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The advantages and disadvantages of direct and indirect calorimetry ☆

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Abstract

Kleiber's definitions of what constitutes direct and indirect calorimetry are accepted as the beginning of a commentary on the advantages and disadvantages of direct and indirect calorimetry in which calorimetry is divided into a number of categories based on the kind of calorimetric measurement. For non-reaction calorimetry such as entropy determinations and differential scanning calorimetry, the only means of measurement is by direct calorimetry. For reaction calorimetry, a preference of direct over indirect calorimetry depends on the accuracy needed and the ability of the experimenter to define the system. The data necessary to correct the observed heat loss in direct calorimetry are often all that are needed to make an indirect calculation of the true heat loss. In general, because they are convenient and inexpensive to use, indirect calorimetric methods are preferable to direct methods. However, when possible, one method can be used to verify the results of the other.

Keywords: Calorimetry; Direct calorimetry; Indirect calorimetry

1. Introduction

The purpose of this commentary is to give briefly some opinions on the advantages and disadvantages of direct and indirect calorimetry. It is not intended to present a thorough review of the literature, but to explore what the two types of

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calorimetry represent and to state preferences where these may exist. A few references are made to articles that represent the first applications of methods in the early history of biological calorimetry, and these are worth reading simply for the pure pleasure of doing so. Selected recent reviews or papers illustrate calorimetric methods in the different categories. More complete references are to be found in the several books or edited volumes of biological calorimetric methods or research papers listed in the General Reference list.

2. Commentary

According to Kleiber [1], "Indirect calorimetry measures the heat production of an animal; direct calorimetry measures the heat loss." The two methods may not give the same value for the same experiment. This is because some of the heat produced by an organism may be absorbed by the activities of the organism. Examples are the heat of evaporation of water from animals during sweating or from the lungs during breathing, or the heat of transpiration from the leaves of plants. However, in theory, if the appropriate corrections can be made for extraneous heat producing or absorbing events during direct calorimetry, the values obtained from direct and indirect calorimetry should be the same. For the purposes of this commentary, direct calorimetry is defined as the *measurement* of heat loss or gain by a system, corrected for as many extraneous heat losses or gains as possible. These latter usually have to be determined by calculation. In the use of indirect calorimetry the heat loss or gain is not directly measured. Indirect calorimetry is therefore defined as the *determination by calculation* of the heat produced by a system, using some method other than the direct measurement of heat loss or gain. Heat is defined as the thermal energy that is exchanged between two masses because of a temperature difference between them. However, with the exception of the determination of heat production by ergometry, all indirect methods of calorimetry depend ultimately on previously made direct calorimetric measurements of one kind or another that are used in the calculation of the heat produced.

Direct calorimetry has an advantage of being effectively a one-step operation resulting in a heat loss measurement. What this actually means may be something else. Unfortunately, except for "no-reaction" calorimetry, most direct calorimetric methods yield "observed" values which, to be meaningful, have to be corrected to give the "true" values. These corrections can be made only if it can be recognized what is going on in the calorimeter and what corrections to apply. These corrections are usually obtained by calculation using other data, and thus constitute an indirect aspect of direct calorimetry. Even if the need for a particular correction can be recognized, the necessary data to accomplish this may not be known. All direct calorimetric methods are theoretically sound with respect to the calorimetric purposes intended. They usually require expensive calorimeters and accompanying instrumentation. In general, all well-designed calorimeters can be considered accurate and precise when used with the highly stable electronic instrumentation

presently available. Instrumentation should not be a problem except for cost. And, all direct methods can be used to measure rates of heat loss as well as total quantities of heat produced.

The methods of indirect calorimetry involve any method of determining heat production other than to measure directly the heat loss from the system being studied. The heat produced is calculated by correlation or by difference. These methods can involve direct bomb calorimetry of the initial and final states of a system being studied, even though the determination of heat production is indirect. However, they usually involve no direct calorimetry at all, which may be a distinct advantage. They may require an accurate knowledge of the thermochemistry of the initial and final states of a system. They also can be used to determine rates of heat production as well as total quantities of heat produced. The use of indirect calorimetric methods frequently results in the determination of the true heat of reaction, requiring no further correction. Aside from the convenience of not having to use a calorimeter, this is certainly one of its principal advantages.

The methods of direct and indirect calorimetry can be divided into several categories, each of which has its own set of advantages or disadvantages. Some of these categories have been much more worked over than others. The references given represent the original development of a category where this is apparent, or selected sources of more information about a category as to method. They may, but need not, represent an exhaustive compilation of sources.

3. Non-reaction calorimetry

This designation is applied because the sample does not react except physically by itself as a result of temperature changes. There are no other reactants and no products. What is measured is physical loss or gain of heat, and this requires a calorimeter.

3.1. Low temperature, adiabatic calorimetry for entropy measurements of biological interest

This is a non-differential type of calorimetry in which known quatities of thermal energy are introduced into a known quantity of sample the temperature of which has been lowered previously to about 5 K. What is measured is the temperature rise per unit mass of sample per unit increment of temperature rise, from which heat capacities can be determined. The measured entropy at the standard temperature of 298.15 K is an observed value. Various impurities will contribute to the value of the observed entropy and for this reason thermochemists have been reluctant to obtain entropy values except for the most pure of crystalline substances, in which case the "observed" value becomes the "true" value. However, everything has an entropy, and there might be some merit in obtaining entropy values for biological polymers and for whole cells. If the composition of a substance is known, it then becomes possible to calculate its standard entropy of formation. Entropy values for many

small molecular weight organic substances of biological interest can be found in papers by Domalski et al. [2], Breslauer [3], and Wilhoit [4]. Entropy data for macromolecular substances are virtually non-existent, except for data on proteins by Hutchens [5]. Work by Putnam and Boerio-Goates [6] represents an example of a modern entropy measurement.

Advantages of direct calorimetry

This is the only experimental method of measuring entropy.

Disadvantages of direct calorimetry

Not only is a calorimeter required, but they are extremely expensive, not commercially available, and must be constructed specifically to order. Expensive electronic instruments are necessary to run the calorimeter, and there is also the cost of liquid helium.

Advantages of indirect calorimetry

Indirect entropy determinations can be made for small molecules by group substitution if their structures are known. This does not require expensive calorimetry.

Disadvantages of indirect calorimetry

Entropy determinations by group substitution cannot be made conveniently with cellular bio-mass, the total monomeric composition of which is usually unknown. And although these methods provide useful estimates, the experimental method is more accurate.

3.2. Differential Scanning Calorimetry (DSC)

This includes differential thermal analysis (DTA) which is a similar, but older technique.

This is a differential type of calorimetry in which two chambers are held at the same temperature while this is being raised, a change in the heat capacity of the sample being detected if the sample absorbs or releases heat as a result of changes in structure as compared to the control. DSC is used mostly to detect changes in molecular configuration or total structure as a function of temperature changes. It can also be used to detect melting or annealing points. There are similarities between these and entropy measurements. Reviews of methods and applications of DSC can be found in articles by Privalov [7], Privalov and Plotnikov [8], and Sturtevant [9]. Some examples of DSC methods are the studies by Privalov and Potkhin [10] on temperature-induced changes in proteins, Schlicher Aronhime [11] on polymorphic transformations, Blume [12] on membranes, Castronuovo [13] on proteins in aqueous solutions, Klump et al. [14] on conformation changes in nucleic acids, Raemy and Lambelet [15] on the thermal behavior of foods, and Münzing [16] on starch in cereals and cereal products.

Advantages of direct calorimetry

What you observe is what you want to measure, provided that the sample is pure.

Disadvantages of direct calorimetry

Apparently none with respect to the purpose of the method.

There does not appear to be an indirect method of doing DSC.

4. Reaction calorimetry

This designation is applied because the loss or gain of heat that is measured is the result of a reaction or process that proceeds from an initial to a final state, or that can be measured as a rate of reaction. Reaction calorimetry can be either direct or indirect.

4.1. Bomb calorimetry (including rotating bomb calorimetry)

This is a non-differential type of calorimetry first described in 1881 by Berthelot [17] and in 1884 by Berthelot and Vieille [18] in which a sample is burned within a thick-walled vessel for the purpose of measuring the heat of combustion. Modern measurements are made adiabatically or ballistically, and expressed as heat loss per unit mass of sample. This information may be useful by itself, as in a quantitative comparison of the heat content of various substances. Otherwise, more must be known about what is being burned. If the composition of the substance is known, measurements can be made with increased accuracy, and heats of formation can be calculated. The various methods of bomb calorimetry have been summarized in books edited by Rossini [19] and by Skinner [20].

Advantages of direct calorimetry

These are the possibility of making the basic measurements from which heats of formation can be calculated, as well as obtaining information as to the caloric equivalents of substances.

Disadvantages of direct calorimetry

Whereas this type of calorimetry has been extremely valuable in obtaining heats of combustion of pure substances of known composition, the general literature shows that there is considerable variability associated with measuring the heat of combustion of biomass. The difficulties may be associated with an inability to account for exactly what is going on in the calorimeter by means of a reaction equation giving all the reactants and products. Also, uncertainties may exist as to what comprises "ash", and what comprises the organic component, so that it becomes equally uncertain what the mass is that loses heat.

Advantages of indirect calorimetry

There is no indirect way to make the basic measurements of heat loss obtained with this type of calorimetry. However, if heat of formation values can be obtained

for the reactants and products in a given reaction or process, it becomes possible to determine indirectly (by calculation) the heat of combustion of a substance. This may be preferable to making the direct, experimental measurement. It is certainly more convenient. Group contribution may also be used if the structure of a substance is known. In this case Thornton's rule may also be applied.

Disadvantages of indirect calorimetry

There are no disadvantages if the requisite enthalpy of formation information is available. However, bomb calorimetry is more simple and direct. It might be expected that the simpler method would be theoretically the more accurate. Practically, the direct and indirect methods of calorimetry appear to be similar in this respect if appropriate data for the latter are available.

5. Catabolic calorimetry

These are direct or indirect calorimetric methods by means of which measurements or determinations can be made of the heat loss resulting from catabolism. By this definition anabolic processes are excluded from the calorimetric system. The classic calorimetric measurement of "basal metabolism", for example, would be included under this definition in that if a metabolizing organism were not growing it would be considered to be catabolizing by means of the oxidation or fermentation of stored substances apart from the fabric of the organism, or of substances that have just been consumed. Except for this latter, it is characteristic of catabolic calorimetry that, if conditions are kept constant, the heat loss is linear over a convenient time period for a calorimetric experiment. This may not apply if an organism has been fed just prior to an experiment. With some growing organisms, the heat loss would be expected to exhibit an exponential increase over time, or to exhibit a lesser, linear heat loss than would be expected from catabolism alone.

5.1. Whole body catabolic calorimetry

This type of calorimetry can be direct or indirect, and implies the use of multicellular organisms. In direct calorimetry plants or animals are placed within a calorimeter. The earliest examples of this method are those described in 1780 by Lavoisier and LaPlace [21] and in 1788 by Crawford [22]. Because of size restrictions such calorimeters are usually small instruments for use with small animals or plants. What is measured is heat loss per unit biomass per unit time. Most of the work during the following century was with respiration calorimeters, such as those used in studies published in 1824 by Despretz [23], in 1894 by Rubner [24], in 1896 by Laulanié [25], and in 1899 by Atwater and Rosa [26]. Because of its importance in studying human or animal metabolism, considerable work has been done since then with respiration calorimeters large enough to accomodate a human or a farm animal. The idea of a "caloric quotient" was introduced in 1911 by Meyerhof [27] and such studies (among many others) culminated in the idea of caloric equivalents

for fats, proteins, and carbohydrates which could be used in indirect calorimetry, and also in calculations made using the respiratory quotient. A good example of this is given on p.125 of Kleiber [1]. The subject of whole body calorimetry in man and animals has been thoroughly reviewed by Lamprecht and Schaarschmidt [28] and by Dauncey [29]. Animals within a calorimeter vessel are not in a natural environment and may experience stress during an experimental run. An interesting attempt to address this with respect to aquatic animals was made by Addink et al. [30] through the use of a flow-through calorimeter. Several studies have been done on whole body calorimetry of plants, and these have been reviewed by Criddle et al. [31]. An example of the modern technical possibility of combined measurements of heat loss, CO_2 production, and O_2 consumption in small plants has been published by Criddle et al. [32]. Whole body calorimetry can include suspensions of small animals, as in the study by Hand [33] on suspensions of *Artemia embryos*.

Advantages of direct whole body calorimetry

Direct measurements can be made of total heat loss or of the rate of heat loss. Such measurements can demonstrate that animals lose more heat when they are active than when they are passive under various conditions of being or of environmental stress, and that under various conditions of basal catabolism different kinds of animals differ in heat loss per unit mass per unit time. With such information, ideas of energy budgets can be formulated.

Disadvantages of direct whole body calorimetry

Corrections must be made with respect to the calorimetry of terrestrial organisms, including those for heat lost by convection, conduction, evaporation, and radiation, where necessary. In addition, the calorimeters required are complex and expensive.

Advantages of indirect whole body calorimetry

Experiments using both whole body calorimetry and respirometry have demonstrated a correlation such that respirometry was soon thought to be as good as, and far more convenient than, whole body calorimetry. With indirect calorimetry, measurements are made of oxygen consumption, carbon dioxide production, and urine excretion or the excretion of other nitrogenous substances. In the open circuit method both the oxygen consumed and the carbon dioxide expired are measured to obtain an RQ. From this, a heat loss as well as ratios of fat and carbohydrate catabolized can be calculated. The quantity of nitrogen excreted is usually taken to be related to the catabolism of protein. In the closed circuit method of indirect calorimetry, only the O_2 consumed is measured. An average RQ of 0.82 is assumed, corresponding to a calorific value of 4.825 kcal (20.188 kJ) per liter of O_2 consumed. Ergometry is the electrical measurement of mechanical work converted into units of heat. It requires no respirometry or calorimetry, but cannot be used except to measure activity above a basal level, i.e., mechanically measurable work has to be done. A distinct advantage is that corrections do not have to be made for heat loss by convection, conduction, evaporation, and radiation, as would have to be done in direct calorimetry where this is appropriate.

Disadvantages of indirect whole body calorimetry

There are no disadvantages with respect to the purpose intended. These methods are routinely used medically or in gymnasiums, as in bicycle ergometry or treadmill ergometry.

5.2. Tissue calorimetry

This includes organ calorimetry; liquid tissues are categorized under non-growing cell suspension calorimetry.

This method is similar to whole body calorimetry except in that the complexity of the system is reduced. Direct or indirect techniques can be used. In direct calorimetry what is measured is heat loss per unit biomass (wet or dry) or per unit biomass per unit time. The samples are small pieces of whole animal tissue or parts of plants that can be placed into a calorimeter vessel. One of the first studies is that of Hill [34], who placed muscle tissue in various stages of metabolism into a differential calorimeter that he designed. A more recent example of muscle tissue calorimetry is that of Daut et al. [35] on the contribution of the sodium pump to the basal metabolism of cardiac muscle. Recent examples of plant tissue calorimetry are those of Seymour [36] on thermogenesis in an arum lily, and Criddle et al. [37] on a number of plant parts. A different form of direct calorimetry was developed in 1902 by Blix [38] and in 1910 by Hill [39], who used thermal junctions to measure the heat output of isolated frog muscle. This is in effect a ballistic type of measurement in which the heat loss is detected and measured before there is appreciable heat exchange with the environment.

Advantages of direct calorimetry

It is possible to compare the total heat loss or rate of heat loss per unit biomass for different tissues, e.g., brain vs. liver, brown fat vs. white fat, apples vs. oranges, etc., or the effects of drugs, pollutants and poisons on different tissues. Other possibilities are, for example, the kind of calorimetry used by Blix [38] and Hill [34] to determine the heat of frog muscle contraction. Fewer corrections are necessary in tissue calorimetry than in whole body calorimetry with respect to the convection, conduction, evaporation, and radiation that would occur with terrestrial animal calorimetry. Unless perfusion techniques are used, gas exchange is usually by diffusion into the tissues. Heat uptake from evaporation is not a problem. In fact, the heat loss that is measured should closely approximate that determined by indirect calorimetry.

Disadvantages of direct calorimetry

These include the surface/volume problem, both with respect to oxygen and substrates or test chemicals. It is best to ensure that all parts of the tissue sample receive oxygen and substrate or test chemicals. This may be more difficult with tissues inside a calorimeter as compared with suspensions of isolated cells, in that they cannot be as easily (if at all) sparged or stirred. Also, during an experiment O_2 and CO_2 within the calorimeter vessel are in the gas phase, whereas in the tissues

they are in the aqueous phase. A correction for this should be made with respect to the observed heat loss, for which the quantities of gases exchanged must be known. If these exchanges are measured, the data for indirect calorimetry are already available, and except for verification, direct calorimetry need not be done.

Advantages of indirect calorimetry

These are similar to those for direct calorimetry, except in that methods of solving the surface/volume problem are usually easier because the sample is not within the confines of a calorimeter vessel. Respiratory quotient values or oxygen consumption values can be obtained, from which values for heat loss can be determined. Conventional Warburg or Barcroft respirometry could be useful here, in that many samples can be run at the same time. However, these techniques are hardly ever used anymore, and electronic methods of O_2 and CO_2 measurement are used to great advantage. There can be a great convenience to indirect calorimetry.

Disadvantages of indirect calorimetry

There are very few disadvantages. Bomb calorimetry of the initial and final states may not be feasible due to only small changes in biomass during the experiments, and rate determinations are not possible by this method. Otherwise, if the equipment is available, indirect calorimetry is the method of choice.

5.3. Non-growing cell suspension calorimetry

This includes cells isolated from tissues.

For the most part, it is direct methods that appear to have been used for studies with suspensions of microorganisms, liquid tissues, or animal cell suspensions. This is similar to tissue calorimetry except that the complexity of the system is further reduced. What is measures or determined is heat loss per unit mass of cells (wet or dry) per unit time. Typically, a substrate is provided but no source of nitrogen, and the heat loss is therefore largely catabolic. Because the cells are suspended and not in clumps as they would be in tissues, oxygen can be provided uniformly to all cells for aerobic studies. For anaerobic studies oxygen can be replaced with an inert gas. Studies of this kind were done early in the development of calorimetry, as in the studies published in 1904 by Rubner [40] on alcoholic fermentation using non-growing suspensions of yeast cells as being most readily available. The heat production of the semen and of the eggs of a sea urchin was studied in 1911 by Meyerhof [41], who also studied the heat production of erythrocytes [42] and bacteria [43]. Calorimetric studies of this kind using non-growing suspensions of cells were almost at the same time replaced by studies involving cells suspended in media of the kind in which they were almost surely growing. Such studies have been revived in recent years with cells that are readily available. For example, Monti [44] has published calorimetric studies of lymphocytes and hybridoma cells, and Monti et al. [45] on blood compatibility of hemodialysis membranes. Clark et al. [46] have

studied hepatocyte thermogenesis. Other studies, such as that by Yamamoto and Aki [47] have made use of washed erythrocytes in drug-induced hemolysis. The use of washed cell suspensions in pharmaceutical calorimetry has been reviewed selectively by Buckton et al. [48].

Advantages of direct calorimetry

The ability to determine total heat loss as a result of the oxidation or fermentation of a substrate is an advantage, provided that the cells are poisoned with an uncoupling agent such as dinitrophenol. Also, the ability to determine, by difference between the heat expected from the total catabolism of the substrate and that actually observed, the quantity of substrate assimilated because of oxidative or fermentative assimilation is an advantage. There are also advantages to being able to determine the effects of drugs on the rates of utilization of substrates.

Disadvantages of direct calorimetry

There are none for the purposes intended.

Advantages of indirect calorimetry

The quantity of oxygen consumed as a result of the oxidation of a given quantity of substrate can be measured. This can be related to the quantity of heat loss. Provided that appropriate $\Delta_f H^{\ominus\prime}$ values are used, no corrections are needed. Rates of heat loss are less easily determined because they are a function of the rate of oxygen consumption.

Disadvantages of indirect calorimetry

There are none for the purposes intended.

6. Metabolic calorimetry

This type of calorimetry includes direct or indirect calorimetric methods by means of which measurements or determinations are made of the heat loss resulting from metabolism. By this definition both anabolism (increase in biomass, or growth) and catabolism are processes that are included within the calorimetric system.

6.1. Whole body metabolic calorimetry

The implication in this category is that the organism being studied can be demonstrated to be growing actively during the time period of the calorimetry. This also implies an ability to separate anabolism from catabolism. Growth in higher organisms is most easily demonstrable in the early stages of development, which would include newborn organisms as studied by Sauer and Visser [49], and germinating seeds as studied by Prat [50]. In either of these two examples the organisms studied are not actually fed during the course of an experiment, the source of energy being endogenous in the form of stored materials: fat and glycogen

in animals, and fat and starch in germinating seeds. Both direct and indirect calorimetric methods can be used. Aspects of plant whole body metabolic calorimetry are covered by Criddle et al. [31].

Advantages of direct calorimetry

These are the same as for whole body catabolic calorimetry.

Disadvantages of direct calorimetry

The observed heat loss may not be very meaningful by itself except in that for growing organisms per unit mass it will be less than for catabolizing organisms. Anabolism is carefully considered in only a few instances in the literature for this category of calorimetry, the paper by Sauer and Visser [49] being one of the exceptions.

Advantages of indirect calorimetry

These are the same as for whole body catabolic calorimetry.

Disadvantages of indirect calorimetry

These are the same as for whole body catabolic calorimetry.

6.2. Tissue culture calorimetry

This category merges indistinctly with the following category in that tissue culture is more or less indistinguishable from cell culture calorimetry.

6.3. Cellular culture calorimetry

This kind of calorimetry comprises direct or indirect methods in which the sample consists of an actively growing suspension of cells. The initial state of a system is that existing before growth begins. The final state is that when growth has ceased as demonstrated by various criteria, following inoculation with growing cells. What is measured or determined is the quantity of heat loss per unit mass of substrate consumed.

Direct methods include non-differential (one vessel) or differential calorimetry (an experimental plus a control vessel) in which cells are grown inside the calorimeter vessel in an appropriate medium. What is measured is heat loss as a function of time if growth is exponential, heat loss per unit mass per unit time if growth is arithmetic, or some measure in between.

There are several indirect methods. One of these is differential bomb calorimetry of the initial and final states of the system, as introduced by Rubner [40], the difference being the heat of growth. Alternatively, the heat of combustion of a unit mass of cells can be determined by bomb calorimetry of the dried cellular substance produced during the growth process, making appropriate corrections for ash. It is assumed that the heat of hydration of this biomass is negligible. Data have to be known as to the kind and quantity of other organic products of the growth process

and their aqueous heats of combustion must be calculated or determined. The heat of combustion of the cells plus those of the aqueous heats of combustion of organic products is then substracted from the aqueous heat of combustion of the substrate to give the heat of the growth process. A second kind of indirect method is to measure the quantity of oxygen consumed by a system, followed by a calculation of the heat of combustion in aqueous solution of the corresponding quantity of substrate. This gives the heat accompanying catabolism. In this method it is assumed that the heat of anabolism is negligible. A third method is to establish the chemical nature and quantities of the substances comprising the initial and final states of a system. With these data an equation can be written representing the growth process. If the $\Delta_f H^{\ominus\prime}$ values for the reactants and products are known (usually by previous bomb calorimetric experiments and solution calorimetry), the heat of growth can be calculated with quite reasonable accuracy. Calorimetric studies of heat flux in animal cells have been reviewed by Kemp [51, 52]. The historical aspects of microbial thermogenesis have been reviewed, by Battley [53], and recent developments in the same subject by Gustafsson [54]. The use of bench-scale calorimetry of cell cultures in biotechnology has been reviewed by Zentgraf [55], and that of large-scale calorimetry of cell cultures by van Stockar and Marison [56].

Advantages of direct cellular culture calorimetry

None, unless enough is known about the system to be able adequately to correct the observed heat of growth.

Disadvantages of direct cellular culture calorimetry

These are the long experimental runs required.

Advantages of indirect cellular culture calorimetry

If the appropriate data are known, the calculated heat of growth is the true heat of growth. And, in general, to correct a direct measurement these data would have to be known anyway.

Disadvantages of indirect cellular calorimetry

It is necessary to determine a $\Delta_f H$ value for one unit formula weight of cells, even if $\Delta_f H^{\ominus\prime}$ values for the other products and for the reactants are known.

6.4. Ecological calorimetry

This is a recently developed field of calorimetry that seems to comprise everything that cannot be otherwise categorized. As with most other categories, both direct and indirect calorimetry can be used. About all that can be done is to examine discrete parts of a given ecosystem, the limiting boundaries of which are up to the experimenter. For direct calorimetry, parts of an ecosystem are studied that are small enough to be placed in a calorimeter. What is measured is the quantity or rate of heat loss. Bomb calorimetry can be used to determine the total quantity of

organic substance present in the initial and final states. Alternatively, part of an ecosystem can be studied using indirect calorimetry by measuring the rate at which O_2 is consumed and CO_2 is produced. The field of ecological calorimetry has been recently reviewed by Reh [57]. An interesting study on what might be called population ecological microcalorimetry has been made by Schutze-Motel [58] on heat loss and thermoregulation in a nest of *Bombus lapidarius*. Drong and Lamprecht [59] have carried out toxicological studies of energy flows in ecological systems.

Advantages of direct calorimetry

In some respects, the calorimetry involved is not dissimilar to whole body calorimetry in that the ecological sample can constitute a "whole body". Direct calorimetry can be used to advantage in making comparative measurements of the rates of heat loss from different parts of ecosystems, or in studying the effects of chemical or physical perturbances on such systems.

Disadvantages of direct calorimetry

As in whole body direct calorimetry, from the measurements alone we do not know what is causing the heat loss that is measured. Similarly, in the direct calorimetry of a part of an ecosystem in a calorimeter, it is not known from the measurements what organisms are causing the heat loss and to what extent. It would be impossible to correct the observed heat loss to obtain the true heat loss because the calormetric system is so undefined.

Advantages of indirect calorimetry

An ability to get an RQ may give some idea as to the general nature of the oxidations taking place within the calorimetric system. Using certain assumptions, it may be possible to deduce how much biomass is present per unit mass or volume of ecosystem (for example, soil).

Disadvantages of indirect calorimetry

It is probable that the results of indirect calorimetry would have to be verified by direct calorimetry, because of the necessity of making various assumptions in order to use the data.

7. Conclusions

It is apparent that for some calorimetric determinations, such as "no-reaction calorimetry", direct calorimetric methods are the only ones possible. For reaction calorimetry measurements, given that the same system can be studied by either direct or indirect methods, the relative advantages of accuracy, simplicity, and convenience of experimentation for either method have to be considered. If it is only needed to know that something is going on, or how fast it is going on, direct calorimetry is preferable in that there is only a qualitative heat loss to be measured.

If quantitive heat losses are to be measured, direct calorimetry is to be preferred, provided that the observed heat loss can be corrected to give the true heat loss. Otherwise, calorimeters are expensive, whereas indirect methods may be considerably less so. And, direct calorimetry is sometimes uncertain and tedious, when appropriate corrections cannot be made or the heat losses accounted for. A good example is flow calorimetry, wherein the change in the environment of the cells as they pass through the flow tubes must be carefully considered. If an explanation needs to be known as to the reason for the heat loss, or if the quantity or rate of heat loss needs to be related to something, then indirect calorimetry may be preferable. This is because the data needed for indirect calorimetry may also be needed to correct the observed heat losses from direct calorimetry. Although it requires much more effort, if the accuracy needed requires this, it may be preferable to use indirect calorimetry to determine what the heat loss should be, and then to confirm this with direct calorimetry, making the appropriate corrections to the observed heat loss.

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