barbital was probably appearing in the perfusate, as the volume of the ventricules is 8 to 12 ml (*9*).

The goats eventually became ataxic and the ataxia in varying degrees persisted for at least an hour after perfusion. Often after the animals had eaten appreciable amounts of feed, they would consume 500 to 1000 ml of water. When water was offered ad libitum during perfusion, the goats would always eat before drinking. Apparently hyperdipsia is secondary to hyperphagia, and the thirst centers were probably not directly affected. Usually the goats became drowsy and dozed occasionally; they could be awakened with varying degrees of difficulty, but would invariably start to eat until they dozed again. Unlike the cats of Feldberg's experiment (*8*), the goats did not eat while recovering from induced sleep.

Since pentobarbital sodium is definitely an inhibitory agent of neural activity, it is anticipated that the hypothalamic ventromedial nuclei (satiety center) are affected. If this is the case and hyperphagia is due to increased hypothalamic lateral-area (feeding center) activity, then in the normal animal some factor or factors, that is, intermediary metabolites, possibly stimulate activity in the satiety center which maintains a balance with the feeding-center activity.

Stimuli that cause increased lateral-area activity intitiate feed intake (*1*). The fact that, in these experiments, feed intake was induced in apparently satiated goats by perfusing the ventriculocisternal system with a pentobarbital solution suggests that in normal, satiated ruminants, as in monogastric animals, the ventromedial-nuclei activity inhibits lateral-area activity and, therefore, promotes satiety. It is not apparent from previous work (*10*) what, if any, receptors may exist in these nuclei that make possible the energy-intake regulation. Perhaps they are only part of a central control with afferent neural communication from receptors in the ruminal area, which, if present, may perceive distention, changes in volatile fatty-acid concentration, and other parameters associated with satiety and hunger.

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11. Supported in part by a grant-in-aid from the National Institute of Neurological Diseases and Blindness (NB-01941) and by the Fund for Research and Teaching, Dept. of Nutrition, Harvard University. C.A.B. is the holder of a postdoctoral fellowship supported in part by the National Institute of Child Health and Human Development. We are grateful to Drs. J. R. Pappenheimer, Tracy Miller, V. Fencl, and J. Nicholl, Jr., for demonstrating their surgical technique for placing ventriculocisternal cannulas. We also thank Sarah Breckenridge and Carol McLaughlin for technical assistance.

25 October 1965

Serum Concentration: Effects on Cycle and X-ray Sensitivity of Mammalian Cells

Abstract. *If the content of serum in the culture medium of exponentially growing Chinese hamster cells is below optimum (15 percent), the doubling time and the resistance to x-irradiation of the cells are increased. In synchronously dividing populations the increase in doubling time is primarily caused by increase in duration of the postmitotic (G_1) phase of the cells; this phase is relatively radiation resistant. The response of the cells growing synchronously is related quantitatively to the response of the cells dividing randomly.*

That the nutritional environment strongly influences the rate of cell growth in culture is widely recognized (*1*). Less well known is the effect of nutritional environment on x-ray sensitivity and on the component phases of the cell cycle. This report deals with the effects of varying serum concentrations in the growth medium in monolayer cultures. In a subline of Chinese hamster ovary cells recently subcloned in this laboratory, the doubling time can be predictably controlled within certain limits by varying the amount of fetal bovine serum (FBS) added to the growth medium [Eagle's minimal essential medium (MEM) plus antibiotics]. Doubling times, as obtained during logarithmic growth from daily cell counts (Coulter counter), compared to the percentage of serum supplement are presented in Fig. 1. If the medium completely lacks serum, the cell number increases by about 50 percent during the first few hours after serum deprivation, and then the cells cease growth entirely. In marked contrast, mouse L cells show only a very limited dependence on the amount of FBS available (Fig. 1). For Chinese hamster cells, supplementing the medium with more than 15 percent of serum favors growth little if at all. Medium containing 15 percent of serum is used as reference and is referred to as standard medium.

In one test of the effect of serum concentration on x-ray sensitivity of the Chinese hamster cells (Fig. 2, closed circles), cells were grown in stock bottles for at least 2 weeks on media containing different amounts of FBS. At a stage of growth when previous experiments had shown the cells to be in ex-

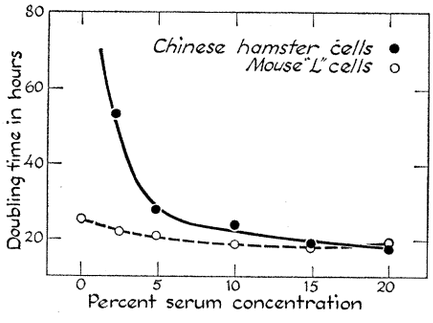

Fig. 1. Mean doubling time of cells during exponential portion of the growth curve. Cells were grown on glass, treated with trypsin (.05 percent, 300:1) and counted on Coulter counter. Without FBS, L cells show short-term (4 to 6 days) growth only. Standard deviations do not exceed ± 5 percent of the mean values.

Table 1. Growth and uptake of C^{14}-thymidine of synchronized Chinese hamster cells.

FBS concentration (%)	Intermitosis * (hr)	Synchrony † (%)	S Phase ‡ (hr)	Interval between S peak and cell division § (hr)	Maximum rate DNA synthesis ‖
2.5			6.1 ± 0.3	6	2.1
5	30	55	6.3 ± 0.4	6	2.2
10	25.5	58	5.9 ± 0.3	6	2.0
15	19.5	58	6.2 ± 0.3	6	2.0

* Determined from hourly cell counts of synchronized populations. † As defined by Engelberg (*10*). ‡ Defined as the time interval between half values of maximum counts (C^{14}-thymidine). § Time interval between the estimated time of the peak rate of DNA synthesis rate and the half value point of the subsequent rapid rise in cell number. ‖ Ratio of counts of C^{14} at peak of DNA synthesis rate for synchronized population to counts for unsynchronized population (standard medium) of same cell number. The time behavior of the counts of the unsynchronized population followed that predicted for exponentially growing cells to ± 15 percent.

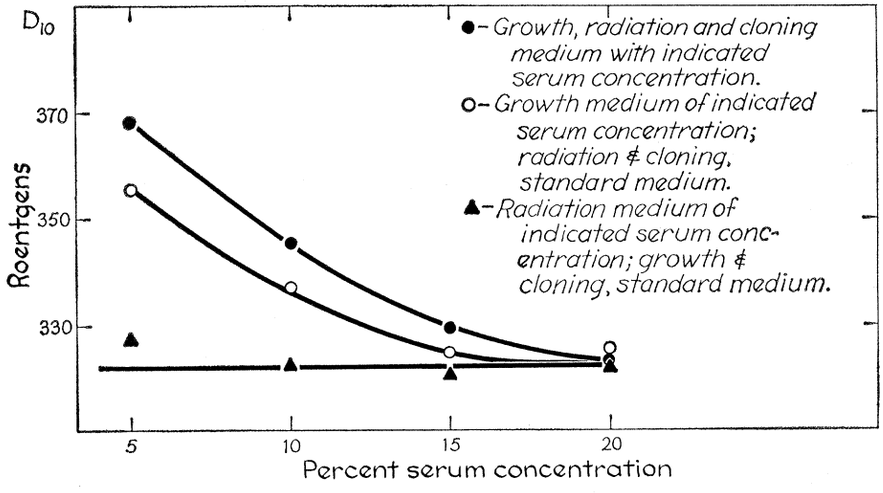

Fig. 2. Radiosensitivity of Chinese hamster cells in media with different FBS concentrations. Below 5 percent FBS, the plating efficiency is low; clones grow diffusely and are difficult to score. Over this range of FBS concentrations, the radiosensitivity of L cells is constant. The controls show fluctuations in D_{10} similar in magnitude to day-to-day variations encountered under essentially identical experimental conditions. Standard errors do not exceed ± 10 r.

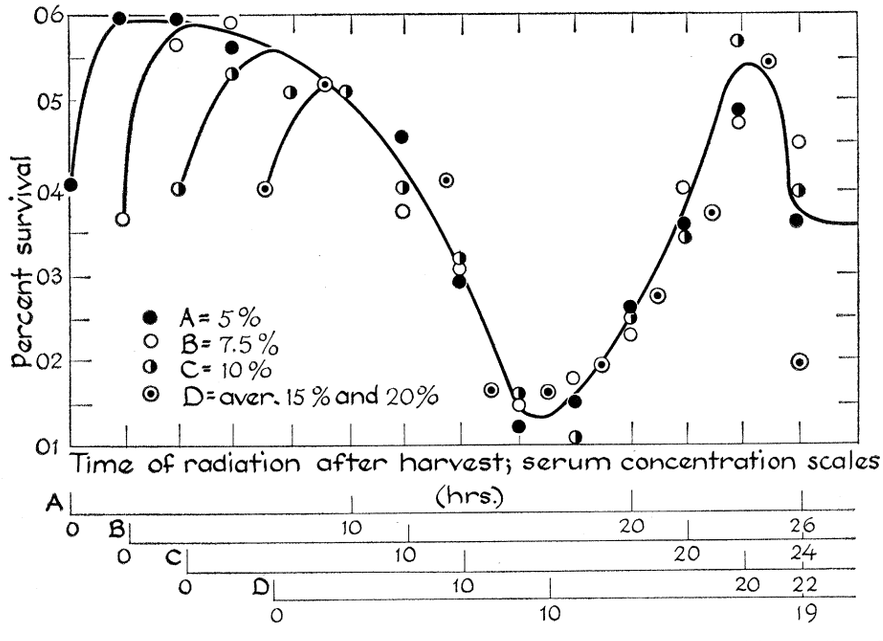

Fig. 3. Cells grown for at least two generations in, and harvested with, media of different FBS concentrations and given 500 r x-irradiation at regular intervals after harvest. Zero of each time scale corresponds to time of harvest. Standard deviations of survival values usually do not exceed ± 5 percent of the mean values.

ponential growth, they were gently treated with trypsin and rapidly planted for irradiation in medium of the same composition as their growth medium. Inoculum sizes were calculated to yield approximately the same number of clones after exposure to doses of 0 to 1000 roentgens. Radiation was delivered from a 120-kv Westinghouse unit drawing 9 ma; the filter was 1.25 mm Al, and the cells were irradiated under conditions of maximum backscatter at a dose rate of 75 r/min. Values of the dose to kill 90 percent on the linear part of the survival curve (D_{10}) and extrapolation number ($\tilde{n}$) were obtained as least-squares estimates from the clonal survival data. In no case did $\tilde{n}$ depart significantly from 2, although the standard error was as much as 0.5. The standard errors of the D_{10}'s in the FBS range of 5 to 20 percent did not exceed 10 r. The plating efficiency in these experiments and those described in the next paragraphs varied between 60 and 95 percent. The lower values tended to be associated with lower concentrations of serum, although not consistently so.

Since De Oca *et al.* (*2*) and Beer, Lett, and Alexander (*3*) have reported serum effects which probably involve repair processes after radiation, two additional sets of experiments were performed to determine whether the changes in D_{10} were attributable to effects before, during, or after radiation. In one group of experiments, cells were grown as before, but the cloning medium was standard. The D_{10}'s so obtained are indicated by open circles (Fig. 2). When both the cloning and growth medium were standard (Fig. 2), the standard medium was replaced by medium having the indicated FBS concentration for only 1 hour prior to and during irradiation. It should be pointed out that because serum coats the cells, the serum concentration in the immediate neighborhood of the cells is in some doubt. It may be inferred from these results, however, that most of the decrease in radiosensitivity as the FBS concentration is lowered from 20 to 5 percent arises from events occurring before irradiation. In Fig. 2 there are no data for the sensitivity of cells grown in 2.5 percent FBS. At that concentration cells cloned poorly. Plating efficiency was low (25 percent), and the clones grew diffusely and proved difficult to score.

Several workers (*4–6*) have shown that the radiation response is a con-

tinuous function of cell position in the cell cycle. Therefore, it seemed reasonable that experiments with synchronized cells could help explain the effects of serum on randomly dividing populations. Cells were synchronized according to the method of Terasima and Tolmach (*4*), except that 3 hours prior to harvest, the cells were placed in a refrigerator at 5°C for 1 hour (*5*). The cells were harvested in media of different FBS concentrations, planted either in T-15 flasks for growth experiments or in plastic petri dishes for studies on C^{14}-thymidine uptake and for exposure to 500-r irradiation at various times after harvest (Table 1). The average intermitotic period (2nd column) is within about 10 percent of the doubling time obtained from randomly dividing cells. A more important result is that the duration of the DNA synthetic phase (4th column), its position in the cell cycle with respect to the onset of the following mitosis (5th column), and the peak rate of DNA synthesis (6th column) are essentially independent of FBS concentration. The data on survival after x-irradiation show that the cells were sensitive at the time of harvest and then entered an insensitive phase. The duration of this insensitive phase increased as FBS concentration decreased. The response of all the cells during the last 15 hours of their cycle was almost identical and, therefore, also independent of FBS concentration. Sinclair and Morton (*5*) have reported on the survival of synchronized Chinese hamster cells whose generation time was about 12 hours. The response of their cells was quite similar to ours, if only the last 12 hours (Fig. 3) are used for comparison. Figure 3 is drawn to accentuate this common behavior during the later part of the cell cycle.

The concurrent measurement of C^{14}-thymidine uptake (0.008 μc/ml, administered to cells for 1 hour) showed that a sharp rise in the rate of DNA synthesis preceded the rise in survival by about 1 hour. While some variation in G_2 and M cannot be ruled out by these data, the major contribution to the serum-induced variation in doubling times is the change in the duration of G_1. Such a dependence has been suggested (*7*) on the basis of autoradiographic studies on mouse epithelial cells and has been confirmed in varying degrees and in various tissues by others (*8*).

The growth cycle data and the radiation response of the synchronously growing hamster cells can be used to calculate the response of the exponentially growing cultures (*9*). At FBS concentrations of 5, 10, and 15 percent, the experimentally obtained D_{10}'s were 368 ± 10, 346 ± 8, and 322 ± 8 r, as compared to the calculated (*9*) values of 362, 342, and 331 r. The excellent agreement between the experimental and calculated values of D_{10}'s leads to the conclusion that the serum-induced change in D_{10}'s is a result of redistribution of cells around the cell cycle as the duration of the G_1 phase of the cycle changes with FBS concentration.

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29 November 1965

Patterns of Alkaline Phosphatase in Developing Drosophila

Abstract. *Electrophoretic study of alkaline phosphatase in developing* Drosophila *shows that different stages are characterized by the appearance and disappearance of organ-specific enzyme bands. A new electrophoretic variant, from adult hindgut, is controlled by the second chromosome locus* Aph-2.

Alkaline phosphatase has long been the subject of ontogenetic and cytochemical studies. The discovery of gene-controlled electrophoretic variants of this enzyme in *Drosophila melanogaster* larvae (*1*) raises the possibility of using this system for the study of the genetic control of enzyme regulation in a diploid organism. We have studied phosphatase electrophoretic patterns during development from egg to adult and have discovered new electrophoretic variants.

Flies of the Woodbury (New Jersey) strain were grown in half-pint milk bottles at 25°C on standard medium of corn meal, dextrose, and agar to which live yeast was added. To collect eggs of uniform age, about 50 to 75 females were allowed to lay eggs on petri plates containing 2 percent agar smeared with a honey-yeast mixture. The first clutch of eggs, collected for 2 hours, was discarded.

Homogenates of many eggs (50 to 100) and first instar larvae (10 to 15) were required to produce adequate intensity of staining, but enzyme bands of subsequent stages were produced from homogenates of single whole flies or organs. All samples were ground in microscale homogenizers in 0.03 ml of distilled water, and the crude homogenates were loaded into Buchler vertical starch-gel molds. Tris-HCl, 0.05*M*, at *p*H 8.6, was used as buffer in both vessels and gels. Electrophoresis was carried out at 7 to 9 volt/cm for 2 to 4 hours at 4°C. Sliced gels were stained (*2*) for 18 hours at room temperature (23°C). Frozen sections for histochemical staining, 7 to 8 μ thick, were cut from unfixed and Formalin-fixed whole larvae (*3*) and incubated for 1 hour in the same staining solution. Figure 1 demonstrates characteristic electrophoretic patterns in different developmental stages. The main enzyme bands have been numbered in order of appearance during development. Identity of bands in different stages is based on identity of electrophoretic mobility and other criteria.

Band 1 is a faint band present in all stages of development. Bands 2, 3, and 4 appear in that order, are all strongly developed in mature eggs (18 to 20 hours after fertilization), and persist through all three larval instars.

Evidence points to larval hypodermis