THE USE OF D_2O^{18} FOR MEASURING ENERGY METABOLISM IN *COLUMBA LIVIA* AT REST AND IN FLIGHT

Eugene A. LeFebvre

Many meaningful problems in avian ecology and physiology could be aided by studies of total energy metabolism on birds living under natural environmental conditions. The classical methods of direct and indirect calorimetry thus far employed (e.g., Benedict, 1938; Benedict and Riddle, 1929; Brody, 1945; Kleiber, 1950; Pearson, 1950; and Peters and Van Slyke, 1946) are not suited for reliable measurements of metabolism of unconfined birds in steady flight or for relatively long periods of time under natural conditions.

Lifson *et al.* (1955) proposed a method for determining carbon dioxide production (and hence energy expenditure, given supplementary information) in unrestricted animals by means of isotopically labeled water (D_2O^{18}). The method was validated, by necessity, on confined rats and mice on a regular food regime in the laboratory (Lifson *et al.*, 1955; McClintock and Lifson, 1958a, b), and on fasting rats (Lifson and Lee, 1961), and hereditarily obese mice (McClintock and Lifson, 1957a, b). In these studies the doubly labeled water determination provided estimates of CO_2 output with errors of only 5–10 per cent. This procedure appeared to be applicable to energy studies in birds.

The present investigation was initiated to test the validity of the method by comparing in birds the D_2O^{18} determinations of CO_2 production with CO_2 production as it is customarily obtained in the laboratory, and to measure the energy expenditure of flight by means of the isotope method and by material balance. Flight-energy determinations have been attempted under laboratory conditions by Pearson (1950, 1954) and Lasiewski (1962) on hummingbirds. The estimates by Odum (1960a, b) and Odum *et al.* (1961) on flight ranges of some passerine birds have implicitly required an indirect calculation of flight-energy expenditure. Other workers (Zeuthen, 1942; Brown, 1951, 1961; Wilkie, 1959; Raspet, 1960; Nisbet *et al.*, 1963) have used aerodynamic considerations for deducing indirectly the energy requirements involved in bird flight.

RATIONALE OF THE METHOD

Fundamental to the isotopic method is the fact that the oxygen of respiratory CO_2 is in isotopic equilibrium with the oxygen of the body water (Lifson *et al.*, 1949). Therefore, although the hydrogen of the body water is lost mainly as water, the oxygen is lost both in water and in the CO_2 produced. The turnover rate for body water oxygen, therefore, is sig-

nificantly higher than that for hydrogen and the difference in the two turn-over rates is proportional to the CO_2 produced. The respective turnover rates are obtainable by labeling the two components of the body water with stable isotopes of hydrogen (deuterium) and oxygen (oxygen −18). In the case in which an animal is given isotope and then allowed to drink unlabeled water during the experimental period, the isotopic turnover rate (K_*) is calculated (from an equation given by Lifson *et al.*, 1955) from the change in specific activity (S_*) of the initial and final blood or body water samples:

$$K_* = \Delta \ln S_* / \Delta t \qquad \text{(Equation 1)}.$$

Because the rate of CO_2 plus water output is equal to the product of the total body water (N) and the fractional turnover rate (K) of the body water oxygen (in this case, of the isotope, or K_{O*}), and the rate of water loss alone is equal to the product of the total body water and the fractional turnover rate of the body water hydrogen (in this case, of deuterium, or K_D), the rate of CO_2 production (rCO_2) can be derived (from an equation given by Lifson *et al.*, 1955) as follows:

$$rCO_2 = N/2 \ (K_{O*} - K_D) \qquad \text{(Equation 2)}.$$

These relationships are based on the following assumptions, which simplify the calculations: (1) total body water is constant; (2) hydrogen and oxygen of body water are lost only as water and CO_2 output; (3) all rates remain constant; (4) storage is negligible; (5) isotopes enter all compartments of the body water; (6) isotopes are lost at the specific activity attained in the body water; and (7) there is no isotope reentry.

The theoretical basis for the D_2O^{18} method and the assumptions involved are discussed by Lifson *et al.* (1955). Published reports have explored certain aspects of the method in greater detail (Lee and Lifson, 1960; Lifson and Lee, 1961). The reader is referred to these papers and to LeFebvre (1962) for a critical analysis of the method and of potential errors introduced by deviations from the assumed conditions.

I. Validation Experiment

Method.—The first experiment was designed to compare CO_2 production determined isotopically ("Calculated CO_2") with CO_2 output determined simultaneously by an accepted laboratory procedure ("Observed CO_2") (cf. Lifson *et al.*, 1955). The "Observed CO_2" production was measured by placing the experimental bird (pigeon, *Columba livia*) in an open-circuit system. Air entering the metabolism chamber was dry and CO_2-free; the water and CO_2 were removed respectively by means of "Ascarite" and "Drierite" absorbers in a series of "U" tubes preceding the chamber. Air flow was maintained between 600 and 1,000 cc per minute. Expired CO_2 was absorbed from the exiting air in 30 per cent KOH contained in a series of three flasks. Total

CO_2 absorbed by the KOH was determined by Van Slyke manometric analysis. Analyses of KOH solutions for CO_2 content were compared periodically with standard solutions; the technique yielded an average error within $\pm$ 1 per cent. Two preliminary recovery tests indicated that total recovery of CO_2 in the metabolism train was about 3 per cent less than actual production.

Starting with pigeon number NB13, the lower portion of the metabolism chamber was packed in dry ice to hold the water condensate and droppings in a frozen condition. This prevented decomposition of the excreta and probably reduced isotopic reentry due to water reentry.

The experimental bird was acclimatized in the metabolism chamber for several days and was provided with 1.5 per cent H_2O^{18} (atom per cent excess) for drinking water. Following the consumption of a sufficient volume of H_2O^{18}, an injection of 99.5 per cent D_2O (excess) was given (intravenously or intraperitoneally). These amounts provided a concentration of about 0.5 per cent for H_2O^{18} and 1 per cent for D_2O in the body water. On the basis of preliminary experiments, one hour was allowed for distribution of the isotope throughout the body water compartment, after which time an initial blood sample was withdrawn via heart puncture.

The bird was returned to the chamber and provided with fresh water and food. The chamber was sealed, the air flow adjusted, and the CO_2 collection begun. At the end of four days (three days, in the case of two birds) the bird was removed from the chamber for a final blood sample. The CO_2 collecting flasks were saved for analysis. The "Calculated CO_2" was then determined: the average total body water was considered, as in the experiments of Lifson *et al.* (1955), as the average of the initial and final body weights and the final per cent body water as determined by oven desiccation of the sacrificed bird to constant weight. It was assumed that the per cent body water did not change significantly during the course of the experiment. The analytical error in the estimate of final per cent body water was within 1 per cent and average total body water was accurate to $\pm$ 5 per cent. The blood water was analyzed by mass spectrometry for specific activity of the isotopes. The measured change in specific activity was used to calculate the turnover rates using equation (1); these values were then substituted in equation (2) which assumes the validity of all assumptions for the rate of CO_2 production. The largest single source of error in this method is probably in the determination of the specific activity and the resultant turnover rates, K_D and K_{O*}, since the calculation involves relatively small differences between large values. Lifson *et al.* (1955) cite an example in which a discrepancy of only 0.003 atom per cent in the determination of the final S_{O*} would produce an error of 4 per cent in the calculated CO_2 production. In samples of both the validation study and the flight study, wide discrepancies in the duplicate readings for specific activity sometimes occurred. Such errors generally result from incomplete distillation of the blood water and subsequent dilution of the sample due to fractionation effects (LeFebvre, 1962: 9–11). Accordingly, in the mass spectrometer analysis, whenever a difference occurred in duplicate samples which exceeded 2 per cent, only the higher reading was used (*cf.* McClintock and Lifson, 1958a) unless other data indicated the higher result was due to contamination or other error. Whenever possible, additional samples were tested in place of these which were variable.

Results.—The Van Slyke CO_2 determinations ("Observed CO_2") and D_2O^{18} CO_2 determinations ("Calculated CO_2") are compared in Figure 1 and Table 1. There is considerable variation in the errors observed although a majority of the comparisons agrees within 10 per cent. Lifson
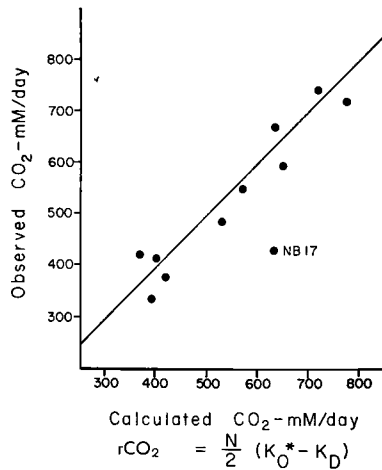
$$rCO_2 = \frac{N}{2}(K_O^* - K_D)$$

Figure 1. Comparison of the CO_2 production obtained by the Van Slyke manometric analysis ("Observed CO_2") and by D_2O^{18} method ("Calculated CO_2").

et al. (1955) discussed the magnitude of error involved in the general analytical procedures and concluded that the discrepancy between observed and calculated values can largely be accounted for in the isotopic measurements rather than in the assumptions of the rationale.

TABLE 1

COMPARISON OF CO_2 PRODUCTION MEASURED BY VAN SLYKE ANALYSIS AND D_2O^{18} TURNOVER

Specimen	Van Slyke determination (mM/day) (observed CO_2)	D_2O^{18} calculation (mM/day) (calculated CO_2)	Per cent error $\left(\dfrac{observed\text{-}calculated}{observed}\right)$
NB11	409	400	− 2.2
NB12	333	389	+16.8
NB13	419	368	− 12.2
NB14	483	525	+ 8.7
NB15	372	414	+11.3
NB16	716	772	+ 7 8
NB17*	425	631	+48.5*
NB18	591	648	+ 9.6
NB19	665	638	− 4.1
NB20	544	565	+ 3.9
NB21	742	718	− 3.2

Mean ± S.D. (S.E.)   8 ± 5 (±2)

* Data from pigeon NB17 are excluded from the statistics on the basis that the error represented is about five standard deviations away from the mean error (Fisher, 1934).
See LeFebvre, 1962:76–77 for summary of data and sample protocol for estimating CO_2 output from isotopic data.

TABLE 2

SUMMARY OF THE AVERAGE ERRORS PROVIDED BY THE D_2O^{18} CALCULATIONS COMPARED
WITH THE VAN SLYKE MANOMETRIC DETERMINATIONS OF CO_2 PRODUCTION IN BIRDS
AND MICE

	Birds *		*Mice* **
	A $rCO_2 = N/2\ (K_{0*} - K_D)$	B $rCO_2 = N/2.08\ (K_{0*} - K_D)$ $- .015\ K_D N$	
Mean ± S.D.	8 ± 5 per cent	8 ± 8 per cent	7 ± 7 per cent
S.E.	2 per cent	3 per cent	2 per cent
Alg. Mean	+4 per cent	−6 per cent	−3 per cent
(N)	(10)	(10)	(15)

* See LeFebvre (1962) for complete table comparing observed and calculated CO_2 outputs in pigeons when basic equation is modified by corrections for simplifying assumptions.
** Lifson *et al.* (1955).

The comparisons of the calculated and observed rates of CO_2 output indicate that the D_2O^{18} method provides results in birds comparable to those obtained in small mammals (Lifson *et al.*, 1955). Table 2 provides a summary of the mean errors obtained in this experiment and in the original study on mice (Lifson *et al.*, 1955). The mean error (column A) is 8 per cent ± 5 per cent (S.D.) and standard error of the mean is 2 per cent. In Lifson *et al.* (1955) the mean error was 7 per cent ± 7 per cent (S.D.). It should be noted that Lifson used an equation corrected for invalidity of one of the simplifying assumptions (fractionation effects). In column B, the comparable values which would be obtained using the corrected equation are listed. Little real difference in the error resulted; only the algebraic bias differs between the two calculations. In the flight experiment to be considered next, I have arbitrarily computed the rate of CO_2 output using equation B, because the published results previously cited have all been based on this equation.

In the validation experiment reported here, the D_2O^{18} method was found to be as accurate for CO_2 determinations for birds in a steady state of body composition as for mice and rats, and therefore is applicable to avian energy studies. In measuring the energy cost of flight, one would expect a non-steady state of body composition, particularly with respect to water and fat. However, Lifson and Lee (1961) found the isotopic determinations satisfactory (mean error ± S.D.: 4 per cent ± 4 per cent) for fasted rats in which body water decreased exponentially with time, and it may be assumed a similar situation holds true for birds. Actually, as will be noted later, the total body water did not decrease greatly during flight and the error from failure to adjust for changing body water was not as great as might be anticipated.

II. Flight Experiment

Method.—For an accurate determination of CO_2 production in flight, it is desirable to obtain blood samples immediately before and after an uninterrupted flight. To convert the CO_2 output to the equivalent energy expenditure, it is necessary to know the composition of the "fuel" burned during the flight. The use of trained racing pigeons as experimental animals makes it possible to approach these requirements. Such individuals are conditioned to fly directly to their home lofts from selected release sites and are conditioned to do so after being deprived of food for 24 hours or more before racing. Upon reaching the home loft area, a good racer usually goes quickly into the loft where it may be caught.

Preliminary tests indicated that pigeons in good physical condition could fly a 300-mile route in a time sufficient for accurate measurement of the decrease in specific activity. The anticipated technique for obtaining blood samples via heart punctures did not appear to affect the flight performance or behavior of the pigeons used in the preliminary tests. Following the actual flight experiment, each individual bird was dissected and examined for any gross indication of damage to the heart. None was evident; this agreed with earlier observations on anesthetized pigeons which demonstrated no apparent damage or trauma following heart puncture.

The flight experiment involved 31 birds. The pigeons had all flown in 300-mile races and some had participated in 500-mile events. Condition of the animals was comparable; all had been in good health and had been exercised frequently prior to the experiment. The flight route was familiar to each experimental animal from its racing experience. Of these birds, 22 (referred to as the experimental or flight group) were released at Allerton, Iowa, a distance of 300 miles directly south of St. Paul, Minnesota, and were allowed to return to their home lofts located at the southern edge of the St. Paul metropolitan area. Nine additional pigeons selected at random (referred to as the preflight group) were maintained under conditions identical to those of the flight pigeons and were sacrificed just prior to release of the homing birds to obtain preflight data on water, fat, and protein composition. These data were used to estimate the initial water, fat, and protein levels in the experimental pigeons for purposes of estimating the energy cost of flight from material balance for comparison with the isotopic determination. Lipid was extracted from an aliquot of dehydrated carcass with diethylether in the Soxhlet apparatus, and nitrogen content was determined on the residual sample by the Kjeldahl method. Both techniques provided determinations with less than one per cent error in recovery tests. Total lipid and nitrogen determinations, however, were undoubtedly less accurate.

The pigeons were kept in individual cages measuring $12 \times 12 \times 12$ inches and they were given a mixture of 99.5 per cent D_2O and 1.5 per cent H_2O^{18} orally until a predetermined minimum amount had been consumed. Three days were required to load the birds with isotopic water. Food was provided only on the first evening (29 July 1961) and was removed the following morning. Water was removed from the cages of the flight and preflight groups after dark on the second evening (30 July). On the third morning (31 July), the weather conditions and predictions appeared favorable enough to warrant releasing the experimental birds.

Prior to release, the flight birds were weighed and initial blood samples were obtained via heart puncture. All were then released as a group. Following the flight, the birds were recaptured as they entered the home lofts, weighed, and the final blood samples obtained. Each bird was killed, placed in a plastic bag, and subsequently used for determination of total body water and total lipid content by desiccation and ether

TABLE 3

CO_2 Production and Energy Expenditure of Flight Calculated from Isotopically Labeled Water

Specimen	Mean wt (g)	$\Delta t_{(bld)}$ (hr)	Δt (flight) (hr)	Average velocity (mph)	Calc. CO_{2*} (mM/hr)	Energy** (kcal/hr)
1285	398	8.6	(8.3)	36	159	24
2660	367	9.8	(9.2)	33	166	25
3208	345	8.1	(7.2)	42	97	14
4082	395	9.3	(8.4)	36	168	25
4084	387	9.4	(8.4)	36	175	26
2029	432	9.3	(8.4)	36	142	21
4012	361	12.1	(11.3)	25	102	15
4051	390	8.1	(7.5)	40	188	28
Mean ± S.E.	384	9.3	8.6	36	150 ± 11	22 ± 2***

* Corrected for fractionation only: $rCO_2 = \frac{N}{2.08} (K_{O*} - K_D) - .015\ K_D N$ (Lifson *et al.*, 1955).
** At RQ = 0.707; 0.1485 kcal/mM CO_2 (Peters and Van Slyke, 1946).
*** Converted to weight-specific basis: mean value ± S.E. = 58 ± 9 kcal/kg/hr.

extraction. Because of the conditioning of these individuals, it was reasonable to assume that all flights were non-stop unless evidence showed otherwise. The energy requirement for flight was calculated by using the caloric equivalent for CO_2 produced, with the assumption that in the flying bird only fat was being metabolized at an R.Q. of 0.71. This assumption was supported by the material balance results. Fasting quotients are obtained in pigeons and doves after 24 hours of food deprivation (Benedict and Riddle, 1929: 518). Since all food was removed from the holding cages about 36 hours prior to the flight release, these birds were in a fasting condition.

Results.—Thunderstorms occurred late in the day of the flight experiment and apparently forced most of the pigeons down. Of the 22 birds released, only 8 returned by evening of that day. Of these, 4 (nos. 2660, 4084, 4082, and 2029) showed indications of having landed (mud on feet, food in crops); the other 4 (nos. 1285, 3208, 4012, and 4051) apparently flew non-stop. Table 3 shows the time lapse from release to capture, average flight speed, calculated CO_2, and energy expenditures obtained for these eight pigeons. The mean energy expenditure per hour as calculated from the mass spectrometer data was 22 kcal ± 2 (S.E.). Sample calculations of the energy expenditure and raw data used for computations of Table 3 for one individual and values are provided in LeFebvre (1962: 41, 80–84). In the calculations of flight-energy requirements, the time interval between initial and final blood samples, $\Delta t_{(bld)}$, was used to represent the flight time. This use introduces some difference in the final values but the maximum so obtained (specimen no. 3208) was approximately + 2 per cent.

A comparison was then made between the caloric expenditures obtained from the D_2O^{18} method and the energy expenditures calculated from the estimated initial lipid content and the ether-extracted final lipid content obtained on each pigeon. Of the last two values (see Table 4), the former

TABLE 4

Summary of Preflight Per Cent Body Composition and Change of Composition
for Eight Experimental Pigeons

Speci-men	Live carcass* (g)	Preflight estimates**			Postflight determinations***		
		Total body water (g)	Fat (g)	Protein (g)	Δ Total body water (g)	Δ Fat (g)	Δ Protein (g)
1285	362.9	231.5	43.1	62.9	+ 4.1	−22.8	+7.2
2660	332.8	212.3	39.5	57.7	− 8.5	−12.3	+1.5
3208	334.7	213.5	39.8	58.1	− 7.5	−23.6	− 2.0
4082	367.7	234.6	43.7	63.8	− 13.6	−19.2	+6.3
4084	366.2	233.6	43.5	63.5	− 4.3	−24.6	+5.5
2029	412.4	263.5	48.8	71.3	− 14.3	−18.6	+0.5
4012	352.9	225.2	41.9	61.2	− 26.2	−21.1	− 1.6
4051	367.8	234.7	43.7	63.8	− 5.8	−25.3	+4.2
				Mean ± S.E.	10.5 ± 3.0	20.9 ± 1.5	3.6 ± 2.3

* Live carcass = total live weight − feather weight; total body water therefore represents body water of the carcass assuming body water of feathers was negligible or normally was not part of the physiologically active body water compartment.

** Based on average per cent body composition determined from nine preflight pigeons (see text) and the initial body weights of each experimental pigeon.

*** Based on actual postflight measurements on each individual (see text).

was obtained from the per cent body composition of the nine "preflight" pigeons; the latter value was determined for each sacrificed flight pigeon following the experiment. The estimated fat loss (protein loss was negligible) was used to calculate energy expense of flight by assigning a caloric value of 9.5 kcal per g fat loss (King and Farner, 1961: 219). The total caloric expenditure divided by the time, $\Delta t_{(bld)}$, yields the energy required per hour of flight. These data are presented in Table 5. The mean metabolism estimated from the computed fat loss was 22 ± 2 (S.E.) kcal/hr. In this experiment the two methods for determining the energy metabolism of steady flight, one relatively direct, the other, an accepted material balance method, showed close agreement (*cf.* Tables 3 and 5).

There is no apparent difference in the results obtained from the four probable-stop birds and the four non-stop individuals. The average estimated flight speed of the probable-stop birds was close to that of the other pigeons, suggesting that non-flight time was relatively short. Furthermore, the energy expenditures, whether derived from the D_2O^{18} data or the fat-loss data, were consistent with the relative flight speeds with the exception of one pigeon for each set of data (no. 3280, Table 3; no. 2660, Table 5). The exceptions noted do not invalidate the results from the seven remaining individuals in each set of data.

The data on changes in body composition of the flight pigeons (Table 4) are of special interest. The fat and protein losses were as expected, but the water loss during this experiment was unexpectedly small (mean = 10.5 g/bird/8.6 hr plus metabolic water, or 20.9 g fat × .107 g water/g fat, =

TABLE 5

ENERGY EXPENDITURE OF FLIGHT DETERMINED FROM THE ESTIMATED FAT LOSS
IN FLIGHT PIGEONS

Specimen	Average velocity (mph)	Fat loss (g)	Caloric equivalent of fat loss (kcal/hr*)	$\Delta t_{(bld)}$ (hr)	Energy* (kcal/hr)
1285	36	22.9	217	8.6	25
2660	33	12.3	117	9.8	12
3208	42	23.6	224	8.1	28
4082	36	19.2	182	9.3	20
4084	36	24.7	235	9.4	25
2029	36	17.6	167	9.3	18
4012	25	21.1	200	12.1	17
4051	40	25.0	238	8.1	29

Mean ± S.E. 22 ± 2**

* If Δt (flight) were used to determine caloric cost/hr, a slight correction would be necessary resulting in a mean energy expenditure of 24 ± 2 kcal/hr.
** Converted to weight-specific basis: mean value ± S.E. is 57 ± 10 kcal/kg × hr.

32.9 g/bird/8.6 hr or 3.8 g/bird/hr). It is generally believed that the increased heat production associated with flight in birds is dissipated primarily by evaporative cooling (e.g., Yapp, 1956, 1962; Eliassen, 1963: 234–235) and estimates of water loss based on this concept suggest relatively high rates (Zeuthen, 1942). Zeuthen's (1942) calculated rates appear too high; as noted by Raveling (Master's dissertation, Univ. of Minnesota, 1963: 45) following Zeuthen, a 290 g pigeon would lose 190 g of water in an eight hour flight! The estimation of energy requirements in migrating Blackpoll Warblers (*Dendroica striata*) by Nisbet et al. (1963: 136) suggests that metabolic water can compensate for water loss during flight. Odum et al. (1964) present evidence for homeostasis in the nonfat components in the body during migration. Perhaps dehydration is not as great in birds in flight as has been generally accepted.

TABLE 6

COMPARISON OF ENERGY EXPENDITURE OF STEADY FLIGHTS WITH THEORETICAL
ESTIMATIONS DERIVED FROM VALIDATION EXPERIMENT DATA

Specimen	Calculated resting metabolism (kcal/hr*)	Flight metabolism (resting kcal/hr × 8) (kcal/hr*)	D_2O^{18} data (kcal/hr)
1285	2.79	22	24
2660	2.57	21	25
3208	2.42	19	14
4082	2.77	22	25
4084	2.71	22	26
2029	3.02	24	21
4012	2.53	20	15
4051	2.73	22	28
Mean ± S.E.	2.69 ± 0.07	22 ± .5	22 ± 2

* Calculations are provided in LeFebvre, 1962: 80–84.

Discussion

The vadidation of the D_2O^{18} method for determining CO_2 production and energy balance in birds provides a basis for examining certain current theoretical work in avian bioenergetics.

Odum (1960a) has estimated the flight ranges for several bird species based on the actual lipid contents of the birds at the time of collection and some inferred value for flight-energy requirements. The energy cost of flight was calculated by using Brody's (1945: 916) ratio for energy cost of sustained strenuous work to the energy cost of *resting* metabolism (*cf.* Brody, 1945: 352 and 916, resting = 1.5 basal). Brody's (1945) investigation of the work capacities of horses led to his conclusion that strenuous work requires up to eight times the resting energy requirements, a conclusion similar to that made for humans by Dill (1936). Pearson (1950) measured the oxygen consumption in the Allen's Hummingbird (*Selasphorus sasin*) which provided a calculated resting requirement of 0.06 kcal/g/hr for one 3.5 g male, while the calculated hovering flight (strenuous work) requirement was 0.40 kcal/g/hr (assuming average flight weight of 3.5 g). These data provide a strenuous work energy to resting energy ratio of about 7, suggesting that Brody's (1945) relationship may apply equally well to birds. Although Pearson's (1950) original figures are high compared to those given in Lasiewski's recent studies (1962, 1963), this *ratio* is substantiated by Lasiewski's data.

This relation can be tested further with the data obtained in this study. If the observed values (Van Slyke) of CO_2 output are used to estimate the resting metabolic rate for each of six pigeons of the validation experiment, the mean energy expenditure in the metabolism chamber is 7 + 1 (S.E.) kcal/kg/hr (LeFebvre, 1962: 85). This value, which I shall assume is a reasonable one for approximating the resting metabolism, compares well with the resting metabolic rate one could calculate from standard metabolic rates recorded for pigeons (*cf.* King and Farner, 1961: 224). A resting metabolic rate for each flight experiment pigeon is found by:

$$7 \text{ kcal/kg/hr} \times \text{mean weight (kg)} \times \Delta t_{(bld)}.$$

The estimated resting metabolism is multiplied by the factor 8 to arrive at an estimated energy requirement for strenuous work (Table 6). The resulting mean caloric expenditure of 22 + 0.5 (S.E.) kcal/hr may be compared with that obtained directly from the D_2O^{18} data. These data further suggest (along with Pearson's and Lasiewski's work) that Brody's (1945) ratio for the energy required for sustained strenuous work to resting energy determined originally for mammals applies to birds generally, and specifically to hummingbirds and pigeons. Hemmingsen's (1960: 61) analysis of the proportionality of maximal energy metabolism to body weight provides

TABLE 7

Comparison of the Flight Energy to Resting Energy Ratios Derived From D_2O^{18} Method and Zeuthen's (1942) Theoretical Calculations

Specimen	Average speed (mph)	Flight energy D_2O^{18} method (kcal/hr)	Estimated resting energy (kcal/hr)	Ratios flight energy/ resting energy	Zeuthen's ratios (modified) flight energy/ resting energy
4012	25	15	2.53	5.9	5.9
2660	33	25	2.57	9.7	13.8
4082*	36	24*	2.82*	8.5	16.5
4051	40	28	2.73	10.3	21.6
3208	42	14	2.42	5.8	23.0
3208**	42	28	2.42	11.6	23.0

* The metabolic rates for four specimens (1285, 4082, 4084, 2029) were combined to obtain average values. All had average speeds of 36 mph.

** The D_2O^{18} value for flight energy required per hour appears to be unusually low, suggesting a mistaken value. Because the fat loss determination shows such close agreement, on the average, I used the energy expenditure/hour represented by the fat-loss data for an alternative estimate for no. 3208.

tacit agreement that the ratio of energy required for strenuous work to basal (and resting) energy requirements is similar in birds and mammals.

Zeuthen (1942) in a study on the respiratory needs of birds has incidentally provided another set of interesting figures on the energy requirements of bird flight based on aerodynamic consideration. The flight metabolism as a multiple of the resting metabolism which his data provide can be compared with those obtained in the D_2O^{18} flight experiment. For this purpose I have used Zeuthen's figure for flight to resting ratio for a speed of 25 mph and have extrapolated to obtain similar ratios for speeds represented by my pigeons. These results may then be compared to my flight energy to resting energy ratios listed in column (5) of Table 7. The agreement is only approximate, as one would expect considering the uncertainties involved in each set of calculations. Zeuthen's (1942) figures are derived from theoretical considerations and are judged by him to be only approximations good within ± 50 per cent. It should be emphasized, too, that in the data for pigeons the speeds recorded may or may not represent close approximations to the true ground speed of each bird.

The significance of the isotopic method for ecological studies is obvious. The energy requirements of many wild organisms may be determined with greater certainty whenever the assumptions involved in the method are validated. Energy flow in ecosystems or main components of ecosystems are amenable to more direct determinations. Thus, aspects of the environmental physiology of wild species may be investigated with minimum manipulation of the organism. In birds and mammals the energy cost of various activities of interest to investigators (e.g., migration, hibernation, incubation, etc.) may be determined directly without the need for greatly altered or artificial environmental situations.

Summary

The D_2O^{18} method for determination of CO_2 production and other components of the energy balance (food intake, water turnover, and gaseous exchange) had been shown to be applicable to laboratory mammals by other workers. The method was extended in this investigation to pigeons for the purpose of validating this approach for metabolism studies in birds. The validation studies performed on ordinary common pigeons and racing pigeons gave a mean calculated CO_2 production which is 8 per cent $\pm$ 2 per cent (S.E.) that of the observed CO_2 production obtained by collection in concentrated KOH solution and analyzed by the Van Slyke manometric method. For the energy expenditure of flight, a mean caloric requirement of 22 ± 2 (S.E.) kcal/hr was obtained for eight racing pigeons averaging 384 g in weight and flying a distance of 300 miles. Four of these birds apparently flew the entire route non-stop, while four showed evidence of having stopped (although the average flight speed suggests this was for a relatively short time). The calculations based on data obtained in this investigation indicate that energy expenditure in flight (sustained, strenuous work) is approximately eight times the energy expenditure of a resting domestic pigeon. This agrees with data obtained for man and large domestic mammals and supports previous estimates of the energy expenditure of sustained flight in small birds.

The results suggest further considerations of this method for studies of metabolism in birds.

Literature Cited

Benedict, F. G. 1938. Vital energetics: a study in comparative basal metabolism. Carnegie Inst. Washington, Publ., **503**: 1–215.

Benedict, F. G., and O. Riddle. 1929. The measurement of the basal heat production of pigeons. II: physiological technique. J. Nutrition, **1**: 497–536.

Brody, S. 1945. Bioenergetics and growth. New York, Reinhold.

Brown, R. H. J. 1951. Flapping flight. Ibis, **93**: 333–359.

Brown, R. H. J. 1961. Flight. Pp. 289–305 *in* Biology and comparative physiology of birds, Vol. 2, A. J. Marshall (ed.). New York, Academic Press.

Dill, D. B. 1936. The economy of muscular exercice. Physiol. Rev., **16:** 263–291.

Eliassen, E. 1963. Preliminary results from new methods of investigating the physiology of birds during flight. Ibis, **105:** 234–237.

Fisher, R. A. 1934. Statistical methods for research workers. Fifth edit. London, Oliver and Boyd.

Hemmingsen, A. M. 1960. Energy metabolism as related to body size and respiratory surfaces and its evolution. Rep. Steno Mem. Hosp. and Nord. Insulin Lab., **9:** 1–110.

King, J. R., and D. S. Farner. 1961. Energy metabolism, thermoregulation and body temperature. Pp. 215–288 *in* Biology and comparative physiology of birds, Vol. 2, A. J. Marshall (ed.). New York, Academic Press.

Kleiber, M. 1950. Calorimetric measurements. Chapter 6 *in* Biophysical research methods, F. Uber (ed.). New York, Interscience.

Lasiewski, R. C. 1962. The energetics of migrating hummingbirds. Condor, **64:** 324.

Lasiewski, R. C. 1963. Oxygen consumption of torpid, resting, active, and flying hummingbirds. Physiol. Zool., **36:** 122–140.

Lee, J. S., and N. Lifson. 1960. Measurement of total energy and material balance in rats by means of doubly-labeled water. Amer. J. Physiol., **199:** 238–242.

LeFebvre, E. 1962. Energy metabolism in the pigeon (*Columba livia*) at rest and in flight. Ph.D. thesis, Univ. of Minnesota, 85 pp. Minneapolis, Minnesota.

Lifson, N., G. B. Gordon, and R. McClintock. 1955. Measurement of total carbon dioxide production by means of D_2O^{18}. J. Appl. Physiol., **7:** 704–710.

Lifson, N., G. B. Gordon, M. B. Visscher, and A. O. Nier. 1949. The fate of molecular oxygen and the source of the oxygen of respiratory carbon dioxide, studied with the aid of heavy oxygen. J. Biol. Chem., **180:** 803–811.

Lifson, N., and J. S. Lee. 1961. Estimation of material balance of totally fasted rats by doubly-labeled water. Amer. J. Physiol., **200:** 85–88.

McClintock, R., and N. Lifson. 1957a. Applicability of the D_2O^{18} method to the measurement of the total carbon dioxide content of obese mice. J. Biol. Chem., **226:** 153–156.

McClintock, R., and N. Lifson. 1957b. CO_2 output and energy balance of hereditary obese mice. Amer. J. Physiol., **189:** 463–469.

McClintock, R., and N. Lifson. 1958a. Determination of the total carbon dioxide outputs of rats by the D_2O^{18} method. Amer. J. Physiol., **192:** 76–78.

McClintock, R., and N. Lifson. 1958b. CO_2 output of mice measured by D_2O^{18} under conditions of isotope re-entry into the body. Amer. J. Physiol., **195:** 721–725.

Nisbet, I. C. T., W. H. Drury, Jr., and J. Baird. 1963. Weight-loss during migration. Bird-Banding, **34:** 107–159.

Odum, E. P. 1960a. Lipid deposition in nocturnal migrant birds. Proc. XII Int. Orn. Congr.: 563–576.

Odum, E. P. 1960b. Premigratory hyperphagia in birds. Amer. J. Clin. Nutr., **8:** 621–629.

Odum, E. P., C. E. Connell, and H. L. Stoddard. 1961. Flight energy and estimated flight ranges of some migratory birds. Auk, **78:** 515–527.

Odum, E. P., D. T. Rogers, and D. L. Hicks. 1964. Homeostasis of the nonfat components of migrating birds. Science, **143:** 1037–1039.

Pearson, O. P.   1950.   The metabolism of hummingbirds. Condor, **52:** 145–152.
Pearson, O. P.   1954.   The daily energy requirements of a wild Anna Hummingbird. Condor, **56:** 317–322.
Peters, J. P., and D. D. Van Slyke.   1946.   Quantitative clinical chemistry: interpretations. Vol. 1. Second edit. Baltimore, Williams and Wilkins.
Raspet, A.   1960.   Biophysics of bird flight. Science, **132:** 191–200.
Wilkie, D. R.   1959.   The work output of animals: flight by birds and by manpower. Nature, **183:** 1515–1516.
Yapp, W. B.   1956.   Two physiological considerations in bird migration. Wilson Bull., **68:** 312–319.
Yapp, W. B. 1962.   Some physical limitations on migrations. Ibis, **104:** 86–89.
Zeuthen, E.   1942.   The ventilation of the respiratory tract in birds. Kgl. Danske Videskab. Selskab., **17:** 1–50.

Minnesota Museum of Natural History, University of Minnesota, Minneapolis 14, Minnesota.