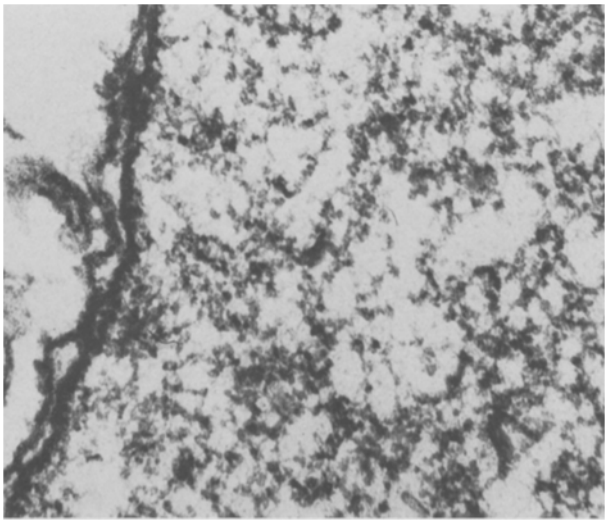

Fig. 2. Rat liver nucleus after heparin treatment. The contact of nucleosomes with the nuclear envelope and the pore complex. × 100,000.

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

after fertilization and before the 1st cleavage division. In addition, the extent of neural defects in embryos which were irradiated during the 1st 45 min after fertilization was substantially greater than in embryos which were UV irradiated just prior to the 1st cleavage division. Albino eggs displayed a similar change in sensitivity to irradiation. In normal pigmented eggs several changes in the surface coat take place after activation. These include various changes in the pigmentation pattern of the egg[5]. A role for these alterations in the pigmentation pattern in diminishing the sensitivity of the egg to UV can, therefore, be eliminated by the results of this series of experiments. Perhaps changes in the distribution of other egg cytoplasmic components, which are known to follow fertilization[6], are responsible for the drop in UV sensitivity.

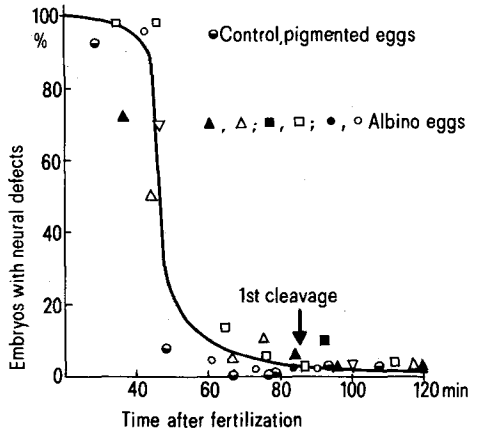

Fig. 1. Change in sensitivity to UV following fertilization. *Xenopus laevis* eggs were artifically inseminated, chemically dejellied, and irradiated at various times after fertilization, as previously described[7]. Solid symbols indicate a UV-dose (254 nm) of 10,000 ergs/mm^2, and open symbols represent a UV-dose of 20,000 ergs/mm^2. For each sample point 10–20 eggs were irradiated. Pigmented and albino eggs display similar temporal sensitivities to UV.

Despite the convenience of UV irradiation as an experimental probe, it is important to establish that its effects are on a specific region of the egg, or on a specific developmental event. In the experiment described in figure 1, as in previous experiments from our laboratory[7] the entire vegetal hemisphere was irradiated, although the UV target is believed to reside in the dorsal half of the vegetal hemisphere[3]. The possibility that the effects of UV on the vegetal hemisphere cytoplasm of the egg might be transmitted to the animal (ectoderm) portion of the embryo was examined by a series of tissue transplantation experiments. A large portion of the ectodermal (animal half) tissue of blastula stage embryos which had been irradiated at the uncleaved egg stage was exchanged with similar tissue from an non-irradiated embryo. The design of the experiment, as well as the result, are displayed in figure 2. It can be seen that transferring ectoderm from irradiated embryos to non-irradiated embryos did not affect the recipient embryo's capacity to undergo neural morphogenesis. As well, the capacity for irradiated embryos to undergo neural morphogenesis was not improved by substituting their ectoderm with normal, unirradiated ectoderm. The control experiments described in figure 2 indicate that effects of the grafting technique did not obscure an interpretation of the data. It would appear, therefore, that irradiation effects on the vegetal hemisphere of the uncleaved eggs are not transmitted to the ectodermal tissue. This conclusion is important, for it validates the interpretation of previous experiments that UV-irradiation destroys an egg cytoplasmic localization which is a component of the neural induction system of the gastrula stage embryo[3,8].

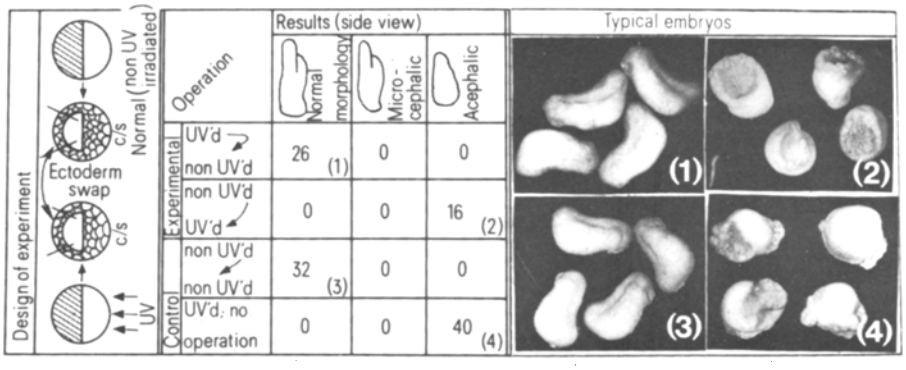

Fig. 2. Effects of UV at the vegetal hemisphere of the egg on the capacity for ectodermal tissue to participate in neural development. Eggs were irradiated with large doses (approx. 20,000 ergs/mm^2) of UV and at the late blastula stage approximately 30% of the ectoderm tissue (animal hemisphere) was exchanged between irradiated and control embryos. With such large doses of UV all embryos were acephalic, rather than microcephalic. *Rana nigromaculata* eggs, which are larger than *Xenopus* eggs and therefore more suitable for grafting experiments, were employed in this experiment. All the embryos which were operated on survived, and are included in the tabulations. Photographs on the right of the figure are indexed to display representative embryos at the developmental stage at which the results were tabulated.

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