CXXXV. A DIFFERENTIAL RESISTANCE THERMOMETER FOR BIOLOGICAL USE¹.

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THE need has arisen for a micro-calorimeter suitable for the measurement of heat-production in biological material dissolved or suspended in a small amount of liquid. With this object in view a differential resistance thermometer of the type used by Watson and Henderson [1928] to measure the heating effects of thorium products has been constructed. The following is a description of an apparatus designed in order to measure the heat produced by about 1 g. of resting anaerobic muscle in about 2 cc. of Ringer's solution. The details of the arrangements could readily be altered and made suitable for many other purposes.

Principle of the method.

The resistance of a metal to an electric current varies as the temperature of the metal is altered. *A*, *B*, *C* and *D* in Fig. 1 are the resistances of the arms of

Fig. 1.

a Wheatstone bridge. When the relationship $AC = BD$ holds good, no current passes through the galvanometer *G*. If now the opposite arms *B* and *D* be heated, their resistance will alter and the bridge will no longer be balanced. This change in balance is the basis of the method. It is not measured directly

¹ A brief account of this was given before the Biochemical Society, but a more detailed description seems desirable.

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by galvanometer deflection, but the bridge is re-balanced by means of an external variable resistance in parallel with one of the other two arms. The value of this compensating resistance which is necessary to restore the equilibrium of the bridge gives a measure of the heat which has been produced.

Details of arrangement.

In the actual apparatus (Fig. 2) each of the four arms of the Wheatstone bridge consists of No. 47 s.w.g. double silk-covered copper wire, having a resistance of about 200 ohms.

Fig. 2.

Arms *A* and *C* are wound on a silver-plated copper tube 7 cm. long and 1 cm. in diameter, with walls 0.15 mm. thick. By means of rubber bands and silk threads (1) this is mounted upon glass tubes (2) which carry the leads from the coils through a rubber stopper (3). This is then placed in a paraffined glass test-tube (4) which is enveloped in a portion of the inner tube of a bicycle tyre (5). Through the rubber stopper a glass tube (6) passes, whose diameter is slightly greater than that of the metal tube.

Arms *B* and *D* of the bridge are arranged in exactly the same manner in another similar paraffined tube. Both of the large test-tubes are sealed into a cork (7) which fits into a silvered Dewar flask (8) 5" in diameter and 10" deep. Passing through this cork into the interior of the Dewar flask are a high-sensitivity mercury thermometer (9) and two glass tubes (10).

Great care is taken to ensure that all the joints between test-tubes and cork and cork and Dewar flask are completely watertight, and this is especially important with regard to the fitting of the rubber stoppers into the test-tubes. The presence of the merest traces of water inside the tubes is sufficient to render the apparatus useless.

The space between the corks and the rim of the Dewar flask is filled with paraffin wax of melting-point 50° . In this paraffin are embedded the soldered connections completing the Wheatstone bridge. The four leads from the bridge are taken off through a glass tube (11) which is also embedded in the paraffin. The Dewar flask with its contents is then placed in paraffin wax in a cylindrical zinc container, and the whole is immersed in a constant temperature water-bath so that the only portions protruding from the water are the glass tubes (6) by means of which material can be lowered into the metal tubes; the glass tubes (10) by which water of the desired temperature can be introduced into the flask; and the glass tube (11) through which the copper leads pass.

The arrangement of the electrical connections to the four resistances A , B , C and D is shown in Fig. 3. As already indicated, the resistances are soldered together in the form of a Wheatstone bridge. Two leads go to a 2 v. accumulator (Ac); the circuit can be opened or closed by the switch (S_1). One accumulator lead passes through a milliammeter (MA), and the other through a variable resistance (R_1) by means of which the current through the bridge can be regulated accurately. The two galvanometer leads pass through a reversing switch (S_2). One of them also goes through a copper tap key (T) which when closed connects the galvanometer to the bridge circuit, and when open completes the circuit of a galvanometer damping resistance (R_2). Another variable resistance (R_3) is a shunt resistance in parallel with resistance C . The resistances A , B , C and D are made of such magnitude that when a current is passing through the bridge and the shunt resistance is set at about 9000 ohms, no current goes through the galvanometer. This variable resistance (R_3) is capable of accurate adjustment to 0.1 ohm.

The instrument is calibrated by the production of a known amount of heat, for which purpose a small heating coil is constructed and mounted as shown in Fig. 4. The coil (H) is made of cotton-covered constantan wire of 46 s.w.g., and has a total resistance of 32.10 ohms. It is sealed into a small glass bulb (Z), through the capillary neck of which pass leads of No. 47 s.w.g. copper which are soldered to the heating coil. On emerging from the capillary, these leads are in turn soldered to 24 s.w.g. copper wires passing through a rubber bung, by which the weight of the whole contrivance is supported. The capillary and bulb are attached to another rubber stopper which is of such a size as to fit exactly into a small glass tube (V), which in turn slips into the metal tube around which the resistances B and D are wound. The tube (V) and its contents can be lowered through the glass tube (6) in Fig. 2.

A duplicate heating coil *etc.* is made for the other metal tube. The function

of this duplicate coil is merely that of keeping the heat capacity of both halves of the Wheatstone bridge the same, and for this reason it is not necessary that it be constructed with such care. The copper leads from the duplicate are not attached to anything.

Fig. 3.

Fig. 4.

The connections to the heating coil are made as shown in Fig. 5. H is the heating coil, and L_1 its leads of 47 s.w.g. copper. R is a constantan resistance equal to the coil resistance, and L_2 are dummy leads of No. 47 s.w.g. copper, whose resistance is the same as that of L_1 . These dummy leads in the actual apparatus are enclosed in the bulb and capillary together with the heating coil and its leads. P is a potentiometer, S a copper switch, W a slide-wire, and Ac a 2 v. accumulator. The position of the sliding contact T on the wire W governs the amount of current passing through H .

When points 1 and 2 on the switch are connected to points 3 and 4 respectively a current passes through H , and the amount of this current is known

by measuring the potential difference between the ends of the resistance. The measurement is made on the potentiometer, which in this position of the switch is connected to the heating coil. If points 1 and 2 are connected to points 5 and 6, the potentiometer is no longer connected to the heating coil. The

Fig. 5.

current from the slide-wire passes, under these circumstances, through an external resistance R equal to H , and then through the dummy leads L_2 ; it does not, however, actually pass through the heating element H .

The galvanometer in use is a moving-coil instrument made by the Cambridge Instrument Company. It has an internal resistance of 14.6 ohms, and at 1 m. scale distance gives a deflection of 600 mm. per milliamp. It can be read to 0.2 mm. at a scale distance of 2 m.

Constant temperature water-bath. The bath is essentially the same as that used by Meyerhof. It has been described by Blaschko [1928]. Small alterations have been described by Meyerhof, McCullagh and Schulz [1930]. The temperature of the bath is constant to within 0.002° .

Procedure.

Calibration. The current across the bridge is adjusted to 4 milliamp. and the temperature of the water in the Dewar flask brought to equilibrium with that in the bath by passing water through the glass tubes (10, Fig. 2).

When a large series of experiments is being carried out over a period of several days, the bridge current and the automatic thermoregulator are permitted to run continuously. This results in a constant zero, *i.e.* the amount of resistance in the shunt required to balance the bridge is in this way kept at the same value.

Although the whole system is made of copper, certain unavoidable thermoelectric effects arise. These are eliminated by averaging the readings obtained when the galvanometer current is direct and when it is reversed.

3 cc. of water are placed in each of the two small glass tubes *V* (see Fig. 4 and text). The heating coil and the duplicate are placed firmly into the tubes, which by means of the leads are then lowered through the glass tubes ((6), Fig. 2) into their positions in proximity to the resistances *A* and *C*, and *B* and *D*, of the Wheatstone bridge. When the system has attained thermal equilibrium, as indicated by the amount of shunt resistance required to balance the bridge remaining constant, the value of this resistance is noted. This gives the zero point. It is affected very slightly by changes in room temperature; the error due to this is constant and is allowed for. A current is now passed through the heating coil (*H*, Figs. 4 and 5) and the potential difference between the ends of *H* is determined.

From the two equations

$$i = E/R \quad \text{and} \quad C = 0.239 i^2 R t,$$

where *i* = current in ampères, *E* = potential difference in volts, *R* = resistance in ohms, *t* = time in seconds, and *C* = heat in gram calories during time *t*, we obtain

$$C = \frac{0.239 E^2 t}{R}.$$

In the case of the apparatus being described the resistance of the heating coil is 32.10 ohms, and we wish to express the heat as gram calories per hour, hence

$$\begin{aligned} \text{Gram calories per hour} &= \frac{0.239 \times 3600 \times E^2}{32.10} \\ &= 26.8 E^2. \end{aligned}$$

Thus, if a current is passing through the heating coil and the potentiometer reading is 0.100, the heat production is $26.8 \times (0.1)^2$, or 0.268 g. cal./hour.

After the heating current has been passed for about 30 minutes the system comes to an equilibrium, as the heat loss is equal to the heat production. Two arms of the bridge are now at a higher temperature than the other two arms and for this reason the shunt resistance will have to be adjusted to a new value in order to bring the galvanometer to zero. In this manner the amount of resistance required in the shunt to balance the bridge can be ascertained for any heat production.

The heat produced by a known weight of muscle can now be measured very rapidly. The glass tube containing the heating coil is withdrawn, and the water is removed and replaced by the muscle, the weight of which should be of the order of 1 g. Ringer's solution is added in such quantity as will bring the heat capacity to its original value. (The specific heat of the muscle is about 0.825, and that of Ringer's solution about 0.995.) The heating coil is replaced, and the whole, after being cooled to a temperature rather below that of the bath, is again lowered into the apparatus. The attainment of the equilibrium condition is hastened by the use of the heating coil until the bridge is obviously approaching its state of balance. When the balance is very nearly reached the switch (*S*, Fig. 5) is put in the position where points (1) and (2) are in contact with points (5) and (6). The sliding contact *T* is then adjusted to the position it would occupy if the amount of heat being produced by the muscle were being produced by the heating coil. This results in the passage of a current through the dummy leads of the coil, of the same magnitude as that which passes through the actual leads during calibration. Thus the heat production in the leads need receive no other consideration.

The heat production of the muscle is known from the amount of resistance necessary in the shunt to balance the bridge after complete equilibrium has been established.

In some cases it is simpler to calibrate the apparatus after the experiment. If the muscle is not required for subsequent chemical measurements it can be killed by placing the small tube containing it in boiling water. The tube and contents are then cooled, dried with ether and replaced in the apparatus. After complete equilibrium has again been reached the changes in resistance which occurred during the experiment are reproduced by passing a suitable current through the heating coil.

Sensitivity. A heat production in 3 cc. of water of 1 cal. per hour results in the shunt resistance having to be changed 145.2 ohms in order to balance the bridge. The bridge can be balanced accurately to 0.1 ohm. It can be shown mathematically that 0.1 ohm. represents a temperature change of about $3^\circ \times 10^{-5}$. By increasing the bridge current or amplifying the galvanometer current the sensitivity could be greatly increased.

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