The Distribution of Esterase in the Nervous System and other Tissues of the Insect *Rhodnius prolixus*

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With one plate (fig. 1)

SUMMARY

Cholinesterase in *Rhodnius* is limited to the neuropile, the nerve-roots, and the larger nerves. None is present in the axons; it seems to be confined to the interneuronal cytoplasm, the product of the glial cells. The intensity of the reaction is greatest in the synaptic regions and appears to be proportional to the amount of interneuronal material.

The ganglion cells contain traces of a non-specific esterase; and larger amounts of non-specific esterase occur in the glial layer between the cells. A similar enzyme is plentiful within the perineurium cells.

Non-specific esterases occur in many other tissues: salivary glands and alimentary canal, pericardial cells, haemocytes, oenocytes, dermal glands and epidermal cells, germ-cells and fat-body. Esterase is absent from the muscle endplates. The cytoplasmic localization and the reaction of these enzymes to inhibitors are described.

In the fat-body, each droplet of fat has a single well-defined 'cap' of esterase, presumably lipase. It is suggested that this controls the transfer of triglycerides to and from the storage vacuoles. Esterase is not associated with the mitochondria; but there is evidence that the enzyme may be disposed as fine filaments, particularly over the surface of the nucleus. Some of these widely distributed 'esterases' may be cathepsins.

THE central nervous system of insects is rich in acetylcholine and cholinesterase (Corteggiani and Serfaty, 1939); and the organophosphate insecticides are commonly believed to owe their toxicity to the inhibition of cholinesterase and the consequent accumulation of acetylcholine in excess (Chadwick and Hill, 1947; Kearns, 1956; Spencer and O'Brien, 1957). The fact that acetylcholine is not toxic when injected into insects led to some doubt as to whether this could be the primary mode of action of the organophosphate compounds, and it was suggested that the poisoning of other esterases might be the cause of death (Hopf, 1954; Lord and Potter, 1954). More recently it has been found that the sheath around the ganglia and nerves acts as a barrier both to acetylcholine (Twarog and Roeder, 1957) and to non-ionized inhibitors of cholinesterase (O'Brien, 1957). At the present time, therefore, it seems probable that inhibition of cholinesterase is a main cause of the toxicity of the organophosphate compounds in insects as in mammals (Spencer and O'Brien, 1957), although it does not necessarily follow that that is their sole mode of action.

All this has led to an extensive study of the esterases of insects (Metcalf and others, 1955, 1956), but little is known about the histological or cytological

[Quarterly Journal of Microscopical Science, Vol. 99, part 4, pp. 441–450, Dec. 1958.]

distribution of these enzymes. The present work forms part of a reinvestigation of the histology of the nervous system of the insect, which is in course of publication. It has been extended to include a few observations on the esterases in other tissues.

Material and Methods

All the observations have been made on the blood-sucking bug *Rhodnius prolixus* Stål.

Three methods for esterases have been used: (i) the acetylthiocholine method of Koelle as modified by Gomori (1952); (ii) the naphthol AS acetate method as described by Pearse (1954); (iii) the 5-bromoindoxyl acetate method of Holt and Withers (1952) as described by Pearse (1953), using copper sulphate 10^{-3} M as oxidation catalyst. The indoxyl method proved to be the most generally useful. Various inhibitors were used in conjunction with these tests: eserine 10^{-5} M; paraoxon (E 600) 10^{-4} M; tetra-*iso*-propylpyrophosphoramide (*iso*-OMPA) 10^{-4} M; 1:5-bis-(4-trimethylammonium-phenyl) pentane-3-one di-iodide (62.C.47 of Messrs. Burroughs and Wellcome) 10^{-4} M; and silver nitrate 10^{-2} M.

Whole organs or 15-μ frozen sections of tissues fixed for 24 h in cold neutral formaldehyde solution were placed in the substrate mixtures for 1–16 h at room temperature and ultimately mounted in Canada balsam, unstained or after staining with borax carmine or Bismarck brown. Other tests were made on fresh tissues using methods (ii) and (iii) with the reagents made up in isotonic saline. Exposure was then for 15–45 min and was followed by fixation in formaldehyde, sometimes preceded by brief fixation in osmium tetroxide.

Distribution of Esterase in the Nervous System

If the brain or the fused thoracic and abdominal ganglia, after fixation in cold neutral formaldehyde for 16 h, are washed in distilled water for half an hour and then placed for 6–24 h in the 5-bromoindoxyl acetate mixture, an intense blue coloration develops in the central parts of the ganglia, in the roots of the nerves as they pass through the layer of ganglion cells, and in the larger nerves (fig. 1, A, D).

The intense reaction in the ganglia is confined to the neuropile. With the osmium ethyl gallate method of staining (Wigglesworth, 1957) the neuropile stains dark and the ganglionic layer is relatively pale. Fig. 1, B, E shows that the distribution of this staining agrees exactly with that of the esterase.

At a higher magnification of frozen sections of ganglia similarly treated it is seen that the axons are completely colourless; the reaction is confined to the material between the axons (figs. 1, F; 2, A). So that in cross-sections of the nerves or nerve-roots the axons are more or less ringed with the deposit of indigoid dye (compare Snell, 1957).

The structure of the neuropile will be described elsewhere (Wigglesworth, 1958) but it may be stated here that it consists of large and small axons and interaxonal material. Since the contents of the axons always give a negative

esterase reaction, the intensity of the blue coloration is proportional to the amount of interaxonal material present (fig. 1, F). The most deeply coloured regions are those which stain most darkly with osmium ethyl gallate; in these regions the nerve-filaments are exceedingly fine and therefore there is relatively more interneuronal material, containing double membranes and mitochondria. This is believed to be the cytoplasm of the large glial cells. 'Synaptic vesicles' within the nerve endings also contribute to the dark staining with osmium and ethyl gallate.

In addition to the neuropile there is a less intense reaction in the layer of ganglion cells and in the perineurium. The cytoplasm of the ganglion cells is always completely negative (apart from some minute granules over the surface of the nuclei, which will be discussed later). The deposit of indigoid dye is in the form of fine granules and thin sheets in the glial substance between the cells and along the nerve processes coming from them. In the case of very large ganglion cells the glia is invaginated deeply into the cell-body in the form of 'mesenteries'. The esterase reaction may sometimes extend in the form of fine granules into these mesenteries.

In the perineurium cells below the fibrous sheath of the ganglia (the perilemma) there are numerous fine granules of indigoid dye scattered through the cytoplasm and concentrated around the nuclei.

In the post-cerebral organs, there is a very weak reaction in the form of a few scattered granules in the corpus cardiacum and a somewhat stronger reaction in the hypocerebral ganglion; the corpus allatum is negative.

If the fresh unfixed ganglia and nerves are immersed in the 5-bromoindoxyl acetate mixture in isotonic saline they become blue within a few minutes. But even after one hour the colour is almost wholly confined to the perineurium; there is usually no reaction within the neuropile of the ganglia or between the axons in the nerves (fig. 2, B). In the perineurium cells there are fine granules, some scattered through the cytoplasm but most concentrated over the surface of the nucleus, so that this appears outlined in blue. It would appear that under these conditions the substrate material will penetrate the perilemma to reach the perineurium cells but it does not reach the enzymes in the deeper parts of the nerves or ganglia. But if the ganglia or nerves are cut across before immersion, the enzymes show the same distribution as in the fixed material. Under these conditions the indigoid dye is deposited in very small granules in the neuropile.

Similar results are obtained with acetylthiocholine. This is far less penetrating than bromoindoxyl acetate and even in formaldehyde-fixed ganglia the reaction is almost limited to the perineurium; and when the ganglion is cut through the reaction is confined to the exposed surface. But the distribution is as described: most intense in the neuropile and between the axons in the nerves, but positive also in the perineurium cells and between the ganglion cells. In 15-μ sections of the ganglia the weak reaction in the perineurium and cellular layers is almost invisible and the esterase appears confined to the neuropile.

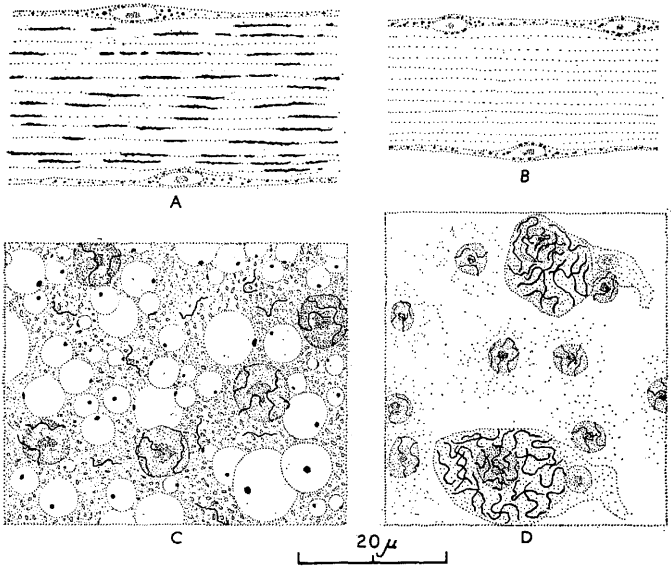


FIG. 2. A, distribution of esterase in perineurium and between axons of nerve. 5-bromoindoxyl acetate after formaldehyde fixation. B, nerve after exposure to 5-bromoindoxyl acetate in the fresh state. Esterase reaction limited to the perineurium cells. C, whole mount of fat-body after exposure to 5-bromoindoxyl acetate in the fresh state. Esterase reaction in form of 'caps' on each fat vacuole and fine filamants over the surface of the nuclei and among the mitochrondria. D, epidermis treated as C. Esterase reaction in form of very fine filaments over the nuclei of the epidermal cells and larger filaments throughout the cytoplasm of the dermal glands.

THE ACTION OF INHIBITORS ON ESTERASE IN THE NERVOUS SYSTEM

Identification of true cholinesterase rests on the effect of selective inhibitors. Whole ganglia or frozen sections, after formaldehyde fixation, were immersed in the solution of inhibitor for half an hour and then transferred to the substrate mixture to which more inhibitor had been added. Four inhibitors were tested on the central nervous system, using 5-bromoindoxyl acetate as substrate. The results were confirmed with acetylthiocholine.

(i) Eserine 10^{-5} M is an inhibitor of the cholinesterase group of enzymes. It completely inhibits the intense reaction in the neuropile and between the axons in the nerves, leaving only some scattered granules of dye. But the reaction in the perineurium and between the ganglion cells is not affected, so that now the neuropile appears paler than the rest of the ganglion (fig. 1, c).

(ii) Tetra-*iso*-propylpyrophosphoramide (*iso*-OMPA) 10^{-4} M is a specific inhibitor for the 'pseudo-cholinesterase' which occurs in the central nervous

system of mammals (Pepler and Pearse, 1957). It has no apparent effect on the esterases in the neuropile, perineurium, or ganglion layer in *Rhodnius*.

(iii) 1 : 5-bis-(4-trimethylammonium-phenyl)pentane-3-one di-iodide (62.C.47) 10^{-4} M is a specific inhibitor of the true acetylcholinesterase of mammals (Pepler and Pearse, 1957). In the ganglia of *Rhodnius* it acts just like eserine: it inhibits the reaction in the neuropile and between the nerve axons but has no effect on the esterase of the perineurium and cellular layer.

(iv) *oo*-diethyl *o*-*p*-nitrophenyl phosphate (paraoxon; E 600) 10^{-4} M inhibits a much wider range of esterases in mammals (Aldridge, 1953). In *Rhodnius* it completely inhibits the reaction in the neuropile, and very largely, but not completely, inhibits that in the remainder of the ganglion.

From these results it appears that the abundant enzyme in the neuropile and between the nerve axons is a specific acetylcholinesterase. There is no evidence of an enzyme with properties resembling those of 'pseudo-cholinesterase' as found in mammals. The esterase between the ganglion cells and in the perineurium cells clearly does not belong to the cholinesterase group (although it does appear capable of hydrolysing acetylthiocholine to some small extent).

Esterase in Other Tissues

Enzymes hydrolysing acetylthiocholine are confined to the nervous system in *Rhodnius* but enzymes hydrolyzing 5-bromoindoxyl acetate and naphthol AS acetate are widely distributed in other organs and tissues. They have been studied in fresh and formaldehyde-fixed material. The 5-bromoindoxyl acetate penetrates into the cells very readily and gives better cytological results.

If the recently fed 4th-stage larva of *Rhodnius* is slit along each side of the abdomen and the tergites and sternites, after separation from the alimentary canal, are immersed for 30 min in the isotonic substrate without previous fixation, the indigoid dye is deposited in many tissues.

Fat-body. The lace-like fat-body becomes blue within ten minutes. Microscopic examination of the fresh tissue shows that each fat droplet, from the smallest (measuring one or two microns in diameter) to the largest (measuring 20 μ or more) has a small point or cap which is the site of the reaction (figs. 1, H; 2, C). If the tissue is fixed in formaldehyde and mounted in Canada balsam it can be seen that in addition to the conspicuous 'caps' on each fat vacuole, there are exceedingly fine blue filaments applied to the surface of the nuclei and a few similar filaments and fine granules elsewhere in the cytoplasm (figs. 2, C; 1, L). When examined fresh the 'caps' on the fat droplets are even and sharply defined; after fixation in formaldehyde they are somewhat wrinkled and distorted; after fixation in Carnoy they break up into irregular clumps of dye in a pale blue ground substance.

If the fat-body, after exposure to the substrate, is fixed for 2 min in 1% osmium tetroxide, and then for 5 min in saturated picric acid, kept in Farrants's medium for several days (so that the fat droplets become pale again) and then stained with borax carmine and Sudan III and mounted in Farrants's

medium, it is possible to see the mitochondria very faintly, as well as the stained fat droplets and nuclei. It is then apparent that the fine blue filaments are much more slender than the mitochondria among which they lie. In these osmium-fixed preparations the 'caps' on the fat droplets retain the sharp outline which they have in fresh preparations.

After formaldehyde fixation the same reactions can be obtained but the 'caps' on the fat droplets are irregular in outline and partially broken up, and there are no uniform filaments on the surface of the nuclei, but only rows of granules.

With naphthol AS acetate identical 'caps' appear on many of the fat droplets in fresh and fixed preparations (fig. 1, G). But no filaments can be seen in either.

The salivary glands and alimentary canal give a strongly positive reaction in fresh and fixed preparations. This takes the form of very fine dispersed granules which are most densely concentrated on the surface of the nuclei.

The Malpighian tubules give an intense reaction, which appears in the fresh material within 2 min after immersion, in the form of fine granules dispersed throughout the cell, but concentrated on the surface of the nuclei, and a diffuse blue coloration of the striated border.

Ovary. The rudiment of the ovary as it exists in the 4th-stage larva reacts strongly in the fresh state. The cells of the developing duct and the thick mantle of mesodermal cells covering the germ-cells are negative; but the germ-cells themselves contain great numbers of blue filaments. These are concentrated around the nuclei and in some of the cells are confined to the surface of the nuclear membrane (fig. 1, K).

The epidermis and dermal glands give a positive reaction (fig. 2, D). In the ordinary epidermal cells there are just a few very slender filaments applied to the surface of the nuclei. In the principal gland-cell of the dermal glands the filaments are somewhat stouter and besides being applied to the surface of the nucleus they run in all directions in the substance of the cell. They have a more or less uniform size and appearance in all the dermal glands.

Pericardial cells. The reaction in these cells is particularly conspicuous after formaldehyde fixation. It takes the form of very fine granules evenly dispersed among the rounded inclusions in these cells, with some concentration around the nuclei.

Haemocytes and oenocytes. The haemocytes contain fine granules, often concentrated around the nucleus. In the oenocytes the reaction is mostly confined to filaments which are rather denser over the nuclear membrane but are also dispersed throughout the cytoplasm (fig. 1, J). The whole reaction is weak in the newly fed insect. But as growth proceeds and the oenocytes enlarge and become charged with their lipoprotein product (Wigglesworth, 1933, 1947) the reaction increases in intensity, so that by seven days after feeding (in the 4th-stage larva) the oenocytes stand out conspicuously by reason of their blue colour, and by this time the whole cytoplasm is filled with blue granules and filaments.

Muscles. The muscles are completely non-reactive apart from the fine granules and filaments which sometimes develop on the surface of the nuclei in the fresh preparations. There is no sign of any positive reaction in the muscle endplates, where cholinesterase is so conspicuous in vertebrates (Chessick, 1954; Couteaux, 1955; Holt, 1954).

In most of these tissues, but particularly in the fat-body and oenocytes, the intensity of the reaction increases during the moulting cycle when reserves of all kinds are being deposited in the cells.

The Action of Inhibitors on the Esterases of Non-nervous Tissues

Eserine, *iso*-OMPA and 62.C.47, inhibitors of the cholinesterase group of enzymes, have no apparent effect on any of the enzyme sites described in the last section.

Paraoxon (E 600) at 10^{-4} M, besides causing complete inhibition of the cholinesterase of the nervous system, brings about a partial inhibition of many of the other enzymes, such as that in the Malpighian tubules, alimentary canal, pericardial cells, and fat-body. But in no case was this inhibition complete.

On the other hand, silver nitrate 10^{-2} M causes complete inhibition of the esterases of the fat-body, gut, and Malpighian tubules, but fails to inhibit that in the pericardial cells.

Discussion

Esterase in the nervous system. The three histochemical tests employed clearly reveal a number of quite different 'esterases'. The only enzyme which is inhibited by eserine and by 62.C.47 is that which occurs so abundantly between the axons in the peripheral nerves and nerve-tracts in the ganglia, and particularly in the neuropile. There is little doubt that this enzyme is a true cholinesterase.

In the nerves this cholinesterase is located in the interaxonal material which is the product of the neuroglial cells; the axon contents seem always to be negative. In the neuropile the reaction is most intense in those regions where the interneuronal material is most abundant. In these places (as studied with the electron microscope) the axons and dendrites are exceedingly fine and the material between them is filled with double membranes ('endoplasmic reticulum') which are largely responsible for the deep staining with osmium and ethyl gallate. This cytoplasm, which also contains mitochondria, appears to be the product of the neuroglial cells and it seems likely that this is the site of the cholinesterase.

In the rat and other mammals (Koelle, 1954; Giacobini, 1956; Pepler and Pearse, 1957) the specific cholinesterase occurs within the cell-bodies of the neurones and along the axons and dendrites of many groups of neurones; the non-specific cholinesterase in neurones and in certain of the gliocytes. The conditions in *Rhodnius* resemble more closely those described in the frog. In

the frog, Shen, Greenfield, and Boell (1955) found that in all cases the enzyme-rich regions are centres with dense and elaborate interneuronal connexions, most of which (apart from motor neurones) contain few cells. They conclude that the enzyme is associated primarily with synaptic connexions and not with cell-bodies. In the optic lobes of the frog cholinesterase appears as the synapses become mature (Boell, Greenfield, and Shen, 1955).

The contents of many of the terminal axons and dendrites in the neuropile of *Rhodnius* also stain with osmium and ethyl gallate (whereas throughout most of their course the axons are almost unstained, apart from the numerous mitochondria which they contain). As seen with the electron microscope, this dark staining is due to the abundant spherical inclusions about 250 Å in diameter, the so-called 'synaptic vesicles'. In vertebrates these are commonly regarded as the site of acetylcholine production.

When fragments of the ganglia in the fresh state are immersed in the indoxyl reagent the deposit of dye appears in very fine granules. It is impossible to say whether such granules represent preformed organelles; it is perhaps safer to regard them as chance deposits of dye. Smallman and Wolfe (1956) and Wolfe and Smallman (1956) found that in homogenates of the central nervous system of insects, cholinesterase is present in both soluble and particulate fractions. Metcalf and others (1956) showed that the cholinesterase was largely particulate, the 'aromatic esterase' largely soluble.

Muscle endplates. The sole-plate of vertebrate muscle is the site of an active esterase which is thought to be concerned with the rapid breakdown of the transmitter substance, acetylcholine (Couteaux, 1955; Chessick, 1954). In insects the nature of the transmitter substance is unknown; the endplates in *Rhodnius* appear not to be the site of any esterase.

Esterase in the fat-body. It has always been difficult to picture how the transfer of fatty material to and from the individual fat droplets is brought about. The present observations show that each droplet has its own sharply localized 'cap' of esterase.

This cap has an identical appearance whether revealed with 5-bromoindoxyl acetate or with napthol AS acetate. The enzyme is not inhibited by 10^{-4} M eserine and is only partially inhibited by 10^{-4} M E 600 (paraoxon). There can be little doubt that this is a genuinely localized enzyme site and it seems most probable that the enzyme in question is a true lipase. Lipase of the pancreas will hydrolyse 5-bromoindoxyl acetate (Pearson and Defendi, 1957) and is not inhibited by E 600 (Aldridge, 1954). It is suggested that at these enzyme sites, localized reversible hydrolysis of triglycerides controls the transfer of fat to and from the storage vacuoles.

The significance of the filaments on the surface of the nuclei and among the mitochondria is more doubtful. Are these filaments preformed structures, the site of an 'esterase', or are they mere deposits of indigoid dye that happen to have assumed a filamentous form? It is quite common to see the indigoid dye separating out in crystalline filaments which are certainly artifacts; but that does not prove that the filaments under discussion are also artifacts. The ques-

tion must remain undecided, but the evidence in support of their being preformed structures is as follows:

(i) In the fat-body they have a uniform thickness, which is the same in every cell.

(ii) They have this even appearance only when the substrate is applied to fresh material. In fixed material the reaction product appears in the form of granules; and, as we have seen, the caps of esterase on the fat droplets likewise tend to be broken up in fixed material.

(iii) Filaments applied to the surface of the nucleus occur in many cells treated in the fresh state. These filaments have a characteristic size in each type of cell; they are almost invisibly fine (probably 0·1 μ thick, or less) in the epidermal cells, larger in the fat-body cells, and larger still in the dermal glands and in the germ-cells of the developing ovary. On the other hand, they do not occur in all cells. For example, in the Malpighian tubules there are small granules scattered through the cell; there are denser around the nuclear membrane, but filaments are absent.

One can do no more than speculate on the nature of the enzyme represented by the filaments. It might be a peptidase connected with protein synthesis; for, as Pepler and Pearse (1957) and Hess and Pearse (1958) have pointed out, 5-bromoindoxyl acetate is readily hydrolysed by intracellular cathepsins, and these enzymes are resistant to inhibition by E 600 but are completely inhibited by silver nitrate. Or it might be concerned in phospholipid metabolism.

The same uncertainty surrounds all the other sites of 'esterase' action that have been described. It is interesting to note the abundant filaments in the oenocytes and in the germ cells of the ovary where active synthesis of protein and lipid is in progress.

The abundant esterase in the pericardial cells is of interest, for the resistance of this enzyme to inhibition by silver nitrate suggests that it is of a different nature from the other enzymes described. The pericardial cells actively segregate colloidal particles of dyes, haemoglobin, and other foreign matter introduced into the blood. They are often compared with the reticuloendothelial system of mammals; otherwise their function is not known.

I am indebted to Dr. A. G. Everson Pearse for permitting me to see the paper by Dr. R. Hess and himself before publication and for the supply of 5-bromoindoxyl acetate, and to Dr. D. Prescott of the Wellcome Foundation Ltd., for a sample of 62.C.47.

POSTSCRIPT

While this paper was a proof, Holt and Withers (1958), using an improved substrate (5-bromo-4-chlorindoxyl acetate) on fat cells of the rat, have reported stained organelles in the cytoplasm close to the nuclei.

REFERENCES

ALDRIDGE, W. N., 1953. Biochem. J., **53**, 110.
—— 1954. Ibid., **57**, 692.

BOELL, E. J., GREENFIELD, P., and SHEN, S. C., 1955. J. exp. Zool., **129**, 415.
CHADWICK, L., and HILL, D., 1947. J. Neurophysiol., **10**, 235.
CHESSICK, R. D., 1954. J. Histochem. Cytochem., **2**, 258.
CORTEGGIANI, E., and SERFATY, A., 1939. C.R. Soc. Biol., **131**, 1124.
COUTEAUX, R., 1955. Int. Rev. Cytology, **4**, 335.
GIACOBINI, E., 1956. Acta physiol. scand., **36**, 276.
GOMORI, G., 1952. *Microscopic histochemistry*. Chicago (University Press).
HESS, R., and PEARSE, A. G. E., 1958. Brit. J. exp. Path. (in the press).
HOLT, S. J., 1954. Proc. Roy. Soc. Lond. B, **142**, 160.
—— and WITHERS, R. F. J., 1952. Nature, **170**, 1012.
—— —— 1958. Proc. Roy. Soc. Lond. B, **148**, 520.
HOPF, H. S., 1954. Ann. appl. Biol., **41**, 248.
KEARNS, C. W., 1956. Ann. Rev. Entom., **1**, 123.
KOELLE, G. B., 1954. J. comp. Neurol., **100**, 211.
LORD, K., and POTTER, C., 1954. J. Sci. Food Agr., **5**, 490.
METCALF, R. L., MARCH, R. B., and MAXON, M. G., 1955. Ann. ent. Soc. Amer., **48**, 222.
—— MAXON, M., FUKUTO, T. R., and MARCH, R. B., 1956. Ibid., **49**, 274.
O'BRIEN, R. D., 1957. Ibid., **50**, 223.
PEARSE, A. G. E., 1953. *Histochemistry*. London (Churchill).
—— 1954. Int. Rev. Cytology, **3**, 329.
PEARSON, B., and DEFENDI, V., 1957. J. Histochem. Cytochem., **5**, 72.
PEPLER, W. J., and PEARSE, A. G. E., 1957. J. Neurochem., **1**, 193.
SHEN, S. C., GREENFIELD, P., and BOELL, E. J., 1955. J. comp. Neurol., **102**, 717.
SMALLMAN, B. N., and WOLFE, L. S., 1956. J. cell. comp. Physiol., **48**, 197.
SNELL, R. S., 1957. Nature, **180**, 378.
SPENCER, E. Y., and O'BRIEN, R. D., 1957. Ann. Rev. Entom., **2**, 261.
TWAROG, B. M., and ROEDER, K. D., 1957. Ann. ent. Soc. Amer., **50**, 231.
WIGGLESWORTH, V. B., 1933. Quart. J. micr. Sci., **76**, 270.
—— 1947. Proc. Roy. Soc. Lond. B, **134**, 163.
—— 1957. Ibid., **147**, 185.
—— 1958 (unpublished work).
WOLFE, L. S., and SMALLMAN, B. N., 1956. J. cell. comp. Physiol., **48**, 215.

FIG. 1 (plate). A, whole ganglion (fused meso- and metathoracic and abdominal ganglia) of 4th-stage larva showing esterase reaction in neuropile, nerve roots, and nerves. 5-bromo-indoxyl acetate.

B, horizontal section of ganglion showing dark staining of neuropile with osmium and ethyl gallate.

C, esterase reaction in whole ganglion in the presence of eserine 10^{-5} M. The reaction in the neuropile and nerves has been inhibited. 5-bromoindoxyl acetate.

D, whole brain of 4th-stage larva treated as A. Esterase reaction confined to neuropile.

E, horizontal section of brain showing staining of neuropile with osmium and ethyl gallate.

F, frozen section of ganglion fixed in formaldehyde solution, showing absence of esterase in the axons, positive reaction between the axons, and intense reaction in the synaptic regions. 5-bromoindoxyl acetate.

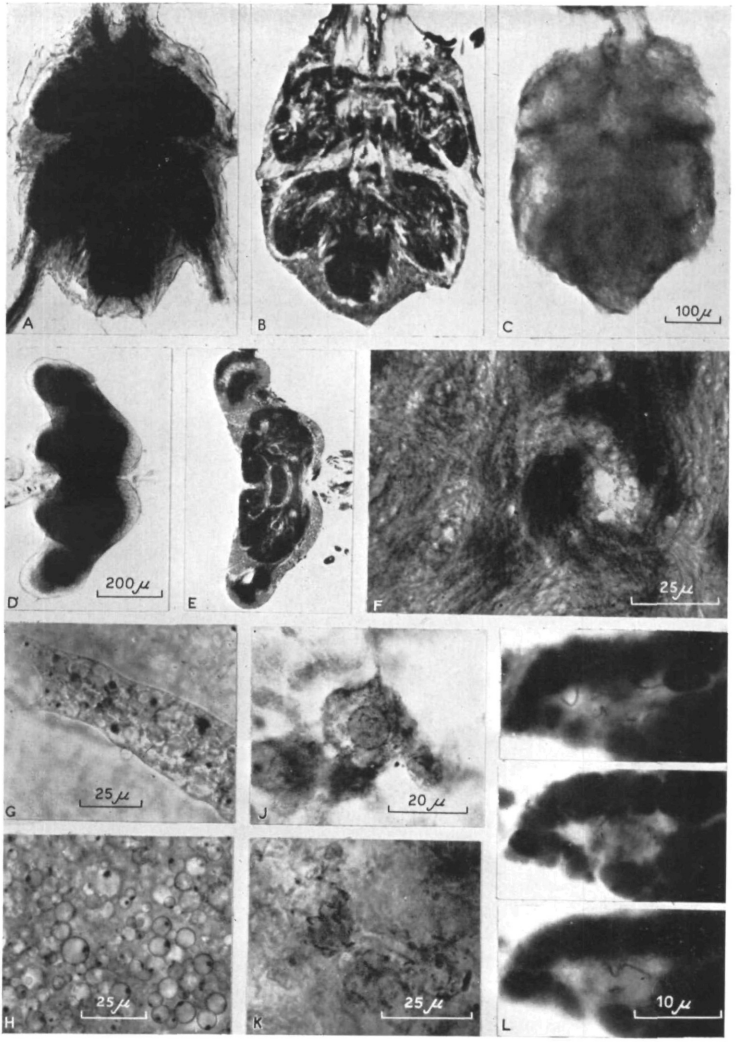
G, fat-body mounted whole; esterase confined to a small area on each fat droplet. Naphthol AS acetate after formaldehyde fixation.

H, fresh unfixed fat-body showing a sharply defined 'cap' of esterase on each fat droplet. 5-bromoindoxyl acetate.

J, esterase in the form of filaments on the nucleus and in the cytoplasm of oenocytes. 5-bromoindoxyl acetate.

K, three large germ-cell nuclei in ovary rudiment, showing esterase in the form of filaments over the surface. 5-bromoindoxyl acetate.

L, a single nucleus in the fat-body after exposure in the fresh state of 5-bromoindoxyl acetate, showing filaments close to the nuclear membrane. Above, lower surface of nucleus; middle, optical section of nucleus; below, upper surface of nucleus. The surrounding fat has been darkened by osmium.



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