

fatty livers caused by poisoning with carbon tetrachloride is under discussion^{2,7,13-25}.

Riassunto. Dopo avvelenamento acuto di ratti con CCl_4 somministrato con dose singola per via orale l'incorporazione in vivo di P^{32} nei fosfolipidi del fegato è alterata precocemente. A 80 min dalla somministrazione del tossico si osserva un forte aumento delle P^{32} -lisolecitine, mentre risulta bloccata la sintesi di acido fosfatidico.

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Ontogenetic Fate of the Neurosecretory Cells in the Larval Brain of *Sarcophaga ruficornis* (Fabricius, 1794) (Diptera: Cyclorrhapha)

Neurosecretory cells have been stained selectively in the whole brain of insects, diverse invertebrates and vertebrates by applying directly to the brain techniques used for revealing such neurons in sections¹. Their study in situ in the brain of the post-embryonic developmental stages of *Sarcophaga ruficornis* disclosed the fate of the neurosecretory cells in ontogeny. This information, being presented here, is based on an examination of about 450 brains, stained with Cameron and Steele's modification of Gomori's paraldehyde-fuchsin technique, as adapted¹, and mounted in canada balsam, either as such (Figures 1, 3, 5, 6) or after appropriate dissections (Figures 2, 4, 7). (From the brains the neural lamella, also being PF-positive, was always cut away.) It was verified on sections, in the transverse, longitudinal and horizontal planes of about 400 brains, stained according to CAMERON and STEELE². The flesh-flies were reared at 28°C ($\pm 1^\circ\text{C}$) and 70% RH.

Each cerebral hemisphere of the brain of all the three larval instars contains four (1, 2, 3, and 4) groups of PF-positive neurosecretory cells, of which the disposition in the first-instar larva is shown in Figure 1. Their comparison with the neurosecretory cell groups of the third-instar larva of *Lucilia caesar*, in which six groups are present², shows the existence of close correspondence in the topography, staining reactions and number of neurosecretory cells comprising the groups (Table), as also of two differences. One difference is due to the absence in *S. ruficornis* larva of neurosecretory cells in the locus of the transient group 6 of *L. caesar* larva. The other difference stems from the splitting up of the cluster of neurosecretory cells at one locus into two groups (3 and 4), mainly on differences in their staining reactions in the diapausing, but not in the non-diapausing, larva of *L. caesar*². But, one group (2) of the third-instar larva of *S. ruficornis*, which develops directly, corresponds to two groups (3 and 4) of the non-diapausing larva of *L. caesar*. As the larva of *S. ruficornis* differs from the diapausing larva only of *L. caesar*, as much as the two types of larvae of the latter fly differ from each other, this difference is nullified.

In the photomicrographs neurosecretory cells of groups 1, 3, and of both the hemispheres of the brain are distinct; all cells of group are not evident as their compact arrangement in tiers prevents their reproduction at one focus.

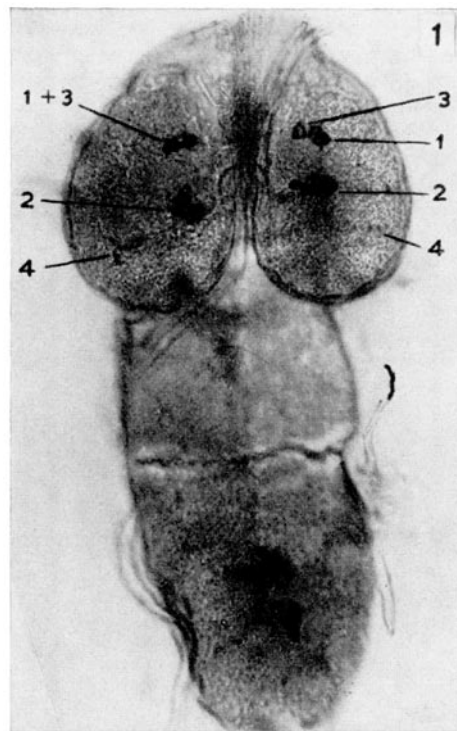


Fig. 1. Brain and ventral ganglion of first-instar larva showing all four groups of neurosecretory cells. $\times 170$.

- ¹ G. S. DOGRA and B. K. TANDAN, *Quart. J. micr. Sci.* **105**, 455 (1964).
- ² M. L. CAMERON and J. E. STEELE, *Stain Tech.* **34**, 265 (1959).
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If the total number of neurosecretory cells in the larval brain is taken into consideration, only four cells are less in that of *S. ruficornis* larva (Table).

In Figure 1 of the whole brain of the first-instar larva, all the groups are clearly visible, but group 4, owing to small amounts of the neurosecretory material in the cell bodies, is relatively indistinct. In the brain of the third-instar mature larva and of the white pupa, the groups show a change in their position. Groups 1 and 2 in each hemisphere come to lie closer to each other, group 3 moves away from group 1 to which it was contiguous, becoming anterior, and group 4 becomes anterolateral (Figure 2); because of its position group 4 was outside the field of this photomicrograph. This reorientation of the cell groups continues. Between two to four days after the white pupa stage, groups 1 and 2 of each side approach each other very closely and move towards the mid-line. While these particular groups come to occupy the anterior, dorsal margin of the brain (protocerebrum), group 3 moves ventrad. During this period, group 4 ceases its activity and fades away. In Figure 3 this position of groups 1 and 2 is distinct; group 3, slightly ventral in position, is evident only on the left side. Between four to six days after the white pupa stage, groups 1 and 2 of each protocerebral lobe merge with one another to form a composite group; concurrently, the resulting composite groups of the two sides also merge together. In Figure 4, among the numerous neurons, on each side of the mid-line, the two larger cell bodies are of group 1 and the remaining smaller ones are of group 2; group 3 of each side is slightly farther away and lies on the basal side of the protocerebrum. In Figure 5 the natural relationships of groups 1 and 2 are faithfully reproduced; group 3 of each side, being ventral in position, is out of focus and is shown in Figure 6 of the same preparation. In the next five days of the metamorphic phase, the median composite cluster undergoes further - but slight - reorientation so that by the end of metamorphosis it assumes an anterodorsal position. In the newly emerged fly (about 10-11 days after the white pupa stage), a small and variable number of cells of the median cluster are stained. However, larval group 3 of each side, ventral in position, is distinctly revealed, but within 24 h its activity ceases and the group fades away. In older flies 24-26 cells are stained in the median cluster and this number agrees with the combined number of larval groups 1 and 2 of the two cerebral hemispheres (Table). In Figure 7 of the dissected pars intercerebralis of a six-day-old female fly, the median cluster distinctly

comprises cells of different sizes. On each side of the mid-line, the two larger cells (in focus only on the left side) belong to the larval group 1 and the remaining smaller cells belong to the larval group 2. This distinction is due to the small amounts of the NSM in the cell bodies.

The number of neurosecretory cells in adult *S. ruficornis* is more than in the related *Calliphora erythrocephala* of which the adult has two median groups of 8-10 neurosecretory cells (total 16-20)⁴. Reasons for this difference

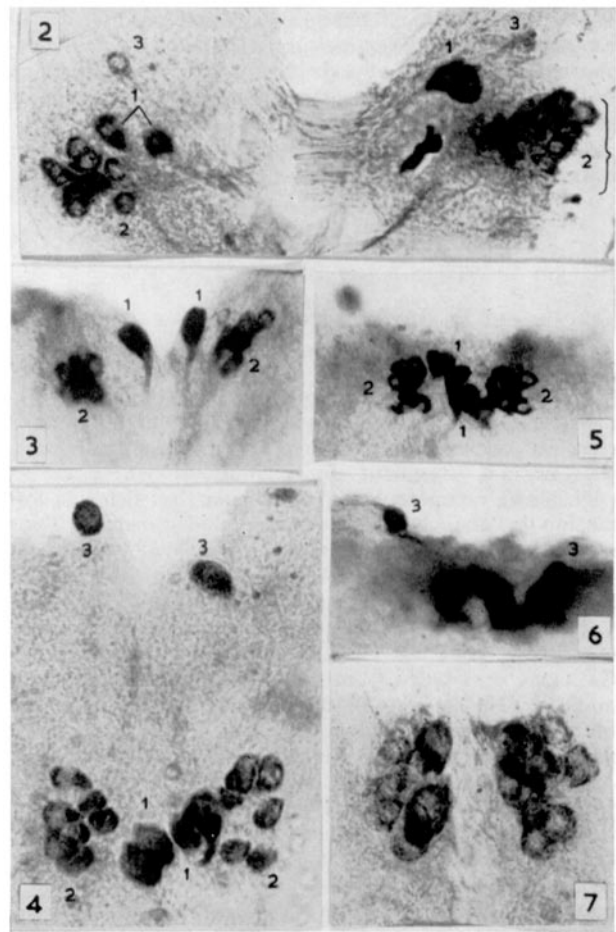


Fig. 2. Dissected portion of the brain of third-instar mature larva showing groups 1, 2, and 3 only (black object on right-hand side, medial to the cells, is a foreign particle). $\times 235$.

Fig. 3. Pars intercerebralis of the protocerebrum of a whole brain of the stage two days after the white pupa stage. $\times 160$.

Fig. 4. Dissected pars intercerebralis of the protocerebrum of an animal four days after the white pupa stage, mounted so that the anterior (and also ventral) surface of the protocerebrum is in contact with the coverslip. $\times 270$.

Fig. 5. Pars intercerebralis of the protocerebrum of a whole brain of an animal six days after the white pupa stage. The solitary cell on each side representing group 3 is out of focus and is shown in Figure 6 of the same preparation. $\times 142$.

Fig. 7. Dissected pars intercerebralis of the protocerebrum of a six-day-old female fly. $\times 225$.

Showing homology in the PF-positive neurosecretory cell groups in the third-instar larval brain of *S. ruficornis* and *L. caesar*

Groups	Number of neurosecretory cells in the two hemispheres of the brain	Groups
	<i>Sarcophaga ruficornis</i>	<i>Lucilia caesar</i> ^a
1	2 + 2 = 4	2 + 2 = 4
2	10-11 + 10-11 = 20-22	4 + 4 = 8
3	1 + 1 = 2	7 + 7 = 14
4	4 + 4 = 8	1 + 1 = 2
		4 + 4 = 8
	Total of 4 pairs of groups = 34-36	Total of 5 pairs of groups = 36
		2 + 2 = 4
		Total of 6 pairs of groups = 40

⁴ M. THOMSEN, Pubbl. Staz. zool. Napoli 24, Suppl., 46 (1954).

might have become clear from a comparison between the larval neurosecretory cells of the two species of flies. But a comparison is not possible because in the larval brain of *C. erythrocephala* the number of median cells, although given as about 12–14, proved difficult to decide⁵.

Thus in *S. ruficornis*, during ontogeny the fate of the four groups of neurosecretory cells in each hemisphere of the larval brain is not the same. Whereas cells of groups 1 and 2 are destined for transformation into neurosecretory cells of the adult fly, groups 3 and 4 are transient⁶.

Résumé. Il y a quatre groupes, 1, 2, 3 et 4, de cellules neurosécrétrices dans chaque demi-sphère du cerveau larvaire du *Sarcophaga ruficornis*. Pendant la métamorphose, les groupes 1 et 2 deviennent antérieurs dorsaux et forment des cellules neurosécrétrices médiales de la

mouche adulte; le groupe 3 devient ventral mais s'évanouit après l'éclosion de la mouche; le groupe 4 s'évanouit aussi, mais plus tôt.

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August 20, 1964.

⁵ M. THOMSEN, Dan. Biol. Skr. 6, 1 (1951).

⁶ We are grateful to Prof. BERTA SCHARRER for a critical review of the typescript; to Prof. K. K. NAYAR, Trivandrum, for generous advice and to Prof. M. B. LAL for his unceasing interest in our work.

Quelle est la durée nécessaire pour déclencher des inductions neurales chez le Poulet?¹

Les recherches de JOHNEN²⁻⁴, de DENIS⁵ et de GALLERA⁶ ont précisé le temps que prend l'induction neurale chez les Amphibiens. La durée de contact entre l'inducteur normal (fragment de la voûte archentérique) et l'ectoblaste compétent suffisante pour déclencher la formation de l'ébauche neurale varie considérablement d'une espèce à l'autre (demi-heure pour l'*Axolotl* et 16 h pour *Triturus alpestris*). Elle ne dépend pas de l'inducteur, mais de la réactivité de l'ectoblaste (JOHNEN⁷).

Jusqu'à présent des recherches analogues n'ont pas été entreprises chez les Oiseaux. Notons, cependant, que GALLERA et IVANOV⁸ ont récemment démontré que le nœud de Hensen mis en contact avec l'ectoblaste d'un blastoderme au stade du prolongement céphalique n'est plus capable de provoquer des inductions neurales. Il semble donc que chez les Oiseaux le feuillet externe perd très vite ses compétences. Compte tenu de cette observation et de la rapidité du développement embryonnaire des Oiseaux, on pourrait s'attendre à ce que le processus de l'induction exige peu de temps. Les investigations que nous allons relater démontrent que c'est le contraire qui est vrai.

Les expériences sont faites sur de jeunes blastodermes de White Leghorn cultivés in vitro selon la technique de NEW⁹, légèrement modifiée par GALLERA et CASTRO-CORREIA¹⁰. La région antérieure de la ligne primitive achevée, dénudée de l'endoblaste, sert d'inducteur. Ce greffon est placé, pour des périodes plus ou moins longues, dans une niche pratiquée dans le rempart vitellin en avant de l'aire pellucide. Le greffon est toujours implanté sa face ventrale contre l'ectoblaste de l'hôte. Comme hôte, nous employons en général des blastodermes au stade de la ligne primitive moyenne. Après un laps de temps déterminé (5 à 10 h 30), le greffon est détaché de l'ectoblaste, auquel il adhère étroitement, et retransplanté sur une autre région de l'aire opaque, à gauche de l'aire pellucide. Dans sa nouvelle position, le greffon se développe normalement et fournit de la corde, des somites et une ébauche neurale rudimentaire. Si la retransplantation a eu lieu avant que l'embryon hôte n'atteigne le stade du prolongement céphalique, le greffon induit la formation d'une ébauche neurale secondaire dans l'ectoblaste sus-jacent.

12 greffons ont été laissés dans leur site primitif durant 5 h; ils n'ont provoqué aucune induction. Dans 7 cas, le contact entre le matériel greffé et l'ectoblaste a été maintenu pendant un temps un peu plus long (6 h environ), 4 de ces greffons ont déclenché, dans l'ectoblaste sus-jacent, la formation de structures d'aspect plutôt neuroïdal que neural. Il s'agit de minuscules plaquettes d'ectoblaste épaissi et pluristratifié. De la face ventrale de ces plaquettes se détachent des cellules qui rappellent celles de la crête neurale (Figure 1). 8 greffons ont été laissés à leur place primitive de 8 h 30 à 10 h 30. Tous ont induit des structures neurales typiques. Ce sont des plaques neurales en forme de raquette dont la région antérieure est élargie et se déprime en profonde et large gouttière de caractère cérébral (Figure 2). Dans tous ces cas, nous avons observé un excès d'éléments provenant des crêtes

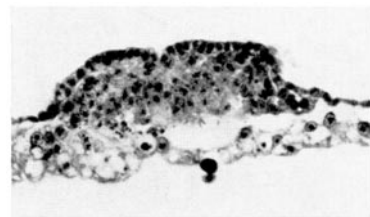


Fig. 1. Structure neuroïdale induite par le greffon qui a été laissé en contact avec l'ectoblaste durant 6 h. Le rempart vitellin s'est reconstitué au-dessous de la structure induite. Remarquer les éléments de la crête neurale (à droite de la Figure).

¹ Travail subventionné par le Fonds national suisse de la Recherche scientifique.

² A. G. JOHNEN, Proc. Acad. Sci., Amst. [C] 59 554 (1956).

³ A. G. JOHNEN, Arch. Entwickl.-Mech. Org. 153, 1 (1961).

⁴ A. G. JOHNEN, Arch. Entwickl.-Mech. Org. 155, 302 (1964).

⁵ H. DENIS, Ann. Soc. roy. zool. Belg. 87, 501 (1957).

⁶ J. GALLERA, J. Embryol. exp. Morph. 7, 487 (1959).

⁷ A. G. JOHNEN, Arch. Entwickl.-Mech. Org. 155, 314 (1964).

⁸ J. GALLERA et I. IVANOV, J. Embryol. exp. Morph. 12, 693 (1964).

⁹ D. A. T. NEW, J. Embryol. exp. Morph. 3, 326 (1955).

¹⁰ J. GALLERA et J. CASTRO-CORREIA, C.r. Soc. Biol. 154, 2014 (1960).