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STUDIES ON NUCLEI. II

EFFECTS OF X-RAYS ON DEOXYRIBONUCLEOPROTEIN FROM RAT THYMUS

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SEVERAL lines of evidence [7, 24] indicate that deoxyribonucleic acid (DNA) and deoxyribonucleoprotein (DNP) are likely sites of radiation damage at a molecular level. In this paper the high sensitivity to X rays of a nuclear gel obtained from rat thymus is investigated, and the relation of this sensitivity to chromosome structure is discussed.

Various investigators [11] have detected alterations in the physical and chemical properties of DNA and DNP after irradiation *in vitro*. However, changes were observed only after very large radiation (kiloroentgen) exposures, far exceeding those which cause marked effects in the organism from which the DNA was isolated; further, no observable effects were seen in DNA isolated from tissues immediately after heavy irradiation. It has been argued that the high X-ray exposures necessary to produce measurable changes reflect merely the inadequacy of available analytical techniques to measure the subtle molecular changes responsible for biological effects [9].

An alternative reason for the insensitivity of isolated DNA and DNP to X-ray exposures that often cause biological damage (10–500 r) may be that DNP exists in the cell in the form of a very labile complex whose properties are not reflected in isolated preparations. There is some evidence suggesting that this is the case. Crude unpurified DNP extracts have been obtained with 1 *M* NaCl that differed in their properties from solutions of isolated and purified DNP [2, 28] and exhibited viscosity losses after low X-ray exposures ([3], and K. V. Shooter cited in Ref. [19]). Somewhat similar observations have been made on extracts prepared in distilled water [21, 28]. The available evidence also indicates that irradiation of the tissue lowers the viscosity of DNP extracts prepared from it [14, 17]. These observations suggest that crude

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DNP extracts are very sensitive to X-rays, but suitable methods for measuring changes have been lacking. The method described in the first paper of this series has been found sufficiently reproducible to warrant a systematic study of radiation effects.

MATERIALS AND METHODS

Sample preparation.—Rat thymus homogenates and DNP extracts were prepared from adult male Sprague-Dawley rats as described [18]. To prepare buffered extracts for the studies on chemical protective agents, we added 0.15 *M* sodium phosphate buffer, pH 6.8, to the 1.43 *M* NaCl solution. Results of viscosity measurements are expressed as η/η_0, which is the ratio of the specific viscosities of the irradiated and the control samples.

Irradiation.—The X-ray source was a General Electric Maxitron (250 kvp, 30 ma, 3 mm Al). Exposures were measured with a Victoreen meter. For comparative studies X-ray doses were administered to the whole animal, the excised thymus tisue, homogenates of excised thymus, or NaCl extracts of the homogenates. The effect was always measured as viscosity reduction in the final NaCl extract. The extracts in 1 *M* NaCl were exposed in 2-dram extraction vials at 25–27°C. Homogenates were exposed in 30-ml beakers in an ice bath. By placing the samples receiving the longer exposures under the X-ray beam first, irradiation of all the homogenate samples was completed simultaneously. For studies on the tissue *in vitro*, the thymus was excised, divided into 6 equal parts, which were weighed and placed in cold NaCl solution in an ice bath. Three of the thymus samples were irradiated, and three served as controls. In all of the *in vitro* studies, the samples were rotated on a turntable during irradiation to assure uniform exposure. Whole animals were X-rayed in a tubular wire cage (18 × 24-mesh window screen) ~7 cm in diameter and 25 cm long.

Radiation protective agents.—All the agents tested for protective action with the extract were adjusted to pH 7 with NaOH. With the exception of mercaptoethylamine (MEA) and mercaptoethylguanidine (MEG) the compounds were added in 1.43 *M* NaCl at the beginning of the extraction period. A 0.2 ml aliquot of MEA or MEG solution was added to the extracts just before irradiation, and the samples were mixed by gently inverting several times.

The following chemicals synthesized in this laboratory [13] were kindly supplied by Drs. David Doherty, Raymond Shapira, and William Burnett, Jr.: MEG, guanidoethyldisulfide, hydroxyethylthiuronium · Br, guanidinoethanol, and diethylaminoisothiuronium dihydrobromide. The MEA was marketed under the trade name of Bécaptan (Labaz).

Viscosity measurements.—Viscosity measurements were made in a specially modified Cannon-Fenske viscometer, as described previously [18].

Radiation studies

Aftereffects.—The DNP extracts were extremely sensitive to X-rays, and a decrease in viscosity could be detected after an exposure to as little as 10 r. Dose curves were sigmoid (Fig. 1). The magnitude of the viscosity decrease at

a given exposure depended on the time lapse after irradiation as well as on exposure, since the viscosity of X-rayed samples continued to fall for several hours (Fig. 2). The viscosity fell rapidly for the first 2 hours and then continued to decline more slowly for the next 2–3 hours. An essentially constant

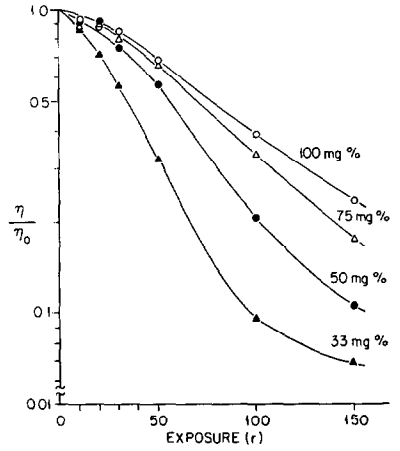

Fig. 1.

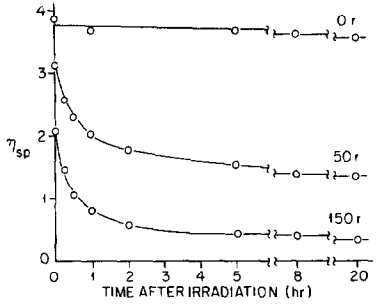

Fig. 2.

Fig. 1.—Dose curves at different extract concentrations. 2.0-ml aliquots of serial dilutions of a 1:333 homogenate were extracted for 12 hours with 4.0 ml of 1.43 *M* NaCl. The samples were exposed at a dose rate of 50 r/min. All viscosities were determined 4–5 hours after irradiation.

Fig. 2.—Radiation aftereffect. Extracts consisted of 2.0 ml of a 1:350 homogenate extracted with 4.0 ml of 1.43 *M* NaCl for 12 hours. The extracts were irradiated to the indicated exposures at a dose rate of 50 r/min., and viscosities were measured at the indicated intervals after irradiation. Measurements for zero time were obtained about 1–2 minutes after irradiation.

value was reached after about 5 hours. The time sequence was thus very similar to that in the aftereffect reported by Taylor *et al.* [32, 33] in X-irradiated DNA solutions. The magnitude of the aftereffect increased with exposure (Fig. 2). Curves obtained 5 hours after irradiation differed only in slope from curves that were obtained immediately after irradiation. Since no change in pH occurred during the experiments (as measured electrometrically), it was not a cause of the viscosity decrease.

Because the viscosity of the irradiated samples changed so rapidly for the first few hours after irradiation, routine measurements were always made when the aftereffect was maximum, i.e., after 4–5 hours.

Dose curves for extracts.—A detailed investigation was made of the relation between viscosity decrease and X-ray exposure, particularly at low doses. Sigmoid dose curves were obtained (Fig. 1). The loss in viscosity seemed to be linear over the initial portion of the curve, but additional points at low ex-

posures revealed the sigmoid character of the curves. In the curves in Fig. 1, tailing is evident only in the lower curve, but it was invariably observed at higher exposures in all concentrations studied. This may be seen by referring to the control curves in Fig. 4.

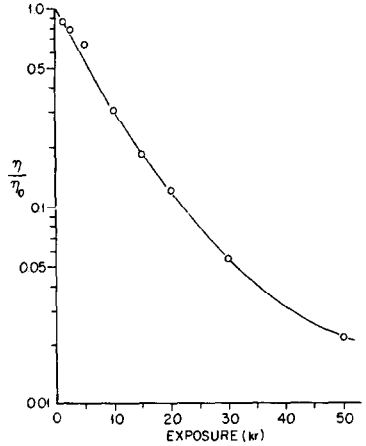

Fig. 3.

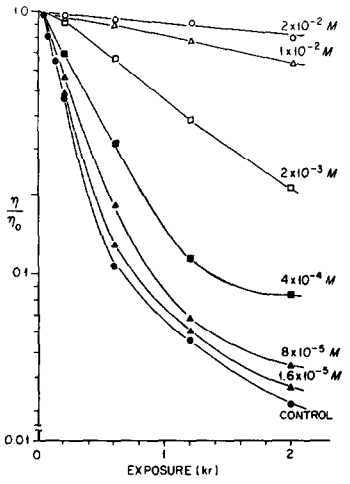

Fig. 4.

Fig. 3.—Dose curve for irradiation of homogenate. Immediately after irradiation samples of a 1:375 homogenate were added to 2 volumes of 1.43 *M* NaCl, and the viscosity of the extracts was determined after 12 hours. Dose rate was 750 r/min.

Fig. 4.—Protective action of aminoethyldisulfide (AED). Aliquots of a 1:333 homogenate were extracted for 12 hours with 2 volumes of 1.43 *M* NaCl containing sodium phosphate buffer (0.15 *M*, pH 6.8) and AED. The exposure rate was 100 r/min. Viscosities were measured 4–5 hours after irradiation.

Dose curves for homogenates.—Irradiation of the homogenates before extraction lowered the viscosity of extracts prepared from the irradiated breis. The exposure required to reduce the viscosity to 50 per cent of the value in unirradiated samples was 6 kr, compared to the 78-r exposure necessary to produce a comparable effect when 1 *M* NaCl extracts of thymus were irradiated. A dose curve for a homogenate is shown in Fig. 3.

Irradiation of thymus tissue in vivo *and* in vitro.—Excised thymus tissue was less sensitive to X-rays than the homogenate, and an exposure of 20 kr caused only a small decrease in the viscosity of extracts from irradiated tissue. The same exposure of the homogenate reduced the viscosity of extracts to 12 per cent of the value for extracts from unirradiated samples (Fig. 3). The effects of X-irradiation of the thymus *in vivo* on the viscosity of DNP extracts from irradiated tissue were also investigated (Table I). The experi-

ment was a typical "nested" or repeated sampling design, and statistical analysis of the data showed that the component of variation contributed by the differences between groups is significant; i.e., the groups differed from one another. There was no evidence of differences among animals within groups although, in general, differences between homogenates from animals within groups made a significant contribution to total variation.

TABLE I. *Extracts of thymus irradiated* in vivo.

Rats were exposed to whole-body irradiation, sacrificed immediately, and two 1:333 homogenates prepared from each thymus. Extracts were then prepared by a 12-hour extraction with 1 *M* NaCl. Dose rate was 2238 r/min. There were four rats in each group.

Exposure (kr)	η_{sp}
0	3.53 ± 0.22
20	3.41 ± 0.15
40	2.00 ± 0.39

TABLE II. *Irradiation of frozen samples.*

The extracts were prepared by extracting a 1:360 homogenate with 2 volumes of 1.43 *M* NaCl for 12 hours. The samples were frozen at −20°C, irradiated in the frozen state, thawed at room temperature (25°C), and their viscosities measured 4–5 hours after irradiation. In the experiment on the homogenate, a 1:333 homogenate was frozen at −20°C, irradiated, and the viscosities of 12-hour extracts of the thawed homogenate measured. Samples maintained at 0°C served as controls. Dose rate for extracts was 100 r/min. and for homogenate 1000 r/min.

	Dose (r)	η_{sp} Frozen	Unfrozen
Extract	0	2.54	1.43
	250	0.16	0.67
Homogenate	0	3.06	1.27
	3,000	2.65	1.26
	10,000	1.33	1.54
	15,000	0.81	1.31

Tests for indirect action: effects of concentration, freezing, and chemical protective agents.—The effects of X-rays on the extracts seemed to be largely indirect, since the effectiveness of a given exposure was reduced by increasing the concentration of the DNP extract (Fig. 1), by freezing, and by the addition

of chemical protective agents. These constitute the classical methods for testing indirect action [22]. However, direct effects may make some contribution since the X-ray exposure required to produce a 50 per cent decrease in viscosity is not directly proportional to concentration.

TABLE III. *Irradiation of DNP extracts in the presence of protective compounds.*

All extracts were prepared by a 12-hour extraction of 1:375 homogenates with two volumes of 1.43 *M* NaCl containing 0.15 *M* sodium phosphate, pH 6.8. All compounds were present at the time of irradiation. Dose rate was ~100 r/min. Mouse data were collected from the literature and represent 21-day survival [1] or 30-day survival [12, 27] after otherwise lethal X-ray exposures.

Additive	Dose-reduction factors		Mouse survival data	
	2×10^{-3} *M*	2×10^{-4} *M*	Survival (%)	Ref.
Sodium acetate	1.08	—	0–10	1
Sodium formate	3.03	1.10	60	1
Sodium azide	8.78	1.85	40	1
Sodium cyanide	1.60	—	50–70	1
Thiourea	9.77	2.29	[a]	1
Hydroxyethylthiuronium · Br	9.81	2.19	0	12
Guanidinoethanol	5.21	1.52	[b]	12
Diethylaminopropyliso-thiuronium · ^{2}HBr	11.75	2.28	[c]	12
Mercaptoethylguanidine	7.30	2.37	96	27
Aminoethyldisulfide	4.97	1.73	100	1
Guanidoethyldisulfide	10.97	—	90	27
Glycine	1.00	—	0	1
Mercaptoethylamine	7.00	—	97	1

[a] Weakly protective in mice but highly toxic.
[b] No data available.
[c] Higly variable.

It was difficult to demonstrate unequivocally that freezing prevented the X-ray induced viscosity losses in the extracts, since freezing itself produced sizable viscosity decreases. Since freezing caused larger viscosity changes than irradiation, low X-ray exposures could not be used. With high exposures, it could be shown that the irradiated frozen extracts had a viscosity midway between those of frozen unirradiated samples and unfrozen irradiated ones (Table II).

Very similar results were obtained with homogenates (Table II). At exposures of 10 and 15 kr, extracts irradiated in a frozen state had a higher

viscosity than unfrozen irradiated samples. However, at 3 kr, freezing caused a larger loss in viscosity than did irradiation.

It seemed advisable to use buffered extracts in the study of protective compounds since viscosity was so markedly affected by changes in pH and

TABLE IV. *Irradiation of the homogenates in the presence of protective compounds.*

The two experiments were performed under identical conditions on different homogenates. Samples of 1:350 homogenate were given an exposure of 20 kr (750 r/min.). Irradiation was done in an ice bath. All agents present at a concentration of 0.01 *M*. For viscosity determinations, 2.0-ml samples of homogenate were extracted for 12 hours with 1.43 *M* NaCl. All pH's were adjusted to 6.8–7.2.

Additive	Exp.	η_{sp} Unirradiated	η_{sp} Irradiated	$\frac{\eta_{sp} \text{ final}}{\eta_{sp} \text{ initial}}$
None	I	4.43	0.69	0.15
	II	4.13	0.58	0.14
Mercaptoethylguanidine	I	4.10	3.62	0.88
(MEG)	II	4.22	4.22	1.01
Guanidoethyldisulfide	I	—	—	—
(GED)	II	4.80	2.00	0.42
Mercaptoethylamine	I	4.47	3.26	0.73
(MEA)	II	4.76	4.07	0.86
Aminoethyldisulfide	I	4.88	2.14	0.44
(AED)	II	—	—	—
Tris buffer	I	4.44	0.66	0.16
	II	4.24	0.72	0.17
Phosphate buffer	I	4.21	0.85	0.20
	II	4.24	0.59	0.14

many of the substances to be tested were acidic. Sodium phosphate and Tris buffer were considered. Tris buffer was highly protective and gave a dose reduction factor of 9.5 at 0.01 *M*, i.e., in the presence of 0.01 *M* Tris buffer, the X-ray exposure required to give a certain viscosity decrease was 9.5 times that necessary for the same decrease in unprotected samples. Phosphate buffer (0.10 *M*, pH 6.8) was used in the study of the protective agents and was added to the 1.0 *M* NaCl extraction medium. It approximately doubled the X-ray exposure necessary to cause a 50 per cent viscosity decrease in the extracts.

Of the large number of compounds which were screened for protective

action in animals we selected only a few for study here; four compounds —2-mercaptoethylamine (MEA), 2-aminoethyldisulfide (AED), 2-mercaptoethylguanidine-HBr (MEG), 2-guanidoethyldisulfide (GED)—were studied in detail. Both MEA [5] and MEG [12] afford mammals considerable protection from lethal X-ray doses. Dose curves obtained for the four compounds at a variety of concentrations were similar to those given for AED in Fig. 4. The initial portions of the dose curves formed a family of nearly straight lines with slopes decreasing with increasing concentration. The dose-reduction factor is the ratio of exposures required to produce a 50 per cent loss in viscosity in the presence and the absence of the compound under study.

The compounds listed in Table III were studied at selected concentrations only, and dose-reduction factors were obtained as shown. None of the compounds tested caused any appreciable changes in the viscosity of unirradiated samples. The dose-reduction factors varied from 1 for glycine to 12 for diethylaminopropylisothiuronium. There was marked variation between similar compounds; e.g., formate but not acetate protected. The disulfides (GED and AED) gave large dose-reduction factors and, in fact, GED gave a larger value than its reduced form, MEG. However, the dose-reduction factors obtained for MEG and MEA were undoubtedly too low. To minimize oxidation, these compounds were added to the extracts just before irradiation, and it was impossible to obtain adequate mixing without shearing the samples enough to cause a decrease in viscosity. Consequently, mixing was not thorough. This point was demonstrated clearly in preliminary experiments in which GED was added just before irradiation and gave dose-reduction factors about equal to those reported for MEA and MEG.

Some of the compounds were also tested for their effectiveness in preventing the viscosity decrease resulting from direct irradiation of the homogenate. A single concentration and exposure were used, so that dose-reduction factors were not calculated; instead, the fraction of the initial viscosity is reported (Table IV). The pattern was similar to that obtained with the extract. MEG and MEA and their disulfides afforded considerable protection from X-ray induced viscosity losses. Tris buffer, which in the extracts gave a fairly large dose-reduction factor, was an exception and was ineffective in the homogenate.

DISCUSSION

Effect of X-rays on DNP extracts.—This study established that the viscosity of crude nucleoprotein extracts is decreased by very low X-ray exposures (of the same order of magnitude as the doses producing biological effects). The

radiation effects will be discussed first before we attempt to relate them to biological events.

The dose curves obtained with the extracts are sigmoid (Fig. 1), except at very low concentrations of DNP, where the first part of the curve is logarithmic. Exponential curves have been reported for the depolymerization of DNA and DNP solutions by X-rays [8, 23, 31–34]. An exponential curve would be expected for the random degradation of a linear polymer [4]. It has been proposed that the DNP of the cell exists as a highly cross-linked gel [9, 11, 18]. This type structure requires multiple breaks before viscosity changes become evident, which would explain the shoulder observed in the dose curves with higher concentrations of DNP. The more nearly exponential curves obtained in dilute solutions indicate that the crosslinks which are believed to be loosened by high salt concentrations [18] are now reduced to a negligible number, which leaves an essentially linear system of DNA molecules linked end to end. The tailing observed in the dose curves at high exposures probably resulted from autoprotection. Released by radiation, fragments that are too small to contribute significantly to the viscosity might still react with activated solvent molecules and thus function as a protective agent. Errera [17] observed similar effects in irradiated DNP extracts and concluded that they resulted from protection by the breakdown products of irradiation.

Direct and indirect effects.—The action of X-rays on the extracts had a large indirect component in agreement with previous studies [8, 23, 30], since increasing the concentration of DNP, freezing, or the addition of chemical protective agents all reduced the effectiveness of X-rays in lowering the viscosity of the extracts. Unfortunately, the data from the present study do not permit a precise estimate of the relative contribution of direct and indirect effects. Several observations indicate, however, that direct effects played some part. The X-ray exposure necessary to produce a 50 per cent decrease in viscosity was not proportional to concentration as one would predict if the effects of X-rays were entirely indirect. Also, irradiation of frozen samples, where X-ray effects should be direct, still produced a small viscosity decrease. When protective agents were added to the extracts, the maximum protection at the highest concentrations studied was still below that of unirradiated samples. As a limit, one can say that the direct effects did not exceed the viscosity loss at the highest concentration of the protective agents studied (Fig. 4) and that they did not amount to more than a few per cent of the total.

The effects observed after irradiation of the homogenate were also indirect

in part, since freezing or the addition of chemical protective agents reduced the effectiveness of X-rays (Table IV). No attempts were made to modify radiation effects in intact thymus tissue, but it has been suggested that any effects on DNA *in vivo* would be largely direct because of the high DNA concentration and the presence of protective substances [25]. Exposure of the tissue to 40 kr, which produced a considerable viscosity decrease, was considerably below the figure of 100 kr found by Drew [15] and Ephrussi-Taylor and Latarjet [16] as a threshold value for direct effects on pneumococcus-transforming principle. The physical properties of DNA irradiated in the dry state were affected by similar exposures [29].

Chemical protective agents.—A marked protective effect was observed with several compounds that are effective in the intact animal. However the data are insufficient to allow any correlation to be made between chemical structure and protective action in the extracts. It is interesting, however, that disulfides and sulfhydryl compounds were equally effective, since the disulfides could not function by reducing the oxygen tension as may occur *in vivo* [20]. The simplest explanation of the action of the compounds tested would be a competition for activated solvent molecules. This is consistent with the diversity of the compounds having activity and with the fact that none of the compounds had any appreciable effect on the viscosity of unirradiated samples. It is clear from the data in Table III that there is no good correlation between protection of the extracts and the dose-reduction action in animals reported in the literature. Although cyanide was the only compound reported to give good protection in animals that did not protect the extracts, other compounds (such as Tris buffer) not protective in animals, or only slightly so, gave large dose-reduction factors in the extracts. The usefulness of the techniques reported here for preliminary screening of compounds before testing them in mice is therefore open to question, although it should be stated that it would be more reasonable to compare our *in vitro* effects with protection against genetic damage in the whole animal.

Biological implication.—This study was undertaken to precisely determine the sensitivity of the extracts to low X-ray doses in a range producing biological effects. The extracts were exquisitely sensitive to X rays; and, if one could extrapolate from results obtained in dilute DNP extracts back to the cell, the discrepancy between the doses necessary to produce *in vitro* and biological effects would, in fact, be largely resolved. Unfortunately, serious objections can be raised to such a comparison. The effects of X rays on the extracts were largely indirect so that the high concentrations of DNP in the cell and presence of compounds such as proteins would be expected to attenuate any

effects. The data clearly show that DNP within the cell was less sensitive to irradiation than that in the extracts. When the homogenate or tissue was irradiated directly, the X-ray exposure necessary to produce a given viscosity decrease was raised by a factor of 700 for the tissue. The great sensitivity of the extract was probably partly caused by its great dilution.

However, the observation that one can produce any effect at all by irradiation of the DNP while it is still present in the nucleus is of considerable interest, in view of the failure of numerous previous workers to detect any changes in DNA isolated *immediately* after irradiation *in vivo* [9]. The dose to the brei (500–1000 r) just sufficient to produce a detectable change in the viscosity of the extracts agrees well with previous observations on DNP extracts [6, 10, 17].

The studies on excised thymus tissue would more nearly approximate the condition of the DNP *in vivo* than studies on the extracts and homogenate, but they are subject to the possible objection that the interior of the tissue was anoxic. Anoxia is known to reduce the radiosensitivity of tissues [19]. In this study to avoid this objection, whole animals were irradiated, and the thumus was removed and extracted immediately after irradiation to avoid as far as possible necrotic changes in the tissue after irradiation. It must be recognized that the whole-animal exposures (20–40 kr) used here exceeded those used in previous attempts to demonstrate alterations in isolated DNA after *in vivo* irradiation [9, 10, 25, 26].

Thus far it has been assumed that the action of X rays is a consequence of a primary ionization either in the solvent near the molecule or on the target molecule. Another possibility should be considered. In crude preparations of the sort used, it is also possible that the X-ray action is the release and activation of enzymes capable of degrading the DNP gel. Heavy X-irradiation has been shown to increase the DNAase activity of animal tissues [4]. This point has not been investigated in detail, but the data suggest that this is not the case in the extracts. One would expect that any quantity of enzyme released, unless inactivated again, would eventually reduce the viscosity of all irradiated samples to the same level. That this was not the case is clearly demonstrated in the aftereffect studies where the delayed viscosity loss was proportional to exposure.

These studies suggest certain conclusions concerning the statistical structure of the chromosomal substance. The extracts studied here are sufficiently dilute to allow each DNA molecule if in true solution to behave independently. The phenomenally high viscosity observed must therefore be caused by the presence of bonds or bridges between DNA molecules that persist in 1 *M*

NaCl. The question is, how are these links arranged? If the linkages are random and a gel in the ordinary sense is formed, then a limit exists beyond which the gel cannot swell without breaks occurring (i.e., dissolving). In the present instance the original nuclear gel would be required to swell by a factor of 1000 or more to fill the solution. If each nucleus expanded to form a "gel-island", then at some dilution where these islands were no longer in contact, the viscosity should drop sharply. This has not been observed. Further, the effects of radiation should be to produce a dose-viscosity curve that resembles a so-called "multihit curve." This is because the first breaks within a gel structure have relatively little effect. However, at sufficiently low DNP concentrations this is not observed. Rather, the curve with low concentrations of DNP is that observed when linear polymers are broken by radiation. The radiation studies therefore add additional support to the view that the nuclear gelwork consists of strands of DNA interlinked by proteins or other substances to form long strands, which in the cell are thought to be held together laterally by salt linkages that dissociate in 1 *M* NaCl.

SUMMARY

1. One molar NaCl extracts of rat thymus are highly sensitive to X rays, and an exposure of 10 r causes a detectable loss in viscosity. An aftereffect was observed, and the viscosity of irradiated samples continued to decrease for 4–5 hours after irradiation. Dose curves were sigmoid. The effects of X rays on the extracts were largely indirect. Freezing, the addition of chemical protective agents, and increasing the concentration of the gell all had a dose-reducing effect. There seemed to be a small contribution from direct effects not exceeding a few per cent of the total.

2. Except for cyanide, all of the compounds tested that are known to protect animals from the lethal effects of irradiation also protected in the extracts. However, several compounds that had no protective action on animals gave large dose-reduction factors in the extracts. No good correlation exists between chemical agents protecting animals and those giving dose-reducing effects in the extracts.

3. The high sensitivity of the extracts to X-irradiation appeared to be a direct consequence of the high dilutions employed. Direct irradiation of the homogenate and excised tissue required, respectively, 100 and 700 times the X-ray dose necessary to produce the same effect in the extracts. The higher doses probably reflect protection from indirect effects by the high concentration of DNP in the cell nucleus and by protective substances such as proteins.

The fact that one can demonstrate an effect by direct irradiation of the tissue is of interest, since no effects on DNA have been detected after irradiation *in vivo*.

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