RNA SYNTHESIS IN THE MOUSE OOCYTE

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ABSTRACT

RNA synthesis in the oocyte and granulosa cell nuclei of growing follicles has been studied in the mouse ovary. The RNA precursor [^{3}H]uridine was administered intraperitoneally to adult mice and the amount of label incorporated into ovarian RNA was quantitated autoradiographically using grain-counting procedures. Uridine incorporation into the nucleus is low in oocytes of small, resting follicles but increases during follicle growth and reaches a peak prior to the beginning of antrum formation. Thereafter uptake rapidly declines and is very low in the oocytes of maturing follicles. Uridine incorporation into granulosa cell nuclei, in contrast to that found in the oocyte, increases gradually during most of the period of follicle growth.

Qualitative studies of the activity of endogenous, DNA-dependent RNA polymerases have also been made in fixed oocytes isolated from follicles at different stages of growth. Polymerase activity is demonstrable in the nucleolus and nucleoplasm of oocytes from growing follicles, but is absent from maturing oocytes of large follicles.

INTRODUCTION

Oocyte growth in the rodent ovary is characterized by active incorporation of RNA and protein precursors (Roversi and Silvestrini, 1963; Oakberg, 1967, 1968; Baker et al., 1969), by the appearance of lampbrush chromosomes in the nucleus (Baker and Franchi, 1967; Miller and Bakken, 1972), and by the accumulation of basophilic material in the cytoplasm (Vincent and Dornfeld, 1948; Mintz, 1965). As yet, however, no attempt has been made to investigate the specific patterns of genetic activity during the various growth phases of the oocyte and its follicle envelope. The present work quantitates the relative changes in [^{3}H]uridine incorporation into the oocyte and granulosa cells during follicle growth in the mouse ovary and indicates the stages at which the production of some of the different RNA species becomes important. RNA synthesis has also been measured autoradiographically by determining the activity of endogenous DNA-dependent RNA polymerases in the oocyte and granulosa cells after isolation and fixation.

MATERIALS AND METHODS

[^{3}H]*Uridine Administration*

12 wk-old female mice of the Bagg albino strain were used in this study. Each animal received a single intraperitoneal injection of 100 μCi of [5,6-^{3}H]uridine (43.3 Ci/mM, Radiochemical Centre, Amersham, England), in saline. 1 h later the mice were killed and the ovaries removed and transferred to Bouin solution for 30 min. The fixed specimens were embedded in paraffin using conventional methods. One ovary from each mouse was serially

sectioned at 5 μm and all sections were mounted on gelatinized slides. The incorporation of [^{3}H]uridine into RNA was tested by treating a number of slides with RNase (1 mg/ml distilled water) for 4 h at 37°C.

Preparation of Isolated Oocytes

Mature oocytes were collected from the fallopian tubes of superovulated mice (Gates, 1971). Oocytes from growing and mature follicles were obtained from the ovaries of juvenile or adult females. Ovaries were dissected out in saline and examined under a dissecting microscope. Follicles were punctured and the liberated oocyte was transferred briefly to fresh saline containing 0.1% Pronase (E. Merck AG, Darmstadt, W. Germany), to loosen the adhering granulosa cells. Finally the oocyte was placed on a glass slide in a small drop of saline and immobilized by flooding with absolute ethanol/acetone (1:1, v/v). The slides were then drained and air dried. Using this procedure, the oocyte and a portion of the follicle cell envelope became firmly attached to the slide surface. The retention of some granulosa cells with the oocyte permitted the detection of some of the signs of follicle degeneration. The absence of pyknotic nuclei in the adhering granulosa cells, for example, suggested that the follicle was not atretic. The slides were reimmersed in ethanol/acetone fixative for 5 min, air dried, and stored in a desiccator at 4°C until required (Moore and Ringertz, 1973).

Assay for Endogenous RNA Polymerase Activity

60 oocytes isolated by the above procedure from the ovaries of 10 mice were assayed for RNA polymerase activity using the method described previously (Moore, 1971, 1972; Moore and Ringertz, 1973). The reaction solution contained, in 0.5 ml, 50 μmol Tris-HCl buffer (pH 7.9), 6 μmol 2-mercaptoethanol, 75 μmol sucrose, 300 nmol each of ATP, CTP, and GTP, 2 nmol [^{3}H]UTP (47 Ci/mM, Radiochemical Centre) 4 μmol $MgCl_2$, 1 μmol $MnCl_2$, and either 0.04 M (low salt assay) or 0.4 M ammonium sulphate (high salt assay).

The reaction was terminated by rinsing slides in distilled water and transferring them to methanol/acetic acid fixative (3:1 v/v) for 30 min. Specimens were treated with 5% TCA for 5 min to remove unincorporated nucleotides and were washed in running water for 30 min.

Autoradiography and Grain-Counting Procedures

Ovary sections and isolated oocytes were coated with Ilford K2 nuclear emulsion (Ilford Ltd., Ilford, Essex, England) diluted 1:1 with distilled water. After suitable exposure in light-proof boxes, the autoradiographs were developed in fresh amidol solution and fixed in a saturated solution of sodium thiosulphate. Sections were stained with Harris haematoxylin and isolated oocytes with Giemsa's.

Ovary sections were examined using a Zeiss projection microscope situated in a darkroom. Each section was scanned for oocytes which had been cut so that the plane of the section passed through the largest cross section of the follicle. If the follicle showed no morphological signs of atresia, the growth stage was determined using the classification of Pedersen and Peters (1968). The number of silver grains over the nucleolus, the nucleoplasm, and the cytoplasm of the oocyte was then counted. Grain counts were also performed over each of four granulosa cell nuclei adjacent to the oocyte. The projected outlines of the oocyte nucleus, nucleolus, and cytoplasm and those of the four granulosa nuclei were drawn on white paper and moved outside the perimeter of the ovary section so that the background grain counts could be made over equivalent volumes of emulsion. The difference between the number of grains over each cell structure and the background was recorded as the net grain count.

Follicle Classification

In the [^{3}H]uridine study which involved the use of serially sectioned ovaries, follicles were classified according to the size of the oocyte, the number of granulosa cells in the largest cross section of the follicle, and the morphology of the follicle (Pedersen and Peters, 1968). Three major follicle types were distinguished: small follicles, oocyte diameter ≦20 μm with 1–20 granulosa cells; medium follicles, oocyte diameter 20–70 μm with 21–200 granulosa cells; large follicles, oocyte diameter 70 μm with 201 to >600 granulosa cells. Details of the classification are incorporated in Table I and Fig. 1.

RESULTS

[^{3}H]Uridine Incorporation

1 h after [^{3}H]uridine injection, tritium label in the mouse ovary was mainly concentrated in the oocyte and follicle cell nuclei. The amount of cytoplasmic incorporation was not greater than that found over the background. Grains were almost wholly absent from sections which had been treated with RNase prior to application of the emulsion, indicating that the label was incorporated into RNA. Table I summarizes the results of grain counts made on oocytes and granulosa cells in two experiments involving five ani-

TABLE I

*Mean Grain Number over Oocyte and Granulosa Cell Nuclei 1 h after Injection of [^3H]Uridine**

	Follicle size:	small		medium			large		
	Granulosa cell no.:	<20	≦20	21–60	61–100	101–200	201–400	401–600	>600
Exp. no.	Follicle type:	2	3a	3b	4	5a	5b	6	7
I (one ovary)	Grains/oocyte nucleus	9 ± 1 (32)‡	13 ± 1 (33)	28 ± 2 (34)	29 ± 2 (33)	42 ± 3 (11)	30 ± 3 (6)	9 ± 2 (12)	7 ± 1 (2)
	Grains/nucleolus	1 ± 0	4 ± 1	9 ± 1	9 ± 1	14 ± 2	6 ± 2	3 ± 1	0
II (four ovaries)	Grains/oocyte nucleus	8 ± 0 (119)	11 ± 1 (139)	19 ± 1 (137)	26 ± 1 (112)	31 ± 1 (97)	24 ± 2 (57)	7 ± 1 (26)	1 ± 2 (8)
	Grains/nucleolus	2 ± 0	3 ± 0	5 ± 0	7 ± 0	8 ± 1	5 ± 1	3 ± 1	1 ± 1
I	Grains/granulosa cell nucleus	—	2 ± 0 (108)	3 ± 0 (92)	3 ± 0 (100)	5 ± 1 (36)	6 ± 1 (20)	6 ± 1 (40)	5 (4)
II	Grains/granulosa cell nucleus	1 ± 0 (476)	1 ± 0 (556)	2 ± 0 (548)	2 ± 0 (448)	2 ± 0 (388)	3 ± 0 (228)	3 ± 0 (104)	2 ± 0 (32)

* The amount of [^{3}H]uridine incorporated into each oocyte was estimated from autoradiographs of ovary sections which included the largest cross section of the follicle.

‡ Figures in parentheses refer to the number of oocytes over which grain counts were made.

mals. The first records the grain numbers obtained from one ovary and the second the pooled results of counts from ovaries of four mice which were treated and processed together. The rate of uridine incorporation into the oocyte during growth is similar in the two experiments. Oocytes in small resting follicles (types 2 and 3a) show relatively little uridine uptake into the nuclear and nucleolar compartments. Incorporation increases markedly, however, as the follicle leaves the resting pool and commences to grow (type 3b follicles). This trend continues until the follicle has developed to the trilaminar stage (type 5a follicles). Oocyte nuclear and nucleolar grain numbers reach a peak at this stage but decline during further follicle growth, which includes the initiation of antrum formation (types 5b and 6). Incorporation is very low in maturing follicles which contain a large antrum (type 7).

Uridine incorporation into the granulosa cells also varies during follicle growth (Table I), although the changes are quite small at all stages of development. As in the oocyte nucleus, uridine uptake increases gradually in the granulosa cells during follicle growth but is maintained at a relatively high level in large follicles in which incorporation into the oocyte is low.

It should be realized in connection with these studies that the size of the oocyte nucleus increases during growth. Thus grain counts are related only to the amount of label present in a 5-μm thick slice of the cell. In small oocytes, this includes a considerable portion of the nucleus, but, as the oocyte grows, the section represents a progressively smaller part of the total volume. Fig. 1 shows the results of grain counts on the four ovaries of exp. II together with the changes in cross-sectional area of the same oocyte nuclei during follicle growth. The increase in nuclear size during oocyte growth reinforces the observation that the amount of uridine incorporated is also increasing. The subsequent decline in labeling of oocytes of large follicles may be more directly related to that found at the stage of peak incorporation since no significant change in the size of the oocyte nucleus occurs in follicles between types 5a and 7.

RNA Polymerase Activity

The incorporation of [^{3}H]UMP into the nuclei of oocytes isolated from adult and juvenile ovaries demonstrates the presence of endogenous, DNA-dependent RNA polymerase activity. The label is removed by RNase, indicating that the label was incorporated into RNA. Polymerase activity is demonstrable in both the nucleolus and nucleoplasm of oocytes isolated from growing follicles (Figs. 2 and 3), but is absent from the nuclei of oocytes of large follicles (Figs. 6 and 7) and from the chromosomes of ovulated oocytes which have resumed meiosis (Fig. 9 *a*). These conclusions were confirmed in oocytes assayed for RNA polymerase activity in the presence of a high salt

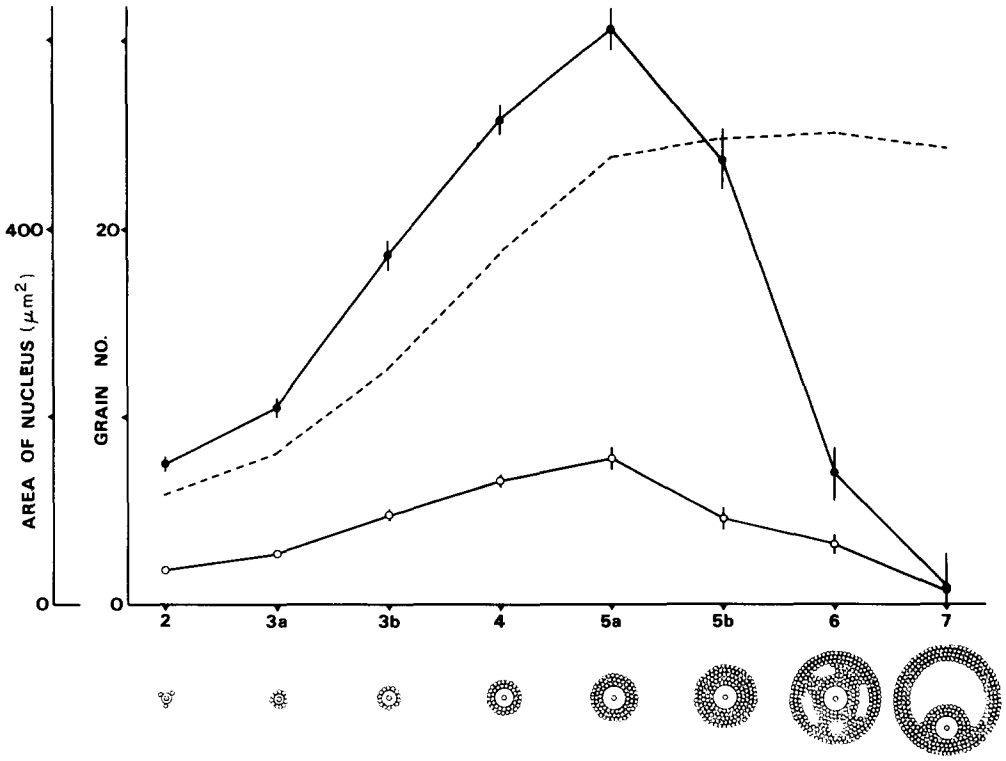


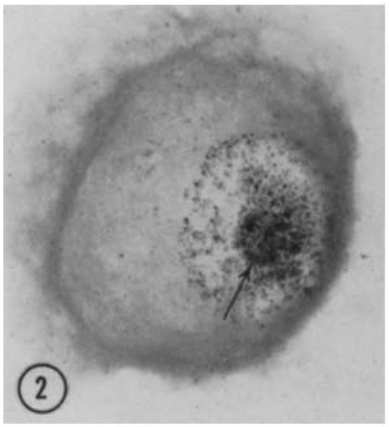
FIGURE 1 [^{3}H]uridine incorporation into the mouse oocyte at different stages of follicle growth expressed as the mean number of grains over the nucleus. Open circles, grains over the nucleolus; closed circles, grains over the whole of the nucleus. Vertical lines represent the standard error of the mean. The broken line depicts the change in area of the oocyte nucleus during growth.

concentration. Model studies have shown that 0.4 M ammonium sulphate considerably enhances nucleoplasmic polymerase activity in fixed cells (Moore and Ringertz, 1973). In oocytes from growing follicles, an increase in [^{3}H]UMP incorporation is observed under these conditions (Figs. 4 and 5), but in those of mature follicles no labeling is evident (Fig. 8). Incorporation of [^{3}H]UMP is also demonstrable in the granulosa cells of growing and mature oocytes (Figs. 3 and 7) and may be considerably stimulated when the ammonium sulphate concentration is increased (Figs. 4 and 8). Polymerase activity is also present in the cumulus cells of oocytes which have been induced to ovulate in the presence of pregnant mare serum gonadotrophin and human chorionic gonadotrophin (Fig. 9 *b*).

DISCUSSION

The RNA precursors [^{3}H]uridine and [^{3}H]UTP have been used to detect the changes in genetic activity of the mouse oocyte during growth. Using autoradiography it has been shown that [^{3}H]uridine incorporation into the oocytes of small, resting follicles is relatively low, but increases as the follicle begins to grow. RNA synthesis reaches a peak in oocytes of multilayered follicles prior to the development of an antrum. With the appearance of follicle fluid and the formation of a definitive antrum, RNA synthesis declines and both uridine and UMP uptake is very low or absent in oocytes which are approaching maturity (see also Oakberg, 1968).

In general, RNA synthesis in the mature oocyte appears to be reduced or absent in many species (Brachet, 1967; Davidson, 1968). In the mouse it has been suggested that the low incorporation of uridine into maturing oocytes may be partly due to their inaccessibility, surrounded as they are by layers of follicle cells and antral fluid (Baker et al., 1969). However, it is clear from the present study that oocytes which had been isolated from large follicles and which were therefore permeable to ribonucleoside triphosphates after fixation (Moore and Ringertz, 1973) showed no RNA polymerase activity (Figs. 6–8).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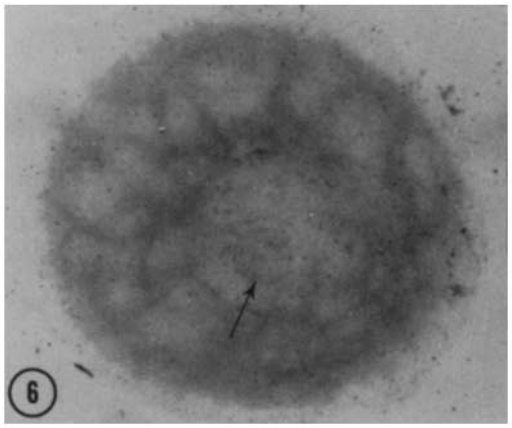
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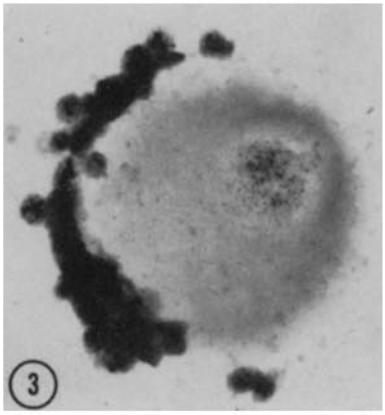
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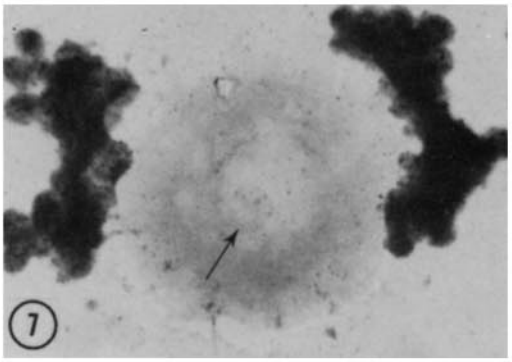
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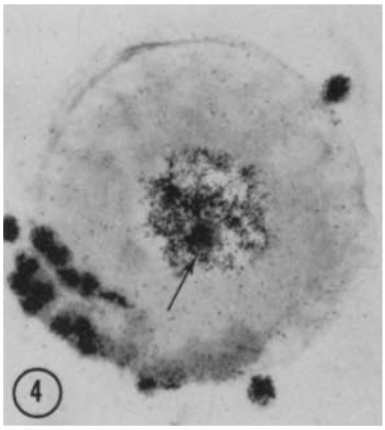
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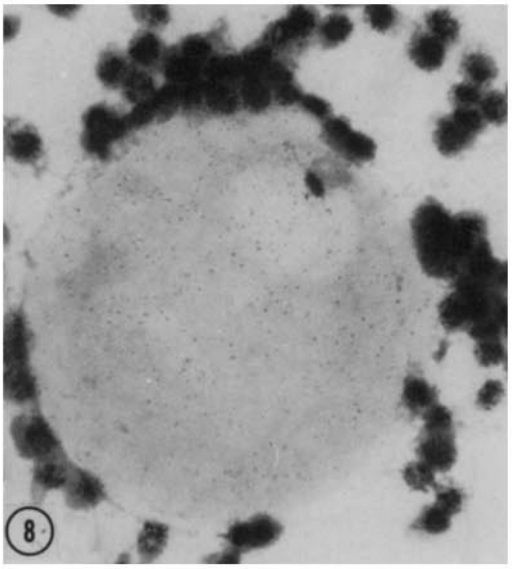
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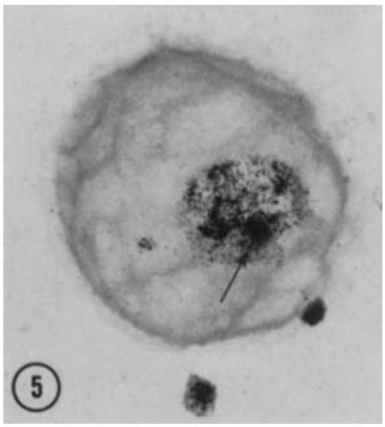
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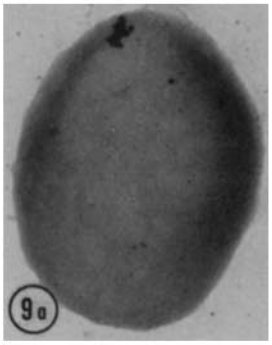
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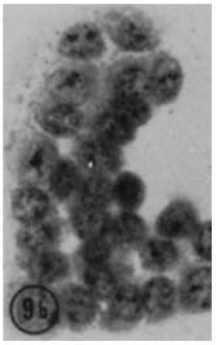
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Support for the view that RNA may be synthesized in maturing oocytes has come from the studies of Bloom and Mukherjee (1972) who demonstrated that [^{3}H]uridine is actively incorporated into the nuclei of isolated mouse oocytes in culture. Similarly, Brown and Littna (1966) have demonstrated that *Xenopus* oocytes synthesize RNA after hormonal treatment to induce ovulation. The latter observations may be reconciled with the present studies since these do not exclude the existence of a short, preovulatory burst of RNA synthesis in the oocyte. Nevertheless the behaviour of mouse oocytes during maturation in vivo may be very different from that found in vitro,(Cross and Brinster, 1970).

The mechanism by which RNA synthesis is suppressed in large oocytes which have retained the dispersed chromatin configuration of their earlier, active state is presently being studied in this laboratory. Recent investigations indicate that antral fluid aspirated from mature cow follicles contains a substance which strongly inhibits DNA-dependent RNA polymerase activity in vitro. Similarly Crippa (1970) and Crippa et al. (1972) have reported that a protein factor isolated from the germinal vesicles of amphibian oocytes inhibits the transcription of ribosomal genes.

The RNA synthesized in the oocyte nucleus is presumably both metabolized during growth and contributes to a store of ribosomes and developmental information to be used after fertilization (Mintz, 1964). There is certainly evidence to show that the amount of RNA in the cytoplasm of the mouse oocyte increases during growth (Mintz, 1965), but the involvement of this RNA in directing postfertilization development has not been clarified. Some indication of the nature of the RNA contained in the oocyte cytoplasm may be gained by examining the potential of the newly formed embryo to develop in the absence of new RNA synthesis. Embryos of amphibia and a number of invertebrates, which have been cultured in the presence of actinomycin D to suppress RNA synthesis, progress through the cleavage stages apparently normally (Davidson, 1968). Thus in these species, early development appears to depend upon a large store of preformed RNA. By contrast, the two-cell mouse embryo is unable to cleave if RNA synthesis is inhibited (Mintz, 1964, 1965). This suggests that the amount of developmental information stored in the oocyte is quite small and that the expression of new information early after fertilization is vital for cleavage. In the mouse embryo, RNA polymerase activity which is sensitive to α-amanitin is first evident at the two-cell stage (Moore, unpublished observations; see also Knowland and Graham, 1972).

In the absence of a contribution of polymerases by the spermatozoon at fertilization, the initiation of RNA synthesis in the embryo must presumably rely on enzymes which have been sequestered in the oocyte. Although RNA polymerases have yet to be isolated from mature mammalian oocytes, both the nucleolar and nucleoplasmic forms have been found in amphibian oocytes (Crippa et al., 1972; Wassarman et al., 1972).

FIGURES 2–9 Mouse oocytes which have been isolated from follicles at different growth stages or collected from the fallopian tubes after superovulation. The oocytes shown have been assayed for RNA polymerase activity together and exposed to autoradiographical emulsion for similar periods of time. × 425.

FIGURES 2 and 3 Oocytes from growing follicles. Incorporation of [^{3}H]UMP into the nucleolus (arrow) and nucleoplasm is evident. In Fig. 3, the adhering granulosa cells are labelled.

FIGURES 4 and 5 Oocytes from growing follicles assayed in the presence of 0.4 M ammonium sulphate. Both oocyte and granulosa cell nuclei are heavily labelled.

FIGURES 6 and 7 Oocytes from large follicles. There is no [^{3}H]UMP incorporation into oocyte nuclei but granulosa cell nuclei (Fig. 7) are labelled.

FIGURE 8 Oocyte from a large follicle assayed in the presence of 0.4 M ammonium sulphate. The oocyte nucleus is not labelled but there is heavy labelling over the granulosa cell nuclei.

FIGURE 9 (*a*) Ovulated oocyte which has resumed meiosis. There is no incorporation of label into the chromosomes. (*b*) Corona cells of oocyte shown in *a* showing label incorporation over the nuclei.

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