Effect of Irradiation and of Various Reagents on Viscosity of Nucleoprotein Solutions.* (18584)

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The effects of X-radiation in (a) producing chromosome breaks *in vivo* and (b) reducing the viscosity of nucleic acid and nucleoprotein solutions *in vitro* are well known. Because of the partially radiomimetic effects of H_2O_2, it was considered of interest to test the effects of H_2O_2 *in vitro* on nucleoprotein solutions, using the relative viscosity of the solutions as criterion of effect. Because of the known effects of cysteine and related compounds in reducing radiation mortality, a study was included of the effects of these compounds on nucleoprotein viscosity.

Experimental. Thymus chromosomes were prepared by the method of Mirsky and Ris (1), except that the final suspension in 0.14 M NaCl was made such that each ml represented one gram of initial thymus. Immediately before use, one volume of this suspension was mixed with an equal volume of N/10 NaOH. Of this 1:1 mixture, 5 ml were pipetted into a 25 ml Erlenmeyer flask containing 1 ml of the substance being tested. Molarities listed in the table are those of the agent as added; in the final mixture the molarity is thus 5/6 of that shown. Chemicals to be tested were used as received, with no attempt at purification. They were dissolved in slightly less than the proper volume of H_2O, brought to pH 8.0, and made to volume with H_2O. Materials of limited solubility were filtered before use.

Procedure. The mixture of 1:1 chromosomes-NaOH and agent to be tested was put into an Ostwald viscometer in a 37°C water bath. After an equilibration period of 15-20 minutes, the passage time was measured. The viscometers were kept in the 37° bath, and the passage time was again measured at the times shown.

Results. Effect of hydrogen peroxide. In preliminary experiments, the effect was tested of 0.1 M H_2O_2 on 5 volumes of thymus chromosomes solution. For comparison, 0.1 M NaCl was used. The viscosity of these solutions under the influence of H_2O_2 was consistently slightly higher than under the influence of the NaCl. In succeeding experiments, however, it was realized that if H_2O was used for comparison, then H_2O_2 was without effect, while 0.1 M NaCl or 0.1 M KCl reduced the viscosity slightly. These differences, however, were all so slight that this question was not further pursued.

Effect of Cysteine. The striking effect of cysteine on the viscosity of solutions of thymus chromosomes is indicated in the data of Tables I, II and III. There are included viscosity data taken at various times after mixture of the chromosome suspension with the NaOH and the cysteine solution, since this effect is considerably magnified with the passage of time.

Effect of the concentration of thymus on the "cysteine effect". As the alkaline solution of thymus chromosomes is further diluted with N/10 NaOH, the effect of cysteine in increasing the viscosity is lost. This is illustrated in Table I. This diminution in cysteine effect is apparently due to nucleoprotein (thymus chromosome) concentration rather than to the level of the initial viscosity, since preparations of varying initial viscosity lost the cysteine effect at the same degree of dilution, rather than at the same viscosity.

Effect of the concentration of cysteine on the "cysteine effect". Cysteine at 0.05 M or 0.06 M is ideal for demonstrating the effect of cysteine in increasing the viscosity of alkaline solutions of thymus chromosomes. If the cysteine concentration is decreased to 0.01 M or less, the viscosity increase is much less striking; if the cysteine concentration is increased to 0.1 M, the viscosity increase in a

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1. Mirsky, A. E., and Ris, H., *J. Gen. Physiol.*, 1947, v31, 1, 7.

TABLE I. Effect of Concentration of Thymus on the "Cysteine Effect."

Exp. No.	Incubation (hr)	Thymus dilution / Agent added	Relative viscosity 1:1 CySH*	1:1 H_2O	1:125 CySH*	1:125 H_2O	1:2 CySH*	1:2 H_2O
1	0		9.3	5.9	4.6	4.7	3.1	3.3
	1		14.5	6.3	5.1	4.8	3.1	3.3
	2		16.8	6.5	5.2	4.8	3.1	3.2
	4		19.2	6.5	5.3	4.8	3.1	3.2
2	0		11.1	7.2	5.9	5.7	3.6	3.6
	1		17.4	8.0	6.7	5.8	3.6	3.6
	2		21.4	8.2	6.9	5.8	3.7	3.7
	4		26.2	8.4	7.3	5.8	3.6	3.6

* CySH = cysteine.

TABLE II. Effect of Cysteine and Related Agents on Viscosity of Alkaline Solution of Thymus Chromosomes.

Incubation (hr)	Relative viscosity in presence of .05 M substance shown: H_2O	Cysteine	Glutathione	Cystine*	Cyanide	Thiocyanate	Ascorbic acid	BAL	Thiourea	Urea	Thiouracil*	Uracil*
0	11.1	49.2	50.8	10.3	20.1				11.2			
4	14.4	116.0	>120	12.7	43.0				15.2			
0	5.3	6.6					5.1	5.3				
4	5.6	12.2					10.1	6.0				
0	12.5	15.7				14.0			12.3	12.0	12.4	11.7
4	13.9	33.4				25.9			14.6	14.6	14.0	14.7

* Cystine, uracil, and thiouracil were filtered saturated solutions, appreciably more dilute than the stated .05 M.

TABLE III. Effect of Cysteine on X-ray Reduction of Nucleoprotein Viscosity.

Sample No. / Treatment	1 X-rayed in presence of cysteine	2 X-rayed in absence of cysteine	3 Not X-rayed; cysteine added	4 Not X-rayed; cysteine not added
(a) "Prophylactic" effect.				
Viscosity, zero time	10.4	6.8	11.3	11.5
3 hr	71.3	6.7	86.7	12.0
Sample No. / Treatment	1 X-rayed; cysteine added	2 X-rayed; cysteine not added	3 Not X-rayed; cysteine added	4 Not X-rayed; cysteine not added
(b) "Therapeutic" effect.				
Viscosity, zero time	9.9	6.9	11.2	11.0
1½ hr	25.8	7.1	62.8	12.2

X-rayed in layer ca 1 cm deep in beaker covered with parafilm and packed in cracked ice. Administered 50000 r at 20 r per sec. 250 KV. 15 milliamperes. No filter used.

short time is so great as to make the use of Ostwald viscometers impractical.

Effect of time of incubation. It will be noted from Tables I and II that the viscosity of the cysteine-containing solutions increases with the passage of time. If the cysteine solution is replaced by water the rise in viscosity is extremely small, if present at all. Occasionally the viscosity in the presence of cysteine has been observed to rise continuously for several hours, and then, at about 4 hours, to begin to decrease. This phenomenon has been attributed to bacterial contamination, with subsequent degradation of the nucleoprotein.

It has also been observed that a given

saline (0.14 M NaCl) suspension of thymus chromosomes, kept in the ice box over a period of time, produced less and less viscous solutions when aliquots are removed and diluted with alkali. Although the decrease in viscosity thus produced is sometimes very large, it is not accompanied by any change in the protein concentration of the solution; that is to say, it does not represent bacterial or autolytic proteolysis. A possible explanation for the increased viscosity of alkaline solutions upon incubation lay in the effect of the repeated "shearing" occasioned by the repeated sucking up of the solutions through the viscometers; this might conceivably so orient asymmetric molecules as to increase viscosity. This was tested, however, and found to be not so; the mere process of determining the viscosity does not effect the viscosity.

Cysteine effect in molar salt solutions. All work described in the rest of this report was performed on 1:1 (v/v) mixtures of N/10 NaOH and chromosome suspensions. Mirsky and Ris(1) fractionated such chromosomes by placing them in a solution of 1 M NaCl. It was felt of interest to determine whether cysteine would increase the viscosity of such salt solution of chromosomes, as well as of alkaline solutions. This was found to be the case, but there was also observed some sedimentation in these preparations, hence alkaline solutions continued to be used. It is of interest that chromosomes diluted with 1 M NaCl were appreciably more viscous than if diluted with the same volume of 0.1 M NaOH.

Effect of other chemical agents on the viscosity of alkaline solution of thymus chromosomes. The other chemicals tried were those that have been tested as therapeutic or prophylactic agents for radiation toxicity and whose effects would seem in some way related to the reducing properties of cysteine. These data are summarized in Table II, in which, for the sake of brevity, only the initial and the 4-hour figures are given. For comparison, the effects of cysteine and water controls have been included.

Effect of cysteine on x-ray reduction of viscosity of chromosome solutions. For reasons discussed below, it was considered of interest to determine whether cysteine had any effect on the reduction of nucleoprotein viscosity by irradiation. In one experiment (Table IIIa), the cysteine was added to a portion of the alkaline nucleoprotein solution before X-radiation of the solution with 50,000 r. The data indicate that, percentage wise, the cysteine largely protects the viscosity of the solution. Using as the basis of calculations the "inherent viscosity" of the solute, *i.e.*, the relative viscosity of the solution minus one, it will be noted that 3 hours after completion of the irradiation, the viscosity of the solution irradiated in the absence of cysteine is 48% lower than its non-irradiated control, while the decrease in the presence of cysteine is 18%. In the next experiment (Table IIIb) the cysteine was added shortly after X-radiation with 50,000 r. In this case, after 1½ hours, the viscosity of the irradiated solution was 45% lower than its non-irradiated control in the absence of cysteine, and 60% lower in the presence of cysteine. In a third experiment, an attempt was made to determine whether the protective effect of cysteine diminished if the cysteine were added after a longer post-irradiation period. This proved not to be the case; rather the cysteine effect was greater if the cysteine were added later. This increased effect, however, is difficult to interpret, since the same is true in the case of non-irradiated nucleoprotein solution.

Discussion. The possibility was considered here that the effect of cysteine in increasing the viscosity of nucleoprotein solutions may be involved in the mechanism of cysteine protection against X-radiation lethality. Thus if X-ray sensitive nucleoproteins are in some way stabilized, *e.g.*, if the nucleic acids are somehow more highly polymerized, these nucleoproteins may be more resistant to irradiation. This suggestion, however, is not entirely borne out by the results reported. Thus thiourea, reported by Limperos and Mosher(2) to reduce X-ray mortality, does not increase the viscosity of alkaline solutions

2. Limperos, G., and Mosher, W. A., *Science*, 1950, v86, 112.

of thymus chromosomes, while thiocyanate, reported by Herve and Bacq(3) actually to *increase* X-ray lethality, was found here to increase nucleoprotein viscosity. On the other hand, most of the agents tested did have parallel activity in their effects on nucleoprotein viscosity and *in vivo* X-ray toxicity. Among these are cysteine(4,5), cyanide(6), and glutathione(5), which respond positively in both tests, and cystine(5), thiouracil (7,8), and ascorbic acid(5), which give negative results in both tests, although ascorbic acid does show a positive effect in the nucleoprotein viscosity test after a prolonged period of time.

In addition, the parallelism holds true for the experiments on the effect of cysteine on X-ray reduction of nucleoprotein viscosity. Patt *et al.*(4) have shown that cysteine protects rats from the lethal action of X-rays only if administered shortly before irradiation. Similarly, it was found here that cysteine protects nucleoprotein solution from viscosity reduction by X-rays much more effectively if added prior to the irradiation.

The possibility must remain, therefore, that some such phenomenon as this cysteine augmentation of nucleoprotein viscosity is a partial explanation of the reduction in X-ray lethality by cysteine and some other agents. The exceptions noted may represent entirely separate phenomena. Thiourea, for example, may protect against X-rays by reducing the metabolic level of the whole organism, in line with the ideas of Blount and Smith(7) and others. No explanation is here offered for the paradoxical behavior of thiocyanate.

Summary. 1. Cysteine, glutathione, cyanide, and thiocyanate greatly increase the viscosity of alkaline solutions of nucleoprotein (thymus chromosomes), while urea, uracil, thiourea, thiouracil, cystine, and BAL do not do so. Ascorbic acid increases the viscosity only after prolonged incubation. Hydrogen peroxide is without effect on the viscosity. 2. If cysteine is added to the alkaline nucleoprotein solution before X-radiation, the expected reduction in viscosity is largely prevented. If the cysteine is added after X-radiation, it is much less effective in this respect. 3. The suggestion is made that these phenomena may be relevant to the question of the mechanism of cysteine and some other agents in increasing resistance of organisms to X-ray lethality.

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Chemical Constituents of Skeletal Muscle from Normal Subjects and Patients with Rheumatic and Non-Rheumatic Diseases.* (18585)

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In the course of an investigation(1) of the incidence of lymphocytic lesions in skeletal muscle of patients with arthritis, biopsy specimens of fresh human muscle became available for simultaneous chemical analysis. Though

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