Deoxyribonucleic acid synthesis in phage-infected *Bacillus megaterium* KM

The observation that the synthesis of deoxyribonucleic acid (DNA) in *Escherichia coli* B infected with phage T2 requires prior protein synthesis was made by COHEN AND FOWLER[1] and has subsequently been confirmed and extended by other workers[2–4]. This requirement for protein synthesis before DNA synthesis can commence is demonstrated by the need, after phage infection, for a period in the presence of amino acids and the absence of chloramphenicol or amino acid analogues before infected cells can synthesize DNA. Several functions have been suggested for the protein made during this period, two of which will be mentioned here. Information carried in the DNA might have to be transferred to protein before more DNA could be made, as suggested by HERSHEY[4] as one of several possibilities. Secondly, the protein might be connected with the metabolism of 5-hydroxymethylcytosine (HMC) which appears only in certain phages.

The fact that prior protein synthesis is not required for DNA synthesis in all phage infections makes the first possibility less likely[5]. It was found that the prior protein synthesis was required only during infection with T2, T4 and T6, phages containing HMC, and not during infection with phages T1, T3, T5 and T7. The suggestion was therefore made that the requirement for prior protein synthesis was connected with the appearance of HMC in the infecting phage. However, it has since been found that the stock used as T5 was not authentic and further studies with a genuine stock of T5 supplied by Dr. S. S. SPICER showed a requirement for prior protein synthesis during infection with this phage also, although it does not contain HMC. Other characteristics of the infection were markedly different from those of T2 infection and will be the subject of a separate report.

During a study of *Bacillus megaterium* KM infected with phages of the "M" series of FRIEDMAN AND COWLES[6] it was found that prior protein synthesis was required during infection with M4. The response of DNA synthesis to the addition of chloramphenicol during infection with phage M4 was very similar to that of *E. coli* B infected with T2, as shown in Fig. 1. In both cases addition of chloramphenicol at zero time almost completely inhibited DNA synthesis but at later times the addition had progressively less effect. The concentration of chloramphenicol used inhibited the incorporation of ^{14}C-leucine by more than 95%.

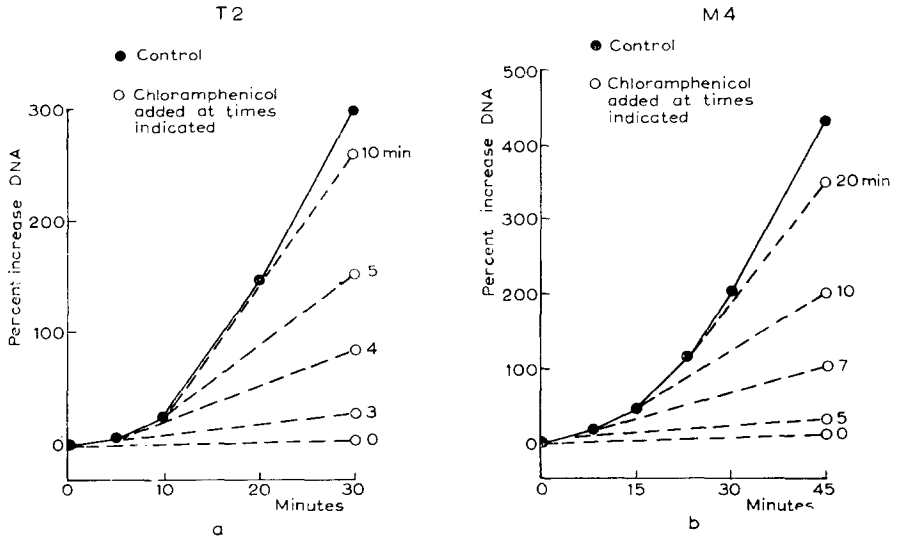

Fig. 1. (a) *E. coli* B was infected with T2 (multiplicity = 5; 6% rods uninfected) in N-free buffer and diluted into peptone–yeast extract medium at 0 min. The suspension was incubated with shaking at 37°. Chloramphenicol (20 μg/ml final concentration) was added at the times indicated. Samples were precipitated with $HClO_4$ (0.2 *N* final concentration), washed once with 0.2 *N* $HClO_4$ and suspended in 1 ml 0.5 *N* $HClO_4$. DNA was estimated by adding 2 ml diphenylamine reagent and developing the colour for 18 h at 30°[2]. This procedure gave the same result as a full fractionation by the method of OGUR AND ROSEN[10]. (b) *Bac. megaterium* KM was infected with M4 (multiplicity = 7; 4% rods uninfected) in N-free buffer and diluted into peptone–yeast extract medium at 0 min. The suspension was incubated with shaking at 34° and samples treated as in (a) except that incubation was continued for 45 min.

The nucleic acid of phage M4 was analysed to find whether HMC or cytosine was present. A sample of M4 was hydrolysed with 88% (v/v) formic acid, used by WYATT AND COHEN[7] to isolate HMC, and the purine and pyrimidine bases were separated on paper chromatograms developed in isopropanol/HCl[8]. The spots were located by u.v. photography and the spot corresponding to cytosine from thymus-DNA cut out and eluted. The "cytosine" from phage M4 was identical with cytosine from thymus-DNA as regards u.v. absorption spectra in 0.1 *N* HCl and in 0.1 *N* NaOH and R_F value on paper chromatograms developed with *n*-butanol/NH_3[9]. HMC is markedly different from cytosine by these criteria.

Thus although phage M4 does not contain HMC, the DNA metabolism of cells infected with it is similar in its response to chloramphenicol to that of *E. coli* B infected with T2, an HMC-containing phage. It is therefore unlikely that the requirement for prior protein synthesis is connected with the presence of HMC in the infecting phage. The reason for the requirement or non-requirement may rather be connected with the absence or presence of genetic homology between the phage and the host bacterium.

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Radiation-induced alteration of splenic-DNase II activity in sucrose and aqueous homogenates

Several theories[1,2,3] have been proposed to explain the increase in desoxyribonuclease II (DNase II) activity per mg wet weight in the lymphoid tissues of irradiated rats, but none of the proposed mechanisms has been established as the *modus operandi*. Since it has been shown that irradiation of isolated mitochondria with large doses of X-rays or γ-rays increases the measurable activity of the DNase II[4], it was considered of interest to determine whether or not damage of DNase-containing subcellular particulates in lymphoid tissue irradiated *in vivo* might account for all or part of the observed increase in enzymic activity. Therefore, the DNase II activity of the spleen of non-irradiated and control rats was assayed in separate portions of tissue homogenized in media which either preserve or destroy particulate subcellular structures.

Male Sprague-Dawley rats each weighing 180–200 g were either sham-irradiated or exposed to 756 R of X-rays, using radiation factors previously described[5]. 24 h later, spleens and thymus glands were removed, weighed and bisected. One-half of each organ was homogenized in distilled water and the other half in 0.44 *M* sucrose using a loosely fitting glass homogenizer. The homogenized tissues were centrifuged, washed and resuspended before assay in hypertonic sucrose solution (1:100, w/v). The conditions of incubation described by KOWLESSAR *et al.*[5] were used. Since sucrose interfered with the diphenylamine reaction used in the above procedure, the concentration of the products of enzymic degradation of desoxyribonucleic acid (DNA) was determined on the basis of the optical density read at 300 mμ in the Beckman spectrophotometer[4].

The results of this study are presented in Table I. It will be noted that the specific activity of the splenic tissue homogenized in 0.44 *M* sucrose was considerably lower than that of the remainder of the organ homogenized in water. In the case of the spleens of irradiated animals, the activity of the sucrose homogenate was about half of that of the aqueous preparation, whereas, in the non-irradiated tissue, the DNase activity in sucrose was one-third of that in water. The irradiated tissue samples were more active than the corresponding control tissues homogenized in the same medium, but the magnitude of the respective changes differed for the preparation