serum, which reaches a maximum in late summer[12] thereafter declining, to parallel closely the seasonal variation in fat metabolism.

γ-Aminobutyrylcholine, isolated from pig brain extracts[13], is a very poorly active cholinergic compound compared with ACh. It is not hydrolysed by AChE and is only poorly hydrolysed by PsChE. Its exact function is unknown but it is possible that it is a functionless by-product of the choline ester synthetic pathway.

J. W. Clitherow
M. Mitchard
N. J. Harper

School of Pharmacy,
Chelsea College of Science and Technology,
Manresa Road,
London, S.W.3.

[1] Hawkins, R. D., and Mendel, B., *Brit. J. Pharmacol.*, **2**, 173 (1947). Hawkins, R. D., and Gunter, J. M., *Biochem. J.*, **40**, 192 (1946). Mendel, B., and Myers, D. K., *Nature*, **176**, 783 (1955). Pittoni, A., and Pasetto, N., *Atti e mem. accad. patavina sci. lettere ed arti*, Pt. 2, **65**, 132 (1953). Myers, D. K., *Biochem. J.*, **55**, 67 (1953).
[2] Bergmann, F., and Wurzel, M., *Biochim. Biophys. Acta*, **13**, 251 (1954).
[3] Cullumbine, H., *Handbuch der Experimentellen Pharmakologie*, edit. by Eichler, O., and Farah, A., **15**, 505 (Springer-Verlag, Berlin, 1963).
[4] Karczmar, A. G., and Koppanyi, T., *J. Pharmacol.*, **116**, 245 (1956).
[5] Whittaker, V. P., *Physiol. Rev.*, **31**, 312 (1951).
[6] Burgen, A. S. V., and MacIntosh, F. C., *Neurochemistry*, edit. by Elliot, K. A. C., Page, I. H., and Quastel, J. H., 311 (C. C. Thomas, Springfield, Illinois, 1955).
[7] Whittaker, V. P., *Handbuch der Experimentellen Pharmakologie*, edit. by Eichler, O., and Farah, A., **15**, 1 (Springer-Verlag, Berlin, 1963).
[8] Koelle, G. B., *Handbuch der Experimentellen Pharmakologie*, edit. by Eichler, O., and Farah, A., **15**, 187 (Springer-Verlag, Berlin, 1963). Sawyer, C. H., and Everett, J. W., *Amer. J. Physiol.*, **148**, 675 (1947).
[9] Berry, J. F., and Whittaker, V. P., *Biochem. J.*, **73**, 447 (1959).
[10] Lynen, F., *Harvey Lectures*, **48**, 210 (New York, Academic Press, 1952–53); *Fed. Proc.*, **12**, 683 (1953). Lynen, F., and Ochoa, S., *Biochim. Biophys. Acta*, **12**, 299 (1953). Green, D. E., *Biol. Rev.*, **29**, 330 (1954).
[11] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, **79**, 255 (1933).
[12] Strelitz, F., *Biochem. J.*, **38**, 86 (1944).
[13] Holmstedt, B., and Sjöqvist, F., *Biochem. Pharmacol.*, **3**, 297 (1960). Kewitz, H., *Naturwiss.*, **46**, 495 (1959); *Arch. exp. Path. Pharmak.*, **237**, 308 (1959).

Laminarinases in Soil and Litter Invertebrates

Laminarinases, enzymes hydrolysing β-D-(1→3)-glucans, are widely distributed among micro-organisms[1–3]. Chesters and Bull[1,2] examined their occurrence in 160 fungi. Eighty per cent of the species showed laminarinase activity. Fifty-six per cent of them had laminarinases of the endohydrolytic type (yielding laminaridextrins as initial products), 25 per cent of the exohydrolytic type (yielding glucose as the sole initial product and only later laminaridextrins), and 19 per cent were intermediary, with both laminarinase types.

The presence of laminarinase in the digestive tract of animals has received little attention. Myers and Northcote[4] report a powerful laminarinase from *Helix pomatia*, and Bailey and Clarke[5] from *Entodinium* species of the sheep rumen.

Since, however, snails and slugs seem unique among soil and litter animals in possessing a very wide range of carbohydrases hydrolysing polysaccharides[6] a number of soil and litter invertebrates of widely different systematic position and feeding types were tested for laminarinase activity: Oligochaetes: *Cognettia sphagnetorum* (Vejd.), *Lumbricus rubellus* Hoffm., *L. terrestris* L.; Isopods: *Porcellio scaber* Latr.; Chilopods: *Lithobius forficatus* Koch; Diplopods: *Schizophyllum sabulosum* (L.), *Glomeris marginata* Koch; Insects: *Ectobius silvestris* (Poda), *Pterostichus niger* Fabr., *Philonthus* sp., *Phyllopertha horticola* L.; Gastropods: *Arion ater* L., *A. circumscriptus* Johnst.

Homogenates of guts and gut contents were extracted in phosphate–citric acid buffer, *p*H 4·95, and used as a source of enzymes, insoluble laminarin (Light and Co.) as a substratum. The products of hydrolysis were identified by paper chromatography.

All species showed very strong laminarinase activity yielding glucose as the main product of hydrolysis.

Two samples of laminarinase, associated with other enzymes, were obtained as an almost white powder by half-saturating the homogenates of *Arion circumstriptus* and *Porcellio scaber* with ammonium sulphate, centrifuging, dialysis against tap water, precipitation of enzymes by double the volume of acetone at 0° C, and redissolving the dried powder in water.

Dominant activities of the enzyme solution were laminarinase and β-glucosidase. Laminarinase activity extends over the *p*H-range 2·25–7·6 but is very weak below *p*H 3·0 and above 7·4. The activity curve is unimodal with a peak at *p*H 4·9–5·1 (*Arion circumscriptus*) or 5·2–5·7 (*Porcellio*). The dominant constituent of the laminarinase is of exohydrolytic type yielding large amounts of glucose and only traces of intermediary products (R_{gl} 0·81 and 0·43, descending chromatography, ethyl acetate/pyridine/water, 12 : 5 : 4).

The experiments suggest laminarinases to be widely distributed among soil and litter invertebrates.

C. Overgaard Nielsen

Molslaboratoriet,
Femmøller,
Denmark.

[1] Chesters, C. G. C., and Bull, A. T., *Biochem. J.*, **86**, 28 (1963).
[2] Chesters, C. G. C., and Bull, A. T., *Biochem. J.*, **86**, 31 (1963).
[3] Chesters, C. G. C., and Bull, A. T., *Biochem. J.*, **86**, 38 (1963).
[4] Myers, F. L., and Northcote, D. H., *J. Exp. Biol.*, **35**, 639 (1958).
[5] Bailey, R. W., and Clarke, R. T. J., *Nature*, **198**, 787 (1963).
[6] Nielsen, C. O., *Oikos*, **13**, 200 (1962).

Role of Catalase and Peroxidase in the Metabolism of Leucocytes

Leucocytes are known to be rich in catalase[1] and myeloperoxidase[1–3], yet very little is known concerning the role of these enzymes in leucocyte metabolism. In a recent investigation by Iyer *et al.*[4], evidence was presented for the formation of hydrogen peroxide by guinea pig exudate leucocytes. An obvious physiological function of catalase or peroxidase, therefore, might be to regulate the intracellular concentration of hydrogen peroxide which, if allowed to accumulate, could become toxic to the cells.

In order to assess the role of catalase in the metabolism of leucocytes a relatively specific inhibitor of catalase activity, 3-amino-1,2,4-triazole (AT), was used. This compound has been shown to inhibit the catalase activity of liver and kidney but not of the erythrocyte[5,6].

In the work to be presented here the effect of AT on leucocyte catalase *in vivo* and *in vitro* in comparison with other tissues was examined. An attempt was also made to ascertain a role of catalase in respiration and aerobic glucose utilization of leucocytes.

Male guinea pigs of *NIH* strain, weighing 350–500 g each, were used. Liver and kidney homogenates were prepared as described previously[7]. Erythrocytes were obtained by centrifuging heparinized blood at 100*g* for 15 min. After removal of the plasma and buffy layer, the erythrocytes were washed with saline to remove any traces of AT, and then lysed in 49 volumes of distilled water.

Suspensions rich in polymorphonuclear leucocytes (80 per cent) were obtained from peritoneal exudates of guinea pigs injected intraperitoneally with 10 ml. of a 12 per cent solution of sodium caseinate 16–18 h prior to killing, as described by Stahelin *et al.*[8]. The leucocytes were removed from the exudate by centrifugation at 250*g* for 5 min, washed once with 5 ml. 0·9 per cent saline, and finally suspended in 1 ml. of distilled water and homogenized by 50 passes in a glass-'Teflon' homogenizer with a motor-driven pestle. For enzymatic assays the