replication, and the observed inhibition of synthesis follows. This formulation would appear to explain satisfactorily the irreversible bacteriocidal action of Mitomycin C.

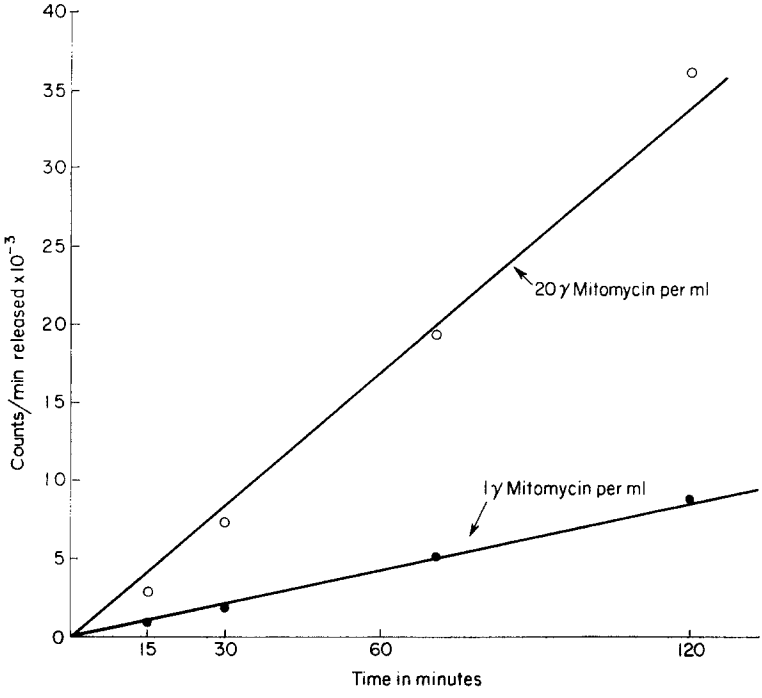

Fig. 1. Time course of appearance of [^{3}H]thymidine in acid-soluble fraction.

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E. REICH
A. J. SHATKIN
E. L. TATUM

Laboratory of Biochemical Genetics, The Rockefeller Institute, New York 21, N.Y. (U.S.A.)

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Thymine starvation and enzyme synthesis

Cultures of *E. coli* strain 15T– suffer progressive and roughly parallel losses of viability and capacity to form the inducible enzyme β-galactosidase after about one generation

Abbreviations: RNA, ribonucleic acid; TMG, methyl-β-O-thiogalactopyranoside.

of growth in thymineless medium[1,2]. Net syntheses of protein and RNA are somewhat less sensitive to thymine starvation[3]. These findings would indicate that when a cell is rendered incapable of replication it also becomes incapable of synthesizing specific proteins. We have recently obtained a divergent result for the enzyme alkaline phosphatase, which suggested another possibility.

The effects of thymine starvation on phosphatase[4], β-galactosidase[5], β-galactosidase permease[6], and protein[7] formation and on viability are presented in Fig. 1.

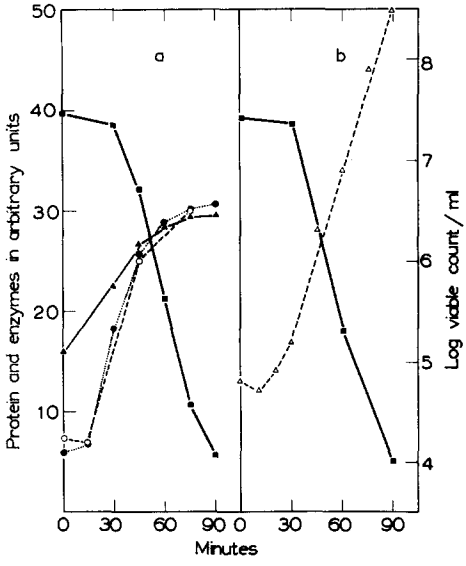

Fig. 1a. Formation of β-galactosidase, β-galactosidase permease, and protein, and decline in viability during thymine starvation. The medium utilized in all experiments was the synthetic glycerol-casamino acids H medium, supplemented with thymine, which has been described previously[10]. Such alterations as were made are as noted below. The inducer for the β-galactosidase enzymes was $5 \cdot 10^{-3}$ *M* TMG. The arbitrary unit of β-galactosidase (●—●) = 0.275 enzyme units/ml cells[5], as measured at 37°. The arbitrary unit of β-galactosidase permease (○—○) = accumulation of 2 counts/min/ml cells during 15 min incubation from a suspension containing $3 \cdot 10^4$ counts/min/ml radioactive TMG ($5 \cdot 10^{-3}$ *M*). The arbitrary units of protein (▲—▲) = 12 μg protein/ml. ■—■, viability. Fig. 1b. Formation of alkaline phosphatase and decline in viability during thymine and phosphorus starvation. The arbitrary unit of phosphatase (△—△) = 13.3 enzyme units/ml cells[4].

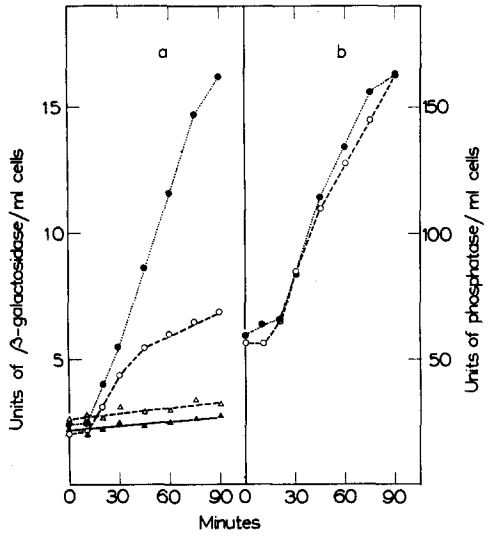

Fig. 2a. Formation of β-galactosidase by thymine-starved cells. The cells (about $3 \cdot 10^7$/ml) had been incubated for 60 min without thymine; the residual viability was then 5%. The cells were resuspended at the same density in medium devoid of an energy source (Cultures I and II, ●—●, ○—○), or in medium containing glycerol and lactate (Cultures III and IV, ▲—▲, △—△). Cultures I and III were induced with $5 \cdot 10^{-3}$ *M* TMG, Cultures II and IV with 0.1% lactose. Fig. 2b. Formation of phosphatase by thymine-starved cells. The cells (about $3 \cdot 10^7$/ml), were incubated without thymine for 60 min, then resuspended at the same density in medium devoid of an energy source (Culture I, ●—●), or in medium containing glycerol and lactate (Culture II, ○—○).

The phosphate starvation required for phosphatase synthesis clearly did not protect the cells from thymineless death. It is apparent that the synthesis of phosphatase, but not that of the β-galactosidase and the permease, progressed well beyond an hour of thymine starvation, at which time the viability of the cultures had declined to a negligible figure.

It will be noted that at the point at which β-galactosidase synthesis has virtually ceased the rate of protein synthesis has diminished considerably. This suggested that decreased anabolic activity of the cells might have led to an accumulation of the products of catabolic reactions within the cells. As previously discussed, these products would repress the formation of an enzyme (in this case β-galactosidase) whose activity would augment their already large metabolic pools (glucose effect)[8,9].

To test this hypothesis it was necessary to place thymine-starved cells under conditions in which this overproduction of metabolic intermediates could not occur. Accordingly, a culture of 15_{T-} in the logarithmic phase of growth was deprived of thymine until 90% of the cells were unable to form colonies. The cells were then incubated in the usual medium or in medium from which glycerol and lactate, the major energy sources, had been omitted, and either TMG or lactose was added as inducer. In another experiment cells similarly treated were incubated with or without glycerol and lactate in a medium devoid of phosphate. β-Galactosidase formation by the cells induced with TMG or lactose, and phosphatase formation by the cells deprived of phosphate were then measured (Fig. 2, a and b). The two cultures (III and IV) which were resuspended in the presence of the excellent energy source glycerol produced no β-galactosidase. Culture I, in medium containing no energy source and with TMG as inducer produced β-galactosidase at a rapid and linear rate for more than an hour after the cells had ceased to be viable. Culture II, resuspended in the same poor medium as Culture I, but with lactose as inducer, produced the enzyme almost as rapidly as Culture I for 30 min, then the rate of synthesis progressively declined, probably because sufficient glucose was being formed from the lactose to cause repression again. The rate of phosphatase formation, however, was the same in both media. It would seem, therefore, that the apparent loss of ability to synthesize β-galactosidase which has been seen to coincide with thymineless death is not an irreversible result of nuclear damage, but is due to repression. It is conceivable that an analogous situation may have existed in cultures being progressively inactivated by ^{32}P decay, in which capacity for synthesis of specific enzymes was found to decline at about the same rate as viability[10]. These experiments will be repeated with induction performed in the absence of energy source.

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Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. (U.S.A.) ELIZABETH MCFALL
BORIS MAGASANIK

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