steady increase in synthesis when the irradiation is performed in nitrogen-bubbled medium, followed by a break to a lower rate of synthesis at about 60 minutes. The rate was apparently much less when the irradiation took place in oxygen. The results show that this type of experiment includes both the degradation after radiation and also synthesis. If correction is made for the amount degraded (data of Fig. 1), then the corrected (solid) lines are obtained. Synthesis continues at an unchanged rate for both conditions of irradiation, but the cessation of synthesis occurs earlier when the medium is oxygenated. In Fig. 4 we show the same kind of data for a dose of 20 kr. The effect is much more marked; there is a depression of synthesis early in the experiment and a subsequent leveling off. A comparison of Figs. 3 and 4 indicates that the effect produced, in oxygen, by 10 kr would have been approximately duplicated by 15 kr in nitrogen. Thus the dose reduction factor is of the order of 1.5 and certainly very much less than that found for the process of degradation. It is interesting that the cessation of synthesis seems to be associated with the division time of the culture, occurring a little later.

When the cells were irradiated on filters, a condition corresponding to a full oxygenation, the label was given only after irradiation so that correction for degradation was not necessary; the results were in agreement with the aforementioned corrected values for oxygen.

Since these effects are observed at doses which are of the same order as those observed for the loss of colony-forming ability, the most commonly observed effect of ionizing radiation—the dose for 37 percent survival for these cells is 3 kr in oxygen and 12 kr in nitrogen—it is quite attractive to suppose that one of the primary damaging actions of ionizing radiation might be on the DNA. The oxygen effect is characteristic of the effect of ionizing radiation on living cells, and, because it is observed so clearly in the case of DNA degradation, the hypothesis that a considerable part of the oxygen effect is due to this degradation is attractive. The lesser effect of oxygen on the synthesis of DNA indicates the presence of a second process. The fact that the amount of DNA degraded when radiation occurs with oxygen present does not exceed 50 percent suggests that a part of the DNA is especially sensitive to this type of radiation. We suggest that one strand of the DNA is more sensitive than the other.

ERNEST C. POLLARD
PHILLIP M. ACHEY
Biophysics Department, Pennsylvania State University, University Park

References and Notes

1. L. H. Gray, A. D. Conger, M. Ebert, S. Hornsey, O. C. A. Scott, *Brit. J. Radiol.* **15**, 72 (1959).
2. P. Howard-Flanders, *Advan. Biol. Med. Phys.* **6**, 553 (1958).
3. E. Pollard and P. Macaulay, *Radiation Res.* **15**, 120 (1961).
4. J. H. Stuy, *ibid.* **14**, 57 (1961).
5. B. Miletic, Z. Kucan, L. J. Sasel, *Intern. J. Rad. Biol.* **7**, 141 (1964).
6. B. Miletic, Z. Kucan, D. J. Novak, *Nature* **202**, 106 (1964).
7. E. Pollard and C. Vogler, *Radiation Res.* **15**, 109 (1961).
8. D. Billen, *Biochim. Biophys. Acta* **72**, 608 (1963).
9. The *E. coli* 15 T-L- was obtained from Frederick Forro of Yale University.
10. R. B. Roberts, D. B. Cowie, P. H. Abelson, E. T. Bolton, R. J. Britten, "Studies of biosynthesis in *E. coli*," *Carnegie Inst. Washington, Publ. No. 607* (1953).
12. We thank Dr. Stan Person for very helpful discussions. Work supported by AEC contract AT(30-1)2804.

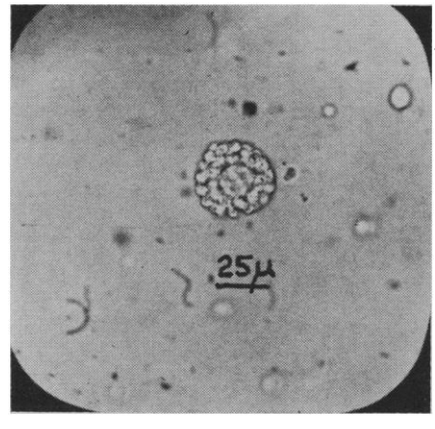
15 July 1964

Enzymatic Dissection of the Mammalian Ovary

Abstract. *Incubation of the ovaries of rats and mice with the proteolytic enzyme "pronase" caused the step by step dispersal of cellular elements from the tissue. The first subunits to be liberated were the outermost follicles or corpora lutea or both. Next, these subunits were dispersed. It is possible to obtain isolated single follicles in all stages of development: corpora lutea, isolated cell layers from within follicles, suspensions containing free cells, and ova in all phases of maturation.*

Proteolytic enzymes have been utilized for the dispersal of both embryonic and adult tissues (*1*). Pronase, a partially purified protease from the actinomycete *Streptomyces griseus* (*2*), has been used for removal of the zona pellucida (*3*), and in the dispersal of several types of tissue (*4*).

To obtain isolated follicle cells, the ovaries of 30 rats and mice were



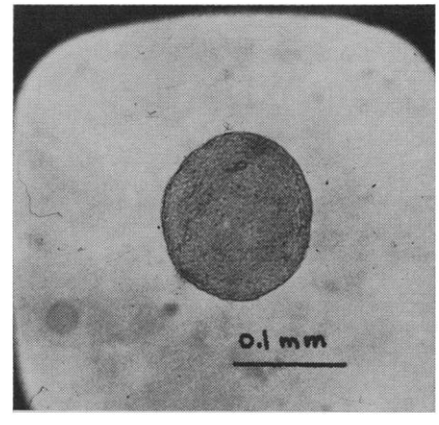


Fig. 1 (left above). Isolated primary follicle, unstained. The ovum is surrounded by follicle cells and can be seen in the center of the structure.

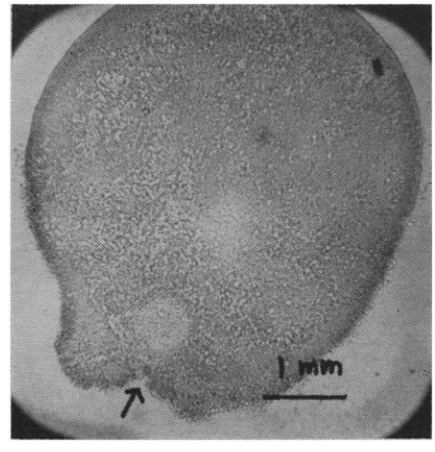


Fig. 2 (right above). Growing follicle, unstained. The size corresponds to that of a follicle at the beginning of antrum formation. The ovum is not visible, owing to the number of cell layers making up the structure.

Fig. 3 (left). Graafian Follicle, unstained. The follicle is in the process of dispersal (arrow). The ovum can be seen as the pale area near the indicated zone of dispersal.

studied (13 were immature and 17 were undergoing estrus cycles). The animals were killed by cervical dislocation; the ovaries were removed, dissected free of fat and oviducts, chilled, washed, and exposed for varying intervals to 0.5 percent pronase made up in a Ca^{++}- and Mg^{++}-free balanced salt solution at *p*H 6.8 to 7.0. The ovaries were placed in the enzyme solution at 37.5°C and were agitated every 10 minutes. As dispersal of the ovary occurred, samples of fluid containing cellular materials were taken and examined microscopically. Cell viability was assessed by testing the dispersed material with nigrosin (*5*).

When the ovaries were taken from immature animals at ages close to the onset of vaginal opening, the elements initially found in the fluid were single whole follicles. These follicles were the outermost follicles of the ovary. After release, each follicle dispersed; the outer cell layers were liberated first. Concurrent with the dispersal of the liberated follicles from the periphery, follicles from the interior of the ovary were released. Disaggregation of the follicle took place only after release from the ovary.

The treatment of ovaries from animals in the follicular phase of the estrus cycle yielded follicles in all stages of maturation (Figs. 1–3). It was possible to obtain intact follicles with large antra but these were more susceptible to dispersal or to rupture than were the less mature follicles with small antra or those at stages prior to antrum formation.

Corpora lutea, when present, were released first, then follicles from the interior. Suspensions of luteal cells were obtained by subjecting the liberated corpora lutea to the enzyme.

When enzymatic degradation of the ovary was attempted with other proteolytic enzymes (trypsin, chymotrypsin, pancreatin, collagenase, elastase) suspensions of cells appeared without the step by step breakdown that took place with pronase.

After dispersal the cellular elements were collected by either mild centrifugation or on Millipore filters, washed with saline, and resuspended in a balanced salt solution containing 0.5 percent nigrosin. In most instances only 10 to 15 percent of the free cells allowed the dye to permeate, and no dye penetration occurred in the whole follicles or corpora lutea.

Any of the tissues isolated may be suitable for study. Embryonic cells isolated by pronase have been used to establish cell cultures (*6*). Other data indicate that the ovarian elements obtained by pronase digestion can be grown in culture and may retain their function.

HOWARD S. GROB*
Department of Biological Sciences, Hunter College, New York

References and Notes

1. L. M. Rinaldini, *Intern. Rev. Cytol.* **7**, 587 (1958).
2. M. Nomoto, Y. Narahashi, M. Murakami, *J. Biochem. Tokyo* **48**, 593, 906 (1960); pronase obtained from Calbiochem Inc., Los Angeles, Calif.
3. B. Mintz, *Science* **138**, 594 (1962); R. B. L. Gwatkin, *Proc. Natl. Acad. Sci. U.S.* **50**, 576 (1963).
4. R. B. L. Gwatkin and J. L. Thompson, *Nature* **201**, 1242 (1964); H. Guthwin and I. Hazen, personal communication.
5. J. P. Kaltenbach, M. H. Kaltenbach, W. B. Lyons, *Exptl. Cell Res.* **15**, 112 (1958).
6. B. W. Wilson and T. L. Lau, *Proc. Soc. Exptl. Biol. Med.* **114**, 649 (1963).
7. We thank Miss A. E. Schimenti for technical assistance.
* Present address: Department of Physiology and Pharmacology, College of Dentistry, New York University, New York.

7 July 1964

Conduction of the Action Potential in the Frog Ventricle

Abstract. *Cardiac muscle fibers are made up of individual cells joined end to end; it has been suggested that the action potential must be conducted from cell to cell by some specialized mechanism. The alternative is that the electrical resistance is low at the junctions between the cells, so that the fiber behaves like a cable. The shape and time course of the foot of the action potential and the conduction velocity in the frog ventricle fit the predictions made from the cable theory.*

The conduction of the action potential in nerve and skeletal muscle is understood from core conductor theory; that is, the tissue is regarded as a long cable, with the cell membrane separating solutions of relatively low resistance on the inside and outside of the fiber. When one region of the fiber is excited, a current flows from the excited membrane through the intracellular solution and outward through the resting membrane. This local current flow depolarizes the resting membrane and leads to excitation (*1*). Many cardiac electrophysiologists have assumed that the same principles apply to conduction in the heart. A difficulty arises because the fibers of cardiac muscle are made up of individual cells, separated one from the other at the intercalated discs (*2*). The interposed membrane might add a large resistance to longitudinal current flow and prevent the flow of local currents.

Some studies suggest that the membranes at the intercalated discs are barriers to current flow (*3*, *4*). One possibility is that transmission across the discs is achieved by the release of a chemical (*5*); most of the evidence for specialized transmission mechanisms comes from experiments on the frog ventricle. On the other hand, the discs must have quite a low resistance for electrical transmission to occur (*6*).

If the fibers in the frog ventricle do behave like a cable, the initial depolarization at the earliest part of the rising phase (the foot) of the action potential would be generated by local current flow from excited regions of the fiber. The foot of the action potential should be exponential, and the time constant, t_c, of the foot of the action potential will be given by:

$$\frac{2\lambda^2}{v\,[v\tau + (v^2\tau^2 + 4\lambda^2)^{1/2}]} = t_c \qquad (1)$$

where λ is the space constant, τ is the time constant of the membrane, and v is the conduction velocity (*7*). Our experiments were conducted to see whether the time course of the foot of the action potential in the frog ventricle could be predicted by Eq. 1. To do this we had to know the time constant of the foot of the action potential, the space constant, the time constant of the membrane, and the conduction velocity.

The foot of the action potential of the isolated frog ventricle in Ringer solution was recorded by using glass capillary microelectrodes filled with 3*M* KCl, a capacity-compensated preamplifier, and an oscilloscope. The ventricles were stimulated electrically with 2 msec square waves applied between a cathode in the tissue and an anode in the Ringer solution. The sample record in Fig. 1*A* shows that the rapid upstroke of the action potential is preceded by a slower depolarization—the foot of the action potential. In Fig. 2, the semilogarithmic plot of the time course of the foot of another action potential shows that the depolarization is exponential. The time constant t_c was determined from plots such as that shown in Fig. 2. Conduction velocity (v) was estimated by assuming that the action potential was conducted along a direct line between the stimulating and recording electrodes. In some of the experiments conduction velocity was slowed by cooling the tissue.

The membrane time constant, τ, was