tion, of these nerve endings. Furthermore, a baskettype axon terminal between steroid-producing cells and enclosing cells was described by the same author. In the present observation, 2 kinds of synaptic vesicle-like structure were discernible, but the existence of 2 kinds of nerve endings was uncertain as yet. Further observation on this is necessary.

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Neurosecretory products diversity in the pars intercerebralis of insects

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Summary. The pars intercerebralis of insect brain, which has numerous physiological functions, contains more neurosecretory cell types than previously thought. There are 2 distinct types among the A cells. In addition to these cells, there are azocarminophilic cells, the C(r) cells, which are not apparent when using 'classical' staining methods.

The pars intercerebralis of insect brain regulates numerous phenomena, such as, for example, water content, trehalose metabolism, reproduction, etc. The origin of the neurohormones implicated in these regulations has been considered to be located in the neurosecretory cells, as demonstrated by the classical methods, chrome hematoxylin-phloxin and paraldehyde fuchsin. After oxidation, these methods allow 2 large categories of neurosecretory materials to be distinguished: neurosecretory materials stained by chrome hematoxylin and paraldehyde fuchsin (A type) and materials retaining acidic stains such as phloxin, light green (B type).

In this paper we attempted to study 2 problems. Firstly, whether there are several types of A cells and how to recognize them; secondly, to discuss another type of neurosecretory cell, which we will call C(r). In addition we have investigated in previous publications and by our own research whether these cells exist in various orders.

Materials and methods. In order to study A cells, we employed Bouin fixative and the following stains: chrome hematoxylin-phloxin (CHP), paraldehyde fuchsin (PF), alcian blue-alcian yellow, paraldehyde thionin-phloxin (PTh-Ph) using Panov's method and paraldehyde thionin-paraldehyde fuchsin (PTh-PF) according to the following method: oxidation under standard conditions, staining for 10 min by PTh, washing, dehydration, staining for 2 min by PF, washing with 95% alcohol, dehydration and mounting. The study of C(r) cells was carried out using Bouin and Helly fixatives, each being followed by both azan and CHP staining.

Results and discussion. The neurosecretion of A cells. The staining of the neurosecretory materials by CH or PF is based on the affinity of these stains for the acidic groups appearing after oxidation, but this affinity permits no clear distinction between them. However, the literature indicates the possibility that there are 2 categories of A cells. In particular, by staining with PTh followed by phloxin, Panov[1] noticed that among the secretions of A type, certain ones are strongly colored by the thionin, while others situated in cells of a similar aspect, retain thionin weakly and have at times a weak affinity for the phloxin.

On the basis of Panov's observation, we tried, using various staining methods, to characterize the substances in question. The best results were obtained with PTh-PF, certain cells containing substances stained by thionin (A1 cells) while others containing fuchsinophilic material (A2 cells). This latter material appears as weakly thionin positive in the staining by PTh-PH.

After applying Ravetto's alcian blue-alcian yellow method, which permits the recognition of strong and weak acids, we again observed a very fine distinction between 2 substances: blue blue-green ones and yellow yellow-green ones. When comparing, by the double staining method, sections treated alternately by PTh-PF and by alcian blue-alcian yellow, a correspondance appeared between the thionin and alcian blue positive material and between fuchsin and alcian yellow positive material. Thus the material stained by thionin is rich in strong acids, while that stained by fuchsin is rich in weak acids.

A cells containing 2 neurosecretory materials have already been reported in Orthoptera[2,3], in Neuroptera and Mecoptera[4], in Diptera cyclorrhapha[5], and in Heteroptera[6-8]. Our research has demonstrated their occurrence in Dictyoptera (figure 1, a), Orthoptera (figure 1, d, e), Hymenoptera (figure 2, f), Coleoptera (figure 2, c), Diptera cyclorrhapha (figure 2, d) and Nematocera (figure 2, e), Heteroptera (figure 2, g), which indicates the generality of this phenomenon.

The neurosecretion of C(r) cells. After using certain fixatives such as Helly, followed by azan staining, a particular neurosecretory material was demonstrated by Raabe in the tritocerebron of various insects[9], in ventral nerve cord ganglia and in the perisympathetic organs[10]. This neurosecretory material containing strongly basic proteins does not appear after Bouin fixative and retains neither chrome hematoxylin nor paraldehyde fuchsin. It has little affinity for phloxin under normal oxidation conditions; after weak oxidation, however, a certain phloxinophilia appears in some species. Previously named C type by Raabe[10], we suggest designating it C(r) in order to avoid any confusion with the C cells, having A type affinity, as described by

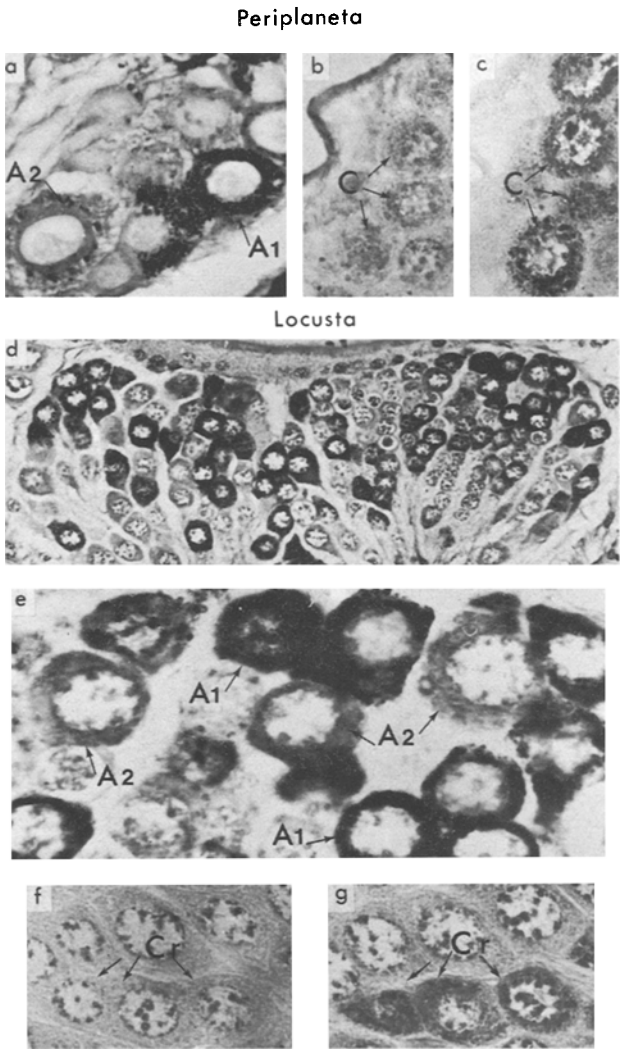

Fig. 1. Various neurosecretory cell types in the pars intercerebralis of insects. *Periplaneta americana:* A1 and A2 cells (a) (PTh-PF), C cells after staining with CHP (b) and azan (c). *Locusta migratoria,* general view of the pars intercerebralis (d), A1 and A2 cells (PTh-Ph) (e), C cells after staining with CHP (f) and azan (g). Bouin fixative (a, d, e); Helly fixative (b, c, f, g). CHP: chrome hematoxylin-phloxin; PTh-PF: paraldehyde thionin-paraldehyde fuchsin; PTh-Ph: paraldehyde thionin-phloxin.

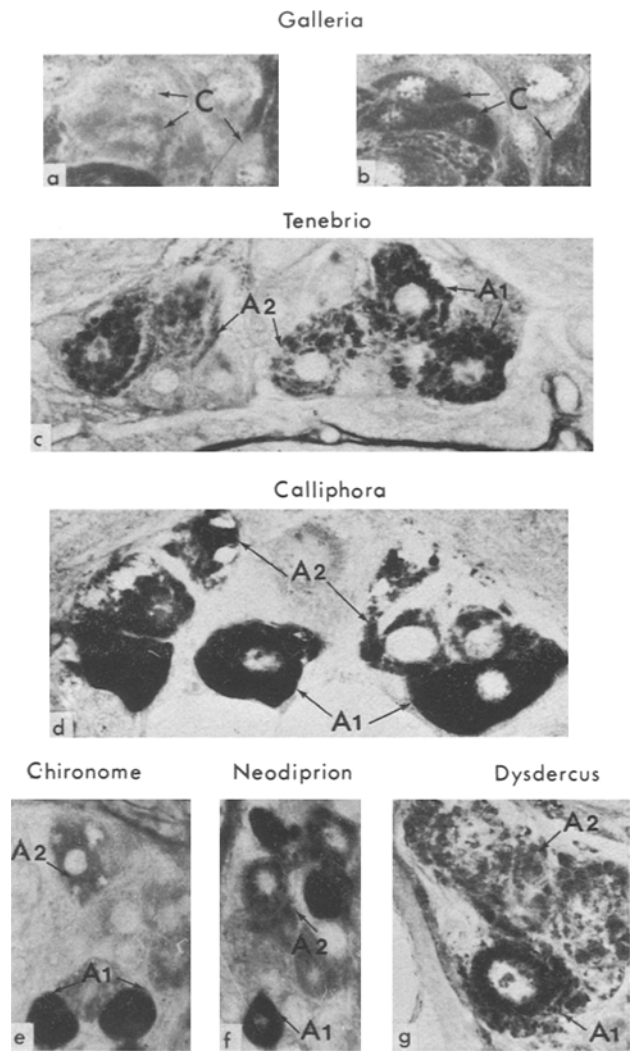

Fig. 2. C cells in the adult female of *Galleria;* CHP (a), azan (b); A1 and A2 cells in the adult of *Tenebrio* (c) and *Calliphora* (d), the larva of *Chironomus* (e), the adults of *Neodiprion* and *Dysdercus* (f, g) (PTh-PF). Bouin fixative (c, d, e, f, g); Helly fixative (a, b).

Johansson[12], Highnam[13] and Girardie[14]. C(r) neurosecretory material was revealed in pars intercerebralis of some species, *Galleria* (Lepidoptera)[15] (figure 2, a, b), *Musca* (Diptera)[16], *Carausius* and *Clitumnus* (Phasmoptera)[11]. Our present research demonstrates its presence in the pars intercerebralis of Dictyoptera (figure 1, b, c), Orthoptera (figure 1, f, g) and Heteroptera. The C(r) cells are located among A cells in Dictyoptera and Orthoptera. It seems that they correspond to the B cells described in *Locusta*[13-14].

Concluding remarks. The literature furnishes scattered reports concerning the presence in the pars intercerebralis, of 2 kinds of A type neurosecretory cells. Our research shows that this phenomenon is present in a large number of species and that the differences observed lie in different proportions of strong and weak acids appearing after oxidation.

In addition the pars intercerebralis of insects contains a particular type of neurosecretory cells designated here as C(r) cells. They are present in numerous orders.

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