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CYTOPHOTOMETRIC STUDY OF TOTAL PROTEIN DYNAMICS IN GASSERIAN GANGLION NEURONS IN KERATITIS

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Cytophotometric studies have shown that the functioning of neurons is accompanied by changes in their nucleic acid and protein content. This rule applies not only under physiological [1, 3-4, 9-10], but also under pathological conditions. For example, changes in the protein content in Gasserian ganglion neurons have been described in experimental traumatic pulpitis [8].

Since the trigeminal nerve participates in the regulation of functions of the cornea and other structures of the eye, the writers postulated that neurons of the Gasserian ganglion, one of the main structures in the trigeminal nerve system, may undergo structural and metabolic changes after injury to the peripheral portion of the reflex arc. Such a situation evidently arises in keratitis, induced by neurogenic, traumatic, bacterial, viral, chemical, and physical factors. To test this hypothesis the investigation described below was undertaken to determine the total protein content in neurons of the Gasserian ganglion during the development of keratitis due to burns.

TABLE 1. Mean Relative Percentages of Different Groups of Cells in Control and in Rabbits with Various Stages of Burn Keratitis

Group of cells	Control	Stage I	Stage II	Stage III	Stage IV
1	14	17	4	4	13
2	51	48	31	33	42
3	33	33	45	44	28
4	2	2	20	19	17

KEY WORDS: Gasserian ganglion; total protein; keratitis.

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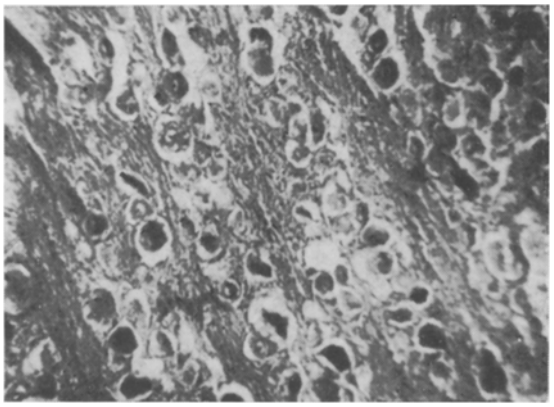

Fig. 1

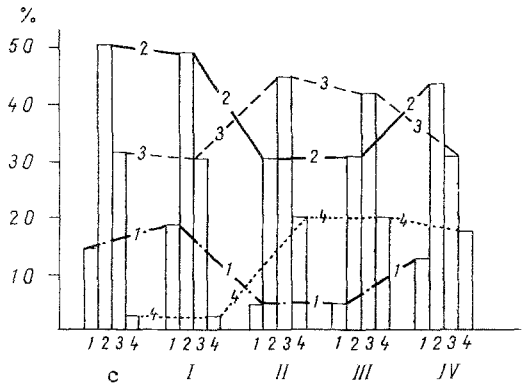

Fig. 2

Fig. 1. Gasserian ganglion of rabbit. Stained by Bonhag's method for total protein. 100 ×.

Fig. 2. Percentage distribution of Gasserian ganglion neurons with different total protein content in control (C) and in different stages of keratitis (I-IV). 1-4) Groups of cells differing in protein content. Ordinate, number of cells (in %).

EXPERIMENTAL METHOD

Experiments were carried out on 24 chinchilla rabbits weighing 2.5-3 kg: 20 experimental and four control animals. Experimental keratitis was produced in the animals by measured cauterization of the cornea with an electrically heated flat nichrome coil, 5 mm in diameter, for 2 sec. The animals were killed by air embolism on the 2nd, 5th, 15th, and 31st days, corresponding to stages I, II, III, and IV of keratitis [7].

The animals were autopsied immediately after sacrifice. The Gasserian ganglia were removed from the skull not later than 5 min after sacrifice and fixed in Carnoy's fluid. The Gasserian ganglia of experimental and control animals were embedded in the same paraffin wax block and sections, 10 μ thick, were mounted on slides. After dewaxing, the sections were stained for total protein by Bonhag's method [6].

Cytophotometry was carried out with the FMÉL-1 photometric attachment on the ML-2 microscope with stabilized source of light. Standard conditions were observed during photometry and a 20 × objective, 10 × ocular, and the 0.5 diaphragm of the FMÉL-1 attachment were used. The area photometrized was 315 μ^2. In the control and at each stage of keratitis 1000 cells were photometrized. The numerical results were subjected to statistical analysis. Optical density was expressed in relative units (RU), which corresponded to the strength of the current in microamperes.

EXPERIMENTAL RESULTS

Gasserian ganglion neurons are characterized by high intensity of staining for total protein, revealed as fine grains uniformly distributed throughout the cytoplasm of the nerve cells. In their appearance the neurons were almost circular in shape, thus greatly simplifying photometry (Fig. 1). It was found during photometry that the optical density of the neurons varied from 32 RU. In view of the reciprocal relationship between light transmission and concentration of substance in the cell, the lowest values of light transmission corresponded to the highest protein concentration, and vice versa. To study the character of distribution of cells differing in their protein content, four groups of cells with different optical densities were distinguished: group 1 — from 32 to 40 RU, group 2 — from 41 to 50 RU, group 3 from 51 to 60 RU, group 4 — 61 RU or more.

The data in Table 1 were used to plot a graph (Fig. 2) showing the dynamics of the protein content in the neurons at different stages of development of burn keratitis. Figure 2 shows that the number of cells with the highest protein content (groups 1 and 2) fell in stages II and III of burn keratitis by 67-75% and was gradually restored until stage IV. The number of cells with the smallest protein content (groups 3 and 4) increased in stages II and III. This pattern was revealed by construction of a histogram.

TABLE 2. Mean Total Protein Content in Cells Differing in Optical Density, Depending on Stage of Keratitis

No.	Control		Stage I		Stage II		Stage III		Stage IV	
	n	*M*	*n*	*M*	*n*	*M*	*n*	*M*	*n*	*M*
1	100	46,94	100	46,09	100	53,87	100	52,43	100	48,03
2	100	47,57	100	47,22	100	55,23	100	53,71	100	50,45
3	100	48,07	100	47,63	100	53,60	100	52,75	100	48,61
4	100	46,91	100	46,09	100	53,49	100	52,80	100	47,98
5	100	47,32	100	46,666	100	54,08	100	52,47	100	49,53
6	100	47,32	100	46,53	100	53,06	100	52,35	100	48,88
7	100	47,82	100	48,33	100	52,87	100	52,13	100	49,60
8	100	48,13	100	47,65	100	55,01	100	53,59	100	49,16
9	100	46,73	100	46,22	100	53,98	100	52,96	100	50,51
10	100	48,34	100	47,57	100	54,01	100	52,98	100	48,90
M	48,01		47,64		53,44		53,55		50,39	
$\pm m$	0,60		0,90		0,86		1,02		1,06	
P			>0,05		<0,05		<0,05		>0,05	

As Table 2 shows, only stages II and III were characterized by higher values of optical density of the cells, reflecting their total protein content; stage IV of keritiitis, according to this parameter, showed a **return** toward the control. However, the number of cells with the lowest protein content (group 4) in stage IV was more than 8 times greater than in the control (Fig. 2).

The results show conclusively that under normal conditions the total protein content in neurons of the Gasserian ganglion is irregularly distributed. Some neurons have a high protein content, others low. The mean number of these cells is 16% of the total. Most cells (84%) have an intermediate level of protein content. On the basis of data in the literature [3-5, 9, 10] and the results of the writers' previous photometric investigations of the histamine and serotonin content in mast cells [2] a similar explanation of the functional state of the neurons depending on their protein content was suggested. Cells with the highest protein content can evidently be regarded as being in a state of relative functional rest. They can be considered as a reserve. Cells with the lowest protein content are in a state of maximal functional loading. They are close to a state of "exhaustion." The remaining neurons occupy an intermediate position as regards functional state. In stage I of burn keratitis (1st-3rd days) no significant changes took place in the distribution of cells with different protein content. In stage II (4th-9th days) and in stage III (10th-23rd days) the number of "reserve" cells was reduced by more than two-thirds and the number of cells with a comparatively high protein content also fell considerably (Fig. 2; 1 and 2). In these same stages the number of cells with minimal and average protein content rose sharply (by 9-10 times; Fig. 2, 3 and 4). Later (24th-31st days) the normal distribution of cells with different protein content was restored.

The results suggest that the dynamics of the total protein content in Gasserian ganglion neurons during keratitis due to burns, as described above, reflects the dynamics of injury and repair of the peripheral portion of the reflex arc of one branch of the trigeminal nerve.

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MORPHOLOGICAL AND FUNCTIONAL REACTIONS OF PEYER'S PATCHES DURING TRAUMATIC SHOCK AND THE EARLY RECOVERY PERIOD IN DOGS

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Modern views on the role of the endotoxin component in the pathogenesis of shock [8-10, 12-14], the well-known views of Selye on involution of the thymicolymphoid system during exposure to stress, the clear changes in regional and distant lymph nodes under the influence of trauma [5, 6], and the complete absence of information on the response of Peyer's patches to trauma all indicate the need for a study of this problem. Interest in morphological and functional changes in Peyer's patches in the course of traumatic shock and the early recovery period after shock is due primarily to the localization of these structures, which are regional in relation to endotoxin derived from the intestine, and also to the possible role of Peyer's patches in differentiation of lymphocytes in the B-cell direction.

EXPERIMENTAL METHOD

Traumatic shock was produced by Cannon's method in albino rats. Standard shock-producing trauma was applied to the animals and, in 50-60% of cases, proved fatal. The times of investigation corresponded to periods of traumatic shock (1 — original background, 2 — erectile phase, 3 — beginning of the torpid phase, 4 — 1 h, 5 — 3 h after trauma) and the early recovery period after shock (6 — 1st day, 7 — 3rd day, 8 — 5th day after trauma). Material for histological study was fixed in Carnoy's fluid and sections were subsequently stained with hematoxylin and eosin, with methyl green and pyronine "g" by Brachet's method, and with gallocyanin for RNA. Reactions for alkaline and acid phosphatases (AlP and AcP, respectively) by Gomori's method were conducted on cryostat sections, fixed beforehand for 24 h in formalin—calcium mixture. The level of enzyme activity was judged from the intensity of the histochemical reactions.

EXPERIMENTAL RESULTS

Peyer's patches in intact animals are localized in the intestinal mucosa and submucosa and are lymphoid formations with high cell density (Fig. 1a). The pale center is somewhat eccentric in situation and displaced toward the serous membrane. The density of small lympocytes around the pale center decreases toward the intestine. Reticulum cells and various intermediate forms of cells predominate in the cupola of the patch, located above the lymphoid zone of the follicle. A few plasma cells can be seen beneath the epithelium of the cupola.

Infliction of standard shock-producing trauma led to definite changes in the cytoarchitectonics of the Peyer's patches as early as in the erectile stage of shock, characterized by dilatation and congestion of blood vessels of capillary type and increased AlP activity (Fig. 1b) in their endothelium. At the beginning of the torpid phase, development of which was judged from the appearance of shock hypotension, some decrease in the density of small lymphocytes was observed in the peripheral portions of the follicle in the Peyer's patches, against the background of microcirculatory disorders, and this was accompanied by an increase in the number of macrophages with signs of phagocytosis in the pale center (Fig. 1c) AlP activity in the macrophages of the pale center, the peripheral zones of the patch, and also in the reticulum cells of its cupola, was distinctly increased (Fig. 1d). Further development

KEY WORDS: Peyer's patches; traumatic shock; early recovery period after shock; endotoxin.

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