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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Incidence and type of malformations following single irradiation with and without Miracil-D treatment of 8-day mouse embryos

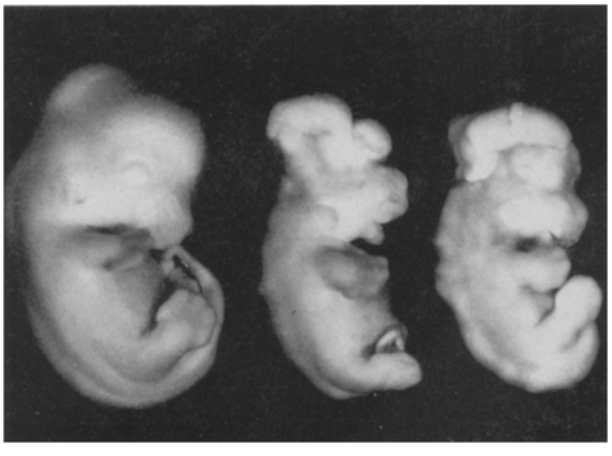
	Untreated	Irradiation with 50 R	Miracil-D treatment (70 mg/kg)	Miracil-D and irradiation with 50 R
No. of mothers	11	10	7	11
No. of implantations	137	124	93	125
Resorptions	14 (10.2%)	8 (6.5%)	27 (29.0%)	38 (30.4%)
No. of normal fetuses	120 (97.6%)	110 (94.8%)	48 (72.7%)	28 (32.2%)
No. of abnormal fetuses	3 (2.4%)	6 (5.2%)	18 (27.3%)	59 (67.8%)
Growth retardation	3 (2.4%)	5 (4.3%)	1 (1.5%)	19 (21.8%)
Anophthalmia	—	—	1 (1.5%)	11 (12.6%)
Microphthalmia	—	1 (0.9%)	3 (4.6%)	21 (24.1%)
Exencephaly	—	—	13 (19.7%)	18 (20.7%)

Macroscopic examination on day 13 of gestation.

After the combined treatment a high percentage of fetuses with growth retardation and eye defects occurred, while the incidence of exencephaly remained on the same level as after Miracil injection alone (20%).

Discussion. As shown by the present study, Miracil-D under the conditions used had a distinct embryotoxic and teratogenic effect on mouse embryos. The most striking visible effect of this chemical treatment was the high incidence of exencephaly. Exencephaly in mice may result after irradiation at any time from 0.5 to 9.0 days of pregnancy[16,17], with a peak in radiosensitivity around the 8th day.

The same brain defect was described by MOORE[18] after administration of hycanthone, another anti-schistosomal chemical, to pregnant mice. With this compound, which is closely related to Miracil-D, MOORE could induce eye anomalies as well as exencephaly in mouse embryos. It seems, therefore, that both Miracil and hycanthone are teratogenic drugs acting possibly in a related way through similar effects on DNA and RNA synthesis[19]. However, the mechanism of action of these drugs on embryonic development is not known. ROSI et al.[20] stated that Miracil-D underwent extensive metabolic transformation, and they assume that the hydroxymethyl derivate, which is identical with hycanthone, is the biologically active metabolite of Miracil.



Effects of Miracil-D-injection (70 mg/kg, i.m., 1 h before irradiation) and whole-body X-irradiation of 50 R on day 8 of gestation. Left, control. Middle and right, exencephaly, anophthalmia and spinal defects. × 5.

The experimental results show that Miracil plus X-irradiation leads to an impressive synergistic effect on the production of visible malformations in mouse embryos. Also, growth inhibition in 13-day-old fetuses is more pronounced after both irradiation and chemical pretreatment. The apparent difference between the effects of Miracil given alone and the combined treatment is the 6-fold increase of eye anomalies in the latter group. There is again a high, but not increased, frequency of exencephaly, suggesting that this damage could be produced by Miracil alone. It is surprising, therefore, that only eye development was more severely influenced by a combined treatment, indicating different sensitivities of the involved tissues to these agents. Radiation eventually affected ocular cells in such a way as to facilitate the effect of the chemical, but further investigations concerning this hypothesis are required.

Zusammenfassung. Die kombinierte Behandlung von Miracil-D und Röntgenbestrahlung bei 8tägigen Mausembryonen führte zu einer hohen Missbildungsrate. Es handelt sich nicht um eine additive, sondern synergistische Wirkung der beiden Agentien.

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