increased, the frequency of generation of ID was more markedly reduced, and the total duration of ID and the life-span of the foci were shortened (Table 2, series 4). This effect of combined administration may be compared to that of separate administration of these preparations in high doses: of valproate in a dose of 150 mg/kg or of glutapyrone in a dose of 80 mg/kg.

Glutapyrone and sodium valproate in doses of 1-100 mg/kg exerted no effect on electroshock-induced seizures in mice. Glutapyrone in a dose of 5 mg/kg, administered 1 and 3 h prior to electroshock, markedly (by 49.9 and 42.3%, respectively; $p<0.05$) enhanced the antiepileptic activity of phenobarbital: ED_{50} of phenobarbital was 35.5 mg/kg, whereas against the background of glutapyrone it was 17.8 and 20.5 mg/kg ($p<0.05$). Sodium valproate under similar experimental conditions had no effect on the anticonvulsive activity of phenobarbital.

The results of our study attest to the advisability of administering glutapyrone in combination with valproate and phenobarbital, since this results in the potentiation of the effect of each preparation for much lower doses. This reduces the likelihood of adverse effects, which is especially important in the case of prolonged treatment.

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Restoration of the Mast Cell Population in Rat Mesentery

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Key Words: *mast cells; mesentery; subpopulation; associability*

There is no consensus on the genesis and role of the mast cells (MC) of the connective tissue in the normal life of the human and animal organism. Much more information is available in reports dedicated to the role of MC in pathological processes. The theory of a "local adaptation syndrome" [7] with a leading role being played by connective-tissue MC, which were considered to be unicellular glands, arose in the 70s. According to the Selye theory, the biologically active substances secreted by MC provide for the local adaptation of tissue to stress effects, just as glucocorticoids of the

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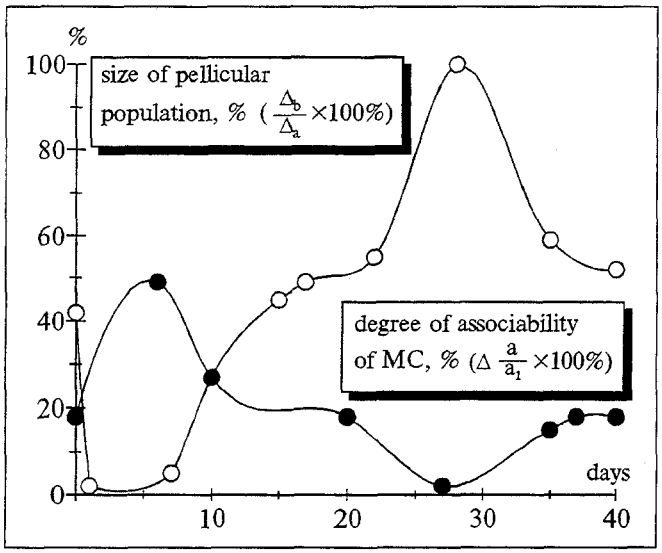

Fig. 1. Time course of number of MC in vessel–free zones of mesentery and the degree of their associability in the paravascular zone.

adrenal glands "switch on" the mechanisms of the general adaptation syndrome [7]. It was also suggested that the MC regulate tissue homeostasis and are the "final link" in the adaptive reaction of tissue at the cellular level [2]. Chronic inflammation and some other pathological processes are accompanied by an increase of the number of MC in tissues, while in acute inflammation and allergic reactions of the reagin type their decrease is noted. The recovery of every damaged tissue also results in the restoration of the MC system. The process of regeneration of the MC population after its depletion has been insufficiently studied.

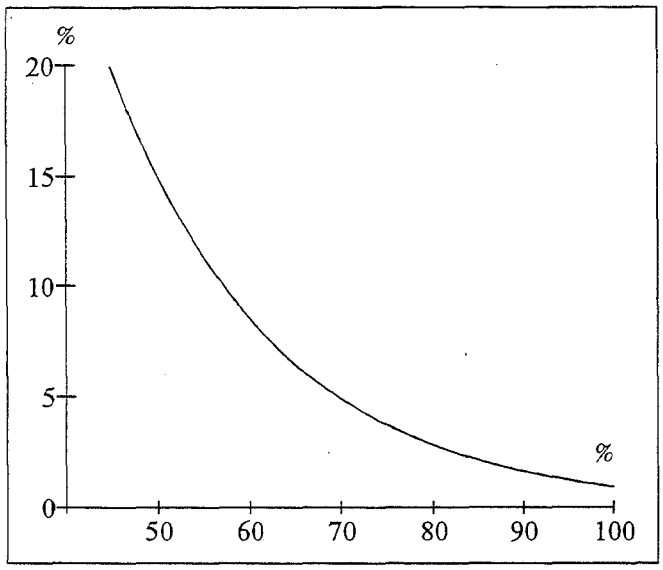

Fig. 2. Correlation between the number of MC in vessel–free zones and degree of their associability in paravascular zones. Abscissa: size of pellicular subpopulation ((Δb/Δa)·100%); ordinate: the degree of associability of MC in the paravascular zone (Δ·(a^1/a)·100%)

This investigation was undertaken to study the processes of restoration of the MC population in rat mesentery after osmotic damage of the cells by i.p. injection of distilled water.

MATERIALS AND METHODS

The total population of mesenteric MC was divided into two subpopulations: the paravascular subpopulation, occurring in the vicinity of blood vessels, and the pellicular subpopulation, situated far away from vessels in "mesenteric windows" - spaces between two rather large vessels detected visually. Damage was induced in MC by a single i. p. injection of distilled water using a method described elsewhere [6]. Examinations of both MC populations were performed at different times (from several hours to 40 days after injection). Control material was taken from intact rats. Collection, fixation, and staining of the material were performed according to Zelichenko's method [1]. The mesenteric MC were examined under a light microscope (oc. ×7, obj. ×20) . Quantitative estimation of both MC populations in different experimental periods was performed using the following parameters:

1) Δ·(a^1/a)·100% – is the mean degree of associability of MC on the slide. Associability means the microscopically detected contacts between two or among several cells. There were mainly paired structures (Fig. 3). (a^1/a)·100% – is the degree of MC associability in a visual field (the relative number of associated MC), where a denotes the total number of cells in the paravascular zone, and a^1 denotes the number of associated MC in the same visual field.

2) (Δb/Δa)·100% – is the relative number of MC in a "pellicle", where Δb is the number of MC in a "pellicle" and Δa is the number of MC in the paravascular zone.

RESULTS

The virtual disappearance of MC from vessel-free zones was noted one hour after i.p. injection of distilled water. The cells of the paravascular zone, on the other hand, did not undergo the destructive effect of osmosis in the period studied. Further study of the size of the two populations revealed that there were no MC in the vessel-free zones of mesentery 1 day after injection and the degree of associability of these cells in the paravascular zones was 20-25 % (normally 15-20%) . The cells began to appear in the mesenteric vessel-free zones on the 5th-7th day after injection, and at this time their content was 2-5% of the total population (normally 40-50%). At the same

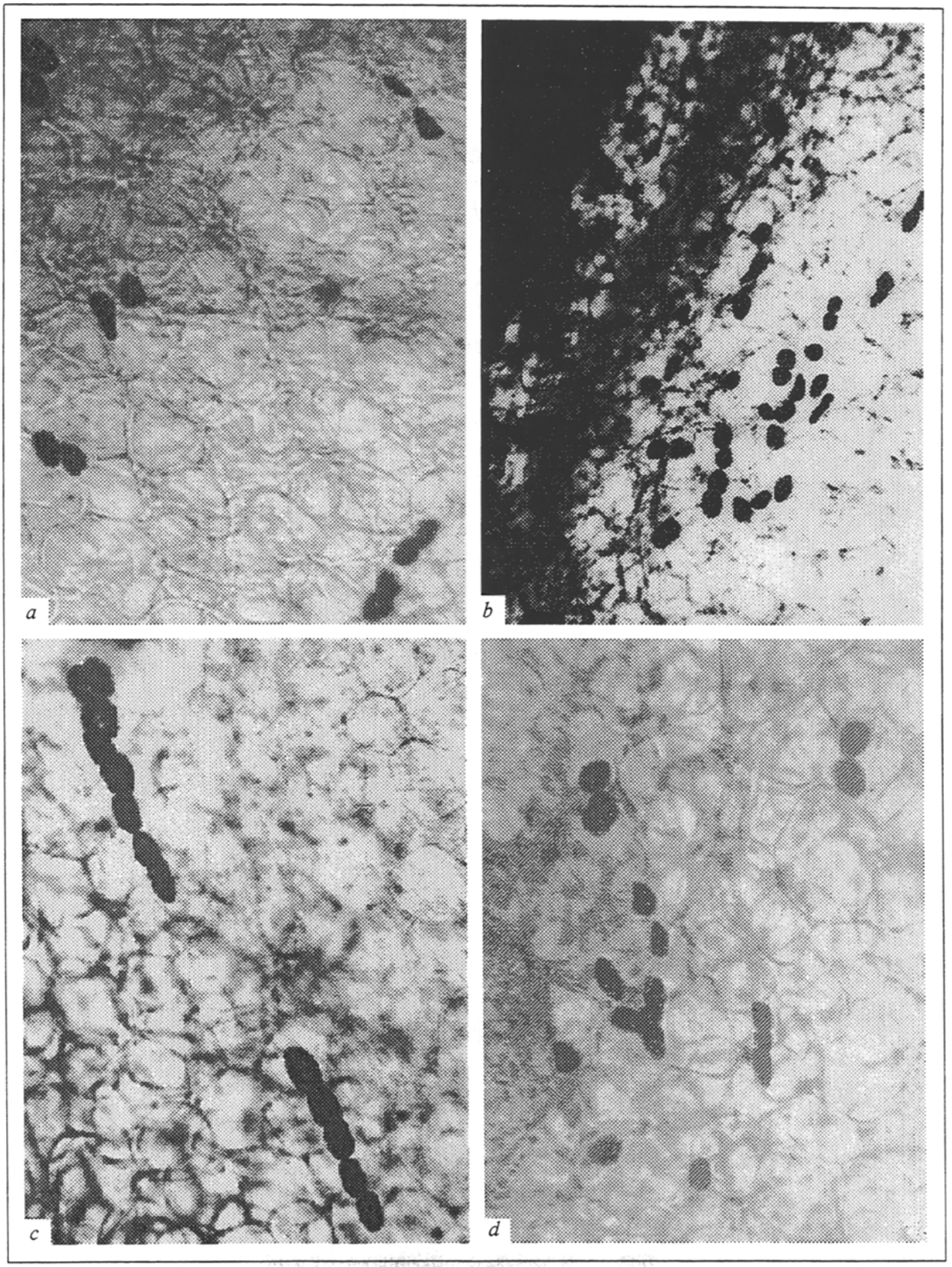

Fig. 3. Rat mesenteric MC in the paravascular zone on the 5th–7th day after i.p. injection of distilled water; a–d) different variants and different degrees of associability of MC. Light microscopy (obj. ×20, oc. ×7). Stained with toluidine blue.

time the content of the associated MC in the paravascular zone reached 40-50%, i.e., about one half of the cells situated along the blood vessels occurred in the associated state. The degree of association of the cells was lower in the paravascular zone on the 10th day after water injection and the number of cells rose in the vessel-free zone. On the 14th-15th day both the degree of associability of the cells along the vessels and the size of the population of the "mesenteric windows" reached the baseline level. From the 20th-21st day the number of MC in a "pellicle" began to increase once more and the degree of MC associability began to drop. Toward the 28th day the maximal colonization of the vessel-free zones occurred (90-100%) and the degree of associability of MC in the paravascular zone approached zero. Both indexes reached the baseline level on the 38th-40th day after injection.

Analyzing the parameters characterizing the quantitative changes in the MC subpopulation of rat bowel mesentery after osmotic impact, we drew the following conclusions:

1) The disappearance of MC from the vessel-free zones of mesentery induced by distilled water coincides with the beginning increase in the number of MC associations along the blood vessels (the first stage).

2) The second stage (5th-7th days) is related to the onset of the recovery of the total population of mesenteric MC. In this same period the degree of MC association in the paravascular zone reaches its maximum and cell colonization starts in the vessel-free zones emptied by the hypoosmotic impact of water.

3) The third stage is characterized by the final occupation of the "mesenteric windows" and by a drop of the degree of associability of MC in the paravascular zone (the 10th day).

4) At the fourth stage the parameters studied return to the baseline level (14th-15th day) (Fig. 1).

Thus, a constant inverse dependence was found between the numbers of MC in the vessel-free zones of the mesentery and the degree of associability of cells situated near the vessels.

In order to ascertain whether the obtained phenomena are due to the damaging effect of hypoosmia or whether they reflect the state of both subpopulations under natural conditions, we examined both subpopulations in mesenteries of intact rats. The findings confirmed that an analogous relationship exists in the intact mesentery as well (Fig. 2).

Damage to the mesenteric MC population by distilled water was performed previously [4,6]. Fawcett's study [6] is close methodologically to our investigation. This author studied the dynamics of recovery of the MC in rat mesentery from the 1st to the 45th day after i.p. injection of distilled water, followed by the administration of different doses of substance 48/80, which is a classical liberator of histamine from MC granules. The content of MC in the mesentery dropped sharply on the 5th-6th day after injection [6]. However, there are no data on different MC subpopulations, although these are mentioned in this report. Fawcett [6] assumed that the recovery of the MC mesenteric pools is probably due to the immature "nongranulated" cells of connective tissue situated near the blood vessels. These cells were stained with alcian blue and were at stage I - II of maturation according to Combs' classification [5]. Young wondered whether the replenishment of the mesenteric MC pool occurs due to the peritoneum itself or whether the cells of the free peritoneal fluid are their precursors too [8]. But this question has been not clarified. It has been convincingly demonstrated, using mouse radiation chimeras [3], that only some mesenteric MC are the descendants of hemopoietic cells, the other cells probably having local precursors.

Our findings suggest that the loss of MC in the mesentery provoked by the damaging factor (namely, hypoosmia in our experiments) and their natural decrease probably due to the end of their life cycle induce their new formation in the paravascular zone (Fig. 3). The nature of the links in the cell associations of this mesenteric region remains unclear and could be interpreted "at will" due to the diversity of their shapes. However, mathematical analysis testifies that the visually determined associability of the MC situated near blood vessels is found precisely where the number of MC in the vessel-free zones is sharply decreased. The latter MC are not protected by adipose tissue and adventitia and are thus more vulnerable to different impacts. The new formation is probably followed by the migration of MC to the region impoverished in MC from the parts of the mesentery rich in blood vessels. Since we studied the state of the MC population in rat bowel mesentery over a long period [1], we can confirm the migration by the fact that the MC in the paravascular zones usually have cytoplasmic processes filled with granules with the nucleus behind them. Scattered MC frequently found far from the vessels are oval or spindle-shaped, more rarely round. These are solitary cells, for the most part, pairs being noted infrequently. The colonization of the mesentery by MC is not an uncontrolled process, but rather strictly regulated. This is indicated by the inhibited formation of associations of these

cells in the paravascular zones when the optimum degree of occupation is achieved in zones far from the vessels.

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Calcium-Binding Properties of Plasma Proteins in Patients with Aseptic Necrosis of Bones after Cadaveric Kidney Transplantation

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Key Words: *plasma proteins; protein calcium-binding properties; aseptic necrosis of bones; kidney; allotransplantation*

Corticosteroid therapy is believed to be responsible for osteopathies developing in the long term after cadaveric kidney allotransplantation and manifested mainly in aseptic necrosis of the caput femoris [4,5]. The mechanisms by which corticosteroids disrupt osseous metabolism and cause focal necrosis in bones are still unknown [6,8]. Impairment of many tissue processes is known to be related to an elevated intracellular calcium concentration [5]. Previously we demonstrated that after cadaveric kidney allotransplantation patients show a high incidence of disorders of plasma protein Ca-binding properties which create a tendency toward hypercalcemia and toward an increase of calcium transport from the blood to tissue cells [2].

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We studied the calcium-binding properties of plasma proteins in aseptic necrosis of the bones after cadaveric kidney allotransplantation. Our task was to reveal the mechanism and quantitative parameters of calcium binding by plasma proteins in patients with aseptic necrosis developing after cadaveric kidney allotransplantation during prednisolone and azathioprine immunosuppression.

MATERIALS AND METHODS

Fifteen patients aged 15 to 48 who had undergone cadaveric kidney allotransplantation followed by two-component immunosuppressive therapy (prednisolone and azathioprine) and 36 healthy controls were examined. Eight of the fifteen patients presented with manifest clinical and x-ray signs of aseptic necrosis of one or both femur heads (group 1) and 7 had no signs of bone system involvement (group 2). The posttransplantation period was

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