ISSN 0326-551X.

CUADERNOS DE HERPETOLOGIA

VOLUMEN 6 - NUMERO 2 - 1991

The Arsenic Action during the *Bufo arenarum* gonad development (Anura: Bufonidae).

Dora RENGEL and Armando PISANO

ASOCIACCION HERPETOLOGICA ARGENTINA

EDITORES: DINORAH D. ECHEVERRIA y JORGE D. WILLIAMS

Mayo 1991

The Arsenic Action During The *Bufo arenarum* Gonad Development (Anura: Bufonidae).[1]

Dora Rengel,[2] and Armando Pisanó[2]

[1] This work was supported by grant PID. 3129600/88 from CONICET, Argentina.

[2] Members of Research Career CONICET, Argentina. Laboratorio Investigaciones Embriológicas (LABINE). CONICET. Montevideo 126. 4º piso (1019). Buenos Aires, Argentina

ABSTRACT:

The Arsenic Action During The Bufo arenarum Gonad Development (Anura: Bufonidae).

Bufo arenarum ovulations have been kept in potassium bibasic arsenate from the fertilization until 26mm larva. The gonad in development, in the presence of the toxic, suffers signifficant alterations in its volume, shape and structure. It does not have the typical naillike morphology; on the other hand, it is presented as a spherical mass of reduced volume, that has, as demostrated by the histological analysis, very few oocytes or gonia submerged in a very scarce quantity of stroma. The hypothesis of this study was that it would be due to a succession of events whose cause would lie on the damage that the arsenic determine at the somatic layers level.

INTRODUCTION:

Several authors (Ferm and Carpenter,1968; Ferm *et al.*, 1971; Hood, 1972. etc.) have studied the effect of the arsenic on mammals during development and have described particular teratogenic actions in ectodermic and mesodermic layers as well as in their respective derivatives.

Analogous results have been obtained in an amphibian, *Bufo arenarum*, in which more serious anomalies could be noted when the arsenic doses have been greater (Vega and Pisanó, 1980, 1983).

Provided the gonad stromal component, at least in amphibians, is grown either at the coelomic epithelium expense (*cortex*) as well as at the mesomere's mesenchyme, which would constitute the *medullar* part (Vannini, 1950; Witschi, 1967; Marchant-Larios and Villalpando, 1981), the question arises whether the *Bufo arenarum* gonad will be altered by arsenic during its differentiation. Because of this statement a series of experimental trials have been carried out.

MATERIALS AND METHODS:

Oocytes of *Bufo arenarum* were obtained by the intraperitoneal injection of a homolog hypophysis suspension in 1 ml. distilled water. Fertilization was carried out *in vitro*. The control group was maintained in standard Holtfreter solution.

The experimental group were exposed to 50 mg/l of potassium bibasic arsenate (Sigma) in Holtfreter solution from fertilization until the larvae reached 26 mm. total length; on about 35 days after from the fertilization, they were fed and reached the stage X of Martin *et al* (1985). The solutions were renewed every day until the larvae started feeding themselves; from that moment the normal solution, and the toxic one, were changed every over five days. Tadpoles were fed with boiled lettuce.

The water temperature was kept in the toxic solution (ph 6.8) were fixed in Bouin for three hours (1 h 30 min. at 4º and 1 h 30 min. at room temperature) and embedded in waxparaffin. Sections embedded in paraffin were cut at 5 um and stained with haematoxylin and eosin. Those larvae having dropsy in ventral areas or serious ill-shapes as acephaly or chondrodystrophy were discarded.

RESULTS

Control larvae:

Macroscopically, the gonad appears as a nail and, along its run, three zones named *progonad, mesogonad* and *metagonad* can be identified (Ponse, 1949). The first zone, the progonad, has very transparent walls, the macroscopic observation individualizes in its inner part small yellowish spherical corpuscles that the histological analysis recognizes as oogonia in multiplication or oocytes in growth (fig.1).

The next zone, the mesogonad, shows it self opaque and histological seried sections, showed that it has gonias in celular division (fig.1). In the metagonad, of filiform aspect, the histological analysis recognizes a very small amount of gonia submerged in the stroma mass. The results are similar to those noted by Pisanó and Pizarro (1958, 1962) in a study about the development of the *Bufo arenarum* gonad. Moreover, it is important to point out that every larva of each group presents a similar female features until shortly after the metamorphosis, since *Bufo arenarum* belongs to an *indifferentiated race* (acording to the nomenclature proposed by Witschi, 1930).

Experimental larvae:

Gonads of treated larva are observed macroscopically as a sub-spherical mass of varying volume between one specimen and another, and sometimes, between two gonads of the same specimen (fig. 4-6). They have lost the caracteristic nail physiognomy, and its

volume is very small particularly in the larvae whose mesonephros, as demostrated by histological analysis, appears more or less anomalous. Serial sections of the gonad reveal that in most cases it has few gonia submerged in few stroma (fig. 2).

On the other hand, if a gonad seems to be colonized by a little number of germinal cells, the other can have only and exclusively, a mass of cells that constitute a desorderly stroma (fig. 3).

In other larvae it is possible to see that if the reduced gonad takes place in anomalous position, the parallelism between the two gonads is lost. It can happen that the gonad may be adhered in a greater or lesser degree, to the corresponding mesonephros border, or else it may be located in positions somewhat distant from the dorsal mesentery. With a strict constancy, when the gonad is very anomalous, in its macroscopic and histologic aspects, the cytological mesonephros waste becomes quite evident, even when the other organs appeared normal.

It has been already described in other studies (Vega and Pisanó, 1980, 1983) the melanic pigment quantity that is accumulated in a very disorderly way in various areas of the larva, and it is not a rare case to find pigment gathered in bulky clots in the proximity of the anomalous gonadal structure (fig. 2).

DISCUSSION:

As pointed out by Burns (1956) "a local interaction between medulla and cortex is the basic mechanism in the differentiation of the gonad"; also, an amphibian gonad not yet with sex differentiation, consist of two parts, that is, by the *cortex* and the *medulla*. The first is formed at the expense of the coelomic epithelium, the second originates by migration and differentiation of mesomeric cell, that is, of the same material that will originate the mesonephros. The germinal cells, of a frequently discussed origin (Pisanó, 1979), in *Bufo arenarum* have been recognized (Rengel and Pisanó, 1981). As a matter of fact after migrating through the dorsal mesentery, the germinal cells take place in the two gonad anlage placed on both sides of the dorsal mesentery and multiply colonizing them (Pisanó and Pizarro, 1958, 1962).

According to experimental data (Rengel, *et al*, 1985) in the present study comparison of normal larva and the larva treated with potassium bibasic arsenate demostrates both macroscopically as well as microscopically, that the gonad has suffered significantly alterations in its volume, shape and structure. As a matter of fact, besides the reduced volume, gonads constituted with a very small quantity of stroma, and very few germinal elements, can be obtained. One should ask oneself if the precence of very few germinal cells represents the consequence of a reduced stroma; that is to say, if the limited quantity of this last tissue, represents an obstacle for the multiplication of the primordial germinal cells and, threfore, the cause of an inadequate colonization of the gonad.

The described anomalous condition leads to the formation of a gonad that has lost every morphological characteristic.

Since the constitution of the progonad has not taken place, the mesogonad and the metagonad will not be able to take place either, according to the scheme put forth in this paper.

It is pointed out that these two latter regions will be constituted not only at the expense of an active multiplication of the stroma cells, but also by presence of germinal cells (Pisanó and Pizarro, 1962). On the other hand, one should ask oneself if the gonad location, more or less different from that one occuring in the normal one, is due to a direct action of the arsenic on the somatic cells that will constitute the outline of the gonad, or due to a defect caused by the arsenic in the mesomeres. The mesodermic layer according to studies also performed on mammals, would represent an embryonal layer that the arsenic can easily alter (Ferm, 1967, 1977; Ferm and Carpenter, 1968; Hood, 1972; Hood and Bishop, 1972).

According to Vega and Pisanó (1980, 1983) when the development of *Bufo arenarumm* is submited to the action of arsenic solution the larvae evidences themselves histological anomalies to the skin level, intestine and specially mesonefros.

That last data results of interest, because it permits suppose that the toxic substance used did not have a direct action over the germinal cells or the germinal plasm. Althrough unsubstantiated in the literature, the autors express the hypothesis that the effect is not direct, since in most cases, although in a very reduced number, germinal cell are found. Coordinating the results it would seem that the gonad's anomalous construction, in most cases of a very small volume, it would be due to a succession of events whose cause would lie on the damage that the arsenic determines at the somatic layers level.

The migration of the germinal cells through the dorsal mesentery does not seem to have been interrupted: it is corroborated by the fact that they are found, although in reduced number, in one or both gonads. It would rather seem that they had some obstacle to multiply and allow a normal development of the gonad; we attribute this obstacle to the scarceness o stroma, inhibited by the action of the arsenic. The interpretation of the picture described represents only a hypothesis: however, we consider that it is the closest to reality.

Acción del arsénico durante el desarrollo de la gónada de Bufo arenarum.

Resumen:

Se han mantenido, en forma crónica, en presencia de arseniato de potasio, ovulaciones de Bufo arenarum desde la fertilización hasta el estadío de larva de 26 mm.

La gónada a lo largo del desarrollo experimenta significativas alteraciones en su volumen, forma y estructura. Pierde el típico aspecto claviforme y se presenta esférica de reducido volumen que, en la mayoría de los casos y en función del estadío, posee, según lo demuestra el estudio histológico, pocos ovocitos o gonios sumergidos en una escasa cantidad de estroma.

Se emite la hipótesis que el arsénico no actuaría directamente sobre las células germinales, sino que destruiría de manera más o menos drástica el tejido mesonéfrico, uno de los principales constituyentes celulares para la formación de la parte somática de la gónada.

REFERENCES:

BURNS, R.K., 1956. Urogenital system. In *Analysis of development*. Willer B.H., Weiss P.A.and Hamburger V, Saunders Company Philadelphia and London, 475 pages.

FERM, V.H., 1967. The use of the golden hamster in experimental teratology.Lab. Anim. Care *17* : 452-462.

FERM, V.H., 1977. Arsenic as teratogenic agent. Environ Health Perspect*19* : 215-217.

FERM, V.H., and Carpenter, S.J., 1968. Malformations induced by sodium arsenate J.Reprod. Fertil. *17* : 199-201.

FERM, V.H., SAXON, A. and SMITH, B.M., 1971. The teratogenic profile of sodium arsenate in the golden hamster. Arch. Environ. Health *22* : 557-560.

HOOD, R.D.,1972. Effects of sodium arsenate on fetal development. Bull.Environ Contam. Toxicol. *7* : 216-222.

HOOD,R.D.,and BISHOP,S., 1972. Teratogenic effects of sodium arsenate in mice. Arch. Environ Health *24* : 62-65.

MARTIN, M.C., NUÑEZ, A.M. de and TOMATIS, M.E., 1985. Metamorfosis en Anfibios: I. Desarrollo metamórfico en larvas de *Bufo arenarum* Hensel (Amphibia; Anura). Hist. Nat. *5* (32): 289-302.

MARCHANT-LARIOS, H. and VILLALPANDO,I., 1981. Ultrastructural events during early gonadal development in *Rana pipiens* and *Xenopus laevis*. Anat. Rec. *199* : 349-360.

PISANO, A., 1979. El determinante germinal en la dinámica embriológica. Acta Phys. Lat. Amer. *29* : 7-13.

PISANO, A. and PIZARRO, N., 1958. Observaciones sobre el desarrollo de la gónada de *Bufo arenarum*. Rev. Soc. Arg. Biol. *34* : 175-184.

PISANO, A. and PIZARRO, N., 1962. Caratteristiche morfologiche della gonada di *Bufo arenarum* durante lo sviluppo. Arch. Zool. Ital. *47* : 167-176.

PONSE, K., 1949. La différenciation du sexe et l' intersexualité chez les Vertébrés. Librairie de L'Université F. Rouge, & Cie Lausanne.

RENGEL, D., and PISANO, A., 1981. Sterile gonads after removal of vegetative cytoplasm from the eggs of *Bufo arenarum*. Acta Embryol. Morphol. Exp. *2* : 121-129.

RENGEL, D., VEGA, D. and PISANO, A., 1985. Acción del arsénico durante el desarrollo de la gónada de *Bufo arenarum* . Com. Biol. *3* (4) 507

VANNINI, E., 1950. Organogenèse des gonades et déterminisme du sexe chezles Amphibians et les Amniotes. Arch. Anat. Microsc. Morphol. Exp. *39* :295-313.

VEGA, D.E. and PISANO, A., 1980. Teratogénesis experimentalmente provocada por acción del *As* . Rev. Soc. Arg. Biol. *56* : 55-58.

VEGA, D. E. and PISANO, A., 1983. Estudio experimental del *As* . como agente teratógeno durante el desarrollo. Bol. Estudios Médicos y Biológicos *32* : 417-425.

WITSCHI, E., 1930. Studies on sex differentiation and sex determination in Amphibians. Jour. Exp. Zool. *56* (2): 149-165.

WITSCHI, E., 1967. Biochemistry of sex differentiation in vertebrate embryos. In *The biochemistry of animal development* . R.WEBER (Editors.). Academic Press, New York, 193-225.

DESCRIPTION OF FIGURES

Fig. 1: Sagittal section through the normal gonad. pr: progonad; ms: mesogonad; metagonad Bar: 80 um.

Fig. 2: Gonads of treated larva. Transversal section. Note few germinal cells and the pigment. go: gonad. Bar: 25 um.

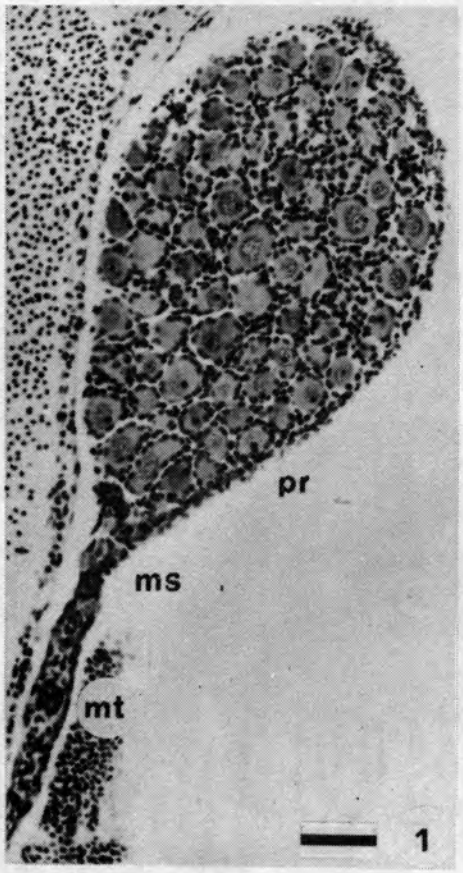
Fig. 3: Gonads of treated larva. Transversal section. Note the different structure of each gonad. Arrow: one germinal cell. Bar: 40 um.

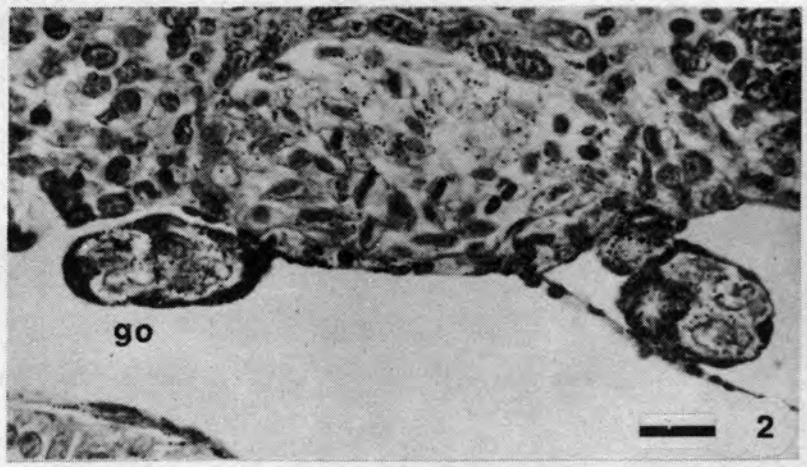
Fig. 4: Gonads of treated larva (premetamorphic stage); its have last the neil physiognomy and its volume is very scantily. Bar: 0,45 um.

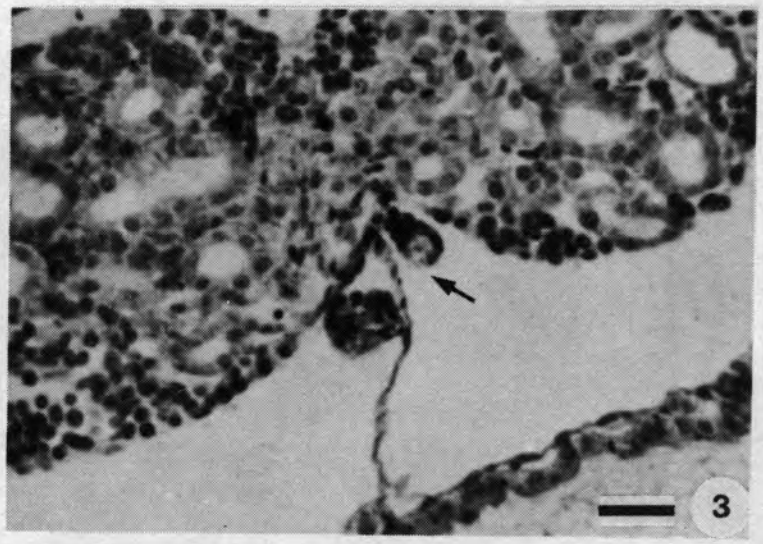
Fig. 5: Gonads of normal larva (premetamorphic stage); note the voluminous progonad. Bar: 0,45 um.

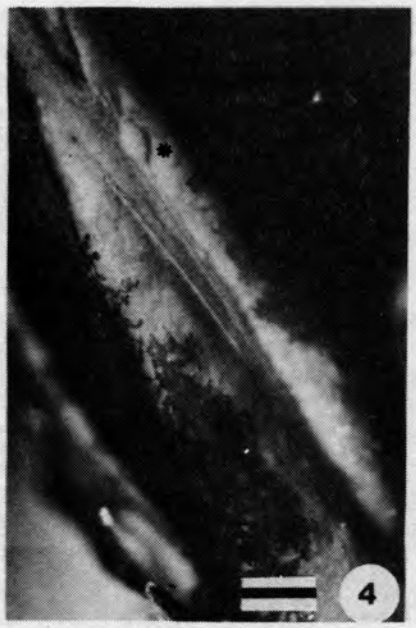
Fig. 6: Gonads of treated larva (premetamorphic stage); note the reduced progonad. Bar: 0,45 um.

SEDE de la ASOCIACION HERPETOLOGICA ARGENTINA
MUSEO DE LA PLATA
Casilla de Correo 745 (1900) La Plata ARGENTINA

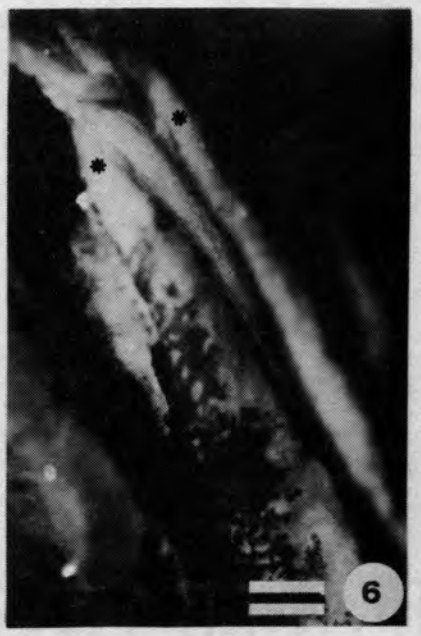

pr
ms
mt
1


go
2


3


4


5


6