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Early onset of a large pool of previtellogenic oocytes and cyclic escape by vitellogenesis : the pattern of ovarian activity of *Xenopus laevis* females and its physiological consequences

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Summary. During the first year of life of the *Xenopus laevis* female, a large stock of previtellogenic oocytes (about 250 000) is built up in the ovaries ; these cells have a low growth rate and metabolic activity. A quantitative analysis of the developing ovary showed that the first vitellogenic wave occurred in two succesive phases with very different activities. In the adult, these vitellogenic oocytes represented the equivalent of four clutches. Shortly after laying, a new population of rapidly growing synchronous oocytes was recruited among medium-sized cells ; the growth of these oocytes slowed down progressively and was achieved only at the moment of the following spawning period. Thus it appeared that the functioning of the *Xenopus* ovary was cyclic and discontinuous like that of temperate climate anurans, with a high level of physiological activity restricted to a short period following egg laying. The cells of the previtellogenic pool did not exhibit atresia and seemed sufficient to ensure the reproductive capacity of the female during its whole life.

Introduction.

The ovaries of sexually mature amphibians living in nature undergo marked changes in morphology, weight and activity throughout the year. This pattern of cyclic ovarian activity has been described in detail in the temperate zone anurans, *Rana pipiens* (Smith, 1955 ; Mizell, 1964), *Rana temporaria* (Smith, 1955), *Bufo bufo* (Jorgensen, 1973a) ; this pattern of activity is also seen in temperate urodeles like *Taricha torosa* (Miller and Robbins, 1954). In most of these amphibians, which normally have only one breeding period each year, four phases can be distinguished during the annual cycle :

1) the large ovaries contain maximum-sized oocytes ready to be ovulated at the beginning of the breeding season (generally in spring) ; at the end of the mating period, when the eggs have been oviposited, there is an abrupt drop in the weight of the ovaries which at that time contain mainly small vitellogenic and previtellogenic oocytes ;

2) the ovaries seem quiescent during the few months after spawning since their weight and appearance remain constant. The length of this resting period can vary according to several factors which may be of internal or environmental origin ;
3) ovarian weight increases considerably, corresponding to the rapid, synchronous vitellogenic growth (mainly due to yolk accumulation) of a fixed number of oocytes ;
4) ovarian weight remains constant over a long period of post-vitellogenic stasis ; the growth of full-grown occytes is over before winter and the animals enter hibernation with a complete stock of future eggs.

In contrast, the reproductive activity of many amphibians living in rainy tropical regions is said to be acyclic or continuous since adults are in breeding condition throughout the year (Inger and Greenberg, 1963 ; Inger and Bacon 1968).

The pattern of the reproductive activity of *Xenopus laevis* is not totally clear (Deuchar, 1975). According to seasonal variations in ovarian weight, culminating in July, it seems obvious that there is only one breeding season which extends over several months from July-August to November-December (Gitlin, 1939 ; Brown, 1970). However this is considerably longer than that observed in temperate climate amphibians (less than one month). On the other hand, as outlined by Wasserman and Smith (1978), it is currently observed that « animals imported from South Africa at almost any time of the year contain oocytes of all size classes » and may be artificially stimulated to ovulation and oviposition by the injection of steroids or gonadotropins. It has also been concluded that oogenesis in *Xenopus laevis* is a naturally continuous and asynchronous process, unlike that in temperate anurans (Dumont, 1972). As outlined recently by several authors (Tokarz, 1978 ; Jones, 1978), very few observations concerning the organization and functioning of the *Xenopus laevis* ovary have been published, and this lack of information could hamper the biological interpretation of well-described molecular events.

The present paper presents a quantitative description of the onset of the activity of the adult *Xenopus laevis* ovary during the second year of life since we have previously demonstrated (Callen *et al.,* 1980b) that the vitellogenic period of the first oogenetic wave occurs mainly at that time. Our present results confirm the growth kinetics of the oocytes observed previously. Moreover, we have been able to accurately estimate the whole pool of previtellogenic cells found in adult females and identify the time at which the pool was stabilized. Finally, we have analysed the process of ovulation and the recruitement of new oocytes by studying the size frequency distribution of vitellogenic oocytes at various times after laying. These results suggest that the oocyte flow through vitellogenesis is not a continuous process in *Xenopus laevis.* The pattern of ovarian development and the hormonal control of oocyte recruitment and growth have been analysed extensively in only one other anuran species, *Bufo bufo* (Jorgensen, 1973 a, b,1974, 1975 ; Billeter and Jorgensen, 1976). The important results obtained in this animal will also be examined in the discussion.

Material and methods.

Animals. — The experiments were carried out on two classes of animal : (1) sexually mature females at least 3 years old, weighing about 200 g and with a minimal body size (mouth to cloaca) of 11-12 cm ; the animals were purchased from Serea (France) ; (2) young growing females of known age (post-metamorphic) bred in the laboratory (for details see Callen *et al.,* 1980b) or kindly provided by the « Laboratoire de Biologie de la Reproduction », University of Rennes, France.

Ovary analysis. — The ovaries of sacrificed animals were excised, rinsed and maintained in Barth's buffer (Gurdon, 1968). The ovarian lobes were carefully examined and counted. Lobes with typical dimensions were fixed in cytological Helly fixative for 4 h, then thoroughly washed with water. These samples could be kept for several months in water at 4 °C.

The vitellogenic oocytes were manually separated from freshly dissected or fixed ovarian lobes, classified according to morphological criteria (Dumont, 1972) and pooled in homogeneous size classes (size interval : 100 μm). The numbers and dimensions of the oocytes were estimated directly under a binocular lens using a micrometer eye-piece.

In order to obtain the absolute number of previtellogenic oocytes in the ovarian lobes, the envelopes of fixed half-lobes were hand-cleaned of all follicles having a diameter larger than 250-300 μm and then mounted in water between a slide and coverslip. The outline of these flattened envelopes was drawn on millimetric paper at a 10/1 scale and their whole surface estimated by a planimetric method. These preparations were then observed with a microscope and all the oocytes contained within a standard field of known area (3.9 mm^2) were counted. The whole surface of the lobe was scanned and since contiguous fields were successively analysed, we could calculate significant mean oocyte density.

For the kinetic experiments, the females were partially ovariectomized after anesthetization with MS 222 (Tricaine methane sulfonate, Sandoz). The amount of ovarian tissue removed at each surgical operation was equivalent to about half an ovarian lobe.

Analysis of clutch size. — The females were stimulated to lay eggs with one or two injections (into the dorsal lymph sacs) of human chorionic gonadotropin (HCG). The whole clutch was collected and the unfertilized eggs stored at 4 °C or in dilute Helly fixative. One to two thousang eggs (about 10-20 % of the clutch) were manually separated with forceps, precisely counted and then centrifuged at 2 500 × g for 15 min. The whole clutch was also centrifuged in the same conditions and the total number of eggs laid at one time by a female was estimated by comparing egg pellet volumes.

Results.

1. *Size frequency distribution of the vitellogenic population.*

Size distribution into 100-μm classes was analysed for the vitellogenic population (cells ranging between 300 and 1 275 μm in diameter) of 16 growing females 1 to about 2.5 years old and 5 old adult animals.

Five types of female were distinguished according to the diameter of largest oocytes found in their ovaries. Specific patterns of frequency distribution, characteristic of the developmental stage of the ovaries of each type, are described in table 1.

The distribution observed in the youngest actively growing females (types A and B) showed that oocytes ranging from 500 to 900 μm in diameter were less abundant ; a small peak of oocytes of about 1 000 μm in diameter developed progressively. When female age increased (types C to E), the relative size of this peak of large oocytes not only augmented but also shifted progressively towards the biggest possible diameter (1 275 μm). The final pattern characteristic of adults was found in type D animals having a large number of banded stage VI oocytes ; in the last type of olds animals, we only observed a relative increase in the frequency of these 1 250-1 275 μm cells.

2. *Absolute number of previtellogenic and vitellogenic oocytes in growing or adult females.*

In order to estimate the size of the previtellogenic and vitellogenic populations contained in the ovaries, we counted the total number of sex cells in samples of fixed ovarian tissue of several animals representative of the five types defined in the preceding section.

After the whole ovaries were dissected, the number of ovarian lobes, their dimensions and size range variation were determined ; some lobes considered as « mean lobes » were then fixed *in toto* and subsequently analysed. During fixation the normally transparent previtellogenic oocytes became opaque and were thus easy to score (fig. 1). Due to progressive hardening of the tissue, each flattened lobe could be dissected into two equal halves. The vitellogenic oocytes, including those of about 300 μm, were carefully removed from the ovarian envelope

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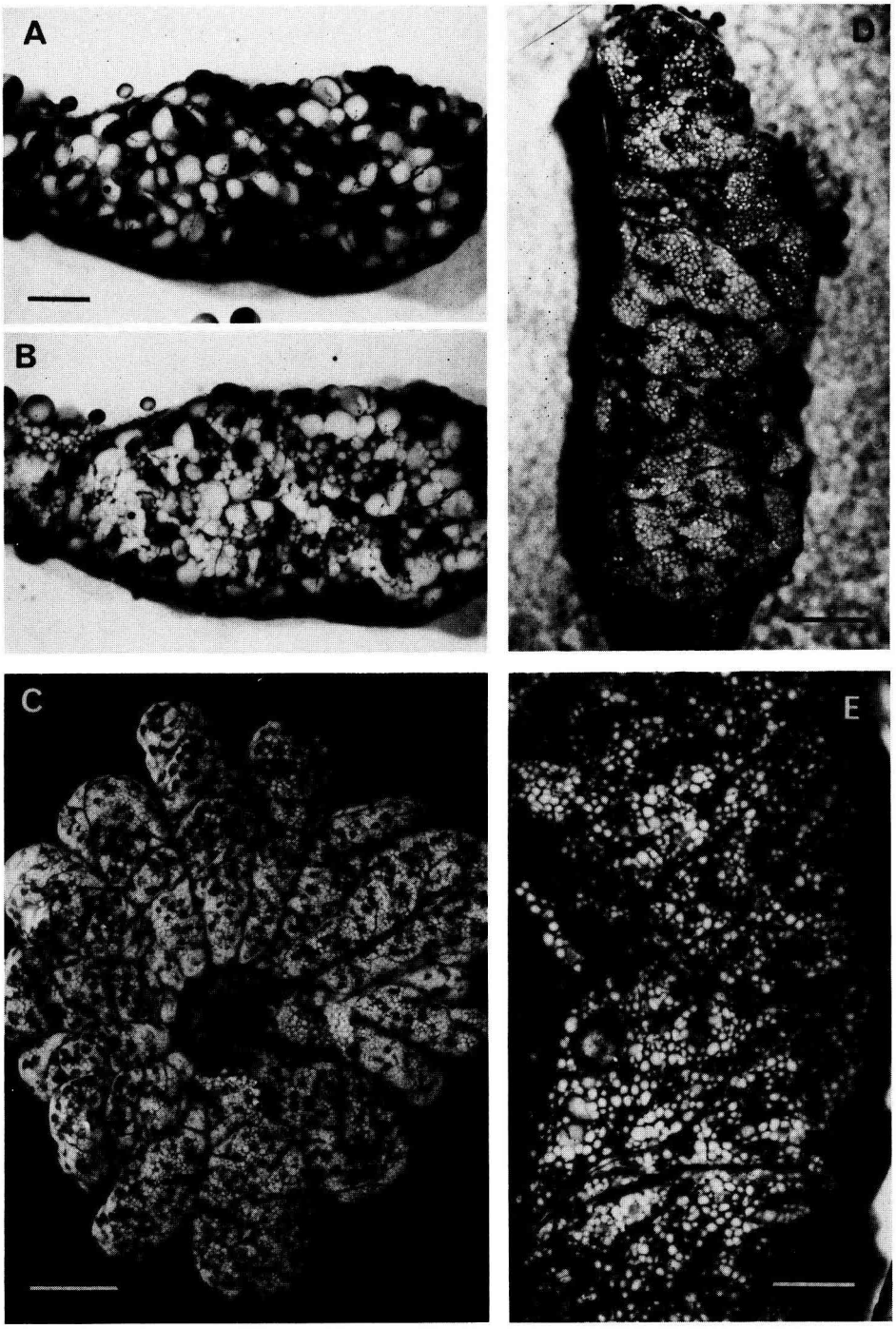
FIG. 1. — *Ovarian lobes of* Xenopus laevis *females fixed in toto with Helly fixative.*

A and B. The same ovarian lobe observed before and after fixation. The vitellogenic oocytes are only visible on the unfixed preparation ; after fixation, the numerous transparent previtellogenic oocytes are opaque and conspicuous. Bar : 2 mm.

C. General view of the whole ovary of a starved female (see text). Bar : 5 mm.

D. Fixed ovarian lobe of a type A female. Bar : 2 mm.

E. Part of a fixed ovarian lobe of a type E female. Bar : 2 mm.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with curved needles and counted (fig. 2a, b) ; the empty envelopes were observed with a microscope and all the oocytes directly counted in each examined field (fig. 2c). As a rule, about 50 % of the whole surface of each half-lobe was analysed in this manner ; consequently, the mean oocyte density we calculated was representative of the whole lobe. The smallest sex cells, unambiguously

TABLE 1

Size frequency distribution of vitellogenic oocytes in growing or adult Xenopus laevis *females.* The 21 animals studied are distributed into 5 classes according to the maximal size of the oocytes in their ovaries. The percentages were obtained by analysing oocyte samples ranging from 500 to 1 000 cells per female. The total number of vitellogenic oocytes for one mean ovarian lobe has been estimated. Also see table 2.

— Female type — Number of females	A_4	B_5	C_3	D_4	E_5
Maximal oocyte size (μm)	1 000-1 050	1 050-1 150	1 150-1 225	1 225-1 250	1 250-1 300
(b)	82 (a)	86 (a)	100 (a)	110 (a)	118 (a)
1 200-1 300			6.1	24.7	30.5
1 100-1 200		4.0	21.0	12.7	8.5
1 000-1 100	11.1	14.8	12.6	3.0	3.6
900-1 000	9.2	5.7	5.1	2.4	2.0
800-900	4.6	4.5	2.8	2.1	2.9
700-800	6.2	4.5	3.2	2.5	3.7
600-700	6.4	4.6	4.3	3.8	4.5
500-600	7.8	7.0	5.0	6.5	5.1
400-500	11.4	11.9	12.8	11.3	6.9
300-400	43.1	42.6	26.9	30.8	32.8
Total number of oocytes (c)	500 ± 100	600 ± 100	900 ± 100	1 100 ± 200	1 400 ± 200

a) Animal size from head to cloaca (mm) ; mean value ; b) oocyte size (μm) ; c) per mean ovarian lobe.

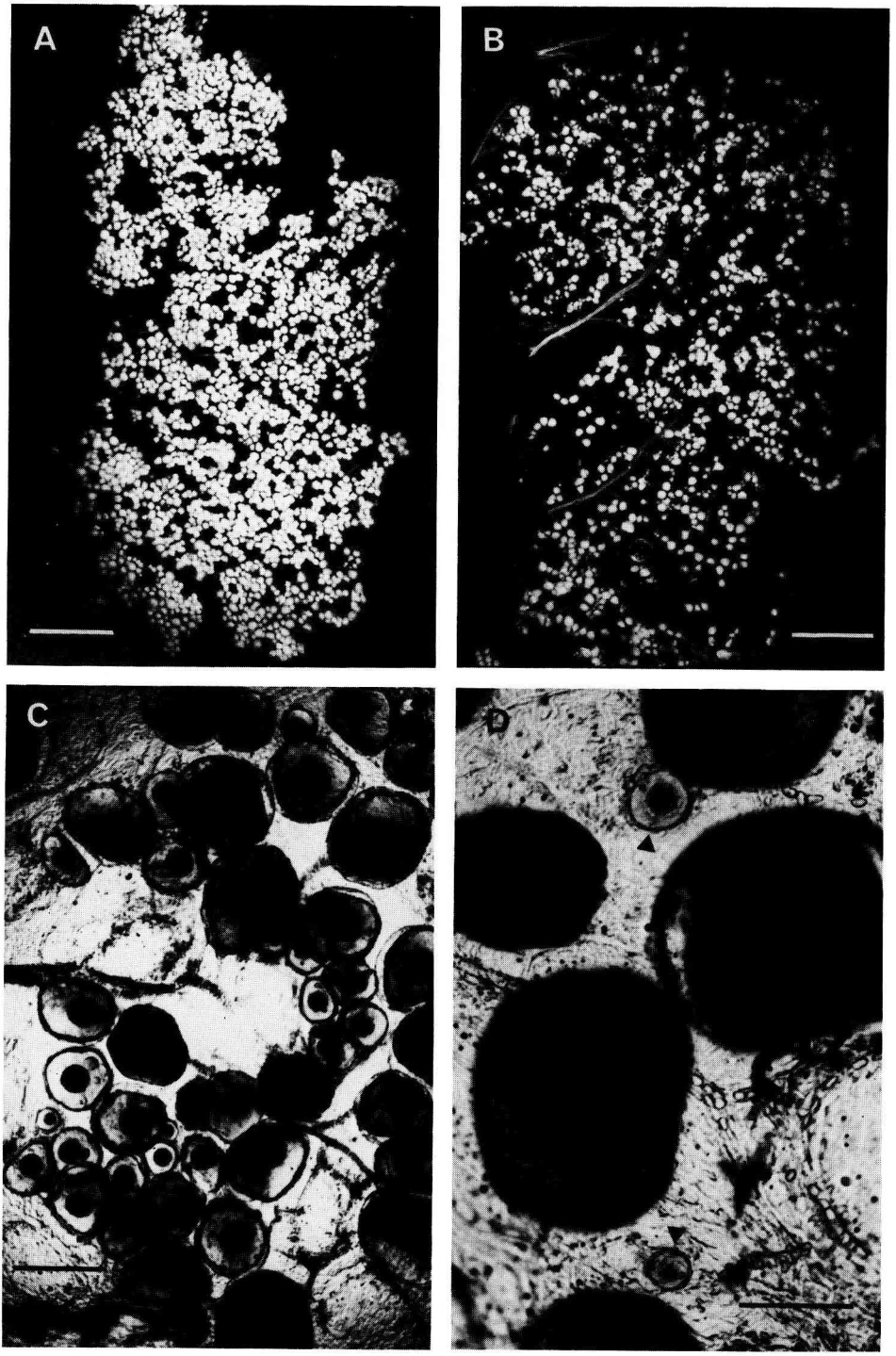
FIG. 2. — *Fixed ovarian envelopes emptied of their vitellogenic oocytes and mounted in water between a slide and coverslip. Microscopic observation.*

A. Fixed envelope of the half of an ovarian lobe from a type B female ; mean previtellogenic oocyte density : about 30 cells/mm^2. Bar : 2 mm.

B. Part of the fixed envelope of half a lobe of a starved female ; mean previtellogenic oocyte density : about 12 cells/mm^2. Bar : 2 mm.

C. Microscopic observation of a fixed envelope ; the mitochondrial mass of some oocytes is clearly visible. The central hole was the site of a large oocyte that was removed. Bar : 200 μm.

D. Detail showing the smallest oocytes found in the envelopes (▲) ; the two cells have diameters of 40 and 58 μm, respectively. Bar : 100 μm.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identified as oocytes by this method, were early diplotene cells 40-50 μm in diameter (fig. 2d). For such an analysis we used 14 lobes representative of the ovaries of 10 females ; the results are presented in table 2. Each lobe contained an average population of about 6 300 oocytes (extreme values : 4 000-10 000), and we observed an average of 38 lobes for the two ovaries. The mean total number of oocytes larger than 40-50 μm was around 240 000-250 000 per animal. This stock was already constituted at the end of the first year of life (type A females), so the mean density of the previtellogenic cells decreased regularly from 30-34 to 8-12/mm^2 when the size and the surface of the lobes augmented in growing animals (fig. 1b). Finally, the vitellogenic oocytes always represented a small proportion (15-25 %) of the total number of cells. Taking into account the absolute number of these oocytes per mean ovarian lobe (see estimate in table 1) and the percentage of each size class, the changes in the whole vitellogenic

TABLE 2

Quantitative analysis of whole ovarian lobes of growing (types A to D) *or large* (type E) Xenopus laevis *females.* The mean previtellogenic oocyte density of each half lobe was determined by microscopy. Only one half (*) of some lobes was analysed, and the total number of oocytes was calculated assuming that the two halves were identical (true in most cases).

Female type	Number of lobes (a)	Lobe size (mm) (b)	Lobe surface (mm^2)	Vitellogenic oocytes	Previtellogenic oocyte density (oo/mm^2)	Number of oocytes per lobe	Number of oocytes per female (c)
A	33	6 × 14	194	511	28.6	6 060	199 980
B	39	6 × 12.5	170	440	31.7	5 830	237 315
		7 × 14	183	499	31.9	6 340	
B	41	8 × 16	193	640	30.0	6 430	251 740
		7 × 14	231	609	22.7	5 850	
B	38	8 × 16	263*	700	22.2	6 540	248 520
C	35	9 × 17.5	217*	539	16.7	4 160	177 275
		12.5 × 18	405	588	13.3	5 970	
C	37	10.5 × 24	720*	1 562	8.2	7 470	276 390
D	41	9 × 18.5	376*	768	13.5	5 840	258 915
		10.5 × 21	491*	1 092	11.6	6 790	
D	35	9 × 20	327	950	14.8	5 790	202 650
E	38	11.5 × 18	555*	710	17.0	10 140	385 320
E	42	11 × 25	520*	988	8.6	5 460	229 320

(a) For the two ovaries ; (b) width × length ; (c) mean value when two lobes were analyzed.

population were shown to be a function of female age (fig. 3). The increase and the progressive shift of a large oocyte peak are clearly shown in the diagram of figure 3.

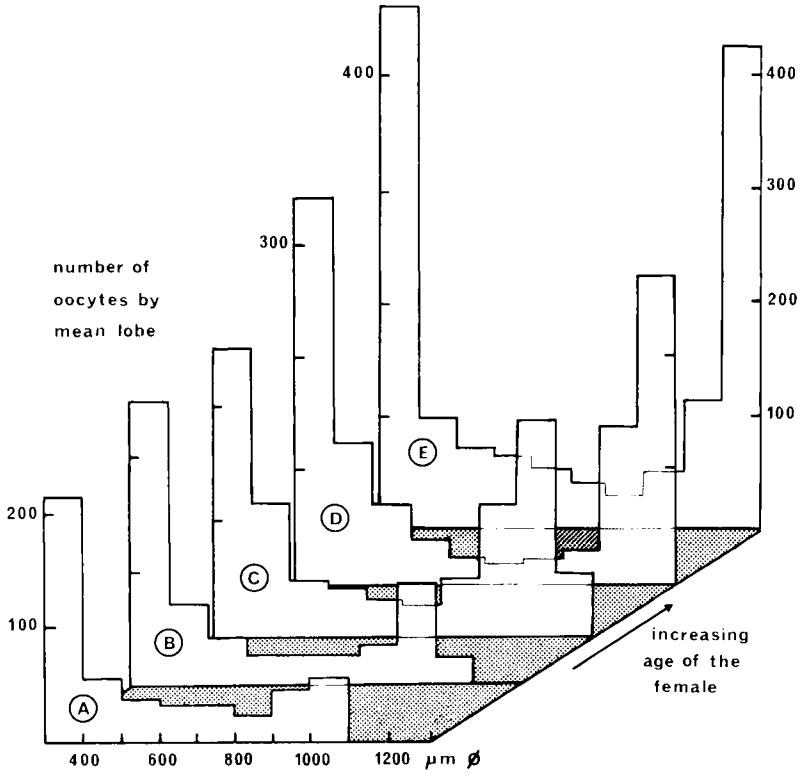

FIG. 3. — *Diagram showing the onset of the vitellogenic population during the first oogenetic wave in growing* Xenopus laevis *females ;* the values are given for a mean ovarian lobe (38 per female). The diagram includes (1) the percentage of each oocyte class within the 5 female types defined in table 1 (A to E) and (2) the absolute number of oocytes estimated per mean ovarian lobe.

3. *Size of the oocyte pool in relation with female growth rate.*

It has been shown (Callen *et al.,* 1980b) that the oocyte growth of a female is not exactly correlated with its body growth since the ovaries of animals starved during the first year of life contain smaller oocytes than expected for their actual age. In addition, early starvation is known to inhibit the process of compensatory regeneration (in terms of cell number) of ovarian tissue in *Bufo bufo* (Billeter and Jorgensen 1976). Consequently, we analysed, in the same way, the ovaries of 3 females which had exhibited a very reduced growth rate and only reached a length of 6-7 cm at 26-28 months of age. The results of our observations on 9 lobes are presented in table 3. There were several striking differences with the controls : (1) the two ovaries contained a smaller average number of lobes (29)

and (2) the total number of oocytes per lobe was significantly reduced (3 000 *vs* 6 300). In conclusion, the whole population of sex cells calculated for these females was only about 88 000 or approximately 1/3 of that of the controls.

TABLE 3

Quantitative analysis of ovarian lobes of Xenopus laevis *females which were starved during the first year of life* (same comments as in the legend of table 2).

Female number	Number of lobes (a)	Lobe size (mm) (b)	Lobe surface (mm^2)	Vitellogenic oocytes	Previtellogenic oocyte density (oo/mm^2)	Number of oocytes per lobe	Number of oocytes per female (c)
1	29	3.5 × 10	104	240	14.9	1 790	73 370
		8.5 × 14	203	587	12.1	3 250	
		6.5 × 12.5	210	641	12.9	3 350	
		6.5 × 12.5	140	320	10.1	1 730	
2	32	5 × 14	143	611	20.4	3 530	91 410
		5.5 × 13	158	509	17.0	3 190	
		5.5 × 11	104	350	14.4	1 850	
3	26	8 × 14	252	802	12.3	3 900	98 670
		7.5 × 14	299*	784	12.7	3 690	

a) For the two ovaries ; b) width × length ; c) mean value.

4. *Size frequency distribution of the vitellogenic population after egg laying.*

We analysed the reproductive pattern of *Xenopus* in two ways.

— Clutch size was first determined for the animals bred in our laboratory conditions. Eight large adult females were induced to lay eggs after HCG injection (500 IU) ; half the population responded after only one injection and the other half after two successive injections at a 48-h interval. The average size of the clutch (11 000 eggs ; extreme values : 5 500-17 000) appeared to be independent of female sensitivity to the hormone.

— Some females were periodically partially ovariectomized or sacrificed and the size frequency distribution of the vitellogenic oocytes in the ovaries analysed. The results obtained with 9 females examined over a period of 6.5 months after laying are shown in table 4 ; as a control, one female was operated just before and 2 months after laying. In these experiments : (1) ovarian distribution showed a large peak of oocytes 1 000-1 200 µm in diameter three weeks after laying ; in other words, their size frequency distribution was intermediate between those of type B and C animals (as defined in section 1) ; (2) this peak progressively shifted with time towards the largest diameters, so the animals examined 1.5-2.5 months after laying belonged to type C and those observed after 4-4.5 months were

intermediate between the patterns of types C and D ; (3) during the period considered, the ovaries did not totally recover the pre-laying oocyte distribution pattern since the ovary organization of the oldest animals (with respect to spawning) did not show the typical peak of oocytes larger than 1 200 µm that characterized type D or E.

TABLE 4

Size frequency distribution of vitellogenic oocytes (Ø > 600 µm) *in the ovaries of 9 females sacrificed or ovariectomized at various times after spawning.* The values obtained by Keem *et al.* (1979) for 2 females analysed after 1.5-2 months are also given ; they compare well with our 2-month old females. These distributions may be compared to the distribution in the ovaries before spawning calculated on type E females (see table 1).

Oocyte size (µm)	Before spawning (5 ♀) (a)	3 weeks (1 ♀)	2 months (1 ♀) (a)	2.5 months (2 ♀)	4-4.5 months (3 ♀)	6.5 months (2 ♀)	1.5-2 months (2 ♀) (b)
1 200-1 300	55.2 (47.3)	8.0	15.8	17.2	33.5	33.4	8
1 100-1 200	15.4 (13.0)	24.3	32.5	44.5	34.0	25.8	33
1 000-1 100	6.5 (8.9)	28.0	16.5	9.0	13.0	13.8	17
900-1 000	3.6 (10.6)	12.8	6.4	4.0	7.5	6.7	12
800-900	5.2 (7.7)	7.4	5.2	4.6	4.8	4.9	
700-800	6.7 (5.9)	7.8	6.8	4.4	4.2	6.1	30
600-700	8.1 (6.5)	11.8	16.7	16.0	3.2	9.0	

a) Results obtained for the same female which had been ovariectomized before and after spawning ; (b) results published by Keem *et al.* (1979).

Discussion.

1. *Changes in the rate of ovarian development with female age.*

The distribution of vitellogenic oocytes in young growing females (types A and B ; table 1) was consistent with the kinetics of oocyte growth already described, i.e. the less abundant classes (500 to 900-µm oocytes) consisted of cells that increased in size very rapidly. The progressive accumulation of oocytes larger than 1 000 µm coincided with a large decrease in their growth (about one year is necessary for oocyte diameter to increase from 1 000 to 1 200 µm ; Callen *et al.*, 1980b). The ovaries of young females are thus a dynamic system with a true continuous oogenetic flow, the relative numbers of different-sized cells reflecting the length of their life-time within each size interval.

In contrast with females of types A and B, older ones (types C and D, 1.5 to 2.5 years old) harbored large oocytes of even greater diameter (1 250 instead of

1 000 to 1 200 μm). The shift of this peak of large oocytes, combined with the absence of a sizeable increase in medium-sized cells, indicates that the rate of ovarian development was different and that the production of vitellogenic cells was gradually reduced. If it were not so, one could expect only a relative (and absolute) increase of 1 000-1 100-μm cells, but not the accumulation of larger ones. Moreover, the absolute number of vitellogenic oocytes did not increase in the same way as a function of time ; the first 500 oocytes in each mean lobe of class A females were produced during an early 4-5-month period, whereas the next 600 did not appear before 16-18 months (type D females) (see fig. 3 in Callen *et al.,* 1980b).

In conclusion, almost half of the vitellogenic cells that constituted the adult ovary underwent a rapid synchronous development in young females at the end of the first year of life, and about the same number of oocytes was produced at a reduced rate over a period spanning the second long vitellogenic growth phase.

2. *How many oocytes are there in the ovaries ?*

In a previous paper, we suggested that previtellogenic oocytes constitute a large sex cell reserve in the ovaries of adult females (Callen *et al.,* 1980b) ; in the present work, the sexual population in growing or adult animals was directly measured. The mean value (240 000-250 000 diplotene cells) appeared to be independent of animal age and this stock was constituted as early as the end of the first year of life ; 20-25 % of these cells were vitellogenic and about 6-7 % were large oocytes (15 000-16 000 cells larger than 1 200 μm in type E females).

Such a quantitative analysis in female amphibians has only been conducted in one other anuran species *(Bufo bufo)* by Jorgensen (1973a, b ; 1975). A large pool of previtellogenic oocytes (about 40 000) is established in wild *Bufo* females a few months after metamorphosis, largely before sexual maturity is attained (during the third year) ; this pool is so large that it appears to be constant throughout adult life (Billeter and Jorgensen, 1976). Since 3 000-4 000 eggs are laid annually, it appears that in the toad, as in *Xenopus,* the total number of ovarian follicles corresponds to several years of egg production, even in immature females.

The size frequency distribution of *Bufo* previtellogenic oocytes is similar to that described for *Xenopus* (Callen *et al.,* 1980b) ; the largest cells in this population are about 500 μm in diameter (Jorgensen, 1973b), whereas in *Xenopus* they reach only 400 μm. Moreover, the weight increase of the ovaries during their early growth phase is mainly due to an increase in the number of previtellogenic oocytes that, one after the other, reach a diameter of 300 μm and then remain in that state (Billeter and Jorgensen, 1976) ; this also holds true for *Xenopus* since we observed that (1) « the size of the young ovary increases significantly without increase of the diameter of the largest previtellogenic cells : 150 μm » and (2) the growth of these previtellogenic cells is biphasic, the first rapid growth phase spanning only 1.5 months (Callen *et al.,* 1980a,b). In summary, oocyte growth is very similar in both wild *Bufo* females and in laboratory bred *Xenopus* females and occurs in two phases : it is first rapid and then slow, leading to the early onset of

a large previtellogenic pool in the ovaries ; later, the oocytes progressively escape from this pool throughout another biphasic period of vitellogenic growth (Callen *et al.*, 1980b ; Callen, 1984).

3. *Pulses in adult ovarian activity.*

Our laboratory bred females produce average clutches of 11 000 eggs ; this is in good agreement with the observations of Wallace *et al.* (1970). From this mean clutch value, we estimate that 70 % of the largest oocytes found in type E animals were expelled from the ovaries during oviposition. Actually, we checked by ovariectomy that females which had just laid abundant clutches still contained oocytes larger than 1 200 µm. Lastly, we estimate (table 1) that the vitellogenic population of steady-state adult ovaries represents about 4 potential clutches (fig. 4).

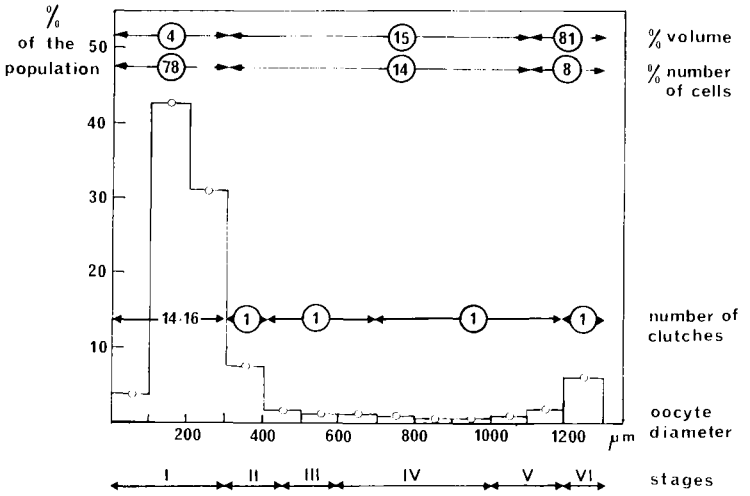

FIG. 4. — *Organization of the whole ovary in adult* Xenopus laevis *females* (type E). With each oocyte stage are shown (1) the volume percentage in the ovary and (2) the total number (%) of cells for 3 classes of oocyte : previtellogenic (up to 300 µm), medium-sized (300 to 1 100 µm) and large (> 1 100 µm). The approximate number of layings represented by these 3 classes is also given (one mean clutch = 11 000 eggs). The graph includes the distribution of previtellogenic oocytes presented separately in Callen *et al.* (1980b).

This is different from what is known in temperate climate anurans like *Rana* or *Bufo* whose post-spawning ovaries are entirely depleted of all pigmented oocytes and resemble the juvenile state, *i.e.* ovarian weight is reduced to 9-10 % (Mizell, 1964 ; Smith, 1955 ; Jorgensen, 1973a) ; in *Xenopus leavis* this value reaches 31 % (Gitlin, 1939). This figure is obtained if we admit that all oocytes larger than 1 200 µm, and only those, are laid in natural conditions. This is in agreement with the results we obtained in laboratory conditions. Our analysis of the ovaries of post-spawning adult females shows that a significant population of 1 000-1 100-µm oocytes appeared within in a few weeks and progressively shifted

to the largest diameters. A new wave of synchronous vitellogenic cells then appeared as in growing animals. From table 1 we can estimate that this new population was recruited from the pool of medium-sized oocytes, including those with a diameter of 600-700 μm. It is noteworthy that such cells (1) undergo very rapid growth during the first oogenetic wave (Callen *et al.,* 1980b) and (2) that they actively (and transiently) incorporate vitellogenin *in vitro,* but only when they are dissected from females which have just laid eggs (Wallace *et al.,* 1970). However, after 6 months, the females have still not returned to the pre-spawning ovarian pattern. This is consistent with two independent observations : (1) the final growth of large oocytes is a very slow process since it takes about one year for them to double their previous volume and (2) ovarian weight increase during the vitellogenic period of the annual cycle of animals living in nature is achieved exactly before the next spawning season (Gitlin, 1939). Thus we suggest the following model : after a short period of recruitment and growth, a sufficient number of cells increases to 1 100 μm in diameter ; an increase from 700 to 1 100 μm is reached after 1.5-2 months (Keem *et al.,* 1979 ; Callen *et al.,* 1980b). The growth of these cells is completed at a lower rate during the following 10 months. Since, at the beginning of this process, the ovaries already contain many oocytes of about 1 200 μm, their weight increase due to newly recruited cells (as a function of time) is very progressive, as observed in nature by Gitlin (1939).

This developmental pattern seems different from that of *Rana* or *Bufo*. After a short resting phase of 1-3 months following spawning, their ovaries grow rapidly over 4-5 months so their maximal size is reached in autumn ; during hibernation, this size remains unchanged (Mizell, 1964 ; Smith, 1955 ; Jorgensen, 1973a). In spite of differences between the two ovarian patterns, the occurrence of a final period of slow growth at the cellular level is observed in both *Xenopus* and *Bufo.* This might be important for complete oocyte differentiation in order to prepare future maturation. In summary, the ovarian pattern we describe for *Xenopus laevis* appears to be similar to that of temperate climate cyclic anurans. On the contrary, oogenesis is classically presented in this organism as a continuous and asynchronous process, leading to the existence of oocytes in all developmental stages at all times of the year, with a balance between oogonial production and atresia (Dumont, 1972 ; Wasserman and Smith., 1978 ; Keem *et al.,* 1979). The physiological consequences of the functioning model we suggest have to be analysed.

The large stock of small oocytes in females older than one year consists of cells of reduced physiological activity. Similarly, between two recruitement and growth periods, all classes of oocytes in the adult ovary show a low metabolic activity ; rapidly growing cells (late stage III, stage IV, early stage V) are found only for a short time after spawning. This is especially true for laboratory maintained adult animals which do not spawn annually ; their ovaries are probably in an arrested physiological state, though they contain medium-sized oocytes. Actually, many metabolic processes have been shown to be greatly stimulated in medium-sized oocytes of post-spawned females compared to controls : protein synthesis, transcription (on amplified nucleolar rDNA or lampbrush

chromosomes), tyrosinase activity, amino acid uptake, pinocytosis of vitellogenin (see reviews in Dumont, 1972 ; Callen, 1984).

As demonstrated by Jorgensen (1974, 1975) for *Bufo,* we consider that the presence of the largest oocytes in the ovaries of *Xenopus* prevents the recruitment and development of smaller cells ; the loss of most of the large oocytes after laying probably modifies some hormonal balance and leads to a new oogenetic wave. Such a feedback model would explain the regulation of the number of cells recruited at each ovarian cycle ; it would not be fixed *a priori* but finally determined by the number of large oocytes reaching a certain critical size. As a working hypothesis, the difference in ovarian organization between *Xenopus* and temperate climate anurans (the constant presence of medium-sized oocytes) could be explained by a differently situated size borderline between specific hormone-producer or sensitive oocyte stages.

4. *Atresia and oogonial proliferation.*

The ovaries of a one-year old, laboratory-bred *Xenopus laevis* female contain a number of oocytes equivalent to 18-20 clutches ; a similar observation was made by Jorgensen (1973a, 1975) for young *Bufo* females living in nature. As outlined by that author, « this finding is not consistent with the assumption that oocytes take three years to differentiate from the oogonia », and if previtellogenic oocytes « do not normally become atretic, their mean life-time must be several years ». We have independently arrived at the same conclusion for *Xenopus laevis* (Callen *et al.,* 1980b). These observations raise two important questions with respect to the reproductive process in amphibians : does atresia affect this large stock of small oocytes, a balance being maintained between atresia and oogonial multiplication and, if not, is there any classical oogonial proliferation in this group ?

Atresia is believed to be rare for small oocytes (Barr, 1968 ; Guraya, 1969), and the factors leading to atresia in large oocytes (hypophysectomy, starvation) leave the former population intact (Smith, 1955 ; Jorgensen, 1973a). In normal conditions, large atretic follicles are only produced by mature oocytes left behind in the ovary after spawning (Lofts, 1974 ; Tokarz, 1978 ; Jones, 1978). However, considering the puzzling existence of the large pool of small oocytes in *Bufo* and the fact that medium-sized cells may become atretic, it was suggested that « atresia of small previtellogenic oocytes may be more extensive than generally believed » and that the importance of the phenomenon should be reexamined (Jorgensen, 1973b ; Billeter and Jorgensen, 1976). As a matter of fact, these cells (350-650 μm in diameter) were considered as previtellogenic only because they could not be stimulated to rapid vitellogenic growth by gonadotropins (Jorgensen, 1973a). In *Xenopus,* we know that small stage II oocytes (300-450 μm) which have just entered vitellogenesis (1) do not grow rapidly (Callen *et al.,* 1980b), (2) are mainly included in the large resting stock of small oocytes (like most of the cells assumed to be previtellogenic in *Bufo*) and (3) are not recruited immediately after spawning. This *Bufo* oocyte population is probably equivalent to vitellogenic stage II oocytes in *Xenopus,* and we suggest

that true previtellogenic cells do not exhibit atresia in this organism. In the ovarian envelopes of *Xenopus* we have extensively examined, we have never found atretic stage I oocytes ; moreover, unlike *Rana* or *Bufo*, the atresia of vitellogenic oocytes is uncommon in *Xenopus,* even after spawning since most of the largest oocytes that remain in the ovaries look healthy and do not regress. This absence of atresia in small oocytes and the necessary ageing they undergo within the ovaries are indirectly confirmed by an observation we made previously in the course of a study of the « mitochondrial mass », characteristic of stage I in *Xenopus leavis*. As the female ages, the relative size and compactness of this structure decrease considerably ; the small oocytes of adult females never contain actively growing mitochondrial masses indentical to those found in very young animals (Callen *et al.,* 1980a).

In summary, the 18-20 potential clutches in the ovaries of a *Xenopus laevis* female may be retained and used throughout the animal's life (around 20 years ; Arnoult and Lamotte 1968). This should hold true for *Bufo* since (1) its ovaries contain 10-12 potential clutches and (2) its eggs are probably laid every second year, as suggested by ethological and biological results obtained independently by Heusser (1968) and Jorgensen (1975). Thus, the early previtellogenic pool should be sufficient to ensure more than 20 years of active sexual life in *Bufo* females without regeneration. It is generally admitted that there is oogonial proliferation in adult amphibians, giving rise to successive generations of oocytes around breeding time (Franchi *et al.,* 1962 ; Jorgensen, 1973a ; Lofts, 1974 ; Tokarz, 1978). This concept implies that there is an exact balance between oocyte loss due to laying and annual oocyte production resulting from oogonial mitoses. Lastly, it is widely accepted that each oocyte takes 3 years to differentiate, the pattern being the same for all oocytes throughout a female's life-time (Grant, 1953 ; Smith, 1955 ; Wischnitzer, 1966).

Obviously, observations on the previtellogenic pool of *Bufo* or *Xenopus* are not easily reconciled with this rigid ovarian pattern ; moreover, there is no evident need to replenish the ovary after each spawning season. Most cytological or experimental evidence concerning the problem of active oogonia in adults is based on very early studies (*e.g.* Gaupp, 1904, cited in Smith, 1955) ; some of these should now be reinterpreted (those of King, 1908 or Humphrey, 1931, cited in Franchi *et al.,* 1962). More recently, Mizell (1964) and Miller and Robbins (1954) claimed that periodic oogonial proliferation does exist in amphibians but they did not give any cytological evidence of this. The presence of active oogonia in adult *Xenopus* females will be very difficult to demonstrate since only 5 % of the total number of cells of a steady-state ovary (about 300 per lobe) would be generated by oogonia each year ; actually these small new oocytes 15-20 μm in diameter would be difficult to recognize among the rest of the population.

On the contrary, biochemical and cytological results obtained recently on green water frogs *(Rana esculenta, R. lessonae, R. ridibunda)* strongly suggest that after the first hibernation of the froglets, there is no more oogonial multiplication, and there is none in any of the adults analysed so far (Tunner, 1980 ; Tunner and Heppich, 1981). This should be related to the previous observations on *Bufo* (the restricted period of compensatory growth of

the ovary after partial ovariectomy) and ours on *Xenopus* where a reduced previtellogenic pool is present in starved young females. In conclusion, the extent of oogonial proliferation during the first months after metamorphosis seems very important to the further development of the ovaries in both species.

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Résumé. *Mise en place précoce d'un stock important d'ovocytes prévitellogéniques et vitellogenèse cyclique : le mode de fonctionnement de l'ovaire chez le Xénope et ses conséquences physiologiques.*

Pendant la première année de la vie de la femelle chez le *Xenopus laevis,* un stock important d'ovocytes prévitellogéniques (environ 250 000) est mis en place dans les ovaires ; ce stock est constitué de cellules ayant une croissance et une activité métabolique réduites.

L'étude quantitative du développement de l'ovaire adulte a montré que la première vague de vitellogenèse se déroule en deux phases successives d'activité très différentes ; l'ensemble des ovocytes vitellogéniques ainsi mis en place représente l'équivalent de quatre pontes.

Très peu de temps après la ponte, une nouvelle population d'ovocytes à croissance rapide et synchrone est prélevée parmi les cellules de taille intermédiaire et mise en route ; la croissance de ces cellules, progressivement ralentie, sera terminée seulement au moment de la saison de ponte suivante.

Le fonctionnement de l'ovaire chez le Xénope est donc cyclique et discontinu comme chez les anoures tempérés : en dehors d'une courte période située après la ponte, toute son activité physiologique est extrêment réduite.

Les cellules constituant le pool prévitellogénique n'étant pas l'objet d'atrésie sont en principe suffisantes pour assurer l'activité sexuelle de la femelle pendant toute sa vie.

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