ELECTRON-AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN THE NUCLEOLUS

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A compensatory increase in the number of DNA-synthesizing hepatocytes was induced in mice by administration of CCl_4. After short-term (20-30 min) labeling of the hepatocytes with [^{3}H]thymidine several different types of distribution of labeled DNA in the nucleus were found. In one type the label was localized almost entirely in the body of the nucleolus. DNA replication in the body of the nucleolus showed certain particular features: very weak labeling of the remaining structures of the nucleus at this period, including heterochromatin bound with the nucleolus, synchronization of DNA synthesis in different nucleoli of the same nucleus, a decrease in the electron density of the body of the nucleolus, and a high concentration of label above it.

KEY WORDS: nucleolus; DNA synthesis; chromatin.

Attempts to determine the distribution of DNA replication sites in the nucleus at individual stages of the synthetic (S) period have been undertaken in electron-autoradiographic experiments. The results have shown that DNA synthesis takes place at different times in different zones of chromatin. The clearest differences in the time of synthesis are found in the central zone of the nucleus, occupied chiefly by euchromatin, and the peripheral zone, where a belt of heterochromatin is located along the inner nuclear membrane [5-7, 9-10]. It has been seriously argued that synthesis in the first zone takes place at the beginning of the S period and in the second zone at its end [8, 9, 10]. Information on DNA synthesis in the nucleolus relates only to perinucleolar chromatin which, according to available data, replicates simultaneously with heterochromatin located along the nuclear membrane [7, 8]. It has not yet been discovered when and how replication takes place in the euchromatin located in the body of the nucleolus (the nucleoloneme), i.e., the main productive element of the nucleolus for ribosomal RNA is synthesized in this structure [3]. Observations pertaining to this problem are described in this paper.

METHODS

Twice a week 0.2 ml of a 40% solution of CCl_4 in peach oil was injected subcutaneously into noninbred albino mice for 5 months. On the 2nd day after the last injection of CCl_4, five animals received an intraperitoneal injection of [^{3}H]thymidine in a dose of 20 μCi/g (specific activity 22 Ci/mmole). The liver tissue was fixed 20-30 min after injection of thymidine in 2.5% glutaraldehyde solution and 1% OsO_4 solution in phosphate buffer, pH 7.4. Electronautoradiographs were prepared from pieces of liver embedded in Epon by the method described previously [4].

RESULTS

The cell cycles of the hepatocytes in the material thus obtained were not synchronized, and for that reason different forms of distribution of grains of reduced silver in the labeled nuclei were found in each animal. These different forms were as follows: 1) Grains of silver were distributed above the euchromatin of the central extranucleolar part of the nucleus. The nucleolus, the perinucleolar heterochromatin, and the peripheral zone of the nucleus adjacent

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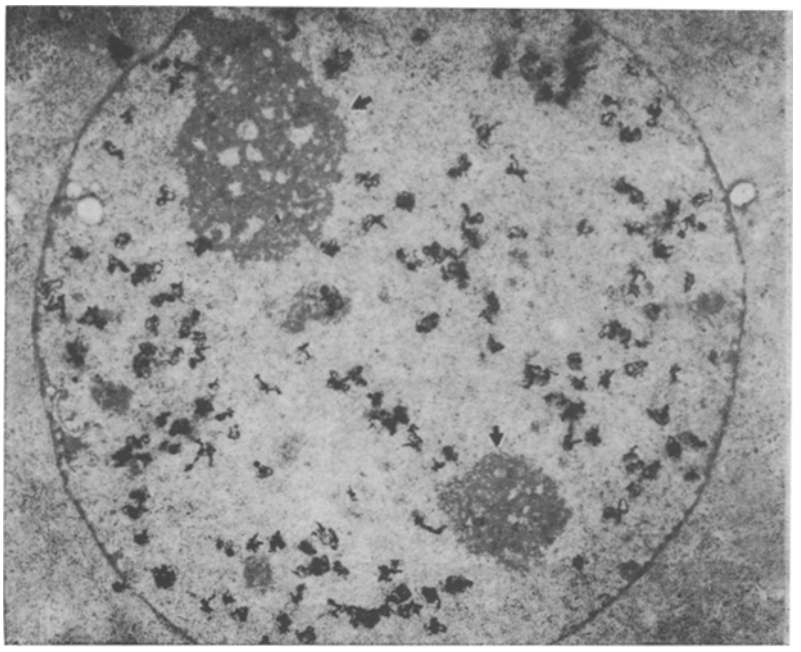

Fig. 1. DNA synthesis in nucleus of mouse hepatocyte almost free from heterochromatin. Grains of silver distributed above euchromatin; nucleoli (arrows) unlabeled (20,000 ×).

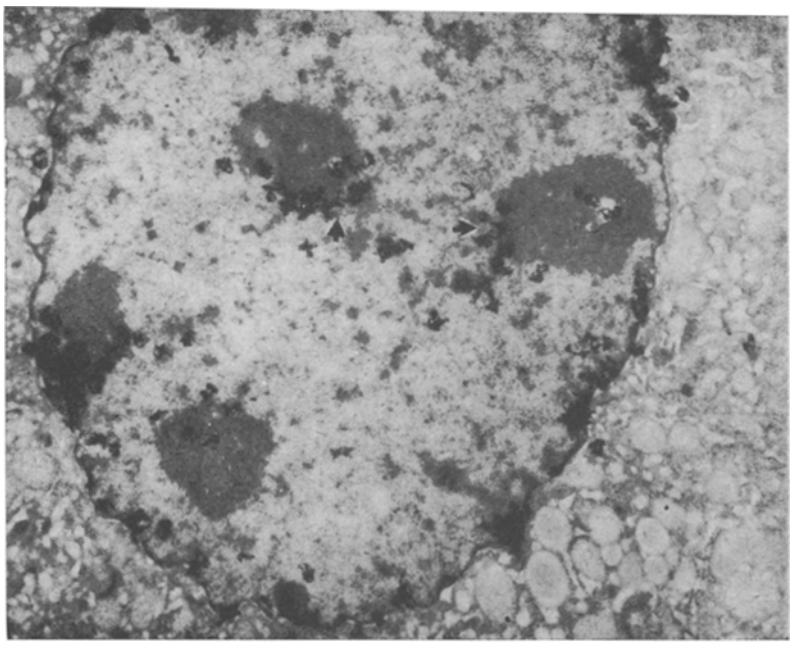

Fig. 2. Mouse hepatocyte. DNA synthesis in heterochromatin distributed along nuclear membrane and bound with nucleoli (arrows) (20,000 ×).

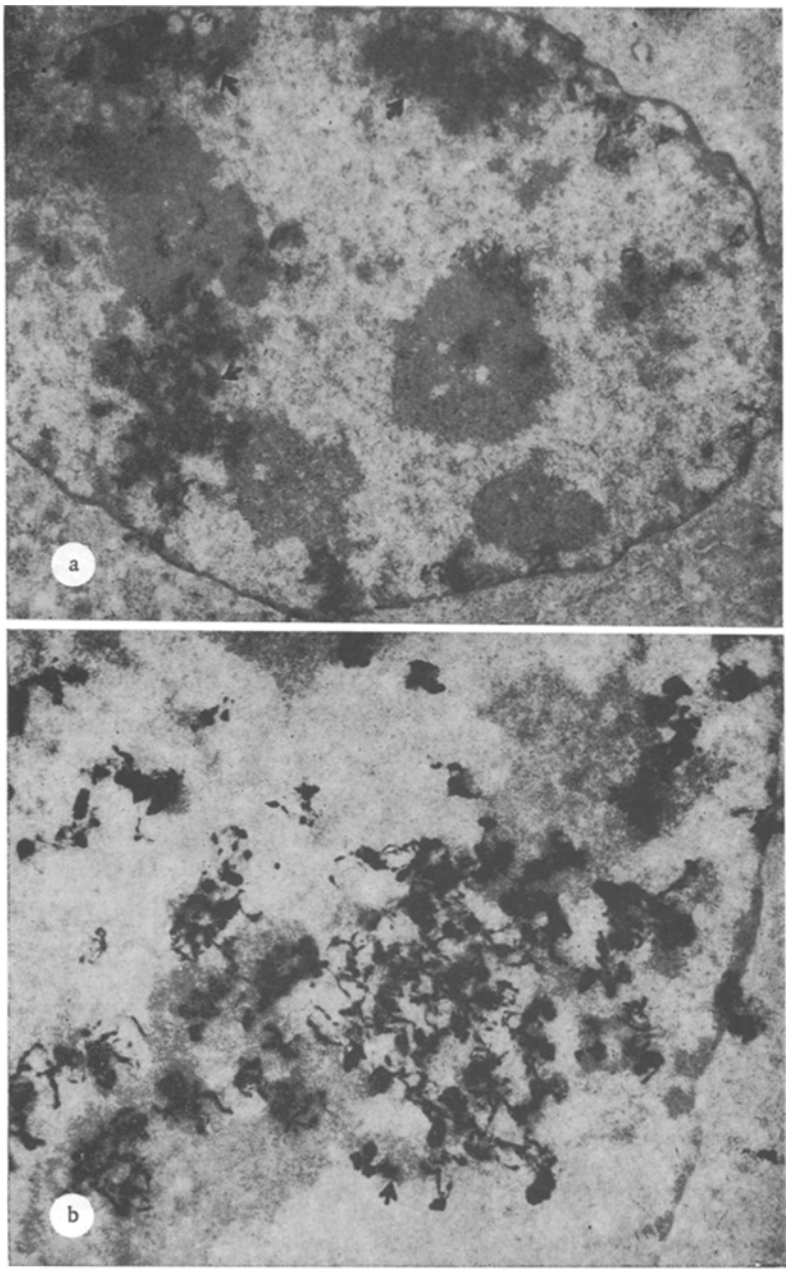

Fig. 3. Synthesis of intranucleolar DNA in mouse hepatocyte: a) synchronized DNA synthesis in three nucleoli (arrows); high concentration of grains of silver above region corresponding to position of nucleoloneme of each nucleolus; weak labeling of remainder of nucleus (25,000 ×). b) Reticulum of nucleoloneme (arrow) in region of concentration of grains of silver (40,000 ×).

to the membrane were virtually free from label (Fig. 1). Heterochromatin was almost completely absent from nuclei of this type, and small concentrations of it were found only in the perinucleolar region. 2) The second form differed from the first in the more appreciable accumulation of heterochromatin, in the form of chromocenters scattered over the nucleus. Around them there were numerous grains of silver. Occasionally chromocenters of this sort, with perichromatin label, were bound to the nuclear membrane. The nucleolus and the heterochromatin bound with it were not labeled. 3) Grains of silver were concentrated near massive accumulations of heterochromatin, distributed on the nuclear membrane, and also near the heterochromatin of the nucleolus. The small chromocenters were unlabeled (Fig. 2). 4) Very dense labeling was observed only above the body of the nucleolus (Fig. 3). The remaining territory of the nucleus, including heterochromatin bound with the nucleolus, was almost

unlabeled (Fig. 3a). The body of the nucleolus itself in such preparations had lost much of its electron density and could be distinguished only under high power (Fig. 3b). This was the most uncommon type of distribution of the grains of silver.

The first three types of distribution of grains of silver confirmed existing data on the unsynchronized replication of chromatin located in the central zone of the nucleus and chromatin concentrated near the nuclear membrane. This is supported by the fact that the first and third types were never combined in the same nucleus, when labeled thymidine was present in the cell only for short times.

The fourth type of distribution of grains is the most interesting. Several conclusions regarding the particular features of DNA synthesis in the nucleoloneme can be drawn from Fig. 3 and from other autoradiographs in the writers' possession. First, this synthesis is not synchronous with replication of the perinucleolar heterochromatin. This is shown not only by the fourth, but also by the third variant (Fig. 2). Furthermore, at the time of synthesis of intranucleolar DNA, DNA synthesis is inhibited or, perhaps, stopped altogether not only in the perinucleolar heterochromatin, but also in all the other regions of the nucleus. Examination of Fig. 3a suggests that all the few centers of concentration of label in the nucleus reflect the distribution of scattered material of the nucleolus, and that the remaining chromatin was unlabeled. Nevertheless, the suggestion that DNA synthesis in the nucleoloneme takes place in general outside the S period seems improbable. If replication of euchromatin in the nucleolus takes place in the course of the S period, the increase in the duration of contact of the cell with [^{3}H]thymidine ought to be connected with the spread of the replication process to other regions of chromatin, and it would be impossible to find the fourth type of distribution. Otherwise, i.e., if nucleolar euchromatin replicated outside the S period, isolated labeling of the body of the nucleolus only could also be found even after long contact of the cells with [^{3}H]thymidine. However, in the experiments in which thymidine was present in the animal's body for 1 h, pictures of this sort were not found. Most probably the intranucleolar DNA replicates at a special moment, measured in minutes, of the S period. It will be clear from Fig. 3a that DNA synthesis takes place simultaneously in different nucleoli of the same nucleus.

Two other characteristic features distinguishing DNA replication in the body of the nucleolus also call for attention. The first — a decrease in the electron density of the nucleoloneme — is attributed to decondensation of the intranucleolar chromatin and a decrease in the RNA content, possibly due to cessation of the synthesis of this substance on templates occupied with replication. The first is the extremely high density of labeling of the nucleoloneme, much higher than the density recorded during replication of euchromatin in other zones of the nucleus (Fig. 1). This feature may indicate repeated copying of the genes during replication of nucleolar DNA, whereas during replication of the remaining DNA the number of genes is simply doubled. Indirect indications of the existence of many identical genes of ribosomal RNA in the nucleolus is given by observations made previously in our department. First, nucleoli can increase the intensity of their RNA synthesis much more than the remaining territory of the nucleus in the acute period of compensation of injuries caused by CCl_4 [1, 4]. Second, information has been obtained to show that during the chronic action of CCl_4 part of the active euchromatin of the nucleolus is converted into inactive heterochromatin, and certain nucleoli of the cell may completely die, yet the cell as a whole remains viable [2]. The material basis for this wide range of adaptive capacity of the hepatocyte nucleoli is evidently amplification of the genes of ribosomal RNA, which takes place by repeated copying of these genes in each S period.

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GLIAL—VASCULAR REACTIONS TO ANGIOTENSIN II IN THE RAT BRAIN

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Reactions of the glia and blood vessels in the sensomotor cortex of adult rats to intraperitoneal injection of angiotensin II were studied electron-microscopically. Repeated injections of the hormone led to edema of increasing severity of the astrocytes, constriction of the lumen of the capillaries, and changes in the structure of their endothelium. It is suggested that these disturbances may be the cause of the cerebrovascular insufficiency and of functional changes in the CNS.

KEY WORDS: brain capillaries; astrocytic glia; angiotensin II.

The structure of the brain capillaries corresponds to the general plan of capillary structure. However, unlike capillaries of other organs, those of the brain have close topographic connections with glial cells. Staining nerve tissue by various methods has shown that processes of astrocytes make contact with the vascular wall [1, 4]. The astrocytic glia is considered to cover 85% of the surface of the brain capillaries [6].

Besides its action on neurons of the CNS, angiotensin II (A-II) also has a direct action on the smooth muscles of blood vessel walls, increasing their tone [5, 7]. It is not yet clear what takes place in the capillary part of the vascular system, in particular, what changes take place in glial—vascular interrelations, which correspond to the level of function of the blood—brain barrier [3].

With these facts in mind, and also considering that a considerable disturbance of functions of the CNS and of visceral regulation persists for a long time after administration of A-II ceases [2], it was decided to study the effect of A-II on the ultrastructural organization of the blood vessels and glia in the cerebral cortex.

METHODS

Experiments were carried out on 24 male albino rats weighing 180-200 g. A-II (Hypertensin, from Ciba) was injected intraperitoneally in a dose of 0.05 μg/kg body weight daily for 1, 3, 7, 14, and 21 days. Intact animals served as the control. The rats were decapitated 5 and 15 min after injection of the hormone on the 1st day and 15 min after its injection on the other days. Pieces of the sensomotor cortex were fixed in glutaraldehyde, postfixed with OsO_4, dehydrated in alcohols of increasing concentration and acetone, and embedded in Araldite. Sections were cut on the LKB Ultrotome, stained with lead citrate by Reynolds' method [8], and studied in the JEM-100B electron microscope. Material from three animals was used at each time. Grids with 6-8 blocks were examined from one animal.

RESULTS

The capillary reaction is one of the early responses of the brain to injection of A-II. Hyperemia of the vessels could be observed macroscopically in the brain of the rats as early as 5 min after a single intraperitoneal injection of A-II. Dilatation of the capillary net-

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