hatched egg-shells yield little or no triene. This suggested that triene compounds were autoxidised during incubation and that the reaction was inhibited in the presence of porose area secretion. This was confirmed by comparing the stability of triene in the wax, with and without porose area secretion, under various other conditions (Table); the reaction induction periods[10] were significantly longer in those with secretion. The shorter induction periods found under laboratory bench conditions probably result from the influence of light as an initiator of autoxidation[11]. Typical spectra for the autoxidation are in the Figure; absorptions assigned to triene (294, 306, 320 nm) are initially eliminated followed by those in the 220–250 nm region. The removal of absorptions at 220–250 nm reveals that other compounds are also autoxidised, although not as readily as those containing the triene chromophore.

ATKINSON et al.[12,13] have recently shown that *cis, cis*-6, 9-heptacosadiene, the major hydrocarbon of the cuticular wax of the cockroach, *Periplaneta americana*, in contrast to its apparent stability when undisturbed on the cuticle, undergoes extreme autoxidative changes when isolated from the cuticle. On the cuticle the *o*-dihydric phenols involved in sclerotization effectively block such autoxidation. A study of the egg waxes of ixodid ticks has now yielded significant amounts of conjugated triene steroids also susceptible to autoxidation. We have shown that the cattle tick, *B. microplus*, inhibits autoxidation of these steroids and other compounds in its egg waxes by the incorporation of a natural antioxidant during deposition of the wax layer by Gené's organ. The secretion from the porose area is the source of that antioxidant.

Zusammenfassung. Es wird die sekretorische Natur der Area porosa in Ixodesarten bestätigt, jedoch nicht die Annahme, dass das Sekret als Schmiermittel für die normale Funktion des Genéschen Organes notwendig ist. Das Sekret wird offenbar in den wachsartigen Überzug des Eies aufgenommen und verhindert dort die Autoxydation der unbeständigen $\Delta^{2,4,6}$-trien-Steroide.

P. W. ATKINSON and K. C. BINNINGTON

CSIRO, Divison of Entomology, Long Pocket Laboratories, Indooroopilly (Queensland, Australia 4068),
21 December 1972.

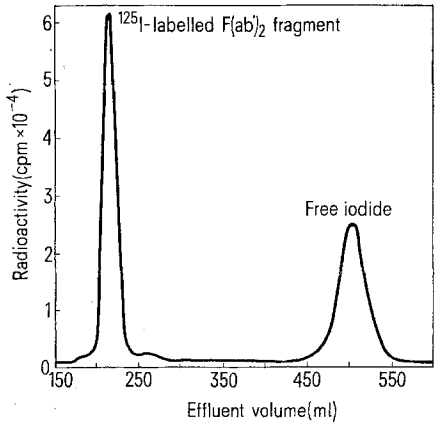
[10] J. BETTS, Q. Rev. chem. Soc. *25*, 265 (1971).
[11] A. POPOV and N. YANISHLIEVA, Izv. Otd. Khim. Nauki, Bulg. Akad. Nauk *2*, 369 (1969).
[12] P. W. ATKINSON, W. V. BROWN and A. R. GILBY, Insect Biochem., in press (1973).
[13] P. W. ATKINSON and A. R. GILBY, Science *168*, 992 (1970).

Insolubilized Lactoperoxidase for the [^{125}I]-Labelling of Proteins

The use of lactoperoxidase as a catalyst for iodination by hydrogen peroxide and carrier-free [^{125}I] iodide provides a mild system for labelling proteins to high specific activity with minimum denaturation[1]. Separation of soluble lactoperoxidase (mol. wt. 84,000) from some labelled protein of comparable molecular weight may become a problem, but is easily accomplished with an insolubilized enzyme. Here we present details for the preparation of such an enzyme derivative and describe as an example the labelling of antibody fragments of the type F(ab')$_2$ (mol.wt. 106,000).

Methods. Enzacryl AA[2] (50 mg) suspended in 2 *N* HCl (5 ml) was diazotized by the dropwise addition of 4% (w/v) $NaNO_2$ (2 ml) followed by further stirring for 15 min and centrifuging (all operations for enzyme coupling were carried out at 0°C). The pellet was washed with 4 portions (8 ml) of 0.05 *M* sodium phosphate, pH 7.4 and was resuspended in the same buffer (1 ml) containing lactoperoxidase[3] (1 mg). Coupling was allowed to proceed with stirring for 60 h. After centrifuging, residual diazonium groups were reacted with 0.01% (w/v) phenol in 10% (w/v) sodium acetate (8 ml) for 15 min. Unbound enzyme was removed by 6 washings (20 min each with vigorous stirring) with 0.5 *M* NaCl in 0.05 *M* sodium phosphate, pH 7.4 (6 ml) followed by 3 brief washings with 0.05 *M* sodium phosphate, pH 7.4. Finally, the orange-coloured derivative was suspended in the same buffer (1 ml).

The enzyme suspension (0.4 ml) was mixed with a preparation of F(ab')$_2$ fragments[4] (10 mg) in the same buffer (1 ml). The mixture was transferred to a disposable tube containing carrier-free Na [^{125}I] (2 mCi in 20 µl of water; concentration of iodide, 0.05–0.1 m*M*)[5]. To this was added 0.88 m*M* H_2O_2 (0.2 ml), and the suspension was stirred for 15 min at 22°C. The reaction mixture was filtered through cottonwool by aspiration with a Pasteur



Separation of the reaction products on Sephadex G-100 following [^{125}I]-labelling of F(ab')$_2$ antibody fragments using an insolubilized preparation of lactoperoxidase. For experimental details, see text.

[1] J. J. MARCHALONIS, Biochem. J. *113*, 299 (1969). – H. RAJANIEMI, P. TUOHIMAA and M. NIEMI, Histochemie *23*, 342 (1970). – J. I. THORELL and B. G. JOHANSSON, Biochim. biophys. Acta *251*, 363 (1971). – J. J. MARCHALONIS, R. E. CONE and V. SANTER, Biochem. J. *124*, 921 (1971).
[2] Enzacryl AA (Koch-Light Laboratories Ltd., Colnbrook, Bucks., England) is a copolyacrylamide containing aromatic amino groups. A booklet on enzyme insolubilization is available from the manufacturer (R. EPTON and T. H. THOMAS, An introduction to water-insoluble enzymes, 1971).
[3] Calbiochem, Löwengraben 14. CH–6000 Luzern 5.
[4] C. C. KUENZLE, R. R. PELLONI and M. H. WEIBEL, Experientia *28*, 1522 (1972).
[5] Eidg. Institut für Reaktorforschung, CH–5303 Würenlingen.

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.

C. C. KUENZLE and M. DÖBELI

Laboratory of Molecular Psychobiology, Department of Pharmacology and Biochemistry and Division of Reproductive Biology, Department of Physiology, School of Veterinary Medicine, University of Zürich, Winterthurerstrasse 260, CH-8057 Zürich (Switzerland)
27 December 1972.

[6] Pharmacia, Uppsala, Sweden.

[7] This work was supported by grant No. 3.709.72 (to C.C.K.) from the Schweizerische Nationalfonds zur Förderung der wissenschaftlichen Forschung.

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.