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Short Communication

An Improved Technique for the Demonstration of Glycogen Depleted Skeletal Muscle Fibres

P. Wirtz[1]*, W. Wallinga-de Jonge[2], and A.J.M. Vermorken[3]

[1] Department of Cytology and Histology, Medical Faculty, University of Nijmegen, Geert Grooteplein 21[n], NL-6500 HB Nijmegen, The Netherlands
[2] The Bio-Information Group, Twente University of Technology
[3] Research Unit for Cellular Differentiation and Transformation, University of Nijmegen, The Netherlands

Summary. Glycogen depleted skeletal muscle fibres can be distinguished from non-depleted fibres with the periodic acid Schiff (PAS) reaction. In this paper the method of Meijer (1968) for the histochemical demonstration of phosphorylase activity is described as an efficient technique to increase the contrast between both groups of fibres.

In 1968, Kugelberg and Edström described a histochemical method to localize skeletal muscle fibres belonging to a single motor unit by depletion of their glycogen. The authors concluded that "contractions produced striking changes in the phosphorylase activity and glycogen content, which varied in different types of fibres". Thus, the pattern of histochemically demonstrated activity of phosphorylase and the glycogen content in tissue sections depends on the "functional state" of the muscle at the moment of sacrifice. Several authors used the method of Kugelberg and Edström (1968), in which the Periodic Acid Schiff (PAS) reaction served to localize the muscle fibres belonging to one motor unit, after repetitive stimulation, in their investigations of physiological characteristics of single motor units. However, the contrast between depleted and non-depleted fibres is often not sufficient for reliable registration. Pre-incubation during $1^1/_2$ to 3 h for α-glucan-uridine-diphosphate-glucosyl-transferase (UDPG-transferase) increased the contrast between both groups of fibres with the PAS reaction (Pool et al. 1978).

The same year that Kugelberg and Edström published their work on the characteristics of motor units and pointed to the strong relation between muscle glycogen and histochemically detectable phosphorylase activity, Meijer (1968a, b) described a method in which dextrans were used as a glucosyl acceptor for the demonstration of phosphorylase activity in infarcted, i.e. glycogen depleted, heart muscle. The author recommended the

* To whom offprint requests should be sent

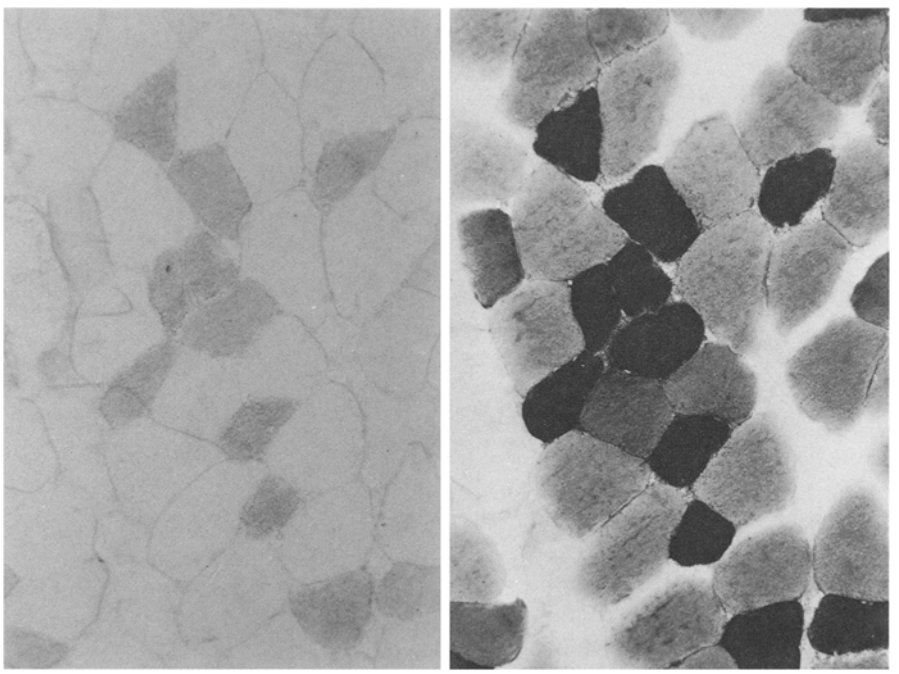

Fig. 1. Cross section through the extensor digitorum longus of the rat. The majority of the fibres have been depleted for glycogen by repetitive stimulation of the nerve. Left: PAS reaction; right: phosphorylase reaction using dextran of a mean mol.wt. of 40,000. Incubation time 10′. There is a marked increase in contrast with the phosphorylase reaction

use of unbranched high molecular weight dextrans in the incubation medium, since the effect of lower dextrans (e.g. mol.wt. 40,000) was not convincing even after incubation times of one or two hours.

We applied the techniques of Meijer and Takeuchi (described by Lojda et al. 1979) to glycogen depleted extensor digitorum longus muscles of the rat using dextrans of a mean mol.wt. of 40,000, 110,000 and 200,000, and glycogen as a glucosyl acceptor. Pre-treatment with periodic acid for 10′, incubation times of 10′, 20′ and 60′, and Schiff for 15′ were used. The contrast between depleted and non-depleted fibres was optimal after a short incubation time of 10′. The PAS reaction served as a control (Fig. 1). Thus the phosphorylase technique of Meijer is advantageous compared with that of Pool in that the reaction time is markedly decreased, which in addition to saving time is also favourable for the preservation of the morphology of the tissue.

Contrary to the findings of Meijer with glycogen depleted heart muscle, we did not find the use of dextrans of a high mol.wt. (110,000 and 200,000) advantageous to overcome the decrease in phosphorylase activity caused by glycogen depletion in skeletal muscle of the rat. After short incubation times (10′, 20′) there was no marked improvement of phosphorylase activity in depleted fibres compared with dextran of a mol.wt. of 40,000. After an incubation time of 60′ the difference between normal and depleted fibres

became less pronounced, but there still remained a contrast in staining intensity. Possibly dextran is not the most suitable glucosyl acceptor. In addition there could exist essential kinetic differences of α-glucan phosphorylase between skeletal muscle fibres and the heart muscle.

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