hydrocortisone and the production of invertase activity (Table 1).

Each organ culture was assayed for activity of lactase as a measure of physiological condition of the tissue. Values for activity of this enzyme were from 20 to 60 percent of the specific activity usually found in fresh intestinal mucosa from rats of this age. The tissue, therefore, maintained relatively normal enzymatic activity during the culture period. It would seem that the appearance of invertase in the intestine of the young rat is a result of hormonal action directly upon intestinal mucosal cells.

There was no decrease in activity of lactase compared with untreated controls, either in the intact intestine after administration of hydrocortisone, or in organ cultures grown in the presence of hydrocortisone. In fact, either normal or slightly elevated activity was found. If the effect of hydrocortisone on the activity of intestinal invertase was due to the maturation of the tissue, then a decrease in activity of lactase would have been expected (*8*). Thus, hydrocortisone does not seem to act solely by hastening normal maturational changes in the intestine. It must also act by a mechanism as yet unknown. This hormone-enzyme interrelationship should be a suitable system for studying the regulation of a developmental process (*12*).

RUTH G. DOELL
NORMAN KRETCHMER
Department of Pediatrics,
Stanford University School of Medicine,
Palo Alto, California

References and Notes

1. J. Ibrahim, *Z. Physiol. Chem.* **66**, 19 (1910); L. S. Fomina, *Vopr. Med. Khim.* **6**, 176 (1960).
2. L. B. Mendel and P. H. Mitchell, *Am. J. Physiol.* **21**, 81 (1907); C. B. Bailey, W. D. Kitts, A. J. Wood, *Can. J. Agr. Sci.* **36**, 51 (1956); A. Dahlqvist, *Nature* **190**, 31 (1961).
3. R. G. Doell and N. Kretchmer, *Federation Proc.* **22**, 495 (1963).
4. D. Miller and R. K. Crane, *Biochim. Biophys. Acta* **52**, 293 (1961).
5. F. Moog, *Federation Proc.* **21**, 51 (1962).
6. "Special Glucostat," Worthington Biochemical Corp., Freehold, N.J.
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
8. R. G. Doell and N. Kretchmer, *Biochim. Biophys. Acta* **62**, 353 (1962).
9. S. L. Clark, Jr., *J. Biophys. Biochem. Cytol.* **5**, 41 (1959).
10. O. Barnabei and F. Sereni, *Biochem. Biophys. Res. Comm.* **9**, 188 (1962); L. Goldstein, E. J. Stella, W. E. Knox, *J. Biol. Chem.* **237**, 1723 (1962).
11. R. P. Cox, and C. M. McLeod, *J. Gen. Physiol.* **45**, 439 (1962); H. M. Nitowsky, F. Herz, L. Luha, *Federation Proc.* **21**, 161 (1962); F. Moog and M. H. Kirsch, *Nature* **175**, 722 (1955).
12. Supported by a grant from the John A. Hartford Foundation and in part by research grant AM-03501 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

23 September 1963

DNA Segregation in Escherichia coli: Observations by Means of Tritiated Thymidine Decay

Abstract. Escherichia coli, *strain 15T-L-, inactivation by H³-thymidine decay was used in a study of DNA segregation. Two characteristics of the inactivation curves due to H³-thymidine decay, the initial slope and stable fraction, enable the mode of segregation to be defined. The results suggest that the segregation pattern is consistent with a semiconservative model of DNA replication. The data on the stable fraction indicate that the DNA of strain 15T-L-, under our experimental conditions, may be organized into one duplex structure. Since colony-forming ability was used as the means of assay, it is possible to compare these experiments with those in which DNA segregation is examined on a structural basis.*

Since Hershey *et al.* (*1*) discovered that the decay of radiophosphorus following its incorporation into the DNA of bacterial viruses causes death of the viruses, the technique of "radioactive suicide" has become widely used as a tool in research (*2*). "Radioactive suicide" is taken as a measure of the destruction of functional biological units by accumulated radioactive decay. Fuerst and Stent (*3*) were the first to suggest that from such experiments one might obtain information about the segregation of DNA in bacteria. In a series of experiments with *Escherichia coli,* they obtained survival curves for cells which had been fully labeled with P^{32} and for cells that had been fully labeled and then allowed to grow for varying times in the absence of P^{32}. The assay used was one of colony-forming ability. Their data indicated that, under their experimental conditions, the segregation processes were not simple enough to permit the pattern of DNA segregation to be defined unequivocally.

Recently, it was shown (*4*) that H^3-thymidine, when incorporated into *E. coli* 15T-L-, a strain which requires thymidine and leucine, causes death by radioactive decay at a rate comparable with that for P^{32} decay. In contrast to P^{32}, H^3-thymidine is incorporated nearly exclusively into the bacterial DNA. Therefore, we studied the segregation of DNA in *E. coli* 15T-L-, using H^3-thymidine decay, using procedures similar to those of Fuerst and Stent (*3*). Our results suggest that the pattern of segregation is indicative of semiconservative DNA replication.

The pattern of DNA segregation may be understood from an analysis of the possible modes of replication for a one duplex or two stranded structure. As stated by Delbruck and Stent (*5*), the scheme is (i) conservative if the original duplex molecule remains intact and produces a completely new two-stranded molecule; (ii) semiconservative if the chains separate without breakage and each serves as the template for the formation of a complementary chain; (iii) dispersive if the chains are broken or destroyed in the process.

These models of DNA replication allow theoretical predictions with regard to the survival curves for fully labeled cells, and for fully labeled cells that have been allowed to grow for n generations in nonradioactive medium, if the mode of organization of DNA within the cell is of a simple nature. These theoretical predictions for cells containing one duplex structure are summarized in Table 1. For fully labeled cells the survival relation is the same for all three models and is $N/N_0 = e^{-\alpha\lambda N^* t}$ (symbols are defined in the title of Table 1). For fully labeled cells grown for n generations in cold medium the initial slopes for the conservative, semiconservative and dispersive models, when compared with that for the fully labeled cells should be in the ratio of 1, ½, $½^n$, respectively. In addition, for the conservative and semiconservative models the survival curves should show leveling at a point dependent both on n and on the model.

We made a brief examination of the survival of cultures of *E. coli* 15T-L-, which had been fully labeled with H^3-thymidine and then grown for various lengths of time in cold thymidine. We observed that the survival curves were similar in character to the semiconservative category in Table 1, indicating that, for this type of cell and for this

type of suicide, it should be possible to extend these experiments to obtain information on DNA segregation.

A parent culture of *E. coli* 15_{T-L-} in the logarithmic phase of growth was diluted and grown for approximately five generations in the presence of H^3-thymidine (*6*), to a final titer of 7×10^8 cells/ml. Cell counts during growth were determined by the use of a Coulter counter (*7*). The cells were then filtered at 37°C to remove exogenous H^3-thymidine, diluted, and regrown in the presence of nonlabeled thymidine. Equal portions were removed at various intervals corresponding to particular numbers of cell divisions (n) in nonlabeled medium; these samples were filtered, and stored at 8°C to accumulate H^3-thymidine decays. More specific details of the procedure for labeling bacteria with H^3-thymidine have been described previously (*4*).

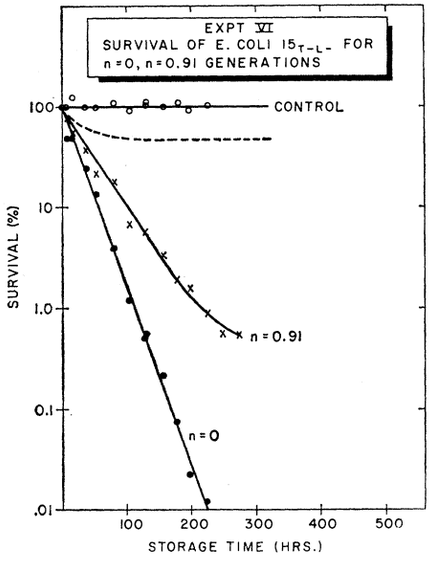

Fig. 1. Test for the conservative model of DNA replication; fully labeled cells were grown for 0.91 cell divisions in the absence of H^3-thymidine. The dashed survival curve is calculated assuming a conservative model of DNA synthesis and predicts leveling at approximately 50 percent survival and an initial slope equal to that for fully labeled cells.

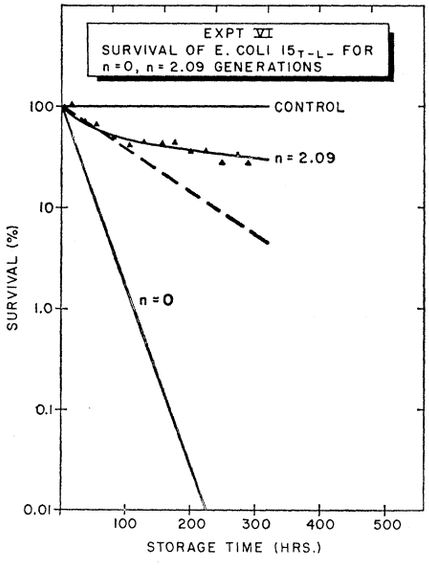

Fig. 2. Test for the dispersive model of DNA replication; fully labeled cells were grown for 2.09 cell divisions in the absence of H^3-thymidine. The dashed survival curve is calculated on the assumption of a dispersive model of DNA replication which predicts no leveling. The leveling shown in these data is indicative of semiconservative DNA replication.

Survival data, as a function of storage time are shown in Fig. 1 for nonlabeled cells, fully labeled cells, and for fully labeled cells grown for 0.91 cell divisions in nonlabeled medium. The purpose of this figure is to show that the data clearly do not fit the expectations of conservative replication. There is no loss of colony-forming ability in the unlabeled control during the period of storage. The survival curve designated $n=0$ is for fully labeled cells. Inactivation follows first order kinetics at least as low as 0.01 percent survival. The survival curve designated $n = 0.91$ is for fully labeled cells grown in cold thymidine for 0.91 cell divisions occurring after labeling. The conservative model requires that at cell division the parent DNA is inherited by only one of the daughter cells. After 0.91 cell divisions approximately one new cell has been formed per original cell. Thus, according to the conservative model given in Table 1, nearly one-half of the cells should have no label and leveling should, therefore, occur at approximately 50 percent survival. Furthermore, for the conservative model, inactivation should occur at a rate equal to that for fully labeled cells. These predictions on stable fraction and initial slope for conservative replication are shown by the dashed line in Fig. 1. It is clear the data do not fit the line, and we conclude that no appreciable fraction of the population segregates labeled DNA in the manner expected from conservative replication.

Additional data on the inactivation of cells of the same culture, but grown for 2.09 cell divisions after labeling, are shown in Fig. 2, which presents evidence against the dispersive model as well as presenting evidence in favor of the semiconservative model. The solid lines for the control and for fully labeled cells are the same lines shown in Fig. 1 and are included for reference. The data for $n = 2.09$ indicate that there is a fraction of the population that is stable and is consistent with the predictions of the semiconservative model. The dashed line is a theoretical line of slope $\frac{1}{2}^{2.09}$ predicted from the model of Table 1 assuming dispersive

Table 1. The relations between the survival of radioactive cells and models for the replication of DNA in cells containing one duplex structure. Symbols: α = efficiency of killing/decay; λ = radioactive decay constant for H^3 (disintegrations per hour per H^3 atom); N^* = number of H^3-thymidine molecules per cell for fully labeled cells; t = time (hours); n = number of cell divisions after labeling; N = number of viable cells at time t; N_0 = numbers of viable cells at time $t = 0$.

Semi-conservative	Conservative	Dispersive
Survival relations for fully labeled cells (N/N_0)		
$e^{-\alpha\lambda N^* t}$	$e^{-\alpha\lambda N^* t}$	$e^{-\alpha\lambda N^* t}$
H^3 atoms per cell after n generations in absence of H^3-thymidine		
$N^*/2$ (for $n>1$)	N^*	$N^*/2^n$
Fraction stable after n generations in absence of H^3-thymidine		
$\frac{2^n-2}{2^n}$	$\frac{2^n-1}{2^n}$	0

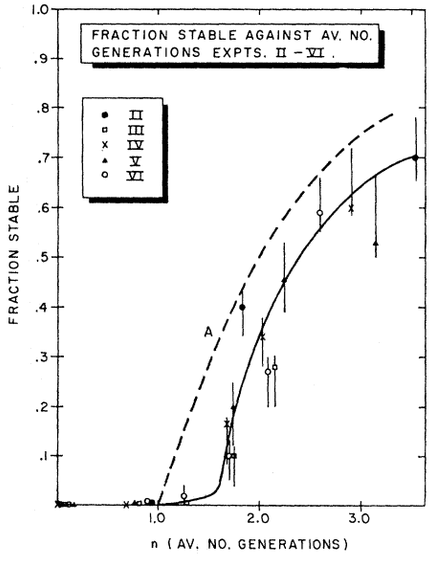

Fig. 3. The fraction of cells that are stable to H^3-thymidine decay following growth for n generations in the absence of label. The error limits on each point are statistical and reflect the maximum possible experimental error in the determination of F. If a population of cells contains one duplex structure the stable fraction is given by curve A. The solid line through the data demonstrates that there is a fraction of cells stable for $n>1$.

replication. It is clear that the data do not fit this line.

It is not clear from Fig. 2 alone, however, that a stable fraction exists whose proportions are those predicted by the semiconservative model. To determine the stable fraction more precisely we had to choose between measuring either a few survival curves for longer storage times, or many survival curves for several cultures, but over shorter storage times. We chose the latter procedure and such data from five sets of experiments comprising a total of 19 inactivation curves, where n ranged from 0.69 to 3.5, are summarized in Fig. 3. The fraction of stable cells, F, obtained from each survival curve, is plotted as a function of the number of cell divisions occurring after labeling (n). There is a general increase in F with increasing n. This is demonstrated by the solid line through the data and means that, for more than one cell division after labeling, a stable fraction of cells exists. This indicates that the mode of replication of DNA is not dispersive regardless of the manner in which the DNA is organized in the cells. A comparison between the predictions of the semiconservative model of Table 1 (which assumes one duplex structure as the minimum DNA content per bacterium) and experiment is also indicated in Fig. 3. The dashed curve A represents the predicted stable fraction according to the relation $F=(2^n-2)/2^n$ whereas the solid line represents the best fit to the experimental data. The deviation of the stable fraction in Fig. 3 from curve A could be due to the fraction of cells that did not contain the minimum amount of DNA when the labeling period ended, but were part way through the synthesis of the second duplex. Analysis in terms of assumptions for numbers of DNA duplexes greater than one is possible. Our results suggest only that for 15_{T-L-}, under our experimental conditions, the simplest model for the organization of DNA is one duplex for cells that have just completed division. The single hit inactivation curves might be taken as further confirmation of this view.

We emphasize that the assay used in these experiments is one of biological function. Thus, it is interesting to compare our results with those previously reported for the segregation of DNA, when this was examined on a structural basis. Our conclusion, that DNA replication in *E. coli* is semiconservative, is in agreement with the density gradient studies of Meselson and Stahl (*8*) on *E. coli*, and with the H^3 autoradiographic studies of Forro and Wertheimer (*9*) on the same organism.

STANLEY PERSON
MARY OSBORN
Biophysics Department, Whitmore Laboratory, Pennsylvania State University, University Park

References and Notes

1. A. D. Hershey, M. D. Kamen, J. W. Kennedy, H. Gest, *J. Gen. Physiol.* **34**, 305 (1951).
2. See, for example, G. S. Stent and C. R. Fuerst, *Advan. Biol. Med. Phys.* **7**, 1 (1960).
3. C. R. Fuerst and G. S. Stent, *J. Gen. Physiol.* **40**, 73 (1956).
4. S. Person and H. L. Lewis, *Biophys. J.* **2**, 451 (1962).
5. M. Delbruck and G. S. Stent, in *The Chemical Basis of Heredity*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1956), pp. 699–736.
6. The H^3-thymidine used in these experiments was at a specific activity of nearly 3.0 c/mM. It was purchased from Schwarz BioResearch, Orangeberg, N.Y., and New England Nuclear Corp., Boston, Mass.
7. Coulter Electronic Corporation, Hialeah, Fla.
8. M. Meselson and F. W. Stahl, *Proc. Natl. Acad. Sci. U.S.* **44**, 671 (1958).
9. F. Forro and S. Wertheimer, *Biochim. Biophys. Acta* **40**, 9 (1960).
10. We thank Marshal Edgell and Hazel Lewis for several stimulating discussions and, in particular, Professor Ernest Pollard for the interest he has shown in our work. We are indebted to the people of the U.S. for supporting this research through the auspices of the U.S. Public Health Service.

7 August 1963

Oncogenic Effect of Human Adenovirus Type 12, in Mice

Abstract. *Undifferentiated malignant tumors were induced at the site of injection of human adenovirus type 12 into newborn mice of the C_3H_f/G_s strain, but not of the DBA_f or A_f strains. The tumors were grossly and histologically similar to those induced by this virus in hamsters, but appeared in a smaller percentage of injected mice than hamsters.*

Our previous observations of the oncogenic effect of human adenovirus type 12, injected into newborn hamsters (*1, 2*) were confirmed and extended by Huebner *et al.* (*3*). Newborn hamsters were selected for testing the oncogenicity of human viruses because of their greater sensitivity to the oncogenic effect of murine polyoma virus (*4*) than even the mouse, the species of origin of the polyoma virus, and because of their relatively poor defense against the growth of normal tissue and tumor tissue transplants from other species, including man (*5*). Having induced the formation of tumors in hamsters by injection of a particular human virus, we wished to determine whether tumors could be induced by this virus in other species also. We therefore injected adenovirus type 12 into newborn mice of three different strains.

Human adenovirus type 12, strain Huie, was propagated in HeLa cells in our laboratory, and cell-free filtrate from the infected tissue culture was prepared as described previously (*1*). Mice of the C_3H_f/G_s, DBA_f, and A_f

Table 1. Relative tumor incidence in mice and hamsters injected at birth with equal doses of adenovirus type 12.

Route of injection	No. of animals with tumor/ No. of animals surviving more than 30 days	Site of tumor	Age at death from tumor (days)	No. dead without tumors (or missing) *	No. alive without tumors
		Mice of strain DBA_f			
Intrapulm.	0/4			3 (533–544) †	1 (776) †
Intraperit.	0/4			4 (528)	
Subcut.	0/5			4 (536–678)	1 (770)
		Mice of strain A_f			
Intrapulm.	‡0/7			‡3 (183–618)	4 (771)
Intraperit.	‡0/3			‡3 (540–647)	
Subcut.	0/6			4 (474–704)	2 (769–771)
		Mice of strain C_3H_f/G_s			
Intrapulm.	3/7	Thorax	61, 81, 145	3 (261–537)	1 (776)
Intraperit.	1/4	Abdomen	111	3 (466–675)	
Subcut.	0/2			2 (611–663)	
		Syrian hamsters			
Intrapulm.	26/27	Thorax and liver	35 – 157	1 (156)	

* A few mice could not be accounted for. † Ages, in days, given in parentheses. ‡ Two old mice of strain A_f were found at autopsy to have round lung adenomas of the type that occur spontaneously in this strain, but these are not included in the results.