mented with 10% FCS the final glucose concentration is 2.5 mg/ml. Aliquots of this medium were taken and enhanced with glucose to yield a final glucose concentration of 3.5, 4.5, 6.5 or 11.5 mg/ml.

Pooled blastocysts were randomly assigned to the culture media and left undisturbed until examined 48 h later. Daily observations were then made at 24 h intervals until a period of 5 days post-culture was reached. The results are presented in tabular form. Embryonic development for each observed event was expressed as the rate of embryos that reached a particular stage of development to the total number of embryos that possed the potential to reach that stage. The stages of development examined were a) hatching from the zona follucida, b) attachment to the collagen substratum, c) trophoblastic cell outgrowth and d) differentiation of the inner cell mass to the egg cylinder stage.

As seen in the table, increasing the glucose concentration above the control value of 2.5 mg/ml can influence in vitro mouse embryogenesis. At 3.5 mg/ml there is a significant increase in the number of blastocysts attaching to the substratum as well as the number exhibiting trophoblastic cell outgrowth. At 4.5 mg/ml there was a significant decrease in the number of embryos reaching all stages of development. Raising the glucose concentration to 6.5 or 11.5 mg/ml did not induce any significant changes with respect to the control concentration and/or the concentration of 3.5 mg/ml. The reason for the marked reduction in embryogenesis at 4.5 mg/ml is not clear but may be related to altered membrane permeability at this concentration. The improvement of attachment and trophoblastic cell outgrowth at 3.5, 6.5 and 11.5 mg/ml reflects a higher glucose need at these stages of development. Klebe[12] has previously reported that glucose is required for the in vitro attachment of cells to the substratum. It appears from this data that media which contain the routine concentration of 1.0 mg/ml of glucose do not allow optimum attachment or trophoblastic cell outgrowth. Raising the concentration above 3.5 mg/ml does not significantly increase the number of embryos reaching a particular developmental stage and may be harmful in some cases (i.e. 4.5 mg/ml). This research thus demonstrates that the routine concentration of glucose utilized for in vitro mouse embryogenesis at these developmental stages should be increased to a final concentration of 3.5 mg/ml. It also demonstrates that the glucose concentration of the fetal calf serum utilized for experiments of this type should be measured so that the experimentor has knowledge of the final glucose concentration in the experimental media.

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Electron microscopic observation of nucleosomes in ultrathin sections of heparin-treated nuclei

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Summary. Isolated rat liver nuclei display nucleosomes (γ-bodies) in common ultrathin sections after treatment of nuclei with heparin. The masking of nucleosomes in intact chromatin by some extranucleosomal component is suggested.

There is considerable evidence that chromatin is composed of nucleoprotein particles named γ-bodies[1] or nucleosomes[2]. The majority of investigations, including electron microscopic visualization, were performed on the sheared chromatin or isolated nucleosomes. The nucleosomes have not been distinguished, as a rule, in embedded material[3]. The only known exclusions are the nuclei with extensive chromatin decondensation, that display chromatin subunits in untreated ultrathin sections[4,5]. It therefore seems likely that pretreatment of nuclei with agents, causing loosening of chromatin, may be a useful method for visualization of nucleosomes. This study deals with such an attempt. For chromatin decondensation, we have used heparin - a natural polyanion, which is known to discharge histones from their complex with DNA[6].

Material and methods. Rat liver nuclei were isolated by the sucrose method[7]. The nuclear pellets were treated with 0.05% sodium heparinate diluted in isotonic saline (pH 7.0) at room temperature for 2-10 min. The nuclei were then fixed by adding glutaraldehyde to the incubation medium to the final concentration of 2.5%, for 1 h, postfixed with osmium tetroxyde (2%) for 2 h, block-stained with uranyl acetate during dehydration and embedded in Epon. Ultrathin sections were made with LKB ultratome, contrasted again with uranyl acetate and lead citrate according to

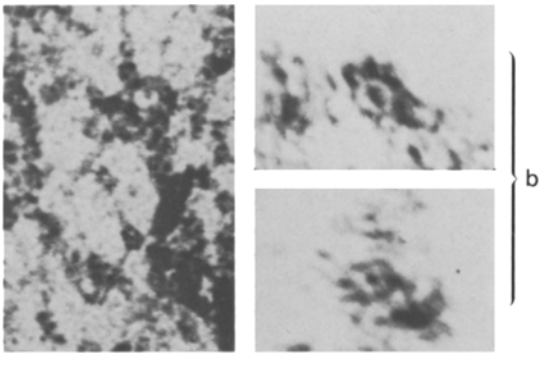

Fig. 1. Nucleosomes in rat liver nuclei after heparin treatment. *a* Many nucleosomes with connective threads. × 300,000. *b* Nucleosomes with the remnants of some extranucleosomal material. The central granule of nucleosome is seen. × 600,000.

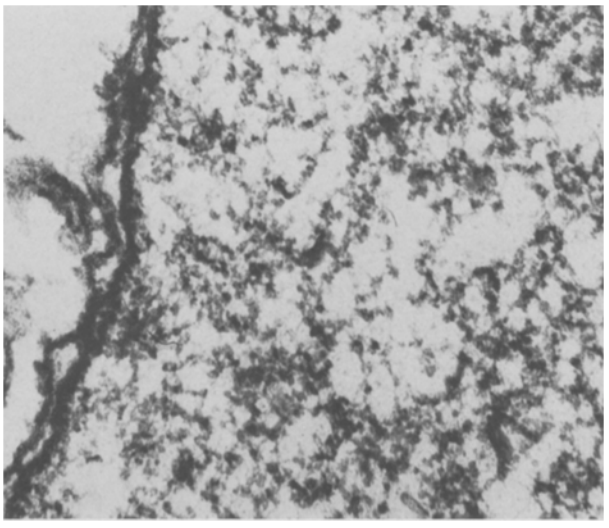

Fig. 2. Rat liver nucleus after heparin treatment. The contact of nucleosomes with the nuclear envelope and the pore complex. × 100,000.

Reynolds[8] and photographed with EMB-100L (Sumi, USSR) electron microscope at 75 kV.

Results and discussion. After heparin action, the chromatin is completely dispersed and many nucleosomes are seen (figure 1). The ultrastructure of nucleosomes is identical with that observed on isolated chromatin[9]. Namely, each nucleosome represents a ring-shaped structure of ~ 80 Å in diameter. A dark granule about 15-20 Å in size is seen in the centre of the ring. The connecting threads, i.e. the histone-free DNA segments between the nucleosomes, are also seen. The length of the connective threads, on account of their various directions in the plane of the ultrathin section, cannot be definitely determined. Twisted threads or short-toothed processes with high electron density radiate from some nucleosomes. They probably represent the remnants of some extra-nucleosomal chromatin component, removed under the influence of heparin. The attachment of nucleosomes to the nuclear envelope can also be observed (figure 2).

The data presented demonstrate the possibility of revealing the nucleosomal organization of chromatin on ultrathin sections of the whole nuclei after artificial decondensation of chromatin by heparin. It is likely that inability to visualize the particles within the compact chromatin depends not on the tight packing of nucleosomes, but rather on the masking of them by some extra-nucleosomal component of chromatin. The latter is probably composed, in part, of the histone H1. It is known that this fraction of histones is not present in the nucleosomes, but localizes outside of them[10].

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Further characterization of the effects of ultraviolet irradiation of the amphibian egg

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Summary. UV-irradiation of the vegetal hemisphere of amphibian eggs leads to developmental abnormalities in neural morphogenesis. The possibility that the egg's transient sensitivity to irradiation could be due to pigmentation changes was examined in albino eggs. The tissue specificity of the effects of irradiation was analyzed by exchanging the ectoderm between irradiated and control embryos.

A variety of evidence from experimental embryology indicates that the amphibian egg contains cytoplasmic components which are required for the neural induction events of later development[2]. 1 or more of those components can be destroyed by UV-irradiation of the fertilized, uncleaved egg[3,4]. Since UV is such a convenient experimental tool, and promises to contribute to our understanding of primary embryonic induction, the characteristics of the effect of UV on the uncleaved egg are being examined in detail. Previous results[3] revealed that the period after fertilization during which the neural induction component(s) of the uncleaved egg is sensitive to UV is restricted to the first $\frac{2}{3}$ of the time period between fertilization and the first cleavage division. That dramatic change in sensitivity to UV correlates with pigmentation changes which occur in the surface coat of the egg. In the 1st experiment reported in this communication, a direct determination was therefore made of whether those pigmentation changes could shield the target of UV-irradiation. Such a shielding effect could be perceived if albino (pigmentless) eggs did not display the dramatic drop in sensitivity shown by normal pigmented eggs.

A series of time course experiments were carried out in which pigmented eggs and several clutches of albino eggs were irradiated at various times after artificial insemination. After irradiation the eggs were permitted to develop to the muscular response stage (Nieuwkoop-Farber st. 25), fixed, and scored for extent of neural morphogenesis. Embryos which displayed a substantial diminution in the external size of anterior axial structures, including the forebrain, optic primordia, and cement gland were scored as defective. Histological analysis of embryos displaying those external signs of neural defects has previously shown that the notochord and neural tube are severely diminished in size[3]. Figure 1 displays the results of experiments in which eggs from several different albino females were irradiated. In the control series (pigmented eggs), the sensitivity to UV changed dramatically during the period