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

**Su Nam, Han**

*Dept. of Veterinary Medicine, College of Agriculture, Seoul National University*
*Suwon, Korea*

=國文抄錄=

## 白鼠臟器에서의 Chromatin의 分離와 그 RNA 合成能에 미치는 X-線全身照射의 影響에 關한 硏究

서울大學校 農科大學 獸醫科

**韓 壽 南**

近年 高等動物細胞에 있어서 遺傳子의 本體인 DNA에서 RNA를 經過해서 特異的인 蛋白質의 生合成에 到達하는 經路에 對해서는 많은 硏究에 의해서 確立되어졌으나 그 調節機構에 對해서는 不明한 點이 많다. 個體, 器官, 細胞內構造 及 DNA의 準位에서의 放射線의 障害에 對해서도 硏究되고 있으나 所謂 放射線感受性 及 非感受性의 各臟器에서 分離한 Chromatin (DNA-Histone-殘餘蛋白의 高次構造結合體)에 對한 DNA, RNA, 全蛋白質과 遺傳修飾體라고 생각되는 Histone-蛋白의 化學組成을 檢出했으며 兼해서 chromatin의 生物活性인 RNA 合成能(priming activity)에 對한 放射線의 影響을 調査하는데 意義가 있다.

電離放射線 照射에 의해서 生體의 DNA의 合成阻害가 잘 알려진 事實이나 分化한 生體組織에서의 DNA의 合成보다도 一般代謝에 重要한 役割을 한다는 것도 생각된다.

細胞의 代謝는 內分泌系等의 "Effector-DNA-RNA-蛋白合成이라는 情報轉遺機構에 의해서 制禦되어 있다.

이 硏究는 放射線生物學上 重要한 것은 論할 必要도 없으며 放射線同位元素標識化合物을 使用하여 生化學的으로 推究하였다.

### 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 highest in the irradiated tissue, and the lowest in the chromatin control tissue. RNA and total protein ratio of chromatins from brain, liver, testis and spleen declined with experimental organs.

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

3. RNA: DNA ratio of chromatin showed a 1.5～2 times increase 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 chromatins showed a

higher value in testis and the activity was greater in organs with higher metabolic activity.

6. Inhibition of Actinomycin D observable in chromatin for testis, liver, spleen and brain declined without relationship between irradiated and non-irradiated conditions. Ammonium sulfate in DNA of chromatin from histone showed increased priming activity with dissociation by Electrostatics. It may give different effect of ammonium sulfate on stimulation by property of chromatins.

7. It is suggested that the results support a proposal that the higher sensitivity of radioactive in testis, spleen by irradiated showed a increase and decrease lower-sensitivity of radioactive from brain, liver than did priming activity under the radioactive conditions.

## I. Introduction

It has been known that DNA which is a major component regulating genetic function in higher animals gives genetic massage 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 pertained 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 chro matins and sequence of DNA may have some influence on radiological aspects, on selective sensitivity, radiological inhibition, priming 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 (1000 r) of X-ray on the regulation of genetic function of rats was studies. The function was analysed by estimating proteins and DNA-depe-ndent RNA biosynthesis, in vitro, from $C^{14}$-ATP to assess a priming activity for RNA and RNA polymerase obtained from Escherichia 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 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 rats 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 experimental at 96 hours after the irradiation 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 was isolated from spleen, liver, testis and brain of the experimental rats. Rat organs were frozen in liquid nitrogen immediately after slaughter. The frozen tissue was broken into small pieces and stored at −70°C before use.

About five g of frozen rat organs were homog-

enized by means of the 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 85V.

The homogenate was next filtered through four layers of cheese cloth and then through two layers of Miracloth (Chicopea Mfg. Co., Millton. N.J.) followed by centrifuged at 1,500×g for 15 minutes. The sediment was sucessively washed with 40 ml. of saline-EDTA and 40 ml. of tris buffer (0.05 M, pH 8), at 1,500×g for 15 minutes.

The final sediment was homogenized by hand (Teflon homogenizer) in 20 ml. of tris buffer and then sedimented at 10,000×g 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 three hours in the Spinco SW 25 head centrifuge.

Approximately 70% of the DNA in the suspension was recovered in a state of gelatinous pellet. The pellet was resuspended in 0.01 M tris buffer (pH 8), and dialyzed against the same buffer for overnight. The dialyzed suspension was next sheared in a Virtis homogenizer at 40 V for three minutes, stirred for 30 minutes and then centrifuged at 10,000×g for 30 minutes.

Approximately 80% of the DNA in the sheared suspension was recovered in the supernatant fraction. The 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 Eschrichia coli, strain B, and purified according to the method of Chamberlin and Berg(1962) up to their fraction 3 (F 3). In some experiments the enzyme was further purified by DEAE-cellulose chromatography up to the fraction 4(F4).

3. Incubation and assay for RNA synthesis

The following ingredients were incubated; these are 4 mM $MgSO_4$, 1 mM $MnSO_4$ $\beta$-Mercaptoethanol, 0.4 mM each GTP, UTP, and CTP, in 40 mM tris buffer (pH 8), 0.1 $\mu$C per tube: one of the tubes was received $C^{14}$-ATP, and the other received 30~70$\mu$g RNA polymerase and the other of 5~30 $\mu$g DNA equivalent to chromatin. This gives a final volume of 0.5 ml. The tubes were incubated at 37°C for 20 minutes under shaking at 150 cycles/minute, after the incuba tion. The reaction was stopped by the addition of 10% trichloroacetic acid, and the acid-insoluble material was collected by filtration through a membrane filter. The pellet was washed for three times with 5 ml. of cold 5% trichloroacetic acid, and then with 95% ethanol, followed by glutinous ether. The residue was dires, suspended in formic acid on alumium planchets, and subjected the determination of radioactivity by a gas-flow counting system.

4. Chemical analysis

Deoxyribonucleic acid and Ribonucleic acid were fractionated by the Schmidt-Tannhauser procedure (1959). DNA was determined by the Diphenylamine method (Burton, 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 standard.

Histone contained in chromatin suspension was added to 5 M NaCl and this was made to the final concentration of 2 M NaCl, and stirred for one hour in ice water. This was added to 1~4 N HCl to made 0.25 M concentration. This was incubated at 0°C, with occasional shaking the centrifuged at 14,000× g for 30 minutes, and the supernatant (histone fraction) was added 50% trichloroacetic acid, and centrifuged at 3,000 ×g for 5 minutes. The precipitate was washed with ethanol, and dried.

To the dried material added was 1 N NaOH in order to neutralize and the amount of protein

contents was determines by the method of Lowry et al. (1951) using brovine serum albumin (Sigma) as a standard.

## III. Experimental Results

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

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

The results indicated that it can be observed that different tissues vary greatly with respect to their total protein; DNA ratio and RNA: DNA ratio. There was essentially no detectable RNA and the amount of total protein was considerably less than in any other chromatin examined.

In this chromatin, it found a value of spleen 1.44, liver 2.46, testis 2.33, brain 2.85 for the total protein: DNA ratio. Which is no significant was observed in the same organs between control and irradiated (Fig.1).

2. The RNA synthesis in vitro directed by chromatin obtained from organs of Rats.

In this experiment, in order to obtained effect

**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

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

| | Incorporation of $C^{14}$-ATP cpm/tube | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Control | | | Irradiated* | | |
| DNA/tube, as chromatin | 9μg | 18μg | 30μg | 9μg | 18μg | 30μg |
| Spleen | 1,279 | 1,961 | 2,473 | 1,659 | 2,159 | 2,861 |
| Liver | 4,772 | 5,365 | 5,938 | 4,736 | 4,624 | 5,221 |
| Testis | 6,500 | 8,036 | 7,678 | 6,989 | 8,148 | 7,834 |
| Brain | 1,516 | 1,630 | 1,922 | 1,141 | 1,418 | 1,508 |

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

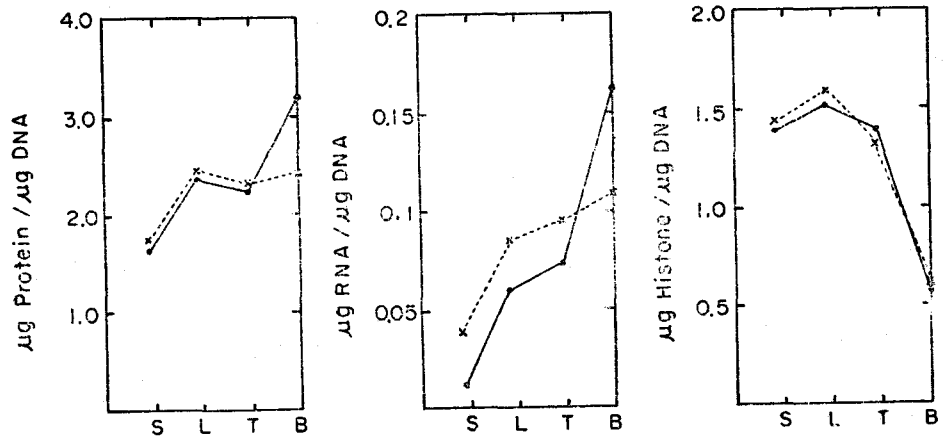



**Fig. 1.** Ratio of components (RNA, DNA, and Protein) of chromatin isolated from rat organs
S.: Spleen L.: Liver T.: Testis B.: Brain
—— Control. ····· Irradiated

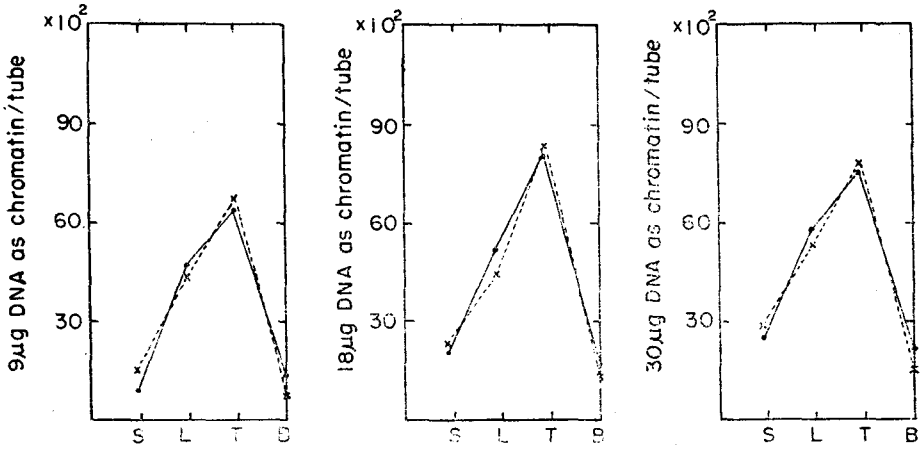



**Fig. 2.** RNA synthesis in vitro directed by chromatin from organs of rat.
S.: Spleen L.: Liver T.: Testis B.: Brain
—— Control. ····· Irradiated.

of irradiated was observed between each organs, and RNA synthesis in vitro directed by chromatin from organs of irradiated.

The results indicated that it observed RNA: DNA ratio of brain decreased by irradiation. It was found a value of spleen 4.70, liver 2.14, testis 1.27, brain 0.69 for the histone-protein: residual protein ratio, whereas a substantial difference in the 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 Actnomycin D on the RNA synthesis in vitro was carried out. Actinomycin

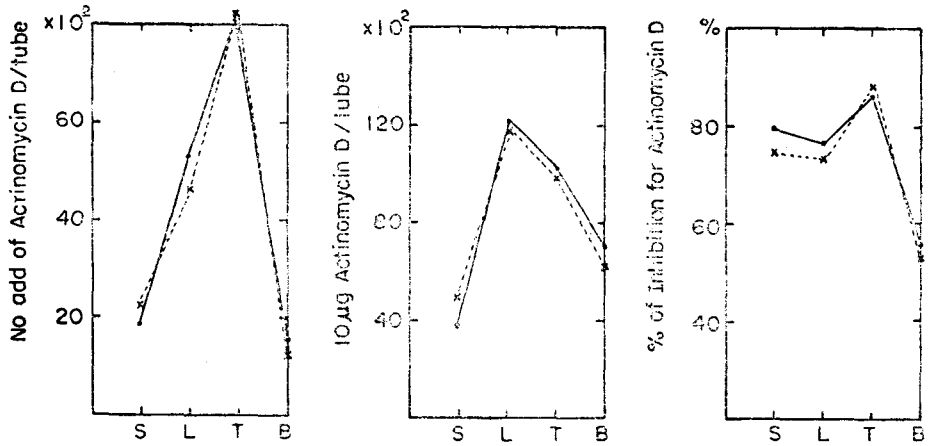



**Fig. 3.** Effect of actino mycin D on the RNA synthesis in vitro directed by chromatin from organs of rats.
S.: Spleen L.: Liver T.: Testis B.: Brain
—— Control. ····· Irradiated.

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

| Organ | Treatment | Incorporation, cpm/tube | | |
|---|---|---|---|---|
| | | no addition | Actinomycin* | % of Inhibition |
| Spleen | Control | 1,961 | 395 | 79.9 |
| | Irradiated** | 2,159 | 529 | 75.5 |
| Liver | Control | 5,365 | 1,259 | 76.5 |
| | Irradiated | 4,624 | 1,205 | 73.9 |
| Testis | Control | 8,036 | 1,018 | 87.3 |
| | Irradiated | 8,148 | 1,006 | 87.7 |
| Brain | Control | 1,630 | 702 | 56.9 |
| | Irradiated | 1,418 | 629 | 55.6 |

*10$\mu$g/tube

**1.000 r, Whole body irradiation
30$\mu$g RNA Polymerase and 18$\mu$g DNA as chromatin/tube

D has been shown to inhibit DNA-dependent RNA synthesis both in intact cells and in vitro.

The results indicate that effect of Actinomycin D on the RNA synthesis in vitro directed by chromatin from organs of the control and the irradiated rats was elevated of inhibitiory action on the testis showed a higher value among the test organs.

It is seens that Actinomycin D 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 by chromatin from organs of control and irradiated rats.

In this experiment, in order to stimulating effect of ammonium sulfate in vitro on RNA synthesis was carried out priming activity by chromatin from organs of control and irradiated rats.

**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 | $(NH_4)_2SO_4$ 32mM | $(NH_4)_2SO_4$ 80mM |
| Spleen | Control | 2,429(100) | 4,281(176) | 4,905(202) |
| | Irradiated** | 3,401(100) | 4,738(139) | 5,648(166) |
| Liver | Control | 6,041(100) | 9,731(161) | 11,270(187) |
| | Irradiated | 5,595(100) | 8,527(152) | 11,460(205) |
| Testis | Control | 7,163(100) | 9,959(139) | 12,450(174) |
| | Irradiated | 6,475(100) | 8,249(127) | 11,370(175) |
| Brain | Control | 1,565(100) | 1,801(115) | 2,023(129) |
| | Irradiated | 1,023(100) | 1,354(132) | 1,595(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 indicate that it can found stimulating effect of ammonium sulfate in vitro on RNA synthesis directed by chromatin from organs of control and irradiated rats was increase

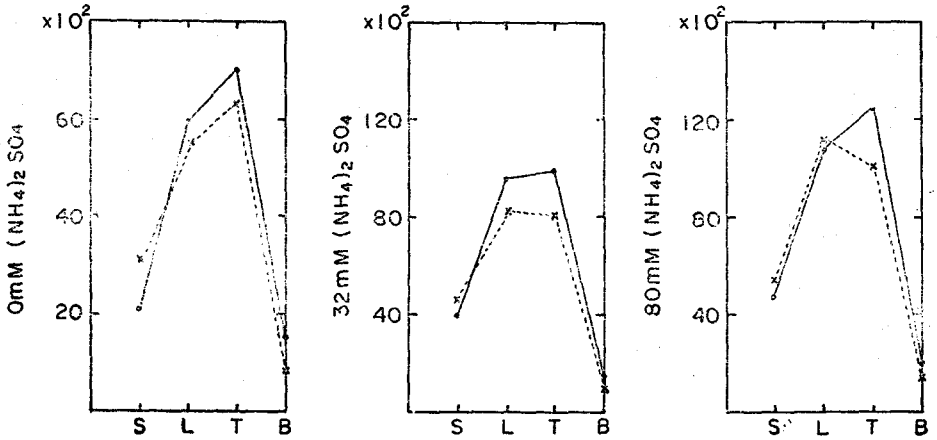



**Fig. 4.** Stimulating effect of $(NH_4)_2SO_4$ in vitro on RNA synthesis directed by chromatin from organs of rats.

S.: Spleen L.: Liver T.: Testis B.: Brain
—— Control. ····· Irradiated.

of priming activity from spleen, liver, and testis those of control rats, but it was slight in the brain.

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 ofchromatin.

## 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 radiation sickness and the lethality.

Irradiation of DNA with 1,000 r under a condition identical with those used in the present experiments has been shown to produce single and double strand breaks in the DNA, which are perhaps accompained by small single-strand regions (Harrington, 1963, Harrington and Ricanati, 1963).

The change in priming activity caused by X-ray irradiation, therefore, may attributed to the production of cross-links.

The following reaction was employed to the priming activity of chromatin in RWA 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 Chamberlis and Berge method from the E. coli strain.

The chromatin of each organs under priming activity of the complete reactory system to some extent. How affected the priming activity in present experiment, it suggest that the endogenous RNA polymerase activity in the sample of chromatin was cannot be found an the incorporation of $C^{14}$-ATP was not used with RNA polymerase in the reactory system.

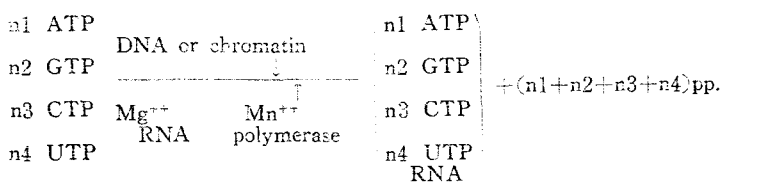
The amount of RNA from bound to the chromatin was increased in the irradiated animal. It seens that this is not in accordance with view of radiological inhibition. But it is conceivable that the RNA synthesis in spleen, testis should be increased temporating for some sort of necessity such the recovery of damage. And that is was expected in the future studies.

The effect of the irradiation on priming activity was unique, in that priming activity was decreased 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 dissociation due to the weak stimulating effect of ammonium sulfate, seens to indicated that brain chromatin from itself showed low priming activity compared with others which are known by residual protein involved.

It is true that the inhibition of DNA synthesis under the exposure to ionizing radiation therefore, showed that the separated one of intact tissue may have a important role in the general metabolism than DNA synthesis from tissue. In particular, the metabolism of cell now available, which pertain to the problem of the mechanisms of regulation of genetic transcription in endocrine system cell, is sufficient to suggest that "Effect-DNA-RNA-protein synthesis" and mechanism, may be alterative to the mechanism of selective repression postulated for genetic function.

Graph 1.



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