J. Reprod. Fert. (1968) **17,** 395–398

FOLLICULAR CELL SUPPORT OF OOCYTE MATURATION: PRODUCTION OF PYRUVATE *IN VITRO*

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(*Received* 28*th May* 1968)

Recently, Biggers, Whittingham & Donahue (1967) demonstrated that cells liberated from mouse ovarian follicles can support oocyte maturation *in vitro*. This maturation involves progression from late prophase (germinal vesicle stage) of the first meiotic division to metaphase of the second meiotic division (metaphase II). Oocyte maturation occurred *in vitro* when cultured either with follicular cells in a glucose medium or without the cells but in the presence of pyruvate or oxaloacetate. These observations suggest that follicular cells cultured in a glucose medium may be forming pyruvate and/or oxaloacetate. In this paper we have measured the amount of pyruvate produced by these cells.

Oocytes and follicular cells from 8- to 15-week-old CF-1 mice (Carworth Inc.) were liberated from their follicles by needle puncture, washed and cultured in 75-μl drops under oil as previously described, using a Krebs–Ringer bicarbonate salt solution with or without glucose for all steps (Biggers *et al.*, 1967). The appropriate drops contained fifteen to twenty oocytes free of surrounding cells, and approximately equal numbers of follicular cells, determined by visual inspection, sufficient to cover the bottom of the microdrop. Three to four mice were used in each experiment. After 17 to 20 hr at 37° C, eggs were scored by microscopy for the occurrence of maturation and 10- or 15-μl aliquots of the culture fluid were used for a fluorometric assay of pyruvate (Lowry, Passonneau, Hasselberger & Shulz, 1964). Measurements were made in 10 × 72-mm Pyrex test tubes containing 0·002 mM-DPNH (Calbiochem) and 1 μg of heart LDH (Calbiochem) in 1 ml of 100 mM-phosphate buffer, pH 7·0. A Farrand model A-3 fluorometer was used. Pyruvate molarity is based on the sodium salt. Hormonal stimulation was accomplished by superovulatory injections (15 units each) of pregnant mare's serum gonadotrophin (PMSG) (Equinex, Ayerst Laboratories) and human chorionic gonadotrophin (HCG) (APL, Ayerst Laboratories); 46 hr after giving PMSG, mice with a pro-oestrous vaginal smear were given HCG. Follicular cells and eggs were obtained from mice killed 30 min after the HCG injection.

Results (Table 1) demonstrate that: (a) maturation (54 to 95% of the oocytes; mean 80%) occurs only in the combined presence of glucose and follicular cells; (b) pyruvate is formed only in the presence of follicular cells; (c) these

cells require glucose in order to support the maturation of oocytes included in the same microdrop; (d) the pyruvate concentration in the twelve drops with glucose and follicular cells ranges between 0·077 and 0·170 mM, with a mean of 0·132 mM; (e) stimulation *in vivo* with PMSG and HCG does not affect the amount of pyruvate produced *in vitro*. Means of 0·156 and 0·152 mM are found, with and without stimulation, respectively; and (f) the cultured cells represent a population with a homogenous morphology and appear similar to follicular cells of sectioned material.

TABLE 1

PYRUVATE FORMED BY FOLLICULAR CELLS AND OOCYTES AFTER 17 TO 20 HR IN MICRODROPS OF A KREBS–RINGER BICARBONATE SALT SOLUTION

Medium	*Follicular cells*	*Oocytes*	*Pyruvate concentration (mM)*			*% of oocytes maturing to metaphase II*	
			(1)	(2)	*Mean*	(1)	(2)
Experiment 1							
Glucose	−	−	0·002	0·000	0·001		
Glucose	−	+	0·005	0·000	0·003	0	0
Glucose	+	−	0·077	0·098	0·088		
Glucose	+	+	0·078	0·098	0·088	95	78
No glucose	−	−	0·000	0·000	0·000		
No glucose	−	+	0·001	0·000	0·001	0	0
No glucose	+	−	0·009	0·019	0·014		
No glucose	+	+	0·010	0·015	0·013	0	0
Total eggs examined						62	60
Experiment 2							
Glucose	−	−	0·003	0·010	0·006		
With hormones	−	+	0·007	0·000	0·004	0	0
With hormones	+	−	0·146	0·166	0·156		
With hormones	+	+	0·147	0·164	0·156	78	83
No hormones	−	+	0·000	0·000	0·000	0	0
No hormones	+	−	0·136	0·167	0·152		
No hormones	+	+	0·133	0·170	0·152	54	89
Total eggs examined						56	60

Fifteen to twenty oocytes were placed in each drop and scored for maturation at termination of culture. Each experiment was performed in replicate (1) and (2) on separate days.

The follicular cells may, of course, be producing several substances capable of supporting maturation. To test whether the pyruvate concentration in those drops supporting maturation is by itself sufficient to support this maturation, a pyruvate dilution experiment was carried out starting with 0·25 mM, an amount previously shown to support maturation (Biggers *et al.*, 1967). When this is done, only 14% of the oocytes mature when the initial pyruvate concentration is lowered to 0·10 mM. However, there is a loss of pyruvate to the surrounding oil environment. After 17 to 20 hr of culture, 23, 42, 68 and 71% of the pyruvate is lost from the drops when the initial concentrations are 0·25, 0·125, 0·062 and 0·031 mM, respectively. Since the kinetics of pyruvate formation are unknown, we cannot state the amount of pyruvate present at any time except at the termination of culture. At termination the amount recovered from drops with follicular cells corresponds to the amount recovered when concentrations

between 0·125 and 0·250 mM are added at the beginning of culture. These latter quantities support a high level of maturation, with 67 to 88% of the oocytes reaching metaphase II. These results suggest that there is sufficient pyruvate formed by the follicular cells to support the level of maturation (mean of 80% of the oocytes) attained in those drops with follicular cells and glucose.

The greater than two-fold range in pyruvate concentration may be due to variation in the cell number. The visual inspection of the cells permits control within an experiment, but not between experiments. This is borne out by the observation that the wide range occurs between experiments (conducted at different times) and not within experiments where the range is small. Within the 0·077- to 0·170-mM range, there is no correlation between the number of oocytes maturing and the amount of pyruvate present.

Under natural conditions, the oocyte remains arrested at the germinal vesicle stage until a gonadotrophic stimulus triggers maturation and subsequent ovulation. This stimulus is of short duration, lasting 30 min in the rat (Everett, 1956). An increase in the synthesis of RNA and protein in rabbit follicles has been reported (Civen, Brown & Hilliard, 1966) and oxygen consumption and succinic oxidase activity are increased *in vitro* in rat follicular cells (Ahren, Hamberger & Hamberger, 1965). Nevertheless, the nature of this stimulus on the follicle remains largely unknown. The present experiments suggest that it does not alter the amount of pyruvate produced by the follicular cells. However, the mere act of liberating these cells from the follicle may alter them so that they can utilize the glucose in the Krebs–Ringer medium, resulting in pyruvate formation. In a similar way, the gonadotrophic stimulus may alter these cells in the follicle, enabling them to utilize energy sources in the follicular fluid to form pyruvate. Possible energy sources include lactate and glucose that have been found in bovine follicular fluid (Lutwak-Mann, 1954). This idea of the follicular cells maintaining the oocyte in a nutritional state wherein maturation is impossible has been expressed before (Pincus & Enzmann, 1935). Alternatively, the gonadotrophins may modify the oocyte by allowing it to use pyruvate already being produced by the follicular cells. In either case, the oocyte would mature in the presence of pyruvate produced by these supporting cells.

We are indebted to Dr J. D. Biggers and Dr V. A. McKusick for their interest and support of this work. Supported by a grant from the Population Council and grants GM 10189-06, GM 00795-10, and 5 T01 HD 109-02 from the National Institutes of Health.

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