not justified; for example, each of the polar areas has a value of about 120 milligal-megameters, and each of the equatorial belts a value more than twice as great.

The presence of a third harmonic of the amplitude (*2*) indicates a very substantial load on the surface of the earth. Following the arguments of Jeffreys, we may calculate the values of this load and the minimum stress required in the interior to support it. We find a crustal load of 2×10^7 dy/cm^2. We can choose between assuming that stresses of approximately this order of magnitude exist down to the core of the earth, or that stresses of about 4 times that amount exist in the uppermost 700 kilometers only (*3*, p. 199). These stresses must be supported either by a mechanical strength larger than that usually assumed for the interior of the earth or by large-scale convection currents in the mantle (*5*).

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27 January 1959

Synchronized Cell Division and DNA Synthesis in a Lactobacillus Acidophilus Mutant

Abstract. Withdrawal and subsequent restoration of deoxyriboside to a deoxyriboside-requiring mutant leads to synchronous cell division. There is a time lag between restoration and the first synchronous division; during this time the amount of deoxyribonucleic acid (DNA) per cell increases in stepwise fashion, and mass per cell increases linearly with time. The onset of cell division is heralded by cessation of DNA synthesis and an abrupt increase in rate of mass synthesis.

The intracellular events leading to cell division are under intensive study currently, and many approaches have been employed. One of the most promising methods is the induction of synchronized division in a population whose members normally divide randomly in time. Synchronization has been accomplished by means of temperature shifts (*1*), fractionation of cell popoulations (*2*), and addition of thymine to a thymineless *Escherichia coli* mutant (*3*). All methods are subject to the objection that the process of induction may produce abnormal divisions, and that the biochemical events preceding these divisions may have no relation to those preceding normal divisions. This objection can be overcome partly by comparison of results from as many methods of synchronization as possible and by use of a method which has specific and reasonably predictable biochemical effects.

The method described here (*4*) employs a mutant with a block at the deoxyriboside level of DNA synthesis, and synchronization is accomplished by withholding deoxyriboside from otherwise normally nourished cells and subsequently restoring it. The direct effect of this method is specific inhibition of DNA synthesis; the indirect effect of inhibition of DNA synthesis is small during the period of abnormal nutrition, as judged by high viability and continuing growth in the present instance. Ribonucleic acid synthesis continues in the absence of DNA synthesis (*5*). A major indirect effect is, of course, inhibition of cell division.

The mutant strain employed in these studies was *Lactobacillus acidophilus* R26. This strain grows in synthetic medium and has a specific requirement for deoxyriboside or deoxyribotide. Hoff-Jorgensen (*6*) has used this mutant for quantitative bioassay of DNA. The synthetic medium (LSM) was the same as Hoff-Jorgensen's, but with the addition of 10 mg of uracil and 0.5 mg of pyridoxine per liter, as suggested by Siedler *et al.* (*7*). Thymidine was used to satisfy the deoxyriboside requirement. Where indicated, thymidine was supplied at a concentration of $3 \times 10^{-6} M$, an amount just sufficient to give maximum growth.

For the majority of experiments, cells were prepared by growing them for 16 hours in LSM with thymidine. These cells were harvested, washed, and suspended at about 10^7 cells per milliliter in synthetic medium lacking thymidine. The suspension was stirred at 37°C, and aliquots were withdrawn for viable cell counts and turbidity and DNA determinations. Viable cell counts were made by the indirect method; suitably diluted aliquots were plated on nutrient agar, and the number of viable cells per milliliter was determined from colony counts. Turbidity measurements provide a direct estimate of cell mass rather than of number of cells (*8*). Turbidity (optical density at 500 mμ in the range of 0 to 0.4) was found experimentally to be proportional to cell protein per milliliter (determined by the Folin phenol method), irrespective of cell size. This is important because the cells elongate during thymidine starvation and break up into smaller units when division starts. De-

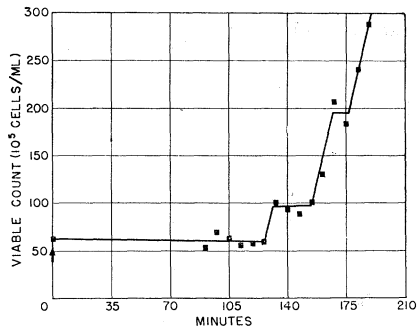

Fig. 1. Synchronous cell division. The time of addition of thymidine is indicated by the arrow.

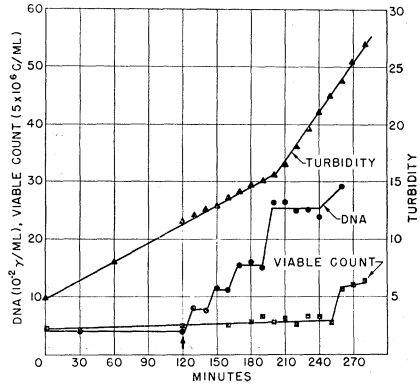

Fig. 2. Variation of DNA, turbidity, and viable cell count with time in a synchronized culture. Incubation in the absence of thymidine started at time 0; addition of thymidine is indicated by the arrow.

oxyribonucleic acid determinations were made by the Ceriotti method as modified by Keck (*9*). Cells were washed twice in cold 5-percent trichloroacetic acid and extracted in hot trichloroacetic acid, and the extract was analyzed for DNA.

Figure 1 shows two cycles of synchronous division. The cells were incubated in the absence of thymidine for 3 hours, then thymidine was added, at time 0 in the figure. There is a lag of about 2 hours before the first division. Two or three synchronous divisions follow the lag in rapid succession, the later ones being less distinct than the earlier. One synchronous division cycle takes about 30 minutes, which is much less than the 1½-hour generation time of nonsynchronous log phase cells.

Viability as a function of time during the course of starvation for thymidine has been determined, but the curve is omitted here for brevity. Death, presumably due to "unbalanced growth" (*3*), begins to appear only after 5 or 6 hours' starvation, if excessive aeration is avoided.

Figure 2 shows the results of simultaneous determinations of DNA, turbidity, and viable cell count. There is no net synthesis of DNA in the absence of

deoxyriboside, but synthesis starts immediately after addition of thymidine and proceeds stepwise. Three to four steps, followed by a long cessation of synthesis immediately prior to the first synchronous division, have appeared in all repetitions of this experiment. Deoxyribonucleic acid increases four- to eightfold before the first division. This is in line with the results of Jeener and Jeener (*5*), who demonstrated, by staining this mutant under conditions of deoxyriboside starvation and reactivation, that the cells can have many more than the normal two nuclei before they begin dividing. The fact that DNA more than doubles before division probably means, then, that the number of nuclei per cell more than doubles. On this basis the steps in the DNA curve may represent nuclear divisions. The appearance of cell division is correlated not with the amount of DNA multiplication, which is variable, but with the temporary cessation of DNA synthesis.

Well before the first division and at about the time of appearance of the DNA plateau, the rate of cell mass synthesis, as measured by turbidity, abruptly triples or quadruples and continues at the new rate through the first division cycle. Cell mass is synthesized linearly with time, rather than exponentially, throughout both the starvation and the reactivation phases. It is evident that the linear rate and the abrupt shift from one rate to another are similar to the results of mass measurements of single *Schizosaccharomyces* cells (*10*) if it is kept in mind that division may begin well before the daughter cells separate to form two viable units. It is tempting to assume that the simultaneous cessation of DNA synthesis and increase in the rate of protein synthesis are coordinated and decisive events in the sequence leading to cell division.

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30 September 1958

Cholecystokinin Activity of Urine

Abstract. In the urine of men and animals was found a factor which evokes the contraction of the gall bladder. This factor increases in the urine after the application of a secretogenous stimulus to the duodenum and decreases after resection of the duodenum. A urine concentrate was prepared which has an effect similar to that of cholecystokinin administered intravenously and which was therefore called urocholecystokinin.

Ivy and Oldberg (*1*) reported the isolation of cholecystokinin from the duodenal mucosa. Intravenous administration of this hormone leads to contraction of the gall bladder without alteration of the blood pressure.

In assays on dog gall bladder *in situ* (*2*) and on guinea pig gall bladder *in situ* (*3*) I found that human urine injected intravenously also caused contraction of the gall bladder. Urine from a healthy fasting person evoked less contraction of the gall bladder than the same amount of urine from the same person after a fat-containing meal (Tables 1 and 2). Vagotomy of the experimental animal did not alter these results.

One milliliter of urine from duodenectomized guinea pigs caused less contraction of the gall bladder in the assay on guinea pigs than 1 ml of urine from normal control guinea pigs (Table 2). One milliliter of urine from guinea pigs given 1 ml of 0.1*N* HCl in the duodenum caused a greater contraction of the gall bladder than 1 ml of the urine from control guinea pigs.

The urine factor which evokes the contraction of the gall bladder can be adsorbed on benzoic acid (*4*). This method was modified in the following way: To 10 lit. of urine was added, drop by drop, 1.5 lit. of saturated ethanolic solution of benzoic acid. The precipitate formed was filtered off, and 1.5 lit. of acetone was added in order to dissolve the benzoic acid that was present. After further purification the precipitate was dried and pulverized. The dry powder was dissolved in physiological saline solution, at *p*H 3, and was administered intravenously.

The concentrates eluted from the benzoic acid did not alter the blood pressure. The urine factor causing the gall bladder contraction is thermolabile and does not stimulate duodenal motility.

These experiments show that there is evidence of a relation between cholecystokinin, Sandblom's blood factor (*5*), and the urine factor that evokes contrac-

Table 1. Assay on dog gall bladder *in situ*.

Material	Quantity	Intragall-bladder pressure level* (cm)	Standard error of the mean	No. of tests
Human urine from fasting persons	10 ml	0.7	± 1.6	15
Human urine from persons 2 hr after drinking 50 ml of olive oil	10 ml	1.1	± 0.25	6
Urocholecystokinin (benzoic acid adsorbate from human urine)	50 mg	0.8	± 0.18	14
Urocholecystokinin (benzoic acid adsorbate from human urine)	100 mg	1.5	± 0.17	14

* Water manometer.

Table 2. Assay on guinea pig gall bladder *in situ*.

Material	Quantity	Intragall-bladder pressure level* (cm)	Standard error of the mean	No. of tests
Human urine from fasting persons	1 ml	1.8	± 0.3	40
Human urine from persons 2 hr after drinking 50 ml of olive oil	1 ml	3.0	± 0.27	10
Urine from normal guinea pig	1 ml	4.2	± 0.32	12
Urine from duodenectomized guinea pig	1 ml	3.5	± 0.38	7
Urine from guinea pig given 1 ml of 0.1*N* HCl in the duodenum	1 ml	5.4	± 0.35	8
Urocholecystokinin (benzoic acid adsorbate from human urine)	5 mg	3.4	± 0.26	10
Urocholecystokinin (benzoic acid adsorbate from human urine)	10 mg	6.5	± 0.28	10

* Elevation of the gall in the pipette.