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ONE-STAGE METHOD OF OBTAINING ENRICHED FRACTIONS OF RAT BRAIN NEURONS AND GLIAL CELLS

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KEY WORDS: fractions of neurons and glia; biochemistry, morphology; cell respiration.

The object of this investigation was to devise a simple, rapid, and nontraumatic method of isolating fractions of neurons (NF) and glial cells (GF) from the brain of one small animal. Existing methods are lengthy (several hours), laborious, traumatic [6-8, 10-12, 15], and require several small animals; in addition, the fractions are contaminated with organoids [9]. In the method now suggested these disadvantages are reduced to a minimum: the NF and GF can be obtained in the course of 7-10 min.

EXPERIMENTAL METHOD

Hydrodynamic Method of Preparation of the Initial Cell Suspension. The brain of noninbred albino rats weighing 200-250 g, without the cerebellum, was placed in the receiver of a microblender (MRTU-2; Odessa Experimental Factory) and treated with Krebs–Ringer solution (KRS), pH 7.4, in a ratio of tissue to solution of 1:25-1:100. The receiver was placed in the microblender, the revolving shaft of which was immersed in the liquid. The blades of the shaft were replaced by paddles of the same size. The electric motor was switched on and the contents of the receiver mixed at a speed of 200-300 rpm for 30-50 sec. The use of a 0.28 M solution of sucrose gave a smaller yield (for example, that of NF was reduced by 33-50%).

Filtration Method of Obtaining Cell Fractions. The initial suspension, in a volume of 100 ml, was passed through a column (the fractioner) consisting of Plexiglas rings between which screen filters with gaskets were fixed (Fig. 1). The suspension first passed through a screen with pore size of 400-600 μ (the first filter), then one of 200 μ (second filter), and so on. After the suspension, 100 ml of KRS was passed through the column. The fractioner was dismantled and the material removed from the screens with the aid of a small brush, the filters having been immersed in beakers containing cold KRS.

Tissue respiration was studied polarographically [5], the K^+ and Na^+ concentrations on a flame photometer (FPF-58), and protein as described in [13]. The solid residues of the

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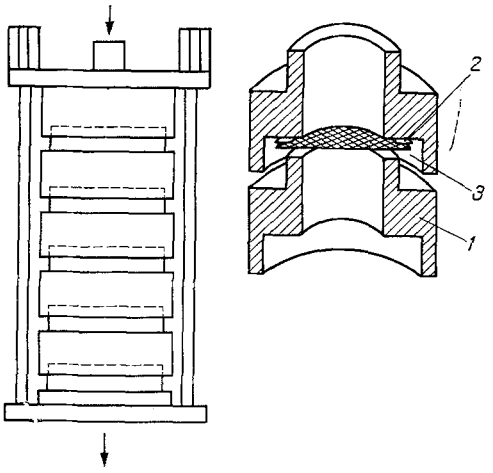

Fig. 1. External appearance of fractioner (left) and assembly of its rings in the working position (right). 1) Ring; 2) gasket; 3) screen filter.

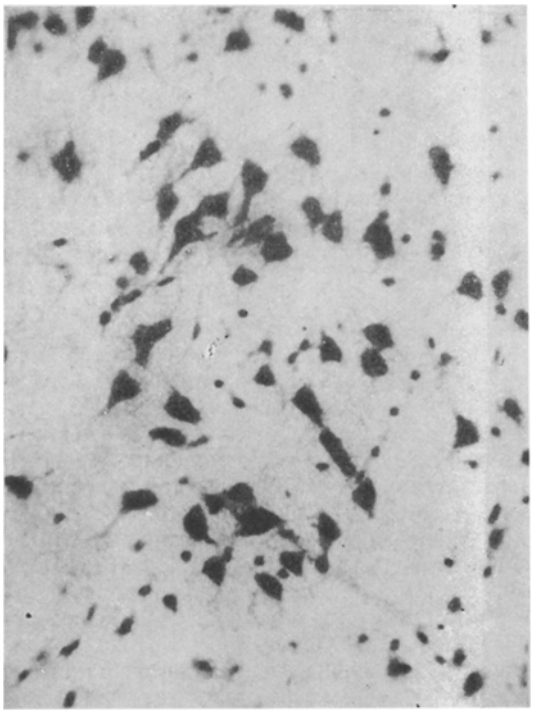

Fig. 2. Enriched fraction of neurons; 200×.

fractions were obtained by centrifugation at 1500g for 10 min. Sections and films were stained with thionine.

Mean values were calculated from the results of six experiments, and the standard error did not exceed 10% of the mean. Data from the literature were obtained from the data bank of the "Biomed" information retrieval system [4].

EXPERIMENTAL RESULTS

The method is based on the property of the tissues of breaking up initially into separate cells under the influence of hydrodynamic forces [2].

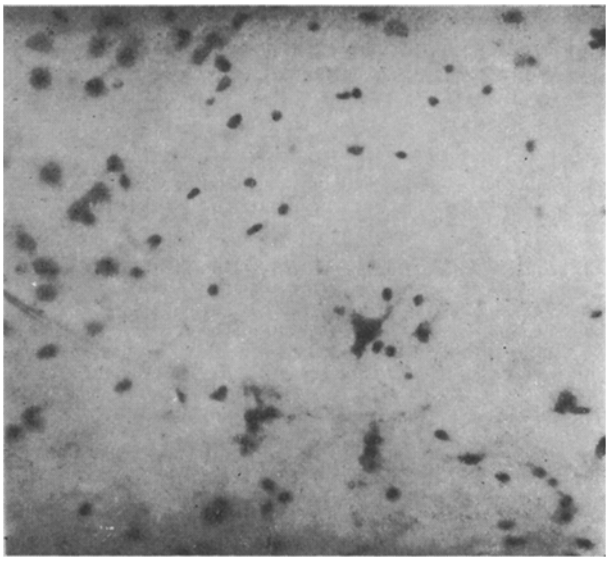

Fig. 3. Enriched fraction of glial cells; 200×.

TABLE 1. Morphological and Biochemical Characteristics of Enriched Fractions of Neurons and Glial Cells Obtained from a Suspension of Whole Rat Brain Cells by Filtration

Parameter	Enriched NF	Enriched GF
	pore size of screen, μ	
	200	100
Purity, %:		
ratio of numbers of cells	60	90
ratio by weight	90	90
Protein content of fraction from 1 g brain tissue, mg	8—10	3,0—3,5
Respiration on succinate, activity in ng·atoms O_2/min/mg protein:		
without ADP	53,0	51,6
with ADP	46,0	35,9
Content of ions: μeq/mg protein:		
K^+:		
KRS	0,13	0,13
0,28 M sucrose solution	0,16	0,13
Na^+:		
KRS	2,42	2,84
0,28 M sucrose solution	0,26	0,29

The protein content of rat brain is 108 mg/g [3], the residue obtained after centrifugation of the original suspension contained about 35 mg/g tissue, the combined NF + GF contained 11-14 mg, and the residues on screens Nos. 1 and 3 contained 27-33 mg. Consequently, the brain cells account for less than 50% of the protein, and the neurons for 25-30% of all brain proteins.

The diameter of the large neurons of the rat brain varies from 100 to 130 μ [14]. In the present experiments neurons (NF) were held up by screen No. 2 (pore diameter 200 μ), and in our opinion this is explained by the presence of the proximal parts of the projecting processes on the isolated cells (Fig. 2). The ratio of the number of neurons to the number of visible contaminating cells in NF was about 60:40, and in GF 90:10 (Table 1). The radii of the neurons in NF were more than 5 times greater than those of the gliocytes (Fig. 2). The area of 60 neurons was about 1500, and that of 40 gliocytes was 40 conventional units.

Correspondingly, the ratio of the neurons (and, consequently, their volume [1]) in NF was 97% of the area of all structures visible on the photograph. The weight of the neurons in NF, i.e., the purity of this fraction, can be taken to be over 90%. GF is illustrated in Fig. 3. The NF and GF were not contaminated by subcellular structures, for the pore size of the filters is much greater than the size of the organoids of the disintegrated cells, which are removed by the flow of liquid. The residue on the filter with a pore size of 20 μ contained large nuclei, small cells, and fragments of myelin; the filtrate included synaptosomes, mitochondria, small nuclei, and erythrocytes.

The NF and GF possessed endogenous respiration (15 and 12 ng·atoms O_2/min/mg protein, respectively); respiration was stimulated on the addition of succinate, the intracellular K^+ concentration was maintained within narrow limits despite differences in its concentration in the isolation media (Table 1). If sucrose solution was replaced by KRS the Na^+ level in the cells was increased tenfold, evidence probably that the transport of this ion is independent of the state of energy processes and of the gradient mechanism of Na^+ transport. ADP inhibited cell respiration on succinate (Table 1), although it is known that succinate has the opposite effect in the case of oxidation by mitochondria. This phenomenon can evidently be explained on the grounds that sodium succinate metabolism by isolated cells is not identical with the process of succinate oxidation by isolated mitochondria.

The filtration method of sorting cells can be used to obtain homogeneous populations of cells of other organs.

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