The amount of DNA was determined by the diphenylamine method[10]. Calf Thymus DNA Type 1 Sigma was used as a standard. In samples treated with DNase (Bovine Pancreas DNase I, Sigma) DNA was no longer detectable by the same method.

The protein content of granules was determined by the LOWRY method[11]. Bovine serum albumin (Fraction V, Sigma) was used as a standard. Estimations of the number of cells in analyzed areas refer to counts made on fixed and stained control preparations.

Owing to non-removal of the area opaca, the 0-h stage does not exactly correspond to the more advanced ones. However, this addition was necessary because of the difficulty in defining the areas exactly at this stage, and preliminary analyses had shown the DNA content/cell to be the same in both areas. Judging from electron micrographs of the stage in question, the resemblance between cells from the two areas is still manifest at this time, especially with regard to yolk granule content. Furthermore, the area pellucida comprises at least 55% of the total number of cells of the 0-h blastoderm, which implies that dissimilarities between the areas in the features analyzed here are likely to be minimal.

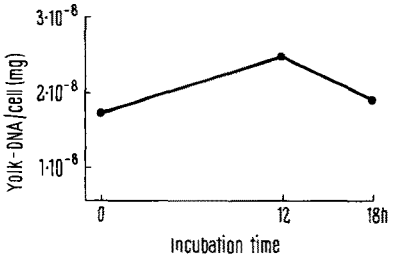


Fig. 3. Abscissa: Incubation time (h); ordinate: Yolk-DNA/cell (mg). Each point represents an average value from at least 300 embryos.

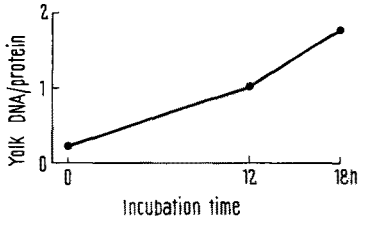


Fig. 4. Abscissa: Incubation time (h); ordinate: Yolk DNA/protein. Each point represents an average value from at least 300 embryos.

Figure 3 shows that during the period investigated the DNA present in the yolk granules undergoes an initial increase in amount followed by a decrease. This temporary increase at first seems puzzling, considering the cytological observation of a gradual disappearance of yolk granules from the developing embryo cells, but is in accordance with autoradiographical analyses which have revealed a considerable reduplication of yolk granule DNA during the very earliest hours of incubation[5]. As yet, however, there is no definite information as to whether this synthesis runs parallel with or is dependent upon nuclear DNA synthesis. Actually the amount of yolk granule DNA/cell at the 0-h stage corresponds to the total nuclear DNA content/cell at the same stage. It should be noticed that the decrease of yolk granule DNA is noticeable immediately before gastrulation in the chick embryo, which may indicate commitment in the morphogenetic events taking place during this process and/or in the subsequent organisation of early organ development. In Figure 4 is shown the result of protein analyses of the isolated yolk granules. The increase of the DNA/protein ratio – although large – seems natural and implies that, with degradation of the granules, utilization of the protein components proceeds more rapidly than the decline of DNA itself. This finding indicates some independence between DNA and protein in the granules. It may to some degree warrant the recent suggestion that yolk granule DNA may code for yolk catabolizing enzymes[8].

*Zusammenfassung.* Nachweis, dass der DNS-Gehalt der Dottergranula in Hühnerembryonalzellen in der frühesten Entwicklungsperiode (0–18 h) temporär ansteigt.

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## Histochemical Evidence for a Somatotopic Organization of the Rubrospinal Projection in the Rat

Loss of acetylcholinesterase (AchE) activity from the cell body of a neurone following severance or ligation of the axon has been reported by several authors [1–5, 7, 9, 11, 12]. The majority of such studies have involved the severance of axons in the peripheral part of the nervous system. The present study uses the thiocholine technique to demonstrate changes in the AchE content of neurones in the red nucleus of the rat following rubrospinal tractotomy.

Under Sodium Pentobarbital anaesthesia and using aseptic conditions, the rubrospinal tract was severed at the vertebral level C4 in one group of 11 rats and between the vertebral levels T9 and T13 in another group of 18 rats. After survival times ranging between 1 to 76 days the ani-

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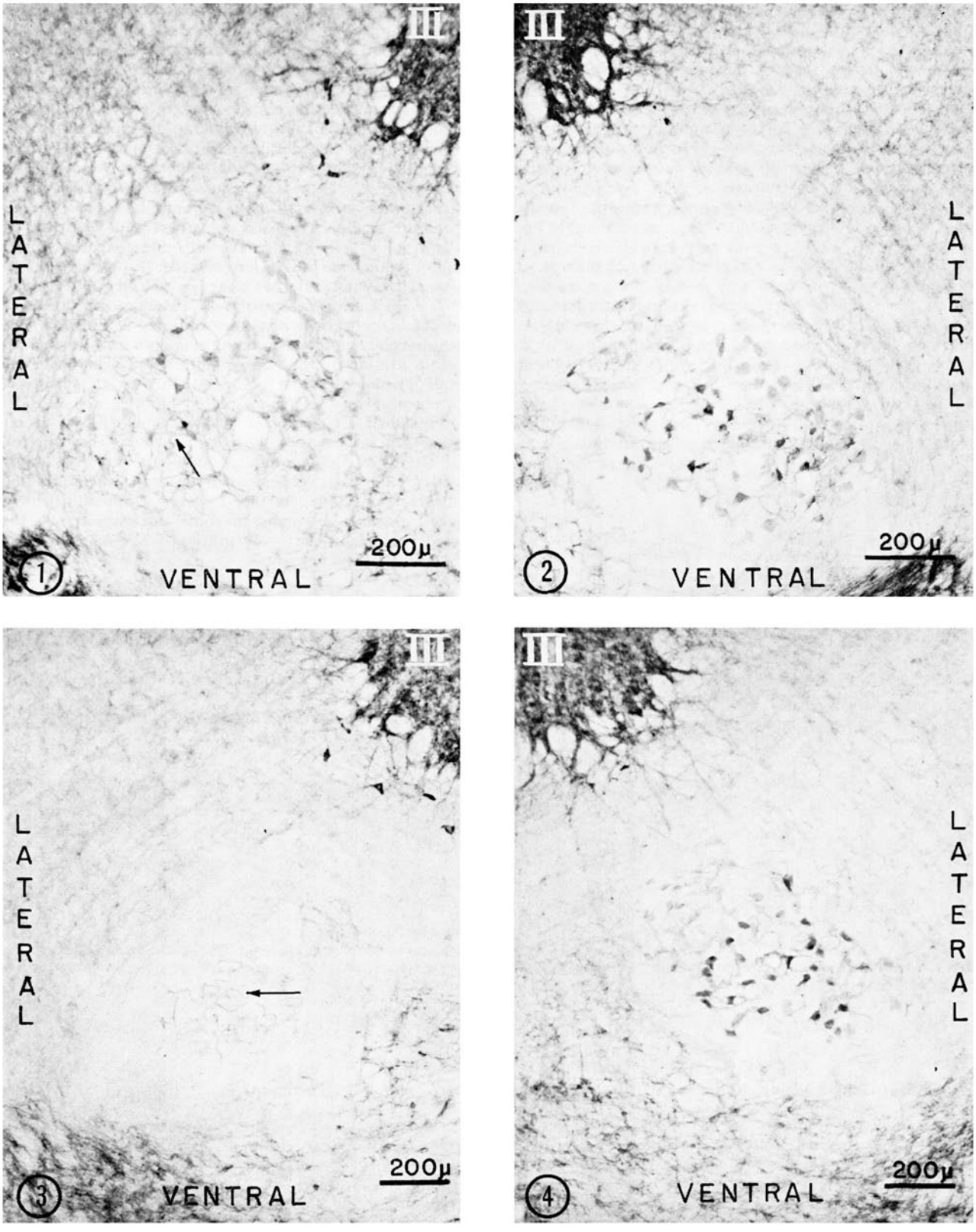


Transverse sections through the red nucleus of the rat stained for acetylcholinesterase (AchE) activity. III, oculomotor nucleus.

Fig. 1. Left (experimental) red nucleus of rat 32 days following severance of the right rubrospinal tract at the spinal cord level T9. Note reduction of cellular AchE activity in the ventral half of the nucleus (arrowed). Compare with control side.
Fig. 2. Right (control) red nucleus from same section as Figure 1.
Fig. 3. Left (experimental) red nucleus (arrowed) of rat 26 days following severance of the right rubrospinal tract at the spinal cord level C4. Note almost complete absence of cellular AchE activity in the nucleus at this level. Compare with control side.
Fig. 4. Right (control) red nucleus from same section as Figure 3.

mals were perfused with 10% formalin in isotonic sodium sulphate. The brain and spinal cord were removed and the portion of the spinal cord containing the lesion was embedded in paraffin wax. The extent of the lesion was verified in cresyl violet stained serial sections. After appropriate fixation frozen sections (80 μm thick) were cut from the midbrains in a plane transverse to the cranio-caudal axis. These sections were stained for AchE activity using the thiocholine technique of LEWIS[6]. Ethopropazine HCl was used to inhibit butyrylcholinesterase activity.

Using a projection microscope a comparison was made of the distribution of heavily and moderately stained cells in both red nuclei in each animal in which the rubrospinal tract had been severed. On the ipsilateral or control side these cells were seen to be restricted to the magnocellular caudal half of the nucleus.

Following low thoracic lesions a loss of cellular AchE activity was observed after 21 days survival and reached its maximal extent by 55 days. This loss of enzyme activity was restricted to cells in the ventral part of the caudal half of the contralateral red nucleus (compare Figure 1 and Figure 2). Following cervical lesions a loss of cellular AchE activity was observed after 4 days survival and was fully developed by 14 days. The loss of enzyme activity affected more cells in the caudal half of the contralateral nucleus, not only in the ventral part but also in more dorsal regions (compare Figure 3 and Figure 4).

It is thus possible to deduce that dorsal levels of the caudal half of the red nucleus project to between levels C4 and T8 of the spinal cord and that the more ventral part of the caudal half of the nucleus projects to between levels T9 and T13 of the spinal cord. POMPEIANO and BRODAL[8] have shown that the rubrospinal projection is somatotopically organized in the cat. The present study suggests that this may also be the case in the rat. This suggestion is at present under confirmation using retrograde degenerative techniques. SHUTE and LEWIS[10] have expressed the opinion that in the case of neurones whose axons lie entirely within the central nervous system no change could be reliably detected in the AchE content of the cell bodies following axotomy. At least in the red nucleus of the rat the present study shows this is not the case. The use of the thiocholine technique as a neuroanatomical tool adds an interesting dimension to cholinesterase histochemistry[13].

*Résumé.* La technique de l'acétylthiocholine démontrant l'activité de l'acétylcholinesterase fût employée pour indiquer la perte régionale d'enzyme des cellules nerveuse du noyau rouge du rat à la suite d'une lésion du faisceau rubrospinal. Les lesions aux niveaux thoraciques inférieurs du cordon médullaire donnent lieu à une perte d'enzyme des cellules dans la partie ventrale de la moitié caudale du noyau rouge. Cependant, les lésions du faisceau aux niveaux cervicaux du cordon médullaire donne lieu à une perte d'enzyme de la partie ventrale ainsi que de la partie dorsale de la moitié caudale du noyau. On suggère que cela indique une organization somatotopique de la projection rubro-spinale chez le rat.

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## Chromosomal Heteromorphism and Female Heterogamety in the Marbled Newt *Triturus marmoratus* (Latreille, 1800)[1]

Investigations on *Triturus marmoratus* lampbrush chromosomes have revealed that in all oocytes of the females studied in the present work bivalent I is characterized by a peculiar morphological asymmetry, which depends on the fact that the 2 partner chromosomes are not identical either in length or in the morphology of the lateral structures: actually some loops inserted at homologous sites show different sizes and matrix texture on the 2 partners: therefore they may be considered to be heteromorphic loops. Among these, the most constant heteromorphic loop is that made with dense matrix (indicated as b in Figure 1) which is inserted on the longer partner chromosome. In addition some globules appear in a heterozigous condition. Within the heteromorphic region chiasmata have not been observed in the several oocytes studied so far; 1, or at times 2 chiasmata are present on the left subterminal region which presumably corresponds to the short arm of the chromosome; occasionally, a single chiasma may also be encountered next to the right terminal region (Figure 2). Thus, the mean chiasma frequency for this bivalent is the lowest in the complement of the species, although the other 11 elements are shorter (Table).

The peculiar morphology of bivalent I from *T. marmoratus* is comparable to that already described in bivalent I from *T. cristatus*[2]. Since the 2 species may undoubtedly be considered closely related on the basis of both their phenotypic[3] and cytotaxonomic[4] characters, bivalent I of *T. marmoratus* can share the significance already ascribed to *T. cristatus* lampbrush bivalent I, i.e. it would consist of the 2 heterochromosomes Z and W[5].

Owing to these cytological observations, which seem in keeping also with the results yielded by recent studies on the lampbrush chromosomes of *Pleurodeles waltlii* and *P. poireti*[6,7], *T. marmoratus* would therefore be endowed with a genetic constitution ZZ (♂): ZW (♀). This interpretation obviously needs experimental support. Nonetheless, the ZZ (♂): ZW (♀) mechanism of sex determination has been already assessed within urodele amphibians – either by cytological studies or by experimental sex-reversal and further analysis of the sex ratio of the offspring of individuals with a sex-reversed phenotype (cf.[8]) – in the suborders[9] Cryptobranchoidea, Sala-

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