The segment beyond the break may be similar, positively accelerated, or possibly linear. In our CD rats, the break occurred as early as 18 weeks, but in the Wistar rats it was generally not seen before 24 weeks.

Similar breaks might occur at later ages in CD rats whose curves currently appear to be continuous. Indeed, in the curve plotted in Fig. 1 (bottom), one might predict a discontinuity or a prolonged plateau beginning at about 31 or 32 weeks, since the first derivative of the function equals zero in that region. If this logic is applied to the sample data of Fig. 2, however, only in the case of CD-16 does occurrence of the break tend to agree with expectation. For the most part, discontinuities appear sooner than predicted (*5*).

The earliest age at which a discontinuity can be expected in heterozygous animals, such as the CD and Wistar rats, would appear, at present, to set the upper limit of the age range over which an extrapolation technique of this type could be usefully employed. If inbreeding were to yield more uniform individual growth curves, or if, through other means, the discontinuities could be eliminated or better understood, it is possible that the useful age range and predictive power of the technique might be extended.

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References and Notes

1. The data of Fig. 1 (top) were recorded by one of us (M.K.) at Columbia University during his tenure as post-doctorate research fellow of the National Institute of Mental Health under sponsorship of F. S. Keller. Similar findings were obtained with four additional Wistar rats.
2. The decline is not related to the *ad lib.*–feeding weight preceding rhythm feeding. In the seven rats observed, the rank-order correlation between this weight and per cent *ad lib.*–feeding weight on the 21st day was 0.179.
3. This work was facilitated by grant B-1273 from the U.S. Public Health Service to John R. Whittier. The CD rats, specific pathogen-free until shipment, are derived from Sprague-Dawley stock. All Wistar and CD rats referred to in this paper were treated alike, weighed on a dietary scale, and fed Purina Laboratory Chow in meal form. In our colony areas, temperature usually ranged from 75° to 78°F, and relative humidity varied between 40 and 50 percent.
4. The latter possibility was called to our attention by C. A. Slanetz while this report was being prepared. See L. M. Zucker, "Growth criteria," in *Rat Quality: A Consideration of Heredity, Diet and Disease* (National Vitamin Foundation, Inc., New York, 1953), pp. 3–22.
5. When parabolas are fitted to the data of CD-16 and 17 for weeks 12 through 20, and CD-13 and 20 for weeks 12 through 18, $dy/dx = 0$ for the respective ages of 20.8, 24.5, 25.8, and 22.8 weeks. Discontinuities seen in Fig. 2 appear at the respective ages of 21, 21, 18, and 19 weeks.

28 November 1953

Autoradiographic Study of Uptake of Tritiated Glycine, Thymidine, and Uridine by Fruit Fly Ovaries

Abstract. Synthesis of DNA occurs in the nurse cell nuclei of *Drosophila melanogaster* in an asynchronous manner, whereas synthesis of RNA occurs in all these nuclei simultaneously. Synthesized RNA is concentrated in the plasmosomes; subsequently nuclear RNA enters the cytoplasm of the nurse cell and eventually the oöplasm. Protein synthesis occurs in the nucleoplasm and cytoplasm of all the cells in the egg chamber.

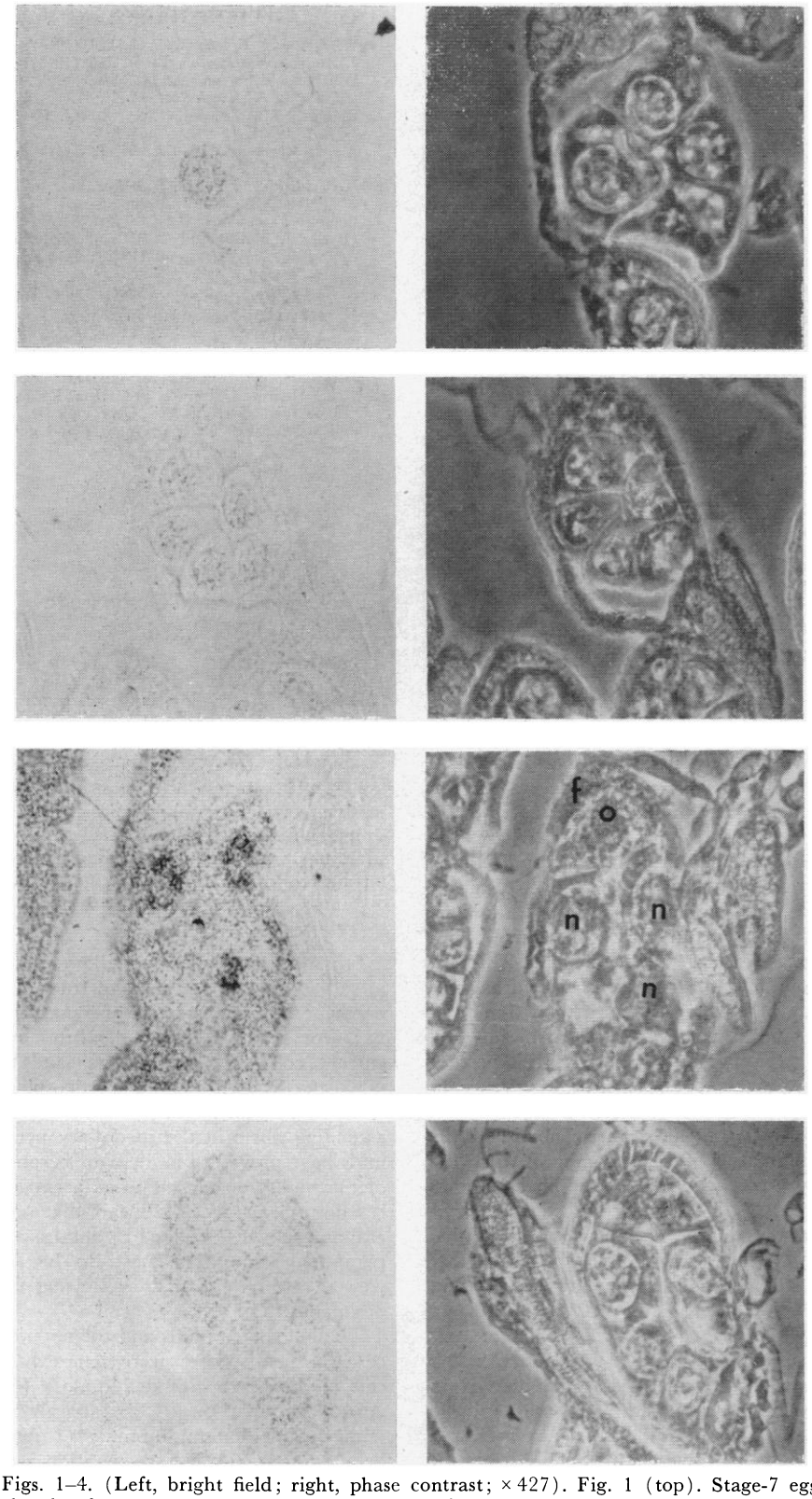
Six-hour-old, Oregon-R, wild type, adult, female *Drosophila melanogaster* were starved for 24 hours and then allowed to feed for 1 hour on a gram of yeast paste (two parts autoclaved, dry baker's yeast/three parts sterile water) containing either 25 μc of uridine-H^3 (1/40), 25 μc of thymidine-H^3 (1/87), or 250 μc of glycine-$2H^3$ (1/3720). The numbers in parenthesis refer to the ratios between radioactive and nonradioactive molecules in the respective radioactive solutions before the addition of yeast. The tritium atom of thymidine or uridine is attached to a carbon of the pyrimidine ring; the tritium atoms of glycine are attached to the amino carbon atom. Subsequently the flies were etherized (for the first time) and placed under insect Ringer's solution, and their ovaries were removed. The ovaries were fixed for 20 minutes in Kahle's fluid, dehydrated, infiltrated first with celloidin and then with paraffin and sectioned at 6 to 8 μ. The sections were mounted on albuminized slides, the paraffin was dissolved away, the tissue was then covered with stripping film (Kodak autoradiographic A.R.10), dried, and left exposed for 3 weeks at 3°C. The film was then developed and the preparation was coated with immersion oil (R.I.. 1.46) and viewed under bright-field and phase-contrast optics.

The silver grains observed occur above those molecules (presumably certain types of protein, RNA and DNA) made within the last hour of the fly's life which after Kahle fixation are insoluble in water, ethanol, benzene, methylbenzoate, and paraffin and which have glycine, uridine, or thymidine as precursors. Ovaries from flies fed glycine-$2H^3$ contained about 4 times, and ovaries from flies fed uridine-H^3 about 3 times, as much tritium as ovaries from flies fed thymidine-H^3 labeled, dead yeast.

The developing egg consists of a 16-cell nest surrounded by an envelope of follicle cells. The 16 cells are daughters which arise from four consecutive divisions of an oögonium. Fifteen of the daughter germ cells differentiate into nurse cells and nourish the most posterior, daughter germ cell which becomes the oöcyte. Intercommunication of cytoplasm between all members of the 16-cell cyst is made possible by pores in the walls separating adjacent cells (*1*). The development of the nest of 16 cells has been subdivided into a series of consecutive stages, ending with stage 14, the mature, ovarian, primary oöcyte (*2*). During the first seven stages all 16 germ cells grow at roughly identical rates. During stages 8 through 11 vitellogenesis occurs, and the oöcyte grows at a much faster rate than previously, at the expense of the nurse cells, which shrink and eventually degenerate. The follicular epithelium secretes first during stages 8 to 11 the vitelline membrane about the oöcyte and next during stages 11 through 13 the chorion. The ovaries observed contain oöcytes in stages 1 to 8 and 14 (plus an occasional one in stages 9 and 13). Stage 14 oöcytes (presumably formed prior to the first labeled meal) gave no autoradiograph.

It is known from the work of J. J. Freed [summarized by Schultz (*3*)] that nurse cell nuclei undergo a series of endomitotic doublings of DNA. In the case of the nurse cell nuclei observed in the thymidine study, the densities of the autoradiographs increased with increasing nuclear volume. However, not all the nuclei in an egg chamber showed an autoradiograph (Fig. 1), which indicates a nonsynchronous synthesis of DNA among the 15 nurse cell nuclei of an egg chamber. Tritium from thymidine was also localized in follicle cell nuclei.

On the other hand, tritium from labeled uridine was found in all the nurse cell nuclei in an egg chamber, which indicates that RNA synthesis is going on simultaneously in all the nurse cell nuclei of an egg chamber. Tritium from ingested uridine was distributed nonhomogeneously in nurse cell nuclei, and in large chambers (like those at stages 7 and 8) tritium can be shown to be localized mainly in the plasmosomes. The term *plasmosome* refers to an RNA-containing nucleolus. The tritium appeared first in the nurse cell nuclei (Fig. 2), and it subsequently appeared in the nurse cell cytoplasm as well, but at lower concentrations (Fig. 3). Similar densities of silver grains are found above the cytoplasm of nurse cells and the follicular epithelium. In stage-7 and -8 chambers the tritium in the follicular epithelium was concentrated to a greater extent in the nuclei than in the cytoplasm, but this nonhomogeneity in distribution cannot be demonstrated in earlier chambers because of the small size of the follicle cells. Under our conditions little tritium from uridine accumulated in yolky oöplasm and none in the oöcyte nucleus. In stage-13 oöcytes, tritium



Figs. 1–4. (Left, bright field; right, phase contrast; ×427). Fig. 1 (top). Stage-7 egg chamber from the ovary of a fly fed thymidine-H^3. The section passes through three nurse cell nuclei, only one of which gives an autoradiograph. Fig. 2 (upper middle). Stage-7 egg chamber from the ovary of a fly fed uridine-H^3. The section passes through five nurse cell nuclei, all of which give an autoradiograph. Fig. 3 (lower middle). Stage-8 egg chamber from the ovary of a fly fed uridine-H^3. The section passes through three nurse cell nuclei (*n*) and through yolky oöplasm (*o*). The density of developed grains is greatest above the nurse cell plasmosomes, next greatest above nurse cell nucleoplasm and cytoplasm and the cytoplasm of the columnar follicle cells (*f*), and least above the oöplasm. Fig. 4 (bottom). Stage-8 egg chamber from the ovary of a fly fed glycine-$2H^3$. Tritium is distributed homogeneously throughout the chamber.

from ingested, labeled uridine was localized in the epithelium surrounding the developing chorionic appendages.

In the case of ovaries labeled with tritium from ingested glycine, a homogeneous distribution of silver grains is seen above the chambers in stages 1 to 8 (Fig. 4). The concentration of grains rises with increasing chamber size. For example, autoradiographs above stage-8 chambers had 5 times as many grains per unit area as did those above stage-2 chambers. Since the stage-8 chamber has a volume 100 times that of a stage-2 chamber, the tritium content must be 500 times greater. In a late stage-9 chamber yolky oöplasm has about one-half as much tritium as the cytoplasm of nurse and follicle cells. Nurse-cell plasmosomes showed more tritium than the surrounding nucleoplasm. Stage-9 oöplasm may contain less tritium from ingested glycine than the cytoplasm of adjacent follicle cells because of the barrier provided by the newly synthesized vitelline membrane. Glycine can now enter the oöcyte only by way of the nurse cell chamber. Stage-13 oöcytes show an autoradiograph above the degenerating nurse cell nuclei and above the epithelium surrounding the developing chorionic appendages (*4*).

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References and Notes

1. See R. C. King and R. L. Devine, *Growth* 22, 299 (1958).
2. See R. C. King, A. C. Rubinson, R. F. Smith, *ibid.* 20, 121 (1956).
3. J. Schultz, *Cold Spring Harbor Symposia Quant. Biol.* 21, 307 (1956).
4. This work was supported by the U.S. Atomic Energy Commission (contract No. AT(11-1)-89, project 12), the National Science Foundation (research grant NSF-G 4816) and by the graduate school of Northwestern University. Valuable technical assistance was performed by H. Pakeltis and A. Bartha. The labeled uridine and glycine were supplied by the New England Nuclear Corp. The labeled thymidine was supplied by Schwarz Laboratories.

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An Auxin-like Action of Coumarin

Abstract. Coumarin, usually regarded as an inhibitor of growth processes in plants, markedly stimulated the elongation of excised segments of *Helianthus* hypocotyls. Substitution in the molecule of hydroxy-, methyl-, or chloro-groups, in the neighborhood of the unsaturated bond in the lactone ring, markedly altered the growth-promoting activity.

Coumarin has long been known to be an inhibitor of germination and root growth (*1–3*). It has also been reported that coumarin inhibits auxin-induced elongation, as measured by the *Avena*