PHYSIOLOGY

Role of Adenosine Triphosphate in Muscular Contraction

Loaded frog's stomach muscle at first relaxes and then contracts when treated with dinitrophenol (DNP)[1,2]. It was presumed that the relaxation and the subsequent contraction produced by DNP were due to depletion of stores of adenosine triphosphate. In the present research ATP, ADP and AMP have actually been estimated under the foregoing conditions.

These experiments were performed on circular strips of the stomach muscle of the frog *Rana tigrina*. Two sets of experiments were performed. In the first set two lightly loaded pieces of muscle from the same stomach were used; one of them, which served as a control, was suspended in Ringer solution and the other in Ringer solution containing 0·5 mM DNP. After 90 min both pieces were analysed for phosphate esters when the DNP-treated muscle had contracted after preliminary relaxation. In the second set of experiments, two pieces of muscle were similarly suspended in Ringer solution containing DNP, and one of them, which served as a control, analysed after 90 min, and the other was changed to Ringer solution and analysed after the next 120 min. To estimate the phosphate esters, the ether-extracted cold trichloroacetic acid extracts of tissue were chromatographed on paper using the solvent system of Krebs and Hems[3], and the esters were located by the acid molybdate spray of Burrows *et al.*[4]. The spots were cut out and wet washed[5] and analysed according to Bartlett's heating method[6] but read at 660 mμ (ref. 7).

DNP, after an initial stimulation, causes the loaded frog's stomach to relax and then contract again; on removal of DNP, the reverse happens, the muscle first relaxes and then contracts (Fig. 1). There are two variations from this usual result; the tonic contraction *B* may be little marked, suggesting the presence of some inhibitor in the muscle, and the relaxation *Y* may also be little marked, the contraction *B* passing into *C* without intervening relaxation. During the tonic contractions *A* and *C*, the muscle contracts when stimulated with electric current and acetylcholine, and shows spontaneous contractions; during *B* there was no response to stimulation and no spontaneous activity. *A* and *C* are relaxed by adrenaline and cyanide, but *B* is resistant to both.

The phosphate ester content in μmoles/g wet wt. of control muscles not treated with DNP was: ATP 1·512 ± 0·058, ADP 1·561 ± 0·053, AMP nil. That of 10 muscles treated with DNP for 1·5 h and in a contracted state was: ATP 0·114 ± 0·014, ADP 1·446 ± 0·085, AMP 1·650 ± 0·079, indicating a decrease of ATP by 92 per cent. In the second set of experiments, the phosphate ester content of 10 muscles treated with DNP was: ATP 0·104 ± 0·012, ADP 1·407 ± 0·079, AMP 1·634 ± 0·083. That of 10 muscles afterwards changed to Ringer and kept for 2 h therein was: ATP 0·809 ± 0·058, ADP 1·642 ± 0·065, AMP 0·539 ± 0·067, showing a recovery of about 50 per cent in the ATP content.

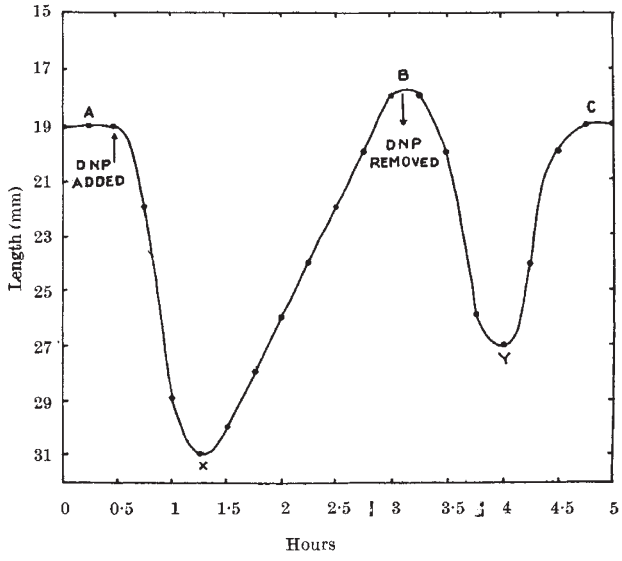

Fig. 1. Effect of 0·5 mM DNP on loaded frog's stomach muscle

These results suggest the two functions of ATP in unstriated muscle: (1) to provide energy for phasic contraction, active relaxation[1,2] and one kind of tone which is susceptible to asphyxia and metabolic inhibitors; (2) to keep the muscle relaxed. These probably correspond to two similar functions of ATP in the glycerol-extracted striated muscle[8].

In these estimations, an unidentified nucleotide phosphate ester band with R_F value 0·22 appeared and disappeared with the ATP band.

Inderjit Singh
N. V. Raju

Department of Physiology,
Medical College,
Agra, India.

[1] Singh, I., *Nature*, **195**, 83 (1962).
[2] Singh, I., *Arch. Intern. Phys. Biochem.*, **70**, 547 (1962).
[3] Krebs, H. A., and Hems, R., *Biochim. Biophys. Acta*, **12**, 172 (1953).
[4] Burrows, S., Grylls, F. S. M., and Harrison, J. S., *Nature*, **170**, 800 (1952).
[5] Eggleston, L. V., and Hems, R., *Biochem. J.*, **52**, 156 (1952).
[6] Bartlett, G. R., *J. Biol. Chem.*, **234**, 467 (1959).
[7] Singh, I., and Sarma, T. J., *Proc. Ind. Acad. Sci.*, **52**, 43 (1960).
[8] Weber, H. H., *Ann. N.Y. Acad. Sci.*, **81**, 409 (1959).

Inhibition of Thyroid Function by a Dithiocarbamoylhydrazine

Paget, Walpole and Richardson[1] described some biological effects of 1-α-methylallylthiocarbamoyl-2-methylthiocarbamoyl-hydrazine ('ICI 33828') in rats, dogs and monkeys and postulated a selective and reversible inhibition of pituitary gonadotrophic function. Similar results have since been obtained in mice[2]. In view of these findings we decided to investigate the effect of the compound on pituitary thyrotrophic function.

The effect on the goitrogenic action of methylthiouracil was investigated in rats using the increase in thyroid weight as an index of thyrotrophin production by the pituitary. The mean thyroid weight in a group treated with methylthiouracil and 'ICI 33828' was significantly less than that in a group given methylthiouracil and saline; but the treated group also showed a significant fall in total body-weight. Since the precise relationship between changes in total body-weight and thyroid-weight was not known these results were inconclusive. Attempts to modify the experiment so as to reduce the loss in body-weight were unsuccessful.

We decided, therefore, to investigate the rate of discharge of thyroid hormone labelled with iodine-131 from the gland in rats and mice treated with 'ICI 33828'. An injection of carrier-free $Na^{131}I$ was given intraperitoneally and after 48 h potassium perchlorate was added to the drinking water to prevent recirculation of iodine and to discharge inorganic iodide from the gland. On the following day and for either two or three days thereafter half the animals were injected subcutaneously with a suspension of 'ICI 33828' and half with the suspending medium alone. After 24 h the animals were killed and the radio-iodine content of their thyroid glands assessed.

It can be assumed that, at the beginning of treatment, the mean iodine-131 content of the thyroid glands of the mice in the control and treated groups is not significantly different. After any given time the mean iodine-131