TABLE 1. Changes in Width of Synaptic Space 3 and 24 h after Injection of DSIP (M ± m)

Conditions	Width of space, mm
Normal (230 synapses)	22,867±0,2596
3 h of DSIP (200 synapses)	24,5534±0,2464*
24 h of DSIP (290 synapses)	23,9688±0,2829*

Legend. *$p < 0.05$: Differences statistically significant.

chosen points on synapses cut through the center, where pre- and postsynaptic membranes were clearly visible. The results in Table 1 show that 3 h after injection of DSIP the width of the synaptic cleft was increased by 10.7%, which is highly significant ($p < 0.0001$). After 24 h this parameter fell to 10.4%, but still remained significantly greater than in the control ($p < 0.0022$).

It has to be pointed out that the synaptic cleft is a special form of intermembrane contact, and the chemical components of the synaptic cleft are responsible for the firm adhesive properties of this junction [3]. Even slight variations in the distance from the presynaptic to the postsynaptic terminal are possible only in the case of a considerable change in structure of the cytoskeleton of the pre- and post-synapse, It thus becomes clear that even a very small increase in width of the synaptic cleft will be evidence of activation of synapses of this type on the bodies of pyramidal neurons under the influence of DSIP. Activation of axo-somatic synapses thus revealed correlates with an increase in the GABA content and activation of the GABA-forming enzyme glutamate decarboxylase, and with the slow-wave activity recorded in the cerebral cortex [6].

LITERATURE CITED

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NUCLEAR BODIES IN RAT HEPATOCYTES: DYNAMICS OF APPEARANCE AND FORMATION DURING AGING AND FUNCTIONAL LOADS

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KEY WORDS: chromatin; ultrastructure; aging; blood loss; enterosorption.

The morphological equivalent of the genome is chromatin, and the state of expression of genetic information can be judged from changes in the fine structure of chromatin. For instance, the change from heterochromatin to euchromatin indicates activation of nucleic acid synthesis, as may be clearly demonstrated by electron-autoradiographic studies [4]. Besides the periodic condensation and decondensation of chromatin, and changes in conformation of the nucleoli and interchromatin granules, which have been adequately studied, the so-called nuclear bodies (NB), or foci with altered

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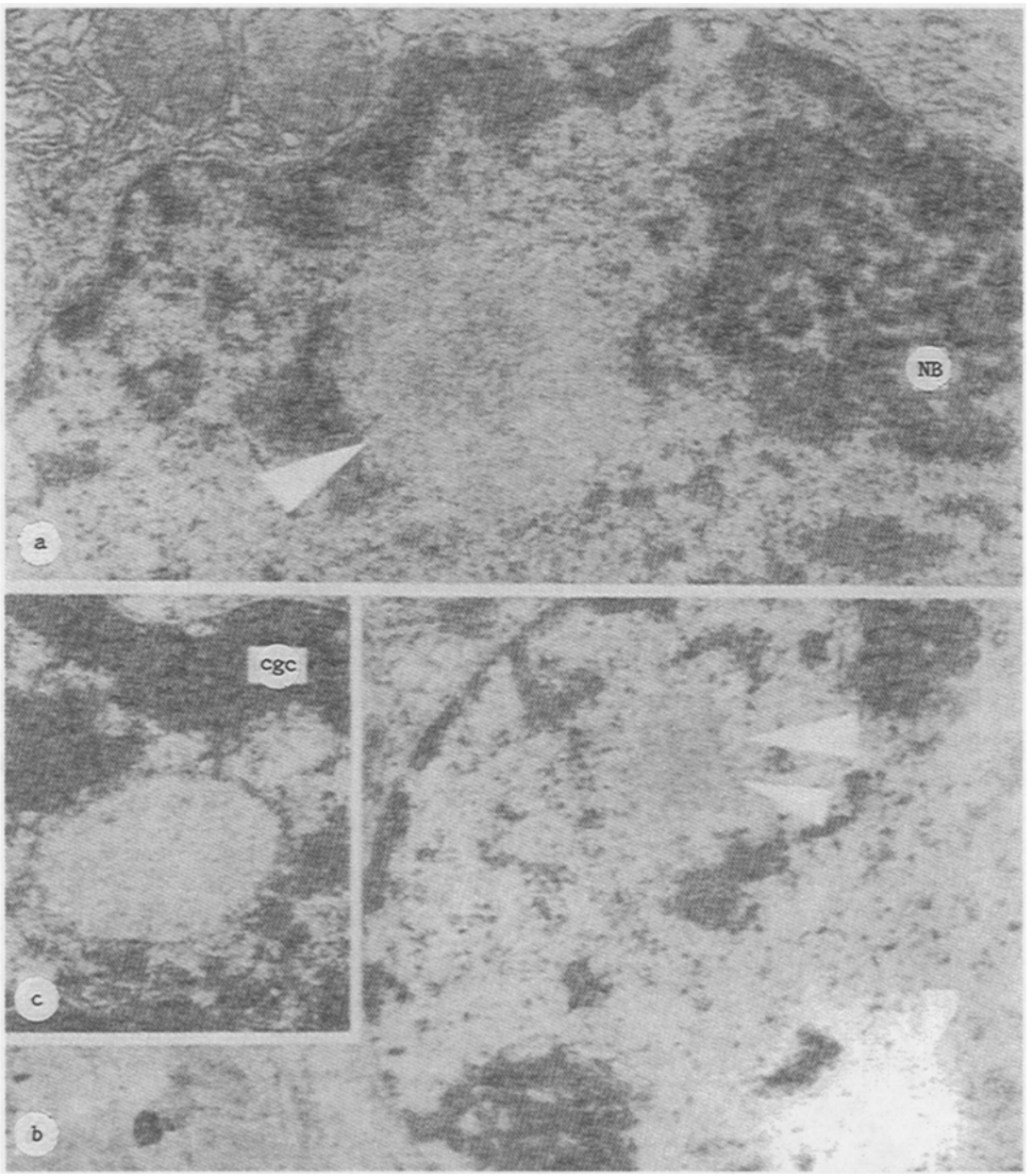


Fig. 1. NB in HC of old rats under different conditions: a) NB in the form of chromatin filaments with spherical formations (CF SF) surrounded by fibrillar layer (→). HC of intact rat aged 24 months with hyperplasia of ribosomes and polysomes. 30,500×; b) Two NB in HC of a 30-month-old rat 30 months after enterosorption. NB below is surrounded by a fibrillar layer (→). That shown above passes invisibly into surrounding chromatin (→→) 15,000×; c) NB with fibrillar layer (→) among concentrations of masses of coarse granular chromatin (cgc). Apoptosis of HC of rat aged 24 months 10 h after blood loss. Magnification 30,000×.

chromatin structure [1, 7], also have been observed. The nature of NB is far from clear, but one thing is evident, that is that they reflect functional changes in the genome, as is shown by the regularity of their appearance and variation of their architectonics depending on age and exposure to external factors. The aim of this investigation was to study this problem.

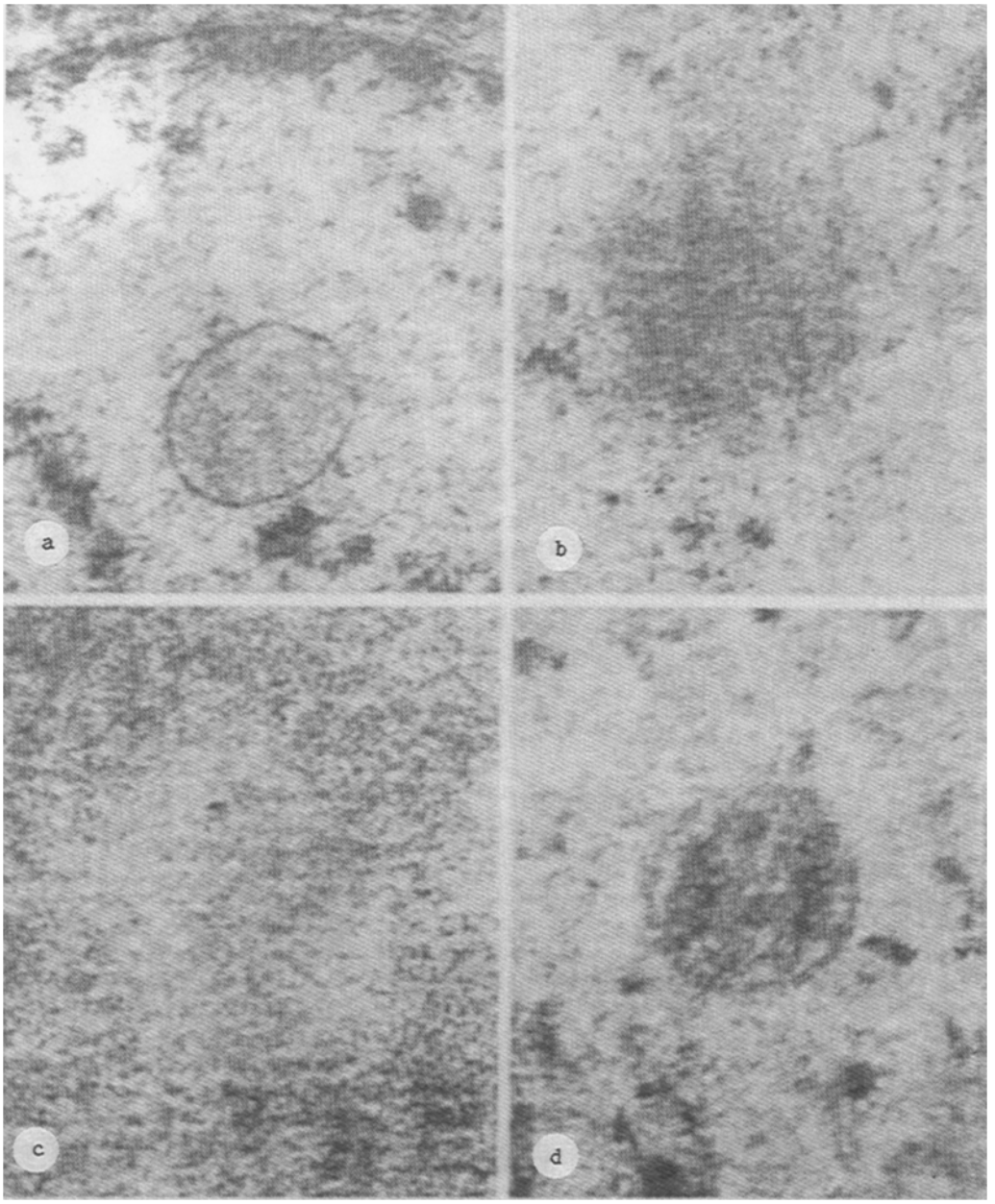


Fig. 2. Modification of structures of NB at different times after blood loss in HC of old rats aged 24 months. a) NB with fibrillar layer located in edematous karyoplasm with chromatin undergoing lysis, 2.5 h after blood loss. Magnification 30,000; b) NB in the form of freely lying CF SF with invisible transition into surrounding chromatin, 5 h after blood loss. 33,000×; c) NB in form of CF SF with fibrillar layer against background of granular osmiophilic chromatin. 10 h after blood loss. Jb_x) 40,000; d) NB in form of condensed core with fibrillar layer, 24 h after blood loss. 27,000×.

EXPERIMENTAL METHOD

Experiments were carried out on 105 male Wistar rats in four age groups: 8, 24-25, 30, and 38 months. Some animals were exposed to blood loss (posthemorrhagic anemia), intensifying synthesis of plasma proteins in the hepatocytes. This procedure was carried out at different times, after an interval of 15 min, 2.5, 5, 10, and 24 h, and 7 days, by the method described previously [1]. Another procedure, aimed at reducing the action of enterotoxins, the quantity of which

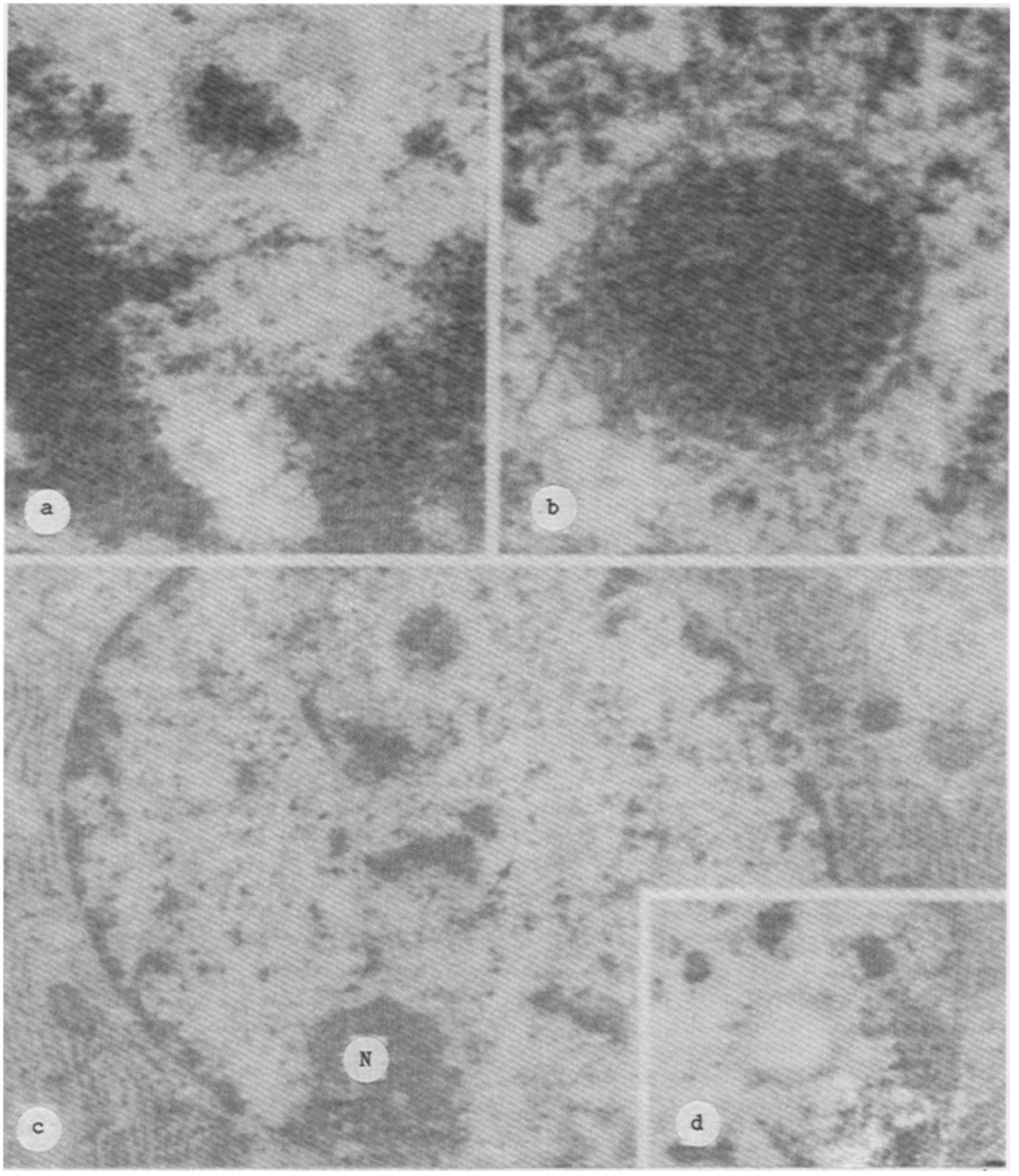
increases during aging, was enterosorption, which is nowadays used clinically in the treatment of several diseases and has been suggested for experimental prolongation of life [2, 6]. Methods of electron microscopy were described previously [1].

EXPERIMENTAL RESULTS

NB were observed in the liver only in hepatocytes (HC), where in old control rats they were found in every 8-10 nuclei, but were absent in endothelial cells, stellate reticuloendotheliocytes, perisinusoidal cells, and pit-cells. Morphologically they consist of circular-oval formations from 1 to 3 μ in diameter, distributed either paracentrally or close to the karyolemma, but never in contact with it (Fig. 1a, b). Sometimes two NB of different structure were found in the same nucleus (Fig. 1b). In binuclear HC NB could be observed in each nucleus. NB were frequently observed in HC with a hypertrophied nucleus and hyperplastic cytoplasmic structures (Fig. 1a), and also in cells undergoing cloudy-swelling degeneration and apoptosis (Fig. 1c). In the last case they were surrounded by large concentrations of condensed chromatin near the karyolemma. The main component parts of NB are fine, haphazardly and loosely arranged chromatin filaments with spherical formations about 0.01 μ in diameter. Similar structures in molecular biology, observed in the study of isolated chromatin, have been called graphically pearl necklaces, and they reflect the nucleosomal level of organization of chromatin, which gives access to the use of genetic information. Outlines of NB in some cases are uneven, and blurred, changing unnoticeably into the surrounding chromatin (Fig. 1b). In most observations, however, NB around the periphery were surrounded by a densely packed, thin fibrillar layer or halo on the section, creating the impression of finality of shape of the NB against the background of differences in the degree of aggregation of a surrounding chromatin (Figs. 1, 2). NB were seen less frequently in the form of a concentration of coarse granular chromatin. In some cases granules occupied only part of the area of section of NB, whereas the rest consisted of loosely packed fibrils (Fig. 3a), and in the rest the whole surface of the section through NB was occupied by coarsely granular chromatin (Fig. 3b). In all observations, however, NB with granular chromatin were surrounded by a fibrillar halo of varied thickness, and not once were they observed without it. Their size, by contrast with NB with a fibrillar component, did not exceed 1 μ. Granular NB with fibrillar surrounding were observed in HC of the rats after adrenalectomy and exposure to ionizing radiation [8]. NB consisting of amorphous material with small round inclusions and microtubular structures have been described in chronic hepatitis B. Delta-antigen has been discovered in them by an immunoelectronic method [9].

Posthemorrhagic anemia, causing marked hypoxic damage to HC 2.5 h after blood loss, also affects the structure of NB. In cells exposed to hydropic degeneration, NB were found in the translucent edematous karyoplasm of the lysed chromatin (Fig. 2a). Later, elements of NB lost the clarity of their outlines, and began to show signs of lysis. In the later stages after blood loss, especially after an interval of 10 h, during the period of stability or of long-term compensation, accompanied by an increase in the number of binuclear HC and by the appearance of mitotic activity, the number of cells with NB increased significantly compared with the control in old rats, where they were observed on sections, one to every three or four nuclei. Meanwhile their true number must have been much greater, if allowance is made for the thickness of the ultrathin section and the size of NB. It is important to note that NB at this time of exposure appeared first of all in HC in rats aged 8 months, which had no such cells in the intact state. The number of MB without a fibrillar halo increased first of all (Fig. 2b), followed by the number surrounded by a halo. In the latter case they were found more frequently in HC undergoing cloudy swelling degeneration and apoptosis (Figs. 1c and 2c). It must be pointed out that the structure of NB showed no significant damage under these circumstances, as in the case of hydropic degeneration. During this period NB with densely packed fibrils, in which no spherical structures could be seen (Fig. 2d), and NB with a granular component (Fig. 3a) also appeared after blood loss.

Fig 3. NB in stages of condensation and intranuclear inclusions in HC of old rats: a) NB in the form of partly condensed core with fibrillar layer 10 h after blood loss. 36,000×; b) NB with fibrillar layer and coarse-granular chromatin in core after enterosorption. 40,000×; c) two NB in HC after enterosorption. Upper NB in the form of granular chromatin and a fibrillar layer, lower NB in the form of CF SF. Small lipid drop next to it (→). 12,000×; d) Lipid drop in nucleus surrounded by fibrillar halo. 16,000×; e) Stage of NB formation: I) CF SF, lying freely in karyoplasm; II) CF SF with fibrillar layer; III) CF SF and partially condensed chromatin, surrounded by fibrillar layer; IV) completely condensed coarse-granular chromatin with fibrillar layer. Legend: NB) nuclear bodies; HC) hepatocyte; N) nucleolus; CF SF) chromatin filaments with spherical formations; cgc) condensed granular chromatin.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b
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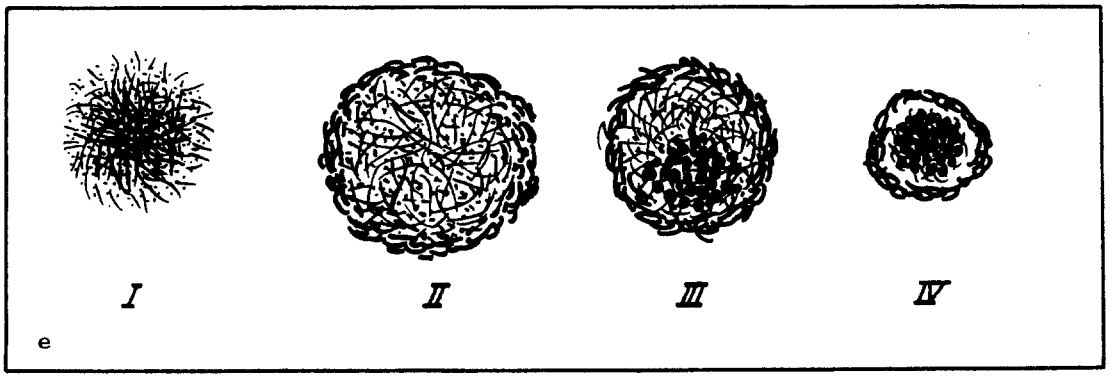
I
II
III
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e

Anterosorption did not cause any marked change in the number of NB in the old rats, but larger NB with a granular component in the center and nucleus, each containing two NB with different structures, were found among them (Figs. 1b and 3b, c). In HC of the old rats enterosorption caused redistribution of components of the cytoplasmic reticulum in the form of reduction of its smooth part and hyperplasia of its rough part [2]. This depends correspondingly on switching of the loci of the genome, which may perhaps be reflected in the formation of NB with the particular features described above.

During the study of NB they must be differentiated from intranuclear inclusions, which are often found during aging in various cell populations [5], but which usually are not reactions of chromatin. In HC, those of them that are observed most frequently are lipid inclusions, entering the nucleus during mitosis. Just like NB, they are surrounded at their periphery by chromatin fibrils, but unlike the latter, they have a homogeneous core (Fig. 3d).

When the facts described above are analyzed, a sequence of formation of NB can be imagined, with distinction of four conventional stages (Fig. 3e). These structural transformations of chromatin are evidently accompanied by functional changes in the corresponding regions of the genome. In stages I and II of NB formation the chromatin is decondensed to the nucleosomal level of organization, and this may facilitate transcription or replication, since the genetic information is available. When condensing into stage III and IV of NB formation, the chromatin in them loses its template activity, as a result of repression of genetic information, or fragmentation of its carrier. Other nucleic acid synthesis is in fact carried out in NB at stages I and II, or whether all that takes place in them is maximal repacking of DNA without any processes of template assembly on it, i.e., whether a sterile flower is formed, can be decided only after electron-microscopic autoradiography. Indirectly, at least as regards activity of the chromatin in MB or its readiness for activity, it is indicated by the fact that these foci increase considerably in number in stages I and II, during the period of stable long-term compensation after blood loss, when synthetic activity is maximal. Under these circumstances they are often observed in HC with signs of intracellular ultrastructural hyperplasia, with cloudy-swelling degeneration, evidence of inadequate hyperfunction, and with apoptosis. The latter, according to our suggested hypothesis, is an alternative process to mitosis [3], and it is therefore energy-dependent and, as a rule, requires additional RNA and protein synthesis. Meanwhile, if lysis occurs as early as in the stage of hydropic degeneration, NB are never seen at all in HC, as they undergo colliquative necrosis, which is associated with complete breakdown of all synthetic processes.

The genesis of chromatin condensation in stages III and IV of NB formation is varied in manner. On the one hand, the appearance of its coarse granular formation may be the result of repression of genetic information, but on the other hand, it may be the results of aggregation, due to internucleosomal cleavage of the chromatin filament by Ca^{2+}, Mg^{2+}-dependent endonucleases, which is similar to what happens in apoptosis [3]. The role of the fibrillar halo, possibly a product of the expressed genes, is likewise unclear; it may also be a material limiting access to genetic information or blocking the further spread of chromatin decondensation.

Since they appear in HC only in old rats in the control, MB can be regarded as a sign of aging. In that case, the appearance of foci of chromatin reorganization in HC in rats aged 8 months after blood loss suggests that aging takes place, not because of time, but during time, faster or slower depending on different conditions. This is in agreement with the findings of Sarkisov et al. [4], who state that morphological changes in elements of cells arising as a result of experimental procedures are identical to those subsequently appearing of their own accord in cells of intact animals with the passage of a certain length of time. The uniform structural changes in chromatin during aging and in posthemorrhagic anemia are evidence that the genome is involved in these processes and that the pathogenetic stages of the disease and of aging are related.

Thus MB in hepatocytes of intact rats appear only during aging. Variations of their architectonics depend on the form of the external influence and take place in definite stages. It is linked with the time elapsing after the beginning of exposure to the external factor and it is linked with changes in conformation of chromatin.

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MORPHOLOGICAL AND BIOCHEMICAL INVESTIGATION OF THE LIVER IN SYSTEMIC ENDOTOXEMIA

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KEY WORDS: liver; metabolism; endotoxin.

Endotoxemia, which accompanies various diseases (septicemia, diffuse peritonitis, gangrene, etc.), leads to the development of multiple organ failure in 40-85% of surgical patients [1, 6, 14]. An important stage in the pathogenesis of injury to organs and tissues, including the liver, is systemic endotoxemia as a result of destruction of the saprophytic and pathogenic intestinal microflora [4, 12].

The aim of this investigation was to study the structure and metabolism of the liver tissue of dogs in the course of systemic endotoxemia.

EXPERIMENTAL METHOD

Experiments were carried out on 11 mongrel dogs weighing 13-18 kg, which received an intravenous injection of *E. coli* lipopolysaccharide (LPS) in a dose of 2 mg/kg, and each dog underwent liver biopsy 0, 1, 3, 5, and 7 h thereafter. Each biopsy specimen was divided into two parts, one of which was fixed in 10% neutral formalin and embedded in paraffin wax. Microtome sections were stained with hematoxylin and eosin. From the other part of the biopsy material a homogenate was prepared, in which the content of albumin, total protein, bilirubin, and glucose and also activity of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GTP), and alkaline phosphatase (AlP) activity were determined using an "Express-550" automatic biochemical analyzer (Ciba Corning, England). The numerical results were subjected to statistical analysis by computer.

EXPERIMENTAL RESULTS

Microscopic study of the liver in endotoxemia showed progressive changes in the microcirculatory system and parenchyma of the organ. Only 1 h after injection of LPS, irregular congestion of the portal vessels, especially branches of the portal vein, was noted. In individual portal tracts there were small areas of hemorrhages, which increased in size after 3 h, and by 5-7 h they had even spread to the periportal regions. After 3 h uneven dilatation and congestion of the sinusoidal vessels and hepatic venules, aggregation of erythrocytes, and foci of leukocytic stasis were observed. After 5 and

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