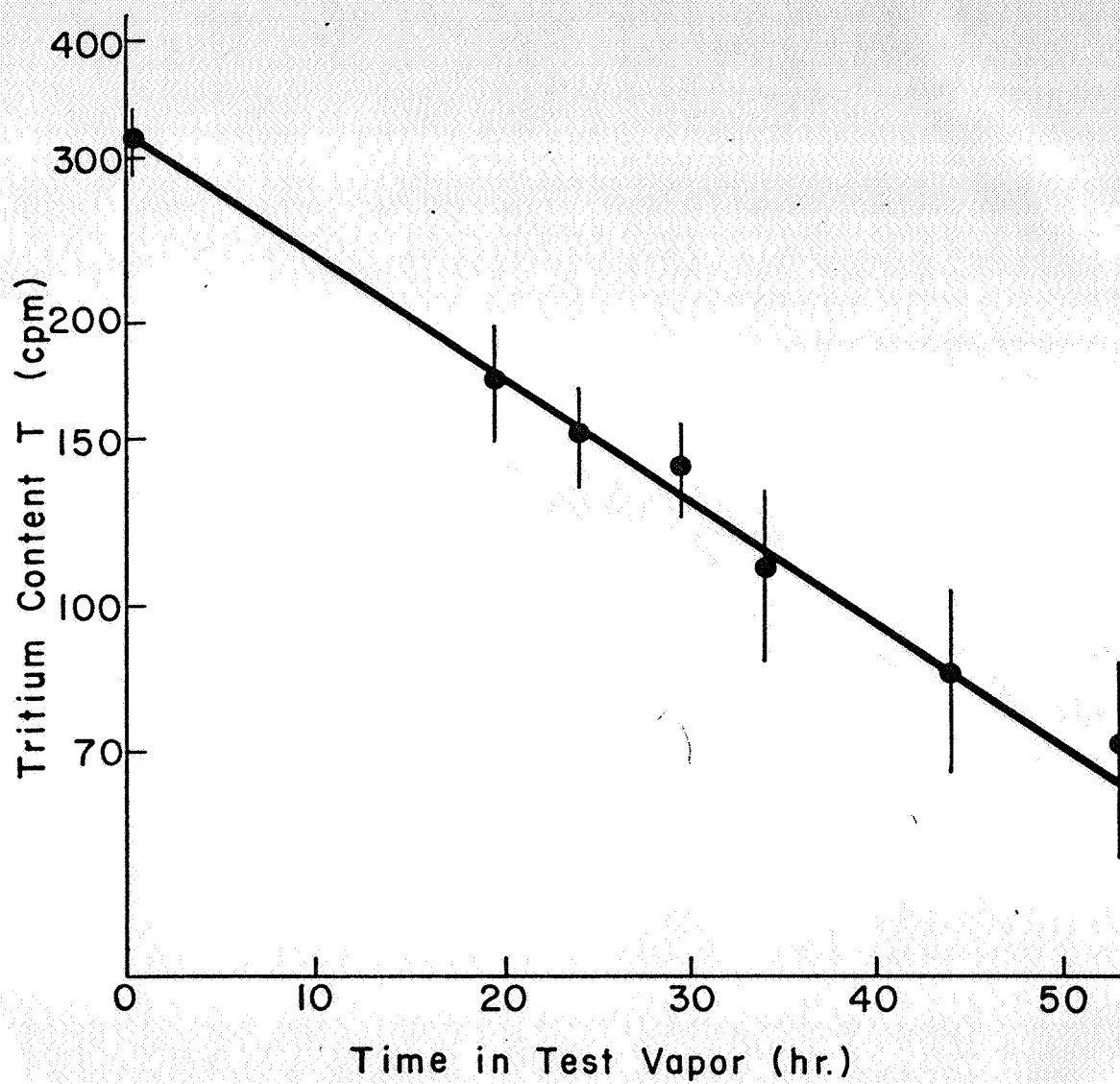


Figure 5. Mean and standard deviation of tritium content of mites held in water vapor activity 0.755 at 25°C for various time intervals.

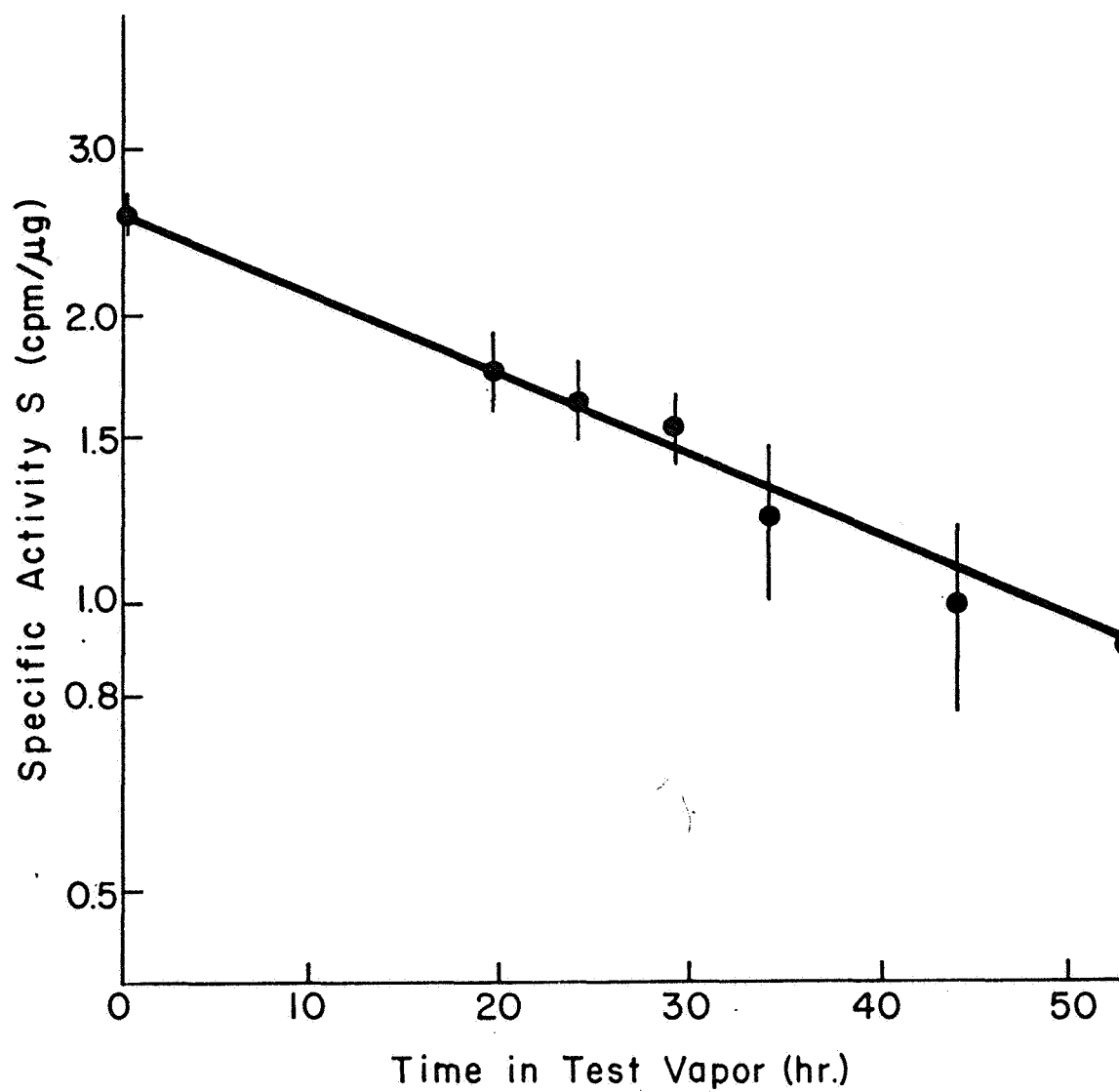


Figure 6. Mean and standard deviation of the specific activity of mites held in water vapor activity 0.755 at 25°C for various time intervals.

TABLE 1
SUMMARY OF RATE CONSTANTS

T (°C)	a_v	Salt	$k_m \pm s_m$ (% / hr)	$k_s \pm s_s$ (% / hr)	$k_T \pm s_T$ (% / hr)
25 (air)	0.07	NaOH	2.9 ± 0.3	(0.1 ± 0.3)	3.0 ± 0.3
	0.225	KAc	2.5 0.4	(0.5 0.5)	3.0 0.4
	0.325	MgCl ₂	2.0 0.3	1.1 0.4	3.1 0.3
	0.43	K ₂ CO ₃	2.2 0.2	1.2 0.3	3.3 0.2
	0.53	Mg(NO ₃) ₂	2.2 0.4	1.0 0.3	3.2 0.5
	0.625	NH ₄ NO ₃	2.0 0.2	1.5 0.2	3.5 0.3
	0.715	NaCl+KCl	1.6 0.2	1.8 0.1	3.4 0.2
	0.755	NaCl	1.1 0.3	2.0 0.2	3.1 0.3
	0.80	KBr	0.8 0.3	2.2 0.4	3.0 0.5
	0.85	KCl	0.7 0.3	3.1 0.2	3.8 0.2
	0.92	KNO ₃	0	3.8 0.2	3.8 0.2
	0.92(s)	KNO ₃	0	3.7 0.6	3.7 0.6
	1.00	-	0	5.5 0.5	5.5 0.5
25 (CO ₂)	0.225	KAc	8.2 ± 0.8	1.2 ± 0.4	9.4 ± 0.8
	0.43	K ₂ CO ₃	7.8 0.6	2.1 0.8	9.9 0.6
	0.625	NH ₄ NO ₃	6.6 0.8	2.6 0.5	9.2 0.6
	0.85	KCl	1.8 0.8	5.8 0.5	7.6 0.5
	1.00	-	2.1 0.2	6.4 0.4	8.5 0.4
15 (air)	0.44	K ₂ CO ₃	1.1 ± 0.2	(0.4 ± 0.3)	1.5 ± 0.2
	0.70	NH ₄ NO ₃	0.7 0.2	1.2 0.2	1.9 0.2
	0.85	KBr	(0.1 0.2)	1.2 0.2	1.3 0.1
	0.93	Na ₂ SO ₄	0	3.1 0.3	3.1 0.4
	0.93(s)	Na ₂ SO ₄	0	3.6 0.6	3.6 0.6
	1.00	-	0	3.1 0.2	3.1 0.2
35 (air)	0.87	Na ₂ SO ₄	0.7 ± 0.4	4.6 ± 0.5	5.3 ± 0.8
	0.87(s)	Na ₂ SO ₄	0.7 0.4	4.3 0.8	5.0 0.7

All initial conditions were 25°C, $a_v=0.93$. $a_v(s)$ indicates sorption HTO from vapor. ($k+s$) determined from mass balance relation.

of water in the mite with both sorption and transpiration by differential processes. All rate constants were found to vary with temperature, water vapor activity, and concentration of carbon dioxide (Table 1).

The net mass loss rate constant, k_m , may be multiplied by the water mass to obtain the mass loss rate in dimensions of mass per unit time. The corresponding net mass flux may be determined by dividing the net mass loss rate by the area of the exchange surface, as is sometimes reported. The conversion of activity to other units is given by Wharton and Devine (1968). The magnitude of k_m in air at 25°C varies from zero at an activity difference of 0.1 to 3 %/hr at an activity difference of 1.0 with a relative minimum at an activity difference of 0.65 (Figure 7). Under other experimental conditions k_m can be made significantly greater than zero, i.e. an increase in mass can be observed, in activity differences less than 0.1 (Wharton and Kanungo, 1962). A relative minimum in the net mass loss rate constant appears in data reported by Loveridge (1968) for dead Locusta migratoria and in the data of Winston and Nelson (1965) for the clover mite Bryobia praetiosa. It is interesting to note that these three organisms have tracheal systems while two organisms that do not show the relative minimum in the variation of k_m with the difference in activity of water, Dermacentor variabilis larvae and Dermatophagoides farinae do not have tracheal systems. (Personal communication of Willi Knülle and G. W. Wharton.)

The rate constants in a high concentration of carbon dioxide were determined at five values of water vapor activity to demonstrate the extreme effect of carbon dioxide as an environmental parameter of water exchange and to determine the effect of anesthetization with carbon

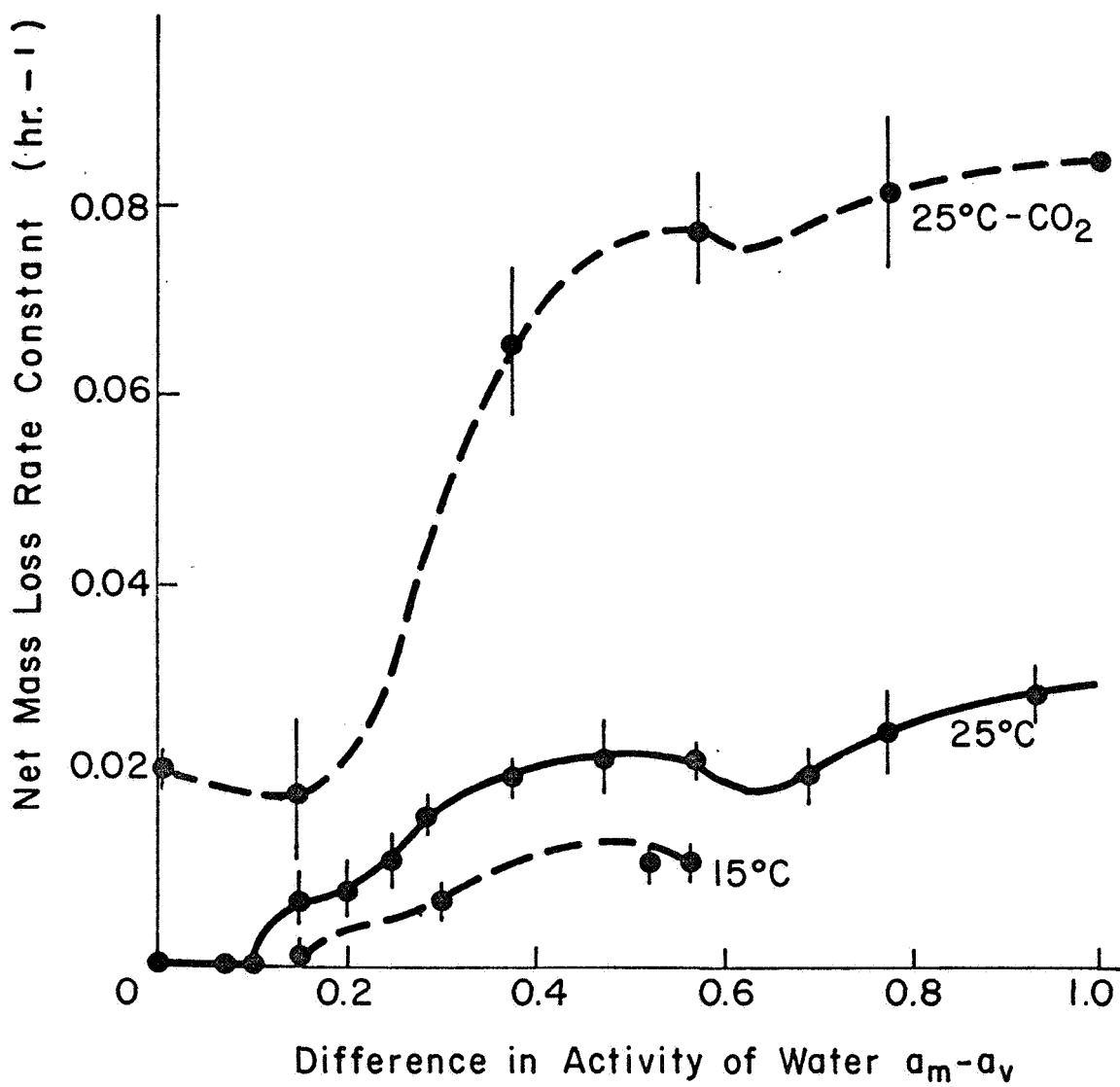


Figure 7. Net Mass loss rate constants at various activities of water vapor at 15°C, 25°C and at 25°C in CO₂.

dioxide on the water mass and tritium content during weighing. This loss of water and tritium is at most a few per cent during the time required for weighing. The variation of k_m with difference in activity of water in high concentrations of carbon dioxide is similar in detail to the variation in air (Figure 7). The magnitude of k_m is relatively constant at 2 %/hr in activity difference 0 to 0.2 and increases rapidly with activity difference to a maximum of 8.5 %/hr in an activity difference of 1.0, therefore equilibrium mass cannot be maintained in high concentrations of carbon dioxide. The concentration used in these experiments was not precisely determined but may be estimated at 90%. The variation of k_m with carbon dioxide concentration was not determined.

The net mass loss rate constants in air at 15°C are about one half as large as those in air at 25°C (Figure 7). These data indicate a critical equilibrium activity somewhat lower at 15°C than at 25°C.

A conventional measure of permeability, the ratio of net mass loss rate constant to the difference in activity of water between mite fluids was calculated for each of the k_m determined (Appendix B). The permeabilities vary considerably with the activity of water in the vapor (Figure 8). The calculated permeabilities are zero when k_m is zero, i.e. over the range of equilibrium water vapor activities. The increase in the net mass loss permeability in high concentrations of carbon dioxide over that in air is approximately a factor of three over most of the range of water vapor activity. The ratio of the permeabilities, or the rate constants, at 25°C to those at 15°C is about two.

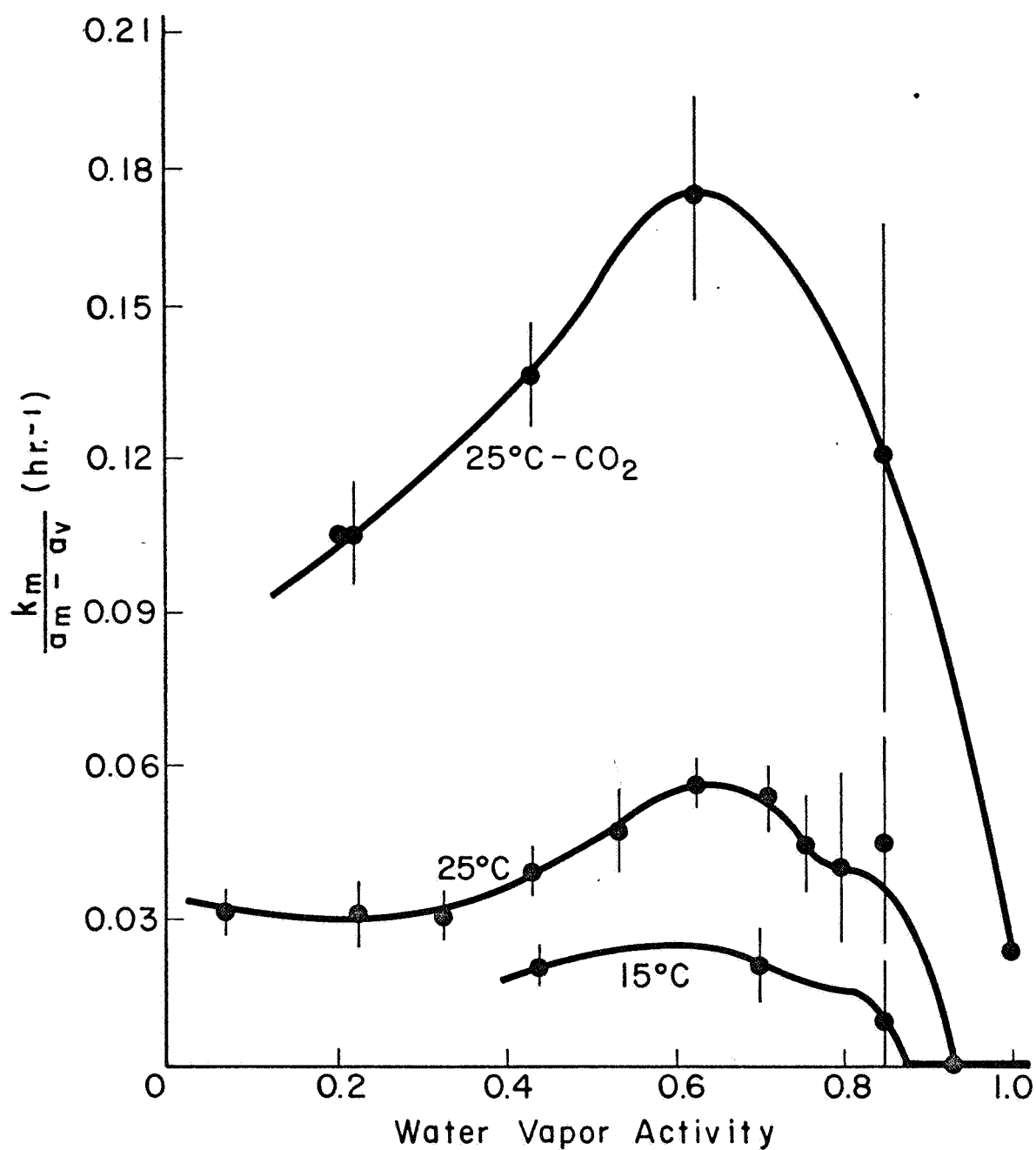


Figure 8. Net mass flow permeability, $\frac{k_m}{a_m - a_v}$, at various water vapor activities in air at 25°C and at 15°C and in carbon dioxide at 25°C.

The independently determined rate constants for transpiration and sorption account for the observed variations in the net mass loss rate constant. The variation of k_m with a_v at low vapor activities is associated with variation in the sorption rate constant alone, but in a_v greater than 0.70, k_m reflects the variations of both transpiration and sorption.

In air at 25°C the rate constant for transpiration, k_T , increases with water vapor activity from 3 %/hr in $a_v=0$ to 4 %/hr in $a_v=1.0$ with a relative minimum of 3 %/hr $a_v=0.80$ having a fractional decrease of twenty per cent from the extrapolated trace of the lower values of k_T (Figure 9). In water vapor activity approaching 1.0 the constants increase to five per cent per hour, possibly as a result of ingestion of condensed water.

The variation of the rate constant for sorption, k_s , with water vapor activity is considerably different than that of the rate constant for transpiration (Figure 9). The sorption rate constant in low water vapor activities extrapolates to 4 %/hr at $a_v=1.0$, but in the range of $a_v=0.33$ to $a_v=0.53$ the value of k_s decreases from 1.1 %/hr to 1.0 %/hr. The latter value is 1.5 %/hr below that of the extrapolated trace of the lower values of k_s . In vapor of $a_v=0.53$ to $a_v=0.80$, k_s increases almost linearly with a_v , but in vapor of a_v more than 0.8, the rate of increase is greater (Figure 9). Sorption is known to equal or exceed transpiration as water balance requires in water vapor of greater than the critical value 0.90 (Wharton and Kanungo, 1962).

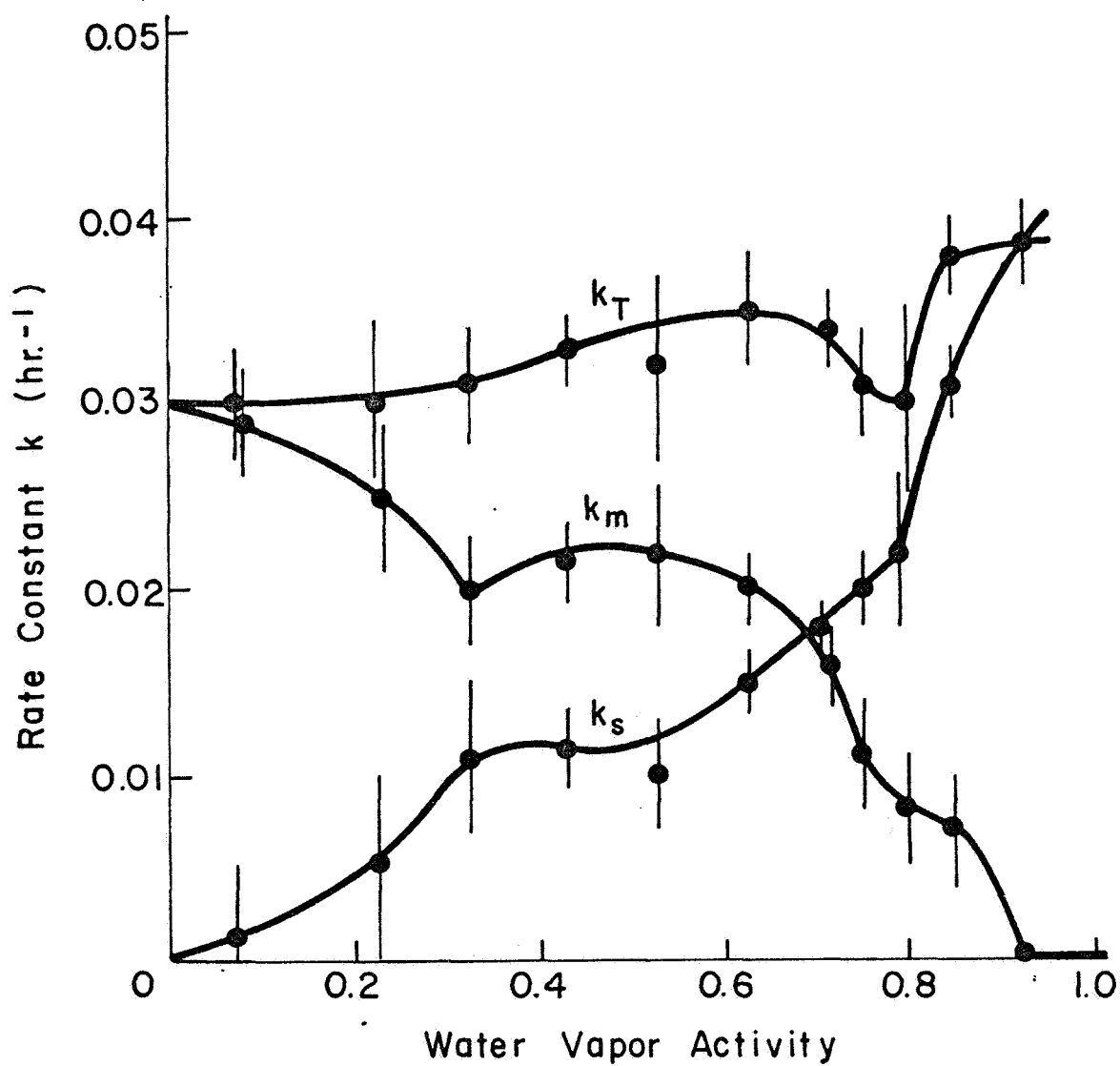


Figure 9. Rate constants for water mass, k_m , transpiration, k_T , and sorption, k_s , at various activities of water vapor at 25°C.

Rate constants of transpiration and sorption were determined as described above in an atmosphere composed of 90% carbon dioxide in air. Water vapor activities in the carbon dioxide atmosphere were assumed to be equal to those in air over the same salt solutions as given by Winston and Bates (1960). The traces of the rate constants indicate the same general variations under the assumed water vapor activities as were observed at the corresponding activities in air (Figure 10). The relative minimum of k_T in $a_v=0.8$ may be somewhat more pronounced than that observed in air. The increase in the sorption rate constant with water vapor activity is not as great in the higher activities in the carbon dioxide atmosphere as it was in air. The data are insufficient to confirm a relative minimum in k_m at $a_v=0.35$ in the carbon dioxide atmosphere.

The increase in the rate constants in the carbon dioxide atmosphere over those in air (Appendix C) were computed with confidence intervals:

$$s_{\Delta k} = \left[s_{k(\text{CO}_2)}^2 + s_{k(\text{air})}^2 \right]^{1/2}$$

The change in transpiration, Δk_T , decreases uniformly from 6.5 %/hr at $a_v=0.4$ to 3.0 %/hr at $a_v=1.0$. The increase in sorption, Δk_s , is relatively constant with a_v except in the region of $a_v=0.8$ where a broad maximum occurs (Figure 11). The trace of Δk_m exhibits a minimum at $a_v=0.8$ associated with the maximum in Δk_s .

The fractional increase in the rate constants in the carbon dioxide atmosphere over those in air, $\Delta k/k$, (Appendix C) were computed with confidence intervals given by:

$$\left(\frac{s_{\Delta k}}{\Delta k} + \frac{s_k}{k} \right) \frac{\Delta k}{k}$$

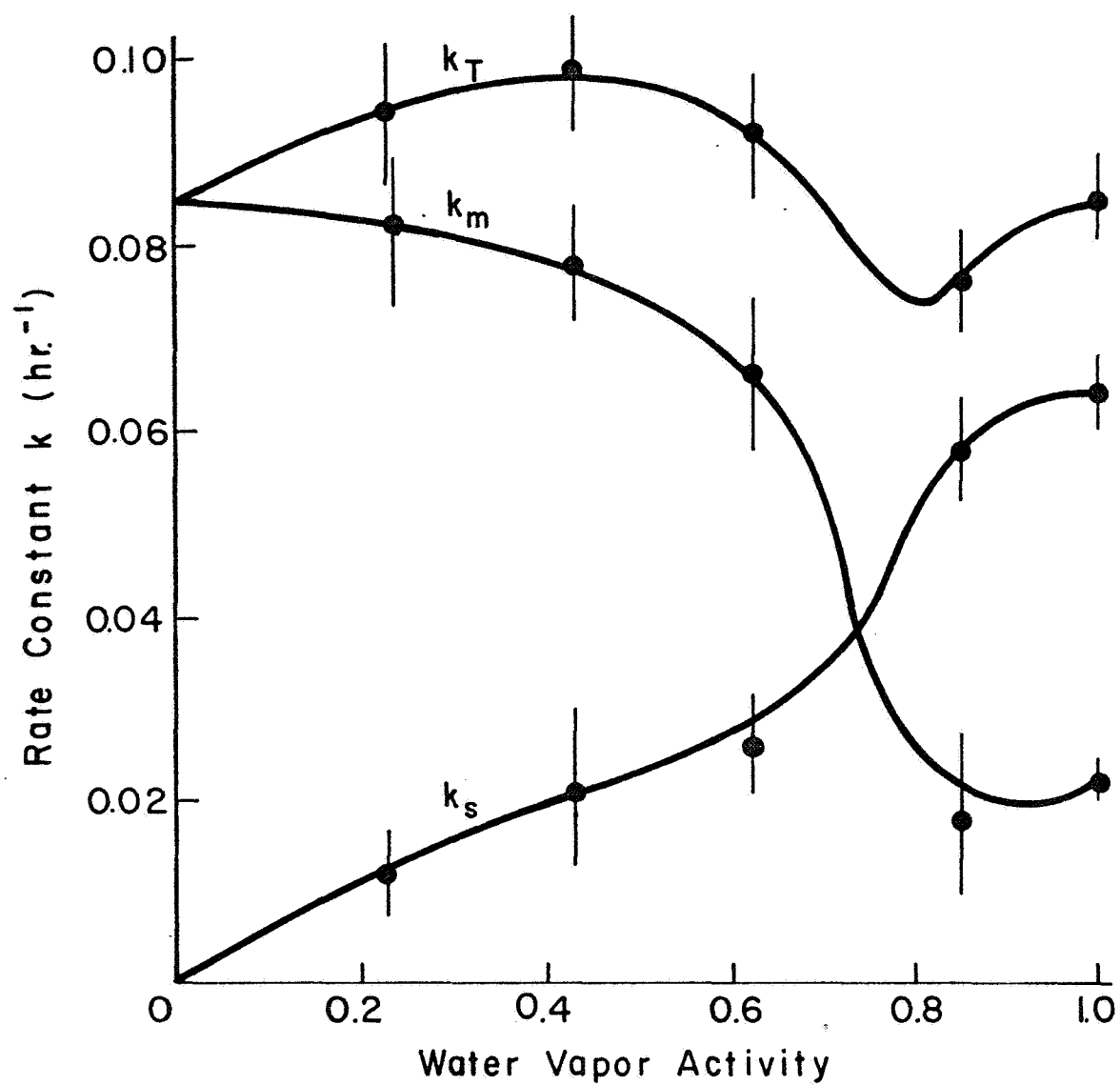


Figure 10. Rate constants for transpiration, k_T , sorption, k_s , and net mass loss, k_m , at various activities of water vapor at 25°C in carbon dioxide.

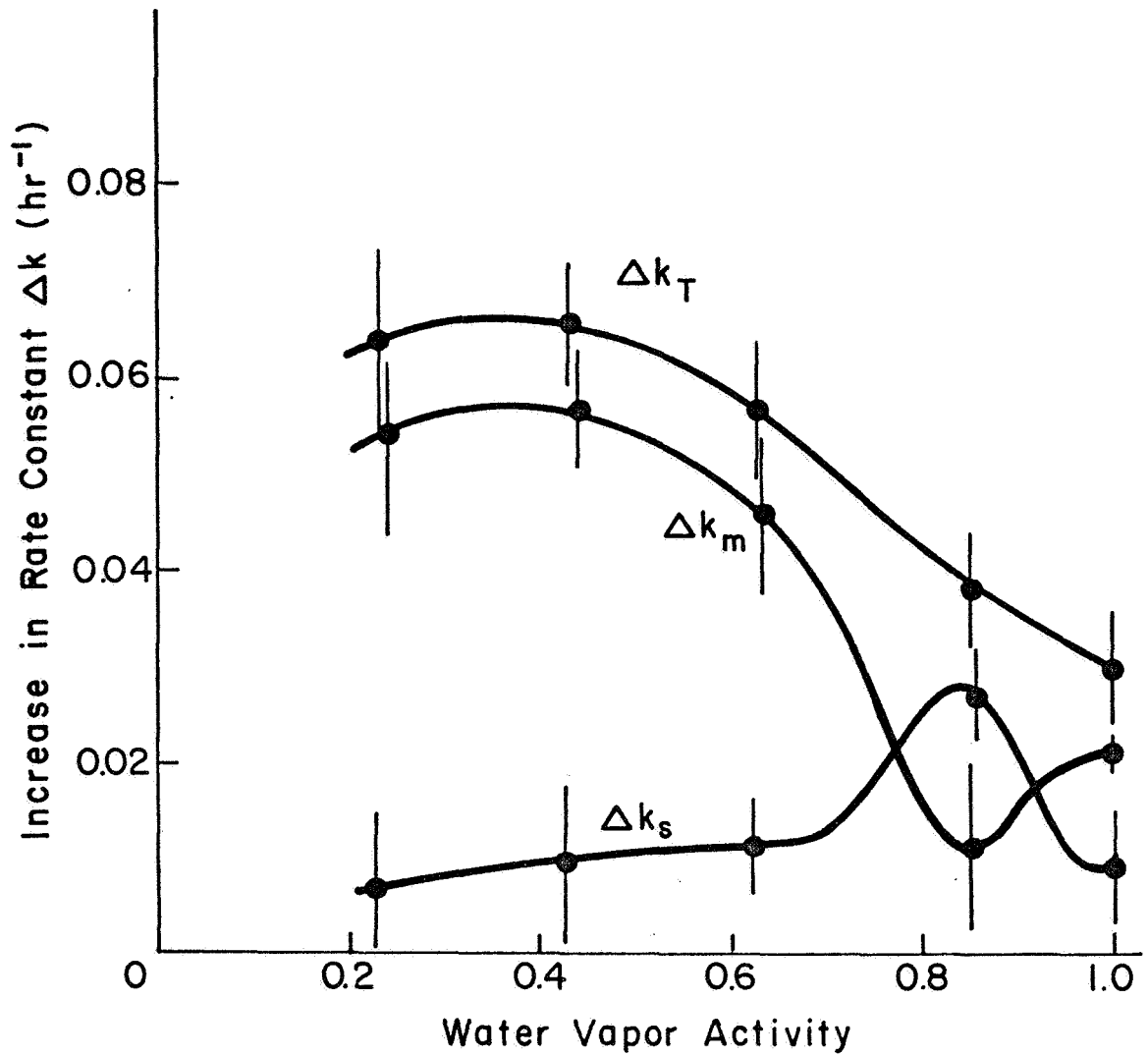


Figure 11. Increase in rate constants for transpiration, Δk_T , sorption, Δk_s , and net mass loss, Δk_m , in carbon dioxide over those in air at various activities of water vapor at 25°C.

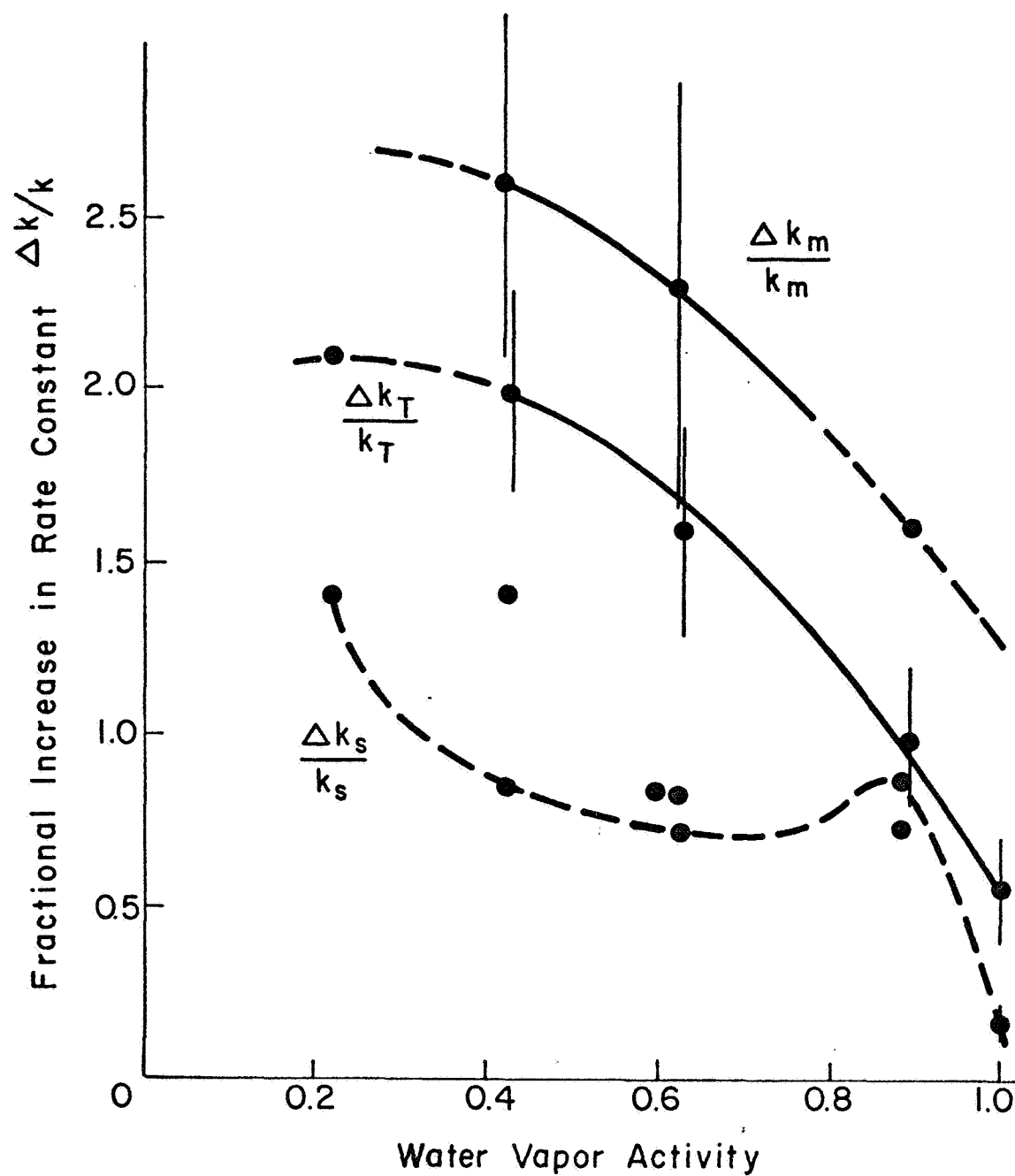


Figure 12. Fractional increase in the rate constants for transpiration, $\Delta k_T/k_T$, sorption, $\Delta k_s/k_s$, and net mass loss, $\Delta k_m/k_m$, at various activities of water vapor in carbon dioxide over those in air at 25°C.

The fractional increase in the rate constant is also the fractional increase in the corresponding permeability. All of the $\Delta k/k$ tend to decrease as water vapor activity increases although the values for $\Delta k_s/k_s$ have large confidence intervals (Figure 12).

Rate constants for water exchange in air at 15°C were measured to determine the temperature coefficients of the rate constants and to elicit independent variations of the rate constants. The variations with water vapor activity at 15°C are, to the extent determined by the few data points, essentially the same as those at 25°C in air. The values of the rate constants at $a_v=0.93$ and $a_v=1.0$ are suspiciously high and may result from ingestion of condensed water. The critical equilibrium activity, the extrapolation of k_m to zero, appears to be slightly lower at 15°C than at 25°C in air.

The increase in the rate constants at 25°C over those at 15°C was computed for each of the measured values at 15°C with confidence intervals similar to those calculated for the increase in the carbon dioxide atmosphere (Appendix D). The increase in transpiration is approximately 1.5 %/hr over most of the range of a_v with a minimum of 0.7 %/hr at $a_v=0.85$. The increase in the rate constant for sorption is about one half as large as that for transpiration (Figure 14).

The fractional increases in the rate constants at 25°C over those at 15°C were calculated with confidence interval similar to that determined for the increase in the carbon dioxide atmosphere (Appendix D). The fractional increases in transpiration and sorption have similar variation with water vapor activity (Figure 15).

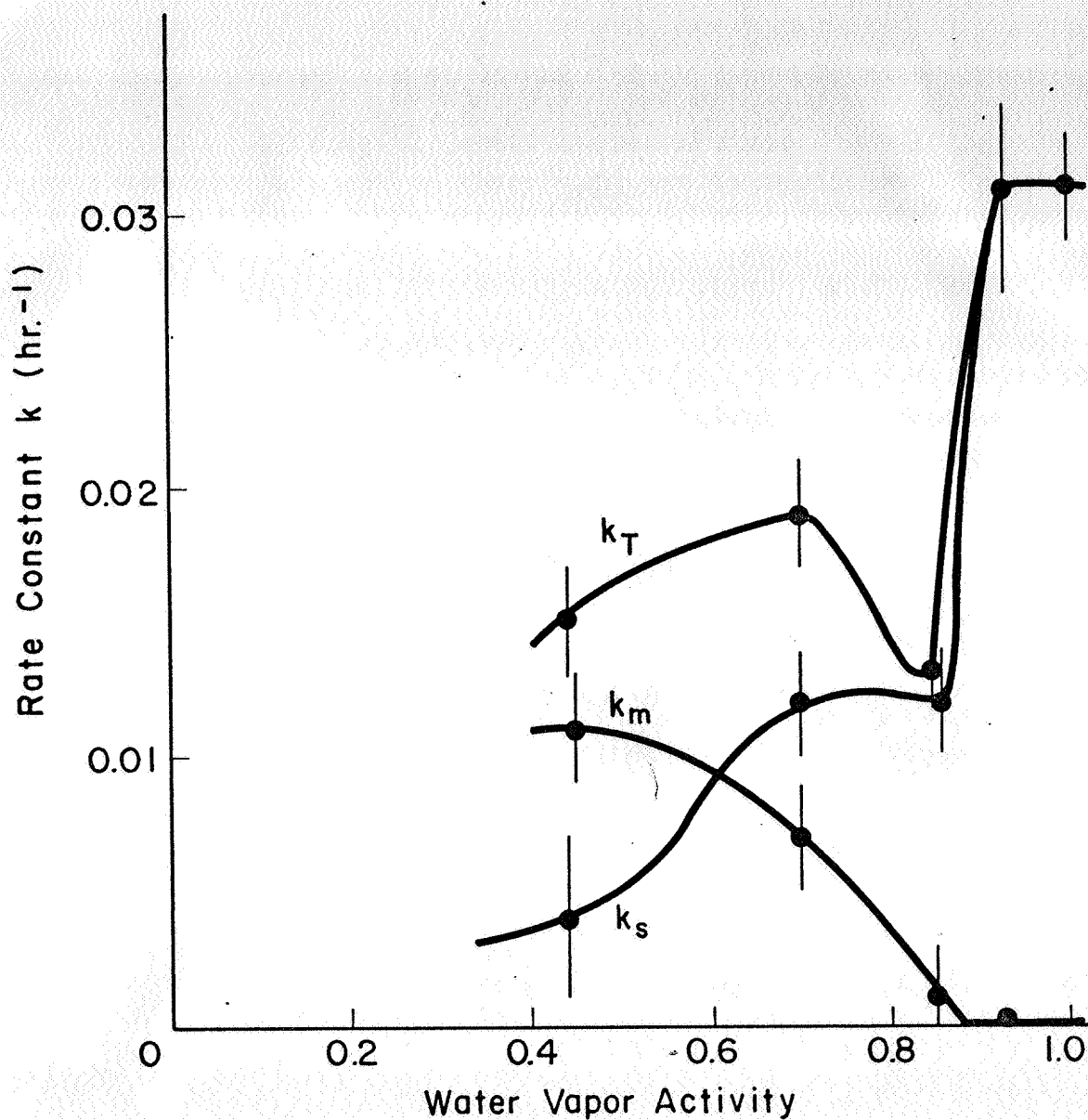


Figure 13. Rate constants for transpiration, k_T , sorption, k_s , and net mass loss, k_m , at various activities of water vapor at 15°C .

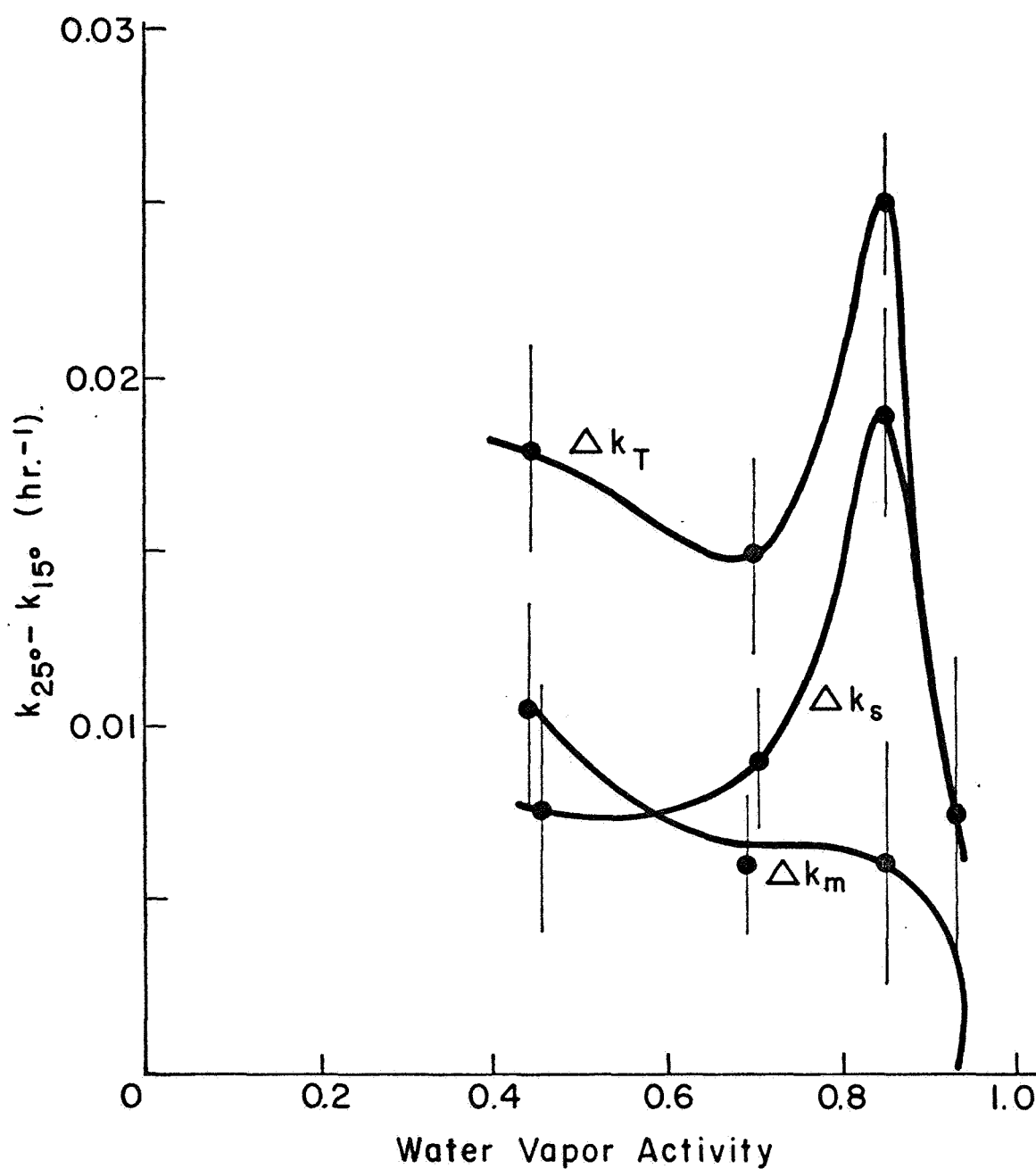


Figure 14. Increase in the rate constants for transpiration, Δk_T , sorption, Δk_s , and net mass loss, Δk_m , at various activities of water vapor at 25°C over those at 15°C.

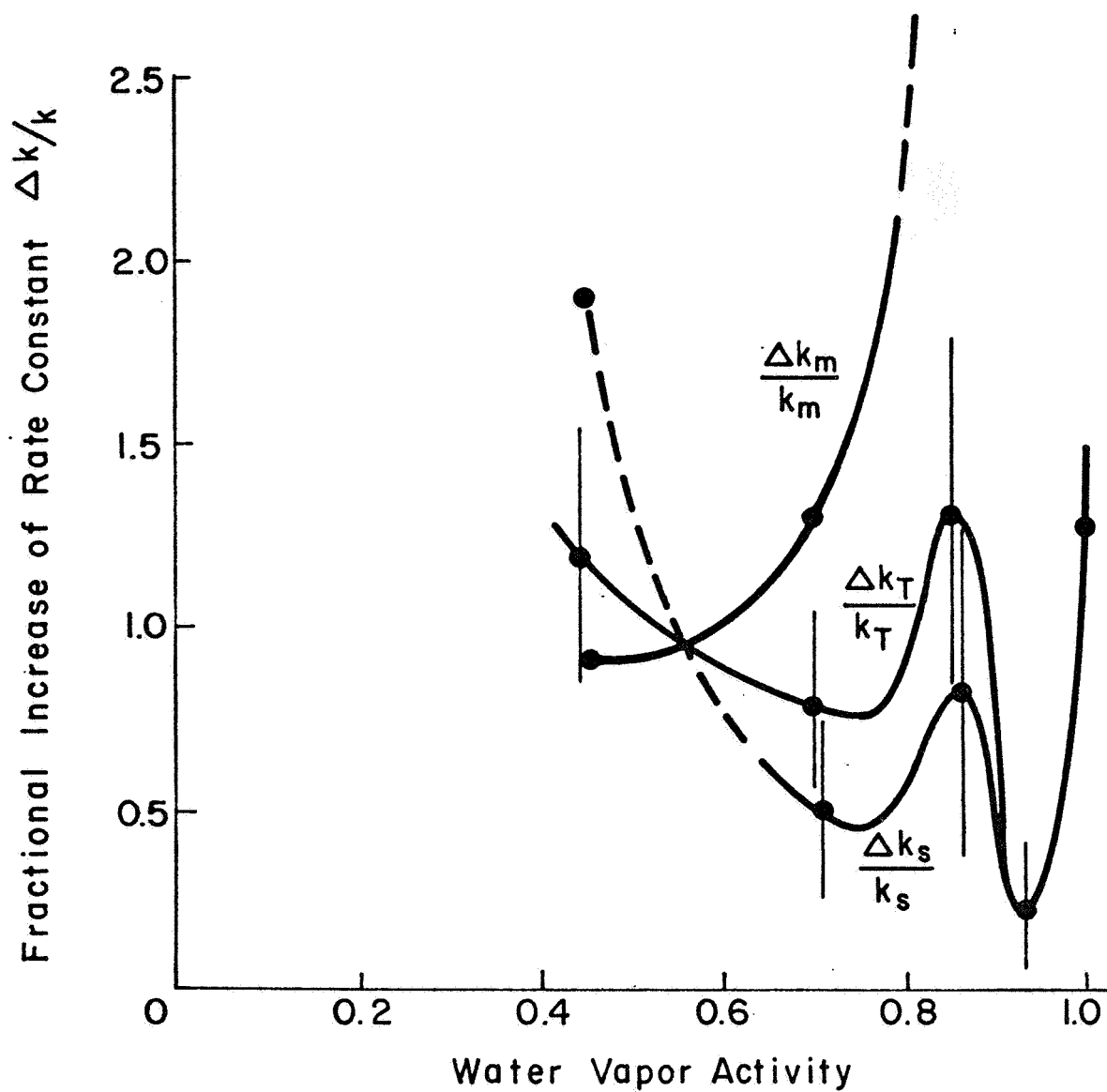


Figure 15. Fractional increase in the rate constants for transpiration, $\Delta k_T/k_T$, sorption, $\Delta k_s/k_s$, and net mass loss, $\Delta k_m/k_m$ at various activities of water vapor at 25°C over those at 15°C.

The permeability, k/a , was computed for each measured rate constant (Appendix B). The values for k_T/a_m are identical to the associated values for k_T because a value of 1.0 was used for the activity of water in the mite fluids. The permeability to sorption in air at 25°C is uncertain in low values of a_v but may equal or exceed that of transpiration. The minimum value of k_s/a_v occurs at $a_v=0.5$ corresponding to the minimum observed in the rate constant at that activity (Figure 16). The variation of the permeability to sorption is remarkably independent of that of transpiration in water vapor activity less than 0.8. A numerical measure of this variation is given by the asymmetry.

Asymmetry, as defined here by $k_s a_m / k_T a_v$, is the ratio of the permeability to sorption to that of transpiration where the permeabilities are the totals of all water transport processes. This measure of asymmetry is less than unity over most of the range of water vapor activity at 25°C (Figure 17), and must necessarily exceed unity in the range of equilibrium water vapor activity.

The permeabilities in the carbon dioxide atmosphere are larger than those in air but their variations with water vapor activity are similar (Figure 18). The permeability to transpiration shows a greater fractional increase than that of the permeability to sorption (Figure 12). The permeability to sorption never exceeds that of transpiration in high concentrations of carbon dioxide and the associated asymmetry is less than unity in all water vapor activities. The asymmetry to water exchange in the carbon dioxide atmosphere is depressed about twenty per cent from that in air and shows somewhat similar variation with water vapor activity (Figure 19).

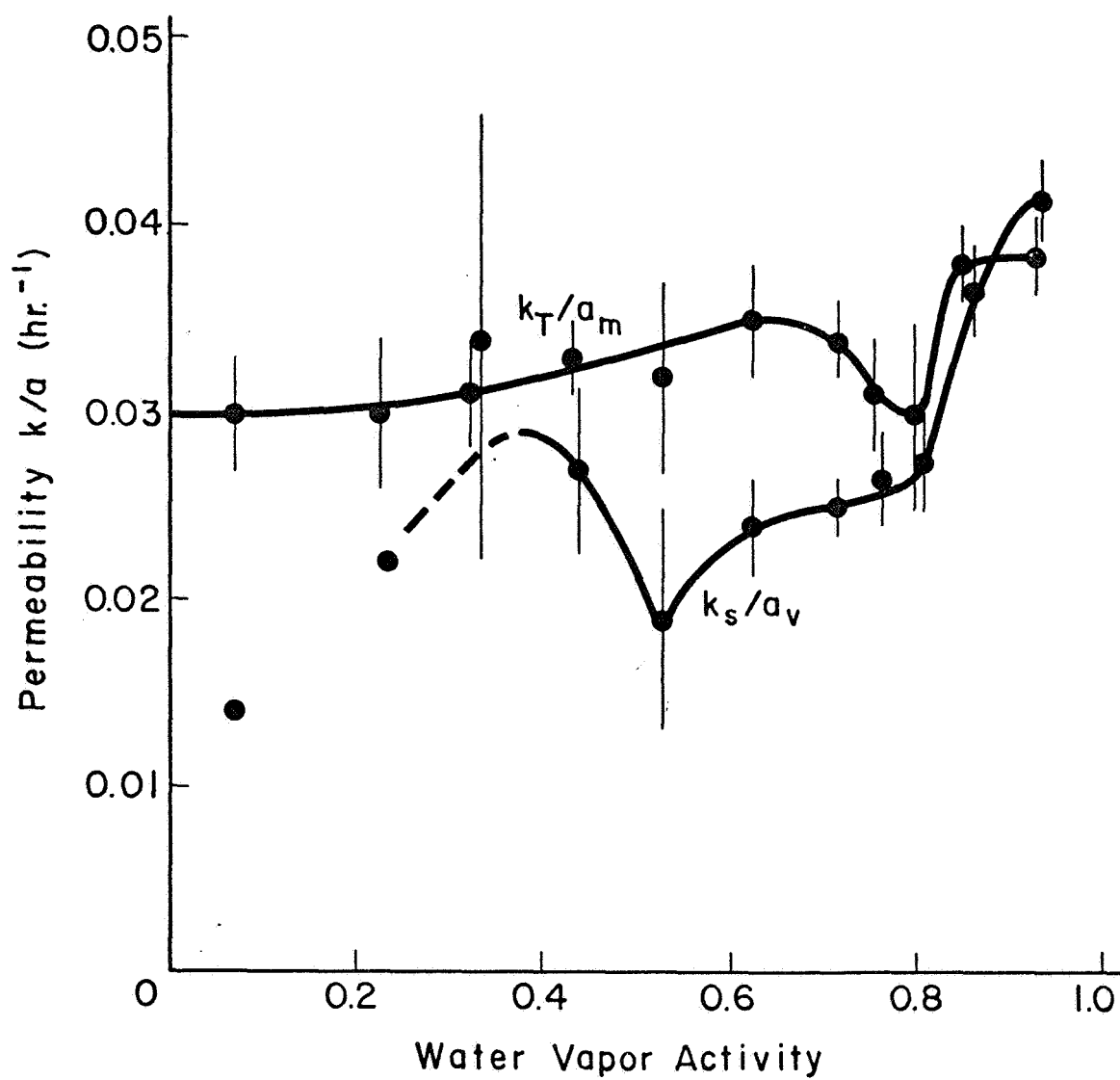


Figure 16. Permeability to transpiration, k_T/a_m , and to sorption k_s/a_v , at various activities of water vapor at 25°C.

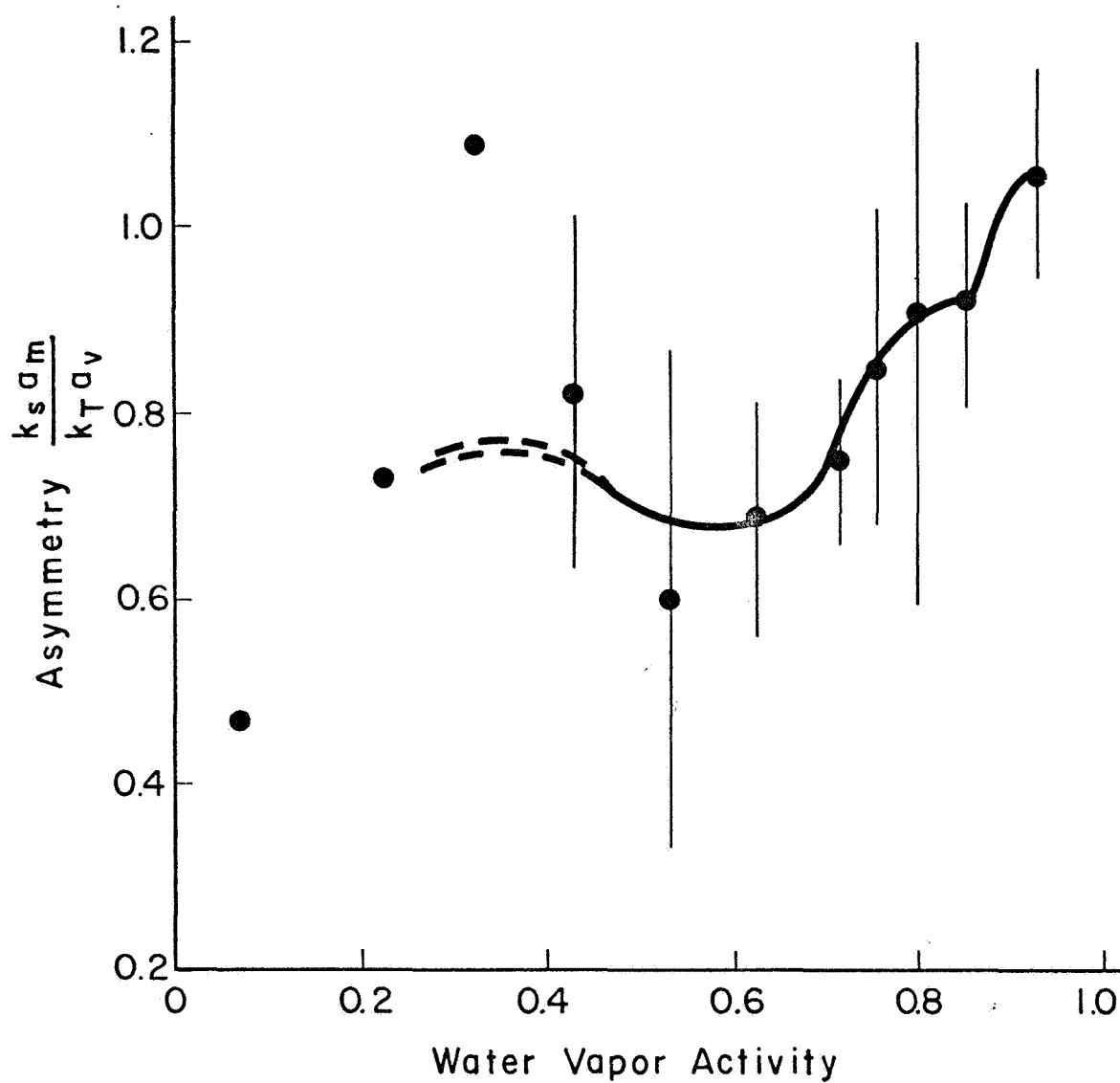


Figure 17. Asymmetry of water exchange with vapor at various activities of water vapor at 25°C.

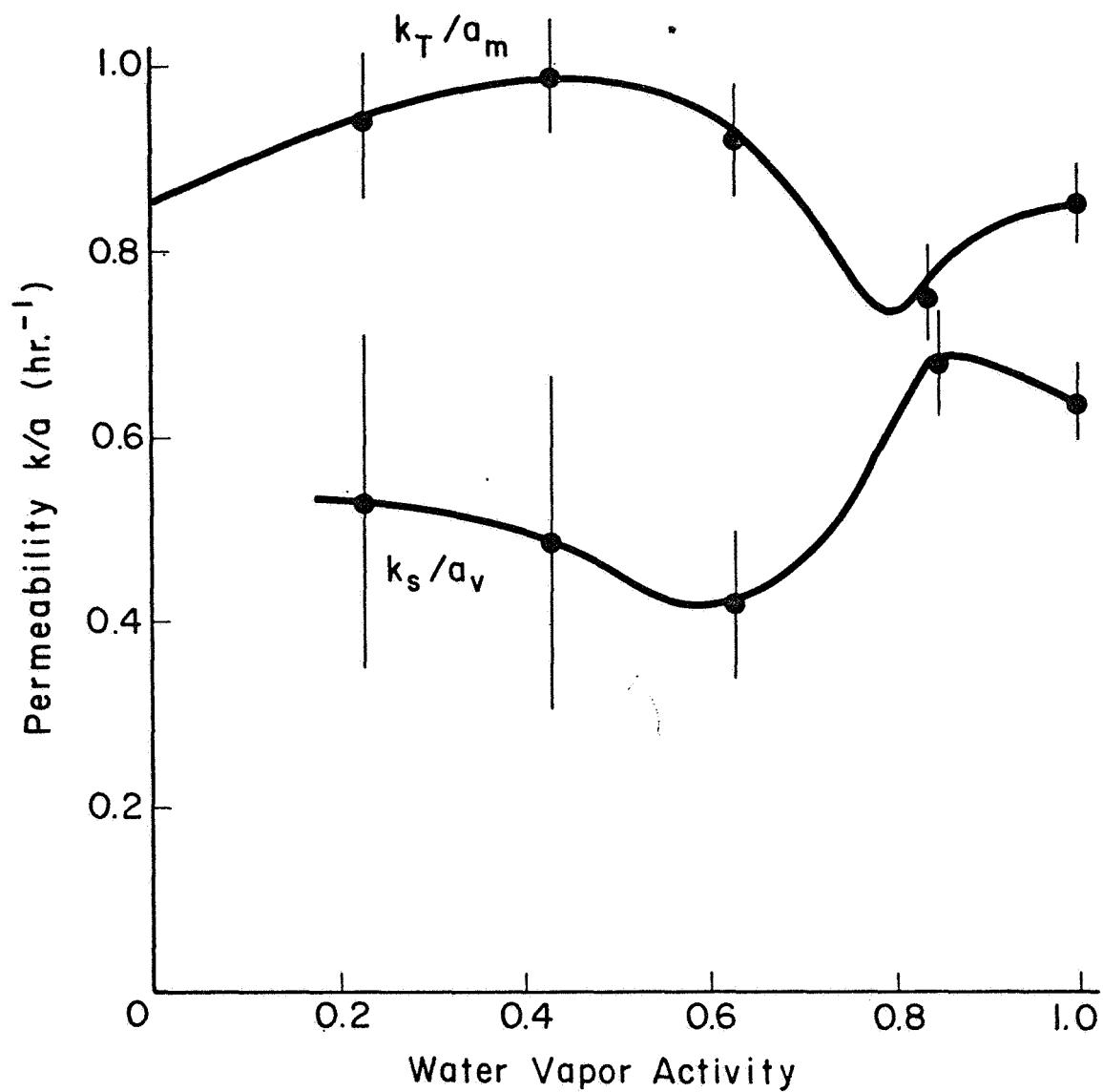


Figure 18. Permeability to transpiration, k_T/a_m , and to sorption, k_s/a_v , at various activities of water vapor at 25°C in carbon dioxide.

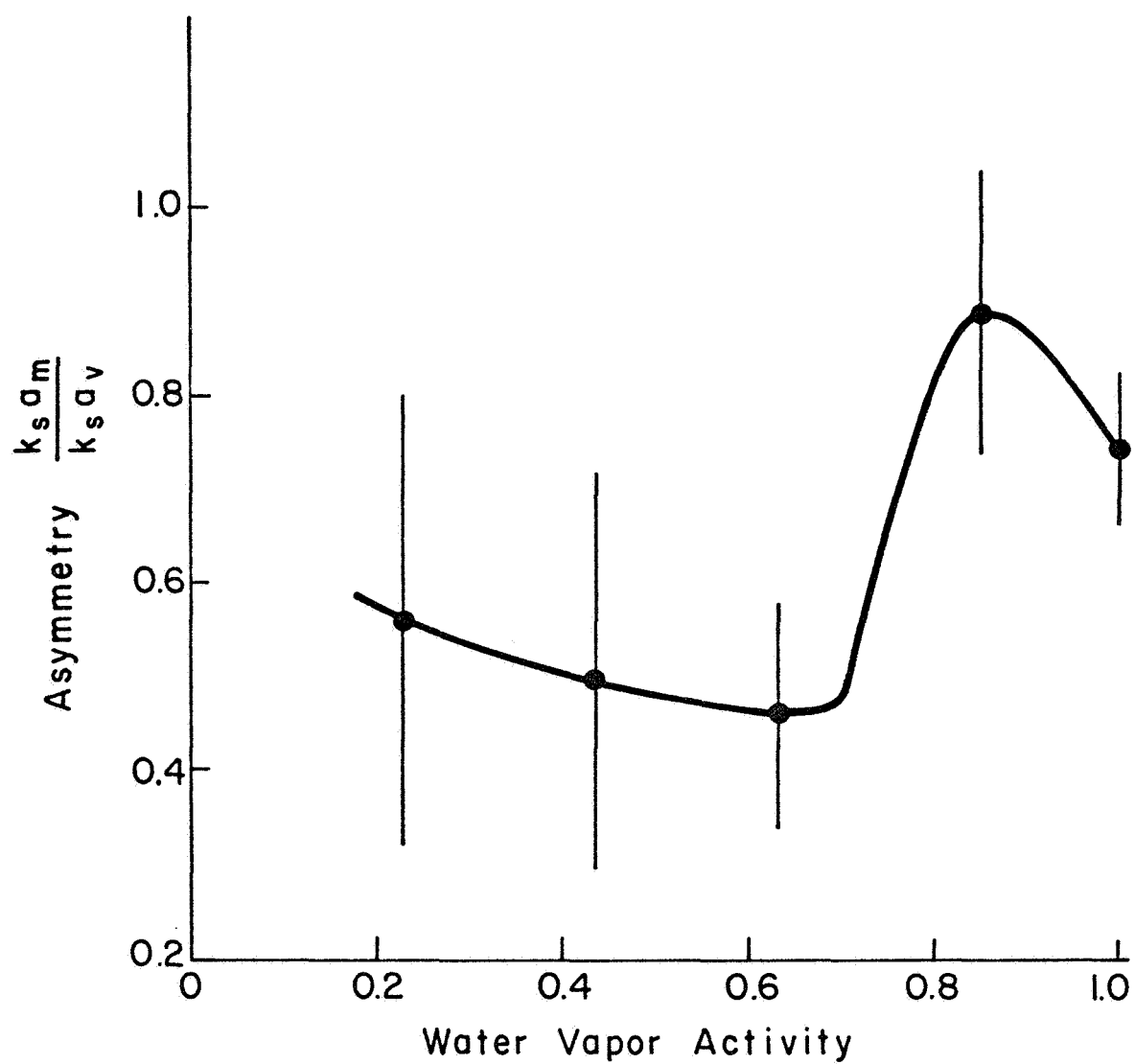


Figure 19. Asymmetry to exchange of water with vapor at various activities of water vapor in carbon dioxide.

The permeabilities to sorption and transpiration in air at 15°C both show greater increases with water vapor activity than were observed at 25°C. At 15°C the two permeabilities are more nearly equal over a wide range of water vapor activity (Figure 20). The ratio of the permeability at 25°C to that at 15°C is equal to the ratio of the rate constants. These were determined to be approximately two for both transpiration and sorption over the range of water vapor activity 0.4 to 0.8 (Figure 15).

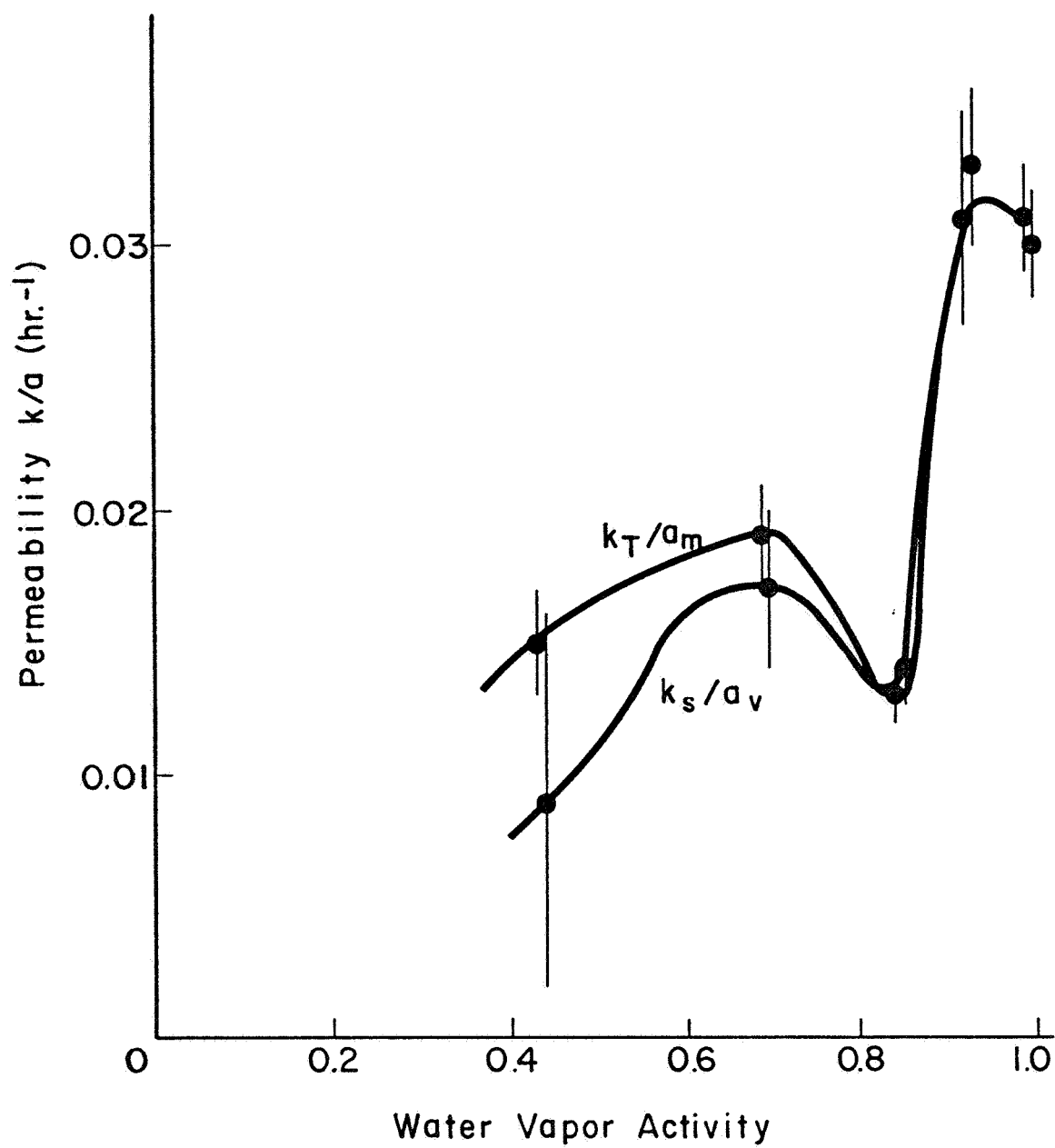


Figure 20. Permeability to transpiration, k_T/a_m , and to sorption k_s/a_v , at various activities of water vapor at 15°C.

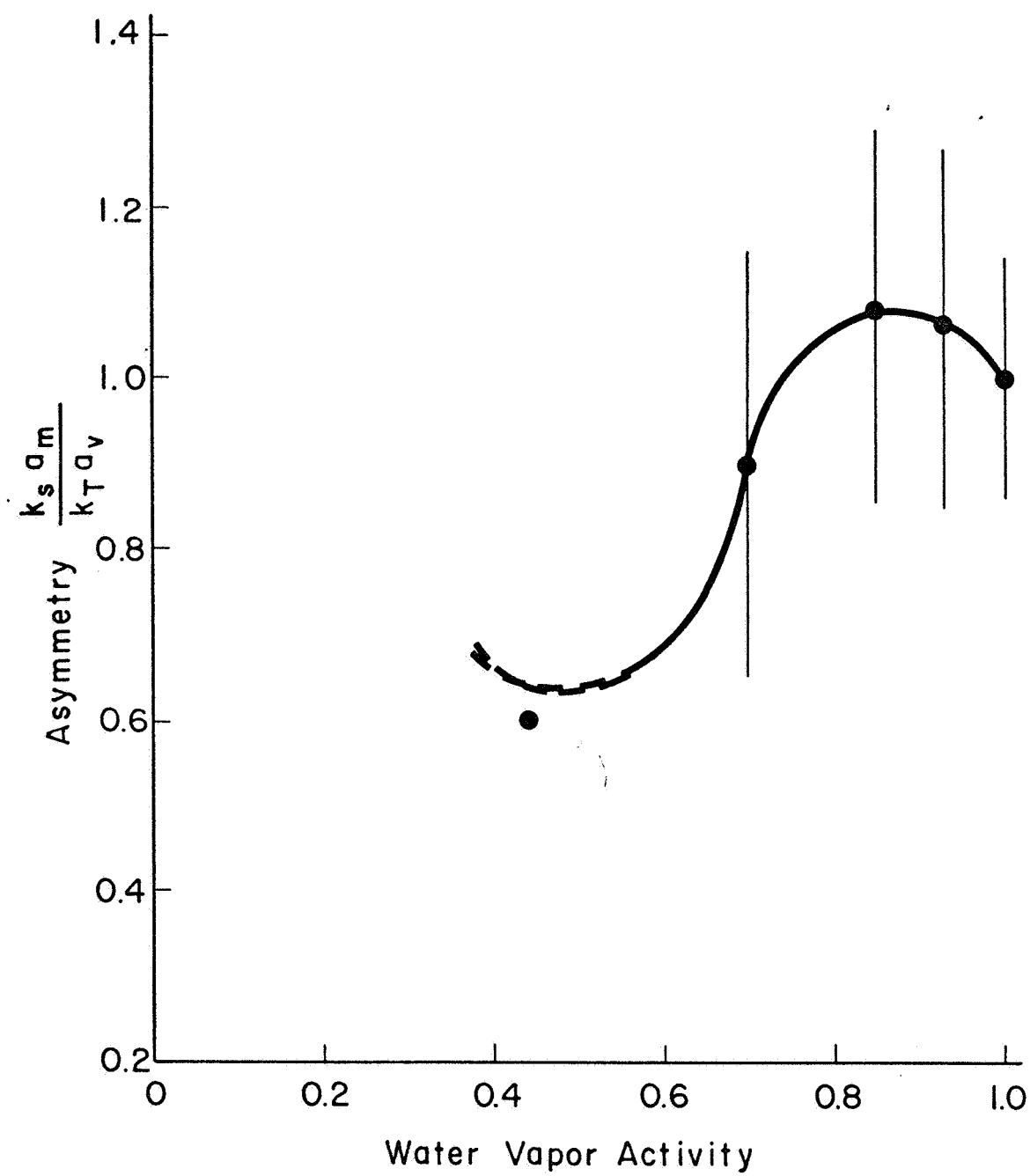


Figure 21. Asymmetry to water exchange with vapor at various activities of water vapor at 15°C in air.

CHAPTER V

DISCUSSION

The preceeding graphs constitute an empirical model of the water exchange system of Laelaps echidnina adult females. From these graphs mathematical expressions can be developed to describe the observed transpiration and sorption.

Examination of the trace of k_T versus a_v (Figure 9) reveals points of inflection at $a_1=0.75$, $a_2=0.85$, and possibly at $a_3=0.50$. These values are the roots of the equation for the second derivitive of k_T with respect to a_v :

$$\begin{aligned}\frac{d^2 k_T}{d a_v^2} &= b_1 (a_v - a_1) (a_v - a_2) (a_v - a_3) \\ &= b_1 a_v^3 - (a_1 + a_2 + a_3) a_v^2 - (a_1 a_2 + a_2 a_3 + a_1 a_3) a_v - a_1 a_2 a_3\end{aligned}$$

Integration of this expression with respect to a_v gives the first derivitive:

$$\begin{aligned}\frac{d k_T}{d a_v} &= b_1 \left[\frac{a_v^4}{4} - \frac{(a_1 + a_2 + a_3)}{3} a_v^3 - \frac{(a_1 a_2 + a_2 a_3 + a_1 a_3)}{2} a_v^2 \right. \\ &\quad \left. - a_1 a_2 a_3 a_v + C \right]\end{aligned}$$

Examination of the trace of k_T versus a_v (Figure 9) reveals a maximum at $a_4=0.65$ and a minimum at $a_5=0.80$. These values are the roots of the first derivitive of k_T with respect to a_v and may be used to determine the constant of integration above. The resulting equation

is an eighth order expression of the form:

$$\frac{d k_T}{d a_V} = \left[f'(a_V) - f'(a_4) \right] \left[f'(a_V) - f'(a_5) \right]$$

where f' is the first four terms of the previous expression evaluated for the indicated a_V . The first derivative must again be integrated with respect to a_V to obtain an equation for k_T where the initial condition, $k_T = 3 \text{ \%/hr}$ at $a_V = 0$, is used to determine the constant of integration. The resulting ninth order polynomial is of little use in identifying the water exchange mechanisms, the active and passive components of the observed rate constants, or the contributions of the integument and tracheal system to the total water exchange.

The discussion above presented eight hypothetical components of water exchange resulting from active transport and diffusion through the tracheal system and the cuticle for both transpiration and sorption. These components may be resolved from the observed total transpiration and sorption with the aid of certain assumptions designed to eliminate some of the variables.

The terms for active and passive transport may at first be combined in order to determine the tracheal and cuticular components of transpiration and sorption. These four terms may then be further resolved into the active and passive components. The acceptable model of tracheal and cuticular components is derived by elimination of hypotheses which permit resolution of the total rate constants but which produce contradictions between the results and the observations.

The significant components of water transport in the mite may be represented by:

$$k_t^- = \text{tracheal transpiration}$$

$$k_t^+ = \text{tracheal sorption}$$

$$k_c^- = \text{cuticular transpiration}$$

$$k_c^+ = \text{cuticular sorption}$$

These rate constants are related to the measured rate constants by:

$$k_s = k_t^+ + k_c^+$$

$$k_T = k_t^- + k_c^-$$

The two independent equations involve four variables of which two must be eliminated by hypotheses. One set of hypotheses which permits the solution of these equations is:

- 1) Assume the tracheal exchange to be symmetric in all conditions:

$$\frac{k_t^+}{a_v} = \frac{k_t^-}{a_m} \quad \text{with } a_m = 1$$

- 2) Assume the cuticular transpiration, k_c^- , to be constant with respect to water vapor activity (a suggestion offered by Prof. Willi Knülle).

- 3) Assume that as water vapor activity decreases, the cuticular sorption approaches zero more rapidly than tracheal sorption.

The equations for the component rate constants are:

$$k_c^- = k_T - k_s \frac{a_m}{a_v} \quad a_v < 0.35$$

$$k_t^- = k_T - k_c^-$$

$$k_t^+ = k_t^- \frac{a_m}{a_v}$$

$$k_c^+ = k_s - k_t^+$$

By iteration of values for k_c^- the resulting values of k_c^+ can be made to approach zero in low water vapor activities. Under these assumptions all components are affected by temperature and carbon dioxide (Table 2). That the increase in cuticular transpiration, $k_c^-(CO_2) = 5 k_c^-(air)$, should be greater than the increase in tracheal transpiration, $k_t^-(CO_2) = 2.2 k_t^-(air)$, seems improbable. This result also contradicts an observation of Knulle that cuticular transpiration is unaffected by carbon dioxide in an acarine that has no tracheal system (personal communication).

TABLE 2

COMPONENT RATE CONSTANTS EVALUATED FOR SYMMETRIC TRACHEAL EXCHANGE AND CUTICULAR TRANSPIRATION CONSTANT WITH RESPECT TO WATER VAPOR ACTIVITY. Rate constant dimensions are %/hr.

a_v	25°C in Air				25°C in CO ₂				15°C in CO ₂			
	k_c^-	k_t^-	k_t^+	k_c^+	k_c^-	k_t^-	k_t^+	k_c^+	k_c^-	k_t^-	k_t^+	k_c^+
0.07	1.0	2.0	0.14	0.00								
0.225	1.0	2.0	0.45	0.05	5.0	4.4	1.0	0.2				
0.325	1.0	2.1	0.68	0.43								
0.43	1.0	2.3	0.99	0.15	5.0	4.9	2.1	0.0				
0.44									0.5	1.0	0.44	0.0
0.53	1.0	2.1	1.1	0.20								
0.625	1.0	2.5	1.5	0.00	5.0	4.2	2.6	0.0				
0.70									0.5	1.4	0.98	0.32
0.715	1.0	2.4	1.7	0.15								
0.755	1.0	2.1	1.5	0.50								
0.80	1.0	2.0	1.6	0.60								
0.85	1.0	2.8	2.4	0.70	5.0	2.5	2.2	3.5	0.5	0.8	0.68	0.52
0.925	1.0	2.8	2.6	1.2								
0.93									0.5	2.6	2.4	0.7
1.00					5.0	3.7	3.7	2.7	0.5	2.6	2.6	0.5

The hypotheses of the first solution may be extended to include the assumption that cuticular transpiration in a high concentration of carbon dioxide is equal to that in air. If, in addition, tracheal exchange is assumed to be symmetric the values for the water exchange components in air are identical to those determined in the first case but the values for cuticular sorption are significantly negative (Table 3). This contradicts the hypothesis of separate components of transpiration and sorption through the cuticle and indicates that tracheal exchange cannot be symmetric if cuticular transpiration is constant with respect to both water vapor activity and carbon dioxide concentration.

TABLE 3

COMPONENT RATE CONSTANTS EVALUATED FOR SYMMETRIC TRACHEAL EXCHANGE AND CUTICULAR TRANSPIRATION CONSTANT WITH RESPECT TO BOTH CARBON DIOXIDE CONCENTRATION AND WATER VAPOR ACTIVITY. Rate constants are in dimensions of %/hr.

a_v	25°C in CO ₂			
	k_c^-	k_t^-	k_t^+	k_c^+
0.225	1.0	8.4	1.8	-0.6
0.43	1.0	8.9	3.8	-1.7
* 0.625	1.0	8.2	5.1	-2.5
0.85	1.0	6.6	4.5	-0.7
1.00	1.0	7.5	8.5	-2.1

An acceptable solution for the components with cuticular transpiration constrained to be constant with respect to both water vapor activity and carbon dioxide concentration results from the assumption of symmetric cuticular exchange. In this case the tracheal exchange is very asymmetric. As water vapor activity approaches zero the tracheal sorption must approach zero more rapidly than cuticular sorption. The limit of permeability to sorption as water vapor activity approaches zero is then the permeability of the cuticle to sorption, which by hypothesis is equal to the permeability to transpiration through the cuticle. The equations for the components are:

$$k_c^- = a_m \lim_{a_v \rightarrow 0} \frac{k_s}{a_v}$$

$$k_t^- = k_T - k_c^-$$

$$k_c^+ = \frac{a_v}{a_m} k_c^-$$

$$k_t^+ = k_s - k_c^+$$

The superscripts indicate transpiration, -, and sorption, +; the subscripts indicate tracheal, t, and cuticular, c, flow. By iteration of values for k_c^- the value of k_t^+ may be made to approach zero with a_v . (Table 4). The values of cuticular sorption may be regarded as minimal, for the case of constant cuticular transpiration, because an active transport pump in the cuticle must produce an asymmetry favoring sorption. The upper limit of k_c^+ is, by hypothesis, k_s in air. The ratios of the tracheal components in carbon dioxide to those in air are acceptable under the present assumptions (Table 4).

TABLE 4

COMPONENT RATE CONSTANTS EVALUATED FOR CUTICULAR EXCHANGE SYMMETRIC AND CUTICULAR TRANSPIRATION CONSTANT WITH RESPECT TO BOTH ACTIVITY OF WATER VAPOR AND CARBON DIOXIDE CONCENTRATION. Rate constants are in dimensions of %/hr.

a_v	25°C in Air				25°C in CO ₂			
	k_c^-	k_t^-	k_c^+	k_t^+	k_c^-	k_t^-	k_c^+	k_t^+
0.07	1.0	2.0	0.07	0.03				
0.225	1.0	2.0	0.22	0.28	1.0	8.4	0.22	0.98
0.325	1.0	2.1	0.32	0.78				
0.43	1.0	2.3	0.43	0.72	1.0	8.9	0.43	1.7
0.53	1.0	2.2	0.53	0.47				
0.625	1.0	2.5	0.62	0.88	1.0	8.2	0.62	2.0
0.715	1.0	2.4	0.71	1.1				
0.755	1.0	2.1	0.76	1.2				
0.80	1.0	2.0	0.80	1.4				
0.85	1.0	2.8	0.85	2.3	1.0	6.6	0.85	5.0
0.93	1.0	2.8	0.93	2.8				
1.00	1.0	4.5	1.00	4.5	1.0	7.5	1.0	5.4

These results (Table 4) are consistent with the empirical model for water exchange in Laelaps echidnina but are not unique solutions for the components. The hypotheses of symmetric cuticular exchange and constant cuticular transpiration must be regarded as a tentative model on which to base further experimentation and alternative analyses.

The observed rate constants may be resolved into components of diffusion and active transport represented by:

$$k_d^- = \text{transpiration by diffusion}$$

$$k_d^+ = \text{sorption by diffusion}$$

$$k_p^- = \text{transpiration by active transport}$$

$$k_p^+ = \text{sorption by active transport}$$

The term k_p^- represents that portion of the pump efflux which escapes to the atmosphere and the term k_p^+ represents the influx of atmospheric water through the pump. The actual flow of water in a pump cycle may be greater than that exchanged with the body fluids and atmosphere. The components are related to the observed rate constants by:

$$k_s = k_d^+ + k_p^+$$

$$k_T = k_d^- + k_p^-$$

These two independent equations involve four variables of which two may be eliminated by hypotheses.

If diffusion is assumed to be symmetric and the pump efflux is assumed to be constant with respect to water vapor activity the value of k_p^+ approaches zero more rapidly than k_d^+ and the components are:

$$k_p^- = k_T - \frac{a_m}{a_v} k_s \quad \text{for small } a_v$$

$$k_d^- = k_T - k_p^-$$

$$k_d^+ = \frac{a_v}{a_m} k_d^-$$

$$k_p^+ = k_s - k_d^+$$

These assumptions and relations are mathematically identical to those of the first case considered for tracheal and cuticular components. The numerical values of the components for symmetric diffusion and constant active transpiration are therefore identical to those for the components of symmetric tracheal exchange and constant cuticular transpiration respectively (Table 2). This relation is strictly numerical and does not require the tracheal transport to be entirely by diffusion or the active transport to be entirely through the cuticle. The increase in active transpiration (k_c^- in Table 2) in carbon dioxide over that in air is now greater than the corresponding increases in both transpiration by diffusion (k_t^- in Table 2) and active sorption (k_c^+ in Table 2). The increase in diffusion with carbon dioxide may be associated with tracheal exchange where the spiracles are presumed to open under the influence of carbon dioxide.

If the assumption is made that the pump operates at constant maximum efficiency in water vapor of activity below the critical equilibrium value and that the pump is unaffected by carbon dioxide the resulting equations for the components are mathematically identical to those in the second case considered for cuticular and tracheal components where $k_p^- = k_c^-$, $k_d^- = k_t^-$, $k_d^+ = k_t^+$, and $k_p^+ = k_c^+$ (values in CO_2 are given in Table 3, those in air are given in Table 2). These relations are only numerical and do not require identification of active transport entirely with the cuticle. Under these assumptions the active sorption values are negative in carbon dioxide, a contradiction similar to that derived above.

The acceptable solution for the resolution of active and passive components of the observed rate constants is similar to that determined for cuticular and tracheal components. Diffusion need not be symmetric in composite, concentration dependent membranes as was assumed in the preceeding argument. The active transport pump efficiency may be reduced by carbon dioxide but probably not increased, thus the assumption of constant permeability of the pump is relatively sound. The pump may then be assumed to be symmetric with respect to some activity, a_p , of the pump efflux. The value of a_p is probably somewhat lower than the critical equilibrium activity and so the assumption of symmetry with respect to $a_p=1.0$ gives an upper limit to k_p^+ :

$$\frac{k_p^-}{a_p} = \frac{k_p^+}{a_v} > \frac{k_p^-}{a_m}$$

$$k_p^- < \frac{a_m}{a_v} k_p^+ = a_m \lim_{a_v \rightarrow 0} \frac{k_p^+}{a_v} = 0 \quad \frac{k_s}{a_v}$$

Under these assumptions the diffusion is very asymmetric in favor of transpiration while the pump is asymmetric towards sorption. In low values of water vapor activity the sorption by diffusion must approach zero so that k_p^+ approaches the value of k_s . The components are then:

$$k_p^- = \frac{a_m}{a_v} k_s \quad \text{low } a_v$$

$$k_p^+ = \frac{a_v}{a_m} k_p^-$$

$$k_d^- = k_T - k_p^-$$

$$k_d^+ = k_s - k_p^+$$

These equations are mathematically identical to those determined for constant cuticular transpiration with symmetric cuticular exchange. The numerical values for the pump components correspond to those given for cuticular components and the values of the diffusion components correspond to those given for the tracheal components (Table 4). Active transpiration is constant at 1 %/hr at 25 °C in air and in carbon dioxide. Active sorption is equal to a_v %/hr at 25 °C in air and in carbon dioxide. Transpiration by diffusion varies with water vapor activity from 2 %/hr to 3 %/hr in air at 25 °C and from 6.6 %/hr to 8 %/hr in carbon dioxide. Sorption by diffusion approaches zero with water vapor activity at 25 °C in air. These values are consistent with existing information on water balance in Laelaps echidnina and with the observations of Knulle and Wharton on acarines that have no tracheal systems.

It remains to relate the four components of active and passive transport to the four components of cuticular and tracheal exchange. The assumptions in the preceeding analyses are contradictory in that cuticular exchange and active transport cannot both be symmetric if cuticular diffusion is not constant. A trivial solution results if cuticular exchange is entirely active and tracheal exchange is entirely passive (Table 4). Alternative solutions for the eight components require further information with which to relate or eliminate certain components. The ratio of tracheal to cuticular exchange would give a valid solution, another results from the ratio of pump to diffusion components.

CONCLUSIONS

The exchange of tritiated water between the mite Laelaps echidnina and the surrounding vapor was observed to be a first order rate process for both uptake of HTO from vapor by initially untritiated mites and for loss of HTO to untritiated vapor by initially tritiated mites. All tritium, absorbed from vapor as HTO, was found to remain as water in the mite. Both content and specific activity of HTO in the mite fluids have characteristic rate constants which were theoretically and experimentally demonstrated to be the rate constants for transpiration of water from the mite and sorption of water from vapor, respectively. These rate constants were found to vary independently and significantly with the activity of water vapor, with temperature, and in a high concentration of carbon dioxide.

The water loss of the mite in a constant desiccating atmosphere was also observed to be a first order rate process with a rate constant equal to the difference in the rate constants for sorption and transpiration. This time dependence of water mass was also derived from the theory of diffusion for surface limited evaporation from solids. The rate constant for water mass can be as much as 3 %/hr in air at 25°C, 1 %/hr in air at 15°C, and 10 %/hr at 25°C in a high concentration of carbon dioxide. All variations of the mass loss rate constant were identified with corresponding variations in the rate constants for sorption and transpiration.

The increase in the rate constant for transpiration in carbon dioxide over that in air varies from 3 %/hr to 7 %/hr in proportion to the difference in activity of water between mite fluids and vapor. The increase in sorption in carbon dioxide over that in air is a constant 1 %/hr over most of the range of water vapor activity, but values to 2.5 %/hr were indicated in vapor of activity around 0.85.

The ratios of the rate constants at 25°C to those at 15°C were about two with that of transpiration slightly higher, having a maximum value of 2.3 in water vapor activity 0.85. This value of Q_{10} could be associated with membrane diffusion or an active process.

Permeability of the water exchange barrier to sorption, the ratio of the sorption rate constant to the water vapor activity, and permeability to transpiration, the ratio of transpiration rate constant to activity of water in the mite fluids, were observed to vary independently and significantly with water vapor activity, temperature, and carbon dioxide concentration. The permeability to sorption was observed to have a minimum of water vapor activity of 0.5 under all experimental conditions.

Hypothetical models were developed to resolve the observed values of transpiration and sorption into components through the major water exchange surfaces, the cuticle and tracheal system. The hypothesis of cuticular transpiration constant with respect to both water vapor activity and carbon dioxide concentration is acceptable only if tracheal exchange is not symmetric; cuticular exchange may be symmetric in this case.

Similar models were examined for the determination of active and passive transport components of the observed rate constants. The plausible assumption of constant active transpiration is acceptable if the active exchange is symmetric but not if the passive, i.e. diffusion, exchange is symmetric. The components of active and passive transport can be further resolved into their contributions to exchange through the tracheal system and cuticle. These hypothetical models are not unique solutions of the transport components but are regarded as tentative models on which to base further experimentation and analysis.

The variations of the rate constants with total atmospheric pressure may permit determination of the diffusion components of water exchange. Microtubules inserted into the trachea of anesthetized mites would permit determination of the cuticular and tracheal components of water exchange. The response of the rate constants to an oscillating water vapor activity and the associated determination of the transfer function may give further information on the system components. In an open vapor train the transpiration of HTO from the mite could be continuously monitored to observe variations in transpiration with time. The electrostatic pump hypothesized by Beament could perhaps be inactivated by a radio frequency field of broad frequency band and moderate intensity which would probably not affect a carrier molecule pump.

APPENDIX A

TABLE 5

SUMMARY OF DATA 25 °C

Activity of Water Vapor a_v	Exposure Time t	Chamber	Number of Mites	Water Mass $m \pm s_m$ (ug)	Tritium Content $T \pm s_T$ (cpm)	Specific Activity $S \pm s_s$ (cpm/ug)
0.925	+ 5 d	eq	15	122 $\pm$ 1.4	422 $\pm$ 40	3.47 $\pm$ 0.13
0.07	-19.5 h	vt	11	61 $\pm$ 9.5	199 $\pm$ 42	3.26 $\pm$ 0.30
0.07	-25.0 h	vt	16	55 $\pm$ 11	189 $\pm$ 36	3.44 $\pm$ 0.30
0.925	+ 6 d	eq	8	121 $\pm$ 9.2	314 $\pm$ 27	2.59 $\pm$ 0.13
0.07	-20.0 h	vc	8	65 $\pm$ 11	178 $\pm$ 15	2.74 $\pm$ 0.17
0.07	-30.0 h	vc	19	55 $\pm$ 10	139 $\pm$ 24	2.53 $\pm$ 0.20
0.925	+ 6 d	eq	12	113 $\pm$ 22	594 $\pm$ 130	5.24 $\pm$ 0.39
0.225	-23.0 h	vc	12	66 $\pm$ 10	331 $\pm$ 41	5.01 $\pm$ 0.48
0.925	+ 9 d	eq	24	107 $\pm$ 14	513 $\pm$ 53	4.80 $\pm$ 0.26
0.225	- 9.0 h	vc	14	80 $\pm$ 12	395 $\pm$ 54	4.92 $\pm$ 0.32
0.925	+ 6 d	eq	15	110	469	4.24
0.225	-11.0 h	vc	19	79	324	4.10
0.925	+ 4 d	eq	17	118 $\pm$ 12	508 $\pm$ 61	4.29 $\pm$ 0.22
0.325	-19.2 h	vt	13	79 $\pm$ 8.5	263 $\pm$ 32	3.35 $\pm$ 0.30
0.325	-27.5 h	vt	13	64 $\pm$ 12	198 $\pm$ 33	3.09 $\pm$ 0.41
0.325	-32.5 h	vt	11	62 $\pm$ 15	154 $\pm$ 37	2.50 $\pm$ 0.40
0.325	-42.5 h	vt	9	55 $\pm$ 12	159 $\pm$ 33	2.92 $\pm$ 0.39
0.925	+ 6 d	eq	8	102 $\pm$ 16	446 $\pm$ 59	4.38 $\pm$ 0.28
0.325	-24.0 h	vt	9	64 $\pm$ 14	210 $\pm$ 52	3.35 $\pm$ 0.68
0.325	-31.0 h	vt	8	62 $\pm$ 23	195 $\pm$ 29	3.14 $\pm$ 0.85

SUMMARY OF DATA 25°C CONTINUED

Activity of Water Vapor a_v	Exposure Time t	Chamber	Number of Mites	Water Mass $m \pm s_m$ (ug)	Tritium Content $T^* \pm s_{T^*}$ (cpm)	Specific Activity $S \pm s_s$ (cpm/ug)
0.925	+ 6 d	eq	20	112 $\pm$ 10	337 $\pm$ 23	3.03 $\pm$ 0.19
0.43	-24.0 h	vt	15	64 $\pm$ 13	145 $\pm$ 45	2.37 $\pm$ 0.62
0.43	-32.0 h	vt	18	59 $\pm$ 9	117 $\pm$ 19	1.98 $\pm$ 0.23
0.925	+ 6 d	eq	9	109 $\pm$ 7.0	370 $\pm$ 22	3.40 $\pm$ 0.22
0.53	-10.0 h	vc	7	84 $\pm$ 10	278 $\pm$ 56	3.34 $\pm$ 0.24
0.53	-13.0 h	vc	9	86 $\pm$ 9.3	258 $\pm$ 26	3.01 $\pm$ 0.08
0.53	-19.0 h	vc	13	66 $\pm$ 11	195 $\pm$ 39	2.95 $\pm$ 0.36
0.53	-19.0 h	vt	8	62 $\pm$ 8.3	186 $\pm$ 50	3.02 $\pm$ 0.45
0.53	-22.0 h	vc	14	66 $\pm$ 13	187 $\pm$ 29	2.88 $\pm$ 0.42
0.53	-22.0 h	vc	8	65 $\pm$ 11	182 $\pm$ 13	2.83 $\pm$ 0.63
0.53	-12.0 h	vt	9	77 $\pm$ 11	230 $\pm$ 28	2.98 $\pm$ 0.23
0.925	+ 6 d	eq	10	108 $\pm$ 6.1	309 $\pm$ 38	2.80 $\pm$ 0.18
0.53	-17.0 h	vt	7	73 $\pm$ 8.3	185 $\pm$ 27	2.55 $\pm$ 0.29
0.53	-17.5 h	vc	12	78 $\pm$ 12	195 $\pm$ 10	2.62 $\pm$ 0.39
0.53	-26.0 h	vc	8	69 $\pm$ 11	148 $\pm$ 29	2.14 $\pm$ 0.15
0.53	-36.0 h	vc	5	49 $\pm$ 14	111 $\pm$ 34	2.25 $\pm$ 0.43
0.925	+12 d	eq	14	120 $\pm$ 10	969 $\pm$ 48	8.08 $\pm$ 0.42
0.53	-16.0 h	vc	6	91 $\pm$ 19	532 $\pm$ 48	5.86 $\pm$ 0.83
0.925	+ 6 d	eq	20	112 $\pm$ 10	337 $\pm$ 23	3.02 $\pm$ 0.19
0.625	-24.0 h	vt	15	68 $\pm$ 10	143 $\pm$ 26	2.11 $\pm$ 0.33
0.925	+ 6 d	eq	20	114 $\pm$ 16	661 $\pm$ 68	5.81 $\pm$ 0.33
0.625	-18.0 h	vt	19	80 $\pm$ 13	365 $\pm$ 43	4.54 $\pm$ 0.50
0.625	-31.0 h	vt	18	57 $\pm$ 11	200 $\pm$ 62	3.50 $\pm$ 0.70
0.625	-42.0 h	vt	5	45 $\pm$ 7.0	120 $\pm$ 69	2.80 $\pm$ 0.65

SUMMARY OF DATA. 25 °C CONTINUED

Activity of Water Vapor a_v	Exposure Time t	Chamber	Number of Mites	Water Mass $m \pm s_m$ (ug)	Tritium Content $T^* \pm s_{T^*}$ (cpm)	Specific Activity $S \pm s_s$ (cpm/ug)
0.925	+ 6 d	eq	13	110 $\pm$ 16	655 $\pm$ 72	5.96 $\pm$ 0.44
0.715	-22.0 h	vt	13	82 $\pm$ 9.3	328 $\pm$ 49	3.97 $\pm$ 0.36
0.715	-29.0 h	vt	14	69 $\pm$ 11	240 $\pm$ 62	3.46 $\pm$ 0.63
0.715	-35.5 h	vt	8	63 $\pm$ 17	182 $\pm$ 14	2.90 $\pm$ 0.66
0.925	+ 6 d	eq	8	121 $\pm$ 9.2	314 $\pm$ 27	2.58 $\pm$ 0.13
0.755	-19.5 h	vt	12	100 $\pm$ 13	176 $\pm$ 26	1.78 $\pm$ 0.18
0.755	-24.0 h	vt	16	93 $\pm$ 5.6	153 $\pm$ 18	1.66 $\pm$ 0.16
0.755	-29.0 h	vt	16	91 $\pm$ 14	141 $\pm$ 15	1.55 $\pm$ 0.13
0.755	-34.0 h	vt	16	88 $\pm$ 13	110 $\pm$ 23	1.24 $\pm$ 0.26
0.755	-44.0 h	vt	20	84 $\pm$ 6.5	84 $\pm$ 19	1.01 $\pm$ 0.23
0.755	-53.0 h	vt	16	78 $\pm$ 8.8	71 $\pm$ 17	0.92 $\pm$ 0.26
0.925	+ 5 d	eq	20	114 $\pm$ 16	661 $\pm$ 68	5.81 $\pm$ 0.33
0.755	-19.0 h	vc	17	100 $\pm$ 16	369 $\pm$ 61	3.69 $\pm$ 0.29
0.755	-31.0 h	vc	18	74 $\pm$ 17	201 $\pm$ 79	2.69 $\pm$ 0.80
0.755	-42.0 h	vc	13	70 $\pm$ 14	142 $\pm$ 43	2.08 $\pm$ 0.50
0.755	-50.0 h	vc	11	63 $\pm$ 14	155 $\pm$ 15	2.44 $\pm$ 0.52
0.925	+ 6 d	eq	12	113 $\pm$ 22	594 $\pm$ 130	5.24 $\pm$ 0.39
0.80	-17.0 h	vc	14	97 $\pm$ 14	315 $\pm$ 64	3.26 $\pm$ 0.37
0.80	-23.0 h	vc	9	82 $\pm$ 20	174 $\pm$ 20	2.06 $\pm$ 0.47
0.80	-33.0 h	vc	13	75 $\pm$ 16	154 $\pm$ 42	2.04 $\pm$ 0.36
0.925	+ 9 d	eq	11	102 $\pm$ 18	534 $\pm$ 45	5.23 $\pm$ 0.53
0.80	-22.5 h	vc	16	87 $\pm$ 13	274 $\pm$ 67	3.15 $\pm$ 0.70
0.80	-30.5 h	vc	17	89 $\pm$ 12	237 $\pm$ 48	2.66 $\pm$ 0.71
0.80	-37.5 h	vc	12	89 $\pm$ 9.0	215 $\pm$ 51	2.42 $\pm$ 0.43

SUMMARY OF DATA 25 °C CONTINUED

Activity of Water Vapor a_v	Exposure Time t	Chamber	Number of Mites	Water Mass $m \pm s_m$ (ug)	Tritium Content $T^* \pm s_{T^*}$ (cpm)	Specific Activity $S \pm s_s$ (cpm/ug)
0.925	+ 6 d	eq	20	112 $\pm$ 10	337 $\pm$ 23	3.02 $\pm$ 0.19
0.85	-24.0 h	vc	14	96 $\pm$ 15	135 $\pm$ 22	1.40 $\pm$ 0.18
0.85	-32.5 h	vc	15	94 $\pm$ 15	104 $\pm$ 20	1.13 $\pm$ 0.26
0.85	-47.0 h	vc	14	85 $\pm$ 15	55 $\pm$ 18	0.65 $\pm$ 0.27
0.925	+ 6 d	eq	12	113 $\pm$ 22	594 $\pm$ 130	5.24 $\pm$ 0.39
0.85	-16.5 h	vt	11	96 $\pm$ 12	282 $\pm$ 27	2.94 $\pm$ 0.45
0.925	+ 4.5 d	eq	4	99 $\pm$ 8	1430 $\pm$ 40	13.5 $\pm$ 0.50
0.925	-24.0 h	vc	17	95 $\pm$ 12	165 $\pm$ 35	1.79 $\pm$ 0.47
0.925	+24.0 h	eq	15	112 $\pm$ 15	985 $\pm$ 58	8.80 $\pm$ 1.50
0.925	+28.0 h	eq	16	103 $\pm$ 13	836 $\pm$ 49	8.16 $\pm$ 1.11
0.925	-28.5 h	vc	12	106 $\pm$ 22	509 $\pm$ 185	4.78 $\pm$ 1.35
0.925	+34.0 h	eq	9	108 $\pm$ 16	1031 $\pm$ 142	9.56 $\pm$ 1.60
0.925	-33.5 h	vc	9	111 $\pm$ 19	380 $\pm$ 76	3.42 $\pm$ 0.85
0.925	+48.0 h	eq	9	100 $\pm$ 15	1101 $\pm$ 111	11.0 $\pm$ 1.4
0.925	-48.5 h	vc	10	102 $\pm$ 9.6	249 $\pm$ 20	2.44 $\pm$ 1.12
0.925	+ 6 d	eq	11	118 $\pm$ 8	1302 $\pm$ 85	11.1 $\pm$ 0.60
0.925	+26.0 h	eq	15	105 $\pm$ 15	744 $\pm$ 53	7.10 $\pm$ 1.35
0.925	-27.0 h	vc	16	114 $\pm$ 14	488 $\pm$ 180	4.35 $\pm$ 1.40
0.925	-44.0 h	vc	5	106 $\pm$ 10	231 $\pm$ 96	2.18 $\pm$ 0.90
0.925	+44.0 h	eq	19	115 $\pm$ 12	1030 $\pm$ 106	9.00 $\pm$ 0.84
0.925	+54.0 h	eq	19	108 $\pm$ 17	1041 $\pm$ 119	9.68 $\pm$ 1.03
0.925	+ 9 d	eq	17	107 $\pm$ 14	509 $\pm$ 53	4.75 $\pm$ 0.36
1.00	-22.0 h	vc	14	119 $\pm$ 16	136 $\pm$ 59	1.14 $\pm$ 0.43
1.00	-29.5 h	vc	16	111 $\pm$ 10	99 $\pm$ 42	0.89 $\pm$ 0.45
1.00	-47.5 h	vc	26	115 $\pm$ 8.3	28 $\pm$ 32	0.24 $\pm$ 0.13

eq - HTO equilibration chamber; vc - vapor chamber; vt - vapor train

SUMMARY OF DATA 25°C, CO₂

Activity of Water Vapor a_v	Exposure Time t	Chamber	Number of Mites	Water Mass $m \pm s_m$ (ug)	Tritium Content $T^* \pm s_{T^*}$ (cpm)	Specific Activity $S \pm s_s$ (cpm/ug)
0.925	+11 d	eq *	12	107 $\pm$ 8	496 $\pm$ 38	4.64 $\pm$ 0.28
0.225	-15.0 h	vt	12	28 $\pm$ 9	108 $\pm$ 34	3.85 $\pm$ 0.44
0.925	+ 6 d	eq *	15	110	469	4.26
0.43	-16.0 h	vc	8	32	99	3.06
0.625	-16.0 h	vc	14	38	108	2.84
0.925	+ 6 d	eq *	15	110	469	4.26
0.85	-11.0 h	vc	18	81	212	1.82
0.85	-18.0 h	vc	10	80	122	1.52
0.925	+11 d	eq *	12	107 $\pm$ 8.0	496 $\pm$ 38	4.64 $\pm$ 0.28
1.00	-15.0 h	vt	22	80 $\pm$ 9	140 $\pm$ 10	1.74 $\pm$ 0.36
1.00	-20.0 h	vt	26	70 $\pm$ 10	86 $\pm$ 24	1.28 $\pm$ 0.27

* The equilibration chamber contained HTO vapor in air.

SUMMARY OF DATA 15 °C

Activity of Water Vapor a_v	Exposure Time t	Chamber	Number of Mites	Water Mass $m \pm s_m$ (ug)	Tritium Content $T^* \pm s_{T^*}$ (cpm)	Specific Activity $S \pm s_s$ (cpm/ug)
0.925	+ 9 d	eq *	17	107 $\pm$ 14	509 $\pm$ 53	4.75 $\pm$ 0.36
0.44	-22.5 h	vt	15	84 $\pm$ 11	347 $\pm$ 39	4.15 $\pm$ 0.4
0.44	-30.0 h	vt	20	82 $\pm$ 14	338 $\pm$ 48	4.11 $\pm$ 0.45
0.925	+ 9 d	eq *	11	102 $\pm$ 18	534 $\pm$ 45	5.23 $\pm$ 0.53
0.70	-17.0 h	vt	17	90 $\pm$ 19	376 $\pm$ 68	4.18 $\pm$ 0.50
0.70	-25.0 h	vt	13	89 $\pm$ 10	353 $\pm$ 54	3.98 $\pm$ 0.30
0.925	+ 9 d	eq *	11	102 $\pm$ 18	534 $\pm$ 45	5.23 $\pm$ 0.53
0.85	-23.0 h	vt	12	93 $\pm$ 14	386 $\pm$ 41	4.15 $\pm$ 0.63
0.85	-31.0 h	vt	15	103 $\pm$ 14	357 $\pm$ 42	3.47 $\pm$ 0.27
0.85	-38.0 h	vt	16	104 $\pm$ 12	369 $\pm$ 41	3.56 $\pm$ 0.41
0.85	-49.0 h	vt	16	84 $\pm$ 11	231 $\pm$ 59	2.76 $\pm$ 0.52
0.925	+ 9 d	eq *	20	105 $\pm$ 26	264 $\pm$ 47	2.50 $\pm$ 0.21
0.925	-9.0 h	vc	15	123 $\pm$ 10	231 $\pm$ 25	1.87 $\pm$ 0.12
0.925	-18.5 h	vc	16	117 $\pm$ 16	195 $\pm$ 38	1.66 $\pm$ 0.15
0.925	-26.0 h	vc	8	111 $\pm$ 25	134 $\pm$ 31	1.21 $\pm$ 0.21
0.925	-44.5 h	vc	15	98 $\pm$ 11	56 $\pm$ 20	0.58 $\pm$ 0.14
0.925	+16.0 h	vc	13	114 $\pm$ 15	117 $\pm$ 31	1.02 $\pm$ 0.19
0.925	+25.0 h	vc	24	115 $\pm$ 21	173 $\pm$ 40	1.50 $\pm$ 0.29
0.925	+37.5 h	vc	17	124 $\pm$ 25	207 $\pm$ 58	1.67 $\pm$ 0.41
0.925	+52.0 h	vc	14	117 $\pm$ 21	239 $\pm$ 58	2.05 $\pm$ 0.51
0.925	+92.0 h	vc	18	115 $\pm$ 11	270 $\pm$ 21	2.34 $\pm$ 0.13
0.925	+ 9 d	eq *	7	107 $\pm$ 14	522 $\pm$ 53	4.89 $\pm$ 0.16
1.00	-18.0 h	vt	20	101 $\pm$ 10	288 $\pm$ 46	2.86 $\pm$ 0.20

* The HTO equilibration chamber was at 25 °C.

APPENDIX B — TABLE 6
VALUES OF PERMEABILITY AND ASYMMETRY

T (°C)	a _v	$\frac{k_T + s_T}{a_m}$ (%/hr)	$\frac{k_s + s_s}{a_v}$ (%/hr)	$\frac{k_m + s_m}{a_m - a_v}$ (%/hr)	$\frac{k_s a_m}{k_T a_v} \left[1 + \left(\frac{s_T s_s}{k_T k_s} \right) \right]$
25 (air)	0.07	3.0 ± 0.3	1.4 ± 5.6	3.1 ± 0.3	0.42 ± 1.7
	0.225	3.0 ± 0.4	2.2 ± 2.2	3.2 ± 0.5	0.73 ± 0.82
	0.325	3.1 ± 0.3	3.4 ± 1.2	3.0 ± 0.4	1.1 ± 0.84
	0.43	3.3 ± 0.2	2.7 ± 0.5	3.8 ± 0.4	0.82 ± 0.20
	0.53	3.2 ± 0.5	1.9 ± 0.6	4.7 ± 0.9	0.59 ± 0.28
	0.625	3.5 ± 0.3	2.4 ± 0.2	5.3 ± 0.5	0.68 ± 0.13
	0.715	3.4 ± 0.2	2.5 ± 0.1	5.6 ± 0.7	0.74 ± 0.09
	0.755	3.1 ± 0.3	2.65 ± 0.3	4.5 ± 1.2	0.86 ± 0.17
	0.80	3.0 ± 0.5	2.75 ± 0.5	4.0 ± 1.5	0.92 ± 0.32
	0.85	3.8 ± 0.2	3.65 ± 0.2	4.7 ± 2.0	0.96 ± 0.12
	0.925	3.8 ± 0.2	4.15 ± 0.2	0	1.09 ± 0.12
	0.925(s)	3.7 ± 0.6	4.00 ± 0.6	0	1.08 ± 0.35
	1.00	5.5 ± 0.5	5.5 ± 0.5	0	1.00 ± 0.18
25 (CO ₂)	0.225	9.4 ± 0.8	5.3 ± 1.8	10.6 ± 1.0	0.56 ± 0.24
	0.43	9.9 ± 0.6	4.9 ± 1.8	13.7 ± 1.0	0.50 ± 0.22
	0.625	9.2 ± 0.6	4.2 ± 0.8	17.6 ± 2.1	0.46 ± 0.12
	0.85	7.6 ± 0.5	6.8 ± 0.6	12.0 ± 5.3	0.89 ± 0.15
	1.00	8.5 ± 0.4	6.4 ± 0.4	0	0.75 ± 0.08
15 (air)	0.44	1.5 ± 0.2	0.9 ± 0.7	2.0 ± 0.4	0.60 ± 0.56
	0.70	1.9 ± 0.2	1.7 ± 0.3	2.3 ± 0.7	0.89 ± 0.25
	0.85	1.3 ± 0.1	1.4 ± 0.2	0.7 ± 1.3	1.08 ± 0.24
	0.93	3.1 ± 0.4	3.3 ± 0.3	0	1.06 ± 0.23
	0.93(s)	3.6 ± 0.6	3.8 ± 0.6	0	1.05 ± 0.34
	1.00	3.1 ± 0.2	3.0 ± 0.2	0	(1.00) ± 0.14
35 (air)	0.87	5.3 ± 0.8	5.3 ± 0.6	5.4 ± 0.3	1.0 ± 0.26
	0.87(s)	4.3 ± 0.8	5.0 ± 0.8	5.0 ± 0.3	1.0 ± 0.43

APPENDIX C -- TABLE 7

INCREASE OF RATE CONSTANTS, PERMEABILITIES, AND ASYMMETRIES IN CO₂ OVER THOSE IN AIR

a_v	$\Delta k_T = \frac{\Delta k_T}{a_m}$ (%/hr)	Δk_s (%/hr)	Δk_m (%/hr)	ΔA	$\frac{\Delta k_s}{a_v}$ (%/hr)	$\frac{\Delta k_m}{a_m - a_v}$ (%/hr)
0.225	+6.4 ± 0.9	+0.70 ± 0.64	+5.7 ± 0.9	-0.17 ± 1.1	+3.1 ± 2.8	6.8 ± 1.1
0.43	+6.6 ± 0.6	+0.95 ± 0.82	+5.7 ± 0.6	-0.32 ± 0.30	+2.2 ± 1.9	10 ± 1
0.625	+5.7 ± 0.7	+1.1 ± 0.5	+4.6 ± 0.8	-0.22 ± 0.18	+1.8 ± 0.8	12 ± 2
0.85	+3.8 ± 0.6	+2.7 ± 0.5	+1.1 ± 0.9	-0.07 ± 0.19	+3.2 ± 0.6	73 ± 60
1.00	+3.0 ± 0.6	+0.9 ± 0.6	+2.1 ± 0.2	-0.25 ± 0.20	+0.9 ± 0.6	∞

APPENDIX C --- TABLE 8

FRACTIONAL INCREASE OF RATE CONSTANTS, PERMEABILITIES AND ASYMETRIES IN CO₂ OVER THOSE IN AIR

a_v	$\frac{\Delta k_T}{k_T}$	$\frac{\Delta k_s}{k_s}$	$\frac{\Delta k_m}{k_m}$	$\frac{\Delta A}{A}$
0.225	+2.8 ± 1.6	+1.4 ± 2.7	+2.1 ± 0.7	-0.23
0.43	+2.0 ± 0.3	+0.83 ± 0.84	+2.6 ± 0.5	-0.39
0.625	+1.6 ± 0.3	+0.73 ± 0.40	+2.3 ± 0.6	-0.32
0.85	+1.0 ± 0.2	+0.87 ± 0.22	+1.6 ± 2.0	-0.07
1.00	+0.55 ± 0.16	+0.16 ± 0.12	∞	-0.25

APPENDIX D -- TABLE 9

INCREASE OF RATE CONSTANTS, PERMEABILITIES, AND ASYMMETRIES AT 25°C OVER THOSE AT 15°C

a_v	$k_T = \frac{\Delta k_T}{a_m}$ (%/hr)	Δk_s (%/hr)	Δk_m (%/hr)	ΔA	$\frac{\Delta k_s}{a_v}$ (%/hr)	$\frac{\Delta k_m}{a_m - a_v}$ (%/hr)
0.44	$+1.8 \pm 0.3$	$+0.75 \pm 0.36$	$+1.0 \pm 0.3$	+0.22	$+1.7 \pm 0.8$	$+1.8 \pm 0.53$
0.70	$+1.5 \pm 0.3$	$+0.60 \pm 0.22$	$+0.90 \pm 0.28$	-0.15	$+0.86 \pm 0.31$	$+3.0 \pm 0.9$
0.85	$+1.7 \pm 0.5$	$+1.0 \pm 0.3$	$+0.60 \pm 0.34$	-0.15	$+1.2 \pm 0.35$	$+4.0 \pm 2.3$
0.93	$+0.70 \pm 0.45$	$+0.70 \pm 0.36$	0	+0.03	$+0.75 \pm 0.39$	0
1.00	$+2.4 \pm 0.5$	$+2.5 \pm 0.5$	0	0	$+2.5 \pm 0.5$	0

APPENDIX D — TABLE 10

FRACTIONAL INCREASE OF RATE CONSTANTS, PERMEABILITIES, AND ASYMMETRIES AT 25°C OVER THOSE AT 15°C

a_v	$\frac{\Delta k_T}{k_T}$	$\frac{\Delta k_s}{k_s}$	$\frac{\Delta k_m}{k_m}$	$\frac{\Delta A}{A}$
0.44	+1.2 ± 0.34	+1.9 ± 2.3	+0.91 ± 0.42	+0.36
0.70	+0.79 ± 0.23	+0.50 ± 0.26	+1.3 ± 0.76	-0.17
0.85	+1.3 ± 0.48	+0.83 ± 0.43	+6.0 ± 15	-0.14
0.93	+0.23 ± 0.17	+0.23 ± 0.14		+0.03
1.00	+0.77 ± 0.22	+0.81 ± 0.21		0

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