Fine Features of Pituicytes in the Neural Lobe of the Green Frog, *Rana esculenta*

The pituicyte is a specialized glia-like cell, typical of the hypophyseal neural lobe[1,2] and deriving from the differentiation of ependymal cells, according to WINGSTRAND[3]. It is well known that pituicytes have not only a trophic function but are also actively involved in the final stages of neurosecretion[4,5]. This report follows previous notes regarding the fine structure of such cell types[6,7] and particularly deals with pituicytes peculiar to the neural lobe of *Rana esculenta*.

Materials and methods are the same as previously described[8]. The neural lobe was dissected out through a localized interruption of the palatine vault.

In the neural lobe of *Rana esculenta*, the pituicytes occupy an area remarkably reduced as compared to other animal species, mainly due to their relatively low number. Actually each pituicyte seems to be slightly increased in volume in comparison with other neural lobes.

The nucleus appears quite irregular in shape because of many peripheral projections and invaginations (Figure 1). The chromatin shows a homogeneous aspect which becomes typically granular close to the inner aspect of the nuclear membrane (Figure 2). The nucleolus is generally small in size. The cytoplasm forms a more or less developed perinuclear halo from which emanate many thin branched processes sometimes reaching the perivascular space (Figures 2 and 3). It contains some free ribosomes and few cisternae of the rough endoplasmic reticulum, several tubule-vesicular profiles of the smooth endoplasmic reticulum, scattered mitochondria, dense bodies, abundant Golgi complexes and prominent filamentous bundles which permeate the whole cytoplasm, often forming a perinuclear bundle (Figure 2). The neurosecretory fibres surround all the pituicytic surface, with which they often establish typical synaptoid contacts[9,10] (Figure 3).

Filaments which we find in the pituicytes of *Rana esculenta* are often lacking in other animal species, where it is generally possible to observe a certain number of microtubules[11]. In this connection the possibility must be

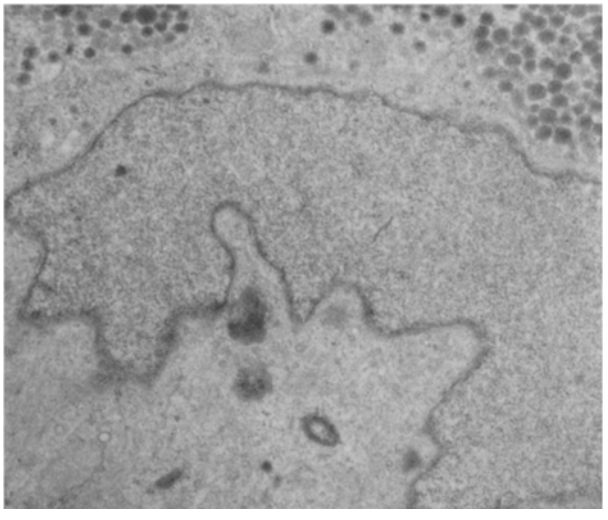

Fig. 1. Neural lobe, showing the irregular shape of a pituicytic nucleus. ×10,000.

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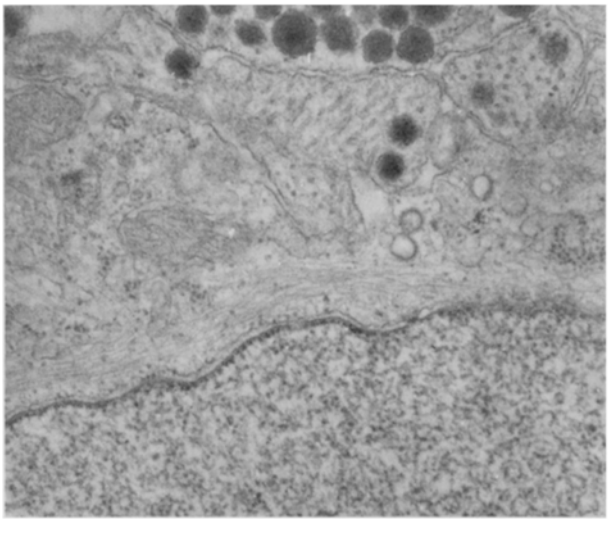

Fig. 2. Neural lobe. A typical filamentous bundle runs round a part of the nucleus. Note the granular aspect of chromatin close to the inner side of the nuclear membrane. ×20,000.

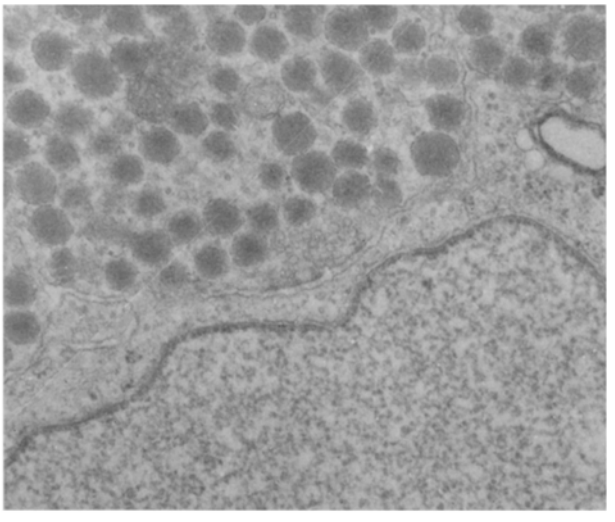

Fig. 3. Neural lobe. A fibre containing neurosecretory granules is localized quite near the nuclear membrane of a pituicyte. A typical synaptoid contact – note the accumulation of synaptoid vesicles and the thickening of the presynaptic membrane – is observed interposed between the neurosecretory ending and the pituicyte. ×20,000.

emphasized of interconversion between filaments and microtubules under experimental conditions[12]. As far as pituicytic filaments are concerned, it is possible that they play a mechanical cytoskeletal role in a manner similar to other neuroglial cell types[13]. Nevertheless a dynamic role to control the movement of cytoplasmic organelles cannot be ruled out.

Finally lipid droplets are lacking in *Rana esculenta*, in contrast to rodents in which they have been involved in the final stages of neurosecretion, i.e. in the neurohormonal release[14–16].

Riassunto. Sono state esaminate le caratteristiche ultrastrutturali del lobo neurale di *Rana esculenta*, in particolare dei pituiciti nei quali si osserva la presenza di numerosi fasci filamentosi.

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Combined Effects of Miracil-D and Radiation on Mouse Embryos

It has been reported that 1-diethylaminoethylamino-4-methyl-10-thioxanthenone (Miracil-D, Lucanthone) is an effective drug against schistosomiasis in man[1,2]. Experimental studies with Miracil-D showed an increased rate of chromosome aberrations and chromosome loss in *Drosophila* germ cells[3–5], and also in human leukocytes in vitro[6]. This drug, when combined with fractionated X-irradiation, induced a significant increase in the frequency of chromosome loss in *Drosophila*[7]. It also caused an enhancement of X-ray damage in HeLa cells[8]. It was found that Miracil-D inhibited nucleic acid synthesis in bacteria[9,10], slime mold[11] and HeLa cells[12] without influencing the protein synthesis.

In our previous experiments, the combined treatment with X-rays and chemicals has shown that a higher rate of congenital abnormalities was associated with an increased incidence of chromosome aberrations in rat embryos[13,14]. Since we presume that a synergism on embryonic damages could be due primarily to the effects on chromosomes, we are interested to investigate the effect of Miracil-D on development of embryos and on radiation-induced embryonic malformation in mice.

Materials and methods[15]. Virgin female NMRI-mice, 2 months old, were caged with fertile males of the same stock. The day of finding spermatozoa or vaginal plug was designated as day 0 of pregnancy. Pregnant mice were divided into 4 groups: 1. untreated, 2. irradiated, 3. Miracil-injected, 4. Miracil-injected and irradiated. On day 8 of pregnancy, X-irradiation was given (without anesthesia) with a single dose of 50 R (200 kV, 12 mA, 1 mm Al filtration, 32.5 cm target-to-subject distance, and dose rate 136.6 R/min). The dose rate was determined with a Victoreen-r-dosimeter placed in a plexiglas phantom inserted into the same lucite chamber in which the mice were irradiated. The chemical was freshly dissolved in sterile water (70 mg/kg body weight) and injected i.m. 1 h before irradiation. The animals were maintained in a temperature-regulated room at 22°C with a 12 h light-dark cycle. Water and food (NAFAG: No. 194) were available ad libitum. On day 13 of gestation all experimental animals were killed and the fetuses were removed, stored in Bouin's fixative, and subsequently examined with a stereoscopic microscope for external anomalies. Only live fetuses were examined for malformations, but the number and position of dead implantations were recorded. The results were analyzed statistically with Student- and χ^2-tests.

Results. The experimental results of this study are summarized in the Table. The number of fetuses with external abnormalities was not significantly different between untreated animals and those receiving a single radiation dose of 50 R on day 8 of pregnancy. The number of resorptions after intrauterine irradiation was even smaller than that of the untreated group, but the difference is not significant. Miracil-D given in the dosage of 70 mg/kg proved to be harmful, since a statistically significantly higher number of malformations can be observed ($\chi^2 = 25.04$, $p < 0.001$). Also, the rate of resorptions increased remarkably from 10.2% in the control to 29.0% when the mothers were treated with Miracil. In the group with the combined treatment (Miracil and X-irradiation), only 32.2% of the live fetuses were without visible external anomalies. The difference in the frequency of abnormalities compared with the Miracil group is about 40% ($\chi^2 = 24.68$, $p < 0.001$).

In untreated fetuses, a low frequency of growth retardation (2.4%) was found, and this frequency was only slightly increased after irradiation with 50 R. Offspring of drug-treated mothers showed malformations in the head region, mainly in form of eye anomalies and exencephaly (Figure). One finding that is of special interest is the high number of fetuses with exencephaly after Miracil treatment. This kind of brain damage was never observed in irradiated mice fetuses in this study and must therefore be a response to the drug administration.

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