Hexadactyly case at a *Rana* kl. *esculenta* sample from the north-western part of Romania

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Abstract. At 17 June 2006, in a habitat close to Gherţa Mică locality (47°56'0" N, 23°14'0" E, Satu-Mare County, Romania) we had found a sample of *Rana* kl. *esculenta* which presented hexadactyly at both of its posterior feet. The captured sample of edible frog had fully formed extra (sixth) toes, with phalanges (bones). The hexadactyly was perfectly symmetrical at both of the posterior feet. At this individual we did not discovered any other malformations, the biometrical characters situating in the variations limits of the other green frogs from the studied habitat. A symmetric hexadacytly can be a result of atavism.

Keyewords: *Rana kl. esculenta, hind limb, symetric, hexadactyly*

Since 1995 herpetologists have paying more attention to the phenomenon of malformations' and anomalies' occurrence at amphibians. Today's reports are coming from all countries of the world to the North American Reporting Center for Amphibian Malformations (NARCAM 2006). Scientific publications which relate malformation cases at amphibians are known since 1920 (Hovelacque 1920, see in: McCallum 1999). After 1995, when in Minnesota (USA) (see in: Meteyer 2000) there was discovered a large number of frogs with malformations, the number of scientific papers dealing with this subject had raised significantly (e.g. Kaiser 1997, Meteyer et al. 2000, Johnson et al., 2001; about Romanian amphibian populations see e.g. Szekely & Nemes 2003, Nemes 2005). At amphibians, malformations and anomalies may appear due to errors in early development (e.g. Meteyer 2000), infections (e.g. see in: Johson et al. 2006) or due to traumas caused by predators (.g. Nemes 2002) or human activity (e.g. cut hand, foot see in: Sas et al. 2005).

At 17 June 2006, in a habitat close to Gherţa Mică locality (47°56'0" N, 23°14'0" E, Satu-Mare County, Romania) we had found a sample of *Rana* kl. *esculenta* which presented hexadactyly at both of its posterior feet. The provenance habitat of the mentioned frog is an approximate 2 km long channel at the edge of a forest. This habitat is used as a breeding site by many species of amphibians (*Triturus vulgaris*, *Triturus cristatus*, *Rana lessonae*, *Rana esculenta*, *Rana dalmatina*). Frogs belonging to the *Rana esculenta* complex were captured from all the lengthiness of the habitat in several outcomes. However, we identified only a single individual with such anomaly. The captured sample of edible frog had fully formed extra (sixth) toes, with phalanges (bones). The hexadactyly was perfectly symmetrical at both of the posterior feet. At this individual we did not discovered any other malformations, the biometrical characters situating in the variations limits of the other green frogs from the studied habitat (Sas – unpublished data).

Cases of polydactyly were observed at many species of amphibians (e.g. Dubois 1979, Vorobyeva 1999, Lada 1999). Even in Romania there is a remark of polydactyly at a frog from the *Rana esculenta* complex (Andrei 1985). In the polydactyly cases the extra digit can be rudimentary, or fully formed. In our case, the collected *Rana* kl. *esculenta* sample, had a fully formed extra (sixth) toes, with phalanges (bones). After the specialty literature, abnormalities are usually unilateral, and when bilateral, asymmetrical (see in: Johnson et al. 2001). It is an important fact, that in our case the hexadactyly was a symmetrical one. Amphibians have no drastically modified hand and foot bones, have a high variability in the number of hand and foot bones, and probably show polydactyly (Galis et al. 2001). It is now well know that early tetrapods had more than five digits. On the basis of developmental data, that the ancestral condition of the tetrapod hand was at least seven digits, including the usual five digits, prepollex/prephallux and the postminimus; especially the digits of the extinct amphibians with up to eight digits (see in Galis et al. 2001). In this manner a symmetric hexadacytly can be a result of atavism.

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Aspecte cu privire la capacitatea de reglare a gluconeogenezei hepatice în ficatul perfuzat de şobolan

Aspects regading the capacity of gluconeogenesis regulation of the rat perfused liver

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Abstract. The liver, also known as „the living laboratory of the organism", is a vital organ, fulfiling a variety of functions, such as gluconeogenesis, which is glucose biosynthesis starting from non-glucidic precursors such as: lactate, faty acids, glycerol and amino acids, an extremely important biochemical process for the animal organism especially under starvation conditions, intense activity or/and pathological states (Paşca C., Kis E. 1999).

By combining the liver perfusion technique and electronic microscopy techniques, we have been able to show that the liver can synthetize the necessary glucose even under starvation conditions, from the lactate-piruvate mixture that has been perfused (Mokuda and Sakamato 1997; Parrila and colab. 2003; Ross and colab. 1976; Sumida and colab. 1993; Sumida and colab. 2006). We have also highlighted the CCCP(carbonil-cianid-m-clorophenylhydrazone) effect on the gluconeogenesis process, at two different final concentrations :2 µmols and 50 µmols in the Krebs-Ringer serum.

The CCCP declutches the oxydative phosphorilation, making the proton gradient fade; if the concentration is low – 2 µmols – the effect lasts in time, and at high concentration – 50 µmols – it has an irreversible inhibiting effect on the gluconeogenesis.

Cuvinte cheie*: gluconeogeneză, precursori neglucidici, CCCP, lactat-piruvat, inaniţie.*
Keywords: *gluconeogenesis, non-glucidic precursors, CCCP, lactate-piruvate mixture, starvation conditions.*

In order to demonstrate the possibility of the liver to synthetise glucose we using the liver perfusion technique (Désy F., Burelle Y., Bélanger P., Gascon-Barré, Marielle and Lavoie J. M. 2001) experiments were undertaken on rats weighing 100-300 g, put to starvation for 48 hours, but with free acces to water.

The perfusion device is based on a Wolkoff's et. colab. (Wolkoff A. W., Johansen K. L. and Goeser T. 1978) device, but has been adapted to our study, the main change being the way of keeping steady the flow in the cannula, using a peristalmic pump, not letting it drop.

The liver perfusion technique appears to have an important advantege, that is the fact that it mostly assures the necessary physiological conditions, which can be found within the living animal (Wolkoff şi colab. 1978). After the preparation of the Krebs-Ringer serum, the cannulation will be fulfiled by following these particular steps:

Also, we using the glucose dosage method (Changani K. K., Jalan R., Cox I. J., Ala-Korpela M., Bhakoo K. S., Taylor-Robinson S. D. and Bell J. D. 2001) This method is specific for glucose as the glucosoxidase only oxydates the glucose.

In order to demonstrate the possibility of the liver to synthetise glucose the liver was provided with a lactate mixture, with a final concentration of 2 mM in the Krebs serum, as well as piruvate with a 0.1 mM final concentration in the Krebs serum.

After the lactate and piruvate injection, the liver immediately begins the glucose synthesis, reaching a medium concentration of 70µmols/hour/100g boby weight. By interrupting the precursors supply a rapid descease of glucose synthesis is detected.

As a conclusion, we can say that under starvation conditions the liver is able to supply the organism with the necessary glucose.

After the 2µmols CCCP is introduced a rapid decrease of the glucogenesis can be observed, until

reaching a certain level (close to the value before the precursors perfusion) as well as its maintenance at this level as long as the CCCP persists.

After the remove of CCCP, gluconeogenesis will gradually come back to the previous values, those registered before adding CCCP.

In another experiment we have observed the effect of CCCP at a 50 µmols concentratie.

At this concentration, the CCCP completely and irreversibly inhibited the gluconeogenesis, so as not even after the CCCP supply stop, the glucose was not synthetized.

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Studiul influenţei tratamentului cu ciclofosfamidă asupra hematiilor din sângele embrionului de găină

A study upon the influence of cyclophosphamide treatment on the red blood cells of the chicken embryo

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Abstract. The aim of this study is to show the effect of cyclophosphamide on the developing red blood cells of the 3-4 days old chicken embryo, when the hematopoiesis is at its peack, located at the vitelline sack level.

I have chosen to work with the chicken embryo red blood cells because they have an intense mitotic activity as well as a tumoural cell-like behaviour.

It is vital to know the particularities of the cell cycle of the healthy and tumoural cells, keeping in mind that most of the cytostatics act upon the cell which are developing their cell cycle (Menkes B., Prelipceanu O., Checiu I., Căpălnăşan I. 1979).

The cyclophosphamide is not stage-dependent, as it acts in all the stages of the cell cycle, its mutagen effect being accompanied also by a cell cycle stopping (Paşca C., Crăciun C., Ardelean A. 2000).

Cyclophosphamide supply determines retrenchment of the cell division, transforming the normal cells into multinucleated cells, with normal ploydia. The cyclophosphamide is a cytostatic using for cancer therapy (Schiavoni G., Mattei F., Di Pucchio T., Santini S. M., Bracci L., Berardelli F., Proietti F. 2000).

Reshearches have done lots of studies along the years both on mice and rats, concerning the effects of cyclophosphamide on: thymus and burse fabricio (Giurgea R., Toma V., 1977), stromal cells of bone marrow (Anton E. 1997), pulmonary thrombocytopoiesis (Sulkowki S., Sulkowska M., Musiatowikz B. 1997).

Cuvinte cheie: ciclofosfamidă, citostatice, hematii, hematopoieză, sac vitelin.
Keywords*: cyclophosphamide, cytostatics, red blood cells, hematopoiesis, vitelline sack level.*

We have incubated hen eggs – hybrids between different races (mixed race) – of maximum 5 days.

The second day, the eggs were opened, as follows:

The administration of the cyclophosphamide took place in the 4th day of incubation.

The cyclophosphamide solution was made with bidistilled water.

We have administrated 0.5 ml cyclophosphamide / chicken embryo.

Before that, we have sterilized this solution in a 0.2 µ diameter porus filter.

From the surviving embryos we have sampled blood coming from the corio-alanthoid vessels, in the

5th, 6th and the 7th day of incubation, smears were made.

The smears were coloured using the May-Grundwald-Giemsa method, as follows:

- May – Grundwald 3 min;
- tap water 1 min;
- Giemsa 30 min. The Giemsa solution was dilluted: 4 drops colour substance/1 ml tap water;

As a summary, we can say that by studying the embryos to which we added 50 µm cyclophosphamide and which we have examide after 3 days, we can observe the following characteristic changes:

- the gathering of the metaphase cells exhibiting unusual morphological properties (red blood cells more wide in diameter (double) and others with a single nucleus but a large volume; the red blood cells exhibit a heavy polychromatophilia; there are oval red blood cells, acidophilic and with a normal aspect, as well as a large number of non-mature, round, basophil).

- the suppression of the cell multiplication and the overturning of these mitotic cells back into cells with more than one nucleus and with abnormal ploydia;

- the disappearance – after 24 hours – of those cells which are not viable, as well as the beginning of a great regeneration of the lost red blood cells, when young, hemocitoblastic, basophile cells appear, probably belonging to a final series, as the activity in the vitelline sack begins.

This study enabled us to show a clear effect of the cyclophosphamide upon the red blood cells of the chicken embryo as well as the minute type of action, very important for the optimum concentration / time balance in obtaining a therapeutic effect

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