Recent developments in interferon research: Conclusion

H. K. Hochkeppel

Pharmaceutical Research Laboratories, Ciba-Geigy Ltd, CH-4002 Basel (Switzerland)

Even though we know today that interferons are involved not only in antiviral, but also in antiproliferative, antibacterial and antiparasitic defense mechanisms, we do not understand why these molecules may also be associated with or participate in certain diseases such as, for example, certain autoimmune diseases or AIDS. In addition, it is still a mystery why interferons, within the complex network of cytokines, stimulate certain cellular growth or differentiation processes and inhibit others. We are just beginning to open the black box of the mechanism of action and are far from understanding these complex events. This is partly due to the constantly growing cytokine network in which interferons are embedded and the tightly interlinked actions within the network, some members of which have only been described in recent years and are less thoroughly understood than interferon itself. The mechanism of action of interferon can no longer be studied in isolation from the other cytokines, which makes research in this field much more complicated than anticipated a few years ago. Interferons remain a fascinating group of molecules, within the superfamily of cytokines, and are very likely to provide as many surprises in the future as they have in the past.

Research Articles

Different intrafusal fiber composition of spindles in sheep and pig extraocular muscles[1]

G. Lalatta Costerbosa, P. A. Scapolo, A. M. Barazzoni, M. L. Lucchi and R. Bortolami

Institute of Veterinary Anatomy, University of Bologna, Via Belmeloro 12, I-40126 Bologna (Italy)
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Summary. Histochemical profiles of intrafusal fibers have been examined in muscle spindles of extraocular muscles of sheep and pig. Results show that in the sheep the intrafusal content presents, in addition to chain fibers, at least one bag_1 and one bag_2 fiber, whereas in the pig almost all the spindles are bag_1-fiber spindles.
Key words. Extraocular muscles; sheep; pig; histochemistry.

The extraocular muscles (EOMs) of both sheep and pig contain large numbers of muscle spindles[2-5]. The cell bodies of these stretch-sensitive afferents lie in the trigeminal ganglion and their localization has been established by retrograde transport of horseradish peroxidase and electrophysiological techniques[6,7]. The structure of EOM spindles in the sheep has been examined by Harker[8], and Kubota[9] has recently observed pig EOM spindles using the electron microscope. While examining the histochemistry of the fibers of EOMs of sheep and pig in our laboratory, we observed a difference between the intrafusal muscle fibers of the two species. The purpose of the present study was therefore to investigate and compare the histochemical profile of the intrafusal fibers in the EOM spindles of sheep and pig. Some morphological details about these spindles are also given. A preliminary report has been presented[10].

Materials and methods

The rectus superior (RS) and the obliquus superior (OS) muscles were rapidly dissected from 3 sheep and 3 pigs which had been sacrificed with an overdose of sodium pentobarbital. The muscles were frozen by immersion in isopentane cooled with liquid nitrogen. Serial cross sections were cut at 10 µm on a cryostat microtome at − 20 °C and processed for histochemical demonstration of myosin ATPase (m-ATPase) with alkaline and acid preincubation according to system A by Snow et al.[11]. Formalin fixation according to Hayashi and Freiman[12] was also performed on serial sections for 5 min at 4 °C, pH 7.8; these slides were then extensively rinsed prior to incubation (30 min at room temperature, pH 9.4). The nuclear bag_1, the nuclear bag_2 and the nuclear chain intrafusal muscle fibers were identified according to their staining properties with the two ATPase reactions[13].

Results and discussion

The RS and OS muscles presented a large number of spindles in both the species examined. There was a compartmentalization of receptors; spindles were found mostly in the region containing the highest concentration of small fibers, i.e. in the orbital region of the RS, and

along the whole outer side in the OS muscle. Several spindles showed some form of contact with one another, and either paired spindles or parallel spindles could be identified on the basis of the characteristics reviewed by Richmond and Abrahams[14] for the spindles of dorsal muscles of the cat neck.

Sheep. Seventy whole spindles were examined in both the RS and OS muscles (tables 1–3). Most spindles contained the complement of intrafusal fibers characteristic of muscle spindles (fig. 1): one or two nuclear bag_1 fibers which stained moderately darkly with acid ATPase but lightly with alkaline ATPase; one bag_2 fiber with both acid- and alkaline-stable ATPase activity; and several chain fibers which stained lightly with acid ATPase, whereas with alkaline ATPase they stained darkly. The bag_1 fiber was often thicker than the chain fibers but sometimes thinner than the bag_2 fiber. The characteristic staining profiles of all intrafusal fibers were consistent throughout the juxtaequatorial and intracapsular polar regions, with few exceptions. Some chain fibers failed to reverse their ATPase staining fully following acid preincubation, as was also recently described in rat limb skeletal muscles[15]. Occasionally the bag_1 fiber stained with a moderate intensity under conditions of alkaline preincubation at the intracapsular polar region. Staining for ATPase was totally absent in the equatorial region of both bag and chain fibers.

Pig. Seventy whole spindles were examined in both the RS and OS muscles. In almost all the spindles only one bag fiber was observable, together with a varying number of chain fibers (tables 1–3).

The histochemical profile of the unique bag fiber present in the EOMs of this animal is shown in figure 2. After acid preincubation the bag fiber exhibited a moderate to high ATPase activity. After preincubation at alkaline pH and after formalin fixation the bag fiber exhibited low ATPase activity; only occasionally, at the intracapsular polar region, the intensity of staining of the bag fiber for alkaline ATPase was moderate rather than low. Chain fibers in the pig displayed a pattern of staining for ATPase that was identical to that of the sheep spindle, including the fact that some chain fibers displayed an ATPase activity which was stable after both acid and alkaline preincubation (fig. 3).

It was also observed in both sheep and pig that with a certain frequency the capsule of the spindle protruded with an extension into the spindle to form septa between the intrafusal fibers (fig. 4). Such septa, however, never extended throughout the entire length of the spindle. They were already observed in EOMs of the sheep ultra-

Table 1. Intrafusal fiber contents of muscle spindles in RS muscle of the sheep and pig

	Bags	Chains																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	Total
Sheep																			
	1			1				1											2
	2		3	2	7	11	6	9	4	5	2		1					1	51
	3			1		2	1	7		1		1	1	1	1		1		17
	total		3	4	7	13	7	17	4	6	2	1	2	1	1		1	1	70
Pig	1	2	11	6	12	10	6	10	2	3	1	1	2				1		67
	2			1				1		1									3
	3																		
	total	2	11	7	12	10	6	11	2	4	1	1	2				1		70

Data shown here were taken from the juxtaequatorial region of the spindles.

Table 2. Intrafusal fiber contents of muscle spindles in OS muscle of the sheep and pig

	Bags	Chains																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	Total
Sheep																		
	1			2	1	3	1	2	2									11
	2	1	2	5	10	6	8	6	3	1		2				1		45
	3				1	3		2	1	1	1	1		1		2	1	14
	total	1	2	7	12	12	9	10	6	2	1	3		1		3	1	70
Pig	1	2	7	9	14	12	6	7	3	2	2		1	1	1	1		68
	2										1							1
	3									1								1
	total	2	7	9	14	12	6	7	3	3	3		1	1	1	1		70

Data shown here were taken from the juxtaequatorial region of the spindles.

Table 3. Number of intrafusal muscle fibers/spindle in the rectus superior and obliquus superior muscles of the sheep and pig

	Sheep		Pig	
	Bag	Chain	Bag	Chain
Rectus superior	2.214 ± 0.478	6.771 ± 3.084	1.043 ± 0.203	5.27 ± 2.893
Obliquus superior	2.043 ± 0.600	6.314 ± 3.250	1.042 ± 0.264	5.41 ± 2.975

Values are means ± SD. Data shown here were taken from the juxtaequatorial region of the spindles.

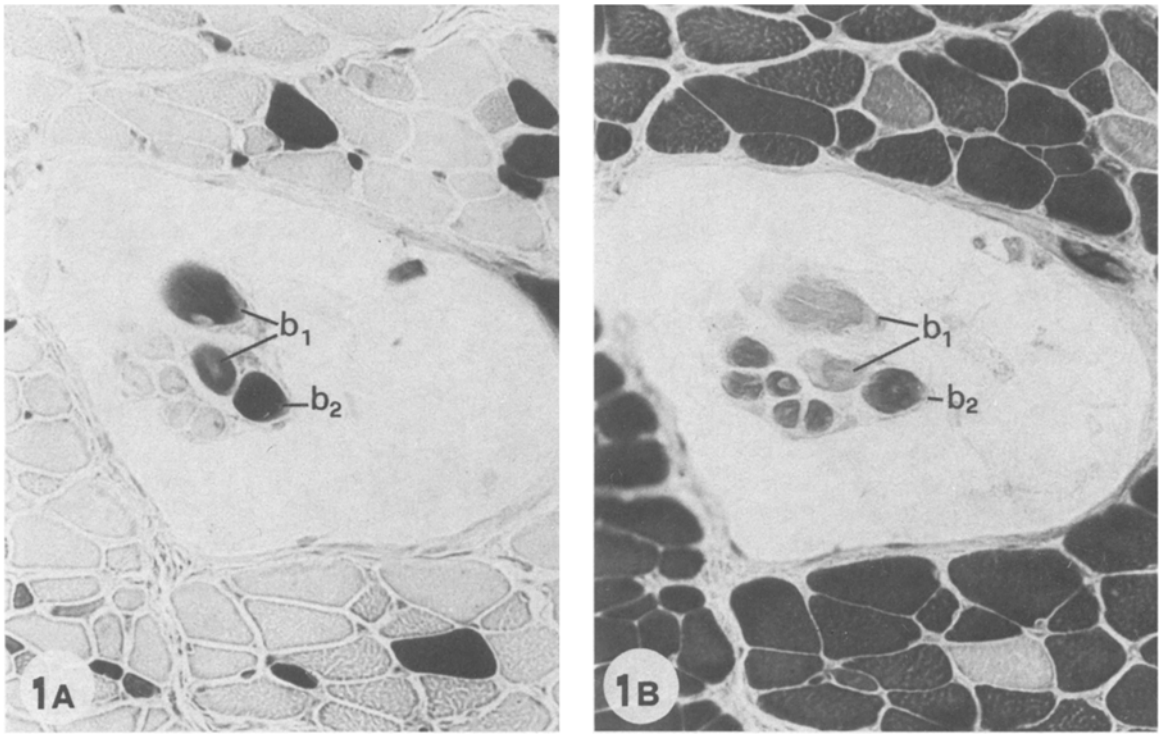


Figure 1. Transverse sections of sheep rectus superior muscle showing a spindle with two bag_1 (b_1), one bag_2 (b_2) and five chain fibers. *A* m-ATPase, pH 4.56 preincubation. *B* m-ATPase after formalin fixation. The unmarked intrafusal fibers are chain fibers. ×375.

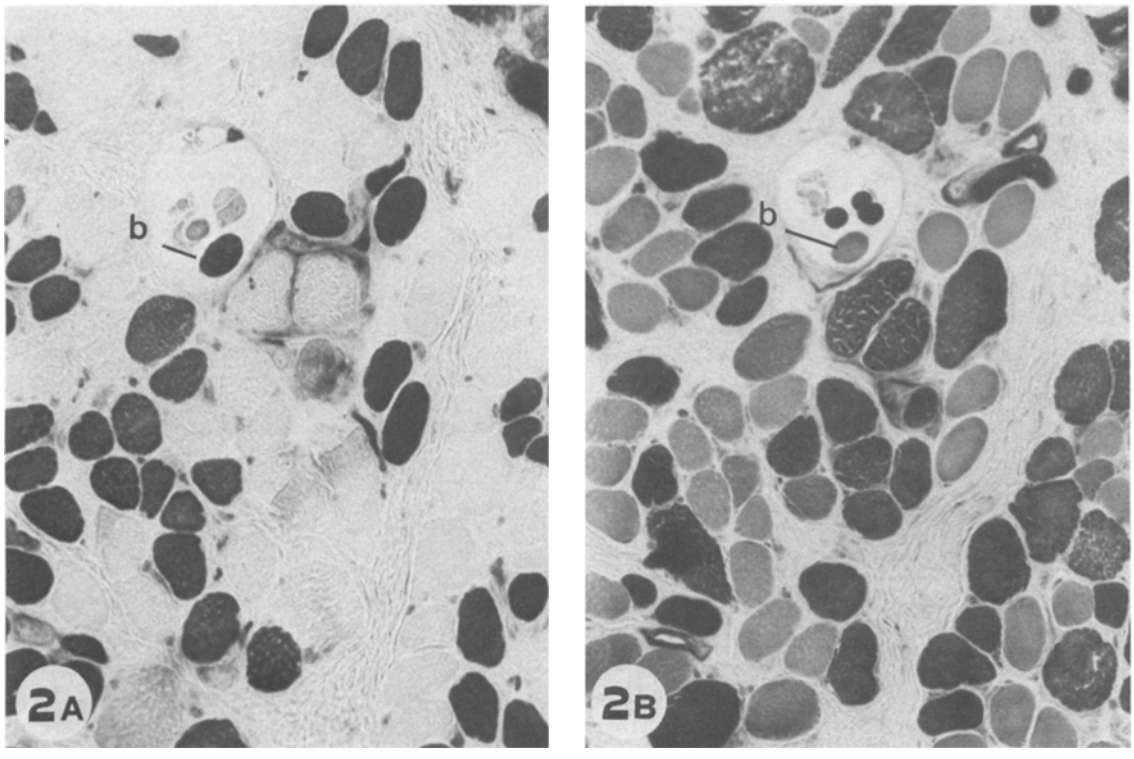
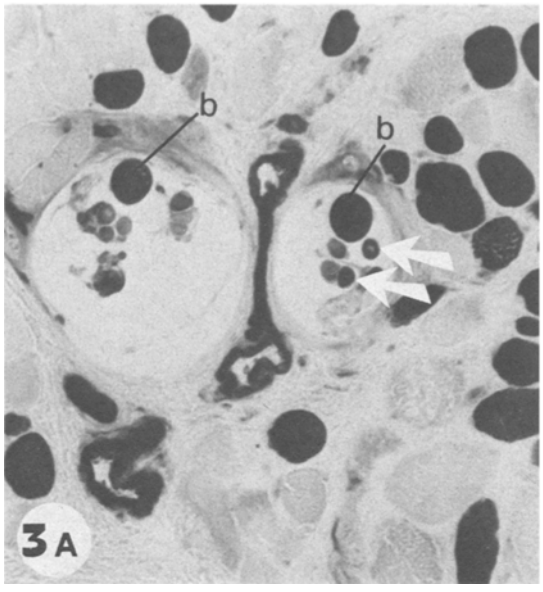


Figure 2. Transverse sections of pig obliquus superior muscle showing a spindle with one bag (b) and three chain fibers. *A* m-ATPase, pH 4.5 preincubation. *B* m-ATPase after formalin fixation. ×160.



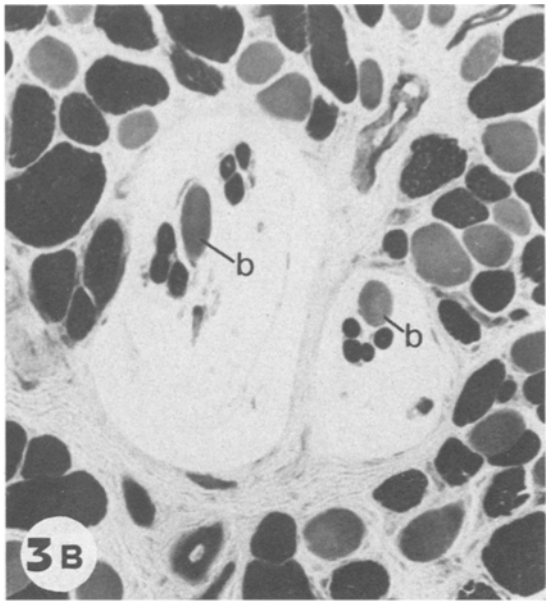
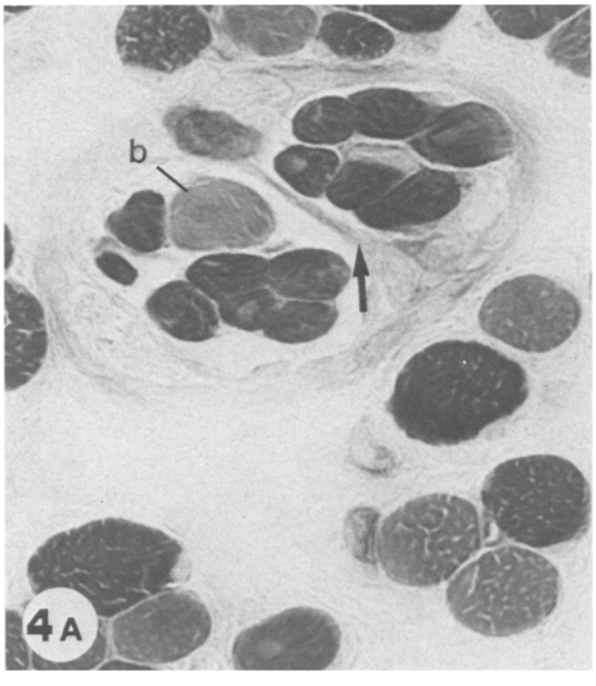


Figure 3. Transverse sections of pig obliquus superior muscle. Two paired spindles are visible. *A* m-ATPase, pH 4.6 preincubation. *B* m-ATPase after formalin fixation. Note in *A* some chain fibers (arrows) which fail to reverse their ATPase staining. b = bag fiber. ×160.



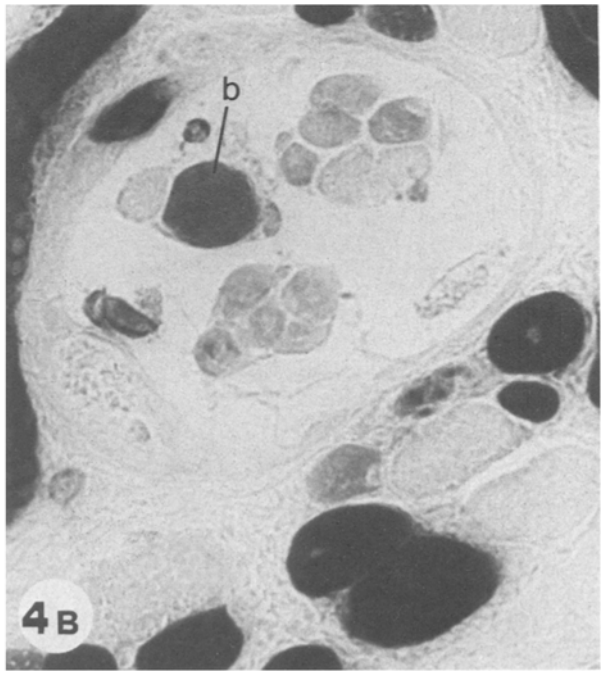


Figure 4. Transverse sections of pig rectus superior muscle spindle through intracapsular polar region *A* and juxtaequatorial region *B*. In *A* the capsule forms a septum (arrow) between intrafusal fibers. In *B* the septum is no longer visible. *A* m-ATPase, pH 10.2 preincubation. *B* m-ATPase, pH 4.55 preincubation. b = bag fiber. ×500.

structurally by Scalzi and Price[16] and have now been shown in the EOMs of pig as well. In the spindles of both the species studied it was noted that one or two chain fibers were often thin and short, and did not extend past the juxtaequatorial region, whereas all the other fibers extended beyond the ends of the spindle capsule, appearing as histochemically differentiable types of extrafusal fibers. In some cases, it was possible to note that in the intracapsular polar region either a bag or a chain fiber occupied a position that was more and more lateral and then appeared to leave the spindle, as shown in figure 5. In conclusion, the present results provide evidence that EOMs of the sheep and pig contain two different kinds of muscle spindle. In the sheep the spindle generally contains three types of intrafusal fibers that are comparable in their histochemistry to the bag_1, bag_2 and chain fibers

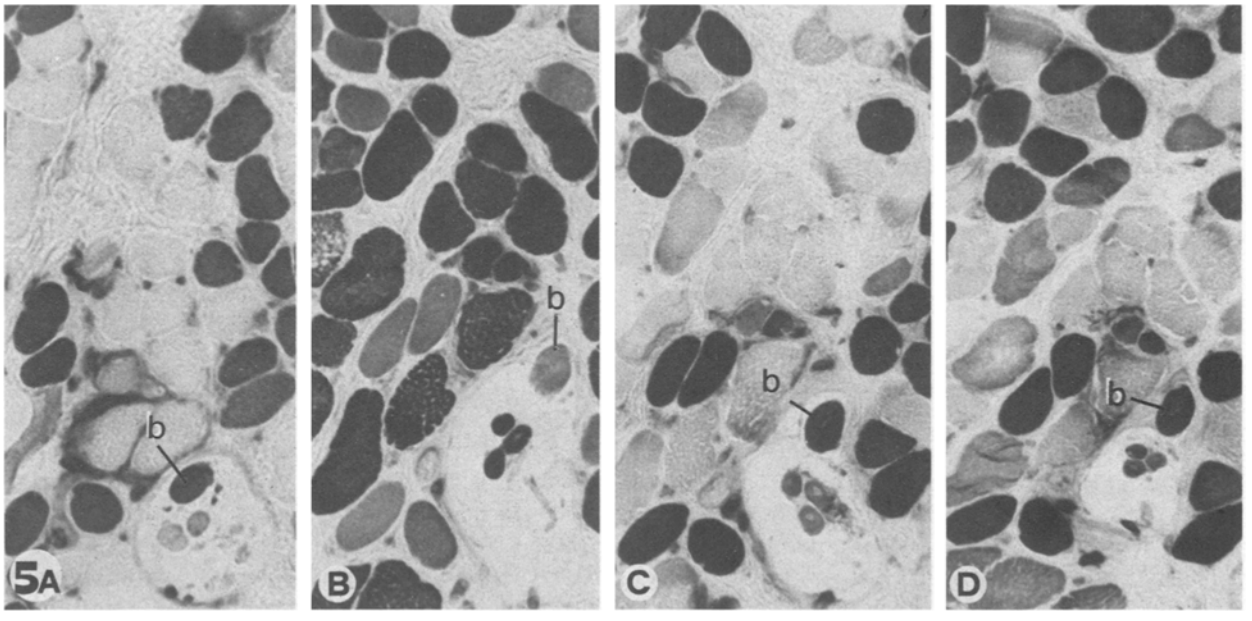


Figure 5. Transverse sections of pig obliquus superior muscle. Note that the spindle presents the bag fiber that moves laterally (*B* and *C*) and leaves the intracapsular space (*D*). *A* m-ATPase, pH 4.5. *B* m-ATPase, after formalin fixation. *C* m-ATPase, pH 4.65. *D* m-ATPase, pH 4.7. b = bag fiber. ×160.

in skeletal muscle spindles, whereas in the pig almost all the spindles contain a single bag fiber and a grouping of chain fibers. Since the unique bag fiber of the pig presents histochemical characteristics which do not completely fit those of bag_1 or bag_2 fibers, further studies will be necessary to better define which type of bag fiber is present in EOM spindles of the pig and its physiological significance.

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Binding property of rat and *Limulus* C-reactive proteins (CRP) to mercury

A. Agrawal and S. Bhattacharya

Environmental Toxicology Laboratory, Department of Zoology, School of Life Sciences, Visva-Bharati University, Santiniketan-731 235, West Bengal (India)
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Summary. The C-reactive proteins (CRP) from both rat and *Limulus* were found to bind mercury (Hg) in both in vivo and in vitro conditions. CRP has high-affinity binding sites for Hg as evidenced by the loss of free sulfhydryl groups, arrested mobility in polyacrylamide gel electrophoresis, and the consumption of CRP in the serum after Hg administration. The binding was tight as it could not be inhibited either by the addition of cysteine or EDTA. By using a direct titration method it was shown that binding of Hg to CRP was saturable at a molar ratio of Hg/CRP = 13.11. The possibility that CRP may act as a scavenger for Hg is discussed.
Key words. C-reactive protein; mercury; cell necrosis.