glucin, are illustrated in Fig. 4. In this case distinct aggregates were not present, although the shape of the erythrocytes differs a little from the control (Fig. 1). The difference consists mainly in the presence of traces of the projections and folds seen in such a distinct form in Fig. 3b.

The results of this investigation, together with those of the writer's previous studies, using transmission electron microscopy [2] thus show that aggregation of erythrocytes is a unique process of intercellular interaction, characterized by a definite structural dynamics, especially in relation to intercellular contact. The most demonstrative feature here is the formation of surface folds and projections, increasing the total area of intererythrocytic contacts and thereby increasing the mechanical strength of the aggregates and, correspondingly, their "pathogenicity" in relation to maintenance of the normal blood microcirculation.

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HISTOCHEMICAL STUDIES OF MICROVASCULAR EFFECTORS REGULATING THE BLOOD SUPPLY TO THE CEREBRAL CORTEX

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KEY WORDS: pial arteries; adrenergic and cholinergic innervation; enzyme activity in vessel walls; regulation of the microcirculation.

Biomicroscopic studies have revealed active regions in the system of pial arteries which are effectors of regulation, whose function determines the intensity of the blood supply to microareas of the cerebral cortex and redistribution of the blood flow between them. These effectors were found to be sphincters in the branches of the pial arteries, precortical arteries, and pial arterial microanastomoses, located between terminal branches of the pial arteries [1, 2]. The object of this investigation was to study the adrenergic and cholinergic innervation and histochemical properties of smooth-muscle cells in the region of these microvascular effectors, and also in the initial segments of the radial intracerebral arteries.

EXPERIMENTAL METHOD

Experiments were carried out on 12 rabbits of both sexes weighing 2.5-3.5 kg. The animals were killed by air embolism and the brain was quickly removed from the skull. The pial vessels were studied in preparations of the pia mater which contained the whole branched system of pial arteries and veins and also fragments of intracerebral radial arteries 100-400 μ long.

Acetylcholinesterase activity was studied with the use of acetylcholine iodide as substrate and tetraisopropylphosphoramide as inhibitor of butyrylcholinesterase [4].

To obtain specific green fluorescence of catecholamines, 1.5% glyoxalic acid solution was dropped on the isolated pia mater [8]. The preparations were dried in a jet of air for 5-20 min, incubated for 5 min at 80°C, mounted in mineral oil, and examined in the Orthoflux fluorescent microscope (Leitz).

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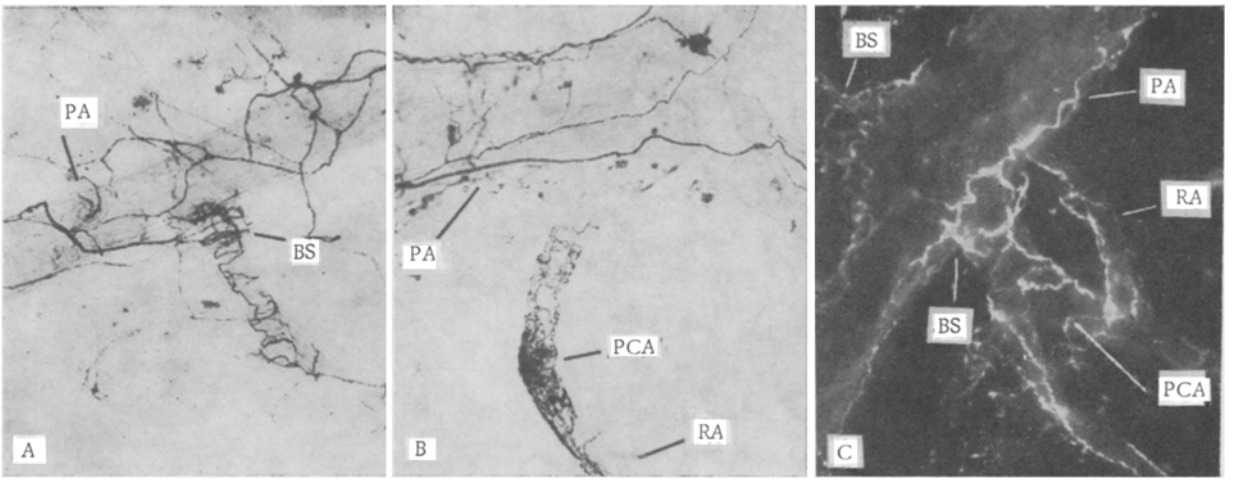

Fig. 1. Innervation of different parts of the pial arterial system (100 ×), A) Cholinergic nerve fibers in region of pial artery and its bifurcation sphincter; B) cholinergic fibers in region of precortical artery, radial artery, and large pial artery (PA); C) adrenergic fibers in region of bifurcation sphincters of pial, precortical, and radial arteries. Here and in Fig. 2; PA) pial artery; BS) bifurcation sphincter; PCA) precortical artery; RA) radial artery.

Activity and localization of certain enzymes in the media of the pial vessels also were studied: activity of phosphorylases I, II, and III [7]; glycogen was used as the substrate and the film of pia mater was incubated for 1 h at 37°C. To study ATPase, CTPase, and GTPase activity the film of pia mater was fixed for 1-2 h in formalin cooled to 4°C, then incubated at 22°C for 40 min [9]. Succinate dehydrogenase and lactate dehydrogenase activity also were investigated [5, 6].

EXPERIMENTAL RESULTS

The pial arteries and sphincters of the branches contained a varied innervation throughout their length. Cholinergic and adrenergic nerve fibers were most richly represented in the region of pial arteries over 100 μ in diameter. With an increase in the diameter of the vessels the density of innervation decreased and the ratio between fibers of different thicknesses changed. Arteries over 100 μ in diameter contained more thick and thin fibers running in longitudinal and transverse directions. With a decrease in the diameter of the arteries the number of thick fibers was sharply reduced (to 1 or 2) and their direction was mainly longitudinal. However, in the region of bifurcation sphincters the density of innervation increased considerably, as could be seen particularly clearly in vessels under 100 μ in diameter (Fig. 1A, C).

Investigation of dehydrogenase, phosphorylase, and phosphatase activity in the region of the bifurcation sphincters showed no difference compared with the neighboring pial arteries. In some cases, however, in constrictions of the pial arteries and at the bifurcation sphincters dehydrogenase and phosphatase activity was increased (Fig. 2A). Phosphorylase was an exception, for its activity was not increased in the constricted segments of the vessels (Fig. 2B). This indicates that increased phosphatase and dehydrogenase activity in the constrictions of the vessels was not due to thickening of their walls.

Precortical arteries, microanastomoses, and radial arteries are located in the region of terminal ramifications of the pial arteries. Although in most cases these vessels are directly continuous with one another and are about equal in diameter, their innervation varies greatly. In the region of precortical arteries the cholinergic and adrenergic innervation was much more abundant than in the neighboring pial arteries (Fig. 1B, C). Radial arteries, the direct continuation of precortical, were accompanied by one or two nerve fibers running longitudinally (Fig. 1B, C).

Phosphorylase and dehydrogenase activity in the region of the precortical arteries and microanastomoses was lower than in the larger pial arteries. ATPase and CTPase were distributed relatively uniformly along the course of the terminal branches in the pial and precortical

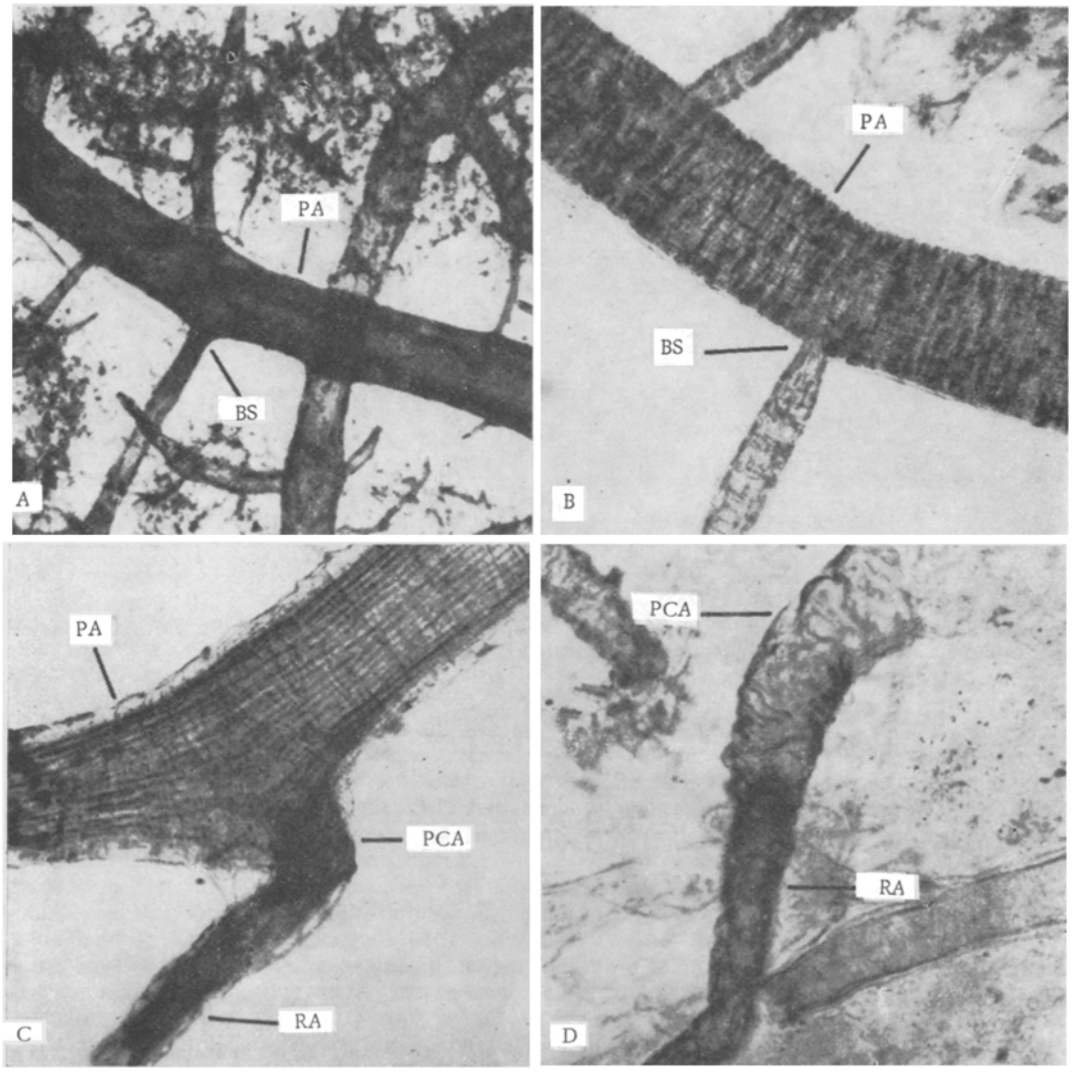

Fig. 2. Enzyme activity in walls of pial arteries. A) High succinate dehydrogenase activity in region of construction of bifurcation sphincter of pial artery compared with the rest of its course (100 ×); B) phosphorylase activity in segment of large pial artery, its bifurcations, and sphincters at their beginning (100 ×); C) high GTPase activity in region of precortical and radial arteries (200 ×); D) high ATPase activity in region of radial artery compared with precortical artery (200 ×).

arteries. GTPase, whose activity was much higher in the walls of the precortical arteries than in the adjacent pial arteries, despite the fact that the diameter of the former was much smaller than the latter, and their walls contain in many cases only one or two layers of smooth-muscle cells, was an exception. This fact also indicates that the activity of the above-mentioned enzymes is not connected with the thickness of the vessel wall. Phosphatase activity was high in the walls of the radial arteries (Fig. 2C, D), although it is still not clear whether this activity was connected with muscle fibers, i.e., with the motor activity of the vessels, or with the adventitia of these arteries, which includes in its composition many processes of glial cells.

The results thus indicate that the histochemical composition of enzymes investigated in this study in different parts of the pial arterial system is in general identical. A high density of innervation was found in the region of the bifurcation sphincters and precortical arteries. In other organs strong positive correlation has been demonstrated between the density of innervation and the magnitude of the neurogenic responses of the blood vessels [3]. It can accordingly be concluded that a neurogenic mechanism must play an important role in the regulation of the lumen of active segments of the pial arteries.

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QUANTITATIVE ULTRASTRUCTURAL CHARACTERISTICS OF RAT CARDIOMYOCYTE MITOCHONDRIA DURING HYPOKINESIA

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As a result of many ultrastructural studies the nature and dynamics of changes in the various components of the myocardium during hypokinesia have been described [1, 6, 8]. Nevertheless, the structure of the organelle systems, such as the mitochondrial apparatus of the cardiomycocytes, in hypokinesia, has not yet been fully investigated. Among investigations devoted to this problem [1, 2], there have been only isolated quantitative studies [6].

The object of this investigation was to study quantitative structural and functional indices of cardiomyocyte mitochondria of rats kept for 30 days under conditions of hypokinesia.

EXPERIMENTAL METHOD

Experiments were carried out on rats (9 control and 18 experimental) aged 6-8 months. To create conditions of hypokinesia the rats were placed in restraining cages. The rats were killed on the 10th, 20th and 30th days of the experiment. Pieces of left ventricular myocardium were fixed in 1% osmium tetroxide solution and embedded in Araldite. Sections were cut on UMTP-I and KV-III microtomes and photographed in UEMV-100B and JEM-7A electron microscopes. The following indices were determined on photographic prints under a magnification of 30,000: 1) the mean number of mitochondria per unit area (100 μ^2) of cardiomyocyte, 2) the relative area (in %) occupied by mitochondria, 3) the mean number of cristae per mitochondrion, 4) the mean length of all cristae in a mitochondrion, 5) the mean area of a mitochondrion, 6) the density index of the mitochondrial cristae (the ratio of the mean length of all cristae to the mean area of a mitochondrion. Indices 3-6 were studied separately in the perinuclear, myofibrillary, and subsarcolemmal zones of the perikaryon and overall mean indices for the cardiomyocytes were calculated. This approach was based on the assumption that corresponding to functional differences between these zones [4] the mitochondria may perhaps differ in function, resistance, and reactivity, and may react differently to conditions of hypokinesia. The indices stated above were chosen because they were sufficiently informative on the structure and function of the cardiomyocyte mitochondrial system [7].

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