"EXPERIENCE TRITON" ON BION 10 : STUDY OF PEPTIDASE-1 EXPRESSION IN EMBARKED *PLEURODELES* FEMALES AND DETECTION OF GENETIC ABNORMALITIES IN THEIR PROGENY.

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ABSTRACT

On Bion 10, the "Experience Triton" pools several experiments engaged by different laboratories. Our laboratories study in *Pleurodeles*: - first: the expression of peptidase-1 in embarked animals; the electrophoretical patterns of the enzyme in ovary, gut and muscles of flight samples were similar to controls but they were slightly different for kidney and heart. - second: possible genetical abnormalities in the offspring of females submitted to space environment. Embarked females (N=6) and synchrone females (N=5) were used as donors of ovaries both to castrated adult females and juvenile castrated males. 2 recipient females and 7 recipient males are still alive.

1. SCIENTIFIC OBJECTIVES

The Satellite Biocosmos 10 (or Bion 10) was launched from Plessetsk on December 29, 1992. It landed in Central Asia on January 10, 1993. This 1-day flight, dealing with life sciences experiments, was conducted by the I.P.B.M. (Moscow). The "Experience Triton" pooled several experiments conducted by different laboratories. Our laboratories have studied : 1- the expression of an enzyme, the peptidase-1, in embarked animals. 2- the possible genetic abnormalities in the progeny of females which were submitted to space environment.

2. EXPERIMENTAL PROCEDURE

The newts selected for these experiments belonged to the *Pleurodeles waltl* species (urodele amphibian). A group of 60 adult females, one year old, issued from the same progeny provided by the Institute of Developmental Biology of Moscow, was divided in 3 batches. The animals of the first batch (N=15) were embarked. Those of the second one (N=15) used as "synchrone controls" were to be submitted on earth to the same variations of temperature that the embarked females. The third batch (N=30) comprised castrated females. A group of 15 castrated juvenile males was provided by one of the French laboratories. Castrated males and females were used as recipients of transplanted ovaries.
The flight of Bion 10 was cut short because of a sudden increase of temperature on board. During the flight, the embarked animals were thus exposed to a high temperature (> 32°C) for about 48 h. Sixteen hours after the Bion 10 landing, the embarked females arrived at the laboratory in Moscow. Of fifteen females, only six were still alive. In contrast, the fifteen synchrone control females were all in good health. It is to notice that the ambient temperature for ground controls was always kept 2°C beneath Bion 10 ambient temperature. As planned for "Experience Triton" all embarked females were sacrificed for organ sampling.

3. PEPTIDASE-1 EXPRESSION IN THE EMBARKED FEMALES

3. 1 The peptidase-1

In *Pleurodeles*, the peptidase-1 is a dimeric ,polymorphic enzyme, sex-linked and encoded by two genes (Pep-1A and Pep-1B) located on the Z and W sex chromosomes (Refs. 1, 2). Pep-1B has an allelic form Pep-1ß (Ref. 3). Males are genotypically Z_AZ_A and females are Z_AW_B or $Z_AW_ß$. The sexual genotype of an animal can be determined by the electrophoretic pattern of the peptidase enzyme of erythrocytes (Ref. 4). Presence of the band corresponding to the AA homodimer characterises Z_AZ_A males. A Z_AW_B genotype corresponds to 3 electrophoretic bands, the AB band being inserted between the AA and BB bands. In the same manner, the electrophoretical pattern of a genotypic $Z_AW_ß$ female consists in 3 bands corresponding to the AA and ßß homodimers and the Aß heterodimer (Refs. 3, 5).
The aim of the project selected by the Russian scientific committee was to detect: 1) some possible alterations of the expression of peptidase-1 using electrophoresis analysis ; 2) changes in the activity of this enzyme in different organs.

3.2. Material and methods

Organ samples. peptidase-1 activity was measured in five organs: heart, trunk muscles, ovaries, kidney and mid-gut, comparatively in adult females embarked on Bion 10 and their ground controls (synchrone controls). All the females had a Z_AW_B sexual genotype.

Peptidase-1 preparation. Within 20h after the landing of Bion 10 , small pieces of the selected organs were excised from the animals and kept frozen in dry ice in buffer A (50mM Tris HCl pH 8.5, 25 mM NaCl, 5 mM $MgCl_2$). Samples were carried from Moscow to France for analysis. Each sample was homogenized in buffer A. After centrifugation at 17000 rpm for 30 min., the supernatant was discarded and the pellet was resuspended in buffer A and stored at 4°C.

Protein determination. Protein concentration in extract fractions was determined by the method of Lowry (Ref. 7) by measuring absorbancy at 750 nm.

Enzyme activity assay. Peptidase-1 activity was measured according to the method developed by Nicholson and Kim (Ref. 6) using Valyl-leucin as a substrate (Ref. 2).

Gel electrophoresis. The samples were run on an horizontal 12 % starch gel , according to the technique of Wright *et al*

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(Ref. 5) modified by using a Tris-citrate pH 8 buffer. The whole gels were stained using the peroxidase reaction.

3.3 Results

The electrophoretic patterns of peptidase-1 in ovary, muscles and gut issued from the embarked or synchrone animals displayed the 3 characteristic bands which correspond to the sexual and enzymatic genotype Z_AW_B (Fig 1). In heart and kidney samples, the two bands corresponding to the heterodimer AB and to the homodimer AA were clearly revealed ,while a third band corresponding to the BB dimer appeared very fainly.

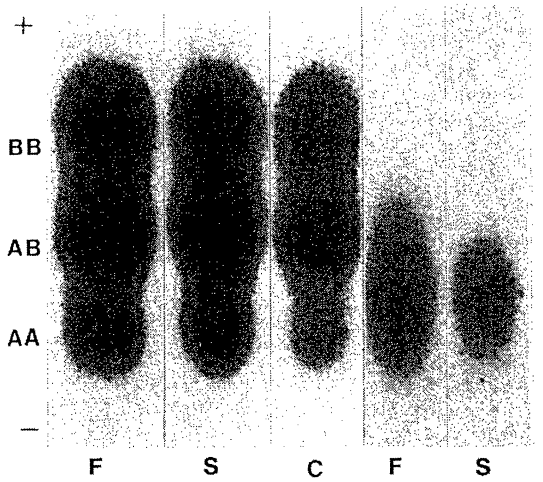

Figure 1 : Peptidase-1 electrophoretic patterns. From left to right: the first two patterns correspond to muscle, ovary and gut and show 3 strips (AA, AB & BB); The central pattern was established from erythrocytes (control). the two others showing 2 strips (AA & AB) correspond to heart and kidney (C: control; F: flight; S: synchrone).

The specific activity of peptidase-1 of the different samples was compared using the Student t test ($p<0.05$) (Table 1).
No statistical significant difference was observed between flight and synchrone samples for ovary, gut, heart, and muscles. In contrast, the enzyme activity in kidney was significantly higher in the flight sample than in the synchrone sample .

3. 4. Discussion

The difference between the electrophoretic patterns of the experimental and control heart and kidneys samples might be explained by an undetectable activity of the BB homodimer, rather than the repression of the B gene because the AB band was observed.

Peptidase-1 activity was not the same in the different organs of control animals. The highest activity was found in ovary and gut, the lowest in muscles and kidney, while an intermediate level was observed for the heart. A space flight does not seem to modify the activity of peptidase-1 in most of these organs, except for the kidney in which the specific activity seems to be slightly increased (significant change at $p<0.05$).
In short, peptidase-1 expression appears unaltered in most of the studied organs of animals maintained under microgravity conditions for 12 days. In this respect, that enzyme can be efficiently used as a marker of the enzymatic and sexual genotype of *Pleurodeles* experimentally submitted to the effects of space environment.

Table 1. Peptidase-1 activity in different organs of *Pleurodeles* following Bion 10 flight. Activity in nmole/mn/mg of protein (mean ± SD).

Organs	Flight batch N0	Flight batch Specific Activity	Synchrone batch N0	Synchrone batch Specific Activity
Muscle	6	22,9 ± 6,7	9	22,5 ± 21,9
Heart	5	39,8 ± 23,8	8	44,5 ± 10,8
Ovary	5	141,0 ± 48,9	9	204,6 ± 61,6
Gut	6	71,6 ± 25,3	9	55,6 ± 16,8
Kidney	6	43,7 ± 11,3	9	29,7 ± 12,2

4. DETECTION OF GENETIC ABNORMALITIES IN THE PROGENY OF THE EMBARKED FEMALES

4.1. Scientific objectives

The purpose of this project was to study both caryological abnormalities and morphological malformations occuring during organogenesis in the progeny of females embarked on Bion 10.

4.2. Protocol of ovary transplantation

The flight procedure of "Experience Triton" made necessary to sacrifice the embarked females just after landing. In order to maintain the ovaries in fully functional conditions we have proposed a surgical procedure based on the graft of these organs into recipient *Pleurodeles* remained on earth and kept in laboratory rearing conditions(Refs. 9, 10). The protocol consisted in grafting the ovaries of the embarked female into recipient castrated males. These grafts induce these genetic males to differentiate into females. Mating of the host animals with standard males can then provide descendants issued from the oocytes previously submitted to space environment.

As in mammalian females, there is a discontinuity between the ovaries and the genital tract (oviducts) in *Pleurodeles* females. This allows the anatomical system "grafted ovary/genital tract" to be physiologically functional. When juvenile ovaries are grafted into recipient juvenile castrated females, the anatomical discontinuity is maintained and the genital apparatus is functional as we have demonstrated by ten control experiments.
In *Pleurodeles*, the two mullerian ducts are present both in juvenile females and males. They differentiate into oviducts in adult females. In adult males, mullerian ducts remain at a vestigial state, however, their development into oviducts can be hormonally induced. We have shown that this occurs after grafting a juvenile ovary into a juvenile castrated male. As a consequence, an ovary grafted into a castrated male induces a complete oviduct differentiation. Moreover, a juvenile castrated male which has received an juvenile ovary reverses in a fully functional female and becomes able to lay ova originating from the grafted ovary.
Laparotomy of the host animals allows to oversee the evolution of the grafted ovaries. Because of the slow development of ovaries, the success of this experiment cannot be ascertain before a minimum delay of one year after the ovary transfert. Twelve control transplantations, using sexual genotypic males were successfull . Two years after the ovary transplantations, recipient males were crossed with standard males and laid fertilized eggs which developed normally. Thus, both castrated females and juvenile males can successfully be used as hosts for grafted ovaries (Fig. 2).

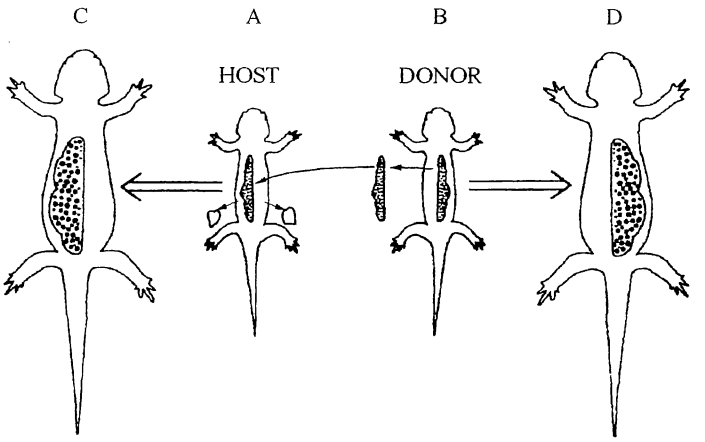

Figure 2 : Principle of ovarian transplantation in Pleurodeles .

A : Castrated juvenile male recipient of a transplanted ovary
B : Unilaterally castrated juvenile female
C : Animal A two years later : adult phenotypic female
D : Animal B two years later : adult female

Peptidase-1 can be used as a marker in order to distinguish between male and female tissues. This enzyme allows to make sure that an ovary grafted into a castrated male is actually that of a female and not an uncompletely removed testis which developed after its reversion into ovary. Mating of host animals (genetic females or males) with standard males can then provide descendants issued from the oocytes that have been previously submitted to space environment.

According to the protocol, thirty juvenile males originating from French laboratories were castrated three months before the launching of Bion 10. Peptidase-1 genotypes of these males and of sixty juvenile females of the same progeny were determined. The females were sent to Moscow in order to constitute the batches of embarked females and ground synchrone controls. All these juvenile females died before the launching of Bion 10 and were then replaced by adult females of a local strain. Thirty adult females of the same local strain were selected to be used for ovary transplantations.
Due to their small size, most of the juvenile males could not be used as recipients of large size ovaries excised from the embarked females. The protocol for ovary grafts was thus modified: the ovaries originating from embarked adult females were grafted both on adult castrated females and on juvenile males. In a maximum delay of 20h after the landing of Bion 10, ovaries of embarked females were cut in three parts. The anterior and central parts, which contain mainly mature oocytes, were grafted into adult castrated females. The posterior part, of a smaller size, and which contains oogonies and growing oocytes, was grafted into juvenile males (Fig. 3).
The same kind of transplantations was effected using synchrone females as donors.The all host animals were then transferred to Paris and bred in laboratory conditions. Many recipient animals died a few weeks after transplantation. All the animals which survived until the end of january 1993 were still in good health in september 1993. Results are shown on Table 2.

4.3. Discussion, conclusion
At the present time, only two adult females, recipient of anterior pieces of ovaries provided by an embarked female and a synchrone female,are still alive.Their morphological pattern is similar to that of castrated females of the same age. In fact, the success of the graft of ovaries of adult females is not ascertain because the two donors of ovaries have been submitted to abnormal temperatures (during the Bion 10 flight and on ground). Nevertheless, if the ovaries developed, progeny might be obtained in a few months.

Table 2. Transplantations of ovaries

	Transplantations in adult castrated females with anterior and central part of ovaries from		Transplantations in juvenile castrated males with posterior part of ovaries from	
	flight batch females	synchrone batch females	flight batch females	synchrone batch females
Number of operared animals (I.93)	10	5	5	5
Number of survival animals (IX.93)	1	1	3	4

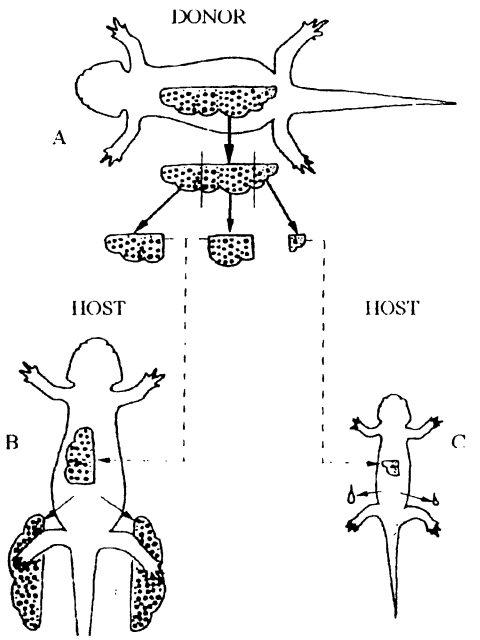

Figure 3 . Actual experimental procedure applied in " Experience Triton "

A : Embarked femaleor synchrone female
B : Castrated adult female recipient of a transplanted ovary (anterior or medium part)
C : Castrated juvenile male recipient of a transplanted ovary (posterior part)

In contrast, the seven juvenile males which received posterior pieces of ovaries provided by embarked and synchrone females, are still alive. Their morphological pattern is similar to that of standard females of the same age.

It can be hypothetized that the different survival rates in these two groups of host animals is due to the presence or absence of full grown oocytes into the grafted pieces of ovaries. Control experiments have shown that necrosis of full grown oocytes, but not of oogonies or growing oocytes, provokes the death of recipient animals. In addition, oogonies and growing oocytes do not seem to be affected by an abnormal increase of the ambient temperature.

These results clearly show that this kind of grafts requires the use of juvenile animals as hosts. This experimental protocol can be easily adapted to study the effects of space environment on other kinds of organs or animals.

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