

compétence cérébrogène (d'ailleurs le contraire serait difficilement concevable puisque, dans le développement normal, l'induction du cerveau précède celle de la moelle). Etant donné que les effets de l'action inductrice

4	3	2	Stade des hôtes
16	26	18	Nombre des greffons nodaux jeunes (st. 3)
16 (100%)	26 (100%)	18 (100%)	Nombre d'inductions cérébrales
16	26	18	Nombre des greffons nodaux âgés (st. 5+)
3 (18,7%)	16 (61,5%)	12 (66,7%)	Nombre d'inductions médullaires

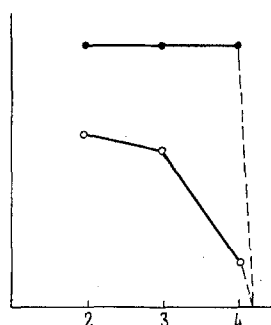


Fig. 4. Graphique représentant les fréquences des inductions cérébrales (●) et des inductions médullaires (○) en fonction de l'âge des blastodermes hôtes. Nos hôtes les plus âgés étaient au stade 4. Nous savons pourtant que, ce stade passé, la compétence neurogène de l'ectoblaste chute très brusquement, comme nous l'avons démontré dans un travail précédent (GALLERA et IVANOV<sup>6</sup>).

doivent dépendre à la fois du pouvoir inducteur du greffon et de la durée de son contact avec l'ectoblaste encore compétent, nos résultats, résumés dans le Tableau et visualisés par notre graphique, s'expliquent aisément.

Sans entrer dans les détails, ce qui dépasserait le cadre de cette note, rappelons que nos observations et conclusions concordent parfaitement avec les données expérimentales acquises de longue date chez les Amphibiens. En effet, chez ce groupe de Vertébrés, le flux inducteur libéré par la plaque préchordale, inducteur normal du cerveau antérieur, se propage plus largement et plus rapidement que les substances inductrices provenant de la région postérieure de la voûte archentérique, lesquelles sont responsables de la formation de la moelle épinière. Par conséquent, au moins chez les Amphibiens, l'induction du cerveau exige moins de temps que celle de la moelle (JOHNEN<sup>6-8</sup>).

**Summary.** Two grafts of the Hensen's node, one young (stage 3) and the other older (stage 5+), were implanted into area opaca of the same blastoderm. The hosts were at stages 2, 3 and 4. The young grafts always elicited the formation of the brain. On the contrary, old grafts only induce very small spinal cords. Moreover, the frequency of spinal cord induction is lowest in the oldest hosts.

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<sup>6</sup> A. G. JOHNEN, Arch. EntwMech. Org. 153, 1 (1961).

<sup>7</sup> A. G. JOHNEN, Arch. EntwMech. Org. 155, 314 (1964).

<sup>8</sup> Travail subventionné par le Fonds national suisse de la Recherche scientifique.

## The Dorsal Horn of the Avian Spinal Cord, a Re-Examination

In an earlier publication the substantia gelatinosa of the chick spinal cord was described as a well-defined band extending from the medial side of the dorsal horn, down the lateral side and ending near the base of the posterior column. The substantia was said to contain primarily small Nissl free neurons with an occasional large cell present (BRINKMAN and MARTIN<sup>1</sup>). This report will show that the area so described can be subdivided on the basis of morphological and embryological criteria into 2 distinct regions.

**Materials and methods.** Chick embryos and post hatched chicks were sacrificed and the spinal cords fixed in Carnoy's B fluid for Nissl staining or in De Castro fixative for block Cajal staining. The cords were embedded in paraplast, sectioned serially at 15  $\mu$  and mounted on glass slides.

Other 5-9-day-old chick embryos were labelled with thymidine H<sup>3</sup>, dose 10  $\mu$ c, and sacrificed 1-9 days later. The cords were then embedded in paraplast, sectioned serially at 5  $\mu$ , mounted on glass slides, pre-stained with Hematoxylin and processed via the dipping method for radioautography.

**Results.** In transverse sections of spinal cord a bundle of incoming fibers, presumably from the dorsal root, divides the gelatinous area into a large lateral and small

medial region (Figures 1 and 2). Morphologically these regions differ as follows: firstly the layer of cells capping the dorsal horn, Waldeyer's layer or Rexed's lamina I, does not extend medially beyond the incoming fiber bundle, thus the medial egg-shaped region abuts directly against the white matter of the posterior column; secondly the large neurons described earlier (BRINKMAN and MARTIN<sup>1</sup>), have been found only in the lateral region and thirdly the medial, unlike the lateral region, is traversed by a dense network of heavy fibers. Developmentally, differences were also noted in the time of appearance of neurons in the substantia. In radioautographs it was found that neurons in the medial region were formed between 5-6½ days of incubation while those of the lateral region were formed between days 6-8 (Figures 3 and 4).

**Discussion.** At this point it seems relevant to ask what constitutes the substantia gelatinosa? ROLANDO<sup>2</sup>, who first described this region, referred to it simply as the

<sup>1</sup> R. BRINKMAN and A. H. MARTIN, Experientia 25, 962 (1969).

<sup>2</sup> S. ROLANDO, cited in CLARK (1859).

posterior one-third of the dorsal horn. Later CLARKE<sup>3</sup>, whose description is generally accepted as the classical definition, narrowed the extent of the gelatinous substance to a thin curved lamina capping the dorsal horn. Recently REXED<sup>4</sup> published a cytoarchitectonic scheme for the spinal cord of the cat and a comparison of REXED's laminar organization and CLARKE's description of the substantia reveals that CLARKE included laminae I, II

and III. More recent investigations have established both a cytoarchitectonic and physiologic difference between laminae I and II and this has resulted in lamina I no longer being considered part of the substantia. Certain workers, in particular REXED<sup>4</sup> and RALSTON<sup>5</sup>, feel that the same line of reasoning can be applied to lamina III, i.e. the cytoarchitectonics and afferent connections of II and III are sufficiently different to warrant lamina II

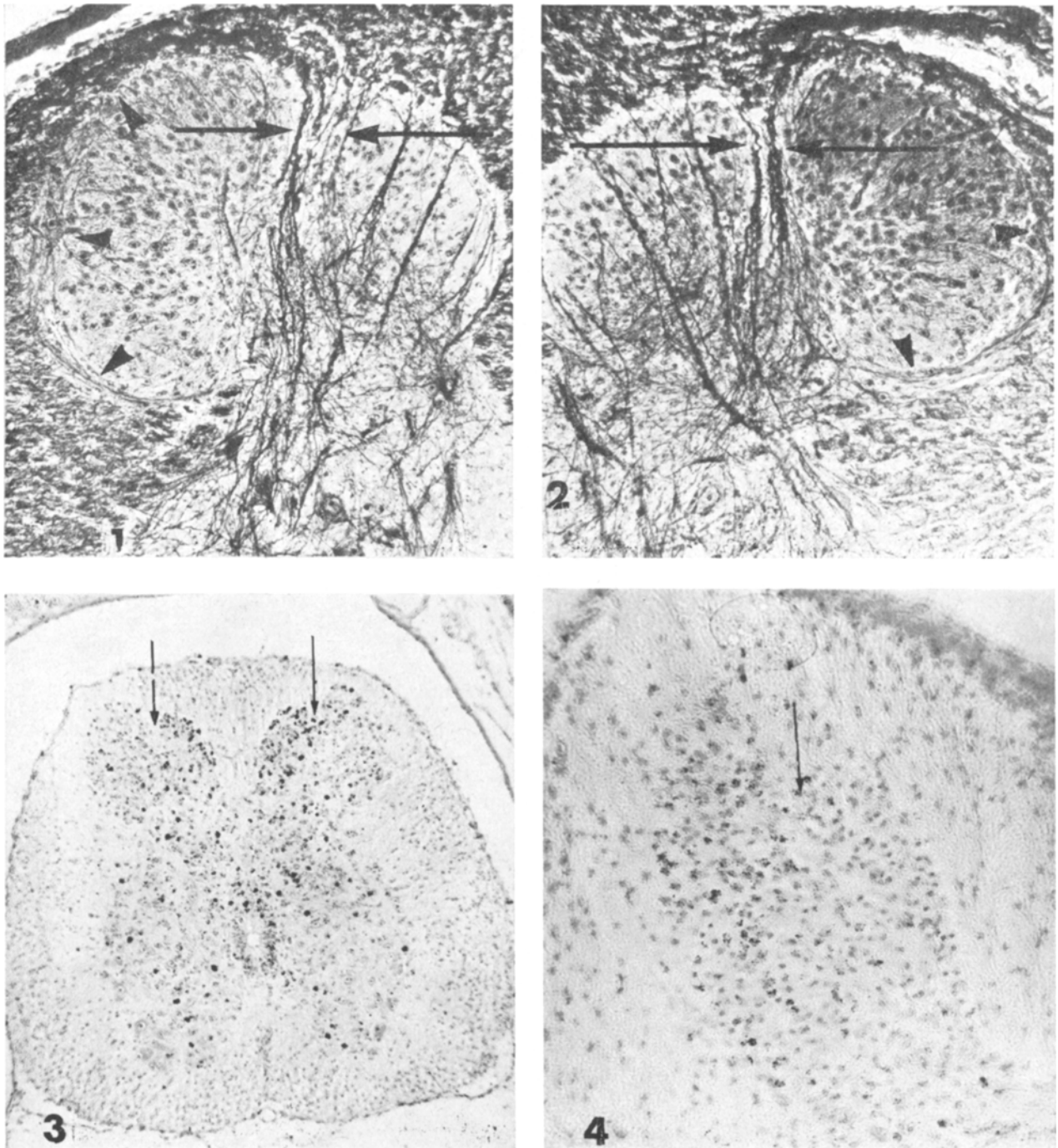


Fig. 1-4 are from the spinal cords of chick embryos incubated for 14-18 days.

Fig. 1 and 2 are transverse silver stained sections which show the incoming fiber bundle (arrows) and marginal cells (arrowheads). Fig. 3 and 4 are radioautographs in which labelled cells are seen in the medial region of the dorsal horn (Figure 3) and in the lateral region (Figure 4).

alone being designated as the substantia. Others, SZENTAGOTHAI<sup>6</sup>, and HEIMER and WALL<sup>7</sup>, grant that while there is a slight difference in the cytoarchitectonics of laminae II and III the fiber connections of these 2 laminae are so similar that they both must be included in a definition of the substantia. The results of the present study add little clarification to this problem except to indicate that the area of the chick cord designated the substantia can also be subdivided by differences in morphology and development into discrete lateral and medial regions.

While the boundaries of the substantia remain in question, there appears to be general agreement with the theory, postulated by SZENTAGOTHAI<sup>6</sup>, that the substantia is an intrinsic system. The findings of this study, however, reveal that the large neurons, found in the lateral region of the substantia, send their axons into the posterior commissure in the area of the cornu commissuralis of Marie (CAJAL<sup>8</sup>). That these axons may simply re-enter the dorsal horn at a more rostral level cannot at this time be disproved but it is interesting to speculate that the large neurons may in fact send their axons to higher brain centers. At present experiments, utilizing degeneration techniques, are underway to test this possibility<sup>9</sup>.

**Résumé.** Basée selon des critères morphologiques et embryologiques, la substance gélatineuse de la moëlle épinière des Oiseaux peut se diviser en régions latérale et médiale. On suppose que la substance gélatineuse peut être connectée avec les centres supérieurs du cerveau par les grands neurones que l'on a trouvé dans la région latérale.

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<sup>3</sup> J. L. CLARKE, Phil. Trans. R. Soc. 149, 437 (1959).

<sup>4</sup> B. REXED, J. comp. Neurol. 100, 297 (1954).

<sup>5</sup> H. J. RALSTON III, Z. Zellforsch. 67, 1 (1965).

<sup>6</sup> J. SZENTAGOTHAI, J. comp. Neurol. 122, 219 (1964).

<sup>7</sup> L. HEIMER and P. D. WALL, Expl. Brain Res. 6, 89 (1968).

<sup>8</sup> R. Y. CAJAL, *Histologie du Système Nerveux* (C.S.I.C. Madrid 1952).

<sup>9</sup> Supported by W.A.R.F. No. 135-4419.

## Acquisition of an Embryonal Biochemical Feature by Rat Hepatomas

Glutathionase splits glutathione (GSH) by  $\gamma$ -glutamyl transpeptidation<sup>1,2</sup>. The enzyme is present in high activity in the liver of some species, e.g. rabbits or guinea-pigs, but in the liver of some other species as e.g. rat, GSH-ase activity was not detected<sup>3,4</sup>. We demonstrated recently<sup>5,6</sup> a very high GSH-ase activity in various rat hepatomas, in contrast to normal adult rat liver where the activity was very low. Two methods were used for the assay of GSH-ase: 1) polarographic, using GSH as substrate and glycylglycine as the acceptor of the  $\gamma$ -glutamyl group<sup>5</sup>; 2) colorimetric, using a synthetic substrate  $\gamma$ -glutamyl-*p*-nitroanilide (Boehringer Mannheim Corp.), which liberates after the transfer of the  $\gamma$ -glutamyl group, a colored product *p*-nitroaniline<sup>7</sup>. The fact that high GSH-ase activity was observed in rat hepatomas which differed not only in regard to the chemical nature of the inducing carcinogens (3'-methyl, 4-dimethyl aminoazobenzene, DAB, and derivatives of 2-acetyl amino-fluorene), but also in regard to the rate of growth (from 4 days to 7 months between transfers) and the degree of differentiation, suggested that the activation of GSH-ase may be an intrinsic feature of rat liver carcinogenesis ('carcinotropic effect')<sup>8</sup>. In accord with this contention, no activation of GSH-ase was observed in regenerating or tumor-bearing rat liver<sup>5,6,8</sup>. Feeding carcinogenic 3'-Me,DAB (0.06%) in a semisynthetic diet increased GSH-ase activity markedly, while feeding non-carcinogenic 2-Me,DAB did not product any substantial change in this activity. Figure 1 shows these effects after 20 days of azodye feeding, using a polarographic assay.

Looking for the nature of GSH-ase activation during carcinogenesis in rat liver, we found that in distinction to adult rat, the embryonal and neonatal livers contained a very high GSH-ase. At 1 h after birth the activity on unit of weight basis was as high, and on unit of DNA almost as high, as in Morris Hepatoma 5123A; at 16 h it was even higher on dry weight basis, while on DNA basis it was slightly lower. The activity between 24-48 h seemed not

to change much, but then the activity rapidly declined; at 5 days after birth it was still several times higher than in normal adult rat liver, but 10 days after birth the activity was as low as in adult rat. Figure 2 illustrates this trend, using colorimetric method; polarographic method gave qualitatively the same results.

These observations indicate that as a result of malignant transformation, a neonatal biochemical feature is re-activated in rat liver, whereas differentiation during normal development leads to the suppression of this embryonic character. We do not know yet whether the activation of GSH-ase during rat liver carcinogenesis is simply a manifestation of the proliferation of primitive cells ('stem cells') which cannot differentiate<sup>9</sup> or whether there is a reactivation (derepression) in the parenchymal cells of a latent property which is normally repressed in the adult state. The first alternative is accessible to testing with a new histochemical reaction for  $\gamma$ -glutamyl transpeptidase<sup>10</sup> which showed some  $\gamma$ -G-ase ( $\gamma$ -glutamyl transpeptidase, GSH-ase) activity in endothelial cells of periportal vessels, in the bile duct epithelium and in Kupffer cells, while no activity was detected in hepatic

<sup>1</sup> C. S. HANES, G. H. DIXON and G. E. CONNELL, in *Glutathione* (Ed. S. COLOWICK; Academic Press, New York 1954), p. 145.

<sup>2</sup> H. WAELSCH, in *Glutathione* (Ed. S. COLOWICK; Academic Press, New York 1954), p. 151.

<sup>3</sup> E. E. CLIFFE and S. G. WALEY, Biochem. J. 79, 118 (1961).

<sup>4</sup> C. E. NEUBECK and C. V. SMYTHE, Arch. Bioch. 4, 443 (1944).

<sup>5</sup> S. FIALA and A. E. FIALA, Naturwissenschaften 56, 565 (1969).

<sup>6</sup> S. FIALA and M. D. REUBER, Gann 67, No. 3 (1970).

<sup>7</sup> M. ORLOWSKI and A. MEISTER, J. biol. Chem. 240, 338 (1965).

<sup>8</sup> S. FIALA, A. E. FIALA, B. DIXON and E. S. FIALA, in preparation.

<sup>9</sup> S. FIALA, Neoplasma 15, 607 (1968).

<sup>10</sup> A. M. RUTENBERG, H. KIM, J. W. FISCHBEIN, J. S. HANKER, H. L. WASSERKRUG and A. M. SELIGMAN, J. Histochem. Cytochem. 17, 517 (1969).