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ULTRASTRUCTURAL MODIFICATIONS INDUCED BY DIRECT ACTION OF CU^{2+} UPON EARLY CHICK EMBRYO

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INTRODUCTION (online version)

Teratological testing of sulphonate phtalocyanine (an alimentary blue dye synthetized by the Center of Chemisty, Timisoara), shown a strong malformative effect of this compound upon early chick embryo (48 hours of incubation), (Sandor, Checiu, Prelipceanu, 1985).

Dye administration on day 2 of incubation (44-48 hours) revealed a high rate of embryo mortality and abnormal modification of caudal segment or even a total absence of caudal tail bud. Living embryos until day 7 of incubation showed a normal development of the anterior body part (head and trunk) in contrast with posterior body part which presented an abnormal position of posterior limbs, tail and trunk aplasia.

The dye with the some name produced in Germany did not show (in the some experimental conditions) a malformative effect. The only difference between the two dyes was the presence of Cu^{2+} in our compound.

It is well known that chemicals and physics factors (X rayes, insuline, hypoxy, D-Actinomycine, sucrose, etc.) are noxious, inducing malformations of caudal segment (tail bud, urogenital and anorectal abnormalities) associated with cardiac, facial and SNC malformations (Landauer 1953, Shepard 1973)

Abnormalities of esophagus, urogenital and anorectal region associated with those of caudal axial skeleton and posterior limb buds are involved in caudal dysplasia syndrome (Duhamel 1961 cited by Roux and Martinet 1962). This syndrome is frequent (1:1000) in children of diabetic mothers (Warkany 1971).

Experimental works on mice suggested implication of genetic factors in pathogenesis of this syndrome (Frye et all.1964 cited by Warkany 1971)

Previous investigations (Checiu et all. 1966) revealed a caudal malformative syndrome in chick embryos induced by Cu^{2+}.

It is well known capacity of some heavy metal ions to affect the formation and desintegration reaction of free radicals.

The aim of this paper is to present a morphological study of caudal malformative syndrome (Checiu et all. 1999) and an experimental approach to a possible cellular and molecular mechanism of Cu^{2+} in teratogenesis. Also, by electron microscopically investigations we tried to show the target cellular organite for Cu^{2+}

MATERIAL AND METHODS

The experimental work was caried out on chick embryos incubated at 38^0C. The embryos were stained in ovo (on windowed eggs, 44-48 hours of incubation) by vital dye (neutral red 1:1000) for somites number determination.

100 µl of $CuCl_2$ 30 mg% was injected into the subembryonic cavity. The injection was made at embryos at: 30 hours of incubation (7-8 somites), 40 hours (10-13 somites), 48-50 hours (15-20 somites) and 60 hours (25-30 somites). For each mentioned above group, 3 embryos were injected.

The effects induced by $CuCl_2$ were controlled at 6,12, 24, 48, 72, 96 and 120 hours (7^{th} day of incubation) ater injection.

Treated embryos were classified in: normal living embryos; malformated living embryos, dead non-malformated embryos and dead malformated embryos. All the embryos were photographied.

For electronic microscopical investigations, 30 embryos (10-20 somites) were injected with l00 µl, 30 mg% $CuCl_2$ into subembryonic cavity. Contol group (30 embryos) was stained with neutral red after this and treated in the same way with Chick Ringer solution.

At 6, 12 and 24 hours after treatment, embryos were prefixed in 2% glutaraldehyde solution 1 hour at 4^0 C, postfixed in 1% osmium tetraoxide solution,1 hour at 4^0 C, washed in phosphate buferred 3 times, infiltrated and embeded in Vestopal W. Sections were contrasted with uranyl acetate and lead citrate and examined in electron microscope Tesla BS-500, at Babes-Bolyai University, Cluj-Napoca.

RESULTS AND DISSCUTIONS

The malformative pattern induced by Cu^{2+} showed that 100 µl from 30 mg% $CuCl_2$ solution injected into the subembryonic cavity is

responsible for various malformations of caudal region in 90% of living embryos.

Taking in account that all the structures from embryonic caudal region are modified we will use the term of "caudal malformative syndrome".

A high mortality rate (85%) was induced by high concentrations (50 mg%), while the low concentrations (20 mg%), induced molformations in 59% of living embryos.

It is to be mentioned that the younger embryos (5-10 somites) were dead, while the older ones (over 20 somites) were dead or without malformations after treatment.

At 6 hours after $CuCl_2$ injection, the first caudal caudal malformations are present:the caudal neuropore still wide open, the sinuous aspect of neural tube in their posterior half, retardation of amniotic folds closure.

At 24 hours (day 3 of incubation) after $CuCl_2$ injection, some embryos are dead and the living ones show the following malformations: wide open caudal neuropore, the absence of caudal primordium and alantoic bud.

At 96 or 120 hours after CuCl2 injection (day 5 and 6 of incubation) the embryos show a normal development at the anterior half of body and different degree of caudal malformation syndrome in the posterior half of body. At 7 day of incubation (5 days after treatment) the living embryos showed taillness and dysgenesis of the limbs. In some cases the tail primordium was rudiment with an abnormal position of the limb buds.

The microscopic examination of malformed embryos fixed at 24 hours after treatment revealed the following pathological changes: disorganization of the ventral part of caudal primordium with structural modifications of neural tube (ventral area), of paraxial mesoderm and of caudal end of notochord. At 48 hours after treatment, the caudal region is high modified: the complete disorganization of the caudal end of neural tube and notochord; the somitic, paraxial mesoderm is keeping their shape (the general plan of organization) but the cells from medium-ventral region show necrosis and cellular degeneration.

The electron microscopically investigations confirm the microscopic results.

At 6 hours after treatment the cells from ectoderm, mesoderm and endoderm showed a normal aspect both in treated and control embryos. Fig.1

At 12 hours post injection some ultrastructural modifications are to be noted: vacuolisation of mitochondria in ectodermal , mezodermal and endodermal cells and in neural tube. Fig.2

At 24 hours after treatment the above mentioned modifications are still present. Picnotic nucleus and residual bodies are present in neural tube and in all cells layers. Fig. 3.

At 48 hours after treatment many residual bodies, lysosomes, necrotic cells and vesiculated mitochondria are present in mezoderm and notochord. Fig.4

CONCLUSIONS

The ultrastructural modifications of mitochondria found in all experimental groups suggest a possible noxious effect of Cu^{2+} upon cellular respiration which in time may induce cell death. It is not clear if these modifications are induced by the free radicals generated in excess by Cu^{2+}.

The fact that all these malformations appear only if the cooper administration is made in embryos with a certain degree of development (10-20 somites). This is an argument to consider other mechanisms as some gene activity involved in organogenesis in the moment of Cu^{2+} administration.

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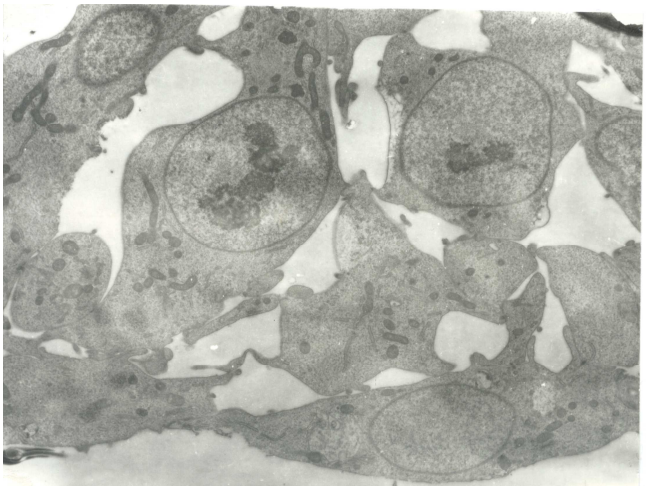

FIG.1a. Ectoderm, 6 hours after treatment, normal aspects, 5320 X

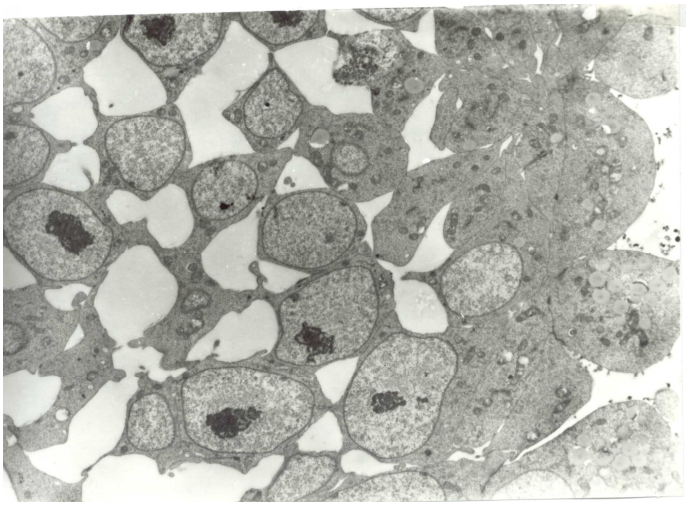

FIG.1b. Mezoderm, 6 hours after treatment, normal aspects, 4370 X.

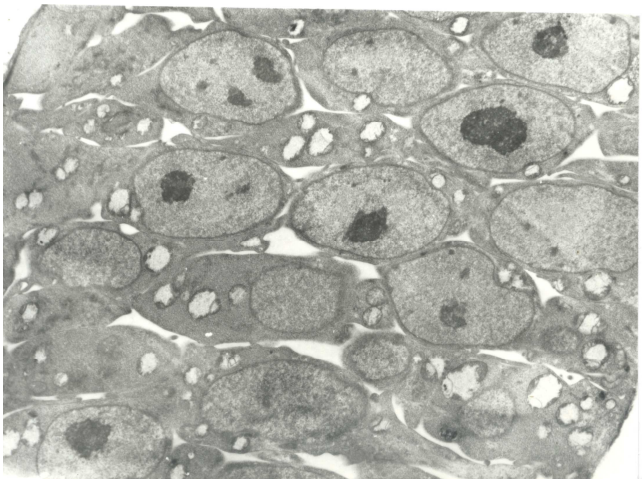

FIG.2a. Ectoderm, 12 hours after treatment, vacuolisation of mitochondria. 4750 X

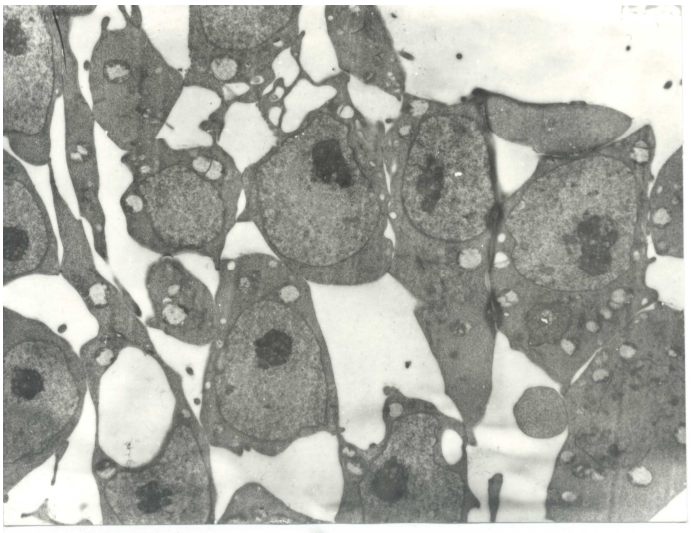

FIG.2b. Mezoderm,12 hours after treatment, vacuolisation of mitochondria. 4750 X

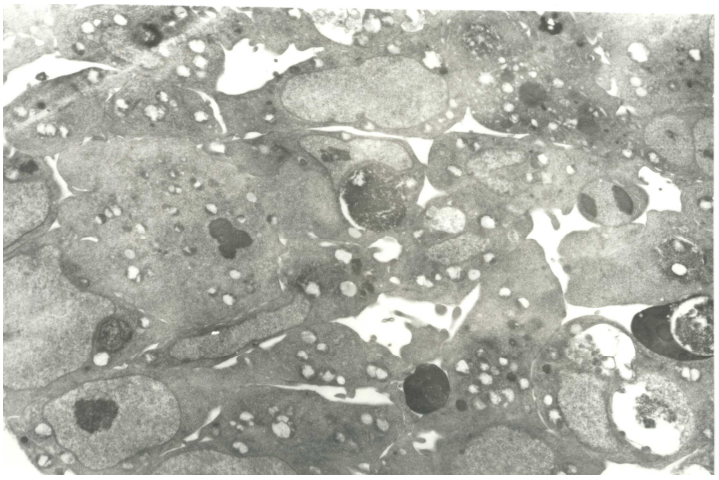

FIG.3a. Neural tube, 24 hours after treatment, vacuolisation of mitochondria, picnotic nucleus and residual bodies. 5510 X

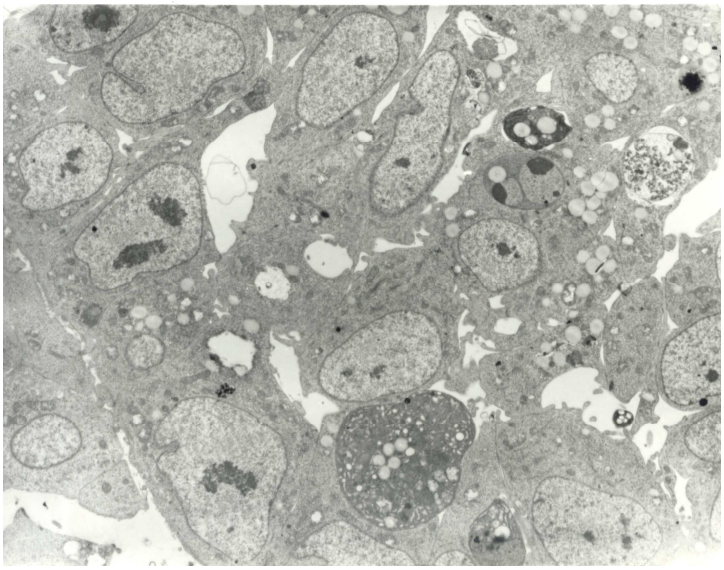

FIG.3b. Endoderm, 24 hours after treatment, vacuolisation of mitochondria, residual bodies. 4370 X

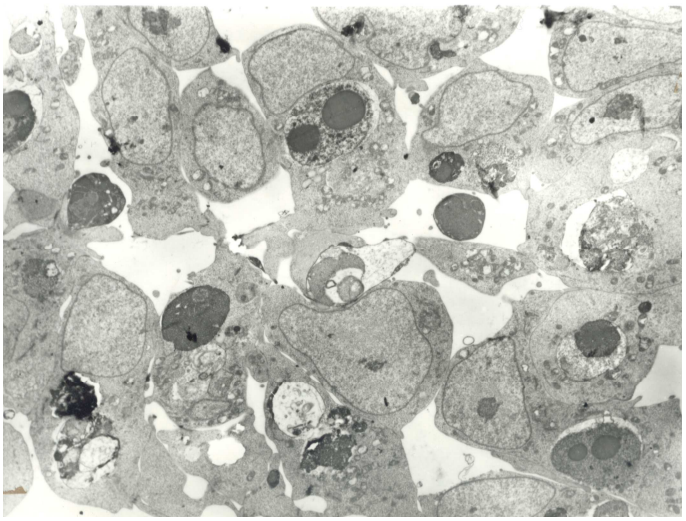

FIG.4a. Mezoderm, 48 hours after treatment, residual bodies, lysosomes, necrotic cells and vesiculated mitochondria. 4180 X.

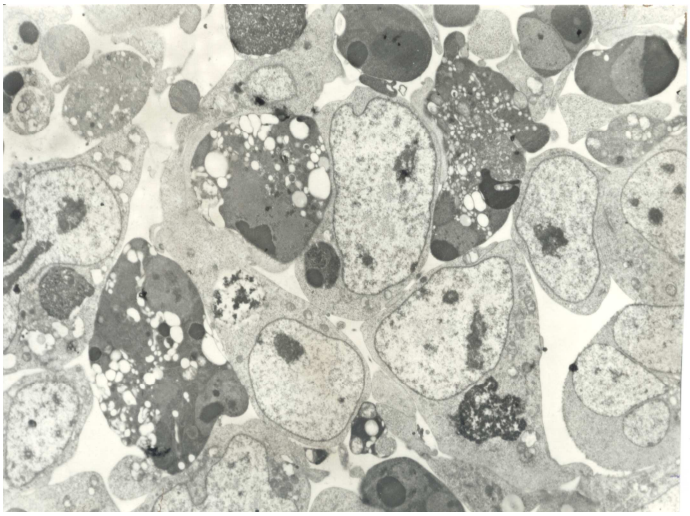

FIG. 4b. Notochord, 48 hours after treatment, residual bodies, lysosomes, necrotic cells and vesiculated mitochondria. 4370 X.