4. Activity was measured by placing animals individually in petri dishes on platforms resting on knife-blade edges. Rocking of platforms would activate microswitches (sensitive to 2.5 g) which were connected to multievent, continuous recorders. All experiments were performed in a Hotpack environmental chamber at Ann Arbor, Michigan; for other details see (*2*). Single units of activity (equal to tripping the microswitches once) were often recorded throughout the day. Thus, it was necessary to establish some criterion for "initiation of activity." This was set at three units per hour; examination of many experiments showed that this criterion in most cases signaled the initiation of an animal's sustained nocturnal locomotor activity. Experimental animals were obtained by H. G. M. Jopson near Bridgewater, Virginia. They were kept in total darkness for 3 days after collection. Then half had their eyes removed, and both groups were placed in petri dishes (day 1). Eyes were removed with scissors after protruding them with slight pressure beneath the head; anesthetics or cold treatment were not used for immobilization since this treatment disturbed the rhythm. All animals were autopsied after experimentation to ascertain that retinas had been completely removed. Temperature of animals did not differ between light-on and light-off conditions at the level of the organisms in any of the experiments, as determined with a thermometer sensitive to 0.1°C.
5. Pactra enamel (black), a fast-drying, opaque paint was used. After the paint dried, the overhead fluorescent lights were turned on, and the time (within 3 minutes) it took for the animals to move was recorded. In most cases, this involved actual movement of the entire animal from one part of the dish to another; in a few instances it meant twitching of the tail or movement of a single limb. All of these were considered to be movement for the purpose of this experiment. To reduce movements due to the endogenous rhythm, all experiments were performed on freshly caught animals from 10:00 a.m. to 3:00 p.m., well before the typical periods of maximum movement at nightfall. See (*4*).
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Human Oocytes: Maturation in Chemically Defined Media

Abstract. *Human oocytes from ovarian follicles resume meiosis in F10, a defined medium, in numbers comparable to that obtained in medium containing serum. Several simple Krebs-Ringer media also support maturation, which suggests a similarity of nutritional requirements between human and mouse oocytes. Fewer oocytes reach metaphase II when the investing follicular cells are removed prior to culture.*

Human oocytes, when released from ovarian follicles and cultured in medium supplemented with serum, resume meiosis and progress from the germinal vesicle stage through the first meiotic metaphase (metaphase I) to metaphase II with first polar body formation (*1*). This maturation normally occurs in vivo just before ovulation. While oocytes of other mammals will also mature in medium with serum, thus far the only one whose oocytes have been matured in chemically defined media is the house mouse (*2*). In this species, pyruvate or oxaloacetate in a Krebs-Ringer salt solution with bovine serum albumin supports maturation, which suggests the operation of certain metabolic pathways in the oocyte. Mouse follicular cells, when included in the culture system, utilize other energy sources to support maturation, probably by liberating pyruvate into the culture medium (*3*). This report, based on a study of 426 oocytes, describes the first successful use of chemically defined media to support maturation of human oocytes and assesses the need for the presence of the follicular cells (cumulus) during maturation.

In comparing the ability of different media to support maturation, two difficulties were encountered—(i) the number of oocytes obtained by puncturing all visible follicles ranged from 0 to 66 per ovary, with a mean of 10, from 90 patients; (ii) the patients for elective gynecologic surgery varied in age, race, stage of menstrual cycle, and pelvic pathology. Therefore, oocytes from each patient were randomly divided into experimental and control groups under paraffin oil in the collection medium (the medium with the fewest components in each experiment). The control oocytes were cultured in a medium that in our hands had previously supported maturation. In the experiments in which cumulus cells were detached from the oocyte, all cells surrounding oocytes of the experimental group were removed by sucking the oocyte in and out of a drawn Pasteur pipette. Each group of oocytes was washed twice in the appropriate culture medium and finally transferred to microdroplets (two to six per droplet) of this medium (*p*H 7.0) under paraffin oil (*2, 4, 5*).

After 43 to 47 hours at 37°C, oocytes were taken from culture and the cumulus removed from those not previously so treated. Oocytes were then fixed, stained, and examined for the nuclear stage attained (*2*). In only one instance was a polar body not identified when a metaphase II group was

Table 1. Comparison of the number of human oocytes maturing in defined and undefined media. Defined medium, F10 with 4 mg of bovine serum albumin per milliliter; undefined medium, 199 with 15 percent fetal calf serum.

Patient	Medium	Number of oocytes cultured	Number of oocytes maturing at 43 to 47 hours		
			Metaphase I	Metaphase II	Total (I and II)
1	199	19	1	9	10
	F10	13	0	9	9
2	199	9	0	6	6
	F10	9	1	3	4
3	199	17	3	3	6
	F10	11	3	6	9
4	199	4	1	2	3
	F10	5	1	1	2
Total	199	49	5 (10.2%)	20 (40.8%)	25 (51.0%)
	F10	38	5 (13.2%)	19 (50.0%)	24 (63.2%)
Probability [$\chi^2(1)$]			>.75	>.25	>.25

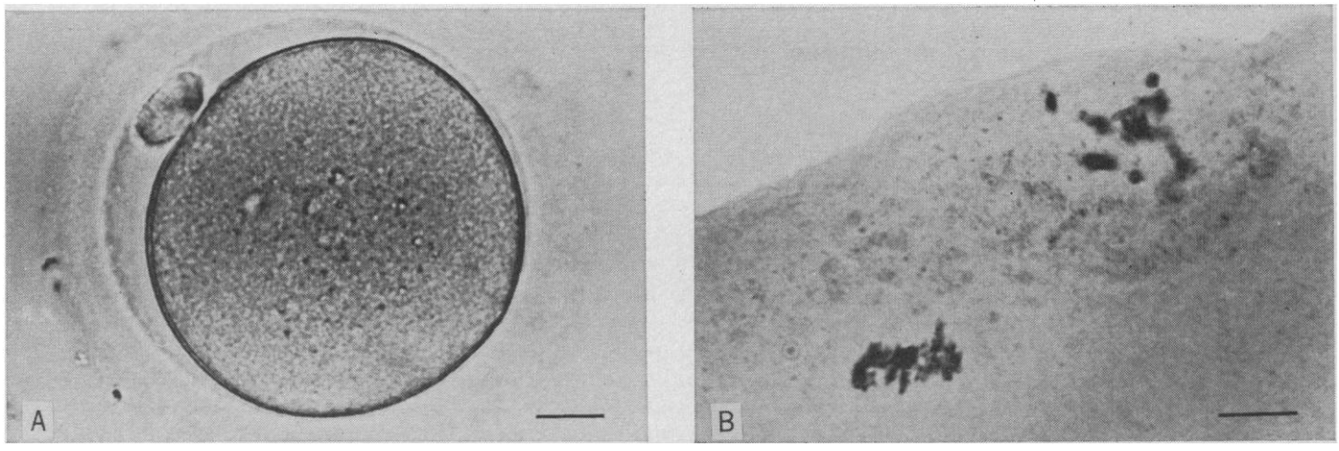

Fig. 1. (A) A living human oocyte after 43 hours of culture without cumulus in 1.0 m*M* pyruvate Krebs-Ringer medium. The first polar body is at the upper left adjacent to the oocyte proper. Both are surrounded by the zona pellucida. Scale, 20 μm. (B) A human oocyte fixed and stained after 43 hours in medium F10 with intact cumulus. An organized metaphase II group of chromosomes is present and in the upper right is the scattered chromatin of the first polar body. Scale, 10 μm.

found. Sixty-nine oocytes, from a number of patients, were similarly examined upon recovery from the ovary, and all were found to have a germinal vesicle, sometimes degenerated, which indicates that very few oocytes obtained in this manner mature while still in the follicle.

We found that 50 percent of 156 oocytes with attached cumulus reached metaphase I (17 percent) or metaphase II (33 percent) in the defined medium F10 (*6*) (Fig. 1). Forty-six percent of 13 oocytes also matured to metaphase I (31 percent) or II (15 percent) in the simple, defined Krebs-Ringer medium, containing pyruvate, lactate, and glucose, used by Whitten and Biggers (*7*) to culture mouse zygotes to blastocysts and by Whittingham (*8*) for the fertilization of mouse oocytes. A modification of the Krebs-Ringer medium developed by Brinster (*9*) to support mouse embryo cleavage, and containing 0.25 or 1.0 m*M* pyruvate (*5*) as the energy source, also supported human oocyte maturation (Fig. 1). Only three of 28 oocytes matured in the 0.25 m*M* pyruvate medium. In a single experiment in which we used 1.0 m*M* pyruvate, 16 percent of 25 oocytes with attached cumulus reached metaphase I (16 percent) and II (0), while 54 percent of 22 oocytes with cumulus removed reached metaphase I (36 percent) and II (18 percent). The small number of patients and oocytes used to test these simpler media prevents a conclusion about the nutritional requirements for maturation. The results do suggest, however, that the human oocyte may be similar to the mouse oocyte, in which pyruvate appears to be the key nutrient (*2*). The apparent arrest at metaphase I of some oocytes in the various media is unexplained.

To compare the ability of oocytes to mature in medium 199 (*10*) with serum as previously used (*1*) and the defined (serum-free) medium F10 used in this study, 87 oocytes were cultured (Table 1). There was no significant difference between these two media in the number of oocytes maturing to metaphase I or II, or both. The lower percentage of oocytes maturing in media 199 and F10 (51.0 percent and 63.2 percent) compared with the previously reported figure of 80 percent of 89 oocytes (*1*) may be due to differences in selection of oocytes for culture. We placed into culture all oocytes recovered from the ovary, including those obviously degenerated or necrotic.

In nine experiments with medium F10, oocytes with the cumulus cells still attached (control group) progressed in greater numbers at all stages than those from which they were removed. Seventeen percent and 13 percent of 71 oocytes with cumulus attached and 9 percent and 6 percent of 64 oocytes with cumulus removed matured to metaphase I and II, respectively, but only the difference at metaphase II was significant [$P < .066$ by $\chi^2(1)$]. Several explanations for these results may be suggested. Removal of the cumulus may have injured some oocytes. Also, the cumulus may be required to supply unique substances to the oocytes. A third possibility is that the cumulus may detoxify substances in the medium inhibitory to maturation of the denuded oocyte.

The use of defined media opens the way for investigation of human oocyte metabolism as well as oocyte-follicular cell interactions. Techniques for interrupting these processes could lead to newer methods of contraception. Moreover, by analogy with the mouse (*2*), the in vitro requirements for oocyte maturation may give clues to the metabolic pathways emerging in the cleaving human embryo.

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