Status of Adrenergic Nerve Supply to the Ventricular Myocardia in Experimentally Produced Massive Pulmonary Embolism

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The production of massive pulmonary embolism in dogs initially results in elevated norepinephrine levels in the stellate ganglion and ventricular myocardia. Six hours after its onset, destructive changes in the stellate ganglion nerve cells are more pronounced than at 1 h and their functional activity is decreased, as are norepinephrine levels in the adrenergic neurons and their terminals. A characteristic feature of compensated massive pulmonary embolism is the presence of many neurons showing pronounced hyperchromatosis and pyknomorphic shrinkage.

Key Words: *experimental massive pulmonary embolism; sympathetic nervous system; stellate ganglion*

Alterations in the state of the sympathetic nervous system (SNS) and in cardiac work are closely interrelated and interdependent. We have shown previously that acute massive pulmonary embolism (MPE) is accompanied by considerable hemodynamic shifts in the pulmonary and systemic circulations [1] and by structural and metabolic changes in the ventricular myocardia, and that these changes are most strongly marked when cardiac insufficiency has developed [4,5]. The purpose of the present work was to study the state of stellate ganglion (SG) neurons and of adrenergic nerve plexuses of the ventricular myocardia in experimental MPE complicated or uncomplicated by cardiac insufficiency.

MATERIALS AND METHODS

The experiments were conducted on a total of 59 mongrel dogs (body weight 15-20 kg) under natural ventilation (their chest was not opened) and general anesthesia achieved by fractional intravenous administration of thiopental sodium (20 mg/kg) following premedication with Promedol (10 mg/kg) injected intramuscularly. The procedures used to catheterize the heart and vessels, record hemodynamic parameters, and produce acute MPE were described in our earlier article [1].

The dogs with MPE were divided into three groups. The first two groups consisted of dogs without detectable signs of circulatory insufficiency (animals with compensated MPE). In these groups, material for neurohistological study was taken after euthanasia with a lethal dose of thiopental sodium 1 h or 6 h after MPE production. The third group comprised dogs in which the onset of MPE was followed by the rapid development of fatal cardiac failure (animals with decompensated MPE); after their death tissue samples were taken for morphological study. Control dogs were also divided into three groups. After catheterization (without embolization) they were immobilized on the oper-

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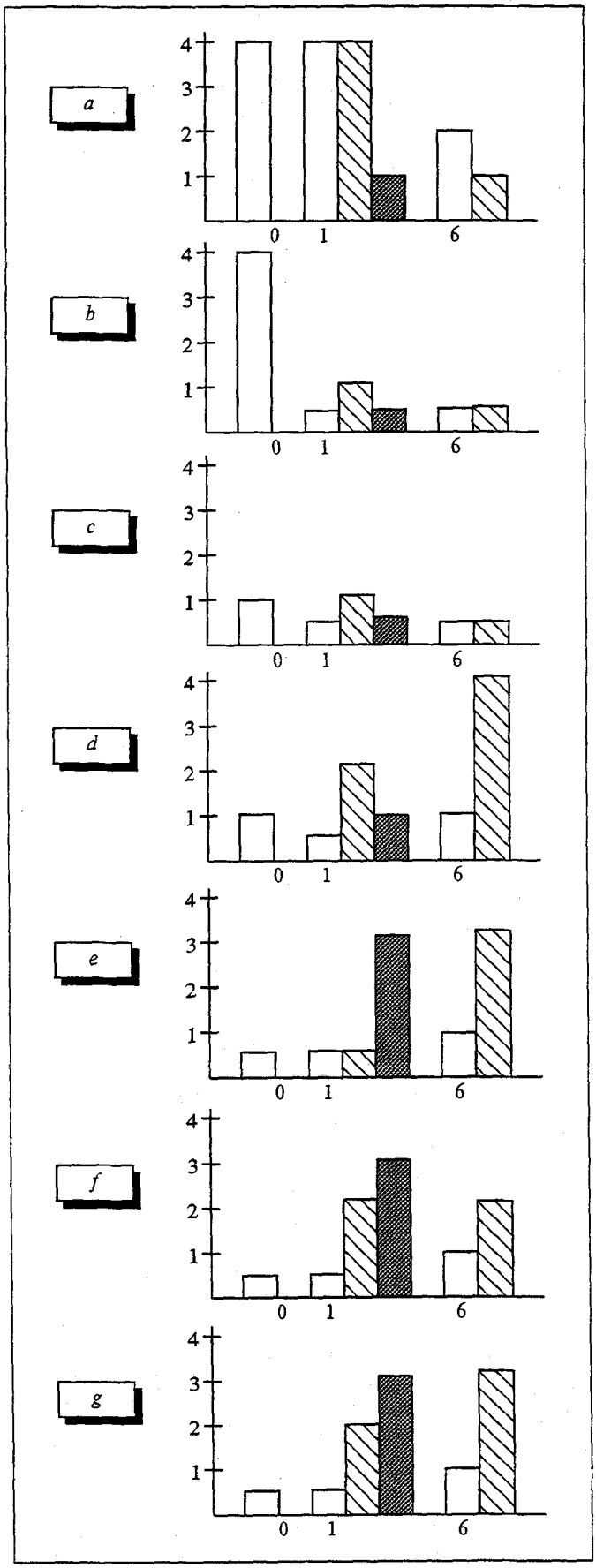


ating table and euthanized either immediately (zero-time controls) or 1 h or 6 h (1-hour and 6-h controls) after the immobilization, this being followed by the collection of autopsy material.

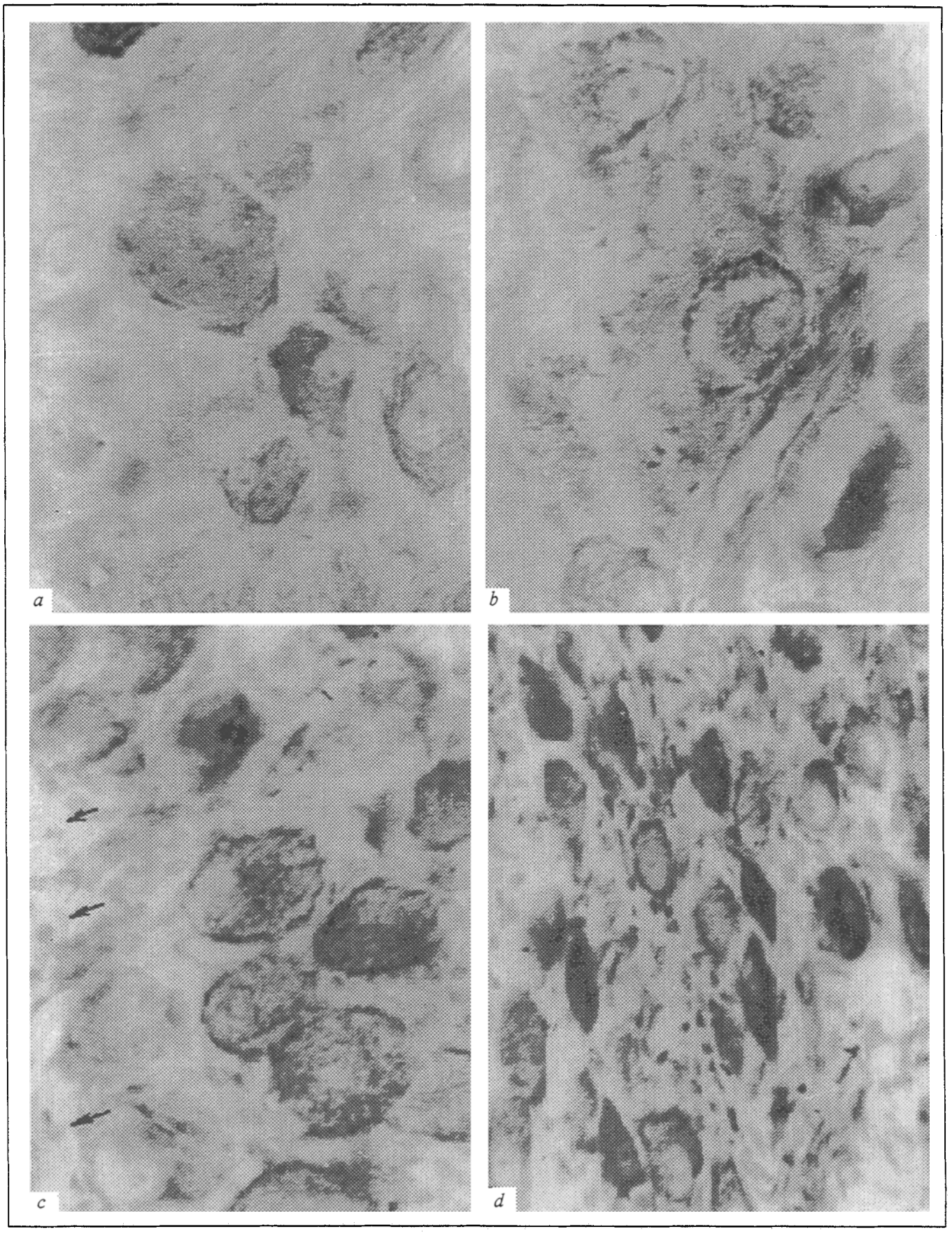
For histological studies, tissue samples from the middle part of the SG were fixed in 10% neutral formalin buffered according to Lillie, and embedded in paraffin. The state of SG neurons was assessed in 5-7 μ sections stained with thionin by Nissl's method and in sections stained with hematoxylin-eosin. For neurohistochemical studies, material dissected out from the middle part of the SG and from the middle third of the right and left ventricular wall was used; the samples were frozen with dry ice, and 25-μ sections were prepared at -15° to -20°C. For the detection of norepinephrine (NE) histochemically in the SG neurons and myocardial nerve plexuses, the sections were incubated in a 2% glyoxylic acid solution [7,10] and then examined under a fluorescence microscope. The relative area occupied by adrenergic nerve plexuses in the myocardium was calculated as described by Stropus [3], using a planimetric ocular grid with 960 nodal points. The results were treated statistically by Student's *t* test.

RESULTS

A large proportion of SG neurons from control dogs in which material for study had been taken immediately after immobilization (zero-time controls; Fig. 1) were characterized by acute swelling (lysis of cytoplasmic basophilic substance and greatly increased sizes of the cell and its nucleus; Fig. 2, *a*) and by so-called "primary irritation" (lysis of basophilic substance in the center of an enlarged cell with nuclear ectopia; Fig. 2, *b*). Such neurohistological changes are usually interpreted as reversible and indicative of enhanced neuronal activity [2,9] and, in particular, of intensified NE synthesis. In

⇦ Fig. 1. State of stellate ganglion neurons in dogs with MPE and controls. *a*) unchanged state; *b*) acute swelling; *c*) "primary irritation" (axonal reaction); *d*) hyperchromatosis and pyknomorphic shrinkage; *e*) ischemic changes; *f*) "severe" changes; *g*) shadow cells. (Neurohistological changes were classified according to Yarygin [9].) Ordinate: degree of expression of the state or characteristic considered: *1*) slight; *2*) moderate; *3*) considerable; *4*) very considerable. Here and in Fig. 4: abscissa: time in hours after completion of catheterization. White bars: control dogs; black bars: decompensated MPE; hatched bars: compensated MPE.

⇨ Fig. 2. Pathomorphological changes in SG neurons as revealed with thionin staining after Nissl. *a*) acutely swollen neuron (×400); *b*) neuron with signs of "primary irritation" (×400); *c*) "severely" changed neurons, with shadow cells shown by arrows (×256); *d*) neurons with hyperchromatosis and pyknomorphic shrinkage (×160).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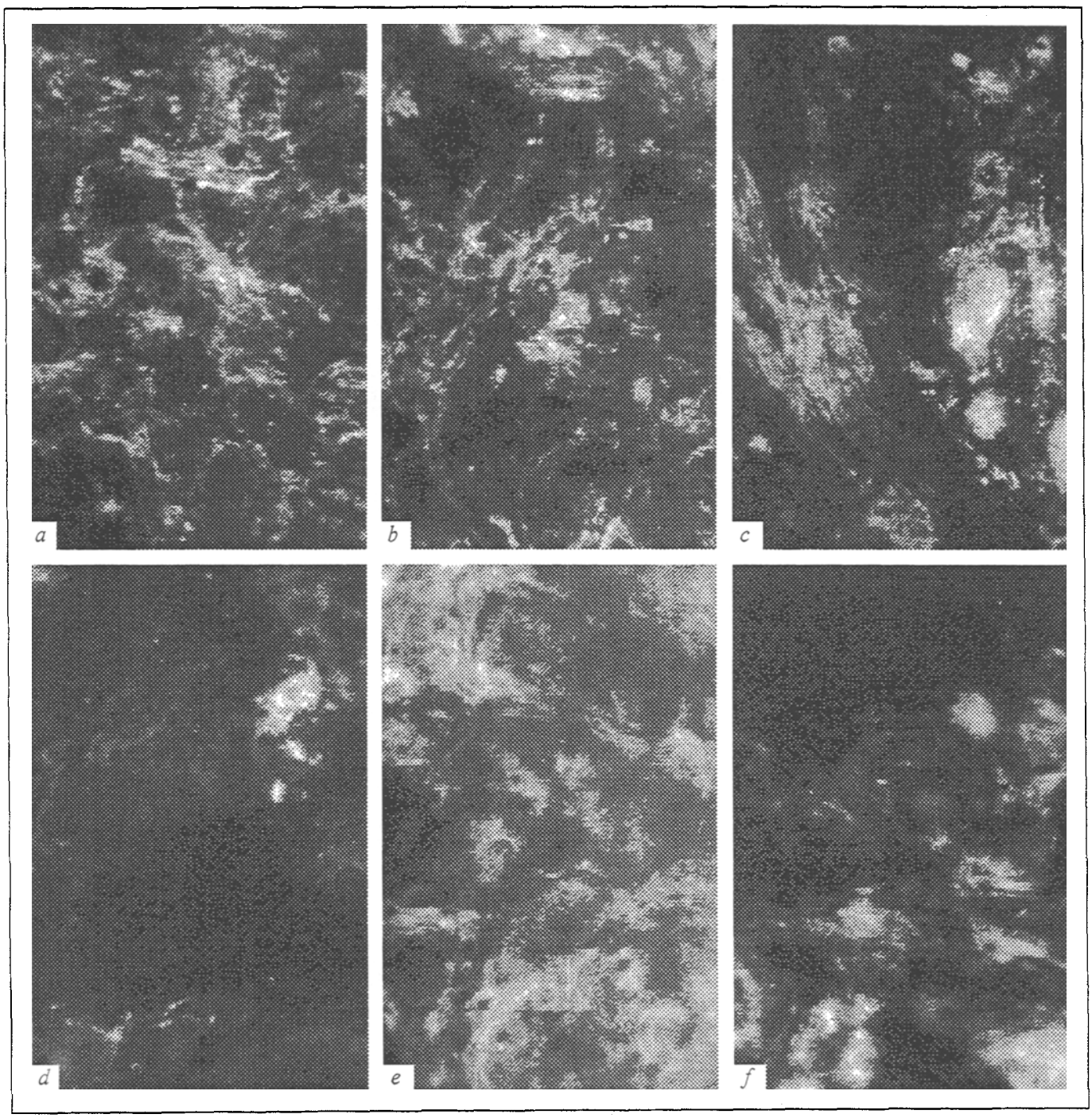

Fig. 3. Norepinephrine content of stellate ganglion neurons, as revealed by histofluorescence; ×140. *a*) to *f*) dogs from the respective groups: *a*) control, zero time; *b*) control, 1 h; *c*) compensated MPE, 1 h; *d*) decompensated MPE, 1 h; *e*) control, 6 h; *f*) compensated MPE, 6 h.

addition to nerve cells with functional changes, large numbers of unchanged neurons were detected in the SG, as were relatively high NE levels in SG cells (Fig. 3, *a*) and in the adrenergic nerve plexuses of the ventricular myocardium (Fig. 4).

In the 1-hour control group, virtually all of the SG neurons were unchanged (Fig. 1). NE levels in SG cells (Fig. 3, *b*) and in the nerve plexuses of the ventricular myocardia (Fig. 4) were lower than in the zero-time controls. In the 6-hour control group, the number of unchanged neurons was lower than in the preceding group, and some neurons showed destructive changes (Fig. 1); NE levels in SG cells (Fig. 3, *e*) and in the adrenergic terminals of ventricular myocardia (Fig. 4) were also lower.

Thus, while the animal was anesthetized and remained immobilized, there was a marked reaction on the part of the SNS, involving activation of NE synthesis so that NE levels were high both in the SG neurons and in the myocardial nerve plexuses, particularly those of the right ventricle. The presence of NE at higher levels in this ventricle was apparently due to a greater density of adrenergic nerve plexuses therein as compared to the left ventricle [3,8]. One hour later (1-hour control group), neurohistological changes seen at zero time were virtually indetectable, indicating that these changes were functional and reversible and that less NE was being synthesized, with a consequent lowering of its levels in the ST and ventricular myocardia. By the 6th hour (6-hour control group), no neurons with changes indicative of their heightened functional activity were detectable, but nerve cells with changes attesting to their severe damage were observed. As a result, still less NE was synthesized than after one hour and its levels in the adrenergic neurons and their terminals were also lower. Taken as a whole, the results of this control experiment support our hypothesis that the SNS is not activated by the 1st or 6th hour in control dogs and that the lowered content of NE in myocardial adrenergic plexuses is due to its diminished synthesis [6]. This led us to the important conclusion that the results of histological examination of material taken from an animal immediately after its anesthetization and immobilization should not be used for characterizing the so-called "normal state" or for making comparisons with the results obtained for other groups of animals.

Shortly (1 h) after MPE production, many SG neurons were unchanged (Fig. 1), but, in contrast to the respective (1-hour) control group, a large proportion of SG cells had heterogeneous changes. The signs of "primary irritation" and acute swelling seen in a number of neurons pointed to their elevated functional activity accompanied by a rise of NE levels in the SG (Fig. 3, *c*). By the 6th hour of compensated MPE, the number of unchanged SG cells was sharply reduced (Fig. 1), and no signs indicative of heightened neuronal activity were present. Many nerve cells showed evidence of ischemic damage [2,9]. For example, the basophilic substance had disappeared; the cytoplasm had become homogeneous and turned pale blue when stained with thionin and bright pink (it is described as "red neuron" by Schoene [11]) upon staining with hematoxylin-eosin; and the nucleus appeared dark, of irregular shape, and decreased in size. Many of the neurons had developed "severe" changes such as karyorrhexis and a homogenized cytoplasm with the presence of large basophilic granules at the periphery of the cell body (Fig. 2, *c*). Large numbers of dead neurons (shadow cells) with overt signs of karyocytolysis were also seen. The most conspicuous neurohistological changes (Fig. 2, *d*) were hyperchromatosis (the cytoplasm stained dark blue by Nissl's method) and pyknomorphic shrinkage (the basophilic substance was dense and stained dark blue with thionin, while the cell body and nucleus had decreased in size and some of them were elongated). The SG neurons with these changes seen by the 6th hour of compensated MPE had lowered NE levels (Fig. 3, *f*).

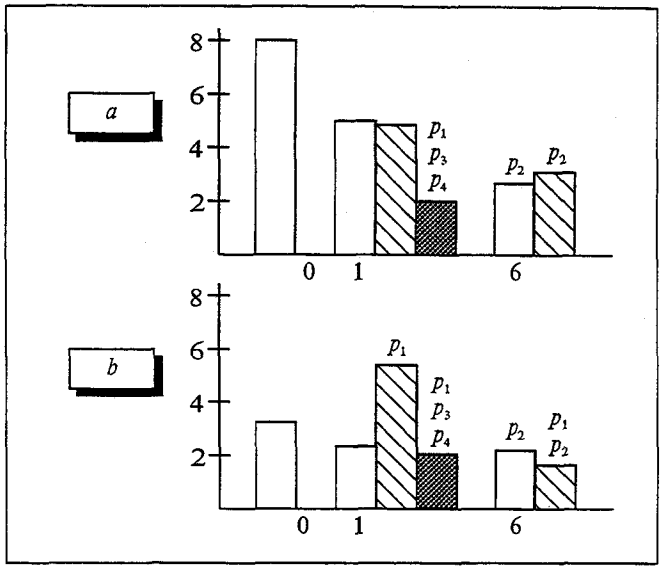

Fig. 4. Density of adrenergic nerve plexuses in the right (*a*) and left (*b*) ventricular myocardia in the test (MPE) and control groups. Ordinate: relative area (in %) occupied by adrenergic nerve plexuses in the myocardium. Significant differences (at $p<0.05$) are shown between the 1-hour and 6-hour control groups and the respective test groups (p_1), between the two test groups 1 and 6 h after onset of MPE (p_2), and between dogs with decompensated and compensated MPE by the 1st and 6th hours (p_3 and p_4, respectively).

The study of SG neurons in MPE thus brought to light three significant facts. First, the functional activity of SG neurons initially increases, and the activation of NE synthesis results in elevated levels of this neurotransmitter in the myocardium and, in particular, the adrenergic nerve plexuses of the left ventricle (Fig. 4). No rise in NE is recorded for the right ventricle, apparently because more NE is released from the nerve endings than is synthesized and taken up by neurons. These results suggest that the rate of NE release from nerve endings in the right ventricle is greater than in the left ventricle, which is most likely due to a greatly increased afterload on the right ven-

tricle myocardium and its intensified work after the onset of MPE [1]. Second, destructive changes in the adrenergic neurons are more pronounced and the functional activity of these cells declines by the 6th hour of MPE, with a fall of NE levels in the SG and in the nerve plexuses of the ventricular myocardia (Fig. 4). Third, as the duration of compensated MPE increases, so does the number of neurons exhibiting hyperchromatosis and pyknomorphic shrinkage. It is believed by some [9] that these changes reflect inhibition of metabolic processes in the nerve cell and its "conservation" and may sometimes be reversible.

The state of SG neurons in the test dogs that had rapidly developed cardiac failure after MPE production was in general similar to that observed for dogs with a relatively prolonged compensated MPE and was largely characterized by irreversible destructive changes (Fig. 1, *e*, *f*, and *g*), with a greatly decreased NE level in the SG (Fig. 3, *d*) and ventricular myocardia (Fig. 4). These findings support our earlier conclusion that the rate of such changes is an important factor in determining the outcome of the pathological process, i.e., whether a state of compensation or decompensation will ensue [6]. The only substantial differences between the compensated and decompensated states lie in the magnitude of hyperchromatosis and pyknomorphic shrinkage (Fig. 1, *d*), which are slight in the decompensated state of MPE and very considerable in the compensated state. The presence of many SG neurons with pronounced hyperchromatosis and pyknomorphic shrinkage may therefore be considered to be a characteristic feature of compensated MPE.

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