with the pattern of control established for the somewhat larger adaptive changes of the tyrosine-α-ketoglutarate transaminase (*2*). A corticoid-induced "metabolic state" (*4*), perhaps the basis for the "permissive" action of cortisone, is considered to be required for these two adaptive responses to the substrate stimuli. In contrast, the tryptophan pyrrolase (peroxidase-oxidase) level is also increased by corticoids, but the substrate is a sufficient stimulus by itself to adaptively increase this enzyme level (*5, 6*).

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6. Acknowledgement is made of grants by the U.S. Atomic Energy Commission and the U.S. Public Health Service.

29 January 1959

Growth of Body Weight and Manipulation of Food Motivation

Abstract. Consideration was given to the possible use of individual growth curves to estimate *ad libitum*–feeding weights as part of technique for producing specified degrees of food deprivation. In heterozygous animals, this possibility was found to be feasible but limited by the occurrence of discontinuous growth functions.

In experiments said to deal with "motivation" for food or requiring "hungry" animals as part of the procedure—for example, to maintain operant responding with food reinforcement—some type of operational specification of food deprivation must be selected. A common technique for manipulating this variable is rhythm feeding, where animals are allowed to eat *ad libitum* for a fixed time interval T every H hours. In Fig. 1 (top), sample data obtained from 12½-month-old, random-bred, male Wistar albino rats show some effects of this technique on body weight when the values of T and H are, respectively, 1 and 23 hours. The daily weight immediately before feeding is expressed as percentage of *ad lib.*–feeding weight, where this base value is the rat's average weight for the 10 days of continuous feeding immediately preceding the start of the rhythm schedule (*1*).

Body weights decrease over successive days of the procedure, and the extent of the decline differs among individual rats (*2*). This suggests that, if one wishes to produce a constant degree of deprivation from rat to rat, a better technique may be deliberate reduction of body weight to a specified percentage of the *ad lib.*–feeding weight. However, *ad lib.*–feeding weight changes with age, and it is frequently necessary to initiate relatively prolonged studies with rats that are still growing. Therefore, in attempting to hold such a percentage fairly constant with the passage of time, one might wish to recompute this weight at regular intervals, using the changing base weights. But once a deprivation procedure is launched, how are we to know the weights that would have prevailed with increases in age, had the animal been permitted to feed freely?

It seemed to us that one answer lay in the *ad lib.*–feeding weights to be *expected* in an animal at various ages. These might be determined by extrapolation from an appropriate equation fitted to some of the animal's prior age-weight data. To examine the feasibility of this notion, we maintained daily age-weight records for rats (all males) feeding *ad libitum* in our colony areas, and for each animal, individual weekly mean weights were computed for successive weeks of age. Plots of typical individual growth data, treated in this manner, are presented for heterozygous males of the Charles River CD strain (*3*).

The lower plot of Fig. 1 shows that satisfactory prediction of *ad lib.*–feeding weight is possible. The smooth curve drawn through the data points is for the equation

$$y = -9.82 + 34.77x - 0.56x^2$$

fitted by the method of averages to the initial data, which are shown as filled circles. Weight values obtained later, shown as open circles, are in fair agreement with the extrapolated portion of the curve. The other curve, labeled *D*, is drawn through the average weekly weights at which this rat might have been maintained in order to keep it at 80 percent of his predicted *ad lib.*–feeding weight.

Continuous functions prevailing over the age range reported here were found to be rare. The bulk of the individual growth curves we obtained appear to be discontinuous functions of the type seen in Fig. 2. The first segment is negatively accelerated. It may be a parabola, as in Fig. 1, but it may be a function of other forms, such as

$$y = c - ae^{-bx}$$

and (*4*)

$$\log y = a - b(1/x)$$

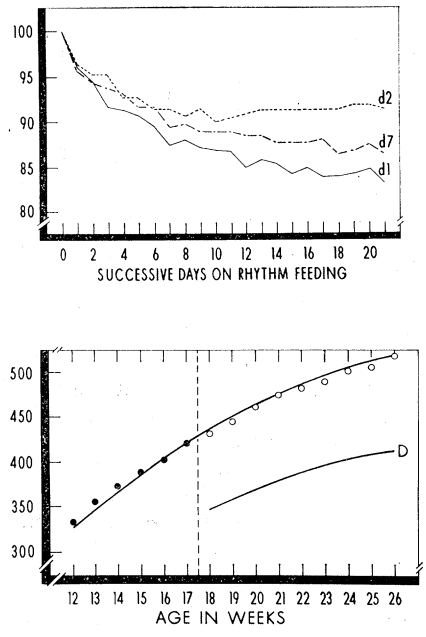

Fig. 1. (Top) Percentage *ad libitum*–feeding weight before eating as a function of successive days on a 23-hour feeding rhythm. Data from male Wistar rats. (Bottom) Individual weekly mean body weight in grams as a function of age in weeks for a male CD rat.

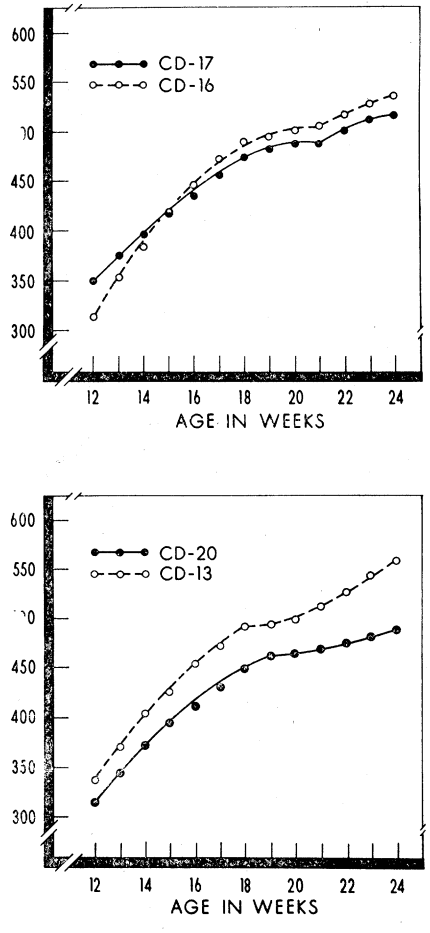

Fig. 2. Individual weekly mean body weight in grams as a function of age in weeks. Data from male CD rats.

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.

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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