RVATION OF ABNORMAL MITOCHONDRIA IN WHITE MUSCLE FIBRES OF RN⁻ CARRIER PIGS

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MARY

The RN⁻ gene affects meat quality by increasing the glycogen content of muscle. Glycogen localization and myofibre ultrastructure udied in the *Longissimus dorsi* muscle from pigs suspected to be RN⁻ carriers (called RN⁻ pigs) and pigs considered as normal (called S). White fibres from RN⁻ pigs showed an excess of glycogen in the sarcoplasm and exhibited abnormal mitochondria. Mitochondria ed scattered particles of glycogen or showed disorganised cristae. No abnormality was visible in red fibres.

Oxygen uptake was measured polarographically using a Clark electrode at 25 °C on mitochondria isolated from *Longissimus dorsi* s of rn⁺ pigs and RN⁻ pigs. Citrate synthase activity was determined. The respiratory control tended to be higher in mitochondria isolated N⁻ pigs as compared to rn⁺ pigs (mean values were respectively 2,5 and 1,8). Respiratory activity was higher in mitochondria from RN⁻ Citrate synthase activity was 0,20 µkat / g of fresh muscle in RN⁻ pigs and 0,14 µkat / g in rn⁺ pigs. Thus, the RN⁻ gene seems to be ated with some alterations in the glycolytic and oxidative metabolisms of skeletal muscle. However, the primary cause of these alities remain unclear.

RODUCTION

The RN⁻ gene affects meat by increasing the glycogen content of muscle, inducing the production of "acid meat" (Monin, 1989 ; dez *et al*, 1992). Glycogen levels are increased by about 70 p.cent in white muscles from RN⁻ carrier pigs (referred to as RN⁻ pigs), as ed with rn⁺rn⁺ homozygotes (referred to as rn⁺ pigs). A comparative ultrastructural study of muscle tissue from RN⁻ and rn⁺ pigs has that the difference in glycogen level was mainly observed in white fibres. The distribution of glycogen particles was similar in tissues oth RN⁻ and rn⁺ pigs but, both amount and density of the glycogen particles were higher in white muscle fibres of RN⁻ pigs as compared n⁺ pigs (Estrade *et al*, 1992). Moreover, some mitochondria showed abnormal ultrastructure in white fibres. These mitochondria owed scattered particles of glycogen and disorganized cristae. The aim of this study was to assess the possible relationship between structural abnormalities of mitochondria, and the functional activity of these organelles in order to verify if the oxidative metabolism is ted by the RN⁻ gene. The mitochondrial respiratory capacity was evaluated on mitochondria isolated from *Longissimus dorsi* muscle of od of rn⁺ pigs. Two different substrates (glutamate plus malate or succinate) were used, each of them providing electrons to a specific site respiratory chain .

TERIAL AND METHODS

Animals. The glycolytic potential was determined on muscle biopsies from 25 pigs of an experimental herd including RN⁻ pigs. Six RN⁻ pigs h glycolytic potential between 226 and 269 µmol / g fresh muscle and 6 normal pigs with a glycolytic potential between 108 and 157 µmol sh muscle were retained for the study.

Animals were slaughtered by pairs at around 100kg liveweight by electronarcosis and exsanguination in an experimental slaughterhouse, pair including 1 RN⁻ pig and 1 rn⁺ pig. Around 50 g of *Longissimus dorsi* muscle were taken for preparation of mitochondria; 2 g were ed in liquid nitrogen and kept at - 80°C for citrate synthase activity determination. Muscle samples were taken by shot biopsy on 8 mal pigs, i.e 4 RN⁻ pigs and 4 rn⁺ pigs, for microscopic study.

Electron microscopy. Small blocks (1-2 mm^3) were cut from the muscle biopsies, fixed for 2 h at 4°C in 2.5 % glutaraldehyde in 0.1 M phosphate buffer pH 7.0 and post-fixed in 1 % osmium tetroxide in the same buffer for 1 h at 4°C. The specimens were dehydrated through an

ethanol gradient and embedded in epoxy resin. Glycogen was stained on ultrathin sections by the acid-thiocarbohydrazide (TCH)-proteinate method (PATAg) according to Thiéry (1967).

The specificity of the staining reaction for polysaccharides was evaluated by omitting the oxidation by periodic acid in control sections. observations were made with a Philips EM 400 Electron microscope at an accelerating voltage of 80 KV.

Preparation of mitochondria. About 15 min after slaughter, 50 g of *Longissimus dorsi* muscle were removed from the animal quickly immersed in 200 ml homogenizing buffer (saccharose, 0.15 M ; mannitol, 0.1 M ; tris HCl, 20 mM ; EDTA, 1 mM ; BSA, 0.1 7.5). All operations were carried out at 1-4°C. Five grams of muscle were dissected free of fat and connective tissue and minced in 20 ml same buffer with a domestic mincer for 15 sec, and homogenized using a Potter. The homogenate was filtered on a sieve and centrifuged x *g* for 10 min. The supernatant was centrifuged at 10000 x *g* for 15 min. The pellet was suspended in 20 ml homogenezing buffe centrifuged at 1000 x *g* for 15 min. The pellet containing the mitochondria was suspended in 1 ml of buffer.

Mitochondrial Respiration. The oxidation of various substrates was measured polarographically at 25°C with a Clark electrode. Abo µl of mitochondria pellet were mixed in a medium comprising : 0.15 M saccharose ; 0.1 M mannitol ; 20 mM tris HCl ; 1 mM EDTA ; KH2PO4 ; MgCl2,6H2O 5mM; pH 7.5 and the substrate in a total volume of 1.2 ml. The respiratory capacity was determined in the pre of pyruvate plus malate or succinate (final concentration 0.5M) and ADP at the concentration of 20 mM. The respiratory control index was calculated by dividing the rate of oxygen utilization in state 3 (in the presence of ADP) by the rate in state 4 (after exhaustion of AD phosphorylation) as described by Chance and Williams (1956). The basal metabolic rate (mitochondrial state 4 respiration) was expres nat.O /min /mg prot. The protein concentration was estimated by the method of Bradford (1976).

Citrate Synthase Activity. One gram of frozen muscle was homogenized in 19 ml of buffer (63 mM glycylglycine ; 500 mM saccha 6.2 mM EDTA ; 125 mM NaF ; 5 mM dithiothreitol ; pH 7.4). Citrate synthase activity was determined on the supernatant obtained centrifugation of the homogenate, by the technique of Srere (1969).

RESULTS

Electron microscopy. White fibres of muscle from RN^- pigs showed abnormal mitochondria, surrounded by an excess of gly granules (Fig.1). Some mitochondria contained glycogen particles (Fig.1a). These particles were scattered and free or enclosed in vesicles. Other mitochondria showed irregular cristae (Fig.1b). The proportion of these abnormalities was variable from one fibre to anoth some white fibres, all mitochondria were seen with disorganised cristae, with a low proportion of them containing glycogen particles. fibres of RN^- pigs, as well as in both types of fibres in rn^+ pigs, no abnormal mitochondria was found.

Mitochondrial respiration. Fig 2 illustrates the RCI values for the oxidation of glutamate plus malate or of succinate by mitochondria RN^- and rn^+ pigs. No significant difference in the value of the RCI was observed between mitochondria from RN^- and rn^+ pigs. Neverth RCI was slightly higher in mitochondria of RN^- pigs for oxidation of both substrates as compared with rn^+ pigs.

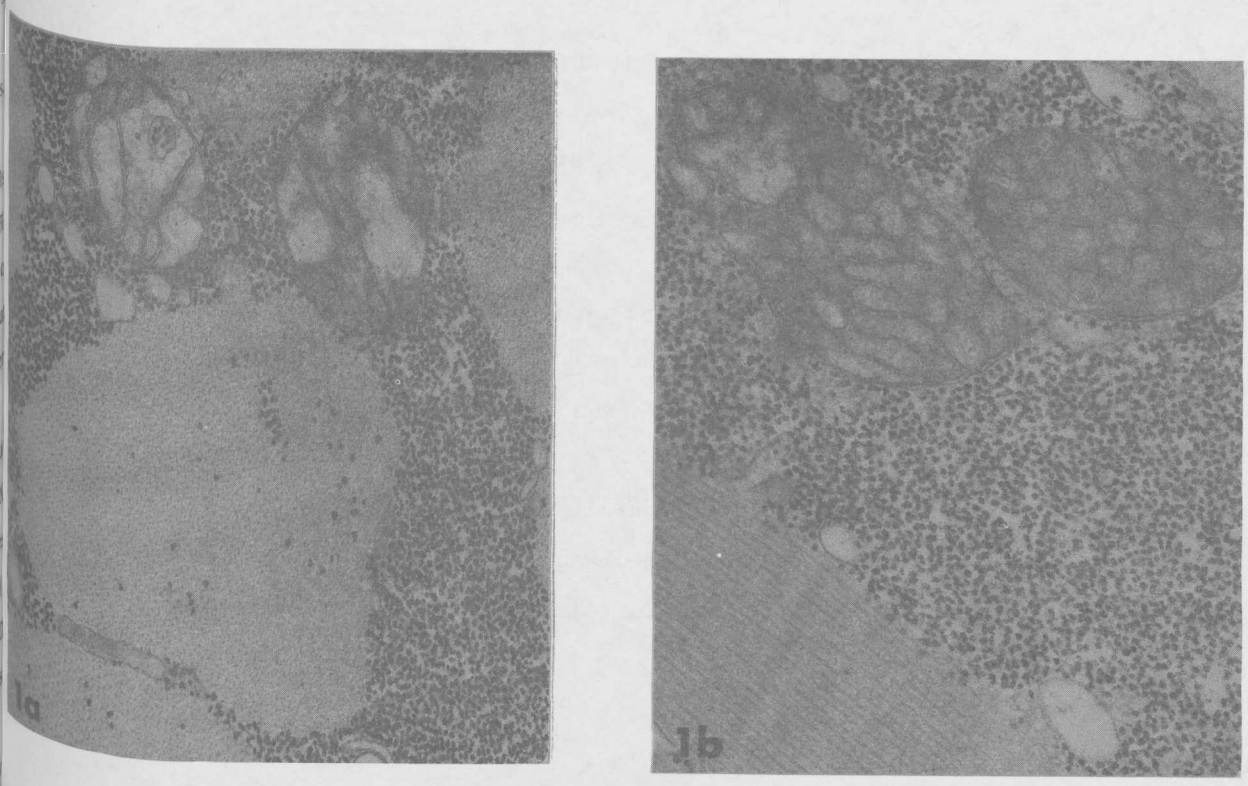
Basal metabolic rate was slightly higher in mitochondria from RN^- pigs as compared to rn^+ pigs for both substrates (Fig. 3).

Citrate synthase activity. Citrate synthase activity was higher in muscle from RN^- pigs as compared with muscle from rn^+ pigs values were respectively 0.20 ± 0.05 µkat / mg protein and 0.14 ± 0.03 µkat / mg protein ($P < 0.05$).

DISCUSSION

Glycogen is present in the mitochondria of various animal species (Personne and Anderson, 1970). In the muscular cell, the presen glycogen particles in mitochondria is considered as abnormal and is associated with pathology. Both types of ultrastructural abnormalities here, i.e. glycogen particles in mitochondria and disorganized cristae, have been already seen in mitochondrial myopathies. The functi defaults associated with these ultrastructural abnormalities have been shown in several cases to be due to deficiencies in respiratory enz (Coquet, 1991). These lesions were occasionnally associated with an increase of glycogen. The mechanisms of this pathology remain un (Morgan-Hughes, 1982).

In this study, a mitochondrial lesion was suspected on the basis of the morphological findings but the present biochemical study



: Electron micrographs of PATAg-stained sections from *Longissimus dorsi* biopsies of RN⁻ pigs. Fig 1a : Transversal section. Glycogen
ules are seen in the sarcoplasm and within mitochondria (arrows). Fig 1b: Longitudinal section. Mitochondrial cristae are irregularly shaped
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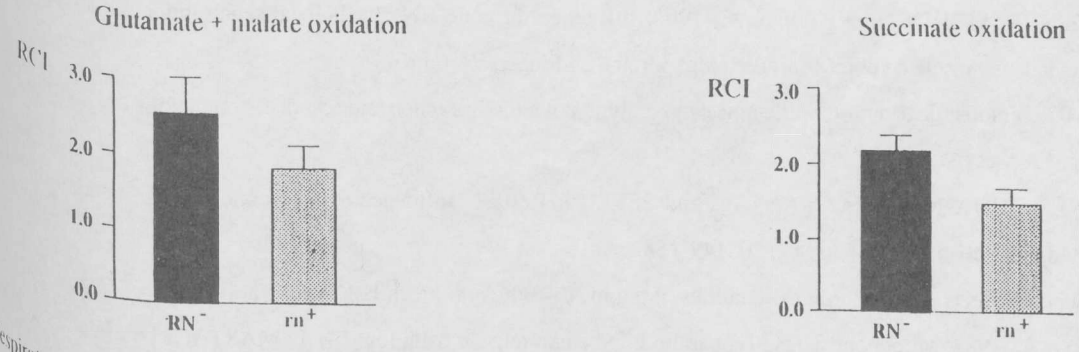
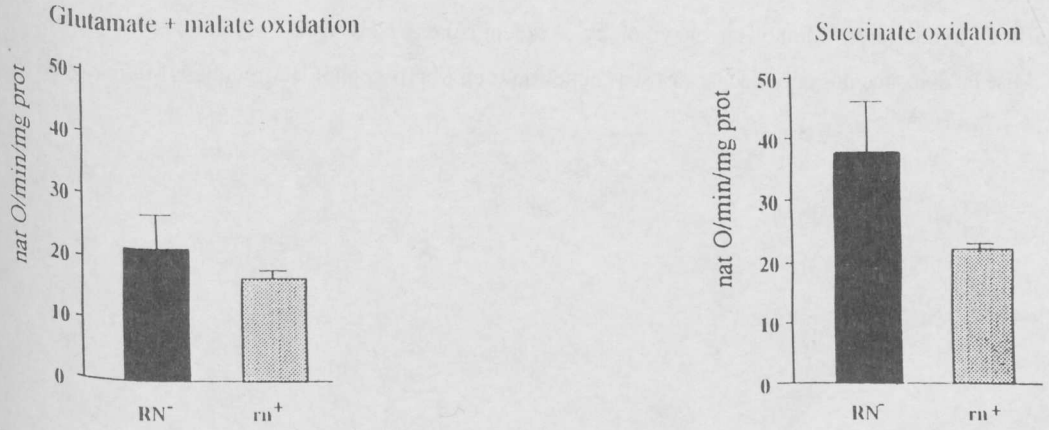


Respiratory control index (RCI) for the oxidation of glutamate + malate or succinate by *Longissimus dorsi* mitochondria from RN⁻ and
ligs. (Vertical bars indicate SE)



Basal metabolic rate for the oxidation of glutamate + malate or succinate by *Longissimus dorsi* mitochondria from RN⁻ and rn⁺ pigs
tical bars indicate SE).

not confirm is assumption. No significant difference in the value of the RCI was observed between RN^- and rn^+ pigs. Citrate synthase activ
was higher in Longissimus dorsi muscle from RN^- pigs as compared to rn^+ pigs. This latter result is in good agreement with those of Monin
al (1987). It suggests that either mitochondria of *Longissimus dorsi* from RN^- pigs show a reenforced citrate synthase activity or the amount
mitochondria is higher in RN^- pigs than in rn^+ pigs. Two hypotheses could explain the discrepancy between ultrastructural and biochemi
studies :

1) as mitochondrial ultrastructural abnormalities were seen only in a part of the mitochondria of white fibres and the biochemical study w
realized on a homogenate of muscle, the proportion of deficient mitochondria could be not sufficient to be revealed in a study from the mus
homogenate, 2) the number of these organelles was increased in RN^- muscles for instance by an adaptative mechanism in order to equilibra
some deficient mitochondria.

The morphological appearance of mitochondria is considered to be an expression of their metabolic state. The both abnormalities, affectin
glycogen level and mitochondrial ultrastructure were observed in the sarcoplasm of the white fibres from RN^- pigs. Considering that
oxidative and glycolytic metabolism are interdependent to some extent, there may be a relationship between a high glycolytic potential and
disfunction of a part of mitochondria in RN^- white fibres.

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