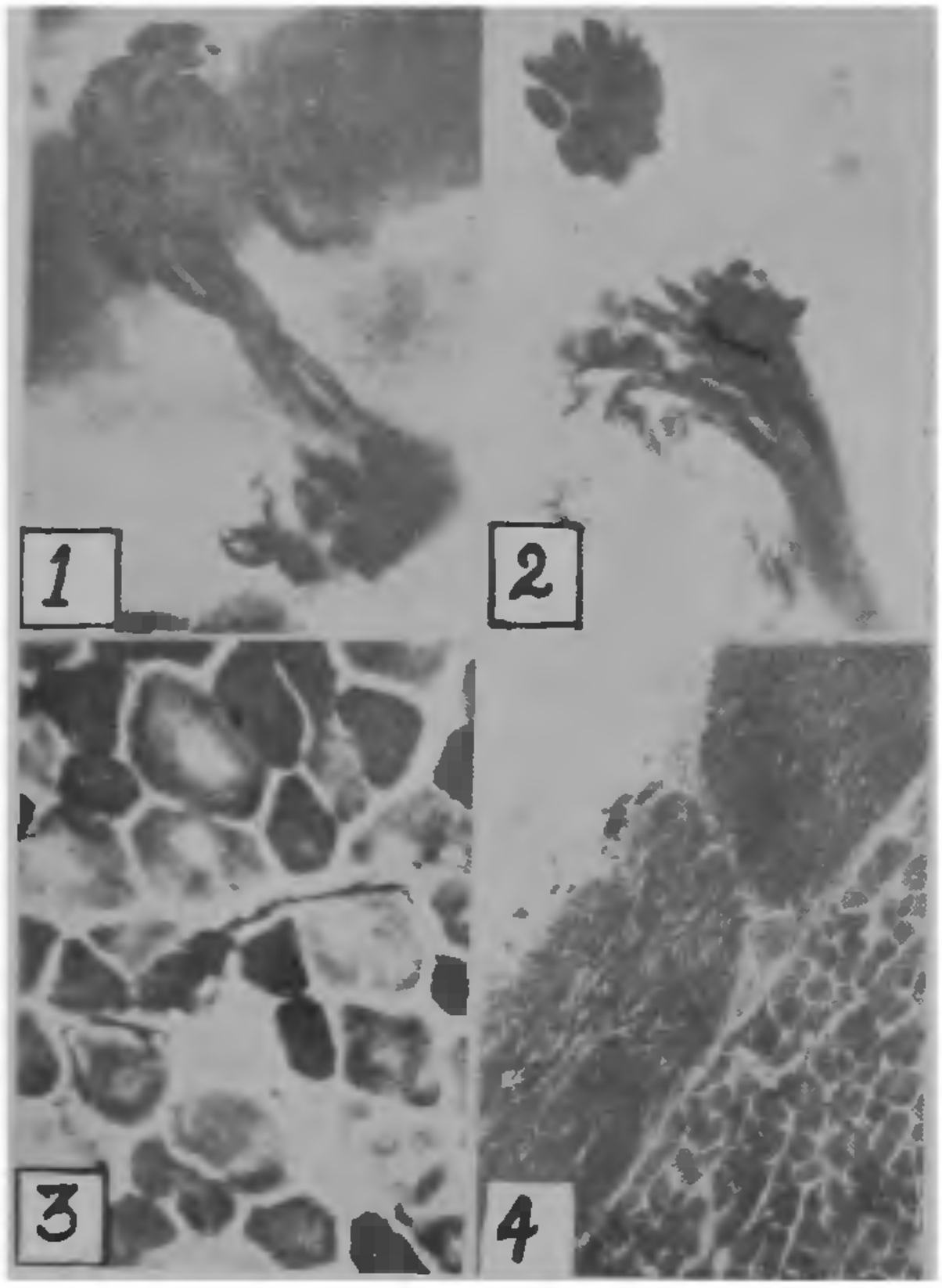
A HISTOCHEMICAL STUDY OF THE INNERVATION PATTERN IN MAMMALIAN SKELETAL MUSCLE BIOPSY

A STUDY of gross muscle fibre innervation is essential for the proper evaluation of skeletal muscle pathology[1,2] and also in the general study of motor units in skeletal muscles. Most of the histological/histochemical procedures employed for the study of muscle fibre innervation are elaborate and time-consuming.



FIGS. 1-4. Fig. 1. Longitudinal section of rat gastrocnemius showing the gross innervation pattern of red (darkly stained) and white (lightly stained) muscle fibres, × 200. Fig. 2. Same as in Fig. 1, showing innervation in a region containing only white muscle fibres, × 200. Fig. 3. Same as in Fig. 1. Note the distinct staining of the heterogeneous population of muscle fibres, and of the nerve fibres, × 50. Fig. 4. Same as in Fig. 1. Note the staining of the cross-section of the two branches of the sciatic nerve, and also of the muscle fibres, × 50.

More recently muscle innervation has been studied by combining the acetylcholinesterase and silver impregnation technique[3], and also by methylene ablue staining[4]. In the present investigation we have used a procedure that is rapid and serves the twin purpose of muscle fibre type identification and the visualization of their gross innervation.

16μ thick fresh-frozen sections of the muscle pieces were fixed for 5 min in 1% cobalt chloride and calcium chloride made in 10% neutral buffered formalin. This fixative was found particularly suitable for the specific staining of nerves (fibres) in muscle sections, besides preserving intramuscular lipids to good effect. Following fixation, the sections were thoroughly washed for 1 min in running tap-water, and then for 1 more min in distilled water. Washed sections were stained for 10 min in a saturated alcoholic solution of Sudan Black B, rinsed in two $\frac{1}{2}$ min changes of 30% alcohol, and then further washed in running tap-water for 2 min. Finally, the sections were mounted in glycerine-jelly. The overall processing time of the fresh frozen sections was never more than 20 min. For a better evaluation of the results of the present technique, both longitudinal and cross-muscle sections were used. By thie technique, however, the nerve fibres could not be precisely traced till the motor end-plate area. Otherwise, it was possible to observe the gross course of nerve fibres along the various muscle fibre types (Figs. 1–3). Whole nerve cross-sections could also be stained with good effect (Fig. 4).

It may be mentioned here that the present technique may prove useful in studying the fibre type innervation pattern of mixed or predominantly white muscles, and not of predominantly or exclusively red muscles (fibres) stain as dark with Sudan Black B as the nerves (fibres) do, and thus the overall staining contrast of the nerve fibres is insignificant. For the reason of its quickness and reproducibility, this technique may possibly be used for the biopsy studies of gross skeletal muscle innervation.

This study was supported in part by a research grant from the U.G.C., New Delhi, to one of us (C.L.T.).

Muscle Physiology and Histochemistry Unit,
Department of Zoology,
University of Delhi, Delhi 110007,
October 21, 1976.

P. KUMAR.
C. L. TALESARA*.

* Address all correspondence to Dr. C. L. Talesara.

1. Adams, R. D., Denny-Brown, D. and Pearson, C. N., *Diseases of Muscle : A Study in Pathology*, 2nd Edn., Harper and Row Publishers, New York, 1965.
2. Woolf, A. L., In: *Disorders of Voluntary Muscle*, Edited by Walton, J. N., J. and A. Churchill Ltd., London, 1969.
3. Ip, M. C. and Vrbova, G., *Z. Zellforsch.*, 1973, 146, 261.
4. Kobneliussen, H. and Waerhaug, O., *Z. Anat. Entwickl. Gesch.*, 1973, 140, 73.