4.0), followed by an equal volume of concentrated elutriant (1.9*M*, *p*H 4.8). The results (Table 1) clearly show that No does not elute before Es.

An elution position for No relative to tracer quantities of Y, Sr, Ba, and Ra was obtained for a heated (80°C) column, 0.2 cm in diameter by 2 cm long, with the concentrated elution solution. The composite result for 13 experiments appears in Fig. 1. Under these conditions nobelium does not exhibit the slightest resemblance to the +3 actinides. In similar tracer experiments, Es, Cm, Am, and Ac were eluted in the Y position, which is promptly after the first free column volume; the same was true for Th, Pa, Pb, and Ce.

For study of the coprecipitation behavior of nobelium fluoride we used the residue-adsorption technique (*11*). A drop of 0.1*M* HCl containing the tracers to which No was to be compared, plus about 5 μg of each of the various charge-state carriers Ba, La, and Zr, was used to dissolve the No atoms from the catcher foil. Two drops of 40-percent HF was added for conversion to the fluorides, and the solution was taken to dryness. The plate was then washed with H_2O, and both residue plate (LaF_3 fraction) and H_2O washes (BaF_2 fraction) were analyzed. The results (Table 1) suggest that the solubility of nobelium fluoride is more like that of BaF_2 than of LaF_3.

Assuming that the nobelium was exhibiting a +2 valence under the above conditions, we attempted to oxidize the No^{2+} to No^{3+} which should form a less soluble fluoride. The procedure was the same as that just described except that the oxidant ceric nitrate was substituted for the Zr. After oxidation with Ce^{4+}, the distribution was in favor of the LaF_3 phase (Table 1).

Yttrium-90 can be separated from ^{90}Sr at room temperature by elution from a $SrSO_4$ column with H_2SO_4 (*12*). Generally the more soluble sulfates are eluted before the less soluble ones. We used a similar column for study of the behavior of nobelium sulfate; in each experiment the No, tracer activities to which No was to be compared, and about 5 μg each of Zr and La were converted to the sulfate form on the platinum catcher foil and transferred to the column in the elutriant, 6*M* H_2SO_4. The mass of Zr and La added was the same as the mass of Ce^{4+} and Ce^{3+} used in later oxidation experiments, in order to duplicate the mass effects on the column. The elution from a column 0.2 cm in diameter by 1 cm long was carried out in two fractions; the results (Table 1) show No to be more strongly adsorbed on the column than are Es and Am.

Similar experiments under oxidizing conditions used Ce^{4+} (Table 1); the No was eluted with Es and before Am. This apparent change in solubility of nobelium sulfate is consistent with a change in valence state from +2 to +3.

These tracer experiments show that the divalent ion is the most stable species for nobelium in aqueous solution; thus the element exhibits chemical behavior substantially different from those of the other actinides. This finding appears to confirm Seaborg's prediction in 1949 (*13*) of a possible stable +2 state for element 102, due to the special stability of the $5f^{14}$ electronic configuration.

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Kinetics of Protein Synthesis in Enucleate Frog Oocytes

Abstract. *Kinetics of protein synthesis were measured in oocytes of* Rana pipiens *enucleated at various times during and following pituitary-induced maturation. These enucleated oocytes were capable of the same rate of protein synthesis as nucleated controls, even when rates were measured many hours after enucleation.*

In amphibians, full-grown oocytes result from an extended period of oogenesis, during which synthetic activity is intense and the oocytes undergo extensive growth. Throughout this period, the oocytes remain in prophase of the first meiotic division. At the end of oogenesis, the full-grown oocytes remain essentially dormant in the ovary until they are stimulated to continue the developmental process. In response to hormone stimulation, synthetic activity and meiosis resume, ovulation occurs, and the eggs undergo cytoplasmic maturation; that is, they acquire the ability to be fertilized and continue development.

During oogenesis, oocytes of *Xenopus laevis* synthesize and accumulate a significant quantity of template-active RNA which persists in the full-grown ovarian oocyte (*1*). They also are reported to synthesize appreciable amounts of DNA-like RNA during the period of ovulation and maturation (*2*). Ovulated, but unfertilized, frog eggs reportedly contain RNA capable of stimulating protein synthesis in vitro (*3*). Several lines of evidence (*4*) indicate that amphibian development can continue at least to the blastula stage in the absence of a functional nucleus. And, activated, enucleated eggs of *Rana pipiens* are as capable of protein synthesis as fertilized controls (*5*). These reports suggest that the amphibian egg, prior to fertilization, synthesizes and accumulates long-lived gene products used later in development.

Protein synthesis is initiated in *R. pipiens* oocytes prior to fertilization or artificial activation. Initiation results from hormone stimulation in the adult female (*6*). We now describe the effect on protein synthesis of enucleation at various times during the hormone-induced maturation period. Our purpose was to identify possible intervals during this period in which nuclear transcription may be obligatory for subsequent protein synthesis.

Adult *Rana pipiens* were collected in the spring as they came out of hibernation, refrigerated immediately upon arrival, and maintained at 4°C until used. Ovulation and associated maturation were induced in mature females by the intraperitoneal injection of an anterior pituitary suspension. Injected animals were maintained at 18° ± 0.5°C. To obtain eggs at times before ovulation, we pithed hormone-stimulated females, excised portions of ovarian lobes, and removed individual oocytes from their ovarian follicles with watchmaker's forceps. After ovulation, jelly-covered, uterine eggs were stripped from frogs. Generally, uterine eggs cannot be obtained in substantial numbers until after the first meiotic division. However, we found that uterine eggs from some animals could be obtained still in first meiotic metaphase. These eggs were immature and could not be fertilized, but they were useful for enucleation studies.

In *R. pipiens*, enucleation can be effected before germinal vesicle breakdown (*7*) or at metaphase of the first or second maturation divisions (*8*, *9*). The latter enucleation process (*9*) was shown to be always successful by enucleation of fertilized controls, which developed as androgenetic haploids. Removal of the germinal vesicle was verified visually.

Relative rates of protein synthesis were determined from the incorporation kinetics of tritiated L-leucine into oocyte proteins. The label was introduced by microinjection, and the materials and procedures were the same as those described previously (*10*). We demonstrated that incorporation in this system follows the general equation

$$P^* = \alpha(1 - e^{-\beta t}) \qquad (1)$$

where P^* is activity incorporated into oocyte proteins, t is the time after injection, and α and β are constants. We also demonstrated that the incorporation rate constant, β, is directly related to the rate of protein synthesis (*10*). In each experiment reported here we compared protein synthetic rates in enucleated eggs and nucleated controls by comparing their respective values of β. The actual values of β were obtained by a computer fit of each set of incorporation data to Eq. 1.

All manipulations were carried out at a temperature between 18° and 20°C in a modified Steinberg's (*11*) solution. Protein synthesis measured in Steinberg's solution is very low in ovarian oocytes from an unstimulated frog and in eggs from females in the first few hours after pituitary stimulation (*6*). Furthermore, protein synthesis at these early times is stimulated by increases in the KCl content of the operation medium (*12*). Since we wished to measure only the capacity of enucleated oocytes for protein synthesis, rather than what actually occurs *in situ*, we supplemented the Steinberg's solution with KCl to a final concentration of 5×10^{-3} mole/liter.

Figure 1 contrasts the kinetics of incorporation observed in ovarian oocytes injected with tritiated leucine immediately after removal from an unstimulated female and the kinetics obtained with eggs taken from a frog 2 days after pituitary stimulation and then activated. The curve shown for the activated eggs indicates the best fit by computer of Eq. 1 to the experimental data. The value of β from this curve is 0.045 min^{-1}. Incorporation in the ovarian oocytes was so slow that the rate constant could not be evaluated.

Table 1 shows the values of β computed from a number of experiments with eggs taken at times both before and during the pituitary-induced maturation period. Usually, the variation in uptake kinetics observed between animals is sufficiently large that no relevant comparison of synthetic rates can be made between different experiments. The significant comparison is made, within each experiment, between the enucleated eggs and their nucleated controls.

Incorporation in oocytes that were not stimulated with hormones varied with the time they were left in vitro before injection. Neither the enucleated oocytes nor their nucleated controls showed a determinable rate of incorporation when they were injected immediately (experiment 1). However, those left for 6 hours in the incubation medium before injection (experiment 2) were capable of significant incorporation. The enucleated eggs were capable of incorporation comparable to that of controls, even though they had been without a nucleus for the entire period. Similarly, removal of the germinal vesicle from stimulated oocytes had no effect on their capacity for protein synthesis, either when they were tested immediately or 6 hours after enucleation (experiments 3 to 6).

Enucleation of oocytes at first meiotic metaphase did not alter their rate of protein synthesis, whether they were injected immediately or 20 hours later (experiments 7 and 8). In the latter

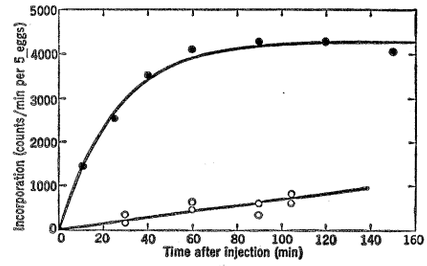

Fig. 1. Kinetics of incorporation of tritiated L-leucine into *Rana pipiens* oocytes. Each egg was injected with 22 mμl at an activity of 1 mc/ml, 5 c/mmole. (Open circles) ovarian oocytes from an unstimulated frog; (closed circles) eggs stripped from a frog 49 hours at 18°C after pituitary stimulation; activated by injection.

case, both the enucleated and the control oocytes continued to undergo cytoplasmic maturation during the 20-hour period, and both were capable of being activated at the time of injection. Enucleation at second meiotic metaphase (experiments 9 and 10) requires prior activation of the eggs. Thus, the regular controls in these experiments were artificially activated at the same time. In experiment 9 two controls were used.

Table 1. Value of the rate constant, β, and its standard error in enucleated and nucleated eggs at various times after pituitary stimulation. Each experiment represents a different frog. All eggs taken 24 hours or later were uterine eggs. In all cases except (†) control eggs were removed from the frog at the same time as those taken for enucleation. Experiments 1 to 6, enucleated by removal of germinal vesicle; experiments 7 and 8, enucleated at first meiotic metaphase; experiments 9 and 10, enucleated at second meiotic metaphase.

Experiment No.	Hours after stimulation at which		β (min^{-1})
	Eggs enucleated	Eggs injected	
1 *	0	0	< 0.002
	Control	0	< 0.002
2 *	0	6	0.021 ± 0.003
	Control	6	$.020 \pm .005$
3	6	6	$.049 \pm .015$
	Control	6	$.051 \pm .020$
4	6	12	$.023 \pm .009$
	Control	12	$.034 \pm .010$
5	10	10	$.021 \pm .005$
	Control	10	$.032 \pm .007$
6	10	16	$.012 \pm .002$
	Control	16	$.010 \pm .012$
7	24	24	$.123 \pm .019$
	Control	24	$.097 \pm .012$
8	24	44	$.027 \pm .007$
	Control	44	$.030 \pm .005$
9	36	49	$.037 \pm .006$
	Control	49	$.025 \pm .005$
	Control †	50	$.020 \pm .002$
10	49	55	$.045 \pm .003$
	Control	55	$.040 \pm .003$

* No pituitary stimulation. † These eggs were stripped from the frog just before injection.

One group was stripped from a frog at the same time as the enucleated eggs; the others were stripped from the same frog 13 hours later, and activated at that time. The regular control group, activated and left 13 hours, would have been early blastulas if they had been fertilized at the time of activation. In no case was protein synthesis in the enucleated eggs significantly different from that in the controls.

On the basis of these observations, we conclude that, prior to hormone stimulation, the cytoplasm of the *R. pipiens* oocyte has the capacity for independent protein synthesis. It appears that the oocyte cytoplasm retains this capacity throughout the period of pituitary-induced maturation and well beyond the time when the egg is capable of being fertilized or artificially activated. We have been unable to demonstrate any contribution of the nucleus to this protein synthetic capability; in all cases, the enucleate cytoplasm is as capable of supporting protein synthesis as the intact egg.

From this study we cannot say that the nucleus has no role in the control of synthetic events during this period. We do not know whether the enucleate and the nucleate oocytes had identical patterns of protein synthesis. Whatever function the nucleus may have, however, its presence is not essential to the continuation of protein synthesis.

There are two possible explanations for our observations: (i) Long-lived templates may be synthesized during oogenesis and function throughout the period of maturation and early cleavage. (ii) Templates of cytoplasmic origin may be synthesized *de novo* during maturation. Of course both of these mechanisms could function concomitantly. However, Davidson *et al.* (*1*) calculated that, in *Xenopus*, the template RNA synthesized during hormone-induced maturation (*2*) is only a small fraction of the amount already present in the full-grown ovarian oocyte. If this is generally true for amphibians, it is likely that the capacity for protein synthesis we observed in enucleate oocytes is due to the presence, in the cytoplasm, of long-lived templates synthesized and accumulated during oogenesis.

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Ouabain and Streptomycin: Their Different Loci of Action on Saccular Hair Cells in Goldfish

Abstract. *Ouabain, when applied to the periotic spaces, that is, to the basal end of the saccular hair cells, suppressed microphonic potentials of the inner ear in goldfish. In contrast, streptomycin produced such an effect only when it was applied directly into the endolymph, that is, to the hair-bearing ends of the hair cells.*

In glandular and epithelial cells in which the luminal and basal surfaces are usually functionally and morphologically quite different, drugs could affect the cells differently depending on whether they were applied from the inside (luminal side) or outside (*1*). The results indicate that ouabain, an inhibitor of (Na + K)-activated adenosine triphosphatase, stops active transport of Na^+ in the frog skin, gall bladder, goose salt-gland, and other organs when it is applied to the side of cells toward which Na^+ is actively transported (*2*). We have studied the effect of locally applied ouabain on the activity of hair cells in the goldfish sacculus, that is, the inner ear. We took microphonic potentials as an indication of hair-cell activity and placed special emphasis on the different effects of the drug when administered on the luminal and on the extraluminal surfaces. We also studied the effect of streptomycin and kanamycin, two well-known ototoxins, for, when applied systemically, they cause severe degeneration of hair cells (*3*). Local application of streptomycin in the lateral line organ causes an immediate abolition of microphonic potentials (*4*).

A goldfish (about 12 cm long) was anesthetized with ethyl *m*-aminobenzoate (MS 222 Sandoz Ltd., Basel) and placed in a fish chamber (*5*). The dorsal part of the skull was removed, and the exposed brain was largely sucked away to disclose underlying structures. Before recording, we immobilized the fish with an intramuscular injection of gallamine triethiodide (about 1 μg per gram of body weight). The gills were continuously supplied with running water through a mouthpiece. Microphonic potentials were recorded through a low-resistance micropipette electrode (tip, 1 μm) filled with 3*M* KCl. When such an electrode was inserted across the macula into the saccular lumen, we recorded negative microphonic potentials whose frequency was twice that of the sound (*6*). For sound stimulation, tone pips were applied through a loudspeaker placed about 30 cm from the fish. An arrangement was made so that the sound wave appeared, on repeated sweeps, at the same place, that is, with the same phase relations, on the oscilloscopic screen. This permitted the use of a digital computer (ATAC-401, Nihonkoden Kogyo Co.) for averaging microphonic potentials.

Drugs or ions were applied locally to the sacculus. For an extraluminal application, 0.03 to 0.1 ml of test solution was dropped onto the base of the skull so as to submerge the saccular macula under the solution. To keep the concentration constant, we sucked the fluid away from the base of the skull every 5 minutes and replaced it with fresh solution. An intraluminal application was made through a micropipette (tip, about 20 μm) inserted into the saccular capsule. The micropipette was connected to a microsyringe through polyethylene tubing, and the solution was driven out by pressure. The infusion rate was most often 0.1 to 0.3 μl/min. A fairly rapid exchange of the saccular endolymph may be expected