Pronuclear Ovum from a Patient Using an Intrauterine Contraceptive Device

Abstract. *Ova were sought in the washings of the excised reproductive tracts of 48 patients after they had ovulated. From 24 patients using no contraceptives, two unfertilized ova were recovered. From 24 patients using intrauterine coils, one unfertilized and one fertilized pronuclear ovum were recovered. The latter ovum was at the earliest stage of development recorded for the human.*

The ovum shown in Figs. 1 and 2 was recovered from the oviduct of a 33-year-old patient who had undergone vaginal hysterectomy for uterine prolapse. Four months before the operation the patient had delivered her fifth child, and she had used a Margulies 5J intrauterine coil continuously for 2 months prior to the operation. She had had intercourse 100, 75, and 30 hours before the operation, which was performed on day 16 of the menstrual cycle.

Neither ovary appeared to have ovulated, and no ovarian biopsy was taken. The right oviduct was arbitrarily removed along with the uterus. Five milliliters of 0.85 percent sodium chloride were flushed through the oviduct from the uterine end and collected in a watch glass. A dense mass of cells measuring 2 by 2 by 3 mm was observed. A few drops of hyaluronidase (150 units/ml) were added, and, after waiting an hour at room temperature, the cell mass was broken apart with fine glass probes under a dissecting microscope.

A vitellus measuring 103 μ in diameter was then observed, and although several dozen granulosa cells remained attached, the zona pellucida could not be seen distinctly under the dissecting microscope. However, when the ovum was compressed lightly under a cover slip, the zona pellucida was visible under phase contrast as a granular ring pierced by granulosa cell processes (Fig. 1).

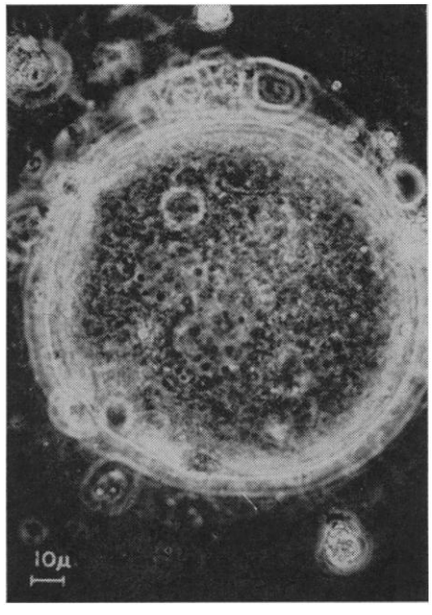
Two pronuclei were near the center of the vitellus, and, after lacmoid (resorcinol blue) staining, the tail of the fertilizing spermatozoon and two pronuclei were observed in detail (Fig. 2). This stage of development has not previously been clearly seen in a human ovum.

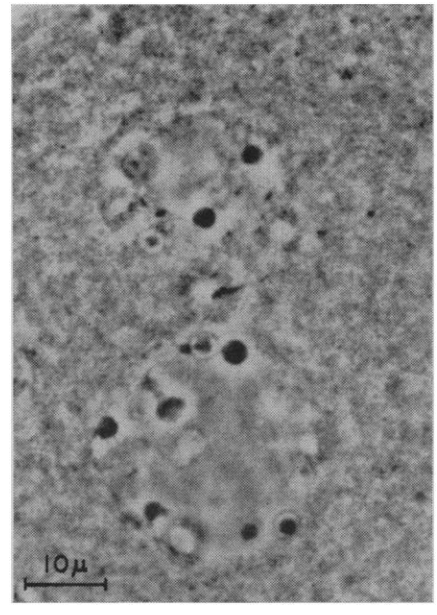
The endometrium (Fig. 3) was developed to postovulatory day 3 according to the standards of endometrial dating (*1*). There was no evidence of infection, hemorrhage, or fibrosis of the endometrium resulting from the use of the intrauterine coil.

Among 23 patients operated upon while they were using the intrauterine contraceptive, one unfertilized ovum was recovered from an oviduct. From a control group of 24 patients using no contraceptives, two unfertilized ova were recovered. Factors that might have contributed to the low rate of ovum recovery were: selection of suitable candidates, predicting the time of ovulation, organ manipulations inherent in the operative procedure, and techniques of ovum recovery from the excised reproductive tract.

The intrauterine contraceptive device, formerly condemned as a dangerous foreign-body abortifacient, is being intensively reevaluated. Recent studies (*2*) have shown the intrauterine device to be safe and effective, but how it prevents pregnancy is unknown. Tietze (*3*) found the incidence of tubal pregnancy to be unusually low in patients using intrauterine contraceptives. This suggests that there may be a block to fertilization, or alternatively, an interference with ovular development, or altered transport of the developing ovum in the oviduct. Spermatozoa have been recovered from the oviduct above the intrauterine device (*4*), but fertilization of the ovum in the oviduct has not previously been reported.

It is clear that the ovum described herein was fertilized by a spermatozoon that had ascended the endometrial cavity past the coil. Whether this pronuclear ovum would have continued to develop and then would have passed into the uterus at the proper time to permit implantation is unknown. How-





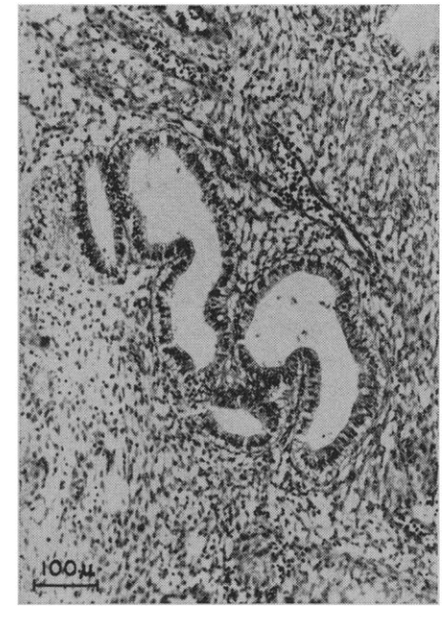


Fig. 1 (left). Living ovum from human oviduct after removal of most of the granulosa cells (phase contrast microscopy). Fig. 2 (middle). The same ovum as that shown in Fig. 1 after staining with lacmoid; showing pronuclei and sperm tail. Fig. 3 (right). Section of endometrium showing early postovulatory response.

ever, since it is already known that pregnancy can occur in very rare instances in spite of the coil, our findings may be an example of such a rare occurrence.

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5. A more extensive paper concerned with the pronuclear ovum will be published at a later date. Supported by USPHS grant HD-00673-01, by the Ford Foundation, by the National Foundation, and by the Association for the Aid to Crippled Children.

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Lymphocyte Lifetime in Women

Abstract. *The lifetime of the lymphocyte in hematologically normal women is 530 ± 64 days. This estimate, made from studies on 25 women who had received radiation therapy for cervical carcinoma, is based on the rate of disappearance of lymphocytes with acentric chromosome fragments.*

Ottesen (*1*) studied the incorporation of P^{32} into the DNA of the lymphocytes of two hematologically normal women. He found that the lymphocytes could be separated into two groups: a minor one with a survival time of 3 to 4 days, and a major one with a survival time of 100 to 200 days. Hamilton (*2*) studied the incorporation of adenine-8-C^{14} into the DNA of patients with chronic lymphocytic leukemia and also found two rates of disappearance of radioactive DNA; the slower rate indicated a half-life of about 300 days. However, Hamilton (*2, 3*) has pointed out that the length of survival of the radioactive DNA may be due to reutilization of the DNA rather than to a long-lived lymphocyte. We have measured the lifetime of the lymphocyte in hematologically normal women by another method and obtained a value for the average life of the lymphocyte of 530 ± 64 days. This value indicates a longer lifetime for the normal lymphocyte than had been supposed.

Our method is based on the fact that radiation produces acentric chromosome fragments in the lymphocytes. Such chromosome fragments are almost never found except in the cells of people who have been exposed to radiation. Moreover, since acentric fragments lack the centromeres that control chromosome movement on the mitotic spindle, they will not be distributed by the spindle to the daughter cells; indeed they will be lost to the nuclei and degenerate in the cytoplasm (*4*) at the first cell division after radiation. Thus an acentric chromosome fragment in the lymphocyte is assumed to mean that the lymphocyte has not divided between the time the patient was exposed to radiation and the time of observation; and the rate of decrease of lymphocytes with acentric fragments provides a direct measure of the lifetime of the lymphocyte.

The 25 patients in this study had all been treated for cervical carcinoma by delivery of 6 to 8000 rads of radiation to the paracervical triangle over a period of about 1 month. From samples of peripheral blood the leukocytes were cultured for 72 hours, and chromosome preparations were made by standard techniques. Linear regression analysis of the relation between lymphocytes with acentric chromosome fragments and time after radiation therapy (Fig. 1) shows that the percentage of lymphocytes, y, with acentric fragments is related to the number of days, x, after therapy by the equation

$$y = (16.5 \pm 1.2) \exp[-(0.00189 + 0.00023)]x$$

From this relation we obtain the estimate of an average life of 530 ± 64 days or about 18 ± 2 months.

Buckton *et al.* (*5*) have described the persisting of chromosome aberrations in patients who have received radiation therapy for ankylosing spondylitis. The extent of their data on cells with acentric chromosome fragments does not allow a good estimate of the lymphocyte lifetime. Their data on the rate of decrease of so-called unstable cells—cells with acentric fragments, dicentrics, or rings—is extensive, but there is a significant probability that the dicentrics and rings will survive one or more cell divisions; therefore, the average life of 29 months of the unstable cells shown by their data must be taken as an upper limit for the lifetime of the lymphocyte. The average life of 18 months, estimated from the rate of disappearance

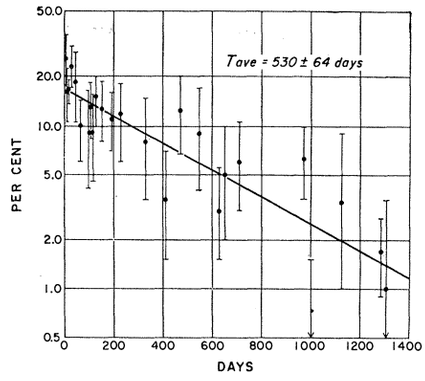

Fig. 1. Percentage of lymphocytes with acentric chromosome fragments plotted against the time after the end of radiation therapy. The vertical lines through each point are the 80 percent confidence intervals.

of lymphocytes with acentric chromosome fragments, is about 40 percent lower than the upper limit of 29 months, estimated from the rate of disappearance of unstable cells. The discrepancy between the two estimates can be accounted for by assuming a probability of about 40 percent per cell division that a dicentric or ring chromosome will survive intact. A preliminary estimate of this probability based on our own data is 33 percent.

A minor lymphocyte component with a survival time of 3 to 4 days (*1*) would not be detectable from our data, which were obtained after the end of a relatively long therapy period. To the extent that an undetected short-lived lymphocyte did influence our data, its effect has been to cause an underestimate of the average lifetime of the long-lived lymphocyte.

Whether the average life of the long-lived lymphocyte is a function of sex, age, or other conditions in man remains to be determined.

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