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DIRECT AND INDIRECT CALORIMETRY OF MEDIUM SIZED ANIMALS

I.H.D. LAMPRECHT

Institut für Biophysik, Freie Universität, D-1000 Berlin 33

ABSTRACT

Direct and indirect calorimetry of medium sized animals is described and in its technique compared with calorimetry of small animals and with whole-body calorimetry in man. Among the indirect methods discussed are: determination of oxygen consumption/carbon dioxid production in open and closed systems, isotope dilution techniques, combustion calorimetry and estimation of metabolism from heart rate. The group of medium sized experimental animals used in the direct calorimetric measurements included rats, rabbits, skipmunks, chinese hamsters and dogs. Most instruments for medium sized animals apply the gradient layer principle proposed by Benzinger and Kitzinger (ref.1).

INTRODUCTION

Animal calorimetry started 200 years ago when Lavoisier and Laplace published their "Memoire sur le Chaleur" with the description of the famous ice calorimeter and the results on respiratory metabolism and heat production of guinea pigs. At the same time Adair Crawford determined the combustion heats of biologically interesting material and only some years later the heat production of living matter. He was the first to clearly state that oxygen consumption is roughly proportional to heat production (ref.2), the fundamentals of modern indirect calorimetry. But then it took more than 100 years before the next essential experiments on animals were performed (ref.3, ref.4).

The above stated sentence of Crawford, namely that there is a good correlation between oxygen consumption and heat production led to the opinion that (energy) metabolism is a biological combustion of energy rich compounds at low temperature and that direct calorimetry may be substituted by the cheaper and easier indirect one. That meant that oxygen consumption and carbon dioxid production of an animal were determined and the respiration quotient RQ of the metabolism calculated as the ratio of the produced mol of carbon dioxid divided by the mol of consumed oxygen. This

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quotient ranges between 1.0 for the combustion of carbohydrates and 0.71 for fats. As the heats of combustion of the different classes of organic substrates are known, heat production can be easily calculated (ref.5).

In recent times "indirect" calorimetry has broadened to further methods, such as combustion calorimetry - mainly in connection with ecological questions and the energy flow through complex biological systems -, isotope dilution techniques and the determination of the heart rate and the concentrations of oxygen in the venous and arterial blood. Each method has its advantages and can - and perhaps always should - be combined with the direct calorimetry.

INDIRECT CALORIMETRY

Determination of gas metabolism

Animal calorimetry mainly utilizes indirect methods among which the calculation of heat production from oxygen consumption and carbon dioxid production is the most frequently applied procedure. One has to distinguish between open-circuit and closed-circuit set-ups for the air flow in the system where concentrations of oxygen and carbon dioxid and the humidity are continuously monitored. In some cases a small quantity of air is collected in an anesthesia rubber bag and analyzed from time to time (see f.i. ref.6). In all of these cases the animal may be placed in a special respiration chamber or connected to a mask or a hood. These methods were intensively applied by Brody (ref.7) for animals of all sizes from elephants to sheep, and by other authors for much smaller probands such as lizards and snakes (ref.8).

To calculate heat production from gas metabolism different formulae are given in literature. Brouwer (ref.9) found for mammals

$$H(kJ) = 16.17\ V{:}O_2(l) + 5.02\ V{:}CO_2(l) - 2.17\ V{:}CH_4(l) - 5.99\ W{:}N(g)$$

and Romijn and Lokhorst (ref.10) for birds

$$H(kJ) = 16.20\ V{:}O_2(l) + 5.00\ V{:}CO_2(l) - 9.93\ W{:}N(g)$$

where W:N is the weight of nitrogen in urinary excreta. For many animals $V{:}CH_4$ can be neglected. Kleiber (ref.5) states the relation

$$H(kJ) = V{:}O_2\ (14.25 + 7.86\ /\ RQ)$$

where RQ is the respiration quotient. In all three formulae V: incates the volume of the exchanged gases in liters.

The application of these formulae to the metabolism of different animals may be found in the literature: for the kestrel (Falco tinnunculus) and for mice (Mus musculus) (ref.11), as well as for lambs (ref.12) and yearling steers of appr. 300 kg (ref.13). Corresponding measurements were performed on lizards and snakes (ref.14), on armadillos (ref.15), on rats under the influence of several drugs (ref.16) and on the non-shivering thermogenesis in young pigs and its alteration by beta-blockers (ref.17), just to cite a few papers on variously sized animals found in the literature.

Heart rate and metabolism

The correlation between heart rate and oxygen consumption or heat production is also used as an indirect method of calorimetry. This technique was applied for different animals among them rodents (ref.18), bats (ref.19, ref.20) and sheep (ref.21). A typical relationship is given by

$$dV/dt = (HR \times SV) \times (Ca - Cv)$$

where dV/dt is the rate of oxygen consumption, HR the heart rate, SV the stroke volume, Ca and Cv the oxygen content in the arterial or venous blood, resp. (ref.21, ref.22). Nagasaka and his coworkers showed that heat production changed from

$$M\ (W/qm) = 0.194\ HR\ (bpm) - 17.7$$

for resting rats to

$$M\ (W/qm) = 0.307\ HR\ (bpm) - 70.9$$

under physical restraint within minutes (ref.23).

Combustion calorimetry

Combustion calorimetry of biologically interesting material dates back till Crawford (1779) who published essential figures of the heat of combustion. Later on this method was used for energy calculations in microbial cultures as well as for higher organisms or ecological systems. Heat contents between 18.4 and 29.3 kJ/g ash free material were obtained for 17 species from protozoans (Ciliata) up to fish (ref.24). Campbell et al. (ref.25) compared the combustion heat of granary weevils (Sitophilus granarius L.), their feces, exuvia and food with the respiration rate of the animals to calculate the bioenergetics of their life. Higher animals were investigated by several authors. In birds migrating over long distances an energy content of 33.1 to 34.7 kJ/g ash free material was found before migration and only 23.8 to 31.o kJ/g at their spring arrivals while lean nonmigrants ranged from 25.1 to 28.9 kJ/g (ref.26). In both groups nonfat body component amounted to 23.0 kJ/g. Nestling starlings (Sturnus vulgaris L.) exhibited a constant caloric value of approx. 21.8 kJ/g dry weight during their development, whereas water and ash content changed considerably (ref.27). These investigations were performed to determine the energy flow through the system. The same intention stimulated the experiments on young and adult armadillos (Dasypus sabanicola) and their metabolic exchange (ref.28).

Isotope dilution techniques

Two isotope dilution techniques were introduced in the literature to evaluate the rate of metabolic activity. Lifson et al. (ref.29) discuss the double-labelled water method to determine the carbon dioxid production, to elucidate the pathway of the molecular oxygen and to identify the sources of the oxygen bound in the expired carbon dioxid. For these experiments, which were performed on mice and rats, labelled molecular oxygen and labelled water were used. In the second, the carbon dioxid entry rate technique (ref.30), the carbon dioxid production is also measured and with the estimated RQ transformed to energy units. Both methods have been applied for large animals, but are expensive and suffer some disadvantages, f.i. if variations in the rate of carbon dioxid production during time periods shorter than a few hours are of interest. In the most advanced form (ref.31), 5 ml/h labelled sodium bicarbonate in saline solution is continuously infused into the jugular vein of sheep and the same amount of parotid saliva is collected. As the surgical interference is small this inexpensive and simple technique could be applied for medium sized animals, too.

Other indirect methods

A quite exotic way of indirect calorimetry was performed on fetal lambs in utero (ref.32). In this case, thermistors were placed into fetal and maternal vessels and amniotic fluid and microheaters into the inferior vena cavae of the lambs. The fetal arterial temperature was 0.54°C higher than that of the maternal blood and increased by max. 0.5°C when the heaters (2 to 7 Watts) were switched on. From the temperature differences a fetal heat production of 2.9 to 3.3 W/kg was calculated.

A broad survey about alternative methods to the calorimetric chamber for large animals may be found (ref.22). The author suggests that a combination of an oxygen electrode with an ECG electrode introduced into the vena cava could be an attractive new technique of indirect calorimetry, especially if the animals carry their battery-powered equipment with them and move freely in their normal surroundings.

DIRECT CALORIMETRY

Calorimetric techniques used for investigations both of small and of large domestic animals (or man) were applied for medium sized animals, too. Some of the commercially available calorimeters fitted well for the experiments on small animals such as insects (ref.33 to ref.38), tadpoles of frog and toad (ref.39) and little vertebrates like lizards (ref.40) and snakes (ref.41) with weights between 4 and 18 g.

On the other end of the scale there are the "whole body" calorimeters with less than 2 cbm (ref.42 to ref.44) or even rooms in which a person can remain for more than one day (ref.45, ref.46). Many of these instruments are based on the gradient layer system introduced by Benzinger and Kitzinger (ref.1), while others depend on temperature and hygrometric measurements between inlet und outlet air (ref.47). Most of the calorimeters can be used for direct and indirect determinations of heat production and heat loss, so that all components of heat exchange with the surroundings can be evaluated simultaneously (ref.48, ref.49). Some of these calorimeters may be used as prototypes for investigations with medium sized animals. A procedure with heat-flow meters as used in human experiments (ref.50) does not seem to be applicable for other animals, except for pigs and nude laboratory animals because of fur or feathers.

In determining the heat loss of an animal, different processes have to be taken into account: heat loss by radiation, by convection, by conduction and by water evaporation (ref.5). The first component of heat loss depends on the temperature difference between the surface of the animal and that of the calorimeter. It is also governed by the character of these surfaces but not by the medium between them. The heat loss by convection is sometimes taken as the major calorimetric signal when the temperature difference of the inlet and the outlet air is measured and transformed to power units via the flow rate (ref.47). Heat loss by conduction may play an essential role for animals in their usual environment when a firm contact of a large surface to the cooler ground is strived for in summer or avoided in winter. Heat loss by conduction plays a minor role in calorimetry or is even excluded by the construction of the instrument. However, heat loss by evaporation of water is an important factor in the animal's heat balance. Due to the difference in vapor pressure of the evaporating surface (skin, tongue) and the surrounding air, this factor can be calculated from the increase in water content of the air passing through the calorimeter. Modern "whole body" instruments are well suited to determine all these factors of an animal's heat loss independently.

Only one commercially available calorimeter is described in the literature in its application to investigations of medium sized animals. It is a gradient layer type instrument (SEC-A, THERMONETICS/San Diego CA) used by Nagasaka and his group in many different experiments, including the indirect and direct determination of heat production in mongrel dogs (ref.51) and rabbits (ref.52), and in a study of the influence of huddling on the heat loss of young dogs (ref.53). At 15^{o}C the authors observed a strong linear decrease in both total and dry heat loss with the number of dogs being together in the calorimeter (up to 3). However, at 20^{o}C this linear decrease was only observed between 1 to 2 individuals. All further animals did not change the output. The same instrument was applied after slight modifications to evaluate the sensible and insensible heat loss from single rats after burn injuries (ref.54, ref.55).

Other gradient layer calorimeters for simultaneous determinations of heat production by indirect calorimetry and partitioned heat loss by direct means can be found in the literature. Lawton et al. (ref.56) describe a calorimeter for the rapid measurement of heat productions and heat losses in small animals (ref.57). Hammel and Hardy (ref.58) designed an instrument to study the thermoregulatory response in dogs. Richards et al. (ref.59) report on energy expenditure after injury in animals. Using a gradient layer calorimeter of the Benzinger type, they observed an increase in heat production by 5 % after injury. Combined direct and indirect calorimetric investigations of rats with burns (ref.60) or with fractures of the femur (ref.61) were also performed. One can even determine calorimetrically the influence of sleep and wakefulness (ref.62) and of diurnal patterns in the heat production and the heat loss of rats under controlled conditions (ref.63 to ref.65).

Pivorum (ref.66) described a gradient calorimeter constructed of many flux plates to study the normothermic and the hibernating heat production of Eastern chipmunks (Tamias striatus). He found a linear relationship between the environmental temperature and the heat loss for normothermic conditions and a reduction to only 2 - 12 % of this value for hibernating chipmunks.

Not only mammals but birds also have been investigated calorimetrically, although in most cases by indirect means. Lundy (ref. 67) gives a survey about poultry calorimetry with special interest on thermal insulation by feathers. Heat production of birds defeathered or with oiled feathers was twice as high as that of the fully feathered individuals for ducks and cockerels. Pickwell (ref. 68) developed a simple calorimetric box of 54 l volume in which the heat lossed by the investigated animal was compensated by circulating cooling water. This instrument was used to determine the energy metabolism in Peking ducks during submergence asphyxia. To this end the duck's beak was fitted into an air-tight rubber mask which could be filled with water to simulate the duck "diving". Simultaneously, heat loss, heart rate, ECG and temperatures of different body parts were monitored. It could be shown that Peking ducks are able to reduce their energy metabolism to less than 10% and their heart rate to 5 - 8 % of resting levels and to recover from diving periods of up to 10 min (ref.69).

The group of Nagasaka from Kanazawa, Japan, made a number of interesting calorimetric investigations on various small animals. They developed a compact calorimeter of approx. 3.5 l with two heat flow plates and a sensitivity of 1.47 V/W (ref.70). This instrument offered so much space that it did not interfere with the animals's vasomotor, respiratory, behavioral and diurnal activities (ref.71). The evaporative heat loss of the animals determined by the air flow through the box was only 1 % and so could be neglected in comparison to other factors. The total heat loss of rats amounted to 6.5 W/kg during daytime and 8.8 W/kg at night. Moreover, the influence of different drugs on the heat balance and on the radiative, convective, conductive and evaporative heat loss of rats was investigated (ref.72, ref.73).

A very simple and inexpensive calorimeter was recently proposed which consists of a warming/cooling box as used for picnics, with a volume of 8 l and a sensitivity of approx. 20 mV/W (ref.74). It was first tested with chinese hamsters, hedgehogs and guinea pigs and proved its applicability. Since then it has been further developed to include oxygen sensors and thermocouples so that simultaneous measurements of oxygen consumption and heat loss in a closed system can be performed. It may also be used in similar determinations as an open system, when air is continuously flowing through the box. Experiments on the Japanese quail (*Coturnix coturnix*) are currently been undertaken.

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