# Observations on the isolated phrenic nerve diaphragm preparation of the rat

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*Commentary by*

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Edith Bülbring's paper is part of a study that she and J.H. Burn and others were carrying out to try to explain the mechanism underlying what was called the 'Orbeli effect'. The Russian physiologist, Orbeli, had shown over 20 years earlier that when the sympathetic nerves to the blood vessels of a fatigued skeletal muscle of a frog were stimulated, the amplitudes of the fatigued contractions were substantially increased. Bülbring developed the isolated phrenic nerve-diaphragm strip preparation of the rat in order to determine whether any of the complex effects of adrenaline on contractions might be secondary to an action on the muscle blood flow. Such effects would of course be absent in the isolated preparation.

The actual results obtained and described in this paper remain difficult to interpret, and are in any case of much less importance than the development of the preparation itself, which was the first mammalian isolated nerve-skeletal muscle preparation to be described. Its success is dependent upon its thinness, which makes it relatively easy to oxygenate and to maintain an appropriate ionic balance across the fibre membranes, although not all workers have been convinced that oxygenation and ionic balance remain adequate in isolated skeletal muscles generally (see, for example, Maclagan, 1962). The development of other isolated muscle preparations has, of course, followed, for example, the isolated innervated tenuissimus muscle of the cat and the isolated triangularis sterni muscle of the mouse. But Bülbring's isolated phrenic nerve-diaphragm of the rat gave impetus to them all, and since 1946 the preparation has been used in a myriad of research experiments, and formed the basis of a multitude of laboratory classes.

In later work, the fibre composition of the rat diaphragm was determined. According to Close (1972) the muscle is composed of 60% fast-contracting red fibres, 20% fast-contracting white fibres, and 20% slow-contracting intermediate fibres, all of them of the focally-innervated 'twitch' type. Electrophysiological analysis of endplate potentials, miniature endplate potentials, the respective currents, their quantal contents and the size of the acetylcholine store, have been made (see, for example, Hubbard, 1972), and a technique for measuring acetylcholine release from the stimulated nerve has been developed (Wessler & Kilbinger, 1986). In their study of the mechanism of action of botulinum toxin, Burgen *et al* (1949) developed a method for injecting acetylcholine retrogradely into the blood vessels of the isolated diaphragm. The prejunctional action of botulinum toxin was shown by its failure to block acetylcholine-induced contractions at the same time as it prevented the muscle response to nerve stimulation.

The isolated phrenic nerve-diaphragm preparation was formerly used for the bioassay of tubocurarine. A full description is given in Burn (1950). The muscle is especially sensitive to tubocurarine and other members of the bisbenzylisoquinolinium type of neuromuscular blocking agent, but, like other rat muscles, it is relatively insensitive (compared with human beings) to other types of neuromuscular blocking drugs, including those of the aminosteroidal type (e.g., pancuronium,) and especially to the depolarizing blocking drugs such as suxamethonium (Zaimis, 1953; Bowman, 1964). Furthermore, 'depolarizing blocking drugs' act by a non-depolarizing mechanism that is quite different from their action in human patients (Ireson et al, 1969).

Despite these pharmacological differences from the responses of human muscles, the experimenter who is familiar with the preparation can use it to learn, and to teach, a great deal about neuromuscular and muscle physiology and pharmacology.

Edith Bülbring, before her sad death a few years ago, must have been pleased to know how her simple preparation had gained such world-wide utility.

## References


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