AN IMPROVED METHOD FOR PREPARING BRAIN CELL SUSPENSIONS

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Received 14 July 1970

1. Introduction

A mechanical-enzymatic method for preparing suspensions of free-floating neurons, glia and débris was recently described [1]. Although the suspensions obtained showed high metabolic activity, relatively low yield of cells called for improvements of the technique. A modification of the method is described producing metabolically active cells in yields 5 to 10 times higher.

2. Materials and methods

2.1. *Preparation of tissue*

Rats, about one month old, were killed by a blow on the neck. Brains were removed, and cortices were carefully cleaned of white matter. The cortices were washed in Krebs-Ringer phosphate buffer to remove blood cells, and placed on a large watch-glass filled with 5 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 10 mM glucose. 0.02% collagenase, chromatographically purified (Worthington), 0.1% hyaluronidase, type I (Sigma) and 1% BSA were added, and the tissue was torn in small pieces with two forceps. The solution obtained was poured into a glass vessel for incubation at 37° in an atmosphere of 100% O_2 for 60 min, with constant shaking. At the end of the incubation the resulting tissue suspension was poured into a bag of nylon mesh, pore size 500 μm, placed inside a glass vessel (nylon bags were shaped by a hot iron). The vessel was mounted on a ball vibrator (Westin and Backlund, Stockholm) suspended freely in air by its gas hose and shaken at a high frequency for 5 min, constantly moving the nylon bag up and down in the vessel to assist the sieving effect. At the end of the period, all remaining tissue was forced out of the bag into the surrounding medium. Similar sieving process, but avoiding any forcing, was then repeated with bags of pore sizes 200, 135 and 65 μm in a total time of 5 min. After each sieving process, the vessel used was rinsed with 5 ml of ice-cold Krebs-Ringer buffer and this was added to the main solution. The cell suspension obtained was placed on ice and once filtered through a double layered 65 μm mesh to remove capillaries [2]. The resulting suspension was immediately centrifuged at 300 *g* and 4° and washed twice with Krebs-Ringer buffer. This method is referred to as the new collagenase-hyaluronidase method. For comparative experiments, the following techniques were used: mechanical-enzymatic method with collagenase-hyaluronidase [1], sieving method [3], and homogenization with 10 strokes in a Potter-Elvehjem teflon glass homogenizer.

2.2. *Microscopy*

Methylene blue was used for staining. Cell counts were taken on a hemocytometer.

2.3. *Measurement of respiration and amino acid incorporation into protein*

Respiration and incorporation experiments were performed in a Warburg respirometer in Krebs-Ringer phosphate buffer, pH 7.4, containing 10 mM glucose, 1.0 mM leucine and 10 μl (10 μCi) ^{3}H-leucine (15.2 Ci/nmole). Incubation was continued for 90 min under pure O_2. Experiments were terminated by immediately centrifuging at 4° and homogenizing the resulting pellets in cold 5% TCA. After centrifugation

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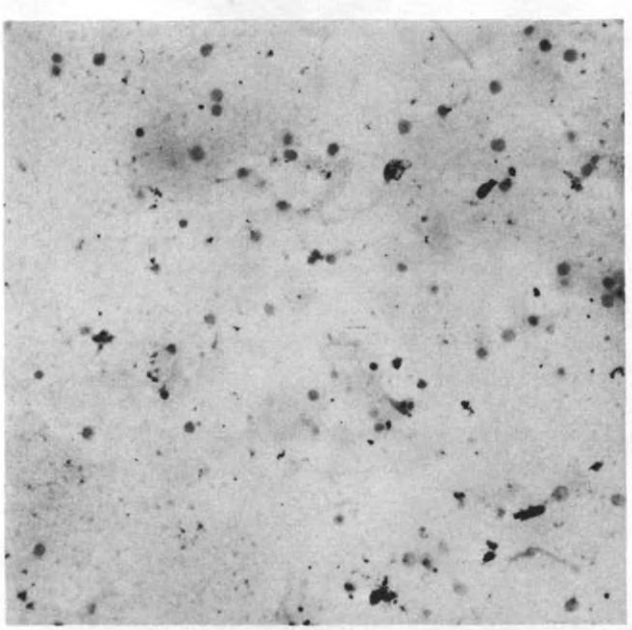

Fig. 1.

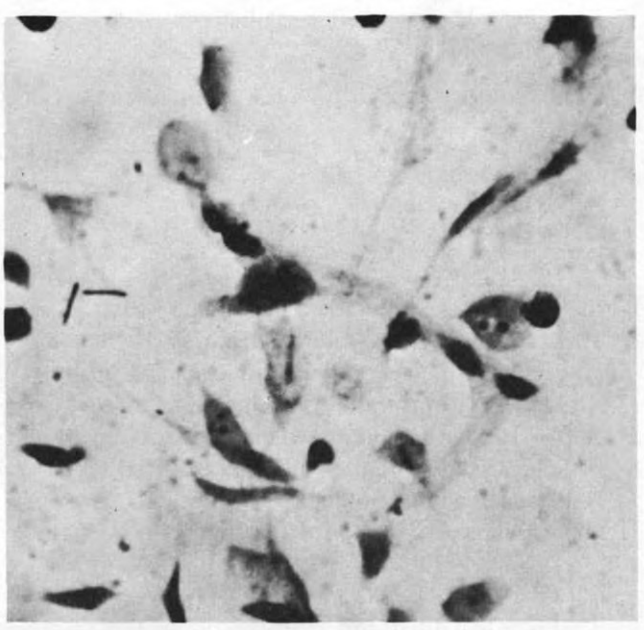

Fig. 2.

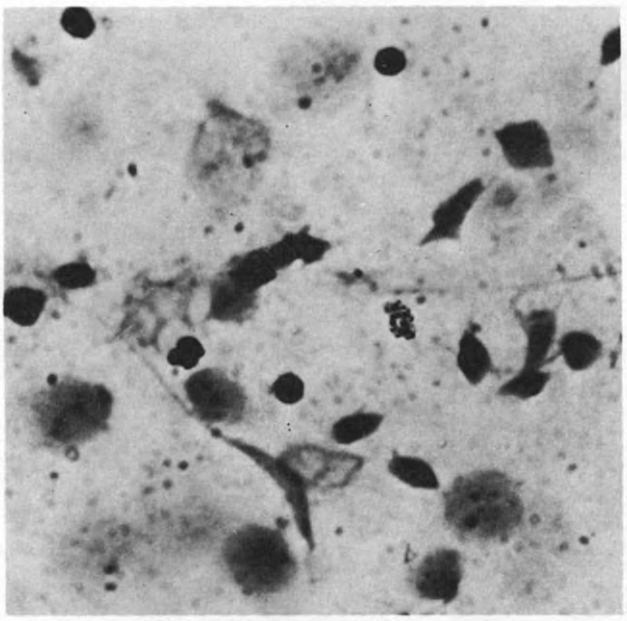

Fig. 3.

Light microscopy of mixed cell suspensions prepared by collagenase-hyaluronidase. Methylene blue staining.
Fig. 1. General view of the suspension (X 100).
Fig. 2. Several neurons and glial cells, small capillary in the center (X 200).
Fig. 3. Neurons and glia (X 400).

Table 1
Yield of neurons by different dispersion methods.

Method	Number of isolated neurons per rat brain cortex (in millions)
Collagenase-hyaluronidase	15
Mechanical-enzymatic	2
Sieving	1

the precipitates were washed three times with cold 5% TCA, twice with absolute ethanol, once with ethanol-chloroform, once with ethanol-ether and finally with ether. The precipitate was dissolved into 0.5 N NaOH. The NaOH solution was rapidly mixed with Cab-o-sil in Bray's solution and counted in a Packard two-channel liquid scintillation spectrometer. Protein was measured by Lowry's method [4].

3. Results and discussion

By the new method, free-floating neurons can be obtained in a yield of 15×10^6 and glia in a yield of 12×10^6 per rat brain cortex (400-500 mg wet weight). These figures are higher than those obtained by mechanical-enzymatic or sieving method (table 1), but are close to those reported by Norton [5]. Morphologically (figs. 1–3) most neurons retain only stumps of their processes, although neuronal nuclei, freed of cytoplasm, are produced only in small numbers indicating gentleness of the technique [3]. Glial cells have retained their processes to a large extent.

The technique described produces metabolically active cells (table 2). Respiratory values are close to those measured by the mechanical-enzymatic method and are higher than those reported for the sieving method [1,3,6].

Table 2
Metabolic activities of brain cell suspensions and homogenates.

Method	Respiration (nmoles O_2/ hr/mg prot)	Protein incorporation (dpm/mg prot)
Collagenase-hyaluronidase	850 ± 90 (5)	4410 ± 100 (5)
Mechanical-enzymatic	1000 ± 30 (7)	2220 ± 310 (7)
Homogenate	160 ± 80 (3)	1580 ± 230 (3)

Incorporation of radioactive leucine into protein is higher than that observed for any other large-scale preparation technique [1].

Acknowledgement

This study has been supported by the Sigrid Jusélius Foundation.

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