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### **QUANTITATIVE ANALYSIS OF THE MYELINATED AXONS IN THE FASCICULUS GRACILIS AND FASCICULUS CUNEATUS. LIGHT MICROSCOPY STUDY**

### **ANÁLISIS CUANTITATIVO DE LOS AXONES MIELINIZADOS EN LOS FASCÍCULOS GRACIL Y CUNEIFORME: ESTUDIO AL MICROSCOPIO ÓPTICO**

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**SUMMARY:** The fasciculus gracilis and fasciculus cuneatus of the spinal cord of mutant Wistar rats (*Rattus norvegicus*) were studied by light microscopy at the level of the first and sixth cervical segments in order to determine the extent of myelination at zero, 1, 3, 5, 10, 15, 30 and 120 days of postnatal life. The fasciculus gracilis was found to be practically nonmyelinated at birth, with effective myelination activity starting after the 5th day of age. At the 20th day of age, myelination was more than 50% completed compared to adult 120-day old animals. At birth, the fasciculus cuneatus had myelin fibers and always presented a greater extent of myelination than the fasciculus gracilis at each stage assessed. At the 20th day of age, more than 3/4 of fasciculus cuneatus were myelinated comparing to adult 120-day old animals at level of the first cervical segment. Thus, the myelination of the fasciculus gracilis and fasciculus cuneatus of the spinal cord of mutant Wistar rats was processed as the normal rat, demonstrating that the mutation of this animal, which expressed as a hairless skin phenotype, did not significantly affect the myelination process.

**KEY WORDS:** 1. Myelination; 2. Fasciculi gracilis and cuneatus; 3. Axons.

#### **INTRODUCTION**

The spinal cord is the portion of the central nervous system presenting the lowest degree of complexity in terms of structural organization (CROSBY et al., 1962).

During the process of nervous system maturation in vertebrates, many axons are enveloped by a myelin sheath (DUNCAN, 1934; BUNGE, 1968). The myelination of nerve fibers is an effective process for the maturation of the nervous system which starts during life, continues after birth and is related to the rapid neural development of the individual.

For adequate neuronal activity, a rapid conduction pathway for action potentials over long distances is essential. In vertebrates, the rapidity of nervous impulse conduction is achieved by myelin-sheathed large-caliber axons sheathed. Myelination is a pre-requisite for the rapid conduction of nervous impulses, and is therefore of high functional importance (SCHWAB & SCHNELL, 1989). Myelination favors an increase in the velocity of conduction of action potentials by permitting the jumping conduction of nervous impulses (STAMPFLI, 1954; REMAHL & HILDEBRAND, 1990).

Thus, myelination represents one more event in the direction of the functional emancipation of the nervous system, providing living beings with the full conditions needed to perform all the neural tasks of their species.

The objective of the present study was to estimate myelination of the fasciculus gracilis and fasciculus cuneatus of mutant *Rattus norvegicus* Wistar rats, at level of the first and sixth cervical segments from birth to 120 days of postnatal life.

## MATERIAL AND METHOD

The study was conducted on 27 mutant Wistar rats (*Rattus norvegicus*), examined groups of 3 at the following ages: zero, 1, 3, 5, 10, 15, 20, 30, and 120 days.

The animals were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg body weight) and perfused through the left ventricle with a heparinized saline solution, at room temperature for 10 minutes, followed by perfusion with a modified Karnovsky fixing solution, containing 3% glutaraldehyde and 3% paraformaldehyde in 0,1M sodium phosphate buffer pH 7,4, for 20 minutes.

The spinal cord was dissected, removed, and sectioned at the level of the first and sixth cervical segments and the fragments were immersed in the same fixative for 24 hours at 4°C. The material was then washed in 0,1M phosphate buffer, postfixed in 1% osmium tetroxide for 2 hours, contrasted *en bloc* with an uranyl acetate solution at 4°C overnight and then dehydrated in a growing ethanol series up to absolute concentration. The fragments were embedded in Spurr resin (SPURR, 1969) in a incubator at 60 to 70°C for three days.

The material was cut into 5µm thick sections and stained with toluidine blue. The slides were examined and photographed with a NIKON HFX photomicroscope. To calculate the percentage of myelination, 10 photomicrographs were obtained for each level (C1 and C6) and for each age studied, five of them for the fasciculus gracilis and five for the fasciculus cuneatus.

The criterion used to analyse the fasciculus cuneatus and fasciculus gracilis of the rat spinal cord, was to determine the percentage of the extent of myelination for each age investigated using direct counts on the photomicrographs obtained.

## RESULTS

The results are reported in relation to the amount of fibers present in the fasciculus gracilis and fasciculus cuneatus in rats at 120 days of postnatal age, which were considered to have maximal myelination (100%).

Few myelinated fibers were observed in the first and sixth cervical segment of the spinal cord on the day of birth ([Table I](#)). Analysis of the spinal cord at this stage revealed few fibers in the fasciculus cuneatus and a practically nonmyelinated fasciculus gracilis ([Fig. 1](#)).

On the third postnatal day there was little myelination ([Table I](#)) and in some cases both the fasciculus cuneatus and fasciculus gracilis were nonmyelinated, demonstrating that the advancement of the myelination process was negligible from the 1st to the 3rd day of postnatal life ([Table I](#)).

At five days of postnatal life, myelination was still mild ([Table I](#)), whereas at 10 days there was a considerable advance in myelination. The observations made at this age clearly revealed a greater concentration of myelinated fibers in the fasciculus cuneatus, demonstrating its precocity compared to the fasciculus gracilis ([Table I](#)).

At 15 days of postnatal age, the fasciculus gracilis and fasciculus cuneatus of the dorsal funiculus of the spinal cord of the rat could be distinguished in terms of extend of myelination and in terms of the differential caliber of their axons ([Table I](#)).

At 20 days of postnatal age, the fasciculus gracilis present more than 50% completed myelination at the C1 level and the fasciculus cuneatus had already exceeded this rate at both levels ([Table I](#)). The results observed at this age revealed a greater concentration of fibers in the fasciculus cuneatus, these fibers being thicker than those observed in the fasciculus gracilis ([Fig. 2](#)). At 30 days of postnatal age there was greater myelination and fibers with thicker myelin sheaths were present in the fasciculus cuneatus compared to the fasciculus gracilis.

The data obtained at the investigated ages indicate that the fasciculus cuneatus completes its myelination earlier than the fasciculus gracilis, both at the level of the first ([Fig. 3](#)) and sixth ([Fig. 4](#)) cervical segments.

Table I - Myelinated fibers (%) in the fasciculi gracilis and cuneatus at level of the first (C1) and sixth (C6) cervical segments of spinal cord of mutant rats since zero to 120 postnatal life, and their respective mean weights.

Age (days)	Mean weight (g)	Fasciculus gracilis		Fasciculus cuneatus	
		% fibers C1	% fibers C6	% fibers C1	% fibers C6
Zero	5.05	0.052	0.075	3.54	0.18
01	5.43	0.28	0.090	4.43	2.34
03	6.01	0.42	0.17	11.35	5.88
05	8.92	0.95	0.28	12.51	6.26
10	13.60	8.49	6.96	28.96	12.33
15	16.10	25.73	8.14	43.11	16.69
20	21.70	64.39	44.76	78.50	61.57
30	47.80	75.20	63.06	88.27	78.89
120	339.50	100.00	100.00	100.00	100.00

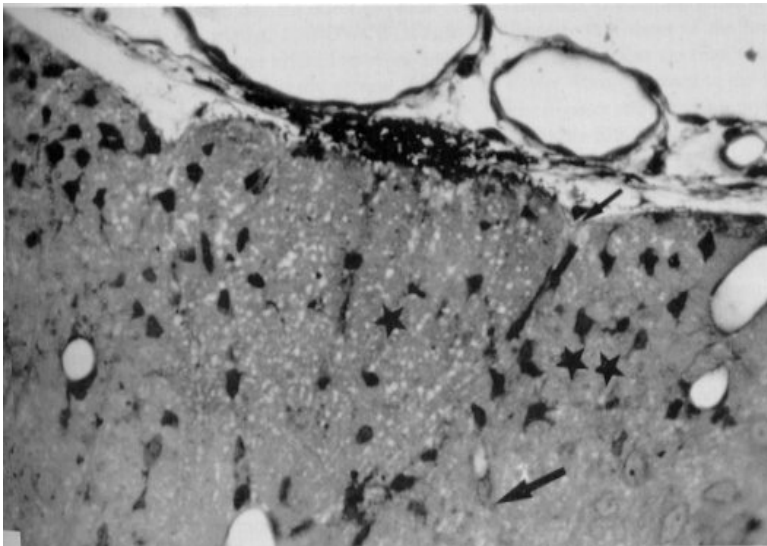


Fig. 1 - Photomicrograph of the fasciculus gracilis (\*) and fasciculus cuneatus (\*\*) at level of the sixth cervical segment of the spinal cord of a rat on the first postnatal day. It can be seen that the fasciculus cuneatus already presents axons with a fine myelin sheath (larger arrow). Complete absence of myelination is observed in the fasciculus gracilis, and the borderline region between fasciculi can also be seen smaller arrow). Toluidine Blue - X 240.

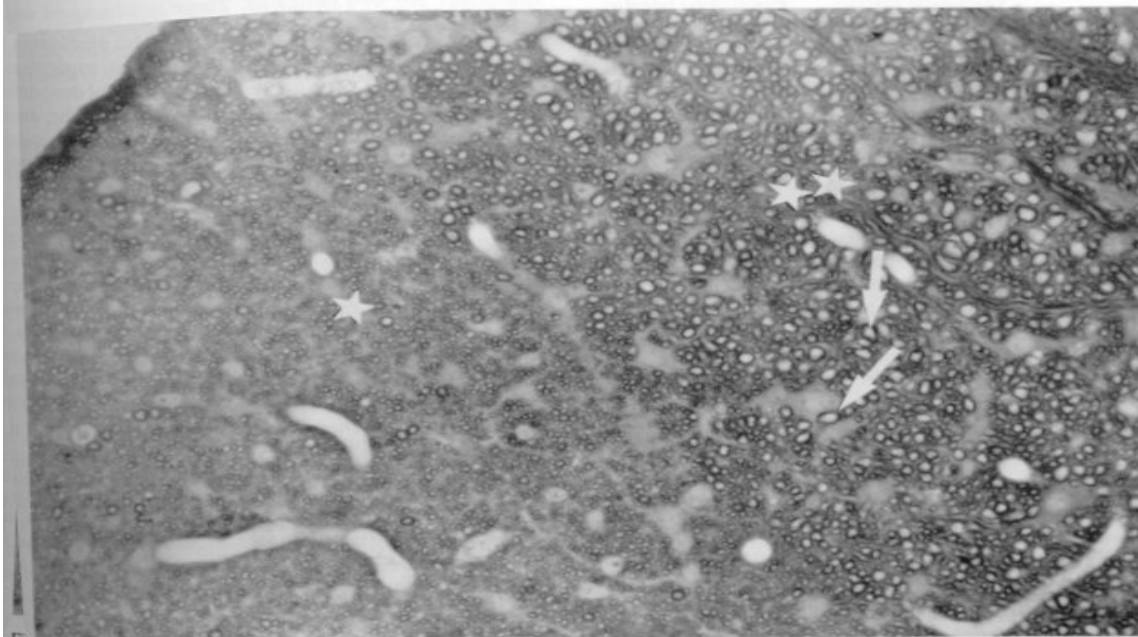


Fig. 2 - Photomicrograph of the fasciculus gracilis (\*) and fasciculus cuneatus (\*\*) in the sixth cervical segment of the spinal cord of the rat at 20 days of postnatal age. Greater axon thickness can be seen in the fasciculus cuneatus (arrows). Toluidine blue - X 240.

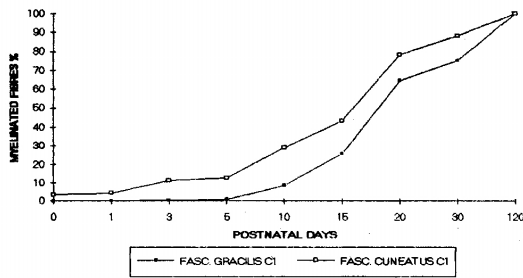


Fig. 3 - Graphic presentation of the percentage of myelinated nerve fibers of the fasciculus gracilis and fasciculus cuneatus at the level of the first cervical segment of the spinal cord of the rat from the day of birth to 120 days of postnatal life.

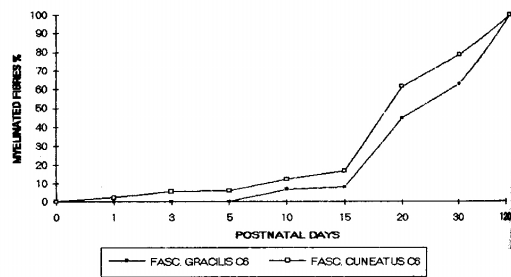


Fig. 4 - Graphic presentation of the percentage of myelinated nerve fibers of the fasciculus gracilis and fasciculus cuneatus at the level of the sixth cervical segment of the spinal cord of the rat from the day of birth to 120 days of postnatal life.

## DISCUSSION

On the basis of the present results, we may state that the acquisition of the myelin sheath by the nerve fibers of the fasciculus gracilis and fasciculus cuneatus of the dorsal funiculus in the first and sixth cervical segments of the spinal cord of the rat occurred at different periods. The myelination of these tracts occurs at relatively long intervals since part of the neural activities is acquired during the animal's permanence in the nest or during the period of strict dependence on the mother. It is known that animals that present little maternal dependence are the most precocious with respect to the full performance of the neural activity of the adult species.

We observed that the extent of myelination for each stage assessed was more advanced in the first than in the sixth cervical segment both in the fasciculus gracilis and in the fasciculus cuneatus. These results agree with those obtained by SCHWAB & SCHNELL who reported the apparent occurrence of a rostrocaudal gradient in the development of myelin of the spinal cord, regardless of whether the nature of the tract was ascending or descending. Due to this myelination gradient and to the earlier development of the fasciculus cuneatus, the anterior extremity of the animal acquires maturity for epicritical sensitivity before the posterior extremity.

Our results demonstrate that myelination starts before birth and continues thereafter since the presence of myelin is already observed at birth and is completed during postnatal development. These data agree with those reported by van der KNAAP et al (1991). Thus, at birth the animal already has some myelinated fibers but this contingent is not yet sufficient for full functional performance, which will become complete only with the advancement of the myelination process during the postnatal phase.

At birth, the myelination activity of the fasciculi is quite weak, with a mean percentage of myelination fibers of 3,54% in the first cervical segment and 0,18% for the sixth segment. No myelinated fibers were observed in some areas, whereas in others, few of such fibers were present. These results agree with those of LANGWORTHY & DUNCAN (1971) who reported that these fasciculi contains few myelinated fibers at birth since the real activity of myelin production is not present until the fifth postnatal day.

On the third postnatal day the effective phase of myelination has not yet started. LANGWORTHY & DUNCAN reported that from three to five days of postnatal life many myelinated fibers are already present. In our study, we found that at this time 12,51% of the fibers were already myelinated in the first cervical segment and 6,26% in the sixth.

At ten days of postnatal life the percentage of myelinated fibers was 28,96% in the first cervical segment and 12,33% in the sixth. The photomicrographs demonstrate that the myelination process has become considerable by this time.

At 15 days of postnatal life, the photomicrographs showed a high degree of myelination in the fasciculus cuneatus. At 20 days, the myelination of the fasciculus cuneatus was almost complete, with 78,50% myelinated fibers in the first cervical segment and 61,57% in the sixth. This implies that at this time more than 2/3 of the animal's fibers are myelinated in this fasciculus.

At 30 days most of the fibers in the fasciculus cuneatus were myelinated in both the first and sixth cervical segments, and at 120 days this fasciculus was fully myelinated.

For the fasciculus gracilis, we found a n average less than 0,1% myelinated fibers both in the first and in the sixth cervical segments on the day of birth. On the first postnatal day we found less than 0,5% myelinated fibers for both segments. During this phase, the fasciculus gracilis was practically nonmyelinated in both cervical segments studied. Negligible myelination was present in both cervical segments on the third day of postnatal life.

In their experiment, LANGWORTHY & DUNCAN also investigated the fasciculus gracilis and found that its axons were smaller in diameter than those of the fasciculus cuneatus in adult animals, and that the time sequence of myelination was slower when compared to the fasciculus cuneatus. These investigators also stated that myelination appeared from the third to the fifth day, with active myelin production occurring from the tenth to the 15th day of postnatal age, and that five days of postnatal age only nonmyelinated fibers could be observed. However, in the present study we obtained an average of 0,95% myelinated fibers in the first cervical segment and 0,28% in the sixth during the same stage. According to data obtained by SCHULTES (1991), myelination is intense at 15 days of postnatal life. In our study, we found a considerable number of myelinated fibers both in the first and sixth cervical segments. The quantitative results for the age of 20 days after birth demonstrate that more than 50% of the fibers are myelinated in the first cervical segment, and similar values are found in the sixth cervical segment. In their experiment, LANGWORTHY & DUNCAN found a high degree of myelination. With these values, at 20 days of age the animals already have a high degree of development of their epicritical neural activities.

At 30 days of postnatal life we observed more than 2/3 completed myelination both for the first and the sixth cervical segments. A photomicrograph of this age in the fasciculus gracilis reveals that this fasciculus is densely settled with myelinated fibers of wider diameter.

The present results demonstrate that the fasciculus cuneatus is more myelinated than the fasciculus gracilis since it always presented a higher extent of myelination at all analysed ages. These relations were observed both in the first cervical segment, where the fasciculus cuneatus presented a more advanced degree of myelination for each stage analyzed, and in the sixth cervical segment.

**RESUMEN:** Fueron estudiados los fascículos grácil y cuneiforme de la médula espinal del *Rattus norvegicus*, mutante de la línea Wistar a nivel del primer y sexto segmentos cervicales, empleando la microscopía óptica, con la finalidad de evaluar el grado de mielinización para las edades cero, 1, 3, 5, 10, 15, 20, 30, 120 días post-natal. Se observó que el fascículo grácil se presenta al nacimiento prácticamente amielínico, con gran actividad de mielinización ocurriendo posterior al quinto día. A los 20 días, a nivel del primer segmento cervical, más de la mitad de la mielinización ya se completó en relación a la mielinización del animal adulto con 120 días. Al nacimiento, el fascículo cuneiforme es portador de fibras mielinizadas, manteniéndose siempre con mayor grado de mielinización que el fascículo grácil para cada edad evaluada. A los 20 días, el fascículo cuneiforme, a nivel del primer segmento cervical, se presentó con más de 3/4 de la mielinización completa en relación al animal adulto de 120 días. En este estudio se observó que hay un gradiente de mielinización rostro-caudal, pues el grado de mielinización fue siempre mayor en el primer que en el sexto segmento cervical. Así, la mielinización de los fascículos grácil y cuneiforme de la médula espinal del *Rattus norvegicus* mutante de la línea Wistar, se presentó en su mayor parte, concordante con los resultados obtenidos por otros investigadores, demostrando que la mutación ocurrida en este animal, la cual se expresó bajo el fenotipo de piel glabra, no afectó de forma significativa el aspecto de la mielinización.

PALABRAS CLAVE: 1. Mielinización; 2. Fascículos grácil y cuneiforme; 3. Axón.

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