

Although we have no direct evidence regarding the function of these organs we suggest that they are involved in the sequestration and storage of some substances from the haemolymph, and that these substances may be toxic.

**Summary.** The nodes formed by the mid-ventral tracheal anastomoses in abdominal segments 3–6 are modified into conspicuous glandular organs in larvae of certain Lepidoptera. They comprise clusters of extremely large cells penetrated by an extensive lacunar system opening onto the tracheal wall. These cells appear to sequester substances from the haemolymph which may be conjugated

with a lipid synthesized within them and the product excreted into the lacunar system, ultimately passing into the tracheal lumen.

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### Glycogen in Epidermal Nerve Terminals of *Lacerta sicula* (Squamata: Reptilia)

Epidermal nerve endings in reptiles were first described in turtle skin by HULANICKA<sup>1</sup>. She describes them to be 'conspicuous buttons, lying just beneath the stratum corneum, ovally shaped, their longer axis parallel to the skins surface'. JABUREK's<sup>2</sup> light-microscopical study revealed them to be located 'intracellularly'. Recent electron-microscopic work<sup>3–5</sup> shows that axons ascend through the intercellular space of the epidermis, penetrate the uppermost keratocytes (just beneath the  $\alpha$ -keratin layer) and enlarge to several  $\mu$ m-sized 'discoid terminals' (a term which we prefer to VON DÜRING's 'bubble receptor' since they are not gas-filled). One axon can give rise to two or even more<sup>2</sup> discoid terminals (Figure 1). These terminals seem, in the section, to be surrounded by two membranes: the outer is part of the plasmalemma of the keratocyte, the inner, thicker one, part of the axolemma (Figure 2). They contain numerous mitochondria, and are densely filled with particles<sup>3,5</sup>, which the UAc-PbCi staining (Uranyl acetate – Lead citrate) (Figures 1 and 2) shows to be 250–370 Å in diameter (mean diameter 300 Å). They are not membrane-bound. In comparison, free ribosomes are approx. 200 Å in size. Higher resolution (Figure 2) reveals no further details. Earlier work<sup>3–5</sup> presumes those particles to be glycogen. In order to verify the glycogen nature of these particles periodic acid-lead citrate (PA-PbCi) stain and  $\alpha$ -amylase digestion were applied.

The PA-PbCi stain<sup>6</sup> (Figure 3) is specific for polysaccharides: Following oxidation by PA, aldehyde groups appear in the polysaccharide chains which may be detected by Pb ions. The particles are 250–350 Å in size (mean diameter 300 Å), thus corresponding to glycogen  $\alpha$ -particles or rosettes. The high magnification (Figure 3) shows  $\beta$ -particles (mean diameter 70 Å, range 50–100 Å). Between the  $\beta$ -particles we find contrasted material (Figure 3), according to DE BRUIJN<sup>7</sup> nonparticulate glycogen. DE BRUIJN claims it to be a separate entity composed of oligosaccharides or glycogen-enzyme complexes of the glycogen metabolism and relates it to the  $\gamma$ -particles described by DROCHMANS<sup>8</sup> (discussion see below).

Enzymatic digestion with  $\alpha$ -amylase according to MONNERON and BERNHARD<sup>9</sup> brings the final proof that these particles are glycogen. Figure 4 shows the successive stages of digestion: undigested  $\alpha$ -particles are still recognizable; soon a brightening is observed in the center of the particle; with the proceeding expansion of the bright center the  $\alpha$ -particle looks like a membrane-bound vesicle (these are abundantly seen); finally no trace of  $\alpha$ -particles can be observed: large areas where particles would most likely be found exhibit only a homogenous matrix. We believe all those different structures to be stages in the

digestion of the  $\alpha$ -particle, because of the identical size of  $\alpha$ -particles and vesicle-like structures (250–350 Å, mean diameter 300 Å) and absence of vesicle-like structures in undigested material (Figures 2 and 3). The big holes of variable diameter can be interpreted as follows: Figures 2 and 3 show that  $\alpha$ -particles often aggregate closely contacting each other. Since the thickness of the sections corresponds to about 3 average  $\alpha$ -particle diameters (800–1000 Å), the holes may be sites of such aggregates arranged vertically which are completely digested. Backing this explanation is the fact that the holes are often surrounded by an electron-dense boundary corresponding to the membrane-like structure of the  $\alpha$ -particle digestion stage described above.

These electron-dense boundaries, respectively membrane-like structures, may represent limit-dextrins aggregated peripherally by what cause so ever.  $\alpha$ -Amylase breaks  $\alpha$ -1 $\rightarrow$ 4 glycosidic linkages – with preference internal ones – but cannot split the  $\alpha$ -1 $\rightarrow$ 6 bonds (branching points) contained in the limit-dextrins<sup>10</sup>. Due to the aldehyde groups appearing following oxidation by PA, the limit-dextrin stains with Pb ions. But increased incubation brings them to disappear as seen in Figure 4, since the  $\alpha$  1 $\rightarrow$ 6 bonds may split without enzymatic activity, thus giving to the  $\alpha$ -amylase the opportunity to break further  $\alpha$ -1 $\rightarrow$ 4 linkages (glycogenolysis occurs even in control sections which are treated with trypsin – inhibitor but in a much slower manner: they need about 10 times the incubation time to show the same effect as do the sections digested by  $\alpha$ -amylase). Our interpretation considering the electron-dense boundaries is backed by THORNELL's<sup>11</sup> statement that  $\alpha$ -amylase treated sections remain stainable with PA-TSC-SP (periodic acid-Thiosemi-carbazide-Silver proteinate), which reveals polysaccharides<sup>12</sup>. Supposing these boundaries were glycogen-enzyme complexes which may be present between the  $\beta$ -particles according to DE BRUIJN<sup>7</sup>, they would have to be visible even in extended incubation

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<sup>3</sup> M. VON DÜRING, *Z. Anat. EntwGesch.* 141, 339 (1973).

<sup>4</sup> M. VON DÜRING, *Z. Anat. EntwGesch.* 145, 299 (1974).

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<sup>6</sup> M. M. PERRY and C. H. WADDINGTON, *J. Cell Sci.* 1, 193 (1966).

<sup>7</sup> W. C. DE BRUIJN, *J. Ultrastruct. Res.* 42, 29 (1973).

<sup>8</sup> P. DROCHMANS, *J. Ultrastruct. Res.* 6, 141 (1962).

<sup>9</sup> A. MONNERON and W. BERNHARD, *J. Microsc.*, Paris 5, 697 (1966).

<sup>10</sup> E. E. SMITH, P. M. TAYLOR and W. J. WHELAN, in *Carbohydrate Metabolism and its Disorders* (Ed. F. DICKENS; Academic Press, London 1968), p. 89.

<sup>11</sup> L. E. THORNELL, *J. Ultrastruct. Res.* 47, 153 (1974).

<sup>12</sup> J.-P. THIÉRY, *J. Microsc.*, Paris 6, 987 (1967).

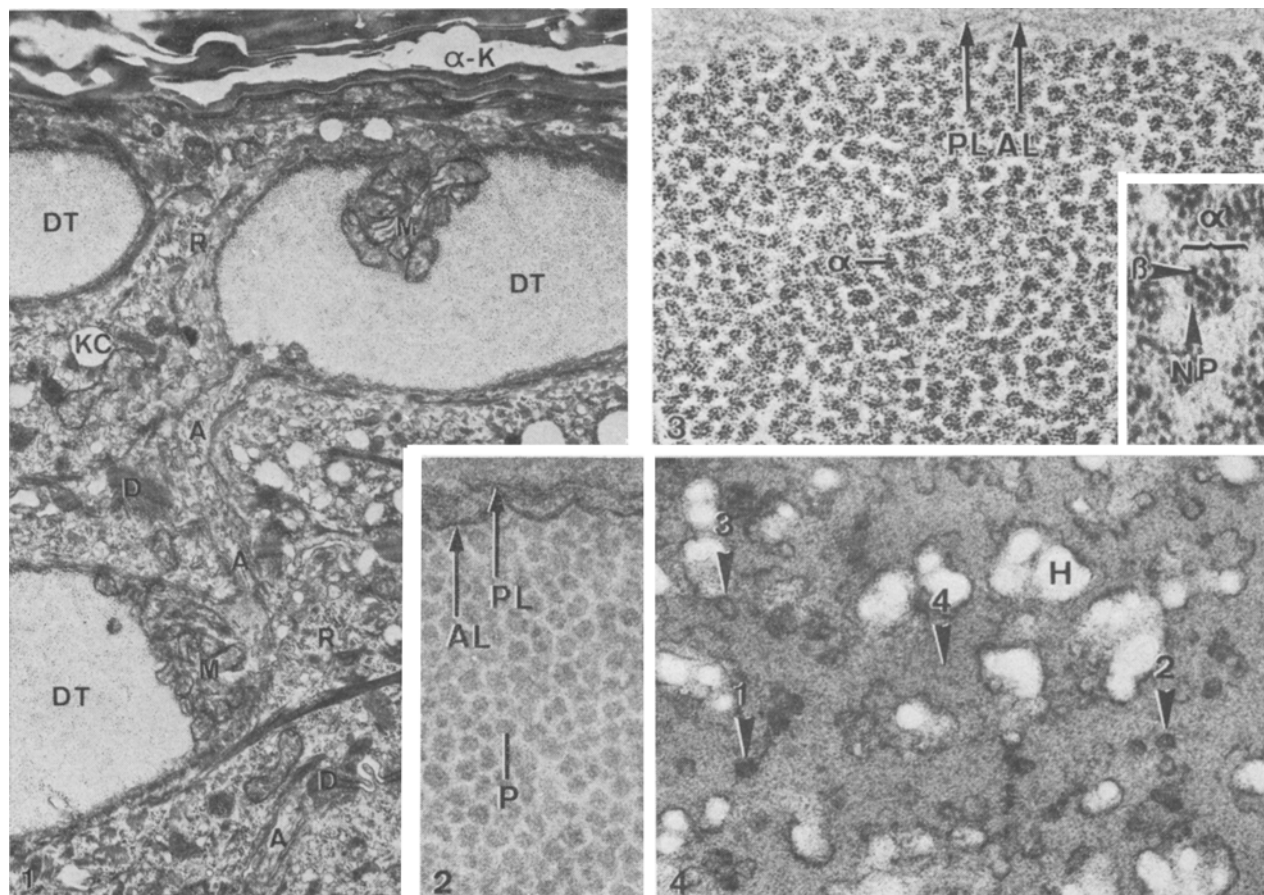


Fig. 1. One axon (A) giving rise to two discoid terminals (DT).  $\alpha$ -K,  $\alpha$ -keratin layer; D, desmosome; KC, keratocyte; M, mitochondria; R, free ribosomes. UAc-PbCi.  $\times 12,500$ .

Fig. 2. Section from a discoid terminal, showing the 300 Å particles (P). AL, axolemm; PL, plasmalemm. UAc-PbCi.  $\times 100,000$ .

Fig. 3. Section from a discoid terminal showing  $\alpha$ - and  $\beta$ -particles.  $\alpha$ ,  $\alpha$ -particle;  $\beta$ ,  $\beta$ -particle; NP, nonparticular glycogen. PA-PbCi.  $\times 100,000$ . Inset  $\times 300,000$ .

Fig. 4. Section from a discoid terminal showing the digestion stages of particles by  $\alpha$ -amylase. 1, undigested particle; 2, beginning of digestion revealed by brightening of center; 3, proceeding digestion (vesicle-like structure); 4, completed digestion (no structures preserved); H, holes (explained in the text).  $\alpha$ -Amylase.  $\times 100,000$ .

time because the protein component of the enzyme would stain with UAc. From this one can conclude that the contrasted material between the  $\beta$ -particles (corresponding to DROCHMAN'S<sup>8</sup>  $\gamma$ -particles according to DE BRUIJN<sup>7</sup>) could be oligosaccharides. Further study of these electron-dense structures will be carried on.

As far as we know, such an aggregation of glycogen in nerve terminals was nowhere else observed, although its occurrence in nervous tissue is long known. Apart from numerous mitochondria, the discoid terminal is completely filled out with glycogen. The mitochondria lie mostly in the periphery of the terminal, very often in the neighbourhood of the entering axon (Figure 1). But such a localization may be due to artefacts of the fixation process. Occasionally areas free of  $\alpha$ -particles appear inside the discoid terminals. This phenomenon may be caused by fixation artefacts too. Nothing is known about the function of such an amount of glycogen in nerve terminals. SOTELO and PALAY<sup>13</sup> describe in the brain of the rat swellings of dendrites filled with mitochondria and glycogen (the latter to a much less extent than found in our material) claiming them to be growing dendritic tips. The suggestion that glycogen and mitochondria indicate nerve fibre growing may be tempting since the

squamate epidermis is shed in periodic sloughings and the epidermal nerves have to be restored, a subject which we will study later on. Another interpretation could arise from the following observation made in an earlier paper: the ascending axons show occasionally glycogen-filled areas or even swellings<sup>5</sup>. This glycogen could be on the way from its synthetization-site, e.g. the perikaryon<sup>14</sup> to the site of energy consumption, e.g. the receptor represented by the discoid terminal.

**Summary.** Proof is given that the granula occurring in the epidermal discoid nerve terminals of *Lacerta sicula* consist of glycogen. Staining with PA-PbCi shows 300 Å sized  $\alpha$ -particles and 70 Å sized  $\beta$ -particles. The electron-dense boundary appearing after digestion with  $\alpha$ -amylase consists of limit-dextrins.

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<sup>13</sup> C. SOTELO and S. L. PALAY, *J. Cell Biol.* 36, 151 (1968).

<sup>14</sup> C.-H. BERTHOLD, *J. Ultrastruct. Res.* 14, 254 (1966).