

Location of Hormone Production in the Ovary of the Guppy, *Poecilia reticulata*

In many fish species the ovary is a hollow, thin-walled, sack-shaped organ in which numerous follicles are embedded in a connective tissue stroma. Each follicle consists of an oocyte surrounded by a single layer of cubical granulosa cells and a flat outer theca in which internal and external layers of cells can be distinguished.

Growth does not always result in maturation of the follicles, but often ends with degeneration of the oocyte. Such degeneration is followed by a proliferation of the granulosa cells and the ultimate resorption of the oocyte. These atretic structures have been named preovulatory corpora lutea¹ due to their morphological resemblance to mammalian corpora lutea. It has been suggested that these are the source of female sex hormone(s) in fishes, but postovulatory corpora lutea are also thought to produce sex hormone(s) in some species (HOAR²). The sex hormone in teleosts are reported to be steroid in nature³⁻⁴.

In viviparous *Poecilia reticulata*, fertilization and gestation take place inside the follicle wall, and thus postovulatory corpora lutea are not formed. Preovulatory corpora lutea, however, are found with great regularity. Since these do not seem to synthesize steroids, LAMBERT and VAN OORDT⁵ prefer to call them corpora atretica. A histological and histochemical study seems to indicate that the corpora atretica represent degenerating steroidogenic tissue⁶⁻⁸. In the guppy the corpora atretica contain coarse droplets of Schultz positive sudanophil lipids, and the enzymes non-specific esterase and acid phosphatase⁵. It is possible, therefore, that the follicle wall forming the corpora atretica actively produces steroids prior to follicular atresia.

An indication of an active general metabolism has indeed been observed in the granulosa cells by the demonstration of three enzymes of the citric acid cycle, i.e. isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase, using the methods given by PEARSE⁹. Using the method of RUDOLPH and KLEIN¹⁰, the presence of glucose-6-phosphate-dehydrogenase, one of the enzymes of the so-called hexose-monophosphate shunt, has also been demonstrated. This is the most important source of NADPH, required for steroid synthesis.

Furthermore, granulosa cells also appeared to contain small droplets of Schultz negative sudanophil lipids and the enzyme 3β -hydroxy-steroid-dehydrogenase (demonstrated by the method of LEVY et al.¹¹).

Recent investigations by BARA^{12,13} pointed towards a glucose-6-phosphate-dehydrogenase activity in the follicular layer and some enlarged theca cells in the ovary of the mackerel, *Scomber scomber*. The enzyme 3β -hydroxy-steroid-dehydrogenase was not observed in the granulosa cells, but was restricted to the theca cells. Our observations, however, seem to indicate that in the guppy the granulosa layer of normal follicles can produce steroids, and is a possible source of female sex hormone(s) in this species¹⁴.

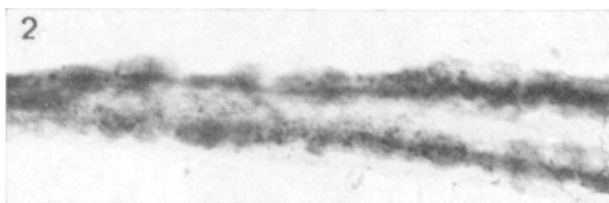


Fig. 2. Detail of the wall of two mature follicles; 3β -hydroxy-steroid-dehydrogenase is present in the granulosa layer. $\times 600$; $25\ \mu$.

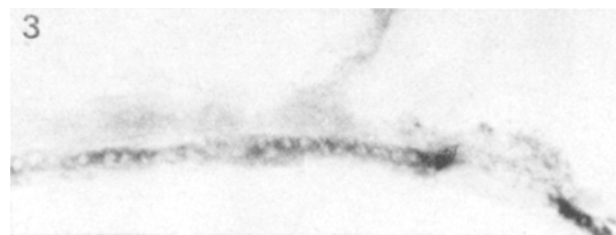


Fig. 3. Detail of the wall of a mature follicle showing a malate-dehydrogenase activity in the granulosa cells. $\times 450$; $10\ \mu$.

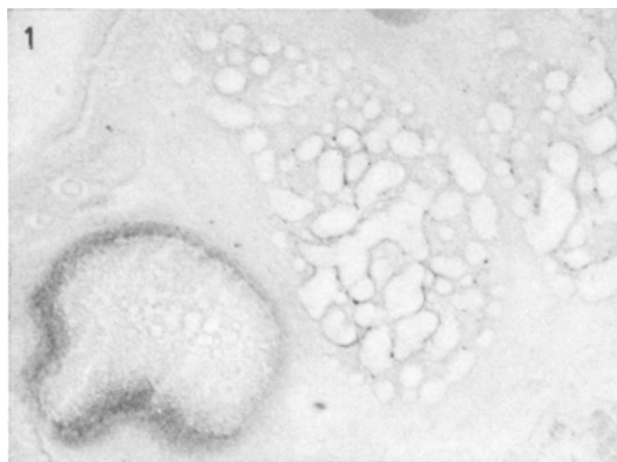


Fig. 1. Part of the ovary of a guppy showing a corpus atreticum on the right and a young follicle on the left. The dark band in the latter results from a positive 3β -hydroxy-steroid-dehydrogenase reaction in the granulosa layer. $\times 100$; $25\ \mu$.

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¹² G. BARA, *Gen. Comp. Endocr.* 5, 284 (1965).

¹³ G. BARA, *Experientia* 21, 638 (1965).

¹⁴ Thanks are due to Dr. P. G. W. J. VAN OORDT for his advice and to Miss N. E. GIEL for her technical assistance.

Zusammenfassung. Enzym-histochemische Untersuchungen weisen darauf hin, dass die sogenannten Corpora lutea praeovulationia im Ovarium von *Poecilia reticulata* keine Steroide produzieren und deswegen als Corpora atretica betrachtet werden müssen. Demgegenüber steht fest, dass in den Granulosazellen, die die Oozyten umgeben, die Enzyme 3 β -Hydroxysteroid-Dehydrogenase, Glucose-6-phosphat-Dehydrogenase und einige Enzyme

des Krebszyklus nachzuweisen sind. Infolgedessen können die Granulosazellen als Steroid-produzierende Zellen angesehen werden.

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Evaluation of the Maternal Role in Survival of Suckling Mice¹

In mice, GRUNEBERG has stated that, apart from malformations, suckling mortality is determined by maternal vigor²; however, it is not clear whether this influence is exerted pre- and/or postnatally. The present report describes experiments in which the maternal influence was tested by foster-nursing mice from strains characterized by high suckling survival to mothers from strains with a lower nursling survival, and vice versa.

Nurses were mice of the AKR/Sp, PL/Sp and C57BL/Sp strains. Non-foster-nursed and foster-nursed mice were males and females of these strains. The procedures followed have been described previously³. Data were analyzed using Chi square with Yates correction. Results for male and female sucklings were pooled inasmuch as no significant sex differences were found.

When mice were raised by their own mothers, survival of C57BL sucklings was higher than that of PL ($P < 0.05$) and AKR ($P < 0.001$) nurslings. Survival of PL infants was higher ($P < 0.001$) than that of AKR infants (Table).

When C57BL mice were foster-nursed, survival was decreased and was independent of the strain of origin of the foster mother (Table). When AKR mice were suckled by PL and AKR foster mothers, survival was also decreased (Table). However, when AKR mice were raised by C57BL dams and when PL infants were foster-nursed, survival was unchanged (Table). Only when AKR infants were foster-nursed did survival appear to depend on the foster mother's strain of origin and follow the same order of survival as found when these mothers raised their own progeny. While it is true that survival of AKR mice raised by AKR and PL foster mothers was lower ($P < 0.001$) than that of sucklings raised by C57BL nurses, no significant differences in survival were found when survival of mice foster-nursed by PL and AKR dams was compared, although survival of PL sucklings was higher than that of AKR mice when both were raised by their own mothers (Table). These data suggest that all the mothers transmitted potentially noxious substances in their milk and that sensitivity to these materials by sucklings was dependent on their strain of origin.

If differences in survival of foster-nursed sucklings were due only to their genotype, one would expect that the survival of mice raised by foster mothers from the same strain would be similar to that of mice raised by their own mothers. While this was the case with foster-nursed PL mice, it was not so with C57BL and AKR mice whose survival was decreased. These observations suggest the possibility that the nurslings must have become somewhat conditioned, during their in utero existence, to materials that were similar to ones that appeared later in

the milk of their own mothers. Not only did these materials appear to differ from mother to mother within a given strain, but conditioning of the fetuses to them may have varied inasmuch as survival of mice raised by their own mothers was not 100%. The data suggest that maternal vigor exerted postnatally was not the only factor concerned with nursling survival, but that the strain of origin of the sucklings and non-genetic maternal influences exerted in utero must also be considered.

Although the present experiments clarify some of the factors concerned with nursling survival, they do not provide any information about the mechanism through which they operate. Recently, some observations have been made in our laboratory that contribute to the understanding of this mechanism. (1) Significant positive associations have been found between survival and thymus mitosis and between survival and thymus

Survival of foster-nursed suckling mice

Strain of mouse suckled	Foster mother	No. of litters	No. of mice at onset of nursing	Mice weaned		P Foster-nursed vs. non-foster-nursed
				No.	%	
PL	None	34	138	107	78	
	AKR	10	47	41	87	NS ^a
	PL	20	94	79	84	NS
	C57BL	23	91	76	84	NS
C57BL	None	19	104	92	88	
	AKR	38	216	96	44	< 0.001
	PL	24	112	57	51	< 0.001
	C57BL	18	93	52	56	< 0.001
AKR	None	26	152	91	60	
	AKR	6	30	5	17	< 0.001
	PL	9	54	14	26	< 0.001
	C57BL	42	213	112	53	NS

^a NS signifies not significant.

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