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Absolute rates of protein synthesis during meiotic maturation of mammalian oocytes *in vitro*

(oogenesis/mouse/[^{35}S]methionine incorporation/methionine pool/ovulation)

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ABSTRACT **Measurements of the rates of incorporation of [^{35}S]methionine into protein and the specific activities of endogenous free methionine pools have been used to calculate the *absolute* rates of protein synthesis in mouse oocytes during spontaneous meiotic maturation *in vitro*. Fluorodinitro[^{3}H]benzene was used to determine the specific activity of the oocyte's free methionine pool. It was found that the absolute rate of protein synthesis decreased from 43 to 31 pg/hr per oocyte during meiotic progression from dictyate to metaphase II (meiotic maturation), while the size of the intracellular free methionine pool decreased from 61 to 35 fmol per oocyte during the same period. Comparable measurements made on ovulated mouse oocytes that had undergone meiotic maturation *in vivo* strongly suggest that the decrease in the absolute rate of protein synthesis observed during meiotic maturation *in vitro* is physiologically significant. An alternative method that depends upon differential expansion of the oocyte's endogenous methionine pool was also used to determine absolute rates of protein synthesis. The results of these experiments are in excellent agreement with those obtained by using fluorodinitro[^{3}H]benzene, indicating that the oocyte's free methionine pool is not compartmentalized.**

During the process of oogenesis, oocytes of many animal species undergo meiotic arrest prior to the completion of chromosomal reduction, and it is in this state that they undergo tremendous growth. The length of time that oocytes remain in this arrested state and the nature of the stimulus that reinitiates meiosis are species dependent (1–3).

In the mouse, nearly all oocytes have arrested at the diplotene ("dictyate") stage of prophase of the first meiotic division by 5 days *post partum*, and they remain in dictyate until just prior to ovulation, a period extending from several weeks to more than a year. The resumption of meiosis can be mediated by a hormonal stimulus *in vivo* (4) or by the release of oocytes from their ovarian follicles into a suitable culture medium (5–8). The oocytes undergo nuclear progression from dictyate to metaphase II and remain at this stage of meiosis in the oviduct, or in culture, until fertilization or parthenogenetic activation takes place. The period of time during which meiosis progresses from dictyate to metaphase II is termed the period of "meiotic maturation." The process of meiotic maturation is characterized by dissolution of the nuclear membrane (germinal vesicle breakdown), condensation of diffuse chromatin into distinct bivalents, separation of homologous chromosomes and emission of the first polar body, and arrest at metaphase II. Mouse oocytes matured and fertilized *in vitro* have developed into viable fetuses following transplantation to the uteri of foster mothers (9).

Recently, we described the results of experiments that employed high-resolution two-dimensional electrophoresis to examine protein synthesis during meiotic maturation of mouse oocytes *in vitro* (10). These experiments showed that the pattern of protein synthesis changed markedly during this period of oogenesis and that virtually all of the changes observed took place subsequent to breakdown of the oocyte's germinal vesicle (GV). We also found that nearly all of the changes in the pattern of protein synthesis took place in *anucleate* oocyte fragments during culture *in vitro*, suggesting that concomitant transcription of the nuclear genome is not necessary to initiate these changes (11).

In this report we describe the results of experiments designed to determine whether the absolute rate of protein synthesis changes during meiotic maturation of mouse oocytes *in vitro*. Because a change in the incorporation of a radioactively labeled amino acid into protein cannot be interpreted as a change in the absolute rate of protein synthesis unless the specific activity of the amino acid pool is known, we have measured the sizes and specific activities of the free methionine pools in mouse oocytes during meiotic maturation *in vitro*. The rates of incorporation of [^{35}S]methionine into acid-precipitable material have also been determined throughout meiotic maturation and converted into absolute rates of protein synthesis. We have also carried out experiments to determine whether the total, or some fraction of the total, intracellular free methionine pool of mouse oocytes serves as a precursor for protein synthesis. While the absolute rate of protein synthesis has been reported for ovulated mouse eggs (12), we know of no previous report of such measurements made throughout the period of meiotic maturation. These results are compared with those reported for oocytes obtained from nonmammalian animal species.

MATERIALS AND METHODS

Collection and Culture of Mouse Oocytes. Fully grown oocytes were obtained from adult (6–12 weeks of age), randomly bred, female Swiss albino mice (CD-1, Charles River Labs) by puncturing isolated ovaries with fine steel needles under a dissecting microscope (13). Oocytes containing an intact GV and free of cumulus cells were harvested with a mouth-operated micropipet and washed in culture medium (14) containing $N^6,O^{2'}$-dibutyryladenosine 3′:5′-cyclic monophosphate (Bt_2cAMP) (Sigma) at 100 μg/ml (15, 16). Ovulated oocytes were obtained from animals injected with 5 immunizing units of pregnant mare's serum (Sigma) and 5 immunizing units of human chorionic gonadotropin (Sigma) 48 hr later (13). Cells were cultured in either plastic dishes (Falcon) or embryological watch glasses in 200 μl of medium under paraffin oil at 37° in a humidified atmosphere of 5% CO_2 in air.

Determination of [^{35}S]Methionine Incorporation into

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Abbreviations: GV, germinal vesicle; Bt_2cAMP, $N^6,O^{2'}$-dibutyryladenosine 3′:5′-cyclic monophosphate; FDNB, fluorodinitrobenzene; DNP, dinitrophenyl.

Oocyte Proteins. Oocytes were harvested and cultured in 200 μl of medium containing 35–50 μCi of [^{35}S]methionine (New England Nuclear, >500 Ci/mmol) and 0.5 μg of methionine, as described above. At 30-min intervals, over a period of 5 hr, replicate samples containing 5–10 oocytes each were removed from culture. These samples were processed as previously described (8) to determine the dpm of [^{35}S]methionine present in acid-soluble and acid-precipitable material.

Determination of the Specific Activity of the Methionine Pool. A modification of the procedure described by Regier and Kafatos (17) has been used to determine the specific activity of the free methionine pool of mouse oocytes. This method is based upon the reaction of methionine with fluorodinitrobenzene (FDNB). The specific activity of [^{35}S]methionine is determined by reaction with [^{3}H]FDNB and measuring the ratio of ^{35}S to ^{3}H in the resulting dinitrophenyl (DNP)-methionine after purification by two-dimensional chromatography.

After 2.5 and 5 hr of culture in 200 μl of medium containing 35–50 μCi of [^{35}S]methionine and 0.5 μg of methionine, approximately 100 oocytes were harvested, extensively washed in phosphate-buffered saline containing 0.3% polyvinylpyrrolidone-40 (Sigma), and transferred in about 2 μl to an acid-washed test tube. The sample was frozen and thawed four times and dried under reduced pressure; 100 μCi of [^{3}H]FDNB (New England Nuclear, >20 Ci/mmol) in 100 μl of benzene was added to the sample, and the benzene was removed under N_2. Ten microliters of absolute ethanol and 15 μl of 0.2 M $NaHCO_3$, pH 9.1, were then added, the tube was tightly stoppered and incubated in the dark at 40° for 12 hr. After the addition of 1 μg of DNP-methionine, the sample was dried under reduced pressure, the residue was taken up in 100 μl of distilled water, and the aqueous phase was extracted twice with 200 μl of ether. The aqueous phase was acidified with 15 μl of 1 M HCl and the DNP-amino acids were extracted with 200 μl of ether. The ether phase was dried under N_2. The residue was dissolved in 15 μl of acetone and spotted on a 7.5 × 7.5 cm polyamide plate (Cheng Chin, Accurate Chemical and Scientific). The DNP-amino acids were resolved by two-dimensional chromatography using 44% formic acid in the first dimension and 1-butanol/glacial acetic acid (9:1, vol/vol) in the second dimension. The DNP-methionine spot, located by its yellow color, was scraped from the polyamide plate and eluted for 3 hr with acetone (100 μl) and 1 M HCl (10 μl). Following elution, the extraction solvent was removed under a stream of N_2 and the residue was dissolved in 15 μl of acetone, spotted, chromatographed, and eluted as described above. Following a third chromatography, the DNP-methionine was eluted into acetone (100 μl)/glacial acetic acid (10 μl) and transferred to a scintillation vial. The solvent was removed under an infared lamp and 10 ml of 0.6% 2,5-diphenyloxazole (PPO) in toluene was added. A background sample was prepared in an identical manner, using 2 μl of the oocyte final wash solution.

Kinetic Analysis of Methionine Pool Size and Absolute Rate of Protein Synthesis. Oocytes were cultured in 200 μl of medium containing 50 μCi of [^{35}S]methionine and various amounts of unlabeled methionine. At hourly intervals, over a period of 5 hr, 5–10 oocytes were removed from each group, washed extensively with fresh medium, transferred to a Microfuge tube, and frozen and thawed four times. Twenty microliters of bovine serum albumin (1 μg/μl) was added to each sample, followed by 20 μl of ice-cold 10% trichloroacetic acid, and the samples were placed on ice for 10 min. Samples were centrifuged at 13,000 × *g* for 5 min, the supernatants were collected, and pellets were washed with ice-cold 5% trichloroacetic acid. Supernatants and washes were combined for each sample and assayed by liquid scintillation counting in 10 ml of 0.6% diphenyloxazole/0.2% Biosolv (Beckman) in toluene at 75% efficiency. Pellets were dissolved in 1 M NaOH (20 μl) for 1 hr at 37°, acidified with 1 M HCl (50 μl), and assayed by liquid scintillation counting in 5 ml of Aquasol (New England Nuclear) at 69% efficiency.

Amino Acid Analysis of Total Oocyte Proteins. Oocytes were collected, washed extensively in phosphate-buffered saline containing polyvinylpyrrolidone-40 (3 mg/ml), transferred to a microfuge tube, and frozen and thawed four times. Ten micrograms of polyvinylpyrrolidone-40 (2 μl) was added, protein was precipitated with 20 μl of ice-cold 10% trichloroacetic acid, and the precipitate was recovered by centrifugation and washed with 10% trichloroacetic acid. The sample was then dried under reduced pressure, hydrolyzed in 100 μl of 6 M HCl for 4 hr at 145°, dried once again, and applied to a Beckman 121M amino acid analyzer in 0.063 M citrate buffer, pH 2.2. To determine total methionine as methionine sulfone, the trichloroacetic acid-precipitated protein was oxidized with 25 μl of performic acid for 5 hr (18), and the reaction was terminated with 3.7 μl of HBr. The sample was dried under reduced pressure, hydrolyzed, and applied to the amino acid analyzer as described above.

Analysis of ^{35}S-Labeled Amino Acids in Total Oocyte Proteins. To determine whether all of the ^{35}S incorporated into mouse oocyte proteins was present in methionine, or whether some had been metabolized to cysteine, oocytes were cultured for 2.5 and 5.0 hr as described above. Acid-insoluble material was obtained from about 50 oocytes as described above and oxidized with 25 μl of performic acid for 6 hr (18), and the reaction was terminated with 3.75 μl of HBr. The sample was dried under reduced pressure and hydrolyzed with 100 μl of 6 M HCl for 4 hr at 145°. The HCl was removed under reduced pressure, and the residue was dissolved in 20 μl of distilled water and chromatographed on Whatman DE-81 paper strips with 100 mM NH_4HCO_3. The R_F values of methionine sulfone and cysteic acid in this chromatographic system were 0.41 and 0.22, respectively. Chromatograms were cut into 1-cm segments and analyzed by liquid scintillation counting in 0.6% diphenyloxazole in toluene.

RESULTS

Meiotic Maturation of Mouse Oocytes *In Vitro*. Meiotic maturation takes place spontaneously when oocytes from adult mice are released from their ovarian follicles into a suitable culture medium (5–8). The time sequence of meiotic maturation *in vitro* can be approximated as follows: GV breakdown takes place within the first 5 hr, metaphase I is reached in 5–10 hr, and metaphase II is reached in 10–15 hr. Under the experimental conditions used in this study, approximately 80% of the oocytes placed in culture underwent GV breakdown within 3 hr and, of these, approximately 70% subsequently emitted first polar bodies. Mouse oocytes cultured continuously in Bt_2cAMP (100 μg/ml) did not undergo GV breakdown or the subsequent morphological events associated with meiotic maturation (19, 20).

Determination of Absolute Rates of Protein Synthesis. The absolute rates of protein synthesis and sizes of the methionine pools during spontaneous meiotic maturation of mouse oocytes *in vitro* are presented in Table 1. These values were calculated from the rates of incorporation of [^{35}S]methionine into protein and the corresponding specific activities of intracellular free methionine pools as determined by the [^{3}H]FDNB method (Table 2). Oocytes arrested in dictyate of the first meiotic prophase by Bt_2cAMP synthesized protein at the rate of 43

Table 1. Absolute rates of protein synthesis and sizes of methionine pools during meiotic maturation of mouse oocytes

Design of experiment	Number of experiments	Absolute rate (range), pg protein/hr per oocyte	Pool size (range), fmol Met/ oocyte	Absolute rate (avg. ± SD), pg protein/ hr per oocyte	Pool size (avg. ± SD), fmol Met/ oocyte
Dictyate; FDNB*	5	38–50	50–84	42.9 ± 4.4	61 ± 18
Dictyate; kinetic*	8	37–50	32–91	41.8 ± 5.5	56 ± 19
GV breakdown–metaphase I; FDNB†	2	35–38	67–71	36.3 ± 1.4	69 ± 2
Metaphase I–metaphase II; FDNB‡	4	26–35	27–43	31.1 ± 3.3	35 ± 6
Metaphase II (ovulated); FDNB§	4	30–36	57–82	33.0 ± 2.2	74 ± 12

* Oocytes were cultured for up to 5 hr in medium containing [^{35}S]methionine and Bt_2cAMP.
† Oocytes were cultured for 5 hr in plain medium and then those oocytes that had undergone GV breakdown were transferred to medium containing [^{35}S]methionine for up to 5 hr of culture.
‡ Oocytes were cultured for 5 hr in plain medium, and those oocytes that had undergone GV breakdown were transferred to fresh plain medium for an additional 5 hr of culture and finally were transferred to medium containing [^{35}S]methionine for up to 5 hr of culture.
§ Ovulated oocytes were cultured for up to 5 hr in medium containing [^{35}S]methionine.

pg/hr per oocyte during the first 5 hr of culture.* During the same period the size of the oocyte's free methionine pool, measured by isotopic dilution, remained constant at 61 fmol per oocyte (240 μM). As meiotic maturation progressed to metaphase II *in vitro*, the absolute rate of protein synthesis decreased to 31 pg/hr per oocyte, while the size of the intracellular free methionine pool decreased to 35 fmol per oocyte (138 μM). Analogous experiments were carried out with ovulated oocytes arrested at metaphase II. These oocytes synthesized protein at the rate of 33 pg/hr per oocyte during 5 hr of culture *in vitro* and had a free methionine pool of 74 fmol per oocyte (291 μM) (Table 1). The latter result suggests that the decrease in the absolute rate of protein synthesis during meiotic maturation of mouse oocytes *in vitro* is not simply due to the decrease in the size of the intracellular free methionine pool.

Conversion of [^{35}S]methionine into [^{35}S]cysteine during the course of these experiments could represent a potential source of error in the measurements. Accordingly, ^{35}S-labeled acid-insoluble material obtained from oocytes cultured for 2.5 and 5.0 hr was oxidized, hydrolyzed, and chromatographed under conditions capable of resolving methionine sulfone and cysteic acid. Analysis of these chromatograms showed that more than 98% of the ^{35}S incorporated into acid-insoluble material was present as methionine sulfone.

Kinetic Analysis of Absolute Rates of Protein Synthesis. Because the absolute rates of protein synthesis reported above were calculated using specific activities for *total* free methionine pools ([^{3}H]FDNB method), these rates would be subject to serious error if a smaller compartment of the pool (i.e., "kinetic pool") actually served as precursor for protein synthesis. To examine this possibility, a method analogous to that described by Ecker (21) was employed. This method for determining absolute rates of protein synthesis depends upon differential expansion of a cell's amino acid pool, such that apparent rates of incorporation are altered while absolute rates remain the same. Consequently, absolute rates of protein synthesis and sizes of intracellular free methione pools may be calculated by solving the following pair of simultaneous equations:

$$dI_1/dt = R \times SA_1 = R \times L_1/(P + G_1)$$
$$dI_2/dt = R \times SA_2 = R \times L_2/(P + G_2),$$

in which dI_1/dt and dI_2/dt are the observed rates of [^{35}S]-methionine incorporation into acid-insoluble material at two different methionine concentrations, L_1 and L_2 are the dpm of [^{35}S]methionine in the acid-soluble material at two different methionine concentrations, G_1 and G_2 are the fmol of methionine taken up by the oocytes at two different methionine concentrations, P is the size of the active endogenous free methionine pool, and R is the absolute rate of protein synthesis. We have applied this method over a 100-fold range of methionine concentrations in the medium (0.1–10 μg/ml) and found that R remained constant, even though the intracellular free methionine pool was expanded as much as 5-fold (Table 3). The rates of protein synthesis and sizes of free methionine pools determined by this kinetic method were in excellent agreement with those determined by the [^{3}H]FDNB method (Table 1). These results strongly suggest that the *total* intracellular free methionine pool serves as precursor for protein synthesis in mouse oocytes.

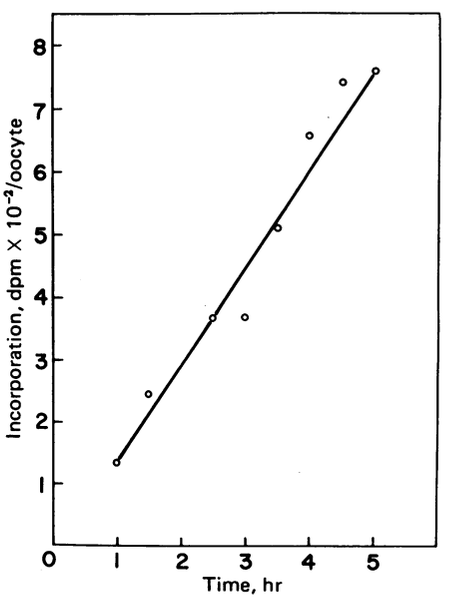

FIG. 1. Kinetics of incorporation of [^{35}S]methionine by mouse oocytes *in vitro*. Oocytes were isolated, cultured, and processed to determine amounts of trichloroacetic acid-soluble and insoluble [^{35}S]methionine as described in *Materials and Methods* and in the legend to Table 2. The amount of acid-soluble [^{35}S]methionine reached a steady-state value of 4961 dpm/oocyte after 1 hr of culture.

* Bt_2cAMP does not alter the absolute rate of protein synthesis in mouse oocytes. The presence of Bt_2cAMP has no effect on the size of the oocyte's endogenous methionine pool (compare lines 1 and 3 in Table 1), on methionine uptake into the acid-soluble fraction, or on the rate of incorporation of [^{35}S]methionine into protein. Therefore, because the specific activity of the precursor and its incorporation rate are unaffected, the absolute rate of protein synthesis is not altered by Bt_2cAMP.

Table 2. Sample calculation of absolute rate of protein synthesis and methionine pool size by using the [^{3}H]FDNB method*

Sample†	Observed cpm		Corrected cpm‡		dpm§		Methionine,¶ fmol	*SA*,‖ dpm/fmol
	^{3}H	^{35}S	^{3}H	^{35}S	^{3}H	^{35}S		
2.5 hr	3891	1807	3099	1743	6886	2640	132.0	20.0
5.0 hr	2190	994	1617	947	3593	1434	69.1	20.8

* The equation $R = (dI/dt)/SA$, in which dI/dt is the dpm of Met incorporated into protein/hr per oocyte and SA is the specific activity of Met (dpm/mol) in the amino acid pool, was used to calculate R, the moles of Met incorporated into protein/hr per oocyte. For example, using the data shown in Fig. 1, dI/dt = 158 dpm/hr per oocyte and SA was determined by the FDNB method, using oocytes taken from culture at 2.5 and 5.0 hr. Because dI/dt = 158 dpm/hr per oocyte and SA = 20.4 dpm/fmol, R = 158/20.4 or 7.8 fmol Met incorporated/hr per oocyte. R was converted to 42.9 pg of protein synthesized/hr per oocyte by using values of 2% for the average Met content of oocyte proteins (determined by amino acid analysis) and 110 for the average molecular weight of an amino acid. The pool size was calculated by using the equation $SA_{exp}/SA_{med} = A/(A + B)$, in which SA_{exp} is the experimentally determined specific activity of the Met precursor, SA_{med} is the specific activity of the Met in the medium, A is the fmol of Met taken up by an oocyte from the medium (dpm in acid-soluble material/SA_{med}), and B is the size of the endogenous free Met pool. In this example SA_{exp} = 20.4 dpm/fmol, SA_{med} = 26.8 dpm/fmol, and A = 185 fmol; therefore, B = 58 fmol of Met/oocyte.

† After 2.5 and 5.0 hr of culture in medium containing [^{35}S]methionine (250 μCi/ml), nonradioactive methionine (2.5 μg/ml), and Bt_2cAMP (100 μg/ml), 120 and 60 oocytes, respectively, were collected.

‡ The observed cpm and background cpm were corrected for spillage (1% spillage of ^{3}H into the ^{35}S channel and 27% spillage of ^{35}S into the ^{3}H channel). The average background after three successive chromatography steps was 305 cpm of ^{3}H and 26 cpm of ^{35}S. These values were subtracted from the observed cpm to obtain the corrected cpm.

§ Counting efficiencies, determined by internal standardization, were 45% and 66% for ^{3}H and ^{35}S, respectively. Corrected cpm divided by counting efficiency gave dpm.

¶ ^{3}H dpm divided by the specific activity of [^{3}H]FDNB (52 dpm/fmol) gave fmol Met.

‖ ^{35}S dpm divided by fmol Met gave SA (dpm/fmol) of the intracellular free Met pool.

DISCUSSION

Results of experiments carried out with echinoderms and amphibians have shown that during oogenesis, oocytes synthesize and/or accumulate a variety of macromolecules and organelles—e.g., yolk, histones, tubulin, enzymes, regulatory factors, ribosomes, mitochondria, and informational RNA—that are utilized during early embryogenesis (22). Consequently, it is generally accepted that much of the developmental program of the early embryo is laid down during oogenesis.

In contrast to the wealth of available biochemical information concerned with oogenesis in lower animal species, there is a paucity of such information concerned with oogenesis in the mammal. That this is the case is unfortunate, because differences in the reproductive physiology of mammalian as compared to nonmammalian species probably impose differences on the biochemistry of oogenesis. For example, the results of several lines of experimentation suggest that while early development of nonmammalian species is directed by RNA templates accumulated during oogenesis, early mammalian development depends upon concomitant transcription of the nuclear genome (22–24).

In order to evaluate the contribution of the oocyte to the progress of early embryogenesis in the mammal, we have begun to determine the absolute rates of synthesis, degradation, and accumulation of protein during oogenesis in the mouse. We know of no other determination of absolute rates of protein synthesis in any mammalian oocyte during meiotic maturation. We have found that the absolute rate of protein synthesis in fully grown, meiotically arrested, mouse oocytes is 43 pg/hr per oocyte and that the rate decreases to 31 pg/hr per oocyte during spontaneous meiotic maturation *in vitro*. Because the absolute rate of protein synthesis in ovulated mouse oocytes, 34 pg/hr per oocyte, is nearly identical to that in oocytes matured *in vitro*, 31 pg/hr per oocyte, it would appear that the decrease observed during spontaneous meiotic maturation is of physiological significance.

The absolute rate of protein synthesis in fully grown mouse oocytes compares quite favorably with that reported for amphibian oocytes when these data are expressed as pg of protein synthesized/hr per pl of oocyte cytoplasm.† On the other hand, the 28% decrease in the absolute rate of protein synthesis during meiotic maturation of mouse oocytes *in vitro* is in marked contrast to the severalfold increase reported for progesterone-induced, maturing, amphibian oocytes (26–28). It is possible that the modest decrease in the absolute rate of protein synthesis during meiotic maturation of mouse oocytes reflects the degradation of oocyte RNA, because the rate of RNA synthesis appears to decrease dramatically following GV breakdown (25).

† This calculation assumes an average value of 30 ng/hr per oocyte for the absolute rate of protein synthesis in amphibian oocytes (3, 25), 43 pg/hr per oocyte for the rate in mouse oocytes, and 1300 μm and 85 μm for the average diameters of amphibian and mouse oocytes, respectively. Using these values, rates of 0.172 pg of protein/hr per pl for the mouse and 0.034 pg protein/hr per pl for the amphibian can be calculated. If yolk platelets comprise 90% of the cytoplasmic volume of the amphibian oocyte, then the actual rate of protein synthesis is 0.34 pg of protein/hr per pl of "active" cytoplasm.

Table 3. Sample calculation of absolute rate of protein synthesis and methionine pool size by using the kinetic method*

Methionine, μg/ml	dI/dt, dpm/hr per oocyte	L, dpm/oocyte	SA_{med}, dpm/fmol Met	G, fmol Met/oocyte
0.25	831	13,897	240	58
2.50	167	8,033	30	268

* Oocytes were cultured for up to 5 hr in medium containing [^{35}S]methionine and Bt_2cAMP; for a description of the nomenclature see *Results*. The data shown in Fig. 2 are used in this sample calculation. Because $(dI_1/dt)/[L_1/(P + G_1)] = (dI_2/dt)/[L_2/(P + G_2)]$, $831/[13{,}897/(P + 58)] = 167/[8033/(P + 268)]$; therefore, P = 55 fmol Met/oocyte. Substituting this value in the equation $R = (dI_1/dt)/[L_1/(P + G_1)]$, $R = 831/[13{,}897/(55 + 58)]$ = 6.8 fmol Met incorporated/hr per oocyte.

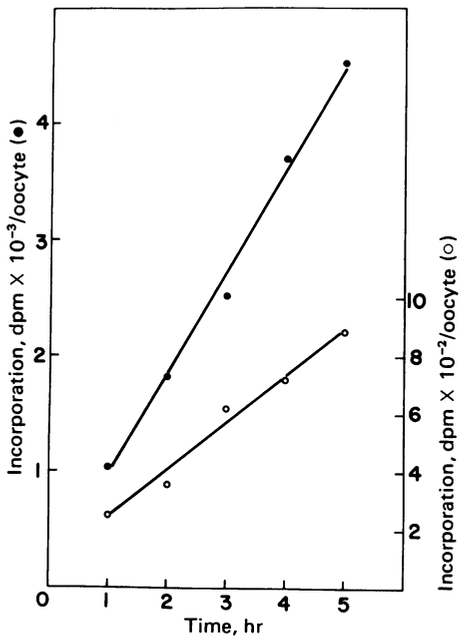

FIG. 2. Kinetics of incorporation of [^{35}S]methionine by mouse oocytes *in vitro*. Oocytes cultured in medium containing 50 μCi of [^{35}S]methionine and either 0.25 (●) or 2.5 (○) μg of Met/ml were processed to determine amounts of trichloroacetic acid-soluble and insoluble [^{35}S]methionine. These data are tabulated in Table 3.

To determine absolute rates of protein synthesis it is necessary to know the specific activity of the true precursor pool; in essence, this requires the determination of the specific activity of aminoacyl-tRNA. While this is not feasible with mouse oocytes, due to limited amounts of material, the coupled [^{3}H]FDNB and kinetic approach taken in this report circumvents this problem. The results presented here indicate that the sizes of the total endogenous free methionine pool and the kinetic free methionine pool used for protein synthesis are the same. Therefore, the free methionine pool of the mouse oocyte is not compartmentalized. This is in contrast to the situation in amphibian oocytes, in which amino acids are apparently compartmentalized to various degrees (3, 21; R. J. Shih, reported on p. 91 of ref. 22).

The decrease in the size of the oocyte's free methionine pool during meiotic maturation *in vitro* probably represents a culture artifact, because oocytes matured *in vivo* did not show this decrease. Once again, mouse and amphibian oocytes behave differently, because meiotic maturation of amphibian oocytes is accompanied by large increases in the sizes of their amino acid pools (29).

The experiments described in this report demonstrate that, with the advent of microtechniques and the availability of radiolabeled compounds of high specific activities, quantitative biochemical studies of mammalian oogenesis are feasible. In addition to the measurements reported here, we have been able to determine the absolute rates of synthesis of several specific proteins during oogenesis in the mouse using the methodology described (unpublished data). We feel that the differences noted here between meiotic maturation of mammalian and nonmammalian oocytes underscore the potential problems that may be encountered in extrapolating the results of experiments on lower species to mammals.

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