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Studies on the Chromatin Isolated from the Organs of Animals Received Whole-body X-ray Irradiation

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I. Introduction

It has been known that DNA which is a major component regulating genetic function in higher animals gives genetic messages to RNA and it acts as a template in the protein biosynthesis. However, a detailed function of the genetic components was not known.

The present data now available, which are pertinent to the problem of regulating function of genetic loci RNA are not sufficient to suggest the mechanism of selective protein biosynthesis postulated in higher animal cells. In particular, the results observed in the studies on rat chromatins suggest that preparation of chromatins and se qence of DNA may have radiological inhibition, activity at specific genetic loci (histone-protein) and chemical components.

According to the above mentioned, the effect of whole-body X-ray irradiation with lethal dose(100 r) of X-ray on the regulation of genetic function of rats was studied. The function was analysed by estimating proteins and DNA-dependent RNA biosynthesis, *in vitro*, from C^{14}ATP to assess a priming activity for RNA and RNA polymerase obtained from Eacherichia coli.

The purpose of this paper is to demonstrate that a number of chromatin obtained from spleen, liver, testis and brain of rats can be a template for DNA-dependent RNA synthesis catalyzed by RNA polymerase.

II. Materials and Methods

C 14-adenosine triphosphate (ATP) was obtained from Japan Radioactive Association.

Male Wistar rats weighing 200-270 gm were used. The rats were conditioned for two weeks prior to use, and they were divided into two groups, 20 rats being distributed in each group.

Ten rats from each group were separated and used as control, and the remainders were subjected to whole-body X-ray irradiation. The animals of first group received 1000 r at the ratos dose of 10.5 r per minute by a 200 K Vp deep-therapy machine with 0.5 mm Cu and 0.5 Al filters.

The each group consisted of ten rats were sacrificed at 24 hours from the irradiation. However, the irradiation for the period longer than hours was eliminated from the experimental design because of their extremely poor physiological conditions.

Immediately after the sacrifice of the animals, four organs were removed. The organs obtained from 20 rats were washed by means of Kreb Ringer's solution and stored at -70°C after cooling by liquid nitrogen.

Chromatins were prepared from spleen, liver, testis and brain by Dahmus and Bonner method and examined within one week of storage 0°C.

1. Isolation of Chromatin

Chromatin were isolated from spleen, liver, testis and brain of the experimental rats. Rat organs were frozen in liquid nitrogen immediately after slaugher. The frozen tissue was broken into small pices and stored at -70°C before use.

About 5 g of frozen rat organs were homogenized by means of Waring Blender with 100 ml. of saline-EDTA (0.075 M-NaCl and 0.024 M sodium-EDTA, pH 8) for 1.5 minutes at 85 V.

The homogenete was next filtered through four layers of cheese cloth and then through two layers of Miracloth (Chicopea Mfg. Co., Millton., N.j.) followed by centrifugation at 1500 xg for 15 minutes. The sediment was sucessively washed with 40ml. of saline-EDTA and 40 ml. of tris buffer (0.05 M, pH8), at 1500 xg for 15 minutes.

The final sediment was homogenized by hand (Teflon homogenizer) with 20 ml. of tris buffer and then sedimented at 10,000 xg for 15 minutes. This procedure was repeated twice. The final sediment was suspended in 15 ml. of tris buffer.

The final homogenate in 11.6 ml. volume was mixed with 58 ml. of 1.7 M-sucrose solution. And centrifuged at 25,000 r.p.m. for hours in the Spinco SW head centrifuge.

Approximately 70% of the RNA in the suspension was recovered in a state of gelatious pellet. The pellet was resuspended in 0.01 M tris buffer (pH 8), and dialyzedagainst the same buffer overnight. The dialyzed suspension was next sheared in a Virtis homogenizer at 40 V for 3 minutes, stirred for 30 minutes and then centrifuged at 10,000 xg for 30 minutes.

Approximately 80% of DNA in the sheared suspension was reccovered in the supernatant fraction. This chromatin could be stored in an ice box for at least one week without any appreciable change in its properties.

2. Preparation of RNA polymerase

RNA polymerase was prepared from early phase cells of Escherichia coli, strain B, and purified according to the method of Chamberlin and Berg (1962) up to the stage of fraction 3 (F. 3). In some experiments the enzyme was further purified by DEAE-cellulose chromatography up to the fraction 4 (F. 4).

3. Incubation and assay for RNA polymerase

The final concentrations of individual in 0.5 ml. of incubation mixture were as fellows: 4 mM $MgSO_4$, 1 mM $MnSO_4$, -Mercaptoethanol, 0.4 mM each GTP, UTP, and CTP, in 40 mM tris buffer(pH 8), 0.1 uC C14-ATP, 30-70 ug RNA polymerase and 5-30 ug DNA, equivalent to chromatin.

The tube containing the reaction mixture incubated at 37°C for 20 minutes under shaking at 150 cycles/minute. The reaction was stopped by the addition of 10% trichloroacetic acid, and acid-insoluble material was collected by filtration through a membrane filter. The pellet was wash three times with 5 ml. of cold trichloroacetic acid, and then with 95% ethanol, followed by ether. The glutinous residue was dries, suspended in formic acid on aluminumplanchets, and subjected the determination of radioactvity by a Gas-flow counting system.

4. Chemical analysis

Deoxyribonucleic acid and ribonucleic acid werfe factionated by the Schmidt-Tannhauser procedue(1959) DNA was determined by the diphenylamine method (Bufton, 1956). using calf thymus DNA (sigma) as a standard, and RNA, by the orcinol reaction (Dische and schwarz, 1937) using yeast RNA(sigma) as a standrad.

Histone contained in chromatin suspension was added to 5 M NaCL solution and this was made to the final concentration of 2 M NaCL, and stirred for one hour in ice water. To this was added 14 N HCl to make 0.25M concentration. This was incubated at)°C with occasional shaking, the centrifuged at 14,000 xg for 30 minutes, the supernatant (histone fraction) was added 50% trichloroacetic acid and centrifuged at 3,000 xg for 5 minutes. The precipitate was washed with ethanol, and dried.

To the dried material was added to 1 N NaOH in order to neutralize and the amount of proteid contents was determined by the method of Lowry et al. (1951). using bovine serum albumin (sigma) as standrad.

III. Experimental Results

1. The ratio of components in chromatin with respect to DNA, RNA, total protein, and histone-protein.

In order to obtain the specific activity of substances, an extensive purification was carried out and recovery of each component was not corrected at the end of purification proceture.

Table 1. Ratios of RNA, DNA, and protein in chromatin obtained from organs of rat

Organ	Treatment	Ratio of protein/DNA	RNA/DNA	Histone/DNA
Spleen	Control	1.67	0.0217	1.41
	Irradiated	1.74	0.0442	1.47
Liver	Control	2.44	0.0694	1.63
	Irradiated	2.47	0.0910	1.79
Testis	Control	2.29	0.0849	1.41
	Irradiated	2.36	0.101	1.33
Brain	Control	3.21	0.168	0.613
	Irradiated	2.48	0.129	0.665

* Total protein (Histone+Residual protein)
** 1,000 r, Whole body irradiation

The results indicated that different tissues vary greatly with respect to their total protein: DNA ratio and RNA: DNAratio. There was essentially no detectable RNA and the amount of total protein was considerably less than in

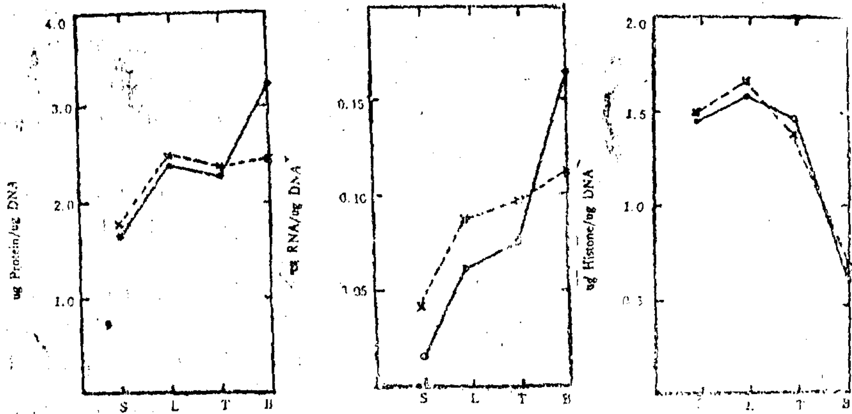


Fig. 1. Ratio of Component(RNA, DNA, and Protein of Chromatin isolated from Rat Organs S. Spleen. L. Liver. T. Testis. B. Brain. ....Contro ________Irradiated.

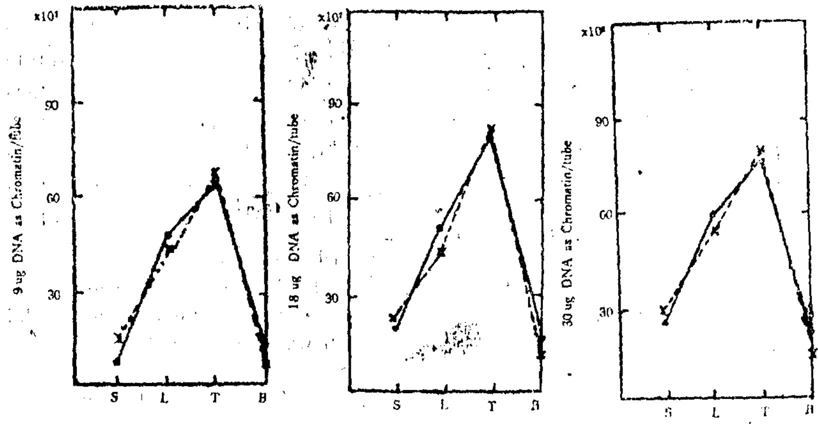


Fig. 2. RNA Synthesis *in vitro*, Directed by Chromatin from organs of Rat S. Spleen L. Liver T. Testis B. Brain ....Control. ________Irradiated.

Table 2. RNA synthesis *in vitro*, directed by chromatin obtained from organs of rat

Treatment	Incorportation of C^{14}-ATP cmp/tube					
	Control			Irradiated		
DNA/tube, as chromatin	9 μg	18 μg	30 μg	9 μg	18 μg	30 μg
Spleen	1279	1961	2473	1659	2159	2861
Liver	4772	5365	5938	4736	4624	5221
Testis	6500	8036	7678	6989	8148	7834
Brain	1516	1630	1922	1141	1418	1508

* 1,000, r. Whole body irradiation
30 μg RNA polymerase/tube

any other chromatin examined.

In this chromatin, values were spleen 1.44, liver 2.46, testis 2.33, brain 2.85 for the total protein: DNA no significant difference was observed between the control and irradiated in the same organs.

2. RNA synthesis *in vitro* directed by chromatin obtained from organs of rats

In this experiment, was carried out to observe the effect of irradiated on the *in vitro* synthesis of RNA directed by chromatin in different organs.

The results indicate that RNA:DNA ratio of brain decreased by irradiation. It was found that the histone-proteein:residual protein ratio for spleen 4.70 liver 2.14 testis 1.27 and brain 0.69 was resulting, where as a substantial difference in he ratio was observed between each organs it was not effected by irradiation.

The RNA synthesis *in vitro*, directed by chromatin from organs of irradiated rat was always higher than those of control and the DNA as chromatin isolated from

testis fraction showed the highest.

3. The effect of Actinomycin D on the RNA synthesis *in vitro*, directed by chromatin from organs of control and irradiated rats

The effect of Actinomycin D on the Rna synthesis *in vitro*, was carried out. Actinomycin D has been shown to inhibit DNA-dependent RNA synthesis both in intact cells and *in vitro*.

Table 3. Effect of Actinomycin D on the RNA *in vitro* directed by chromatin from organs of control and irradiated rats

Organ	Treatment	no addition	Actinomycin*	% of Inhibition
Spleen	Control	1961	395	79.9
	Irradiated	2159	529	75.5
Liver	Control	5365	1259	76.5
	Irradiated	4624	1205	73.9
Testis	Control	8036	1018	87.3
	Irradiated	8148	1006	87.7
Brain	Control	1630	702	56.9
	Irratiated	1418	629	55.6

* 10 μg/tube

** 1,000 r, Whole body irradiation
30 μg RNA polymerase and 18 μg DNA as chromatin tube

The results indicate that Actinomycin D elevated the inhibitory action on the RNA synthesis *in vivitro* directed by chromatin from organs of the control and irradiated rats, the testis which showed a higher value among test organs.

It seems that Actinomycin S is capable of displacing all but firmly bound protein from the DNA.

4. The stimulating effect of ammonium sulfate *in vitro* on RNA synthesis directed chromatin from organs of control and irradiated rats.

The stimulation effect of ammonium sulfate, *in vitro* on RNA synthesis was investigated.

Table 4. Stimulating effect of $(NH_4)_2SO_4$ *in vitro* on RNA synthesis directed by chromatin from organs of control and irradiated

Organ	Treatment	Incorporation(cpm/tube) & % of Activation***		
		$(NH_4)_2SO_4$ OmM	$(NH_4)_2SO_4$ 32 mM	$(NH_4)_2SO_4$ 80 mM
Spleen	Control	2429(100)	4281(176)	4905(202)
	Irradiated**	3401(100)	4738(139)	5648(166)
Liver	Control	6041(100)	9731(161)	11270(187)
	Irradiated	5595(100)	8527(152)	11460(205)
Testis	Control	7163(100)	9959(139)	12450(174)
	Irradiated	6475(100)	8429(127)	11370(175)
Brain	Control	1565(100)	1801(115)	2023(129)
	Irradiated	1023(100)	1354(132)	1595(156)

* final concentration

** 1,00 r, Whole body irradiation

*** Value of incorporation without addition of $(NH_4)_2SO_4$ was taken as 100 %

The results indicated that ammonium sulfate on RNA synthesis directed by chromatin was caused an increased in the priming activity of spleen, liver, and testis from control and irradiated rats, but the effect was slight in brain.

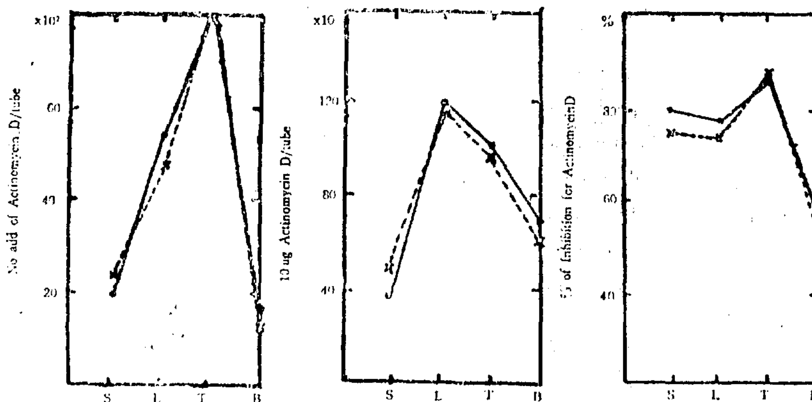


Fig. 3 Effect of Actinomycin D on the RNA synthesis *in vitro* directed by Chromatin from Organs of Rats
S. Spleen L. Liver T. Testis B. Brain ....Control. ___Irradiated.

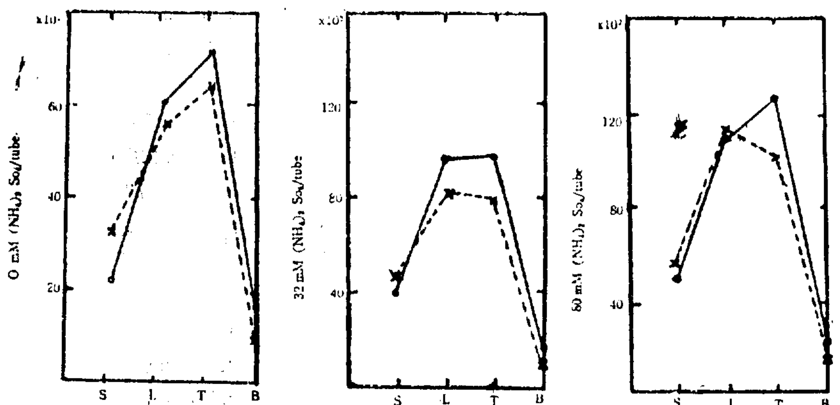


Fig. 4. Stimulating effect of $(NH_4)_2SO_4$ *in vitro* on RNA synthesis directed by Chrom atin from Organs of Rats
S. Spleen L. Liver T. Testis B. Brain ....Control. ________ Irradiated.

Ammonium sulfate may cause the dissociation of histone electrostatical to DNA as in chromatin, thus increasing the priming activity and the stimulating activity of ammonium sulfate different in by the property of chromatin.

IV. Discussion

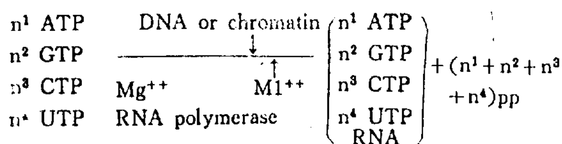
Whole body irradiation of rats results in, at the early stage, a simple reaction to the physical stimulus of irradiation. However, the late reaction of irradiated organs contributed to the development of radioation sickness and the lethlity.

Irradiation of DNA with 1000 r under a condition identical with those used in the present experiments has been shown to proceduce single and double srand breaks in the DNA, which are perhaps accompained by small single-strand regions (Harrington 1963, Harrington and Ricanati 1963).

The change inpriming activity caused by X-ray irradiation, therefore, may attribute to the production of cross-links.

The following reaction was employed the priming activity of chromatin RNA synthesis *in vitro*, by incorporation of 8-C^{14}-ATP into RNA.

Graph 1. All reaction formulas are schematically given in graph 1.



RNA polymerase was preparated by ch amberlis and Berge merge method from the E. coli strain.

The chromati of each organs shows the priming acivity of the complete reaction system to some extent. The complete lack in incorporation of C^{14}-ATP in the reaction system without RNA polymerase appears to be due to the absence of endogenous RNA polymerase in the chromatin preparation used in these experiment.

The amount of RNA from bound to the chromatin was increased in with the view of radiological inhibition. But it is conceivable that the RNA synthesis in spleen and testis should be increased temporating for some sort of bound such the recovery of damage. Their problem remains to be solved in future.

The effect of the irradiation on priming activity was unique, in that priming activity was decrased when high concentrations of DNA were used in the assay mixture and it was increased when the low concentrations were used.

It was shown by ammonium sulfate that Histone: DNA ratio of brain chromatin indicated a half value of those of other organs. A little amount of histone obtained by dissociation due to the weak stimulating effect of ammonium sulfate, seems to indicate that brain itself showed low priming activity compared which are known by residual protein involved.

It is true that the inhibition of DNA synthesis under the exposure to ionizing radiation may have more inportant role in the general metabolism than the DNA synthesis in more developed tissue. Cell metabolism is

controlled by the "effector-DNA-RNA-protein synthesis" which contstiutes the mechanism of transcription of genetic information in the endocrine system.

V. Summary

1. Within experimental chromatin, the total protein: DNA ratio did not vary in the same organs of control and irradiated rats. However, the amount of RNA and total protein associated with the DNA varied considerably among the different types of chromatin.

In particular, the content of chromatin was the control tissue. RNA and total protein ratio of chromatins from brtain, liver, testis and spleen declined with experimental organs.

2. There was the same quantitative relationship between the amount of RNA and the amount of histone-protein associated with DNA in chromatin.

3. RNA: DNA ratio of chromatin showed 1.5-2 times increas in the irradiated organs except brain. However, RNA: DNA ratio was decreased in chromatin by irradiation.

4. Histone-protein:residual protein ratio was greatly varied among the organs. However, the effect was not found by irradiation.

5. Priming activity of chromatin showed a higher value in testis and the activity was greater in organs with higher metabolic activity.

6. Inhibition of Actinomycin D is observable in chromatin from testis, liver, spleen and brain declined without relationship between irradiated and non-irradiated conditions. Ammonium sulfate showed increased priming activity by the electrostatic dissociation of DNA and histone in chromatin on the stimulation depending on property of chromatins.

7. It is suggested that the results support a proposal that testis and spleen of highly sensitive to irradiation should an increase in the priming activity whereas brain and liver of lower sensitivity decreased in the activity

VI. References

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