XXXVII. AN ISOTHERMAL CALORIMETER[1].

BY HEMMIGE BILIGIRIRANGACHAR SREERANGACHAR
AND MOTNAHALLI SREENIVASAYA.

From the Department of Biochemistry, Indian Institute of Science, Bangalore.

(*Received November 26th, 1934.*)

AMONG the existing isothermal calorimeters for the measurement of the heat liberated during reaction, dilution or solution are the compensation device of Wasserman [1930], where the heat liberated is balanced by the cooling caused by diluting a concentrated solution of ammonium nitrate, and the isothermal calorimeter of Hirobe [1926] in which the temperature change is prevented by adding simultaneously with the progress of the reaction, the necessary quantity of an indifferent substance at a different temperature. Coon and Daniels [1933] recommend a calorimeter particularly suitable for measuring the heat changes accompanying prolonged reactions like the hydrolysis of ethyl acetate and the fermentation of yeast.

While the above instruments are cumbersome and require skilled and elaborate manipulation, the original ice calorimeter of Bunsen [1870] remains one of the most simple and successful devices in the field. For biochemical reactions Bunsen's calorimeter cannot be employed since the reactions proceed extremely slowly at 0°, but if ice could be replaced by a substance melting at 25–30° the instrument might find extended application in biochemistry. The substance chosen should satisfy the following requirements: (1) a reproducible and sharp melting-point, (2) stability at the temperature of melting without any decomposition on repeated meltings and coolings, (3) indifference towards the mercury used in the capillary, (4) appreciable volume change during the melting of the solid and (5) low latent heat of fusion contributing towards a greater sensitivity of the instrument.

Diphenylmethane, which has been tried in our present study, meets most of these requirements, in that it is a stable hydrocarbon melting at 24·5° and has a latent heat of fusion of 26 cal./g. *p*-Bromotoluene (M.P. 28·5°), *tert*butyl alcohol (M.P. 25°), *iso*quinoline (M.P. 24·5°) and diphenylether (M.P. 28°) are suggested as possible alternatives.

The instrument and its manipulation.

The instrument (Fig. 1*a*) (kindly made to our design by Messrs Greiner and Freidrichs) consists of a reaction tube *A* surrounded by a bulb *B* which, in turn, is thermally insulated by a vacuum jacket *C*. The tube *D* leading to the bottom

---

[1] A preliminary report of this paper was presented before the Joint Session of the Society of Biological Chemists, India, and the Association of Economic Biologists held at Coimbatore in October 1932 (*Proc. Soc. Biol. Chemists, India*, 1932, **18**). It formed part of a thesis submitted by one of the authors (H. B. S.) for the Associateship of the Indian Institute of Science, Bangalore. While this paper was being prepared for publication, there appeared a note on this subject by Schukarew *et al.* [1934].

of the bulb B, communicates with the capillary L through the standard ground glass joint J. The capillary tube leading out of the apparatus is bent at right angles towards its extreme end and can be made whenever necessary to dip into mercury contained in a crucible.

The bulb B is evacuated and filled with diphenylmethane. The tube D is then filled with mercury and the capillary inserted at the ground joint when the column of mercury fills the capillary. When the instrument is not in use the capillary end is kept dipping into mercury so that changes in surrounding temperature will not effect a break in the mercury column and necessitate refilling of the apparatus.

The instrument after filling is placed in a Dewar flask containing melting diphenylmethane. Initially part of the liquid inside the calorimeter is frozen by evaporating dry ether in the reaction tube A, by means of a current of dry air. The calorimeter is then allowed to remain in the Dewar flask containing melting diphenylmethane for 48–72 hours so as to attain thermal equilibrium with the surroundings.

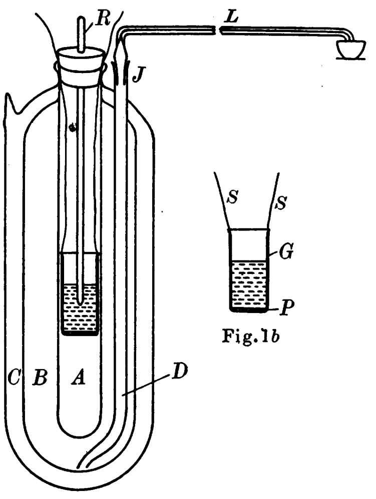

Fig. 1b

Fig. 1a

Experimental procedure.

Before the commencement of an experiment, 1 ml. of pure dry ether is introduced into the reaction tube and evaporated by a current of dry filtered air. By this means, a uniform incrustation of solid diphenylmethane is formed on the outer walls of the reaction tube. The mercury meniscus in the capillary can be adjusted to any desired position by withdrawing the mercury cup at the end of the capillary and continuing the operation of cooling. The interior of the reaction tube is then dried by means of a swab of clean cotton wool.

The "housing" of the reagents. The "housing" of the two reacting liquids separately in the calorimeter offered considerable difficulty. Before mixing they should be kept separate inside the calorimeter and allowed to attain the temperature of the melting diphenylmethane. The mixing operation itself should not involve any disturbance in the thermal equilibrium of the whole system. After several trials, the arrangement illustrated in Fig. 1*b* was found to be satisfactory. One of the solutions is introduced directly into the reaction tube while the other is contained in a separate small glass tube G closed at one end with a thin layer of paraffin wax P and suspended in the reaction tube by means of a non-conducting string S of cotton. A glass rod R which passes through the cork closing the reaction tube dips into the liquid contained in G. The projection of the glass rod over the cork is covered with felt to minimise transference of heat from outside to inside. After the calorimeter has attained thermal equilibrium as shown by the steadiness of the creep of the mercury column, the glass rod is gently pushed in, breaking the paraffin bottom of the glass tube G and effecting a mixture of the two liquids. That the operation of mixing itself does not lead to any detectable disturbance in the thermal equilibrium of the calorimeter can be shown by mixing distilled water in the reaction tube with water in the suspended glass tube; the position of the mercury column remains undisturbed.

The heat of reaction produced is measured by the shift in the mercury meniscus along the capillary. Before an experiment is begun, the rate of flow of mercury is determined. When this is minimum and fairly steady, the liquids are mixed and measurements continued. The shift of mercury column due to the heat of reaction is obtained by allowing for the already determined creep.

Fig. 2 gives a graphical representation of the course of heat evolution during a few typical experiments. Curve *A* is characteristic of all reactions like the neutralisation of HCl with NaOH wherein the heat liberation is instantaneous.

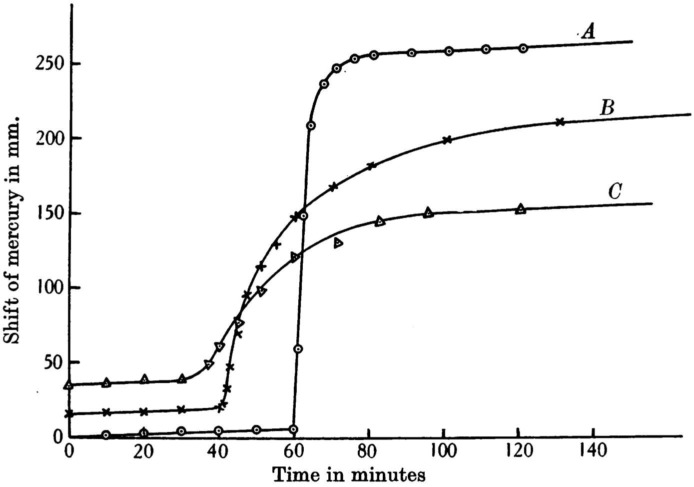

Fig. 2. *A*, neutralisation of HCl with NaOH. *B*, *C*, quinol-peroxidase system (two concentrations of substrate.)

B and *C* represent the curves characteristic of reactions, particularly enzymic reactions, where heat generation is gradual. In all three cases there is first a flat portion of the curve corresponding to a uniform change in the initial conditions of the calorimeter, then either a steep or a gradual rise corresponding to either a rapid or slow liberation of heat due to reaction and finally again, a flat portion showing the completion of the reaction and the attainment of a state of thermal equilibrium. By the use of a thermostat maintained at the melting temperature of diphenylmethane, more steady conditions could be obtained in these various stages.

Calibration of the instrument.

The calorimeter is calibrated by conducting standard reactions which evolve known amounts of heat. Dilution of sulphuric acid, neutralisation of hydrochloric acid with sodium hydroxide and double decomposition of barium chloride and sodium sulphate were all carried out in the calorimeter and the results obtained are given in Table I. The heats liberated in each case have been calculated from the values given in Standard Physical Tables.

It will be observed from the results in Table I that the calorimeter gives reliable and concordant results as tested by three independent reactions. The average quantity of heat corresponding to 1 mm. shift of mercury column is 0·063 cals.

Table I.

Reaction	Mercury shift mm.	Heat liberated cals.	Heat liberated per mm. shift of mercury cals.
1. Dilution of 49 % sulphuric acid	153		0·063
2 ml. of acid + 1 ml. water	155	9·58	0·062
	154		0·062
Mean	154		0·062
2. Neutralisation of HCl with NaOH	254		0·066
2 ml. 0·62N acid +	252	16·76	0·067
2 ml. 0·65N alkali	253		0·066
Mean	253		0·066
3. Double decomposition of $BaCl_2$ and Na_2SO_4	25		0·059
2 ml. 2 % Na_2SO_4 +	25	1·48	0·060
2 ml. 3 % $BaCl_2$	24		0·061
Mean	25		0·060

Application to biochemical reactions.

The heat changes accompanying the enzymic hydrolysis of a few substrates, urea, sucrose, amygdalin and quinol, have been investigated in the calorimeter. While the amounts of heat in the cases of urease-urea, invertase-sucrose and emulsin-amygdalin systems, as determined by our present calorimeter are too small to be accurately estimated, the instrument has shown itself suitable for a study of the peroxidase-quinol system (Table II).

Table II. *Peroxidase-quinol.*

Quinol mg.	Quantity of enzyme	Shift of mercury column mm.	Heat liberated per g. of substrate cals.
91·0	2 ml. potato extract + 1 ml. 1 % H_2O_2	93·0	64·4
81·6	,,	90·5	70·4
80·2	,,	89·0	69·8

The mean value obtained for the liberation of heat by the peroxidase-quinol system is 68·2 cals. per g. of quinol. In the case of the other three systems, urease-urea, emulsin-amygdalin and invertase-sucrose, the heat changes involved have been found to be too small to be measured with adequate accuracy. If, for example, the value for sucrose hydrolysis be accepted as 11·21 cals. per g. of sucrose, as determined by Brown and Pickering [1897] and Dixon and Ball [1920], then the expected value under the conditions of our experiment should correspond to a shift of 9 mm. in the mercury column which can be detected by the present calorimeter but not measured with sufficient accuracy. Obviously, those reactions which are accompanied by heat changes corresponding to shifts of less than 15–20 mm. of mercury cannot be investigated by the instrument in its present form. By employing a capillary with a smaller bore, the sensitivity of the calorimeter can be increased and reactions involving smaller heat changes studied. An attempt in this direction is being made. The calorimeter is now being employed for measuring the heat liberated during the respiration of small insects, germination of seeds and a few typical fermentations.

SUMMARY.

1. An isothermal calorimeter using diphenylmethane, which is very simple in operation, is described.

2. The calorimeter has been calibrated with the help of the following standard reactions: (*a*) dilution of sulphuric acid, (*b*) neutralisation of HCl with NaOH, and (*c*) double decomposition of Na_2SO_4 and $BaCl_2$.

3. The heat changes accompanying a few enzymic reactions have been investigated; the peroxidase-quinol system yields an average value of 68·2 cals. per g. of substrate.

4. The possibility of employing the calorimeter for the investigation of other biological reactions, *e.g.* respiration, germination and fermentation, is indicated.

In conclusion, we wish to express our grateful thanks to Mr G. Gundu Rao, Chemist, Mysore Sugar Factory, for many helpful suggestions in the course of the work and to Dr M. A. Govinda Rau, Department of Physics, Indian Institute of Science, for his constructive criticisms in the preparation of the paper.

REFERENCES.

Brown and Pickering (1897). *J. Chem. Soc.* **71**, 783.
Bunsen (1870). *Poggendorf's Ann.* **141**, 1.
Coon and Daniels (1933). *J. Physical Chem.* **37**, 1.
Dixon and Ball (1920). *Sci. Proc. Roy. Dub. Soc.* **16**, 153.
Hirobe (1926). *J. Faculty Sci. Imp. Univ. Tokyo*, **1**, 155.
Schukarew, Krivobabko and Schukarewa (1934). *Physik. Z. Sowjetunion*, **5**, 722.
Wasserman (1930). *Z. physikal. Chem.* **146**, 409.