Quantitative Determination of Cholinesterase Activity in Individual End-plates of Normal and Denervated Gastrocnemius Muscle

It has been reported[1,2] that as late as two months after denervation of the gastrocnemius muscle the end-plates still reacted positively to a histochemical test for cholinesterase. The test was made by the thiocholine method of Koelle and Friedenwald[3]. Recent development of the diver technique for quantitative determination of cholinesterase activity in individual cells[4,5] has made it possible to record quantitatively the observed histochemical reaction of the end-plates after denervation.

The cholinesterase activity of end-plates from the diaphragm and the gastrocnemius muscle was first determined in normal animals. Albino mice were killed by strangulation. The diaphragm or the gastrocnemius was removed and placed for 10 min. in the incubation medium for histochemical visualization of end-plates. The medium used was essentially similar to that recommended by Koelle[6]; but the required volume was made up with 0·9 per cent saline instead of 25 per cent sodium sulphate. Inspection of the gross substance after incubation revealed the accumulated end-plates as whitish bands extending across the muscle. A small piece of the histochemically active area was cut out with scissors and was placed under the microscope on a hollow slide containing 0·9 per cent saline. Single end-plates were dissected free with the aid of a micromanipulator and were placed in divers for quantitative determination of cholinesterase activity[5]. A $5 \times 10^{-3} M$ acetylthiocholine iodide solution was used as substrate and the measurements of activity were carried out at 25° C. The activity was calculated as the amount of carbon dioxide liberated from the bicarbonate buffer during 1 hr.

Table 1. Cholinesterase Activity of End-Plates isolated from Normal Diaphragm and Gastrocnemius Muscle and from Denervated Gastrocnemius Muscle

Mouse No.	End-plates isolated from	Cholinesterase activity of single end-plates (expressed in 10^{-5} μl. carbon dioxide per hr.)
	Diaphragm	
4		400
5		320, 350
6		420*
7		450*
	Gastrocnemius	
8		580, 500
10		1,000, 1,000
11		740, 750
12		740, 1,200, 710
	Gastrocnemius: days after denervation	
13	75	200
14	80	510, 430
15	85	170, 170

* In these experiments two end-plates instead of one were placed in the diver; the respective readings were 940×10^{-5} and 900×10^{-5} μl. carbon dioxide per hr.

Table 1 shows that end-plates isolated from gastrocnemius muscle had higher cholinesterase activity than end-plates from the diaphragm, and that the variations in the enzymic activity were considerable. It is further seen that 80 days after gastrocnemius denervation the end-plates still displayed appreciable cholinesterase activity. (The denervation was performed under ether anæsthesia; a section of the left sciatic nerve, about 10 mm. in length, was excised at mid-thigh level.)

More detailed study of the cholinesterase activity variations in end-plates and a correlation with the cholinesterase activities of the associated neurites will be attempted in later investigations.

M. Brzin
J. Zajicek

Institute of Radiopathology,
Stockholm 60.

[1] Gerebtzoff, M. A., and Vandermissen, L., *Ann. d'Histochim.*, **1**, 221 (1956).
[2] Bergner, A. D., *Brit. J. Exp. Path.*, **48**, 160 (1957).
[3] Koelle, G. B., and Friedenwald, J. S., *Proc. Soc. Exp. Biol. and Med.*, **70**, 617 (1949).
[4] Zajicek, J., and Zeuthen, E., *Exp. Cell Res.*, **11**, 568 (1956).
[5] Zajicek, J., *Acta Physiol. Scand.*, **40**, Supp. 158 (1957).
[6] Koelle, G. B., *J. Pharmacol.*, **103**, 153 (1951).

Metabolism of the Perfused Cat Brain during Metrazol Convulsions and Electroshock

There are a number of reports in the literature that during metrazol convulsions the rate of cerebral oxygen consumption increases[1–3]. It is also generally accepted that normally cerebral oxidations proceed almost exclusively at the expense of glucose. In a previous report[4], however, it was demonstrated with the aid of radioactive glucose that, in the case of cat brain perfused with 'simplified blood' (bovine red blood corpuscles suspended in Ringer's solution containing albumin), only about 30 per cent of the carbon dioxide produced by the brain comes from the blood glucose, while the rest of the carbon dioxide is derived from the combustion of endogenous substrates. This communication reports experiments showing an increase in production of carbon dioxide by the perfused cat brain following convulsions induced by metrazol and following electroshock. The results indicate that the major part of the excess carbon dioxide is derived from unknown endogenous substrates.

Isolation of the cerebral circulation of the cat was carried out according to the method described by Geiger and Magnes[2]. Perfusion of the brain at a constant rate of blood flow and the experimental technique generally were as described previously[5]. After 34 min. perfusion, 0·6–1·2 ml. of a 1 per cent solution of crystalline metrazol in saline was injected into the perfusing blood just before it entered the carotid arteries. The injection took about 1 min. Convulsive activity was almost immediate and continued for variable short periods after the injection. It was always accompanied by typically convulsive electrocortical activity and a slight transient fall in cerebral vascular resistance.

Results from a typical experiment are given in Fig. 1. It may be seen that the convulsive episode was accompanied and followed by an increased rate of carbon dioxide production, and the percentage of carbon dioxide produced by the brain, which is derived from the blood glucose, dropped considerably. Recovery was complete in about 12 min.

The passage of electric current through the brain (10 volts, 60 cycles from a condensor stimulator) by means of electrodes placed on the exposed skull produced similar metabolic changes. The current was applied three times during the course of 2 min. for a total of 30 sec. Each time the current was switched on an immediate and rapid rise of cerebral vascular resistance was evidenced by an increase in perfusion pressure, which returned to its previous value very rapidly when the current was switched off. During the passage of current the animal showed severe

© 1958 Nature Publishing Group