Columns 2 and 3 represent the average measurements (in mm) of neurosecretory cells

Stages	Cells	Nuclei
1-day-old	0.0075 × 0.006	0.003 × 0.003
3-day-old	0.012 × 0.009	0.0045 × 0.0045
5-day-old	0.0165 × 0.0075	0.0045 × 0.0045
8-day-old	0.0195 × 0.012	0.0075 × 0.0075
12-day-old	0.0225 × 0.018	0.0105 × 0.0075

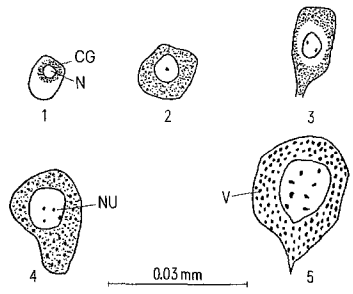

Fig. 1–5. Camera lucida diagrams of neurosecretory cells from the brain of female *S. bullata* at different stages.

production of larvae in the case of larviparous insects. The object of this brief communication is to throw some light on the correlation, if any, existing between the activity of the brains' neurosecretory cells and production of larvae in a larviparous flesh-fly *Sarcophaga bullata*.

Specimens of *S. bullata* were reared on mammalian kidney, sugar and water in the laboratory. The larvae pupate after 3 days of larval life. The pupal period lasts for 12 days. The adult females on their 11th/12th day of emergence from pupae lay larvae on the flesh. Brains from females of different ages were dissected out, fixed and processed for histological details, as also for the detection of nucleic acid(s), by standard methods[6,7].

The neurosecretory cells occur in many places in the brain of this insect but those in pars intercerebralis are more distinct. These cells, along with their nuclei, increase in size gradually as the fly becomes old (see Table).

The cellular activities, as observed during different stages of growth of the fly, can be inferred from the figures. In a 1-day-old fly the cytoplasmic granules (CG) are perinuclear in position, leaving a clear gap along the periphery of the cell (Figure). These become uniformly distributed in the extranuclear zone (Figure 2) in a 3-day-old fly. In a 5-day-old fly, these granules tend to accumulate on the periphery of the cell leaving a perinuclear gap (Figure 3). However, in a 8-day-old fly these granules, amidst which have meanwhile developed a few vesicles, appear to be uniformly distributed (Figure 4). The progressive increase in the number of vesicles, presumably at the expense of the granules, is marked hereafter, so much so that the whole of the cytoplasm of the neurosecretory cell becomes crammed with vesicles in the 12-days-old flies (Figure 5).

The nucleus (N) is without nucleolus in the first day of the emergence of the fly but in due course the nucleoli (NU) appear in variable number in different stages (Figures 2–5). Intense reaction for RNA in cytoplasmic constituents and nucleoli, and DNA in nuclear chromatins have been observed in all the stages.

In as much as the adult fly initiates and completes larvaelaying during the 11th–12th day of its emergence from the pupal phase, the observed activity of neurosecretory cells is seemingly correlated with the development and laying of larvae. The persistence of vesicles in the cells beyond the 12th-day stage indicates their implication in yet other metabolic activities.

The abundance of RNA in the neurosecretory cells of *S. bullata* suggests that synthesis of neurosecretory material is coupled with a high rate of protein synthesis. Similar results have been derived from work with *Periplaneta*[5,8].

Résumé. L'activité des cellules neurosécretrices dans le cerveau de la femelle de *Sarcophaga bullata* est en corrélation avec le développement des larves dans la femelle et leur position.

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A Radioautographic Study of Neural Induction in the Chick Embryo

GALLERA and OPRECHT[1] and LAVARACK[2] used histochemical technique to study the correlation between RNA content and morphogenesis in early chick embryos. They showed that the content of basophil cytoplasmic material was always high in morphologically active regions, e.g., Hensen's node and neural folds. LAVARACK[2] further showed that there was an increase in basophilia in the ventral portion of the neural groove adjacent to the notochord and that the intensity of the staining here was much greater than in the underlying notochord. These findings together with those obtained from studies on amphibiar embryos have led BRACHET[3,4] to suggest that induction involves a rather massive passage of ribonucleoprotein-containing particles (microsomes) from the inductor into the reacting tissue.

The present work was undertaken to examine radioautographically whether there is any marked transfer of

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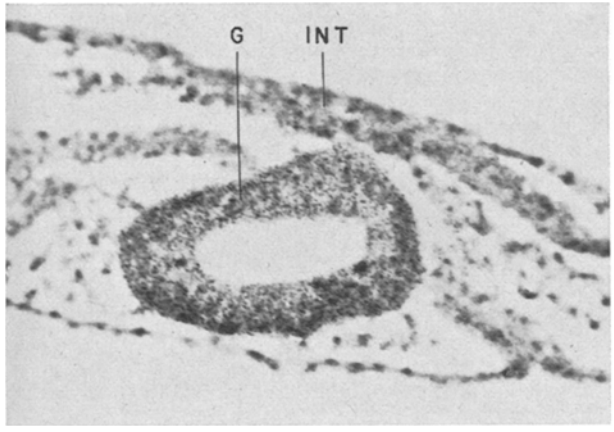

Fig. 1. A transverse section of a blastoderm with a labeled graft which has undergone self-differentiation and has induced a thickened neural ectoderm. The host axis is not included in the photograph. Note there is no appreciable amount of grains in the induced neural tissue. ×210. G, graft; INT, induced neural tissue.

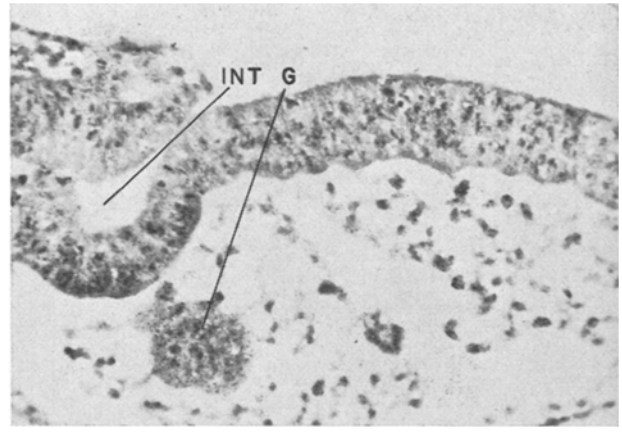

Fig. 2. A transverse section of a blastoderm with a labeled graft which has induced a well-developed neural tube, but has failed to undergo self-differentiation. The host axis is not included in the photograph. Note the induced neural tube has a relatively large number of grains on the side adjacent to the labeled graft but practically none on the other side. ×210. G, graft; INT, induced neural tissue.

RNA or part of it from the inductor (Hensen's node graft) to the competent tissue (ectoderm).

Materials and methods. Fertile hen's eggs were incubated at 37.5 °C for 16–19 h to obtain embryos at stages 3–4[5]. The embryos were cultured in vitro by SPRATT's[6] technique. A Ringer-albumen-agar medium[7] was used to culture all the embryos and will be referred to as the basic medium.

The grafts used in this study were prepared as follows: Isolated stage 3_+ blastoderms were grown on the basic medium containing 5 µc/ml uridine-H^3 (New England Nuclear Corp., Boston, Mass.) until they reached stage 4. The node area (size 0.3 × 0.3 mm) was excised and washed several times in chick Ringer's solution. The ectoderm was then removed from the isolated node area and the remainder (mesoderm with its underlying endoderm) was used as a graft. Each graft was inserted between the ectoderm and endoderm of a host embryo at stage 4 using GRABOWSKI's[8] technique. All the host embryos were grown for 19–21 h on the basic medium, washed several times in Ringer's solution, fixed in absolute ethanol-glacial acetic acid (3:1), embedded in paraffin, sectioned at 5 µm, and mounted on slides. The slides were placed in distilled water or in 80 µg/ml ribonuclease (Worthington Biochemical Co., Freehold, N.J.), incubated at 37 °C for 1 h, covered with Kodak NTB-2 liquid photographic emulsion using the dipping technique of KOPRIWA and LEBLOND[9], and exposed for 5–14 days at 4 °C. No noticeable increase in the number of grains in host tissues were found by increasing the exposure time. The emulsion was developed for 6 min in Kodak D-19 at 20 °C and fixed for 10 min in Kodak acid-fixer. The sections were stained with either hematoxylin-eosin or methyl green-pyronin, dehydrated through a graded ethanol series, and mounted in Permount.

Results. Of the 36 embryos, 25 showed neural inductions ranging from a thickened neural ectoderm to a well-developed neural tube (Figures 1 and 2). The induced neural tissue was often in close contact with the graft and was sometimes attached to the host neural tube. Those grafts, which failed to give recognizable neural induction, were usually surrounded by mesoderm and endoderm of the host. The overall results showed that there was no clear-cut correlation between the degree of differentiation and the inducing capacity of the graft. In all cases the grafts showed a high uridine-H^3 uptake (Figures 1 and 2). Ribonuclease digestion removed 60–70% of labeled material, the remainder being almost exclusively associated with the nuclei. The notochord, mesenchyme, and foregut associated with the induced neural tissue had a relatively large number of grains and thus appeared to be derived mainly from the graft. The host, except the portions which were in close contact with the graft (Figure 2), showed little or no labeled material. In addition, the concentration of grains in cells which had been induced to develop into neural tissue was similar to that in others which had failed to respond to the graft.

Discussion. This study showed, in contrast to BRACHET's[3,4] idea, that there was no massive transfer of RNA or part of it from the inductor to the competent tissue, regardless of whether or not an induction process took place. The possibilities remain, however, that 1. the inductively active RNA might be synthesized before or after the labeling period and this is transported during the time of incubation and 2. only a very small amount of RNA (undetectable by the radioautographic technique used in this study) might be necessary to trigger a sequence of reactions which result in the activation of appropriate gene(s) responsible for neural differentiation. On the other hand, SIRLIN et al.[10] and PANTELOURIS and MULHERKAR[11] showed that methionine-S^{35} was transferred, though not on a large scale, more actively from the labeled graft into cells which were induced to develop into neural tissue than into others which failed to respond to the graft. They concluded that there might be some selective migration of proteins from the inductor into the competent tissue. If this were the case the inductively active substance in ribonucleoprotein would be protein rather than RNA. Further work along this line is presently under investigation in our laboratory.

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Zusammenfassung. Gebiete aus dem Hensen'schen Knoten wurden implantiert, wobei dessen DNA-RNA mit Uridin-H^3 markiert wurde. Die induzierten Neuralanlagen enthielten kaum markierte Substanzen, und wenn, dann nur im Bereich der Kontaktstellen, woraus folgt, dass andere Substanzen für die Induktion verantwortlich sein müssen.

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Thrombin-Induced 'Macrophage Disappearance Reaction' in Mice

As previously shown in guinea-pigs, macrophages disappear from the peritoneal fluid of sensitized animals after reinjecting the animal with specific antigen[1]. This macrophage disappearance reaction (MDR) is one of the in vivo manifestations of delayed hypersensitivity. Endotoxin elicited a similar effect[1] which was thought to be the result of the natural hypersensitivity state to endotoxin[2,3]. FORBES[4] demonstrated that endotoxin treatment caused the disappearance of macrophages from the peritoneal fluid of mice, too. As anticoagulants, heparin and warfarin, inhibited the MDR in guinea-pigs[5], it was anticipated that the coagulation system, or at least some of its components, plays a role in this in vivo reaction. In the present study the effect of intraperitoneal thrombin injection was examined on the peritoneal differential cell count of mice.

Materials and methods. Throughout the experiments 8–14-week-old randomly bred male BALB/c mice were used, and in each series of experiment the treated and control animals were from the same age. At various intervals after giving thrombin or saline i.p., the animals were anesthesized with ether and the peritoneal cavity was washed out with 3 ml of TC 199 solution containing 5 units/ml heparin and 2% normal inactivated rabbit serum. Total and glass-adherent cell-count was determined. The glass-adherent cells were separated as follows: small chambers were mounted on microscope-slides; the walls of the chambers were formed by two half cover slips of 0.4 mm thickness and this was covered by a Bürker cover slip. The cell suspension was incubated in these chambers for 60 min at 37 °C in a humid atmosphere, then the cover-slips were removed and the slides were allowed to stand in a reverse position for 10 min in a TC 199 solution.

After staining with Giemsa-stain, absolute and differential cell counts were determined. The adherent mononuclear cells were regarded as macrophages and the number of lymphocytes was calculated from the difference of the total and adherent cell numbers.

For fibrinogen determination[6], the peritoneal cavity was washed out with 3 ml of citrate solution (0.38% Na-citrate, 0.8% NaCl).

Results and discussion. Table I shows that 2 (NIH) units of thrombin (Topostasin, Hoffmam-La Roche) did not appreciably decrease the peritoneal fibrinogen content, although the total cell count diminished, which could be prevented by heat-inactivating the thrombin. 10 U of thrombin significantly reduced both the fibrinogen level and the cell number.

Table II shows the effects of 2 and 10 U of thrombin on the differential cell count of the peritoneal cells. The great majority of the macrophages disappear from the peritoneal fluid, while the number of lymphocytes diminishes less markedly. The effect of 10 U of thrombin lasts at least for 5 h and after 28 h the cell picture becomes normal again (Table III).

Table I. Effect of thrombin on the peritoneal fibrinogen level and cell count

Treatment[a]	No. of animals	Fibrinogen (μg)	Cell-count ($\times 10^6$)
Salt	6	122 ± 8	14.2 ± 1.5
Thrombin, 2 U (inactivated[b])	6	127 ± 18	14.0 ± 1.2
Thrombin, 2 U	6	122 ± 36	10.5 ± 2.0
Salt	5	86.5 ± 6	13.9 ± 1.8
Thrombin, 10 U (inactivated[b])	5	114 ± 42	13.1 ± 2.4
Thrombin, 10 U	5	21 ± 7	8.3 ± 2.0

[a] 1 h before sacrificing the animals. [b] Thrombin was inactivated at 70 °C for 15 min.

Table II. Effect of thrombin on the peritoneal cell-count

Treatment[a]	Cell-count $\times 10^6$ per mouse				
	Total	Adherent	Macrophages	Lymphocytes	Granulocytes
Salt	16.9 ± 0.2	5.23 ± 0.65	4.90 ± 0.52	11.6 ± 2.5	0.33 ± 0.11
Thrombin, 2 U	8.4 ± 2.1	1.26 ± 0.26	1.01 ± 0.27	7.1 ± 1.9	0.25 ± 0.05
Thrombin, 10 U	8.1 ± 0.7	0.81 ± 0.16	0.57 ± 0.24	7.3 ± 0.5	0.24 ± 0.08

[a] 1 h before sacrificing the animals; 5 mice per group.

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