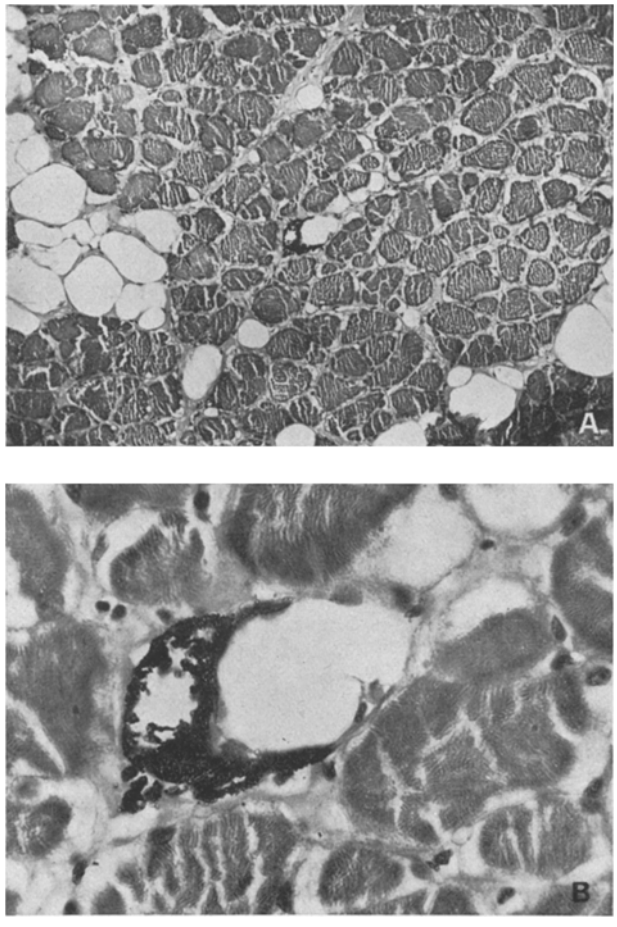
Electron Spin Resonance Spectra of White, Cardiac and Various Red Skeletal Muscles of the Carp, *Cyprinus carpio* L.

A number of experimental results suggest that the majority of free radicals associated with the enzyme reactions in tissues are localized in the mitochondria[1-3]. As the mitochondrial content is higher in the red muscles than in the white ones, these two types of muscle should give electron spin resonance (ESR) signals of different intensities. Such a discrepancy has already been observed between heart and skeletal muscle in rabbit[4], guinea-pig[5] and rat[6]. But these works have not taken into account the occurrence of two types of skeletal muscle. A re-examination of these results thus appeared desirable. In this work we have compared, by ESR spectrometry, various striated muscles of the carp in which differences in sarcoplasmic proteins have been investigated recently[7]. In fish, the mitochondrial content of the red muscle of the lateral line is much larger than that of the white muscles, according to electron microscopic studies[8]; the other red skeletal muscles appear to correspond to the same aerobic type of muscle[7]. The signals from several red skeletal muscles have been measured as well as those given by the cardiac and white muscles. In the course of this study, histological preparations have been made in order to correlate some of the results with the structure of the muscles.



Transverse section of the carp red muscle of the lateral line stained by hematoxylin-eosine. A) In the center of the picture, a blood capillary is situated among striated muscle fibres; empty lipid cells are also visible (×150). B) The same capillary is seen at a higher magnification (×750); it is surrounded by melanocytes.

Materials and methods. Mirror carps (*Cyprinus carpio* L.) weighing usually 2–2.5 kg were anaesthetized in a 75 mg/l solution of tricaïne methanesulfonate (MS-222 Sandoz) for 30 min. They were killed by decapitation and skinned. The following red skeletal muscles were dissected: the red muscle of the lateral line (m. superficialis trunci), the anterior supracarinales muscle situated between the head and the first dorsal fin, the adductor superficialis and abductor superficialis muscles of the pectoral fin, the ventral adductor of the tail. In the case of the red muscles of the pectoral fin, the colour of which decreases from the external to the internal part, the external reddest part was taken. For a more detailed account, the reader is referred to a previous publication[7]. As melanine gives a well-known strong ESR signal[4], dissection was carried out in order to avoid the interference of this pigment. The silvery dermal layer which sometimes remains attached to the red muscle of the lateral line and gives, according to some controls, a strong ESR signal, was discarded by removing the external part of this muscle. Samples weighing about 150 mg were immediately introduced into quartz tubes with a 3 mm diameter and dipped into liquid nitrogen. The spectra were taken with a Varian Associates type E-3 spectrometer using a cavity refrigerated at 110 °K. The field strength was set at 3260 G, with a scan range of ± 100 gauss. During scanning, the time constant of detection was 1 sec, and the scan time was 4 min. The modulation frequency was 100 kHz, and the amplitude 4 G. The microwave power was 5 mW. The receiver gain used was of 10^6. In view of the very low intensities recorded, the surfaces of the absorption curves were not evaluated but the peak heights were measured and corrected to 1 mg fresh tissue per mm of length. Small fragments of the superficialis trunci muscle and of the abduc-

Amplitudes of ESR signals of various carp muscles at 110 °K

	Number of samples	Arbitrary units and standard deviation
White muscle	8	0.9
Cardiac muscle	9	3.2 ± 0.6
Red skeletal muscles:		
Abductor superficialis	9	2.1 ± 0.4
Adductor superficialis	9	2.1 ± 0.5
Anterior supracarinales	8	3.1 ± 0.5
Ventral adductor of the tail	7	3.1 ± 1.0
Superficialis trunci	6	5.9 ± 1.8

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tor superficialis muscle of the pectoral fin were fixed in Bouin fluid. Paraffin sections were stained by hematoxylin-eosine. Discoloration by H_2O_2 has been used as a specific histochemical test for melanine.

Results. The amplitudes of the ESR signals, expressed in arbitrary units, are given in the Table. The intensity observed in the white muscle is too low to be significant. But the cardiac and the red skeletal muscles show signals which are easily distinguished from the background fluctuations. In the case of the adductor and abductor muscles of the pectoral fin, few determinations (not reported in the Table) have also been made on the internal parts which do not appear to contain myoglobin; the spectra did not differ from that of the white muscle. The amplitude of the ESR signals parallels, therefore, the intensity of the colour of the muscle and, according to a cross-section examination of the abductor muscle of the pectoral fin, the decrease in the fibre size. The figure obtained in the case of the red muscle of the lateral line is, however, much higher than that corresponding to the other red muscles.

Histological sections of the red muscle of the lateral line and of the abductor superficialis muscle of the pectoral fin have been compared. As shown in the Figure, the former contains pigmented cells. They are usually situated along blood capillaries but also occur along conjunctive tracts. These cells contain melanine granules which are discolored by H_2O_2. On the other hand, we did not observe any melanocytes in the abductor superficialis muscle. The high amplitude signal obtained for the red muscle of the lateral line is thus due to the fact that it contains melanine granules. It is interesting to note that in amphibians the presence of this pigment has been observed in the heart and various skeletal muscles[9]. In fish muscle, its localization thus seems to be much more restricted.

In conclusion, determinations of ESR signals together with histological observations have led us to note a peculiarity of the fish red muscle of the lateral line. This muscle differs from the other fish red muscles in the presence of some melanocytes located along its blood capillaries and conjunctive tracts. The other red skeletal muscles and the cardiac muscle give signals of about the same intensity which are definitely higher than the very low signal obtained for the white muscle. The parallelism usually observed between mitochondrial content and ESR signal intensity in various tissues appears valid also in the case of the two types of skeletal muscle[10].

Résumé. Les mesures de résonance paramagnétique électronique effectuées jusqu'à présent sur le muscle n'ont pas tenu compte de la différentiation de ce tissu en muscles blancs et rouges. Nous avons comparé les signaux du muscle blanc, du muscle cardiaque et de divers muscles rouges de carpe. Le muscle blanc présente une très faible intensité; le myocarde et différents muscles rouges donnent des valeurs plus élevées en accord avec leur plus grande teneur en mitochondries. Le muscle rouge de la ligne latérale se distingue toutefois des autres muscles rouges par un signal environ 2 × plus intense dû, comme le montrent des examens histologiques, à la présence dans ce cas de mélanocytes situés le long de faisceaux conjonctifs et de capillaires sanguins.

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Enzymatic Hydroxylation of Anthranilamide in Rat Liver

A new metabolic pathway from anthranilic acid, one of the tryptophan metabolites, to conjugates of 5-OH-anthranilamide and 3-OH-anthranilamide was suggested to be present by the authors.

Anthranilamide has been isolated and identified as a new metabolite of anthranilic acid in our laboratory[1,2] and when anthranilamide was injected to the rat, two main metabolites supposed to be conjugates of 5-OH-anthranilamide and 3-OH-anthranilamide[3], could be also observed in organs such as liver and kidney as well as in urine. Considering the above discoveries, it is of interest to study the hydroxylation and the conjugate formation of the anthranilamide.

It has been reported by KASHIWAMATA et al.[4] that an enzyme which is capable of hydroxylating anthranilic acid to 5-OH-anthranilic acid is present in the microsome of rabbit liver, but the hydroxylation activity in that of rat liver was found to be negligible[5].

An *Achromobacter* sp. has been shown be able to oxidize anthranilic acid via 5-OH-anthranilic acid by LADD[6], and CAIN[7] also reported that in *Nocardia opaca*, 5-OH-anthranilic acid was an metabolite of anthranilic acid.

The hydroxyl-derivatives of anthranilamide has been observed in the urine of rabbit by BRAY et al[8] and in both liver and urine of rat by SUTAMIHARDJA et al.[3], but the enzymatic hydroxylation in vitro could not be demonstrated until now.

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