Influence of Certain B-Vitamin Antimetabolites on Ovulation *in vitro* in the Frog, *Rana pipiens.** (26057)

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Induction of ovulation in a typical vertebrate classically requires cooperation of hypophyseal FSH and LH to rupture follicular walls and effect release of ova(1). In frogs, where *in vitro* procedures can be used conveniently, evidence indicates that glycolysis, tricarboxylic acid cycle, and cytochrome-cytochrome oxidase systems are essential for ovulation and that reactions leading to extrusion of ova are endergonic(2). Various steroids appear to play a supporting role in ovulation (3,4) and may even substitute for pituitary factors entirely(5). The foregoing information as well as experiments using unfractionated frog pituitary extracts and mammalian FSH and LH(6,7) suggest that *sensitization* of ovarian tissue may be a distinct process from *ovulation itself*. Data of this communication, obtained using certain antimetabolites in connection with ovulating fragments of excised frog ovary, also support a concept of sensitization as separate from ovulation.

Methods. Ovaries and pituitary glands were dissected from Vermont frogs shipped to the laboratory at frequent intervals. Preparation of pituitary extracts consisted of grinding several fresh glands at a time in a small mortar, lysing any remaining cells with a small quantity of distilled water, and diluting to desired concentration with standard Ringer's solution. Ovaries were cut into lobes and placed at random in Petri dishes with 50 ml Ringer's fluid containing pituitary extract and any vitamin or antimetabolite to be tested. Numbers of eggs ovulated were recorded after 22-24 hours at 22-24°C. Possibility of spontaneous ovulation (*i.e.*, without added pituitary stimulation *in vitro*) was checked by placing ovarian lobes in plain Ringer's fluid (Ringer controls), but in no case were any ova extruded without pituitary influence. Standard responsiveness of each ovary was determined by exposing one lobe to a concentration of from 0.1 to 0.5 pituitary gland (pituitary controls), exact dosage depending on the season. Responses to identical dosages of pituitary may vary considerably from animal to animal, undoubtedly due to unknown and immeasurable differences in physiological state. Unless pituitary controls extruded at least 15 eggs upon optimal stimulation, sensitivity of ovarian tissue was not considered sufficient to provide reliable data.

Results. Preliminary tests showed (Table I) that addition of B vitamins to ovulating media neither enhanced nor impaired responses to pituitary extract. In experiments run during December (Table IIA), antimetabolites of nicotinic acid (αpa, p3sa, and acetyl pyridine) markedly inhibited ovulatory responses to pituitary extracts. About 25% of this inhibition could be removed by simultaneous inclusion of 5×10^{-4} M nicotinic acid. In contrast, antimetabolites of thiamine (oxythiamine), biotin (desthiobiotin), folic acid (aminopterin), pyridoxine (desoxypyridoxine) and pantothenic acid (pantoyl taurine) showed no inhibition of ovulation. From these data it appears that metabolism of nicotinic acid is in some way intricately involved in ovulation, but that other B vitamins are not directly implicated.

During April, *pipiens* ovary is at its peak of sensitivity, and a retest of nicotinic acid

TABLE I. Effect of B Vitamins on Ovulation *In Vitro*.

Vitamin (5×10^{-4} M)	No. of exp.	No. of eggs released in Pituitary controls	Pituitary + vitamin
Nicotinic acid	4	173	163
Thiamine	5	265	278
Biotin	2	76	80
Folic acid	7	271	306
Pyridoxine	3	142	130
Calcium pantothenate	3	51	43
Inositol	4	187	199
Riboflavine	6	303	290

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TABLE II. Effect of B-Vitamin Antimetabolites on Ovulation *In Vitro*.

Antimetabolite	No. of exp.	No. of eggs released in: Pituitary controls	Pituitary + .001 M antimet.	Pituitary + .0005 M antimet.	Pituitary + .0001 M antimet.
A. December Exp.					
α-Picolinic acid (αpa)	5	329	1	83	117
Pyridine-3-sulfonic acid (p3sa)	6	439	0	1	34
Acetyl pyridine	4	167	2	30	73
Oxythiamine	2	57	74	65	56
Desthiobiotin	2	196	194	166	208
Aminopterin	2	106	117	105	113
Desoxypyridoxine	2	91	80	99	94
Pantoyl taurine	4	130	124	125	136
B. April Exp.					
α-Picolinic acid	6	253	244	—	291
Pyridine-3-sulfonic acid	4	361	213	—	303
Acetyl pyridine	3	140	142	—	187

analogues at this time revealed a lack of inhibition for αpa and acetyl pyridine, and a much reduced inhibitory activity for p3sa (Table IIB). A retest of antimetabolites of other B vitamins at this time confirmed our December results.

Discussion. It is not surprising to find that ovulation was impaired during winter months by analogues of nicotinic acid, for it is now well known that DPN plays an important and almost ubiquitous function in release of cell energy. In early winter most frog ovarian tissue is only moderately sensitive to pituitary factors, and intermediate to large dosages are still required for maximal ovulation. In April, nicotinic acid analogues had lost most or all of their ovulation-inhibiting capacity. This is the breeding season for this species in the wild, and only minimal dosages of pituitary extract are required for optimal ovulatory response of laboratory animals at this time, either *in vivo* or *in vitro*. Between December and April, then, a sensitization of ovarian follicles has occurred so that ovulation may proceed not only more rapidly but also in response to smaller dosages of ovulatory factors. From data provided above, it appears that nicotinic acid or DPN is required during winter months while sensitization of follicles proceeds, prior to actual ovulation and during the time that FSH is exerting its principal effect. Metabolism of nicotinic acid or DPN appears to be unnecessary in ovulatory reactions induced in April after sensitization has presumably already occurred. Time studies(8) bear out this same scheme, since beginning of ovulation *in vitro* in early winter may require 14-18 hours but only 6-8 hours in April. Difference in time may well be explained by a necessity for 8-10 hours of sensitization time in winter, and absence of this requirement in spring.

Summary. Ovulation *in vitro*, as induced by pituitary extracts, was inhibited by antimetabolites of nicotinic acid in December experiments but not in April. Results suggest that metabolism of nicotinic acid may participate in sensitization of frog ovary which occurs gradually during the hibernation months from December to April, but not directly in ovulation itself. Other B vitamins were not implicated.

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