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RELATIONSHIPS BETWEEN AGE, BODY WEIGHT, METABOLIC RATE, RESPIRATORY QUOTIENTS, AND TIME OF DAY IN THE DOMESTIC RABBIT

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ABSTRACT

Metabolic rates (RMR) and respiratory quotients (RQ) were measured in domestic rabbits to determine how these parameters vary with age, body weight, and time of day.

The RQ increased with age indicating change in substrate metabolism from fat to carbohydrate. The RQ decreased from morning to afternoon, probably indicating a shift from the absorptive to post absorptive state.

The RMR increased from morning to afternoon and decreased with age. The exponential equation relating weight to metabolic rate (RMR = M) for growing rabbits is very close to the interspecific relationship, $W^{0.75}$:

$$M = 4.67\ W^{0.765}$$

INTRODUCTION

This experiment was designed to (i) determine the effects of growth and diurnal rhythm on metabolic rate and respiratory quotient (RQ) in the domestic rabbit while simultaneously (ii) developing and evaluating a technique for measuring metabolic rate. The technique used measures the O_2 consumption and CO_2 production as a method of determining heat production, or metabolic rate in kcal $\times$ kg^{-1} hr^{-1}. The metabolic rate so determined is differentiated from other measurements of energy use by designating it R. Conditions of the experiment, animals kept at 20°C with *ad lib* diet, provide data for resting metabolic rates (RMR) and not basal metabolic rates (BMR). The measurement of R does not include energy deposited as tissue (P).

Changes in metabolic requirements (energy needs) with growth are usually thought to be due to changes in body size and other undefined changes occurring with growth, as well as energy deposited as tissue. The values measured in this experiment deal with changes due to difference in body size and changes during the growth process and not with actual tissue production.

Experiments on animal size as it affects oxygen consumption have been done. The inverse relationship between size and metabolic rate, especially across species, is well known. A recent review by Hart (1971) reports a correlation between resting metabolism and body weight in rodents that can be characterized by the equation $M = 3.8\ W^{0.75}$, where M is metabolic rate in kcal/day and W is body weight in kg. Goldstone and Steffens (1967) report experimental basal metabolic rate values for exponents as high as 0.933. Hart noted that there is considerable variation within species.

A study relating age, body size, and body composition to basal metabolism has been reported on albino rats (Conrad and Miller 1956). These workers tried to separate the decline in metabolism due to increase in size from the decline in metabolism due to a systematic decline in tissue metabolism with increasing age. They followed the oxygen consumption of the animals from birth to maturity and found the decline in metabolic rate with age is more rapid than would be expected according to the $W^{0.75}$ rule. A variety of data included in this report suggests that metabolic differences occur with changes in age. For example, metabolically inert fat increases with age. Also there is a decrease in percentage of body weight of highly metabolic organs such as the liver, brain, heart, and kidneys. However, the authors concluded that results to date do not prove that conditions accompanying growth are a major factor causing high metabolic rate in immature animals.

This experiment measures the RMR of growing rabbits to determine whether or not an "additional growth factor" can be established. The RMR measurements are used to establish an exponential relationship with body size during growth $M = aW^b$. The exponent b is then compared to the expected adult b. A significant difference in exponents could be attributed to a "growth factor."

The time of day that metabolic measurements are made is relevant since metabolic rates tend to follow a diurnal rhythm (Aschoff and Pohl 1970). The animals were monitored from morning to afternoon, and changes in RMR and respiratory quotient (RQ) were followed. RQ is calculated by dividing volume CO_2 produced by volume O_2 consumed and gives and indication of substrate being metabolized.

Age variations in RQ values, an important aspect in growth metabolism, have been neglected in small animals. Parker (1968) followed the change in RQ with age in the Red-winged Blackbird and reported a decrease in values from 0.884 at 0 to 3 days to 0.754 at 7 to 9 days for marsh nestlings and 0.826 and 0.727, respectively, for the same age groups in upland nestlings. This experiment allows us to explore these relationships in rabbits. RQ values were monitored as they changed with age and time of day for two purposes: (i) RQ values, a ratio of CO_2 produced to O_2 consumed, are essential for the accurate conversion of O_2 consumed to kcal/kg energy used (Hawk, Oser, and Summerson 1954), and (ii) RQ values are an indication of the energy balance of an animal, i.e., substrate being metabolized or the percent of fat and carbohydrate being oxidized.

METHODS

Experimental animals in this study were crossbred strains of California and New Zealand hares. These included two mature rabbits (1 to 1½ years old), one 2-month-old rabbit, four 3-month-old rabbits (littermates), and two 4-month-old rabbits (littermates). Availability of animals determined these age groups. The young animals were tested at approximately 2-month intervals up to the age of 250 days. Two adults were also tested.

Rabbits were given Purina Rabbit Chow and water *ad lib.* They were kept on a 12:12 LD (light/dark) schedule. Temperature was maintained between 21° and 22°C throughout the experiment. The trials were run from 18 August 1971 to 19 February 1972, and from 10 am to 5 pm (1000 to 1700) MST. Activity was limited by the size of the metabolism cages. The cages had interior dimensions of 51 × 30 × 36 cm and are housed in

Plexiglas experimental chambers (51 × 66 × 66 cm). The conditions would provide for a resting metabolic rate (RMR) and not a fasting or basal metabolic rate (BMR).

Oxygen consumption and CO_2 production were monitored continuously in an open flow system (Fig. 1). Carbon dioxide concentration (in %) was measured using a Beckman Infrared Analyzer, Model 315A. Oxygen concentration (in %) was measured using a Beckman Paramagnetic Oxygen Analyzer, model F3. Output from both gas analyzers was recorded on a strip chart. Barometric pressure, total volume of gas withdrawn from the system, flow rate, and temperature were monitored manually on a specific schedule during each experiment.

Accuracy of the entire system was determined by burning an alcohol lamp in the Plexiglas chamber and calculating the theoretical quantity of O_2 consumed and CO_2 produced. These data were compared with actual measurements and showed close agreement (Appendix I). Equilibration time within the chamber was determined by measuring time from the beginning of the experiment to the point at which the percentage of O_2 and CO_2 reached stable levels on the chart paper. Values before equilibration was reached were ignored.

Calibration of the CO_2 and O_2 analyzers were performed at weekly intervals during the data collection using standardized gas mixtures. The slopes of separate weekly regression equations do not differ from the slope of the overall regression equations. The regression equations used in the calculations were (Campion and Steinhorst 1972*a*):

$$O_2 = 20.9509 - 0.05778\,X + 0.000083\,X^2, \quad (R^2 = 0.952) \qquad (1)$$

$$CO_2 = 0.00977 + 0.034209\,X + 0.00152\,X^2, \quad (R^2 = 0.953) \qquad (2)$$

where X is the strip chart measurement.

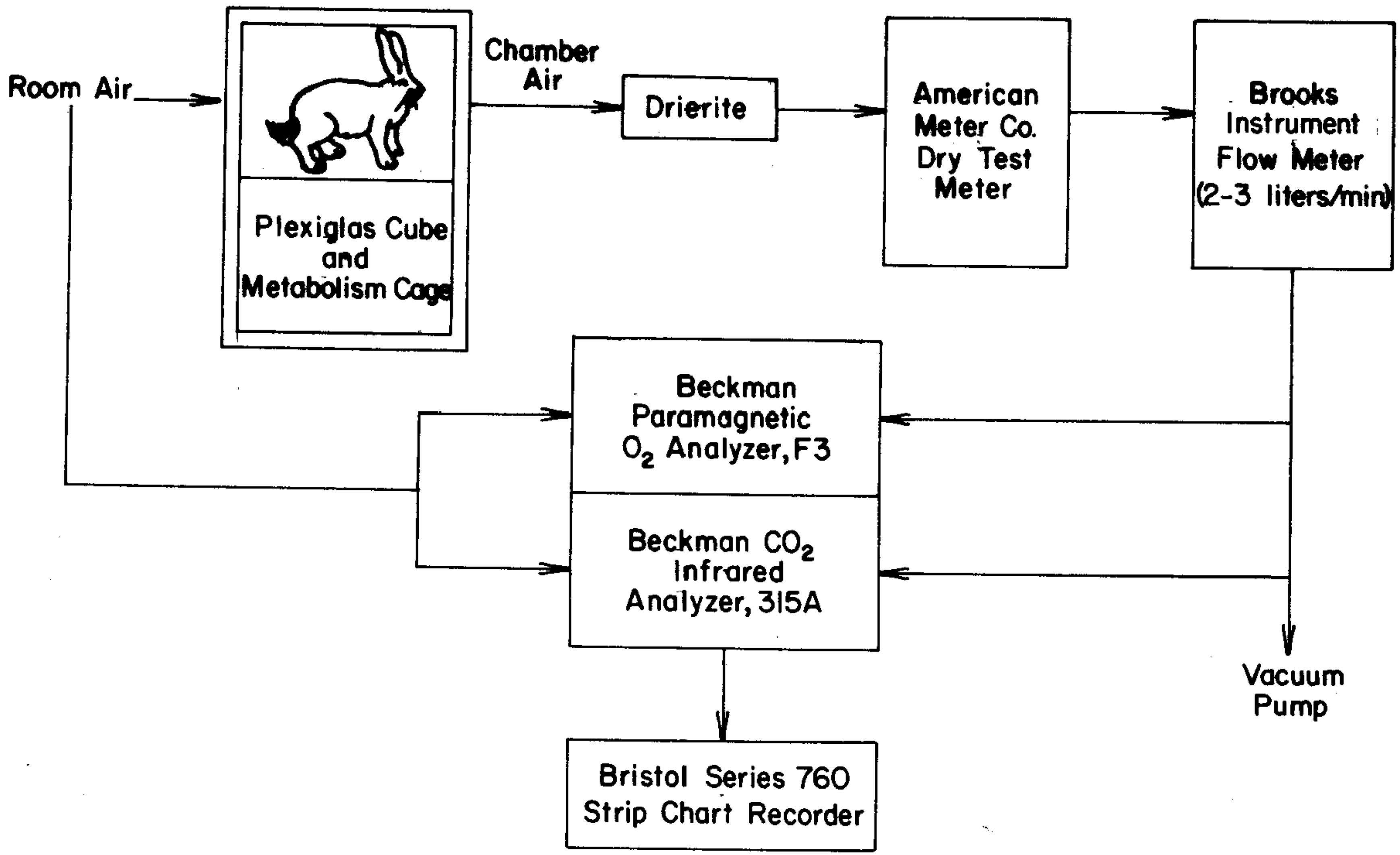

Fig. 1. Metabolic flow through test apparatus.

To determine the CO_2 production and O_2 consumption, the following equations were used:

$$\text{Total volume } (CO_2 \text{ or } O_2) = \Delta(CO_2 \text{ or } O_2) \times \text{time} \times \text{flow rate} \times \text{ambient barometric pressure} \quad (3)$$

where

ΔCO_2 (%) and ΔO_2 (%) were computed as shown in equations (4) and (5):

$$\Delta CO_2 = CO_{2_{out}} - CO_{2_{in}} \quad (4)$$

$$\Delta O_2 = O_{2_{in}} - O_{2_{out}} \quad (5)$$

Flow rate (liters/min) was determined by measuring the total volume (liters) passing through the system during the time span of the experiment:

$$\text{Flow rate} = \text{Total volume/total time} \quad (6)$$

Ambient barometric pressure is computed as:

$$\text{Ambient barometric pressure} = \text{mm Hg}/760 \text{ mm Hg} \times 273/(273 + {}^\circ C) \quad (7)$$

The airstream was dried before volume or gas measurements were taken so that corrections for humidity were ignored. Temperature and barometric pressure conditions were monitored hourly.

The data from equations (4) and (5) were used to obtain liters of gas per body weight per hour and to derive the respiratory quotient:

$$RQ = \Delta CO_2/\Delta O_2 \quad (8)$$

A regression equation derived from data found in Hawk et al. (1954) was employed to determine liters of O_2 consumed/kcal produced:

$$\text{Liters } O_2/\text{kcal} = 3.8163 + 1.229\ RQ + 0.00148\ RQ^2$$

Using this calculation kcal × kg^{-1} hr^{-1} were computed.

These calculations were carried out on a Hewlett-Packard 9100B computer (Appendix I). A comparable program listing for a CDC 6400 computer is included in Appendix II.

RESULTS

The results of this study were analyzed by least squares analysis of variance relating RQ and RMR with age and time of day. RMR as it changes with weight was also calculated. These analyses (Campion and Steinhorst 1972*b*) showed that:

1. RQ is significantly related to age ($p > 0.01$), both linearly and quadratically. Fig. 2 shows a plot of composite values at each age. Even though the points do not yield a smooth curve, the data show the general trend of increasing RQ with age. Values of 0.842, 0.762, and 0.707 for age groups of 53, 75, and 125 days increase to 0.877, 0.911, and 0.906 for age groups of 180, 221, and 280 days.

2. Using composite age groupings, RQ was found to vary with time of day ($p > 0.10$) in a linear fashion, decreasing from morning to afternoon (Fig. 3). This indicates that

$$RQ = 1.006 - 0.0130 \text{ (time of day/100, } R^2 = 0.65)$$

Nonprotein metabolism shifts from 57.5% carbohydrate (42.5% fat) in the morning to 26.3% carbohydrate (73.7% fat) in the afternoon.

3. Metabolic rate (RMR) expressed in kcal × kg^{-1} hr^{-1} is related to time of day ($p > 0.05$) increasing from morning to afternoon (Fig. 4).

$$RMR = 2.455 + 0.110 \text{ (time of day/100, } R^2 = 0.95)$$

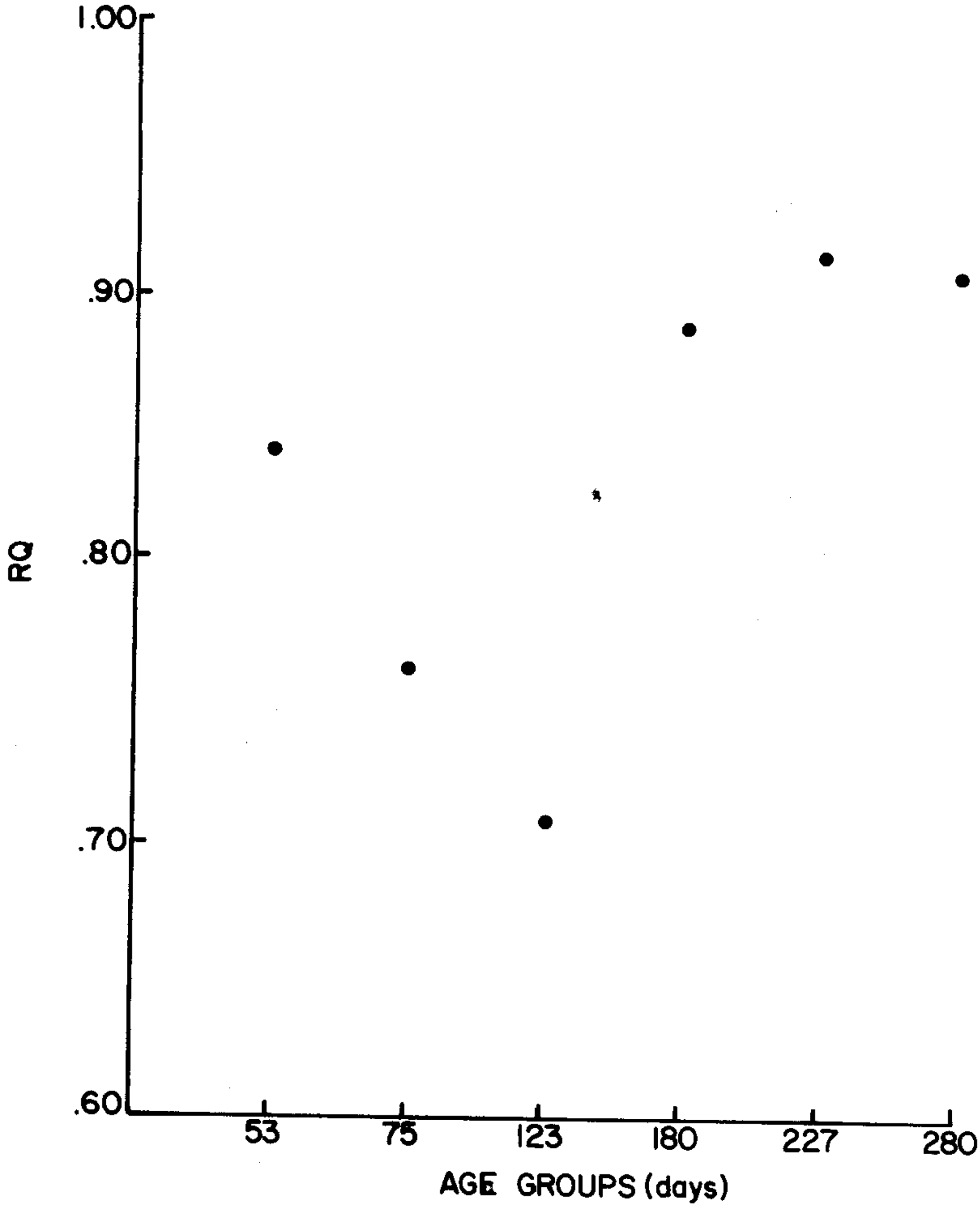

Fig. 2. RQ of domestic rabbit compared to age.

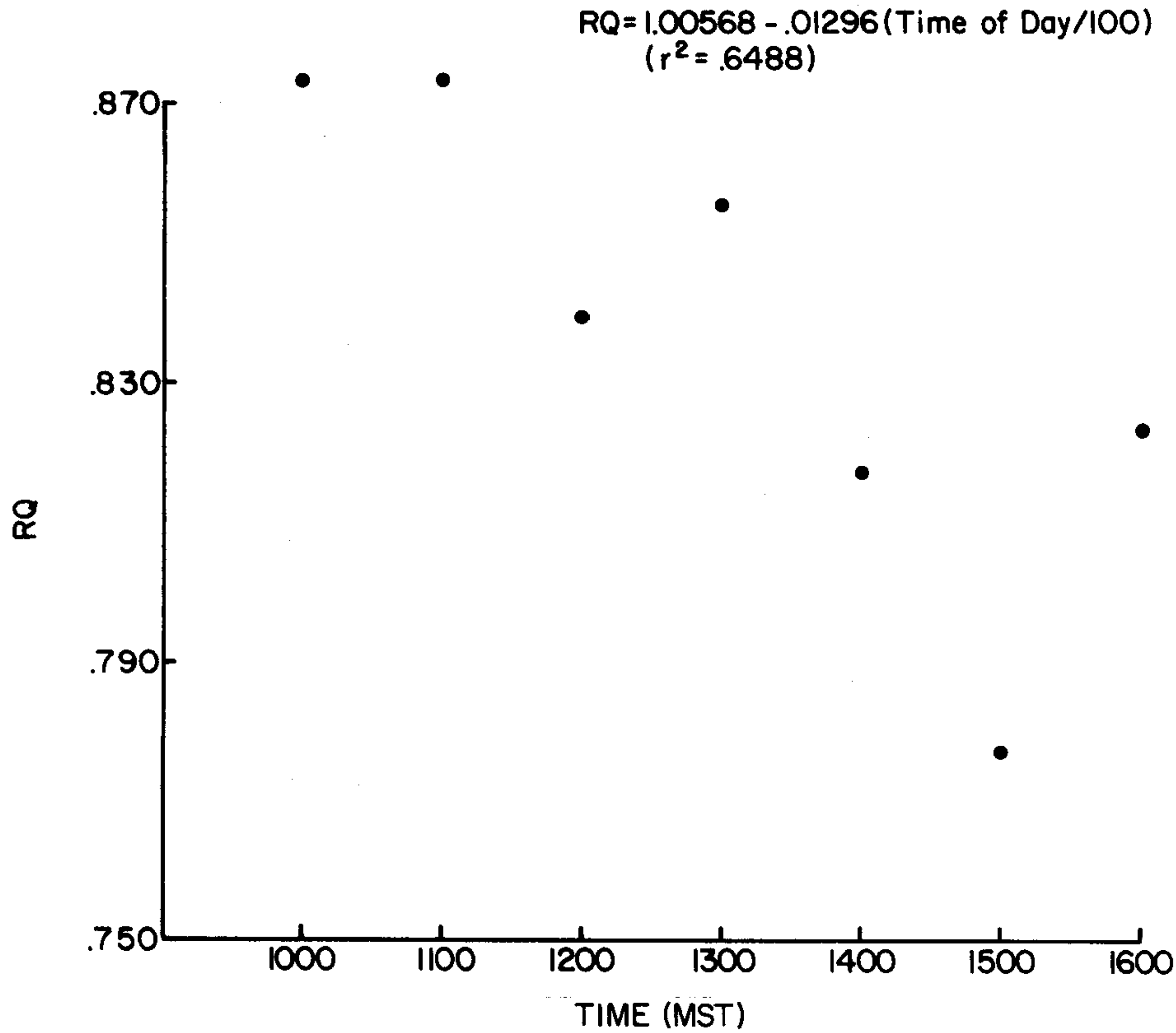

Fig. 3. RQ of domestic rabbit compared to time of day.

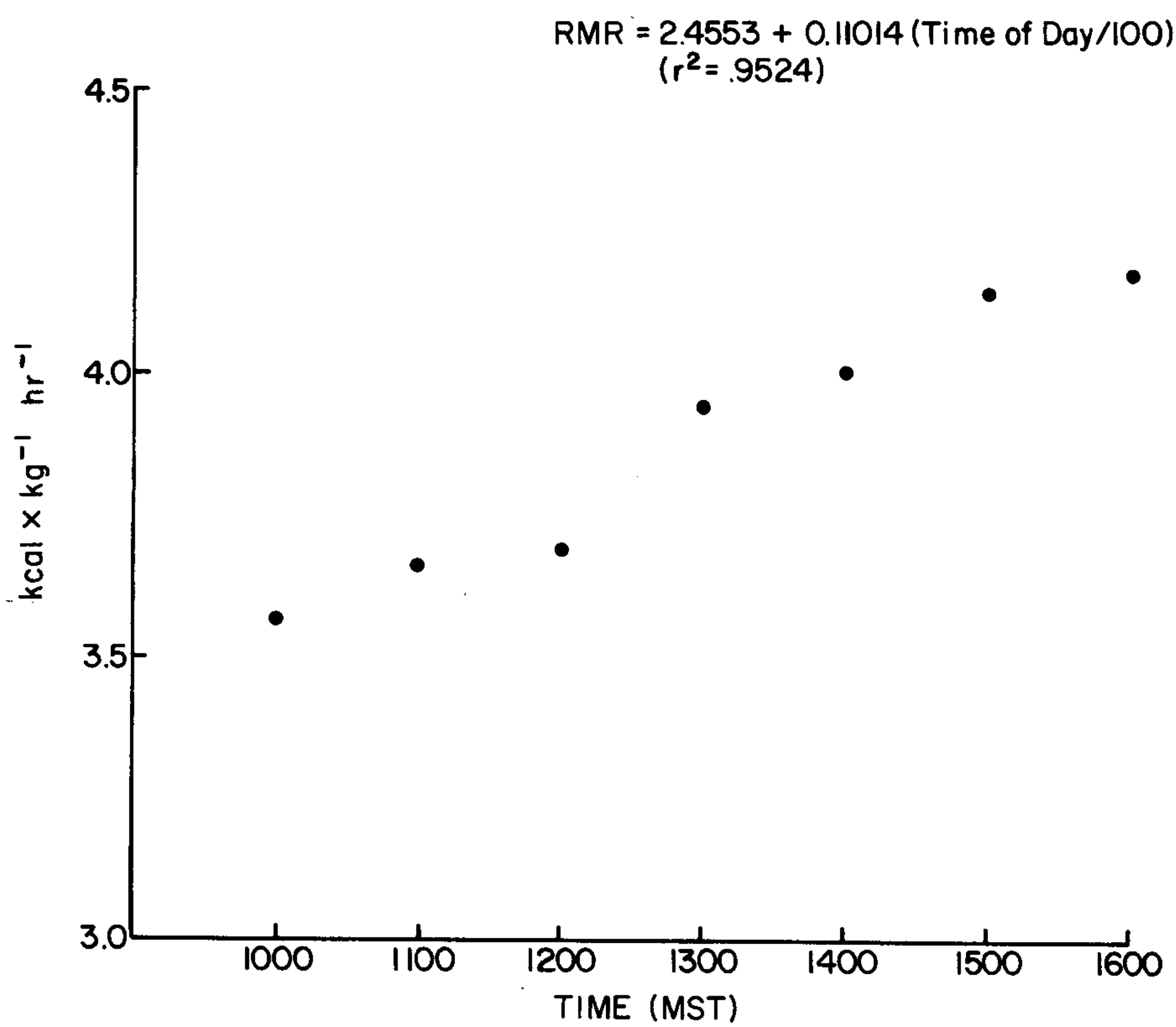

Fig. 4. Energy expenditure levels of domestic rabbit during daylight hours.

4. Metabolic rate (RMR) expressed in kcal × kg^{-1} hr^{-1} decreases with age in a quadratic manner (Fig. 5) where

$$RMR = 7.238 - 0.032 \text{ Age} + 0.00006 \text{ Age} \quad (R^2 = 0.53)$$

5. Fig. 6 shows that although there are significant fluctuations in metabolic rate, they are small compared to the effects of age on metabolism.

6. The exponential relationship, $M = aW^b$ where M = metabolic rate as total kcal/animal × hr and W = body weight in kg was also calculated:

$$M = 4.67\ W^{0.765},\ (R^2 = 0.75)$$

The correlative equation standardizing the data to a kcal/unit body weight/unit time or kcal × kg^{-1} hr^{-1} was determined, $M/W = aW^{b-1}$:

$$M/W = 4.67\ W^{-0.233}$$

DISCUSSION

The values obtained from the experiments on adult rabbits at 21°C agree very closely with other work on the rabbit (Table 1). The data are also comparable to that from a study on changes in food consumption with age in the European hare (Pilarski 1969). Animals in the 50- to 60-day old age group consumed 217 kcal × kg^{-1} 24 hr^{-1} in food. Fecal material eliminated in the same 24 hr period had an energy value of 43 kcal × kg^{-1} 24 hr^{-1}. Total kcal metabolized/kg in the 24-hr period equals 217 - 43 = 174 kcal × kg^{-1} 24 hr^{-1}. Converting this value to an hourly basis yields 7.2 kcal × kg^{-1} hr^{-1}. The mean value from the present experiment in the 53-day old age group is 6.12 kcal × kg^{-1} hr^{-1}. Considering the difference in experimental technique there is good agreement between the two values.

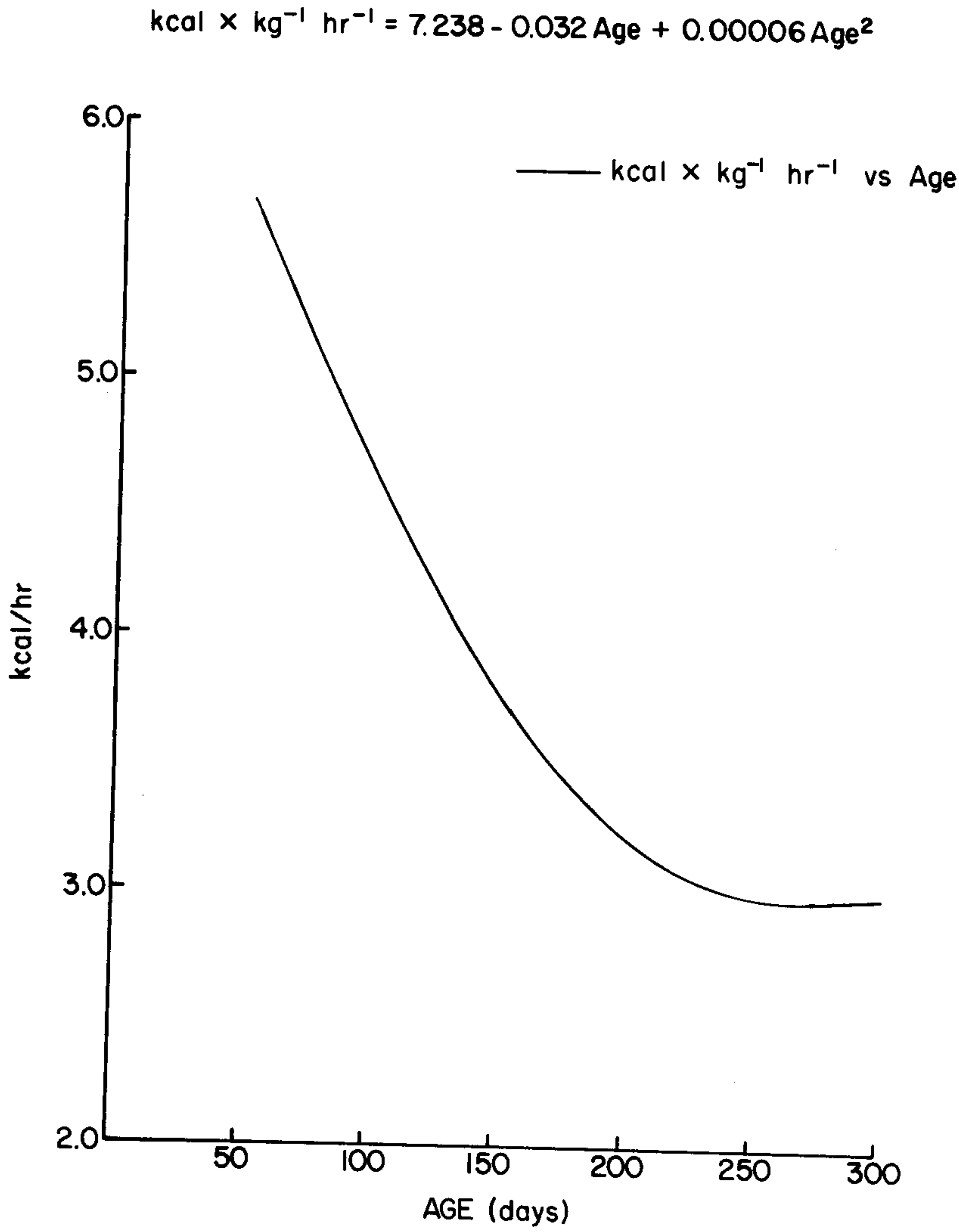

Fig. 5. Change of RMR of domestic rabbit as a function of age.

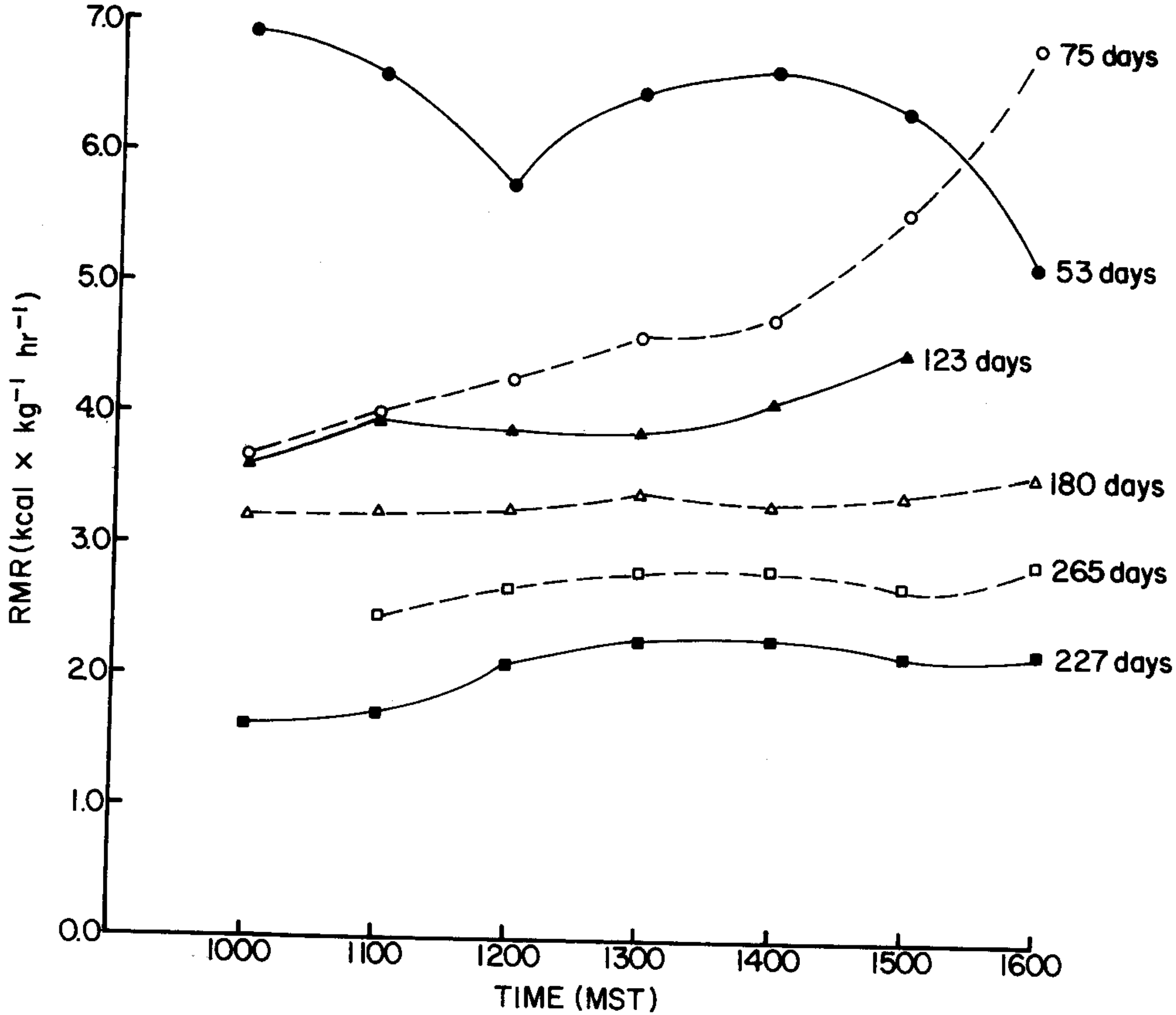

Fig. 6. Isobar graph showing relationships between kcal × kg^{-1} hr^{-1}, time of day, and age.

Table 1. Comparison of data in this experiment with previous work.

Liters $O_2 \times kg^{-1}\ hr^{-1}$	Animal weight in kg	Data source
0.85	2.54	Hart and Heroux (1955)
0.80 - 1.20	1.2 - 1.9	Hart, Pohl, and Tener (1965)
0.75 - 0.79	3.97	Present study
0.53 - 0.89	2.50	Present study

Relationships between age, time of day, RMR, and RQ point to several interesting relationships. The increase in RQ with age shown by this experiment is an indication of the change in substrate metabolism with age. The entire picture of substrate being oxidized is not available because urinary nitrogen was not determined, and it is impossible to separate protein from nonprotein metabolism. In nonprotein metabolism, the ratio of carbohydrate to fat increased with age. This would have two possible explanations:

1. Neonatal rabbits are known to rely heavily on deposits of brown adipose tissue for thermoregulation during their early developmental period (Heim and Kellermayer 1967). The RQ values would indicate that immature rabbits continue to rely upon fat stores even after the initial developmental period.

2. The shift in energy sources also may indicate a shift in energy balance with age. Older, larger animals require less energy for heat production and thermoregulation so, instead of using fat stores, they begin to deposit fat and use existing carbohydrate and carbohydrate stores.

The decrease in RQ from morning to afternoon would seem to indicate a pattern of eating and related metabolic substrates. It indicates a shift from the absorptive state (predominant carbohydrate utilization) in the morning to the post-absorptive state in the afternoon (predominate use of fat stores). This could be part of a normal diurnal pattern or it could be a result of the animal's unwillingness to eat during the experimental period. To determine the actual basis of this pattern, consecutive 24-hr periods would need to be measured.

The increase in RMR from morning to afternoon may result from the rabbits circadial pattern (Aschoff and Pohl 1970). Again, a 24-hr experimental period would be useful to characterize the complete pattern.

The change in metabolic rate with increasing age (and increasing size) substantiates decreasing metabolic rate changes per unit body size as the animal matures. This will be discussed more thoroughly in another section.

Fig. 5 puts the relationships discussed above into perspective. The fluctuations in RMR and RQ with time of day are really small when compared to the changes with age.

The exponential equation for the growing rabbits, $M = 4.67\ W^{0.765}$ is very close to values for RMR discussed by Hart (1971) for between species relationships. Since a relationship between RMR and body weight for nongrowing animals was not established, we must rely on the between species relationship which may or may not be valid for rabbits. The higher energy as determined in the growing colony could be attributed solely to the smaller body size and not necessarily "additional growth requirements."

This experiment points out the necessity of establishing and knowing a "baseline" adult relationship between body weight and metabolic rate to determine the effect of body size on the metabolic rate. Then, by establishing the immature relationship, any difference between metabolic rate changes expected from smaller body size and actual metabolic changes could be attributed to an "additional growth factor." Such information may be somewhat trivial for certain studies; e.g., those attempting to establish phylogenetic relationships among groups of organisms. However, those studies concerned with definition of ecosystems and interactions among components of the system cannot ignore these definitions. A case in point is provided by Miller (1974) where definition of adult and neonate energetics of *Microtus ochrogaster* gives some insight into how this species may regulate its population site in the grassland ecosystems. Thus we feel it is important to have a clear understanding of the energetics changes throughout the life history of given animal species or groups.

RECOMMENDATIONS

Areas in which design and techniques should be improved include constant monitoring and recording of temperature and flow measurements The resolution of time-related metabolic changes would be increased by shortening equilibration time (using smaller chambers). Animals should be monitored for sampling periods arranged to characterize a circadial rhythm, i.e., 24- to 48-hr periods.

This experiment has emphasized the need for establishing adult RMR to body weight relationships before attempting to define costs of growth above simple difference in body size and tissue production.

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APPENDIX I

PROGRAM LISTING FOR HP 9100B COMPUTER

Regression values for O_2 -- Stored in memory cells: +A, +B, +C.

Regression values for CO_2 -- Stored in memory cells: -A, -B, -C.

Flow values -- Stored in -D.

Clear	+	x→	x	(xε)
Stop	2	d	d	--
Enter x for O_2	0	↑	+	d
Cont	•	x	0	x
x→	9	(x←)	1	6
d	5	--	0	0
↑	--	c	3	x
x	y→	x	--	Print (y)
c	e	(x←)	Print (y)	Print (space)
x	Print (y)	--	e	ε0↑
e	Clear x	a	÷	To
+	Roll ↓	+	Print (y)	00
d	Clear x	d	Clear x	0
y→	Roll ↓	y→	Roll ↓	
d	Clear x	d	Clear x	
↑	Roll ↓	↑	Roll ↓	
b	Stop	(x←)	Clear x	
x	Enter x for CO_2	--	e	
d	Cont	b	+	

APPENDIX II

THEORETICAL CALCULATIONS: CALIBRATION OF METABOLIC TEST SYSTEM

Alcohol consumed = 3.36 g

$$C_2H_5OH + 3\ O_2 \rightarrow 2\ CO_2 + 3\ H_2O$$

$$46\text{ g} \quad 96\text{ g} \quad 88\text{ g} \quad 54\text{ g}$$

(a) $$CO_2 \text{ produced} = \frac{3.36\text{ g ETOH} + OH + 88\text{ g } CO_2}{46\text{ g ETOH}}$$

$$= 6.428\text{ g } CO_2$$

$$22.4\text{ liters} = 1\text{ mole} = 44\text{ g } CO_2$$

$$1\text{ liter} = 1.96\text{ g}$$

$$\frac{6.428\text{ g } CO_2}{1.96\text{ g } CO_2/\text{liter}} = 3.28\text{ liters } CO_2$$

(b) $$O_2 \text{ consumed} = \frac{336\text{ g ETOH} + 96\text{ g } O_2}{46\text{ g ETOH}}$$

$$= 7.012\text{ g } O_2$$

$$22.4\text{ liters} = 1\text{ mole} = 32\text{ g } O_2$$

$$1\text{ liter} = 1.428\text{ g}$$

$$\frac{7.012\text{ g } O_2}{1.428\text{ g/liter}} = 4.910\text{ liters } O_2$$

ACTUAL EXPERIMENTAL CALCULATIONS

$$2.258\text{ liters/min} \times 75\text{ min} = 180.862\text{ liters gas}$$

$$180.48\text{ liters} \times 0.0175\%\text{ d } CO_2 = 3.158\text{ liters } CO_2$$

RQ =

65%

$$180.48\text{ liters} \times 0.0270\%\text{ d } O_2 = 4.862\text{ liters } O_2$$

$$CO_2 = 96.3\%$$

$$O_2 = 99.0\%$$

APPENDIX III

PROGRAM METABOLISM

```
                 PROGRAM METAB (INPUT, OUTPUT)
          C      READ IN CONSTANTS FOR REGRESSIONS, BEGINNING AND ENDING WEIGHTS,
          C      NUMBER OF DATA CARDS, CHAMBER NUMBER
000003           READ 10, CO2A, CO2B, CO2C, O2A, O2B, O2C, AWTBG, AWTED, NDC,NCMB,
                1IDATE
000035           READ 11, IHR,IMIN
000045           WTCRFT = (AWTBG - AWTED)/NDC
          C      SET WEIGHT CORRECTION FACTOR AT BEGINNING WEIGHT
000051           PRINT 20
000054           CORWT = AWTBG
          C      START LOOP TO BE ITERATED FOR EVERY DATA CARD
000056           DO 1 I = 1, NDC
          C      LOOP FOR KEEPING TRACK OF TIME
000057           IHR = IHR
000060           IMIN = IMIN + 25
000062           IF (IMIN.GE.60) GO TO 5
000064           GO TO 8
000065         5 IHR = IHR + 1
000067           IMIN = IMIN - 60
000070           IF (IHR.EQ.24) GO TO 6
000072           GO TO 8
000073         6 IHR = 0
000074         8 CONTINUE
000074           READ 10, CO2AM, CO2EX,O2AM, O2EX, BP, TEMP, FLOW
000116           CORWT = CORWT - WTCRFT
          C      CALCULATE AMBIENT O2 AND CO2 PERCENT
000120           AO2PC = O2A + O2B*O2AM +O2C*O2AM**2
000125           ACO2PC = CO2A + CO2B*CO2AM + CO2C*CO2AM**2
          C      CALCULATE CHANGED O2 AND CO2 PERCENT
000133           EO2PC = O2A + O2B*O2EX + O2C*O2EX**2
000141           ECO2PC = CO2A + CO2B*CO2EX  + CO2C*CO2EX**2
          C      CALCULATE DELTA PERCENT, RQ
000147           DTO2 =(AO2PC - EO2PC)*.01
000152           DTCO2 =(ECO2PC - ACO2PC)*.01
000155           RQ = DTCO2/DTO2
000157           CO2LHR = (DTCO2*(BP/760.)*(273./(TEMP + 273.))*FLOW*60.)/CORWT
000166           O2LHR = (DTO2*(BP/760.)*(273./(TEMP + 273.))*FLOW *60.)/CORWT
000176           REGRQ = 3.8163 + 1.229 * RQ + .0015*RQ**2
000203           CKAL = O2LHR*REGRQ
000206           PRINT 30, (NCMB, IDATE, IHR, IMIN, O2LHR, CO2LHR, RQ, CKAL, TEMP)
000233         1 CONTINUE
000236           STOP
000240        10 FORMAT (8F8.2, 2I4, I8)
000240        20 FORMAT (1H1,10X,*CHAMBER NUMBER*, 7X, *DATE*, 4X,*TIMECODE*, 4X,
                132HCCO2/L/HR/KG        CCCO2/L/HR/KG, 7X,*RQ*,7X,10HKCAL/KG/HR,5X,
                2*CHAMBER TEMP*)
000240        30 FORMAT (1H , 16X, I2, 12X, I6, 5X, 2I2, 7X, F8.3, 12X,F8.3,8X,F5.3,
                1,8X,F6.2, 8X,F6.2)
000240        11 FORMAT  (2I2)
000240           END
```