pipette, and after addition of sucrose (75 mg), carrier KI (0.1 mg) and a trace of bromophenol blue, the filtrate was layered onto a column (2.8 × 62 cm) of Sephadex G-100[6] and eluted with 0.1 m*M* ethylenediamine tetra-acetic acid in 1 m*M* potassium phosphate, pH 7.5. Radioactivity in the collected fractions (2.7 ml) was determined using a well counter.

Results and discussion (Figure). The total radioactivity was almost equally distributed between the $F(ab')_2$ peak (48.9% in the volume 194 to 243 ml) and the peak corresponding to free iodide (45.0% in the volume 432 to 565 ml) with only a minor proportion being associated with polymerized (1.0% in the volume 170 to 194 ml) and degraded (5.1% in the volume 243 to 432 ml) protein material. The fractions eluting between 202 and 232 ml were collected as the pure [^{125}I]-labelled $F(ab')_2$ fragments; the yield was 8.7 mg protein (87%), and the specific radioactivity amounted to 45.4 μCi per mg.

In conclusion, insolubilized lactoperoxidase allows the [^{125}I]-labelling of proteins to high specific activity with results comparable to those reported for the soluble enzyme[1], but in contrast to the latter it offers the advantage of its rapid and effective separation from the labelled material. This ensures the accurate control of reaction times and facilitates the purification of the labelled product irrespective of its molecular weight. Although, conceivably, the enzyme could be re-used, this was never tried in view of the danger of cross-contamination and the low cost of preparation, which would not have warranted the effort associated with repurification[7].

Zusammenfassung. Antikörperfragmente wurden mittels trägergebundener Lactoperoxidase radioiodiert. Dies erleichterte die Abtrennung des Enzyms vom markierten Protein.

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The Measurement of the Costs of Maintenance in Terrestrial Poikilotherms: A Comparison Between Respirometry and Calorimetry

Short-lived terrestrial poikilotherms dissipate about 50% of the assimilated energy in life processes (MCNEILL and LAWTON[1]). Some importance, therefore, attaches to accurate measurement of metabolic costs when constructing energy budgets for such organisms. Conventionally they are estimated by determining the respiratory rate and then multiplying oxygen consumption by an oxycalorific equivalent, to express the value in units of energy. Many refined methods of great sensitivity and precision have been developed in recent years to meet the need for accurate determinations (PETRUSEWICZ and MACFADYEN[2]). Measurement of oxygen consumption requires absorption of the carbon dioxide evolved during respiration, generally by alkali, which, besides reducing the carbon dioxide level to zero, also has the effect of lowering the relative humidity. Thus the conditions within the respirometer will place many of the animals of interest to ecologists in a degree of physiological stress, with unknown consequences for the respiratory rate. Clearly, great advantages would stem from a direct method of estimating energy dissipation in more natural conditions and, since it is manifested as heat, calorimetry recommends itself, particularly as recent developments have produced instruments approaching respirometers in their sensitivity and precision. The most extensive calorimetric investigations of smaller poikilotherms, ranging in size from the fruit fly, *Drosophila*, to the cockroach, *Periplaneta americana* (L) (PRAT[3]) are essentially qualitative, since no correction was made for the heat absorbed in evaporating water from the experimental animals. As this can be a

[1] S. MCNEILL and J. H. LAWTON, Nature, Lond. *225*, 472 (1970).

[2] K. PETRUSEWICZ and A. MACFADYEN, *Productivity of Terrestrial Animals: Principles and Methods* (Blackwell Scientific Publications, Oxford/Edinburgh 1970), p. 112.

[3] H. PRAT, *Calorimetry of Higher Organisms in Biochemical Microcalorimetry* (Ed. H. D. BROWN; Academic Press, New York 1969), p. 181.

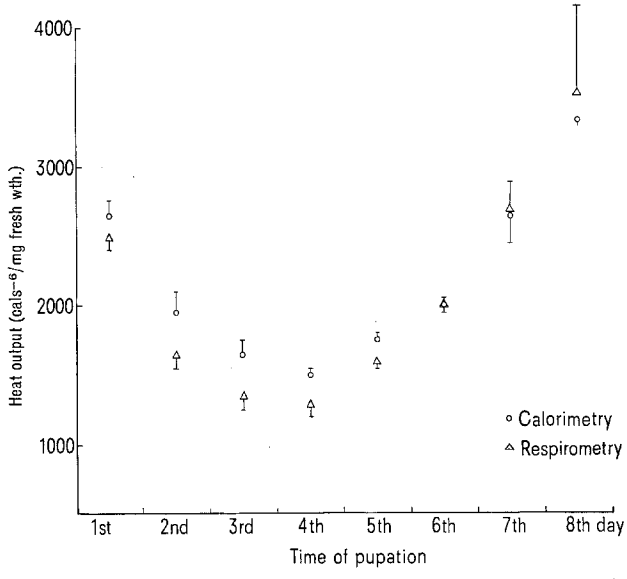
The metabolic energy costs of pupation in *Tenebrio molitor* L.

Age of pupae (days)	No. of readings	Heat output estimated by calorimetry (cals/mg FW[a]/h ± S.E.)	No. of readings	Heat output estimated by respirometry (cals/mg FW[a]/h ± S.E.)
0–1	6	0.002663 ± 0.000095	6	0.002482 ± 0.000070
1–2	4	0.001961 ± 0.000163	4	0.001630 ± 0.000085
2–3	6	0.001673 ± 0.000067	4	0.001370 ± 0.000100
3–4	7	0.001492 ± 0.000063	6	0.001318 ± 0.000104
4–5	6	0.001742 ± 0.000057	8	0.001586 ± 0.000043
5–6	5	0.001997 ± 0.000044	7	0.002024 ± 0.000084
6–7	6	0.002664 ± 0.000203	7	0.002689 ± 0.000179
7–8	2	0.003438 ± 0.000008	3	0.003537 ± 0.000616

[a] FW, fresh weight.

large proportion of the total heat produced, particularly in poikilotherms, it is clear that considerable error can be introduced into the measurements. Indeed, calorimetry has tended to be discounted as an alternative to respirometry for this reason (PETRUSEWICZ and MACFADYEN[2], SOUTHWOOD[4]).

This note is a preliminary account of a comparison between respirometry and calorimetry in measuring the metabolic energy costs of pupation in *Tenebrio molitor* L. The results are summarized in the Table, where the heat output estimated by calorimetry has been corrected for the heat absorbed by evaporation and the heat output estimated by respirometry has been arrived at by multiplying oxygen consumption by IVLEV's[5] oxycalorific equivalent. When heat output is plotted against time (Figure) the familiar U-shaped curve results, and, futhermore, it will be seen that the 2 methods produce very similar curves. Calorimetry apparently gives a slightly higher estimate for the first 5 days of pupation but respirometry does so in the last 3 days. However, at no time are these differences significant. It can be concluded, therefore, that, if proper allowance is made for the heat absorbed by evaporation, calorimetry provides as good a measure of the energy dissipated in metabolic processes as respirometry.



The metabolic energy costs of pupation in *Tenebrio molitor* L.: A comparison between calorimetry and respirometry.

These results can be interpreted in two ways. Firstly, they may be regarded as confirmation that respirometry is a satisfactory method, but it should be remembered that, while *T. molitor* pupae are well adapted to the conditions obtained in the respirometer, many poikilotherms are not and it would be unwise to assume that the results can be extended to cover them all. Alternatively, it can be argued that calorimetry is a viable method which, besides having the advantage of giving a direct measure of energy dissipation, is theoretically capable, unlike the majority of respirometric methods, of accommodating the experimental animals in an environment close to that in which they normally live. Since many of the poikilotherms of interest in production studies are found conditions of high humidity, avoiding the physiological stresses attendant on desiccation, establishment of natural conditions within the calorimeter will have the additional advantage of reducing the correction necessary for the latent heat of vaporization. The recently described modification to the LKB Flow Microcalorimeter (ERIKSSON and WADSÖ[6]), which allows of the oxygenation of the contents of the reaction vessel, suggests that it is not only theoretically, but also practically, possible to establish an atmosphere congenial to any experimental animal. For these reasons calorimetry deserves more favourable consideration as an alternative method for estimating metabolic costs.

A fuller account of these experiments and the methods employed will appear elsewhere[7].

Zusammenfassung. Durch Vergleich respirometrisch und kalorimetrisch bestimmter Daten über den Energieverbrauch während der Verpuppung von *Tenebrio molitor* wird gezeigt, dass die letztgenannte Methode der ersteren ebenbürtig ist, bei feuchtigkeitsliebenden Arten sogar überlegen sein müsste.

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[7] I am most grateful to LKB-Produkter AB., S-161125 Bromma 1, Sweden and their staff for the extended loan of a modified Batch Microcalorimeter.

The Influence of Hydrostatic Pressure on the Rate of Hydrolysis of Acetylcholine and Contractility in the Vagal Heart System

Previously we have shown that hydrostatic pressure blocks the vagal inhibition completely at 5,000 ψ[1]. Our value is considerably lower than that reported by CATTELL and EDWARDS[2], BROWN[3] and EDWARDS and BROWN[4] for inhibition of muscular contraction in the skeletal muscles. We further demonstrated that physostigmine, a specific acetylcholinesterase inhibitor, raises the blocking pressure from 5,000 to 6,500 ψ. We concluded that the effect of hydrostatic pressure on the vagal inhibition is primarily a conformational change of acetylcholinesterase molecule located at the post-junctional membrane.

Over the last decade we have examined rather extensively the activity of this particular in situ enzyme with respect to various physical[1,5,6] and chemical[7–9] parameters. This report is a further investigation on the rate of hydrolysis of acetylcholine by acetylcholinesterase and the extent of volume change in the enzyme molecule as a function of pressure.

Materials and methods. The experiments were performed on isolated vagal heart preparation of frogs, *Rana pipiens*, The heart was perfused with modified Ringer's solution, which has the following composition: NaCl,