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

with respect to the two homogenizing media. Thus, DNase II activity observed after irradiation was 3.4 times greater than the control value if the tissues were homogenized in sucrose solution and 2.2 times higher than the control value when water was used instead of sucrose as the homogenizing medium. As indicated by the ratios (Table I, column 3, line 3), the differences mentioned above are statistically significant at the 5% level. The data suggest that, in sucrose homogenates, a larger fraction of DNase II present in the tissue has become accessible to the substrate as a result of the prior irradiation of the animals.

TABLE I

ENZYMIC ACTIVITY OF AQUEOUS AND SUCROSE HOMOGENATES OF SPLEEN

After homogenization in sucrose or water, all samples were resuspended in sucrose before enzymic activity was assayed. Each group contained 10 animals. The DNase II specific activity is expressed as $\Delta E_{300\ m\mu}$/mg wet tissue. The total activity is the specific activity × wet wt of spleen (mg). *Standard errors are given except where the asterisks denote the use of standard deviation of individual ratios.

		I *Sucrose homogenate*	*II* *Aqueous homogenate*	*Ratio I/II**
DNase II specific activity	A. Control	0.226 ± 0.040	0.641 ± 0.042	0.349 ± 0.122
	B. Irradiated	0.697 ± 0.067	1.416 ± 0.148	0.489 ± 0.130
	Average ratio B/A*	3.39 ± 1.035	2.23 ± 0.340	
Total DNase II	A. Control	12.6 ± 1.9	25.2 ± 4.3	
	B. Irradiated	19.8 ± 2.9	40.3 ± 6.1	
	Average ratio B/A*	1.83 ± 0.802	1.19 ± 0.296	

In evaluating these data, it was considered of importance to determine the degree of activation of DNase II resulting from treatment of tissue homogenates with distilled water. Aqueous homogenates of spleen were subjected, therefore, to the further disruptive action of sonic vibration in order to learn whether an additional increase in enzymic activity would occur. Exposure of aqueous homogenates for 10–20 min to 9 kcycle sonic vibration in a Raytheon Vibrator Unit caused no alteration in DNase II activity. This indicates that homogenization of tissues in water results in a loss of structural integrity sufficient to render a considerably greater quantity, perhaps all, of the DNase II activity accessible to the substrate. Since more vigorous destructive measures might cause even greater activation of DNase II, it cannot be assumed that the measured increase in activity caused by treatment with distilled water necessarily represents the maximal activation possible.

It may be concluded that a moderate increase in the DNase II per mg wet lymphoid tissue presumably due to radiation damage *in vivo* can probably be demonstrated if precautions are taken to preserve the structural integrity of subcellular particulates during homogenization and assay. This increase in activity might well be more striking if the study had been made at the peak of cellular destruction (6–12 h after irradiation) rather than at 24 h when cellular debris has been largely removed. Such a mechanism, if it exists, cannot explain the published reports[1,2,3] of enhanced DNase II activity in lymphoid tissue being maximal 24–72 h after irradiation of the animal. Since distilled water was used as the homogenizing medium in all of these studies, it may be assumed that the measured increase in activity represents an actual increase in the amount of DNase II present per mg of the atrophied tissues.

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