THE EFFECT OF RELATIVE HUMIDITY ON THE METABOLISM OF THE STARVED YELLOW MEALWORM *TENEBRIO MOLITOR* L. (COLEOPTERA, TENEBRIONIDAE) AS MEASURED BY CHANGES IN DRY MATTER AND LIPID COMPOSITION

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TABLE OF CONTENTS

INTRODUCTION 1

MATERIALS AND METHODS 7

Rearing and Maintenance of Cultures 7

Relative Humidity Chambers and Larval Cages 7

Relative Humidity Exposure 8

Dry Matter and Water Content Determination 9

Lipid Analysis 11

Calculations and Statistics 12

RESULTS 14

Total Weight Changes 14

Body Water Change 14

Dry Matter Change 17

Lipid Free Dry Matter Change 22

Total Lipid Changes 24

Fatty Acid Profile 25

DISCUSSION 27

SUMMARY 39

ACKNOWLEDGMENTS 41

REFERENCES CITED 42

VITA 46

APPENDICES 47

Appendix I: Supplementary Experimental Data 47

Appendix II: Description and Data for Statistical Analyses 63

Appendix III: Calculation Methods 72

INTRODUCTION

Nutrients including carbohydrates, fats, and amino acids are oxidized by animals to produce energy, carbon dioxide, and water. The water produced is called metabolic or oxidation water and would obligatorily enter the total water pool of the organism (Edney, 1967). Some insects appear to use metabolic water as the predominant water source for growth and development (Edney, 1967; Fraenkel and Blewett, 1944; Murray, 1968). Certain evidence indicates that "insects under water stress metabolize more food and thus derive more oxidation water than they otherwise would" (Edney, 1967). Increased metabolism and metabolic water production would allow insects to replace larger water losses during exposure to very dry conditions. However, other information indicates that water stress does not increase metabolism. This controversy has been reviewed by Andrewartha and Birch (1954), Buck (1953), Bursell (1964), Edney (1957), and Edwards (1953).

The question of whether insects increase metabolic water production by increasing metabolism has been investigated by several methods. Indicators of metabolic rate including oxygen uptake, CO_2 production, nutritional reserve losses, food utilization, and dry matter loss were measured and compared between groups of insects exposed to a range of relative humidities over a period of time.

Oxygen uptake and/or CO_2 production in relation to humidity have been measured by respirometry for larval Japanese beetles, Popilla japonica (Belluci, 1939); larval and adult lesser grain borers, Rhizopertha dominica (Birch, 1947); adult blow flies, Phormia regina (Buck and Keister, 1949); larval mealworms, Tenebrio molitor (Caldwell, 1925); adult cockroaches, Blatta orientalis (Gunn and Cosway, 1942); adult grasshoppers, Chortophaga viridifasciata (Ludwig, 1937); and adult fruit flies, Drosophilla sp.

(Thompson and Tennant, 1932). Only adult blowflies and larval mealworms appeared to show increased metabolism at low humidity. The results indicated increased oxygen uptake in blowflies and increased CO_2 production in mealworms.

Body fat apparently has been the only nutritional reserve studied in relation to relative humidity, possibly because fat constitutes the primary respirable energy store in some insects. Fat theoretically is the best substrate for production of metabolic water. Fat provides more water per unit weight when completely catabolized than does carbohydrate and protein. Mellanby (1942) has objected to this idea since fat requires more oxygen for oxidation. He concluded that water losses due to respiration would increase if oxygen uptake increased. This would negate any advantage of fat as a source of metabolic water. The question is discussed in detail by Andrewartha and Birch (1954) and Bursell (1964).

To determine the effect of relative humidity on fat loss, the average lipid contents of insects were compared after periods of starvation at various relative humidities. Data were expressed in one of three different ways by various investigators: (1) weight of lipids per insect, (2) total lipids as percentage of total dry matter, (3) total lipids as percentage of insect live weight at the beginning of the experiment. The latter two methods have the advantage of adjusting the final value for differences in beginning weights of the individual insects. Low lipid weights or percentages in insects starved at low humidities indicated increased fat utilization. Experiments of this type have been done with pupae of two species of tsetse flies, Glossina morsitans (Bursell, 1958) and G. tachinoides (Buxton and Lewis, 1934), and with adults of two species of tsetse flies, G. tachinoides (Buxton and Lewis, 1934) and G. palpalis (Mellanby, 1936a). Only adults of

G. tachinoides showed increased fat loss at low humidities.

Nutrient utilization in feeding insects as influenced by relative humidity has been investigated in two studies. Mellanby (1935) stated that humidity had no effect on the increase in dry matter of bed bugs, Cimex sp., fed blood and reared at 0% and 90% R.H. Therefore, low humidity apparently did not increase the amount of nutrients oxidized to water and CO_2 as compared with the amount used to increase dry matter.

Fraenkel and Blewett (1944) reared confused flour beetles, Tribolium confusum; Mediterranean flour moths, Ephestia kuehniella; and dermestid beetles, Dermestes vulpinus, from first instars to pupae at three different humidities. All three species consumed more food per unit amount of pupal dry weight formed at the low humidities. This was interpreted as indicating that more food was oxidized to CO_2 and water and less was used to increase dry matter at low humidities. Therefore, total metabolism over the whole larval period would have been higher at low humidities presumably producing more metabolic water.

Since body dry matter provides the respirable substrated in starving insects, the loss of dry matter with relation to relative humidity should indicate the effect of humidity on metabolism. Buxton (1930) used this method in work on the larval yellow mealworm, T. molitor. His results showed that more live weight was lost at low humidities as compared to high humidities, but the proportion of dry matter to total body weight remained constant. Therefore, he concluded that more dry matter had been lost at the lower humidities with a corresponding increase in metabolism and metabolic water production. According to Edney (1957), Yeager and Munson (1950) and Ludwig and Wugmeister (1953) have found data for cockroachs, Periplaneta americana, and Japanese beetle larvae, Popilla japonica, respectively, which

can be interpreted in a similar manner.

An alternative method for comparing dry matter loss in relation to humidity has been to compute dry matter content of starved insects at the end of the humidity exposure as a percentage of the beginning live weight. If metabolism increases as humidity decreases, the percentage should be lower at low humidities. This method has been used to investigate the effect of humidity on the metabolism of pupae of two species of tsetse fly, G. morsitans (Bursell, 1958) and G. tachinoides (Buxton and Lewis, 1934) and G. palpalis (Mellanby, 1936a); eggs of the blowfly, Lucilla sericata (Evans, 1934a); adult bedbugs, Cimex lectularius (Mellanby, 1932a); larval clothes moths, Tineola biselliella (Mellanby, 1934); and larval mealworms, T. molitor (Mellanby; 1932b, 1936b). None of these studies showed increased dry matter loss in starved insects at low humidities. This indicates that metabolism and metabolic water production did not increase at low humidities. Buxton (1930) also used this method to confirm his results for mealworms.

Evans (1935) found no effect of humidity on the amount of dry matter lost during prepupal and pupal periods of the blowfly, L. sericata. However, he did not indicate how he obtained his values.

In summary, low humidity and/or water stress did not seem to cause an increase in metabolic rate of most of the insects studied. There is conflicting evidence for increased metabolism in the mealworm, T. molitor. Caldwell (1925) stated that larval mealworms produced more CO_2 when desiccated. This would indicate an increased metabolic rate or a change in the respiratory quotient. A respiratory quotient change would indicate a shift in the nutrient reserves being utilized.

Buxton (1930) obtained results which he interpreted as showing that mealworms metabolized more dry matter at low humidities to produce more

metabolic water. He found that mealworm larvae maintained a constant percentage dry matter of live weight during starvation at all humidities of 80% and below at 23 C. Since more total weight was lost at low humidities, more dry matter must have been lost to maintain the constant percentage. Therefore, more dry matter must have been metabolized presumably to produce more metabolic water. Buxton also computed the dry matter content of each larva at the end of the experiment as a percentage of the original live weight. If dry matter loss increased within an experimental group, the average percentage final dry matter of original live body weight should decrease. The average percentages showed no difference at 90% through 30% R.H. However, the values for 20% and 0% were significantly lower. Buxton felt that this confirmed his hypothesis that metabolism increased at the lower humidities to produce more metabolic water.

Mellanby (1932b) repeated Buxton's work at a slightly higher temperature (30 C) and found that mealworm larve did not maintain a constant percentage of dry matter to live body weight in response to decreasing humidity. The average percentage increased at the lower humidities probably because of increased water loss. Further, the average ratios of dry matter to original live weight were not significantly different throughout the range of humidities. This indicated that the metabolic rate and metabolic water production were the same at all humidities. However, Mellanby accepted Buxton's work as valid at the lower temperature and commented that the higher temperature used in his own experiments did not allow the mealworms "to decrease their (metabolic) rate below a certain figure." Therefore, humidity could not affect metabolism at higher temperatures.

In later work Mellanby (1936b) repeated his and Buxton's experiments at a range of temperatures and relative humidities and with larger numbers of

larvae. He stated that "as the body composition of mealworms is so variable and these (Buxton's) results were obtained from rather small numbers (5 - 7 insects per humidity with no replication), it appeared advisable to make further experiments . . ." Mellanby concluded from these later experiments that rate of metabolism was influenced only by temperature and not by humidity. However, he gave few data or details of methods.

Our study was designed to reinvestigate the hypothesis that mealworm larvae, T. molitor, increase metabolism to produce more metabolic water to offset increased water loss at lower humidities. We have done this by estimating the average percentage of the original dry matter lost by groups of larvae starved at various relative humidities. Since fat makes up as much as 17% of the wet weight of the mealworm larva (Finkel, 1948; Moran, 1959) while glycogen is only approximately 2% of wet weight (Mellanby, 1932b; Rousell, 1955), we reasoned that any change in metabolism might be reflected in a change in fat loss. Therefore, the effect of humidity on fat loss and on the qualitative and quantitative total fatty acid composition of starving mealworm larvae was also studied.

MATERIALS AND METHODS

A list of the experimental methods used on each group of insects is given in Appendix I, Table 6.

Rearing and Maintenance of Cultures.

Yellow mealworm cultures were obtained from the Midwest Grain Investigations Laboratory, USDA, Manhattan, Kansas (USDA); from Dr. Carl Rettenmeyer, Department of Entomology, Kansas State University (KSU Entomology); and from the Chemagro Corporation, Kansas City, Mo. through the Department of Zoology, Kansas State University (KSU Zoology). The insects were reared in gallon glass jars in a constant temperature and humidity room at approximately 27 C and 60% R.H. The rearing medium was screened wheatshorts. A piece of paper toweling placed on the medium provided a darkened crawling area. No other food or source of water was provided. All cultures were maintained separately and were not intermixed.

Relative Humidity Chambers and Larval Cages.

Relative humidity chambers were made from 38.1 x 27.9 x 15.2 cm (15 x 11 x 6 inch) clear plastic boxes with lids (Althor Products, Brooklyn, N.Y.). A 25.5 x 35 cm platform was made from 1.3 cm ($\frac{1}{2}$ inch) mesh hardware cloth and covered with fine mesh bolting cloth to prevent fecal material from falling into the relative humidity solution and yet allow for air circulation. The platform was supported in the chamber at a height of approximately 6.5 cm by plexiglass props cemented to the chamber sides. Relative humidities of 30%, 60%, and 90% were maintained by potassium hydroxide solutions (Solomon, 1951). A low relative humidity of approximately 0% was maintained with anhydrous calcium sulfate (Drierite, W. A. Hammond

Drierite Co., Zenia, Ohio). A small crystallizing dish containing potassium hydroxide pellets was also placed in this chamber to absorb carbon dioxide.

Mesh cages to contain individual larvae were made from 4.5 x 9.0 cm pieces of Lumite Saran shade cloth, 32 meshes per inch (Chicopee Manufacturing Corp., Cornielia, Georgia). The shade cloth was folded over and two of the free sides stapled shut with an office stapler making an envelope approximately 4.5 x 4.5 cm.

Relative Humidity Exposure.

Larvae were sieved from the culture medium and placed in a beaker. They were drawn at random from the beaker, weighed, and individually placed in each envelope. The open side of the envelope was stapled shut. Various control groups usually of 25 larvae each were also drawn at this time. Each control larva was weighed and placed in a glass vial for storage at -20 C. Generally larvae weighing less than 70 mg were not used. There was no upper limit on larval weight.

The experimental larvae were placed in the chambers by arranging the envelopes in rows on the platforms. Two groups, each of 25 larvae, were placed in each chamber. Therefore, each experiment had two replicates running concurrently. The chambers were closed and sealed with 3.8 cm (1.5 inch) wide plastic electrical tape and were placed in an environmental cabinet at 23-25 C in total darkness for 21 days. The 28 day exposure period used by Buxton (1930) was impractical because too many larvae pupated or died during the longer time span, and numbers of larvae left were too small for analysis.

At the end of the experimental period, the envelopes were removed from the chambers. Larvae were removed from the envelopes, weighed, placed in

individual glass vials, and frozen for storage. Adults and pupae that developed during the experiment were pooled, placed in beakers, covered with aluminum foil, and frozen for storage.

After removal of the insects, a hygrometer (Abbeon Inc., Jamaica, N. Y.) was placed in each chamber and the chambers were resealed. The hygrometer was allowed to equilibrate for approximately 48 hours although the final reading was approached within a few percent after two hours. Readings indicated that the humidities expected were present within a range of approximately ±2% in the 30%, 60%, and 90% chambers and between 0% and 4% in the low humidity chamber.

Dry Matter and Water Content Determination.

The dry matter and water contents of the experimental larvae and one group of control larvae were determined by either heat drying at 110 C for 72 hours or by freeze-drying. Since heat drying was much faster and less tedious, it was used when fatty acid analyses were not made. In both drying methods larvae were processed individually. Pupae and adults were heat dried and processed in groups.

Freeze-drying was accomplished as follows: a 25 x 16 pyrex dish was placed on dry ice in an insulated box to provide a working surface cold enough to keep the larvae frozen. Since intact larvae would not freeze-dry and often shattered during cutting, they were individually placed in a 80 x 40 mm crystallizing dish set on the working surface and were cut into quarters with a single edged razor blade. The pieces of each larvae were transferred to tared aluminum foil containers. Small fragments were collected with a small stiff brush. The containers were made from 7.5 x 7.5 cm squares of aluminum foil shaped by pressing the foil into a 20 ml beaker with a smaller vial or

beaker. All containers and utensils were prechilled and kept below freezing at all times either with dry ice or in a refrigerator freezing compartment. The cut larvae were stored in a freezer -20 C.

To freeze dry, the foil containers with larvae were placed in two prechilled vacuum desiccators. The desiccators were placed in a plastic foam ice chest packed with dry ice and were attached to an Aminco Freeze-dry Apparatus (American Instrument Co., Silver Springs, Md.) with rubber vacuum hose. Drying lasted 48 hours. All larvae from a single experiment were dried at one time.

After heat or freeze-drying, the insects were stored under nitrogen over Drierite until they reached a constant weight. Dry weight was determined for each larva and for groups of pupae and adults. Water content was determined by subtraction. All weighings were done out of a desiccator since the dried material rapidly absorbed moisture. The dried material was stored either in a freezer at -20 C or in a desiccator under nitrogen. The latter method appeared to cause some degradation of the unsaturated fatty acids and was used in only one experiment.

Freeze-drying efficiency was checked by two methods. One method consisted of heat drying three freeze-dried control larvae. Any additional weight loss indicated incomplete drying although some volatile substances might also be lost. Little additional weight was lost.

The second method consisted of heat drying a replicate group of control larvae. The percentage dry matter of live weight was calculated for each larva from the freeze-dried and heat dried control groups, and mean percentages were compared by the "t" test for unpaired observations. Statistical analyses indicated a significant difference in only one of four samples. The freeze-dried controls were used to estimate the amount of dry matter and water present

in experimental larvae before humidity exposure. The estimates were then used for calculating dry matter and water changes during humidity expsoure. Since any error would be applied equally to the calculations made at each humidity and since this work was concerned only with the relative relationships between changes at different humidities and not absolute values, the difference found between the heat and freeze dired control groups was not considered to be significant.

Lipid Analysis.

Larvae from the undried and dried controls and from each experimental group were pooled within each group into batches weighing approximately 2 g. The lipids were extracted and analyzed according to Valder, et al. (1969). The undried control group served as a check for degradation of lipids during storage and drying. The lipid analysis entailed homogenization in chloroform-methanol, extraction of the homogenate with hexane to determine the amount of total lipids, saponification, methylation, and analysis of the fatty acids by gas-liquid chromatography. No fatty acid analyses were made on heat dried material. Several minor modifications were made in Valder's procedure. They were as follows:

1. 10 ml of distilled water instead of 0.5 ml was added to the chloroform methanol homogenate before extraction with hexane. This procedure gave quicker separation of the layers and less emulsion formation.

2. Chloroform-methanol volume was reduced by about one-half when small numbers of insects were extracted.

3. In later experiments weighing was done by quantitatively transferring lipid residues to tared scintillation vials with diethyl ether. Residues were weighed under nitrogen with vials capped. The smaller containers had

less weight fluctuation than the flasks that Valder used for weighing.

4. Fractionation of lipid classes by column chromatography was not performed.

5. Saponification mixtures were extracted four times with 50 ml portions of diethyl ether before acidification because unsaponifiable material was not separated on a column. The ether was pooled and washed with distilled water until neutral. Water washes were added to the saponification mixture, and the total volume was acidified and extracted.

6. National Institute of Health methyl ester standards were used to calibrate the gas chromatograph and to identify most unknowns. Methyl linoleate (Applied Science Laboratories, State College, Pa.) was used to identify one unknown by comparison of retention times and co-chromatography. Plots of carbon number vs log of retention time were used to tentatively identify other unknown peaks for which standards were not available.

Calculations and Statistics.

The percentage of original live weight remaining at the end of each experiment and the percent dry matter were calculated for each experimental larva. The original dry matter in each larva at the beginning of the experiment was estimated by multiplying the beginning live weight of each larva by the mean percent dry matter found in the dried control group. The original water was estimated by subtraction. From data for original and final dry matter and water contents, weight and percentage changes in the original dry matter and water were calculated for each larva. Mean values for the percentage changes were determined and compared by analysis of variance. Use of percentages in the statistical analyses reduced data variability due to differing insect sizes.

The total original lipid content of each experimental group of larvae, pupae, and adults was estimated by multiplying the total original live weight of each group by the lipid percentage of the dried control total live weight. The total original lipid free dry matter was estimated by subtraction. Total original and final lipid contents were used to calculate total weight and percentage losses in original lipid and to calculate percentage of dry matter loss accounted for by lipid loss. Similar calculations were made for lipid free dry matter. In addition, the estimated percentage of total original dry matter lost was calculated for groups of pupae and adults because individual data were not determined. In calculation of all data on whole groups of insects, values were based on percentages of total weights of the group and not on mean percentages of individual insects. Data was analyzed by analysis of variance as above. Details for all statistical analyses and all calculations are given in Appendices II and III, respectively.

RESULTS

The experiments were designed primarily to study the effect of relative humidity on dry matter and fat losses in starved mealworm larvae and to indirectly measure metabolism and metabolic water production. Small numbers of pupae and adults that developed during some experiments were also analyzed (Appendix I, Table 7). In experiments where no pupae or adults developed, the missing data were estimated statistically by analysis of covariance. Therefore, results for pupae and adults are tentative due to small numbers of insects and missing data.

Total Weight Changes.

Percentage losses of original live weight increased as humidity decreased (Table 1). Live weight loss would include water loss and dry matter loss due to expiration of CO_2, elimination of feces, and exuviae. The results indicate that increase in weight loss at low humidities was due primarily to increased loss of water because dry matter loss did not vary between humidities. Percentage losses of original live weight were not analyzed statistically.

Body Water Change.

Water losses or gains were determined from the estimated percentage change per larvae in original water. Water change was not determined for pupae or adults. Body water of control larvae averaged about 56%. This figure was close to values of about 58% and 61% given by Mellanby (1958) and Urs (1970), respectively. As Buxton (1930) and Mellanby (1932b) pointed out, percentages of dry matter and of water in individual larvae are quite variable.

Water loss by larvae would be expected to be greater at low humidities

Table 1. Body weight and compositional changes as influenced by relative humidity for Tenebrio molitor larvae starved for 21 days. All experimental values are mg/larva unless followed by a percentage sign. Complete data are given in Appendix I, Tables 6-20.

Body components	Weights and percentages	Replications	Calculation 1/	Experimental values for relative humidities 2/ Control	0%	30%	60%	90%	Statistical Signif. 3/
Live weight	Starting weight	8	Avg.	133.4	127.7	125.1	122.3	126.5	N.A.
	Final weight	8	Avg.		104.7	107.7	107.0	120.0	N.A.
	Weight lost	8	Avg.		23.1	17.4	15.3	6.5	N.A.
	% of original weight lost	8	Avg.		17.7%	12.9%	12.2%	5.3%	N.A.
Dry matter	Estimated original dry matter	6	Avg.	59.3	61.3	56.9	56.0	58.3	N.A.
	Final dry matter	6	Avg.		50.2	49.0	46.6	48.2	N.A.
	Estimated dry matter lost	6	Avg.		11.2	7.9	9.4	10.1	N.A.
	% of original dry matter lost	6	Avg.		18.5%	14.7%	17.2%	18.4%	n.s.
	Dry matter % of live weight	6	Avg.	43.4%	44.2%	44.1%	41.5%	37.9%	N.A.
Water	Estimated original water	6	Avg.	77.6	80.3	74.5	73.2	76.3	N.A.
	Final water	6	Avg.		63.2	62.5	65.1	78.2	N.A.
	Estimated water change	6	Avg.		17.1	12.0	8.2	+ 2.0	N.A.
	% original water change	6	Avg.		20.5%	16.2%	10.4%	+ 3.1%	***
	Water % of live weight	6	Avg.	56.6%	55.8%	55.9%	58.5%	62.1%	N.A.
Lipid	Estimated original lipid	8	Avg.	19.5	19.2	18.3	17.9	18.5	N.A.
	Final lipid	8	Avg.		14.8	15.9	14.1	14.7	N.A.
	Estimated lipid lost	8	Avg.		4.4	2.5	3.8	3.8	N.A.
	% of original lipid lost	8	Total		23.2%	14.1%	22.0%	20.5%	n.s.
	Lipid lost as % of dry matter lost	8	Total		44.9%	31.9%	47.1%	46.7%	n.s.
	Lipid % of live weight	8	Total	14.6%	13.7%	14.5%	12.9%	12.2%	N.A.
	Lipid % of dry matter	8	Total	34.0%	32.0%	33.6%	31.4%	32.2%	N.A.

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Table 1 (continued).

Body components	Weights and percentages	Replications	Calculation 1/	Experimental values for relative humidities 2/ Control	0%	30%	60%	90%	Statistical Signif. 3/
Lipid free dry matter (LFDM)	Estimated original LFDM	8	Avg.	38.0	37.1	35.4	34.5	35.8	N.A.
	Final LFDM	8	Avg.		31.7	31.0	30.2	30.9	N.A.
	Estimated LFDM lost	8	Avg.		5.5	4.3	4.4	4.9	N.A.
	% of original LFDM lost	8	Total		14.0%	12.1%	12.0%	13.5%	n.s.
	LFDM lost as % of dry matter lost	8	Total		55.1%	68.1%	52.9%	53.3%	n.s.
	LFDM % of live weight	8	Total	28.5%	29.5%	28.8%	28.2%	25.7%	N.A.
	LFDM of dry matter	8	Total	66.0%	68.0%	66.4%	68.6%	67.8%	N.A.
Number Molts	% of larvae that molted during the experiments	2	Avg.		57.8%	54.2%	80.5%	45.0%	N.A.

1/ Avg.: data calculated from averages of weights and percentages. Total data calculated from percentages of total weights.

2/ There are no estimated values in the control groups. +: percentage or weight gain.

3/ N.A.: not analyzed. n.s.: differences are not statistically significant at p = 0.10. *** p<0.01

due to increased transpiration and evaporation from cuticle and spiracles. Insects at the lowest humidity did show greatest water loss (Table 1). Loss decreased as humidity increased until an overall percentage gain occurred at 90% R.H. This gain is consistent with the results of Buxton (1930), Mellanby (1932b), and Locke (1964) who demonstrated that mealworms can absorb water from air at humidities of approximately 90% or more. Water uptake from subsaturated atmospheres has been found in several other insect species (Locke, 1964).

Differences between means for percentage change in original water were highly significant (Table 1). Therefore, rate of water loss accelerated as humidity decreased. Percentage water of live body weight also showed a decrease with humidity (Table 1).

Two conclusions can be drawn from these results. First, larval mealworms lost water more rapidly as humidity decreased. Consequently, if metabolic rates in mealworms do increase to produce more metabolic water in response to increased water loss, then metabolic rates should have increased at the lower humidities. Secondly, our methods for estimating losses and percentage losses should be sensitive enough to detect differences in dry matter losses of starved larvae because these methods revealed the expected differences in water losses.

Dry Matter Changes.

Larval dry matter changes were determined by percentage loss per larva of original dry matter. Pupal and adult losses were determined by percentage loss from each experimental group's total original dry matter.

If metabolism increased, percentage loss should have increased. Percentage loss should have increased as more nutrients were oxidized to CO_2 and water.

Table 2. Body weight and compositional changes as influenced by relative humidity for Tenebrio molitor pupae which developed from larvae during the 21 day humidity exposure period. All experimental values are in mg/pupa unless followed by a percentage sign.

Body components	Weights and percentages	Replications	Calculation [1]	Experimental values for relative humidities [2] 0%	30%	60%	90%	Statistical Signif. [3]
Live weight	Starting weight	6	Avg.	133.9 [6]	128.1 [7]	132.4	146.1	N.A.
Dry matter (DM)	Estimated original dry matter	6	Avg.	57.4 [6]	55.9 [7]	57.0	63.2	N.A.
	Final dry matter	6	Avg.	38.5 [6]	36.6 [7]	40.0	46.5	N.A.
	Estimated dry matter lost	6	Avg.	18.9 [6]	19.3 [7]	17.0	16.6	N.A.
	% of original dry matter lost [8]	6	Total	34.9%	35.3%	29.8%	26.1%	n.s.
Lipid	Estimated original lipid	4	Avg.	20.0 [4]	20.2 [5]	20.4	22.0	N.A.
	Final lipid	4	Avg.	8.9 [4]	10.9 [5]	12.4	10.7	N.A.
	Estimated lipid lost	4	Avg.	11.3 [4]	9.3 [5]	8.1	11.2	N.A.
	% of original lipid lost [8]	4	Total	56.4%	45.7%	39.5%	52.0%	**
	Lipid lost as % of dry matter lost [8]	4	Total	46.6%	41.6%	43.9%	51.6%	*
	Lipid % of dry matter	4	Total	23.5% [4]	27.2% [5]	28.8%	23.9%	N.A.
Lipid free dry matter (LFDM)	Estimated original LFDM	4	Avg.	40.9 [4]	42.3 [5]	40.9	44.1	N.A.
	Final LFDM	4	Avg.	29.0 [4]	29.7 [5]	30.7	33.4	N.A.
	Estimated LFDM lost	4	Avg.	11.9 [4]	12.0 [5]	10.2	10.8	N.A.
	% of original LFDM lost [8]	4	Total	31.2%	31.0%	25.0%	24.4%	*
	LFDM lost as % of dry matter lost [8]	4	Total	53.4%	58.4%	56.1%	48.4%	*
	LFDM % of dry matter	4	Total	76.5% [4]	72.7% [5]	71.3%	76.1%	N.A.

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Table 2 (continued).

1/ Avg.: data calculated from averages of weights and percentages. Total: data calculated from percentages of total weights.

2/ Control values are the same as in Table 1 and are not listed here.

3/ N.A.: not analyzed. n.s.: differences were not significant at $p = 0.10$. *: $p<0.10$. **: $p<0.05$.

4/ Mean of two values.

5/ Mean of three values.

6/ Mean of four values.

7/ Mean of five values.

8/ Some means include missing data that was estimated statistically.

Table 3. Body weight and compositional changes as influenced by relative humidity for starved *Tenebrio molitor* adults which developed from larvae during the 21 day humidity exposure period. All values are mg/adult unless followed by a percentage sign.

Body components	Weights and percentages	Replications	Calculation[1]	Experimental values for relative humidity[2] 0%	30%	60%	90%	Statistical signif.[3]
Live weight	Starting weight	6	Avg.	127.9 [5]	124.5 [5]	129.3	127.3	N.A.
Dry matter	Estimated original dry matter	6	Avg.	54.6 [5]	54.3 [5]	55.9	55.0	N.A.
	Final dry matter	6	Avg.	32.3 [5]	31.2 [5]	33.4	32.1	N.A.
	Estimated dry matter lost	6	Avg.	22.2 [5]	23.1 [5]	22.5	22.9	N.A.
	% of original dry matter lost[6]	6	Total	40.5%	42.4%	40.7%	41.2%	n.s.
Lipid	Estimated original lipid	4	Avg.	18.1 [4]	18.5 [4]	19.9	19.7	N.A.
	Final lipid	4	Avg.	4.1 [4]	5.1 [4]	6.6	5.3	N.A.
	Estimated lipid lost	4	Avg.	14.0 [4]	13.5 [4]	13.3	14.5	N.A.
	% of original lipid lost[6]	4	Total	76.1%	73.1%	67.2%	73.3%	n.s.
	Lipid lost as % of dry matter lost[6]	4	Total	57.8%	53.6%	54.5%	54.6%	n.s.
	Lipid % of dry matter	4	Total	13.5%[4]	16.3%[4]	18.3%	16.2%	N.A.
Lipid free dry matter (LFDM)	Estimated original LFDM	4	Avg.	36.8 [4]	37.2 [4]	39.8	39.6	N.A.
	Final LFDM	4	Avg.	26.2 [4]	25.6 [4]	28.8	27.5	N.A.
	Estimated LFDM lost	4	Avg.	10.6 [4]	11.6 [4]	11.0	12.1	N.A.
	% of original LFDM lost[6]	4	Total	28.0%	31.4%	27.9%	30.5%	n.s.
	LFDM lost as % of dry matter lost[6]	4	Total	42.2%	46.4%	45.5%	45.3%	n.s.
	LFDM % of dry matter	4	Total	86.5%[4]	83.8%[4]	81.7%	83.9%	N.A.

(continued on next page)

Table 3 (continued).

1/ Avg.: data calculated from averages of weights and percentages. Total: data calculated from percentages of total weights.

2/ Control values are the same as in Table 1 and are not listed here.

3/ N.A.: not analyzed. n.s.: differences were not statistically significant at $p = 0.10$.

4/ Mean of three values.

5/ Mean of five values.

6/ Some means include missing data that was estimated statistically.

This loss would include all oxidizable substrates such as fats, carbohydrates, and amino acids. Other losses of dry matter including feces and exuviae would also be part of the total but would not reflect metabolism.

Statistical analysis showed no significant differences in dry matter loss by larvae, pupae, or adults at humidities tested (Tables 1, 2, 3). Pupae showed a possible trend although it was not significant (Table 2). This trend was probably due to one extremely low value at 90% R.H. and to missing data which were concentrated at 0% and 30% R.H. (Appendix I, Table 9). Our results indicate that metabolism does not increase in response to increased water loss as humidity is reduced. Metabolic water production does not seem to be regulated in larvae, pupae, or adults to compensate for increased desiccation.

Lipid Free Dry Matter Change.

Lipid free dry matter changes were determined from two sets of calculations: (1) estimated percentage loss from total original lipid free dry matter, (2) percentage of total dry matter loss accounted for by lipid free dry matter loss. Statistical analysis indicated no significant differences between percentages for larvae or for adults (Tables 1, 3). Therefore, no detectable humidity effect was evident.

The measures for pupae were somewhat significant (Table 2). The small numbers of pupae involved, the fact that the adults do not show any trend, and the fact that missing data is concentrated in groups at 0% and 30% R.H. indicate that the difference may not be a valid biological effect. Estimated percentage of total dry matter loss accounted for by lipid free dry matter loss showed little relation to low or high humidity. The lowest losses were at 0% and 90% R.H. and the highest was at 30%. However, there seemed to be

a trend with humidity in percentage loss from original lipid free dry matter (Table 2). As humidity increased, loss decreased indicating greater lipid free dry matter utilization at lower humidities. The meaning of such a trend, if it was real, is uncertain. It would probably indicate increased protein metabolism at low humidities since there is little glycogen or total carbohydrates in the mealworm larvae or pupae (Evans, 1934b; Mellanby, 1932b; Rousell, 1955).

It should be noted that lipid free dry matter losses in larvae and pupae were estimated to make up slightly over half of dry matter loss. Lipid would be expected to make up the greater fraction of dry matter loss since there is little carbohydrate in mealworms. However, lipid made up more than half of dry matter loss only in adults (Tables 1, 2, 3). We have data which indicates that cast skins and fecal material might be expected to make up roughly one-half to one-fourth of lipid free dry matter loss (Krchma, unpublished data). This factor would increase the proportion of lipid free dry matter loss in total dry matter loss. Many larvae molted once during the experimental period. Dry weight loss due to weight of cast skins is not weight loss due to metabolism of food stores to water and CO_2. Therefore, the dry matter loss and lipid free dry matter loss should have been adjusted for the weight of the cast skins if these measures were to be true indicators for rate of metabolism. However, the effect due to exuviae probably would average itself out over the long run. The effect should add a constant increment to dry matter loss because the percentage of larvae which molted seemed to be approximately the same at all humidities (Table 1). Data on number of insects molting were collected for only two experiments. The high molting percentage at 60% R.H. might have been due to the small number of larvae in one experiment. All these larvae molted giving a value of 100%.

Data on molting and cast skins should have been collected and compared in all experiments.

Total Lipid Changes.

Lipid is the major respirable substrate of mealworms (Mellanby, 1932b) and has been suggested as the best source for metabolic water because complete combustion of fat produces the largest amount of water per unit weight of all nutrient reserves. Therefore, we examined lipid utilization at various relative humidities by measuring lipid loss. Most lipid loss is probably due to triglyceride catabolism. Triglycerides make up more than 80% of total lipids in yellow mealworms (Krchma, unpublished data; Urs, 1970), and triglyceride fatty acids are probably the primary lipid utilized for energy production.

Lipid changes were determined from two sets of calculations: (1) estimated percentage loss from total original lipid, (2) percentage of total dry matter loss accounted for by lipid loss. Humidity had no significant effect on lipid loss in larvae or adults by either calculation method. Pupae showed a possible humidity effect. Both calculation methods showed that highest lipid loss occurred at 0% and 90% R.H. The small number of pupae available for the experiment (Appendix I, Table 7) and the fact that the adults did not reflect a similar difference in lipid change indicate that the difference in pupae may not be a valid biological effect. However, if the effect was real, it would seem not to be a direct effect due to water loss because groups at 0% and 90% R.H. both show statistically similar percentages of loss (Appendix II, Table 23).

Two groups of larvae showed an apparent increase in lipid during starvation (Appendix I, Table 10). This may be due to groups of insects being so

variable in lipid content that the control group did not accurately estimate the original lipid present. Another possible explanation is that carbohydrate and or protein were converted into lipid. However, it would seem to be inefficient for insects to convert one energy reserve into another. The insect would be more likely to use reserves directly. In addition, only two samples out of many showed a lipid gain. Therefore, an inaccurate estimate of the total original lipid present would be a more reasonable explanation. Even if experiments which gave estimated gains were dropped from the statistical analysis, the means were still not significantly different.

Fatty Acid Profile.

Fatty acids and fatty acid containing compounds such as phospholipids and triglycerides make up approximately 95% of lipid content in mealworm larvae (Krchma, unpublished data; Urs, 1970). Since fatty acids would comprise a major metabolic substrate and a possible major source for metabolic water, the effect of relative humidity on fatty acid composition of mealworm larvae was analyzed.

Analysis of control groups of larvae showed that oleic acid was the predominant acid followed by linoleic, palmitic, myristic, and palmitoleic (Table 4; Appendix I, Tables 14-20). These results are comparable with those of Bieber and Monroe (1969) and Urs (1970) except that the position of linoleic and palmitic acid were reversed in order of predominance in Bieber and Monroe's work, and the relative percentage found by Bieber and Monroe for linoleic acid was about one-half of Urs's and our values. This may be due to the fact that Bieber and Monroe reared their larvae on artificial media.

Humidity did not seem to have any statistically detectable effect on the relative percentage of any of the concentrations of major fatty acids (Table 4).

Also, there were no apparent qualitative differences between various humidities (Appendix I, Tables 14-20).

Table 4. Fatty acid composition of total lipids as influenced by relative humidity for T. molitor larvae starved for 21 days. Relative percentages are averages of three replicates except where noted.

Fatty acids	Experimental values for relative humidities 1/					Statistical Significance 2/
	Control	0%	30%	60%	90%	
Myristic	5.8%	6.3%	N.A.	6.3%	6.6%	n.s.
Palmitic	16.5%	13.0%	N.A.	11.6%	13.0%	n.s.
Palmitoleic	4.6%	5.1%	N.A.	4.8%	5.3%	n.s.
Oleic	49.0%	49.9%	N.A.	50.8%	48.4%	n.s.
Linoleic	21.0%	22.1%	N.A.	23.3%	23.2%	n.s.
Total Minor Fractions	3.2%	3.5%	N.A.	3.2%	3.6%	n.s.

1/ Control is an average of two replications and was not included in the statistical analysis. N.A.: not analyzed.

2/ n.s.: differences are not statistically significant at $p = 0.10$.

DISCUSSION

Our results show relative humidity had no effect on metabolism in starved mealworms. Over a range of 0% to 90% R.H., starved larvae showed no significant differences in losses of dry matter, total lipids, and lipid free dry matter or in fatty acid composition of total lipids. Dry matter and lipid losses in adults and dry matter losses in pupae were also not influenced by humidity. Humidity appeared to have an effect on lipid and lipid free dry matter losses in pupae. However, results for pupae and adults must be considered tentative because of missing data, small numbers of insects, and the fact that differences shown in pupae were not reflected by adults.

Since metabolism was apparently unaffected by humidity, metabolic water production should be the same at all humidities. This confirms the results of Mellanby (1932b, 1936b). Negative results reported by Mellanby (1932b) should not be confused with positive results in his paper which were actually taken from Buxton (1930) and were included for the sake of temperature comparision. Mellanby found no humidity effect at 30 C but assumed that Buxton's results at 23 C did show a humidity effect.

Our results do not support the hypothesis of Buxton (1930) that mealworms increase their metabolic water production as humidity is lowered and water loss increases. Buxton indicated that more dry matter was utilized by mealworms at low humidities for the purpose of producing more metabolic water to maintain a constant ratio of dry matter to body weight and to replace increased water loss. Mellanby (1936b) criticized Buxton's findings because they were based on small numbers of highly variable insects. Examination of Buxton's work indicates that these criticisms are valid since Buxton used only 5-7 insects per humidity with no replication. Mellanby redid Buxton's experiments and found no effect of relative humidity on metabolism.

Caldwell (1925) concluded that desiccated mealworm larvae increased their CO_2 output indicating increased metabolism. The conclusion was based on time necessary for respired CO_2 in an air stream to change the color of a pH indicator solution as judged by visual observation. Small time intervals indicated large amounts of CO_2 respired. Each experiment consisted of a control group of three larvae kept in room air and a desiccated experimental group. In most of the five experiments, starved larvae were weighed and CO_2 output measured daily for six days. Caldwell did not adjust his results for weight of each insect group. If the data are adjusted by dividing time interval by total weight of the insect group, results show that one desiccated group of larvae gave off less CO_2 per unit weight than the control while CO_2 output rates were approximately equal in another experiment. In two experiments CO_2 output was higher in desiccated groups. In the fifth group control insects were fed while desiccated insects were starved. Therefore, the two groups were probably not comparable in the final experiment. In view of the subjective aspect of experimental methods and results from adjusted data, this work does not clearly show increase in rate of CO_2 release by desiccated larvae over total experimental time span. In addition, the experimental method does not take into account possibility of periodic CO_2 release which occurs in some insects (Chapman, 1970).

Results of several studies on insects other then mealworms have been interpreted as showing an increase in metabolism or utilization of a nutrient reserve in response to low relative humidity. Buxton and Lewis (1934) reported an increase in fat metabolism at low humidities in adult tsetse flies, G. tachinoides. They measured fat metabolism by comparing average weights of fat found in starved flies at the end of a period of exposure to various relative humidities. Results were not adjusted for differences in

beginning live weights. Insects with low live weights also had low average fat contents. In addition, only 5-11 insects were used per humidity with no replication. Analysis of another species of adult tsetse fly, G. palpalis, showed no effect of humidity on fat loss (Mellanby, 1936a). Therefore, a change in fat loss in response to humidity is doubtful.

Fraenkel and Blewett (1944) indicated that three species of stored products insects required more food to produce a unit amount of pupal weight when reared at low humidities. Food utilization was measured by determining dry weight lost by food in which an insect or group of insects was reared. Final dry weight of food at end of larval period also included fecal material and frass. Therefore, dry weight lost by the food would not be a measure of food eaten, as indicated by Fraenkel and Blewett, but a measure of food utilization. Dry weight loss from the food media would approximate the sum of food stored as nutrient material, food used to manufacture body materials such as protein, and food catabolized to water and CO_2. Increased food utilization per unit pupal weight at low humidities meant that more food was metabolized to produce energy, CO_2, and water over the total length of the larval period. In general, as humidity decreased, pupal weight decreased; and larval period was extended. The latter fact could account for greater food utilization since insects would be expected to use more food during longer larval periods. However, Fraenkel and Blewett increased larval periods for Mediterranean flour moths by decreasing temperature while maintaining relative humidity at the same level as in other experiments. Food utilization per unit pupal weight was found to be the same at both temperatures for the same humidity. Fraenkel and Blewett concluded that humidity, not larval period, was the major factor influencing net food utilization.

We have analyzed some data of Fraenkel and Blewett and believe

that their results are open to an alternate interpretation. Only data for Mediterranean flour moths and dermestid beetles were examined since data for confused flour beetles were not sufficient for further analysis. If average food utilization per day per mg pupal weight is calculated for the insects, results indicate that insects held at the same temperature utilized essentially the same amount of food per day at all humidities (Table 5). Since food utilization includes both food catabolized and food stored as metabolic reserves, protein, etc., the same daily food utilization at all humidities does not necessarily indicate that metabolism was the same at all humidities. However, there is an obvious difference between averages for food utilization at 25 C and the average at 21 C (Table 5). Apparently metabolic rate and feeding activity were reduced at the lower temperature as might be expected. Therefore, the low temperature experiment does not prove that food utilization is independent of larval period and dependent only on humidity as Fraenkel and Blewett have argued.

Food utilization values determined by Fraenkel and Blewett are a measure of food catabolized plus food stored. Therefore, dry weight of food utilized minus dry weight of pupa should give an estimate of food catabolized to supply energy and to form water and CO_2. This figure was calculated for each insect as food catabolized per day per mg pupal dry weight. Results were averaged, and means tested statistically by analysis of variance (Table 5). Results indicated little difference between means for food catabolized per day at each humidity although the general trend was upward as humidity decreased. The data for Ephestia were not significant. Data for Dermestes were significant at $p = 0.10$ but not at $p = 0.05$. Therefore, on the average there is probably little change in the rate of metabolism in response to low humidities.

Table 5. The effect of relative humidity on food utilization and catabolism in Ephestia kuhniella and Dermestes vulpinus based on the data of Fraenkel and Blewett (1944). Means tested by analysis of variance. Variances tested by Bartlett's Test for homogeniety of variance.

Calculation	Relative humidities 1/ 0%	20%	70%	70% LT2/	Statistical signif.3/
Ephestia kuhniella					
Dry food utilized mg/day/mg dry pupal weight	0.1728	0.1704	0.1740	0.0951	N.A.
Variance	N.A.	N.A.	N.A.	N.A.	N.A.
Range: high value	0.2457	0.2252	0.2005	0.1201	
low value	0.1141	0.1186	0.1490	0.0758	
Dry food catabolized mg/day/mg dry pupal weight	0.1593	0.1514	0.1464	0.0794	n.s.
Variance	0.0014553	0.0005695	0.0001448	N.A.	***
Range: high value	0.2298	0.2043	0.1702	0.1033	
low value	0.1019	0.1032	0.1251	0.0585	
	Dermestes vulpinus				
	30%	50%	70%		
Dry food utilized mg/day/mg pupal dry weight	0.1336	0.1301	0.1338		N.A.
Variance	N.A.	N.A.	N.A.		N.A.
Range: high value	0.1640	0.1517	0.1600		
low value	0.1090	0.1006	0.1150		
Dry food catabolized mg/day/mg pupal dry weight	0.1199	0.1092	0.1078		*
Variance	0.0003985	0.0001090	0.0001237		**
Range: high value	0.1419	0.1304	0.1362		
low value	0.0945	0.0843	0.0937		

1/ N.A.: not analyzed. Low temperature experiment run only for Ephestia.

2/ LT: low temperature experiment at 21 C. All others at 25 C. Low temperature experiment was not included in statistical analyzes.

3/ N.A.: not analyzed. *: $p<0.10$ **: $p<0.05$ ***: $p<0.01$.

Dermestes were significant at $p = 0.10$ but not at $p = 0.05$. Therefore, on the average there is probably little change in the rate of metabolism in response to low humidities.

The range of values for food catabolized per day per mg pupal dry weight increased as humidity decreased (Table 5). Variances for various values were tested by Bartlett's Test for homogeniety of variance and were found to be statistically different. Since mean values were approximately the same, difference in variance indicates that rate of metabolism for an individual insect at a low humidity might be either higher or lower than its expected rate at higher humidity. Differences might be explained by postulating that individual insects respond to low humidity in one of two different ways. Some insects might show increased metabolism possibly due to factors such as increased activity to effect dispersal or increased metabolic rate to produce more metabolic water as Buxton (1930) hypothesized. Other insects might show a decreased metabolism due to a slowing of growth or dormancy under dry conditions. Therefore, averages of metabolic rates for all insects in a group at low humidity would not give a true picture of what was occurring in the population. The high and low rates would cancel each other. This hypothesis accounts for data for Ephestia better than data for Dermestes. In Dermestes low values in the range seemed to be approximately constant while high values increased somewhat.

Another possible explanation for apparently higher metabolic rates may be that some pupae contained less stored fat when reared at low humidities. There is some evidence that fed mealworms given water to drink develop a higher lipid content then mealworms given no water (Mellanby and French, 1958; Mellanby, 1958; Urs, 1970). It is conceivable that the humidity at which an insect is reared may affect lipid content in a similar way. Metabolism is an

enzyme mediated series of reactions. Rates of enzyme reactions depend on amount of enzyme present and not on amount of substrate as long as there is enough substrate to saturate the enzyme. Since fat in an insect is a substrate for metabolism, its amount would not be expected to influence rate of metabolism as long as there was enough fat to saturate the enzymes. Lipid free dry weight might be a better basis for comparison of metabolic rates. An insect with low storage lipid content would give a higher value for metabolic rate if the rate was adjusted only for insect dry weight, as was done here, instead of lipid free dry weight. Therefore, actual rates of metabolism per unit weight of metabolizing tissue could be similar in all groups; and increased variability could be an artifact due to differences in lipid content.

The question arises as to why length of larval period increased and weight of pupae decreased at low humidities in Fraenkel and Blewett's experiments while food catabolized per day per mg dry pupal weight remained approximately constant. One hypothesis might be that more food energy per day at low humidities was needed for increased activity, for increased energy to take up nutrients from very dry food, or for increased energy to extract more water from feces. There is evidence for the final point in insects. Water stress can decrease percentage of water in feces of tsetse flies (Bursell, 1960) and increase solute concentration in the hind gut of cockroaches (Wall and Ochsman, 1970). Since food catabolized per day per mg pupal dry weight was approximately the same at all humidities, increased energy use for active water conservation would have caused less energy to available for synthesis and storage of fats, carbohydrates, and proteins at low humidities. Part of the energy normally used for growth and development would have been used for water conservation. Therefore, development and

weight increase would have been slower, and possibly final weight would have been less. As insect dry weight increased, overall metabolic rate would have increased; but catabolism per day per unit dry weight would not. Food catabolized values would continually stay the same because they were adjusted by insect dry weight, and each increment of dry weight would add a proportional amount to overall metabolism.

To summarize, an increase in metabolic rate at low humidities need not be postulated to explain Fraenkel and Blewett's results. A possible explanation would be that at low humidities available energy was not used for growth and development but for other functions such as water conservation from fecal material. In any case average weight of food catabolized per mg pupal dry weight per day was close to the same at all relative humidities. For any increase in metabolism to be effective in counteracting increased water loss, food metabolism would have to be increased on a daily basis because water loss at low humidities would increase on a daily basis. Therefore, it is doubtful that Fraenkel and Blewett's results show an overall increase in metabolism for insect populations reared at low humidities although some individual insects may have had a somewhat higher metabolism for some reason such as increased activity or increased water conservation.

Buck and Keister (1949) found evidence for adult blowflies, Phormia regina, which might be interpreted as showing a higher metabolic rate at low humidity. They measured O_2 uptake per mg dry weight per hour for a period of 10 hours in 6 and 12 insects exposed to wet and dry conditions respectively. In general, O_2 uptake for flies in the dry condition was higher than uptake for flies in the wet condition. However, examination of the published data leads to the conclusion that there was probably no statistical difference between rates over at least half of the experimental period. Also, few

insects were used and the experimental period was probably too short to draw firm conclusions. Furthermore, Buck and Keister drew no conclusions about influence of humidity on metabolic rate. This experiment was only part of work on effects of humidity on DDT poisoning.

Apparently Edney (1957) interpreted data from Yeager and Munson (1950) and Ludwig and Wugmeister (1953) as supporting the hypothesis that dry conditions may stimulate greater metabolism. Examination of the above two reports on P. americana and P. japonica, respectively, reveals that these studies were concerned with effect of starvation on dry matter and blood volume. Effect of humidity was not studied. Yeager and Munson starved cockroaches at only one humidity which varied from 60% to 40% R.H. Ludwig and Wugmeister minimized effects of desiccation by keeping larvae at a single high humidity. Therefore, the results probably do not support the hypothesis that dry conditions stimulate metabolism.

There has been some speculation whether an increase in metabolic rate to produce more metabolic water would benefit insects at low humidities. Mellanby (1936b, 1942) argued that "in the light of present knowledge, it is difficult to see how an increase in the rate of metabolism could help an insect to withstand desiccating conditions . . . For every molecule of metabolic water produced, at least one molecule of oxygen must be taken in, and under dry conditions more water is lost by evaporation which accompanies respiration than is gained by metabolism." On the other hand, Bursell (1964) states that it cannot be assumed that increased respiration necessarily involves a proportionate increase in water loss. In Glossina flight activity, even in dry air, appears to be relatively economical of water reserves; metabolic rates increase by a factor of 22 during flight, water loss only by a factor of 6 (Bursell, 1959). Both in Aphis (Cockbain, 1961) and in

Schistocerca (Weis-Fogh, 1956) losses of water are fully compensated by metabolic gains during flight at fairly low humidities."

Another aspect of this matter is that metabolism of nutrients to produce water and CO_2 obligatorily produces energy. In biological systems most of this energy is usually incorporated into high energy phosphate esters such as adenosine triphosphate (ATP). The availability of adenosine diphosphate (ADP) to act as an acceptor for high energy phosphate bonds probably limits the rate of reaction and thus rate of oxidation (White, Handler, and Smith, 1959). Since combined concentration of ADP, ATP, and other high energy storage compounds is probably very low in insects, ATP must be converted into ADP for metabolism to continue. Energy is obligatorily released during this conversion because the reaction is exothermic or energy producing. Energy from conversion of ATP to ADP is usually used for mechanical work such as muscle contraction or for chemical work such as synthesis of food stores, proteins, etc. If the energy is not used as work, it must be released as heat. Oxidation and energy producing reactions can also be uncoupled from ATP formation. If uncoupling occurs, oxidation and water production would be independent of ADP concentration. However, uncoupling also produces heat since no high energy chemical bonds are formed to take up energy. Therefore, if insects increased their metabolism to produce more metabolic water, they would probably either have to increase some energy requiring activity to convert ATP to ADP so that rate of metabolism would not be limited by lack of ADP; or insects would have to produce heat. The latter alternative is likely to be detrimental because insects are poikilothermic and because increased temperature would cause increased water evaporation. Increased energy use through dispersal activity or active water conservation would seem more likely.

There is some evidence for both increased activity and increased water conservation. Andrewartha and Birch (1954) and Bursell (1964) have reviewed literature pertinent to these two points. Roberts (1970, 1971) found that low humidity decreased activity in face flies, Musca autumnalis. However, increases in either activity or water conservation does not necessarily mean that overall metabolic rate and energy production in an insect increase. Overall energy available might not increase, but use might shift from growth and development to water conservation or increased activity. Increased larval periods and decreased weight in insects reared at low humidities indicate that this possibility is plausible (see discussion of Fraenkel and Blewett's work above).

To summarize, there would be several possible consequences of increasing insect metabolism to produce more metabolic water. Among these consequences would be increased temperature and thus possible increased water loss if energy production were uncoupled from performance of mechanical or chemical work. Therefore, uncoupling would probably be deleterious to insects. Increase in some energy use would be more likely. However, increase in a particular energy use does not necessarily mean an overall increase in energy production since energy use might shift from one function to another.

From foregoing discussion one can conclude that there is little, if any, evidence to support the hypothesis that insects increase their metabolism at low humidities to produce more metabolic water. However, review of direct evidence against the hypothesis indicates that some of this evidence is also inconclusive for various reasons such as small sample sizes and too few replicates. Also, the question arises as to whether insects in these experiments were under enough water stress to show any difference in metabolic rate. The question of whether insects increase their metabolic rate in response

to low humidities may not necessarily be the same as the question of whether insects increase their metabolism in response to water stress. For instance, from our work we know that mealworms lost water, but we do not know to what degree the insects were stressed. We feel that the evidence is fairly conclusive that there is little influence of humidity on average metabolic rate in populations of insects. However, evidence is not so conclusive that water stress has no effect on rate of metabolism. Feeding experiments such as those of Fraenkel and Blewett (1944) seem to be a better method for analyzing influences of humidity or water stress on metabolic because such experiments should indicate conditions under which an insect could survive and develop and effect of these conditions.

Also, many experiments, ours included, used starved insects and determined effect of humidity on metabolism by measuring dry matter loss. These experiments exposed insects to two stresses, water loss and starvation. Experiments on fed insects should show influence of only one stress, low humidity. For this reason experiments on fed insects may provide a more valid approach although they are more difficult to carry out.

SUMMARY

The hypothesis examined in this work is that certain insect species inhabiting dry environments are able to produce more metabolic or oxidation water at low humidities to offset increased water loss. Critical evaluation of the literature leads to the conclusion that there is no unequivocal evidence to support and little evidence to reject this hypothesis. Most studies have not found humidity to have any influence on metabolism. Data that have been interpreted as supporting the hypothesis are either inconclusive or open to alternate interpretations. However, various indirect indicators of metabolic rate such as locomotor activity and water uptake from fecal material may increase in some insects at low humidities.

Influence of relative humidity on metabolism was reinvestigated by determining effect of relative humidity on water loss, dry matter loss, and lipid metabolism of fasting yellow mealworm larvae, Tenebrio molitor, L. Effects of humidity on dry matter loss and lipid metabolism in mealworm pupae and adults also were studied through larvae that pupated or metamorphosed into adults during the course of experiments on larvae. Data for pupae and adults are tentative because of small numbers of insects, few replications, and missing data due to no pupation or metamorphosis during some experiments.

As would be expected, estimated total water losses or gains in larvae during humidity exposure were dependent on relative humidity. Larvae gained water at 90% R.H. and lost water at lower humidities. Water loss increased as humidity decreased. It follows that metabolism should have correspondingly increased at low humidities if rate of metabolism was indeed affected by increased water loss. In addition, results for water change showed that the experimental methods were sensitive enough to detect significant changes

in average dry matter of larvae because similar methods were used to measure both water change and dry matter loss. Methods used to measure lipid changes in larvae and change of all body components in pupae and adults were probably not as accurate because values were based on percentages of total weights and not mean percentages. Therefore, these results were biased by sample size and variability in weight of individual insects.

Within limitations of experimental methods, relative humidity had no discernible effect on dry matter loss, lipid change, qualitative and quantitative fatty acid profile, or lipid-free dry matter change of fasting mealworm larvae. Humidity had no effect on dry matter losses in pupae and adults as well as lipid and lipid-free dry matter losses in adults.

Differences were detected in lipid and lipid-free dry matter changes in pupae at different humidities. Effect on lipid loss did not vary directly with humidity since results for highest and lowest humidities were statistically similar. Losses in lipid-free dry matter in pupae did increase as humidity decreased. However, in the case of pupal lipid and lipid free-dry matter losses differences are questionable because of small numbers of insects and replicates and because missing data were concentrated in the two lowest humidities.

Therefore, our results do not support the hypothesis of Buxton (1930) that mealworm larvae regulate metabolism and metabolic water production to compensate for increased water loss as relative humidity is decreased. Total body dry matter loss, lipid-free dry matter loss, total lipid loss, and fatty acid composition of total lipids did not change in relation to increasing water loss by larvae.

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VITA

Ludwig John Krchma was born on July 26, 1941, in Kansas City, Missouri. He attended grade school, high school, and college in that city. In 1963 he graduated from Rockhurst College with a Bachelor of Science degree in Education. For the 1963-1964 school year, he taught speech and English at Lillis High School in Kansas City. He returned to Rockhurst College from 1964 to 1966. In September, 1966, he received an N.I.H. Traineeship at Kansas State University.

He is married to the former Alice Elizabeth Davis of Kansas City, Kansas. They have three children: Barbara Renee, Michele Marie, and Laurie Anne.

Appendix I:

Supplementary Experimental Data

Table 6. Methods of analysis used on each experimental group.

Exp. No.	Culture Source	Final Insect Stage	Drying Process 1/	Dry Matter Loss 2/	Lipid and Lipid Free Dry Matter Losses 2/	Water Loss 2/	Fatty Acid Analysis 3/
5.1	K.S.U. Zoology	Larvae	Freeze	Avg.	Total	Avg.	Partial
		Pupae	N.A.	N.A.	N.A.	N.A.	N.A.
		Adults	N.A.	N.A.	N.A.	N.A.	N.A.
5.2	USDA	Larvae	Freeze	Avg.	Total	Avg.	Partial
		Pupae	N.A.	N.A.	N.A.	N.A.	N.A.
		Adults	N.A.	N.A.	N.A.	N.A.	N.A.
6.1	K.S.U. Zoology	Larvae	Freeze	Avg.	Total	Avg.	Partial
		Pupae	Heat	Total	Total	N.A.	N.A.
		Adults	Heat	Total	Total	N.A.	N.A.
6.2	K.S.U. Entomology	Larvae	Freeze	Avg.	Total	Avg.	Partial
		Pupae	Heat	Total	Total	N.A.	N.A.
		Adults	Heat	Total	Total	N.A.	N.A.
7.1	K.S.U.	Larvae	Freeze	Avg.	Total	Avg.	N.A.
		Pupae	Heat	Total	Total	N.A.	N.A.
		Adults	Heat	Total	Total	N.A.	N.A.
7.2	USDA	Larave	Freeze	Avg.	Total	Avg.	Partial
		Pupae	Heat	Total	Total	N.A.	N.A.
		Adults	Heat	Total	Total	N.A.	N.A.
8.1	USDA	Larvae	Heat	N.A.	Total	N.A.	N.A.
		Pupae	Heat	Total	N.A.	N.A.	N.A.
		Adults	Heat	Total	N.A.	N.A.	N.A.
8.2	USDA	Larvae	Heat	N.A.	Total	N.A.	N.A.
		Pupae	Heat	Total	N.A.	N.A.	N.A.
		Adults	Heat	Total	N.A.	N.A.	N.A.

1/ Freeze: freeze dried. Heat: heat dried. N.A.: not analyzed.

2/ Avg.: data calculated from averages of weights and percentages. Total: data calculated from percentages of total weights. N.A.: not analyzed.

3/ Partial: only certain groups at selected humidities analyzed. N.A.: not analyzed.

Table 7. Number of insects available for analysis in each experimental group.

Experiment Number	Larvae Relative Humidities				Pupae 1/ Relative Humidities				Adults 1/ Relative Humidities			
	0%	30%	60%	90%	0%	30%	60%	90%	0%	30%	60%	90%
5.1	18	12	5	10	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5.2	20	18	18	22	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
6.1	1	5	11	15	0	3	2	2	12	8	7	5
6.2	12	9	10	10	0	1	1	8	7	7	11	4
7.1	23	19	19	20	2	4	2	1	0	1	4	4
7.2	19	16	11	10	4	8	11	7	1	1	3	6
8.1	17	16	21	17	5	3	2	3	1	6	1	4
8.2	19	22	21	22	2	1	1	1	3	0	1	2

1/ N.A.: none analyzed.

Table 8. Percentage of original water lost or gained by the larvae during exposure to various relative humidities. All values are losses unless marked with a plus sign. Means are significant at $p = 0.01$. Details of the statistical analysis are given in Appendix II, Table 20.

Experiment Number	Relative Humidities			
	0%	30%	60%	90%
5.1	31.9	23.9	19.0	+ 2.3
5.2	17.8	16.1	11.8	6.4
6.1	29.1	18.5	10.8	+ 3.9
6.2	14.7	17.8	6.1	+ 7.9
7.1	14.6	11.9	5.5	+ 4.0
7.2	15.0	9.1	9.3	+ 6.7
Means:	20.5	16.2	10.4	+ 3.1

1/ +: water gain.

Table 9. Percentage of the estimated original dry matter lost by larvae, pupae, and adults. None of the means in this table are statistically significant at p = 0.10. Details of the statistical analysis are given in Appendix II, Table 21.

Experiment Number	Larvae[1/] Relative Humidity				Pupae[1/] Relative Humidity				Adults[1/] Relative Humidity			
	0%	30%	60%	90%	0%	30%	60%	90%	0%	30%	60%	90%
5.1	15.8	13.5	17.5	14.0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5.2	17.6	10.5	19.0	16.6	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
6.1	27.1	11.8	19.5	24.2	Est.	Est.	27.9	34.0	46.9	47.9	41.3	44.5
6.2	18.3	25.5	18.3	23.7	Est.	40.8	30.6	39.0	47.0	49.0	45.6	48.3
7.1	21.1	15.5	21.1	17.8	37.8	34.6	31.2	27.5	Est.	40.6	41.0	40.3
7.2	10.9	11.6	8.0	14.2	38.1	32.1	29.6	33.9	41.0	43.6	36.6	45.7
8.1	N.A.	N.A.	N.A.	N.A.	26.4	27.3	31.0	24.0	37.5	31.7	31.6	27.6
8.2	N.A.	N.A.	N.A.	N.A.	28.3	39.1	28.6	1.3	30.9	Est.	48.4	40.7
Means:	18.5	14.7	17.2	18.4	34.9	35.3	29.8	26.1	40.5	42.4	40.7	41.2

1/ N.A.: not analyzed. Est.: value was not determined experimentally due to lack or loss of material but was estimated statistically and the value included in the calculations of the means and the statistical analysis.

Table 10. Percentage of the estimated original lipid lost or gained by larvae, pupae, and adults during relative humidity exposure. Values are losses except those marked with a plus sign. Means for larvae and adults are not statistically significant at p = 0.10. The means for the pupae are significant at that same level. Details of the statistical analysis are given in Appendix II, Table 22.

Experiment Number	Larvae 1/ Relative Humidity				Pupae 1/ Relative Humidity				Adults 1/ Relative Humidity			
	0%	30%	60%	90%	0%	30%	60%	90%	0%	30%	60%	90%
5.1	8.1	9.7	13.5	17.9	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5.2	18.9	12.3	30.1	24.6	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
6.1	36.1	17.1	35.3	38.7	Est.	40.1	28.1	46.3	77.5	77.4	63.5	74.9
6.2	29.4	39.3	33.4	35.9	Est.	53.6	41.7	67.4	77.5	80.5	74.0	72.1
7.1	30.1	27.7	35.6	23.4	54.1	42.9	44.4	45.1	Est.	58.6	75.8	65.9
7.2	12.0	2.3	+ 8.6	20.5	57.7	46.3	43.7	49.2	77.8	75.8	55.4	80.2
8.1	10.4	1.5	10.2	+22.2	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
8.2	40.5	2.7	26.8	25.1	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Means:	23.2	14.1	22.0	20.5	56.4	45.7	39.5	52.0	76.1	73.1	67.2	73.3

1/ N.A.: not analyzed. Est.: value was not determined experimentally due to lack or loss of material but was estimated statistically and the value included in the calculations of the means and the statistical analysis. +: percentage gain.

Table 11. Total lipid loss as a percentage of the estimated total dry matter lost for larvae, pupae, and adults. Means for the larvae and adults are not significantly different at p = 0.10. The means for the pupae are significant at that level. Details of the statistical analysis are given in Appendix II, Table 23.

Experiment Number	Larvae Relative Humidity				Pupae 1/ Relative Humidity				Adults 1/ Relative Humidity			
	0%	30%	60%	90%	0%	30%	60%	90%	0%	30%	60%	90%
5.1	17.4	25.4	27.1	47.5	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5.2	37.6	40.3	57.6	51.9	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
6.1	45.0	49.1	59.7	54.0	Est.	Est.	33.7	45.5	55.3	54.0	51.4	56.3
6.2	51.5	52.8	62.3	51.9	Est.	44.7	46.3	58.6	56.0	55.8	55.1	50.6
7.1	49.5	60.3	57.4	47.0	49.2	42.6	48.9	56.3	Est.	49.6	63.6	56.2
7.2	39.4	6.7	0.0	49.9	47.9	45.6	46.7	45.9	59.8	54.9	47.9	55.5
8.1	45.7	9.8	38.6	0.0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
8.2	73.2	10.8	74.4	71.4	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Means:	44.9	31.9	47.1	46.7	46.6	41.6	43.9	51.6	57.8	53.6	54.5	54.6

1/ N.A.: not analyzed. Est.: value was not determined experimentally due to lack or loss of material but was estimated statistically and the value included in the calculations of the means and the statistical analysis.

Table 12. Percentage of the estimated original lipid free dry matter lost by larvae, pupae, and adults during relative humidity exposure. Means for larvae and adults are not statistically significant at p = 0.10. The means for the pupae are significant at that same level. Details of the statistical analysis in Appendix II, Table 24.

Experiment Number	Larvae Relative Humidity				Pupae 1/ Relative Humidity				Adults 1/ Relative Humidity			
	0%	30%	60%	90%	0%	30%	60%	90%	0%	30%	60%	90%
5.1	19.0	14.4	18.4	10.1	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5.2	16.7	9.7	11.8	11.1	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
6.1	22.3	9.0	12.0	16.6	Est.	Est.	27.8	27.8	31.6	33.1	30.2	29.3
6.2	14.2	18.1	10.4	17.1	Est.	34.2	24.9	24.5	31.3	32.8	31.0	36.1
7.1	16.1	9.6	13.8	13.8	29.3	30.3	24.3	18.3	Est.	31.2	22.7	26.9
7.2	8.6	15.0	15.5	9.5	29.0	25.5	23.0	26.9	24.0	28.7	27.9	29.7
8.1	7.0	8.0	9.1	24.4	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
8.2	8.4	12.7	5.2	5.7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Means:	14.0	12.1	12.0	13.5	31.2	31.0	25.0	24.4	28.0	31.4	27.9	30.5

1/ N.A.: not analyzed. Est.: value was not determined experimentally due to lack of loss of material but was estimated statistically and the value included in the calculations of the means and the statistical analysis.

Table 13. Total lipid free dry matter loss as a percentage of the estimated total dry matter lost for larvae, pupae, and adults. Means for the larvae and adults are not significantly different at p = 0.10. The means for the pupae are significant at that level. Details of the statistical analysis are given in Appendix II, Table 25.

Experiment Number	Larvae Relative Humidity				Pupae 1/ Relative Humidity				Adults 1/ Relative Humidity			
	0%	30%	60%	90%	0%	30%	60%	90%	0%	30%	60%	90%
5.1	82.6	74.6	72.9	52.5	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5.2	62.4	59.7	42.4	48.1	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
6.1	55.0	50.9	40.3	46.0	Est.	Est.	66.3	54.5	44.7	46.0	48.6	43.7
6.2	48.5	47.2	37.7	48.1	Est.	55.3	53.7	41.4	44.0	44.2	44.9	49.4
7.1	50.5	39.7	42.6	53.0	50.8	57.4	51.1	43.7	Est.	50.4	36.4	43.8
7.2	60.6	93.3	100.0	50.1	52.1	54.4	53.3	54.1	40.2	45.1	52.1	44.5
8.1	54.3	90.2	61.4	100.0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
8.2	26.8	89.2	25.6	28.6	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Means:	55.1	68.1	52.9	53.3	53.4	58.4	56.1	48.4	42.2	46.4	45.5	45.3

1/ N.A.: not analyzed. Est.: value was not determined experimentally due to lack or loss or material but was estimated statistically and the value included in the calculations of the means and the statistical analysis.

Table 14. Summary tables of the relative percentage concentration of the major fractions from the total fatty acid analysis. Mean No. 1 excludes experiments 6.1 and 6.2 because there were indications that oxidation of the unsaturated acids occurred due to improper storage. Mean No. 2 excludes experiments 6.1, 6.2, and 7.2 for the reasons mentioned above and because the freeze-dried control for 7.2 was not analyzed. Experiments 7.1, 8.1, and 8.2 were not analyzed. Detailed data for total fatty acid analysis is given in Appendix I, Tables 8-12.

Fatty Acid	Experiment Number	Controls 1/		Experimental Humidities 1/			
		UDC	FDC	0%	30%	60%	90%
Myristic	5.1	4.0	5.1	6.7	N.A.	5.0	6.1
	5.2	N.A.	6.4	6.4	N.A.	8.0	5.9
	6.1	5.5	6.4	13.9	N.A.	9.5	N.A.
	6.2	5.4	6.7	8.9	N.A.	8.4	N.A.
	7.2	N.A.	N.A.	N.A.	N.A.	5.8	7.8
	Mean No. 1			6.3		6.3	6.6
	Mean No. 2		5.8	6.5		6.5	6.0
Palmitic	5.1	14.8	17.6	12.3	N.A.	11.4	12.0
	5.2	N.A.	15.4	14.1	N.A.	11.3	14.0
	6.1	19.6	24.0	13.7	N.A.	17.2	N.A.
	6.2	18.1	20.3	15.2	N.A.	14.9	N.A.
	7.2	N.A.	N.A.	12.7	N.A.	12.1	13.0
	Mean No. 1			13.0		11.6	13.0
	Mean No. 2		16.5	13.2		11.3	13.0
Palmitoleic	5.1	4.2	4.2	5.3	N.A.	4.4	5.2
	5.2	N.A.	4.9	5.0	N.A.	5.2	5.0
	6.1	3.7	3.6	6.8	N.A.	5.1	N.A.
	6.2	4.7	5.2	5.7	N.A.	6.1	N.A.
	7.1	N.A.	N.A.	5.1	N.A.	4.9	5.7
	Mean No. 1			5.1		4.8	5.3
	Mean No. 2		4.6	5.1		4.8	5.1

1/ FDC: freeze-dried control. UDC: undried control. N.A.: not analyzed.

(continued on next page)

Table 15 (continued).

Fatty Acid	Experiment Number	Controls 1/		Experimental Humidities 1/			
		UDC	FDC	0%	30%	60%	90%
Oleic	5.1	58.5	53.6	54.0	N.A.	58.1	53.6
	5.2	N.A.	44.3	46.3	N.A.	43.9	44.9
	6.1	53.7	51.1	52.9	N.A.	52.6	N.A.
	6.2	58.2	57.4	56.0	N.A.	57.6	N.A.
	7.2	N.A.	N.A.	49.5	N.A.	50.5	46.7
	Mean No. 1			49.9		50.8	48.4
	Mean No. 2		49.0	50.1		51.0	49.2
Linoleic	5.1	16.3	17.1	19.4	N.A.	19.4	21.2
	5.2	N.A.	24.9	27.7	N.A.	28.0	26.5
	6.1	15.6	6.3	9.8	N.A.	4.8	N.A.
	6.2	11.7	8.4	8.8	N.A.	11.3	N.A.
	7.2	N.A.	N.A.	23.1	N.A.	22.5	21.8
	Mean No. 1			22.1		23.3	23.2
	Mean No. 2		21.0	21.5		23.7	23.9
Total Minor Fractions	5.1	2.2	2.4	2.4	N.A.	1.7	1.9
	5.2	N.A.	4.0	4.6	N.A.	3.7	3.7
	6.1	1.9	8.6	2.9	N.A.	10.8	N.A.
	6.2	2.0	2.0	5.4	N.A.	1.8	N.A.
	7.2	N.A.	N.A.	3.7	N.A.	4.2	5.1
	Mean No. 1			3.5		3.2	3.6
	Mean No. 2		3.2	3.5		2.7	2.8

1/ UDC: undried control. FDC: freeze-dried control. N.A.: not analyzed.

Table 16. Relative percentages of the total fatty acids found in Experiment 5.1. The experimental group at 30% R.H. was not analyzed.

Fatty Acid Carbon Number 1/	Controls		Relative Humidities		
	Undried	Freeze-dried 2/	0%	60%	90%
8	-	trace	-	-	-
* 9	-	trace	-	-	-
10	trace	trace	trace	trace	trace
*10.2	-	trace	trace	-	trace
*11.1	trace	trace	trace	-	trace
12	0.4	0.6	0.6	0.5	0.5
*13	trace	trace	trace	0.1	trace
14	4.0	5.1	6.7	5.0	6.1
*14:1	trace	trace	0.8	0.8	0.5
15	-	trace	-	trace	-
16	14.8	17.6	12.3	11.4	12.0
16.1	4.2	4.2	5.3	4.4	5.2
*16.2	-	trace	-	0.3	0.4
18	1.9	1.7	1.0	trace	0.4
18.1	58.5	53.6	54.0	58.1	53.6
18.2	16.3	17.1	19.4	19.4	21.2

1/ *: tentatively identified by a plot of carbon number vs log of retention time.

2/ Average of two replications.

Table 17. Relative percentages of the total fatty acids found in Experiment 5.2. The experimental group at 30% R.H. was not analyzed. There was no undried control in this experiment.

Fatty Acid Carbon Number 1/	Freeze-dried Control	Relative Humidities		
		0%	60%	90%
10	0.1	0.1	-	0.1
*10:2	trace	trace	-	trace
*11.1	0.1	0.2	0.2	0.2
12	1.1	0.9	0.9	1.0
*13	trace	0.1	0.2	0.1
14	6.4	6.4	8.0	5.9
*14.1	0.4	0.7	0.9	0.7
*15	trace	trace	trace	-
16	15.4	14.1	11.3	14.0
16:1	4.9	5.0	5.2	5.0
*16.2	trace	0.6	trace	0.7
18	2.3	2.0	1.5	0.9
18.1	44.3	46.3	43.9	44.9
18.2	24.9	23.7	28.0	26.5

1/ *: tentatively identified by a plot of carbon number vs log of retention time.

Table 18. Relative percentages of the total fatty acids found in Experiment 6.1. The experimental groups at 30% and 90% R.H. were not analyzed.

Fatty Acid Carbon Number	Control		Humidities	
	Undried	Freeze-dried	0%	60%
** X	-	-	0.7	-
* 9	-	trace	-	0.3
10	-	trace	trace	0.1
*10:2	-	0.2	-	0.2
*11.1	-	-	-	-
12	0.6	0.9	1.2	0.8
** X	-	-	0.4	-
*13	trace	0.2	-	0.3
14	5.5	6.4	13.9	9.5
*14.1	trace	trace	trace	1.0
*14.2	-	0.4	-	0.3
*15	-	0.4	-	0.6
16	19.6	24.0	13.7	17.2
16:1	3.7	3.6	6.8	5.1
*16.2	trace	-	trace	-
18	1.4	2.3	0.6	0.8
18:1	53.7	51.1	52.9	52.6
18:2	15.6	6.3	9.8	4.8
** X	-	2.0	-	2.4
** X	-	2.3	trace	4.1
** X	-	-	trace	-

1/ *: tentatively identified by a plot of carbon number vs log of retention time. **: possible products of oxidation of unsaturated fatty acids due to improper storage. No peaks with the retention times of these peaks were found in the undried controls or in analyses of other experiments. X: unknowns.

Table 19. Relative percentages of the total fatty acids found in Experiment 6.2. The experimental groups at 30% and 90% R.H. were not analyzed.

Fatty Acid Carbon Number 1/	Controls		Humidities	
	Undried	Freeze-dried	0%	60%
* 9	-	-	0.2	-
10	trace	trace	0.1	trace
*10:2	-	-	0.1	-
*11:1	-	-	trace	-
12	0.8	0.8	1.0	0.9
*13	-	trace	0.3	0.2
14	5.4	6.7	8.9	8.4
*14:1	trace	trace	trace	trace
*14.2	-	-	0.3	-
15	-	-	0.3	-
16	18.1	20.3	15.2	14.9
16:1	4.7	5.2	5.7	6.1
*16:2	-	-	-	-
18	1.2	1.1	0.9	0.7
18:1	58.2	57.4	56.0	57.6
18:2	11.7	8.4	8.8	11.3
** X	-	-	0.7	-
** X	-	-	1.6	-
** X	-	-	-	trace

1/ *: tentatively identified by a plot of carbon number vs log of retention time. **: possible products of oxidation of unsaturated fatty acids due to improper storage. No peaks with the retention times of these unknowns were found in the undried controls or in analyses of other experiments.

Table 20. Relative percentages of the total fatty acids found in Experiment 7.2. The experimental groups at 30% R.H. and both the undried and freeze-dried controls were not analyzed.

Fatty Acid Carbon Numbers	0%	60%	90%
10	0.1	0.1	0.1
12	1.0	1.2	1.6
*13	0.1	0.1	0.2
14	5.9	5.8	7.8
*14:1	0.8	1.0	1.2
*14:2	-	trace	-
*15:0	trace	trace	-
16	12.7	12.1	13.0
16:1	5.1	4.9	5.7
*16.2	0.6	0.5	0.8
18	0.9	1.4	1.1
18:1	49.5	50.5	46.7
18:2	23.1	22.5	21.8

1/ *: tentatively identified by a plot of carbon number vs log of retention time.

Appendix II:

Description and Data for Statistical Analyses

Statistical analyses were carried out by two-way analysis of variance to minimize experimental effects. The analyses were done by the Statistical Laboratory, Kansas State University, Manhattan, Kansas by computer. Missing observations were estimated statistically, also by computer. Most tables are reproduced as they appeared on the computer print-out. Treatment is the variability between mean values for the various humidities, and experiments is the variability between the means of the various experimental replicates. The probability listed is the probability of the "F" value being due to random error. Therefore, as the probability of a random "F" value decreases, the probability that the tested means are different increases. $p = 0.10$ was taken as the lowest level of significance. The L.S.D. test was calculated by hand whenever $p<0.10$. The means are ranked below the appropriate table only when $p<0.10$. A horizontal line beneath two or more means indicates that the L.S.D. test cannot show any statistical difference between them. If $0.10>p>0.05$, "t" at the $p = 0.10$ level was used for the L.S.D. test. If $p<0.05$, "t" at the $p = 0.05$ level was used.

Table 21. Analysis of variance table for the mean percentage of the original water lost or gained by the larvae. All figures for the means are losses except those indicated by a plus sign.

		Larvae			
Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	1895.937	631.979	35.905	0.0000
Experiment	5	430.062	86.012	4.887	0.0075
Residual	15	264.023	17.602		
Total	23	2590.018			
Percent R.H.:		0%	30%	60%	90% 1/
Rank of Means:		20.51	16.21	10.42	+3.07

1/ +: percentage gain.

Table 22. Analysis of variance tables for percent dry matter lost for larvae, pupae, and adults.

Larvae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	55.018	18.339	1.284	0.8159
Experiment	5	298.783	59.757	4.184	0.0140
Residual	15	214.218	14.281		
Total	23	568.006			

Pupae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	254.696	84.899	1.399	0.2910
Experiment	5	422.475	84.495	1.392	0.2948
Residual	12	728.436	60.703		
Total	20	1353.917			

Adults

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	10.222	3.407	0.163	0.9197
Experiment	5	555.837	111.167	5.303	0.0071
Residual	13	272.504	20.962		
Total	21	840.113			

Table 23. Analysis of variance tables for percent of original lipid lost.

Larvae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability 1/
Treatment	3	397.148	132.382	1.279	N.S.
Experiment	7	4366.898	623.842	6.028	p<0.005
Residual	21	2173.289	103.490		
Total	31	6937.336			

Pupae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	483.353	161.118	5.456	0.0300
Experiment	3	403.340	134.447	4.553	0.0452
Residual	7	206.706	29.529		
Total	13	1100.937			
Percent R.H.:		60%	30%	90%	0%
Rank of Means:		39.47	45.72	52.00	56.37

Adults

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	151.334	50.445	0.717	0.5693
Experiment	3	107.356	35.785	0.508	0.6874
Residual	8	536.065	70.383		
Total	14	865.496			

1/ N.S.: not significant at p = 0.10. p: probability.

Table 24. Analysis of variance tables for lipid loss as a percentage of dry matter lost.

Larvae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	1264.946	421.662	1.481	0.250
Experiment	7	5917.934	845.419	2.968	0.025
Residual	21	5980.469	284.784		
Total	31	13163.391			

Pupae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	194.628	64.876	4.427	0.0577
Experiment	3	201.116	67.039	4.574	0.0541
Residual	6	87.931	14.655		
Total	12	437.787			
Percent R.H.:		30%	60%	0%	90%
Rank of Means:		41.59	43.90	46.57	51.57

Adults

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	30.678	10.226	0.476	0.7078
Experiment	3	19.836	6.612	0.308	0.8194
Residual	8	171.919	21.490		
Total	14	213.049			

Table 25. Analysis of variance tables for percent of the total original lipid free dry matter lost.

Larvae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	25.331	8.444	0.362	0.7810
Experiment	7	163.284	23.326	1.000	0.4583
Residual	21	489.653	23.317		
Total	31	678.261			

Pupae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	110.907	36.969	3.751	0.0790
Experiment	3	46.283	15.428	1.565	0.2927
Residual	6	59.134	9.856		
Total	12	183.120			
Percent R.H.:		90%	60%	30%	0%
Rank of Means:		24.37	25.00	31.04	31.24

Adults

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	35.307	11.769	2.130	0.1746
Experiment	3	93.389	31.130	5.635	0.0226
Residual	8	44.196	5.524		
Total	14	166.153			

Table 26. Analysis of variance tables for total lipid free dry matter loss as a percentage of total dry matter lost.

Larvae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	1257.767	419.256	1.474	0.2504
Experiment	7	5903.414	843.345	2.964	0.0252
Residual	21	5974.719	284.510		
Total	31	13135.895			

Pupae

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	194.626	64.875	4.427	0.0577
Experiment	3	201.113	67.038	4.575	0.0541
Residual	6	87.927	14.654		
Total	12	437.787			
Percent R.H.:		90%	0%	60%	30%
Rank of Means:		48.42	53.43	56.10	58.41

Adults

Source	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Treatment	3	30.802	10.267	0.475	0.7086
Experiment	3	19.954	6.651	0.307	0.8196
Residual	8	173.097	21.637		
Total	14	214.420			

Table 27. Analysis of variance tables for the relative percentages of the various fatty acids.

Fatty Acids	Source 1/	Deg. of Freedom	Sums of Squares	Mean Squares	F-ratio	Probability
Myristic	Treat.	2	0.187	0.093	0.060	0.9428
	Exp.	2	1.087	0.543	0.348	0.7256
	Resid.	4	6.247	1.562		
	Total	8	7.520			
Palmitic	Treat.	2	4.016	2.008	4.291	0.1011
	Exp.	2	2.296	1.148	2.453	0.2017
	Resid.	4	1.871	0.468		
	Total	8	8.182			
Palmitoleic	Treat.	2	0.336	0.168	1.279	0.3719
	Exp.	2	0.109	0.054	0.415	0.6858
	Resid.	4	0.525	0.131		
	Total	8	0.969			
Oleic	Treat.	2	9.082	4.541	1.296	0.3682
	Exp.	2	159.102	79.551	22.701	0.0066
	Resid.	4	14.017	3.504		
	Total	8	182.176			
Linoleic	Treat.	2	0.082	0.041	0.039	0.9618
	Exp.	2	85.182	42.591	40.698	0.0022
	Resid.	4	4.186	1.047		
	Total	8	89.449			
Total Minor Fractions	Treat.	2	0.269	0.134	0.350	0.7245
	Exp.	2	9.556	4.778	12.427	0.0192
	Resid.	4	1.538	0.384		
	Total	8	11.362			

1/ Treat.: treatment
Exp.: experiment.
Resid.: residual.

Appendix III:

Calculation Methods

Average Percentage of the Beginning Dry Matter Lost by the Larvae. The percentages of beginning dry matter lost were calculated differently for the larvae and the pupae and adults. The values for the larvae were calculated as follows. The dry matter at the start of the experiment was estimated by multiplying the weight of each larvae by the average fraction of dry matter contained in the group of freeze dried control larvae for the respective experiment. The dry matter at the end of the experiment was determined by freeze-drying or heat drying the insect and determining the dry weight. The dry matter loss for each larvae was estimated by subtracting the final dry weight from the estimated beginning dry matter weight. The ratio of estimated dry matter lost to the estimated beginning dry matter was determined for each larva, and the result was multiplied by 100 to determine the estimated percentage of the original dry matter lost by each larva. The average of all the percentages was computed for each experimental group at each humidity. The results were compared statistically. The values calculated here were true means.

Percentage of the Total Beginning Dry Matter Lost by the Pupae and Adults. The total dry matter contained at the start of the experiment by the larvae which either pupated or metamorphosed into adults was estimated by multiplying the sum of the beginning live weights for each group of experimental insects by the ratio of total dry matter to total live weight found in the freeze-dried control group for the respective experiment. The final dry matter was determined by heat drying the various groups of pupae and adults and determining the total dry matter content of each group. The total dry matter lost by each group of insects was estimated by subtracting the total final dry matter from the total estimated beginning dry matter. The ratio of the estimated total lost to the estimated total beginning dry matter weight was calculated and

multiplied by 100 to determine the percentage of the estimated total original dry matter lost by each group of pupae or adults. The values for the groups of pupae and for the groups of adults, respectively, were compared by two-way analysis of variance.

The percentages calculated for the various groups are not true averages for each insect because the values were calculated from total weights and not the weights of individual insects. Therefore, the results are somewhat biased by sample size and variability in the weights of the insects.

Percentage of the Estimated Total Beginning Lipid Lost by Larvae, Pupae, and Adults. The values for percentage of lipid lost were calculated using total weights of groups of groups of insects because lipid extraction of each insect was considered to be too tedious. The total original weight of lipid at the start of an experiment was estimated by multiplying the total starting live weight of a group of insects by the lipid percentage of the total live weight of the appropriate dried control group. The total lipid content at the end of the experiment was determined by lipid extraction. The lipid lost was estimated by subtracting the total final lipid weight from the estimated total beginning lipid for each group of insects. The ratio of the estimated total lipid lost to the estimated beginning lipid was calculated and multiplied by 100 to determine the percentage of the total original lipid lost. The various percentages were then compared statistically. Again, this value is not a true mean and is therefore biased by variability in weight and sample size.

Total Lipid Loss as a Percentage of the Total Dry Matter Lost. This percentage was calculated by dividing the estimated total lipid lost by the estimated total dry matter lost for each group of insects and multiplying by 100. The estimated total lipid lost and the estimated total dry matter lost

for the groups of puape and adults were determined as indicated in the preceding two sections. The estimated total beginning dry matter for the groups of larvae was estimated as in each group of pupae and adults, and the estimated total dry matter lost was determined for each group of larvae. The average percentage of dry matter was not used to calculate the estimated total original dry matter in the groups of larvae because the lipid has been determined on a total weight basis. Therefore, total weights and percentages of total weights were used in all calculations for the sake of consistency.

The Percentage of Lipid Free Dry Matter Lost and the Estimated Total Lipid Free Dry Matter Lost as a Percentage of the Total Weight Lost. The two measurements of lipid free dry matter loss were calculated in a manner similar to the figures for estimated total dry matter lost and estimated total lipid lost. Values for the estimated total beginning lipid free dry matter and the total final lipid free dry matter at the end of the experiment were calculated by subtracting the respective total lipid contents from the respective total dry matter contents for each group of insects. The calculations for percentage of the original weight lost and loss as a percentage of total dry matter lost were then performed as in the two preceding sections.

Water Change. Water change was measured only in the larvae. The original water was estimated by subtracting the estimated dry matter for each larva from the beginning live weight of that larva. The original dry matter was determined by the method outlined in the first section of this appendix. The final water content was calculated by subtracting the final dry matter weight from the live weight at the end of the experimental period. The estimated water lost by each larvae was then determined by subtracting the final weight of water from the estimated original weight of water. The percentage of the original water which had been lost was calculated, and the mean for each group

of larvae was determined. The various values were compared statistically. These percentages were true mean percentages for each group of larvae since the water content and loss for each larvae was measured and estimated separately and an average of the values was calculated.

THE EFFECT OF RELATIVE HUMIDITY ON THE METABOLISM OF THE STARVED YELLOW MEALWORM TENEBRIO MOLITOR L. (COLEOPTERA, TENEBRIONIDAE) AS MEASURED BY CHANGES IN DRY MATTER AND LIPID COMPOSITION

by

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B. S., Rockhurst College, 1963

AN ABSTRACT OF A THESIS

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The hypothesis examined in this work is that certain insect species inhabiting dry environments are able to produce more metabolic water at low humidities to offset increased water loss. Critical evaluation of the literature leads to the conclusion that there is no unequivocal evidence to support or reject this hypothesis. Most studies have not found humidity to have any influence on metabolism. Data that have been interpreted as supporting the hypothesis are either inconclusive or open to alternate interpretations. To examine the hypothesis, the effect of relative humidity on insect metabolism was indirectly determined by measuring the changes in the water, dry matter, lipid, and lipid free dry matter composition of yellow mealworm larvae, Tenebrio molitor, L., starved at a range of relative humidities for 21 days. The effects of humidity on the dry matter loss and lipid metabolism in mealworm pupae and adults were also studied through larvae that developed into pupae or adults during the course of the experiments.

Although the larvae did loose more water at low humidities, there was no indication of any increase in any of the indirect measures of metabolism. The adults also showed no effect of humidity on metabolism. Dry matter loss in the pupae was evidentally not affected. Humidity may have influenced the lipid and lipid free dry matter changes in the puape. However, the effect is questionable because of the small number of puape and experimental replicates and because some missing data was concentrated in the two lowest humidities.

Therefore, our results do not support the hypothesis that mealworm larvae regulate metabolism and metabolic water production to compensate or increased water loss as relative humidity is decreased. The total body dry matter loss, lipid free dry matter loss, total lipid loss, and fatty acid composition of the lipids did not change in relation to increasing water loss by the larvae.