PN 1268

The effect of focussed ultrasound on the permeability of frog muscle

High-intensity ultrasound is now widely used for disintegrating cell suspensions[1], for producting trackless lesions in nervous tissue and for treating Menières' disease in humans[2–4]. The precise mechanism of these biological effects is largely unknown[5]. It is the purpose of this paper to show that focussed ultrasound, causes changes in the permeability of isolated frog sartorii.

Frog sartorii were dissected from pithed frogs (*Rana temporaria*) and tied with cotton thread at both ends. Pairs of muscles from the same frog were soaked in various well oxygenated salines and Na^+, K^+ and water movements followed after treatment by ultrasound of one of each pair. The treatment with ultrasound was carried out with a 5-cm-diameter barium titanate lens driven at 1 Mcycle/sec by a Hartley-type oscillator. The focus was at approx. 4 cm and the intensity in the focal region was approx. 1 kW/cm^2. The muscle was held in a groove cut in a rubber pad which was passed through the sound focus 5 times by means of a microscope mechanical stage adapted for the purpose: each traverse took 5 sec. During the treatment the muscle and its control was bathed in the appropriate saline cooled to 5°. After treatment the muscles were placed in new saline from which they were removed at intervals, blotted on Whatman No. 542 filter paper. The wet weight was determined immediately and the dry weight after heating overnight at 110°. The muscles were then ashed in HNO_3 and Na^+ and K^+ determined by flame photometry.

Muscles soaked in a saline containing Na^+ (120 equiv/l), K^+ (2.5 equiv/l) and glucose (0.013 M) lost K^+ and gained Na^+; this process was markedly accelerated in muscles which had been treated by ultrasound (Fig. 1). The change in ionic balance was accompanied by a gain in the water content of the muscle of about 2–3% but no significant change in the total concentration of salt and of chloride was evident. In further experiments muscles were soaked in saline containing higher concentrations of Na^+ (360 mequiv/l) until Na^+ uptake and K^+ extrusion ceased, after which, treated and untreated muscles were put into frog Ringer; Na^+ loss and K^+ gain was marked in the untreated muscles but was diminished in the treated muscles. Similar results were obtained with muscles which had been soaked overnight by the method of CONWAY[5] in K^+-free isotonic saline (Fig. 2).

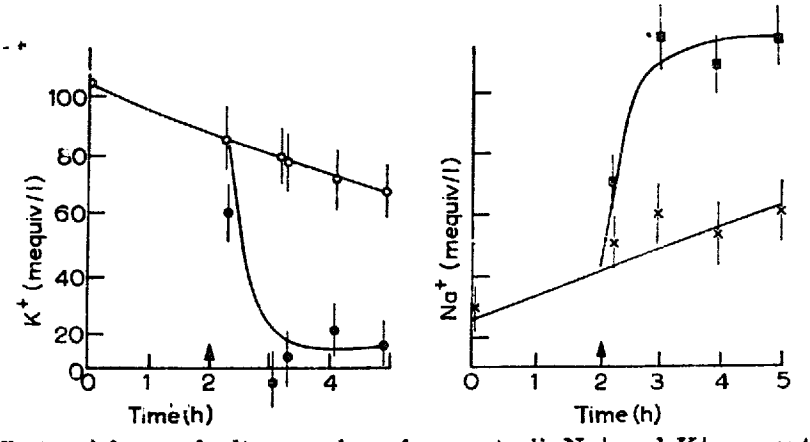

Fig. 1. The effects of focussed ultrasound on frog sartorii, Na^+ and K^+ concentrations. Paired muscles were placed in oxygenated saline containing 120 mequiv/l Na^+, 25 mequiv/l K^+ for 2 h. One of each pair was treated with ultrasound and soaking in saline continued for a further 3 h. Na^+ and K^+ are expressed as mequiv/l of total muscle water. O—O, untreated (K^+); ●—●, treated (K^+); ×—×, untreated (Na^+); ■—■, treated (Na^+).

Concomitant with these experiments, material was prepared from controls and treated muscles for normal histological and electron-microscopical examination. No marked or consistent structural changes were caused by the sound at the intensities used in these experiments.

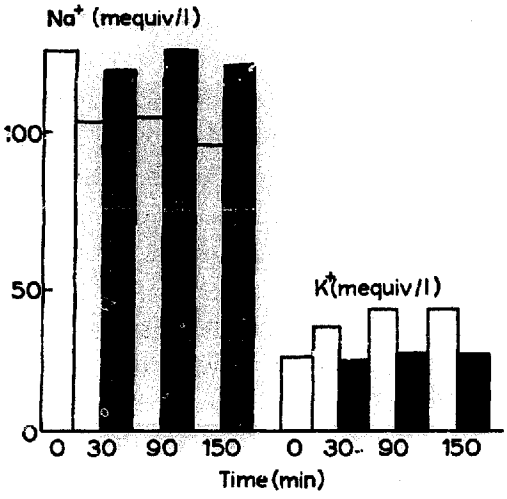

Fig. 2. The effect of ultrasound on Na^+ extrusion by Na^+-loaded frog sartorii. Paired muscles were soaked overnight in saline containing 122 mequiv/l Na^+ and no K^+. One of each pair was treated by ultrasound after which both muscles were placed in saline containing 104 mequiv/l Na^+ and 25 mequiv/l K^+. Each area represents a mean of 4 muscles and concentrations are expressed as mequiv/l. Plain areas are for untreated, and filled in areas are for treated muscles.

It is noteworthy that the muscles often contracted and remained contracted during or immediately after treatment. There was no apparent connection between contraction and the effects on ion movements. From further observation it seems likely that when the muscle is oriented towards the sound beam, so that the nerve which innervates the sartorius receives a high dose of sound the muscle immediately contracts. In normal saline or saline with high Na^+ content most treated muscles contracted, sometimes after only a few minutes of immersion after treatment. This also appeared to be independent of the Na^+ and K^+ movements.

It is evident from these results that the ability of muscle to extrude Na^+ and to retain a high internal K^+ concentration is rapidly diminished by focussed ultrasound. The extrusion of Na^+, depends on concomitant metabolic reactions such as respiration or anaerobic glycolysis which yield available energy[6].

It was found that the respiration of treated muscles was not significantly affected under the conditions which diminished Na^+ extrusion. The anaerobic formation of CO_2 was accelerated in a $NaHCO_3$ buffer up to five-fold and overall lactic acid production in a period of 2–3 h up to three-fold. One possible explanation for this acceleration, is that muscle has become freely permeable to glucose whereas in intact muscle it is known that there is a barrier to free glucose entry.

These experiments, although showing clearly that the permeability of tissue is rapidly altered by treatment with ultrasound, do not allow further clarification of a likely mechanism. In particular neither the heat rise in the tissue nor the dose of sound have been measured and correlated with the observed effects. The results are, however, of interest in connection with the effects of ultrasound in producing nerve block[7] and the immediate effects on motor activity when the brain is treated (2), since

the failure to extrude Na^+ and to maintain a high internal K^+ concentration, would inhibit the conduction of nervous impulses. It is worthwhile to record that it was also found in some preliminary experiments *in vivo*, that rat brain lost K^+ and gained Na^+ and water after treatment with ultrasound. These results on the changes in permeability are also of interest in the treatment of Menières' disease where it is thought that there is a failure to maintain the ionic balance of the vestibular fluids. It is noteworthy that an effect on a biochemical process has been found in the absence of significant structural changes in the muscle. This supports the suggestion that "biochemical lesions" precede the histological changes observed in tissues some days or months after treatment with ultrasound[8].

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A flow chamber for the differential microfluorimeter of CHANCE and LEGALLAIS. Preliminary work with glass-grown ascites cells

Microfluorimetric studies[1,2] have revealed a close relationship between the blue fluorescence of mitochondria in living cells and their pyridine nucleotide content. Coverslip preparations afforded only limited possibilities of biochemical investigation in the microfluorimeter, since the cells were confined in a closed environment which could not be modified once the preparation was sealed. This necessitated the adaptation of a flow chamber for the microfluorimeter, using for biological material glass-grown cells as suggested by CHANCE[3].

The difficulties met with in the use of the microfluorimeter for work with a flow chamber were largely optical, since it was not possible to focus the short working distance darkfield condenser of the microfluorimeter through even a thin layer of